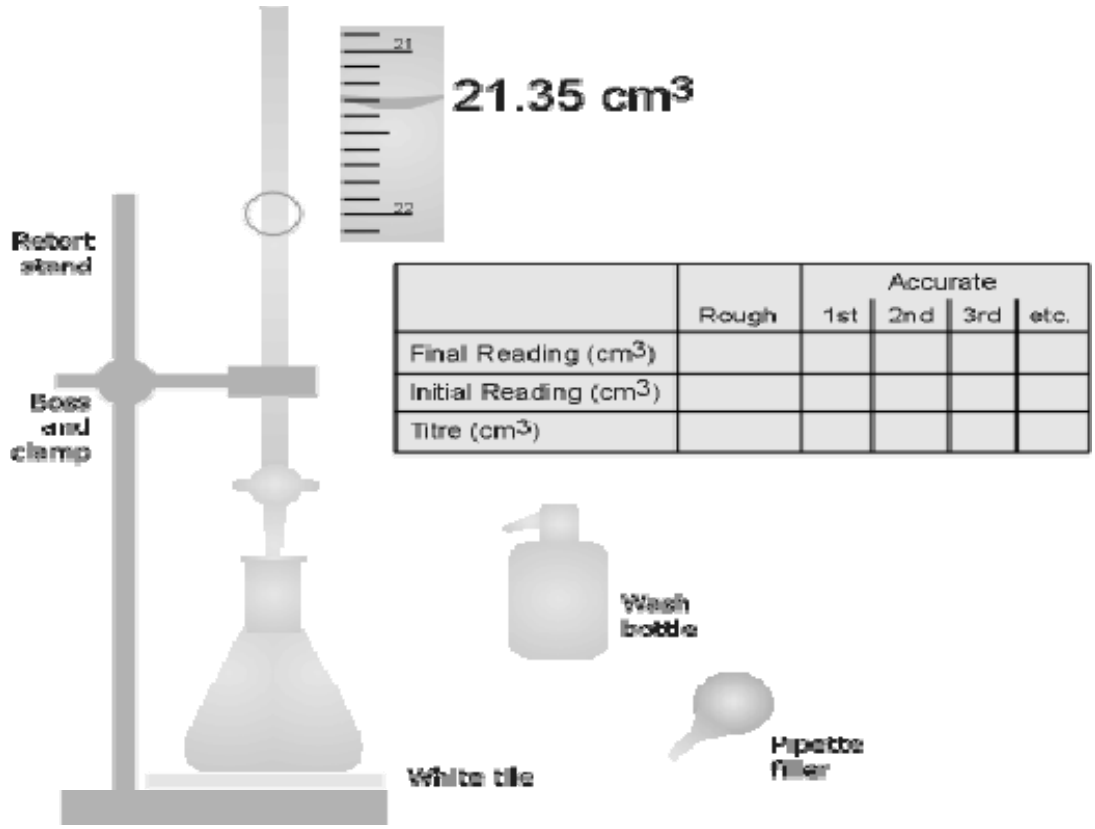
# NEW MEXICO
# WASTEWATER LABORATORY CERTIFICATION STUDY GUIDE

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**UTILITY OPERATORS CERTIFICATION PROGRAM**
SURFACE WATER QUALITY BUREAU
New Mexico Environment Department
P.O. Box 5469
Santa Fe, New Mexico USA 87502
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# NEW MEXICO WASTEWATER LABORATORY CERTIFICATION STUDY GUIDE

*Version I*

Prepared By

Douglas Roby

For The

**UTILITY OPERATORS CERTIFICATION PROGRAM**

SURFACE WATER QUALITY BUREAU
New Mexico Environment Department
P.O. Box 5469
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# INTRODUCTION

This "WASTEWATER LABORATORY CERTIFICATION STUDY GUIDE" has been created as a tool to assist Laboratory Technicians in New Mexico in preparation for taking the New Mexico Wastewater Laboratory Technician Level 1, 2 and 3 certification examinations. This guide is not intended to be a complete reference manual for technical information. Its purpose is to guide the reader to study material for each of the major subject areas for each of the classes. There is no implied claim that this study guide covers every possible point on which an technician may be tested. However, it is intended to be comprehensive in its coverage of the essential information for each examination.

This study guide is divided into eighteen chapters. Each chapter in the study guide has basic and advanced information intended to help the individual prepare for the certification examination.

The certification exams use several texts as reference manuals for exam topics. Each chapter of the study guide contains references to specific chapters of these manuals for those who wish to access more information on the topics covered in that particular section.

References: Standard Methods 18th, 19th, 20th and 21st.
Operation of Wastewater Treatment Plants, Volumes 1, 2 Sacramento

Special thanks to: NMED Utility Operators Certification Program for funding this Manual, and Special thanks to Mr. Robert Gott, NMSU WUTAP, Ms. Monica Peterson, OMI Farmington WWTP and Ms. Patricia Petrak, Tucumcari WWTP Laboratory.

DOUGLAS ROBY
OCTOBER 31, 2007

## NEW MEXICO ENVIRONMENT DEPARTMENT
## SURFACE WATER QUALITY BUREAU
## UTILITY OPERATORS CERTIFICATION PROGRAM

The New Mexico Water Quality Control Commission, through the New Mexico Environment Department (NMED), grants certification for competency to the operators of water and wastewater systems. The Utility Operators Certification Program (UOCP) conducts the testing for certification. Certain requirements must be met before an operator is eligible to take a certification examination.

An operator begins the process by completing a test application from the UOCP. Applications will only be accepted if they are submitted at least 30 days prior to the exam date. A certification officer will review each application to determine if the operator is eligible to take the requested examination. An application must be submitted every time a test is taken. Examinations are given several times a year at various locations around the state. A **fee** of **$25.00** for the WWLT1 and a **fee** of **$30.00** for the WWLT2 and WWLT3 examinations must be paid to **NMED Utility Operators Certification Program**. A check or money order for the proper amount **must** accompany each exam application.

Certificates must be renewed every three years. The renewal date will be the last day of the certificate holder's birthmonth following the third anniversary of the certificate. The UOCP also handles renewal of certificates. The **fee** for renewal is determined by an applicant's level of proficiency as listed in the currently effect Fee Schedule for **each** certificate.

The Utility Operators Certification Program maintains training credits for certified personnel only. Each Laboratory Technician must keep a record of all training credits earned. A record of current training credits may be obtained on the Online Database once an certified technician logs in. Anyone who intends to apply for New Mexico certification must include documentation of training credits when the application is submitted. Certified laboratory technicians that are taking higher level exams may also have to submit training credit documentation to update training record files at the UOCP.

All correspondence, including applications, should be mailed to:

**NMED – Surface Water Quality Bureau**
**Utility Operators Certification Program**
**P.O. Box 5469**
**Santa Fe, New Mexico USA 87502**

Please feel free to call the Utility Operators Certification Program to request information on exam application forms, exam dates and locations, or certification and renewal. The telephone number for the office is **(505) 827-2804**.

# IMPORTANT FACTS ABOUT OPERATOR CERTIFICATION

An operator, as defined by NM Water Quality Control Commission Regulations, is "any person employed by the owner as the person responsible for the operation of all or any portion of a water supply system or wastewater facility. Not included in this definition are such persons as directors of public works, city engineers, city managers, or other officials or persons whose duties do not include actual operation or direct supervision of water supply systems or wastewater facilities."

Under the Utility Operator Certification Act, "a certified operator is a person who is certified by the commission as being qualified to supervise or operate one of the classifications of water supply systems or wastewater facilities". Experience is "actual work experience, full or part-time, in the fields of public water supply or public wastewater treatment. Work experience in a related field may be accepted at the discretion of the commission". Any claim of related experience will be reviewed by the WQCC or its advisory body, the Utility Operators Certification Advisory Board.

The Advisory Board is an appointed "seven-member board from the certified water systems operators and wastewater facility operators to function with the commission to establish qualifications of operators, classify systems, adopt regulations and advise the administration of the Utility Operators Certification Act."

Experience that includes operation, maintenance or repair of water treatment and water distribution systems is accepted based on whether it is full or part-time. The Advisory Board will review and approve experience in other related fields, such as commercial plumbing or utility construction. Credit for part-time experience will be based on the percentage of time devoted to actual operation or maintenance. Full time water or wastewater laboratory experience may be counted as operator experience at a rate of 25% of actual experience. The credit for this experience will be determined by review of the Advisory Board.

## BASIC CERTIFICATION REQUIREMENTS

There are three basic requirements a laboratory technician must meet to qualify for New Mexico certification. All certified laboratory technician must have at least **one year** of actual experience in a laboratory or performing laboratory analysis. All levels of certification require **high school graduation or GED** (see substitutions.) All levels of certification require a certain number of **training credits** in laboratory education or related fields.

**BASIC CERTIFICATION REQUIREMENTS**

| | Experience | Training Credits | Education |
|---|---|---|---|
| Class 1 | 1 year* | 10 | HS Grad or GED* |
| Class 2 | 2 years* | 30 | HS Grad or GED* |
| Class 3 | 4 years* | 50 | HS Grad or GED* |

* - See Substitutions

## SUBSTITUTIONS

One year of additional experience may be substituted for the high school graduation or GED requirement for all classes **except** Class 4. Education may be substituted for experience or training credits in some cases. The education must be in water or wastewater related field. One year of vocational education can be substituted for up to one year of experience. Associates and Bachelor's degrees in a related field may be substituted for up to three years of experience and 50 training credit hours, depending on the amount of actual experience. The criteria for substitution of education for experience are as follows:

No more than one year (30 semester hours) of successfully completed college education in a **non-related field** may be substituted for an additional **six months** of the required experience.

**One year** of approved vocational school in the water and/or wastewater field may be substituted for only **one additional year** of the required experience.

An **associate's degree** for a two-year program in an approved school in the water and/or wastewater field and **six months** of actual experience in that field (which may be accrued before, during, or after the school program) may be substituted for the requirements of any level up to and including **Class 2**. An **associate's degree** for a two-year program in an approved school in the water and/or wastewater field and **twelve months** of actual experience in that field (which may be accrued before, during, or after the school program) may be substituted for the requirements of any level up to and including **Class 3**.

Completion of at least **three year**s of actual experience in the water and/or wastewater field plus **high school graduation** or equivalent, plus **15 semester hours** of successfully completed college education directly related to the water or wastewater field may be substituted for any level up to and including **Class 3**.

A **bachelor's degree** for a major directly related to the water or wastewater field plus **two years** of actual experience in that field may be substituted for any level up to and including **Class 3**.

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# Chapter 1 Introduction

In early 1972, Congress enacted the Clean Water Act providing funds for construction and operation of wastewater treatment systems, and the training of professional wastewater operators. The nation's rivers and lakes had become major wastewater disposal sites and had turned healthy ecosystems into septic and stench filled dump areas. Congress implemented the NPDES (National Pollutant Discharge Elimination System) self-monitoring program requiring industrial and municipal systems to clean up their discharge or face financial and criminal penalties. Operator certification began to be enforced to develop professional operators who understood the importance of the operation, maintenance, and management of wastewater treatment plants. Wastewater systems were required to have a permit to discharge and that permit established limits on the levels of contaminants that could be discharged. Laboratory analysis was required to document the level of these contaminants. Previously, in many instances, laboratory personnel, the operator, and management never communicated and treatment was poor. The NPDES system helped bring the parties together since they all now became responsible for the effluent quality.

## Effects of Discharging Wastes

There are two routes by which pollutants enter receiving waters: point sources, such as a wastewater treatment plant discharge pipes, and non-point sources, such as runoff from feedlots and agricultural fields. In New Mexico, point source discharges can be made to streams, rivers and lakes or can be made to underground aquifers, because both surface and ground water discharges are common. Wastewater treatment plants, and wastewater treatment plant operators, control point source pollution. Non-point source discharges also occur to both surface and ground waters in New Mexico. Because non-point source pollution is not limited to a single outfall pipe, it is very hard to control. The type of pollution, and the impairment of the receiving water are related to the type of waste being discharged. For example, heavy rains may wash animal waste from feedlots and dairies or fertilizer and pesticides from agricultural fields into the river.

The NPDES discharge permit system was established to help minimize the environmental devastation and health effects caused by poor treatment. One environmental problem caused by poor treatment is *oxygen depletion* in the receiving streams. Many aquatic organisms, including fish, need dissolved oxygen (O2) to survive. These types of organisms are referred to as **aerobes** and the environment they live in as **aerobic.** If untreated non-toxic waste is discharged into receiving streams, rivers, or lakes it becomes food for the microorganisms. In a normal ecosystem, the microorganisms will utilize the naturally occurring dissolved oxygen in the water to stabilize the waste and convert it into energy and more microorganisms. As the aerobic bacteria multiply, they require more and more dissolved oxygen to sustain their growing numbers. When the waste is consumed, most microorganisms will die and the oxygen level will return to its original level. However, if the waste concentration is very high or additional waste is added downstream, the digestion process will continue. When the population of aerobic bacteria grows large enough, the oxygen available in the river will be depleted. If this happens, the aerobic microorganisms (and most other aerobic organisms) in the river, die. If all of the dissolved oxygen in a river has been consumed, another type of organism begins to grow in the oxygen depleted environment. **Anaerobic** bacteria live in conditions where there is no dissolved oxygen (septic). Anaerobic bacteria breathe by using the oxygen that is chemically combined with other elements, such as nitrate ($NO_3$), and sulfate ($SO_4$). When anaerobic bacteria use the oxygen from nitrate and sulfate for respiration,

nitrogen gas ($N_2$) and hydrogen sulfide ($H_2S$) gas is released as a byproduct. Hydrogen sulfide, sometimes called sewer gas, smells like “rotten eggs” and is very dangerous due to its explosive, toxic and suffocating characteristics. Furthermore, anaerobic bacteria release other objectionable by-products, such as organic acids, methane gas and nutrients, all of which can further harm the natural environment in the receiving stream. Because of the chain of events that the discharge of organic waste sets into motion, one of the principal goals of wastewater treatment is to prevent as much of the waste from getting into receiving waters as possible.

## Sources of Wastewater

Wastewater consists of two things- waste and water. Wastewater treatment plants are designed to treat the waste. Operators and laboratory technicians are responsible for effectively operating, maintaining, and monitoring the system. Waste originates from a wide variety of industrial, commercial, and residential sources and its characteristics determine what treatment is required. Most commercial and residential wastewaters contain normal "domestic" contaminants such as fecal matter, plant and animal waste, oil and grease, detergents, rags, and sediments such as sand. On the other hand, industrial waste is very unpredictable and can contain toxic chemicals and metals, very strong organic wastes, radioactive wastes, large amounts of sediment, acidic/caustic waste or high temperature waste. Industrial users often have pretreatment permits which limit the type and amount of contaminants that can be discharged into the collection system. These pretreatment limitations help prevent overloading or interfering with the operation of the municipal system.

Additional contaminants can enter the collection system from line breaks, or storm water runoff from streets and parking lots. This wastewater often contains motor oil, gasoline, pesticides, herbicides, and sediment (sand and gravel).

## Wastewater Characteristics

The term wastewater characteristic is used to describe the components and condition of the waste stream. Wastewater is a very complex mixture of all sorts of materials having different characteristics. Wastewater components can be identified specifically but more commonly the mixture is characterized using general characteristics. One way to describe the waste is to divide it into two broad categories: ***organic*** wastes and ***inorganic*** wastes. **Organic Wastes** are those substances that *contain the element carbon (C)* and are derived from something that was once living. Examples include: vegetable and animal food waste, fecal matter, grease, proteins, sugars and paper. **Inorganic Wastes** are those substances that *do not contain carbon* and are derived from something that was not living. Examples include: metals, minerals, sand, acids and bases. Common wastewater tests used to measure the organic concentration is Biochemical Oxygen Demand (BOD) / Chemical Oxygen Demand (COD). These tests will discussed in a later chapter.

### Solids

Another method used to describe waste is to divide it into different solid classifications. Wastewater is 99.9% water and only 0.1% solid but this small solids concentration can be classified by its physical and chemical characteristics. The common categories of solids include:

Total Solids: All the solids present after evaporation of the water.

Total Dissolved Solids (TDS): Solids which pass through a 0.45 micron glass fiber filter. Dissolved solids represent primarily minerals and small organic molecules such as sugar. Most of the TDS is inorganic.

Total Suspended Solids (TSS): Solids which do not pass through a 0.45 micron glass fiber filter. Total suspended solids represent large materials such as plant and animal debris, microorganisms, fecal material and sand. TSS is primarily organic.

Settleable Solids (SS): Settleable solids are part of the total suspended solids and a defined as solids which settle within 1 hour. These solids are generally very large and have a specific gravity that allows them to settle to the bottom of an imhoff cone within 1 hour. These solids usually represent sand, large plant and animal debris and some fecal material. Microorganisms are too light to settle.

Non-Settleable Solids (Colloidal): Non-settleable solids are the suspended solids which do not settle after 1 hour. The majority of these solids don't weigh enough to settle quickly. Within this group is a subgroup called colloids. These solids may be large enough to get caught on the glass fiber filter but do not settle because they carry similar charges. The charges on the colloids repel each other and interfere with settling. Most of these solids are fecal material and microorganisms.

Volatile Solids (VS): Solids which burn off at 550$^{o}$C. This would be similar to burning logs in a fireplace. The organic material making up the logs is combusted to carbon dioxide which blows away. The material remaining after the fire goes out is called ash or fixed solids and represents the inorganic materials present.

Fixed Solids (Ash): Solids which do not burn off at 550$^{o}$C.

Solids analysis will be discussed in a later chapter.

**Nutrients**

Nutrients are substances that are required for the growth of living plants and animals. Major nutrients include nitrogen (N) and phosphorous (P). Both are found in wastewater in various forms. Nitrogen is typically present in the influent in the forms of ammonia (NH3) and organically bound nitrogen. Both nitrogen compounds can be measured by the Total Kjeldahl Nitrogen (TKN) test. Nitrogen may be present in effluent as ammonia, organically bound nitrogen or even nitrite ($NO_2$) and nitrate ($NO_3$). Phosphorous is present in influent and effluent primarily in the form of phosphates ($PO_4$). When large amounts of nutrients are allowed to enter into rivers and lakes, they can cause problems by increasing the growth of plants, such as algae. If the algae growth is extensive, it can choke up the water body. As the lower layers of algae are blocked off from the sun, they die and end up as food for bacteria. This begins the oxygen depletion cycle again.

**Temperature**

Influent wastewater temperature can effect the operation of the wastewater treatment plant. Temperature effects solid settling velocity in clarifiers, microorganism activity and oxygen solubility in aeration basins and receiving stream environments. Seasonal temperature changes can cause the treatment plant to operate less efficiently. This is most evident during cold weather because the growth and activity of the microorganisms in the treatment plant slows down considerably. High temperature discharges can disrupt the natural ecology in surface waters by encouraging the growth of algae and aquatic plants that would not normally be as abundant.

**pH**

pH is a measurement of water's acid or alkaline condition. pH is measured on a scale that spans from 0 to 14 with 7 being the middle, or neutral value. pH values lower than 7 are progressively acidic while pH values that

are higher than 7 are progressively basic, (also called caustic or alkaline). Most living organisms live in a narrow pH range that is near neutral. If an effluent has a pH that is higher or lower than that of the receiving water, the organisms in the receiving water may be killed off. In addition, if the pH of the influent coming into a wastewater treatment plant changes rapidly and significantly, the plant treatment processes may be disrupted. The microorganisms used by the wastewater treatment plant to digest the organics may also be killed off.

**Microorganisms**

The foundation of wastewater treatment is the use of microorganisms to digest the waste. The microorganisms convert the waste into new cells which then form a settleable solid called floc. This floc settles in the secondary clarifier hopefully leaving a clear liquid called supernatant. The clear wastewater effluent can be discharged to the receiving stream after any remaining microorganisms are disinfected. The vast majority of microorganisms used in waste treatment originate in the intestines of warm blooded animals. These microorganisms are called fecal coliforms. The most famous fecal coliform and the one common to human intestines is E. coli. E. coli are beneficial bacteria and are not typically **pathogenic.** However, pathogens may be present in wastewater and the laboratory technician should always take precautions to avoid direct contact with wastewater. There are no "kind" pathogens!!!

**Pathogenic Organisms**

**Any** wastewater can potentially contain disease causing organisms, or **pathogens**. Pathogens can cause extreme illness and even death and should be killed prior to effluent discharge. The operator and laboratory technician are always potentially exposed to these organisms. Common diseases caused by water borne pathogens include; typhoid, hepatitis, cholera, dysentery and polio. Pathogens fall into the following categories:

***Pathogen Type***

Bacteria - Cholera, Shigella, Salmonella
Viruses - Norwalk, Rotavirus, Adenovirus
Protozoa -Giardia lamblia, Cryptosporidium

If pathogens are discharged without treatment into the natural environment, they will pose a danger to anyone that is exposed to them. For this reason, one of the primary goals of wastewater treatment is to disinfect the effluent before it is discharged. Regardless of the method of disinfection, the test used to measure the effectiveness of disinfection is the same. Because so many different types of pathogens could be present, it is not practical to perform laboratory tests to detect each pathogen. Instead, non-pathogenic microorganisms are used to indicate the possible presence of pathogens. The microorganism used to indicate the possible presence of pathogens is E. Coli in the Fecal Coliform group. If E. Coli are detected in water, it indicates waste from humans is present and therefore pathogens could be present. If E. Coli are not present, it is likely all the pathogens have been killed and the wastewater is safe to discharge.

**Toxins**

Several substances in wastewater can be toxic if not properly treated. One of these is ammonia. Ammonia is usually the main form of nitrogen present in raw domestic wastewater, while industrial wastes may or may not contain ammonia. Most people that have owned a fish tank are aware that even small amounts of ammonia can kill aquarium fish. Similarly, large-scale fish kills can occur when effluent containing ammonia is discharged into receiving waters. In the case of point source discharges, ammonia toxicity depends upon the pH and temperature as well as the dilution factor in the receiving water. Warm temperatures and high pH make ammonia much more toxic to fish. If the

discharge is to a small stream where only a little dilution occurs, ammonia can cause serious problems. The laboratory may be required to perform a bio-monitoring test to show the effluent is not toxic to organisms in the receiving stream.

Another toxin of concern is the residual chlorine left over from the disinfection process. If residual chlorine is discharged into a receiving water, even in small amounts, it can also be toxic to fish. For this reason, chlorinated effluents must often be ***dechlorinated*** to eliminate all of the measurable residual chlorine. Many systems have gone to UV light for disinfection to eliminate chlorine residual problems.

Last, with regard to toxins, is the problem of ground water contamination from nitrogen compounds such as nitrate (and ammonia that is converted into nitrate by soil bacteria). If nitrate contamination occurs in an aquifer that is used for drinking water, the nitrate could cause methemoglobinemia, also called *blue babies* syndrome, in infants that drink the water. Methemoglobinemia is a condition where the blood's ability to carry oxygen is greatly reduced. In New Mexico, wastewater treatment plants that discharge to the land are required to have a groundwater discharge permit. This permit usually monitors for total dissolved solids, chloride, nitrate and total kjeldahl nitrogen (TKN).

## Wastewater Treatment Overview

Water that has been discharged by various residential, commercial, and industrial users into a collection system is defined as wastewater. Wastewater consists of every conceivable contaminant from dirty bath water to radiological waste. A treatment system uses different physical, biological, and chemical processes to remove contaminants to avoid harming the receiving stream and downstream users.

**Pretreatment**

In a typical municipal treatment system, the first treatment process is the physical process of screening. A set of steel bars spaced about 1/2" to 2" apart removes large objects such as rags, rocks, rubber products and any other large screwball things put down the sewer. These materials interfere with downstream equipment operation and are collected and removed to the landfill. The next process is generally a grit chamber designed to remove heavy inorganic materials such as sand, eggshells, and coffee grounds. This chamber reduces flow to 1 foot per second to separate grit from heavy organic settleable solids. This gritty material is abrasive to downstream pumps and valves and takes up valuable space in tanks and digesters. Grit is also hauled off to the landfill. The lab technician can measure the volatile solids present in the grit chamber to determine if the grit chamber velocity is acceptable. A high volatile solids concentration would indicate heavy organics are settling and the grit chamber is too slow.

**Primary Treatment**

Following grit removal, the wastewater enters a large tank called a primary clarifier. The flow through the primary clarifier is slowed down even further to allow the heavy organic suspended solids (sludge) to separate from the light suspended solids and floatable solids (scum). The heavy settleable solids (sludge) settle to the bottom of the clarifier, where they are eventually pumped to the digester. The floatable solids (scum) are taken to the landfill. Because the remaining solids are small and light, physical settling is no longer effective because it would take too long. The lighter suspended solids move on to the secondary treatment unit. The lab technician can measure the settleable and suspended solids to determine the tank removal efficiency. The primary clarifier should remove:

90-95% of the settleable solids
40-60% of the suspended solids
20-50% of the BOD

Changes in the % removal may indicate broken clarifier flights or short circuiting.

Volatile solids could also be run to validate that the grit chamber is working correctly. The presence of high fixed solids may indicate the grit chamber was flowing too fast and not allowing the grit time to settle.

**Secondary Treatment**

Secondary treatment involves physical, chemical and biological processes that convert the incoming light suspended solids and dissolved organic components into settleable solids. In secondary treatment the incoming suspended solids and dissolved organic material represent food for microorganisms. As defined earlier, this organic food material is called BOD. As the microorganisms consume the BOD, they convert it into more microorganisms that are then separated from the water by gravity settling. This conversion is accomplished using different types of secondary treatment systems such as; trickling filters, rotating biological contactors (RBCs), activated sludge and even lagoons. When using trickling filters, RBCs and activated sludge systems, the microorganisms are grown in one unit and the solids separation occurs in a secondary clarifier.

It becomes the lab technician's responsibility to monitor the environment in the secondary treatment unit to make sure the microorganisms are "happy". The lab technician will monitor the temperature, the pH, and the dissolved oxygen to make sure the microorganisms are not environmentally stressed. The lab technician will also measure the amount of food present (TSS /BOD) and determine how many microorganisms are needed to digest the food. This would be much like determining how many pizzas to order if you know how many people are coming to dinner. It may be the lab technician's responsibility to observe and identify different types of microorganisms. Using a microscope to identify the different microorganism provides the lab technician with a tool to help control the biological processes. At the end of the secondary process the organics should be fairly well stabilized, the effluent water should be clean, and the water almost ready for discharge into the receiving stream.

**DISINFECTION**

After the effluent has been clarified, it is typically disinfected to lower the number of pathogenic microorganisms. This can be done in several ways including; chlorination, ozonation, ultra violet light exposure and even long detention times in lagoon cells. Chlorination is one of the most common methods of disinfection. However, chlorine has the undesirable potential of causing carcinogens that effect fish and other aquatic life. To reduce the carcinogen potential, chlorine must often be de-chlorinated to prevent residual chlorine from harming organisms in the receiving stream. This is accomplished by adding sulfur dioxide to destroy the chlorine residual. The lab technician will be responsible for monitoring both the fecal coliform and chlorine residual to assure the effluent is acceptable to discharge.

**SOLIDS HANDLING**

The major function of a wastewater treatment plant is to stabilize the waste and clarify the water prior to disposal. Often overlooked is solids handling. The solids that settled in the primary and secondary clarifier must be treated and also disposed of. This aspect of the treatment plant is separated from the liquid wastewater treatment processes and is known as solids handling. Solids handling may involve four components: thickening, digestion, dewatering and sludge disposal or re-use.

**Thickening** is performed to reduce the volume of sludge that must be stored in a digester. Thickening is accomplished by gravity thickeners, centrifuges, belt presses and diffused air floatation (DAF) units.

**Digestion** involves the breakdown of the solids by aerobic or anaerobic microorganisms. Digestion is done in aerobic (aerated) or anaerobic (heated, mixed, not aerated) digesters.

**Dewatering** is just as it sounds, removing water from the solids so they occupy less storage space. Dewatering can be accomplished in many ways including; gravity thickeners, drying beds, centrifuges, belt presses and diffused air floatation (DAF) units.

**Disposal or Re-use** of the solids after they have been digested and dewatered is the final step in the solids handling process. If the sludge is of a high quality and has undergone proper treatment, sludge generated from municipal wastewater treatment plants can be used beneficially as a resource to improve soil quality in various areas including; crop land, landscaping areas and land reclamation sites. If a beneficial use cannot be found, stabilized sludge may be disposed of in municipal landfills, surface disposal sites or, as a last resort, incinerated. Various state and federal regulations exist that pertain to sludge disposal and re-use.

## Lab Technician Responsibilities

In New Mexico, certified operators are responsible for the treatment and disposal of wastewater according to various state and federal regulations. Lab technicians help operators monitor the system. In many small systems, the operator is also the lab technician. In either case, the lab technician has to be able to complete core competencies. The lab technician must

- Collect and preserve samples
- Prepare samples for analysis
- Analyze samples and interpret results
- Operate and maintain equipment and instruments
- Handle chemicals and wastes
- Quality assurance/quality control
- Manage laboratory
- Laboratory safety
- Understand how volumetric, gravimetric, colorimetric and electrometric analysis are performed.

**Levels and Classification**

To assure the lab technician has the core competencies, a laboratory certification plan has been implemented. Based on the results of the job analysis of laboratories in New Mexico the certification classes are as follows:

| | |
|---|---|
| Level 1 | Laboratory Technician/Operators performing wastewater lab tests and lab functions; pH, Dissolved Oxygen, Chlorine residual, Temperature, Total Solids, Total Suspended Solids, and Settleability tests. General duties in the laboratory that incorporate glass care, and lab safety. |
| Level 2 | Laboratory Technician/Operators performing wastewater lab tests and lab functions; all Class 1 analysis plus BOD, COD, Nitrogen, Phosphorus, Coliform bacteria, metals, Inorganic, Organics, and Oil and Grease. |
| Level 3 | Laboratory Technician/Operators performing wastewater lab tests and lab functions; all Class 2 analysis listed above in Class 1and 2 plus Bioassay, Laboratory Management, and NPDES Permit preparation. |

The remaining chapters will address these competencies and help prepare the lab technician for the certification exam.

# Chapter 2 Reagent Water

Pure water- no such thing. Pure water is $H_2O$. Water is the universal solvent and often called aggressive because it dissolves everything. Oils, gases, metals, salts, organics, inorganics all dissolve in water, some more than others. This dissolution of materials of course adds contaminants to the water. Municipal water supplies contain various amounts of these contaminants depending upon the source of the water, surface water or groundwater. Since analysis is the measurement of contaminants in water, the water used to make chemical reagents, dilutions, calibration blanks must be as free of the contaminants as possible. To prepare reagent grade water the contaminant must be removed through the use of various water treatment processes. There is no one treatment process used to remove all contaminants. The most common commercial systems use activated carbon, microfiltration, ultrafiltration and reverse osmosis, followed by deionization or distillation.

Figure: An example of a laboratory reagent grade water production system.

## Granular Activated Carbon

Granular activated carbon is primarily used to remove chlorine residual and organics. Activated carbon has lots of surface area and removes organics primarily by absorption. The efficiency of the absorption is tied to the type of carbon material, the flow rate, and the nature of the organic materials. Not all organic materials are effectively removed. Chlorine residual is effectively removed by activated carbon. Because chlorine is so efficiently removed, living bacteria that escape chlorine become embedded in the downstream carbon and can begin to reproduce. This may become a major source of biofouling in other downstream treatment units.

## Microfiltration

Microfiltration also commonly called a sediment filter removes TSS particles. Most of the cartridge filters are 1-5 micron nominal filters which are used to protect the downstream reverse osmosis unit from TSS fouling. The size of a nominal filter designates the average size of the particle removed. For instance, a 1 micron nominal filter will catch most 1 micron particles but some larger particles will manage to get through and likewise some smaller particles will not. An absolute 1 micron filter will remove all 1 micron particles but is much more expensive. The feedwater to the microfilter passes through the filter, catching particles in the filter matrix.

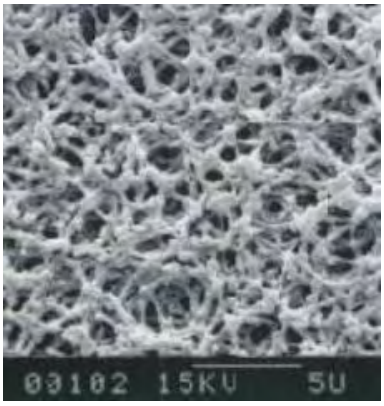
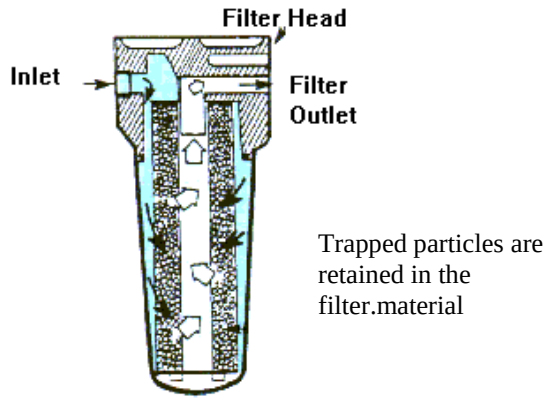


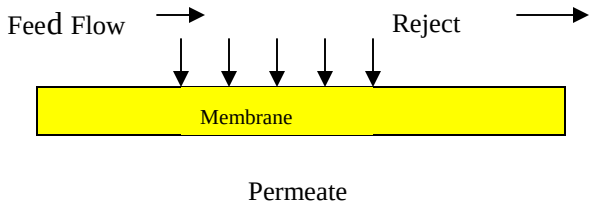

Figure: Notice the different size pores in this nominal filter. The tortuous path through the filter will catch most but some particles will manage to get through.




Microfilter plugging can be monitored by determining the differential pressure across the filter. Some commercial units will have a pressure gauge before and after the filter.
As the filter plugs, the difference in pressure will increase indicating the filter is plugging. Most cartridge filters are removed and replaced with a new filter.

## Ultrafiltration

Ultrafiltration is a cross flow membrane separation process. In a cross flow system, a feed stream is introduced into the membrane element under pressure and passed over the membrane surface. A portion of the feed water passes through the membrane at 90$^{o}$ and is called permeate.




The rejected materials are flushed away in a stream called the reject or retentate. The cross-flow direction scouring the membrane surface and reduce membrane fouling. 90-95% of the feedwater is often recovered as permeate. Ultrafiltration is not as widely used in reagent grade water production as microfiltration but is valuable when organic contaminates are a concern. Ultrafiltration removes particles in the 0.01- 0.1 micron range. Ultrafilters are also classified by the molecular weight of the particle they reject. This molecular weight cut off (MWCO) is helpful to laboratories analyzing for low level organics. Large molecular weight organic contaminants such as those from surface water can be effectively removed by ultrafiltration. Small molecular weight organics are likely to pass through. Microorganisms, including viruses are theoretically all removed by ultrafiltration.

## Reverse Osmosis

Reverse Osmosis is another cross flow membrane separation process. Following the removal of particles by microfiltration or ultrafiltration, the water is forced under pressure through a semi permeable thin film composite (TFC) membrane. RO membranes remove approximately 99% of dissolved ionic materials (TDS) including both multivalent and monovalent salts. Essentially all dissolved and suspended materials rejected by the membrane go to drain. The RO permeate usually flows to a storage tank then shuts off when the tank is full. One of the drawbacks of reverse osmosis is that at least half of the feed flow is rejected with each pass through the RO membrane. This reject flow is required to minimize scaling and fouling on the membrane surface. The RO permeate is considered low grade reagent water (reagent grade Type III). This quality water has very little use in analysis but makes a great economical source of water for deionization and distillation units. The major advantage of using RO is as a pretreatment for deionization and distillation. Because RO removes 99% of the TDS, it extends the service cycle of deionization resin and reduces scaling and carryover problems with distillation. The RO membrane is subject to fouling from TSS, microorganisms, colloidal particles, and scaling. Pretreatment with activated carbon, microfilters, and ultrafilters, extends the life of the RO membrane. Monitoring the percent salt

rejection and conductivity will help identify problems with the RO.

## Deionization

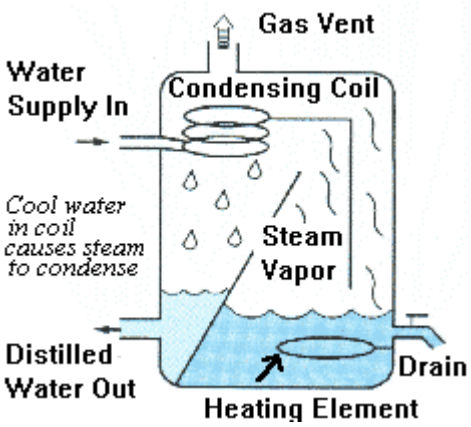
Deionization refers to the removal of ions using exchange resin. There are two major types of resin, strong acid cation resin and strong base anion resin. Strong acid cation resin exchanges all positive ions ($Na^{+}$, $Ca^{+2}$, $Mg^{+2}$) for hydrogen ions (H+). Strong base anion resin exchanges all negative ions ($Cl^{-}$ , $HCO_3^{-}$ , $SO_4^{-2}$ ) for hydroxide ions (OH-). The hydrogen ions (H+) and hydroxide ions (OH-) react to form "pure" water ($H_2O$). This water has now been deionized. The cation resin and anion resin can be purchased either as separate beds or together as a mixed bed. In either case, the resin has a limited capacity. The more ions in the feedwater to the resin, the faster the resin becomes used or exhausted. Once the resin is exhausted it must be regenerated or replaced. The quality of the deionized water (DI water) is ideally monitored continuously using inline meters or at least monitored monthly by testing for conductivity or resistivity, TOC, chlorine residual, and heterotrophic plate count.

A high quality DI water (Type I) will have a resistivity of 18.3 megohms. To reach this level the water must be flowing. Laboratories requiring ultrapure water usually have a continuously flowing/recirculating system with taps at the point of use. Remember "pure" water is very aggressive. As soon as the water leaves the recirculating system, contaminants are immediately introduced. The first contaminant is usually carbon dioxide from the air. Carbon dioxide reacts with the water to form carbonic acid which drops the pH. A pH around 5 should not be alarming. In addition, pH is very difficult to measure in ultrapure water and is seldom accurate. As the water quality deteriorates the resistivity will drop. A resisitivity below 10 megohm indicates the resin is nearing exhaustion and a replacement cartridge should be available or the resin bed should be regenerated. Ion exchange resin is not 100% efficient at removing ions and often a second mixed bed ion exchange cartridge is used as a polishing resin. For trace analysis, the contaminant level in the DI product water should be measured for each specific test. .

## Distillation

Like deionization, the feedwater to a still is usually product water from the RO storage tank. RO product water will drastically drop the maintainance required and reduce the carryover of solids into the distillate. Distillation works by heating the water in a boiler. The steam produced goes through a series of glass tubes and eventually passes a condenser coil containing cooling water. The steam condenses, is collected and taken to the storage vessel. The distillation unit is usually all borosilicate glass to minimize metal corrosion and carryover to the storage tank. Like deionized water, the water quality should be tested monthly for resistivity, chlorine residual, HPC, and TOC and any other constituents for individual tests.

Deionized or distilled water used for microbiological analysis must also be checked annually for cadmium, chromium, copper, nickel, lead, and zinc. These trace metals may be found in plumbing and can be harmful when running microbiological tests.



There are instances where double or triple distilled water is needed to produce water without trace contaminants.

## UV Sterilization

Ultraviolet sterilization often follows deionization or distillation. A lamp composed of mercury is used to produce light with a wavelength of 254 nm. The 254 nm wavelength is considered a bactericidal wavelength which is capable of sterilizing microorganisms. The UV light destroys the microorganisms ability to reproduce but does not cause lysis. For this reason, another 0.2 micron absolute microfilter is often found after the UV to "collect" the sterilized microorganisms.

## Reagent Grade Water

Reagent grade water is classified primarily on its resistivity. For most wastewater laboratory applications, medium-quality reagent water with a resistivity of >1.0 megohm (conductivity <1 uS) is acceptable. Medium grade water is produced by distillation and deionization.

High quality reagent water has a minimum resisitivity of >10 megohms (conductivity <0.1 uS). This quality of water is very unstable and must be produced continuously. This quality reagent water is often in a closed loop system where the water is constantly returned to a polishing mixed bed demineralizer to maintain its high resistivity. Water line drops to the sink from high purity closed loop lines should be very short to reduce chances for biofilm formation.

High-quality water is used for all trace metal and trace organic analysis. In addition to resistivity, this quality water is often tested for specific contaminants to document that there is no background contamination that will affect trace analysis.

# Chapter 3: Safety

Every laboratory manager, laboratory technician, and operator must be committed to working in a safe and environmentally conscious manner. The lifestyle in a laboratory is a very structured one. Learning good safety practices is just as easy as learning bad safety practices. Certain codes of conduct pertaining to your safety and that of others must be observed at all times. Working in a wastewater treatment plant exposes the laboratory technician to a large variety of hazards such as

1. Weather
2. Hazardous chemicals
3. Biohazards
4. Electrical
5. Confined Space
6. Lifting/Back injury

## Weather Hazards

The laboratory technician must often collect samples in inclement weather. Lightning, wind, snow and rain often challenge the laboratory technician. Proper sample collection planning will help reduce the "hurry" associated with collecting samples in bad weather.

Lightning is a major component of storms. If caught outside during a lightning storm, seek proper shelter immediately. General safety tips regarding lightning include:

1. Immediately get away from ponds, clarifiers, aeration basins and other bodies of water.

2. Never use a tree as a shelter.

3. Avoid areas higher than the surrounding landscape such as tops of biotowers, clarifiers, and reservoirs.

4. Keep away from metal objects such as metal stairs and railings.

Strong winds with blowing dust may also be a frequent problem in New Mexico. Strong winds can occur at any time especially during the spring months. Wear appropriate clothing, dust masks, and protective eye equipment when collecting samples during a dust storm.

Snow and rain create the obvious safety hazard of slipping. Wet stairs, icy gratings and a technician carrying sampling equipment is a formula for disaster. In addition, stairways, railing, and gratings can be slippery even on a sunny day. Wastewater overspray from the aeration basin or other treatment units can often create a slipping hazard as well as a biohazard.

## Hazardous Chemicals

Chemical exposure is a serious hazard and may contribute to a variety of serious health effects such as heart ailments, kidney and lung damage, sterility, burns, and rashes. Chemicals also have the potential to cause fires, explosions, and other serious accidents. Because of the seriousness of these safety and health problems, the Occupational Safety and Health Administration (OSHA) has issued a standard entitled the Hazard Communication Act.

The purpose of the Hazard Communication Act is to insure that all chemicals used in the workplace are evaluated and the hazard information is transmitted to affected employers and exposed employees. Chemical manufacturers convey the hazard by means of a label on the container and a form called a Material Safety Data Sheets. (MSDS's) In addition, all covered employers are required to provide the information to their employees by means of container labeling, and other forms of warning, MSDS forms and training. This has been dubbed the "Right to Know Act". The employee has a right to know what hazards are in his/her work area.

## Written Hazard Communication Program

Employers (including lab managers) must develop, implement, and maintain at the workplace a written, comprehensive hazard communication program that includes provisions for container labeling, MSDS sheets, and an employee training program. The written program must contain a list of the hazardous chemicals in each work area and the means the employer will use to inform employees of the hazards.

### Labels

Chemical manufacturers must be sure that hazardous chemicals are labeled, tagged, or marked with the identity, appropriate hazard warnings, and the name and address of the manufacturer or other responsible party.

In the laboratory, each container must be labeled, tagged, or marked with the identity of the hazardous chemical and must show hazard warnings appropriate for employee protection. The warning can be any type of message, words, pictures or symbols that convey the hazard of the chemical. Hazardous chemicals removed from the original manufacturer's container must also be labeled. For instance, alcohol transferred from a large one gallon container into a small capped bottle must be labeled the same as the original container. The NFPA (National Fire Protection Association) labeling system is a good system for secondary labeling. The NFPA labeling system rates the hazards from 0 low to 4 high. Each chemical is divided into 4 categories - fire, reactivity, health (toxicity) and any specific hazard. The four categories are color coded to help segregate the chemicals properly. Don't be fooled by a low number such as 2. Anything labeled 2 or above is extremely dangerous and may pose life threatening conditions.

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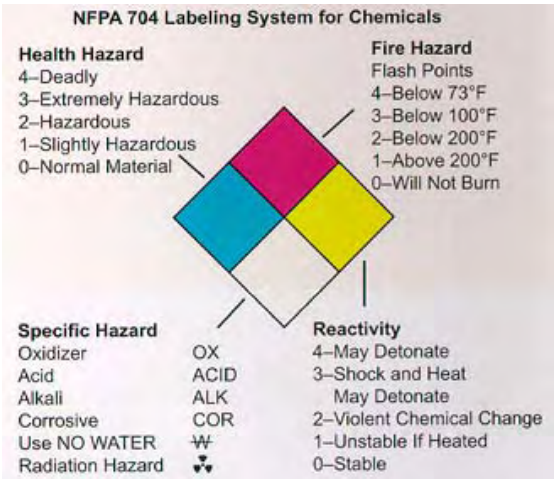



### Material Safety Data Sheets

MSDS sheets are provided by the manufacturer at the time of shipment. For laboratory chemicals the MSDS is a detailed reference for the chemical prepared by the manufacturer. It contains technical, safety and health information about the chemical. The MSDS document must be available to all people who utilize the laboratory including office personnel. All personnel must know what the MSDS is and where they are located. (Usually in the laboratory not the main office.) The laboratory manager is usually responsible for maintaining a set of MSDS sheets for hazardous materials used in the laboratory. Remember, each person using the laboratory has a "Right to Know" about the hazards they are exposed to and they should be readily available during each work shift.

### Chemical Storage

Storage of chemicals presents a safety problem to the lab technician because of the diverse characteristics of the chemicals used. There are oxidizers, corrosives, combustibles, carcinogenics, radioactives, water reactives, light reactives, and general non-reactive chemicals. OSHA regulations describe the proper storage procedures. Chemical suppliers label the chemicals as to their proper storage location. Ideally, storage cabinets should be in cool, well lighted, and well ventilated rooms, separate from laboratory itself. Chemicals

should be dated and inventoried when received and again when opened. Excess, out-dated, or unused chemicals should be discarded appropriately.

**Training**
Employers must establish a training and information program for employees exposed to hazardous chemicals in their work area at the time of initial hiring.

The employee training program must consist of:
1. A discussion of the hazard communication program.
2. How to read and interpret labels, MSDS forms, and how to use the available information.
3. The specific hazards of the chemicals in the work area.
4. Measures the lab technician can take to protect themselves from hazards.
5. Specific procedures required by the employer to provide protection such as work practices and the use of personnel protection equipment (PPE)
6. Methods and observations that the lab tech can take to detect the presence of hazardous conditions. (i.e. smell, color, gas production)

**Personal Protective Equipment**
Clothing is a critical factor in the safety of laboratory personnel. The lab technician must understand that failure to wear or failure of the PPE can cause life threatening conditions. At a minimum, lab technicians must wear:

1. Safety glasses must be worn at all times in areas where chemicals are being used. Splash goggles or face shields must be worn for protection from harmful chemical splash. Safety glasses must fit properly, not obscure the vision and be comfortable. Accidents may be caused by others working in the laboratory and directly impact the lab technician.

2. Pants, full coverage lab coats, aprons, or tyvek-type coveralls must be worn. Routine chemical and microbiological analysis often results in "micro" splashes. Wearing lab coats protects the lab technician and prevents taking the hazardous material home.

3. Protective gloves must be worn when the potential for contact with corrosive, toxic, or microbiological materials exists. Wastewater technicians are always exposed to microbiological hazards and wearing gloves is a must. Ideally, gloves should not be reused. They should be disinfected prior to removal then disposed of in the trash. Hands should be washed with disinfectant after removing the gloves.

4. Closed-toed shoes must be worn in areas where hazardous chemicals are used or stored.

## Biological Hazards
Wastewater laboratories are a great place to find microorganisms. Not only are the samples potential health hazards, but some tests actually grow bacteria. Safe handling of microbiological materials is very important to not only the lab technician but all others who enter the facility (including tour groups). The first barrier to microbiological exposure is disinfectant. The lab bench should be routinely and periodically disinfected. The disinfectant is usually left to dry on the lab bench. The second protection is rubber latex gloves and proper washing procedures. Finally, contaminated materials must be safely disposed of by sterilization before discarding in the trash. The autoclave is used to sterilize contaminated materials before disposal. A written record of the temperature, pressure and time of sterilization must be maintained. After proper sterilization, the materials can be discarded in the trash.

## Electrical Hazards
Water and electricity don't mix. While not a common hazard, the lab technician is exposed to electrical equipment both in the lab and in the treatment plant. A sampling plan should include an evaluation for electrical hazards. For

instance, will a metal pole be used to collect the sample around electrical equipment?

## Confined Space Entry

Confined space sampling is not a common activity for lab technicians but it may occur occasionally and the lab technician must know that specific procedures are required before entering a confined space. Confined space is defined as a space which has:

1. Limited means of entrance and exit
2. Is subject to the accumulation of toxic or flammable contaminants
3. is subject to the unanticipated introduction of liquids or solids
4. Has the potential of developing an oxygen-deficient atmosphere.

Common sources of confined space in wastewater treatment would be collection lines, deep lift stations, tanks, and possibly even pump pits.

A confined space entry procedure shall include the following essential features.

1. Permit systems
2. Testing and Monitoring
3. Labeling and Posting
4. Rescue procedures
5. Training

**Permit Systems**

Entry into a confined space is only allowed by permit authorized in writing by the employer. By completing the permit system, the lab technician will have evaluated the condition of the confined space. Typical permits will contain:

1. Location and description of work
2. Hazards that may exist
3. Isolation checklist
    a. Blank or disconnect
    b. Electrical lockout
    c. Mechanical lockout
4. Special clothing and equipment
    a. PPE
    b. Harness, lines, winch
    c. Tools or collection equipment needed
5. Atmospheric tests required before entry and re-entry
    a. oxygen level
    b. flammable or explosive levels
    c. Toxic substance levels
6. Training required and completed by the lab technician
7. Atmospheric control (ventilation) needed

**Testing and Monitoring**

Once the permit is completed and approved, entry is prohibited until the atmosphere has been tested and confirmed to be safe.

**Label and Post**

All entrances to any confined space shall be posted with a warning sign and copy of the authorizing permit.

**Rescue Procedures**

Rescue procedures shall be designed for each entry and for each participant: worker, standby person, rescue team. Safety equipment such as harnesses, lifelines, atmospheric monitoring equipment must be on site, must be in working condition and must be used if conditions indicate.

**Training**

It is the responsibility of the lab manager to assure that the lab personnel are trained appropriately in confined space entry. Training should include:

1. Rescue techniques and equipment
2. Use of respirators (SCBA)
3. Lockout/tag out procedures
4. Permit system
5. Atmospheric testing and monitoring equipment.

The lab manager should assure that each person is trained and reviewed annually. ***Training is not considered completed until the employee***

***demonstrates proficiency for entering and working in confined spaces. Records of the training must be maintained.***

## Lifting/Back Injury

Back injuries are among the majority of reported injuries. Proper sample planning will help reduce back injuries. One common source of back injury is the lifting of the composite sampler. There is a large difference between an empty and full composite sampler. Failure to use a hoist often results in back injuries. The following recommendations will help reduce back injury.

1. Loads over 25 pounds may require assistance
2. Lifting objects above your shoulder is risky
3. Position yourself so that the load is kept close to your body
4. Lift with your legs
5. Maintain your balance by placing one foot slightly ahead of the other.
6. Complete the lift before turning or twisting your torso with the load.

## General Lab Safety

Because the laboratory personnel are exposed to so many potential hazards, it is essential that all employees be empowered to recognize and remedy any safety problems. The following precautions should help reduce lab accidents.

1. First locate the safety equipment. Know where the fire extinguisher, safety shower, eye wash, fire blanket, first aid kit, and emergency exits are located.
2. Always wear safety glasses. Wear splash resistant goggles at all times. If prescription glasses are needed, purchase safety glasses that fit properly over the prescription glasses.
3. Long hair is a lab hazard. It may get caught in equipment, fall into hazardous chemicals, and may even catch fire in a Bunsen burner. Tie it back.
4. No horse play.
5. No eating, drinking, or smoking in the laboratory. Chemical dust and microorganisms are everywhere in the lab. Inadvertent ingestion of toxic materials can occur through something as simple as placing your pen in your mouth.
6. Do not use lab equipment to store, heat, or hold food or drink.
7. Add acid to water, never the reverse.
8. Assume all chemicals are toxic. Label all chemicals clearly. Never taste any chemical.
9. Discard unlabeled chemicals.
10. Open bottles that are under pressure by using a towel, i.e. Hydrochloric acid, ammonia.
11. Never smell a chemical directly. Use your hand to waft any odors to your nose to prevent damage to your nose and lungs.
12. Use bottle carriers to transport acids, bases, and toxics.
13. Chain gas cylinders. Keep caps on until the cylinder is needed.
14. Read the chemical label twice. There are many chemicals that look and sound similar. Pay particular attention to the suffix such as "ite", "ate", "ide" Using the wrong chemical may give a surprise!!
15. Never return chemicals to the stock bottle. It is better to remove only what you need. Any unused chemicals should be disposed of properly.
16. Broken or chipped glassware should be discarded or fire-polished. To avoid cuts from shattered glass on the floor, wear closed toed shoes. Sandals, flip flops, and old holy tennis shoes are not adequate protection.
17. Never pipet a solution by mouth. Always use a pipet bulb
18. Never open flammable liquids near an open flame. Vapors may escape and create a flash fire. Avoid storing solvents above eye level, or in refrigerators with other chemicals. An explosion proof refrigerator should be used if necessary.
19. Clean and disinfect all lab bench areas before and after use.
20. Always be a good housekeeper. Do not store things where they don't belong. Keep

aisles free from obstruction. Clean up broken glass and spills immediately.

## Safety Equipment

Once the safety equipment is located, it is important for all laboratory personnel to know its function and operation. Equipment that should be found in all laboratories includes:

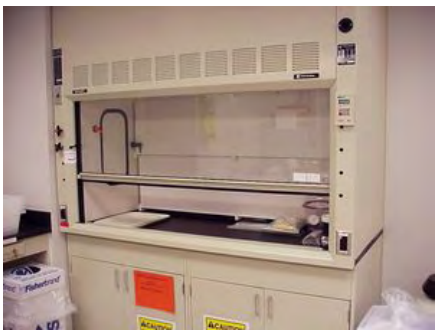
1. Fire extinguisher (ABC)
2. Fume hood
3. Safety shower
4. Eye wash
5. Fire blanket
6. Pipet bulbs

**Fire extinguisher**

The laboratory should be equipped with at least one ABC type fire extinguisher. It should be checked at least annually for proper operation and should always be replaced or recharged if it has been used. The lab manager should be sure all personnel have attended a fire extinguisher training program.

**Fume Hood**

A fume hood is used to remove toxic, flammable, and unpleasant vapors and dust. A hood should include a sink, water spigots, gas and electrical services. The hood is enclosed on 3 sides by a solid barrier and at the front by a safety glass shield which may be raised or lowered. To operate, simply turn on the fans and close the front shield to the desired level. The shield should be lowered far enough to protect the eyes against splashing or explosion. The air flow through the fume hood should be checked for proper operation annually. Fume hoods should not become storage facilities. Chemicals and equipment not in use should be removed from the fume hood.



**Safety Showers**

Emergency showers should be located in a conspicuous area which is quickly and readily reached from anywhere in the laboratory. The shower is activated by pulling on the chain or ring. Water will flow at a very high rate and continue to flow after you release the ring. Showers should be located away from electrical equipment and must have a large drain. In the event of an acid spill, affected clothing must be immediately removed and the area rinsed rapidly. An acid burn can be flushed with a 10-20% sodium bicarbonate solution to neutralize the acid. An alkali (base) burn can be flushed with a saturated solution of boric acid to neutralize the base. The boric acid or sodium bicarbonate should be rinsed off. The burned area should be covered with a dry sterile dressing and the victim transported to a hospital.

**Eyewash Station**

Eye washes are often incorporated with the emergency shower but may be found separately. The water from an eyewash should be at a moderate temperature, aerated, and in soft streams sufficient to wash the eyes without damaging the tissues. If a corrosive or irritating chemical gets into the eyes, the safety glasses should be removed and your face (eyes) placed directly in the streams of water. It is important that eyelids be held open with your hands to allow the water to get to the eye. The eyes should be rinsed a minimum of 15 minutes to thoroughly remove the irritant. The victim should be transported to the hospital for further treatment.

**Fire Blanket**
A fire blanket is used if a lab technician has caught fire. Follow the drop and roll procedure.

**Pipet Bulbs**
All pipets used for laboratory reagents, biological samples, or hazardous liquids should be filled using a pipet bulb to provide the necessary suction. A pipet bulb is a necessary safety device and should be insisted upon in all lab work. Pipet bulbs eliminate contact between the analyst's mouth and the pipet, thereby eliminating accidental aspiration or inhalation. Pipet bulbs are often contaminated, so they must be handled carefully. Be sure to clean any contaminated pipet bulb with distilled water. A pipet bulb with a leaking valve should be cleaned or replaced.

## First Aid
It is important that all lab personnel be aware of first aid measures which should be employed if an accident occurs. Chemical dangers may be in gas, liquid, or solid form. Gases represent the most serious, fastest acting hazard. Very short exposure time may be fatal. Toxic gases require ~~immediate~~ removal from the contaminated area. Gases may be light, heavy, toxic, corrosive, asphyxiating, nauseous, flammable, or explosive. It is important to know the type of gas. In all cases, get the victim to fresh air as soon as possible.

Liquid chemicals present the greatest danger to the eyes and skin. Liquids may splash into the eyes, burn the skin, or absorb through the skin and do internal damage. The most common liquids are the corrosives, acids and bases. Acids and bases must be handled with extreme caution. The addition of water to acid rather than acid to water may cause a violent splashing. Chemical burns are often not noticed until they have proceeded for a long time. If your skin feels sun burned, slippery, or itchy, your skin may be being chemically burned. These burns occur rapidly and are potentially fatal. In case of contact with corrosive liquids, the exposed area should ~~immediately~~ be flushed with copious amounts of water. If the eyes are involved, flushing should be continued for a minimum of 15 minutes and a physician should be called. Another common accident involving liquids occurs during mouth pipetting. Untrained or unskilled pipetters usually get some solution into their mouth. Do not allow any lab personnel to mouth pipet. Ingestion of acids, bases, or toxins can be debilitating and potentially fatal. Lab managers must insist on the use of pipet bulbs.

Solids are usually the least dangerous because the affected person usually has time to wash the material off. The major problem with solids is their slowness in reacting. The damage is not immediately noticed or the contact is not noticed at all. Chemical dust causes this type of problem. Inhalation of toxic dust may go entirely unnoticed until several years later when toxic threshold limits are finally reached. Respirators and fume hoods should be used when working with dusty materials especially carcinogens. First aid for skin contact consists of washing with large amounts of water and calling a physician.

***Safety: When in doubt, use the MSDS sheets to identify the type of potential hazard.***.

**First Aid Kit**
A first aid kit should be available to treat minor burns and cuts. Major accidents require an ambulance and physician care. All lab accidents should be reported to the lab manager and Risk Management Office for documentation. Claims for workman's compensation benefits may be denied if the accident is not reported within 24 hours.

# Chapter 4 Laboratory Equipment

## Laboratory Space Requirements

Sufficient laboratory space is essential for precise and accurate data measurement. While there is no specific design size, the laboratory must have adequate lighting at bench sites, multiple electrical outlets, a source of filtered air, oil-less vacuum, and natural gas. Electrical outlet design is critical and many laboratories are drastically undersized. The facility should be temperature and humidity controlled.

The laboratory should have sufficient bench top area for sample preparation and analysis, storage space for media, glassware, and portable equipment such as pH meter, DO meter, desiccator, oven, water bath, muffle furnace, centrifuge, microscope, and incubator.

Ample floor space is also needed for free standing equipment such as autoclave, refrigerator, BOD incubator, and analytical balance. Space must also be available for safety equipment such as fume hoods, safety shower (with adequate drain) and eye wash.

Bench tops and floors should be of a material that is easily cleaned and disinfected.

When planning for a new laboratory, it is beneficial to seek advice from other laboratories rather than just the architectural firm.

## Temperature Controlled Equipment

### Thermometers

Glass, dial, or electronic thermometers can be used to measure temperature. Mercury filled thermometers have gone out of favor due to the high toxicity of liquid mercury and the spill handling problems associated with a broken thermometer. Accuracy should be checked at least annually against a certified NIST thermometer. Thermometers should be checked over the entire range of intended use to verify the thermometer is accurate at all potential temperatures. Never exceed the maximum temperature rating of the thermometer. Discard thermometers that differ by more than 1$^{o}$C from the reference thermometer. Reference thermometers should be recalibrated every 5 years. Currently, the State of NM Department of Health, Scientific Laboratory Division will calibrate thermometers for free. Maintain a record of the thermometer calibrations. Thermometers not in service should be stored in their protective sleeve. Mercury thermometers should be read at the top of the mercury meniscus.

*Figure: Notice the thermometer correction factor on the label.*

### Drying Oven

Drying ovens generally serve two common purposes, removing moisture and sterilizing. Drying ovens are set at 104 ± 1$^{o}$C to commonly remove moisture from TSS samples. At 104$^{o}$C, the heat will evaporate water but is not hot enough to drive off organic molecules. The laboratory should purchase a forced air oven to avoid differences in temperature within the oven. The temperature in the oven is commonly measured by a digital thermometer on the door panel. This thermometer is convenient but is not acceptable unless it can be calibrated against a NIST thermometer. If the digital thermometer cannot be calibrated, the bulb of a calibrated thermometer graduated in at least 1$^{o}$C increments should be immersed in sand and

placed on each shelf in the oven. The sand will hold the heat and reduce rapid fluctuations in the temperature reading when the door is opened. Calibration-corrected temperatures should be recorded for each thermometer being used. Record at least twice per day during each day the incubator is in use. The readings should be separated by at least 4 hours.

***SAFETY:*** ***Be sure to use asbestos gloves or tongs when removing objects from the oven***.

**Sterilizing Oven**

The drying oven temperature can be increased to 170-180$^{o}$C. At this temperature, objects in the oven for at least 2 hours will become sterilized. Objects commonly sterilized in the sterilizing oven are inoculating sticks and glassware such as pipets. Spore strips can be used to confirm sterilization.

***SAFETY:*** ***Be sure to use asbestos gloves or tongs when removing objects from the oven.***

**Refrigerator**

The laboratory refrigerator is commonly used to preserve samples, and store perishable chemicals (most commonly prepared biological broth). Laboratory refrigerators should maintain a temperature of 1-5 $^{o}$C. Calibrated thermometers should be graduated in at least 1$^{o}$C increments and the bulb immersed in liquid. A thermometer can be inserted into a rubber stoppered Erlenmeyer flask to prevent evaporation. The reading should be recorded at least once per day.

**Dry Air Incubator**

Incubators are similar to ovens except they are primarily used for bacteriological work and require more precise control temperatures. If the temperature varies from the proper temperature or if the wrong temperature is used, good test results may be reported from bad water. As with ovens, forced air incubators are preferred to convection incubators and are less likely to form "hot" spots. Calibrated thermometers should again be inserted in liquid and placed on each shelf of the incubator. The temperature should be 35 $\pm$ 0.5$^{o}$C. Calibration-corrected temperatures should be recorded for each thermometer. Record at least twice per day during each day the incubator is in use. The readings should be separated by at least 4 hours. A thermometer graduated in at least 0.1$^{o}$C increments should be used.

Figure: Dry Air Incubator

**Waterbath**

The water bath incubator is similar to the dry air incubator except the temperature must be at 44.5 $\pm$ 0.2$^{o}$C. To maintain this temperature a recirculating water bath is required with a gable cover. A thermometer graduated in at least 0.1$^{o}$C increments should be used. Record at least twice per day during each day the waterbath is in use. The readings should be separated by at least 4 hours.

Figure: Waterbath

**BOD Incubator**
The BOD incubator is a specially converted refrigerator used in the BOD test. The temperature must be maintained at 20 $\pm$ 1.0$^{o}$C Calibration-corrected temperatures should be recorded for each thermometer being used at least once per day during each day the incubator is in use. A thermometer graduated in at least 0.1$^{o}$C increments should be used.

**Muffle Furnace**
Muffle furnaces heat materials from about 200-1100 $^{o}$C. The most common use of a muffle furnace is to "burn" or "volatilize"off the organic portion of a sample leaving only ash behind. Muffle furnaces usually do not run continuously, so allow a 30 minute warm up time.

***SAFETY:*** ***Be sure to use asbestos gloves and long tongs when removing objects from the muffle furnace.***

**Hot Plates**
Electric hot plates are commonly used by the laboratory to heat solutions in either beakers or Erlenmeyer flasks. They usually have a temperature range of about 50- 350$^{o}$C. Hot plates heat up slowly but also cool down slowly. The amount of heat produced is controlled by an adjustable controller or rheostat (knob). The numbers on the knob are not assigned a calibrated value so the laboratory must calibrate the knob if the temperature is important. To calibrate the numbers on the rheostat, set the beaker containing a high boiling oil such as mineral oil on the hot plate. Turn on the hot plate and measure the temperature at each setting on the rheostat. Record the temperature values for each knob setting.

## Burners
Burners are primarily used for bacteriological work in water and wastewater analysis laboratories. They are used occasionally to heat solutions, dry samples, and soften glass for glass repair. Most burners have an air vent and fuel adjustment valve.

To light a burner, open the air vents. Bring a lit match or striker up the side of the burner to the top and carefully turn on the gas valve. If the gas flow is too fast, the gas may flare up or go out. Reduce the gas flow and repeat the ignition procedure. After the flame is lit, adjust the flame height using the gas valve. If a hot flame is needed the air valve should be completely open and the flame will be blue. If a cooler flame is needed, reduce the air flow and the flame will become more yellow.

***SAFETY: Natural gas burns very clean and the tip of the flame may be almost clear. Be careful not to reach across on open flame especially wearing flammable synthetic materials.***

Burners are also used for glass bending and fire polishing. To bend glass tubing, attach a flame spreader, and gently roll the tubing back and forth until the glass becomes soft. Remove the glass tubing from the flame and gently bend it to the desired shape. To cut a piece of glass tubing, etch the tubing with a triangular file. Using gloves to protect your hands, place your thumbs close together on the opposite side of the tubing from the scratch. Gently push out on the scratch until it snaps. Fire polish the ends by gently and continuously rolling the ends in a flame until the glass begins to soften. Remove from the heat and cool. The ends of the glass should be free from rough, jagged or sharp edges.

***Safety: Fire polished glass may be very hot even though it looks cool.***

## Digestion Apparatus
In addition to hot plates, wastewater laboratories have specially dedicated heating equipment for specific tests such as COD, TKN and phosphates. Heating blocks are not easily adjusted to a specific temperature and are therefore usually dedicated to a specific test. Sealed test tubes containing digesting chemicals are placed in the heating cells. These devices

should be used in a fume hood in the event one of the tubes breaks.

Figure: Hach COD digester

***<u>SAFETY:</u> Be sure to use gloves, goggles and the tube sleeve when removing hot tubes from the digester.***

## Balances

Balances are an essential tool in most wastewater laboratories. Balances are used to determine a variety of solids analysis such as TSS, MLSS, VSS, TDS and to measure chemicals needed to prepare standard solutions and reagents. There are usually two types of balances, the top loading which is used for low accuracy measurement (sensitivity of 0.01 gm) and the analytical balance which is used for high accuracy measurement (sensitivity of 0.0001 gm).

### Top Loading Balance

The top loading balance is used when the weight of a substance or object is not needed to a high degree of accuracy. When an object is placed on the balance pan, the balance will display the weight to the nearest 0.01 gm. This balance is often used to weigh chemicals to prepare reagents or bacteriological broth.

*Figure: Mettler Electronic Top Loading Balance*

To weigh an object on the top loading balance, first clean the pan using a camel hair brush. Next zero the balance by gently pressing the bar. The meter display will show zero. Now carefully place the object or tare on the balance. Read and record the weight to the nearest 0.01 gm. If the object is going to be used as a tare weight, the bar can be pressed again to make the tare weight go to zero. Chemicals can now be added to the tare using a spatula. The weight on the electronic display represents the weight of the chemicals. The maximum capacity of the top loading balance is around 600 gm and should not be exceeded.

### Analytical Balances

Analytical balances are the workhorses of the wastewater laboratory. Analytical balances come in a variety of sensitivities ranging from 0.0001 gm to the highly analytical 0.000001 gm balance.

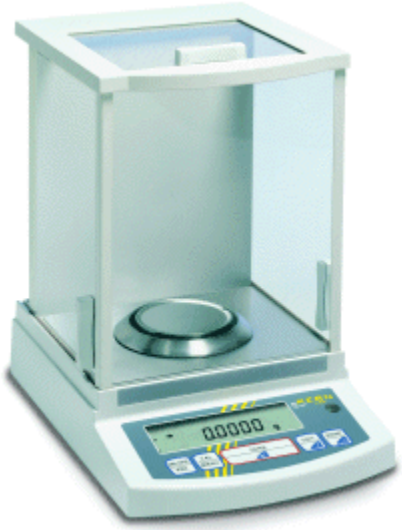


*Figure: Electronic Analytical Balance*

Most wastewater laboratories use the single pan electronic analytical balances having a sensitivity of 0.0001 gm. Because of this very high sensitivity, special handling is required for all objects placed on the pan. The maximum capacity of analytical balances will be around 150 gm.

**Balance Rules**
Because of the high sensitivity of the analytical balance, there are a number of "rules" that should be followed to avoid erratic and inaccurate balance readings.

1. Always use a tare. A tare is any object placed on the balance and used to contain the material to be weighed. The tare prevents contamination of the weighed material. If the pan has not been cleaned properly, dust or previous chemicals may contaminate the weighed material. In addition, chemicals placed directly on the pan may damage (corrode) the balance pan itself. Using a tare also allows chemicals to be easily and accurately transferred from the pan.

2. Balances should be placed in a constant temperature, constant humidity atmosphere. They should be located away from direct sunlight, heating and cooling vents, heavy laboratory traffic, and corrosive fumes. Both the top loading and analytical balances are sensitive enough to be affected by ambient room air currents. Close the balance doors before taking the final weight.

3. Balances should be leveled before using. This is generally a one-time operation performed during installation but should be checked periodically. The balance is leveled when the bubble is in the center of the centering circle. If the bubble overlaps the circle, the balance is out of adjustment and should be corrected by turning the screws on the balance feet.

4. Position the balance on a separate table free from vibration. Special balance tables are available but very expensive. If a table can not be purchased, the balance should be placed in a quiet space (away from the pump room) and given time to stabilize before the reading is taken.

5. Keep the balance clean. Always clean the pan and the chamber with deionized water or alcohol. Clean spills up immediately to prevent corrosion

6. Never put chemicals directly on the pan

7. Never overload the balance. Check the maximum rated capacity prior to use. Replacing the internal controls of an electronic balance is very expensive and may leave the lab without a balance for at least a month or more.

8. Never weigh a warm or hot object. Always cool to room temperature in a desiccator prior to weighing. Hot objects will heat the air within the balance chamber causing the pan to be elevated and giving a lighter weight.

9. Allow the balance reading to stabilize before taking the reading. Electronic balances indicate when the reading is stable.

10. Balances should be calibrated in-house at least monthly using NIST traceable Class 1 weights. Keep Class 1 weights clean and dry at all times.

Figure: Class 1 weights

Never touch the weights with fingers as the oils and acids will change the weight. Handle the weights with forceps or tongs. Class 1 weights should be calibrated every 5 years. Be sure to record and maintain all calibration results.

11. Calibrate and service the balance once a year using a certified balance technician. A good balance service will clean the interior and exterior of the balance and verify that the

balance is working within specification. A good service should show that each quadrant of the pan will produce an identical weight.

12. Always use forceps or tongs to place an object on or off the balance. Modern electronic balances are sensitive enough to weigh an oily fingerprint.

**Sources of Error**

Moisture, temperature, and static electricity are problems that should be considered. Many of the weighings in a wastewater laboratory involve driving off moisture. It is important to pre-dry dishes, filters, and in some cases chemicals before weighing in order to prevent moisture from adding weight that may be lost later in the test procedure. Some chemicals are defined as hygroscopic. Highly hygroscopic chemicals will absorb water under humid conditions. Analytical balances are sensitive enough to measure this hygroscopic water. It is very apparent when a hygroscopic chemical is placed on the balance because the weight never stabilizes and continues to increase. This error can be minimized by placing a small beaker of desiccant inside the balance chamber.

Temperature changes will also affect the weight of material on the balance. If the material is hotter than room temperature, convection air currents in the balance will push the pan upward causing the material weight to be less than the true value. If the material is colder than room temperature, convection currents will cause the material the weigh more than the true value. Materials removed from the drying oven or muffle furnace should never be immediately placed on the balance pan. Allow to cool in the desiccator for sufficient time to reach room temperature.

Static charges from objects coming in contact with the balance can be transferred to the balance weighing mechanism and cause erratic readings. These charges are difficult to remove. Sometimes touching the outside of the balance chamber with a drop of water will reduce the static charge error.

**In-house Balance Calibration**

Each laboratory should calibrate their balances at least monthly using Class 1 weights. Record the Make, Model, and Serial Number of the balance being calibrated. Place a Class 1 weight on the balance and record the displayed weight. Record the value as acceptable if it is within $\pm$ 0.0002 gm. Repeat this process with a minimum of 5 Class 1 weights covering the balance range. For instance, 1.0000, 5.0000, 10.0000, 50.0000 and 150.0000 gm weights could be used. The balance should be recalibrated if the recorded values are outside this allowance. Follow the manufacturers recommended procedure. Class 1 weights should be re-certified every five years. The NM Dept. of Agriculture Metrology office at NMSU will calibrate these weights for a fee. If the balance is frequently out of calibration, it may need to be calibrated more often. Most electronic analytical balances have an internal calibration procedure which takes about 2 minutes and can be performed daily.

## Autoclave

The autoclave is a glorified pressure cooker that uses steam heat to sterilize. The autoclave should maintain a sterilization temperature of 121$^{o}$C during the sterilizing cycle and be able to complete the entire cycle within 45 minutes when a 12-15 minute sterilization period is used.

Figure: Steam sterilizer

The autoclave should have a pressure gauge, a safety valve and a thermometer sensor in the exhaust. It is also common to have a temperature recording device and a digital timer. The sterilization temperature of 121$^{o}$C must be confirmed by using a spore strip, integrator strip or a calibrated high temperature thermometer. Overloading the autoclave by placing objects very close together may prevent complete sterilization and should be avoided. The autoclave is commonly used to sterilize bacteriological media, phosphate dilution water and contaminated microbiological samples.

## Magnetic Stirrers

A magnetic stirrer is a common apparatus used during titrations and to mix solutions. Teflon covered magnets are placed in the flask to be stirred.

Figure: Magnetic stirrer

The solution is added then the speed is slowly increased causing a vortex to form. Do not stir the solution so violently that the solution splashes up the side of the flask. If a large amount of solids needs to be mixed, add the solids slowly as the magnet turns to avoid jamming the magnet. If excessive solids prevent the magnet from spinning, swirl the flask to dislodge the magnet, and then continue. If the magnet begins to wobble excessively or starts vibrating without spinning as the speed is increased, stop the stirrer then restart slowly.

## Desiccator

A desiccator is used to allow hot objects to be cooled without condensation. *A desiccator is NOT used to remove water from samples*. If humidity is allowed to condense on the surface of the object, the weight will be inaccurate. Hygroscopic chemicals such as bacteriologic media are often stored in a desiccator to reduce moisture absorption into the media. Dehydrated bacteriological media should be stored upside down in tightly closed bottles in the dark at less than 30$^{o}$C. If they become discolored and caked they should be discarded.

The desiccator has a pan in the bottom containing a chemical called a desiccant.

Figure: Desiccator

There are a variety of different desiccants but the most common one is called Indicating Drierite. Indicating Drierite will change colors from blue to pink as the material absorbs moisture (humidity). It is important to keep the door closed to prevent excessive humidity absorbance. It is important that the seal on the desiccator be tight. If the seal or lock is poor, moisture will infiltrate the desiccator and

prematurely exhaust it. Once the Drierite turns pink it must be replaced or regenerated. It can be regenerated at 180$^{o}$C for 2 hours. Never place any object directly on the desiccant as it may pick up contaminants.

There is a second type of desiccator that looks like a glass jar. It works the same way but has a sliding top that is sealed by silicone grease. When placing hot objects into the desiccator, be sure to leave the top cracked slightly to allow the heat to escape for a couple of minutes. If the top is closed immediately the hot sample will heat up the air in the desiccator causing the pressure to increase and the expensive lid may pop off.

## Laboratory Grade Water

A source of laboratory grade water is essential. The two most common methods of producing laboratory grade water are distillation and deionization. Distillation involves heating water to produce steam. The steam is then re-condensed to a receiving bottle, leaving the contaminating solids behind. The solids remaining in the boiler will eventually scale the heater elements and require maintenance. Deionization involves passing water thru a demineralization cartridge which removes contaminating ions. When the capacity of the demineralizer cartridge is exhausted, the cartridge must be replaced or regenerated. The life of the demineralizer cartridge can be extended by pretreatment with a cartridge filter and RO membrane. Laboratory grade water must be checked for quality monthly. The most important parameter is conductivity which should be less than 2 uSiemans. Other parameters will be discussed later.

## Ultraviolet Sterilizer

The ultraviolet sterilizer uses high intensity UV light to sterilize. A minimum exposure time of 2-3 minutes is required for sterilization.

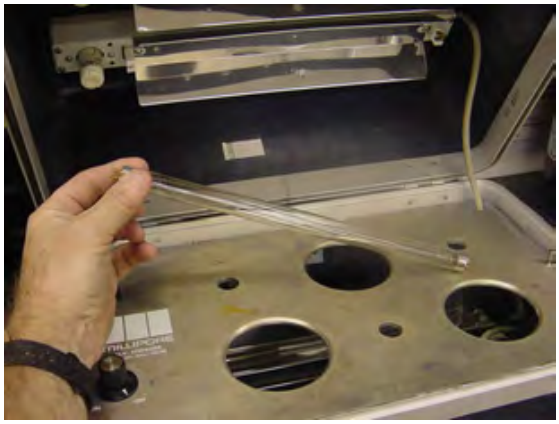
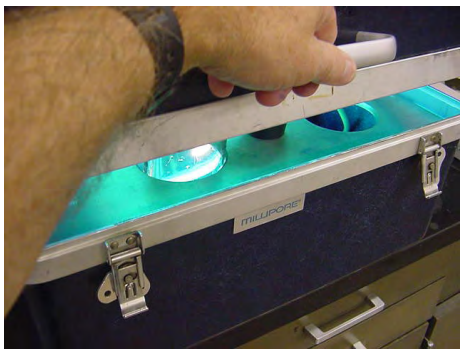


Figure: Ultraviolet lamp

The UV lamps should not be touched with the fingers and should be cleaned monthly using Isopropyl alcohol and a lint free cloth. The lamps should be replaced when they no longer produce a 99% kill. Lamps typically have a shelf life of 2 years.

***<u>SAFETY</u>***: ***Do not look at UV light. The light can damage the retina of the eye.***



## Electronic Instrumentation

The wastewater laboratory may also use a variety of instruments to measure contaminants such as pH, DO, and metals. These instruments all operate by comparing millivolts generated by a standard with millivolts generated by the sample. All instruments must be calibrated frequently against either prepared or purchased standard solutions. Specific meter operation will be discussed in later chapters.

**Light Microscope**
The microscope can be a valuable tool to the wastewater laboratory technician. Monitoring the microbiological population of an activated sludge process can help diagnose the operating condition of the activated sludge process and guide the operator to process control changes. The laboratory technician can use the microscope to identify, count and check on the activity of various types of protozoans and metazoans to:
1. Determine which organisms are present in a WWTP that is running well.
2. Determine if a toxic shock to the activated sludge system has taken place.
3. Provide data for trend charts relating protozoan/metazoan numbers to specific plant treatment parameter.
4. Identify filamentous bacteria types to help in determining the cause of bulking or foaming.

The light microscope is a sturdy instrument which when handled properly can work for many years. The microscope should always be carried using two hands- one on the base and one on the neck. This will help reduce crashing the microscope into the lab bench and misaligning the lenses. The use to the microscope will be discussed in a later chapter.

**Visible Light Spectrophotometer**
Spectrophotometry uses the Beer-Lambert Law to determine the concentration of a compound by creating colors that are proportional to the concentration. This instrument will be discussed in a later chapter.

Figure: Spectrophotometer

**Atomic Absorption Spectrophotometer**
An atomic absorption spectrophotometer is a very expensive high end spectrophotometer used to measure very low levels of metals. It operates in a similar manner as the basic spectrophotometer but measures metals in their atomic state rather than as a molecular compound.

**Inductive Coupled Plasma (ICP)**
This is also a very expensive high end spectrophotometer that measures a nebulized sample into a "plasma" which dissociates molecules into their atomic form. The emission of colors by these ionized materials is measured using a spectrophotometer.

## Maintenance/Service Contracts
In order to produce valid answers, the equipment must be in proper operating conditions. Whenever the equipment calibration cannot be obtained, the data becomes invalid. To avoid this, laboratory personnel can perform both routine maintenance and preventative maintenance. An example of routine maintenance would be to simply press the CAL button on the analytical balance. Preventative maintenance would be to replace the pH electrode or DO membrane at some time interval, long before the data becomes questionable. If the maintenance is beyond the capabilities of the lab technician, a specialist may be needed. These are often expensive but a necessary part of doing business as a laboratory. Some manufacturers offer service contracts. These are also expensive and should be evaluated closely to be sure they cover your maintenance expectations. It is important for all levels of management to understand the role and cost of maintaining laboratory equipment. The laboratory must have sufficient budget to cover maintenance and repair cost. Laboratory data is often required to meet Federal law and intentional filing of invalid data carries civil and criminal penalties. You, the lab technician, are responsible for the data you generate!!

# Chapter 5 Gravimetric Analysis

Gravimetric analysis involves the use of the analytical balance. Common laboratory practices include weighing chemicals for standard and reagent preparation and solids analysis such as Total Suspended Solids, Mixed Liquor Suspended Solids, and Total Dissolved Solids.

## Weighing Solids

One of the basic skills required by a lab technician is the preparation of reagents used in a variety of analyses. To weigh a chemical on an analytical balance, the technician should first place a "tare" on the balance. A "tare" can be any clean, dry object capable of holding the chemical. Common objects that can be used as tares are weighing paper, weighing bottles, weighing boats, or even beakers.

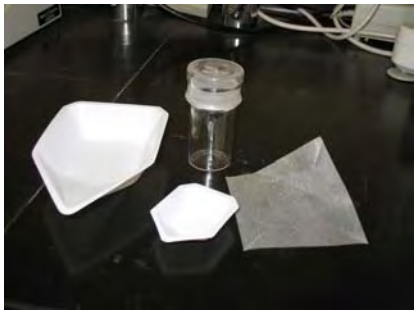


Figure: Various tare materials

If the weighing is only a one time operation, the tare can be zeroed by pressing the zero button/bar. The tare weight will now read zero.

Figure: Tare zeroed

The chemical can now be carefully added directly to the tare using a small spatula. Place the bottle containing the dry chemical through one door of the balance and the spatula through the other door to reduce spilling. After the dry powder has been measured, the balance doors should be closed to verify the weight is correct. Add or remove small amounts of chemical by using a narrow-tipped spatula. To avoid contamination of the original bottle, excess solids should not be returned to the bottle but rather be discarded appropriately.

## Transferring Solids

Once the solids have been accurately weighed, they must be transferred to a receiving container without loss. Accurate weighing is only useful if all the solids are transferred. Carrying a weighing boat across the room is poor procedure. It is better to remove the tare and solids from the balance and bring the receiving container to the balance area. Small amounts of the weighed powder may be lost by carrying solids across the room. Place a small funnel into the neck of the receiving flask to avoid loss of the powder.

Figure: Use a funnel to transfer solids

Pour and wash the solids into the funnel using a deionized water wash bottle. Avoid splashing or spilling the powder. Recover any remaining powder by rinsing the tare several times with deionized water. Pour the rinses through the funnel. Rinse the funnel with several small

volumes of deionized water. Remove the funnel and now rinse the neck of the container with small volumes of deionized water. All the weighed solids should now be in the container.

Figure: Rinse to recover all the weighed solids

If the container is a volumetric flask, add a magnetic stirrer to the flask and dissolve the powder. Use a magnet retriever to remove the magnet and rinse the magnetic retriever with deionized water as it is removed from the flask. Once the magnet is removed, fill the volumetric flask to the mark with deionized water.

The magnet can now be put back into the volumetric flask again to finish mixing the solution completely.

## Weighing Liquids

Liquids cannot be measured accurately on an analytical balance. The analytical balance is so sensitive; it will measure evaporation of the liquids. If measuring liquids, the first weight displayed should be used.

## Transferring Liquids

Liquids which have been weighed are transferred using the same technique as solids. The liquid should be poured into a funnel; the container, the funnel and the walls of the container should be rinsed down with deionized water.

## Reagent Preparation

Reagents are chemical solutions used in the measurement of some specific analyte (what your testing for). Analytical balances are commonly used to measure the amount of chemical called for in the recipe found in *Standard Methods for the Examination of Water and Wastewater*. Most reagent preparation is straight forward and uneventful, however, it is critical that the lab technician read the complete procedure before beginning preparation. Sometimes the last sentence is the first thing you do!!

## Standard Preparation

Concentrations of solutions are usually written in one of 3 forms - Molarity (M), Normality (N), and mg/L. For most laboratories, it is more economical to purchase standard solutions than prepare them in-house. Major suppliers like Fisher Scientific, VWR, and Ricca have a large variety of solutions commonly used in routine laboratory analysis. While these solutions come pre-standardized, the laboratory may have to verify the concentration upon arrival.

### Molarity

Molarity is a form of concentration not typically encountered in wastewater analysis. It stands for moles/liter and is occasionally found in *Standard Methods* as part of a chemical recipe.

### Normality

Normality is a form of concentration commonly describing the concentration of acids and bases. For example, a 0.02 N concentration of hydrochloric acid is weaker than a 1 N concentration. The normality of a solution is measured by a process called standardization. Standardization is a chemical method of

calibration. In the standardization process, the concentration of an unknown solution is compared against a known concentration using a procedure called a titration. This will be discussed later.

**Mg/L**

The most common form of concentration used in water and wastewater analysis is milligrams per liter (mg/L). A milligram per liter would be defined as the weight of a solid component dissolved in one liter of liquid, namely water. For example, a 40 mg/L total suspended solids concentration would mean that there were 40 milligrams of solid material in each liter of wastewater. A 0.15 mg/L sulfate concentration would mean that 0.15 milligrams of sulfate were dissolved in 1 liter of water.

This is the common unit used for analysis of water and wastewater and would be similar to $1 if we were measuring money. It is seldom feasible to test 1 liter of sample but the answer is always reported as if l liter was tested.

For example, suppose you filtered 100 milliliters (0.1 liter) dried the filter then weighed 20 mg of dry solids using the analytical balance.

The answer could be: $\frac{\text{20 milligrams}}{\text{0.1 liter}}$

However, convention calls for the answer to be reported as mg/L. This would make the reportable answer:

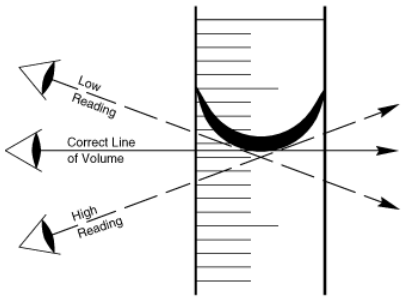
$$\frac{\text{20 milligrams}}{\text{0.1 liter}} = \frac{\text{200 milligrams}}{\text{1 liter}}$$

# Chapter 6 Volumetric Analysis

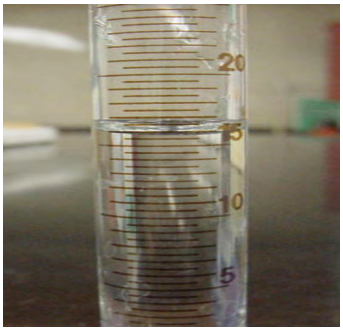
Volumetric analysis involves the measurement of liquids. Since water and wastewater laboratories deal mostly with liquids, it is easier to measure liquids as volumes rather than weigh them. Typical activities of the lab technician would involve measuring volumes and transferring liquids, preparing standard solutions and analysis by methods such as titration and spectrophotometry

When water is placed in a container, there is a chemical attraction between the water and its container. Water tends to adhere to the sides of the container and "climb" the sides. The stronger the attraction, the higher the water will climb. Glass has a stronger adhesive attraction than plastic. This climbing liquid gives a curved and distorted surface called a meniscus.

The water level is measured at the bottom of the meniscus. When measuring liquids, it is important to read the water level at a 90$^{o}$ angle to avoid parallax error. Parallax error occurs when the technician looks at the meniscus incorrectly.




Read the meniscus of the graduated cylinder below. You read _____ ml

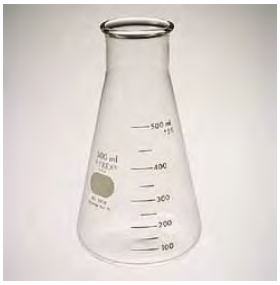



(15 ml)

Glassware manufacturers etch the volume marks on the glassware. Different types of glassware have different calibration marks and accuracy. Glassware can be divided into two general categories - general glassware and measuring glassware.

## General Glassware

The two most widely used pieces of general glassware are beakers and erlenmeyer flasks.



They are used to mix and heat solutions, to hold samples and to provide a container for chemical reactions such as titrations. Many beakers and flasks have graduation markings, however, the markings are only approximate readings, usually within 5%. This inaccuracy delegates these pieces of glassware to measurement of non-critical volumes. Beakers and erlenmeyer flasks can be heated and cooled without concern for distortion of the graduation marks. Glassware marked **Pyrex®** or **Kimax®** can be heated and cooled without regard to temperature shock and resultant glassware breakage.

## Measurement Glassware

All glassware designed for volumetric measurement are labeled either TD or TC. TD glassware is used "To Deliver" while TC glassware is used "To Contain".

**"To Deliver" (TD) Glassware**

Glassware used "to deliver" consists of:

1. Graduated Cylinders
2. Burets
3. Pipets

**Graduated Cylinders**

Graduated cylinders are used to deliver low accuracy volumes. Graduated cylinders are found in volumes of 10, 25, 50, 100, 250, 500, and 1000 milliliters.

In wastewater analysis, graduated cylinders are useful for total solids and total suspended solids measurement because the solids may clog other higher accuracy glassware.

When using a graduated cylinder, select a size closest to the volume needed to reduce the error involved. For instance, if you need to measure 40 ml, use a 50 ml graduated cylinder rather than a 100 ml cylinder. Graduated cylinders are made of high quality borosilicate glass or plastic such as polypropylene. Both polypropylene plastic and glass graduated cylinders can be autoclaved as necessary.

**Burets**

Burets are designed "to deliver" variable volumes. They are made from accurate bore glass tubing with glass or teflon stopcocks and capillary tips. The stopcock is a valve used to regulate the flow of liquid from the buret and with a little practice can deliver 1 drop at a time. Stopcocks are made from either glass or Teflon. Teflon stopcocks are more expensive than glass but eliminate binding problems and contamination from stopcock grease. Cheaper burets still use glass stopcocks and must be lubricated with stopcock grease. When using stopcock grease, only use a very thin layer as the grease will work its way into the bore and plug or restrict the flow of liquid from the buret.

Burets are marked on the side in various graduations. The most common sizes are 25 and 50 mls with graduations divided into 0.1 ml increments. Buret readings can be interpolated to the nearest 0.01 ml but for most analysis, only 0.1 ml accuracy is needed.

**Filling a Buret**

Burets are primarily used for titrations. Titrations require the addition of a controlled and measurable amount of liquid. To accurately measure this volume, the buret must be clean, uncontaminated, and used correctly. To fill a buret correctly and avoid contamination:

1. Close the stopcock

2. Pour approximately 10 ml of the solution to be used into the buret.
3. Drain some of the solution through the buret tip and pour out the remainder to drain.
4. Repeat these steps 3 to 4 times
5. Fill the buret above the zero line, and check for bubbles clinging to the walls of the buret. If possible fill the buret from the original reagent bottle; otherwise, transfer a small amount to a dry, clean beaker. Bubbles on the side of the buret can be released by gently tapping with your finger.

***Safety: Fill the buret over the sink and below eye level.***

6. Withdraw the excess liquid through the tip to remove any air bubbles in the stopcock or capillary tip. A bubble in the tip is an error.

7. Adjust the meniscus to zero by opening the stopcock.
8. Remove any drops clinging to the tip by touching the buret tip to the wall of a waste solution beaker (not the original beaker to avoid contamination). Residual drops on the outside of the buret will give inaccurate results.
9. Place the buret in the buret stand, making sure it is clamped vertically and clamped tightly.

**Care of Buret**
Poor rinsing or cleaning may cause solids to dry on the inside of the buret. A clean buret should produce a smooth sheet of water. If drops of liquid adhere to the inside of the buret, it is dirty and must be cleaned. Small volumes of liquid adhering to the inner glass wall make it impossible to accurately determine the amount of liquid delivered. Wash dirty burets with hot water, soap, and a buret brush. Rinse 10 times with large volumes of tap water, then rinse 5 times with small volumes of distilled water. Wash the buret immediately after use and store inverted on the buret stand with the stopcock open.

Burets should not be used for storage. Always throw the buret contents away when finished for the day and never return them to the original container. If the solution is to be left in the buret for a few hours, cover the buret top with a beaker or cap to prevent dust contamination and liquid evaporation. Never store bases in a buret for any length of time. Bases will cause glass stopcocks to freeze and can etch the glass walls thus altering the buret's capacity.

**Use of a Buret**
To use a buret, simply make sure it is clamped vertically and the stopcock is closed. After filling correctly, turn the stopcock to zero the buret. Record the initial buret reading. To deliver correctly, turn the stopcock slowly to dispense the desired volume. A good technician will be able to control the buret such that only one drop can be delivered if desired. When delivery is completed, record the final buret reading. To determine the volume delivered, subtract the initial reading from the final reading. Be careful to avoid parallax errors.

**Pipets**
The third piece of glassware used "to deliver" various volumes of liquid is called a pipet. There are 2 major categories.

1. Measuring (Mohr and Serological) pipets
2. Volumetric (Transfer) Pipets

Filling any pipet requires suction. All pipets should be filled using a pipet bulb to provide the necessary suction. A pipet bulb is a mandatory safety device and must be insisted upon in all lab work. Pipet bulbs eliminate contact between the analyst's mouth and the pipet, thereby preventing accidental ingestion or inhalation. Pipet bulbs are often contaminated, so they must be handled carefully and cleaned when a solution has accidentally been sucked into the bulb. Clean the pipet bulbs promptly to avoid corroding the internal check valves.

Pipets are used both for measuring and transferring volumes. One major problem encountered with pipets used to measure

wastewater is the presence of large particulate matter. The pipet tip may be too small to allow some solids in and thus filters the sample or the tip may clog when letting liquids/solids out. This will also act as a filter and alter the actual sample concentration.

**Measuring Pipets**

Measuring pipets fall into 2 types:

1. Mohr Pipets
2. Serological pipets

The top pipet below is called a Mohr pipet. Mohr pipets are graduated in various divisions almost to the end of the pipet. No liquid should be delivered past the last graduation. They have very narrow diameter tips and are not very useful in wastewater analysis if suspended solids are present.

Figure: Mohr pipet

Serological pipets are graduated in various divisions all the way to the end of the pipet. These are often called blowout pipets. They have a large bore and work well with most wastewater samples. If solids interfere, the sample can often be homogenized to minimize plugging.

Figure: Serological pipet

***Safety: Serological pipets should be blown out using a pipet bulb not your mouth.***

**Using Mohr and Serological Pipets**

1. Select a dry, clean pipet of appropriate size. If clean, dry pipets are unavailable, rinse with the solution to be used. Avoid contaminating or diluting the solution by dipping a dirty or wet pipet into the original solution. Pour some of the solution into a clean beaker, and then discard after rinsing the pipet.
2. Use a pipet bulb. First squeeze the bulb to remove air, immerse the pipet tip into the reagent then fill the pipet above the calibration mark.

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3. Remove the pipet bulb and place your index finger over the pipet end. These pipet bulbs are not designed to be inserted into the pipet, only set on the top to create a suction.

4. Wipe the pipet tip with a clean cloth or Kimwipe® to remove any drops clinging to the outside of the pipet.
5. Hold the pipet vertically and gradually drain the excess solution into a waste beaker until the meniscus is exactly on the desired mark, usually zero. Never put the excess solution back into the original reagent bottle. If there is a drop still hanging on the tip, gently touch the tip to the side of a waste beaker.

6. To deliver, allow the reagent to drain vertically into the desired receiving container. By gently twisting the pipet, the rate of withdrawal can be controlled. If using the full volume, blow out the last remaining drop in serological pipets but do not go below the last line on Mohr pipets.
7. Discard any excess solution remaining in the pipet.

**Volumetric Pipets**
Volumetric pipets or transfer pipets are special pipets used for accurate work. They have a smaller measurement error than Mohr or serological pipets. Volumetric pipets differ from serological pipets in that they have only one calibration mark. Volumetric pipets come in standard sizes of 1.0, 2.0, 3.0, 4.0, 5.0, 10.0, 15.0, 20.0, 25.0, 50.0, and 100.0 milliliters.

Figure: Volumetric pipet

Most procedures requiring high accuracy use volumetric pipets. If the procedure specifically requires a volumetric pipet, it is implied by using the decimal point in the notation. For instance, if the procedure calls for 5 ml of solution, a serological pipet could be used. However, if the procedure calls for 5.0 ml, a volumetric pipet should be used. Volumetric pipets are commonly used to prepare working standards from stock solutions.

**Using Volumetric Pipets**
1. Use a pipet bulb, suck a few milliliters of the chemical reagent into the pipet to rinse the pipet. Hold the pipet horizontal and turn the pipet to rinse the insides. Drain and discard the solution.
2. Squeeze the pipet bulb to remove air, place the bulb on the end of the pipet, immerse the pipet tip into the reagent then fill the pipet above the calibration mark.
3. Quickly remove the pipet bulb and place your index finger over the pipet.
4. Dry the outside of the pipet tip with a paper towel or kimwipe.
5. Hold the pipet vertically and gradually drain the excess solution until the bottom of the meniscus is on the calibration mark. Do not drain the excess solution back into the original solution.
6. To deliver, allow the reagent to drain vertically into the desired container. Allow the liquid to drain for 5 to 10 seconds after the liquid is dispensed. **Do not blow out.** Touch the pipet tip to the side of the receiving container. There should be a small volume of liquid left in the tip. The presence of this drop is accounted for in the calibration of the pipet and must not be blown out.

**Cleaning Pipets**
1. Store dirty pipets upside down in a pipet basket in soapy water.
2. When full, rinse for 1 to 2 hours in the pipet rinser.

3. Remove and rinse in a distilled water bath 3 times.
4. Let dry. Pipets that do not drain smoothly or leave residual drops inside the pipet are dirty and need more aggressive cleaning with acid.

**"To Contain" (TC) Glassware**

**Volumetric flasks**
Volumetric flasks like volumetric pipets are specially calibrated flasks with a single calibration mark on the neck. Volumetric flasks are used only "to contain" a specific volume, not

to deliver the volume. A 100 ml volumetric flask filled to the mark will **contain** 100 ml but will not **deliver** 100 ml if it is inverted and drained. Commonly available sizes of volumetric flasks are 25, 50, 100, 250, 500, 1000 and 2000 milliliters. Volumetric flasks are used to make reagents and standard solutions. Volumetric glassware is usually calibrated at 20$^{o}$C. Hot liquids should never be added to volumetric flasks and the flask itself should never be heated. Heating will "melt" the glass. While this melting will not be visible it will change the flask's calibration.

Figure: Volumetric flask

**Using a Volumetric Flask**

1. Carefully transfer a known volume or weight to the flask. Volumetric pipets should be used to transfer known volumes. If the substance is a powder, transfer using a funnel. Rinse the powder from the funnel and neck of the volumetric flask several times using distilled water.
2. Fill the flask approximately 3/4 full, stopper, and thoroughly mix. Continue mixing until all the powder has dissolved.
3. Fill to the calibrated mark with distilled water.
4. Stopper and mix by inverting again. Invert a minimum of 10 times to make sure the liquid in the neck gets mixed. Each time allow the trapped air to move through the solution completely. Shaking or twirling is ineffective. Magnetic stirrers are OK but the magnet should be added after filling to the calibration mark. Be careful the magnet does not displace water out the top of the flask.

**Glassware Cleaning**

If handled promptly, most dirty glassware can be cleaned with soap and water. Cleaning should be done as soon as possible to avoid drying solids on the side of the glassware. The standard procedure for glassware cleaning is:

1. Add a ***pinch*** of laboratory detergent to the glassware.
2. Add hot tap water.
3. Scrub the glassware using an appropriate size brush. Avoid scratching the glass. As brushes become used, the metal if often exposed and can easily scratch the glass. Dispose of bad brushes.
4. Rinse ***10 to 20 times with tap water*** to remove the detergent. Allow the water to drain each time.
5. Rinse ***5 times with small amounts of distilled water***. At this point the glassware is clean but has residual tap water that needs to be removed. Distilled or deionized water has few impurities and is used as a final rinse. The glassware does not need to be filled completely when using distilled water. Five to ten precent of the bottle volume is adequate. Again allow time for the glassware to drain between successive rinses.
6. Dry the outside of the glassware and store in a dust free environment.

For more stubborn films or residues such as iron stains, or grease accumulations, stronger cleaning solutions may be necessary. The most common is chromic acid cleaning solution. To prepare this solution, slowly add l liter of concentrated sulfuric acid, with stirring, to 35 ml of saturated sodium dichromate solution. Be sure to use safety goggles, gloves, and an apron when using chromic acid. This solution is very dangerous, requiring special handling and disposal.

# Chapter 7: Sampling

Going out and collecting a sample without first doing some planning may result in a meaningless, contaminated, or unrepresentative sample. No matter how precise lab techniques are, if the sample being testing has not been obtained, stored, or preserved in the proper manner, the results may be invalid and time and material used to test the sample will have been wasted. The greatest lab technician in the world will not obtain meaningful results from a poorly-collected sample. If the results are to be meaningful—that is, if the data is needed to indicate the conditions present when the sample was taken, then some fore-thought to sample collection is necessary. There are many questions to consider before actually collecting a sample. The answers to these questions will help put together a sampling plan which is easier and more effective.

## Prepare a Sampling Plan

A sampling plan should address the following questions.

1. Why is the sample being collected?
2. What tests need to be run on the sample?
3. Where is the sample going to be collected from?
4. How is the sample going to be collected?
5. When does the sample need to be collected and analyzed?

**Why is the sample being collected?**
Wastewater samples are collected for a variety of reasons.

1. Federal and State Regulations
2. Process Control
3. Plant expansion
4. Surcharges

In 1972, Congress passed PL 92500 which became known as the "Clean Water Act". This act provided money for the construction of wastewater treatment plants, operator training, and research. The money had strings attached which required municipalities to monitor the quality of their effluent. As a result a permit system was established by EPA which required municipalities to self-monitoring the quality of their effluent. The NPDES (National Pollutant Discharge Elimination System) system was established and municipalities were required to submit DMRs (Discharge Monitoring Reports). Laboratory tests such as BOD, TSS, pH, and Fecal Coliforms were common to all DMRs.

WWTP operators/managers also run laboratory tests to monitor treatment plant operation. These tests are called process control tests and indicate how well a particular process is working. Lab personnel and/or operators will take samples and run tests to make operational decisions. For example, in a typical wastewater treatment plant there are several places to sample. As wastewater flows through the treatment plant (and through the collection system) its characteristics change. By taking samples at the different treatment basins, information can be gathered to help decide how the plant is operating and whether operational changes need to be made to improve treatment. If sampling and testing procedures are correct, the lab technician can use the test results combined with plant observations and knowledge of the plant operational procedures to formulate a fairly accurate picture or pattern of what is happening in the plant. Operational questions such as:

1. Is the dissolved oxygen level in the aeration basins adequate?
2. How often and how much sludge do you want to waste?
3. Is the chlorine dosage sufficient for disinfection without having too high a residual?
4. Are solids settling well in the primary clarifier?
5. Is the aeration basin removing the BOD effectively?
6. Is there a oil and grease problem in the collection system?

can be answered through laboratory analysis. Timely information gathered from test results will help answer these and other questions.

Laboratory data can also be collected for planning purposes. Are you sampling specifically to justify a new budget or a new procedure or a plant expansion? Collecting laboratory data over a period of time can provide plant managers and operator sufficient evidence to justify building additional treatment units, replacing old units, etc.

Municipalities are often only concerned with the wastewater that arrives at the headworks to the WWTP. Eventually it becomes obvious that the waste contributors must be identified. Larger municipalities establish IPP (Industrial Pre-treatment departments) with full time staff responsible for collecting samples from industry. Industrial samples can play a major role in WWTP operation. Meat packers, food processors, beverage producers, semiconductor manufacturers, etc can all discharge high volumes of high strength, high volume, or toxic materials. Industries are often charged extra when they discharge abnormal amounts. This extra charge is called a surcharge. For example, a coca cola bottling plant may discharge a high sugar waste (BOD of 10000 mg/L). This high level of waste will require additional treatment capacity at the WWTP. If a normal consumer discharges a BOD of 100 mg/L, the industry will be billed more than a normal consumer. Laboratory data will justify the surcharge.

**What specific tests need to be run on the sample?**

Knowing what is going to be sampled will help determine the locations and the number of sampling sites necessary to obtain all the information you want.

A single collected sample can be used for several different tests. Knowing the reason for sampling helps determine what types of tests need to be run and how much sample to collect. Being familiar with the various testing procedures allows the technician to be able to estimate the volume of sample that needs to be collected. Knowing what tests needs to be run also helps budget technician time. Time management is a skill that all lab technicians will have to develop. Lab technicians will often be running several tests at the same time. In order to do this proficiently, the lab technician must have a work plan, taking into account holding times, preservation, drying times, etc. The lab technician may also have to prepare chemicals or reagents ahead of time. Other chemicals may need to be prepared the day of the test because they are unstable and quickly deteriorate in quality.

Knowing the specific tests required also requires the lab technician to understand the wastewater characteristics. Special sampling methods or preservation techniques may be needed. If you are sampling for dissolved oxygen at various depths in a basin, reservoir or lake, there are certain sampling procedures required so that your oxygen levels will be accurate. If the sample contains chlorine, and bacteriological tests need to be performed, a dechlorinating agent will need to be added to remove the excess chlorine so that further disinfection does not occur after the sample has been collected. If samples are very cloudy or turbid, you may want to use a different procedure for some tests to minimize the effects of the turbidity. Refrigerating samples (1 - 6$^{o}$C) will lessen the chance that bacteria will either die-off or multiply, thus ensuring more accurate results from a bacteriological test such as fecal coliform.

**Where are the samples going to be collected?**

If samples are collected from the collection system, which manhole will be used? Does the technician have the necessary collection equipment? Is there a traffic concern? Is there a toxic environment concern? Will additional manpower be needed? Will the sample be well mixed at the sampling point?

If the sample is collected within the treatment plant, the technician should consider the purpose of the treatment unit. Where should TSS samples be collected? Where should BOD samples be collected? Do you collect the sample from the supernatant? Do you collect the sample from a well mixed area? Where is the best place to collect a sample for microscopic examination of the aeration basin?

**How is the sample going to be collected? Are you going to obtain a grab sample or a composite sample?**

## Grab Sample

A grab sample consists of a single container or bucketful of wastewater that is "grabbed" at one specific time. Grab samples indicate the condition of the wastewater at that specific time and may or may not represent the normal conditions. Grab samples are required when the contaminant concentrations change rapidly. For instance, grab samples are required for certain tests, such a pH, D.O. (dissolved oxygen), and bacteriological analyses.

## Composite Sample

A composite sample consists of several grab samples collected from the same spot over a specific period of time and merged into a single sample. A composite sample is more arduous, complicated and usually inconvenient than a simple grab sample. Collecting a sample every few minutes and adding it to a single bottle is tedious, boring, and costly. To help solve this problem, a 24-hour automatic sampler is often used. The automatic sampler consists of a battery pack, a programmable timer, a pump, and as many as 24 bottles.

The automatic sampler can be programmed to draw a certain volume of sample every few minutes and deposit each sample into one of the iced (preserved) bottles.

At the end of the sampling period, the lab technician can retrieve the bottles, bring them back to the lab and create a single composite sample. Analysis can now be performed on a single composite sample that is more representative of the wastewater quality than a grab sample. ***SAFETY: Be sure to handle the automatic sampler safely. A full sampler can weigh as much as 80 pounds.***

**Unweighted composite**

An unweighted composite consists of collecting the same sample volume at a constant time interval. For instance, the technician collects 400 ml every 30 minutes for 6 hours. At the end of the time period, there will be 12 individual bottles representing the wastewater quality over the 6 hour time period. The technician now composites the samples by pouring say 200 ml from each bottle into a large 1 gallon plastic bottle and mixes. The technician now has 2400 ml of a 6 hour composite sample that can be used for analysis. Unfortunately, the sample at 7 am was collected when the water was pretty clear and hardly flowing while the sample at 10:30 was collected when the wastewater was very cloudy and flowing very rapidly. Both these sample are given the same proportion in the composite yet the 10:30 sample represented a higher percentage of the actual wastewater flow. To reduce this unweighted effect, a flow proportional or flow weighted composite is collected.

**Flow weighted composite**

A flow proportional composite is collected using a flow meter connected to the automatic sampler. The flow meter will measure the flow in the wastewater stream. As the flow increases during the day, more samples are collected, thereby collecting a greater volume at the time

when the flow is highest. Using the same example as before, the technician sets the automatic sampler to collect 20 ml every time the wastewater total flow increases by 10,000 gallons. At 7:52, the flow totalizer goes over the 10,000 gallon mark, the sampler is triggered to collect 20 ml and places it in the 1 gallon container in the center of the automatic sampler. At 8:42, the totalizer now records the second 10,000 gallons for the day and again collects 20 ml. As the daily flow increases, the number of samples collected and composited increases. Eventually, at 10:30, the sampler may be collecting samples every 10 minutes. At the end of the 6 hour composite period, the samples in the 1 gallon container represent the composite sample and the technician can begin the analysis immediately.

For labs that do not have sampling flow meters, the waste treatment plant flow totalizer log can be used. In this instance, the automatic sampler will be triggered by time rather than flow. For instance, the sampler was started at 7 am and set to collect 80 ml every ten minutes. At 7:10, 80 ml will be delivered into bottle #1, at 7:20, a second 80 ml will be delivered to bottle #1, again at 7:30, 7:40, 7:50 and finally at 8:00. Bottle #1 will now contain a total of 480 ml. This process will repeat again but the sample will now be added to bottle #2. At the end of the 6 hour composite period, the automatic sampler will contain 6 bottles each containing 480 ml. The technician must now composite the sample by using the WWTP flow meter to determine how much to removed from each bottle.

The formula is:

$$\frac{\text{Flow at time} \times \text{volume of sample needed}}{\text{Total flow during composite period}} =$$

General composite procedure:
1. Estimate the total amount of composited sample that will be needed for analysis. Be sure to collect extra in case of spillage. Doubling the amount needed is a nice safety factor. For instance, the lab needs about 2 liters to run BOD, TSS, and TKN. The technician decides to collect about 4 liters.
2. Collect the samples.
3. Record the flow readings for each time a sample is taken.
4. Add up the flows (it really does not matter what the units of flow are; what is significant is how the flows are proportional to each other)

For example, determine how much sample should be composited from each bottle. Assume 2000 ml should be collected for the analysis

| Time | Flow, gpd | Volume in bottle | Volume to composite |
|---|---|---|---|
| 8:00 am | 40,000 | 880 ml | |
| 9:00 am | 80,000 | 880 ml | |
| 10:00 am | 100,000 | 880 ml | |
| 11:00 am | 200,000 | 880 ml | |
| 12:00 pm | 120,000 | 880 ml | |
| 1:00 pm | 60,000 | 880 ml | |

Answer: The total flow is 600,000 gal. Using the formula, the amount of sample that should be removed from bottle #1 (8:00am) is:

$$\frac{40{,}000 \text{ gpd} \times 2000}{600{,}000 \text{ gpd}} \text{ ml} = 133 \text{ ml}$$

Calculate the amount that should be composited from the remaining samples. Notice that more sample is composited during the higher flows.

| Time | Flow, gpd | Volume in bottle | Volume to composite |
|---|---|---|---|
| 8:00 am | 40,000 | 880 ml | 133 ml |
| 9:00 am | 80,000 | 880 ml | 267 ml |
| 10:00 am | 100,000 | 880 ml | 333 ml |
| 11:00 am | 200,000 | 880 ml | 667 ml |
| 12:00 pm | 120,000 | 880 ml | 400 ml |
| 1:00 pm | 60,000 | 880 ml | 200 ml |

When these volumes are added to the sample container, the final volume will be 2000 ml.

Mix the sample well before analysis. BOD and TSS are often performed on composited samples.

There is another type of composite sample, which involves sampling at different locations such as a compost sludge pile, rather than sampling over a period of time. For example: sludge regulations require that pathogens be destroyed prior to disposal. A composite sample of the sludge is collected by grabbing samples from various areas of the sludge pile then composite them.

## When does the sample need to be collected and analyzed?

Finally, the lab technician needs to know when the sample should be collected and how long the sample can be held before analysis must begin.

For instance, the discharge permit may require the sample be collected over the peak flow period. If the sample is to be a 6 hour composite, the lab technician may need to have the sampler prepared by 8:00 am to be able to collect the composite over the 6 hour peak flow period. Will there be enough time to composite and analyze the sample? In this example, the composite will finish at 14:00. How long will it take to prepare the composite? How long does the analysis take? Can the sample be held until the next morning? The lab technician needs to plan? If the sample cannot be preserved overnight, has the lab technician done preliminary preparation to expedite the analysis?

## Sample Collection

**Safety First!!!** Laboratory safety was discussed earlier, but many of the same precautions are required when collecting wastewater samples. Gloves, goggles, and lab coats are the minimum requirements for a safe sample collection plan. Avoid selecting sampling sites that present a hazard such as leaning over railings, slippery decks, etc.

**Prepare the sample bottles**

Choose sample bottles that will not affect or react with the sample. For most normal wastewater samples, either glass or plastic is acceptable. The most common glass container is composed of borosilicate glass (hard glass). Borosilicate glass is marketed as Pyrex or Kimax. Plastic bottles are generally composed of LPE (Linear Polyethylene) or PP (polypropylene). There are many other types of sampling containers and the sampler should be aware of the composition when analyzing for trace contaminants. Containers may decompose and release contaminants or sorb materials onto the walls of the bottle. The sampler should also be aware of the type of bottle cap used. For instance, caps with metal foil liners should not be used when collecting metal samples. For trace analysis, the type of sample container should be documented.

For microbiological analysis such as total or fecal coliform, the sample bottle must be sterile and contain a dechlorinating agent such as sodium thiosulfate. The minimum bottle size is 120 ml and at least 1" of air space must be present to allow for adequate mixing.

Use amber colored bottles for collecting light sensitive materials, usually organics.

Discard cracked, chipped and etched glass bottles. Discard crazed plastic bottles. Discard bottles which are leaking.

Just like lab glassware, sample bottles should be clean and dry. Clean by using (alconox) detergent and hot water; rinse with tap water 10x to remove all trace of detergent; then rinse five times with small amounts of DI water. In most cases, the sample container should never be rinsed with the sample prior to collection. Pre-rinsing may allow some contaminants to stick to the sides of the container or settle to the bottom prior to the final sample collection. For instance, pre-rinsing with an influent sample

may cause "extra" oil and grease to stick to the sides or "extra" settleable solids to remain in the bottom of the bottle. This would cause a high bias to the sample answer. Pre-rinsing may also rinse out pre-loaded preservatives such as acid. Samples collected for total or fecal coliforms should never be rinsed. Rinsing will remove the dechlorinating agent.

**Site Selection**

Remember, the test result is only as good as the sample collected. Select a sample site that is

1. safe
2. representative
3. uncontaminated.

**Sample Identification**

Once the sample is collected it must be labeled. Label each sample container with an adhesive tag or label. Know in advance what information you will put on the container. An information form preprinted on the tags or labels provides uniformity of sample records, assists the sampler, and helps ensure that vital information will not be omitted. Useful information to put on the sample container includes:

1. Sample identity code.
2. Signature of sampler.
3. Signature of witness.
4. Description of sampling location detailed enough to accommodate reproducible sampling. (It may be more convenient to record the details in the field record book). This may include depth when sampling ponds or clarifyers.
5. Sampling equipment used.
6. Date of collection.
7. Time of collection.
8. Type of sample (grab or composite).
9. Water Temperature.
10. Sampling conditions such as weather, water level, flow rate of source, etc.
11. Any preservative additions or techniques.
12. Record of any test results done in the field.
13. Type of analyses to be done in laboratory.

It's guaranteed, that lab technicians who do not label sample bottles will eventually get the bottles mixed up either in route to the lab or in the lab.

**Preservation, Storage, and Holding Times**

Deliver the sample to the laboratory as soon as possible after collection. Holding time is considered the time interval between collection and analysis. Samples begin to change as soon as they are removed from their environment. The shorter the holding time the more reliable the analytical results will be. If the sample cannot be delivered to the laboratory within 2 hours, it should be preserved. Preservation only helps retard chemical and biological changes that begin immediately after sample collection. The sampler should plan for transportation to the laboratory. If the sample must be shipped, commercial transportation such as FEDEX ® and bus pickup and delivery schedules must be considered.

Some samples cannot be preserved because the sample conditions change quickly. These samples must be analyzed either in situ or on site immediately after collection. Such analyses would be pH, DO, chlorine residual, and temperature.

There are no absolute methods of preservation but the methods used are generally intended to retard biological action, retard chemical changes and reduce volatility of organic molecules. Ice samples that need to be transported to the laboratory or that will be composited. If using an automatic sampler, the bottles in the sampler should be packed in ice during the compositing period. Do not freeze samples or use dry ice. Once the samples reach the laboratory, refrigeration is used to retard biological activity and reduce volatilization of organics until the time of analysis. Be sure to document time of arrival and refrigeration temperature.

Chemical contaminants such as metals are commonly preserved using acid and dropping the pH to below 2. The addition of acid drops the pH and helps retard chemical oxidation and precipitation reactions. Ideally add the acid prior to the sample bottle so that preservation begins immediately. Check the pH and document at the time of collection.
Once the samples have been delivered to the laboratory, it is the responsibility of the lab manager/technician to maintain the preservation and assure timely analysis. Samples that exceed the holding time should be invalidated or identified as such. For composited samples, it is common practice to use the time at the end of the composite collection as the sample collection time.

## Chain of Custody

Most samples are simply collected by the wastewater laboratory technician or operator, labeled and returned to the laboratory for analysis. However, when legal litigation is indicated, it is necessary to have an accurate written record to trace possession and handling of samples from collection through reporting. This chain of custody should be able to identify who handled the sample from collection, to transport, to storage, to analysis, to destruction. An example of a chain of custody form is included in Appendix A

A sample is in "custody" if:

1. It is in one's actual physical possession.
2. It is in one's view, after being in one's physical possession.
3. It is in one's physical possession and then locked up so that no one can tamper with it.
4. It is kept in a secured area, restricted to authorized personnel only.

**Collection**

Field collection records should be completed at the time the sample is collected and should be signed by the collector. Typical field information would be:

a. Date and time
b. Sample location (be detailed)
c. Preservation used
d. Analysis requested
e. Name and signature of collector(s)
f. Pertinent field data (DO, PH, Chlorine residual, temperature)
g. Serial numbers of transport cases, sample log numbers
h. Comments

The sample should have a waterproof label and the information written with an indelible pen. The sample container should be closed, placed in the transport case, then sealed and transported.

**Transport**

Usually samples that are headed for court are not sent to the in-house lab, which means they may be hand delivered, delivered by mail, or delivered by common carrier. The chain of custody form must accompany the sample. If hand delivering, have the receiving person sign the form. If sending by mail, send as registered mail with return receipt requested. If sending by common carrier, keep the sending receipt.

**Laboratory**

Laboratory personnel are responsible for the care and custody of the sample once it is received and be able to testify that the sample was secured in the laboratory at all times from the moment it was received until the time it was disposed. The receiving laboratory will usually designate one person (the custodian) to be responsible for the sample custody. The custodian will maintain a lab book which tracks the movement of the sample throughout the laboratory. The log book will indicate who removed the sample from the custody area, when it was removed, when it was returned, when it was destroyed.

**Disposal**

The laboratory custodian will dispose of the sample when it is certain that the information is no longer required, or the sample has deteriorated. A sample is considered to have deteriorated when the maximum holding time has elapsed.

# Chapter 8 Electronic Measurement

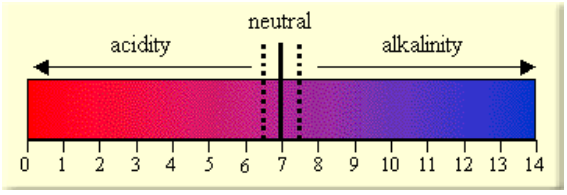
There are a number of sample analyses that can be performed using a meter and electrode. The three most common electronic measurements in wastewater are pH, conductivity, and dissolved oxygen. In electronic measurement, an electrochemical reaction occurs at the end of the electrode. This reaction creates an electrical millivolt current that is sent to the meter which interprets the current and compares it against a known millivolts value. The current is then translated to a value and displayed on the meter screen. All electronic meters must be calibrated -- they don't know anything until you tell them.

## pH

pH is a measure of the acidity or basicity of a sample. Pure water dissociates into hydrogen ions and hydroxide ions.

$H_2O \rightarrow H^+ + OH^-$

Because there are equal concentrations of hydrogen ions and hydroxide ions, the water is considered neutral and the pH is 7.0. If another substance is added to pure water and adds more hydrogen ions there will be a higher concentration of hydrogen ions than hydroxide ions. This solution is called an acid. Likewise, if a sample has more hydroxide ions than hydrogen ions, the solution is called a base.




The pH scale goes from 0-14 with 7 being considered neutral. If the sample pH is below 7.0, the sample is acidic. If the sample pH is above 7.0, the sample pH is alkaline or basic. PH is directly related to the concentration of acids and bases. Each change of 1 pH unit represents a 10 fold change in concentration. For example, a sample with a pH of 2.0 is 1000 times more acidic than a sample with a pH of 5.0. Likewise an acid on the laboratory bench labeled 0.1 N Hydrochloric acid solution is 100 times more concentrated than a 0.001 N solution and may pose a safety hazard. If the pH of the 0.001 N acid is 3.0, what would the pH of the 0.1 N be? (pH =1.0 ) Be sure to wear appropriate gloves, goggles and apron when handling concentrated acids and bases.

**pH Measurement**

The pH meter is used to measure the hydrogen ion concentration of a sample. For routine work, the pH meter must be accurate and reproducible to the nearest 0.1 pH unit. pH is measured by an electrode that is sensitive only to hydrogen ions. For routine lab work, a sturdy combination electrode with either a plastic or glass body should be used. pH papers or pH indicator strips are unacceptable for laboratory pH measurement.

**Electrode Preparation**

A combination electrode contains both a measuring electrode and a reference electrode. The hydrogen ions in the sample create a potential difference which is compared to the reference electrode. This difference is translated into millivolt current and is displayed on the meter. As the electrode ages, potassium chloride inside the electrode is lost and must be periodically replenished. A glass combination electrode usually has a small hole on the side

which is used to fill the electrode with potassium chloride.

Gel filled combination electrodes cannot be refilled and must be discarded when they fail.

pH meters may also have an attachment port for an ATC (automatic temperature compensation) probe. This probe will correct for minor pH errors due to differences in temperature. The pH difference between a sample at 5$^{o}$C and 20$^{o}$C is about 0.1 pH unit. Some pocket pH meters are readily available that automatically compensate for temperature.

**Electrode Storage**

The electrode must **NOT** be stored dry. The best solution to store normal combination electrodes is saturated potassium chloride, (KCl) or if unavailable, use pH 7 buffer. Tap water or deionized water should not be used because the electrolyte within the electrode (KCl) will diffuse out and cause premature aging or additional maintenance. If the electrode is not going to be used for a long time, the electrode can be stored in a large test tube filled with KCl and covered with parafilm to prevent evaporation. Clean the electrode if a biofilm develops in the storage solution.

**Daily pH meter Calibration**

Modern digital pH meters are normally very stable and need to be calibrated only once or twice daily. Calibrate more frequently if meter is unstable or drifts. The pH meter should be calibrated with a minimum of 3 buffers. pH 7.0, pH 4.0 and pH 10.0 buffers are the standard buffers commonly used. It is best to select buffers that bracket the expected sample pH. For example, if the sample pH is usually 7.8, the lab technician should use at least pH 7.0 and pH 10.0 buffers. Sample pH and standard buffers should be measured at the same temperature, generally room temperature. There are many different manufacturers of pH meters and they all require calibration before use. Inexpensive meters may have only one calibration knob, while more expensive meters will have the capability of using multiple buffers.

Always follow the manufacturer's instructions. The following is an example of a typical calibration procedure.

**pH Meter Calibration Procedure**

1a Connect the pH electrode to the pH meter. Remove the electrode from the KCl or pH 7 buffer storage solution; rinse with deionized water and blot dry. TIP: The bulb of the pH electrode must be below the water level. If the meter reading is erratic, check the water depth.

1b. Select the pH mode.

1c. Clear any previous calibration data. Press the **STANDARDIZE** button. Calibrate with at least 3 buffers. Select pH buffers that will bracket the typical sample pH. A third buffer can be used to validate the electrode response. Normal buffers are 4, 7, 10. Purchasing color coded buffers is recommended.

1d Place the electrode in pH 7 buffer. Press the **STANDARDIZE** button and **ENTER** the standardization value. A small 7.0 should appear; indicating the calibration point has been entered.

1e Rinse the electrode with deionized water and blot dry.

1f Place the electrode in pH 10 buffer. Press the **STANDARDIZE** button and **ENTER** the standardization value. A small 10.0 should appear; indicating the calibration point has been entered.

1g Rinse the electrode with deionized water and blot dry.

1h. Place the electrode in pH 4 buffer. Press the **STANDARDIZE** button and **ENTER** the standardization value. A small 4.01 should appear; indicating the calibration point has been entered. .

1i Press the SLOPE button. The slope should be 56 ± 3 mv or (>95% efficient) **QC: Record the slope on the pH Meter Calibration Log.** Press the slope button again to get back to the original pH display.

Corrective Action: If the slope is not within tolerance, recalibrate. If the meter does not calibrate properly, replace the buffers or electrode and try again.

**Automatic Temperature Compensation**
If using an ATC probe, the probe should be placed in the buffers and sample at the time of calibration.

**Troubleshooting pH Meter**
1. Disconnect the electrode and attach the shorting strap.

2. Press the millivolt button. The display should show zero. If the display does not change, the meter needs repair.

3. Gel electrodes generally require no maintenance if kept moist. However gel electrodes have a shelf life of around 3 years. Recording the date the electrode was placed in service helps with troubleshooting. If the electrode response is sluggish or does not adjust to the correct pH, the electrode is about to fail and should be replaced.

4. Glass electrodes fail because of scratches, deterioration, and accumulation of debris on the glass bulb. Rejuvenate the electrodes by cleaning in 0.1 M HCl and 0.1 M NaOH.

Corrective action: If the pH responds slowly or drifts after rejuvenation, replace the electrode.
5. Reference electrodes fail because of clogged liquid junctions. The liquid junction must remain open to avoid slow response and meter drift. If the liquid junction becomes clogged, place in warm deionized water for several hours. Some combination electrodes can be refilled with saturated KCl.
6. If the pH meter does not respond to different buffers (remains at pH 7 all the time), replace the electrode.

**pH Sample Handling**
1. Clean plastic or glass containers can be used
2. Samples should be collected and analyzed within 15 minutes. No sample preservation is permitted. On site analysis is preferred.
3. Minimum sample volume should be 50 ml.

**SAFETY: Use latex gloves when collecting and handling all wastewater samples**

**Sample pH Measurement**
1. Use a calibrated pH meter. The meter should be in "pH" mode.
2. Carefully pour the sample into a 100 ml beaker. If the temperature is significantly different from room temperature, use the Automatic Temperature Compensating (ATC) Electrode if available or adjust the meter manually.
3. Immerse the pH electrode into the solution. Be careful not to break the glass bulb.
4. Swirl the sample gently until the meter reading stabilizes. For poorly buffered samples such as deionized water, immerse in three or four consecutive portions of sample, and then immediately measure the pH in a fresh sample.
5. Report the answer to the nearest 0.1 pH units on the bench sheet.

**Data Management and Records Management**
1. All records must be maintained in three ring binders or bound laboratory notebook.
2. Maintain all calibration records for a minimum of 5 years.

**pH Quality Control**
Decisions on the accuracy of the reported data will be based on the quality control information.

 Sample holding time less than 15 minutes.
Corrective Action: Reject samples and request a resample. Measure on site.
 Record sample date, time, type, sampler, date and time of analysis, analyst.
 Record method used
 Samples must be mixed well, poured quickly, and swirled constantly when measuring the pH
 Calibrate against a minimum of 3 buffers.
 Record slope
 Buffers should bracket the expected sample pH.
 Buffers should be used within the expiration date. Record date of purchase, lot number, and expiration date of buffers used.

Corrective Action: Expired buffers should be discarded. Replace with fresh buffers. Purchase a 6 month supply and rotate stock frequently.

 Store buffers in the dark, at room temperature, and in the chemical supply cabinet.
 Performance evaluation samples should be run at least annually.
 Split samples can be run with a second lab or second lab technician.
 Duplicate sample pH results should be within 0.1 pH units.

## Conductivity

Conductivity or electrical conductance (EC) is a measure of the ability of a solution to conduct an electrical current. Current is conducted by the presence of ionic compounds in the water. The more ions present, the easier it is to conduct the current and therefore the higher the conductivity. Conductivity in wastewater analysis provides a quick estimate of the Total Dissolved Solids of the solution. Conductivity is also used as part of the laboratory QC program indicating the quality of reagent grade deionized or distilled water.

The conductivity cell consists of two platimum electrodes coated with platinum black. The distance between these two wires is called the cell constant. The most important requirement in conductivity measurement is a clean cell. A dirty cell will contaminate the solution and cause the conductivity to be incorrectly measured.

**Conductivity Units**
Conductivity is customarily reported in units of micromhos per centimeter (umhos/cm) or the international units of microsiemans/cm (uS).
1 umhos/cm = 1 uS/cm

**Ions**
Ions are atoms or groups of atoms that have an unbalanced number of electrons and protons. The most common example of an ion is sodium. Sodium in its atomic form has 11 protons and 11 electrons. Unfortunately, the #11 electron is a long way from the nucleus of the atom and is easily lost; leaving only 10 electrons. Because sodium now has 11 protons and 10 electrons, it has a +1 charge. This is the most stable form of sodium and is the only form found in water. There are a large number of minerals in water that are commonly in ionic form; ie. $Ca^{+2}$, $Mg^{+2}$, $Cl^{-1}$, $SO_4^{-2}$, $HCO_3^{-1}$, $Fe^{+2}$, $PO_4^{-3}$, $NO_3^{-1}$

**Non-ions**
There are other compounds in water that do not have a charge. These compounds are usually classified as molecules. Molecules share electrons and don't conduct electrical current well. Two common non-ionic compounds are silica and organic molecules.

**Meter Calibration**
To measure conductivity, the conductivity meter and electrodes must be calibrated.

The conductivity meter is calibrated against a 0.01 N potassium chloride (KCl) solution. This solution is easy to make and will have a reference conductivity of 1412 umhos at 25$^{o}$C. There are many conductivity meter manufacturers so follow the manufacturers recommended calibration procedure.

**Calibration Procedure**

The following is a universal calibration procedure.

1. Clean the conductivity cell with DI water if needed.
2. Adjust the temperature of the standard to 25$^{o}$C. An ATC (automatic temperature compensating electrode) can be used instead.
2. Rinse the electrode with deionized water to remove any cleaning solution.
3. Pour the 0.01 N standard KCl solution into 3 labeled 100 ml beakers.
4. Dip the conductivity cell into beaker #1, swirl slowly. Be sure to immerse the electrode below the hole in the side of the electrode.
5. Dip the conductivity cell into beaker #2, swirl slowly. Do not rinse the electrode with DI water between beakers.
6. Finally dip the conductivity cell into beaker #3. Avoid touching the conductivity cell to the sides or bottom of the container as the standard is swirled slowly.
7. Press the standardize knob/button to adjust/enter the standard value of 1412 umhos.

**Conductivity Sample Handling**

1. Clean plastic or glass containers can be used
2. Samples should be collected and analyzed as soon as possible. The sample may be preserved by refrigeration up to 28 days.
3. Sample volume should be at least 500 ml.

**Conductivity Measurement**

Once the conductivity cell has been calibrated, the sample is measure by following the same procedure as the standard.

1. Rinse the electrode with deionized water
2. Adjust the temperature of the sample to 25$^{o}$C. An ATC (automatic temperature compensating electrode) can be used instead.
3. Pour the sample into 3 labeled 100 ml beakers.
4. Dip the conductivity cell into beaker #1, swirl. Avoid touching the conductivity cell to the sides or bottom of the container.
5. Dip the conductivity cell into beaker #2, swirl.
6. Finally dip the conductivity cell into beaker #3.
7. Record the conductivity value displayed on the meter.

**Low Conductivity Samples**

One of the controls performed on laboratory reagent water is conductivity. Because the conductivity is so low, it may be significantly influenced by temperature, measurement time, mixing, atmospheric gases ($CO_2$). Ideally the conductivity should be measured in a flowing stream of DI water to reduce the holding time errors created by dissolving $CO_2$.

**Electrode Storage/Replatinization**

The platimum black coating on the electrodes of the conductivity cells is extremely important to the cell operation. If the cell constant begins to change, inspect the electrodes for wear or flaking of the platinum black. To help reduce flaking of the electrodes, store the electrodes in deionized water. If the electrode shows wearing or flaking, it can be re-platinized. The re-

platinizing solution is expensive but can be reused many times.

**Quality Control**
Decisions on the accuracy of the reported data will be based on the quality control information.

**Sample QC**
 Record sample date, time, type, sampler, date and time of analysis, analyst and method used
 Samples must be mixed well, poured quickly, and swirled as soon as possible for best results.
 Record the sample temperature and adjusted temperature ($25^{o}C \pm 0.1$)

**Equipment QC**
 Calibrate against a conductivity standard. 0.01 N KCL is the most common standard. Record the date the standard was prepared or purchased, lot number, and expiration date
 Record the cell constant.
A good meter will have the ability to display the cell constant. Record the cell constant. It is normally around 1.0. Record the value in the calibration log book.

Corrective Action:
Replace or replatinize cells that do not show the expected cell constant.

 The conductivity standard used should be within the expiration date. Record date of purchase, lot number, and expiration date.

Corrective Action: Replace expired standards

 Use an automatic temperature compensation electrode.
 Performance evaluation samples should be run at least annually.
 Split samples can be run with a second lab or second lab technician.

## Dissolved Oxygen

Dissolved oxygen is often measured to determine the oxygen level in receiving streams, in the aeration basin, and in the BOD test. The DO meter and electrode work in similar fashion to other electronic meters but unlike the others, this meter must be continually "on" to remain polarized. To be "on" the electrode must be continuously attached to the meter and the meter must be either plugged into an AC outlet or if battery operated, the battery must be good. If the battery fails, or the meter is unplugged, the DO electrode must be repolarized for an hour before being used to measure a sample.

**Selecting a DO meter**
ASK! There are a number of DO meter manufacturers and a lot of headaches associated with DO meters. Talk to a number of other laboratories to find out about membrane replacement, manufacturer support, ease of calibration, and reliability. Purchasing a $1000 meter, then finding out the manufacturer will not support the electrode after a few years is a tough pill to swallow. Are accessories for BOD measurement available, fragile, expensive? When running the BOD test, how many parts will have to be cleaned between samples? Lots of parts will mean lots of potential sources of error.

**Calibrating the DO meter**
DO meters must be calibrated following the manufacturer's instructions. Since oxygen is a gas, it is influenced by the barometric pressure (altitude) and temperature. The manufacturer provides a table that can be used to determine the saturated DO level at different elevations and temperatures. Meters are commonly calibrated against a saturated air solution. The saturated air can be a saturated sponge in the storage sleeve (Orion) or in a BOD with about 1" of water (YSI).

Figure: Laboratory calibration of DO meter with BOD probe

It is recommended that the DO meter calibration be periodically validated using the Winkler Titration Method.

**Membrane replacement**

A functioning membrane is essential to good DO measurement. The membrane allows oxygen to permeate to the electrode where it creates a millivolts current directly proportional to the DO concentration. One of the major advantages of the electrode method is that it can be used continuously. The DO electrode can be submerged in the stream to different depths or in an aeration basin and the DO monitored continuously. However, the membrane does not last indefinitely and must be changed frequently.

Figure: DO electrode, the membrane at the end of the probe requires periodic replacement.

Erratic calibrations or readings are usually indicative of either a bad membrane or bad battery. Maintaining a maintenance log will help the lab technician predict when failure is likely to occur.

Replacing the membrane should only take a couple of minutes. Follow the manufacturer's instructions and be sure to use the manufacturers recommended filling solution. The filling solution is not the same as the KCl used for the pH meter. The electrode should be disconnected from the meter when the membrane is replaced. If the membrane has been replaced properly there should be no air bubbles beneath the membrane and the membrane surface should not be wrinkled.

**Sample Measurement**

The DO should be measured at a representative point. Remember dissolved oxygen is a gas and is affected by turbulence and temperature. Measuring a DO sample at a point of high turbulence will not be representative. Likewise, measuring DO in shallow or quiet areas of a river will be different than in deeper and faster moving areas. A sampling plan will help define what information is needed and where the most appropriate location will be.

Using the DO meter for BODs will be discussed in the BOD chapter.

**Specific Ion Electrodes**

Specific ion electrodes are used in wastewater for such parameters as Total Chlorine Residual or Ammonia. They follow the same rational and techniques as a pH meter which is a specific ion electrode measuring hydrogen ions. These electrodes must be calibrated using prepared standards with each run and the calibration information recorded just like pH.

# Chapter 9 Titration

A titration is a method of determining the unknown concentration of a substance by reacting it with a known chemical substance. A titration requires
1. the reaction between the chemicals to be rapid and complete.
2. few competing reactions
3. the exact quantities of one of the reactants to be known.

There are several types of titrations but the two most common titrations are acid/base titrations and oxidation/reduction titrations.

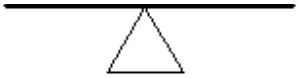
## Titration Theory

Analytically, all titrations are the same. A titration occurs when a chemical called a titrant is placed in a buret. The unknown sample is placed in a beaker or Erlenmeyer flask. A chemical called an indicator is added to the unknown solution and stirred. As the stopcock on the buret is opened, the titrant is added, the unknown sample begins to react with the titrant. The titrant continues to be added until the indicator changes to the expected color. The color change indicates the teeter totter is balanced and the endpoint reached. The endpoint indicates an equivalent amount of titrant and sample have been added and the reaction is complete. The unknown concentration can now be calculated.

## Equivalents

The titration is essentially a chemical balancing act.

Equivalent A = Equivalent B
(4 Quarters) (10 dimes)

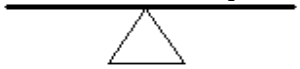


When the equivalents of A equal the equivalents of B, the teeter totter will be balanced and the equivalence point is reached. For example, if 4 quarters are placed on the left side of the teeter totter, 10 dimes could be placed on the right side of the teeter totter. Even though both sides don't weigh the same, they are equivalent because they would both purchase a $1 hamburger. We could then say 4 quarters is equivalent to $1. If one equivalent is $1, how many equivalents would be present if the left side of the teeter totter has 20 quarters?

$$20 \text{ quarters} \times \frac{1 \text{ equivalents}}{4 \text{ quarters}} = 5 \text{ equivalents}$$

How many equivalents would it take to balance the teeter totter?

5 Equivalents = 5 Equivalents

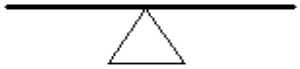


How many dimes would it take to have 5 equivalents on the right side?

$$5 \text{ equivalents} \times \frac{10 \text{ dimes}}{1 \text{ equivalent}} = 50 \text{ dimes}$$

If 37 pesos equals $1, how many pesos would it take to balance 50 dimes?

$$50 \text{ dimes} \times \frac{1 \text{ equivalents}}{10 \text{ dimes}} = 5 \text{ equivalents}$$



$$5 \text{ equivalents} \times \frac{37 \text{ pesos}}{1 \text{ equivalent}} = 185 \text{ pesos}$$

By using equivalents in a titration, chemical reactions do not need to be balanced or even understood. In a titration, equivalents are hidden in the term Normality, N. By definition a 1 N solution contains 1 equivalent per liter, therefore, a 0.02 N solution would contain 0.02 equivalents per liter.

## Standardization

The first step in a titration is the standardization of the titrant. When performing a titration, one side of the teeter totter <u>always</u> needs to be known. By convention, this is usually the solution in the buret. This solution can be prepared or purchased. In either case, the concentration of the titrant must be known or verified if purchased. To standardize the titrant, a primary standard is used. A primary standard is a solution that has special characteristics. The most important characteristics of a primary standard are

1. stable to light. The standard does not change concentration when exposed to light
2. stable to temperature. The standard does not change concentration when exposed to an increase or decrease in temperature.
3. doesn't absorb moisture when weighed (not hygroscopic). This is very important when preparing in-house standards.

In most cases, it is best to purchase primary standards from a reputable supplier than to prepare them in-house.

For example, let's purchase a 0.02 N primary standard solution of sodium carbonate. The 0.02 N indicates the bottle has a concentration of 0.02 equivalents of sodium carbonate per liter. Now pipet 20.0 ml of the sodium carbonate standard solution into a 100 ml Erlenmeyer flask. The flask now contains 0.0004 equivalents of sodium carbonate.

$$\frac{0.02 \text{ equivalents}}{1 \text{ liter}} \times \frac{1 \text{ liter}}{1000 \text{ ml}} \times 20 \text{ ml} = 0.0004$$

One side of the teeter totter is now known.

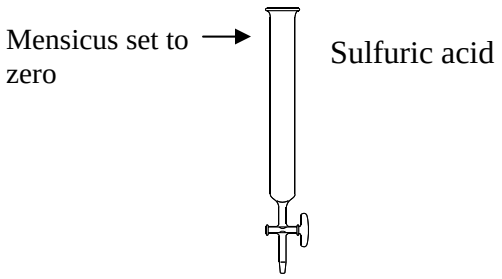
$$0.0004 \text{ equivalent} = \text{Equivalent B}$$

A few drops of methyl purple indicator is added to the sodium carbonate to create a green color.

The right side of the teeter totter is the solution that needs to be standardized. In this example, sulfuric acid will be on the right side of the teeter totter and in the buret. Once we standardize the sulfuric acid it can be used to determine the alkalinity of the anaerobic digester.

The buret is filled from a bottle of sulfuric acid that has been sitting on the shelf for 3 months. The bottle is labeled 0.02 N but since the sulfuric acid is not a primary

standard it must be periodically standardized since it may have deteriorated. First, remember to rinse the buret with small amounts of the sulfuric acid. Bleed the buret tip to remove bubbles, then adjust the meniscus to zero.




The titration now begins as the sulfuric acid is "titrated" into the Erlenmeyer flask. The sulfuric acid titrant should be added as quickly as possible without passing the endpoint and should be swirled to quickly complete the reaction. The flask can be swirled by hand or more conveniently stirred using a magnetic stirrer. If using the magnetic stirrer, the magnetic will be placed in the Erlenmeyer flask and the speed adjusted to mix quickly without splashing the solution onto the side of the flask.

In this example, the endpoint will be purple. As the sulfuric acid is added, the solution in the Erlenmeyer flask will flash purple as the titrant is added. The color will quickly return to green. As the teeter totter comes closer to balancing, (reaching the equivalence point), the purple color will last longer and longer. At this point the addition of the sulfuric acid should be slowed to a drop at a time.

When the purple color finally persists, the equivalence point has been reached, the teeter totter balanced and the standardization is complete. Record the volume of sulfuric acid that was added from the buret.

**Calculations**

All titrations use the same basic formula

| |
|---|
| Concentration A x Volume A = Concentration B x Volume B |

Notice how the formula sets up like the teeter totter. In this example, 20.0 ml of 0.02 N sodium carbonate was used and titrated to the purple endpoint using 24.2 ml of sulfuric acid.

| |
|---|
| 0.02 N x 20.0 ml = Sulfuric acid concentration X 24.2 ml |

Solve the equation to determine the acid concentration.

$$\frac{0.02\ \text{N} \times 20.0\ \text{ml}}{24.2\ \text{ml}} = \text{Sulfuric acid concentration}$$

0.017 N = Sulfuric acid concentration

The sulfuric acid that has been sitting for 3 months has now been standardized. Notice that the concentration from a bottle labeled 0.02 N has deteriorated over the past 3 months to an actual concentration of 0.017N. This decay is why standardization

is essential. All data generated during the past 3 months is invalid.

**Quality Control**
How often should titrants be standardized? There is no uniform answer since all titrants do not have the same stability. The best answer is that standardization should be performed frequently. If the concentration changes between standardizations, all data generated after the last standardization becomes invalid. If the sulfuric acid just standardized had been used during the past three months rather than just sitting on the shelf, any results generated would be incorrect since the normality was incorrect.

Performing the standardization is only half the job. All the standardization information must be recorded.

**QC** Record the lot number, expiration date, purchase date, concentration of the primary standard
**QC** Record the date and time of the standardization
**QC** Record the name of the analyst

**Additional tips**
Some endpoints are difficult to determine. Going past the endpoint by adding more sulfuric acid than necessary will unbalance the teeter totter and give erroneous results. When in doubt, note the buret reading, then add another drop or two. If the color still changes, continue adding a drop at a time until the color persists. If the color does not show any change, use the previous buret reading.

A sheet of white paper placed below the beaker or flask can sometimes help visualize the endpoint color.

A drop of titrant on the tip of the buret is an error. Once the drop has left the buret, the drop has been added to the solution as far as the buret reading is concerned. The drop can be added to the solution by using a DI water squirt bottle to rinse it into the Erlenmeyer flask.

Always validate the normality of a purchased standard and re-standardize any time new reagents are prepared. In this example, the only prepared reagent is the methyl purple indicator. If a new batch has been prepared, re-standardize the sulfuric acid.

## Sample Determination

Now that the sulfuric acid has been standardized, it can be used to measure something else. Lets measure the alkalinity of the anaerobic digester.

Since the sulfuric acid is still in the buret, leave it there. Pipet 25.0 ml of separated digester supernatant into an Erlenmeyer flask, add the stirring bar and a few drops of indicator. Titrate to the purple endpoint by again adding the sulfuric acid quickly then slowing until the endpoint is reached. The endpoint is reached after 39.6 ml of sulfuric acid was added. (Remember to stop the buret at the 25 ml mark then refill!!)

$$\text{Sample concentration} \times 25.0\text{ ml} = 0.017\text{ N sulfuric acid} \times 39.6\text{ ml}$$

Notice, the standardized sulfuric acid concentration (0.017N) is now used in the calculations. Solve.

$$\text{Sample concentration} = \frac{0.017\text{ N sulfuric acid} \times 39.6\text{ ml}}{25.0\text{ ml}} = 0.027\text{ N}$$

The sample alkalinity can now be reported as 0.027 N. However, normality is not a common concentration unit for most

wastewater analysis. The most common unit to report alkalinity concentration is mg/L. In this example, normality can be converted to mg/L by multiplying by 50,000. Changing from N to mg/L could be compared to converting dollars into pesos.

$$0.027\ N \times \frac{50{,}000\ mg/L}{1\ equivalent} = 1350\ mg/L$$

Standard Methods contains many titration procedures but they all follow the same general principles described above.

# Chapter 10 Microscope

The microscope is a great tool to use in a activated sludge wastewater laboratory. Frequent microscopic examination can provide valuable information about the condition of the microorganisms in the activated sludge. Observing and recording the different types of microorganisms can help the wastewater treatment plant operator identify changes that may cause a plant upset.

## Parts of a Microscope

The eyepiece or ocular lens is located at the top of the microscope. It may be monocular or binocular. If it is binocular, there is a slide adjustment to move the eyepieces so the lab tech can see clearly. The eyepiece usually magnifies the object 10x.

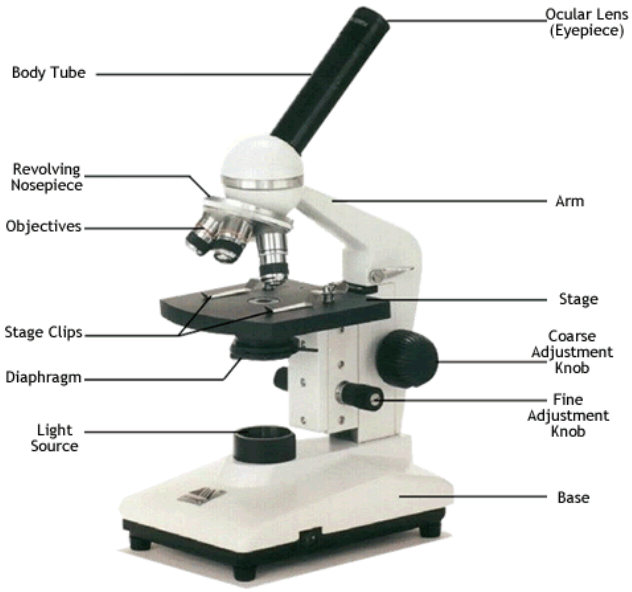



***Figure: Parts of Light Microscope***

The next set of lenses called objective lenses are mounted on a turret or nosepiece. There will be either 3 or 4 objective lenses. The objective lenses are rotated and come in close contact with the microscope stage. Each objective lens will have a different magnification, commonly 4x,10x, 20x, 40x or 100x. The 4x lens is a considered a scanning lens, magnifying the object only a total of 40 times. This lens is often used to locate an object of interest but shows little detail. Once identified, the nosepiece can be rotated to a higher magnification for more detail.

A good microscope will be parfocal and parcentered. Parfocal means that once the object is focused on the slide, the next more powerful objective lens can be swung into place with only a minimum of fine focus adjustment. Parcentered means the object in the center of the viewing area will still be in the center when the turret is rotated to the next more powerful objective lens with minimum adjustment. The 10x, 20x or 40x objective are all used to see the object in more detail. The 100x objective lens is called an oil immersion lens. This lens provides a magnification of 1000 times. Oil is used to reduce light diffraction and improve the resolution, the ability to distinguish between objects that are close together. As light passes upward through the slide, it is diffracted (bent). This bent light is lost and makes visualizing the object more difficult. By using oil, the light is not bent as much, allowing more light to reach the lens and improve the object resolution. The oil should always be cleaned from the lens after each use to reduce the accumulation of dirt on the lens.

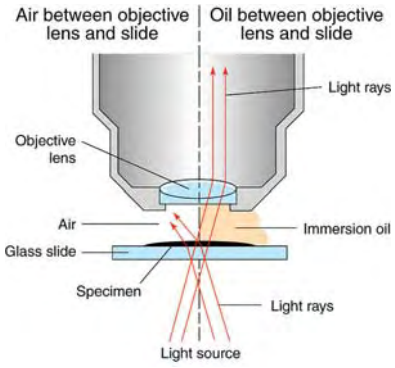



Figure: Oil immersion lens refraction

The microscope stage is the platform that holds the slide. A good microscope will have two slide movement knobs. These knobs allow the

slide to be slowly moved back and forth on the stage. Below the stage are the diaphragm lever and the condenser. The condenser consists of a series of lenses which focus the light on the slide. The condenser is almost always kept close to the stage for maximum image sharpness. The iris diaphragm lever is located between the condenser and the light source. Moving the lever causes the iris to open and close, thus controlling the amount of light reaching the condenser. If too much light is allowed to reach the condenser, the object on the slide gets "burnt out" by decreasing the image contrast. Focusing the object occurs by turning the two knobs on the body of the microscope. The larger knob is the coarse adjustment and should be used to bring the objective lens close to the slide. The fine adjustment knob is used to bring the object into focus. Care should be taken not to bring the objective lenses into contact with the slide. Rapid adjustment using the coarse adjustment knob may crack the slide or worse may crack the objective lens. The safest procedure is to watch the objective lens from the side of the microscope as the lens is lowered to the slide, then look through the microscope as the lens is lifted off the slide using the fine focus knob. Finally, at the bottom of the microscope is the light source. Some microscopes have a knob which can adjust the intensity of the light. The on/off switch is either on the cord or the base.

## Lens Cleaning

It is critical to keep all lenses clean, including the condenser. Frequent wiping with lens paper or Kimwipes is necessary to keep all surfaces free of dust and oily substances. Dust and oil will drastically reduce the microscope resolution. Resolution is defined as the ability to distinguish between 2 objects that are close together. Use only lint-free cleaning tissues and store in a dust free environment hopefully with the dust cover on.

Objective lenses should be cleaned using manufacturer approved solvents. The most common approved solvent is xylene. Apply xylene to the lenses using a cotton swab, then wipe off with lens tissue, and finally blow off any remaining lint with an air syringe (pipet bulb).

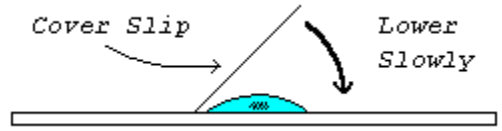
The eyepiece lens gets the dirtiest because it is always contacted by eyelashes and makeup. It should be cleaned frequently. Using a dust cover will help reduce the cleaning frequency.

## Better Viewing Hints

1. Adjust the condenser so it is close to the stage
2. Close the diaphragm lever to almost shut to block out most of the light and prevent glare. As the magnification is increased, slowly open the lever to allow more light in.
3. Always clean the lenses. Always use lens paper to clean the lenses and begin at the lowest objective to avoid contamination with the oil immersion lens.
4. Start scanning for objects of interest using the 4x or 10x lens.

## Preparing and Viewing a Wet Mount

1. Use a clean dry oil-free slide. Dry the slide using lens paper if necessary to avoid scratching the slide.
2. Obtain a freshly collected sample, aerate if needed to keep aerobic microorganisms alive.
3. Place a drop of sample on the slide using a pipet or eyedropper. Place a clean, dry cover slip onto the slide at a 45$^{o}$ angle so that one edge touches the droplet and the water spreads along the edge of the cover slip.




Gently lower the cover slip, squeezing out air bubbles as you go.

4. Carefully blot any moisture off the bottom of the slide. If the bottom of the slide is wet, it will not move freely across the stage.
5. Place the slide on the stage and make sure it is securely held in place.
6. Start at one corner of the cover slip. Use the 4x or 10x objective and work your way up one edge until you reach the top then move over one complete field of view. Start back down until you reach the bottom. Continue until the entire area is viewed. Center any object of interest, then turn the turret to magnify the object.

## Oil-Immersion Objective

1. Focus the object with the high dry objective (20x or 40x). Be sure to focus on an object that is not near the edge of the cover slip because some of the oil may get under the cover slip.
2. Rotate the 20x or 40x objective out of the way but stop before the 100x has swung into place.
3. Take a dropper of immersion oil and allow a drop of oil to fall onto the slide at the point where the light is shinning up from below.
4. Rotate the oil immersion lens (100x) into place. Check the image focus before adjustment using the fine focus knob.
5. Clean the lens with lens paper when finished. Do not use paper towels.

## Safety

1. Always carry the microscope using two hands.
2. Slides and cover slips can break and cause cuts and infection.

## Application

In wastewater analysis, the microscope is a valuable tool in activated sludge process control. Frequent microscopic examination of activated sludge can determine floc structure and identify, count, and check on the activity of various types of protozoans and metazoans.

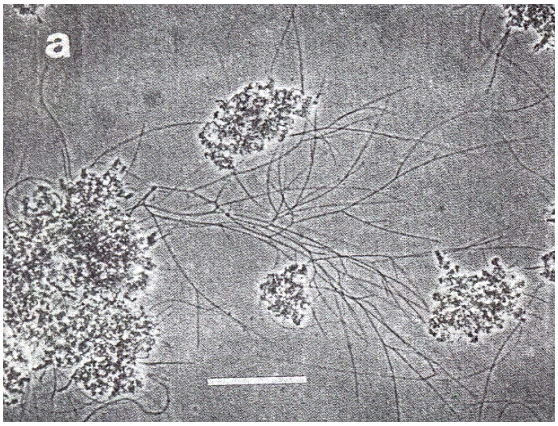



Figure: Compact floc with filamentous interbridging

## Floc Structure

Daily microscopic examination of floc by the laboratory technician can alert the operator to changes in the activated sludge treatment process. Floc types include:

a. Compact, well-formed floc which settles easily, leaving a clear supernatant.
b. Pinfoc -- tiny very compact floc, often with few bacteria that may or may not settle well, but leaves floc suspended in the supernatant.
c. Straggler floc -- loose floc structure, which is characterized by large, amorphous floc which may or may not have filamentous bacteria. Generally settles slowly.
d. Bulking floc -- usually characterized by filamentous bacteria. Filamentous bacteria extend between floc particles, connecting them together, and thus preventing them from compacting and settling well.
e. Dispersed floc-- loose small floc structure, very little organization. Poor settling usually has a very turbid supernatant.

## Microorganism classification

Besides examining the floc characteristics, the types of microorganisms present in activated sludge can also be identified. The quality of the activated sludge can be monitored by identifying, counting, and describing the activity of the organisms present. By doing this, the operator/lab technician can:

a. determine which organisms are present when the treatment system is running well
b. determine if a toxic shock has taken place in the activated sludge system
c. provide data for trend charts relating protozoan numbers to treatment parameters.
d. identify filamentous bacteria types to determine cause of bulking or foaming
e. count filamentous bacteria numbers to predict effective or ineffective treatment.

## Common Microorganisms

***Safety:***
*While microorganisms are commonly taken for granted in wastewater treatment plants, they are opportunistic which means they can use the laboratory technician as a food source. Always disinfect the lab bench and wear gloves when handling wastewater samples. Wear gloves and a mask if there is significant overspray during sampling.*

**Bacteria**
Bacteria are the major work force in waste treatment. There have many different strange names but most fit in the category called heterotrophs.

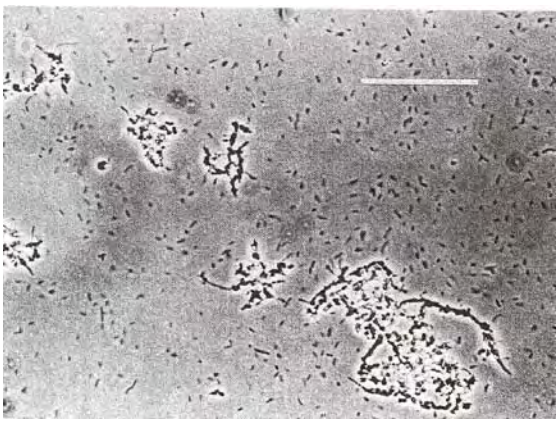


Figure: Bacteria

Under the microscope, bacteria will appear as very small black dots some will be stationary others will be motile. The presence of lots of bacteria indicate very early treatment and high Food:Microorganism ratio (BOD). Bacteria can barely be seen under the 400x magnification (40x objective).

There is a second type of bacteria called filamentous bacteria. They provide similar waste stabilization as normal bacteria except they create problems because they don't settle well. Filamentous bacteria begin to predominate when the environment is not “happy” such as low pH or low dissolved oxygen.

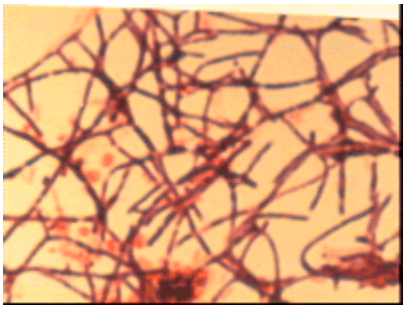


Figure: Filamentous Bacteria

**Protozoans**
Protozoans are one-celled, animal like organisms which include amoeba, flagellates, ciliates, and sporozoans. Protozoans can be free-living or parasitic. Common parasitic protozoans found in water cause amoebic dysentery, Giardiasis, and Cryptosporidasis.

**Amoeba**
Amoeba are very flexible one-celled protozoans that have a cytoplasmic membrane but no cell wall although some are encased in shells. They have pseudopodia (false feet) for both locomotion and food capturing. They feed upon complex organics by surrounding the food with their pseudopodia. Enzymes are used to break down the organics which are then ingested by absorption through the cell membrane. They are quite numerous during plant start-up or during a

plant upset. Their presence indicates a very young activated sludge with a high F:M ratio.

**Flagellates**
These one-celled organisms vary in size and have one or more thread-like flagella attached to their cells at either the posterior or anterior end. The flagella are used to move the organism and if a flagellate has two or more flagella, it tends to move in a corkscrew fashion.

Flagellates dominate when bacterial populations are low and the organic loading (BOD) is high. Their presence also indicates a young activated sludge with a high F:M ratio. The floc usually is light and dispersed. (straggler floc). Flagellates are very small but can be seen under 100x magnification (10X objective)

**Ciliates**
Ciliates are protozoans having hair-like structures (cilia) covering all or part of their cell membrane. The beating cilia either move the cell or cause currents in the water which aid in food gathering. They use bacteria or particulate organic matter as food. The movement of the cilia force the particles into the cell's gullet. Ciliates can be divided into free-swimmng, grazers, and stalked ciliates.

Figure: Free swimming Ciliates

Free-swimming ciliates are present in high numbers when the bacteria population is large. Two types of free swimmers include those that cruise between floc eating whatever particulate matter is available; and grazers, those that usually stay within or on the floc and feed on the floc as cows would munch on grass in a field.

Figure: Grazing ciliate

The presence of free-swimmers indicate the activated sludge system is approaching optimum treatment, although lots of grazers seem to indicate a better treatment than the presence of the cruisers.

Stalked ciliates use a stalk to hold onto the floc. Because they do not have to propel themselves through the water, they do not expend as much energy as the free-swimmers and therefore live in water with lower organic loadings. Since they are attached to the floc, they cannot move to more oxygenated areas, so the dissolved oxygen must be fairly high for them to survive. The rhythmic beating of the cilia at the anterior end (head) produce currents which drive bacteria and organic particles into their gullet. Some stalked ciliates have a contractile protein in their stalk that routinely contracts into a tightly wound coil, then springs out. This "spring action" stirs the water and helps the stalked ciliates gather food.

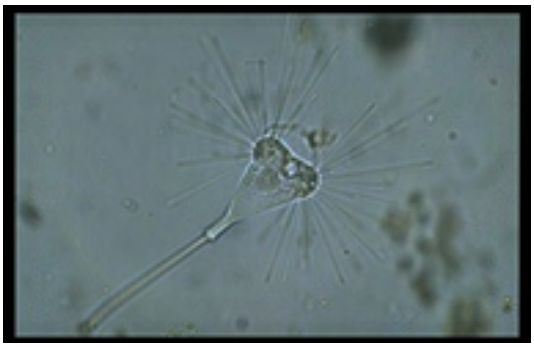


Figure: Stalked ciliate

Stalked ciliates may occur individually or form colonies. They begin to predominate when free-swimming ciliates are unable to compete for food because the bacteria population is small.

Figure: Colonized Stalked Ciliates

When stalked ciliates are present, floc formation is usually good and the effluent is clear. Ciliates are easily visible under 100x and 200x magnification (10x and 20x objective)

**Metazoans**
Metazoans are the largest organisms in the activated sludge system. They include rotifers, nematodes, and bristleworms.

**Rotifers**
Rotifers are found mainly in older activated sludges. At the head end, most rotifers have a conspicuous corona with cilia used both for locomotion and food gathering. Just below the corona is the mouth which is connected to a grinding structure called the mastax. The mastax is usually easily seen in rotifers and can be used to differentiate rotifers from other microorganisms. The foot, at the posterior end has spurs and retractile toes which allow the rotifer to attach to floc.

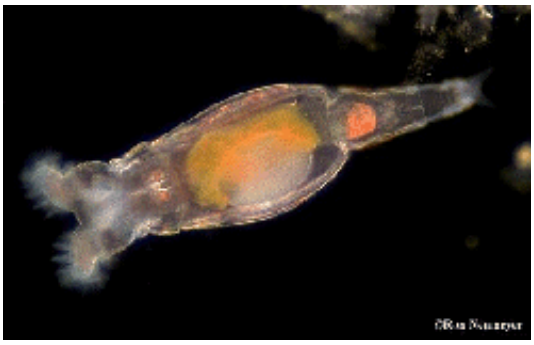


Figure: Rotifer

Rotifers have a high DO requirement and will die when the DO is too low. The presence of stalked ciliates and rotifers indicates a good activated sludge floc and produce a clear effluent.

**Nematodes**
Nematodes commonly found in wastewater are non-segmented roundworms. They have a long, slender body with one end usually sharply pointed while the other end tapers to a blunt tip (little fire hoses). They feed on bacteria, protozoans, ciliates, rotifers, and floc. The presence of nematodes indicates an older sludge. Sludge worms are easily seen under the 4X objective.

**Bristleworms**
Aeolosoma is the segmented worm commonly found in very old activated sludge. It has bristles which extend from its sides and burnt-orange dots on its surface. Bristleworms consume organic sludge.

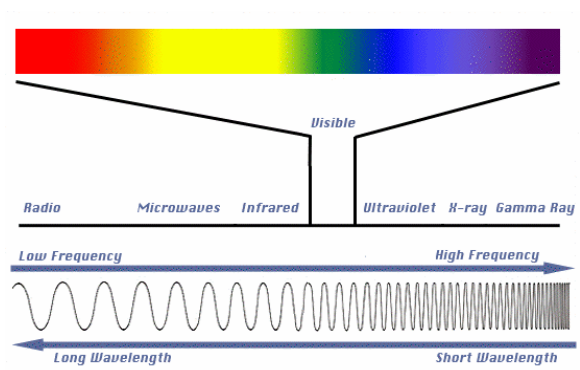
Once again, the microscope can be a valuable tool to help the lab technician identify types of microorganisms and relate them to the effluent quality (BOD and TSS).

# Chapter 11 Spectrophotometry

Spectrophotometry is a process using electromagnetic radiation (light) to measure an unknown analyte concentration. The spectrophotometry described in this section is commonly called UV-Vis Spectrophotometry since wavelengths of light in the visible spectrum are used to determine concentration. Light is described as a photon of energy. The amount of energy in each photon is determined by its wavelength. The shorter the wavelength the more energy the photon has. In UV-Vis Spectrophotometry, wavelength between 400-700 nm are generally used to determine the concentration of samples. Wavelengths shorter than 400 nm fall into the ultraviolet and x-ray range, while wavelengths longer than 700 nm fall into the infrared region.

## Visible Spectrum

The visible spectrum is composed of the colors we see (the rainbow) ranging from red to violet. A typical incandescent light bulb puts out white light which is the result of all the visible colors being combined. By using a prism, this white light from the light bulb can be separated into individual wavelengths.




In spectrophotometry, chemical reactions are created with the analyte of interest. These reactions create a product that has a color. For example:

Iron + phenanthroline → orange color

The orange color is the result of the molecule **absorbing** photons of energy. The more molecules present, the darker the orange color. A spectrophotometer can be used to measure the intensity of the color. The spectrophotometer operates off the Beer-Lambert Law.

## Beer-Lambert Law

The Beer-Lambert Law states that the absorbance of light is proportional to the color intensity. It also states that the intensity of the color is proportional to its concentration. In other words, the darker the color, the more concentrated the solution.

Absorbance = a b Concentration

Figure: This solution contains a small amount of iron

Figure: This solution contains a large amount of iron

The intensity of the orange color must be measured at a specific wavelength. This wavelength is determined by the method used. In this example, the wavelength selected is 510 nm. This wavelength was selected because it

had the highest sensitivity for iron. Each color will have its own special wavelength. For example, COD uses 620 nm, nitrate uses 400 nm, and phosphate uses 880 nm.

## Spectrophotometer Components

The spectrophotometer is a light detecting instrument. It is composed of 6 major components.

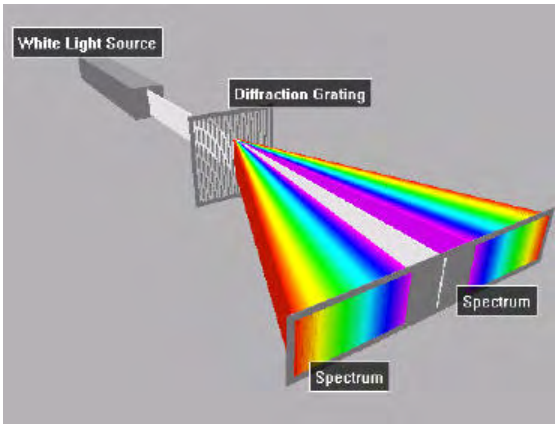
1. White light source
2. Diffraction Grating
3. Monochrometer
4. Exit slit
5. Sample cell holder
6. Light detector

**White Light Source**

This is usually a tungsten lamp that produces a constant white light output. The bulb is fairly cheap and can be run continuously. Remember that white light represents all the different colors.

**Diffraction Grating**

The diffraction grating is a special plate with hundreds of parallel grooved lines. The grooved lines act to separate the white light into the visible light spectrum (rainbow). The more lines the smaller the wavelength resolution. The more lines the more it costs.




**Monochrometer**

Once the light has been diffracted, a digital or mechanical knob is used to turn the diffraction grating and aim the desired wavelength at the exit slit.

**Exit slit**

The exit slit is a small hole that allows only a small amount of the diffracted light to leave the instrument. The smaller the exit slit, the more expensive the spectrophotometer. Common exit slits might be 2 - 20 nm. The exit slit dimension is often called the band pass. If the band pass is 20 nm, 20 nm of light is passing through the slit. If the spectrophotometer diffraction grating is set at 510 nm, then a spectrophotometer with a bandpass of 20 would be allowing wavelengths of light between 500 - 520 nm through the exit slit.

**Sample Cell Holder**

The 510 nm light passing through the exit slit now passes into the sample compartment. The sample compartment holds the sample cell or cuvette.

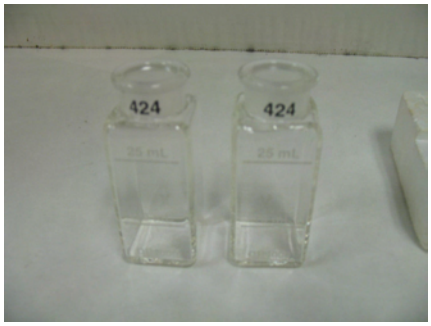



Figure: Matched Sample Cells

The sample cell holds the colored solution. The light is directed through the cuvette. The light energy either reacts with the colored molecules and is absorbed or it passes through the solution and is transmitted. The transmitted light is now sent to the detector. Sample cells should be "matched". Matched sample cells have the same optical properties and produce the same absorbance answer when a cobalt chloride solution is added to the

sample cell. The lab should have a minimum of 2 matched sample cells. One cell is for the reagent blank and the second is for all standards and samples.

**Light Detector**

The transmitted light hits a photomultiplier tube which converts the light energy into electrical energy. The electrical energy causes the electronics within the spectrophotometer to display an answer on the instrument. Unfortunately, the instrument does not know the correct answer because like the pH meter, it has not been standardized. Newer spectrophotometers may have computer programs built in which allow for immediate answer determination. These machines require the computer program to be validated.

## Stock Preparation

Standards are used to calibrate the spectrophotometer. Standards are usually prepared from a stock solution purchased from reputable chemical supply houses. They commonly come as 1000 mg/L or are sometimes labeled in titer form as 1 ml = 1 mg. If the bottle only indicates the titer concentration, convert to mg/L by multiplying by 1000. For example, if the titer is 1 ml = 0.2 mg, then the concentration is 200 mg/L

$$\frac{0.2 \text{ mg}}{1 \text{ ml}} \times \frac{1000 \text{ ml}}{1 \text{ liter}} = 200 \text{ mg/L}$$

The stock solution is usually stable for a year. Discard any stock solution that changes color, shows growth or precipitation.

**Intermediate Standard Preparation**

The stock solution is normally too strong for most spectrophotometric analyses and must be diluted. This diluted stock solution is called an intermediate standard. The intermediate standard has a limited shelf life, usually no longer than 1 month. The intermediate standard is diluted to make working standards which are then used to calibrate the spectrophotometer. It is common practice to make at least 8 working standards to calibrate the spectrophotometer. For example, lets assume the phosphate procedure calls for working standards between 0 - 20 mg/L. The stock phosphate solution is 1000 mg/L. All standards should be prepared using volumetric pipets and volumetric flasks. Since the smallest volumetric pipet is 1.0 ml, pipetting 1.0 ml into a 100 ml volumetric flask will give a working standard of 10 mg/L.

$$1000 \text{ mg/L stock} \times \frac{1.0 \text{ ml}}{100 \text{ ml}} = 10 \text{ mg/L WS}$$

There is a dilemma here since working standards below 10 mg/L are needed but there are no volumetric pipets smaller than 1.0 ml. To avoid this dilemma, the intermediate standard is prepared. The concentration of the intermediate standard can be prepared using the following formula.

$$C_{stock} \times V_{stock} = C_{int.\ std} \times V_{int\ std.}$$

Where:

$C_{stock}$ = stock concentration in mg/L
$V_{stock}$ = volume of stock used
$C_{int.\ std}$ = concentration of intermediate standard
$V_{int\ std.}$ = volume of intermediate standard needed

In this example, lets prepare 250 ml of a 100 mg/L intermediate standard. Calculate how many milliliters of the stock solution will be needed.

$$C_{stock} \times V_{stock} = C_{int.\ std} \times V_{int\ std.}$$

$$1000 \text{ mg/L} \times V_{stock} = 100 \text{ mg/L} \times 250 \text{ ml}$$

$$V_{stock} = \frac{100 \text{ mg/L} \times 250 \text{ ml}}{1000 \text{ mg/L}} = 25.0 \text{ ml}$$

Prepare the intermediate standard by using a 25.0 ml volumetric pipet and a 250 ml volumetric flask. Pipet the stock solution into the flask, then fill to the mark with DI water and mix by inverting several times.

**Working Standard Preparation**

Working standards are the standards that will be used to create the colors needed to calibrate the spectrophotometer. Since 8 working standards are needed to produce a standard curve, the same formula can be used to calculate the volume of intermediate standard needed to prepare the working standards.

$C_{int.\ std} \times V_{int\ std} =. C_{work\ std} \times V_{work\ std}$

$C_{int.\ std}$ = intermediate standard concentration in mg/L

$V_{int\ std}$ = volume of intermediate standard used

$C_{work\ std}$ = concentration of working standard to be prepared

$V_{work\ std.}$ = volume of working standard needed

In this example, lets prepare 100 ml of a 1.0 mg/L phosphate working standard.

$C_{int.\ std} \times V_{int\ std} =. C_{work\ std} \times V_{work\ std}$

100 mg/L x X ml = 1.0 mg/L x 100 ml

$$X\ ml = \frac{1.0\ mg/L \times 100\ ml}{100\ mg/L}$$

X ml = 1.0 ml

Pipetting 1.0 ml using a volumetric pipet into a 100 ml volumetric flask will produce a 1.0 mg/L working standard which can now be used to calibrate the spectrophotometer.

| Volume Inter. Std | Conc. | Absorbance |
|---|---|---|
| 0 ml | 0 | |
| 1.0 ml | 1.0 mg/L | |
| 3.0 ml | 3.0 mg/L | |
| 5.0 ml | 5.0 mg/L | |
| 8.0 ml | 8.0 mg/L | |
| 10.0 ml | 10.0 mg/L | |
| 15.0 ml | 15.0 mg/L | |
| 20.0 ml | 20.0 mg/L | |

The remainder of the chart can be filled in using volumetric pipets available in the lab. .

## Reagent Blank

Notice the table has a "0" standard. This standard is call a reagent blank and will be used to zero the absorbance on the spectrophotometer. The reagent blank is prepared by taking deionized water through the same chemical reactions as the rest of the standards. A reagent blank has the advantage over a deionized water blank because any contaminants in the chemical reagents are ignored. For instance, if the deionized water has some phosphate contaminant, a background color will be produced in the reagent blank because the phosphate reagents will react to produce a color. This background color would be present in all the standards because they were all prepared using the same deionized water. If the reagent blank is used to zero the spectrophotometer, the background color will be subtracted out automatically from all the remaining standards.

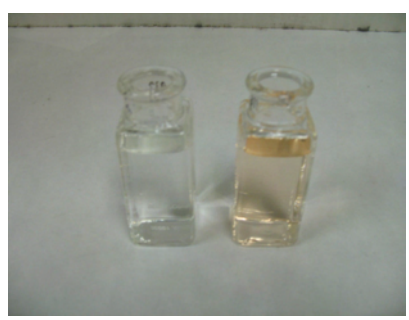


Figure: DI water blank vs. Reagent blank

Matched cuvettes are sample cells that have the same optical properties. Absorbance is a logarithmic function and small errors are magnified tremendously on the absorbance scale. It is important to handle cuvettes carefully to avoid errors which interfere with the absorbance reading. At low absorbance, contaminants such as dust, dirt, fingerprints, scratches, and bubbles interfere with the passage of light to the detector and will potentially cause significant false positive errors in the absorbance reading. The cuvette glass properties and cell positioning can also affect absorbance readings. Differences in glass such as scratches, thickness, and composition, also interfere with light passing through the same point on the cuvette. It is very important that the cuvette be lined up facing the same direction each time. Cleaning with detergent and deionized water should be completed as soon as possible to avoid having solids dry on the sides of the cuvette. Be careful using old brushes with wire to clean the sample cells. As the bristles from the brush wear, the exposed metal will scratch the sides of the sample cell.

## Standard Curve Preparation

To prepare the calibration curve, the working standards are all taken through the chemical procedure and a colored solution is formed. The colored solution should increase in intensity as the concentration increases.

Figure: Cap and fill the volumetric flask, mix by inverting several times.

Figure: Be sure to label each flask with its concentration.

Now that the standards have been prepared following the test procedure, the lab technician should notice an increasing color intensity as the concentration of the analyte increases. This color needs to be measured by the spectrophotometer.

Step 1: Warm up the Lamp
Allow the spectrophotometer lamp to warm up for a sufficient time to eliminate electronic drift. This usually takes 10-20 minutes.

Step 2: Set the wavelength
Select and adjust the spectrophotometer to the desired wavelength. Older spectrophotometers have a knob to manually adjust the wavelength to the correct wavelength. The knob on the right in the picture below is the wavelength adjustment knob. Turn the knob slowly to avoid damaging the diffraction grating mechanism.

The wavelength on newer spectrophotometers is digitally selected.

Step 3: Zero the Spectrophotometer
Transfer the reagent blank to a matched sample cell.

Wipe the sample cell with Kimwipes® (non-scratching) to remove any water spots or fingerprints. It's important that sample cells be clean.

Place the reagent blank in the sample cell holder. Sample cells can be handled at the top without creating an error. The light generally passes through the lower half of the sample cell.

Close the cover to prevent outside light from entering. Press the absorbance button to select the absorbance mode, and then press the zero button to zero the absorbance. At this point the spectrophotometer is seeing the highest amount of light reaching its detector. In other words, no light is being absorbed because there is no color in the reagent blank.

Record the absorbance on the data sheet.

Step 4: Standard Curve Preparation
Using the second matched sample cell, transfer the lowest standard into the sample cell and wipe with a Kimwipe® to remove fingerprints.

Place the sample cell into the sample cell holder as before, close the door and read the absorbance displayed on the spectrophotometer. Record the absorbance on the data sheet.

Continue the same procedure using the same sample cell for the remaining working standards. The sample cell should be rinsed 3x using small volumes of each successive standard. Record the absorbance for each standard.

At this point, the absorbance of the 8 working standards should be recorded on the data sheet.

| Volume Inter. Std | Conc. | Absorbance |
|---|---|---|
| 0 ml | 0 | 0.000 |
| 1.0 ml | 1.0 mg/L | 0.053 |
| 3.0 ml | 3.0 mg/L | 0.112 |
| 5.0 ml | 5.0 mg/L | 0.295 |
| 8.0 ml | 8.0 mg/L | 0.423 |
| 10.0 ml | 10.0 mg/L | 0.582 |
| 15.0 ml | 15.0 mg/L | 0.854 |
| 20.0 ml | 20.0 mg/L | 1.122 |

Step 5: Calibration curve
Now that the absorbencies for the working standards have been determined, a calibration curve must be prepared. Most calibration curves can be plotted on regular Cartesian graph paper. Beer's Law states that absorbance is proportional to concentration, so the y-axis will be labeled **"absorbance"** and the x-axis is labeled "**Concentration**". The scales should be divided up so as to include the highest absorbance and the highest concentration. The units of each scale do not need to be the same but they do need to be proportional. For instance if 1 block is 0.5 mg/L then the second block must be 0.5 mg/L higher (1.0 mg/L). Use as much of the graph paper as possible. Once all the points are plotted, the graph should fall in a reasonably straight line. Use a ruler to draw a line of best fit through the points. If the points do not fall on the line, an acceptable result is a line with an equal number of points above and below the line. Do NOT connect the dots. It is not permissible to extrapolate past the last point 20.0 mg/L (highest working standard) since you have no guarantee that the absorbance continues to be linear.

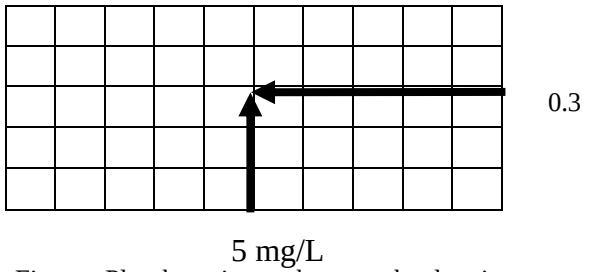
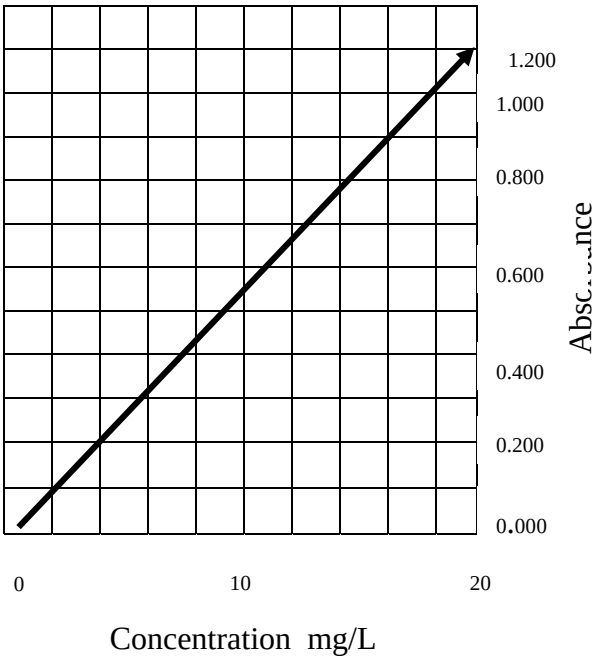



Figure: Plot the point on the curve by drawing a vertical line using the concentration data. Draw a horizontal line using the absorbance data. Make a point where the lines intersect. Repeat for all the remaining standards.

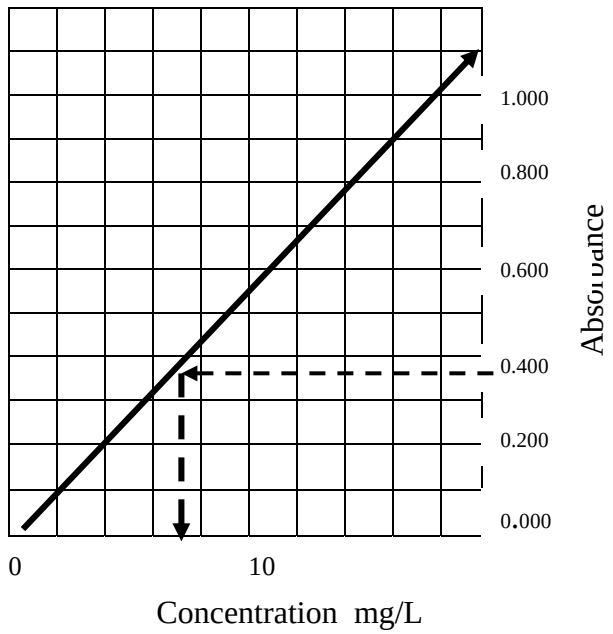



Draw the best fit straight line through all the points. This calibration curve will remain valid as long as the reagents that prepared it are not out of date. A high and low working standard should be run periodically to verify the curve has not changed.

## Sample Measurement

Now that the calibration curve is prepared, numerous samples can be determined over several months. In this example, if the phosphate level in the effluent is to be measured, the sample will be taken through the test procedure to produce a color. The sample will be poured into the sample cell and the absorbance measured. The sample absorbance must fall within the calibration curve range to be able to determine the sample concentration.

Example 1:
The sample has an absorbance of 0.363. To determine the sample concentration, find 0.363 on the absorbance scale and draw a horizontal line until the line intersects the calibration curve. Drop a vertical line and read the concentration directly off the scale.

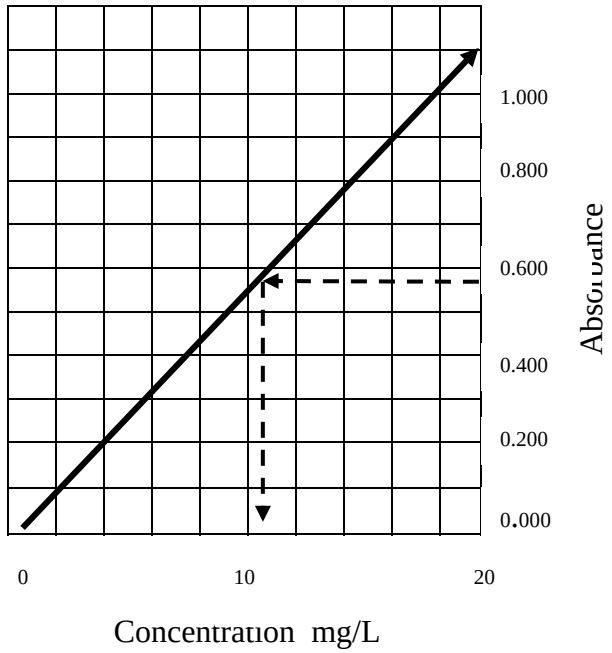



The sample concentration is 7.1 mg/L.

Example 2:
An influent sample is tested for phosphate. The absorbance is 1.334. Using the calibration curve, determine the sample concentration.

Notice the sample absorbance goes beyond the 20 mg/L standard absorbance. The sample is invalid and must be rerun using a diluted sample. In this example, the sample absorbance is just out of range, so a 1:2 dilution will most likely give a sample absorbance that will "hit" the middle of the calibration curve.

To prepare the 1:2 dilution, 50 ml of sample can be mixed with 50 ml of deionized water, then the diluted sample is tested for phosphate. The diluted sample absorbance is now 0.565. What is the phosphate concentration of the sample?




The diluted sample concentration is about 10.8 mg/L. Because the sample was diluted by 2, the sample concentration must be multiplied by 2 to get the sample concentration of 21.6 mg/L

Example 3
The sample absorbance is 0.011. The absorbance is so low, it is difficult to read the answer off the standard curve. Because you can't measure something that isn't there, the answer is reported as "less than" the detection limit. The detection limit can be the limit the manufacturer suggests or the limit the technician is confident of. In this case, the detection limit might be 0.2 mg/L, so the answer would be <0.2 mg/L or <mdl (minimum detection limit.)

## Computer Generated Calibration Curves

Newer spectrophotometers have computer software which has the standard calibration curves built in for many procedures. A reagent blank still must be prepared to zero the spectrophotometer.

Figure: Place the reagent blank in the sample holder

Figure; Close the lid to keep out background light.

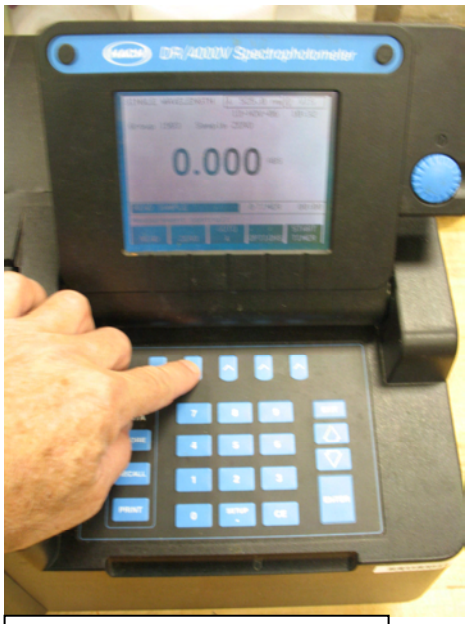



Figure: Press the zero button

The spectrophotometer is now calibrated using the manufacturer's calibration curve. The manufacturer's curve should be validated by running a technician prepared standard to verify the concentration. It is best to use a high and low standard to confirm that the technician's methodology will produce the same answer as the manufacturer's methodology.

The sample concentration can be determined directly from the spectrophotometer if using the manufacturer's calibration curve.

Figure: Place the sample into the sample cell holder, close the lid.

Figure: Press the read button to measure the concentration directly from the spectrophotometer.

## Troubleshooting the Spectrophotometer

There are few problems with the spectrophotometer. The most common errors are usually due to sloppy technique. Failure to wipe sample cells, scratched sample cells, and improperly rinsed sample cells are common mistakes.

The other error associated with spectrophotometry revolves around interfering ions. Other compounds that will react with the reagents to produce an additional color are called interferences. A compound that makes the absorbance higher is called a false positive or positive interference while a compound that makes the absorbance lower is called a false negative or a negative interference.

## Spectrophotometry Quality Control

- ☐ Method used is documented
- ☐ Make and model of spectrophotometer is documented.
- ☐ Reagent blank is used to zero the spectrophotometer.
- ☐ Standard calibration curve is prepared using a minimum of 8 working standards. Absorbances are documented.
- ☐ Record the purchase date, lot number, expiration date of stock solution.
- ☐ Wavelength used is documented.
- ☐ Calibration curve is periodically validated using a high and low working standard. Ideally, this should be performed with each run.
- ☐ Sample cells are free of dirt, scratches, fingerprints. Discard sample cells that are scratched, chipped, cracked.

# Chapter 12
# Biological Oxygen Demand

## Application

The BOD (Biochemical Oxygen Demand) test represents the amount of biochemically digestible organics (food). The test is performed on wastewater and other contaminated samples for various reasons:

**1. To satisfy the requirements of an NPDES permit.**
The monthly average for BOD in the effluent of a WWTP with a NPDES permit usually cannot exceed 30 mg/l.

**2. To evaluate the effectiveness of a treatment unit or process.**
Performing the BOD test on the influent and effluent of a primary clarifier, the activated sludge process, or the full wastewater treatment plant can indicate removal efficiency.

**3. To calculate the organic loadings (amount of food) on a treatment plant or specific treatment unit to determine if the plant or unit is under loaded or over loaded.**
Various organic loadings calculations would be:

a. pounds/day BOD/1000 cubic feet (Trickling Filters)
b. pounds/day BOD /1000 sq feet (RBCs),
c. pounds/day/acre (ponds)
d. F:M ratio (Activated Sludge)

**4. To determine industrial surcharges.**
Industrial wastewaters such as from food processing plants (Coca Cola, chile processors, meat processors) often contain high levels of concentrated organics. Many cities require either pretreatment to lower the BOD or charge an additional amount (surcharge) to compensate for the extra treatment capacity required.

## Oxygen Demand

Measuring the digestible organics directly is difficult, so the amount of oxygen used by the bacteria to digest these organics is measured instead. This digestion process creates an ***oxygen demand***. The BOD test measures the amount of free oxygen ($O_2$) consumed by polluted waters such as wastewater treatment plants, rivers, lakes, industries, etc. The following reactions indicate the types of reactions that create the oxygen demand.

1. Oxygen is consumed by microorganisms to biochemically degrade (oxidize) organic materials (***carbonaceous demand***)

Reaction 1

$$\text{Organics (food)} + O_2 + \text{microorganisms (heterotrophic bacteria)} \rightarrow CO_2 + H_2O$$

2. Oxygen is consumed by microorganisms to oxidize reduced forms of nitrogen such as ammonium or nitrite. (***nitrogenous demand***)

Reaction 2

$$NH_4^+ + O_2 + \text{microorganisms (nitrosomonas bacteria)} \rightarrow NO_2^- + H_2O$$

$$NO_2^- + O_2 + \text{microorganisms (nitrobacter bacteria)} \rightarrow NO_3^- + H_2O$$

3. Oxygen is consumed when reduced inorganic material such as sulfides and ferrous iron are present. These are often created in septic sewer lines.

$$Fe^{+2} + O_2 \text{ ----------> } Fe^{+3}$$

$$S^{-2} + O_2 \text{ ----------> } SO_4^{-2}$$

These reactions can occur spontaneously or can be caused by microorganisms as well.

## Problems created by lack of oxygen

If an organic overload occurs in a wastewater biological treatment unit, the dissolved oxygen (DO) may drop too much because the aeration equipment may not be able to keep up with the rate of oxygen depletion. In other words, the microorganisms use more oxygen than can be supplied. This would result in incomplete stabilization (digestion) of the organics. Low dissolved oxygen (in activated sludge systems) may cause the growth of undesirable filamentous bacteria, which in turn might cause a bulking sludge problem.

If wastewater effluent has a high BOD and is discharged to a receiving stream (river, stream, lake, etc), the bacteria in the receiving stream may deplete the oxygen in the stream (using Reaction 1 again). While the microorganisms are digesting the discharged organics in the wastewater, fish kills or other undesirable conditions may occur if the oxygen in the receiving stream is not quickly replaced. This problem was the primary reason the NPDES permit system was established.

## BOD Test Theory

The BOD test is one of the most difficult tests to run because there are so many variables that effect the outcome. In order for the test outcome to be acceptable the following variables must be controlled.

1. BOD dilution water must be aerated and non-contaminated.
2. The pH environment must be acceptable (6.5 - 7.5)
3. The incubation temperature must be controlled and consistent.(20 $\pm$ 1$^{o}$C)
4. The microorganisms must be viable, must be acclimated to the waste material must be active and must be at acceptable concentration.
5. The sample strength must be sufficient to cause a DO depletion of at least 2 mg/L but leave at least 1 mg/L residual.
6. Toxic materials must be absent. (Dechlorinate)

In summary, the BOD test must create ideal growing conditions which will encourage microorganisms to effectively and efficiently utilize the digestible organic materials (waste).

Running a BOD analysis on a sample consists of placing a portion of a sample (along with prepared dilution water) into an air-tight bottle (300 mls volume) and incubating the bottle at 20 +/- 1 deg C for (usually) 5 days. The dissolved oxygen is measured initially and again after the 5 day incubation. The bottle size, incubation temperature, and incubation period are all specified. Most wastewater and other samples (such as industrial waste waters) contain more organics (food) than the amount of dissolved oxygen available. Therefore, it is necessary to dilute the sample before incubation to bring the oxygen demand and oxygen supply into appropriate balance. The nutrients, magnesium sulfate, calcium chloride, and ferric chloride, are added to the dilution water to maintain ionic strength and to promote bacterial growth. A phosphate buffer is added to help maintain a pH of 6.5 - 7.5, which is desirable for good bacterial growth.

In other words, (Reaction 1) the amount of organics can be measured by measuring the amount of oxygen used up by microorganisms over a 5 day period. In order for the test to work properly, the microorganisms must be healthy and happy. To be happy, they need food

(organics), oxygen, and an acceptable growth environment including normal pH, temperature, nutrients and no toxics).

In the BOD test, microorganisms are charged with eating all the organics (food). In a BOD bottle, organics from a sample are added to dilution water containing nutrients, oxygen, and microorganisms, then capped and incubated at 20$^{o}$C for 5 days. Initially the microorganism level is fairly low, but the environmental growing conditions are excellent, so the microorganisms quickly enter the log growth reproduction phase and begin to consume the organics.

**Growth curve**

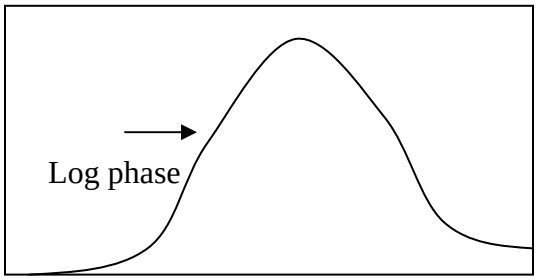



Figure 1: Logarithmic growth

The large number of microorganisms consume the organics and as a result also consume the oxygen.

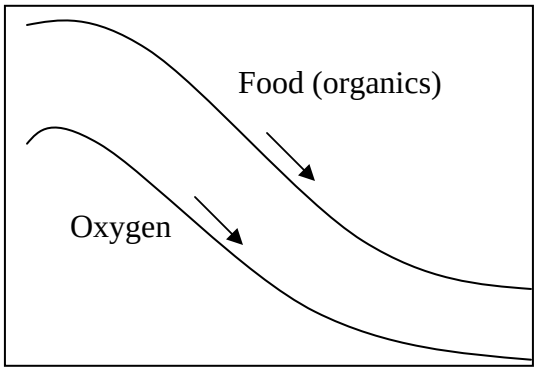



Figure 2: Organics (food) is consumed

If the BOD bottle is capped tightly, no external oxygen can enter. The microorganisms can only utilize the oxygen initially available. Over the next couple of days the microorganisms continue to digest the organics, reproduce logarithmically, and utilize oxygen rapidly

By the 4th day of the BOD test, the organics have begun to become limiting, just barely able to maintain the current microorganism population (stationary growth phase) Figure 3.

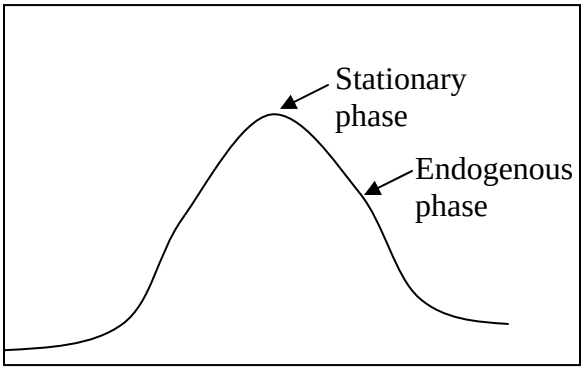



Figure 3: Stationary and endogenous phase

The lack of food causes a slowing in the reproduction rate as well as a decrease in the amount of oxygen used. With the high microorganism population, the remaining organics are quickly consumed and the microorganism enter the endogenous phase. During the endogenous phase, the microorganisms utilize internal food reserves, and many die (endogenous phase). If everything is OK, most of the microorganisms are hungry, but alive and there is still sufficient oxygen left in the bottle to be measured. The amount of oxygen that has been consumed over the 5 days is proportional to the amount of organics (BOD) consumed. The BOD curve in Figure 4 represents the total amount of food used up over the 5 days.

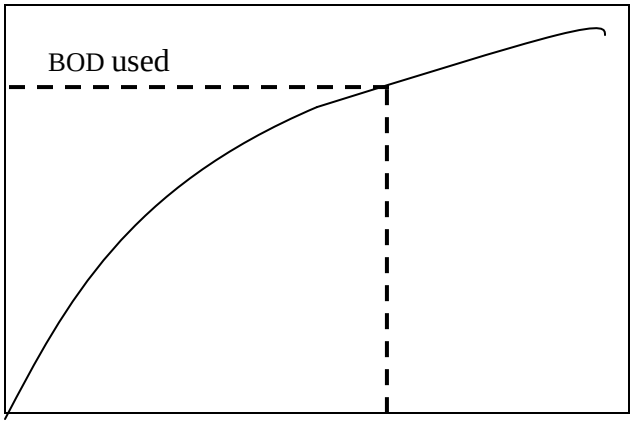



Figure 4: BOD used

**$BOD_5$ vs CBOD**

Complete stabilization of a sample may require a period of incubation too long for practical purposes, therefore, five days has been determined as the standard incubation period.

BOD measures food that create a carbonaceous oxygen demand (Reaction 1) and nitrogenous oxygen demand (Reaction 2). In New Mexico, the NPDES permit generally requires BOD and does not distinguish between carbonaceous and nitrogenous BOD. If the nitrogenous oxygen demand is not desired, an inhibiting chemical may be use to prevent ammonia oxidation. The resulting BOD is called CBOD. With this technique carbonaceous and nitrogenous demands can be measured separately.

$$BOD_5 = CBOD + NBOD$$

Before using inhibitors, the permittee must first apply for a BOD variance from the EPA. The extent of oxidation of nitrogenous compounds during the 5-day incubation period depends on the presence of microorganisms capable of carrying out this oxidation (Reaction 2). Such organisms (nitrosomonas and nitrobacter) are not usually present in raw sewage or primary effluent in sufficient numbers to oxidize significant quantities of nitrogen in the 5-day BOD test.

Newer treatment processes involve nitrification and denitrification. These processes may have a NBOD component.

## BOD Procedure

### Dilution Water Preparation

Dilution water is used to provide oxygen for the bacteria to breath during the 5 day incubation period. It is important to have the dilution water saturated but not super-saturated. There are 3 commonly accepted ways to aerate the water. The water can be:

1. Shaken vigorously for a few minutes.
2. Aerated overnight using an oil-less air source.
3. Allowed to sit in the incubator at 20$^{o}$C for several days.

For example:
10 liters of deionized water is aerated using air from a clean oil-less air supply. A ***CLEAN*** large bore serological pipet is attached to a clean section of tygon tubing. The tubing should not touch the water as it is often left lying around on lab benches and can easily become contaminated. This may easily be a source of error that contributes to bad dilution water. The pipet is inserted into the BOD carboy and allowed to bubble gently overnight. The next morning the pipet and hose are removed and the air turned off. The aerated water is now allowed to set for a minimum of 1 hour to allow any supersaturated air to dissipate.

Passing air through the dilution water may cool the water several degrees. If the air temperature is below 20$^{o}$C the aerated water will become too cold to be acceptable. Cold water holds more oxygen than warm water and will release oxygen as it warms

up. This may create problems with the dilution water blank. Allow any aerated water to stabilize for a minimum of 1 hour before use or longer if the water temperature is still below 20$^{o}$C. Likewise, warm air may raise the temperature of the dilution water and make it difficult to hold sufficient amounts of oxygen.

Nutrients are also added to the water. The four nutrients are Magnesium Sulfate, Calcium Chloride, Ferric Chloride, and Phosphate Buffer. One milliliter of each solution should be added for each liter of dilution water. In this example, if 10 liters of dilution water is being prepared, 10 ml of each nutrient solution should be added. A serological pipet is acceptable for this process. Always add the nutrient to the water. Do not add the nutrients first then the water, since this may cause the nutrient to precipitate out.

* These reagents can be purchased already prepared and can be purchased in individual packets.

The BOD water could also be aerated by allowing the dilution water to equilibrate in a cotton-plugged bottle for several days. The dilution water is usually stored in the BOD incubator, thus avoiding any dissolved oxygen shifts caused by temperature shifts. The potential problem here is the growth of microorganisms in the water during the equilibration period. These microorganisms will become BOD and cause the blanks to be bad.

The least effective method of aerating the BOD water is to vigorously shake a partially filled bottle for a few minutes.
This will provide an aerated water but is not usually saturated. DO meter calibration can be inaccurate when using this aeration method.

**Dilution Water Blank Preparation**
Now that the dilution water containing the nutrients has been allowed to equilibrate to room temperature, the dilution water must be checked for contamination. Checking the dilution water will require the water to be taken through the entire BOD incubation procedure.

1. Fill two or more clean BOD bottles with dilution water being careful to avoid bubbling and supersaturation.

Fill the bottle slowly to avoid excessive agitation and supersaturation of DO

Carefully allow the dilution water to slowly fill the BOD bottle. If the BOD bottle is filled too rapidly, excessive agitation and bubbling may result in a supersaturated water. Avoid using hoses to fill the BOD bottles. Hoses help avoid supersaturation but can easily add contamination.

2. Once the BOD bottle is filled to the neck, measure the initial dissolved oxygen level.
3. Place the glass stopper in the BOD bottle and check to make sure there are no bubbles beneath the stopper.
4. Place a small amount of water above the stopper. This will provide a water seal to prevent air from entering the bottle over the 5 day incubation period. Cap the

bottle to prevent evaporation of the water seal during incubation at 20°C for 5 days in the dark.

5. After 5 days, once again measure the dissolved oxygen.
6. Record data on the BOD worksheet.

Run Blanks in duplicate. Remember the blanks have only nutrients, oxygen and water. There should be <0.2 mg/L drop in dissolved oxygen after 5 days. If the DO drops more than 0.2 mg/L, the technician needs to investigate the cause of the excessive drop. Running duplicates helps identify problems.

**Seed Control**

Many wastewater effluent samples may be partially or completely sterile as a result of chlorination, ozonation, UV sterilization, exposure to toxic chemicals, heat, unfavorable pH, or other factors detrimental to biological activity. In the BOD test, microorganisms are an essential part of the procedure.

$$\text{Organics} + O_2 + \text{microorganisms} \rightarrow CO_2 + H_2O$$
(food) (heterotrophic bacteria)

Validity of the BOD results depends upon the presence of organisms capable of prompt and effective biodegradation. Since harsh environmental conditions may have killed the original bacteria, the sample must be re-inoculated with "seed" bacteria from receiving waters (rivers, ponds), soil suspensions, raw wastewater or lypholyzed commercial seed to provide enough microorganisms to oxidize the waste properly. Raw wastewater has the advantage because it usually has a high number of bacteria that are indigenous to the waste itself. By using bacteria that are already common to the wastewater, there is no delay while other bacteria get acclimated to the food. Using raw wastewater provides numerous organisms but also contain excess organics which must be corrected for.

**How many bacteria are needed?**

The amount of bacteria needed is determined by trial. The bacteria added should be "active" and initiate biochemical activity promptly. If the seed is either low in concentration or unacclimated (doesn't like the type of waste), the BOD reaction is slowed. In other words, if there are not enough microorganisms, the amount of food (organics) eaten is slow and the amount of oxygen consumed is low. On the other hand, if there is sufficient microorganisms but they don't have the correct enzymes needed to digest the food, the amount of food eaten will also be low and the amount of oxygen consumed will again be low.

Figure 5.

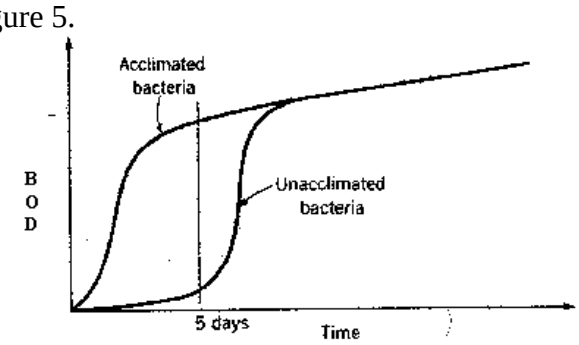



Figure 5: Acclimated seed

Seeding is always an uncertain procedure but very necessary at times. A seed containing viable organisms at a lower energy state (hungry bugs) because of limited food (organics) theoretically is the best available seed source. A microorganism population created from the waste itself should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. (In other words, if you are a vegetarian and like eating carrots, it would not take you long to begin eating (log growth) if they brought you a plate of carrots. On the other hand, if you are a vegetarian and they brought you a plate of barbeque ribs, you would have to think about eating for awhile before eating and you may decide not to eat at all.) The microorganism population should not be stored too long because organism redistribution and die-out become limiting. (If the seed organisms are already hungry, they will begin to die and must be used quickly). This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular waste material under consideration. Different seed sources and locations should be checked out to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

**Seed Collection**

Collect the seed in a 500 ml bottle and let settle at least 1 hour and up to 36 hours. This will allow settleable solids to settle and help assure the seed is homogeneous.

It is very important to allow the settleable solids to settle and the supernatant to be poured off slowly. The more consistent the supernatant (homogeneous) the more consistent and reliable the BOD test results will be. If there are a lot of large suspended solids (organics) in the supernatant, the seed correction and test results will be very erratic.

Figure 6: Settleable solids allowed to settle at least 1 and up to 36 hours

Figure 7: Supernatant carefully poured off the top without disturbing solids.

**<u>Seed Correction Bottle Preparation</u>**

1. Set up 3 or more clean BOD bottles.
2. Add varying amounts of seed to each BOD bottle. For example: add 6, 9, and 12 ml of seed. Use a large bore 10 ml serological pipet.
3. Carefully fill with BOD dilution water. Be careful not to overfill the BOD bottle. Overfilling will cause loss of seed.
4. Record initial dissolved oxygen level for each bottle.

5. Stopper, place a water seal, cap, and incubate at 20°C in the dark for 5 days.
6. Determine the 5 day dissolved oxygen level.
7. Calculate the dissolved oxygen depletion caused by the added seed.

**Seed Correction Calculation**
When seed is added to a BOD sample, it usually contains some amount of "extra" organics. Since the BOD test measures all digestible organics, the extra organics that come with the seed will give a BOD answer that is higher than it should be. This extra organic must be corrected for (subtracted out). Since this test is often used for the NPDES permit, an answer that is too high may cause a violation of the permit.
To make this correction, seed correction bottles are used. The seed correction bottles determine how much of the oxygen used over 5 days was due to the seed itself.

sample organics + $O_2$ + seed → $BOD_1$

seed organics + $O_2$ + seed → $BOD_2$

The BOD test will measure $BOD_1 + BOD_2$

The BOD caused by the seed organics must be subtracted or "corrected".

For example, if 10 ml of seed is added to a BOD bottle, filled with dilution water, then incubated for 5 days.

Initial Dissolved Oxygen = 7.7 mg/L
Final Dissolved Oxygen = 5.4 mg/L
Dissolved oxygen used = 2.3 mg/L

$$\frac{2.3 \text{ mg/L}}{10 \text{ ml}} = \frac{0.23 \text{ mg/L}}{1 \text{ ml of seed}}$$

Each milliliter of seed used 0.23 mg/L of oxygen over the 5 days. Two milliliters of seed would have used 0.46 mg/L of oxygen. Three milliliters of seed would use 0.69 mg/L of oxygen, etc. See if you can calculate the seed correction for the following example?

25 ml of settled seed is added to the BOD bottle, filled with dilution water, then incubated for 5 days. If the initial DO is 8.5 mg/L and the final DO after 5 days is 2.7, what is the seed correction?
(answer: 0.23 mg/L/ml seed.)

How many milliliters of seed would be needed to give a depletion between 0.6 - 1.0 mg/L? (Answer: 3 or 4 ml)

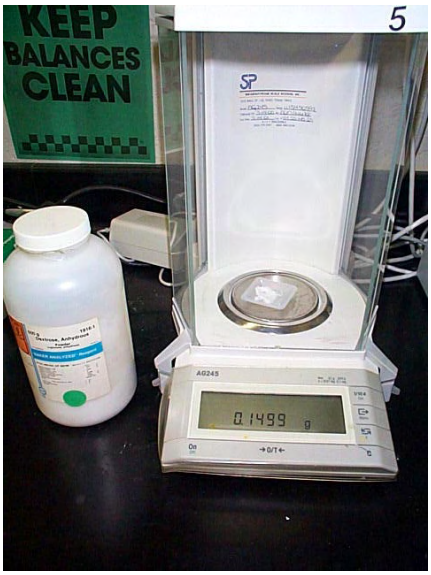
The BOD test suggest the analyst add enough seed so that each standard or sample bottle have a seed correction of 0.6-1.0 mg/L. By targeting this range, there should be ample microorganisms to digest the sample organics (BOD)

**Glucose - Glutamic Acid Standard**
In order to validate the BOD test conditions, reagents, and procedure, it is necessary to measure a solution with a known amount of organics. A solution that has a known value is called a standard. In the BOD test, a solution containing a carbohydrate and a protein is used as the source of organics. The carbohydrate is glucose and the protein is the amino acid glutamic acid. This standard provides a source of food for a wide variety of microorganisms (seed). If everything in the test is acceptable, the BOD

should be 198 ± 30.5 mg/L. ***Results outside this range indicate a problem that requires immediate remediation and invalidates all samples that were also run.***

**Glucose-Glutamic acid preparation**
The glucose and glutamic acid are both powders that must be dried in an oven for an hour at 103°C before being used. Once dried, the glucose and glutamic acid can be cooled and stored in a desiccator.

Each of the powders should be weighed on a calibrated analytical balance.




0.1500 ± 0.0005 gm of each powder is weighed out, then carefully transferred to a 1000 ml volumetric flask.

Be sure to rinse the weighing boats to recover all the powder. Once the powder has been transferred, fill the volumetric flask to the mark with deionized or distilled water. Add a magnet and mix for at least 1 hour to allow the glucose and glutamic acid to completely dissolve.

The glucose - glutamic acid standard should be prepared fresh, on the day of the test. Commercial preparations can be purchased. Be sure to check for growth and contamination before using. Do not exceed the expiration date.

**Glucose-Glutamic Acid Standard Procedure**
1. Set up 2 or more clean BOD bottles.
2. Use a large bore serological pipet to measure the appropriate amount of seed to each BOD bottle. (Remember, the ideal seed should show a drop between 0.6-1.0 mg/L). If the influent is weak, a higher amount of seed may be required.

The GG requires seed to supply the microorganisms needed. Here 3 ml of seed is being added.

3. Use a 6 ml Class "A" volumetric pipet to pipet 6.0 ml of the glucose glutamic acid standard into each bottle. Remember volumetric pipets are not blown out.

6.0 ml of glucose-glutamic acid standard are added to duplicate bottles as a BOD control

4. Carefully fill the remainder of the bottle with BOD dilution water. Be careful not to overfill the BOD bottle. Overfilling will cause loss of seed and standard.
5. Measure the initial BOD.

6. Insert the stopper and place a water seal and cap, and incubate at 20$^{o}$C for 5 days.

Once the BOD bottle has been filled to the neck with dilution water, the initial dissolved oxygen is measured, the stopper twisted in place and covered with a cap to prevent water loss and prevent air from entering for the next 5 days

7. Measure the final DO after 5 days.

**Glucose-Glutamic Acid Calculations**
The calculations for the GG standard will be discussed later. Remember, if the GG standard is not correct, all sample data is invalid.

**BOD Sample Collection**
The BOD sample can come from several sources. It may come from industry, from the influent, the effluent, river, etc. The sample may be a composite or grab sample. The sample can be collected in glass or plastic bottles. Because the BOD test measures organics, preservation is required to minimize degradation of organics during collection and transport. Analysis should begin as soon as possible after collection. If the sample can be analyzed within 2 hours, preservation is not required. If the sample is to be transported or stored for more than 2 hours, it must be transported on ice or refrigerated.

Grab samples should be run within 24 hours and must be run within 48 hours. Store in a refrigerator below 6$^{o}$C.

Composite samples must be stored in a refrigerator or on ice during the entire compositing period. The analysis should begin within 24 hours after the last composite sample has been collected. Samples collected for NPDES permits should be analyzed within 6 hours after compositing.

**Sample Pretreatment**
As discussed earlier, the BOD is a measure of the organic strength of a waste. In order to measure organics, oxygen consumed by the microorganisms is measured at the beginning and at the end of 5 days. In order for microorganisms to grow and metabolize the organics, their environment must be controlled. The factors which need to be controlled are:

1. pH
2. Temperature
3. Toxic disinfectants
4. Supersaturation

**pH Adjustment**
In the BOD test, a **sample** with a pH between 6.0 and 8.5 does not need to be adjusted. If the sample pH is below <6.0 or above pH >8.5, the sample pH must be adjusted by adding either acid or base to bring the sample pH to 6.5-7.5. Be sure to use a calibrated pH meter. The concentration of the acid or base used to adjust the pH should be high enough to avoid significant dilution of the sample. No more than 5 ml of acid or base can be added to one liter of sample (0.5%) for pH adjustment. Samples with extreme pH may not have any viable microorganisms present and must always be seeded.

**Sample Temperature Adjustment**
Temperature affects the metabolism rate of microorganisms as well as the dissolved oxygen concentration. In order to minimize these effects the sample temperature is adjusted to 20 $\pm$ 1$^{o}$C. Often the sample temperature is <6$^{o}$C because it has been composited and refrigerated. It is undesirable and time consuming to let the sample warm to room temperature so it can quickly and efficiently be warmed in a waterbath.

Sample can be quickly warmed to 20oC using a waterbath. Use a thermometer, the sample warms quickly.

**Sample Dechlorination**
As discussed earlier, if this is an effluent sample, it has been subjected to disinfection. If the sample has been chlorinated, there will be few if any microorganisms still present. If the effluent has been disinfected properly, there will be a chlorine residual. If this chlorinated sample is added to the BOD bottle, the residual chlorine will continue to kill the microorganisms and the BOD will be invalid. (If there are no microorganisms present in the BOD bottle, no oxygen will be used and no organics will be consumed).

You get a great answer for your permit but its WRONG.

To prevent the chlorine from killing the microorganisms, the sample must be checked for the presence of a chlorine residual. A 100 ml aliquot of sample is added to an Erlenmeyer flask, 1 ml of the acetic acid , potassium iodide reagents are added. If a blue color is present, chlorine is present and must be destroyed.

The blue color is titrated by a sodium sulfite solution until the color disappears.

$Cl_2 + Na_2SO_3 \text{ ----> } Cl^- + Na_2SO_4$

Once the 100 ml sample has been titrated, record the volume of sodium sulfite used. The 100 ml sample is **DISCARDED.**

Next, determine how much sodium sulfite should be added to dechlorinate the volume of sample for the BOD test. The volume of sulfite added needs to be proportional to the 100 ml aliquot dechlorinated.

For example, if the volume of sodium sulfite titrated was 0.2 ml for 100 ml, how many milliliters of sodium sulfite would be added to dechlorinate 1000 ml of sample.

$$\frac{0.2 \text{ ml}}{100 \text{ ml}} \times 1000 \text{ ml} = 2.0 \text{ ml}$$

This volume is then added to 1 liter of sample. Like pH, the volume of sodium sulfite added can not exceed the 5 ml/1000 ml sample additional volume limit. In this example, 2 ml is acceptable as long as the volume used to adjust the pH was below 3.0 ml. Mix the sample and allow to sit for 20 minutes to allow time for the sodium sulfite to dechlorinate the l liter of sample.

Once the sample has been dechlorinated, it must be checked to verify that dechlorination was accomplished. Repeat the sodium sulfite titration to verify the absence of a blue color.

**Sample Supersaturation**

In New Mexico, it is very possible to have samples with dissolved oxygen levels above 9.0 mg/L. Cold water and photosynthesis contribute to high levels of DO. This excess DO may interfere with the BOD test and should be removed prior to analysis. Warm the sample to 20$^{o}$C while vigorously shaking the sample or by aerating with clean filtered air.

The sample environment has now been adjusted for pH, temperature, and chlorine.

## Effluent BOD Sample

The effluent sample is usually chlorinated and must have gone through the pre-treatment procedures discussed earlier. Since the water has been chlorinated, then dechlorinated, there are few viable microorganisms. This will require that

"seed" be added to the effluent sample. This should be the same seed as discussed earlier.

**Procedure**

1. Determine the approximate sample size needed from the table below. Notice that the lower the expected BOD, the more sample volume needed.

| Volume added | Expected BOD |
|---|---|
| 3 | 200 - 560 mg/L |
| 6 | 105 - 280 |
| 9 | 70 - 187 |
| 12 | 53 - 140 |
| 15 | 42 - 112 |
| 30 | 21 - 56 |
| 45 | 14 - 37 |
| 60 | 11 - 28 |
| 100 | 5 - 15 |
| 300 | 0 - 5 |

After gently mixing the effluent sample, measure out at least three dilutions (using a large bore serological pipet or a graduate cylinder) and add to three clean BOD bottles.

In most cases, samples with consistent BOD's only need 3 BOD dilutions set up. However, if the BOD is truly unknown, a wide range of dilutions should be selected. It is recommended that at least 2 of the BOD dilutions meet the **DO depletion rule.**

## DO Depletion Rule

**The final day 5 DO must drop at least 2 mg/L from the initial DO and be greater than 1 mg/L**

2. Add the appropriate amount of seed to each BOD bottle. Carefully fill the BOD bottles with dilution water.
3. Measure the initial DO.
4. Be careful not to entrap air beneath the stopper, place a stopper and water seal on the bottle and incubate for 5 days at 20$^{o}$C in the dark.
5. Measure the 5 day DO.
6. Calculate the BOD in mg/L.

For example: What volumes of sample should be added to the BOD bottles if the BOD is less than 30 mg/L?

Look at the table! Any sample volume between 30 and 60 milliliters should measure a BOD of 30. The analyst selects 45 ml. What if your BOD was higher than 30 mg/L? By using 30 ml for a second bottle, you would extend your range to as high as 56 mg/L. By using 60 ml, you would extent your low range to as low as 11 mg/L. Therefore, by running at least 3 dilutions of 30, 45, and 60 ml, the analyst would be sure to measure any BOD between 11 and 56 mg/L. More dilutions could be prepared to extend the range.

What sample volumes would you use if the expected BOD was 12 mg/L? Would 30, 60 and 100 ml work?
Yes, the three dilutions would give at least 2 valid results for a sample with a BOD

between 5-56 mg/L. In this example, the 60 ml and 100 ml bottles would both show good DO depletion for a sample with a BOD of 12 mg/L. The 30 ml sample would have no DO left and would not meet the depletion rule.

## DO Meter Calibration

Most DO meters have specific calibration procedures set by the manufacturer. Because oxygen is a gas, it is pressure and temperature dependent. The DO meter must be calibrated against these two variables. As a rule the warmer the water, the less DO the water can hold. Likewise, the colder the water, the more DO the water can hold. This is one of the reasons sample are pre-warmed prior to placing in the BOD bottles. A BOD bottle filled with cold sample will have a different DO than a bottle filled with warm sample. Bottles having different volumes of sample will also potentially have different DO readings, that is why the DO on each bottle must be read initially.

Altitude also affects the amount of DO present. The higher the altitude, the less DO the sample can contain. A laboratory at an elevation of 5000 feet will have less oxygen in the saturated dilution water than a laboratory at sea level. For instance, at sea level, saturated water might have a DO of 8.5 mg/L while the same water at 4000 feet will only have a saturated DO value of 7.5 mg/L.

Problems with DO meters almost always center around the membrane. Whenever problems arise and the DO doesn't "look right", change the membrane. Follow the manufacturers troubleshooting and maintenance procedures. Record the maintenance in the log book.

## BOD Calculations

BOD calculations follow the general formula:

$$\text{BOD} = \frac{\text{DO used by sample x 300}}{\text{sample volume}}$$

Unfortunately, it's not going to appear that easy. The dissolved oxygen used by the sample represents the amount of oxygen consumed by the microorganisms as they digest the organics over the 5 day incubation period at 20$^{o}$C. The organics can come from the sample and from the seed source.

Lets check the Unseeded blanks first! The blanks were filled with aerated BOD dilution water.
How much organics were in the BOD dilution water? No organics should be present in the BOD dilution water.

How many microorganisms were in the BOD dilution water? No microorganisms would be expected to be present in BOD dilution water either.

If there are no microorganisms and no food, there will be very little metabolic activity. If there are no organics and microorganisms present in the BOD dilution water, how much oxygen would you expect to be consumed?

Thus the DO used in the unseeded blanks would be expected to be zero. Because oxygen is temperature and pressure sensitive, a drop of less than 0.2 mg/L is acceptable.

If the unseeded blanks are not acceptable, the test is still valid but an effort to correct the problem must be made.

## Seed Correction Calculations

The seed correction subtracts the amount of oxygen used metabolizing the organics from the seed source. Calculate the seed correction from the following data.

Example 1:
First select all bottles which meet the DO depletion rules. Remember the dilution rule requires the bottle to use at least 2 mg/L of DO but have a least 1 mg/L of oxygen remaining at the end of 5 days. Which bottles meet the DO depletion rule?

| Volume seed added | 3 ml | 5 ml | 8 ml |
|---|---|---|---|
| Initial DO | 7.4 | 7.4 | 7.4 |
| Final DO | 5.9 | 4.1 | 2.5 |
| **Difference** | **1.6** | **3.3** | **4.9** |
| **Correction** | **BAD** | **0.66** | **0.61** |

The 5 ml and 8 ml bottle are acceptable because they dropped at least 2.0 mg/L and the final DO is above 1.0 mg/L. The 3 ml bottle dropped only 1.6 mg/L and does not meet the depletion rule and cannot be used.

What is the seed correction for each acceptable bottle?

$$\frac{3.3 \text{ mg/L}}{5 \text{ ml seed}} = \frac{0.66 \text{ mg/L}}{1 \text{ ml seed}}$$

$$\frac{4.9 \text{ mg/L}}{8 \text{ ml seed}} = \frac{0.61 \text{ mg/L}}{1 \text{ ml seed}}$$

Calculate the seed correction by averaging all bottles that meet the depletion rule. What is the average seed correction?

$$\frac{0.66 + 0.61}{2} = \mathbf{\underline{0.64 \text{ mg/L}}}$$

| Example 2: Calculate the seed correction from the following data. Volume seed added | 5 ml | 10 ml | 15 ml |
|---|---|---|---|
| Initial DO | 8.3 | 8.4 | 8.3 |
| Final DO | 6.1 | 4.1 | 2.5 |
| **Difference** | **2.2** | **4.3** | **5.8** |
| **Correction** | **0.44** | **0.43** | **0.39** |

Which bottles meet the DO depletion rule?
All the bottles meet the depletion rules.

What is the seed correction for each acceptable bottle?

$$\frac{2.2 \text{ mg/L}}{5 \text{ ml seed}} = \frac{0.44 \text{ mg/L}}{1 \text{ ml seed}}$$

$$\frac{4.3 \text{ mg/L}}{10 \text{ ml seed}} = \frac{0.43 \text{mg/L}}{1 \text{ ml seed}}$$

$$\frac{5.8 \text{ mg/L}}{15 \text{ ml seed}} = \frac{0.39 \text{ mg/L}}{1 \text{ ml seed}}$$

What is the average seed correction?

**0.42 mg/L**

## GG Standard Calculations

It's time to find out if all your test conditions, procedures, and lab techniques are acceptable. To do this, a known BOD standard solution containing glucose-glutamic acid is measured. If the glucose-glutamic acid standard results are not within 198 ± 30.5 mg/L, the test is invalid and all data **must be rejected.**

To calculate the G-G concentration, you need to know:

| | |
|---|---|
| **Volume of GG added** | **6.0 ml** |
| **Volume of seed added** | **1 ml** |
| **Initial DO** | **7.8** |
| **Final DO** | **3.2** |
| **DO Difference** | **4.6** |
| **Seed correction** | **0.6** |
| **Corrected DO** | **4.0** |

In this example, the microorganisms used up 4.6 mg/L of oxygen digesting 6 ml of glucose-glutamic acid. However, because seed was used, 0.6 mg/L must be subtracted to correct for the organics that were added with the seed. The final oxygen used up digesting the glucose-glutamic acid is 4.0 mg/L.
Does this bottle meet the oxygen depletion rules?

$$\text{BOD} = \frac{\text{DO used by sample x 300}}{\text{sample volume}}$$

$$\text{BOD} = \frac{(4.6 - 0.6) \times 300}{6.0} = \frac{4.0 \times 300}{6.0}$$

$$= 200 \text{ mg/L}$$

Because the GG result is within 198 $\pm$ 30.5 mg/L, the test conditions validate the sample data. If the result was not within 198 $\pm$ 30.5 mg/L, the test is **INVALID** and all data must be discarded. Determine the GG results from the data in example1.

Example 1:
Six ml of GG and 2 ml of seed were added to a BOD bottle. The seed correction value was 0.44 mg/L per ml of seed. If the initial DO was 7.8 and the final DO was 2.2, what is the BOD for the GG.

| | |
|---|---|
| **Volume of GG added** | **6.0 ml** |
| **Volume of seed added** | **2 ml** |
| **Initial DO** | **7.8** |
| **Final DO** | **2.2** |
| **DO Difference** | |
| **Seed correction** | |
| **Corrected DO** | |

Calculate the seed correction. **(0.88 mg/L)**
Calculate the DO used by just the GG. **(4.72 mg/L)**
Calculate the BOD in mg/L. **(236 mg/L)**
Determine if this is this an acceptable BOD. **(No, the BOD is above 228.5 mg/L)**

Example 2:
Six ml of GG and 5 ml of seed were added to a BOD bottle. The seed correction value was 0.14 mg/L per ml of seed. If the initial DO was 6.8 and the final DO was 2.7, what is the BOD for the GG.

| | |
|---|---|
| **Volume of GG added** | **6.0 ml** |
| **Volume of seed added** | **5 ml** |
| **Initial DO** | **6.8** |
| **Final DO** | **2.7** |
| **DO Difference** | |
| **Seed correction** | |
| **Corrected DO** | |

Calculate the seed correction.
**0.14 mg/L x 5 ml seed added = 0.70 mg/L**

Calculate the corrected DO.
**(6.8 - 2.7) = 4.1 mg/L** This represents the total oxygen used.
**4.1 - 0.70 = 3.4 mg/L** This represents the oxygen used up by just the GG.

Calculate the BOD in mg/L.

$$\textbf{BOD} = \frac{3.4 \times 300}{6.0} = 170 \text{ mg/L}$$

Determine if this is this an acceptable BOD. **Yes**. This value (170 mg/L) does fall within 198 $\pm$ 30.5 mg/L. The results are satisfactory and sample data may be accepted. The BOD is just barely within the acceptable range. If this continues to be a borderline problem, the analyst should troubleshoot the procedure to bring the BOD closer to the mean.

## Seeded Sample Calculations

This is the final step in the BOD test-sample analysis. There are two types of samples typically run.

1. Those with sufficient microorganisms
2. Those with few or no microorganisms

Samples with abundant populations of microorganisms will not require extra "seed". A raw influent sample would be an obvious sample that would have its own population of bacteria often as high as 10,000,000/100 ml.

On the other hand, samples with few microorganisms will require an outside source of microorganisms or "seed". A disinfected secondary effluent is a common sample that requires "seed". A disinfected effluent sample may have no higher than 10 bacteria/100 ml.

**Example 1:** Calculate the sample BOD concentration for a chlorinated WWTP effluent sample with an expected BOD of 20 mg/L. The seed correction was 0.3 mg/L per ml of seed.

| BOD bottle number | 6 | 3 | 51 |
|---|---|---|---|
| Volume of sample added | 30 | 60 | 100 |
| Volume of seed added | 2 | 2 | 2 |
| Initial DO | 7.5 | 7.5 | 7.4 |
| Final DO | 3.9 | 1.1 | 0.0 |
| DO Difference | 3.6 | 6.4 | |
| Seed Correction | 0.6 | 0.6 | |
| Corrected Difference | 3.0 | 5.8 | |
| BOD | 30.0 | 29.0 | |

In this example, three different volumes of sample were placed in the BOD bottles, 30, 60, and 100 ml. The sample volumes selected were based on the anticipated answer. Standard Methods recommends preparing enough bottles so that at least 2 of the bottles will meet the DO Depletion Rule.

| Volume added | Expected BOD |
|---|---|
| 3 | 200 - 560 mg/L |
| 6 | 105 - 280 |
| 9 | 70 - 187 |
| 12 | 53 - 140 |
| 15 | 42 - 112 |
| 30 | 21 - 56 |
| 45 | 14 - 37 |
| 60 | 11 - 28 |
| 100 | 5 - 15 |
| 300 | 0 - 5 |

In the example above, which bottles meet the DO Depletion Rule? Bottles 6 and 3 meet the DO Depletion Rule. Bottle 51

dropped below 1 mg/L, and should not be used.

To calculate the sample BOD, the same formula is used. Since seed was added, a seed correction is necessary.

$$\text{BOD} = \frac{\text{DO used by sample-seed correction} \times 300}{\text{sample volume}}$$

$$\text{BOD} = \frac{(7.5 - 3.9 - 0.6) \times 300}{30} = \frac{3.0 \times 300}{30} = 30.0$$

$$\text{BOD} = \frac{(7.5 - 1{,}1 - 0.6) \times 300}{60} = \frac{5.8 \times 300}{60} = 29.0$$

To calculate the BOD, average the two valid bottles.

$$30.0 + 29.0 = \frac{59}{2} = 29.5 \text{ mg/L}$$

**Example 2**

The lab expects an effluent BOD between 5-30 mg/L. Using the table, select 3 sample volumes to use.

In this example, the technician selects 50, 100, and 300 ml. Complete the table and calculate the sample BOD. The seed correction factor is 0.21 mg/L per ml of seed.

| BOD bottle number | 26 | 33 | 89 |
|---|---|---|---|
| Volume of sample added | 50 | 100 | 300 |
| Volume of seed added | 4 | 4 | 4 |
| Initial DO | 7.7 | 7.5 | 7.4 |
| Final DO | 5.2 | 3.0 | 0.8 |
| DO Difference | | | |
| Seed Correction | 0.84 | 0.84 | 0.84 |
| Corrected DO | | | |
| BOD | | | |

Is the seed correction OK? Yes, the correction falls between 0.6 and 1.0 mg/L

Is the DO depletion on all the bottles OK? No. Bottle 89 has dropped below 1.0 mg/L and should not be used in the calculations. The BOD answer is 10.5.

**Example 3**

Calculate the BOD from the data below. The seed correction is 0.25 mg/L per ml of seed.

| BOD bottle number | 22 | 29 | 55 |
|---|---|---|---|
| Volume of sample added | 10 | 20 | 40 |
| Volume of seed added | 2 | 2 | 2 |
| Initial DO | 8.5 | 8.7 | 9.0 |
| Final DO | 6.6 | 4.9 | 2.1 |
| DO Difference | | | |
| Seed Correction | | | |
| Corrected difference | | | |
| BOD | | | |

Is the seed correction OK? Yes, however, if the seed correction is consistently around 0.5, the volume of seed should be increased from 2 ml to 3 ml in order to bring the seed correction between 0.6-1.0 mg/L

Is the DO depletion on all the bottles OK? No. Bottle 22 has not dropped at least 2.0 mg/L and should not be used in the calculations The answer is 48.8 mg/L.

**Example 4**
Calculate the BOD from the data below. This is a chlorinated effluent. The seed correction is 0.12 mg/L per ml of seed.

| | | | |
|---|---|---|---|
| **BOD bottle number** | 12 | 39 | 58 |
| **Volume of sample added** | 10 | 20 | 40 |
| **Volume of seed added** | 4 | 4 | 4 |
| **Initial DO** | 7.5 | 7.7 | 8.0 |
| **Final DO** | 4.9 | 3.9 | 3.4 |
| **DO Difference** | | | |
| **Seed Correction** | | | |
| **Corrected difference** | | | |
| **BOD** | | | |

You should have calculated BODs of 63.6, 49.8, and 30.9.
Are all the bottles acceptable? Yes
What is the correct BOD to report?
Normally, all the acceptable BOD values are averaged. The average calculated BOD is 48.1 mg/L. However in this example, notice the erratic nature of the data. Notice that as more sample was used in the BOD bottles, the BOD became lower. This is a common picture when dechlorination of the sample has not been performed properly or other toxic materials are present. As more sample is added, more chlorine is added to the BOD bottle. The higher amount of chlorine then kills the seed and results in low answers. The BOD should be repeated if possible. If that is not possible, the highest BOD (63.6 mg/L) should be reported.

## BOD Troubleshooting

The BOD test is only invalidated when

1. BOD incubator temperature is outside $20 \pm 1^{o}C$ or
2. The GG standard is outside $198 \pm 30.5$

Anything else is problematic and must be evaluated.

**Troubleshooting BOD Dilution Water**:
If the dilution water blanks drop more than 0.2 mg/L, the dilution water needs to be evaluated. The BOD test is not invalid. However, the analyst must begin to seek a remedy for the problem. Sources of error include.

1. Poor aeration procedure. (Common error)
2. The dissolved oxygen meter improperly calibrated. (Common error)
3. Bottle filling was too vigorous
4. Dilution water temperature was too cold.
5. Dirty BOD bottles and **stoppers**. It's easy to forget to scrub the stoppers (Common error)
6. The nutrient reagents were contaminated.
7. Deionized water source was contaminated.

The lab technician needs to think about every step taken to prepare the dilution water. Contaminated tubing, dirty pipets, dirty air, dirty bottles could all contribute to a dissolved oxygen change. Be sure the pipet is clean and the hose does not touch the water. Dirty air is not a likely source if an oil-less compressor is used.

The DO meter calibration can be verified by titrating a separate sample using a second method called the Winker Method, Standard Methods 4500-O-C
If the final DO reading is not acceptable, the DO meter should be recalibrated. Perhaps the battery is old, weak. Perhaps the membrane is contaminated. Perhaps the

probe storage container is contaminated.

Filling the BOD bottles too rapidly can cause supersaturation. This "extra" oxygen may come out of solution after 5 days in the incubator. Fill the bottles slowly.

Remember gases are temperature and pressure dependent. The colder the water the more oxygen that can be held. If the dilution water is colder than the BOD incubator temperature (20$^{o}$C), the extra oxygen contained in the cold water will be released during the incubation period. This may create an error. An elevated dilution water temperature, such as room temperature, does not usually create a significant error.

Proper glassware washing must be observed. Be sure to wash with a non-toxic detergent, scrub the bottle thoroughly with a brush, then rinse at least 10 times with tap water to remove the detergent, then rinse 5 times with small quantities of deionized water. The detergent may have an organic component.

Inspect the reagent stock solutions for cloudiness, growth, or precipitation. If present, discard and replace with fresh reagents. Cloudiness is usually indicative of bacterial growth.

The deionized water source should be checked monthly for conductivity, heterotrophic plate count, and chlorine residual. A large heterotroph (bacteria) count in the DI water is undesirable. DI is not sterile but should have only trace microorganism. Perhaps the DI system is contaminated. Is there an activated carbon canister on the system? Is there an RO? All equipment can be a possible source of contamination that will make the dilution water blanks unacceptable.

**Seed Correction Troubleshooting**

The seed correction should have a value between 0.6 - 1.0 mg/L in the glucose-glutamic acid standard and all seeded sample bottles. Selecting the volume of seed to use is basically an educated guess (voo doo for you). When a seed sample is collected, the activity is unknown which makes it difficult to decide how much seed to use. If the results do not fall within the expected range, all is not lost. However, the analyst must now try to adjust the seed to correct the situation. Remember the purpose of the seed is to provide a source of active microorganisms in log phase. Possible causes for bad seed corrections could be:

1. Weak seed activity. Usually indicates a dilute influent strength (low numbers of bacteria). For instance if the seed correction used for the GG standard is less than 0.6 mg/L, the first remedy is simply to add a larger volume of seed. If 3 ml of seed had been previously used, try adding 4 or 5 ml the next time. As an example, if the seed correction is 0.1 mg/L/ 1 ml of seed and 3 ml of seed were added, the seed correction for the GG would have been 0.3 mg/L. Since this is below the desired 0.6 - 1.0 mg/l range, the analyst might add 6 ml next time. This would potentially give a seed correction of 0.6 mg/L. Unchlorinated secondary effluent and receiving streams usually have weak seed activity.

**Glucose-Glutamic Acid Standard**

| | | |
|---|---|---|
| Volume GG used | 6 ml | 6 ml |
| Volume seed used | 3 ml | 3 ml |
| Initial DO | 7.0 | 7.0 |
| Final DO | 3.0 | 3.1 |
| DO used | 4.0 | 3.9 |
| Seed Correction | 0.3 | 0.3 |
| Corrected DO | 3.7 | 3.6 |
| BOD mg/L | 185 | 180 |

Notice the GG value is acceptable, even though the seed activity is low. The test results would be valid but the seed problems should be remedied.

2. Strong seed activity. Usually indicates a high waste strength (lots of bacteria). Same as in #1, except this time the remedy is to lower the seed volume. If 3 ml of seed was giving a GG seed correction of 1.5 mg/L, then reducing the seed volume to 1 or 2 ml would give a seed correction between 0.5-1.0 mg/L. A fresh, normal influent (BOD of 200 mg/L) will usually provide a good seed source and a good seed activity, which is why seed is often collected from the manhole.

**Glucose-Glutamic Acid Standard**

| | | |
|---|---|---|
| Volume GG used | 6 ml | 6 ml |
| Volume seed used | 3 ml | 3 ml |
| Initial DO | 7.0 | 7.0 |
| Final DO | 3.0 | 3.1 |
| DO used | 5.0 | 4.9 |
| Seed Correction | 1.5 | 1.5 |
| Corrected DO | 3.5 | 3.4 |
| BOD mg/L | 175 | 170 |

As before, the GG value is acceptable, even though the seed activity is high. The test results would be valid but the seed problems should be remedied.

3. Erratic seed corrections. This problem usually presents as seed corrections that are sometimes high, sometimes low, and sometimes OK. Collecting the seed at the same time and place often helps eliminate this problem. Since both flow and waste concentration change throughout the day, collecting seed at 8:00 am, then 1:15 pm most likely will not have the same waste strength and therefore, the same bacterial activity. Seed activities as low as 0.05 or as high as 1.5 mg/L per milliliter of seed can be found throughout the day. However, the seed activity may be between 0.2 - 0.4 mg/L if always collected between 8:00 and 9:00 am. Using 3 ml of seed then gives a depletion of typically 0.6 - 1.2 mg/L.

**Seed Correction - Wed. 8:00 am**

| | | | |
|---|---|---|---|
| Volume seed used | 3 ml | 5 ml | 8 ml |
| Initial DO | 7.0 | 7.0 | 7.0 |
| Final DO | 5.0 | 3.8 | 1.6 |
| DO used | 2.0 | 3.2 | 5.4 |
| Seed Correction | 0.67 | 0.64 | 0.67 |
| Average Seed Correction/ ml | 0.66 | | |

**Seed Correction - Thurs. 1:15 pm**

| Volume seed used | 3 ml | 5 ml | 8 ml |
|---|---|---|---|
| Initial DO | 7.0 | 7.0 | 7.0 |
| Final DO | 6.5 | 5.8 | 4.6 |
| DO used | 0.5 | 1.2 | 2.4 |
| Seed Correction | invalid | invalid | 0.3 |
| Average Seed Correction | 0.3 | | |

In the example, both seed corrections are OK but only 1 ml of seed would be needed in the first case, while 2 or 3 ml would be acceptable in the second case.

4. Erratic seed correction bottles. For instance, the analyst has run 3 seed correction bottles and ends up with a seed correction of 0.3 mg/L in bottle #1, 0.5 mg/L in bottle #2, and 0.14 mg/L in bottle #3. Averaging the three values would give a value of 0.31 mg/L. However, the spread is very large. This could be due to the way the seed is handled. The seed collected needs to settle for a minimum of an hour to allow the settleable solids to get out of the way. If the seed is used too soon or the seed is shaken before being poured off, the settleable solids will give erratic results. Let seed settle for a minimum of an hour and DO NOT SHAKE. Filtering the seed sample through a filter might also help reduce settleable solid interferences. A Whatman #1 filter might work.--remember your not trying to filter out the bacteria, only the solids.

5. Erratic seed source, similar to #3. If the seed source is very inconsistent or continually weak, then a reasonable alternative remedy could be commercial seed. Commercial seed is lypholyzed bacteria, that "come alive" when they are rehydrated in dilution water. The activity is pretty constant but often low (0.1 mg/L/1 ml seed). The directions call for 4 ml of seed to be added to the GG bottle, which gives a seed correction of 0.4 mg/L so more commercial seed may need to be added. Trial and ERROR!

**Glucose-Glutamic Acid Troubleshooting**

Here's the big one. All is not well if the GG is bad. The glucose-glutamic acid standard should have a 5 day BOD of 198 $\pm$ 30.5 mg/L. If the results do not fall within this range, all sample data are invalid. The analyst must now determine the cause of the failure and remedy the problem. Possible causes could be:

1. Inactive/low seed. Inactive/low seed means low levels of living microorganisms. Without a living population, the glucose and glutamic acid cannot be metabolized. The GG results are normally low. Remedy: Find another reliable seed source, use commercial seed.

2. Old glucose/glutamic acid. Glucose and glutamic acid normally do not "age" but if the reagents are improperly stored, it is possible that these organic materials can break down. This is not a common problem. Remedy: Keep reagents current and store the stock bottles in a cool, dry area away from light. Discard outdated reagents. Purchase a commercial standard.

3. Improperly dried glucose/glutamic acid. If the GG was not dried, the GG may contain absorbed water (not visible, but may be noticable if crystals stick together). When weighed on the analytical balance, the 0.1500 gm will now contain a small amount of water weight. This will result in less than 0.1500 gm of GG being weighed. A lower amount of GG will most likely result

in a lower GG BOD. Remedy: Dry for an hour in the drying oven at 103$^{o}$C, then store in a properly operating desiccator.

4. Improperly calibrated analytical balance. If the analytical balance is not zeroed or is not calibrated, the actual weight of the GG may be incorrect. Remedy: Verify the analytical balance is calibrated by comparing to Class 1 weights monthly.

5. Dirty BOD bottles. Dirty BOD bottles may cause either high or low GG results. If the "dirt" is toxic detergent residue, the microorganisms added may very well be killed. If the "dirt" is residual organics from a previous sample that was not washed out completely, the GG result will be higher. Remedy: Wash the bottles with hot water and detergent. Use a brush that is capable of getting the corners and the shoulders of the bottle. For samples that are extremely greasy, the bottles may need to be acid washed. Autoclaving the bottles is not really necessary, although it will denature the organics. Rinse the detergent with tap water 10x. It is best to use small volumes of rinse water, multiple times and allow time for the water to drain completely. What often happens is the rinsing is done so fast, that the water in the BOD bottle does not have enough time to drain out before the next rinse water is added. After rinsing with tap water, rinse with DI water at least 5 times. After drying, examine the bottle corners and shoulders. They should look clean. Don't allow the dirty BOD bottles to dry before washing. Leave them full until washing can be done. Its harder to clean your glass after leaving it in the sink for a week isn't it?

6. Contaminated dilution water. If the unseeded dilution water blanks drop more than 0.2 mg/L, the dilution water may be contaminated. Remedy: Correct the contamination problem by cleaning the bottle, replacing any hoses, inspecting reagents, etc.

7. Improperly calibrated DO meter. An improperly calibrated DO meter can give either high or low GG results. Before invalidating the test results, **always** recheck the calibration. While it is unlikely that meter calibration by itself will cause GG failure, it may be part of a number of problems which when added together cause the GG standard to fail. Remedy: Maintain the meter in good working condition by periodically verifying the DO using the Winkler Method and replacing the membrane, battery, and electrolyte periodically.

8. Deionized water. While unlikely, the deionized water could potentially be contaminated with heavy metals. If other samples indicate toxic conditions, repair or replace the DI water source.

## BOD Quality Control

The lab technician should document the following information to support the BOD data. Use the following checklist to determine if sufficient information has been recorded to support the data in court.

**Sample QC**

☐Sample holding time cannot exceed 48 hours. *Document time of collection, arrival, and analysis.*

☐Samples must be preserved on ice or refrigeration until time of analysis. *Document refrigerator temperature*

☐Samples that have been chlorinated must be dechlorinated prior to analysis. *Document volume used to dechlorinate.*

□After samples have been dechlorinated in the lab, the chlorine residual must be rechecked prior to BOD analysis. *Document dechlorination check results.*

□Sample pH must be adjusted to between 6.5-7.5 if the initial pH is <6.0 or >8.5 *Document initial pH, volume of acid/base added.*

□After samples have been adjusted for pH, the adjusted pH must be documented. *Document final adjusted pH.*

□Samples that have been chlorinated must have seed added at the time of BOD analysis. *Document volume of seed added to each bottle.*

□Samples that have had pH adjustment must have seed added at the time of BOD analysis.

□Samples must be warmed to room temperature (20 $\pm$ 3 $^{o}$C) prior to BOD analysis. *Document adjusted temperature.*

□Mix all samples thoroughly just prior to analysis

□Homogenize samples with large amounts of chunky suspended solids

□Effluent samples should be run in duplicate a minimum of 10% of the time.

**Equipment QC**

□BOD incubator must be 20$^{o}$C $\pm$ 1.0$^{o}$C and should be checked and recorded daily when in operation. *Document daily adjusted temperature.*

Corrective Action: Incubator outside the control limits must be adjusted. ***Invalidate*** the BOD sample results

□The incubator thermometer must be immersed in water or glycerol.

□The incubator thermometer must be calibrated at least annually against a NIST certified thermometer. The calibration must include date, thermometer correction factor, serial number, and initials of the person performing the calibration. *Document thermometer calibration.*

□The DO meter must be calibrated at the time of use. *Document the calibration value.* Typical DO should be around 7.0-8.0 if calibration is OK.

Corrective Action: DO meter that does not calibrate, requires maintenance. *Document battery and membrane cap replacement.*

□The analytical balance must be calibrated annually by a certified balance technician. *Document date, balance condition, and name of technician and company.*

□The analytical balance should be checked monthly using Class 1 weights. A series of weights should be selected to cover the range of balance operation. Usually 1, 2, 5,10, 50, and 100 gm weights are used. *Document weight values.*

Corrective Action: If the weights deviate more than 0.0003 grams, the balance needs service. Request balance service.

□pH meters must be calibrated daily, prior to use.

□A minimum of 2 buffers should be used, with a 3rd buffer typically used. *Document calibration.*

Corrective Action: If the pH meter cannot be calibrated or the slope outside acceptable

levels, replace the electrode. *Document the electrode replacement.*

□Validate the DO meter calibration quarterly using the Winkler Azide Method. Check DO meter operation if readings are not within 0.2 mg/L. *Document calibration check*

**BOD Test**

□Dilution water should be checked annually for heavy metals. *Document values for lead, nickel, chromium, copper, zinc, and cadmium.*

□Collect seed on day of test, preferably around 8-9 am. *Document seed collection location and time.*

□Run a seed control set (minimum of 3) with each run of samples. *Document seed correction data.*

□Seed correction factor should be 0.6-1.0 mg/L. Do not invalidate BOD results if the seed correction is outside the range.

Corrective Action: If the seed correction factor is not within 0.6-1.0, adjust the volume of seed added to obtain the desired depletion for the next series of samples.

□Run unseeded blanks in duplicate. *Document test results*.

□Use large bore pipets for pipetting samples. Preferably, use graduated cylinders for large sample volumes

□Unseeded blank DO depletion should be <0.2 mg/L, preferably <0.1 mg/L. Do not invalidate the BOD results if the DO depletion is >0.2 mg/L.

Corrective Action: Check the bottles for cleanliness, recalibrate the DO meter, check the dilution water carboy for cleanliness, replace the aeration hose and pipet, clean the DO electrode in a separate bottle prior to use.

□There should be not air bubbles below the stopper in the BOD bottles at either the beginning or the end of the test. Do not invalidate the BOD results if there are air bubbles in the bottle at the end of the test.

Corrective Action: Fill the BOD bottles before placing the stopper. Check the water seal to be sure water is in the flared part of the bottle above the stopper and no bubbles are below the stopper. Place the plastic cap over the stopper.

□check the sample and dilution water temperature (should be close to 20$^{o}$C), equilibrate the BOD dilution water longer.

□Glucose-glutamic acid standards should be run in duplicate. *Document test results.*

□Glucose-glutamic acid standards should be within 198 $\pm$ 30.5 mg/L after 5 days. If both standards are outside the control limits, the BOD data should be invalidated. However, the analyst should use his judgment and historical data prior to invalidating the sample results if one of the glucose-glutamic acid standards is within the acceptable range.

Corrective Action: Check the other controls such as unseeded blanks and seed control. If these controls are OK, then redry the glucose-glutamic acid. Check the analytical balance operation. Check the preparation procedure. Check DO meter calibration. Check the incubator temperature.

□DO depletion should be >2.0 mg/L and have a final DO >1.0 mg/L

Corrective Action: BOD bottles outside this range should be disregarded and any calculated values should not be used in the answer. Exception: If the sample is very clean and 300 ml of sample is used, calculate and report the results even if the DO depletion is not >2.0 mg/L.

Performance evaluation samples should be run at least annually. *Document results*.

# Chapter 13
# Chemical Oxygen Demand

The COD (Chemical Oxygen Demand) test represents the amount of chemically digestible organics (food). COD measures all organics that were biochemically digestible as well as all the organics that can be digested by heat and sulfuric acid. It is used in the same applications as BOD. COD has the advantage over BOD in that the analysis can be completed within a few hours whereas BOD requires 5 days. The major drawback of the COD test is the presence of hazardous chemicals and toxic waste disposal.

## COD Theory

Like the BOD test, oxygen is used to oxidize the organics to carbon dioxide and water. However, instead of free dissolved oxygen, chemically bound oxygen in potassium dichromate $K_2Cr_2O_7$ is used to oxidize the organics.

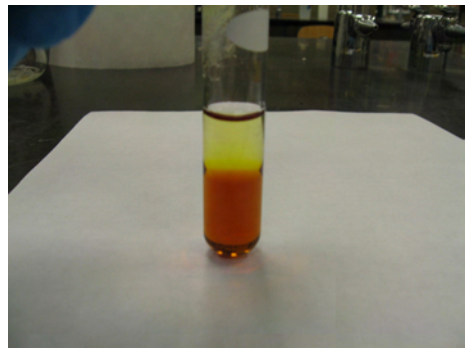
As the potassium dichromate is used up the $Cr^{+3}$ ion is produced. The amount of dichromate used is proportional to the amount of organics present. Likewise, the amount of $Cr^{+3}$ ion present is proportional to the amount of organics digested.

$$\text{Organics} + \underset{\text{(Orange)}}{K_2Cr_2O_7} \rightarrow \underset{\text{(Green)}}{Cr^{+3}}$$

Most labs use the Hach Method to measure COD. This method uses test tubes with pre-measured amounts of potassium dichromate, sulfuric acid, and catalyst. For the digestion to occur, the reaction needs acid, heat, and a catalyst.

The acid is sulfuric acid and is already in the tube. The sample will get very hot when the sample is added to the acid and mixed. Be sure the cap is on tight before mixing and mix just prior to placing in the digestion reactor.

***SAFETY: Be very careful when adding water to acid. This is contrary to normal safety procedure. Be sure to wear goggles, gloves, apron, etc. and do not mix until the cap is tightened on the test tube.***



The heat will be provided by the digestion reactor which is set at 150°C. The sample is refluxed (digested) for 2 hours. During the 2 hours, the organics are oxidized by the acid, potassium dichromate and catalyst.

The catalyst is silver. However, silver must be soluble and will precipitate if chlorides are present in the sample. To prevent silver precipitation, mercury has been added to the reagents in the tube. The mercury will remove the chloride interference.

Once the 2 hour refluxing period is finished, the remaining potassium dichromate is measured using the spectrophotometer.

Since this is a spectrophotometric test, a standard curve can be prepared as discussed earlier. However, this method is so reliable, that the Hach standard curve stored in the spectrophotometer software program is normally used to determine the sample concentration directly. As discussed earlier, a reagent blank and standard must be prepared to zero the spectrophotometer and validate the curve.

## Sample Collection

The sample is usually collected the same as BOD, but is not generally used for NPDES purposes. Most NPDES permits specify BOD because it gives a better representation of the organics that are affecting the receiving stream. The COD sample may be a composite or a grab sample. Because there are no microorganisms involved in this procedure, preservation is usually acidification using sulfuric acid to a pH below 2. Refrigeration is acceptable if BODs are also to be run on the sample. The sample holding time is 7 days, much longer than the 24 hours allowed for the BOD test.

## Sample Handling

The sample volume used for the COD test is 2.0 ml so measuring the sample volume is critical. Be sure to mix the sample well and homogenize if necessary. Pipet quickly to avoid settling errors. CODs can be run on industrial samples that may have high BODs. If the COD strength is greater than 1650 mg/L, the sample must be diluted. Make a 1:2 dilution by measuring 50 ml of sample and adding to 50 ml of deionized water, then add 2.0 ml of the well mixed dilution to the test tubes.

## COD Procedure

### KHP Standard Preparation

1. Place about 5 grams of Potassium Hydrogen Phthalate (KHP) in an aluminum weighing dish.
2. Place the aluminum dish in the drying oven at 110$^{o}$C for 2 hours. Remove the dish from the oven and place in the desiccator until time of use.
3. Weigh 0.4251 gm of dried KHP on a calibrated analytical balance.
4. Transfer completely to a 1000 ml volumetric flask.
5. Fill to the mark with deionized water**.** This prepares a 500 mg/L standard. Store in the refrigerator.

### Select the Method

Hach Chemical Co. uses two methods for COD, a high range 0-1500 mg/L and a low range, 0-150. Use the low range for effluent samples and the high range for all other samples. The procedure for both methods is essentially the same until measuring with the spectrophotometer. The concentration of dichromate in the low level method is 10x lower than in the high level method.

### Blank Preparation

1. Obtain a Hach COD high level tube (Dark orange)
2. Using a 2.0 ml volumetric pipet, add 2 ml of deionized water to the COD tube.
3. Replace the cap and tighten.

***SAFETY: When liquid is added to the tubes, the tubes will become very hot when mixed.***

4. Carefully mix by inverting several times to suspend the powder in the bottom of the tube.
5. Place the tube in the Hach Reactor at 150$^{o}$C.

**KHP Standard Preparation**

1. Obtain a second Hach COD high level tube (dark orange)
2. Using a 2.0 ml volumetric pipet, add 2 ml of the 500 mg/L KHP standard to the COD tube.
3. Replace the cap and tighten.
4. Carefully mix by inverting several times to suspend the powder in the bottom of the tube.
5. Place the tube in the Hach Reactor at 150$^{o}$C.

**High Level Sample**

1. Obtain a third Hach COD high level tube (dark orange)
2. Using a 2 ml serological pipet, add 2 ml of the sample to the COD tube. If necessary, homogenize the sample for 1 minute. If the sample is expected to be >1500 mg/L, the sample can be diluted. Add 2 ml of each dilution to a separate COD tube. For instance, a 1:2 dilution could be made by adding 50 ml of the homogenized sample to 50 ml of DI water. Mix and quickly pipet into the COD tube to avoid settling errors.

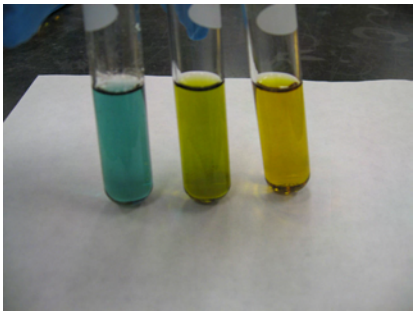


Figure: Sample dilutions. Left sample is >1500 mg/L. All dichromate is gone.

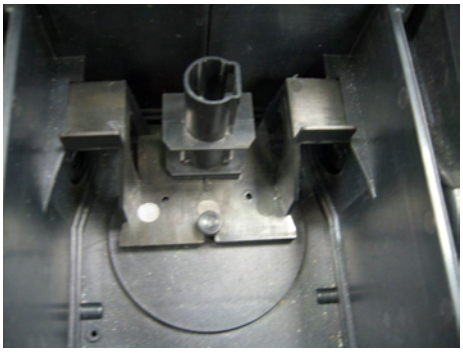
3. Replace the cap and tighten.
4. Carefully mix by inverting several times to suspend the powder in the bottom of the tube.
5. Place the tube in the Hach Reactor at 150$^{o}$C.

**Sample Digestion**

1. Turn on the power switch located in the back of the digestion apparatus. Allow the digestion apparatus to warm up by pressing the infinity switch ( ∞ ).
2. After the blanks, standards, and samples have been placed in the digester, turn the timer to 120 minutes.
   Press the timer button to begin the timed digestion. The digestion apparatus will turn off automatically.

**Sample Measurement**

1. Turn on the Spectrophotometer and allow to warm up for 10 minutes.
2. Insert the Hach COD tube adapter.



3. Set up the spectrophotometer to the correct Hach program (2720). The wavelength should be displayed. Record the wavelength used.

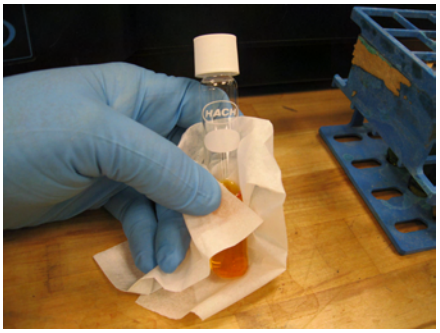



Figure: Wipe with kimwipe® to remove fingerprints, dirt.

4. Using a kimwipe®, wipe off the outside of the COD Blank. Insert the blank into

the sample holder with the Hach name facing forward.

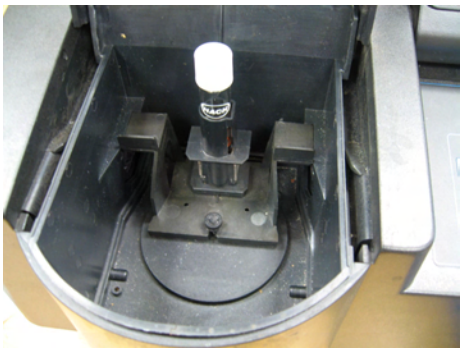


Figure: Insert tube into cell holder

Close the cover and press the ZERO button. The display should now show "0". Record the display value on the data sheet

Figure: Zero the spectrophotometer using a reagent blank.

5. Using a kimwipe®, wipe off the outside of the COD 500 mg/L KHP Standard. Insert the standard into the sample holder with the Hach name facing forward. Close the cover and press the READ button. Record the display value on the data sheet.

6. Using a kimwipe®, wipe off the outside of the sample. Insert the sample into the sample holder with the Hach name facing forward. Close the cover and press the READ button. Record the display value on the data sheet.

## Troubleshooting

Very little goes wrong in this test. Most errors come back to lab technician techniques. The most critical errors occur with the blank and KHP standard.

The spectrophotometer must be warmed up and zeroed using the reagent blank. If the volume of DI water added to the reagent blank is not 2.0, the color of the reagent blank will be either lighter or darker than expected. This error will be most noticeable when using the low range method.

Likewise, if the KHP standard does not fall at 500 $\pm$ 50, the samples should be invalidated. The lab technician should critique the standard preparation procedure.
1. Was the balance calibrated prior to weighting the KHP?
2. Was the standard weighed correctly?
3. Was the powder spilled during standard preparation?
4. Was the KHP volume pipetted correctly?
5. Were the caps on the standard loose during digestion?
6. Were the sample cells wiped clean?
7. Is the correct wavelength being used?
8. Has the KHP standard deteriorated? cloudy?

If the KHP standard and the digestion reactor temperature are incorrect, the data must be invalidated.

## Quality Control

The lab technician should document the following information to support the COD data. Use the following checklist to determine if sufficient information has been recorded to support the data in court.

**Sample**

 Sample holding time cannot exceed 7 days. Corrective Action: Reject samples and request a resample.
 Samples must be preserved with sulfuric acid or refrigeration until time of analysis. Corrective Action: Adjust refrigerator to below 4$^{o}$C. Service the refrigerator if the temperature does not adjust properly.
 pH meters must be calibrated daily, prior to use. A minimum of 2 buffers should be used, with a 3rd buffer typically used. Corrective Action: If the pH meter cannot be calibrated, replace the electrode. Document the electrode replacement.
 Samples must be warmed to room temperature prior to COD analysis.
 Samples with large chunks of non-homogeneous materials should be homogenized for 1-2 minutes for better precision and accuracy. Avoid excessive homogenization which might cause volatilization of some solids.
 Samples must be mixed well and measured quickly to avoid settling errors.

**Equipment**

 Hach Digester must be 150$^{o}$C $\pm$ 2.0$^{o}$C and should be checked. Corrective Action: Digester outside the control limits must be adjusted.
 Immerse the thermometer in the heating block for duration of digestion.
 Calibrate the digester thermometer at least annually against a NIST certified thermometer.
 Use an analytical balance capable of weighing 0.0001 gm to weigh the KHP
Calibrate the analytical balance annually using a certified balance technician.
 Calibrate the analytical balance monthly using Class 1 weights. Select a series of weights which covers the range of balance operation. Usually 1, 2, 5, 20, 50, and 150 gm weights are used
Corrective Action: If the weights deviate more than 0.0002 grams, the balance needs service.
 Calibrate the spectrophotometer using a reagent blank for each run.

**COD Test**

 Prepare fresh KHP standard at least quarterly. Discard if growth or precipitation present.
 KHP is dried and stored in desiccator
 Zero the analytical balance prior to each weighing series
 Use volumetric pipets for standards and blanks. Use large bore serological pipets for samples.
 Performance evaluation samples should be run at least annually
 KHP standard run with each set of samples.
 KHP standard value 100 mg/L $\pm$ 10 for low level, 500 $\pm$ 50 for high level.
Corrective action: If the KHP standards are outside the acceptable levels, new KHP should be prepared and the analysis repeated. Samples should be invalidated.

 Document dates/times
 Document heating block temp
 Document reagents: lot number, expiration date, purchase date.
 Reagent blank used to zero the spectrophotometer
 Document KHP standard-preparation/purchase date, concentration
 Document wavelength used
 Document method used
 Thermometer calibration
 Balance calibration

- PE samples annually
- Make/Model spectrophotometer
- Document time digestion begins and ends on the data sheet.
- Run duplicates 10% of time

# Chapter 14 Solids

In wastewater analysis, if the water is evaporated, solids are left. These solids can be defined in numerous ways such as total solids, suspended solids, dissolved solids, settleable solids, volatile solids, and fixed solids. Total solids are defined as the material left in a container after all the water has been evaporated, usually at 103-105$^{o}$C.

## Total Solids Procedure

1. Wash an evaporating dish with DI water and dry in the drying oven at 103-105$^{o}$C for a minimum of 1 hour. Cool the evaporating dish in the desiccator to balance temperature. The time in the desiccator will vary depending upon the size and number of dishes being cooled. The more dishes to be cooled, the longer the dishes must be in the desiccator prior to the initial weight. Usually a minimum of 45 minutes is needed to cool the dish to room/balance temperature. Weigh the dish on the analytical balance.
2. Transfer the evaporating dish to the analytical balance using forceps and record the initial tare weight.
3. Since this is wastewater, a graduated cylinder is usually a better choice than a pipet to measure 50 ml of sample and transfer to the evaporating dish. Shake the sample well and quickly pour into the graduated cylinder. The sample may be homogenized in a blender if necessary. Measure the volume of sample. Select a volume of sample that will have between 0.0025 - 0.2000 gm. of dry solids. The volume of sample needs to be measured accurately but does not need to be exactly 50 ml. Transfer speed is important, since solids will begin to settle as soon as mixing stops. For instance, shake and pour an influent sample into a 50 ml graduated cylinder, the volume measures 43 ml. Do not try to add more sample to reach the 50 ml mark, since solids in the sample have now begun to settle.
4. Pour the sample in the graduated cylinder into the evaporating dish. Rinse the graduated cylinder with small volumes of DI water and transfer to the evaporating dish.
5. Using tongs, place the dish in the drying oven. If spattering is a concern, lower the drying oven temperature to below boiling (98 $^{o}$C) until the water has evaporated. After the water has evaporated, raise the temperature to 103-105$^{o}$C for a minimum of 1 hour. Remove from the oven and cool to room temperature in the desiccator.

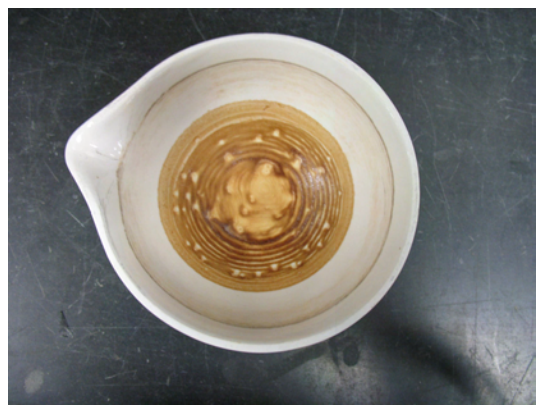


Figure: Notice the solids that remain after evaporation.

6. Record the 1st dry weight.
7. Return the dish to the oven, re-dry for an additional 1 hour at 103-105$^{o}$C. Re-cool in the desiccator and re-weigh.
8. Record the 2nd dry weight. The difference between the first and second weighing should be <0.0005 gm. or less than 4% of the previous weighing to prove the sample was dried completely.

## Total Solids Calculations

| | | |
|---|---|---|
| First dry weight + dish | = | 46.9088 gm |
| Second dry weight + dish | = | 46.9086 gm |
| Difference | = | 0.0002 gm |

The difference of 0.0002 gm is < 0.0005gm indicating the sample has been completely dried.

| | | |
|---|---|---|
| Second dry weight + dish | = | 46.9086 gm |
| Dish weight (tare) | = | 46.8193 gm |
| Difference | = | 0.0893 gm |

The difference of 0.0893 gm is the weight of the dry solids from the 43 ml poured into the dish. The weight of 0.0893 is acceptable since it is between 0.0025 - 0.2000 gm. Weight below 0.0025 gm is statistically invalid, while a weight above 0.2000 gm will likely cause solids to form a crust which interferes with water evaporation.

Determine the total solids by using the formula:

$$\frac{\text{Weight of dry solids}}{\text{Volume of sample}} \times 1000000 =$$

$$\frac{0.0893\ \text{gm}}{43\ \text{ml}} \times 1000000 = 2080\ \text{mg/L}$$

As discussed earlier, the total solids can be reclassified in a number of ways by changing the test procedure. Total solids can be subdivided into Total Suspended Solids (TSS) and Total Dissolved Solids (TDS). Total suspended solids are defined as the portion of the solids that are retained on a 2 um (or smaller) glass fiber filter. Total dissolved solids are defined as the portion of solids that pass through a 2 um (or smaller) glass fiber filter.

## Total Suspended Solids (TSS)

Suspended solids are of interest in a WWTP because they indicate the effectiveness of physical and biological treatment. Solids entering a WWTP are initially separated by reducing the flow so that heavy inorganic suspended solids (grit) can settle in the grit chamber. Measuring solids before and after the grit chamber can help the operator determine the operating condition of the grit chamber. Likewise, total suspended solids can be run on wastewater entering and leaving the primary clarifier. Changes in efficiency may indicate problems with the sludge collection mechanism, changes in flow, changes in water temperature, short circuiting, etc.

Suspended solids leaving the primary clarifier are converted into settleable solids in secondary treatment processes such as trickling filters, SBRs or activated sludge. The suspended solids are digested and entrained with microorganisms as floc, which is heavy enough to settle in the secondary clarifier. Significant reduction of total suspended solids should take place between the primary and secondary clarifier effluents. Changes in the secondary clarifier TSS effluent can be an indication of problems with the secondary treatment processes.

**TSS Theory**

A well mixed sample is filtered through a pre-washed, pre-weighed, glass fiber filter. The suspended solids are retained on the filter. The filter is then rinsed, dried, and weighed. The increase in weight represents the suspended solids.

**Filter Preparation**

There are many manufacturers of satisfactory glass fiber filters. Be sure to purchase filters without organic binders (glue). The filters usually come in packages of 100 or 1000. These filters are not ready to use and must be pre-treated using the same procedure as the sample. All filters must be pre-washed and those that will be used for volatile solids must be pre-volatilized. Pre-washing and pre-volatilizing removes loose materials from the manufacturing process that will give a significantly higher answer.

1. Place the filter onto the filter holder, rough side up, screen side down. Look closely to see the screen.

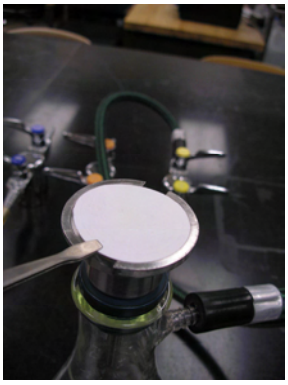


Figure: Place filter on holder rough side up.

2. Turn on the vacuum and quickly pour three successive 20 ml portions of deionized water. Allow the water to be completely removed between each rinse, usually 1-2 minutes is sufficient.
3. Remove the filter from the filter holder and place in an aluminum weighing dish.

Figure: Always use forceps to handle the filter.

4. Place the filter in the drying oven at 103-105$^{o}$C for a minimum of 1 hour. If volatile suspended solids are to be measured, place the filter in the muffle furnace at 550$^{o}$C for 15 minutes after first drying in the drying oven. Cool in the desiccator until at room temperature.
5. Record the first dry weight.
6. Repeat the cycle of drying, volatilizing, cooling, and weighing
7. Record the second dry weight to prove the filters are dry. Dry filters should show a difference in weight of <0.0005gm.

Repeat for **all** filters. Store the filters in the desiccator until needed.

> Note 1: This is the procedure recommended by Standard Methods and makes the assumption that the washing step is effective. The best practices procedure would be to go all the way back and prewash the filters a second time to document the fact that the washing procedure is effectively rinsing out all the particles and then redry the filters to show that the weight has not changed.

**Sample Collection**

TSS samples can be either composite or grab samples and can be collected in either glass or plastic. Samples should be analyzed as soon as possible or preserved by ice or refrigeration to reduce microorganism activity. Holding time is no longer than 7 days.

**TSS Procedure**

1. Set-up. Obtain a clean, dry filter funnel, flask, and vacuum hose. Warm the sample to room temperature. If necessary, the sample can be warmed quickly using a waterbath or hot plate with stirring.
2. Tare Weight Determination. Remove an aluminum dish containing a pre-washed filter from the desiccator. Zero the analytical balance, place the aluminum dish containing the filter on the balance using forceps. **Record the tare weight on the bench sheet.**

Figure: Weigh the dish and filter not just the filter.

3. Remove the filter from the aluminum dish using forceps and place on the filter funnel. Wet the filter with a small volume of reagent water to seat the filter.
4. If warming the sample on a hot plate, stir the sample with sufficient speed to provide a homogenous solution. Stirring too fast may cause particles to separate by size and weight. Pipet the desired volume from a point middepth and midway between the wall and the vortex. Do not pipet from the vortex.

> Note 2: The mixing procedure above is from Standard Methods and by its own admission is prone to large errors. If the point of sample removal is important, the sample is by definition not homogeneous and according to Standard Methods will produce imprecise data. It is important for the technician to obtain a well mixed sample that will provide good precision.

Pipet the sample onto the seated glass fiber filter. Proceed to step 5.

> Note 3: This procedure in Standard Methods may work well for influent samples or poor effluent samples but does not utilize best practices for medium or high quality effluent samples. The procedure requires pipetting the sample onto the filter but also indicates up to 1 liter can be filtered. To pipet 1 liter of effluent using a 25 ml serological pipet would require 40 pipettings which is poor practice. For sample volumes larger than 25 ml, a graduated cylinder should be used.

**Alternate Mixing Procedure**

Shake the sample vigorously. Pour the sample quickly into a graduated cylinder to prevent solids from settling. Choose a volume of sample that will yield between 0.0025 - 0.2000 gm. Influent samples generally require less than 100 ml while clean effluent samples can use up1000 ml. Do not filter more than 1000 ml. Pour quickly and do not try to get a specific volume. Trying to get a specific volume will allow heavy particles to settle and have the same precision problems as stirring.

Measure the volume in the graduated cylinder. **Record the volume on the bench sheet.**

Pour the entire volume into the filter funnel, turn on the vacuum and filter the sample. **TIP:** If the sample does not filter within 1-2 minutes, the sample volume is too high. It will be faster to repeat the test with a smaller volume than wait for the sample to completely filter. Filtration should not take longer than 10 minutes. **TIP:** If the sample filters quickly, a second volume can be added. It is important to add enough sample to achieve a dry solids weight of at least 0.0025 gm.

5. Rinse the with 3 successive 10 ml volumes of deionized water and pour into the funnel.

   Allow complete drainage between washings and continue suction for about 3 minutes.
6. Rinse the filter funnel with deionized water to recover any solids remaining on the sides of the funnel. Turn off the vacuum.

**TIP:** If the vacuum is left "on" for a long period of time, the filter may stick to the funnel base causing a loss of weight when the filter is removed. This can usually be avoided by re-wetting the filter with deionized water then remove as usual.

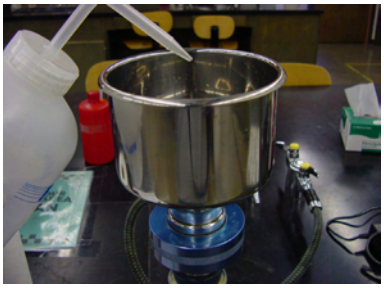


Figure: Rinse with DI water from top to bottom.

7. Remove the filter from the filter funnel using forceps and return to the aluminum dish.

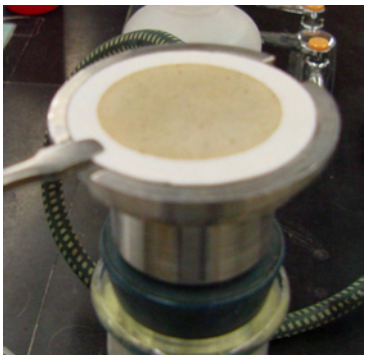


Figure: Notice that the solids have been distributed evenly throughout the filter.

**TIP:** If the filter sticks to the bottom of the funnel, replace the funnel and turn the vacuum back on. With the vacuum on, remove the funnel. Now turn off the vacuum and remove the filter with forceps. Be careful not to tear the filter. If the filter tears, repeat the analysis.

8. Place the aluminum dish in the drying oven at 104°C for a minimum of 1 hour. **TIP:** Place the filter on the top shelf to prevent debris from dropping onto the filter.
9. Remove the dry aluminum dish and filter from the drying oven and place in the desiccator for a minimum of 15 minutes to cool to balance temperature.
10. Remove the dish and filter from the desiccator, place the dish and filter on the analytical balance. Remember to zero the balance. **Record the 1st dry weight on the bench sheet.**
11. Return the aluminum dish to the drying oven at 104°C for a minimum of 1 hour. Remove the dry aluminum dish and filter from the drying oven and place in the desiccator for a minimum of 15 minutes.
12. Remove the dish and filter from the desiccator, place the dish and filter on the analytical balance. **Record the 2nd dry weight on the bench sheet.**

If the difference between the first and second weighings is <0.0005 gm, the filter is dry. If the difference between the first and second weighing is >0.0005 gm, the filter is still wet and must be returned to the drying oven until the weight difference is <0.0005 gm.

**TSS Calculations**

$$\text{TSS mg/L} = \frac{\text{2nd dry weight of solids (gm)} \times 1{,}000{,}000}{\text{Volume of sample (ml)}}$$

Report results to 3 significant figures.

Example 1: The technician quickly pours 730 ml of well mixed effluent into the filter funnel. The tare weight of the filter is 1.5546 gm. After rinsing, drying, cooling, and weighing, the first dry weight is 1.5622 gm. The filter is returned to the oven and dried, cooled, and weighed. The second dry weight is 1.5619 gm. Calculate the TSS

| | |
|---|---|
| First dry weight | = 1.5622 gm |
| Second dry weight | = 1.5619 gm |
| Difference | = 0.0003 gm |

Is the filter dry? Yes, the difference is <0.0005 gm

Second dry weight = 1.5619 gm
Tare weight = 1.5546 gm
Weight of dry solids = 0.0073 gm

Is the weight of the dry solids acceptable? Yes, the weight of solids is between 0.0025-0.2000 gm.

$$\text{TSS} = \frac{2^{nd}\text{ dry weight of solids (gm)} \times 1{,}000{,}000}{\text{Volume of sample (ml)}}$$

$$\text{TSS} = \frac{0.0073\text{ gm}}{730\text{ ml}} \times 1000000 = 10\text{ mg/L}$$

Example 2: The technician quickly pours 41 ml of well mixed influent into the filter funnel. The tare weight of the filter is 1.4603 gm. After rinsing, drying, cooling, and weighing, the first dry weight is 1.4722 gm. The filter is returned to the oven and dried, cooled, and weighed. The second dry weight is 1.4700 gm. Calculate the TSS

First dry weight = 1.4722 gm
Second dry weight = 1.4700 gm
Difference = 0.0022 gm

Is the filter dry? No, the difference is >0.0005 gm. The filter must be returned to the drying oven and be redried until the difference is <0.0005 gm. The redried sample now weighs 1.4699

Second dry weight = 1.4699 gm
Tare weight = 1.4603 gm
Weight of dry solids = 0.0096 gm

Is the weight of the dry solids acceptable? Yes, the weight of solids is between 0.0025-0.2000 gm.

$$\text{TSS} = \frac{2^{nd}\text{ dry weight of solids (gm)} \times 1{,}000{,}000}{\text{Volume of sample (ml)}}$$

$$\text{TSS} = \frac{0.0096\text{ gm}}{41\text{ ml}} \times 1000000 = 234\text{ mg/L}$$

**TSS Troubleshooting**

TSS is generally a fairly simple test with few trouble spots. The most common mistakes involve filter preparation and sample mixing. Running duplicates on a continuous basis provides helpful information and builds confidence in the technician's procedure.

**Blanks are inconsistent**

Methods blanks should be performed on a routine basis, a least once per batch of filters prepared. The method blank is the same as re-rinsing the filters with 100 ml of deionized water. If the filters have been washed properly and consistently, the filters should not lose particles and should have an initial and final dry weight difference close to zero. If there is a difference of >0.0002 gm or duplicate filters are not consistent, the filters may not have been rinsed completely. For instance, if two method blanks are run and the first drops by 0.0001 gm and the second drops by 0.0012 gm, the difference may indicate something is wrong with the way the filters were rinsed. Method blanks can be run with each set of samples to show that rinsing is effective and that sample is not being carried over.

**Not Enough Sample**

The selection of the "right" sample volume is often a guess. The sample volume should ideally add a significant amount of solids to the filter to minimize any balance errors. That is why the minimum weight is supposed to be >0.0025 gm. Analytical balances have an electronic error of $\pm$0.0001 gm. This uncontrollable instrumental error would be a 10% error if the amount of solids was 0.0010 gm. The error caused by the electronic noise goes down as more sample is added to the filter. When at least 0.0025 gm of solid have been added, the error is now a reasonable 4% and will get better as more weight is added. Always add as much sample as possible. If

the sample is still filtering without clogging, and the filter looks clean, add more well mixed sample.

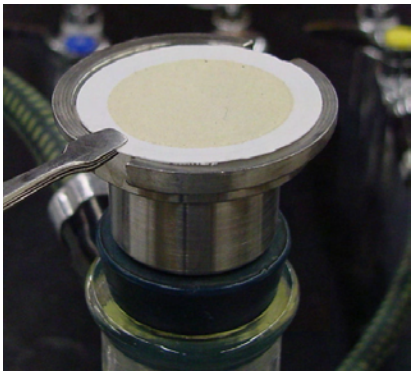


Figure: This filter may not have enough weight. Increase the sample volume to reduce error.

**Too Much Sample**

This is a problem just the opposite of the last one. In this case, too much sample has clogged the filter and the remaining sample is just slowly dripping. Several problems result. First, the filtration time becomes excessive. If the sample is still filtering after 10 minutes, discard the filter and repeat using a smaller volume. Second, a clogged filter will interfere with the rinsing step. The rinsing step should recover any straggler solids from the sides of the graduated cylinder and funnel and rinse out any trapped TDS. Trapped TDS will give a positive bias to the answer. Third, the excessive solids trapped on the filter will form a hard crust on the filter and may prevent water from evaporating completely. This is visible when the filter has a lot of crazing or cracking on it. This is similar to clay. Clay looks dry but when broken into pieces, the inside is still wet. This water weight will also give the sample a positive bias.

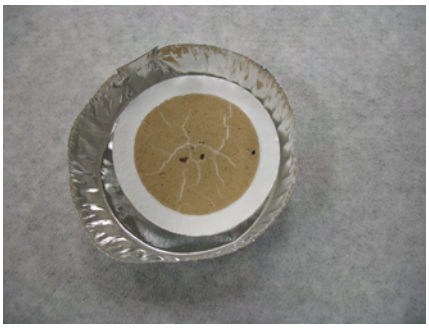


Figure: This cracking indicates too much sample was filtered. Reduce the volume next time.

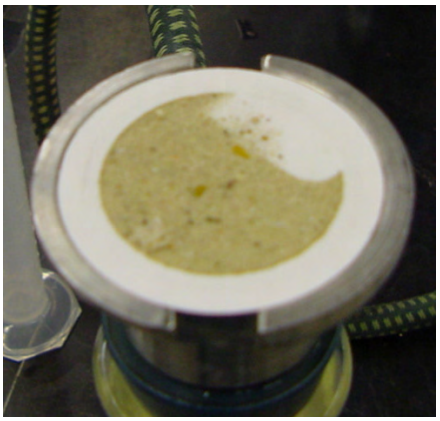


Figure: This sample was poured slowly and results in poor distribution of the solids. High solids in one area may not dry completely.

**Duplicates are inconsistent**

TSS samples should always be run in duplicate. It takes very little time to run a second sample. If the duplicates are consistent, the technician's technique, sample handling, mixing, rinsing, etc. is validated. If the duplicates are inconsistent, sample handling problems may be present. Each sample measured should be mixed and poured completely. For instance, if 67 ml of sample has been measured, all 67 ml should be passed through the filter and rinsed. The sample should not be split, say 25 and 42 ml. While the average of the sample may be correct, the duplicate answers may be far apart. The 25 ml sample may have a TSS answer of 50 mg/L while the 42 ml sample may have a TSS of 84 mg/L. This large difference indicates the solids in the sample settled between the 25 and 42 ml pouring. It

would be better to run a 30 ml sample all at once then mix and pour a second sample of 35 ml. The answers for these two separate samples will be more consistent. Homogenizing the sample may also help improve the precision.

**ODD Stuff**

In spite of the best effort to mix the sample, some samples contain odd stuff such as bugs, twigs, grease balls, etc. These odd particles can give very positively biased samples (high answers). Standard Methods allows you the technician to decide if these materials of truly representative of the sample. If the sample contains a lot of swimming critters, then perhaps they should be included in the TSS filter. If there is only 1 or 2 mosquitos and the technician pours one out onto the filter, perhaps this mosquito should be removed. The same argument applies to grease and oil. Grease and oil stick together and cling to the top of the sample bottle. It is often difficult to adequately mix these materials, so they get pipetted or poured off into the graduated cylinder because they are normally floating at the top of the sample. Is a large grease chunk on one filter representative? Is it a grease chunk? The technician may wish to run the sample and make a note of the abnormal particle.

Note 4: Large particles determined by the lab technician to be "unrepresentative" must be removed prior to analysis. They cannot be removed from the filter after filtration.

## Quality Control

Decisions on the accuracy of the reported data will be based on the quality control information.

**Sample QC**

Sample holding time cannot exceed 7 days.

Corrective Action: Reject samples and request a resample.

Samples must be preserved on ice or refrigeration until time of analysis. *Record the temperature of the refrigerator.*

Corrective Action: Adjust refrigerator to below 6$^{o}$C. Service the refrigerator if the temperature does not adjust properly

Samples must be warmed to room temperature prior to TSS analysis.

Run samples in duplicate 100% of the time if possible. Use approximately the same volume of sample for both. Remix sample between tests.

*Record sample date, time, type, sampler, date and time of analysis, analyst and method used.*

Samples with large chunks of non-homogeneous materials should be homogenized for 1-2 minutes for better precision and accuracy. Avoid excessive homogenization which might cause volatilization of some solids.

Samples must be mixed well and poured quickly.

**Equipment QC**

Drying Oven must be 104$^{o}$C $\pm$ 1.0$^{o}$C *Record the temperature of the drying oven.*

Corrective Action: Incubator outside the control limits must be adjusted. An oven temperature below 103 $^{o}$C may not dry the sample completely. An oven temperature above 105 $^{o}$C may cause some organics to volatilize.

Immerse the oven thermometer in sand to prevent inaccurate temperature readings when the oven door is opened frequently.

Calibrate the oven thermometer at least annually against a NIST certified thermometer. The calibration must include date, thermometer correction factor, serial number, and initials of the person performing the calibration.

*Record the calibration data*
 Use an analytical balance capable of weighing 0.0001 gm.
 Calibrate the analytical balance annually using a certified balance technician. ***Document date, balance condition, and name of technician and company.***
 Calibrate the analytical balance at least monthly using Class 1 weights. Select a series of weights which covers the range of balance operation. Usually 1, 2, 5, 20, 50, and 150 gm weights are used. ***Record weight values on Balance Log***
Corrective Action: If the weights deviate more than 0.0002 grams, the balance needs service. Use another calibrated balance until the next service cycle if possible

**TSS Test QC**
 Pre-wash filters with deionized water and perform at least 2 method blanks on each lot washed.
Corrective Action: If the weight difference is >0.0002 gm, the filter has not been washed completely. **All** the filters in this lot must be rewashed and the process repeated until the difference is within 0.0002 gm
*Document all method blanks.*
 Store pre-washed filters in the desiccator to avoid water absorption.
 Zero the analytical balance prior to each weighing series
*Document the balance was zeroed.*
 Use large bore pipets for small sample volumes and graduated cylinders for large volumes.
 Pipets and graduated cylinders are rinsed with deionized water and the rinse is added to the filter after the sample has been filtered.
 Use sufficient sample to obtain a minimum of 0.0025 gm of TSS on the filter.
Corrective action: If the 2nd dry sample weights <0.0025 gm, invalidate the results and repeat the analysis using more sample volume. Exception: If the weight is <0.0025 gm as a result of filtering 1000 ml, the results are valid and the data reported.
 Filters are checked for dryness. The difference between the 1st and 2nd dry weight is <0.0005 gm. *Document the weights.*
Corrective Action: If the 2nd dry weight has changed by more than 0.0005 gm, the filter is not dry and must be re-dried in the drying oven until the change in weight is <0.0005 gm.
 Filters are placed in the desiccator upon removal from the drying oven.
*Document the condition of desiccator.*
 Performance evaluation samples should be run at least annually.
 Split samples can be run with other nearby facilities
 Duplicate sample TSS results should be within 10% of their average.
Corrective Action: Homogenize samples with large amounts of chunky suspended solids to obtain a more uniform sample. Remix samples between duplicates and measure quickly.
 Perform monthly in-house performance evaluation samples. Weigh 0.1000 gram of dried Infusorial Earth and place in a 1 liter volumetric flask. The answer should be 100 mg/L. Percent recovery should be 100 ± 5%.
Corrective Action: Review all the above QC parameters and repeat analysis until the results are acceptable.

## Volatile Suspended Solids (VSS)

Volatile suspended solids are defined as the suspended solids that can be ignited at 550°C. Volatile suspended solids gives a rough approximation of the amount of organics present in the sample. Volatile suspended solids may be run on grit chamber solids to determine if heavy organics are settling in the grit chamber. Volatile suspended solids are also run to determine the amount of organics in the mixed liquor of an activated sludge system.

If volatile suspended solids are to be run, the glass fiber filters must be pre-ignited in the muffle furnace at 550 $^{o}$C to remove any organic contaminants on the filter. If the filters have been pre-volatilized, the filter used for the TSS sample can be volatilized to give the VSS portion. The difference between TSS and VSS is called ash or fixed solids. The ash represents primarily the inorganic component of the suspended solids.

**VSS Procedure**

1. Pre-heat the muffle furnace to 550$^{o}$C.
2. Place the dry TSS filter in the muffle furnace for 15 minutes.

***Safety: Use long forceps to place and remove the TSS filter from the muffle furnace***.

3. Remove from the muffle furnace and allow to partially cool in the air before placing in the desiccator. Cool to room temperature in the desiccator.
4. Record the weight of the ash on the filter.
5. Calculate the VSS.

**VSS Calculations**

| | | |
|---|---|---|
| Volume filtered | = | 45 ml |
| Weight of TSS filter + dish | = | 1.4983 gm |
| Weight of ash + dish after 550$^{o}$C | = | 1.4956 gm |
| Weight of VSS | = | 0.0027 gm |

$$\text{VSS} = \frac{2^{nd}\text{ dry weight of solids (gm) x 1,000,000}}{\text{Volume of sample (ml)}}$$

$$\text{VSS} = \frac{0.0027\text{ gm}}{45\text{ ml}} \times 1000000 = 60\text{ mg/L}$$

**Fixed Solids or Ash**

From the same data above, the ash can be determined by subtracting the VSS from the TSS. For example, if the TSS was 100 mg/L and the VSS was 60 mg/L, the ash would be 40 mg/L.

## Total Dissolved Solids (TDS)

Total Dissolved Solids are defined as the portion of solids that pass through a 2 um (or smaller) glass fiber filter. The TDS sample can be prepared at the same time the TSS sample is run. The sample which passes through the TSS filter into the receiving flask is the TDS sample. This is called filtrate.

The filtrate is placed in a weighed evaporating dish, evaporated, and dried to a constant weight at 180$^{o}$C. TDS is typically unaffected by the wastewater treatment process. However, excess TDS may interfere with treatment processes. In New Mexico, TDS is required for WWTP's that have groundwater discharge permits. Drinking water has a TDS target of <500 mg/L. If the effluent is percolated through the soil, the TDS can be used as a tracer, to determine the effect of the effluent on the groundwater. TDS samples are often collected from monitoring wells surrounding the effluent disposal area. Will an effluent TDS of 1200 mg/L effect the groundwater having a TDS of 400 mg/L? Yes. The TDS downstream from the WWTP will begin to increase.

**Total Dissolved Solids Procedure**

1. Wash a 100 ml evaporating dish with DI water and dry in the drying oven at 180 $\pm$ 2$^{o}$C for a minimum of 1 hour. Cool the evaporating dish in the desiccator, usually a minimum of 45 minutes, then weigh the dish on the analytical balance. The time in the desiccator will vary depending upon the size and number of dishes being cooled. The more dishes to be cooled, the longer the dishes must be in the desiccator prior to the initial weight.

2. Transfer the evaporating dish to the analytical balance using forceps and record the initial tare weight.
3. The volume of sample can be measured using a graduated cylinder or volumetric pipet. The sample is passed through a glass fiber filter and collected in a clean dry filtration flask. The filter is then rinsed with three successive 10 ml volumes of DI water. The washings are collected in the filtration flask. The contents of the filtration flask are then poured into the weighed evaporating dish. Rinse the filtration flask with small volumes of DI water to recover all the TDS.
4. Using tongs, place the dish in the drying oven at 180 $\pm$ 2$^{o}$C. If spattering is a concern, lower the drying oven temperature to below boiling until the water has evaporated. After the water has evaporated, raise the temperature to 180 $\pm$ 2$^{o}$C for a minimum of 1 hour. Remove from the oven and cool to balance temperature in the desiccator.

5. Record the 1st dry weight.
6. Return the dish to the oven, re-dry for an additional 1 hour at 180 $\pm$ 2$^{o}$C. Re-cool, and re-weigh.
7. Record the 2nd dry weight. The difference between the first and second weighing should be <0.0005 gm. or less than 4% of the previous weighing to prove the sample was dried completely.

**Total Dssolved Solids Calculations**

| | | |
|---|---|---|
| First dry weight + dish | = | 49.9778 gm |
| Second dry weight + dish | = | 49.9774 gm |
| Difference | = | 0.0004 gm |

The difference of 0.0004 gm is < 0.0005gm indicating the sample has been completely dried.

| | | |
|---|---|---|
| Second dry weight + dish | = | 49.9776 gm |
| Dish weight (tare) | = | 49.8193 gm |
| Difference | = | 0.1583 gm |

The difference of 0.1583 gm is the weight of the dry dissolved solids from the 50 ml poured into the dish. The weight of 0.1583 gm is acceptable since it is between 0.0025 - 0.2000 gm.

Determine the total dissolved solids by using the formula:

$$\frac{\text{Weight of dry solids}}{\text{Volume of sample}} \times 1000000 =$$

$$\frac{0.1583 \text{ gm}}{50 \text{ ml}} \times 1000000 = 3170 \text{ mg/L}$$

Notice the volumes of DI rinse water are not added to the sample volume since pure water should not have any effect and is evaporated away.

**Problems**

This procedure works fine for most industrial, influent and monitoring well samples. A small volume of sample (50 ml) can yield reasonable TSS weights yet not generate a large volume of filtrate. The method becomes a problem when clean effluent is used since as much as 1 liter of filtrate may be produced. According to the procedure all the filtrate must be transferred to the evaporating dish which means the evaporating dish must be continually "refilled" with effluent until the effluent is gone, which could take many hours. This

may create a second problem of excessive weight (>0.2000 gm) in the evaporating dish. A low TSS effluent sample could have a high TDS value, ie. TSS of 4 mg/L and TDS of 2700 mg/L.

## Settleable Solids

Settleable solids is defined as the solids that settle to the bottom of an imhoff cone in 1 hour. The test is helpful in monitoring the effectiveness of the clarifiers. The test is usually measured in volume settled rather than weight.

**Settleable Solids Procedure**

1. Shake a representative sample and quickly pour 1 liter into a 1 liter imhoff cone.
2. Start a timer and allow the solids to settle for 45 minutes.
3. At the end of 45 minutes, take a stirring rod or pipet and slowly agitate any solids adhering to the sides of the imhoff cone.
4. Allow the solids to settle for an additional 15 minutes, then measure the volume of settled solids.

Do not include floating solids in the answer. If the solids do not settle uniformly, estimate the volume of water and subtract from the imhoff cone reading.

**Settleometer**

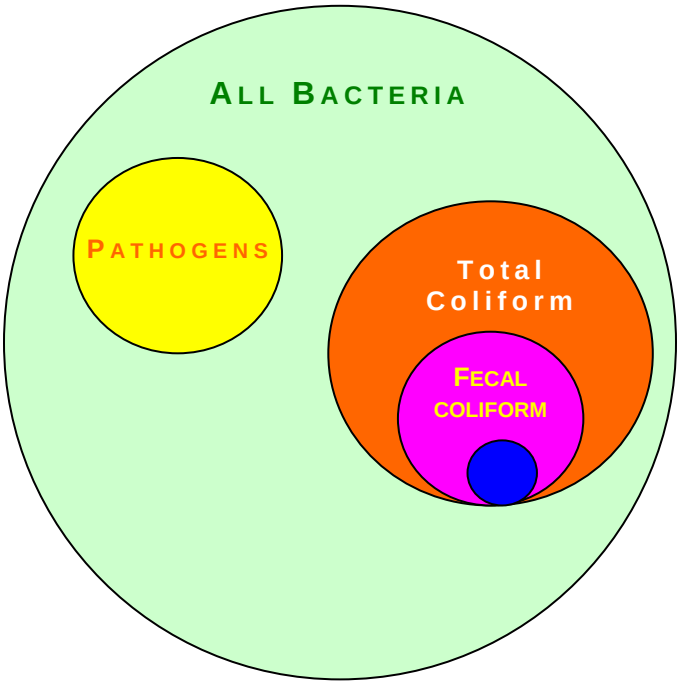
This test is often confused with the settleable solids test but is usually used as a tool to monitor the condition of the activated sludge process.

# Chapter 15 Fecal Coliform

Microorganisms are an integral part of wastewater treatment. They are beneficial yet dangerous. Fecal coliforms originate in the intestines of warm-blooded animals, in this case, primarily humans. Fecal coliforms in the influent of domestic waste are usually several million per hundred milliliters. These bacteria function as decomposers in the secondary treatment process. They serve to metabolize the waste under aerobic conditions then flocculate and settle in the secondary clarifier. Those bacteria which are not trapped in the floc will flow over the secondary weir to the disinfection chamber. At this point, the bacteria are no longer needed and are killed by disinfection with chlorine, UV light, etc.

Coliforms are considered indicator organisms. In wastewater the primary coliform is the fecal coliform which originates in the intestines of warm blooded organisms. Since fecal coliforms are seldom pathogenic under normal circumstances and are easily cultured, their presence indicates the potential presence of pathogens. High levels of fecal coliforms discharged into the receiving stream would indicate that pathogens could also be discharged into the stream. On the other hand, if fecal coliforms are absent, pathogens are also absent since they die quickly outside the host. So why not add lots of chlorine to kill all the fecal coliforms and remove any chance that pathogens might survive? Besides being expensive, chlorine is carcinogenic and harms other organisms in the receiving stream. To reduce the possibility of over-chlorinating, sulfur dioxide has been used to remove excess chlorine prior to discharge. Many wastewater treatment systems have changed disinfection processes to avoid the headaches associated with chlorine. The common alternative is UV light which is very effective at inactivating fecal coliforms and pathogens.

Fecal coliforms belong to the group of bacteria called coliforms (orange circle). The coliform group of bacteria are defined as facultative anaerobic, gram-negative, non-spore forming, rod shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35$^{o}$C.




Fecal coliforms (magenta circle) are members of the coliform group but have the added characteristic of being able to live at the elevated temperature of 44.5$^{o}$C. E. Coli (blue circle) is a specific type of fecal coliform and associated with human waste. Newer discharge permits are requiring analysis for E. Coli rather than the more general fecal coliform.

## Test Theory

A sample is collected and analyzed using aseptic (sterile) technique. A measured volume of sample is filtered through a sterile 0.45µ membrane filter, transferred to an absorbent pad containing m-FC broth, then incubated at 44.5$^{o}$C for 24 hours. Blue/blue gray colonies are counted and reported as colony forming units (cfu) per 100 ml of sample. The method is limited by turbidity in the sample. Excessive

turbidity will reduce fecal coliform recovery, requiring the MPN method to be used instead of the membrane filter method.

## Sample Collection

Unlike other wastewater tests, fecal coliforms must be collected in a clean, sterile borosilicate glass or plastic bottle containing sodium thiosulfate. Presterilized bags or bottles containing sodium thiosulfate can also be used. Sodium thiosulfate is added to remove residual chlorine which will kill fecal coliforms during transit. 0.1 ml of 10% sodium thiosulfate is added to a 120 ml sample bottle prior to sterilization. The minimum bottle size should be 120 ml to allow enough head space (1") for proper sample mixing.

### Collection Procedure

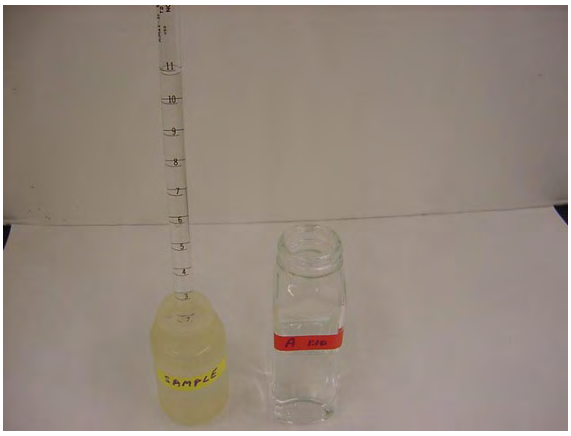
Select a site that will provide a representative sample. Fecal coliform samples are always grab samples and should be drawn directly from the flow stream without using collection devices such as unsterilized dippers or buckets. For example, do not collect a BOD sample then transfer some of the sample to the fecal coliform sample bottle. Keep the sample bottle lid closed tightly until it is to be filled. Remove the cap and do not contaminate the inner surface of the bottle, neck, threads or cap. Fill the container without rinsing, being sure to leave ample air space to allow mixing. Rinsing will remove the dechlorinating agent. All samples should be labeled properly with date and time of collection, sampler's name, and sample collection location. Leaking sample bottles allow for contamination of the sample and should be discarded and the sampling repeated.

### Preservation

Fecal coliform samples should be analyzed as soon as possible after collection to prevent changes to the microorganism population. Fecal coliforms must be transported on ice, if they cannot be analyzed within 1 hour of collection. Fecal coliforms transported at ambient temperature may reproduce and higher bias to the numbers than desired or they may be killed off resulting in lower numbers, if handled poorly such as transport in sunlight. Fecal coliform samples should be stored by the laboratory in a refrigerator until time of analysis. The maximum holding time for state or federal permit reporting purposes is 6 hours.

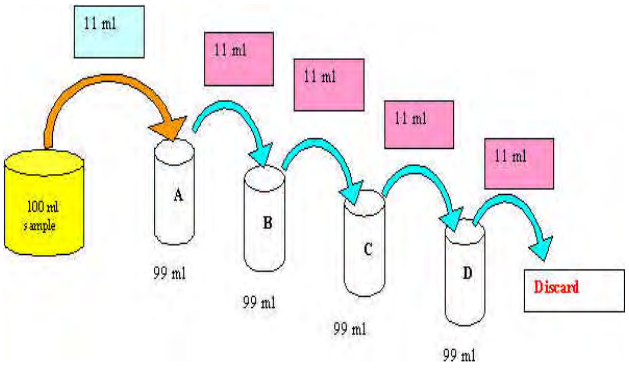
### Dilutions

Ideally, sample volumes filtered should produce a countable plate with 20-60 colony forming units per plate. The lab technician may need to perform several dilutions to reach the desired colony count. If high levels of fecal coliforms are expected, the most common practice is to prepare a serial dilution. A serial dilution dilutes the sample concentration by a factor of 10. The most common dilution is prepared by transferring 11 ml of sample to 99 ml of sterile phosphate dilution water using a sterile serological pipet.



A 1:10 dilution (11ml :110 ml) will reduce the bacteria count in the sample by 10 times. For instance, if the expected sample answer is 4000 cfu/100 ml, a 1:10 dilution will produce a diluted sample containing 400 cfu/100 ml. Both of these samples will produce plates that are uncountable (TNTC) and outside the desired range of 20-60 cfu/100 ml. Therefore, a second serial dilution can be made by pipetting 11 ml of the diluted sample into a second 99 ml bottle of

sterile phosphate dilution water. This will now reduce the colony count from 400 to 40 cfu/100 ml. The 40 cfu/100 ml is a countable plate.




The serial dilution can continue as long as needed. In many instances, if the answer is truly unknown, the technician may make several dilutions to cover the anticipated colony range. Notice in the picture above that each time 11 ml is transferred; the remaining volume is approximately 100 ml which is then filtered.

| Bottle | Volume original sample used | Volume filtered | Dilution | Cfu/100 ml |
|---|---|---|---|---|
| Original | 100 ml | 100 ml | none | 4000 |
| A | 10 ml | 100 ml | 1:10 | 400 |
| B | 1 ml | 100 ml | 1:100 | 40 |
| C | 0.1 ml | 100 ml | 1:1000 | 4 |

The last bottle has 110 ml; so11 ml is discarded, leaving 100 ml. Each serial dilution reduces the amount of original sample by 10, so the colonies in bottle A actually come from 10 ml of sample. The colonies in dilution B come from 1 ml of sample, etc.

For most wastewater treatment plants with a well disinfected effluent, the colony count may be well below the state/federal permit limitations. In this instance, undiluted volumes of 10, 25, 50, and 100 ml will commonly be filtered.

| Volume sample filtered | Cfu/100 ml |
|---|---|
| 100 ml | 92 |
| 50 ml | 48 |
| 25 ml | 26 |
| 10 ml | 9 |

In this example, there are 2 countable plates (50 ml and 25 ml) that will be used in the calculations.

## Test Preparation

Prior to analysis, there are number of preliminary steps that must be performed.

1. Obtain M-FC broth - the broth must be either prepared or purchased. Most labs purchase pre-sterilized 2 ml ampules. The broth sterility, growth characteristics, pH, purchase date, expiration date and lot number should be recorded. Each new lot of media should be tested against the previously acceptable broth to verify acceptable performance. Each lot should be tested for performance with positive and negative control cultures.

   A positive fecal coliform control culture is commonly E. Coli (ATCC 8739 or 25922) An E.Coli pure culture can be purchased, prepared, and stored in the refrigerator. A positive culture should produce blue colonies.

   A negative fecal coliform control culture is commonly Enterobacter aerogenes (ATCC 13048). A negative control culture should not grow.

2. Membrane filters and absorbent pads. Membrane filters are also purchased pre-

sterilized. They must be approved for membrane filtration based on data documenting toxicity, recovery, retention, and absence of growth promoting substances. They must be grid-marked, 47 mm in diameter, and 0.45 micron pore size. They should also be white and composed of cellulose ester. The lot number, date received and sterility must be recorded. Absorbent pads may be included with the membrane filter package or pre-loaded in the culture dish. In either case, the lot number, date received and sterility must be recorded.

3. Culture dishes (Petri dishes). Pre-sterilized disposable, plastic culture dishes (50 mm) are usually purchased. The lids must be tight-fitting. The lot number, date received, and sterility must be recorded.
4. Pipets, Graduated cylinders. Glass pipets should be placed in a stainless steel or aluminum container and sterilizing in a sterilizing oven at 170-180$^{o}$C for 2 hours. Glass graduated cylinders should be wrapped with aluminum foil and sterilized. Pre-sterilized plastic pipets can be purchased in a package or wrapped individually. Reseal packages between each use. Pipets should be accurate to within 2.5% tolerance. Document lot number, date received, and sterility.
5. Sterile Phosphate Dilution/Rinse Water. Prepare or purchase phosphate dilution water. Prepared dilution water should be sterilized for a minimum of 15 minutes in the autoclave at 121$^{o}$C. Volume, pH, and sterility should be checked after sterilization. pH should be 7.2 $\pm$ 0.5 and volume should be 99 $\pm$ 2 ml. Check sterility by adding 50 ml of water to 50 ml of sterile double strength tryptic soy broth (TSB). Incubate for 24-48 hours at 35.0 $\pm$ 0.5$^{o}$C. Record sterility. Measure and record the volume and pH on a second sterile bottle.
6. Membrane filtration apparatus. A plastic, glass or stainless steel, non-leaking, non-scratched, non-corroded and calibrated filtration apparatus should be sterilized for a minimum of 2 minutes in a germicidal (254 nm) UV lamp.
7. Waterbath The waterbath must be turned on and adjusted to 44.5 $\pm$ 0.2$^{o}$C. Waterbath incubators are used because they hold the temperature more accurately than dry air incubators and allow for better selection of fecal coliforms. Calibration-corrected temperatures should be recorded at least twice per day during each day the waterbath is in use, separated by at least 4 hours. Record the date, time, temperature, and technician initials.

Once the equipment and materials are satisfactory, the technician is ready to run the test.

## Test Procedure

1. The lab bench should be disinfected with either alcohol or commercial lab disinfectant to reduce the potential for contamination. The technician should wear appropriate PPE
2. The filtration funnel apparatus is placed in the UV sterilizer and sterilizer for a minimum of 2 minutes. After sterilization, place the filtration apparatus on the filtration funnel until time of use. Record the sterilization time.

**Safety: Do NOT look at the UV light. UV light may cause permanent eye damage.**

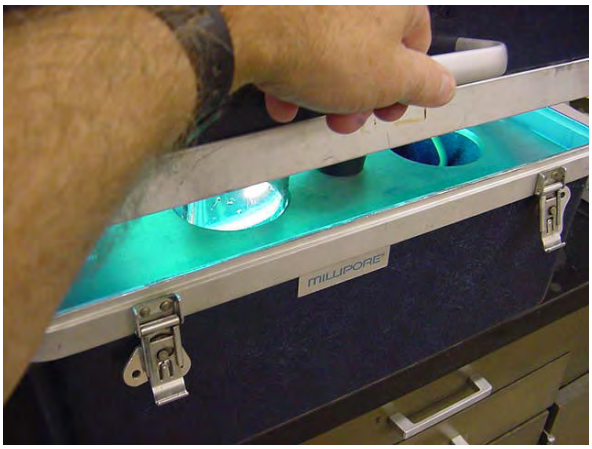


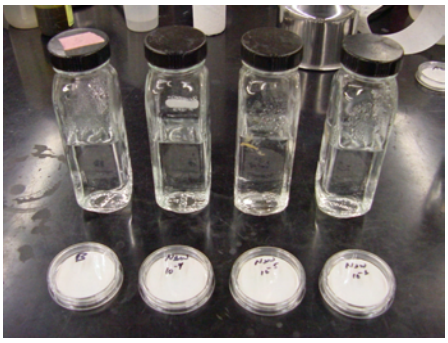

Figure: Sterilize for 2-3 minutes in UV sterilizer.

3. Obtain the number of Petri dishes needed and label. The first dish should be labeled pre-blank and the last dish should be labeled after blank or end control. The pre-blank will be 100 ml of sterile phosphate dilution water. The pre-blank is a sterility control and acts to document that all the equipment is sterile and the technician's technique is aseptic. The after blank is also 100 ml of sterile phosphate dilution water. The after blank helps confirm that filter rinsing has been adequate. Both blanks should show no growth. Any growth on either blank invalidates the test.

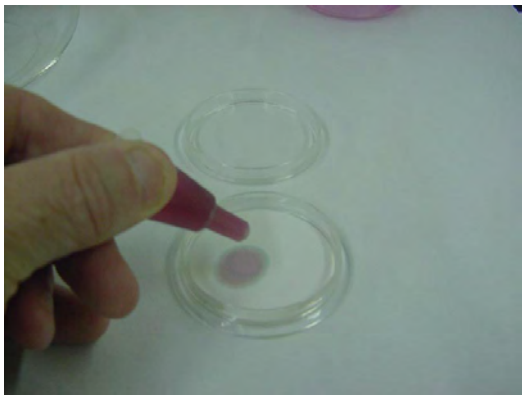
   The remainder of the samples and dilutions are run between the blanks. A test run is defined as an uninterrupted series of analyses.

   Prepare sample serial dilutions if necessary. If using less than 10 ml of sample, add the volume to sterile phosphate dilution water. This will aid in the uniform distribution of bacteria over the entire filter surface.

   For example, the Petri dishes could be labeled.
   1. Pre-blank
   2. 3 ml sample (in sterile phosphate dilution water)
   3. 10 ml sample
   4. 25 ml sample
   5. 50 ml sample
   6. 100 ml sample
   7. After blank



4. Aseptically add 2 ml of m-FC broth to each plate. Pour the broth directly onto the pad without touching the pad. Excessive media should be poured off into the sink. Re-cover each dish.



5. Light a Bunsen burner. Use flat, blunt, non-corrugated forceps. Sterilize the forceps by dipping in 95% ethyl alcohol and igniting in the Bunsen burner flame.

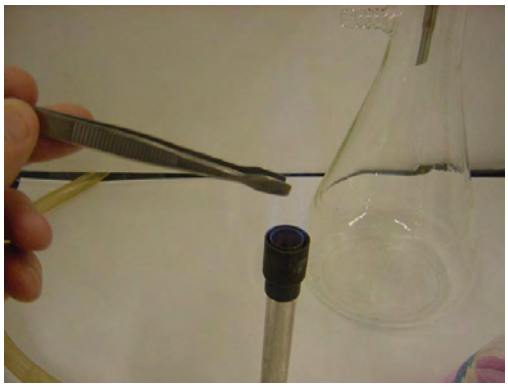


Figure: Do not overheat the tip of the forceps. The membrane will stick to hot forceps and tear.

**Safety:** Hold the tips of the forceps down to avoid burning your fingers with burning alcohol. Do not shake flaming alcohol off the forceps, let it burn off.

6. Aseptically remove a membrane filter and place it grid side up on the filter funnel support.

Figure: Carefully peel back the edges of the membrane filter. Sterile forceps can be used to help separate the filter from its blue backing. The blue backing is discarded.

It is best to hold the upper portion of the funnel in one hand while doing this in order to avoid contamination of the funnel.

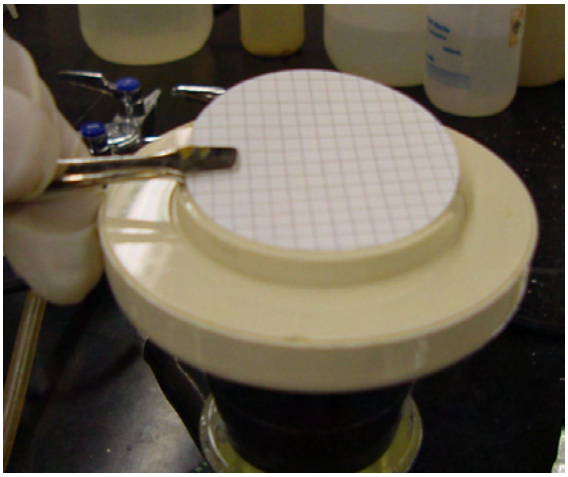


Figure: Place the filter grid side up. Discard if the filter chips, cracks or tears.

Replace the upper portion of the funnel making sure the membrane filter is still centered on the funnel support.

7. Shake a bottle of sterile phosphate dilution water vigorously (the pre-blank). Remove the cap and pour the dilution water into the filter funnel.
8. Turn the vacuum on. After the dilution water has passed <u>completely</u> through the filter, rinse the funnel 2 times with 20 - 30 ml of sterile phosphate rinse water to assure the sample has been rinsed from the funnel.

   Stop between <u>each</u> rinse to allow organisms to get caught on the filter. A rinse bottle can be used. Start rinsing at the top of the funnel and rinse down the sides. Repeat after all the rinse water has been filtered.

9. Turn the vacuum off. <u>TIP</u>: Carefully break the residual vacuum in the flask to prevent tearing the filter during removal.
10. Re-sterilize the forceps by dipping in alcohol and igniting in a Bunsen burner flame.
11. Remove the membrane filter using sterile forceps. Remove the petri dish cover and transfer the filter, grid side up, into the prepared petri dish by gently allowing it to role over the edge onto the m-FC saturated pad.

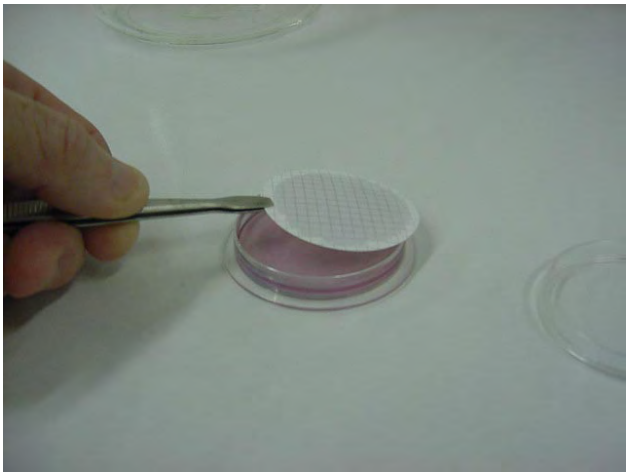


Figure: Set the filter on the back edge and pull forward until the membrane falls onto the absorbent pad.

**TIP:** Light patches on the filter indicate air is trapped beneath the filter. Using sterile forceps, gently lift the membrane filter edge and replace it.

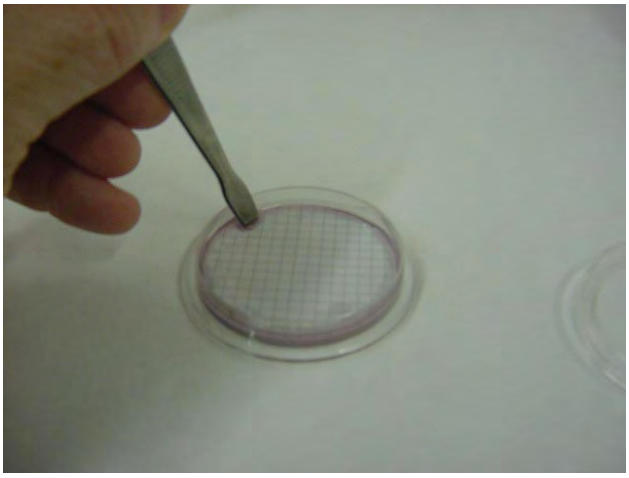


Figure: Gently secure the filter edges to the absorbent pad by running forceps around the perimeter of the Petri dish. Do not touch the area of the filter that contains bacteria.

12. Replace the petri dish cover, making sure it fits snuggly. Label the dish as blank.
13. Repeat the procedure for the remaining samples and after blank. If highly contaminated samples are run, decontaminate the filter funnel after each sample by exposing to UV light again. Alternatively, run an additional sterile phosphate dilution water blank after the filter has been removed. This will prevent carryover between samples.
14. Place the inverted dishes in a Whirlpack bag. All filtered culture plates should be in the waterbath within 30 minutes after filtration.
15. Immerse in the waterbath at 44.5 $\pm$ 0.2 $^{o}$C for 24 $\pm$ 2 hours.

Figure: Squeeze out the air from the bag, roll the bag tightly, and then fold over the edges to seal the bag.

## Colony Counting

After 24 $\pm$ 2 hours, remove the Whirlpack bag from the waterbath. Count all the colonies on each plate with various shades of blue.

The pre-blank must have no growth, indicating reagents, equipment and technique are acceptable. Data is rejected if growth occurs on the pre-blank.

Corrective Action: If growth occurs on the pre-blank, first examine aseptic technique then equipment, media sterility.

The after-blank must also have no growth. Growth on the after-blank usually indicates rinsing technique is poor. Data is rejected if growth occurs on the after blank.

Corrective Action: If growth occurs on the after-blank, examine rinse technique, increase the number or volume of rinses.

The technician should make enough dilutions to have a least 1 plate with a countable number of 20-60 blue colonies. Plates are <u>not</u> rejected if there is <u>not</u> a countable number.

## Reporting

Confluent Growth is defined as a plate having growth covering the entire plate with no distinct colonies.

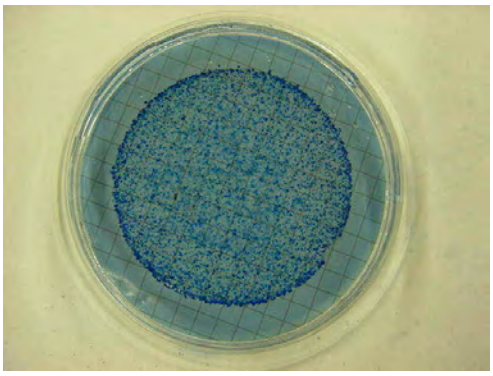


Figure: Confluent growth, distinct colonies are not visible.

Corrective Action: Confluent growth cannot be used for DMR purposes. A resample must be requested.

TNTC is defined as Too Numerous to Count. Plates having excessive growth on the entire plate with distinct colonies are too numerous to count accurately. Plates with greater than 200 colonies are considered TNTC.

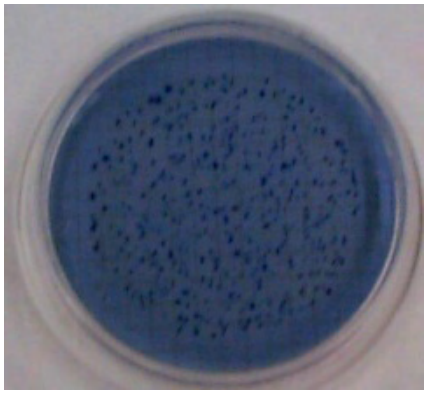


Figure: TNTC, too numerous to count. Individual colonies are visible but accurate counting is unlikely.

Corrective Action: TNTC growth cannot be used for DMR purposes. A resample must be requested and different dilutions selected.

A countable plate is defined as having between 20-60 colonies on the entire plate. These plates are acceptable.

Plates having no colonies are reported as <1 cfu/100 ml.

If no plate has between 20-60 colonies, all the counts are added from the other plates and divided by the total volume of **sample** filtered.

## Fecal Coliform Calculations

$$\text{Fecal coliform} = \text{coliform} \frac{\text{colonies counted} \times 100}{\text{ml sample filtered}}$$

| Example 1: | ml sample filtered | Colonies |
|---|---|---|
| Pre-Blank | | <1 |
| Effluent undiluted | 100 | 94 |
| Effluent 1:10 dil. | 10 | 33 |
| Effluent 1:100 dil. | 1 | 1 |
| After blank | | <1 |

First, examine the blanks. If either blank shows growth, all sample data is invalid and must be rejected. The analysis must be repeated.
If the blanks show no growth, identify the plate(s) with a countable number. In this case, the plate with 33 is the only plate with a countable number. Identify the volume of sample which contained the 33 colonies (10 ml)

Calculate: $\frac{33 \text{ cfu} \times 100}{10 \text{ ml}} = 330 \text{ cfu/100 ml}$

| Example 2: | ml sample filtered | Colonies |
|---|---|---|
| Pre-Blank | | <1 |
| Effluent | 5 ml | 6 |
| Effluent | 15 ml | 28 |
| Effluent | 25 ml | 45 |
| Effluent | 50 ml | 87 |
| Effluent | 100 ml | TNTC |
| After blank | | <1 |

In this example, there are 2 countable plates (28 and 45).

Calculate: $\frac{(28 + 45) \text{ cfu} \times 100}{(15 + 25) \text{ ml}}$ = 182 cfu/100 ml

| Example 3 | ml sample filtered | Colonies |
|---|---|---|
| Pre-Blank | | <1 |
| Effluent 1:10000 | 0.01 ml | <1 |
| Effluent 1:1000 | 0.1 ml | 15 |
| Effluent 1:100 | 1 ml | 95 |
| Effluent 1:10 | 10 ml | TNTC |
| Effluent | 100 ml | TNTC |
| After blank | | <1 |

Since there is no countable plate, total all the coliform counts on **all** filters and report as cfu/100 ml.

Calculate: $\frac{(95+15+ 0) \text{ cfu} \times 100}{(1 + 0.1 + 0.01) \text{ ml}}$ = 9909 cfu/100 ml

Report to 2 significant figures, i.e. 9900 cfu/100 ml

| Example 4: | ml sample filtered | Colonies |
|---|---|---|
| Pre-Blank | | <1 |
| Effluent | 5 ml | <1 |
| Effluent | 15 ml | <1 |
| Effluent | 25 ml | <1 |
| Effluent | 50 ml | <1 |
| Effluent | 100 ml | <1 |
| After blank | | <1 |

There are no countable plates, so report the answer as <1 cfu/100 ml. Report as 1 cfu/100 ml on the DMR.

| Example 5: | ml sample filtered | Colonies |
|---|---|---|
| Pre-Blank | | <1 |
| Effluent 1:10 dil | 10 ml | <1 |
| Effluent 1:100 dil. | 1 ml | <1 |
| Effluent 1:1000 dil. | 0.1 ml | <1 |
| After blank | | <1 |

In this example, there are also no countable plates. Because 100 ml of sample was not analyzed, the result is reported as <10 cfu/100 ml

Calculate: $\frac{(<1) \text{ cfu} \times 100}{10 \text{ ml}}$ = <10 cfu/100 ml

## Quality Control

Fecal coliforms are not predominant in most environments but are certainly predominant in a wastewater treatment plant. Poor lab hygiene can result in erratic laboratory results. Disinfecting lab benches frequently and maintaining general lab cleanliness will help reduce contamination and provide a safe working environment. Be sure to autoclave contaminated materials before disposal in the trash.

In spite of the simplicity of the method, the quality control required for this test is enormous because of the sterility requirements.

**Sample QC**

 Sample bottles. Sterile sample bottles containing 0.1 ml of sodium thiosulfate should be available. Each bottle must be able to contain a minimum of 120 ml and be autoclavable. Sample bottles are sterilized and checked for sterility using tryptic soy broth.

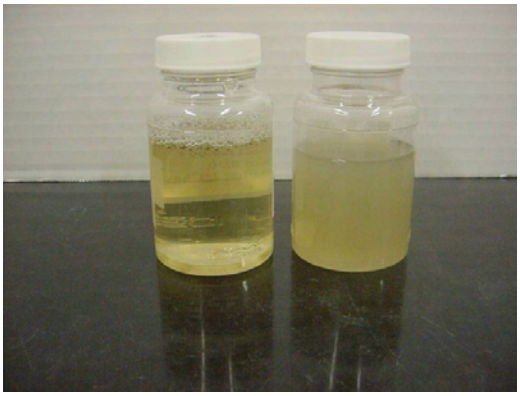


Figure: Growth in TSB indicates a contaminated sample bottle.

If using commercially available sterile bottles, confirm sterility using tryptic soy broth.

QC- record date/time sterilized

QC- record sterility after adding 50 ml of TSB and incubating for 24 hours at 35.0°C

QC- record lot number, sterility, date of purchase, and expiration date for commercially purchased bottles.

Corrective action- If sample bottle shows growth, repeat. If still positive, reject the entire batch and re-sterilize.

 Sample holding time exceeds 6 hours.

QC- Record

- date and time of sample collection
- sample type (grab)
- sampler
- date and time of arrival
- date and time of analysis
- analyst

Corrective Action: Reject samples and request a resample if:

1. holding time exceeded
2. disinfectant present in sample (odor)
3. non-sterile container was used
4. insufficient sample volume

 Samples that will take longer than an hour to analyze must be preserved on ice during transport and refrigerated until time of analysis

QC- record preservation, refrigeration temperature.

Corrective Action: Adjust refrigerator to below 6°C. Service the refrigerator if the temperature does not adjust properly. Reject any sample that shows evidence of freezing. (ice)

 Samples should be run in duplicate 10% of the time.

 Samples must be shaken vigorously to reduce bacterial clumping.

 Sample dilutions are run to obtain a countable plate of 20-60 colonies per plate.

**Equipment QC**

 pH meter calibrated. The pH meter is used to measure the pH of the various bacterial broths.

QC- record buffer lot numbers, purchase date, expiration date, slope

Corrective action- discard expired buffers, recalibrate if pH meter slope is below 95% or above 105% efficiency.

 Top loading balance calibrated. The top loading balance is used to weigh bacterial media and potentially sample volumes. 100 ml of sample can be weighed if a sterile graduated cylinder is not available. Bacterial media is very light and easily spilled. Be sure to clean the balance pan after each use to prevent cross-contamination.

QC- record monthly calibration and sensitivity of 0.1 gm

QC- record annual calibration by service contractor.

Corrective action: service as needed.

 All thermometers calibrated

QC- calibrate annually, record

- serial number of thermometer
- serial number of NIST thermometer
- temperature of lab thermometer
- temperature of NIST thermometer
- temperature correction factor
- date of check
- analyst

Corrective action- discard thermometer if correction factor >1.0°C. Dispose of mercury thermometers properly.

 Waterbath must be 44.5°C ± 0.2°C. The waterbath is used to provide a tighter temperature control with only minor variations.

QC - record twice per day at least 4 hours

QC- record date, time, temperature, and analyst

Corrective action- Waterbath outside the control limits must be adjusted prior to use. Service the waterbath if temperature cannot be adjusted or varies excessively. Be sure the thermometer is immersed to the correct depth in the waterbath to prevent inaccurate reading.

 Autoclave. The autoclave is usually used to sterilize bacterial media, phosphate dilution water, samples bottles, and contaminated materials prior to cleaning or disposal. Be sure materials placed in the autoclave can withstand the temperature. Use autoclavable plastics such as polypropylene.

Safety: Contaminated materials may contain billions of bacteria and a slight cut or poor hygiene when cleaning can have potentially fatal consequences.

QC - Record for each autoclave run

- date/time sterilized
- contents in autoclave
- sterilization temperature
- sterilization time in/out
- spore strip/integrator results
  - -lot number
  - -expiration date
- analyst

Corrective action- discard any prepared media if the sterilization time exceeds 15 minutes or the complete sterilization cycle exceeds 45 minutes. Excessive heating will denature the carbohydrates in the media. Use spore strips or integrator strips for each sterilization cycle to document effective sterilization.

Service the autoclave if spore strip results indicate inadequate sterilization. Re-sterilize contaminated materials if sterilization time is less than 30 minutes. Contaminated materials that have been sterilized (gloves, Petri dishes, etc.) can be disposed of in the general trash.

 Sterilizing oven. The sterilizing oven is usually used to sterilize glassware (pipets and graduated cylinders) and wooden applicator sticks.

QC - Record for each run

- date/time sterilized
- contents in sterilizing oven
- sterilization temperature 170 $\pm$ 10$^{o}$C
- sterilization time in/out (2 hours)
- spore strip/integrator results
  - -lot number
  - -expiration date
- analyst

Corrective action- If spore strip results are invalid, repeat the sterilization process after adjusting the temperature.

 Ultraviolet Sterilizer. The UV sterilizer is primarily used to sterilize the membrane filtration funnel. The UV lamps have a limited shelf life and must be replaced periodically. They should be tested quarterly with a UV light meter or agar spread plate. The lamp should be replaced when the UV intensity is less than 70% of its initial output or if an agar spread plate containing 200-300 colonies, exposed to the UV light for 2 minutes, does not show a count reduction of 99%.

Figure: Pre-UV heterotrophic plate count

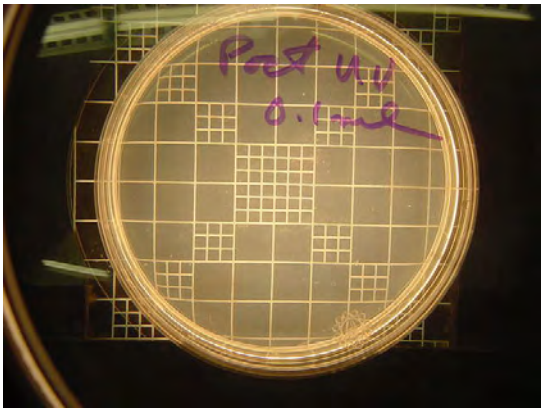
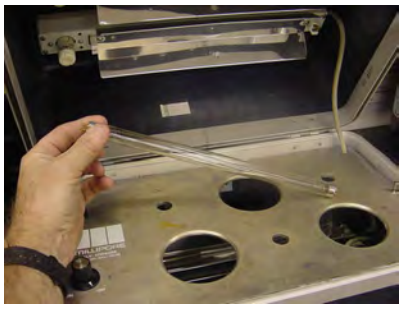


Figure: Post-UV heterotrophic plate count

The UV lamps should be cleaned monthly using ethyl or isopropyl alcohol. Do not touch the glass surface with fingers.



Corrective action- If the lamps do not show 99% kill, replace lamps. Lamps contain mercury, discard safely.

 Sterile phosphate dilution water. The phosphate dilution water used for dilutions, blanks, controls, and rinsing must be sterilized.

Commercially purchased dilution water must also be checked for sterility.
For dilution water bottles,
QC- Record for each batch

- pH after sterilization (7.2 $\pm$ 0.5)
- Volume of dilution water (99 $\pm$ 2 ml)
- Sterility

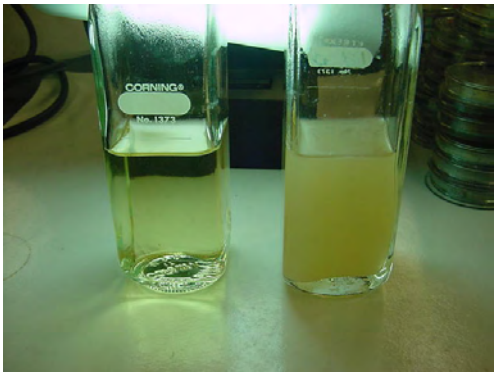


Figure: Phosphate dilution water sterility check.
TSB is added and checked for growth after 24 hours. Left bottle is sterile. Right bottle shows growth, indicating batch should be rejected.

Corrective action- If the pH, volume, or sterility is incorrect, reject the batch. If sterilizing large loads of dilution water in the autoclave, a longer sterilization time may be needed.

 Petri dishes, absorbent pads, membrane filters. Each lot purchased should be verified for sterility. Purchase a 6 month supply and rotate stock.
QC- Record for each batch of Petri dishes and absorbent pads.

- Lot number
- Sterility
- Expiration date

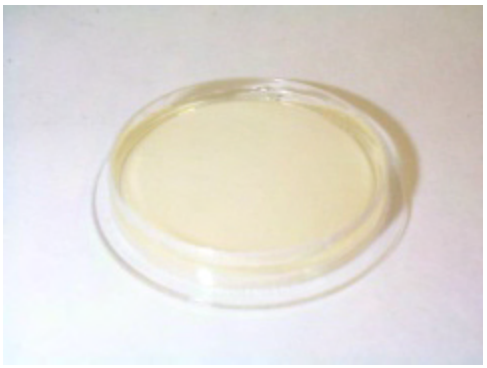


Figure: Sterility is checked by adding 5 ml TSB, incubating and checking for growth.

Corrective action- repeat with a second test. If positive, check with manufacturer.

QC- Record for each batch of m-FC broth

- Date received
- Lot number
- pH
- Expiration date
- Sterility
- Growth characteristics

Each lot of m-FC broth should be shown to be able to differentiate between fecal coliforms and total coliforms. The new lot of m-FC broth should show a comparable recovery to the current lot. Perform a parallel study to document recovery.

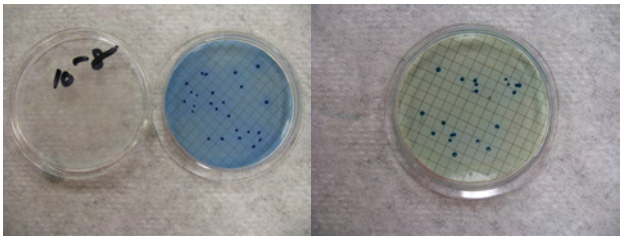



Figure: old broth, new broth at same dilution

 Dehydrated culture media

Dehydrated culture media such as tryptic soy broth should purchased in 1/4 pound bottles. Store is a cool, dry place away from sunlight. The desiccator is a good place to store opened bottles. Opening the factory seal only slightly will allow the seal to be reused and help prevent moisture absorption. Store opened bottles upside down to minimize water absorption. Use opened bottles of media within 6 months, discard media that is caked or discolored.

QC Record for each media

- Date received
- Date opened
- Date expiration
- Lot number

 Filtration apparatus.

The filtration apparatus, even if pre-marked by the manufacturer must be calibrated. Place the blue backing paper from a membrane filter on the apparatus. Measure 97.5 ml of water and pour into the filtration funnel. Mark the lower control value on the side of the funnel. Pipet 5 ml into the funnel giving a total volume of 102.5 ml. Mark the upper control value on the funnel. Recheck annually.

 Reagent Grade water.

The water used to prepare media, blanks, controls, and rinse filters must be of the highest quality and be shown to not contain toxic materials. The reagent grade water must be checked monthly for

QC - conductivity,
heterotrophic plate count
chlorine residual.

QC - It must also be checked annually for heavy metals - chromium, cadmium, copper, zinc, lead, and nickel. Heavy metals may inhibit bacterial growth.

 Inhibitory residue test

The inhibitory residue test is a test that shows the detergent used to wash glassware does not effect the growth of bacteria. Some laboratory

detergents contain bacteriostatic substances which are difficult to rinse out. This test should be performed annually or prior to using a new supply of detergent. The test involves performing heterotrophic plate counts on 3 sets of dishes. The first set follows the normal laboratory glassware washing procedure. The second set follows the normal procedure with additional rinsing to remove residual detergent. The third set is washed but not rinsed. The plate counts are run and the result compared. If an inhibitory effect cannot be eliminated by routine washing, a different detergent should be used.

Detergent residue on glassware can be spot checked using 0.04% bromthymol blue and checking the color reaction. The color should be blue-green. Purple usually indicates detergent has not been rinsed completely, yellow indicates acid has not been rinsed completely.

**Fecal Coliform Test QC**

 Pre-blank sterility control is run for each sample series. A sterility control should be run between each different sample set (influent, effluent) to reduce chances of cross-contamination.

Corrective Action: Any growth on the pre-blank indicates contamination and invalidates the test. There are a number of variables that can contribute to contamination of the pre-blank. Evaluate each source of error.

- Petri dish
- Absorbent pad
- Membrane filter
- UV light
- Phosphate dilution water
- Phosphate rinse water
- Technician technique

 After-blank rinse control run after each sample series.

Corrective action: Any growth on the after-blank also indicates contamination and invalidates the test. If the pre-blank was sterile, the equipment was not a problem. The most likely source of error is carry-over of a contaminated sample due to poor rinsing technique. Re-evaluate the rinsing procedure.

 Petri plates placed in the waterbath within 30 minutes of filtration.

 Negative Controls

Negative controls should be run for each new lot of m-FC broth. A negative control should show no growth of blue colonies. The negative control shows the media will differentiate between fecal and total coliforms. Enterobacter aerogenes is a total coliform and will not produce blue colored colonies on m-FC broth.

Corrective action: If blue colonies are present, either contamination of the Enterobacter aerogenes has occurred or the m-FC broth is defective. Repeat with a second culture of Enterobacter. If still positive, check with the media manufacturer.

 Positive Controls

A positive control should show growth of blue colonies. E. Coli is a fecal coliform and will produce blue colonies. Failure to produce blue colonies may indicate the E. Coli culture is bad or that toxic materials are present which inhibit growth.

Corrective action: If no blue colonies are present, repeat with a second culture of E. Coli. or use raw wastewater. If still negative, check with the media manufacturer.

## MPN (Most Probable Number)

The MPN method (also called Multiple Tube Fermentation Technique) for fecal coliform detection is often used when the sample has excessive turbidity. Excessive turbidity in the

sample will plug the membrane filter, causing poor bacteria recovery and slow filtration times.

The MPN method involves adding the wastewater sample to a series of 5 sets of tubes, each of which contains either lactose broth or lauryl tryptose broth and an inverted tube. The tubes are then incubated at 35 $\pm$ 0.5$^{o}$C for 24 to 48 hours. Each tube is then observed for growth and gas production. If growth and gas production is observed in an inverted tube, the result is positive and the bacteria are **presumed** to be coliform bacteria. A sterile applicator stick or inoculating needle is used to transfer a small portion from the positive tube to a second tube containing EC broth. Each EC tube is incubated in a water bath at 44.5 $\pm$ 0.2C for 24 hours. Tubes which again show growth and gas are now **confirmed** as fecal coliforms. The coliform density is then calculated from statistical probability formulas that predict the most probable number, MPN, of coliforms necessary to produce certain combinations of gas-positive and gas-negative tube in the series of inoculated tubes.

## E. Coli

E. Coli are a more specific indicator of human pollution and possible pathogen presence. Newer discharge permits are requiring measurement of E. Coli rather than fecal coliform. Methods for E. Coli in wastewater are not yet approved and will be added later.

# Chapter 16
# Advanced Instrumentation

Advanced instrumentation is used primarily for analysis of trace contaminants such as metals or hydrocarbons. Most smaller municipalities don't have the need for this expensive equipment unless there is an industrial component to the waste stream. It is usually more cost effective for municipalities to send the occasional sludge sample or industrial pretreatment sample to larger labs that are accustomed to handling trace analysis.

## Units

Up to now, the unit of measure has been primarily mg/L or ppm. When measuring trace contaminants, the concentration in mg/L is too low, for example 0.015 mg/L. To avoid reporting such small numbers, the units are often changed to micrograms/liter (ug/L) or parts per billion (ppb). There are 1000 micrograms in 1 milligram. For example, 0.015 mg/l would be converted to 15 ug/L. For extremely low levels of contaminants, the concentration is reported as nanograms/liter (ng/L) or parts per trillion (ppt). There is a 1,000,000 nanograms in 1 milligram.

## Metals

Metals are analyzed by a variety of instruments. The 3 most common instruments used are:

- Atomic absorption Spectrophotometry (AA)
- Flame photometry
- Inductively Coupled Plasma (ICP)

Metals follow the same definitions as solids.

a. Total metals are defined as the concentration of the metal in an unfiltered sample after vigorous digestion.

b. Dissolved metals are defined as the metals in an unacidified sample that pass through a 0.45u filter.

c. Suspended metals are defined as the metals in an unacidified sample that are retained on a 0.45u filter.

**Sample Collection and Preservation**

The laboratory technician must know what type of metal sample is going to be analyzed prior to collection of the sample. If dissolved metals are needed, the sample should not be preserved with acid as the acid will cause particulate metals to dissolve. Because of the low concentrations of metals being measured, every effort must be made to minimize contamination. Significant sample contamination can come from

- Sample containers
- Sample caps
- Sample collection devices
- Preservative acid
- Filters and filtration equipment

All sample collection materials must be acid washed. A typical cleaning procedure could be:

1. clean with a non-ionic detergent
2. rinse with tap water
3. soak in 1+1 $HNO_3$ or 1+1 HCl for 24 hours
4. rinse with metal free deionized water

The best sample containers, least likely to contain leachable metals, are quartz and Teflon. These containers are expensive and used primarily for critical analysis. Routine samples are usually collected in dedicated polypropylene (PP) or linear polyethylene (LPE).

Preservation for total metals consists of adding concentrated nitric acid to pH <2. If

dissolved metals are to be measured, filter the sample prior to adding nitric acid.

**Preliminary Treatment**
The amount of pretreatment needed will depend upon the type of metals tested and the method used. Consideration must be given not to introduce contaminants into the sample during any pretreatment steps. Because of the trace levels being measured, contamination from reagents, laboratory water, laboratory equipment, and laboratory air can introduce significant error. Blanks should be run to determine background contamination levels. Pretreatment includes:

- Filtration
- Digestion
- Extraction

**Filtration**
Filtration using a preconditioned 0.45u filter is used to separate dissolved and suspended metals. Acidify the filtrate after filtration to preserve the sample.

**Digestion**
Digestion is used to prepare samples for total metals. Organically and inorganically bound metal complexes need to be broken down to release the free metals. There are a variety of different digestion methods. The most common method is nitric acid digestion. The procedure involves measuring 100 ml of well-mixed, preserved sample into a beaker. Five milliliters of nitric acid is added, the beaker covered with a watch glass, then heated to a gentle boil in the fume hood. Evaporate the sample until a precipitate forms or a light-colored clear solution forms. Do not heat to dryness. Wash down the beaker walls with metal free water to recover any metals splashed on the side, filter if necessary, then transfer to a 100 ml volumetric flask. Rinse the beaker with additional metal-free water and add to the volumetric flask. Fill to the mark.

**Microwave Digestion**
The microwave digestion method is recommended for metals analysis using ICP. The microwave digestion method is a closed digestion method,

**Extraction**
Extraction is a process used to concentrate trace levels of materials, in this case metals. Some metals are able to be complexed with an organic chelating agent. The organic chelating agent, most commonly, Ammonium pyrrolidine dithiocarbamate (APDC), chemically binds the metals in the water. APDC is easily dissolved in the non-polar organic solvent, methyl isobutyl ketone (MIBK). Because MIBK is non-polar, it will not dissolve in water and will separate from the water like oil and water. Only small volumes of MIBK are used, so the extracted metals that were in perhaps 200 ml of sample are now concentrated in 10 ml of MIBK. This results in a concentration of metals 20 times. The MIBK is aspirated into the atomic absorption spectrophotometer and the absorbance measured.

## Atomic Absorption Spectrophotometry

Atomic absorption requires the presence of ground state metal atoms ($Me^o$). The initial process which takes place in an atomic absorption spectrophotometer is to create a population of ground state atoms. This is accomplished in a variety of ways, usually classified as flame and non-flame. The simplest and most common method uses a flame to produce these atoms. This atomization process involves 5 steps:
1. nebulization
2. droplet precipitation
3. mixing

4. desolvation
5. compound decomposition

The atomization process begins when a sample that has been pretreated is "sucked" into the machine by an oxidizing gas (air). The liquid sample is converted in a spray of fine mist using a nebulizer. Different manufacturers have different methods for this atomization process. The nebuliziation process breaks the large droplets of sample into a fine mist. The droplets of water that don't shatter sufficiently precipitate out and go to drain. The smaller "mist" droplets then mix with the fuel in a spray chamber. Changes in direction of flow within the spray chamber, create turbulence which ensures a smooth quiet, well-mixing flow into the burner head. The heat of the flame within the burner now desolvates (dries) the remaining water from the sample particles and decomposes the particles into their constituent ground state atoms. Thus somewhere within the flame is a pocket of ground state atoms which can now be measured. The effectiveness of each step influences the number of ground state atoms produced.

**Nebulizers**
Each manufacturer has their own nebulizer design. The design influences the effectiveness of the spray reaching the flame. The most effective nebulizer will create a mist of small drops of uniform particle size. The smaller and more uniform the droplets, the steadier the absorption result will be. Large droplets will be difficult to desolvate and will produce chemical interferences with light passing though the flame.

**Flames**
Different gas mixtures produce different flame temperatures. The most common flame is the air-acetylene flame. The air-acetylene flame produces a flame temperature hot enough to desolvate and decompose most particles into ground state atoms. Some compounds do not decompose as easily or as readily as others, therefore a higher temperature flames (nitrous oxide-acetylene) may be needed to decompose the compound. Likewise, the flame fuel to oxidant ratio can be adjusted. If more fuel is added and less oxidant (air), the flame is called a reducing flame. If more oxidant is added and less fuel is used, the flame is called an oxidizing flame. The procedure usually indicates what the best fuel-oxidant ratio should be, but changing the mixture can affect the number of ground state atoms produced.

**Light absorption**
Once the ground state atoms ($Me^o$) have been produced in the flame, they can be energized by a specific wavelength of light. Each different ground state atom has a number of very specific wavelengths which can react with the ground state electrons and send them to a different electronic states. For example, a ground state sodium atom has one electron in its outer ground state orbital (3s). That ground state electron can be excited by light energy at a wavelength of 589.0 nm. This wavelength is called its resonance line. Only sodium will be able absorb light at 589.0 nm. The resulting electron is now "excited" and is in a different orbital (say 3p). Other atoms work the same way, thus by choosing specific resonance lines different metals can be measured, ie $Co^o$ at 324.7 nm, $Ni^o$ at 232.0 nm, and $Fe^o$ at 248.3 nm. Every atom will have a series of spectral lines specific for its excitation patterns.

**Hollow Cathode Lamps**
Hollow cathode lamps are "lights" that are composed of the element being measured. These lamps emit only the specific

resonance wavelengths of interest. The lamp is energized and the light beam is directed through the flame. The lamp and burner are adjusted up/down, left/right, until the ground state atom cloud is maximized. The instrument now follows the Beer-Lambert Law as before. Blanks, reagent blanks, calibration curves can be run as before.

**Monochromator**

The light from the hollow cathode lamp passes through the sample and the optical system focuses the light on the entrance slit to the grating monochromator. The monochromator is used to select only the specific resonance line emitted by the hollow cathode lamp. For instance, copper may have 10 resonance lines, but only the one at 324.7 nm is desired. The monochromator directs the incoming light through the exit slit, minimizing stray light. The light now passes through to the detector which converts the light energy into electrical energy. The electrical energy is now converted by the detector system into an answer on the display.

Because the number of ground state atoms energized is very low in direct flame AA, the sensitivity of the flame AA methods is not very high. Newer methods increase the ground state atom density which improves the sensitivity and detection limits significantly. The most common are

1. Electrochemical AA, also called graphite furnace
2. Hydride generation
3. Cold Vapor Mercury

All of these methods work off the same atomic absorption theory using the same instrument. However, the method of generating the atoms differs. The graphite furnace uses high electrical current to heat the sample and create ground state atoms. The sample is usually placed in a carbon cup or tube, taken through a drying, ashing, and atomizing stage, then measured using light absorbance as before. No flame or burner is used.

The Hydride method uses chemical reactions with sodium borohydride to create large clouds of gaseous hydrides. The gaseous hydride, representing a large amount of sample, can then be swept into the flame very quickly rather than slow aspiration through the nebulizer. This increases the number of ground state atoms and improves sensitivity and detection limit.

The Cold Vapor Mercury method again used chemical reactions to create atomic mercury vapor. Because the mercury is already in its ground state, the mercury vapor is passed through a tube aligned in the light path. No flame is needed. Because the mercury is concentrated within the tube, the sensitivity and detection limit are also improved.

All methods are subject to the same alignment problems

**Flame Emission Photometry**

Flame emission photometry works on the same similar principle as atomic absorption spectrophotometery. This method is limited to metals that are easily excited such as sodium, potassium, and lithium. In AA, the amount of light absorbed by the ground state atom being excited to a different energy level was measured.

$$\underset{\text{Ground state}}{Na^{o}} \xrightarrow{\text{Light Absorbed}} \underset{\text{Excited state}}{Na^{*}}$$

In Flame emission photometry, standards and solutions are aspirated into the flame. The hot flame evaporates the solvent, atomizes the metal, and excites a valence electron to an upper state. Light is emitted at

characteristic wavelengths for each metal as the electron returns to the ground state. The light emitted by these excited ground state atoms is measured.

$$Na^{o} \xleftarrow[\text{Light Emission}]{} Na^{*}$$

Optical filters are used to select the emission wavelength monitored for the analyte species. Comparison of emission intensities of unknowns to either that of standard solutions, or to those of an internal standard, allows quantitative analysis of the analyte metal in the sample solution.

Follow the instrument manufacturer's operating instruction.

**Interferences**

The flame emission photometer is susceptible to the same problems as AA. Alignment, sample viscosity, fuel and oxidant flow rates, reagent water purity, and solids all can effect the emission readout.

# Chapter 17 Quality Assurance

Lab management really boils down to quality assurance. Quality assurance is the program/plan designed by the laboratory that specifies the methods and procedures required to produce defensible data and acceptable precision and accuracy. Each person in the laboratory should be familiar with the laboratory's Quality Assurance (QA) plan but it is the lab manager who is primarily assigned the task of facilitating the plan and keeping it up to date.

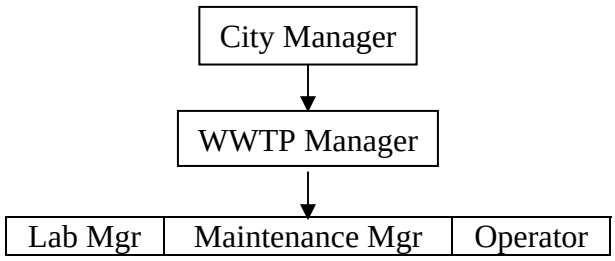
## Laboratory QA Plan

The laboratory QA plan is a written document that includes:

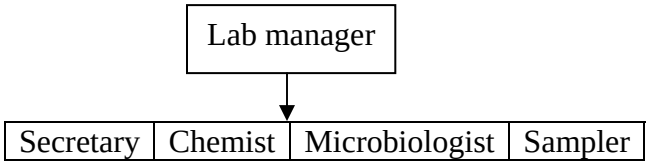
1. Laboratory organizational chart describing the function and responsibilities of each laboratory personnel and identifies the line of responsibility;
2. Field sampling procedures;
3. Laboratory sample handling procedures;
4. Standard Operating Procedures for each analytical method used;
5. Quality control procedures;
6. Data reduction, validation, reporting and verification;
7. Preventative maintenance procedures;
8. Corrective action contingencies; and
9. Recordkeeping procedures.

## Organizational Chart

For most small systems, the organizational chart is pretty simple. The laboratory may be assigned to the WWTP manager.




As the system gets larger, the organizational chart will expand and the lines of communication become less clear.




Each position in the laboratory should have a well defined job description. Each person in the organizational chart should know the job duties of all the other personnel. A clear job description will minimize laboratory communication problems and provide an efficient movement of samples through the laboratory.

## Training Procedures

The lab manager is responsible for assuring the personnel are performing the job duties correctly. This requires training to assure the technician is up to date on regulations and methodology. Cross-training is very valuable in the laboratory. Vacations, and illness can interfere with proper sample processing. The lab manager can set monthly, quarterly, or annual training goals for each employee as well as periodic performance evaluation samples. Each laboratory technician must demonstrate proficiency in the methods they perform.

## Field Sampling Procedures

The lab manager is responsible for the Sampling Plan discussed in chapter 7. The sampling plan is a written description of:

- Sampling procedures;
- Sample locations;
- Safety concerns;
- Preservation;
- Types of containers;
- Cleaning and sterilization procedures;

- Holding times; and
- Shipping and storage procedures.

## Sample Handling Procedures

The lab manager should prepare sample labels or forms that will contain the necessary collection information required by the laboratory. The QA plan should address how samples are checked in when they arrive at the laboratory. Questions that need to be addressed in the QA plan include:

- legibility;
- temperature;
- sample volume;
- type of container;
- preservation;
- holding times; and
- chain of custody for samples likely to be the basis for legal action.

## Laboratory Sample Handling Procedures

Once the laboratory receives the sample, the QA plan should address how samples are processed within the laboratory from storage to analysis to disposal. Questions that need to be addressed in the QA plan include

- Legibility and laboratory notebooks;
- Storage temperatures;
- Isolation from laboratory contaminants;
- Specific sample rejection criteria;
- Holding times;
- Preservation; and
- Chain of custody.

## Standard Operating Procedures

Standard operating procedures describe the analytical procedures used by the laboratory in very specific detail. SOPs should be in sufficient detail that a laboratory technician who is unfamiliar with the method can produce acceptable results. SOPs should describe:

1. Method title and referenced documents;
2. Scope of method and application;
3. Method summary;
4. Materials and equipment needed;
5. Health and safety precautions, including personal protective equipment;
6. Personnel qualifications;
7. Calibration or standardization methods;
8. Sample handling and preservation;
9. Detailed analytical procedure;
10. Calculations and data reduction;
11. Bench sheet and record handling; and
12. Quality control parameters, frequency, corrective actions and troubleshooting.

## Quality Control Procedures

The laboratory QA manual should identify procedures used by the laboratory to demonstrate the laboratory data is valid and defensible. The QA manual will define control samples used and their frequency of use.

### Field Control Blanks

Field control blanks are taken in the field during the sample collection process then taken through the entire analytical process. Field control blanks are usually laboratory pure water that is taken through the entire collection procedure. Field blanks can be used to verify the sampling equipment, the collection environment, the storage, transport, and sample preparation are not introducing errors to actual field samples.

### Field Duplicates

Field duplicates are two separate samples collected at the same time and place under identical circumstances and treated exactly the same through field and laboratory procedures. Results of the duplicates give a measure of the precision associated with the collection and analytical processes.

**Split Samples**
Split samples are portions of the sample taken from the same sample container after thorough mixing or compositing. They are analyzed independently and the results used to document precision.

**Negative Controls**
Method blank (reagent blank) is a solution prepared and diluted with the same reagents and solvents (usually DI water) used in the samples. The method blank can be used to samples calibrate instruments. The method blank is used to provide a zero reading when running a calibration curve on an instrument. A method blank can also indicate if contamination from reagents interferes with the analysis. For instance, the unseeded blanks in the BOD test could be considered a method blank.

Microbiological negative controls are pure solutions of biological cultures prepared from ATCC control cultures which do not produce the desired growth. For example, Enterobacter Aerogenes is a negative control for fecal coliforms.

**Positive Controls**
Blank Spikes are prepared by adding known quantities of a standard to a volume of DI water. The concentration prepared should be typically mid-range for the method used. For example, a 1.0 mg/L spike could be prepared to verify a phosphate curve with a range of 0-3 mg/L.

**Matrix Spikes**
Matrix spikes are prepared by adding known quantities of a standard (blank spike) to a sample prior to analysis. This is also often called standard addition. For instance, a sample is analyzed and found to have a phosphate level of 1.6 mg/L. The 1.0 mgL blank spike could be added, the analysis repeated. The result would be expected to be 2.6 mg/L. The results of the matrix spike is reported as percent recovery. If the percent recovery is outside the established acceptable range, interferences with the method may be present.

Microbiological positive controls are pure solutions of biological cultures prepared from ATCC control cultures which produce the desired growth. For example, Enterobacter Aerogenes is a positive control for total coliforms.

**Calibration Standards**
Primary calibration standards are prepared from NIST traceable stock solutions which can be purchased or prepared in-house from reagent grade chemicals. These standards are used to calibrate instruments, prepare standard curves, and verify any other method of analysis including titration. Calibration standards should be used frequently to verify instrument calibration has been maintained.

**Performance Evaluation Samples**
Performance evaluation samples are samples that are generally purchased as a "blind sample" from an outside source. The sample is analyzed and the results are sent to the supplier for evaluation. These samples should be performed at least annually for each analytical method used.

**Lab Duplicates**
Lab duplicates are two portions taken from the same sample container that are processed and analyzed separately. For instance, running two TSS samples from the same container would be a lab duplicate. Duplicates are generally run on 5-10% of all samples.

Any quality control tests that are suspect, require the laboratory to evaluate the analytical process for errors. The errors

could be from the method used, the instrument used, or personal errors by the analyst. Error analysis will be discussed in another chapter. The QA manual should discuss what corrective actions the laboratory will take to remedy errors.

## Data Reduction, Validation, Reporting and Verification Procedures

Data handling is the process of transforming the raw analytical numbers into reportable numbers. All written records are to be documented in permanent ink. Any errors during the analytical process should be corrected by drawing a single line through the error. The correct value is then entered beside the incorrect entry with the initials and date of the individual making the correction. The technician has the primary responsibility of assuring the data is correct and complete. The technician should verify quality controls are within the control range. If data is outside the acceptable control limits, the technician must either re-analyze the sample or invalidate. If the data is invalidated, it should not be reported. The QA plan should describe how invalidated samples are handled and should describe the procedures that are used to prevent the release of incorrect data. Large labs often have several analysts who are responsible for calculating and verifying data. The lab manager or his designee has the responsibility to review QC and bench sheet data prior to release. Small labs with only one technician do not have luxury so calculation and transcription errors are more likely. The QA plan can identify what corrective actions are to be performed when data cannot be validated. The laboratory manager or designee is responsible for the final verification and distribution of analytical results.

## Preventative Maintenance Procedures

The lab manager is often responsible for setting up a preventative maintenance schedule. Instruments must be properly maintained to ensure they continue to work properly. The QA manual should describe preventative maintenance procedures needed to assure proper operation. Effective preventative maintenance will reduce down-time, poor performance, and "interruption of analysis". For instance, a DO meter that is constantly giving an error message may indicate the membrane needs to be replaced, the battery needs replaced, or the electrode is failing. Consumable materials such as batteries and membranes should be readily available. Replacing the expensive electrode could involve problems associated with budgeting, purchasing, or shipping delays that create a long "out of service" time. An effective lab manager must plan for replacement and repair of lab equipment. Routine maintenance performed by laboratory personnel should be recorded in a logbook for each major piece of equipment. The lab technician should document the problem and the repairs made.

Service contracts on major equipment should be documented.

## Corrective action contingencies

The QA plan should describe how analytical sample results are handled when QC samples are unacceptable. Steps which should be taken in the corrective action process are:

- identify and define the problem
- assign responsibility for investigating the problem
- determine the cause of the problem
- determine actions needed to correct the problem
- implement the corrective action

- establish the corrective action is effective
- management verifies the corrective action effectiveness

## Recordkeeping Procedures

Recordkeeping is often a weakness in laboratory management. The QA manual should describe recordkeeping for all aspects of the laboratory. Recordkeeping not only includes tracking sample and analytical information but also QC information, equipment maintenance, calibrations, reagent purchase/preparation, DMR reports, etc. Recordkeeping should cover the complete process:

Sample collection

↓

Sample analysis

↓

Data Verification

↓

Data Distribution

↓

Data Storage

↓

Data Disposal

After the data has been distributed, it must be stored for some time period. The QA plan should identify where the data will be stored, what form it will be stored in, and how long it will be stored. Most compliance/regulatory data must be stored for a minimum of 5 years and sometimes indefinitely (per state regulation). It can be stored as a hard copy or stored electronically. If it is stored electronically, the QA plan must identify where the copies will be stored, how many copies will be made, how long will the laboratory keep the copies before disposal, how the database be will secured?

The QA manual should address any differences in handling between process control samples, compliance samples and samples headed to court. The data for process control tests may not need to be as tightly documented as a compliance sample used for the DMR. Finally, facilities subject to legal action will need to have all laboratory information defensible. If samples are expected to be part of a legal action, chain of custody procedures will be needed.

# Chapter 18 Quality Control

It's time to go to court. The WWTP has been accused of destroying the downstream ecosystem due to continuous low pH discharge. The only defense is the lab technician data that shows the pH for the past 6 months has been between 7.1 and 7.6. Will the judge believe the lab technician or the downstream user? The judge asks the lab technician for the documentation showing his pH meter was working correctly. "Your Honor, I've been measuring pH for 15 years and I'm sure it's correct!" The judge bellows: "Where's the pH meter calibration data?" The technician responds, "The bugs ate it, your honor!" Guilty! Guilty! Guilty!

In chapter 17, the QA plan identifies what the laboratory was going to do to produce valid and defensible data. Did the lab technician follow the QA plan? No! Did the lab manager check the data? No! The laboratory failed to follow its own QA plan and has no documentation to defend its data. Quality control (QC) is the written documentation demonstrating implementation of the QA plan. The laboratory technician will spend as much as 25-50% of his/her time doing QC work. To decide what information should be recorded, think like a lawyer. The SOP details the analytical procedure, QC answers the question, "Was the action performed?" As an example, let's look at measuring pH. PH is a very quick simple measurement but the QC takes longer to perform than the sample measurement.

For example, the lab technician analyses a sample and reports a pH of 7.5. In order to measure the pH, the technician will have to be able to prove to the judge the meter was calibrated by documenting in writing:

a. What meter was calibrated?
   QC: Make/Model of pH meter
b. Who calibrated the meter?
   QC: Name of analyst
c. When was the meter calibrated?
   QC: Date/Time of standardization
d. What buffer was used?
   QC: pH buffer Lot #
   QC: Date of buffer purchase or preparation
e. Was the buffer valid?
   QC: Date of buffer expiration
f. Did the meter accept the pH values
   QC: pH buffer calibration results
   QC: Measured slope

By writing the information down in a log book or bench sheet, the laboratory will be able to provide defensible data to inspectors and courts.

Laboratories have different methods of documenting quality control but the key is to **WRITE IT DOWN IN INK**. Each SOP should identify QC parameters. Common QC information includes

- Dates/ times of collection, arrival, analysis
- Name/signature of sampler, analyst
- Calibration data
- Standardization data
- Temperatures
- Blanks
- Positive/Negative controls
- Equipment operation/maintenance
- Reagent purchase/expiration dates
- Reagent lot numbers
- Method used
- Performance evaluation sample results

## Error Analysis

Even though all the QC information is recorded, the laboratory must know the result is accurate and precise. Analytical

results are always subject to errors that can affect their validity. Understanding method performance through error analysis and precision, and accuracy will improve measurement quality.

**Systematic Errors**
Systematic errors are errors that are repeated for every measurement that is made. For example, a reagent blank may have a slight background color due to contaminants from the reagents. This color would give a positive bias to any sample containing these reagents. This error would be present in all samples but could be determined and subtracted out from all the sample measurements.

**Random Errors**
Random errors are errors that occur from different sources and add either a positive or negative bias. Random errors can be divided into 2 categories:

1. Determinant errors
2. Indeterminant errors

Determinant errors are errors that can be determined. They can be method errors, instrument errors, or personal errors.

**Method Errors**
Method errors are errors that result because of problems in the method. Methods are developed to determine the answer for some component, for example, phosphate. The method may also cause a reaction between another components, like silica. These competing components may either make the sample answer higher (positive bias) or the answer lower (negative bias). These competing components are called interferences. Interferences create inaccurate results.

**Instrument Errors**
An operating instrument usually produces a signal (background noise) even when no sample is present or when a blank is being analyzed. This electronic noise causes the display to vary. The larger the variance, the more inaccurate the answer. Large variances also affect the detection limit. A common example of electronic noise is the electronic analytic balance or pH meter. The balance has been zeroed but flutters between 0.0001 and -0.0001. This flutter is the result of small electronic fluctuations. If the fluctuations change over time, the equipment may need to be serviced.

**Personal Errors**
Personal errors are attributable to individual mistakes which are consistently made by the analyst. These errors are the result of carelessness, lack of knowledge, or personal bias. These errors are correctable. Improvements in precision and accuracy should be found as errors are eliminated. Examples of personal errors would include

- Using the wrong glassware
- Blowing out volumetric pipets
- Poor pre-washing of TSS filters
- Poor titration endpoint determination
- Failure to calibrate instruments
- Incubators outside the temperature range
- Incorrect calculations

The laboratory manager has the most control over these errors. Prior to assigning samples to a new analyst, the lab manager should document the analyst's capability to perform the method and obtain satisfactory precision and accuracy results.

## Statistical Evaluation

The lab manager should perform precision and accuracy for each method used.

Accuracy is defined as the ability of the analyst to get the correct results.

Accuracy = Truth

This could be considered the bullseye on a target. Hitting the bullseye, means the analyst measured the answer correctly. If the analyst misses the bullseye, the answer was measured incorrectly. The laboratory manager must decide how far from the bullseye will be acceptable before the data can be reported. Accuracy is usually reported as % recovery. As an example, if you go to the bank to cash a $100 check, the bank teller counts back 97 one dollar bills. The bank teller is inaccurate. Is the analyst happy? What if the teller counted out 103 one dollar bills? Is the analyst happy now? How much latitude is allowed? This will be defined as accuracy or percent recovery later.

Precision is the ability of the analyst to get reproducible results. If we again use the bullseye on the target and the technician hits the bullseye he is accurate. The precision question is can he do it again and again. If the analyst consistently hits the bullseye, the analyst is both precise and accurate. (Our bank teller counts out 100 one dollar bills each time)

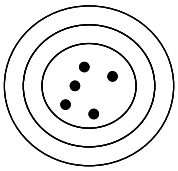


Figure: Precise and accurate

If the technician always hits the top right corner of the target, he is precise but inaccurate. (Our bank teller always counts out 97 dollars)

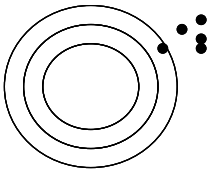


Figure: Precise but inaccurate

If the technician misses equally high, left, right, and low, the technician is accurate but very imprecise. (Our teller counts out 90 dollars one time, recounts 100 the second time, recounts 105 the third time, and finally counts 100 the fourth time. The analyst gives up and assumes 100 is correct (accurate) but the teller is very imprecise.)

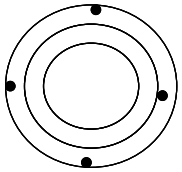


Figure: Imprecise but accurate

Finally, if the technician misses way left, a little right, a little low, and just nicks the target high, he is both imprecise and inaccurate. (Our teller doesn't know what a dollar looks like and needs additional training and he finds another bank.)

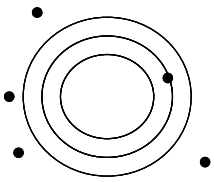


Figure: Imprecise and Inaccurate

Precision is usually reported as standard deviation or relative standard deviation.

## Calculating the Mean

Precision and accuracy data are performed on normal samples. First determine the mean for a sample. To calculate the mean, 10 replicate samples are analyzed. The

average result called the mean is calculated. The mean is the sum of all the sample values divided by the number of samples run.

$$\xi = \frac{\sum X_i}{n}$$

For example: The lab analyst has run 10 TSS tests on a single homogenized influent sample with the following results.

| | |
|---|---|
| 110 mg/L | 100 mg/L |
| 105 mg/L | 90 mg/L |
| 97 mg/L | 104 mg/L |
| 103 mg/L | 96 mg/L |
| 95 mg/L | 100 mg/L |

To calculate the mean, all the values are added then divided by 10.

$$\xi = \frac{1000}{10} = 100 \text{ mg/L}$$

Having tested the sample 10 times, the lab analyst is pretty sure the sample answer is 100 mg/L.

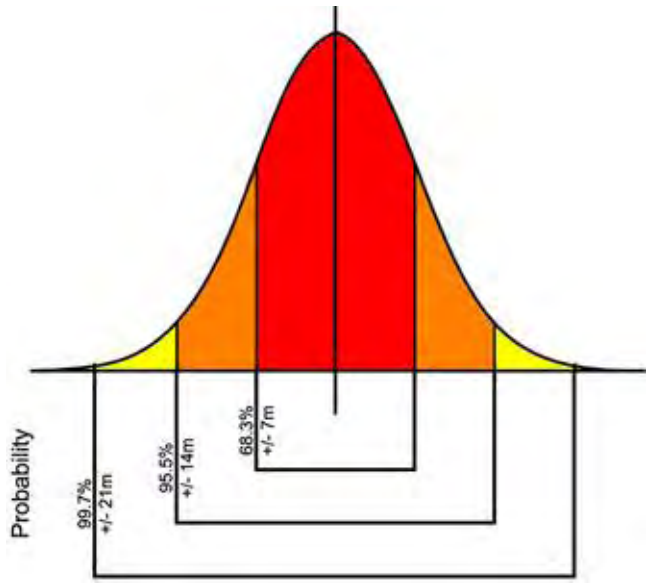
## Calculating Precision

Once the mean has been determined, the analyst should find out how far his data deviates from the mean. The closer the data, the more precise the analyst. The farther the data, the poorer the precision. By calculating precision, the laboratory can check on the analyst's capability and the feasibility of using the method.

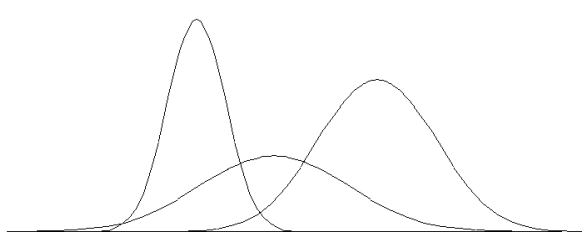
To calculate precision, the standard deviation is calculated from the data used to calculate the mean.

$$S^{n-1} = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n-1}}$$

A scientific calculator with a statistics function can easily perform this calculation. In this example, the standard deviation is ±6 mg/L. Two standard deviations would be ±12 mgL and three standard deviations would be ±18mg/L. The normal distribution of data follows the Gaussian or normal distribution curve.




In a normal distribution of data, most points fall close to the mean. For instance, if the analyst performs 10 replicate titrations, most of the titration volumes should be the same. There will be a few titrations that take a little bit more and few that take a little bit less. These deviations are due to error. The fewer mistakes the analyst makes, the sharper the curve will be. If the analyst makes lots of big mistakes, the curve will be very broad.



The lab manager's goal is to produce as sharp a peak as possible.

A fixed percentage of data points should fall within each standard deviation. Sixty-eight percent of the sample values should fall within 1 standard deviation. In this example, 7 of the 10 values should fall between 94 and 106 mg/L (100 $\pm$ 6 mg/L). Ninety-five percent of the values should fall within 2 standard deviations (100 $\pm$ 12 mg/L) and Ninety-nine percent of the values should fall within 3 standard deviations (100 $\pm$ 18 mg/L).

This data could have been generated for 3 different purposes. First, if this is a new method being used in the laboratory, the standard deviation could be compared with the reference standard deviation expected for the method. If the references coincide, the lab may decide to use the procedure. For example, the TSS procedure might indicate a sample with a concentration of 100 mg/L might have a standard deviation of 5 mg/L. Since the laboratory standard deviation is $\pm$ 6 mg/L, the laboratory may not be performing the method satisfactorily.

Likewise, the laboratory could be using this data to define its capabilities. The precision data is basically telling the lab manager or customer that the lab is 95% confident a sample with a TSS of 100 mg/L will be within 88 - 112 mg/L.

Finally, if the laboratory is using the standard deviation in Standard Methods ($\pm$ 5 mg/L) as its guideline, and the data represents a new analyst's capabilities, the new analyst is not yet ready to perform samples to the laboratories QC levels.

Laboratories use the 95% values to establish control charts.

The process can be repeated at lower levels to establish the standard deviation for low level samples such as effluent.

Example 2: Effluent TSS data

| | |
|---|---|
| 5.4 mg/L | 5.5 mg/L |
| 6.0 mg/L | 6.0 mg/L |
| 5.5 mg/L | 5.8 mg/L |
| 6.0 mg/L | 5.6 mg/L |
| 4.8 mg/L | 5.9 mg/L |

The calculated mean and standard deviation for this effluent data is found to be

$$\xi = \frac{56.5}{10} = 5.6 \text{ mg/L}$$

SD = 0.4 mg/L

The two different standard deviations are difficult to compare numerically but can be compared by percentage. The relative standard deviation (RSD) can be calculated by dividing the standard deviation by the mean and then multiplying the result by 100.

$$\text{RSD} = \frac{\text{SD} \times 100}{\xi}$$

The RSD for the influent data is :

$$\text{RSD (influent)} = \frac{6 \times 100}{100} = 6\%$$

$$\text{RSD (effluent)} = \frac{0.4 \times 100}{5.6} = 7\%$$

Although the SD for the effluent (0.4 mg/L) is lower than the SD for the influent (6.0 mg/L), the RSD is higher. The higher RSD indicates the effluent data is actually less precise than the influent data. Remember precision is the ability to get the same answer, so the higher the RSD, the less precise the answer.

The precision statement should read: In a single laboratory, using wastewater samples at concentrations of 6 and 100 mg/L, the relative standard deviation was between 6% and 7%.

## Accuracy (Percent Recovery)

The laboratory should calculate accuracy to determine whether a method is compatible with a specific sample by using standard additions (spiking). A standard spike is a method used to determine if the sample matrix contains materials that will interfere with the analytical method. If the method either adds or detracts analyte, an accurate answer will not be obtained.

To perform a standard spike, first analyze the unspiked sample to determine the mean. For example, the analyst runs seven replicate total phosphate samples and finds the mean to be 5.0 mg/L.

Next, add a known amount of phosphate standard to a second set of replicates of the same sample and repeat the analysis. Check to see if the additional standard added has been recovered. For instance, the sample above was spiked with 1 ml of a 100 mg/L phosphate standard and analyzed. If the percent recovery of the spiked sample is 90-110%, the method may be deemed acceptable. If the spike result is outside the range, a substance may be present in the sample matrix which interferes with the analytical method.

## Calculating Percent Recovery (Standard Addition)

A lab technician analyzes a 50 ml sample for total phosphate and finds the answer to be 4.0 mg/L. The sample is spiked with 1.0 ml of a 100.0 mg/L phosphate standard. The theoretical concentration of the spiked sample is

$$\text{Theoretical conc} = \frac{(C_u V_u)+(C_s V_s)}{V_u + V_s}$$

Where;
$C_u$ = Original measured concentration of unspiked sample
$V_u$ = Volume of sample to which the spike is added
$C_s$ = Concentration of the standard used to spike
$V_s$ = Volume of standard used to spike

$$\text{Theoretical Conc.} = \frac{(50 \times 4)+(100 \times 1)}{(50 + 1)} = \frac{200 + 100}{51} = 5.9$$

The spiked sample is now analyzed and found to be 5.6 mg/L. The percent recovery is calculated by dividing the original sample answer by the spiked sample answer then multiplying by 100.

$$\text{\% accuracy} = \frac{\text{actual spike}}{\text{theoretical spike}} = \frac{5.6 \times 100}{5.9} = 95\%$$

Spiked samples should be determined at both high and low sample concentrations to show the method is acceptable for the entire method range. A second sample at the high end of the analytical range (15 mg/L) was also spiked with 1 ml of 100 mg/L phosphate standard.

$$\text{Theoretical Conc.} = \frac{(50 \times 15)+(100 \times 1)}{(50 + 1)} = \frac{750 + 100}{51} = 16.7$$

The analyst measures 16.9 mg/L. The second percent recovery is:

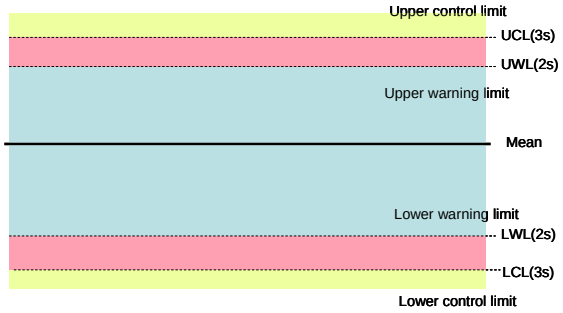
$$\text{\% accuracy} = \frac{\text{actual spike}}{\text{theoretical spike}} = \frac{16.9 \times 100}{16.7} = 101\%$$

Accuracy is reported as:
In a single laboratory, using wastewater at concentrations of 4 mg/L and 15 mg/L, recoveries of 95% and 101% were found. The laboratory manager will have to decide

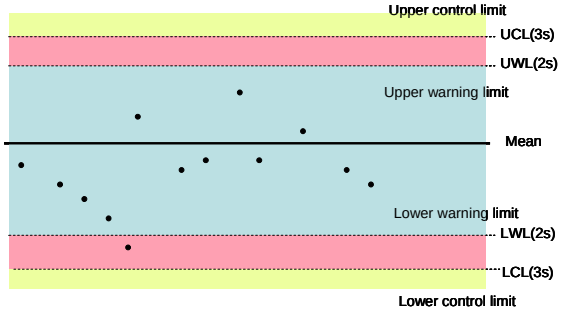
if the % recovery is satisfactory for the method chosen.

## Control Charts

Control charts are visual tools used by the laboratory to track performance. Each time a standard is run, ie GG standard for BOD, the results are plotted on the control chart. Control charts can be used to track both accuracy and precision over a period of time. Accuracy charts can track QC samples such reagent blanks, standards, spikes, etc. The control chart will have several horizontal lines which act as performance limits. WL is the warning limit and is defined as two times the standard deviation (95% confidence level). CL is the critical limit or control limits and defines the outer boundary for the test. It is defined as three times the standard deviation.



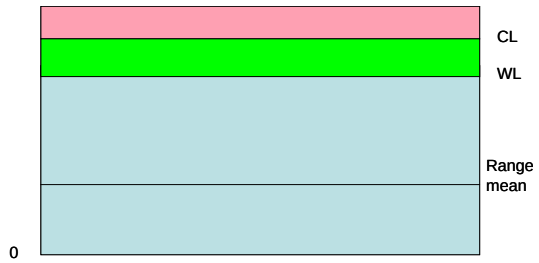

As an example, the GG standard for the BOD test could be plotted. The upper and lower control limits are defined in the procedure as being 198 $\pm$ 30.5. So the lower control limit is 167.5 mg/L and the upper control limit is 228.5 mg/L. Since 30.5 is 3 times the standard deviation, the warning limit would be about 20 mg/L. Each time the BOD analysis is run, the standard result would be plotted on the graph. Any standard result falling below the upper or lower warning limit would be an indication to the laboratory that the test is in danger of becoming invalid and investigation and correction is needed. By using the warning limits, the laboratory can take preventative measures to correct problems before the process is out of control and sample data becomes invalid.




Control charts should be constructed for each instrument and analytical method used.

For procedures where the control limits are not established by the method, the laboratory can establish there own control limits by running 15-20 standards and calculating the mean and standard deviation.

The second type of control chart is a precision control chart. Precision charts commonly track differences between duplicates. As before, if the warning limit or control limit are exceeded, the process is out of control and corrections must be made before continuing with analysis of samples. A minimum of 20 sets of duplicates should be run to determine the warning limit and control limit.




For simplicity, we'll use an example with 10 values instead of 20.
10 sets of a COD standard data were run over 10 weeks.

| | | Range |
|---|---|---|
| Standard 1: | 187, 194 mg/L | 7 |
| Standard 2: | 195, 196 mg/L | 1 |
| Standard 3: | 198, 198 mg/L | 0 |
| Standard 4: | 200, 205 mg/L | 5 |
| Standard 5: | 189, 190 mg/L | 1 |
| Standard 6: | 198, 193 mg/L | 5 |
| Standard 7: | 199, 189 mg/L | 10 |
| Standard 8: | 207, 205 mg/L | 2 |
| Standard 9: | 201, 205 mg/L | 4 |
| Standard 10: | 187, 189 mg/L | 2 |

Calculate the standard deviation of the range values. In this example, s = 3.0 mg/L
To determine the mean range, the table in standard methods requires using the factor 1.128 for duplicates. The mean range is:

Mean range (R) = 1.128(s) = 1.128 (3.0) = 3.4 mg/L

The warning limit is:

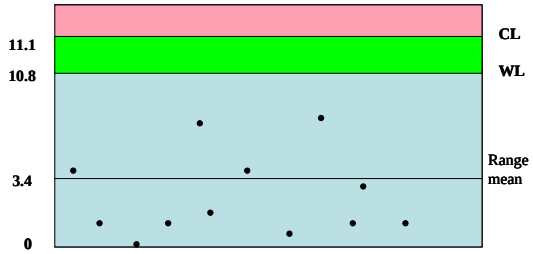
WL = mean range + 2/3 ($D_4$)(mean range)

$D_4$ again comes from the table in standard methods for duplicates.

WL = 3.4 + 2/3 (3.267)(3.4) = 3.4 + 7.4 = 10.8
The control limit is:

CL = ($D_4$)(mean range)

CL = 3.267(3.4) = 11.1 mg/L




Once the control chart is prepared, the range between duplicates can be compared.

**Control limit exceeded**:
If any range exceeds the CL, the measurement must be repeated. (duplicates are too far apart). If the repeat duplicate is still beyond the CL, the analysis must be stopped and the problem corrected. If the repeat duplicate is within the CL, continue analysis. For example: Standard 21 has a range of 15 which exceeds the CL of 11.1. Run another set of duplicates. If the next set has a range of 12, problems must be identified and corrected before more samples can be analyzed.

**Warning limit exceeded**
If 2 out of 3 consecutive range values are greater than the WL, analyze another standard. If the next point does not exceed the WL, continue analysis. If the next point exceeds the WL, problems must be identified and corrected before samples can be analyzed. For example, standards 22, 23, 24 have ranges of 11, 11, 10 and exceed the WL of 10.8. Analyze another standard. If this standard is 11 mg/L, problems must be identified and corrected before samples can be analyzed.

While establishing control charts seems like a lot of work, once established they provide the analyst, lab manager, and inspectors a quick review to show the laboratory data is under control.

# Appendix A

# Chain of Custody Forms

# SAMPLE CHAIN OF CUSTODY RECORD FORM

CHAIN OF CUSTODY RECORD

**ENVIRONMENTAL IMPROVEMENT DIVISION**

| SAMPLE NO. | DATE TAKEN | TIME TAKEN (HOURS) |
|---|---|---|
| SOURCE OF SAMPLE | | |
| REMARKS: (ANALYSES REQUIRED, SAMPLE TYPE, ETC.) | | PRESERVATIVE |

| SAMPLE COLLECTOR | | | WITNESS(ES) | | |
|---|---|---|---|---|---|
| TEAM 1 | TEAM 2 | TEAM 3 | TEAM1 | TEAM 2 | TEAM 3 |
| | | | | | |

I hereby certify that I received this sample with the chain-of-custody sealing tape intact and disposed of the sample as noted below:

| RECEIPT OF SAMPLE | IN POSSESSION OF: | TRANSFERRED TO: | |
|---|---|---|---|
| | Signature of Receiver | DATE | Time Transferred |

I hereby certify that I received this sample with the chain-of-custody sealing tape intact and disposed of the sample as noted below:

| RECEIPT OF SAMPLE | IN POSSESSION OF: | TRANSFERRED TO: | |
|---|---|---|---|
| | Signature of Receiver | DATE | Time Transferred |

I hereby certify that I received this sample with the chain-of-custody sealing tape intact and disposed of the sample as noted below:

| RECEIPT OF SAMPLE | IN POSSESSION OF: | TRANSFERRED TO: | |
|---|---|---|---|
| | Signature of Receiver | DATE | Time Transferred |

**SEE ATTACHED INTERACTIVE FILE:**

SLD CHAIN OF CUSTODY

# Appendix B
Sample Standard Operating Procedures

# STANDARD OPERATING PROCEDURE FOR THE DETERMINATION OF pH

**Prepared by: Lab Rat**

**Date prepared: 7/1/2007**

## SOP - pH

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Section 12: pH Quality Control ..... B5

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Appendix 1 ..... B7

## Section 1: Control Information

The SOP is stored as a Microsoft Word document in the SOP folder as file named SOPpH.doc.

## Section 2: Personnel Qualifications

Technician should assist in the test for at least 1 week and should have performed a witnessed, unassisted pH performance sample before initiating the procedure unsupervised.

## Section 3: Summary of Method Used

The method used is Standard Methods for the Examination of Water and Wastewater, 20th edition, Section 4500-$H^+$-B . This method is applicable to drinking water and wastewater samples. The method consists of placing a glass pH electrode into a well mixed sample and recording the display on a calibrated pH meter.

## Section 4: Materials and Equipment

Fisher Accumet 50
Combination glass electrode
General laboratory glassware
pH 4 buffer
pH 7 buffer
pH 10 buffer

## Section 5: Personal Protection Equipment

Latex gloves and safety goggles should be worn at all times. Personal hygiene should include hand washing with disinfectant upon completion.

## Section 6: Daily pH meter Calibration

The pH meter is used to measure the hydrogen ion concentration of a sample. For routine work, the pH meter must be accurate and reproducible to the nearest 0.1 pH unit. Sample pH and standard buffers should be measured at the same temperature, generally room temperature. The pH meter is normally very stable and needs to be calibrated only once daily. (generally within 3 hours of use). Calibrate more frequently if indicated.

**pH Meter Calibration**

6.1a Connect the pH meter and electrode to channel A. Remove the electrode from the KCl or pH 7 buffer storage solution; rinse with deionized water and blot dry. TIP: The bulb of the pH electrode must be below the water level. If the meter reading is erratic, check the water depth. If using the ATC, insert the ATC in the buffer as well. The meter will adjust for temperature differences automatically.

6.1b Press the **CHANNEL** button and select channel A. Press the **pH** button.

6.1c Press the **STANDARDIZE** button and press 2 to clear any previous data. Calibrate with at least 3 buffers. Select buffers that will bracket the typical sample pH. A fourth buffer can be used to validate the electrode response.

6.1d Place the electrode in pH 7 buffer. Press the **STANDARDIZE** button, and then press 1. Gently swirl the buffer, and then press **ENTER**. The large number

display should show 7.0. In the upper left-hand corner, a small 7.0 should appear; indicating the calibration point has been entered. **Record the lot #, expiration date, and calibration value on the pH Meter Calibration Log**

6.1e Rinse the electrode with deionized water and blot dry.

6.1f Place the electrode in pH 10 buffer. Press the **STANDARDIZE** button, then press 1. Gently swirl the buffer, and then press **ENTER**. The large number display should show 10.0. In the upper left-hand corner, a small 10.0 should appear; indicating the calibration point has been entered. **Record the lot #, expiration date, and calibration value on the pH Meter Calibration Log**

6.1g Rinse the electrode with deionized water and blot dry.

6.1h. Place the electrode in pH 4 buffer. Press the **STANDARDIZE** button, then press 1. Gently swirl the buffer, and then press **ENTER**. The large number display should show 4.01. In the upper left-hand corner, a small 4.01 should appear; indicating the calibration point has been entered. **Record the lot #, expiration date, and calibration value on the pH Meter Calibration Log**
Corrective Action: If the pH 4 buffer does not measure within 0.1 pH units, troubleshoot the electrode.

6.1i Press the SLOPE button. The slope should be 56 $\pm$ 3 mv or (>95% recovery) **Record the slope on the pH Meter Calibration Log. Press the slope button again to get back to the original pH display.**
Corrective Action: If the slope is not within tolerance, recalibrate. If the meter does not calibrate properly, replace the electrode and try again.

## Section 7 Electrode Maintenance

7.1a The electrode must **NOT** be stored dry. The best solution to store the electrode is saturated KCl or pH 7 buffer. Tap water or deionized water should not be used because the electrolyte within the electrode will diffuse out and cause premature aging or additional maintenance.

7.1b Store the electrode in a solution of saturated KCl. Long term storage requires storage in a large test tube covered with parafilm.

## Section 8 Troubleshooting

**Meter**

8.1a Disconnect the electrode and attach the shorting strap.

8.1b Press the millivolt button. The display should show zero.
If the display does not change, the meter needs repair.

**Electrode**

8.2a Gel electrodes generally require no maintenance if kept moist. However gel electrodes have a shelf life of around 2 years. Recording the date the electrode was placed in service helps with troubleshooting.

8.2b Glass electrodes fail because of scratches, deterioration, and accumulation of debris on the glass bulb. Rejuvenate the electrodes by cleaning in 0.1 M HCl and 0.1 M NaOH.
Corrective action: If the pH responds slowly or drifts after rejuvenation, replace the electrode.

8.2c Reference electrodes fail because of clogged liquid junctions. The liquid junction must remain open to avoid slow response and meter drift. If the liquid junction becomes clogged, place in hot deionized water for several hours. Some combination electrodes can be refilled with saturated KCl.

8.3 If the pH meter does not respond to different buffers (remains at pH 7 all the time), replace the electrode.

## Section 9 Sample Handling

9.1 Sample collection is the responsibility of the customer. Clean plastic Nalge bottles are provided to the customer in the size indicated below. Glass containers are also acceptable.

9.2 Samples should be collected and analyzed within 15 minutes. No sample preservation is permitted. On site analysis is preferred.

9.3 Minimum sample volume should be 50 ml.

***SAFETY: Use latex gloves when collecting and handling all wastewater samples***

## Section 10 Sample pH Measurement

10.1 Use a calibrated pH meter. The meter should be on channel A and in "pH" mode.

10.2 Carefully pour the sample into a 100 ml beaker. If the temperature is significantly different from room temperature, use the Automatic Temperature Compensating (ATC) Electrode if available.

10.3 Immerse the pH electrode and ATC into the solution. Be careful not to break the glass bulb.

10.4 Swirl the sample gently until the meter reading stabilizes. For poorly buffered samples such as deionized water, immerse in three or four consecutive portions of sample, and then immediately measure the pH in a fresh sample.

10.5 Report the answer to the nearest 0.1 pH units on the bench sheet.

## Section 11 Data Management and Records Management

11.1 All records must be maintained in three ring binders and filed under the customer's name.

11.2 Maintain all calibration records for a minimum of 5 years.

## Section 12 Quality Control

Decisions on the accuracy of the reported data will be based on the quality control information.

**Sample QC**

12.1 Sample holding time cannot exceed 15 minutes.
Corrective Action: Reject samples and request a resample.

12.2 Record sample date, time, type, sampler, date and time of analysis, analyst and method used.

12.3 Samples must be mixed well, poured quickly, and swirled constantly when measuring the pH

**Equipment QC**

12.4 Calibrate against a minimum of 2 buffers. Generally pH 7, pH 4 and pH 10 are used.

12.5 Buffers should bracket the expected sample pH.

12.6 Buffers used should be within the expiration date. Record date of purchase, lot number, and expiration date of buffers used.
Corrective Action: Expired buffers should be discarded and not used. Replace with fresh buffers. Purchase a 6 month supply and rotate stock frequently.

12.7 Store buffers in the dark, at room temperature, and in the chemical supply cabinet.

12.8 Performance evaluation samples should be run at least annually.

12.9 Split samples can be run

12.10 Duplicate sample pH results should be within 0.1 pH units.

## Section 13 pH Calibration Log Sheet (Appendix 1)

# pH METER CALIBRATION

Make and Model pH meter: _____
Date electrode place in service: _____

| Date | Time | pH 4 buffer | | | pH 7 buffer | | | pH 10 buffer | | | Slope | Name |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lot # | Exp. Date | pH | Lot # | Exp. Date | pH | Lot # | Exp. Date | pH | | |
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# Appendix C

Sample QA Plan Outline
Sample QA Plan-Vermont DEC
Laboratory QA Plan

# Appendix C – QA Plan Outline

**PART 1:** This document has been modified from a QA plan prepared by Paul Gray at the New Mexico Department of Health's Scientific Laboratory Division (SLD).

Title Page (Who are you – When was this written)
Signature Page (the Manual MUST be signed by everyone processing samples every year).
If you have more than a few pages, include a Table of Contents.

This "*Outline*" follows the format of the EPA Checklist that is used during on-site evaluations. The **ONLY** purpose of this "*Outline*" is to ASSIST development of a Quality Assurance Manual. Use the "*Outline*" portion (black letters) to lead you through the process. Replace the RED LETTERS with your own information and descriptions.

## 1. PERSONNEL

### *1.1 Supervisor/Consultant*

Name, Position within Organization, Training, Training relevant to Microbiological Water Testing.

### *1.2 Analyst (or equivalent job title)*

Name, Position within Organization, Training, Training relevant to Microbiological Water Testing.

### *1.4 Personnel Records*

This is a good place for the Organization Chart

## 2. LABORATORY FACILITIES

Describe the facility and equipment.

## 3. DATA QUALITY OBJECTIVES

This is the section where you describe the QA plan the lab will follow to produce valid and defensible data. Describe what QA procedures are used to validate sample results such as method blanks, calibration standards, laboratory fortified blanks, duplicates, matrix spikes, precision and accuracy control charts, etc.

## 4. LABORATORY EQUIPMENT AND SUPPLIES

This section is the Standard Operating Procedures (SOP) for all of the equipment used in your Laboratory.
Leave out any Item that is not used in your Laboratory.
Describe the Item, where it comes from (is it bought ready to use or prepared in your laboratory) its use, what Quality Control procedures are performed, how often and the preventive maintenance done - with a schedule.

***4.1 pH meter***

***4.2. Balance (top loader or pan)***

***4.3 Temperature Monitoring Device***

***4.4 Incubator Unit***

***4.5 Autoclave***

***4.6 Hot Air Oven***

***4.7 Colony Counter***

***4.8 Conductivity Meter***

***4.9 Refrigerator***

***4.10 Inoculating Equipment***

***4.11 Membrane Filtration (MF) Equipment***

***4.12 Culture Dishes (loose or tight lids)***

***4.13 Pipets***

***4.14 Glassware and Plasticware***

***4.15 Sample Containers***

***4.16 Ultraviolet Lamp (if used)***

***4.17 Other lab equipment such as DO meters, Drying ovens, computers and software, microscopes, desiccators, muffle furnaces, etc.***

## 5. GENERAL LABORATORY PRACTICES

Describe the procedures in your laboratory. If an Item is not relevant to your lab, leave it out.

***5.1 Sterilization Procedures***

***5.2 Sample Containers***

***5.3 Reagent-Grade Water***

### *5.4 Dilution/Rinse Water*

### *5.5 Glassware Washing*

### *5.6 General Laboratory Safety Procedures*

## 6. ANALYTICAL METHODOLOGY

This is where you will have a description of how samples are processed in your Laboratory – the Method SOP. Include descriptions (SOP's) for all procedures performed in your laboratory. You must follow Standard Methods and the EPA Manual, but everyone slightly alters the specifics of what is done. You MAY be stricter in your interpretation of the Regulations (sample must arrive at the laboratory within 24 hours rather than 30), but you can NOT be less strict (Incubator temperature ranges from 30-40°C).

### *6.1 General*

### *Media, chemicals*

- What media, chemicals do you use?
- Where does it come from?
- Where is it stored? For how long?
- How do you challenge the media? When?
- How do you dispose of outdated chemicals?

### *6.2 Membrane Filter (MF) Technique* (for total coliforms in drinking water (dw))

### *6.3 Multiple Tube Fermentation Technique (MTF or MPN)* (for total coliforms in dw)

### *6.4 Total suspended solids*

### *6.5 pH*

### *6.6 BOD*

### *6.7 Titration for chloride*

### *6.8 DO*

### *6.9 Heterotrophic Plate Count*

### *6.10 Total Dissolved Solids*

### *6.11 Nitrates*

### *6.12 Ammonia*

## 7. SAMPLE COLLECTION, HANDLING, AND PRESERVATION

### *7.1 Sample Collector*

If you do not collect samples, this Item is not relevant.

### *7.2 Sampling*

Even if you do not collect samples, you should have information available in the Laboratory to hand out to the people who do submit samples to you.

### *7.3 Sample Icing*

### *7.4 Sample Holding/Travel Time*

### *7.5 Sample Information Form*

Include a copy of your submission form.

### *7.6 Chain-of-Custody*

Not required in New Mexico, but a good idea to have written up IF you ever need it.

## 8. RECORDS AND DATA REPORTING

### *8.1 Legal Defensibility*

Are you doing all of the above AND documenting it AND:

### *8.2 Maintenance of Records*

- Keeping your Documentation?
- Where?
- For how long?

### *8.3 Sampling Records*

Refer to the copy of the Sample Submission Form.

### *8.4 Analytical Records*

What information do you maintain on a "Bench Sheet"? A copy of your Bench Sheet would be good.

### *8.5 Preventive Maintenance*

- What do you do to insure that your equipment works as it should?
- How do you document what has been done?

## 9. ACTION RESPONSE TO LABORATORY RESULTS

### *9.1 Notification of normal results*

- When are they notified?
- How are they notified?

### *9.2 Notification of Abnormal Results*

- Who so you notify?
- When are they notified?
- Is there documentation (Contact Log) of that notification?
- What information is kept on the Contact Log?
- How long is the Contact Log kept?

### *9.3 Notification of Invalid results*

- How does the lab invalidate data?
- What happens if invalid data is distributed accidentally?

### *9.4 Lab Certification*

- How does the lab maintain certificiation?
- Who has to be certified?
- What happens if the laboratory obtains unsatisfactory results from analysis of PE samples?
- What happens if the lab is decertified?

## 10. QC Checks and Their Frequency

This section describes what QC information is to be performed and documented. It could be included under the specific equipment indicated in SECTION 4.
This section could discuss Performance Evaluation samples, precision and accuracy procedures, calculations, use of control charts.

# PART 2: SAMPLE QUALITY ASSURANCE PLAN

This QA plan is a modification of the *Vermont Department of Environmental Conservation Laboratory QA plan*. This example is not meant to be an example that can be simply copied and used as your QA plan. The various sections should be customized for the laboratory analysis performed in your lab and the procedures you use. Each laboratory should prepare its own QA plan and SOPs. Most small labs will have a QA plan much simpler than this but should consider each area.

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## 2.0 INTRODUCTION

The City of _____ Laboratory Quality Assurance Manual documents or references documents that describe the laboratory policies and procedures as required by the State of New Mexico Surface Water Quality Bureau and is reviewed prior to the laboratory's annual on-site inspection. This manual can be found in the Office of _____

### 2.1 Objectives and Commitments of Management

The City of _____ relies on its laboratory to provide data, which is the scientific basis for completion of its NPDES and decisions impacting other environmental concerns. The Department's management is committed to provide the necessary resources to insure the laboratory can produce high quality data and maintain its State of New Mexico certification.

**Management's Quality System Policy Statement**

The City of _____ Laboratory is committed to providing consistent, high quality data in a timely manner. Since each analytical result will be used to make important program decisions, each analytical result is of critical importance. It is imperative that the City of _____ Laboratory generate and report data of known quality. Through our
Quality Assurance Plan we have outlined all procedures and precautions taken to insure that reported data is consistently of high quality. City of _____'s commitment to maintain the laboratory's quality objective is demonstrated by the following:

1. An expertly staffed and fully equipped laboratory facility
2. Successful participation in State of New Mexico's approved proficiency testing programs
3. Annual management review of each analytical process
4. Timely reporting of analytical results.
5. Laboratory test results which are supported by quality control data and documented
testing procedures.
6. Systems to inform management if analytical data does not meet all quality control
requirements.

This policy is communicated to all new employees and is constantly reinforced to all employees. It is implemented and maintained by employees at all levels. This policy is documented by management through employee evaluations, by formal reviews of the QA Plan and all revisions, training procedures, internal audits and document control.

## 2.2 Employee Code of Ethics, Training, and Reporting of Unethical Behavior

### 2.2.1 Employee Code of Ethics and Laboratory Fraud

Laboratory fraud is defined as, the deliberate falsification of analytical data or quality control results, where failed methods and contractual requirements are made to appear acceptable.
The City of _____ Laboratory management recognize that employee ethics have
a profound effect on the integrity and quality of the work performed at the City of _____ Laboratory. Policy regarding the acceptable handling, reporting and review of data are outlined in this Quality Assurance Plan (QAP) and Laboratory Standard
Operating Procedures (SOPs). Improperly performed procedures including all steps in calibration, analysis and data reporting, are not tolerated. Examples of unacceptable procedures include: falsifying data, improper calibration, misrepresenting quality control data, manipulation of computer software, data file substitution and concealment of known problems or unethical behavior or action

from laboratory management. Laboratory personnel must remain free of commercial or financial pressure which might influence their technical judgment.

#### 2.2.2 Ethics Training

New full time employees receive information sheets describing City of _____ Policies and Procedure. The policies set standards for Agency employees that include policies on conflicts of interest. Each employee must sign a statement that states they have received the information and they will be responsible for reading the material. Records are maintained with the Personnel Administrator. New laboratory personnel must complete an orientation program at the City of _____ laboratory. Documentation is kept in laboratory training files that states that new employees have read, understood and will use the latest version of the Laboratory's QA Plan and Laboratory SOPs which relates to his/her job responsibilities. Documents that must be read include:

Laboratory Quality Assurance Plan (QAP)
Laboratory Sampling Plan
Relevant Laboratory Standard Operating Procedures (SOPs)
Laboratory Safety Plan

Annual ethics training is required for each employee. Documentation of the training is retained in each employee's continued demonstration of ability folder. The training focuses on issues arising from activities such as hiring, training and supervising staff; handling, analysis and reporting of quality assurance data and legal responsibilities including the potential punishments and penalties for improper, unethical and illegal actions. Annual training reinforces the policies and procedures outlined in the QA Plan. Training may be conducted internally or by an outside source.

#### 2.2.3 Reporting of Unethical Behavior

Employees are required to report any suspected unethical activities to Laboratory management. The reporting can be in writing or verbal. Unethical situations can be reported anonymously through the interoffice mail system. Reporting can be to the Laboratory Director, Supervisor, QA Officer or any other designated person. It then becomes the responsibility of that individual to initiate corrective actions which may include reporting the incident to upper management or the Department of Personnel. Each employee involved in the reporting and receiving of reported information must document the incident, actions taken, information reported and individual the incident was reported about and reported to. This ensures that the individual reporting any suspected unethical behavior has evidence that they have acted appropriately.

## 3.0 LABORATORY REVIEW OF REQUESTS FOR TESTING

### 3.1 Lab Contracting Policy

The City of _____ Lab and clients requesting environmental analysis require an agreement that is written and signed by the project manager and Laboratory Supervisory Issues addressed are:

1. Tests requested, required reporting limits.

2. Turn around times.
3. Enforcement administrative procedures required (Y/N).
4. Method of sample delivery.
5. Laboratory responsibilities for sample preparation.
6. Lead time needed to schedule sample delivery and to secure sampling bottles.
7. Analytical method used. Existing methods have demonstration of lab capability in place. New procedures will require method development and an initial demonstration of lab capability documentation be in place before samples are accepted.
8. Laboratory input on applicability of methods to sampling planned.
9. Report deliverables.

The contract will include a copy of the Laboratory's State certification status. The final agreement will notify the client if the Laboratory possesses the necessary physical, personnel and information resources that are necessary to meet the clients needs. The Laboratory must notify the client in writing if the Laboratory's certification status changes during the life of the contract or if the contract needs to be amended after work
has commenced.

### 3.2 Subcontracting of Analytical Work

Other than providing solicited advice the Laboratory is not involved with program contracts with private laboratories.

## 4.0 LABORATORY ORGANIZATION AND RESPONSIBILITY

The City of _____ Laboratory is an internal service organization, which is charged with providing analytical support to all city departments.

The City of _____ laboratory is organized into three analytical areas:

**The Metals Analysis Section** is responsible for analyzing metals in a wide variety of matrices. It supports a number of Departmental programs including industrial pretreatment, landfill assessment, hazardous waste investigations and lake sediment/fish studies. The center employs ICP/MS and a mercury cold vapor system as the methods of analysis.

**The Inorganic Chemistry and Microbiology Section** is responsible for all non metal/organic analyses performed at the Laboratory. The center supports a number of diagnostic water quality studies, landfill assessments, and departmental investigations. The center supports the monitoring of the wastewater treatment system and swimming water. Analyses are performed using auto analyzers, ion chromatography, a variety of manual chemistry and microbiology methods.

**The Organic Chemistry Section** provides identification for organic materials in a variety of matrices including water, fish, solids and air. The center provides the full complement of organic contaminant analysis required by department programs. These analyses include volatiles, carbonyls, PCBs, and pesticide compounds. Analyses are performed using gas chromatography, gas chromatography-mass spectroscopy and high performance liquid chromatography.

The laboratory analytical sections require considerable administrative support, including a laboratory supervisor, secretary, personnel to manage the safety and quality assurance plans, and laboratory technicians that assist in managing glassware, bottle orders and simple analyses.

The laboratory has 6 permanent positions, and when the budget allows, seasonal technician position(s). The administrative center is assigned 2 positions. The remaining personnel work in the analytical sections. The laboratory director is responsible for all aspects of analysis within their section: instrument control, technical method development, equipment purchases, quality control, supervision and training of seasonal technicians and workload management. Technicians assist in each work sections as work loads require. Position descriptions outlining education and experience requirements are available upon request. In order to ensure that data is of acceptable quality all data is subject to review. The laboratory data review process is described in Section 10.2. Individuals responsible for ensuring data is valid and for routinely assessing measurement systems for precision and accuracy are listed below.

## 4.1 Laboratory Users

### 4.1.1 Program Directors, Project Leaders

- Are responsible for providing the Laboratory and EPA (federally funded projects) with a QA Project Plan which identifies and defines data quality needs in terms of appropriate analytical levels; contaminants and levels of concern; required detection limits; critical samples; and completeness, comparability and representativeness requirements.
- Responsible for scheduling the collection of additional samples to assess precision for matrices the Laboratory has not routinely analyzed and collecting appropriate field quality control samples (Section 11.1).
- Review data for errors and outliers as it becomes available.
- Oversees the sampling process to ensure field personnel are following proper sample collection and preservation steps. Responsible for providing written standards on operating procedures for all aspects of field work.
- Periodically assess data and initiates corrective action when analytical results do not provide useable data i.e. quantitation level is unacceptable for a particular set of low level samples; unacceptable field duplicate, split

sample or instrument blank results or data does not conform to required accuracy, precision or completeness requirements.

- Notfies the Laboratory when Chain of Custody (COC) will be required on a sample set, preferably before the sampling event when possible. Responsible for maintaining proper sample handling, and delivery of COC samples.

## 4.2 Laboratory Positions and Job Duties

### 4.2.1 Laboratory Supervisor

- Responsible for the overall technical quality of the work performed in the Laboratory and for assuring the use of standard methods. Supervises all personnel employed by the Laboratory. Assures that the Laboratory has sufficient personnel having the necessary education, training and technical knowledge and experience for their assigned duties. Assures that the Laboratory has appropriate equipment and supplies.
- Assures that the laboratory has the capacity, facility and resources to perform new work.
- Acts as liaison between laboratory and regulatory agencies (NMSWQB, EPA) and laboratory users.
- Oversees the scheduling of projects and the completion of tasks within the required time schedule and sample hold times. Monitors progress of projects and communicates with laboratory staff and users as required.
- Oversees the transformation of analytical data which may be necessary to meet program needs.
- Provides technical assistance to laboratory users in regard to the selection of appropriate analytical and/or sampling methods. Review QA plans.
- Provides technical assistance to laboratory staff regarding QA problems and method and instrument selection. Reviews laboratory standard operating procedures. Generates and maintains current Standard Operating Procedures (SOPs) for laboratory operation within his/her work area. Assures that all referenced method requirements are part of the SOP. Assures that all SOPs are appropriately detailed for personnel performing a method or step of a method and SOP protocol is followed.
- Reviews all data before it is reported as final. Assures that results from different parameters of a sample correlate.
- Maintains a sample custody and tracking system within the laboratory.
- Performs internal performance evaluation standards.
- Maintains the supply of sample bottles used for sample collection.
- Oversees the Chain of Custody (COC) sample transfer into the laboratory and assures that data handling and COC records are organized and accessible.

### 4.2.2 Laboratory Technician

- Is responsible for the technical quality of work performed.
- Completes required Demonstration of Ability protocols for all procedures/methods prior to undertaking independent analysis.

- Remains current on equipment and methods used in the analysis of samples within their analytical section. Is capable of providing insight into equipment purchases and analytical methods.
- Is capable of resolving technical problems encountered in the analysis of samples.
- Responsible for ordering all consumables needed for methods performed and assuring they meet standards.
- Responsible for equipment maintenance and maintenance contract oversight
- Maintains quality assurance documentation on procedures, equipment, reagents and standards. Initiates corrective action when quality assurance data does not meet pre-established control and warning limits.
- Participates in Interlaboratory Performance Evaluation studies.
- Assures that all data generated is properly reviewed and that all reviewed data meets internal acceptance criteria or is properly flagged.
- Responsible for the generation of data packages that contain all relevant information needed to reproduce a result and for the maintenance of both paper and electronic copies (when applicable) of data for methods performed.
- Reviews Laboratory Quality Assurance Plan revisions and follows protocols and procedures outlined within the Plan.
- Follow SOPs and QA/QC requirements of methods and the Laboratory.
- Informs immediate supervisor when precision and accuracy values are beyond established warning and control limits. Maintains QA/QC records for tests performed.
- Assists in data review for his/her analytical area.
- Is responsible for providing clean glassware and sample containers.
- Monitors the temperatures of refrigeration units, calibrates analytical balances and monitors indicator lights on the Laboratory water system on a daily basis.
- Prepares containers and other sampling items needed by samplers.

## 5.0 QUALITY ASSURANCE OBJECTIVES

Analytical precision and accuracy are assessed through the analysis of reference standards and laboratory generated quality control samples such as analytical duplicates, matrix spikes, and matrix spike duplicate samples. The accuracy and precision of data are related to the procedures used to analyze samples and generate data, the sample matrix being analyzed and the sample concentration. When a method does not specify limits, upper and lower control limits are established using historical Laboratory data as a guideline. When insufficient data is available, default limits are used. Laboratory quality assurance objectives for analytical data in terms of reporting limits (practical quantitation limits) and precision and accuracy, are listed by compound, method in Tables 5.1 Section 14 describes how data quality indicators are calculated. Precision and Accuracy objectives listed are based on upper and lower control limits.

Data outside the upper and lower control limit is flagged and the laboratory supervisor notified. The lower reporting limit for a method is listed as the Practical Quantitation Limit (PQL). The PQL is typically 2 to 10 times the calculated MDL. If accuracy is a problem at the calculated PQL due to background levels of contamination, sample dilution or other issues the PQL will increase. The low calibration standard must be at the method reporting limit (PQL) or lower. Results reported outside the calibration range must be flagged. Occasionally data is reported when the accompanying quality control data are not within established quality control limits, sample hold times have been exceeded, Laboratory clients request data be reported below the Laboratory's established reporting limits or for some other reason the data is not to standard. Sample remark codes are used to alert the data end user to the fact that analytical data accompanied by a remark code may not be appropriate for the intended use.

**Sample Remark Codes ***

| **Remark Code** | **Description** |
|---|---|
| BH | Reported value may be biased high. |
| BL | Reported value may be biased low. |
| C | Colony count outside ideal range. |
| D | Dilution resulted in instrument concentration below PQL. |
| H | Hold time exceeded, value may be in error |
| I | Matrix Interference |
| N | Not processed or processed but results not reported |
| O | Outside calibration range, estimated value. |
| OL | Outside Limit |
| P | Preservation of sample inappropriate, value may be in error. |
| S | Surrogate recovery outside acceptance limits. |
| T | Time not provided or time/date discrepancy |
| W | Sample warm on arrival, no evidence cooling has begun |

* Codes may also be used to qualify quality control data.

" BH: The reported value may be biased high" is used if the analyst determines or suspects that a sample or method bias has elevated the reported values. All samples of the same matrix may be flagged if a representative sample shows a matrix effect.

"BL: The reported value may be biased low" is used if the analyst determines or suspects that a sample or method bias has suppressed the reported values. All samples of the same matrix may be flagged if a representative sample shows a matrix effect.

"C: Colony count outside ideal range" is used when microbiology membrane filtration counts are not within the method recommended ideal counting range.

“D: Dilution resulted in instrument concentration below PQL” is to be used when dilution is necessary to eliminate an interference for the parameter being reported. The interference can be a chemical or physical interference. For multi-parameter methods analysis and reporting from more than one dilution may be required if the interference is only compromising a portion of the chromatography.

“H: Hold Time is exceeded” is used to flag a result when sample or extraction method specified holding times are exceeded.

“I: Matrix Interference” is used if Laboratory data quality objectives can not be met due to a matrix interference and the analyst can not determine if the bias is high or low.

“N: Not processed or processed but results not reported”. The use of this code requires that the parameter related comment field be completed. The “comment field” may list one of the following comments or others: Lab Accident, Sample arrived in unacceptable container, Sample not properly preserved, Insufficient sample volume, Hold Time exceeded, Associated quality control data unacceptable.

“O: Outside calibration range, estimated value” is to be used for reported results that are above or below the calibration standards. It is not used if a sample dilution is made and the diluted sample result is within the calibration range. Laboratory policy requires that sample results be bracketed by standards. If there is insufficient sample volume or for some other reason the sample can not be diluted and reanalyzed to bring a result(s) within calibration range the result must be flagged. A high standard analyzed at or above the concentration of the sample with recoveries within Calibration Verification Criteria may negate the need to flag the data.

“OL: Outside Limit” is used to flag data that is outside the precision or accuracy criteria established by the Lab, or referenced method.

“P: Preservation of sample inappropriate, value may be in error” is to be used when there is a decision made by the Lab to analyze an inappropriately preserved sample rather than reject the sample.

“S: Surrogate Recovery outside acceptance limits” is used to flag surrogate recoveries that are outside laboratory acceptance criteria.

“T: Time not provided” Analysis with a hold time of <72 hours must have the sampling time entered at log-in and the analysis time entered by the analyst. Samples with inadequate time of arrival documentation will be flagged if a decision is made by the lab to analyze an inappropriately labeled sample rather than reject the sample.

“W: Sample warm on arrival, no evidence that cooling has begun” is to be used when samples requiring cooling arrive and are not on ice.

## 6.0 SAMPLE HANDLING

### 6.1 Sample Collection

The laboratory is responsible for collection departmental samples (WWTP). All other departments must properly collect and deliver samples to the laboratory. The laboratory does not accept samples collected by the general public. Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater describes the required containers, preservation and holding times for the parameters analyzed at the laboratory. Sample collection and handling protocols are available for microbiological samples.

### 6.2 Sample Receiving

Most samples are delivered, logged into the Laboratory Logbook, labeled, preserved, subdivided, filtered if necessary and stored in refrigeration units by laboratory personnel responsible for the collection of the sample(s). Sample temperature upon delivery is monitored and the temperature of a representative sample is recorded in the logbook. Dedicated refrigeration units are used for samples that could become contaminated. Sample log in instruction and chain of custody sample handling instructions are described in Section 7.0. A limited number of samples arrive by courier and are logged in at the time of arrival. The Sample Log-In Sheet is used by field personnel to record required information. The Laboratory secretary, supervisor or analyst: insures that required information is provided, documents whether samples requiring thermal preservation have been iced, records the temperature of a representative sample, and labels the samples with the lab numbers. Chemical preservation of samples delivered by courier is generally performed at the laboratory by laboratory staff, however some samples require field preservation. When preservation or sample manipulation (filtration, subdividing) is performed by laboratory staff, a record is made of the processing steps.

### 6.3 Sample Preservation

#### 6.3.1 Temperature Preservation

Samples requiring thermal preservation will be considered acceptable if there is evidence that the chilling process has begun, such as arrival on ice for samples that are delivered to the laboratory the day of collection. If samples require thermal preservation and they are not delivered on the day of collection they will be considered acceptable if the arrival temperature is either within 2°C of the required temperature or the method specified range. For example if the specified temperature is 6°C, sample temperature ranging from above freezing to 6°C is acceptable.

#### 6.3.2 Chemical Preservation

In most instances chemical preservation can be initiated upon delivery of samples to the Laboratory, if samples are delivered the day of collection. If field preservation is required the sample container provided will contain the required preservative. If samples have not been field preserved lab staff must preserve

the samples at the Lab following protocols outlined in Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater . All samples requiring acid preservation must be tested for proper pH prior to or after analysis. Preservation is verified by the analysts and documented on bench sheets or in laboratory notebooks. If a sample(s) was not properly preserved and if the sample has not been analyzed the requesting department may be notified before proceeding if practical (metals samples are an exception, policy is to preserve and wait 16 hours to analyze). If the sample was not properly preserved and the department requests that sample results be released, the analyst must flag data with a "P-preservation of sample inappropriate, value may be in error". The laboratory Supervisor must document in writing the clients decision to report results that do not meet acceptance criteria.

## 6.4 Sample Acceptance and Rejection Policy

### 6.4.1 Sample Acceptance

#### 6.4.1.1 Required Information:

Samples submitted to the laboratory must be accompanied with the following information either electronically or by completing a Sample Log-In Sheet if samples are to be logged in by laboratory staff:

- Customer Identification
- Sample Collector
- Date of Collection
- Time of Collection (time must be entered for all tests)
- Collector's Name
- Preservation
- Sample Matrix (solid, wastewater, drinking water, etc.)
- Customer Sample Identification(s)
- Requested Tests
- Sample Remarks
- Have samples arrived on ice? Yes/No
- Temperature of representative sample ($^{o}$C)

Chain of Custody samples (COC) must follow protocol outlined in the Laboratory QA Plan: Section 7.2 Chain of Custody Procedures. A lab COC Form must be submitted with the samples. If protocols are not followed the laboratory supervisor or his designated representative will refuse samples unless written instructions from the client instruct the laboratory to proceed with analysis. The final Lab Report will document irregularities and the client's decision on how the Lab was instructed to proceed i.e. discard samples or analyze and report results with a qualifier.

#### 6.4.1.2 Sample Labeling:

Samples must be clearly labeled with a unique identification. Labels should be water resistant and indelible ink used. At sample Login the laboratory will label samples with a unique bar code that allow analysts to enter Sample ID #s into laptop spreadsheets or analytical instrument data bases.

#### 6.4.1.3 Sample Containers:

Samples must be collected in appropriate sample containers provided by the laboratory as described in Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater. If the Laboratory Supervisor accepts a sample in a container not provided by the lab a Sample Note or Order Comment must be added to the bench sheet.

#### 6.4.1.4 Sample Holding Times:

Samples must be delivered to the Laboratory to allow sample analysis to be completed within sample hold time. Samplers must schedule pH and chlorine (analyze immediately), Microbiology (6 hour hold time for wastewater samples), BOD5 (24 hour hold time). Sample holding times for other parameters are listed in the Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater.

#### 6.4.1.5 Sample Volume:

Appropriate sample volume must be provided. Containers, which are provided for each test, will provide the appropriate volume if filled. Instructions on filling sterile microbiology bottles, total phosphorus tubes, and volatile vials (solid and water) must be followed. An additional sample volume may be required for some tests to provide the Laboratory with the sample volume required to perform required QC. Samplers are instructed to collect an extra volume of sample and designate the sample as a duplicate and/or matrix spike sample.

6.4.1.6 Sample Preservation:

Sample preservation protocols outlined in Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater must be followed.

### 6.4.2 Sample Rejection:

If a sample is received that is not suitable for testing or insufficient details are provided for the laboratory to proceed the requesting department must be consulted for further instructions before proceeding with analysis.

The laboratory supervisor determines if submitted samples are to be rejected or results not reported for samples that have already been processed. Laboratory staff must immediately inform the supervisor of any non-conformities that may affect the validity of sample results. The Laboratory shall either:

- Retain correspondence and/or records of conversations concerning the final disposition of rejected samples; or
- Fully document any decision to proceed with the analysis of samples not meeting acceptance criteria.

When samples do not meet laboratory requirements, they are either rejected or reported with a comment for the appropriate tests. In either case the program manager is contacted in writing (e-mail or letter) requesting concurrence with the Laboratory's decision to reject the samples or to proceed with the analysis and include an appropriate comment on the laboratory report. If samples are

accepted, analysis data will be “qualified” on the final report. Samples may be rejected for the following reasons:

- Insufficient volume.
- Inappropriate container.
- Sample beyond hold time or laboratory is unable to perform analysis within required hold time.
- Inappropriate sample preservation or sample chilling has not begun for samples requiring thermal preservation.

### 6.5 Sample Containers, Preservation and Holding Times

Preservation, types of containers, and holding time information can be found in Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater

## 7.0 SAMPLE MANAGEMENT

Detailed instructions are available for individuals that are required to login samples. The laboratory technician will assign a unique laboratory control number to track samples through analysis and final report.

### 7.1 Chain of Custody Procedures

#### 7.1.1 Introduction

Before deciding to use chain of custody procedures to insure that laboratory results can be used for litigation and enforcement, the collection and analysis of samples must be part of a well organized plan. The plan will delineate what, where and how the samples are taken and establish the level of quality assurance needed. A plan calling for chain of custody procedures will require documentation of sample integrity from collection to final disposition by the laboratory.

Chain of Custody Procedures are necessary to insure the legal integrity of sample materials collected and submitted to the City of _____laboratory for analysis. The validity of the test results is assured if the Department can show that after the samples were collected, they were kept safe from tampering or chemical contamination. This requires that complete written documentation of the security of the sample from collection to disposition be kept.

#### 7.1.2 Sample Custody

A sample is under custody if

- It is in your possession, or
- It is in your view, after being in your possession, or
- It was in your possession and then you locked it up to prevent tampering, or
- It is in a designated secure area.

### 7.1.3 Submittal of Samples

Any user of the City of _____ laboratory can request chain of custody handling of their samples; these requests presume that the results are likely to be used for enforcement. Whether samples are hand carried or delivered by a courier they must be properly preserved, individually sealed and include a Chain of Custody Record. After receipt of the sample, a copy of this record is returned to the sampler. To track their samples from collection in the field to receipt of a laboratory report, the sampler will use the laboratory's chain of custody transfer record form and will seal the individual sample containers.

Hand Carried – This is the most common approach and is used almost exclusively by department personnel. Unless special arrangements are made, these samples should be submitted Monday through Friday between 8:00 a.m. and 4:30 p.m. and relinquished to the Laboratory Supervisor or his designee. Thermal preservation must be maintained by packing samples with a sufficient volume of ice (blue ice does not cool samples sufficiently). Unless special arrangements are made, overnight delivery is required and samples need to arrive Monday through Thursday before noon. Note: Do not use US Postal Service since delivery is to a central location and not the laboratory building. Samples sent by a package carrier, UPS or Federal Express, are to be addressed to:

City of _____ Laboratory
Attn: Chemistry Laboratory Supervisor
103 South Main Street
North Pole

### 7.1.4 Sample Custody Procedures

- The Laboratory Supervisor must be given advance notice of samples requiring Chain of Custody handling procedures.
- Field personnel must document in a field notebook all details regarding sampling activities. Documentation must include exact information regarding date, time, location, names of people present, unusual events, field measurements, details of sample storage and security, and transfer of samples to others.
- Field personnel are supplied with the proper sampling containers, chemicals for sample preservation, coolers, sample labels, Chain of Custody sealing tape, a Chain of Custody Record, and a listing of acceptable hold times, sampling procedures, and preservation techniques.
- Field personnel must collect samples according to standard procedures and add preservative if required. Samples requiring field preservation must be collected in containers containing the preservative. Field personnel needing to break the seal(s) at the laboratory to add preservation chemicals are asked to transfer custody to the laboratory after the samples are resealed. Lab personnel must be notified if preservation is to be done at the Lab by laboratory staff.

- Field personnel must seal the top of the sampling container with a Chain of Custody Sample Seal, initial the seal, complete the identifying label and store and transport samples in a sealed cooler with ice, if thermal preservation is required. A secure container capable of being sealed is acceptable if thermal preservation is not required.
- At no time are samples to be left unattended unless they have been locked or secured with initialed seals in place.
- The samples must be delivered to the Laboratory Supervisor or designated staff chemist who will accept the samples and perform the following steps.
    1. Verify that correct containers were used and required preservation was performed.
    2. If thermal preservation is required:
        verify that samples arrived on ice and cooling has begun
        record the temperature of a representative sample.
    3. Verify that all samples listed on the Chain of Custody Record form are accounted for.
    4. Verify that all containers are properly sealed and that all seals are intact and the Chain of Custody form and seals are completed correctly.
    5. Accept the samples and sign the Chain of Custody Record form.
    6. Log samples into the laboratory logbook and designate the samples as enforcement. Label the samples with unique label or verify that sample login was completed correctly and samples are properly labeled with unique sample identification numbers.
    7. Store samples in a designated locked refrigerator(s).
    8. Provide the designated individual with the Chain of Custody Record form, to file.
    9. Notify analysts or technical directors responsible for the analysis of the sample(s).

#### 7.1.5 The Chain of Custody Record

- The sampler(s) and/or witness are required to sign the form when samples are collected.
- A witness is not required to be present during sampling to satisfy the Chain of Custody requirements of sampling.
- Enter the name of the laboratory performing the analysis.
- Enter the exact sample location.
- Record the date and time of sample collection and whether the sample was a composite or grab.
- The description and number of containers should include the tests to be analyzed by groups on the slanted lines and the number of containers for each group in the accompanying box; e.g. volatiles, metals, semivolatiles on the slanted line and the number of containers/sample in the box.
- The total number of sample containers per location and any remarks regarding the sample should be recorded.

- When custody is transferred from one person to another, both parties must sign and date this form. If someone other than the person whose signature appears at the top of the form transports the samples to the laboratory, that transfer must be documented on this form.
- If the sample is to leave the laboratory for any reason the sample must be resealed and a Chain of Custody Record form will be reinitiated.

#### 7.1.6 Responsibility of the Analyst

An analyst assigned to perform the required analyses on Chain of Custody samples is expected to follow the procedures listed to insure that the Chain of Custody is maintained throughout the analytical process.

That analyst, using a Sample Sign-Out Sheet, signs a Chain of Custody sample from the refrigerator, removes the container, breaks the seal and removes an aliquot of sample, which is adequate to perform the analyses requested. A majority of containers are designed to provide enough sample for one analysis or a series of similar analyses.

This assumes that the sample will not be analyzed by another laboratory. If enough sample for a valid retest remains in the container after the analyst removes an appropriate aliquot of sample, the container is returned to the Chain of Custody refrigerator for possible re-analyses. An analyst, who is responsible for a subsequent analysis to be performed on an aliquot of this sample, must also document the removal and return of the sample on the sample sign out sheet. If all analyses from the container are complete any remaining samples are placed into long-term storage (Section 7.1.7).

Empty containers and containers of samples in which insufficient volume remain to complete another analysis are discarded. If the client requests that samples be removed from the Laboratory facility, the samples will be resealed and a new Chain of Custody Record will be initiated.

The specific steps to be documented on the Sample Sign-Out Sheet are:

- Laboratory ID #.
- Date and time samples are removed from the refrigerator.
- Amount of sample removed.
- Initials of analyst removing the samples.
- Tests to be performed.
- Can a valid analysis be performed on the remaining sample Y/N? If N, then the remaining sample can be discarded. If Y, the sample is returned to the locked refrigeration unit.
- Date and time samples are returned to the refrigerator.
- Initials of analyst returning the container to the refrigerator or discarding vessel if insufficient sample volume remains.

#### 7.1.7 Long Term Storage of Chain of Custody Samples and Records

When all tests on a sample from a particular container have been completed and if any remaining sample in that container can be used to obtain a valid analysis, that container must be stored as a Chain of Custody sample. Unless the laboratory has been specifically instructed to retain the samples, once hold times for the individual analyses in that container have been exceeded by 30 days, the samples can be removed from the refrigerator and discarded. All paperwork with the exception of field notes, are retained by the laboratory or Public Records. All laboratory records must be kept secure and in confidence to the client. Laboratory policy on record retention described in
Section 10.0 Data Reduction, Validation, Reporting, Tracking and Storage must be followed.

#### 7.1.8 Sample Containers, Preservation and Hold Times

Required containers, preservation and hold times for regulated contaminants are listed in Table 1060:I in 20th edition Standard Methods for Examination of Water and Wastewater

#### 7.1.9 Chain of Custody Record Form

See appendix A.

## 8.0 CALIBRATION PROCEDURES

All instruments and equipment used within the Laboratory are routinely calibrated by laboratory personnel. Analytical balances and thermometers are annually calibrated by an external calibration service. A summary of calibration procedures for individual instruments and tests is provided in this section.

Primary Calibration Standards used for calibration are purchased from a reputable dealer or prepared at the laboratory using reagent grade material. All purchased primary standards are certified by the vendor for purity and identity and when available are NIST traceable. Vendor supplied Certificates of Analysis are retained within analytical sections for a minimum of 5 years.

Calibration Standards (working standards) are dilutions or mixtures of stock standards used to calibrate an instrument. These standards are prepared or restandardized frequently (Section 9.3). NIST traceable reference materials are used when available. Certificates of analysis are retained in analytical centers for a minimum of 5 years.

The calibration range defines how results are reported and samples are processed. Results below the low calibration standard are reported as less than (<) the Reporting Limit (PQL). Results above the high calibration standard must be diluted and reanalyzed so that the instrument reading is within the calibration range.

## 8.1 Organics

### 8.1.1 GC (Volatiles, Pesticides, PCBs, TPH)

An initial calibration curve is prepared for each analyte of interest. Five or more calibration standards are prepared with one of the concentrations at the lower reporting limit (PQL)and the other concentrations corresponding to the expected range of concentrations in real samples. Each standard is injected into the instrument and the area response is tabulated against the concentration. The coefficient of determination is calculated for each curve by the software and is used to judge the curve fit. A coefficient > 0.99 is acceptable. The initial calibration curve must be verified every 12 hours (continuing calibration) by the injection of a mid-range standard. If the response for any analyte varies from the predicted response beyond the acceptance criteria, a new calibration curve must be prepared for that analyte. Acceptance criteria is listed by method in Table 8.1.

### 8.1.2 GC/MS (Volatiles)

An initial calibration curve is prepared for each analyte of interest. Five or more calibration standards are injected. A response factor is calculated as follows:

RF = As X CIS
CS X AIS
As = peak area of analyte or surrogate
AIS = peak area of internal standard
Cs = concentration of the analyte or surrogate
CIS = concentration of the internal standard

The System Performance Check Compounds (SPCC) are checked for the minimum average response factor. The Calibration Check Compounds (CCC) are checked for percent relative standard deviation. The SPCC and CCC checks must fall within the method guidelines. An average response factor is generated for each parameter. One primary ion is used for quantitation. A continuing calibration verification standard (CCV) is run every 12 hours, and the SPCC compounds are checked for response factors, and the CCV compounds are checked for percent relative difference.

The mass assignments of the GC/MS system are determined by calibration with perfluorotributylamine (PFTBA). The system is then hardware tuned to meet method criteria for mass spectra of a 50ng injection of BFB (4-bromofluorobenzene).

### 8.1.3 HPLC (carbonyl compounds)

An initial calibration curve is prepared for each analyte of interest. Five or more calibration standards are injected with one of the concentrations at the practical quantitation limit and the other concentrations corresponding to the expected range of concentration in real samples. Each standard is injected into the instrument and the area response is tabulated against the concentration. The coefficient of determination is calculated for each curve by the software and used

to judge the curve fit. A coefficient above 0.999 for at least 5 of the 6 compounds and >0.995 for one is acceptable. The initial calibration must be verified every 10 injections by running a mid-range standard.

## 8.2 Metals

### 8.2.1 Mercury Cold Vapor Analyzers

Instrument calibration for metals analysis is performed daily. A multi point curve is generated. The calibration curves must have correlation coefficients greater than or equal to 0.995. Calibration verification is monitored by analyzing a second source standard immediately following calibration (Initial Calibration Verification - ICV). A midrange standard is analyzed after every tenth sample, and at the end of the sample run to assure that calibration is maintained throughout the run (Continuing Calibration Verification -CCV). The calibration blank is also reanalyzed immediately following calibration, after every ten samples and at the end of the analytical run. Calibration blank results must be less than one-half the reporting limit (PQL), and the ICV result should be within ±10% of the true value for analysis to continue. The CCV result(s) must be within ±10% of the initial value. Failure of a CCV sample requires recalibration or reanalysis of all samples analyzed after the last passing CCV.

### 8.2.2 ICP-MS

The instrument is tuned with multi-element tune solutions to meet method criteria. A performance report that verifies Mass calibration and resolution is performed daily. The detectors are cross calibrated when necessary. After instrument calibration criteria are met an initial calibration curve is prepared daily for each metal to be analyzed. Three or more standards are analyzed with one of the concentration at the reporting limit and the other concentrations corresponding to the expected range of concentrations in samples to be analyzed. The coefficient of linearity must be >.995. The calibration curve is verified by analysis of mid-range second source standard mixes containing all metals to be quantitated.

Calibration is verified after every 10 samples and at the end of the run by analysis of a mid-range standard. Results must be within ± 10% of expected values.

## 8.3 Inorganic Chemistry

There are several automated and non-automated analyses performed in the inorganic chemistry section. Calibration and calibration verification protocol will vary from test to test. For most tests calibration is verified by the analysis of a working standard (ICV) at the beginning of the analytical run. The Initial Calibration Verification (ICV) should be within + 10% of the true value for analysis to continue. A mid-range Continuing Calibration Verification standard (CCV) is analyzed after every 10 samples. A different concentration CCV is run at the end of the analysis. Results should be within 10% of the true value. Reagent Blanks/Method Blanks must be less than one-half the Reporting Limit (PQL).

For most colorimetric analysis a standard curve consisting of 4 to 6 points and having a correlation coefficient of at least .995 is generated. A typical ion chromatography run will have a standard curve consisting of 4 or 5 points for each ion of interest. A combined ion stock standard is used. The correlation coefficient of the standard curve for each ion should be >.998. The coefficient is calculated by plotting the peak area against the standard concentration using a linear fit.

## 8.4 Support Equipment

### 8.4.1 Thermometers

Thermometers used in the Laboratory are calibrated against a NIST-traceable thermometer. The NIST thermometer is re-certified every 5 years at thermometer readings 4°, 44.5°, 95°, and 122°. Correction factors are taken into consideration when the thermometer is used to determine correction factors of Laboratory thermometers. Correction factors are noted on thermometers if needed. Correction factors, date calibrated, temperatures of both thermometers and thermometer serial numbers are documented in a laboratory notebook.

### 8.4.2 Refrigeration Units

Temperatures within refrigeration units are checked almost daily. Temperatures are recorded in a logbook and should be 0-6°C for refrigeration units and -17° C + 2° for freezers. Thermometers are submersed in an appropriate solution within each unit. If temperatures exceed these limits the unit is monitored and corrective action taken if temperatures remain outside limits.

### 8.4.3 Incubators/Water Baths/Ovens

Microbiology incubator temperatures are checked twice daily when in use. Temperatures must remain within method specified limits. Oven temperatures for tests requiring a specified temperature are checked daily when in use. All temperatures are recorded. The water bath used for the digestion of mercury samples is checked and temperature recorded at the beginning of analysis, and at the end of the digestion and must be 95° + 2° C.

### 8.4.4 Balances

Calibration of analytical balances is performed annually by a calibration service that is ISO 17025 compliant. Calibration is verified monthly with NIST traceable Class 1 weights. Two weights bracketing the expected range of measurements are used, measurements should be within ± .5mg. All weights are recorded in a lab notebook. The NIST traceable weights are verified by the State of New Mexico Metrology Dept. every 3-5 years. Weights are cleaned with 95% ETOH 24 hours before they are checked. Weights must be within the balance tolerance of ± .0002g or the weight tolerance, whichever is greater.

#### 8.4.5 Automated Pipettes and Dispensing Devices

Dispensing devices used as Class A volumetric devices have routine maintenance performed and are calibrated by an external calibration service annually**.** The manufacturers precision and accuracy specifications must be met. Multi-volume dispensing devices have each dispensing head calibrated at a minimum of two volume settings each. NELAC requires that all class A dispensing devices be checked on a quarterly basis. This is performed in-house.

#### 8.4.6 pH Meters

A three point calibration is performed daily and after every 3 hours of continued use. Standards bracket the pH of the samples analyzed. The percent slope of the calibration curve must be >95%. If criteria is not met the meter must be recalibrated using appropriate standards.

#### 8.4.7 Computer Software

Computer software is purchased either to support new instrumentation, to upgrade the performance of existing equipment or to manage the tracking of Laboratory data. Software needs to meet bid specifications which is demonstrated during installation/or training. The IDA files contain relevant data that documents performance.

## 9.0 ANALYTICAL AND OPERATIONAL PROCEDURES

### 9.1 Analytical Methods

All methods commonly used City of _____ Laboratory are approved by 40CFR 141_____. Parameters with corresponding method numbers and references are summarized in Table 5.1 of this manual. Current Laboratory Standard Operating Procedures (SOPs) are available upon request. Technical SOPs describe in detail, routine analytical tasks performed at the laboratory and typically include:

- Method title and referenced documents
- Reagents and preparation
- Method summary
- Definitions
- Health and safety warnings
- Personnel qualifications
- Apparatus and materials
- Interferences
- Calibration and standardization
- Sample preparation and preservation
- Quality control procedures
- Equations, calculations and data reduction procedures
- Data bench sheets and reporting forms
- Deviations from referenced methods
- Troubleshooting
- Deionized Water System Maintenance SOP

#### 9.1.1 Method Review

SOPs for current methods must be reviewed biannually. The most current revision of the referenced method must be reviewed at the time of the biannual SOP review to assure that all method requirements and control limits are being met. The review/revision process must be documented.

#### 9.1.2 Method Revision

If a significant variation to a referenced method is made the Laboratory must first demonstrate the alternative protocol results are comparable. The Laboratory SOP must clearly describe the variance and comparability data must be on file at the Laboratory. To demonstrate comparability the laboratory must, at a minimum, analyze four consecutive representative split sample(s) using the standard method and the alternative protocol. The alternative protocol results must be within 10% of the approved test procedure. Each sample site may be subject to this demonstration of comparability.

The laboratory retains a copy of all archived SOPs. The analysts bench copy of an SOP must be updated whenever a protocol is changed and is the most current version of an SOP. Significant changes, including any change affecting the calibration or quality control acceptance limits must be authorized (initialed and dated) by the Lab Supervisor. The bench copy of an SOP must be officially revised every two years at a minimum.

### 9.2 Laboratory Water

Laboratory water meets or exceeds ASTM Type II Reagent Grade Water requirements. The laboratory's water system is described in the Laboratory Deionized Water System Manual. The SOP also provides a description of the daily, weekly, monthly and yearly water system maintenance and monitoring schedules.

### 9.3 Reagent Preparation, Documentation and Storage

All standards and reagents are prepared from reagent grade materials, primary standards or are purchased from reputable vendors. When standards are purchased the date of receipt is documented on the certificate of analysis and certificates are filed for a minimum of five years. Standards and reagents are prepared using Class A volumetric glassware and calibrated dispensing devices and ASTM Type II reagent water. An electronic log or log books are used to record the receipt of all vendor supplied standards and reagents. The vendor, date received, lot number, expiration date and other pertinent information must be documented in the Standards/Reagent Log. Log books or sheets are utilized to document all information needed to maintain proper traceability of all standards and reagents prepared or purchased by the laboratory. Logs document the date of preparation or opening of purchased standards, expiration date, a list of standards/reagents or solutions used, lot numbers and the

preparer's name (initials). Additional information may also need to be recorded such as pH.

Once a solution is prepared it is labeled with the solution name or description, concentration or normality, preparation and expiration dates and initials of preparer. Documented information must be sufficient to allow traceability to the preparation record which should provide traceability of all ingredients. Expiration dates for standards and reagents are usually specified in methods or by the manufacturer and are adhered to unless degradation prior to this date is observed. Purchased materials are labeled with the date received and opened and the expiration date if more stringent than manufacturer's expiration date. Reagents are stored according to Method or manufacturer's instructions and discarded upon expiration. When expiration dates are not specified the following guidelines are used:

**Stock Standards** used for calibration can be used for 1 year if properly preserved and stored.

**Titrating Solutions** need to be either re-standardized or a new bottle of vendor certified standard opened each month.

**Calibration or Spiking Standards** are dilutions of stock standards used to calibrate an instrument. These standards are to be prepared daily unless specified otherwise in the method SOP. All other solutions are used for no more than a year. They are valid for that length of time only if evaporation is minimized and proper preservation and storage techniques are used. If a bottle is opened often or is much less than half full more frequent preparation may be required. If degradation becomes apparent the solution is discarded immediately and holding times are reduced.

### 9.4 Miscellaneous Procedures

In addition to method specific procedures several operational activities are monitored at the laboratory. Documentation of the monitoring can be found in the following locations:

- Reagent and preparation notebooks
- Instrument maintenance logs
- Instrument service logs
- Laboratory water system maintenance logs
- Balance/refrigeration/incubator monitoring log books

### 9.5 Traceability of Measurements

All measurements are required to be traceable to a national or international standard of measurement when a traceable standard is available. Equipment and measurement devices including balances, thermometers, and dispensing devices, associated with the accuracy of a measurement are calibrated according to protocols outlined in this QA Plan. Reference standards and materials used at the lab or by equipment calibration services are traceability to a national standard. Traceability requires that lab employees document and retain all pertinent information related to a measurement. Records pertaining to

calibration, calibration verification, and analysis must be detailed and traceable to the standards used. All results, information and calculations needed to generate a result must be documented. Record retention will vary depending on the record but must meet lab policy outlined in the QA Plan.

### 9.6 Data Recording and Editing

All written records in notebooks and on bench sheets need to be legible and recorded in permanent ink. Sharpies or other markers should not be used. Corrections must be made by drawing a single line through the incorrect entry. Corrections must be initialed and dated (month-day-year). Writing over an incorrect entry or using white-out, correction tape or erasers is not allowed. Pages may not be removed from bound notebooks. All records must be signed or initialed (electronic or written signatures are acceptable) and the reason should be clearly indicated such as “prepared by”, “reviewed by” or “validated by”.

## 10.0 DATA REDUCTION, VALIDATION, APPROVAL, REPORTING, TRACKING AND STORAGE

All analytical data generated by the City of _____ laboratory is recorded, reported, reviewed and archived according to Laboratory protocols described in this Section of the QA Plan and in Laboratory SOPs. Analytical areas have slightly different data reduction, validation and reporting protocols depending on the means by which the data is generated and specific method requirements.

### 10.1 Data Reduction

Data reduction is the process of transforming raw data into final results that are reported in standard units to Laboratory users. The Laboratory's goal is to minimize the steps needed to transform raw data into reportable results Laboratory SOP's include equations used to calculate results or a reference to the instrument manuals or methods that include the equations, the method of calculation and bench sheets used to record pertinent data.

#### 10.1.1 Manual Integration

Situations arise where the automated quantitation procedures in the GC/MS, GC, HPLC and IC software provide inappropriate quantitations. This normally occurs when there is compound co-elution, baseline noise, or matrix interferences. In these situations, the analyst must perform a manual quantitation. Manual quantitations are performed by integrating the area of the quantitation ion of the compound. This integration shall only include the area attributable to the specific target compound, or internal standard compound. The area integrated shall not include baseline background noise. The area integrated shall also not extend past the point where the sides of the peak intersect with the baseline noise. Manual integration is not to be used solely to meet Quality Control (QC) criteria, nor is it to be used as a substitute for corrective action on the chromatographic system. Manual integration must be documented. Where manual integration has been performed, most software will mark the integrated

area with the letter “M” on the quantitation report. Removal of data computer operational codes, such as the “M” flag is not allowed. A hard copy print-out of the quantitation report will be filed with the modified report if an electronic copy cannot be archived.

## 10.2 Data Validation and Approval

The analyst generating the analytical data has the primary responsibility for its correctness and completeness. It is his or her responsibility to verify that the instrument was calibrated and performing correctly. Analysts are responsible for analyzing the appropriate type and quantity of quality control samples with their daily work. Results must meet pre-established control limits. If control limits are not met the analyst is responsible for reanalyzing samples or documenting, justifying and flagging final analytical results or reported quality control data. If data is deemed unacceptable due to quality issues the data should not be reported. The Laboratory Supervisor must be notified and written notification must be provided to the client. The protocol for reporting data in which pre-established control limits are not met is described in Section 5.0 of this manual. Results can not be approved or released until the validation step is performed. It is the data reviewer’s responsibility to know the frequency and type of quality control samples required and acceptance limits for each method he/she is reviewing; including curve acceptance, continuing calibration and precision and accuracy criteria. If criteria are not met and data are not flagged the data reviewer must return the data to the analyst responsible for flagging results. If the data reviewer feels the data should not be reported due to quality issues it is his/her responsibility to notify the technical director of the analytical center or the Laboratory Supervisor. The Laboratory Supervisor or his designee reviews and approves all data for a given sample before it is released. This final review insures that all QC reporting and data qualifying requirements were met and results from different parameters for a given sample correlate. The dates of data entry, validation, and approval and the name of the employee responsible for each step are tracked. Method specific checks are being incorporated into SOPs as they are revised.

### 10.2.1 Organics

In the organic section, each data set has a data review check off list that must be completed by a second analyst. The following information is verified when applicable. Method specific checks and acceptance criteria will eventually be detailed in each laboratory SOP.

- Checks all worksheet header information for completion. Checks dates (extraction, analysis and calibration). Insures extraction and analysis dates and times are documented and entered.
- Checks initial calibration data against established criteria.
- All criteria for instrument tuning, internal standard areas, retention times, surrogate recoveries and analytical quality control results are checked.
- Checks all method quality control data to assure the correct type and amount of checks are performed and results are within control limits

- Compounds identified on the quantitation report must agree with results reported. All manual integrations must be properly documented.
- All calculations such as total volatile hydrocarbons, soil concentrations, percent recoveries and dilutions are checked.
- Verifies that data is correctly calculated and correct standard concentrations and dilutions have been entered
- All irregularities are properly documented and if necessary data flagged when pre-established control limits are not met.
- Verifies that sample dilution factors are accounted for in manual, instrument calculations.
- Reports only the parameters that were analyzed for on multi-parameter tests.

### 10.2.2 Inorganics/Metals

In the inorganic analytical section, the second analyst checks the following items when applicable. Method specific checks and acceptance criteria will eventually be detailed in each laboratory SOP.

- Analysis date and time (required when hold times are <72 hours) are documented on bench sheets.
- Insures all calibration and continued calibration criteria are met.
- Checks all method quality control data and documentation to insure the correct type and amount of checks are performed and results are within control limits.
- Checks all bench sheets for completion (i.e. Lot # of chemicals, QC identification, Initials, dates and times when required).
- Checks all manual calculations or data entry into calculation programs designed to calculate final results.
- Verifies that dilution factors have been properly accounted for and that standard and spike concentrations are correct.
- Checks to be sure any irregularity is documented and if necessary data flagged when pre-established control limits are not met.

### 10.2.3 Microbiology

Method specific checks and acceptance criteria will eventually be detailed in each laboratory SOP.

- Checks all Data Management System entries against bench sheets for transcription and reporting errors for manually entered information. All dilution calculations are checked. MPN values are rechecked against MPN Tables if the MPN Program has not been used.
- Insures that all data is flagged if the sampling time has not been provided.
- Insures that the date and time of analysis and the chemist initials are entered on bench sheets.
- Checks for completion of required bench sheet information.
- Insures that membrane filtration results are flagged with a “C” Colony Counts

- Outside Ideal Range, if counts are not within method recommended range.
- Insures that documentation of the notification of appropriate contacts has been made when acceptance limits are exceeded for clients that require immediate notification.

## 10.3 Data Reporting

### 10.3.1 Policy

Laboratory staff shall not release results (electronic, paper or verbal) to individuals outside the laboratory. All inquiries for information must be directed to the Laboratory Supervisor who will either obtain written permission (e-mail is acceptable) or forward the request to a Program or Project Manager. All records are held secure and confidential.

### 10.3.2 Final Report Format

Data is transmitted to Laboratory users in one of three ways: paper reports for each lab number, condensed reports when multiple results are reported on a page and electronic reports. Final reports for test data are issued only after internal review has been completed. Electronic transfer of data is an option available to laboratory users that have access to the laboratory network. Condensed and Electronic Reports do not contain all the information presented on paper reports. Clients receiving electronic reports are aware that information such as quality control data and sample and order comments are not presented. If an order has both Organic and Inorganic tests requested two separate reports for the same order ID will be generated. The reports have a different format. The cover page of lab reports have general comments and may have Order specific comments that have been added by the laboratory supervisor. When opinions and interpretations are included in a Report the laboratory documents the basis upon which the opinions and interpretations have been made. Opinions and interpretations are clearly identified as such. If a Report has been revised this will be indicated on the cover page.

## 10.4 Data Tracking and Record Storage

### 10.4.1 General Information

The Laboratory has policies and procedures for the retention and disposal of all quality and technical records (see Summary Table 10.1 Record Storage and Retention Times). The record keeping system allows for the reconstruction of all activities required to produce an analytical result. All records are stored under appropriate conditions for the type of media (electronic or hard copy), and are readily retrievable to individuals that are allowed access. Backup and access policies for electronic files are in place. Records must be legible and held secure and in confidence for a minimum of 5 years. Records may be destroyed after the minimum required hold times have been exceeded. In the event that the Laboratory is closed, all electronic and paper records will be transferred to the State Public Records center.

### 10.4.2 Sample Handling and Receiving

Records are maintained for all procedures and policies pertaining to sample handling and receiving for a minimum of 5 years. Records of any deviations from policies are also retained either on bench sheets.

### 10.4.3 Technical Records

#### 10.4.3.1 Paper Records

Original raw data for calibrations, samples and quality control measures, worksheets, and instrument response records are stored for a minimum of 5 years. Vendor supplied standard certification paperwork is archived at the Laboratory for a minimum of 5 years. If records must be retained indefinitely they are transferred to the State Public Records facility and microfilmed. Once microfilmed the original records are destroyed. Analyst observations and calculations are documented at the time of analysis and retained with the raw data. All written records are documented in permanent ink. Errors in records must be corrected by drawing a single line through the error. The correct value is entered alongside the incorrect entry with the initials and date of the individual making the correction. When results are changed due to reasons other then transcription errors the reason for the correction must be obvious, if it is not, the analyst must document why the documented result has been modified. Laboratory notebooks and logs (paper) are tracked and archived for a minimum of 5 years and can not be destroyed without the Laboratory Supervisor's consent. The notebooks are stored within each analytical section. Information contained in notebooks includes sample processing steps and details such as: extraction and digestion records, instrument maintenance and routine checks, data reduction and transformation steps and standard and reagent receipt and preparations. Earlier revisions of Standard Operating Procedures (SOPs) and Quality Assurance Plans are archived (paper and electronic). The document control system used in this QA Plan (upper right hand corner of page) is also used for lab SOPs.

#### 10.4.3.2 Electronic Records

Electronic logs and bench sheets are stored as both paper and electronic copies in most instances. Electronic Logs that are not printed out and retained with the raw data are periodically archived on CD. A detailed data archiving protocol is under development. Records that are stored or generated by computers must be retained as a hard copy or have a write protected electronic copy.

## 11.0 QUALITY CONTROL SAMPLES AND ROUTINES USED TO ASSESS ACCURACY AND PRECISION

The purpose of this section is to define quality control procedures that are necessary to develop information which can be used to evaluate the quality of analytical data. Quality control (QC) terms are defined and an explanation of how, when and why QC samples are taken or analyzed is provided. This section is intended to be used as a guideline for laboratory users.

## 11.1 Field Quality Control Samples

The results of quality control samples taken in the field reflect the precision and accuracy of the entire process, from sample collection through analyses. Below is a brief description of quality control samples laboratory users should collect when appropriate. Samples may be logged in as “blind” samples if desired. Synonymous terms are provided in parenthesis.

### 11.1.1 Blanks

#### 11.1.1.1 Equipment Blanks

Equipment Blanks are a type of field blank used to determine if contamination has been introduced through contact with sampling equipment or to verify effectiveness of equipment cleaning procedures. Laboratory water free of analyte is transported to the site and processed through the sample collection device, preserved if necessary and returned to the lab for analysis. Laboratory water should not be stored for future use, a hold time of one week is recommended. Do not contaminate the carboys with field equipment. Do not use water from other sources or return water to the carboy. Equipment blanks should be processed whenever contamination is suspected, with each analytical batch or every 20 samples. Corrective action for contamination detected in equipment blanks is addressed by laboratory users evaluating data.

#### 11.1.1.2 Field Blanks

Field Blanks are used to determine if method analytes or other interferences are present in the field environment. This would include contamination from sample bottles, storage, transport and sample preparation. A field blank is usually laboratory deionized water that is transported to the sampling site, opened to the contaminated environment, and processed as a sample (filtration, preservation, etc.). One field blank should be submitted with each analytical batch or every 20 samples or whenever contamination is suspected. Contamination detected in field blanks would need to be evaluated by both field and laboratory personnel.

#### 11.1.1.3 Filter Blanks

Filter Blanks (Cartridge Blanks) are used to determine if method analytes or other interferences are introduced during the filtration or sampling process. Laboratory water is used to rinse the filter and filtration apparatus. Air filter blanks may also be submitted to determine if sample breakthrough has occurred. At least one filter blank should be processed with each sample batch or whenever contamination is suspected.

#### 11.1.1.4 Trip Blanks

Trip Blanks are routinely used when sampling for volatile organic compounds. Volatile organic compounds are most susceptible to this type of contamination. The laboratory supplies samplers with a VOA vial containing acidified analyte free water. The vial is transported to the sampling site and returned to the lab without being opened. Sample contamination from penetration of the Teflon cap by halogenated solvents during transport or at the site can be detected with a trip

blank. Trip blanks are logged into the data management system and are assigned a sample ID number.

### 11.1.2 Precision and Accuracy Checks

#### 11.1.2.1 Field Duplicates

Field Duplicates (duplicate samples, replicate samples) are two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Results give a measure of the precision associated with sample collection, preservation and storage as well as with laboratory procedures. Field duplicate data provides the best measurement of precision from sample collection through analyses. Field duplicates should be taken on 5% of the sample volume. Duplicates are logged in as individual samples and can arrive at the laboratory as “blind” duplicates if the laboratory user desires. A field duplicate should not be confused with a split sample (Section 11.1.2.3).

#### 11.1.2.2 Matrix Spikes

Matrix Spikes are the same as analytical matrix spikes (Section 11.2.4.3) except that spiking is done in the field. Spiking samples in the field is less reliable and more difficult than spiking in the laboratory and is not recommended.
Results from analytical matrix spikes are used to detect matrix interference and measure method accuracy. If a sample is spiked a percent recovery is provided on the Final Laboratory Report. Spiked sample recovery results are useful to laboratory users.

#### 11.1.2.3 Split Samples

Split samples are aliquots of samples taken from the same sample container after thoroughly mixing or compositing the sample. They are analyzed independently and are used to document intra- or interlaboratory precision. Split samples may also be used to request matrix spike analysis for tests requiring two separate samples.

#### 11.1.2.4 Blind Samples

Blind samples are sample(s) submitted to the lab for analysis, the composition or origin of the sample is known to the submitter but unknown to the analyst. Blind samples can be a duplicate sample, blank, proficiency sample, or an interlab comparison sample.

## 11.2 Analytical Quality Control Samples, Solutions and Routines

Results of analytical quality control samples are used to estimate the precision and accuracy of data from sample preparation through analysis. Some of the data are reported with the associated sample result(s). In addition to the quality control samples in which results are released with the associated data there are several types of samples (solutions) that may be analyzed or procedures performed to verify the precision and accuracy of the entire system. Results may be used to verify calibration, identify reporting limitations or to help identify and if

possible correct for instrument, method or sample interferences. Not all of these sample types will apply to every analysis; some are instrument and method specific. Data acceptance criteria are method specified and if no specifications are provided they are based on historical data or internally established.

### 11.2.1 Negative Control (Blank) – Method Performance

The level of analyte of interest detected in the Method, Continuing Calibration, or Initial Calibration Blank is evaluated in relation to the sample result being reported within the batch. The general laboratory policy is to qualify any reported analytical results analyzed in the batch. If the blank is > ½ PQL but <PQL and the results are > 2X Blank no comment is needed. Analyst/Supervisor discretion must be used when reporting results. If there are method or regulatory requirements for qualifying data associated with Blank results those requirements must be followed. If the minimum acceptance limit is exceeded one of the following corrective actions must be taken. They are listed from the most to least desirable course of action.

The source of the irregularity must be found and corrected and the blank and samples re-prepared and reanalyzed.

All associated sample results and the blank(s) must be flagged with a "BH" Remark Code and either an Order Comment or Sample Comment describing the Irregularity must be added.

#### 11.2.1.1 Initial Calibration Blanks – ICB

Initial Calibration Blanks are aqueous solutions prepared and diluted with the same volume of chemical reagents and solvents used in the preparation of the primary calibration standards. They may be used to give a null reading for the instrument response when running a calibration curve and to establish instrument background. The initial calibration blank does not assess for possible contamination during the preparation and processing steps. The ICB is analyzed as a sample at the beginning of the analytical run.

#### 11.2.1.2 Continuing Calibration Blank – CCB

Continuing Calibration Blank is the ICB solution that is reanalyzed throughout an analytical run to assess baseline drift.

#### 11.2.1.3 Method Blank

Method Blank (Laboratory Reagent Blank, Preparation Blank), is a volume of deionized laboratory reagent water carried through the entire analytical procedure including all preparation and processing steps. The Method Blank contains the same reagents as the samples. Analysis of a method blank verifies that interferences from contaminants in solvent, reagents, glassware and other sample processing devices are quantified. A method blank is analyzed at a minimum of 1 per preparation batch (20 samples) for methods that have a preparation procedure.

### 11.2.2 Positive Controls – Method Performance

#### 11.2.2.1 Laboratory Control Samples – LCS

Laboratory Control Samples – LCS (Blank Spike, Laboratory Fortified Blanks) are prepared by adding known quantities of the method analyte(s) to a volume of reagent water. Laboratory Control Samples must be processed at a minimum of 1 per preparation batch (20 samples). The LCS solution is the same solution used for matrix spikes. The LCS must be processed exactly like samples within the analytical batch. The concentration is typically mid-range but must be within the calibration range. LCS results are used to evaluate the total analytical process including all preparation and analysis steps. LCS results are also used to validate or reject matrix spike recovery results since the solution used to spike the LCS is the same solution used for sample matrix spikes.

The results of LCS are reported as a percent recovery. LCS control limits are those established in the referenced method. If there are no established criteria, the lab determines internal criteria based on historical data. If an LCS recovery is outside the control limit the LCS solution is reanalyzed. If the reanalysis of the solution is acceptable a note is made on the bench sheet and results are accepted. Any samples associated with an unacceptable LCS must be reprocessed and re-analyzed or the associated samples and LCS results are to be reported with a data qualifier. For multi-parameter methods, the components to be spiked shall be as specified by the referenced test method or other regulatory requirement.

#### 11.2.2.2 Quality Control Sample – QCS (Certified Reference Material CRM: Standard Reference Material SRM)

Quality Control Sample – QCS can be either an uncontaminated sample matrix, (i.e. fish, soil, ash) spiked with known amounts of analytes or a contaminated sample matrix. The QCS is a NIST certified standard purchased to establish intra-laboratory or analyst specific precision and bias or to assess the performance of the measurement system. QCS results are tracked.

### 11.2.3 Standards – Method Calibration

#### 11.2.3.1 Primary Calibration Standards

Primary Calibration Standards (Primary Standard, Calibration Standard) are prepared from dilutions of a NIST traceable stock standard solution or are prepared in-house from reagent grade materials. The standards are used to calibrate the instrument response with respect to analyte concentration.

#### 11.2.3.2 Initial Calibration Verification Standard – ICV

Initial Calibration Verification Standard – ICV (Second Source Standard, Quality Control Check Sample-QC or C, Initial Performance Check-IPC) is a certified reference standard from a source different then the primary calibration standard. When available they are processed the same as the primary calibration standard and are an independent check on the primary standard used to calibrate the instrument.

ICVs are analyzed immediately following calibration and determine if sample analysis can proceed. The concentration of the ICV is approximately the midlevel of the calibration range. If acceptance limits are not method specified they are established in-house. If the first analysis does not produce an acceptable result the sample may be reanalyzed once. If the second attempt does not generate an acceptable result the analysis of samples may not proceed. The source of the error needs to be determined and corrective actions taken. The Laboratory Supervisor must be consulted and associated data will likely be qualified. The client may be contacted prior to releasing data. The second source standard result generated at the beginning of the run is calculated in percent recovery and tracked.

#### 11.2.3.3 Continuing Calibration Verification Standard – CCV

Continuing Calibration Verification Standard – CCV (Calibration Check Standards, Same Source Standard, Calibration Check Compounds, Calibration Verification Check – CVC, or Continuing Calibration Check Standards – CCC) is a primary calibration standard(s) that is reanalyzed with test samples to verify continued calibration of the analytical system. Continued Calibration Standards are analyzed at the beginning and end of the analytical run and after every 10 samples for large analytical runs. The concentration of the CCV at the beginning and the end must be varied within the calibration range. The CCV is expressed as a percent recovery. Reported results need to be bracketed by acceptable CCVs. If limits can't be met affected samples can be reanalyzed once prior to taking corrective actions.

Under unusual circumstances results may be reported without a passing CCV. The Laboratory Supervisor should be consulted and associated data will likely be flagged. The client may be contacted prior to releasing the data.

#### 11.2.3.4 Internal Standards

Internal Standards are used for some organic methods and ICP/MS technology. The standards are added to every standard, blank, matrix spike, matrix spike duplicate and sample extract at a known concentration prior to analysis. Internal standards are used as the basis for the quantitation of the target compounds for several organic methods. For ICP/MS the internal standard solution is used to monitor the analysis for matrix effects and correct for instrument drift throughout the analysis.

### 11.2.4 Precision and Accuracy Checks – Sample Specific Controls

#### 11.2.4.1 Analytical Sample Duplicate

Analytical Sample Duplicate (Duplicate, Lab Duplicates) are two aliquots taken from the same sample container that are processed and analyzed separately. Results are used to measure analytical precision from sample preparation through analysis for a given matrix. A minimum of 5% of all samples are analyzed in duplicate. When a sample is analyzed in duplicate the first result

recorded appears in the final laboratory report result column. The second result and the relative percent difference (RPD) of the duplicate values are reported in the QC results section of the report. The RPD calculation can be found in Section 14.1 of this manual.

Historical data from the analysis of laboratory duplicates are used by the laboratory to establish precision control limits. If there are method specified limits they must be met. Sample results in which the sample duplicate Relative Percent Difference (RPD) is outside the laboratory control limit must be flagged. If a result is outside the established control limit (OOC) the analyst must:

Flag the QC result that is OOC.

Flag associated sample results with an appropriate Sample Remark Code and provide a Sample or Order Comment if further qualification is needed or warranted.

Notify the Laboratory Supervisor if the analyst is unsure whether results should be reported.

When control limits are exceeded by 5% or more the analyst must take further action to assure that a correctable error was not the cause of the irregularity. The analyst must re-prepare and reanalyze the sample in duplicate (i.e. if the control limit is 10%, analysis resulting in RPDs >15% require reanalysis of the original sample). The analyst is allowed to repeat analysis once. If after re-analysis the RPD falls within the established limit the new result(s) can be reported as long as there is clear documentation and traceability of the reported result. If the RPD is still outside limits and if all other QC within the run are acceptable the analyst can report the initial result(s) if properly qualified. If there is evidence that the analytical system is not in control analysis must stop and results must not be reported. In some situations reanalysis is impossible (insufficient sample volume) or impractical (hold time has been exceeded or there is a known documented interference that can not be corrected for). If the analyst suspects that a processing error occurred that impacts all of the samples analyzed then the entire analytical batch must be reprocessed and reanalyzed. When results for a method are consistently below the reporting limit, precision data is obtained from the analysis of matrix spike duplicates (11.2.4.4) when spiking solutions are available.

#### 11.2.4.2 Instrument Duplicates

Instrument Duplicates are two aliquots taken from the same extract or digestate and analyzed in duplicate. Results are used to measure instrument precision only. The average value of instrument duplicates may be reported, however method precision may not be calculated using instrument duplicates for methods requiring predigestion, extraction or any other sample preparation steps.

#### 11.2.4.3 Matrix Spikes – MS

Matrix Spikes – MS (Laboratory Fortified Sample Matrix) are prepared by adding a predetermined quantity of stock solution of the analyte(s) being measured to a sample prior to sample extraction/digestion and analysis. The stock solution must

be the same solution used to prepare the LCS. The concentration of the spike should be at the regulatory standard level or spiked at a level that will result in a final concentration that is approximately 1.5 times the unspiked concentration. The volume of the spiking solution must be less than 5% of the sample volume being spiked. A portion of the unspiked and the spiked sample are analyzed and a percent recovery is calculated (Section 14.2). Recovery data provides a measure of accuracy for the method used in a given matrix. Recovery results verify the presence or absence of matrix effects and are particularly important when analyzing complex matrices (soil, sludge, sediment or samples with interferences). Five percent of all samples received at the lab are spiked when sufficient sample volume is provided or at a rate specified by the test method or project plan. If a sample is spiked the calculated percent recovery is reported on Final Laboratory Report forms. Samples of some methods cannot be spiked (i.e., chlorophyll, dissolved oxygen, turbidity). Samples to be spiked are selected by the analyst unless they are pre-selected by laboratory users. Acceptance limits for matrix spikes analyzed at the lab will vary depending on the analysis, matrix and sample concentration level. Acceptance limits are either method specified or established from historical laboratory results. The narrower limits must be used. If recovery data is unacceptable, and the laboratory control sample (LCS) is within acceptance limits a matrix interference may be the cause of the irregularity. Sample results associated with a Matrix Spike Recocvery (MS) outside the laboratory control limit(s) must be flagged. If a result is outside the established control limit (OOC) and the LCS is acceptable the analyst must:

Flag the QC result that is OOC.

Flag associated sample results with an appropriate Sample Remark Code and provide a Sample or Order Comment if further qualification is needed or warranted.

Notify the Laboratory Supervisor if the analyst is unsure whether results should be reported.

When control limits are exceeded beyond 10%, the analyst must take further action to assure that a correctable error was not the cause of the irregularity. The analyst must re-prepare and reanalyze the sample and matrix spike (i.e. if limits are 80-120%, reprep and analysis is required if outside 70-130%). The analyst is allowed to repeat analysis once. If after re-analysis the result(s) fall within the established limits the new result(s) can be reported as long as there is clear documentation and traceability of the reported result. If the MS result is still outside limits and all other QC within the run are acceptable the analyst can report the initial result(s) if properly qualified. If there is evidence that the analytical system is not in control, analysis must stop and results must not be reported. In some situations reanalysis is impossible (insufficient sample volume) or impractical (hold time has been exceeded or there is a known documented interference that can not be corrected for). If an interference is suspect, the analyst may spike a series of dilutions to verify and eliminate or reduce the effect of the interference. In some instances analysts are able to eliminate the interference and report uncompromised data. However, if the required dilution is large and the original sample is no longer represented in the diluted sample or

the analyte of interest is diluted below the PQL results may not be reported or reported as <PQL unless properly qualified. The client is responsible for determining the usability of flagged Matrix Spike sample results.

#### 11.2.4.4 Matrix Spike Duplicate – MSD

Method precision can also be calculated from matrix spike duplicates. Matrix spike duplicates are used to estimate method precision for analytes that are frequently found below the practical quantitation limit. A second aliquot of the sample is treated like the original matrix spike sample. The relative percent difference (RPD) of the matrix spike and the matrix spike duplicate is calculated and is used to assess analytical precision. Final laboratory reports indicate when RPD values are calculated from matrix spike duplicates.

#### 11.2.4.5 Surrogates

Surrogates are organic compounds, which are not found in environmental samples, but have similar chemical structures, and extraction and/or chromatography properties. These compounds are spiked into all blanks, calibration and check standards, samples (including duplicate and laboratory control samples) prior to analysis by GC or GC/MS. Percent recoveries are calculated for each surrogate. Surrogate compounds and their acceptable recovery ranges are specified in analytical methods and are listed in Standard Operating Procedures (SOPs) for organic methods. The Laboratory tracks surrogate recovery results and when sufficient historical data is available internal control limits are established. Recovery data is reported with every sample result. When a recovery value is not within acceptance limits calculations and surrogate solutions are rechecked. Samples or extracts may be reanalyzed. If results are still not within suggested limits a flag “S-surrogate recovery outside acceptance limits” must be added next to the surrogate result that exceeds a criterion.

### 11.2.5 Limits

#### 11.2.5.1 Method Detection Limits (MDL)

Method Detection Limit is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. It is determined from repeated analysis of a low level sample in a given matrix containing the analyte at a predetermined level. MDLs are determined annually for most analytes and matrices. The process and formula used to generate MDLs are described in Section 14.4.

#### 11.2.5.2 Practical Quantitation Limit (PQL)

Practical Quantitation Limit (PQL) (Reporting Limit) is the lowest level that can be reliably achieved during routine laboratory operating conditions. The PQL is approximately two to ten times the calculated MDL. PQLs are a preferred reporting limit because MDL values will change each time they are calculated even though the analytical procedures, instruments and sample matrices are the same. By reporting the low level value as less than (<) the established PQL, laboratory users can be certain that any reported value is reliable and that

reporting limits will remain relatively constant. In the organics analytical center a N.D. (not detected) appears on final laboratory report forms rather than <PQL value. However, if a compound is detected at a level that is less than the PQL and the value is no less than one half the PQL; a <"PQL" (of the compound in question) is reported rather than a N.D. For organic results the Laboratory Reporting Limit (PQL) must increase if sample dilution is required. The increase in the PQL will be equivalent to the dilution factor. For multi parameter methods the analyst must make an effort to report results from the least dilute analysis for each parameter. If sample results are reported from two analyses, then the PQL is increased for the parameters reported from the diluted sample. If for some reason it is impractical to increase the PQL at final report time, another form of qualification must be implemented.

#### 11.2.5.3 Instrument Detection Limit (IDL) (ICP-MS only)

Estimated by calculating the average of the standard deviation of three runs on three non-consecutive days from the analysis of a reagent blank solution (which is equivalent to a calibration blank for waters) with seven consecutive measurements per day. Each measurement must be performed as though it were a separate analytical sample. IDLs must be determined at least every three months.

#### 11.2.5.4 Preparation Batch

Preparation Batch is composed of one to twenty environmental samples of the same matrix that are prepared and analyzed together with the same process and personnel, using the same lots of reagents. The maximum time between the start of processing of the first and last sample in a preparation batch is 24 hours.

#### 11.2.5.5 Analytical Batch

Analytical batch is defined as a group of samples (extracts, digestates or environmental samples) that are analyzed together with the same method and personnel, using the same lots of reagents and having a defined set of quality control samples analyzed with the samples. Several preparation batches can be analyzed together in an analytical batch but each preparation batch must have associated QC data.

### 11.2.6 Instrument Checks

#### 11.2.6.1 Tuning Solutions

Tuning Solutions are used to verify that the resolution and mass calibration of the instrument are within required specifications prior to calibration and sample analysis (GC/MS, ICP/MS).

#### 11.2.6.2 Interference Check Solutions (ICS)

Interference Check Solutions (ICS) contain known concentrations of interfering elements. They are analyzed prior to samples to demonstrate that correction equations are adequate (ICP/MS).

## 12.0 AUDITS AND DEMONSTRATIONS OF CAPABILITY

System and performance audits are used to assess the overall effectiveness of the City of _____ Laboratory's quality assurance program. Demonstration of capability must be made prior to using a test method or if there is a change of equipment type, personnel or test method.

### 12.1 System Audits

A system audit is a qualitative evaluation of all components of a measurement system. System audits can be conducted by external auditing authorities (external audit) or can be conducted inhouse (internal audit).
Internal System Audits are a tool to: verify analyst compliance with the laboratories quality policies; to address any on going quality issues; and to highlight technical, equipment or management support needed within the analytical center being audited. It is the responsibility of the Laboratory's QA Officer to plan and organize internal audits within each of the laboratory's analytical sections. Internal audits of each analytical section are performed annually. Audits are conducted by qualified personnel that are independent of the activity being audited. Under certain circumstances a qualified chemist from another organization assists in the audit. Internal audits generally review all aspects of sample analyses from sample preparation to data reporting and review. In some instances the analyst is required to analyze a sample(s) of unknown concentration(s) while the auditor(s) observe. All notebooks and records are checked for traceability. SOPs are reviewed prior to the audit to assure written protocols are being followed. Checklists from external auditing organizations are often used. Previous audit reports are reviewed prior to an internal audit to assure that previously recommended corrective actions have been implemented. An Audit Report summarizing the method(s) reviewed and findings and recommendations is distributed to management and analysts audited. The analysts being audited have an opportunity to add to the Audit Report any comments or recommendations to management that would assist in improving the overall function of the section and the quality of data being generated. If during the course of an audit or at any other time a significant departure from the QA Plan policies, method SOP, or requirements are revealed, the findings will be documented and corrective actions will be required. The need to contact customers will depend on the severity of the departure and the effect the departure had on released data. The Laboratory Supervisor must notify clients in writing if audit findings cast doubt on Laboratory results. Follow-up audit activities shall verify and record the implementation and effectiveness of the correction action taken.

### 12.2 Performance Audits

Performance audits determine quantitatively the accuracy of analytical data. This is primarily accomplished by means of interlaboratory performance evaluations. Laboratory staff analyze reference materials and are rated on their performance. Each proficiency provider has a unique rating system and acceptance criteria

and vary in difficulty. Proficiency samples must be handled in the same manner as real environmental samples. This includes using the same staff, methods, procedures, frequency of analysis, reporting protocol, equipment and facility used for routine analysis. The Laboratory maintains records of the analysis of all PEs for at least 5 years. Proficiency audit results are reviewed by the Laboratory Quality Assurance Officer and distributed to the Laboratory Supervisor, laboratory staff and to laboratory users when requested.

Unacceptable results or trends from proficiency evaluations may necessitate an internal audit of the method in question. The audit is initiated by the Laboratory Quality Assurance Officer who may submit blind check samples for analysis.

**12. 2.1 Water Pollution Study (WP Series)** - semi-annual evaluation. Results are submitted for both methods of analysis when the laboratory reports results by more than one method.

- Trace Metals (aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, iron, lead, mercury, manganese, molybdenum, nickel, selenium, vanadium, zinc, silver, strontium, thallium, uranium)
- Minerals (spec. cond., total dissolved solids, total hardness, calcium, magnesium, total alkalinity, chloride, sulfate, sodium, potassium)
- Nutrients (ammonia as nitrogen, nitrate as nitrogen, total Kjeldahlnitrogen, total phosphorus)
- Demands (COD, 5-day BOD)
- PCB's in water
- Pesticides (chlordane, aldrin, dieldrin, DDD, DDE, DDT, heptachlor, heptachlor-epoxide)
- Volatile Halocarbons
- Volatile Aromatics
- Miscellaneous Parameters (non-filterable residue, total suspendable solids, volatile solids, total residual chlorine, pH, turbidity, nitrite, silica)

## 12.3 Demonstration of Capability

### 12.3.1 Initial Demonstration of Capability (DOC)

#### 12.3.1.1 New Method or Technology DOC

The demonstration of capability must be made prior to using any test method or any time there is a change of instrument type or test method. If there are method specified criteria they must be followed. When the method does not specify a procedure, the following steps should be documented when applicable:

- The lab supervisor of each analytical section shall participate in vendor provided training courses when new technology is employed.
- Demonstration of linearity.
- Method Detection Limit study.
- Accuracy - typically demonstrated by analysis of an internal blind NIST traceable standard at one or more concentrations.
- Precision - repeated analysis of a known sample four times.

#### 12.3.1.2 Precision and Accuracy Assessment

- A quality control sample shall be obtained from an outside source. If not available, the QC sample may be prepared by the Laboratory using stock standards that are prepared independently from those used in instrument calibration.
- The analyte(s) shall be diluted in a volume of clean matrix sufficient to prepare four aliquots at the concentration specified, or if unspecified, to a concentration approximately 10 times the method-stated or Laboratory calculated method detection limit.
- At least four aliquots shall be prepared and analyzed according to the test method either concurrently or over a period of days.
- Using all of the results, calculate the mean recovery (X) in the appropriate reporting units (such as µg/l) and the standard deviations of the population sample (n-1) for each parameter of interest. When it is not possible to determine mean and standard deviations, such as for presence/absence and logarithmic values, the Laboratory must assess performance against established anddocumented criteria.
- Compare the information from above to the corresponding acceptance criteria for precision and accuracy in the test method (if applicable) or in Laboratory-generated acceptance criteria (if there are not established mandatory criteria). If all parameters meet the acceptance criteria, the analysis of actual samples may begin. If any one of the parameters do not meet the acceptance criteria, the performance is unacceptable for that parameter.
- When one or more of the tested parameters fail the acceptance criteria, the analyst must locate and correct the source of the problem and repeat the test for all parameters of interest. Repeated failure, confirms a general problem with the measurement system that must be corrected. For some methods NIST traceable standards are not available.

#### 12.3.1.3 New Analyst Demonstration of Capability (DOC)

A demonstration of ability is required prior to a new analyst reporting results for an established method that has an initial method/instrument DOC on file but has not previously been performed by him/her. The analyst must review all referenced methods, pertinent instrument manuals, and current method SOP. The analyst must observe the current analyst through all aspects of the procedure (sample preparation through reporting). The new analyst must also be observed processing QC samples to assess accuracy and precision by the primary analyst. Requirements vary depending on the complexity of the equipment or procedure to be performed. At a minimum the analyst must be able to accurately analyze a blind Proficiency Sample and meet established internal quality control limits for accuracy and precision.

#### 12.3.1.4 Analyst files are to contain training documentation.

### 12.3.2 Continued Demonstration of Proficiency

Analyst must demonstrate continued proficiency at least once a year for tests they are reporting results for. Analyst training files must contain a copy of a proficiency sample result that is rated acceptable.

## 13.0 PREVENTATIVE MAINTENANCE

Preventative maintenance is scheduled for most analytical equipment within the City of _____ Laboratory to minimize poor performance, instrument down time and subsequent "interruption" of analysis. Major analytical equipment is maintained under service contract, other instruments are maintained by a qualified analytical instrument repair service. Preventative maintenance schedules are listed in Table 13.1. Routine maintenance is performed on all analytical equipment by qualified Laboratory personnel. When it is practical, an inventory of critical replacement parts and spare parts needed for routine maintenance is maintained for each instrument. Logbooks are kept for each major instrument to document instrument problems, repairs and routine maintenance.

**Table 13.1 Laboratory Instrument Maintenance Schedules**
**INORGANIC**

| Instrument | Manufacturer | Model | Contractor | Preventative Maintenance Schedule |
|---|---|---|---|---|
| pH/Millivolt Meter | Orion | 720A | QC Services | 1/year |
| Non-Ratio Turbidity meter | HF Scientific | Micro 100 | QC Services | 1/year |
| Spectrophotometer | Genysis | Thermo Spectronic 10 | QC Services | 1/year |
| Dissolved Oxygen Meter | YSI | 5100 | QC Services | 1/year |
| Fluorometer | Turner | TD-700 | QC Services | As Needed |
| COD Reactor | Hach | 45600 (2) | QC Services | 1/year |
| Centrifuge | International Equipment | EXD | QC Services | As Needed |
| TCLP Extraction Apparatus | Millipore | — | — | — |
| Conductance Meter | YSI | 3200 | QC Services | 1/year |
| Pensky Martin Flashpoint Apparatus | — | — | — | — |
| Oven | Precision | – (2) | QC Services | 1/year |
| Chlorine Pocket Colorimeter | Hach | | — | — |
| Micro Distillation System | Lachat | — | Lachat | — |
| Auto Analyzer Systems | Lachat | QC 8000 | Lachat | As Needed |
| Auto Analyzer System | Lachat | QuickChem FIA 8000 Series | Lachat | As Needed |
| Ion Chromatograph | Dionex | DX 320 | Dionex | 1/year |
| Sonification Bath | Ultra Clean Equipment | — | — | — |

## METALS

| Instrument | Manufacturer | Model | Contractor | Preventative Maintenance Schedule |
|---|---|---|---|---|
| ICP/MS | Thermo-Elemental | X Series | Thermo-Elemental | 1/year |
| Automated Mercury Analyzer | Perkin-Elmer | FIMS100 | Perkin-Elmer | As Needed |
| Microwave Digestion Furnace | CEM | MDS 2100 | CEM | As Needed |
| Hot Block Digestors | Environmental Express | (2) | — | — |
| Water Bath | Precision | — | — | — |

## MICROBIOLOGY

| Instrument | Manufacturer | Model | Contractor | Preventative Maintenance Schedule |
|---|---|---|---|---|
| Autoclave | Castle | 122LS | Castle | 4/year |
| Air Incubator | Boekel | 133000 | — | — |
| Waterbath | Precision | 260 | — | — |
| Air Incubators | Fisher Scientific | 650F(2) | — | — |
| Quanti Tray Sealer | IDEXX | 2X | — | — |

## ORGANICS

| Instrument | Manufacturer | Model | Contractor | Preventative Maintenance Schedule |
|---|---|---|---|---|
| GC/MS system | HP | 6890/5973 (2) | Hardware and Software Systems (HSS)[a] | 1/year |
| GC system | HP | 5890 (TPH only) (FID Detector) | HSS | As Needed |
| GC System (Volatiles) | HP | 5890 (PID/FID Detectors) | HSS | As Needed |
| TurboVap Evaporator | Zymark | 500 (1) | Caliper | |
| HPLC | Waters | 2487 | Waters | 1/year |
| Cryogenic Concentrator | Entech | 7100 | Entech | As Needed |
| Canister Cleaner | Entech | 3100 A | Entech | As Needed |
| Gas Mixing System | Entech | 4600A | Entech | As Needed |
| Pure Air Generator | Aadco | 737 | — | — |
| Autosampler | Entech | 7016CA | Entech | As Needed |
| GC System (Pesticides/PCBs) | HP6890 Dual ECD Detectors | — | Hardware and Software Services (HSS) | As Needed |
| Purge and Trap with Archon Autosampler | Tekmar | 3100 | Varian | As Needed |
| Purge and Trap with Autosampler (Aquatec 70) | Tekmar | 3000 | — | As Needed |

## ANALYTICAL BALANCES

| Instrument | Manufacturer | Model | Contractor | Preventative Maintenance Schedule |
|---|---|---|---|---|
| Balance | Mettler | AE200 | QC Services | 1/year |
| Balance | Mettler | AT400 | QC Services | 1/year |
| Balance | Mettler | PM400 | QC Services | 1/year |
| Balance | OHAUS B | 1500D | QC Services | 1/year |

**MISCELLANEOUS**

| Instrument | Manufacturer | Model | Contractor | Preventative Maintenance Schedule |
|---|---|---|---|---|
| Exhaust Hoods (9) | Peck | — | — | 1/year |
| Microzone Hoods (2) | ENV. Service | — | — | 1/year |
| Refrigeration Units (11) | — | — | — | — |
| Glassware Washer | Steris | — | — | 4/year |
| Water System | U.S. Filter | MilliRo 120 D. I. | Ionpure | 2/year |
| Electronic Pipettes (12) | Rainin | — | — | 1/year |
| Manual Pipettes (7) | Rainin/Eppendorf | — | — | 1/year |

[a] QC Services is ISO 9002 registered, ISO 17025 compliant.

## 14.0 PROCEDURES USED TO CALCULATE AND ASSESS DATA QUALITY

This section describes the data quality indicators that are tracked. Equations for precision, accuracy, completeness and method detection limits are provided. Method specific calculations can be found in Laboratory SOPs.

### 14.1 Precision

Precision is a measure of how well replicate measurements reproduce and can be calculated from laboratory duplicates, instrument duplicates, duplicate analysis of a Laboratory Control Sample (LCS) , method blank duplicates (MBD) or matrix spike duplicates (MSD). Relative percent difference (RPD) is the current measure of precision for most analytes and is calculated as follows:

$$RPD = \frac{(C1 - C2)}{m} \times 100\%$$

where: RPD = relative percent difference
C1 = larger of the two observed values
C2 = smaller of the two observed values
m = mean of two observed values

If calculated from three or more replicates, relative standard deviation (RSD) is calculated rather than RPD:

$$RSD = (s/m) \times 100\%$$

where: RSD = relative standard deviation
s = standard deviation
m = mean of replicate analyses

### 14.2 Accuracy

Accuracy is a measure of how near a result is to the true value and is expressed as a percent bias or percent recovery. Method accuracy is determined from the

analysis of a laboratory control sample, continuing calibration check, quality control check samples or matrix spikes. Method accuracy and matrix effects are assessed by evaluating matrix spike results. The amount of analyte recovered after a sample has been spiked and processed reflects matrix effects upon the accuracy of the method. Percent recovery is calculated from matrix spike results using the following equation:

$$\% R = \frac{100 \times S - U}{Csa}$$

where: %R = percent recovery
S = measured concentration in spiked aliquot
U = measured concentration in unspiked aliquot
Csa = actual concentration of spike added
The above calculation does not take spike volume into consideration. Lab protocol requires that a <5% volume change occurs when a spike is added negating the need to volume correct. Percent bias is another measure of accuracy and is calculated using the following equation:

$$\% B = \frac{100 \times (O-T)}{T}$$

where: %B = percent bias
O = measured concentration of reference material
T = actual concentration of reference material

### 14.3 Completeness

Completeness is defined as the number of measurements judged valid compared to the number of measurements needed to achieve a specified level of confidence in decision making. The number of measurements judged valid must be determined by laboratory users familiar with the collection site, laboratory detection limits, and anticipated sample concentrations. Measurements judged invalid or suspicious by laboratory staff will be
flagged on final laboratory report forms and should be considered in completeness calculation. Laboratory data flags of importance to laboratory users are < “less than” flags and those summarized in Section 5.0 of this manual. Completeness is calculated as follows:

$$\% C = \frac{100 \times V}{n}$$

where: %C = percent completeness
V = number of measurements judged valid
n = total number of measurements necessary to achieve a specified level of confidence in decision making

## 14.4 Detection Limits

### 14.4.1 Method Detection Limits

Method Detection Limits (MDLs) are defined in the Federal Register as “the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte”. The Method Detection Limit is a statistical determination of precision only and is not used as a reporting limit. Method Detection Limit studies are part of a methods initial demonstration of ability if there is a spiking solution available. If a Method requires that MDL studies be performed the study must be repeated at the method required frequency for each sample matrix. This would likely mean that studies would need to be repeated on an annual basis, each time there is a change in the method that affects how the test is performed, or if there is a change in instrumentation. If a frequency of greater than 1/year is suggested rather than required the Laboratory will perform the study 1/year. MDLs must meet method-required limits if specified or must be below the laboratory Practical Quantitation Limit (PQL). If a calculated MDL for a parameter exceeds the PQL then the PQL must be raised until results from a new study justify lowering the PQL. If an MDL study is not required the laboratory may not report to a level lower than the low standard. Method Detection Limits are determined according to the Federal Register Appendix B Part 136, Revison 1.11. A minimum of seven replicates of low level spiked reagent blanks or solid samples are processed and analyzed as described in the reference listed. The standard deviation of the responses is used to calculate the MDL as follows:

MDL = S(t•99) for n replicates

Where: n = number of replicates analyzed
S = standard deviation of the values
t•99 = student’s t value for a one-tailed test at the 99% confidence level for “n” replicates.

A replicate result may not be excluded from the MDL calculation unless it is statistically determined to be an outlier (Dixon’s Test for Outliers; α .05, two sided test). Only the results for the parameters tested to be outliers can be dropped in multi parameter tests. A calculated recovery of 70-130% should be achievable if the MDL is to be used to calculate the PQL. If this level of accuracy is not achieved the concentration of the spike for the study is likely not appropriate and the study should be repeated if practical. This level of accuracy may be difficult to achieve for all parameters in multi-parameter methods. All method detection limit study results must be provided to the QA Officer upon completion.
The following information must be included:

- Date of sample analysis and preparation
- Analyst(s) – sample preparation and analysis
- Parameter/matrix
- Method

- Instrument ID
- Spiking level and level of low standard
- Individual results for all of the replicates (including outliers)
- Recalculated results if outliers were excluded (justification must be failure of Dixon Test for outliers)
- Calculated mean, standard deviation and MDL (precision)
- Accuracy (mean of replicate results)

### 14.4.2 Practical Quantitation Limit

The Practical Quantitiation Limit (PQL) is the laboratory reporting level It is a concentration at which both the accuracy and the precision of a method have been taken into consideration. The PQL is generally 2-10 times the calculated MDL. The PQL may be established from variables other than the calculated MDL since the MDL is only an estimation of method precision. One variable that may be taken into consideration is the concentration of the analyte found in the laboratory reagent blank. If background interference cannot be removed the PQL will be greater than 2-10 times the calculated MDL in order to reflect method inaccuracy at low levels. The laboratory reagent blank processed with the analytical batch should be no greater than 1/2 the concentration of the PQL. Another variable that may effect the Reporting Limit is sample dilution. If a sample dilution is required to remove an interference from an inorganic sample and the diluted result is below the PQL a "<PQL" result may not be reported. In this situation an "N" –sample not processed or processed but result not reported" Sample Remark Code is used and an order or sample comment is added to explain the unusual situation. For organic method the laboratory PQL increases by the sample dilution factor. There are alternative approaches to establishing and validating the PQL that may be more practical and appropriate for a given method. If an alternative approach is utilized the protocol must be approved by the Lab Supervisor and Quality Assurance Officer and the protocol and acceptance criteria clearly described in the Method SOP. The PQL is to be verified annually for each matrix, method and analyte according to a defined, documented procedure. Verification is not required for methods or analytes in which a spiking solution is not available. The validity of the PQL must be confirmed by the successful analysis of a QC sample containing the analyte of concern in each quality system matrix 1-2 times the claimed PQL. A successful analysis is one where the recovery of each analyte is within the established test method acceptance criteria or client data quality objective for accuracy. Unless there are method or client specified acceptance criteria for accuracy the laboratory's criteria for the low level PQL verification is + 20% for Inorganic methods and + 30% for Organic methods. The concentration of the low level standard used to calibrate an instrument must be at or below the PQL.

### 14.4.3 Instrument Detection Limits

Instrument Detection Limits (IDLs) are an estimate of instrument precision. A reagent blank solution is analyzed on three non-consecutive days with seven consecutive measurements per day. Each measurement must be performed as

though it were a separate analytical sample. IDLs are estimated by calculating the average of the standard deviations of three runs. The IDL only defines the instrumental limitations of a method and does not take the precision of processing and analyzing real samples into consideration. IDL studies are required only when they are method specified. A method requirement for performing an IDL study does not eliminate the requirement of annually verifying the Limit of Quantitation (LOQ).

#### 14.4.4 Limit of Quantitiation

The Limit of Quantitiation (LOQ) is a term synonymous to the laboratory's Practical Quantitation Limit (PQL) (see Section 14.4.2). The most commonly used procedure at the Lab for determining and verifying the LOQ is the performance of an MDL study followed by the calculation of a PQL (see Sections14.4.1 and 14.4.2).

### 14.5 Tracking of Quality Control Data

The five QC types: spikes, duplicates, standards, blanks and surrogate data. Each QC Type may have sub-categories. The following terms are defined in Section 11.2 of this Plan.

- Spike: Matrix spikes (MS)
- Laboratory Control Samples (LCS)
- Duplicates: Sample Duplicates (Duplicate)
- Laboratory Control Sample Duplicates (LCSD)
- Method Blank Duplicates (MBD)
- Matrix Spike Duplicates (MSD)
- Standard: Initial Calibration Verification Low Level (ICV Low)
- Initial Calibration Verification Mid Level (ICV Mid)
- Initial Calibration Verification High Level (ICV High)
- Continuing Calibration Verification Low Level (CCV Low)
- Continuing Calibration Verification Mid Level (CCV Mid)
- Continuing Calibration Verification High Level (CCV High)
- Quality Control Standard (QCS)
- Surrogates:
    - Blanks: Method Blank (MB)
    - Continuing Calibration Blank (CCB)
    - Initial Calibration Blank (ICB)

### 14.6 Quality Control Acceptance Criteria

Quality control acceptance criteria were established by reviewing historical data for each method/matrix. The limits meet method specified criteria but are generally narrower. The established limits are annually reviewed and adjusted if necessary. The validity of established limits can be verified by reviewing data. The mean ± 3 standard deviations is used. When acceptance limits are validated the data set may be tested for outliers. Statistical outliers are removed prior to calculating acceptance criteria. If a data set is from a matrix specific measure of precision or accuracy e.g. matrix spike recovery data, and the data set

predominately represents an unusually “clean” or “dirty” matrix from a specific project the Laboratory will look at historical performance and either widen or narrow a calculated acceptance limit to avoid creating an unrealistic window of acceptability. If a method specifies a required acceptance limit the limit must be met unless data is qualified. Laboratory acceptance criteria currently being used are summarized by parameter and matrix in Section 5.0 Quality Assurance Objectives.

### 14.7 Reporting of Quality Control Data

Laboratory analysts assign the appropriate quality control types at the required frequency to a QC Batch. Results are reported by QC Batch at data entry. Relative Percent Difference and Percent Recovery are automatically calculated from the information entered. Laboratory Reports have a “QC Information” summary at the end of each report. The QC information section of the report summarizes all QC data for Matrix Spikes-MS (percent recovery), Analytical Duplicates – Dup (RPD) and matrix spike duplicates –MSD (RPD) for the entire Order by parameter and Sample Number. When Control Limits are exceeded a flag is added to the QC data by the analyst. A sample result qualifier or comment must be added if the failed QC result indicates that a sample result(s) may be compromised.

## 15.0 Corrective and Preventative Actions and Customer Complaints

### 15.1 Corrective Actions

Corrective actions may be initiated as a result of a problem identified through a system or, performance audit, data review or data end user’s request. The process is generally initiated by the Quality Assurance Officer or Laboratory Supervisor and documented on a Quality Assurance Irregularity Report Form (Figure 15.1) by the analyst or Technical Director responsible for the data. The lead analyst has the ultimate responsibility of evaluating the effectiveness of the corrective actions. If a corrective action is ineffective it is the analysts’ responsibility to notify the Laboratory Supervisor. Laboratory management must verify that corrective actions have been effective – by performing a follow-up data review, submitting a proficiency sample or performing other internal audit activities. The steps taken in the corrective action process are:

- identify and define the problem
- assign responsibility for investigating the problem
- determine the cause of the problem
- determine the actions needed to eliminate the problem
- implement corrective action
- establish effectiveness of the corrective action
- management verifies effectiveness of corrective action

Corrective action may also be initiated by an analyst during or after analysis of samples. Laboratory personnel are aware that corrective actions may be necessary if:

- Unacceptable instrument conditions or calibration or continuing calibration data is generated.
- QC data are outside the warning or control limits for precision and accuracy.
- Peak shapes and or baselines are unacceptable.
- Blank(s) contain target analytes above acceptable levels.
- A surrogate recovery falls outside the expected range.

Investigation of problems revealed by the routine analysis of laboratory QC samples are the responsibility of the analyst generating the data or the Technical Director of the analytical center reviewing the data. Quality control sample results and instrument conditions are checked against established limits and deviations are immediately addressed. Predetermined limits for data acceptability beyond which corrective action may be required can be found in Sections 5 and 8. Additional method specific limits or conditions are described in the Standard Operating Procedures for each of the analytical methods used in the laboratory. If an analyst determines that corrective actions have not resolved an irregularity and a data set is compromised it is the analysts responsibility to notify the Technical Director or Laboratory Supervisor immediately. The Technical Director or Laboratory Supervisor must assess the data and determine if the data is to be released and how it will be qualified. This process is likely to require contact with the client(s) and written instructions on how to proceed. The clients instructions must be retained on file.

### 15.2 Non-conforming Work

If at any time it is determined that any aspect of the analytical process has compromised the Laboratory's ability to generate defensible data the analyst must notify the laboratory supervisor immediately. The laboratory supervisor must notify clients in writing (e-mail is acceptable) of the irregularity. This policy applies to situations in which the laboratory supervisor has determined that the significance of the irregularity justifies recalling work that has already been released or when the laboratory has decided that it will not report results that are considered invalid. This policy does not apply to those situations in which a data flag or sample note can be used to qualify the data. Corrective actions described in Section 15.1 must be taken immediately to remedy the situation.

### 15.3 Preventative Actions

All laboratory staff are encouraged to identify opportunities for improvement and notify management if resources are needed. A Preventative Action Form (Figure 15.2) is available to all laboratory staff and is used to document needed improvements and potential sources of non-conformance either technical or pertaining to the quality system in general. Forms are submitted to the laboratory supervisor. Preventative actions and follow-up are documented to assure that the preventative action was implemented and successful.

### 15.4 Customer Complaints

Customer complaint regarding the quality of data or service provided by the Laboratory shall be placed in writing and addressed to the laboratory supervisor (e-mails or letters are acceptable). The laboratory supervisor is responsible for evaluating the nature of the complaint. Once individuals or systems are identified as being deficient a corrective action will be put into place. The laboratory supervisor is responsible for verifying that a corrective action has been implemented and is effective in resolving the customer's complaint. The laboratory supervisor must respond to the written complaint in writing in a timely fashion. Complaints and Laboratory responses are kept on file. If a customer is not satisfied with the Laboratory's response the customer has the option of bringing the complaint to the Laboratory Director's attention.

**Figure 15.1 - Quality Assurance Irregularity Report**

A quality assurance irregularity report should contain the following information.

Date:

Due Date:

Date Returned:

Sample ID Number(s) Involved:

Reason for Initiation:

Description of QA Irregularity:

Name of Employee who Performed Work:

Steps taken to investigate irregularity:

Explanation of probable cause of irregularity:

Steps taken to prevent future occurrence:

Reviewers Comments:

Date(s):

Analyst:

Reviewer:

Laboratory Supervisor:

### 15.5 Preventive Action Plan

A preventive action plan contains the following information:

Date Submitted:

Response Date (2 weeks after submittal date):

Name of Employee Requesting a Preventative Action:

Needed Improvement and Potential Sources of Non-conformance:

Recommended Preventative Action:

Supervisor's Response:

Laboratory Supervisor:

Date:

Employee's Review Response:

Date:

RETURN FORM TO LABORATORY SUPERVISOR

## 16.0 QUALITY SYSTEM REVIEW

### 16.1 Quality Assurance Reports to Management

The Laboratory Supervisor will provide the following QA information:

- Precision/Accuracy Report for each analyte/matrix (annual).
- Laboratory QA Plan Updates (annual or as needed).
- Performance Audit results will be distributed to Laboratory Director as they become available. Irregularity reports issued as a result of performance audit ratings will be provided to the Laboratory Supervisor and maintained in a central location.
- QA office goals and objectives for the upcoming year (annual performance evaluation/work plan).
- Internal system audit reports from each analytical center (annual).
- Standard Operating Procedure (SOP) updates.
- Completed irregularity reports and correspondence related to corrective actions or investigations.
- Will inform Laboratory director when QA information required/requested of analyst is not provided. This may include MDL data, irregularity reports or SOP revisions. The Laboratory Director will also be notified if it is determined analysts are not complying with the QA policies described in this manual, or method SOPs.

### 16.2 Laboratory Director's Review of Quality System

An annual review of the laboratory's quality system must be documented. This review must consider:

- Suitability of policies and procedures.
- Technical director's issues and concerns that have been identified in Internal Audit
- Reports, Preventative Action Plans or Irregularity Reports.
- The outcome of recent internal audits.
- Corrective and preventative actions.
- Assessments by external bodies.
- Results of proficiency tests.
- Changes in the volume or type of work.
- Client feedback/customer complaints.
- Other relevant factors, such as quality control activities, resources and staff training.

# Appendix D

# Sample Laboratory Bench Sheets

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# ANALYTICAL BALANCE CALIBRATION

**Balance:** _____ **Serial Number:** _____
**Date Last Serviced:** _____ **Service Company:** _____

| **Date** | **Class 1 Weights** | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1.0000 gm | 2.0000 gm | 5.0000 gm | 20.0000 gm | 50.0000 gm | 150.0000 gm | Signature |
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# Autoclave Operation Log

| | |
|---|---|
| Phosphate dilution water | - 15 minutes minimum |
| Phosphate rinse water | - 15- 30 minutes |
| Sample bottles | - 15 minutes minimum |
| MPN broth (LST, BGB, TSB, EC) | - 13-15 minutes maximum |
| Contaminated materials | - 30 minutes minimum |

| Date | Contents | Time In | Sterilize Time | Time out | Temp °C | Signature |
|---|---|---|---|---|---|---|
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| Timer Check (twice per year) | | | | | | | |
|---|---|---|---|---|---|---|---|
| Date | Theoretical time | Actual time | Signature | Date | Theoretical time | Actual time | Signature |
| | 30 minutes | | | | 30 minutes | | |
| | 15 minutes | | | | 15 minutes | | |

| Spore Strip check (monthly) | | Purchase Date | Lot # | Expire date: | |
|---|---|---|---|---|---|
| | | DateTSB prepared | | | |
| Date | Negative control (clear, yellow) | Negative control (clear, yellow) | Positive control (cloudy, yellow) | Time at 121°C | Signature |
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# Sample

Biochemical Oxygen Demand Worksheet

Name of Facility :_____
Date of Sampling:_____
Time of Sampling:_____
Sampling location:_____
Type of sample: grab ☐ Comp ☐
Sample preservation ice ☐ refrig ☐
Daily Flow :_____ Peak Flow :_____
Signature of sampler:_____

Date of Arrival _____
Time of Arrival _____
Method Used:

| BOD Incubator Temperature | | | |
|---|---|---|---|
| Initial Temp | °C | Time | |
| Final Temp | °C | Time | |

| Sample Pretreatment | |
|---|---|
| pH meter calibration: Buffers used: | |
| Sample pH:_____ Time meter calibrated:_____ | |
| pH adjusted to:_____ w/_____ | |
| Sample temp:_____ | |
| Chlorine present:_____mg/L Dechlor: | yes ☐ |
| Volume of sulfite used/liter sample:_____ | no ☐ |
| Chlorine detectable in recheck: | yes ☐ |
| | no ☐ |

| DO Meter Calibration | |
|---|---|
| Make and Model: | |
| Initial Meter Standardization | |
| Final Meter Standardization | |

| Seed Preparation | | |
|---|---|---|
| Seed Source | | |
| Date collected | | |
| Time collected | | |

| Glucose-Glutamic acid Prep | |
|---|---|
| Preparation date | |

**BOD Sample Data**

| Unseeded Blanks | | | |
|---|---|---|---|
| Bottle Number | | | |
| Initial D.O. | | | |
| Final D.O. | | | |
| Difference | | | |

| Seed Correction | | | |
|---|---|---|---|
| Bottle no. | | | |
| ml seed | | | |
| Initial D.O. | | | |
| Final D.O. | | | |
| Difference | | | |
| DO/ml | | | |
| Average seed correction | | | |

| Glucose-Glutamic Acid Standard | | |
|---|---|---|
| Bottle Number | | |
| ml standard | | |
| ml seed added | | |
| Initial D.O. | | |
| Final D.O. | | |
| Difference | | |
| Seed correction | | |
| Corrected Difference | | |
| BOD mg/L | | |

| Sample Data | | | | | |
|---|---|---|---|---|---|
| Bottle No. | | | | | |
| ml sample | | | | | |
| ml seed | | | | | |
| Initial D.O. | | | | | |
| Final D.O. | | | | | |
| Difference | | | | | |
| Seed corr. | | | | | |
| Corr. Diff. | | | | | |
| BOD mg/L | | | | | |
| Average BOD | | | | | |

Analyst (Preparation) :_____ Date _____ Time _____
Analyst (Completion) :_____ Date _____ Time _____

Comments:

# CHEMICAL PURCHASE LOG

| Chemical Reagent Name | Date Prepared<br>Date Purchased | Lot # | Expiration Date | Concentration | Amount Purchased |
|---|---|---|---|---|---|
| | | | | | |
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# CHEMICAL OXYGEN DEMAND BENCH SHEET

| | | | |
|---|---|---|---|
| Name of Facility | | Date of arrival | |
| Date of Collection | | Time of arrival | |
| Time of Collection | | Date of analysis | |
| Preservation | | Time of analysis | |
| Sample location | | Method | |
| Name of sampler | | Analyst | |

Digestion Time:

| | | | | | |
|---|---|---|---|---|---|
| Start/On | | Finish/Off | | Temp | |

**<u>Method</u> Low Range (0-150 mg/L)**
**Wavelength: _____**
**Lot number: _____**
**Expiration Date: _____**

**<u>Method</u> High Range (0-1500 mg/L)**
**Wavelength: _____**
**Lot number: _____**
**Expiration Date: _____**

| Blank | **Concentration** |
|---|---|
| 2 ml DI water | |

| Blank | **Concentration** |
|---|---|
| 2 ml DI water | |

| KHP standard<br>Date prepared _____<br>Expected conc. _____ | **COD mg/L** |
|---|---|
| 2 ml KHP Standard | |
| 2 ml KHP Standard | |

| KHP standard<br>Date prepared _____<br>Expected conc. _____ | **COD mg/L** |
|---|---|
| 2 ml KHP Standard | |
| 2 ml KHP Standard | |

| **Sample** | **Dilution** | **COD mg/L** |
|---|---|---|
| | | |
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| **Sample** | **Dilution** | **COD mg/L** |
|---|---|---|
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# CONDUCTIVITY METER CALIBRATION LOG

| Date | Standard Lot # | Expiration date | Calibration Value (Expected) | Calibration Value (Actual) | Cell Constant | Signature |
|---|---|---|---|---|---|---|
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| Maintenance | Description |
|---|---|
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# REAGENT WATER QC LOG

| Date | Conductivity (<2 uS) | HPC (<500 cfu/ml) | Chlorine Residual (<0.1 mg/L) | Analyst |
|---|---|---|---|---|
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|---|---|
| **Suitability Date:** | |
| **Heavy Metal Date:** | |

# SAMPLE FECAL COLIFORM BENCH SHEET

Name of Facility: _____
Date of Sampling: _____
Time of Sampling: _____
Exact Sample location:_____
Sample preservation: _____
Signature of Sampler_____

Date of Arrival: _____
Time of Arrival: _____
Method Used:

Time of Analysis:_____
Analyst:_____

| | Membrane Filter | m-FC Broth | Absorbent Pads |
|---|---|---|---|
| Date of Purchase | | | |
| Lot number | | | |
| Date of Expiration | | | |
| pH | | | |

| Waterbath temperature (44..5 ± 0.2°C) | |
|---|---|
| Time In: | Date In: |
| Temp In: | |
| Time Out: | Date out: |
| Temp Out: | |

| | |
|---|---|
| Filter Funnel Sterilized:<br>(2-3 minutes minimum) | Work area disinfected: |

| Positive control Organism used | Date purchased | Lot number | Expiration date | Result |
|---|---|---|---|---|
| | | | | |
| Negative control Organism used | Date purchased | Lot number | Expiration date | Result |
| | | | | |

| Dish | Sample volume (ml) | Colonies on membrane | CFU/100 ml | Plates used in count |
|---|---|---|---|---|
| Pre-blank | | | | |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| After blank | | | | |

**Fecal Coliform** cfu/100 ml

# GLASS FIBER FILTER WASH BENCH SHEET

| Date: | Manufacturer: | | Lot number: | | Analyst: | |
|---|---|---|---|---|---|---|
| Desiccant color: | | | Balance zeroed | | Balance used: | |
| Initial time in | | | Initial temperature in | | | |
| 1st dry time in | | | 1st dry temperature | | | |
| 2nd dry time in | | | 2nd dry temperature | | | |
| Filter dish number | | | | | | |
| First dry weight | | | | | | |
| Second dry weight | | | | | | |
| Difference | | | | | | |

| Date: | Manufacturer: | | Lot number: | | Analyst: | |
|---|---|---|---|---|---|---|
| Desiccant color: | | | Balance zeroed | | Balance used: | |
| Initial time in | | | Initial temperature in | | | |
| 1st dry time in | | | 1st dry temperature | | | |
| 2nd dry time in | | | 2nd dry temperature | | | |
| Filter dish number | | | | | | |
| First dry weight | | | | | | |
| Second dry weight | | | | | | |
| Difference | | | | | | |

# INHIBITORY RESIDUE TEST

Equipment needed:

Get 1 glass petri dish for blank
Get 19 glass petri dishes/covers
Get 7 plastic petri dishes/covers
Need HPC agar for all the plates
Detergent to be used, diluted to the working concentration normally used.
Bent glass rod, alcohol, burner

Procedure

**Step 1: Plate preparation. Prepare the plates as indicated**

Group A
Wash 6 glass petri dishes in the new detergent and rinse like you normally would, let dry
Label 1 glass petri dish as **Blank**
Label 3 plates as **Group A - l ml**
Label 3 plates as **Group A - 0.1 ml**

Group B
Wash 6 glass petri dishes in the new detergent and rinse 12 times with deionized water, let dry
Label 3 plates as **Group B - l ml**
Label 3 plates as **Group B - 0.1 ml**

Group C
Wash 6 glass petri dishes in the new detergent and do not rinse, let dry
Label 3 plates as **Group C - l ml**
Label 3 plates as **Group C - 0.1 ml**

Group D
Do not wash with detergent.
Label 1 plastic plate as **Blank**
Label 3 plastic plates as **Group D - l ml**
Label 3 plastic plates as **Group D - 0.1 ml**

**Step 2: Sterilization**

Sterilize the 19 glass petri dishes in the sterilizing oven at 180oC for 2 hours or in the autoclave at 121oC for 15 minutes

**Step 3: Melt Agar ( Spread Plate Method)**
Melt the HPC agar as normal and pour into the petri dishes, swirl the agar gently both clockwise and counterclockwise then allow to solidify, then place inverted in the 35oC incubator overnight to pre-dry the agar. The plates should loose 2-3 grams of water weight. I prefer the pour plate method because it keeps spreader colonies down but the agar must be cooled to 46oC to avoid heat shocking the organisms. It also allows for a larger sample aliquot
(1 ml vs 0.5 ml)

**Step 4: Preparation of culture solution.**
1. Need a solution containing between 100-300 colonies, a countable number will probably be fine.
2. Take your enterobacter culture slant tube and add 1-2 ml of sterile phosphate dilution water from a 99 ml sterile phosphate dilution water bottle and swirl to suspend the colonies.

3. Transfer to the 99 ml sterile phosphate dilution water bottle.
4. Make 2 consecutive 1: 10 dilutions (a serial dilution) into 99 ml sterile phosphate dilution water bottles.
5. From here on out it is guess work. I would use the last bottle for all the petri plates. The following steps should take less than 10-20 minutes to complete. I think this is because of the bacterial generation time. So get everything ready
6. Shake the dilution bottle well and using a sterile pipet, place 1 ml and 0.1 ml into each petri plate as labeled.
7. Sterilize the bent glass rod with dipping in alcohol and igniting. Place the glass rod into the petri dish and swirl Repeat the **glass rod sterilization** step **for each plate** to avoid cross contamination.

8. Incubate the petri dishes, inverted, overnight at 35oC

**Step 5 Count the colonies, Document all counts but the ones of interest will be the plates with 30-300 colonies**

| Group A | | Group B | | Group C | | Group D | | Blank | |
|---|---|---|---|---|---|---|---|---|---|
| 1 ml | 0.1 ml | 1 ml | 0.1 ml | 1 ml | 0.1 ml | 1 ml | 0.1 ml | Glass | Plastic |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| Average A = | | Average B = | | Average C = | | Average D = | | | |

If there are no countable plates, repeat with different dilutions.

**Step 6 Compare the results**

$\frac{\text{Average A}}{\text{Average B}}$ x 100% = % (Should be 100 $\pm$ 15%)

$\frac{\text{Average A}}{\text{Average C}}$ x 100% =

$\frac{\text{Average B}}{\text{Average C}}$ x 100% = %

$\frac{\text{Average A}}{\text{Average D}}$ x 100% = %

$\frac{\text{Average B}}{\text{Average D}}$ x 100% = %

A/B comparison indicates if rinsing procedure used by lab is adequate. If the detergent is toxic and is not rinsed adequately the plate counts will be different. Usually B will be higher than A

A/C comparison indicates if the detergent is toxic and the rinsing is inadequate. If the detergent is toxic C will be lower than A as long as the rinsing is adequate. If both A and C are lower than B, then the detergent is toxic and not being rinsed adequately.

B/C comparison indicates if the detergent is toxic. If the detergent is toxic, C will be lower than B.

B/D comparison indicates if the plastic petri dish contains toxics. If D is lower than B or A, then toxic materials in the petri dish are killing the bacteria.

# MAINTENANCE LOG

**Equipment Name: _____**
**Serial Number: _____**

| Date | Description of maintenance<br>ie. repair, replace | Name |
|---|---|---|
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<u>Replacement Part numbers/Supplier</u>

1.
2.
3.
4.
5.
6.

# MEMBRANE FILTER QC

**Petri Dish with Pads**

| Sterility | | Expected Reaction | | | | Incubation Time & Temp | |
|---|---|---|---|---|---|---|---|
| 50x9 mm petri dish and pad soaked in 5 ml 1x TSB | | No growth | | | | $35^o \pm 0.5^oC$ for 24-48 hours | |
| Purchased Date | Lot No. | Sterility | | Status | | Date of analysis | Initials |
| Expiration Date | | Start Time | Finish Time | Accept | Reject | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**Membrane Filters**

| Sterility | | Expected Reaction | | | | Incubation Time & Temp | |
|---|---|---|---|---|---|---|---|
| 50x9 mm petri dish, pad, and filter soaked in 5 ml 1x TSB | | No growth | | | | $35^o \pm 0.5^oC$ for 24 -48 hours | |
| Purchased Date | Lot No. | Sterility | | Status | | Date of analysis | Initials |
| Expiration date | | Start time | Finish time | Accept | Reject | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |
| | | | | | | | |

**mFC Broth**

| Date Purchased | Lot No. | Expiration Date | Sterility | | PH (7.4 ± 0.2) | Control $44.5^o \pm 0.2^oC$ for 24 hr | | Date | Initials |
|---|---|---|---|---|---|---|---|---|---|
| | | | Start time | Finish time (Clear) | | Positive E. coli | Negative E. aerogenes. | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

# M-FC BROTH QC LOG

| Date: | Time: | Analyst: |
|---|---|---|

**Waterbath**

| Time In | Temperature In | Time Out | Temperature Out |
|---|---|---|---|
| | | | |

**Broth**

| | Date Purchase | Lot # | Date Expiration | pH |
|---|---|---|---|---|
| New Broth | | | | |
| Old broth | | | | |

| | E. coli (old broth) | E. Coli (new broth) | | | E. aerogenes | E. aerogenes | |
|---|---|---|---|---|---|---|---|
| Blank | | | | Blank | | | |
| $10^{-4}$ | | | | $10^{-4}$ | | | |
| $10^{-5}$ | | | | $10^{-5}$ | | | |
| $10^{-6}$ | | | | $10^{-6}$ | | | |
| $10^{-7}$ | | | | $10^{-7}$ | | | |
| $10^{-8}$ | | | | $10^{-8}$ | | | |
| End blank | | | | End Blank | | | |

*all values are in cfu/100 ml

| Pure Culture | Purchase Date | Lot # | Expiration Date |
|---|---|---|---|
| E. Coli | | | |
| E. Aerogenes | | | |

# pH METER CALIBRATION LOG

Make and Model pH meter: _____

| Date | Time | pH 4 buffer | | | pH 7 buffer | | | pH 10 buffer | | | Slope | Name |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lot # | Exp. Date | pH | Lot # | Exp. Date | pH | Lot # | Exp. Date | pH | | |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| | | | | | | | | | |

# Phosphate Dilution Water Log

| Date | Sterility (50 ml in TSB) (No growth) | | | | Volume (99ml ± 2 ml) | | | | | | PH (7.2 ± 0.5 ml) | | | | | | Analyst |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Bottle | | | | | | Bottle | | | | | | |
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | |
| Date Stock Phosphate Prepared: | | | | | | | | | | | | | | | | | |

| Date | Sterility (50 ml in TSB) (No growth) | | | | Volume (99ml ± 2 ml) | | | | | | PH (7.2 ± 0.5 ml) | | | | | | Analyst |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Bottle | | | | | | Bottle | | | | | | |
| | | | | | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| Date Stock Phosphate Prepared: | | | | | | | | | | | | | | | | | |

# SAMPLE SPECTROPHOTOMETERY BENCH SHEET

| | | |
|---|---|---|
| Name of Facility | Date of Arrival | Date of Analysis: |
| Date of collection: | Time of Arrival: | Time of Analysis: |
| Time of collection: | Preservation: | Analyst: |
| Sample location | Method used | |
| Name of sampler | | |

| | | | |
|---|---|---|---|
| Stock concentration | | Intermediate Standard concentration | |
| Purchase date | | Date prepared | |
| Lot number | | Lot number | |
| Expiration date | | Expiration date | |

**Standard Curve** Date Prepared: _____

| **Concentration** | **Absorbance** | **Concentration** | **Absorbance** | **Concentration** | **Absorbance** |
|---|---|---|---|---|---|
| mg/l | | mg/L | | mg/L | |
| mg/l | | mg/L | | mg/L | |
| mg/L | | mg/L | | mg/L | |
| mg/L | | mg/L | | mg/L | |

| | Absorbance | Concentration | | LFB conc. actual | |
|---|---|---|---|---|---|
| Reagent blank | | | | LFB conc. theoretical | |
| Laboratory Fortified blank (LFB) | | | | % recovery (85-115%) | |

**Sample**

| Sample description (lab number) | Dilution | Absorbance | Concentration |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

# TEMPERATURE LOG

**Month:_____**

**Equipment name: _____**
**Serial number: _____**

| Date | Temperature | | Time | | Signature |
|---|---|---|---|---|---|
| | AM | PM | AM | PM | |
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
| 8 | | | | | |
| 9 | | | | | |
| 10 | | | | | |
| 11 | | | | | |
| 12 | | | | | |
| 13 | | | | | |
| 14 | | | | | |
| 15 | | | | | |
| 16 | | | | | |
| 17 | | | | | |
| 18 | | | | | |
| 19 | | | | | |
| 20 | | | | | |
| 21 | | | | | |
| 22 | | | | | |
| 23 | | | | | |
| 24 | | | | | |
| 25 | | | | | |
| 26 | | | | | |
| 27 | | | | | |
| 28 | | | | | |
| 29 | | | | | |
| 30 | | | | | |
| 31 | | | | | |

# SAMPLE TITRATION WORKSHEET

| | | | |
|---|---|---|---|
| **Name of Facility** | | **Date of arrival** | |
| **Date of Collection** | | **Time of arrival** | |
| **Time of Collection** | | **Date of analysis** | |
| **Preservation** | | **Time of analysis** | |
| **Sample location** | | **Method** | |
| **Name of sampler** | | **Analyst** | |

**Standardization**

| | | |
|---|---|---|
| **ml of std used** | | |
| **Conc. of std in mg/L** | | |
| **ml titrant** | | |
| **Conc. of titrant, mg/L** | | |

| | |
|---|---|
| **Purchase date standard** | |
| **Lot number** | |
| **Expiration date standard** | |
| **Supplier** | |

**Sample Data**

| | |
|---|---|
| **Ph meter calibrated (if needed)** | Time: |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Sample number** | | | | | | |
| **Sample adj. pH** | | | | | | |
| **ml sample used** | | | | | | |
| **Conc. of titrant** | | | | | | |
| **Initial buret reading** | | | | | | |
| **Final buret reading** | | | | | | |
| **ml titrant used** | | | | | | |
| **Sample conc mg/l** | | | | | | |

**Formula:** $\frac{\text{ml titrant used} \times \text{conc of titrant (mg/L)}}{\text{ml of sample titrated}} = \text{conc of sample}$

# Sample

Total Suspended Solids Bench sheet

Name of Facility :_____

Date of Sampling:_____

Time of Sampling:_____

Sampling location:_____

Type of sample: grab ☐ Comp ☐

Sample preservation ice ☐ refrig ☐

Daily Flow :_____ Peak Flow :_____

Signature of sampler:_____

Date of Arrival _____

Time of Arrival _____

Method Used:

| Drying Oven Temperature | | | |
|---|---|---|---|
| Initial Temp | °C | Time | |
| 1st Temp | °C | Time | |
| 2nd Temp | °C | Time | |

| Analytical Balance Calibration | | |
|---|---|---|
| Balance Make, Model: | | |
| Date last Calibrated: | | |
| Calibrating Company: | | |
| Monthly Calibration Performed | | yes ☐ |
| Balance Zeroed | | yes ☐ |

| Filters Used | |
|---|---|
| Type of Filter Used | |
| Filter Lot # | |
| Filters Prewashed | |
| Filters pre volatilized | |
| Desiccant color | |

| TSS Sample Data | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Location | | | | | | | | |
| Dish number | | | | | | | | |
| Sample volume | | | | | | | | |
| Sample dilution | | | | | | | | |
| 1st weight dry sample + dish | | | | | | | | |
| Tare weight (dish) | | | | | | | | |
| 1st Weight of dry solids | | | | | | | | |
| 2nd weight dry sample + dish | | | | | | | | |
| Tare weight (dish) | | | | | | | | |
| 2nd Weight of dry solids | | | | | | | | |
| TSS mg/L | | | | | | | | |
| Average TSS | | | | | | | | |

| VSS Sample Data | Muffle Time : | | Muffle Temp : | | Desiccated : | | | |
|---|---|---|---|---|---|---|---|---|
| 2nd weight dry sample + dish | | | | | | | | |
| Weight of ash + dish | | | | | | | | |
| Weight of volatile solids | | | | | | | | |
| VSS mg/L | | | | | | | | |
| Average VSS | | | | | | | | |

Analyst :_____ Date _____ Time _____

Comments:

Formula: $\frac{\text{weight solids x 1000000 x dilution}}{\text{volume sample}} =$