

Appendix D

Sample Laboratory Bench Sheets

TABLE OF CONTENTS

Analytical Balance Calibration Log.....	1
AutoclaveOperationLog.doc.....	2
BOD Bench Sheet.....	3
Chemical Purchase Log	4
COD Bench Sheet.....	6
Conductivity Meter Calibration Log.....	7
DI Water QC Log.....	8
Fecal Coliform Bench Sheet.....	9
Glass Fiber Filter Wash Bench Sheet	10
Inhibitory Residue Bench Sheet.....	11
Maintenance Log	13
Membrane Filter QC Log.....	14
m-fc Broth Log	15
pH Meter Calibration Log.....	16
Phosphate Dilution Water Log Bench Sheet	17
Spectrophotometry Bench Sheet.....	18
Temperature Log.....	19
Titration Bench Sheet	20
TSS Bench Sheet.....	21

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	<input type="checkbox"/>
Volume of sulfite used/liter sample: _____		no	<input type="checkbox"/>
Chlorine detectable in recheck:		yes	<input type="checkbox"/>
		no	<input type="checkbox"/>

DO Meter Calibration	
Make and Model:	
Initial Meter Standardization	
Final Meter Standardization	

Seed Preparation		
Seed Source		
Date collected		
Time collected		

BOD Sample Data

Unseeded Blanks			
Bottle Number			
Initial D.O.			
Final D.O.			
Difference			

Glucose-Glutamic acid Prep	
Preparation date	

Seed Correction			
Bottle no.			
ml seed			
Initial D.O.			
Final D.O.			
Difference			
DO/ml			
Average seed correction			

Glucose-Glutamic Acid Standard			Sample Data			
Bottle Number			Bottle No.			
ml standard			ml sample			
ml seed added			ml seed			
Initial D.O.			Initial D.O.			
Final D.O.			Final D.O.			
Difference			Difference			
Seed correction			Seed corr.			
Corrected Difference			Corr. Diff.			
BOD mg/L			BOD mg/L			
			Average BOD			

Analyst (Preparation) : _____ Date _____ Time _____
 Analyst (Completion) : _____ Date _____ Time _____

Comments:

REAGENT WATER QC LOG

Date	Conductivity (<2 uS)	HPC (<500 cfu/ml)	Chlorine Residual (<0.1 mg/L)	Analyst

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: (2-3 minutes minimum)	Work area disinfected:
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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 - 1 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 - 1 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 - 1 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 - 1 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	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

$$\frac{\text{Average A}}{\text{Average B}} \times 100\% = \quad \% \quad (\text{Should be } 100 \pm 15\%)$$

$$\frac{\text{Average A}}{\text{Average C}} \times 100\% =$$

$$\frac{\text{Average B}}{\text{Average C}} \times 100\% = \quad \%$$

$$\frac{\text{Average A}}{\text{Average D}} \times 100\% = \quad \%$$

$$\frac{\text{Average B}}{\text{Average D}} \times 100\% = \quad \%$$

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.

M-FC BROTH QC LOG

Date:	Time:	Analyst:
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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			

Phosphate Dilution Water Log

Date	Sterility (50 ml in TSB) (No growth)					Volume (99ml \pm 2 ml)						PH (7.2 \pm 0.5 ml)						Analyst	
						Bottle						Bottle							
						1	2	3	4	5	6	1	2	3	4	5	6		
Date Stock Phosphate Prepared:																			

Date	Sterility (50 ml in TSB) (No growth)					Volume (99ml \pm 2 ml)						PH (7.2 \pm 0.5 ml)						Analyst	
						Bottle						Bottle							
						1	2	3	4	5	6	1	2	3	4	5	6		
Date Stock Phosphate Prepared:																			

SAMPLE SPECTROPHOTOMETRY 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: _____

<u>Concentration</u>	<u>Absorbance</u>	<u>Concentration</u>	<u>Absorbance</u>	<u>Concentration</u>	<u>Absorbance</u>
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:
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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	

Filters Used	
Type of Filter Used	
Filter Lot #	
Filters Prewashed	
Filters pre volatilized	
Desiccant color	

Analytical Balance Calibration	
Balance Make, Model:	
Date last Calibrated:	
Calibrating Company:	
Monthly Calibration Performed	yes <input type="checkbox"/>
Balance Zeroed	yes <input type="checkbox"/>

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: $\frac{\text{weight solids} \times 1000000 \times \text{dilution}}{\text{volume sample}} =$