Comparison of Methods for Quantifying Bacterial Indicators in an Urban Brackish Water Environment

Bradd J. Haley[1], Elisa Taviani[1], Jinna Choi[1], Christopher J. Grim[1,2], Mario Tamburri[3], Rita R. Colwell[1,2,4] and Anwar Huq[1*]

[1] Maryland Pathogen Research Institute, University of Maryland, College Park, MD, [2] Center of Bioinformatics and Computational Biology, University of Maryland Institute of Advanced Computer Studies, University of Maryland, College Park, MD, [3] Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, One Williams Street, Solomons, MD
[4] CosmosID, 387 Technology Dr. College Park, MD

Summary

The United States EPA and European Community Bathing Water Directive recommend testing the levels of *Escherichia coli* and enterococci in surface waters as proxies for the presence of human enteric pathogens. Similarly, international and United States regulations for ships' ballast water discharge include acceptable limits for *E. coli* and enterococci. In this report we present the results of a comparative study of standard membrane filtration methods and recently developed enzyme substrate methods, Colilert and Enterolert (IDEXX Laboratories, Inc.), for detection of *E. coli* and enterococci in an urban brackish water environment at the Port of Baltimore. Enterolert and Colilert assays showed significant and positive correlations with analogous membrane filtration methods, ρ = 0.60 for modified $mTEC_{44.5ºC}$, and, ρ = 0.55 for mEA-$BEA_{44.5ºC}$. Microbial concentrations were significantly higher for membrane filtration assays incubated at Enterolert and Colilert recommended temperatures (41ºC and 35ºC, respectively), thereby producing stronger correlations, ρ = 0.89 for modified $mTEC_{35ºC}$ and ρ = 0.65 for mEA-$BEA_{41.5ºC}$. These results indicate that the membrane substrate methods tested, Enterolert and Colilert, may overestimate the target bacterial populations because of incubation at reduced temperatures compared to standard methods, most likely by allowing growth of non-thermotolerant or non-fecal bacteria.

Materials and Methods

1 Liter water collected in sterile polypropylene bottle

↓

Transported to laboratory on ice and processed within 4 hours of collection

↓ ↓

Enterolert and Colilert Processing

↓

Incubated at manufacturers recommended temperature

Membrane filtration processing for *E. coli* and enterococci

↓

Incubated at standard temperature for thermotolerant *E. coli* and enterococci as well as temperatures recommended for Enterolert and Colilert processing

Results

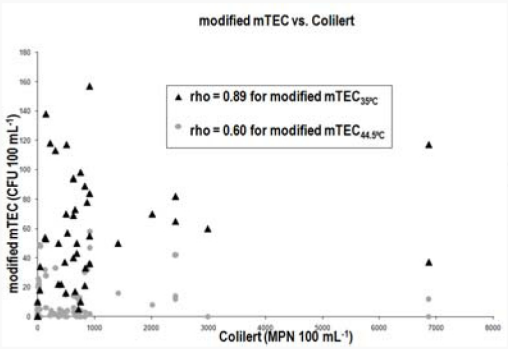

Figure 1. Scatter plot of *E. coli* densities as determined by modified mTEC agar and Colilert assays.

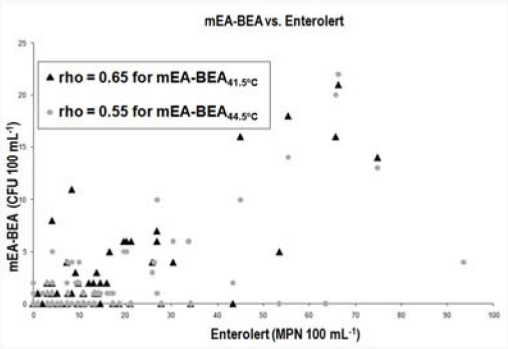

Figure 2. Scatter plot of enterococci densities as determined by mEA-BEA agar and Enterolert assays.

Statistic	Enterolert	mEA-BEA 41.5ºC	mEA-BEA 44.5ºC	Colilert	modified mTEC 35ºC	modified mTEC 44.5ºC	HPC
n	134	116	134	129	116	134	120
Mean	9.9	1.6	1.27	385.8	25.3	6.1	16648
Median	3.5	0	0	12	4	0	454500
Maximum Value	93.5	21	0	6867	157	58	16648.5
Minimum Value	0	0	11.0	0	0	0	21.5
Range	93.5	21	22	6867	157	58	454479
Standard Deviation	16.7	3.84	3.37	977.1	35.9	11.7	74244
Percent Not Detected	37	68	69	36.4	43	53	0

Table 1. Descriptive statistics of bacterial indicator assays.

Assay	Enterolert	mEA-BEA 41.5ºC	mEA-BEA 44.5ºC	Colilert	modified mTEC 35ºC	modified mTEC 44.5ºC
mEA-BEA 41.5ºC	0.65 a	1	0.6	ND	ND	ND
mEA-BEA 44.5ºC	0.55	0.6	1	ND	ND	ND
modified mTEC 35ºC	ND	ND	ND	0.89	1	0.68
modified mTEC 44.5ºC	ND	ND	ND	0.6	0.68	1
HPC	0.39	NS	NS	0.72	0.75	0.54

Table 2. Summary of correlation between methods as determined by Spearman's rank order correlation analysis.

Sampling Location

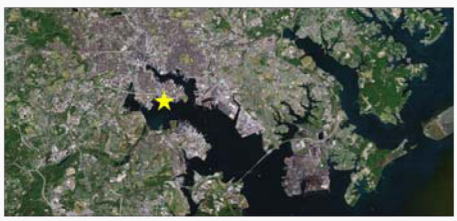

Image credit Google Maps

Water samples were collected twice weekly, primarily in the spring and summer months over a 2 year period onboard the M/V Cape Washington docked at Part Covington, Baltimore, MD

Discussion

Results of this study suggest a significant correlation between the Colilert and modified mTEC assay incubated at 35ºC. However, the USEPA recommended incubation temperature for detecting thermotolerant *E. coli* on modified mTEC agar is 44.5ºC. Thus, although the correlation is strong, the Colilert results most likely reflect the larger numbers of environmentally adapted enteric as well as non-enteric (non-thermotolerant) *E. coli*. Thus following recommendations of the Colilert manufacturer for incubation temperature and the USEPA recommended incubation temperature for modified mTEC will yield results corresponding to different groups of bacteria, thermotolerant *E.coli* with modified mTEC and total *E. coli* with Colilert. All other correlations between IDEXX and MF results showed significant, but weak correlations, an observation also valid for the correlation between HPC and the bacterial indicator assays. These results demonstrate the inconsistency of methods recommended for indicators of fecal pollution in surface waters and support a call for methods that accurately assess the public health safety of bodies of water in the natural environment. This is of particular importance given state, national, and international regulations, such as for the discharge of ships' ballast water, which have incorporated limits for microbial indicators of public health significance. Future work should assess the efficacy of both methods, in parallel, in predicting the presence of human enteric pathogens and stronger consideration should be given to directly testing waters for the presence of pathogens.

Conclusions

Results of standard fecal indicator assays can be inconsistent.

Pathogens of public health relevance to a region should be assayed directly.

Acknowledgements

The authors would like to thank Maryland Port Administration, US Maritime Administration, MERC Testing Team, and Crew of M/V Cape Washington for their help in sample collection.

MPRI MARYLAND PATHOGEN RESEARCH INSTITUTE

CosmosID™ A Biosystematics Company

University of Maryland CENTER FOR ENVIRONMENTAL SCIENCE