Improved Selectivity of *E. coli* using Modified m-TEC: EPA 1603

In March of 2007, the USEPA (United States Environmental Protection Agency) replaced regular m-TEC with modified m-TEC as an approved method for enumerating *E. coli* for NPDES (National Primary Discharge and Elimination) reporting. Why this change occurred may be found by examining the effectiveness of using a chromogenic substrate as opposed to a urea/phenol substrate.

In 1981, A.P. Dufour was credited with developing a membrane filter technique for the enumeration and identification of *E. coli* using m-TEC media.[1] m-TEC is the acronym for membrane thermotolerant *E. coli*. This constituted a time savings over traditional methods involving multiple steps such as gas production from glucose. The widely recognized ability of *E. coli* to produce indole from tryptophane at elevated temperatures was used in conjuction with gas formation by many who were searching for a rapid method of enumerating and identifying *E. coli* as a water quality indicator. [2] One of the problems was the extra day of incubation, and the agents used in the tryptophan test are bactericidal, which precluded further testing of the subject microbes!

In 1986, the EPA recommended that *E. coli* be used as the main bacterial water quality indicator used to monitor the safety of ambient and recreational waters with respect to gastroenteritis and other in kind maladies.[3] To that end, the quest for ever faster and more reliable methods of enumerating and identifying the venerable *E. coli* bacteria continued. The advance of m-TEC brought the incubation time down to one day, (two hours at 35.0 +/- 0.5 degrees C., then 22 hours at 44.5 degrees C.) plus fifteen minutes in a urea/phenol substrate. Certainly a marked decrease in the amount of time for *E. coli* enumeration existed with this membrane filtration method.

So what makes modified m-TEC (method EPA 1603) a preferred method with greater selectivity over regular m-TEC? Upon examining the specific ingredients to each media, we see a great similarity. In fact, besides the urea substrate in situ confirmation with regular m-TEC, there is only one main ingredient change that differentiates regular m-TEC from modified m-TEC, that being the substitution of the indicators bromcresol purple and bromphenol red with the chromogen, 5-Bromo-6-Chloro-3-Indolyl-ß-D-Glucuronide.

Modified m-TEC Ingredients: m-TEC Ingredients:

(per liter of reagent water).

Proteose Peptone No. 3	5.0 g
Yeast Extract	3.0 g
Lactose	10.0 g
Sodium Chloride	7.5 g
Dipotassium Phosphate	3.3 g
Monopotassium Phosphate	1.0 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
5-Bromo-6-Chloro-3-Indolyl-ß-D-Glucuronide	**0.5 g**
Agar	15.0 g

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Dipotassium Phosphate	3.3 g
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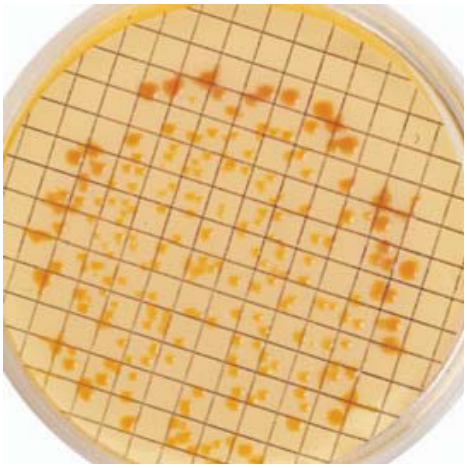
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Bromcresol purple	**0.05 g**
Bromphenol Red	**0.08g**
Agar	15.0 g

Amino acids, carbon, and nitrogen are supplied by the protease peptone. Trace elements, some vitamin complexes, as well as amino acids are found in the yeast. Cell equilibrium is maintained by the sodium chloride which promotes osmotic balance. Fermentable carbohydrates and more carbon come from the lactose. To balance pH in the media, mono and di-potassium phosphate provide buffering. Growth from competing gram positive bacteria is discouraged in both media by the addition of sodium lauryl sulfate and sodium desoxycholate.

Now we've covered all of the ingredients common to both media including the base agar. The difference will occur in the last portion of the membrane filtration procedure. Each of the plates representing m-TEC versus modified m-TEC have been introduced after filtration to the dry incubator for two hours at 35.0 degrees C. +/- 0.5 C. to revive any injured or stressed cells. Then, both are transferred to a wet bath with gable cover at 44.5 degrees C. +/- 0.2 C. for twenty-two to twenty-four hours. Incubation is over, and it's time to remove the plates – but the filter on the m-TEC plate still as to be transferred to a petri dish with a pad soaked with urea and phenol substrate for fifteen minutes (in situ portion). Wait fifteen minutes for the *E. coli* colonies (if present) to turn a yellow-brown color. The bromcresol purple and bromphenol red are the indicator ingredients. The problem is, it can be difficult for the analyst to determine which colonies are not *E. coli*, because you often get competing colonies that are clear, or similar in color and/or morphology to the *E. coli* colonies you are presumeably looking at. Some urease negative colonies may not have been differentially reduced via the high secondary incubation temperature. Competing colonies like Proteus mirabilis can be problematic. The *E. coli* colonies are sometimes not easily distinguishable from competing organisms, and the background color of the m-TEC plate (purple-red) does not create a stark contrast to the colonies (yellow-brown) in some cases.

See example below of m-TEC agar plate with *E. coli* colonies.



Above Photo courtesy of Difco Co.

When we look at *E. coli* colonies on a plate prepared with modified m-TEC, the colonies are a stunning red-magenta color and stand out superbly against the light tan background. Because competing organisms are few and mostly clear, differentiating between *E. coli* and competing organisms is elementary. See the image of *E. coli* colonies on modified m-TEC below.

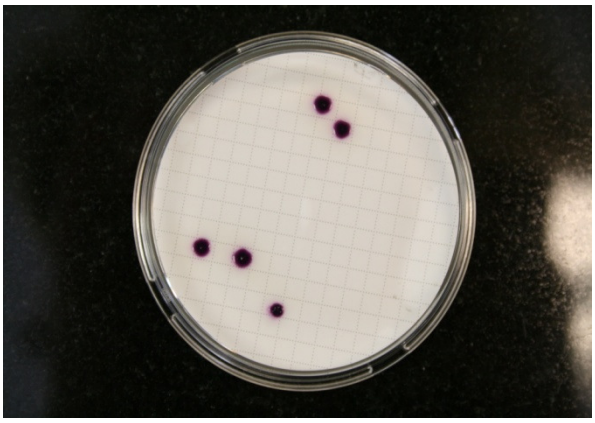


Photo above courtesy of Aquacheck Laboratory, Inc.

Practical considerations such as eye strain are real world examples of difficulties with identification of *E. coli* colonies. The ingredient that makes this possible is a chromogen called, 5-bromo-6-chloro-*B*-D-glucuronide. The coloration of the colonies is evident as soon as they are removed from the gable covered wet bath. Catabolism of the chromogen to form a glucuronic acid and a red-magenta compound is evidenced by *E. coli* that produce the enzyme

B-glucurodinase. The color change can be viewed with a spectrophotometer at 463 nanometers as the colony forms.[4] As you can see from the images above, it is much easier to read and record the colonies grown on modified-m-TEC as compared to regular m-TEC. Selectivity is greatly increased because of the elimination of confusing colonies (competing colonies of similar color and/or morphology) as well as providing excellent contrast which promotes easy visual identification. *Klebsiella pneumoniae* (Kp) may form a light colony with typically mottled morphology. Formation may include individual colonies, or small "spreaders" where colonies run into each other.

In conclusion, superior selectivity of the chromogenic method over the urea substrate method is noted by the significantly increased ease of recognition of red-magenta colonies over the yellow-brown colonies.

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References:

1. Applied Environmental Microbiology – May, 1981 vol. 41 nos. 1152-1158
2. Applied Environmental Microbiology – May, 1981 vol. 41 nos. 1152-1158
3. USEPA 1986 – Ambient Water Quality Criteria for Bacteria: EPA440/5-84-002
4. Sigma-Aldrich – correspondence from Mr. Tom Glendening, Ph.D. Sigma-Aldrich Technical Support Service