Verification Performance of m-ColiBlue24 Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water

Hach Company
5600 Lindbergh Drive
Loveland, Colorado 80539 USA

June 5, 2009

Verification Performance of m-ColiBlue24 Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water

INTRODUCTION

m-ColiBlue24® is a differential membrane filter (MF) medium for the simultaneous detection and enumeration of both total coliforms (TC) and *Escherichia coli* (*E. coli*) in water samples in 24 hours or less on the basis of their specific enzyme activities. The medium has been validated for use with drinking water, source water, and wastewater in single-laboratory and multi-laboratory studies and is United States Environmental Protection Agency (USEPA) approved for use by certified drinking water laboratories (presence/absence) and for regulatory compliance in ambient water and wastewater (enumeration).

SITUATION

Recently, a report from a state hygiene laboratory and two side-by-side verification studies performed in Mexico and Puerto Rico raised questions about whether m-ColiBlue24 may be under reporting the detection and enumeration of *E. coli*. In all three instances, no information was provided as to the root cause of this asserted under-reporting. These instances appeared isolated as laboratories using m-ColiBlue24 for regulatory reporting purposes were not observing or reporting this anomaly. However, Hach Company, in the interest of public health and its customers who use m-ColiBlue24, placed the medium on product hold and began a detailed quality assurance investigation.

INVESTIGATION

Hach Company began an internal investigation with its manufacturing process, formulary, quality control (QC) procedures and results, use model (use methodology), and USEPA protocols[1], for evaluating microbiological methods and media. In this investigation, Hach's QC department observed low recovery of single strain *E. coli* organisms when analyzed in combination with m-Coliblue24 medium and a certain brand of absorbent pads. Hach then contracted with Dr. Doug Rice *et al*., of Colorado State University Environmental Services to conduct an absorbent pad study. Samples were prepared from a stock solution of single-strain colonies of *E. coli*, then analyzed in combination with m-ColiBlue24 and a reference media using multiple lots of different vendor provided absorbent pads.

The results of this study indicated substantially low or no recovery of *E. coli* with the suspect cellulose absorbent pad. Similarly, with all other absorbent pads, reduced recovery was observed from that of the reference media, but in those cases, the coefficient of variation (CV) was statistically similar. Therefore, it was concluded that (1) the absorbent pad was the root-cause of low or no recovery of *E. coli*; and (2) with all other absorbent pads, reduced recovery from that of the reference medium was a result of using single-strain *E. coli* organisms.

---

[1] EPA Method 1604: Total Coliforms and Escherichia coli in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium), EPA 821-R-02-024, United States Environmental Protection Agency, Office of Water, Washington DC 20460; EPA Microbiological Alternate Test Procedure Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods, EPA 821-B-03-004, United States Environmental Protection Agency, Office of Water, Washington DC 20460.

Review of the USEPA protocols for microbiological media evaluation indicated that performance of a method's medium should be based on the analysis of *E. coli* derived from primary treated wastewater effluent and that colony recovery performance should be interpreted from $\log_{10}$-based transformed data using coefficient of variance (a measure of repeatability). It was determined from the state hygiene, Mexico, and Puerto Rico studies that performance of m-ColiBlue24 against a reference medium was based solely on absolute colony recovery.

VERIFICATION OF m-COLIBLUE24 PERFORMANCE

Based on the investigative results from Colorado State University and USEPA protocols for microbiological media evaluation, a performance study was designed and conducted by Dr. Ted Pass *et al*., of Morehead State University to compare and verify the performance of m-ColiBlue24 against USEPA approved reference media, using indigenous strains of *E. coli* from primary treated wastewater effluent. Samples were prepared and analyzed at three different colony forming unit (CFU) ranges (1-10 CFU, 11-30 CFU, and >30 CFU). The results of this study (Appendix A) indicate the recovery of *E. coli* when using m-ColiBlue24 medium is statistically equivalent to all other USEPA approved MF media.

In a final performance evaluation of m-ColiBlue24, Morehead State University prepared and analyzed Webby certification proficiency test (PT) samples[2] against all USEPA approved MF media. The m-ColiBlue24 medium and all other media were found to be "Acceptable" for presence/absence in drinking water and "Acceptable" for enumeration in wastewater (Appendix B).

CONCLUSIONS

The studies from Colorado State University indicates that certain absorbent pads, when used in combination with m-ColiBlue24 medium and testing against single-strain organisms, may yield substantially lower recovery of *E. coli* when compared against another reference medium. When using other absorbent pads, m-ColiBlue24 performance is statistically equivalent to its reference media. Based on this research, Hach has concluded that its product is only compatible with the following branded pads:

- Pall Corporation (e.g., Sterile Petri Dishes with Absorbant Pads)
- Private Label/OEM products manufactured by Pall
- Gelman (now owned by Pall)
- Sartorius cellulose pads and glass fibre pads
- Sartorius BioSart products with the integrated Petri dish

No other pad is compatible with the product and the use of any pad other than those recommended above may result in unreliable, inaccurate data.

[2] Webby Environmental Proficiency Study RR-05226

Verification Performance of m-ColiBlue24 Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water

The verification study from Morehead State University indicates that m-ColiBlue 24 performance is equivalent to all other USEPA approved MF media and its colony recovery and coefficient of variation are statistically indifferent.

Verification Performance of m-ColiBlue24 Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water

Appendix A
Verification of Performance Results[1]

Medium	*E. coli* Count Range (CFU)	Initial *n*	Final *n*[2]	S_r[3]	RSD_r[4] (%)
m-ColiBlue24	<10	20	20	0.13	29.6
m-Endo	<10	20	20	0.41	49.0
modified m-Tec	<10	20	20	0.32	68.1
MI	<10	20	20	0.22	82.7
mFC/NA MUG	<10	20	20	0.23	51.4
m-ColiBlue24	11 - 30	20	20	0.63	53.8
m-Endo	11 - 30	20	20	0.53	40.4
modified m-Tec	11 - 30	20	20	0.47	39.9
MI	11 - 30	20	20	0.52	47.4
mFC/NA MUG	11 - 30	20	20	0.53	48.7
m-ColiBlue24	>30	20	19	1.06	63.1
m-Endo	>30	20	19	0.64	40.2
modified m-Tec	>30	20	20	1.16	66.6
MI	>30	20	20	0.86	49.4
mFC/NA MUG	>30	20	19	1.03	59.8

[1] The values are based on $\log_{10}$ transformed data
[2] These values were obtained using the Grubbs Outlier Statistical Calculation
[3] S_r, Single Operator Standard Deviation, a measure of repeatability
[4] RSD_r (%), Single Operator Relative Standard Deviation (Coefficient of Variance), a measure of repeatability

Appendix B
Proficiency Testing Results (*E. coli*)

Matrix	Medium	Assigned Value P/A or (CFU/100mL)	Result P/A or (CFU/100mL)	Acceptance Limits P/A or(CFU/100mL)	Evaluation
Drinking Water	m-ColiBlue24	P	P	P	Acceptable
	m-Endo	P	P	P	Acceptable
	modified m-Tec	P	P	P	Acceptable
	MI	P	P	P	Acceptable
	mFC/NA MUG	P	P	P	Acceptable
Source Water	m-ColiBlue24	79	90	22 - 136	Acceptable
	m-Endo	79	99		Acceptable
	modified m-Tec	79	78		Acceptable
	mFC/NA MUG	79	106		Acceptable
Control	m-ColiBlue24	30	26	Not Applicable	Acceptable
	m-Endo	30	28	Not Applicable	Acceptable
	modified m-Tec	30	26	Not Applicable	Acceptable
	MI	30	33	Not Applicable	Acceptable
	mFC/NA MUG	30	34	Not Applicable	Acceptable

P – Presence of *E. coli*
A – Absence of *E. coli*