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*Resource Protection Division*



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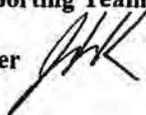
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**MEMORANDUM**

**TO:** Kris Pintado, Standards, Planning and Reporting Team Leader  
**FROM:** Jodey Kougioulis, Quality Assurance Officer   
**DATE:** February 26, 2014  
**SUBJECT:** Triennial Review – Most probable number (MPN)/colony forming units (cfu) enumeration methods and proposed standards reporting revision

**Introduction and Purpose**

The purpose of this memo is to address EPA’s and SWQB staff comments and suggestions regarding the reporting of bacterial concentrations as MPN and to propose suggested revisions to the state’s current reporting language for bacteria criteria which are expressed as colony forming units (cfu) per 100 ml. Currently, the SWQB reports bacteria data as most probable number (MPN) based on the use of IDEXX *Quanti-Tray* (QT) method which is an extended version of the IDEXX Colilert test. MPN and cfu represent different enumeration methods and result in different method specific units, but for purposes of reporting, EPA has used these terms interchangeably. EPA has approved methods for enumeration and allows reporting in either cfu or MPN in federal rule for ambient water (40 CFR, 2003) and for wastewater and sludge (40 CFR, 2007).

**Background and General Description of MPN and cfu.**

The MPN is a statistical estimate of the number of bacteria that, more probable than any other number, would give the observed result; it is not an actual count of the bacteria present. Membrane filtration (MF) methods which produce results expressed as cfu are culture-based and results are quantified by counting the number of colonies that arise from bacteria captured on the membrane filter per volume of water filtered. Although expressed as an actual count of the bacterial colony forming units, the number is still considered an estimate because colonies can be produced by one or several cells that can clump together in the sample. MPN methods are also culture-based with a defined substrate which produces an estimate number (density) of organisms based on the combination of positive and negative test tube results that can be read from a statistical probability MPN table.

**Proposal**

The SWQB currently uses an approved EPA method for sampling and analyzing bacteria levels in its ambient water quality monitoring program and reports these results in MPN. The water quality standards for bacteria criteria are proposed to be revised to reflect SWQB’s current reporting practices and EPA’s approved use of either membrane filtration methods, reported as cfu, or MPN methods, reported as MPN for enumeration of bacteria in ambient water and effluent. This change, if adopted, would allow results to be reported in either cfu or MPN, depending on the analytical method. The most appropriate place to do this may be in 20.6.4.900.D and E of NMAC by adding language similar to the following: “Water quality standards for *E. coli* are expressed in colony forming units per 100 milliliters of water (cfu / 100 ml) or as a Most Probable Number (MPN)”

## **Related Research**

There have been numerous published papers that address the similarities or differences between enumeration results obtained by cfu methods and those obtained by MPN methods. Much of the earlier research concluded that *“there was no significant difference for the enumeration of E. coli between the QT and MF methods”* (Rompré et al., 2002).

More recently published research by Wohlsen et al. (2006) does show a significant difference between the two enumeration methods when using a standard reference inoculum. The use and calibration of a standard reference inoculum of only viable cells still needs to be related to original criteria development which was based on a combination of frequency, magnitude, and duration of exposure to ambient recreational waters, bacterial densities as enumerated by MF, and selected illness rates in response. As stated earlier, this is primarily a reporting revision to acknowledge the programmatic reality that both MPN and cfu can be reported and used to assess against the water quality standard.

## **Staff and EPA Comments, Suggestions, and Initial Review of Bacteria Criteria Reporting**

Responses to both the EPA, SWQB staff, and the proposal justification will need to be clearly communicated in a consistent and coordinated fashion. The need to remain consistent with existing water quality standard language, definitions, and format may limit the expanse of revised language but ultimately the simple proposed revision will communicate the available reporting options for bacteria criteria. Comments from SWQB staff largely focused on the fact that MPN and cfu are enumerated and expressed differently with method specific units and that clear definitions are needed to describe this difference. EPA’s comments and suggestion are largely in concert with the proposed revision and the suggested language will provide the clarity needed for criteria interpretation.

## **SWQB Staff**

1): I have come across several scholarly articles that attempt to correlate MPN to cfu. They are not the same; cfu represents an absolute number of units, whereas MPN represents a theoretical value (often considered the maximum value).

*Response: EPA permits staff and SWQB staff raised issues about the enumeration of bacteria - most probable number (MPN) and colony forming units (cfu) - relative to implementation and assessment of the WQS. The traditional plate tests, including membrane filtration, estimate or count ‘colonies’ of bacteria reported as cfu. These provide a direct count of an indicator organism (E. coli) in ambient water or wastewater based on the development of colonies in/on media and a calculation is still performed. While microscopic counts may be more accurate, it’s costly and time consuming, and there’s still the problem of what’s viable or not. Very few tests are conducted to determine live and dead colonies; in summary exact counts are generally not feasible to obtain. Newer tests such as Colilert (which is used by SWQWB for assessment and monitoring) report data as MPN which is a statistical representation of what level of E. coli is likely present in a sample. While MPN and cfu may not be entirely equivalent, for the purposes of reporting, these terms are currently used interchangeably by the EPA. EPA has approved these methods for enumeration in federal rule for ambient water (40 CFR, 2003) and for wastewater and sludge (40 CFR, 2007). The currently recommended EPA recreational or bacteria criteria for E. coli are expressed as cfu/100 ml measured using EPA Method 1603 or any other equivalent method that measures culturable E. coli. Therefore, the water quality standards are under deliberation to be revised to reflect the use of updated methods for monitoring, assessment and reporting. After much consideration, the most appropriate place to do this may be in 20.6.4.900.D and E of NMAC by adding language similar to the following:*

“Water quality standards for E. coli are expressed in colony forming units per 100 milliliters of water (cfu / 100 ml) or as a Most Probable Number (MPN)”

*References for EPA Method 1603 and EPA’s final rules establishing alternate test procedures could also be included in 20.6.4.901 NMAC as references.*

*Abbreviations for both cfu and MPN are suggested to be included in the WQS definitions.*

2) Similar to the cfu/100mL definition, do we need to make reference to cfu/100mL in the MPN definition? Add the term “most probable number” (under terms beginning with the letter ‘M’).

**Response:** Generally, the definitions seem to stand on their own, e.g., there doesn't seem to be any 'cross referencing' in these definitions. Instead of adding a definition for MPN, the abbreviation for MPN is retained in this section. Please also see the previous discussion in response to bacteria enumeration (under 20.6.4.7.A (3)(a) NMAC), and response below.

"MPN" will be listed under the abbreviations section of the definitions, so it'll be 'defined' in that way. It's also appropriate to add 'MPN' (as an alternate enumeration to cfu) under the criteria section in 20.6.4.900.D and E NMAC (see the new language in that section). As there's not a "full" definition for cfu in the WQS, to be consistent with the rule format, a "full" definition for MPN won't be added. Also, there's really not a concise, easily understood definition for cfu to put into the standards. Both enumeration methods are also fully described in the EPA criteria recommendations and supporting documents, in the methods, and in the scientific literature.

### **EPA Comments and Suggestions**

The Region's concern with the state's current bacteria criteria are related to how the provision reads and its interpretation. The *E. coli* standard that the state uses is expressed as colony forming units (cfu) per 100 ml. In a plain reading, this provision requires a specific test method but does not allow an alternative test. Generally the Region recommends avoiding this type of approach to test methods.

When bacterial Total Maximum Daily Loads (TMDL) are issued, they may specify extremely large numbers of cfu/100 ml as a loading limit. This requires building an equation for calculating the loading limit as expressed in the TMDL into a footnote into NPDES permits. To simplify the process, the Region has consulted with waste water treatment plant operators to determine if the most probable number (MPN) can be used as an equivalent to cfu/100 ml. The general answer is yes, and the Region has been using this approach. NMED inspectors seem to agree with this approach, since they also see the problem in the field. The problem here is that this approach requires the use of a different test method. What the Region suggests is that both the standards and TMDL guidance documents refer to both cfu/100 ml and MPN as equivalent, allowing either generally approved test method to be used to account the level of indicator bacteria in permits.

**Response:** EPA Region 6 has suggested that the water quality standards and the state's TMDL guidance refer to both colony forming units (cfu) and most probable number (MPN), as EPA has approved the use of test methods with results that are expressed in either cfu or MPN. The use of more cost-effective and time efficient methods in which counts are expressed as MPN was approved by EPA as equivalent for testing ambient waters in 2003<sup>[1]</sup>, and for wastewater and sewage sludge in 2007<sup>[2]</sup>. The SWQB is currently using an approved EPA method for sampling and analyzing bacteria levels in ambient water and reporting results in MPN. The currently recommended EPA recreational or bacteria criteria for *E. coli* are expressed as cfu/100 ml measured using EPA Method 1603 or any other equivalent method that measures culturable *E. coli*<sup>[3],[4]</sup>. Therefore, the water quality standards are proposed to be revised to reflect the use of updated methods for monitoring, assessment and reporting. References for EPA Method 1603 and EPA's final rules establishing alternate test procedures will also be included in 20.6.4.901 NMAC as references.

### **Footnotes**

1. U.S. Federal Register - 40 CFR Part 136 Vol. 68, No. 139; July 21, 2003.
2. U.S. Federal Register - 40 CFR Parts 136 and 503, Vol. 72, No. 157; March 26, 2007.
3. EPA, 2012:  
<http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/factsheet2012.pdf>
4. USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA-821-R-02-023

## **References**

Annie Rompre', Pierre Servais, Julia Baudart, Marie-Rene'e de-Roubin, Patrick Laurent (2002). *Detection and enumeration of coliforms in drinking water: current methods and emerging approaches*. Journal of Microbiological Methods 49 (2002) 31–54

*U.S. Federal Register* - 40 CFR Part 136 Vol. 68, No. 139; July 21, 2003.

*U.S. Federal Register* - 40 CFR Parts 136 and 503, Vol. 72, No. 157; March 26, 2007.

USEPA, 2012:

<http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/factsheet2012.pdf>

USEPA. 2002. Method 1603: *Escherichia coli* (*E. coli*) In Water By Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar ( modified mTEC). U.S. Environmental Protection Agency, Office of Water, Washington D.C. EPA-821-R-02-023

Wohlsen, T., Bates, J., Vesey, G., Robinson, W.A. and M. Katouli (2006) Evaluation of the methods for enumerating coliform bacteria from water samples using precise reference standards. *Letters in Applied Microbiology* **42**, 350-356.