

Microbiological Measurement in Wastewater Samples

Hach m-ColiBlue24[®] Technology

Comparison to Other Methods, “How To”,
Analytical Performance, and EPA Approval Status

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m-ColiBlue24[®] EPA Approval – YES!

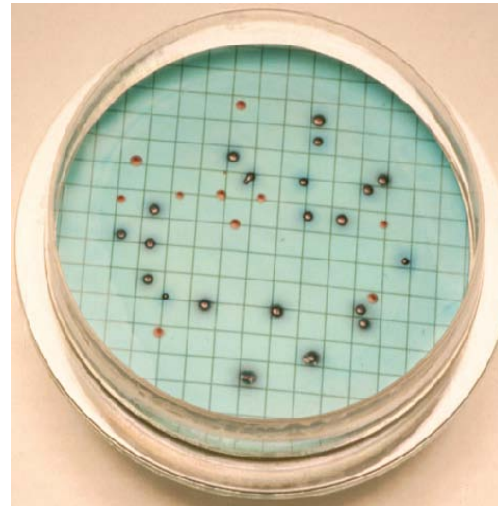
- US EPA Approved in 2000 after rigorous multi-state, multi-water study
 - *Total Coliform*
 - *E. coli*
- Ohio EPA Approved
- Continued round robin and multi-site studies to validate on-going performance of the product line

Microbiology - CFU

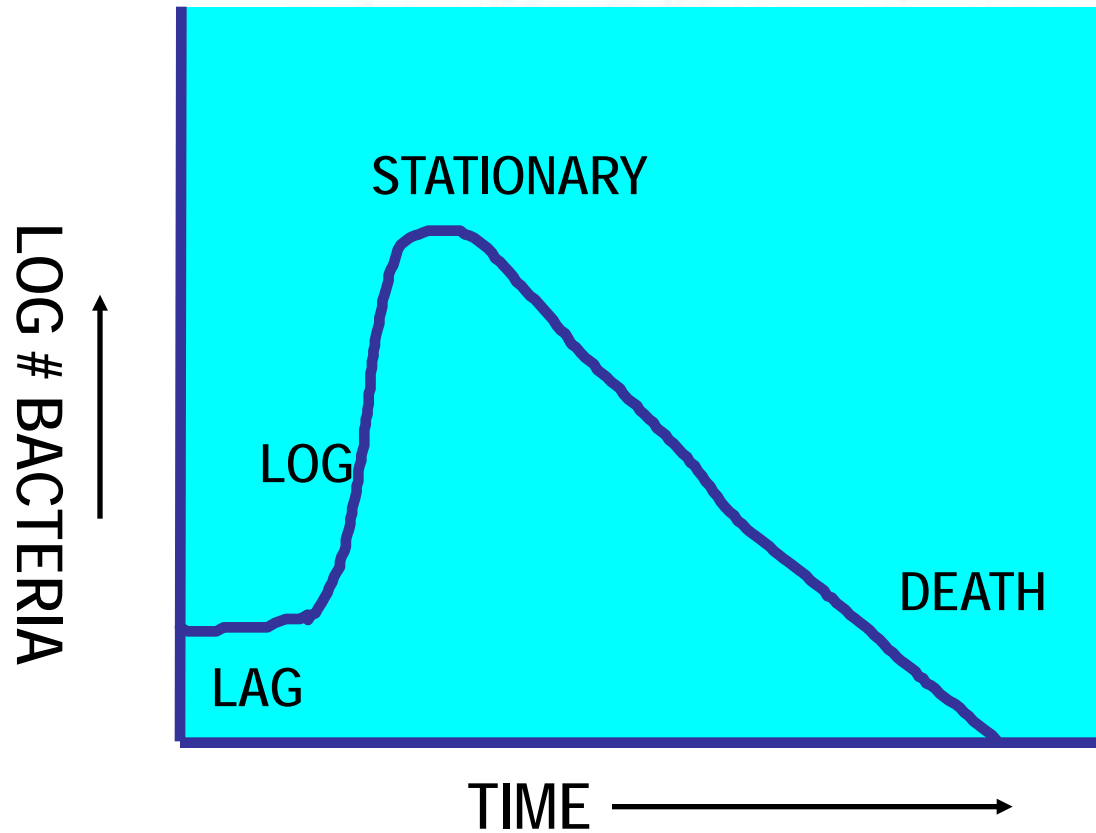
- Colony Forming Unit (CFU) – Microbe capable of reproduction to form a colony. We are interested in detecting bacteria that are capable of growth.
- Sometimes, there are “stressed” bacteria that have had near Inactivation Doses (near death experiences) of treatment but can recover and form colonies
- Several ways microbiology can be Inactivated:
 - Cell wall damage
 - Mitochondrial damage
 - RNA/DNA damage
 - Others

Culture Media Testing

- Time is Required for Reproduction
 - Bacteria reproduce in numbers, not size – Cell division (binary fission)
 - Can double population every 20 -30 minutes
 - Start with 1 cell; after 24 hrs = 30,000,000
- Bacterial growth requires time and temperature, especially when “stressed”, culture media provides a luxurious and selective environment for the targeted microbe to recover and grow



Growth Rate Curve



Two Microbiology Test Types

Presumptive Test: Growth media that facilitates the growth of the target organism...false positives may occur

Confirmation Test: Selective growth of target organism & sometimes a higher temperature incubation.

Hach's Microbiology Portfolio

- Organisms
 - *E. coli*
 - **Total Coliforms**
 - **Fecal Coliforms**
 - Yeast & Mold
 - Total (Heterotrophic) Bacteria
 - Pseudomonas
 - Enterococci
 - Others for diagnostic testing (e.g., acid producing, nitrifying, slime producing)
- Techniques Supported
 - **Membrane Filtration (MF)**
 - Multiple Tube Fermentation (MTF)
 - Most Probable Number (MPN)
 - Presence-Absence Media (P/A)
 - Paddle Testers (Dip Slides)
 - Biological Activity Reaction Test (BART)

Hach Company Media Products

***E. coli* Detection**

- Hach Colitag™ 16
- **m-ColiBlue24®**
- MI Media
- Modified m-TEC

Coliform Detection

- Hach Colitag™ 16
- **m-ColiBlue24®**
- MI Media
- m-Endo
- m-FC
- Paddle Testers (Dip Slides)

Yeast & Mold Detection

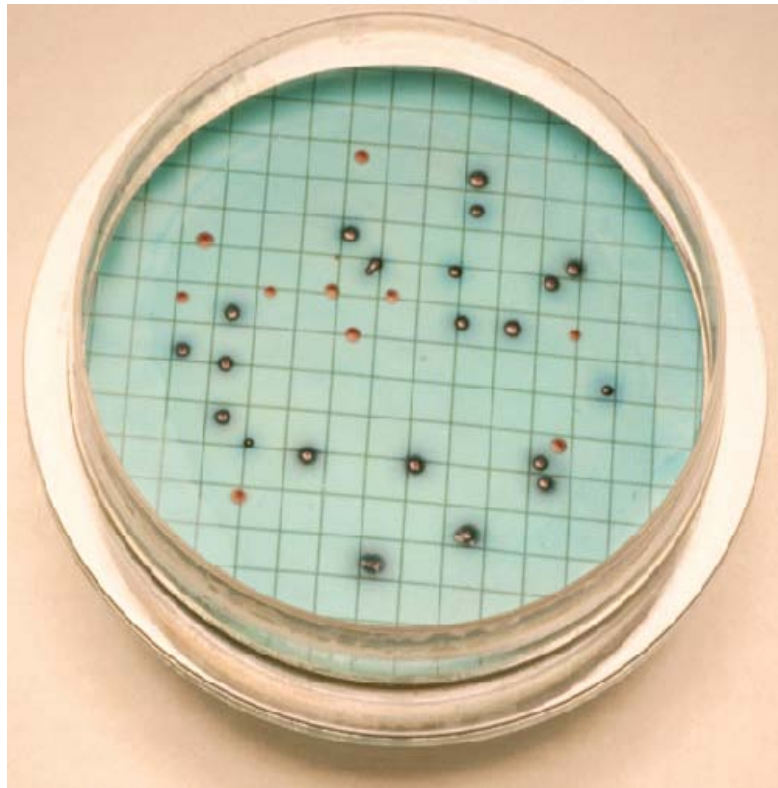
- M-Green YM
- PRY
- Paddle Testers (Dip Slides)

Total Aerobic Bacteria

- m-HPC
- m-TGE
- Tryptic Soy Broth
- Acridine Orange
- Nutrient Agar
- Paddle Testers (Dip Slides)

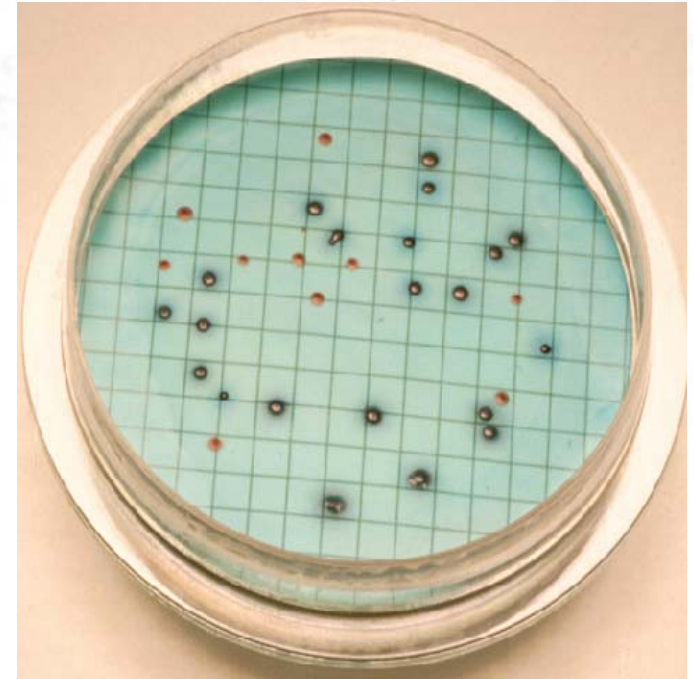
Membrane Filtration (MF) m-ColiBlue24[®] Method

A versatile, accurate, and easy to use system



Membrane Filtration (MF)

- m-ColiBlue24[®] testing medium
 - No confirmation test required
 - Typical sample
 - Potable and nonpotable water
- 100 ml sample + m-ColiBlue24[®] Media
 - **Red** + **Blue** colonies for Total Coliforms
 - **Blue** colonies for *E. coli*
- Incubation Requirements
 - Time = 24 hours
 - Temp = 35 ± 0.5 °C



m-ColiBlue24[®] Media = Confirmation test for *E. coli*



mColiBlue24[®] Media

What is it?

- Nutritive, lactose-based media that contains inhibitory components to eliminate growth of gram-positive organisms and non-coliform bacteria
- Provides easy visualization of coliform growth
- *E. coli* growth and enzymatic cleavage of BCIG result in blue colonies on mColiBlue24[®]
 - BCIG: 5-bromo-4-chloro-3-indolyl- β -D-glucuronide cyclohexylammonium salt
 - *E. coli* produces enzyme β -glucuronidase
 - β -glucuronidase enzyme will hydrolyze BCIG forming a blue color unique to *E. coli*

Step-by-Step: m-ColiBlue24[®]

Preparation of the Work Area

- Common cause of false positives in microbiology: CONTAMINATION!
- Disinfect the work area and equipment
- Wash hands
- Number, label, and date everything before you start
- Use pre-sterilized collection containers (Hach recommends Whirl-Pak[®] bags or presterilized bottles) with or without dechlorinating agent

Step-by-Step: m-ColiBlue24®

Sample Collection/Preservation

- Common cause of false positives in microbiology: CONTAMINATION!
- In sterilized containers (do you need dechlorinating agent?)
- Representative samples...
 - Surface waters: Plunge container mouth down and fill under water (do not rinse container)
 - Taps: Allow spigot to run for 2-3 minutes
- Holding time: No more than 6 hours for non-potable, 30 hours for potable waters (recommend 10° C but do not freeze)

Step-by-Step: m-ColiBlue24[®]

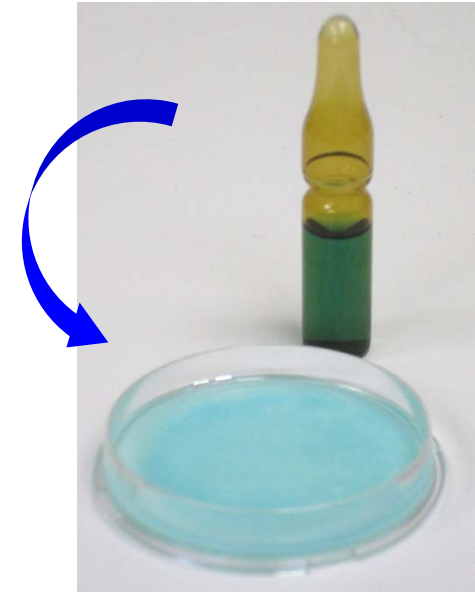
Collect Sample



Potable water can be filtered directly



Non-Potable water dilution series



**Add Media
to plate**

Step-by-Step: m-ColiBlue24[®]

Dilution

- Select sample size to give 20-200 CFU per filter
- Ideal target is 20-80 CFU per filter (ease of counting)
- Mixing: Waste to Ear motion

11mL of Sample into 99mL: Bottle A

11mL of Bottle A into 99mL: Bottle B (10X Dilution)

11mL of Bottle B into 99mL: Bottle C (100X Dilution)

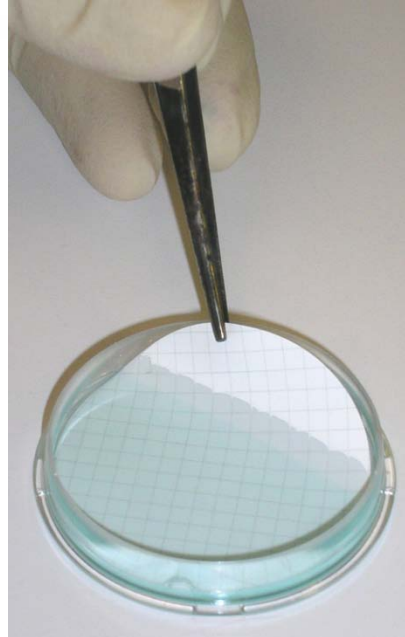
And on...

11mL into 99mL? Why? We're not dealing with uniform distribution.

Step-by-Step: m-ColiBlue24[®]



Filter Samples



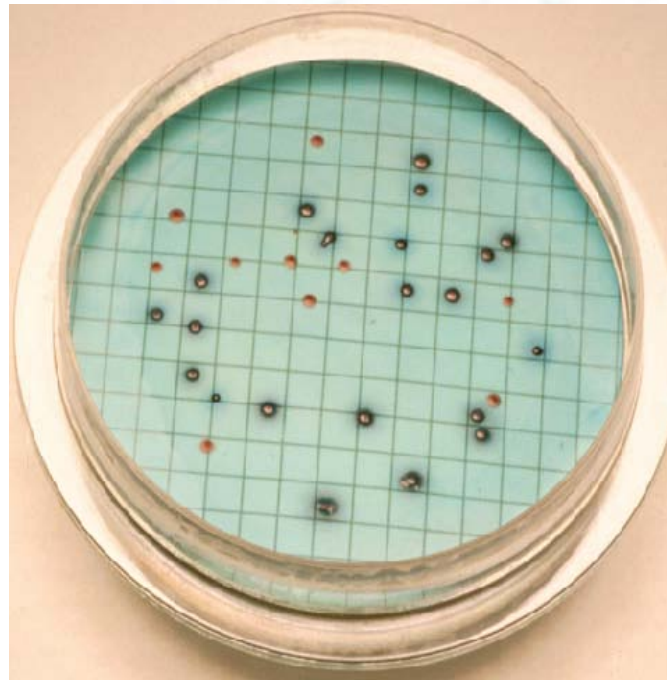
Place Filter onto Media
in Petri Dish



Place Dish in Incubator

Step-by-Step: m-ColiBlue24[®]

- m-ColiBlue Colonies...24 hours later



Red + Blue = Total Coliform
Blue = *E. coli*

Step-by-Step: m-ColiBlue24®

Interpreting Results – Single Filter Test

$$\text{Bacteria colonies per 100mL} = \frac{\text{Bacteria colonies counted}}{\text{mL of Sample}} \times 100$$

Interpreting Results – Multiple Filter Test

$$\text{Bacteria colonies per 100mL} = \frac{\text{Sum of colonies in all samples}}{\text{Sum of volumes (in mL) of all samples}} \times 100$$

- Indistinct colonies: Report as “Confluent growth with or without coliforms”
- High colony density: Report as “Too numerous to count” (TNTC) typically with colonies >200 CFU

Convenient Packaging



Broth

Cost/test ~\$1.50

Agar Plates

Cost/test ~\$3.50

This includes media, absorbent pad, petri dish and membrane filter.

Hach Manufacturing

- Products manufactured under Good Manufacturing Practices
- Facilities on a regular certification cycle
- Rigorous quality control of each lot of media produced

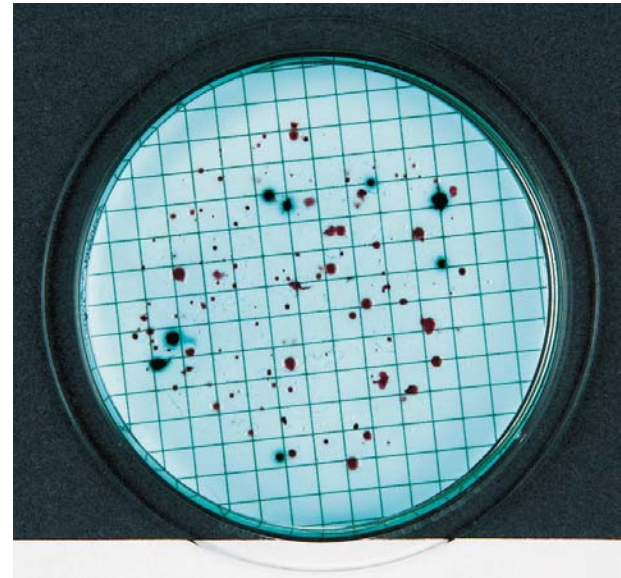


Features of m-ColiBlue24® Media

- EPA-approved media
- Membrane Filtration method
- Detection and differentiation of total coliforms and *E. coli* by color
- 24 hour test
- No confirmation required
- Prepared media in 2 mL plastic or glass ampules or 100 mL bottle or agar plates
- Certificate of Analysis included
- Shelf life is 1 year when stored at 4°C
- Economy and convenience kits available
- Easy to follow procedures and instructions available

Specificity Study

- 25 water samples from 7 states
 - 19 surface waters
 - 3 nonchlorinated primary wastewater effluent
 - 1 potable water during boil water alert
 - 2 potable water spiked with wastewater



Total Coliform Specificity

m-ColiBlue24[®]

m-Endo

98.9%

Sensitivity

96.8%

78.7%

Specificity

80.1%

E. coli Specificity Results

m-ColiBlue24[®]

100%

97.7%

Sensitivity

Specificity

mTEC

97.5%

88%

