Comparison of Coliform Media

AWWA Lab Practice Committee
Overview

- Types of Media
- General Procedures
- Individual Media Properties/Results/Comments
- Comparisons
Types of Media

- Membrane Filtration (m-Endo and m-Coliblue)
- Quanti-Tray
- Presence/Absence
- Colilert
- Colisure
- Colitag
- Ready Cult
- E*Colite
General Membrane Filtration Procedure

* Collect water sample in sterile container
* Prepare media in petri dish
* Filter water through filter and rinse
* Transfer filter onto media and incubate at 35 +/- 0.5°C
* Perform confirmation step (if needed)
m-Endo

- Media can be made in-house or purchased as ampules or pre-made plates
- Incubate for 22-24 hours
- Will need to perform confirmation test for both T. Coliform and E. Coli
  - T. Coliform-Lauryl Tryptose and Brilliant Green Bile
  - E. Coli-EC w/ MUG media or Nutrient Agar w/ MUG
m-Endo Results

* Presumptive
* T. Coliform/E. Coli will be red w/ greenish-gold metallic sheen
Confirmation of T. Coliform

- Use sterile inoculating needle to transfer positive colony from plate to LT and BGB broth tubes
- Incubate tubes at 35 +/- 0.5°C for 24 +/- 2 hours
- Possible incubation for another 24 hours if both samples not shown to be positive after 24 hours
m-Endo Results

* Confirmation of T. Coliform
  * Positive tubes will have gas in inner tubes
  * Positive tubes will have turbidity
m-Endo

- Confirmation of E. Coli
- EC w/ MUG
  - Transfer positive colonies w/ sterile swab/loop into EC/MUG broth tubes
  - Incubate at 44.5 ± 0.2°C for 24 ± 2 hours
- Nutrient Agar w/ MUG
  - Transfer filter from m-Endo pad onto petri dish w/ NA w/ MUG
  - Incubate at 35 ± 0.5°C for 4 hours
m-Endo Results

- Confirmation of E. Coli
- EC w/ MUG
  - Positive tubes will have growth and fluorescence under black light
- Nutrient Agar w/ MUG
  - Positive colonies will fluoresce under black light
Longer time period from initial test to results
Premade ampules had too much media for pads
More time-consuming procedure
May take more operator training
More consumables/equipment needed than other methods
m-Coliblue

- Media come in ampules or pre-made plates
- Incubate for 24 hours
- Count number of positive colonies
T. Coliform colonies will be red

- May need to use oxidase test if many interfering bacteria
- *Pseudomonas, Vibrio* and *Aeromonas* spp. will be red also but are oxidase positive and will be blue w/ oxidase reagent

- E. Coli colonies will be blue
Comments

* Shorter time span to get results
* Able to differentiate between T. Coliform/E. Coli
* Blue colonies can appear gray
* Some did not get good recovery
* Interfering bacteria may cause issues
* Glass ampules may cause injury/contamination when opening
* More consumables/equipment than other methods
Quanti-Tray Procedure

- Collect sample in sterile container (100 mL)
- Mix sample w/ Colilert media
- Pour sample/media into Quanti-Tray
- Seal Quanti-Tray in sealer
- Incubate sample for 18-24 hours at 35 +/- 0.5°C (depending on type of media used)
- Count number of positive wells and use table to obtain MPN
Quanti-Tray Results

- Negative wells will be clear
- T. Coliform positive wells will be yellow
- E. Coli positive wells will fluoresce under a black light
- Count the number of positive wells and use MPN table to get number of T. Coliform/E. Coli
Can have results in 18-24 hours
Have to purchase additional equipment/consumables
Counts up to 2419.6 MPN
Collect sample in sterile container
* Mix media with sample
* Incubate at 35 +/- 0.5 °C
* Use color change/fluorescence to indicate presence of T. Coliform or E. Coli
* Perform confirmation steps (if needed)
Presence/Absence

* Can make media in-house or purchase ampules or bottles w/ media inside
* Incubate samples 24-48 hours at 35 +/- 0.5°C (depending on results)
* Confirmation steps necessary (similar to m-Endo)
  * BGB for T. Coliform
  * EC w/ MUG for E. Coli
Presence/Absence Results

* Presumptive
  * Negative samples will be red/purple
  * T. Coliform/E. Coli positive samples will be yellow/brown with gas production
• Longer time period from initial sample to results
• Some got false positives/negatives
• Packaged media had possible contamination issues (pull tabs on bottles)
• Glass ampules could cause injury/contamination
18 Hour and 24 Hour Media Options

18 Hour

* Incubate in waterbath at 44.5 +/- 0.2°C for 7 minutes or in 35°C waterbath for 20 minutes
* Incubate for 18 hours at 35 +/- 0.5°C

24 Hour

* Incubate for 24 hours at 35 +/- 0.5°C
Colilert Results

- Negative samples will be clear
- T. Coliform positive samples will be yellow
- E. Coli positive samples will be yellow and fluoresce under a black light
Comments

* Sample results in 18-28 hours
* Easiest media to open of all tested
* Fairly simple procedure
Colisure

- Sample should be brought to room temperature
- Incubate for 24 hours at 35 +/- 0.5°C
Colisure Results

- Negative samples will be yellow
- T. Coliform positive samples will be magenta
- E. Coli positive samples will be magenta and fluoresce under a black light
May be good for turbid/yellow/rusty samples
Sample results in 24 hours
Fairly simple procedure
16-48 Hour Procedure
* Incubate in 44.5°C water bath for 7-10 minutes
* Incubate at 35 +/- 0.5°C for 16-48 hours

22-48 Hour Procedure
* Incubate at 35 +/- 0.5°C for 22-48 hours
Colitag Results

- Negative samples will be clear
- T. Coliform positive samples are yellow
- E. Coli positive samples fluoresce under a black light
Comments

- Quickest window from sampling to results
- Samples can stay in incubator much longer
- Concerns about long window
- Media packets difficult to open (some tore)
* Incubate at 35-37°C for 24 hours
* Can incubate at room temperature for 48 hours
* Confirmation step for E. Coli (Kovac’s Reagent)
ReadyCult Results

- Negative samples will be clear
- T. Coliform positive samples are blue-green (whole sample or just upper portion)
- E. Coli positive samples are blue-green and fluoresce under a black light
- Kovac’s reagent makes an immediate red ring in E. Coli positive samples
Good for samples when incubator not available
Kovac’s reagent is hazardous
Some operators did not like color change
Kovac’s reagent will make a ring in T. Coliform only samples if left to sit for long enough (only look for immediate reaction)
Room temperature samples have less intense color change
Media packets are hard to open
* Samples poured directly into E*Colite bags
* Sample pushed through divider to react with media
* Incubate in 35°C waterbath for 10 minutes and then in 35 +/- 0.5°C incubator for 28 hours
* Push sample through second divider to react with bactericide when done
E*Colite Results

* Negative samples will be clear/yellow
* T. Coliform positive samples will be blue or blue-green (even if just in corners)
* E. Coli positive samples will fluoresce under a black light
E*Colite Comments

- Difficult to push sample through divider
- Bags leaked/punctured when pushing sample through divider
- Smelled very bad (for a long time)
- May be good for field sampling (all in one)
- Instructions right on bag
## Cost

<table>
<thead>
<tr>
<th>Media</th>
<th>Average Cost/Test</th>
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<tbody>
<tr>
<td>m-Endo (in house)</td>
<td>$0.25</td>
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<tr>
<td>m-Endo (ampules)</td>
<td>$1.30</td>
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<tr>
<td>m-Endo (plates)</td>
<td>$5.00</td>
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<tr>
<td>m-Coliblue (ampules)</td>
<td>$2.00</td>
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<tr>
<td>m-Coliblue (plates)</td>
<td>$5.10</td>
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<tr>
<td>QuantiTray</td>
<td>$6.50</td>
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<tr>
<td>Presence/Absence (in house)</td>
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<tr>
<td>Presence/Absence (ampules)</td>
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<tr>
<td>Presence/Absence (bottles/reagent)</td>
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<tr>
<td>Colilert 24 Hour</td>
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<td>Coliler 18 Hour</td>
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<tr>
<td>Colisure</td>
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<td>ReadyCult</td>
<td>$5.75</td>
</tr>
<tr>
<td>E*Colite</td>
<td>$4.75</td>
</tr>
</tbody>
</table>
Time On Task (Least to Most)

* Colilert/Colisure/Colitag/ReadyCult
* QuantiTray
* P/A
* Colilert 18/Modified Colitag
* E*Colite
* m-Coliblue
* m-Endo
Time to Train (Shortest to Longest)

- Colilert/Colisure/Colitag/ReadyCult
- Colilert 18/Modified Colitag
- QuantiTray
- E*Colite
- m-Coliblue
- P/A
- m-Endo
Any Questions?

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