IDEXX Summary 12J

Topic Enterolert*250; comparison of the IDEXX method for detection of enterococci in bottled water samples to two European enterococci standard methods

Title A Performance Comparison of Three Methods: Enterolert* 250 Presence/Absence Method, the International Standards Organization (ISO) method 7899-2 and the Ministry of Drinking Water (MoDW) 2006, Part 5 method for the Detection of Enterococci in Bottled Water Samples

Source IDEXX Laboratories, Inc.

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Highlights

- The 121 bottled water samples included in this study originated from 10 different countries around the world and were made up of the following bottled water types – spring water, artesian water, drinking water, still mineral water, and sparkling mineral water
- Untreated primary sewage effluent was used as a seeding source of naturally occurring enterococci. Effluents collected from across the USA and throughout England were used for spiking purposes. Each effluent was inoculated into a bottled water sample to obtain a target concentration of 1 – 10 cfu / 250 mL.
- The following study showed that the detection capabilities of Enterolert 250, for enterococci in various bottled water matrices is equivalent to that of ISO 7899-2 and MoDW 2006, Part 5

A Performance Comparison of Three Methods: Enterolert* 250 Presence/Absence Method, the International Standards Organization (ISO) method 7899-2 and the Ministry of Drinking Water (MoDW) 2006, Part 5 method for the Detection of Enterococci in Bottled Water Samples

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Introduction

Enterolert* 250 is designed to detect Enterococcus species in bottled water samples. It is based on IDEXX's patented Defined Substrate Technology* (DST*). Enterolert 250 provides confirmed presence/absence results in 24 hours. Enterolert 250 uses Ortho-Nitrophenyl-ß-D-Glucoside as a nutrient indicator and incorporates a specifically designed blue background colour in its formulation. When the substrate is metabolized by enterococci, the sample turns from blue to green to indicate a positive result. Any change from the original colour to green is considered a positive result. No ultraviolet light source is required. Enterolert 250 detects enterococci at 1 cfu in 250 mL bottled water samples in 24 hours.

Method

Bottled Water Sample Types

The 121 bottled water samples included in this study originated from 10 different countries around the world and were made up of the following bottled water types – spring water, artesian water, drinking water, still mineral water, and sparkling mineral water (**Table 1**). Enterolert 250 requires that sparkling waters be thoroughly degassed prior to testing. Therefore, all sparkling bottled water samples were manually degassed, by shaking, prior to testing.

Table 1
Bottled Water types, total number of brands and geographical locations used in this study

	Total # of waters	Total # of brands	Water source location				
Bottled Spring (still)	34	10	12 x USA	8 x France	7 x Iceland	2 x Italy	5 x United Kingdom
Bottled Artesian (still)	15	3	3 x Croatia	6 x New Zealand	6 x South Pacific Islands		
Bottled Mineral (still)	18	5	3 x USA	15 x United Kingdom			
Bottled Drinking (still)	17	7	17 x USA				
Bottled Spring (sparkling)	6	1	6 x United Kingdom				
Bottled Mineral (sparkling)	31	7	4 x United Kingdom	8 x France	8 x Italy	11 x Germany	
Totals	121	33					

Analytical Methods

It is optimal, whenever possible, to use natural samples with the target organisms when comparing methods. Due to the limited availability of bottled water samples containing enterococci bacteria, it was necessary to perform the comparison by the inoculation of relevant target and non-target organisms into the water matrix in question.

Untreated primary sewage effluent was used as a seeding source of naturally occurring enterococci. Effluents collected from across the USA and throughout England were used for spiking purposes. Each effluent was inoculated into a bottled water sample to obtain a target concentration of 1 – 10 cfu / 250 mL. Each bottled water sample was then split and analyzed in duplicate using the Enterolert 250 presence/absence method and the reference methods ISO 7899-2 and MoDW 2006, Part 5.

Enterolert 250 presence/absence method was performed as described in the supplied product insert. As previously described, each inoculated bottled water sample was split between the two methods (ISO and MoDW were done using the same sample read at 2 and 4 hours respectively). For Enterolert 250, duplicate 250 mL samples were dispensed into IDEXX sterile plastic 290mL vessels and a blister pack of Enterolert 250 medium was added to each vessel. Enterolert 250 samples were agitated until the medium was completely dissolved and then placed into an air-circulating incubator set at 41°C ± 1°C. Enterolert 250 samples were observed for colour change after 24 and 28 hours of incubation. After the 28 hour observation all Enterolert 250 samples, positive and negative, were confirmed by the confirmation methods listed in **Appendix A, Flowcharts**.

Reference methods ISO 7899-2 and MoDW 2006, Part 5 were performed according to the Standard Test method described in the related ISO and MoDW documentations. As previously described, each inoculated bottled water sample was split between the two methods. For ISO 7899-2 and MoDW 2006, Part 5, duplicate 250mL samples were concentrated by membrane filtration. The membrane was carefully placed on the Slanetz and Bartley agar and agar plates were placed into an air-circulating incubator set at 36°C ± 2°C. Samples were observed after 44 ± 4 hours and all raised colonies which showed a red, maroon or pink colour, either in the centre or throughout the colony was considered typical. If there were typical colonies, the membrane and the colonies were transferred onto a plate of bile-aesculin-azide agar which had been preheated to 44°C. The plates were incubated at 44°C ± 0.5°C for 2 h. All typical colonies showing a tan to black colour in the surrounding medium are considered true enterococci for ISO. The plates were returned to the 44°C incubator for an additional 2 hours. Plates were observed again and any additional typical colonies showing a tan to black colour surrounding the medium were considered true enterococci for MoDW. The confirmed ISO 7899-2 and MoDW 2006, Part 5 membrane filtration count was used to determine the actual enterococci inoculum during testing.

Statistical Analysis

The confirmed ISO 7899-2 and MoDW 2006, Part 5 membrane filtration counts were used to determine the actual enterococci inoculum. Only data sets with an average count falling between 1-10 cfu / 250 mL were used in the data analysis. Once used for the determination of actual inoculum, the ISO 7899-2 and MoDW 2006, Part 5 quantitative data sets were converted to presence/absence data sets so that they could be directly compared to the Enterolert 250 presence/absence data sets. Finally, all the Enterolert 250 and ISO 7899-2 and MoDW 2006, Part 5 results were then populated into a simple 2 x 2 summary table as depicted in **Table 2**. The results in each table were analyzed using the McNemar's test[1, 2], which has a final output of a p-value. Summary of the McNemar's test will be shown in the form

1. McNemar, Quinn (June 18, 1947). "Note on the sampling error of the difference between correlated proportions or percentages". Psychometrika 12 (2): 153–157.
2. McNemar's test is listed as a suitable method for the statistical evaluation of Presence/Absence methods in ISO/DIS 17994:2002.

of a table as depicted in **Table 3.** The p-value was assigned using the chi-square result calculated, with one degree of freedom, from the number of unmatched pairs represented by *b* and *c* in **Table 2**. It was determined that a significance level α of 0.05 would be assigned, requiring 95% confidence of the null hypothesis. Therefore, it can be said, with a 95% confidence level, that data sets with a p-value equal to or greater than 0.05 would be considered statistically similar while data sets with a p-value less than 0.05 would be considered statistically different, as seen in **Table 3**.

Table 2
Example of 2 x 2 table used in McNemar's test

ISO 7899-2 or MoDW 2006, Part 5 Confirmed result	Enterolert 250 Confirmed result	#	Comments
+	+	*a*	Number of matching results
+	-	*b*	ISO or MoDW method more sensitive
-	+	*c*	Enterolert 250 more sensitive
-	-	*d*	Number of matching results
		n = *a* + *b* + *c* + *d*	

Table 3
Example of McNemar's test output based on Table 2

Study	# data points	P-value (α = 0.05)	Conclusion
Enterolert 250 Confirmed Result vs. ISO 7899-2 or MoDW 2006, Part 5 Confirmed Result	n = *a* + *b* + *c* + *d*	y	y ≥ 0.05 = Methods equivalent y < 0.05 = Methods different

Results and Discussion

Enterococci Recovery

121 water samples were inoculated with different untreated primary sewage effluent sources. Of the 121 samples, 107 samples fell within the required spike concentration of 1 – 10 cfu / 250 mL (determined by ISO 7899 colony count). These 107 sample pairs (214 actual data points) were used to populate **Table 4** representing the 24 hour Enterolert 250 results compared to the ISO 7899 results**.**

Of the 121 samples, 101 samples fell within the required spike concentration of 1 – 10 cfu / 250 mL (determined by MoDW colony count). These 101 sample pairs (202 actual data points) were used to populate **Table 5** representing the 24 hour Enterolert 250 results compared to the MoDW results**.**

Table 4
Enterococci Recovery of ISO 7899-2 vs. Enterolert 250 after 24 hour

ISO 7899-2 Confirmed result	Enterolert 250 Confirmed result	#
+	+	212
+	-	2
-	+	0
-	-	0
		n = 214

Table 5
Enterococci Recovery of MoDW 2006, Part 5 vs. Enterolert 250 after 24 hour

MoDW 2006, Part 5 Confirmed result	Enterolert 250 Confirmed result	#
+	+	200
+	-	2
-	+	0
-	-	0
		n = 202

Of the 214 data points for ISO 7899-2, the number of confirmed Enterolert 250 positive results matching the ISO 7899-2 confirmed positive results were 212 after 24 hours of incubation. The 2 sample difference in the performance of Enterolert 250 and ISO 7899-2 were found to be 1 false negative and 1 true negative at 24. By 28 hours one of the vessels had turned positive.

Of the 202 data points for MoDW 2006, Part 5, the number of confirmed Enterolert 250 positive results matching the MoDW 2006, Part 5 confirmed positive results were 200 after 24 hours of incubation. The 2 sample difference in the performance of Enterolert 250 and MoDW 2006, Part 5 were found to be false negatives at 24 hours. By 28 hours both vessels had turned positive.

Results listed in **Table 4 and 5** were subsequently used in the statistical analysis by the McNemar's test. The statistical analysis indicated that the Enterolert 250 confirmed positive results after 24 hours of incubation were equivalent to the ISO 7899-2 and MoDW 2006, Part 5 confirmed positive results, summarized in **Table 6.**

Table 6
Enterococci - McNemar's test output based on Tables 5 & 6

Study	# data points	P-value ($\alpha = 0.05$)	Conclusion
Enterolert 250 at 24 hours vs. ISO 7899-2	214	0.4795	Methods equivalent
Enterolert 250 at 24 hours vs. MoDW 2006, Part 5	202	0.4795	Methods equivalent

Conclusions

The following study showed that the detection capabilities of Enterolert 250, for enterococci in various bottled water matrices is equivalent to that of ISO 7899-2 and MoDW 2006, Part 5, and is further summarized in **Table 7**.

Table 7
Summary of Enterolert 250 performance in detection of target organisms

Study	# data points	P-value ($\alpha = 0.05$)	Conclusion
Enterolert 250 at 24 hours vs. ISO 7899-2	214	0.4795	Methods equivalent
Enterolert 250 at 24 hours vs. MoDW 2006, Part 5	202	0.4795	Methods equivalent

References

- ISO/TR 13843:2000(E) Water Quality – Guidance on validation of microbiological methods. Geneva: International Organization for Standardization.

- ISO 17994:2004 Water Quality – Criteria for establishing equivalence between microbiological methods. Geneva: International Organization for Standardization.

- ISO 7899-2 Water quality — Detection and enumeration of intestinal enterococci — Part 2: Membrane filtration method

- The Microbiology of Drinking Water (2002) - Part 5 - A method for the isolation and enumeration of enterococci by membrane filtration

Appendix A
Methods used to confirm Enterolert 250 presence/absence samples

Flowchart
Confirmation steps performed on positive and negative Enterolert 250 samples inoculated with primary effluent