IDEXX
Literature Cover Sheet

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IDEXX #: 12C

Topic: Enterolert™ evaluation

Title: Evaluation of Enterolert™ for the Enumeration of Enterococci in Recreational Bathing Waters

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Highlights:

- 138 Marine and freshwater recreation water samples were analyzed with Enterolert and the mE MF method.
- Enterolert had false positive rate of 5.1% vs mE MF's 10.0%.
- Enterolert had a false negative rate of 0.4% vs. mE MF's 11.7%.

Evaluation of Enterolert™ for the Enumeration of Enterococci in Recreational Bathing Waters

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ABSTRACT

Enterococci density is the best bacterial indicator of health risks associated with exposure to fecal pollution during recreational bathing activities. **Enterolert**™ (IDEXX Laboratories Inc., Westbrook, ME.), a new semi-automated, most probable number (MPN) method, has been developed as an alternative to the standard membrane filter (MF) procedure for enumeration of enterococci in bathing waters. This system was evaluated and compared to the standard MF method by testing 138 marine and freshwater samples obtained from routinely monitored recreational bathing areas. Statistical analysis of parallel test results showed a strong linear correlation (r=0.97) and no significant difference (p=0.63 by paired t-test analysis) between the two methods. In addition, a total of 501 positive and negative Quanti-Tray™ wells were cultured for the presence of enterococci to determine actual false positive and false negative rates, which were found to be 5.1% and 0.4%, respectively. Samples with variation in comparative test results (5.8% of samples tested) had minimal effect on sample quality reporting status. Significant time savings were achieved due to less set-up, incubation (24 versus 48 hours) and reading times. We concluded that **Enterolert** is a more sensitive, specific, cost and time saving alternative to the MF procedure for determining the microbiological quality of recreational bathing waters.

Membrane filter methods have been used extensively to test various waters for microbiological indicator organisms and the mE method of Levin et al.(13) is the standard membrane filter method used to test recreational bathing waters for enterococci levels.(1,17) Although this method was shown to efficiently recover enterococci from marine and estuarian waters, the false positive and false negative rates were found to be 10.0% and 11.7%, respectively. Recently, a new semi-automated MPN method, Enterolert, has been developed that can enumerate enterococci in bathing waters in significantly less time than the MF procedure while requiring less manipulation and quality control testing. The test system is based on Defined Substrate Technology(9) which has been used successfully to test fresh (6,8) and marine (15) waters for fecal indicator organisms. Enterolert utilizes a nutrient indicator substrate, 4-methylumbelliferyl-β-D-glucoside, that fluoresces when metabolized by enterococci. Methylumbelliferyl (MU) substrates have the advantage of being highly sensitive and specific, non-carcinogenic, and easily detected with ultraviolet light sources. Consequently, test systems using MU have been designed for environmental monitoring of fresh and marine waters for fecal pollution.(2,11) In this study we have evaluated this new methodology and compared it to the standard MF technique using samples obtained from routinely monitored tidal marine and freshwater recreational bathing areas.

CONFIRMATION PROTOCOL: (FIGURE 2) Optimum growth of enterococci occurs at 35°C and most strains of enterococci grow at 45°C, tolerate 6.5% NaCl, and hydrolyze esculin in the presence of 40% bile salts (bile-esculin medium).(10,14) Using these characteristics, a protocol was designed to determine the presence of enterococci in individual Quanti-Tray wells.

SAMPLING: A total of 138 fresh and marine water samples were collected by the Connecticut State Department of Environmental Protection/Bureau of Water Management and Monitoring or local health departments for routine monitoring of tidal marine and inland freshwater public bathing areas. Dates of collection were from 6/12/95 through 9/11/95 and all samples were received and tested within 30 hours of collection.

STATISTICS: A comparison of the Enterolert and MF methods for statistical correlation was done using InStat, a statistical software program (GraphPad Software, Inc.).

EFFECT OF TEST METHOD ON SAMPLE WATER QUALITY DETERMINATION:

The overall esult distribution of the total sample population (N=138) is shown in Figure 3. These parallel test results were then grouped by enterococci density based on the established State of Connecticut single sample cut-off limit for microbiological acceptability (FIGURE 4). By classifying samples as either *not acceptable* (enterococci density exceeds the established single sample cut-off value of 61 enterococci/100 ml) or *acceptable* for recreational bathing use, the overall effect on the final water quality of the total sample group was determined (TABLE 2).

feature makes reading the test easier and more objective. Less training and experience is required to read the reactions and the potential for errors is decreased. This feature may also be contributing to the greater sensitivity and specificity of Enterolert, as compared to the MF method, because of the greater accuracy in reading the test.

Quality control: We have found that **quality control testing and reagent handling for Enterolert is less as compared to the MF method.** In our laboratory, for example, each batch of **mE and EIA prepared agar plates** must be checked for pH, sterility, and performance acceptability and shelf life of the prepared agar plates is two weeks (refrigerated). Maintenance of **sterile membrane filter apparatus** and testing **buffered rinse water** sterility at the beginning and end of each filtration series is required. On the other hand, the Enterolert nutrient powdered media is stored at room temperature (up to one year from date of manufacture) and this allows larger amounts of to be kept on hand and loss due to media expiration is reduced. In addition, Enterolert only requires presence/absence testing of the single reagent lot using defined target levels of positive and negative control organisms. The **Quanti-Tray itself does require a greater amount of incubator space** because of its size (approx. 260x120x9mm) relative to the standard MF petri dish (50x9mm). Each Quanti-Tray takes the space of approximately eight MF plates and this is a consideration if laboratory incubator space is limited and large numbers of samples are routinely tested.

positive results. The use of the Enterolert to screen the recreational bathing areas used in this study would have resulted in an overall 2.9% decrease in closure rates as compared to using the MF method. These issues are of primary concern to public health authorities involved in maintaining and monitoring recreational bathing areas. If the enterococci levels in a public recreational bathing area are found to exceed the standards for acceptability, the bathing area is kept closed until the microbiological quality of the water is shown to be acceptable through repeat sampling and testing. Closure time translates into significant revenue losses, especially during peak season, in bathing areas that charge a fee for use. Our study demonstrates that using Enterolert would allow these areas to be opened for use more quickly.

We have found that Enterolert can provide results sooner than the membrane filter technique with an increase in sensitivity and specificity. The resultant decrease in turnaround time translates into cost savings and allows officials to more quickly respond to potentially hazardous public health situations. These characteristics make this methodology a practical choice when testing recreational bathing waters for microbiological quality.

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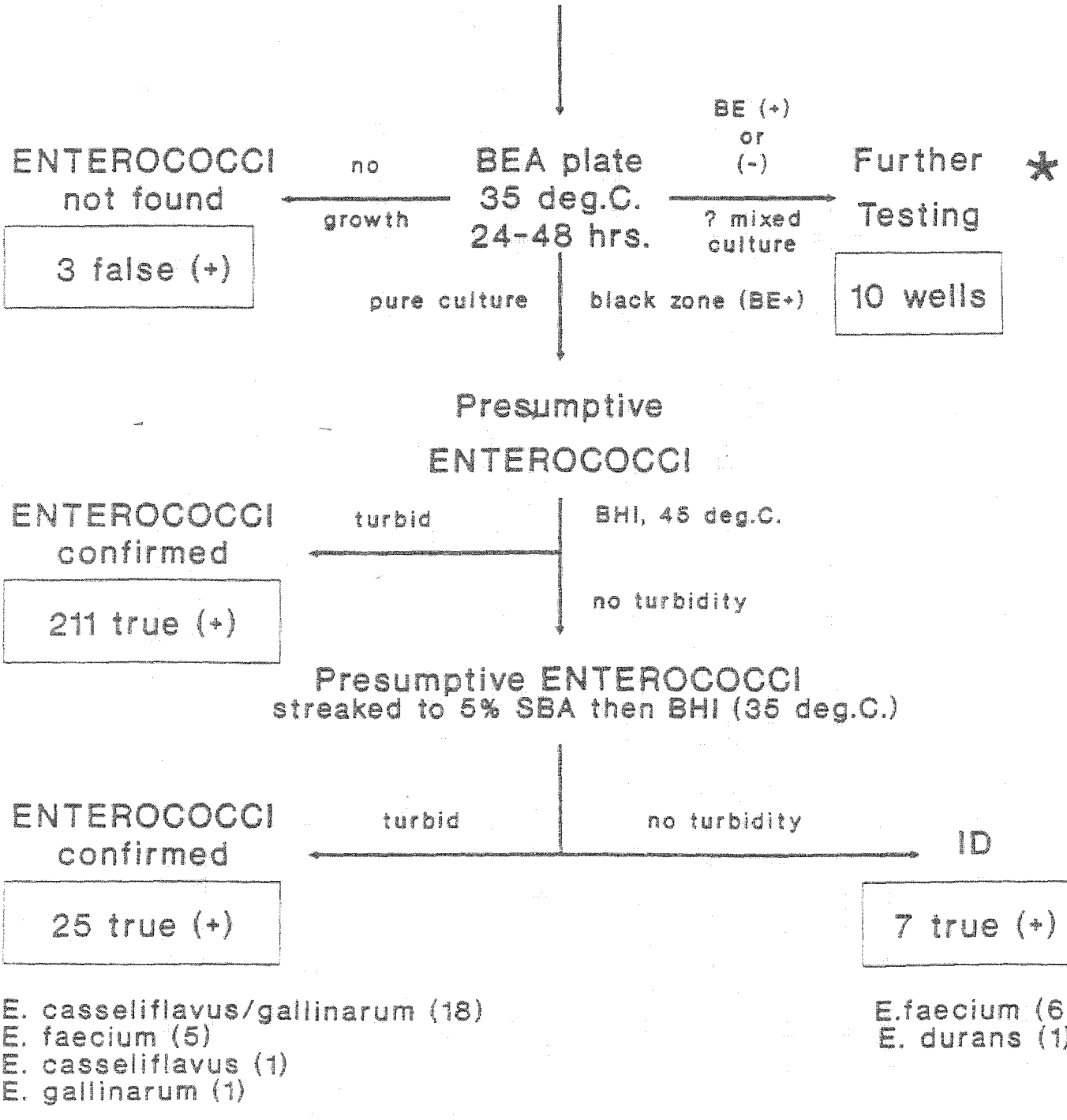
FIGURE 2
QUANTI-TRAY WELL CONFIRMATION PROTOCOL



SAMPLE TEST RESULT DISTRIBUTION

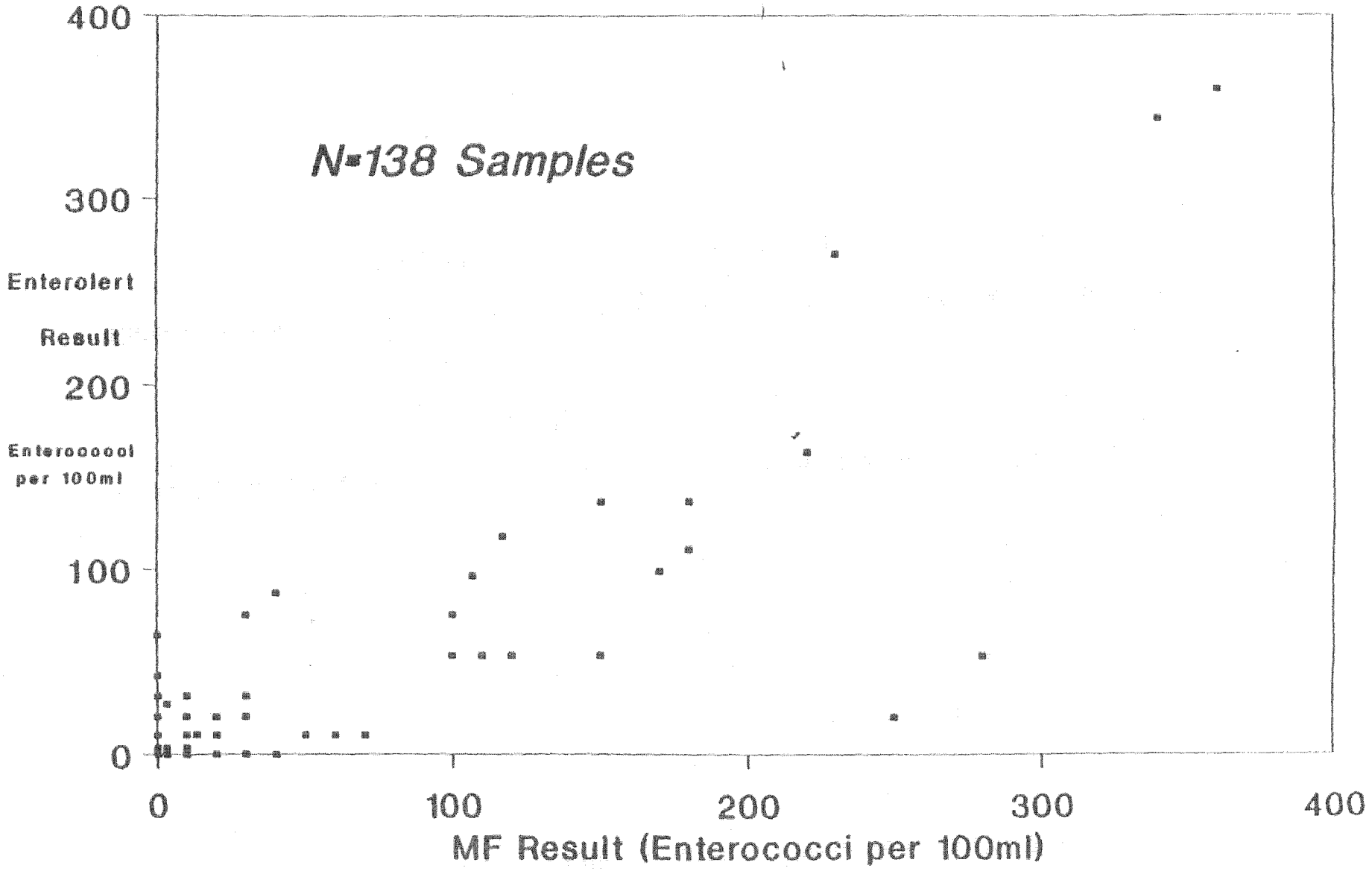


TABLE 1. Enterolert Test Well Confirmation: False Positive and False Negative Rates

Positive wells (N=256)		Negative wells (N=245)	
True Pos.	False Pos.	True Neg.	False Neg.
243 (95.3%)	13 (5.1%)	44 (99.6%)	1 (0.4%)

Enterolert sensitivity (%) =Probability that a positive Quanti-Tray well contains enterococci

= # Tp/ (# Tp + # Fn) x 100 = (243/243 + 1) x 100= **99.6%**

Enterolert specificity (%) = Probability that a negative Quanti-Tray well does not contain enterococci

= # Tn/ (# Tn + # Fp) x 100 = (244/244 + 13) x 100= **94.9%**

SUMMARY OF PROCEDURE COMPARISON

Membrane Filter vs. Enterolert

	MEMBRANE FILTER	ENTEROLERT
False positive rate	10.0%[1]	5.1%
False negative rate	11.7%[1]	0.4%
Quality Control:		
Media	Test each batch of plates: sterility and positive (*E. faecalis*) and negative (*E. coli & S. aureus*) controls	Test each lot once: with target levels of positive (*E. faecalis* and negative (*S. marsescens A. viridans*) controls
Equipment & Supplies	Buffered rinse water, membrane filters (each lot) MF apparatus (UV), glassware, pipettes	Dilution water, glassware, pipettes
Media storage	Prepared plates: 2 weeks refrigerated	Enterolert reagent: one year @ room temp.
Time study	approx. 16 min./sample	approx. 2 min./sample
Incubation time	48 hours	24 hours
Reading Test	zone size & intensity on EIA variable depending on colony size and density	more objective: positive or negative fluorescence
Sample turbidity	Particulate matter may affect recovery and reading of test	not a factor
Incubator space requirements	Media plate dimensions (approx. 50 X 9 mm)	Quanti-Tray dimensions (approx. 260 X 120 X 9 mm)

[1] Levin et al. 1975. Appl. Microbiol. 30:66-71