Come See Us On The Road This Spring/Summer !

Our sales staff will be traveling to several shows and events in the next few months. If you're planning to attend any of the following events, stop by to see us and say hello. Planned events are listed on our web site at: www.envexp.com/news/upcoming_events.asp.

Rocky Mountain Water Quality Symposium, *Denver, CO, April, 25, 2008, Website - http://www.rmwqaa.org/*

SAS Atomic Spectroscopy Workshop, *Westmont, IL, May 8, 2008, Website - http://www.Varianinc.com*

Triangle Chromatography Symposium, *Raleigh, NC, May 15, 2008, Website - http://www.rtpnet.org/tcdg/symp25.html*

North Carolina Lab Technology Day (Sponsored by NCWOA, NCAWWA-WEA, CLANC, State Lab of Public Health, NC DWQ & NC DEH), *Raleigh, NC, May 20, 2008, Website - http://www.ncwoa.com/news.htm*

Florida Society of Environmental Analysts (FSEA) Spring Meeting *St. Pete Beach, FL, May 21-23, 2008, Website - http://www.fsea.net/*

NYAAEL/PaAAEL (New York and Pennsylvania Associations of Approved Environmental Laboratories) 2008 Annual Convention and Exposition, *Syracuse, NY, July 20-22, 2008, Website - http://www.nyaael.org/*

Virginia Lab Practices Conference, *Charlottesville, VA, Aug 4-5, 2008, Website - http://www.vaawwa.org/events/events.html?id=206,*

NEMC (National Environmental Monitoring Council) *Aug 11-15, 2008, Washington, DC Website - http://www.nemc.us/*

WEASC (South Carolina Water Environment Association) Laboratory Workshop, *Columbia, SC August 21, 2008, http://www.weasc.org/*

EXPRESS NEWS

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Improved Selectivity of E. Coli Using Modified m-TEC: EPA 1603

By Scott Bradley, Laboratory Director, Aquacheck Laboratory, Inc.

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. The need for this change can best be understood by comparing results obtained using a chromogenic substrate as opposed to those obtained using 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 tryptophan at elevated temperatures was used in conjunction with gas formation by many who were searching for a rapid method of enumerating and identifying *E. coli* as a water quality indicator.[2] This technique required an extra day of incubation. In addition, 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°C., then 22 hours at 44.5 °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. The only ingredient change that differentiates regular m-TEC from modified m-Tec is the substitution of the indicators bromcresol purple and bromphenol red with the chromogen, 5-Bromo-6-Chloro-3-Indolyl-ß-D-Glucuronide (see chart below).

Modified m-TEC Ingredients: (per liter of reagent water)		***m-TEC Ingredients: (per liter of reagent water)***	
Proteose Peptone No. 3	*5.0 g*	*Proteose Peptone No. 3*	*5.0 g*
Yeast Extract	*3.0 g*	*Yeast Extract*	*3.0 g*
Lactose	*10.0 g*	*Lactose*	*10.0 g*
Sodium Chloride	*7.5 g*	*Sodium Chloride*	*7.5 g*
Dipotassium Phosphate	*3.3 g*	*Dipotassium Phosphate*	*3.3 g*
Monopotassium Phosphate	*1.0 g*	*Monopotassium Phosphate*	*1.0 g*
Sodium Lauryl Sulfate	*0.2 g*	*Sodium Lauryl Sulfate*	*0.2 g*
Sodium Desoxycholate	*0.1 g*	*Sodium Desoxycholate*	*0.1 g*
5-Bromo-6-Chloro-3-Indolyl-ß-D-Glucuronide	*0.5 g*	*Bromcresol purple*	*0.05 g*
Agar	*15.0 g*	*Bromphenol Red*	*0.08g*
		Agar	*15.0g*

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

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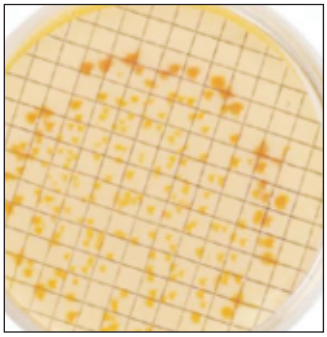
carbohydrates and more carbon come from the lactose. To balance pH in the media, mono and di-potassium phosphate provide buffering. Growth from competing gram-positive bacteria is discouraged in both media by the addition of sodium lauryl sulfate and sodium desoxycholate.

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°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°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 has 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 presumably 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.

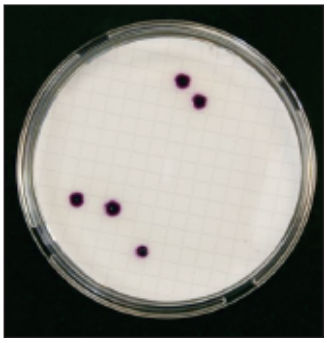
When we look at *E. coli* colonies on a plate prepared with modified m-TEC, the colonies are a stunning red-magenta color and stand out superbly against the light tan background. Because competing organisms are few and mostly clear, differentiating between *E. coli* and competing organisms is elementary.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

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 below, 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.



The top image shows an m-TEC agar plate with E. coli colonies. Photo courtesy of Difco Co.



By contrast, the image at right shows the E.Coli colonies on a plate prepared with modified m-Tec. The bright red/magenta colonies are much easier to see and count. Photo courtesy Aquacheck Laboratories, Inc.

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*

To order a free sample of modified m-Tec plates, call Environmental Express, 800.343.5319, or visit our web site, www.environmentalexpress.com. Part # MMTecS.

"Overseed" the GGA Standard when doing CBOD?

By Perry Brake

The latest editions of* Standard Methods *include the following guidance:

"**Para 8.** When nitrification inhibitors are used [i.e., when doing CBOD], GGA test results falling outside the 198 ± 30.5 control limit quite often indicate use of incorrect amounts of seed. Adjust amount of seed added to the GGA test to achieve results falling within this range."

CBOD results would likely be lower than BOD results for the GGA solution because the nitrogen in the glutamic acid is not fully oxidized to nitrate when nitrifying bacteria are inhibited. So in effect, paragraph 8 is saying to "adjust" by adding extra seed, or "overseeding" the GGA test when doing CBOD to get results closer to 198 mg/L. Apparently, overseeding does cause higher CBOD results, although this author is unsure why since adding additional seed would result in a corresponding increase in the seed correction and theoretically, no change in the net DO depletion. But whether or not it works is not the question. The question is why should one attempt to make CBOD results equal BOD results?

The difference between CBOD and BOD is that oxidation of nitrogen in a sample is not optimized when doing CBOD. Accordingly, a sample containing reduced forms of nitrogen... such as glutamic acid... is ***expected*** to yield lower results than would a BOD test on the same sample. So why attempt to make CBOD and BOD results equal?

At one time, *Standard Methods* implied that analyzing a GGA sample was intended only to check effectiveness of the seed. In the latest editions, *Standard Methods* correctly states that GGA test is used to "check seed effectiveness and ***analytical technique***". When doing CBOD, checking analytical technique includes making sure the inhibitor is doing its job. If CBOD results for a sample are essentially the same as BOD results for the same sample, one would have to conclude that there are no nitrifying bacteria in the seed to inhibit, or that the sample contains no reduced forms of

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