E. coli Test Method Help Sheet

Goal: Provide information to help compliance staff respond to questions from externals (operators, labs, etc.) and verify compliance with testing methods, procedures, and standards during inspections.

General Information

s. NR 219.04 Table A, Wis. Adm. Code - Bacteria Testing Standards

s. NR 219.04 Table F, Wis. Adm. Code – Sample Maximum Holding Times (as soon as possible, but test started no later than 8 hours after collection)

s. NR 219.037(3), Wis. Adm. Code – Exclusion for test to be performed by a certified or registered lab (allows for test to be done "in house")

WDNR_2020_Bacteria_Fact Sheet_E Coli

*Note that the information from the *WDNR 2020 Bacteria Fact Sheet* has been included within this help sheet. The *2020 Fact Sheet* may be given to externals, while this help sheet is intended for internal use only.

How to Perform Serial Dilutions: https://www.youtube.com/watch (theory) and https://www.youtube.com/watch (demonstration)

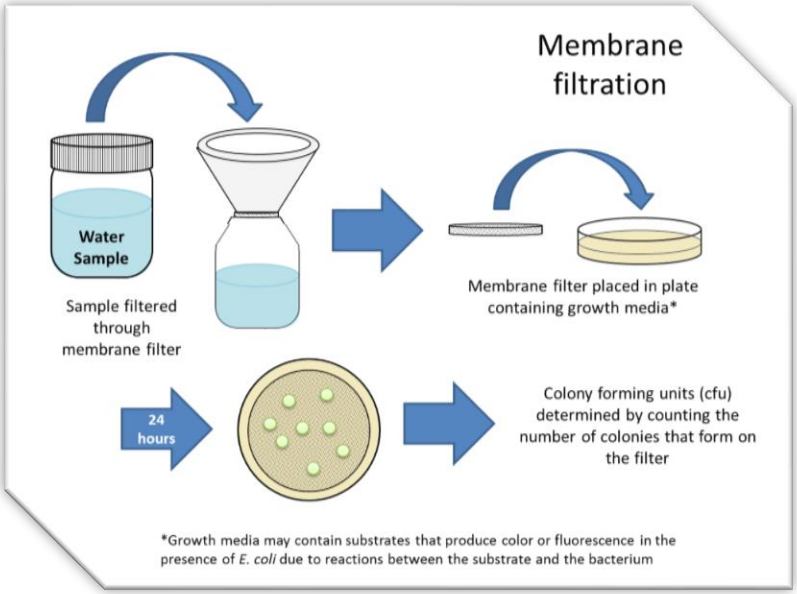
Direct and Indirect Measurements of Bacteria: https://www.youtube.com/watch

Membrane Filtration Technique

In the membrane filtration approach, a water sample is filtered through a membrane. The membrane is then placed on culture media that is selective for *E. coli*. Because the bacteria are retained on the surface of the filter, they grow on the media and develop into a visible colony.

The number of colonies that are formed are counted and reported as the colony forming units (CFUs).

mColiblue-24® by Hach Company is a commercially available culture media that can be used to quantify *E. coli* via the membrane filtration approach.



Approved Methods (Single Step)
- EPA Standard Method 1603
- HACH mColiblue-24 (commercial technology)

Approved Methods (Double Step)
- SM 9222B-2015
- SM 9222I-2015


method_1603_2014.pdf


Hach membrane filter technique.pdf

Analytical Approach	Standardized Test Method	Commercial Technology	Advantages	Disadvantages
Membrane filtration:			• Readily available • Used to establish EPA's *E. coli* criteria[1] • Results can be compared directly to fecal coliform results • Media less costly	• Labor and material intensive • Require high degree of technical skill to evaluate results • Additional analysis may be needed for samples with high turbidity, high levels of noncoliform bacteria, or organisms stressed by chlorine
Single-step	EPA 1603	mColiBlue-24®		
or				
Two-step	SM 9222B-2015 SM 9222I-2015	N/A		

1. Membrane filtration was used to quantify *E. coli* in EPA's 1986 Ambient Water Quality Criteria for Bacteria. The EPA used the 1986 *E. coli* data in their 2012 Recreational Water Quality Criteria because new *E. coli* data was not collected as part of the epidemiological studies.

SM = Standard Methods for the Analysis of Water and Wastewater

Links to Instructional Videos

How to Video: https://www.youtube.com/watch

Pour Plate vs. Spread Plate: https://www.youtube.com/watch

Difference in HACH *E. coli* versus fecal coliform methods
- Different broth (mColiBlue24®) and a lower dry incubator temperature (35 deg C).

Main Differences Between the Two Methods (EPA Standard and HACH)
- HACH mColiblue24® is a variant of the EPA 1603 Standard
- "Broth" - 1603 Standard uses a modified mTEC agar while HACH uses the mColiblue-24® broth
- Incubator Temperature - 1603 Standard uses 44.5±0.2 °C while HACH uses 35±0.2 °C

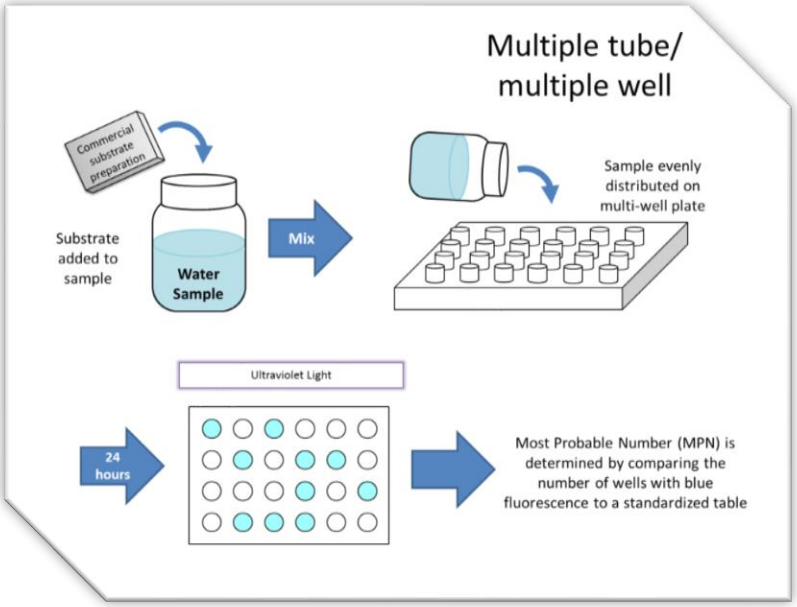
If using mColiblue24® broth, make sure the HACH method is being followed.

"Word on street" is that the 1603 method, after the recent changes in 2014, is a little too time/effort intensive with the verification procedures. So other methods such as the HACH method or MTMW is used instead.

Questions to Ask:
1. Is the test performed in house or sent to a lab?
2. How quickly is the test started after sample collection?
3. Which method is used? (Ask for a demonstration/talk through the testing process.)

Multiple Tube/Multiple Well (MTMW)

In the multiple tube/multiple well approach, a water sample is mixed with a commercial reagent containing methylumbelliferyl-β-glucuronide (MUG). *E. coli* enzymatically cleaves MUG, forming a fluorescent product. Samples are distributed into a multi-well plate. After incubating for 24 hours, the most probable number (MPN) is estimated from the number of wells that are positive for the presence of bacteria growth using a standardized table. The MPN is a statistical estimate of the mean bacteria density.



Colilert®and Colilert-18® by IDEXX Technologies are commercially available kits that can be used to quantify *E. coli* via the multiple tube/multiple well approach.

Approved Methods

- SM 9223B-2016
- AOAC 991.15
- Colilert® & Colilert-18® (commercial technology)

Links to Instructional Videos

https://www.idexx.com/en/water/water-products-services/colilert/

https://www.idexx.com/en/water/water-products-services/quanti-tray-system/

Multiple Tube: https://www.youtube.com/watch

Differences Between Colilert Equipment/Kits

- Colilert® vs Colilert-18®
  - 24 hrs vs 18 hrs for incubation time
  - Both Colilert® and Colilert-18® are used to quantify *E. coli* in drinking water
  - Colilert-18® is also used to quantify fecal coliform in wastewater (when incubated at 44.5±0.2 °C)
- Quanti-Tray vs Quanti-Tray/2000
  - Used for counts up to 200 vs 2,419
- Quanti-Tray/Legiolert: only used to quantify *Legionella pneumophila*

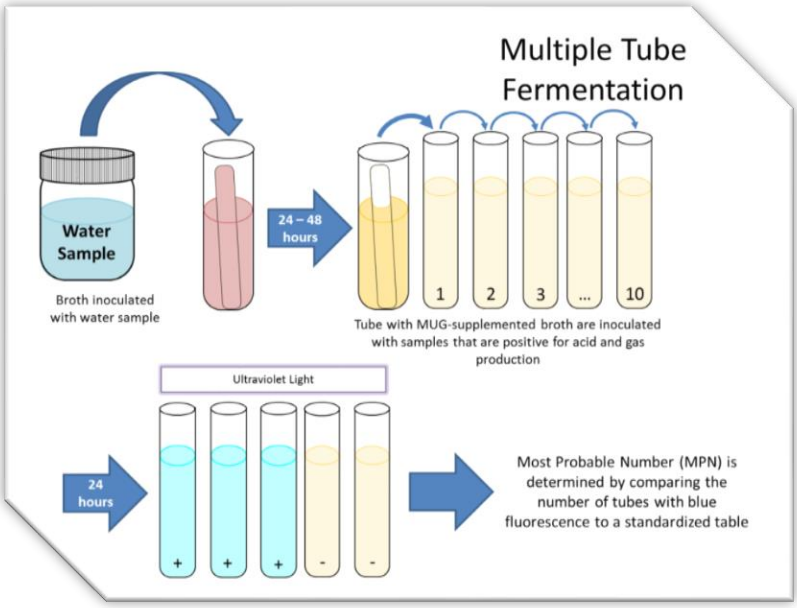
*Note: a facility may need to perform a dilution of their sample in order to quantify their *E. coli* counts using the Quanti-Tray method.

Analytical Approach	Standardized Test Method	Commercial Technology	Advantages	Disadvantages
Multiple tube/ multiple well	SM 9223-B-2016 AOAC 991.15	Colilert®[1] Colilert-18®[1]	• Commercially available • Standardized media and procedure • Less labor, material, and time intensive • Requires minimal technical skill to evaluate results	• May yield higher values than membrane filtration methods[2] • Reagent more costly[3] • Requires specialized equipment

1. The advantages listed are specific to the Colilert® technologies.
2. Potential causes of discrepancies may include: (1) a greater-than-average false-positive rate with Colilert®; (2) a high number of false negatives with membrane filtration; (3) the ability for Colilert® to detect injured and viable but non-culturable bacterial cells while these cells cannot be detected via membrane filtration.
3. Facilities may potentially get a discount on Colilert which could make MTMW cheaper than membrane filtration after the initial upfront costs of equipment.

SM = Standard Methods for the Analysis of Water and Wastewater
AOAC = Association of Analytical Chemists

Multiple Tube Fermentation



The multiple tube fermentation approach is a two-step process. First, a water sample is added to test tubes containing bacteria growth media and incubated for 24-48 hrs. Tubes that are positive for the production of acid and/or gas are then added into a series of tubes with media containing MUG. After 24 hours, the tubes are examined for fluorescence.

The bacteria level is reported as the most probable number (MPN). The MPN is estimated from the number of tubes that are positive for the presence of bacteria growth using a standardized table.

This approach is not used frequently as the precision is low unless a large number of samples are collected, and it is more labor and time intensive than the other approaches.

Approved Methods

- SM 9221B.3-2014
- SM 9221F-2014

Not commonly used and so additional details not provided at this time.

Analytical Approach	Standardized Test Method	Commercial Technology	Advantages	Disadvantages
Multiple tube fermentation	SM 9221B.3−2014 SM 9221F−2014	N/A	• One of the first approved methods for quantifying *E. coli*	• Not commonly used • Labor and time intensive • May underestimate bacterial density

SM = Standard Methods for the Analysis of Water and Wastewater

REPORTING RESULTS

Results shall be reported on the DMRs in #/100 mL. If a dilution of the effluent sample is performed in order to quantify their result, the facility will need to calculate the number of colonies per 100 mL.

For example, a sample volume of 25 mL (with 75 mL of distilled water), produces a count of 75 colonies. The reported result on the DMR would be 300 #/100 mL.

$$\frac{75\ \#}{25\ mL} \times 100 = 300\ \#/100\,mL$$

If a Too Numerous to Count (TNTC) occurs, then an asterisk (*) should be recorded for that day on the DMR and a new sample obtained immediately. One of following changes should be made to the test method:

- If the membrane filtration method is being used, to reduce interference from overcrowding, the dilution series should be changed, or a smaller portion of the sample should be filtered such that a countable number is obtained.
- If the multiple tube/multiple well method is being used, the dilution series should be changed, or a different count tray should be utilized which has a higher range.

All less than (<) daily values should be reported on the DMR as less than whatever the dilution series would indicate. For example, if counts from all filters in a dilution series are zero, report the count for the fecal coliform as a less than value. Calculate the number of colonies per 100 ml that would have been reported if there had been one colony on the filter representing the largest filtration volume. For example, sample volumes of 25, 10- and 2-ml produced colonies of 0, 0, and 0 respectively. The count would be reported as <4 colonies per 100 ml.

$$\frac{<1\ \#}{25\ mL} \times 100 = <4\ \#/100\,mL$$

Additional Resources

- Analytical Test Methods and Procedures. *Wisconsin Administrative Code,* Chapter NR 219, 2020. https://docs.legis.wisconsin.gov/code/admin_code/nr/200/219.pdf
- Bain RE, *et al*. 2015. Evaluation of an inexpensive growth medium for direct detection of Escherichia coli in temperate and sub-tropical waters. PLoS One 10(10): e0140997.
- Bain, R. *et al.* A summary catalogue of microbial drinking water tests for low and medium resource settings. *International Journal of Environmental Research and Public Health*, 2012, 9: 1609-1625. http://www.mdpi.com/1660-4601/9/5/1609/pdf
- Buckalew, D. W. *et al.* A long-term study comparing membrane filtration with Colilert® defined substrates in detecting fecal coliforms and *Escherichia coli* in natural waters. *Journal of Environmental Management*, 2006, 80: 191-197.
- Clark DL, *et al*. 1991. Comparative study of commercial 4-methylumbelliferyl-beta-D-glucuronide preparations with the Standard Methods membrane filtration fecal coliform test for the detection of Escherichia coli in water samples. *Applied and Environmental Microbiology* 57(5): 1528-1534.
- Edge, T. A. and Boehm, A. B. (2011). Classical and molecular methods to measure fecal bacteria. In Sadowsky and R. L. Whitman (Eds.), *The Fecal Bacteria* (241-273). Washington, DC: American Society for Microbiology.
- Hach Company. *m-ColiBlue24® Broth, Plastic Ampules, PK/50*. http://www.hach.com/m-coliblue24-broth-plastic-ampules-pk-50/product?id=7640249626&callback=pf
- Hamilton, W. P. *et al*. comparison of commercially available E*scherichia coli* enumeration tests: Implications for attaining water quality standards*. Water Research*, 2005, 39: 4869-4878.
- IDEXX Laboratories. *Colilert®*. https://www.idexx.com/en/water/water-products-services/colilert/
- Guidelines Establishing Test Procedures for the Analysis of Pollutants. *Code of Federal Regulations*, 40 "CFR" 136, 2014. http://www.ecfr.gov/cgi-bin/text-idx?SID=b104ff3b9795753b09a5aac5af6eaf95&mc=true&node=pt40.25.136&rgn=div5
- Mannapperuma WMGCK, *et al*. 2011. Comparison of bacteriological methods for detecting and enumerating total coliforms and Escherichia coli in water. *Research Journal of Microbiology* 6(12): 851-861.
- Olstadt, J. et al. A comparison of ten USEPA approved total coliform/*E. coli* tests. *Journal of Water and Health*, 2007, 267-282.
- State of Oregon – Department of Environmental Quality (2003) Memorandum: *E. coli* methods and holding times. http://cwwuc.org/reference/prehearingstmt/Exhibit5.pdf
- State of Washington – Department of Ecology (2011) *Alternative bacteria source identification using Colilert®/Quanti-Tray 2000 test methods in irrigated agricultural watersheds.* http://www.svid.org/images/November%2017%20Final%20%20Report%20for%20Contract.pdf
- United States Environmental Protection Agency (1986) Ambient Water Quality Criteria for Bacteria. https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OW-2007-0808-0001&disposition=attachment&contentType=pdf
- United States Environmental Protection Agency (2012) Recreational Water Quality Criteria https://www.epa.gov/sites/production/files/2015-10/documents/rwqc2012.pdf