IDEXX
Literature Cover Sheet

IDEXX #: 12E

Title: Enterolert™ - A Rapid Method for the Detection of Enterococcus spp.

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Source: IDEXX Laboratories

Topic: Enterolert

Highlights:

- Enterolert was evaluated in parallel with the MF method to determine the level of agreement. 821water samples from different geographic areas were tested. The correlation coefficient between these two methods was 0.91.

	MF	MPN	Enterolert
Time to Result	48 - 72 hours	48 - 72 hours	24 hours

	False Positives	False Negatives
Enterolert	5%	1%
MF	10%	12%

* see the following pages for more highlights

Enterolert™ - A Rapid Method for the Detection of Enterococcus spp.

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ABSTRACT

Enterolert™ - A Rapid Method for the Detection of Enterococcus spp.

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Enterolert defined substrate system is a rapid 24-hr test that detects enterococci in water. Enterolert uses 4-methylumbelliferyl-ß-D-glucoside as the defined substrate nutrient-indicator. This compound, when hydrolyzed by enterococcus ß-glucosidase, releases 4-methylumbelliferone which exhibits fluorescence under a UV_{365nm} lamp. The sensitivity of Enterolert presence/absence test was evaluated with 25 enterococcus strains; ~ 96% (24/25) at 1 - 3 cfu/100 ml were detected within 24 hrs of incubation at 41 °C. In the Quanti-Tray™ format, Enterolert detected ~ 95% (121/127) enterococci in 24 hrs. The specificity of the test was examined using 17 non-enterococci bacteria; these bacteria did not show cross reactivity at levels above 10^5 cfu/100 ml within 48 hrs.

Evaluation on 821 water samples from different geographic areas showed good agreement between the Enterolert-MPN and the membrane filtration method. The correlation coefficient between these two methods was 0.91 (N = 766, Range = 0 - 200). About 95 % (1576/1658) Enterolert-positive isolates were confirmed to be enterococci. The recovery rate for Enterolert at 24 hrs was 99 % (849/854). Enterolert is rapid, accurate, easy-to-use, and requires minimal hands-on time.

INTRODUCTION

Enterococcus is a valuable bacterial indicator for monitoring fecal pollution in water. Enterococcus density in recreational waters has been shown to be related to the risks of swimming-associated gastroenteritis. The United States Environmental Protection Agency recommended that enterococci be the bacterial indicator for fresh and marine waters (*Federal Register*, 1986). Current guidelines for enterococci density are 33 enterococci/100 ml for fresh water and 35 enterococci/100 ml for marine water.

Current methods for the analysis of enterococcus density include the multiple-tube technique for most probable number (MPN) and the membrane filter technique (MF). Both techniques are labor-intensive, tedious, and require a minimum of 48 - 72 hours before results can be obtained.

We developed a defined substrate-Enterolert system which detects enterococci in water within 24 hours. Enterolert uses 4-methylumbelliferyl-ß-D-glucoside as the nutrient-indicator. This compound, when metabolized by enterococci, releases 4-methylumbelliferone and glucose. 4-Methylumbelliferone exhibits blue fluorescence when viewed under a long-wavelength ultraviolet lamp. The glucose moiety is further metabolized by enterococci to promote their growth. Non-enterococci are suppressed by the incorporated antimicrobial agents and cannot metabolize this nutrient-indicator.

The Quanti-Tray system, previously shown to effectively quantify coliform bacteria and *Escherichia coli* using Colilert®, was used in conjunction with Enterolert to enumerate enterococci in water.

In this study, a total of 127 enterococci and 17 non-enterococci were tested to evaluate the sensitivity and specificity of Enterolert. The Enterolert system was also evaluated in parallel with the MF method to determine the level of agreement between these two methods. A total of 821 water samples including drinking water, bottle spring water, river and lake water, sewage effluent, and marine water from different geographic areas were evaluated. Enterolert results were confirmed by selective isolation and biochemical identification by the Vitek™ bacterial identification system.

ENTEROLERT PROTOCOL

Presence/Absence Assay Protocol

- Collect 100 ml water sample.
- Add Enterolert reagent, Mix.
- Incubate at 41 °C for 24 hours.
- Read (UV-365nm lamp).

Quantification Assay Protocol

- Collect 100 ml water sample.
- Add Enterolert reagent, Mix.
- Pour the solution in a Quanti-Tray.
- Seal the tray with the Quanti-Tray sealer.
- Incubate at 41 °C for 24 hours.
- Count the number of fluorescent wells.
- Refer to the 51-well Quanti-Tray MPN table.

RESULTS

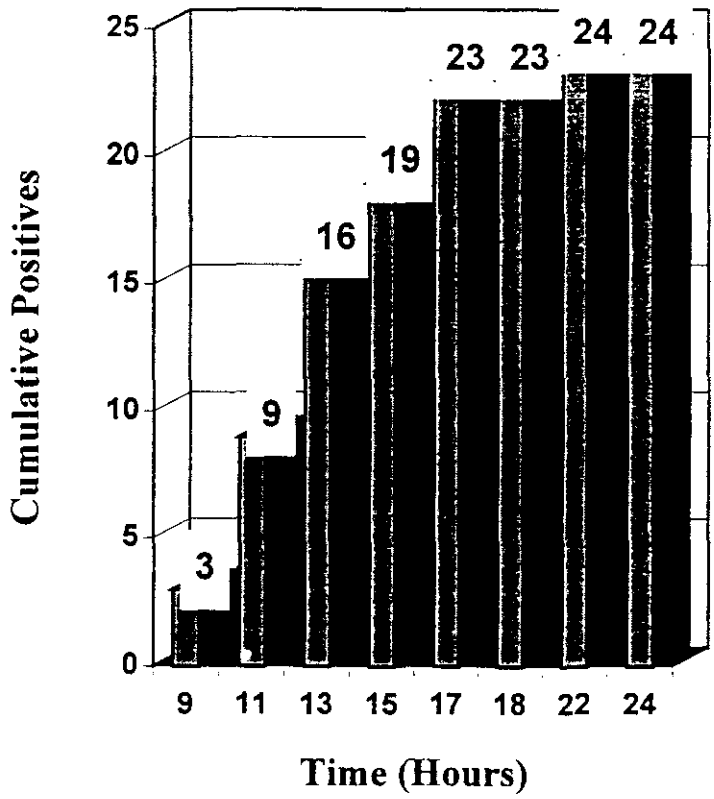
Selectivity of Enterolert[2]

Strains	*Inoculum*[1]	*Fluorescence*
Acetobacter xylium ATCC 23769	1.0×10^6	-
Aerococcus viridans ATCC 10400	2.9×10^6	-
Aeromonas hydrophila ATCC 35654	2.0×10^5	-
Escherichia coli ATCC 29522	3.0×10^6	-
Enterobacter cloacae ATCC 13047	3.5×10^6	-
Klebsiella penumoniae ATCC 9997	3.7×10^6	-
Lactococcus lactis ATCC 11454	1.5×10^6	-
Micrococcus luteus ATCC 4698	5.2×10^5	-
M. roseus ATCC 186	2.0×10^5	-
Pediococcus spp.	1.8×10^6	-
Planococcus citreus ATCC 14404	5.0×10^6	-
Pseudomonas aeruginosa ATCC 16145	7.4×10^6	-
Serratia marcescens ATCC 43862	1.0×10^6	-
Staphylococcus aureus ATCC 9144	1.0×10^6	-
Streptococcus bovis ATCC 9809	8.4×10^6	-
S. equinus ATCC 9812	4.8×10^6	-
Tetragenococcus halophilus ATCC 13621	7.8×10^5	-

1. Enterolert was challenged with non-enterococcus bacteria at the indicated colony forming units per 100 ml sample volume.

2. Samples were incubated at 41°C and monitored for fluorescence development for 48 hrs.

Enterolert Evaluation with 25 Enterococci Strains



Enterolert sensitivity was evaluated using 25 enterococci in deionized water. The inoculum was 1 - 3 cfu/100 ml. The samples were incubated at 41 °C and monitored for fluorescence development at the indicated time. Twenty three strains were positive within 17 hours. Twenty four strains were positive before 22 hours. Only *Enterococcus hirae* ATCC 8043 was not detected within 24 hours.

Enterolert Sensitivity in Quanti-Tray Format[1]

Strains	20 hr	22 hr	24 hr	> 24 hr	ND[2]	# Tested
E. faecalis	10	3				32
E. faecium	10	3	2	2	2	67
E. casseliflavus	2	3	3			16
E. durans	1	2	1	1		10
E. gallinarum			1			1
E. hirae				1		1
# positives	24	11	7	4	2	
# cumulative positives	103	114	121	125		127
% cumulative positives	81 %	90 %	95 %			100 %

1. The sensitivity of Enterolert in the Quanti-Tray format was evaluated using 127 environmental enterococcus isolates in deionized water. The inoculum was 0 - 30 cfu in 100 ml water. The 100 ml water was equally distributed into 51 Quanti-Tray wells to achieve a theoretical 1 cfu/well. The sample tray was incubated at 41 °C and monitored for fluorescence development at the indicated time. 114 strains (90%) were positive at 22 hours and 121 strains (95%) were positive at 24 hours. These environmental isolates were identified by API® 20 Strep system. The identities of these strains included 32 *E. faecalis*, 67 *E. faecium*, 16 *E. casseliflavus*, 10 *E. durans*, 1 *E. gallinarum*, and 1 *E. hirae*.

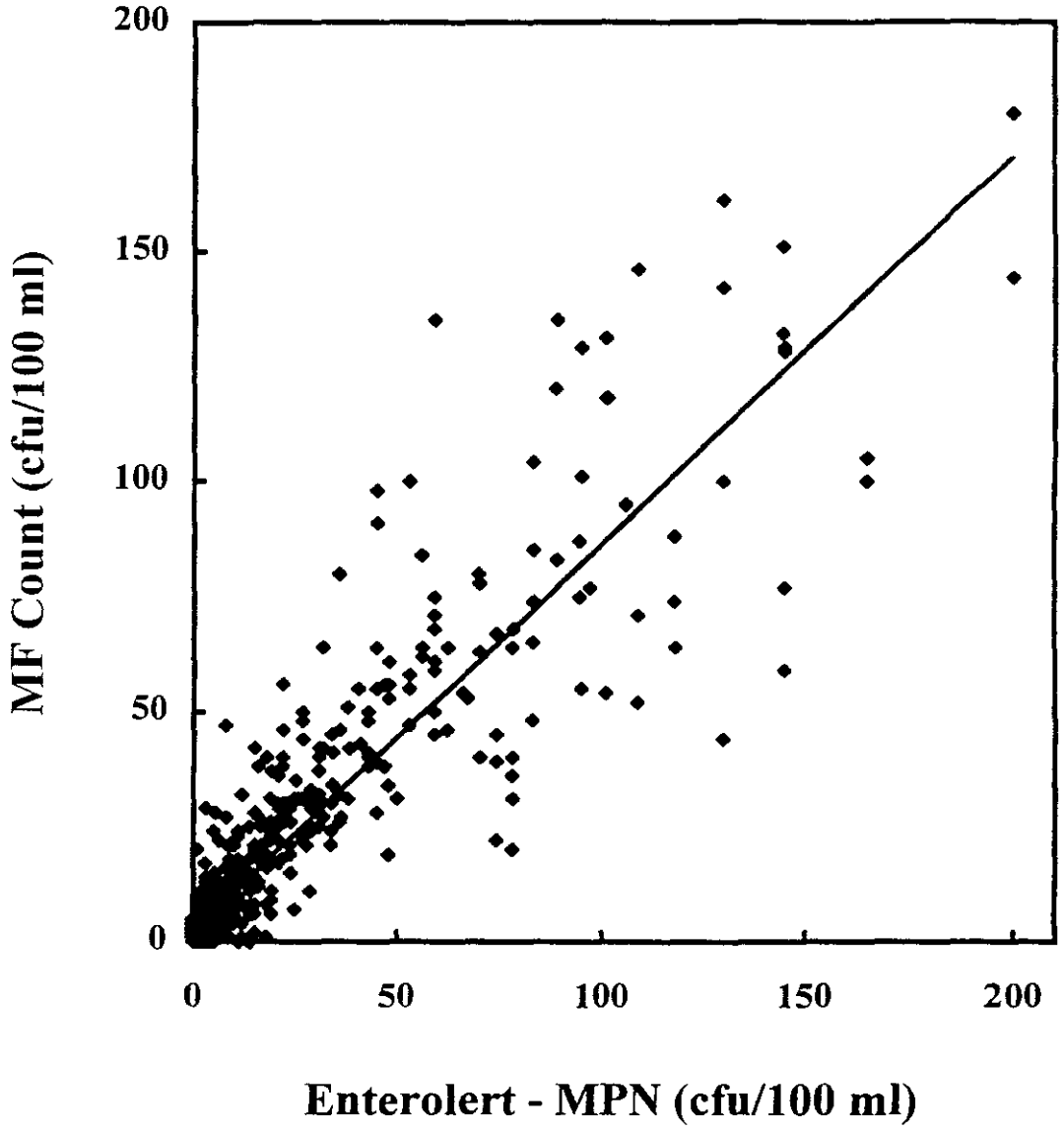
2. not detectable.

Comparison of Enterolert-MPN *vs.* MF[1]

Range	# Samples	
MF > 200; Enterolert > 200	51	
MF > 200; Enterolert < 200	1	
MF < 200; Enterolert > 200	3	
MF < 200; Enterolert < 200	766	r = 0.91
Total	821	

[1.] Defined substrate Enterolert system in the Quanti-Tray format was performed in parallel with the membrane filtration technique.

[2.] Water samples used in this evaluation included 21 bottled spring water, 110 treated (chlorinated) drinking water, 76 untreated raw water (river and lake water), 247 sewage effluents, and 367 marine water. Fresh water samples were tested without dilution. Marine water samples were tested using a 10 X dilution procedure (i.e. 10 ml marine water in 90 ml sterile deionized water).



Correlation between Enterolert - Quanti-Tray MPN values and membrane filtration counts for 766 water samples within the counting range of 0 - 200 (Enterolert < 200; MF < 200). The correlation coefficient between these two methods was 0.91 (Y = 0.43 + 0.98 X).

Accuracy of Enterolert

	Enterolert	
Confirmation	**# Positive Wells**	**# Negative Wells**
enterococci	1576	5
non - enterococci	82	849
% false positive rate	4.9 %	
% false negative rate	0.6 %	
% Accuracy	96.4 %	

Enterolert results (positive and negative wells) were confirmed by steps of selective isolation and biochemical identification using a combination of bile-esculin agar (35 °C), brain heart infusion 6.5% NaCl broth at 45 °C and/or 35 °C, and Vitek bacterial identification system. About 95 % (1576/1658) Enterolert-positive isolates were confirmed to be enterococci (false positive rate ~ 5 %). The false negative rate for Enterolert at 24 hrs was ~ 1 % (about 99 % recovery rate).

SUMMARY

- Enterolert detects ~ 95 - 96% of enterococci within 24 hours of incubation at 41 °C
- No cross reactivity was observed when Enterolert was challenged with 17 non-enterococcus bacteria
- Evaluation on 821 water samples showed good agreement between Enterolert Quanti-Tray MPN values and MF counts (N = 766; range = 0 - 200; r = 0.91)
- Enterolert is accurate (~ 96 % accuracy) with a false positive rate of ~ 5 % and a false negative rate of ~ 1 % as compared to the 10 % false positive and 12 % false negative rates for the MF method (Levin, M.A. et. al, 1975).
- Enterolert, coupled with Quanti-Tray system, allows rapid detection and quantification of enterococci in water (bottle water, drinking water, river water, lake water, sewage effluents, and marine water).
- Benefits of Enterolert:

  Rapid (24 hrs results)
  Accurate
  Less than 45 seconds hands-on time
  Ready-To Use Reagent (No media preparation)
  Easy interpretation