Enterolert*-DW and Quanti-Tray* System Quantitative Detection of Enterococci in Drinking Water

ISO 13843 method performance validation
Updated August 6, 2019

IDEXX

Executive summary

The Enterolert*-DW method is a test specifically designed for the most probable number (MPN) enumeration of species of *Enterococcus* from potable water. Enterolert-DW is presented as a powdered reagent in blister-pack format for testing 100 mL water samples, or smaller sample volumes made up to 100 mL. Enterolert-DW is based on the Defined Substrate Technology* (DST*). As enterococci grow in the medium, they will metabolize an enzyme substrate specific for β-glucosidase and produce a detectable signal that changes the medium color from blue to green, which is indicative of the presence of confirmed enterococci in water samples. The Enterolert-DW test was characterized according to ISO 13843:2017.

The method was challenged with isolates derived from naturally contaminated samples. Assessment of the sensitivity, specificity, and selectivity of Enterolert-DW was achieved through the identification of isolates recovered from positive green wells and negative blue wells for each sample. The results of evaluations for sensitivity, specificity, selectivity, and counting and method uncertainties are summarized in table 1.

Table 1. Test results for all parameters

Parameter	Result
Sensitivity	91.9%
Specificity	93.0%
Selectivity	42.1%
False-positive rate	9.8%
False-negative rate	7.0%
Efficiency	91.7%
Counting repeatability	3.4%
Counting reproducibility	3.1%
Method repeatability	15.4%
Method reproducibility	17.5%–33.8%

The Enterolert-DW method was compared with the ISO 7899-2:2000 and the *Microbiology of Drinking Water* (MoDW) 2012 Part 5 membrane filtration methods following the requirements of ISO 17994:2014. Data from 402 routine drinking water samples (197 from chlorinated supplies and 205 from unchlorinated supplies) were collated and analyzed. The outcomes of the ISO 17994 two-tailed analyses for both chlorinated drinking water and unchlorinated drinking water as well as for the combined data for the two comparisons are summarized in tables 2 and 3.

These analyses showed the Enterolert-DW/Quanti-Tray* method gives overall higher recoveries in comparison with ISO 7899-2. Against MoDW 2012 Part 5, the overall outcome was that the two methods were “not different,” with the results from Enterolert-DW trending higher for the chlorinated samples but lower for the unchlorinated samples.

Table 2. Outcomes of ISO 17994 analysis of paired-count data for Enterolert-DW versus ISO 7899-2 for chlorinated and unchlorinated drinking water

Comparison	MRD%	SD	*W*	X_L	X_U	Conclusion
Chlorinated waters	28.6	81.0	11.5	17.0	40.1	Enterolert-DW higher
Unchlorinated waters	4.5	53.8	7.5	-3.0	12.0	Methods not different
All data	16.3	69.5	6.9	9.4	23.2	Enterolert-DW higher

MRD% = Mean relative difference expressed as a percentage
SD = Standard deviation

Table 3. Outcomes of ISO 17994 analysis of paired-count data for Enterolert-DW MoDW 2012 Part 5 for chlorinated and unchlorinated drinking water

Comparison	MRD%	SD	*W*	X_L	X_U	Conclusion
Chlorinated waters	14.4	81.1	11.6	2.9	26.0	Enterolert-DW higher
Unchlorinated waters	-9.0	53.0	7.4	-16.4	-1.6	MoDW Part 5 higher
All data	2.5	69.1	6.9	-4.4	9.4	Methods not different

MRD% = Mean relative difference expressed as a percentage
SD = Standard deviation

I. Introduction

Enterolert-DW is based on the DST and designed to detect enterococci in drinking water samples. As enterococci grow in the liquefied medium, the organisms will metabolize an enzyme substrate specific for β-glucosidase and produce a detectable signal that changes the medium color from blue to green, which is indicative of the presence of confirmed enterococci in water samples.

The Enterolert-DW and Quanti-Tray system (Enterolert-DW/Quanti-Tray) is used for the detection and enumeration of enterococci in water samples. A blister pack of Enterolert-DW reagent is added to a sterile vessel containing the water sample. After the powder reagent is added, the vessel is shaken to dissolve the powder. The dissolved sample/medium mixture is added to a Quanti-Tray or Quanti-Tray*/2000 and then sealed with the Quanti-Tray* Sealer PLUS. The tray is incubated at 41°C ±0.5ºC for 24 hours. The quantification of confirmed enterococci in the sample is determined by counting the number of green-colored wells on the Quanti-Tray using the MPN statistics.

This report presents the performance characterization of Enterolert-DW/Quanti-Tray for the enumeration of enterococci from drinking water samples with respect to the requirements of ISO 13843:2017.

II. Scope of application of Enterolert-DW/Quanti-Tray system

The Enterolert-DW/Quanti-Tray system is intended for the analysis of drinking water samples. It aims to be comparable in performance to ISO 7899-2:2000 Water Quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method (ISO 7899-2) and *The Microbiology of Drinking Water* (2012) - Part 5 – Methods for the isolation and enumeration of enterococci (MoDW 2012 Part 5) in drinking water applications. The Enterolert-DW/Quanti-Tray system provides quantitative, confirmed results for the detection of enterococci in drinking water samples within 24 hours. It is also effective in the detection of enterococci in fresh and marine bathing water matrices, the ISO 13843 performance characterization of which is presented in a separate report.

III. Target organism identification

Enterolert-DW detects enterococci based on their β-glucosidase activity using the DST. When enterococci are present in the test sample, the medium changes color from blue to green. The Quanti-Tray system provides quantitative results based on the MPN statistics.

A. Pure culture challenge

Typical reactions of Enterolert-DW were characterized by challenging the medium using pure cultures of reference strains from the American Type Culture Collection (ATCC) and environmental isolates from the IDEXX culture collection. Target *Enterococcus* bacteria, as well as nontarget Gram-negative and Gram-positive bacteria, were tested. Typical results for these reference and environmental strains are listed in table 4.

Table 4. Strains of *Enterococcus* and selected Gram-negative and Gram-positive bacteria used to test typical positive and negative reactions in Enterolert-DW

Bacterium	Source	Reaction in Enterolert-DW
Enterococcus faecalis[1]	ATCC 33186	Green
Enterococcus faecalis	ATCC 29212	Green
Enterococcus faecalis	Waste water	Green
Enterococcus faecium[1]	ATCC 35667	Green
Enterococcus faecium	Raw water	Green
Enterococcus hirae	ATCC 8043	Green
Enterococcus hirae	Water	Green
Enterococcus hirae	River water	Green
Enterococcus durans	ATCC 19432	Blue (due to no growth)
Enterococcus durans	Water	Green
Enterococcus casseliflavus	Waste water	Green
Enterococcus avium	ATCC 35665	Green
Enterococcus avium	Water	Green
Enterococcus gallinarum	ATCC 49573	Green

Bacterium	Source	Reaction in Enterolert-DW
Escherichia coli†	ATCC 25922	Blue (no color change)
Klebsiella oxytoca†	Water	Blue (no color change)
Enterobacter aerogenes†	Water	Blue (no color change)
Serratia marcescens[1]‡	ATCC 43862	Blue (no color change)
Pseudomonas aeruginosa§	Cooling tower water	Blue (no color change)
Pseudomonas putida†	Hospital water	Blue (no color change)
Elizabethkingia meningoseptica†	Water	Blue (no color change)
Shewanella putrefaciens†	Water	Blue (no color change)
Aerococcus viridans[2]	Water	Green
Lactococcus garvieae[3]	Water	Green
Streptococcus bovis[4]	ATCC 8177	Green
Staphylococcus aureus§§	ATCC 6538	Blue (no color change)
Staphylococcus aureus‡	ATCC 25923	Blue (no color change)

[1] These strains are used by IDEXX for routine quality control of Enterolert-DW.
[2] *Aerococcus viridans* is closely related to the enterococci and several strains are β-glucosidase-positive.
[3] *Lactococcus garviae* is β-glucosidase-positive and is closely related to the enterococci, previously being known as *Enterococcus seriolicida*.
[4] *Streptococcus bovis* is closely related to the enterococci but is a slow/weak producer of β-glucosidase.
† Up to 10^8 CFU/100 mL ‡ Up to 10^6 CFU/100 mL § Up to 10^5 CFU/100 mL §§ Up to 10^4 CFU/100 mL

B. Sensitivity, specificity, false-positive/false-negative rates, efficiency, and selectivity studies (ISO 13843 clause 6.2)

In terms of microbiological methods not needing confirmatory tests, these characteristics are defined by ISO 13843 as:

Sensitivity: The fraction of the total positives correctly assigned in the presumptive count.

Specificity: The fraction of the total negatives correctly assigned in the presumptive count.

Selectivity: The ratio of the number of target colonies to the total number of colonies in the sample volume.

For the Enterolert-DW/Quanti-Tray system, these characteristics relate to the number of wells that were positive for enterococci that actually contained target bacteria as well as the number of negative wells that were truly negative for the target bacteria. Assessment of sensitivity, specificity, and selectivity of the Enterolert-DW/Quanti-Tray system was achieved through the identification of bacterial isolates from positive and negative wells of tested samples. The samples were prepared by seeding the drinking water samples with either chlorinated or unchlorinated sewage effluent samples. In this study, false positives and false negatives of the Enterolert-DW/Quanti-Tray were determined by the selective isolation and bacterial identification from 163 positive wells and 186 negative wells selected at random from samples collected in 49 locations in the United States, United Kingdom, Italy, Belgium, and Spain. The VITEK* II biochemical identification system (bioMérieux, Inc.) was used. Results are detailed in Appendix A and Appendix B, respectively.

1. Calculation of sensitivity, specificity, selectivity, false-positive rate, and false-negative rates

For each parameter, the identification data was divided into four categories:

a = Number of typical wells from which growth is obtained that is identified as being the target organism (i.e., *Enterococcus* spp.) by an external identification test (true positives)

b = Number of atypical wells from which growth is obtained that is identified as being the target organism (i.e., *Enterococcus* spp.) by an external identification test (false negatives)

c = Number of typical wells from which growth is obtained that is identified as not being the target organism (i.e., bacteria other than *Enterococcus* spp.) by an external identification test (false positives)

d = Number of atypical wells from which growth is obtained that is identified as not being the target organism (i.e., bacteria other than *Enterococcus* spp.) by an external identification test (true negatives)

The sensitivity, specificity, selectivity, efficiency, false-positive rate, and false-negative rates from the data were calculated as follows:

Sensitivity = a / (a + b)

Specificity = d / (c + d)

False-positive rate = c / (a + c)

False-negative rate = b / (b + d)

Selectivity = a / (a + b + c + d)

Efficiency (*E*) = (a + d) / (a + b + c + d)

2. *Enterococcus* spp.

Isolates were obtained from all of the 163 positive wells subcultured and *Enterococcus* spp. were identified from 147 (90.2%) wells. The identities of the non-*Enterococcus* isolates from the 16 positive wells are listed in table 5.

Table 5. False-positive bacterial identification using VITEK II

False-positive ID	Occurrences
Lactococcus garvieae	5
Lactococcus garvieae/lactis	7
Lactococcus raffinolactis	1
Elizabethkingia meningoseptica	3

Lactococcus garvieae is a β-glucosidase-positive species and is very closely related to the enterococci having been redesignated in 1996 after previously being named *Enterococcus seriolicida*.

3. Negative wells

One hundred and eighty-six (186) negative wells were subcultured, all of which yielded colonies for identification. These were screened for aesculin hydrolysis on bile aesculin azide agar (BAAA), and those showing aesculin hydrolysis were identified by VITEK II. Thirteen isolates hydrolyzed aesculin on BAAA, all of which were identified as a species of *Enterococcus*, representing five species, as listed in table 6.

Table 6. False-negative bacterial identification using VITEK II

False-negative ID	Occurrences
Enterococcus avium	5
Enterococcus casseliflavus	1
Enterococcus faecalis	1
Enterococcus faecium	4
Enterococcus hirae	2

4. Determination of characteristics related to sensitivity and specificity

From the data above, the values for calculating the categorical characteristics of Enterolert-DW are:

a = True positives = 147
b = False negatives = 13
c = False positives = 16
d = True negatives = 173

The performance characteristics of Enterolert-DW for drinking water samples, as defined by ISO 13843, are listed in table 7.

Table 7. Enterolert-DW sensitivity, specificity, false-positive rate, false-negative rate, selectivity, and efficiency for drinking water samples

Parameter	Results
Sensitivity	91.9%
Specificity	93.0%
False-positive rate	9.8%
False-negative rate	7.0%
Selectivity	42.1%
Efficiency	91.7%

The outcomes of this assessment indicate that the Enterolert-DW/Quanti-Tray system is sensitive and specific (91.9% and 93.0%, respectively) for the detection of enterococci in drinking water samples. The method is also acceptably selective with a value of 42.1%, which is better than the guidance value of >10% suggested by ISO 13843 for colony count methods. With a calculated efficiency value of 91.7%, the data suggests that the method is highly efficient. The identification data also indicates that Enterolert-DW/Quanti-Tray recovers a broad range of *Enterococcus* species.

IV. Uncertainty of measurement

Repeatability and reproducibility are two estimates of the reliability that can be achieved with an analytical method. These can be assessments of the whole method using appropriate natural samples or of the counting uncertainty associated with reading the results of a method. The result produced by any method will be dependent upon the ease with which a count of colonies or positive MPN reactions can be made by analysts. This will be affected by the distinctiveness of colonial morphology of target and nontarget organisms on an enumeration agar or by the clarity of positive and negative reactions in broth-based MPN tests. Assessments of counting uncertainty can, therefore, provide an indication of any potential problems that could occur with the wide adoption of a method.

Repeatability (r) and reproducibility (R) are defined as follows:

> Repeatability: Closeness of the agreement between the results of successive measurements of the same measure and carried out under the same conditions of measurement.
>
> Reproducibility: Closeness of the agreement between the results of measurements on the same measure and carried out under changed conditions of measurement.

A. Counting uncertainty (ISO 13843 clause 6.7)

When the above definitions are applied to counting of microbiological colonies on an agar plate or positive reactions in an MPN test, the same or changed condition is the counting analyst. Thus, in this context, repeatability is the agreement in counts obtained by repeated counting by one analyst, and reproducibility is the agreement in counts obtained by repeated counting by two or more analysts. The assessment of reproducibility is generally more informative than the assessment of repeatability.

ISO 13843 recommends that, when using pure cultures, uncertainties (u_{rel}) should normally be less than ±0.03 (i.e., not more that ±3% deviation). Counting uncertainty studies were conducted by IDEXX analysts using 30 drinking water samples spiked with sewage effluent. The data are presented in Appendix C. The counting was done so that each analyst was not aware of the other analysts' counts, and the number of positive wells was recorded separately for each analyst. The resulting MPN values were recorded as whole integers. The counting was undertaken after 24 hours of incubation at 41°C ±0.5°C. The calculated uncertainties (relative standard deviations, or RSDs) for positive wells from Enterolert-DW/Quanti-Tray trays are presented in table 8.

Table 8. Enterolert-DW repeatability and reproducibility for spiked drinking water samples

Counting uncertainty	Relative standard deviation (u_{rel})
Repeatability (r)	3.4%
Reproducibility (R)	3.1%

B. Method uncertainty (ISO 13843:2017 section 6.4)

Method repeatability is the agreement in counts obtained by repeated analysis of a selection of samples by one analyst, and method reproducibility is the agreement in counts obtained by repeated analysis of selected samples by two or more analysts.

Assessment of method repeatability and reproducibility of the Enterolert-DW/Quanti-Tray method was conducted on tap water samples taken from three taps in different places (10 from each site) and inoculated with sewage effluent. Assessment of method reproducibility of the Enterolert-DW/Quanti-Tray method was conducted on 80 samples of drinking water inoculated with filtered sewage effluent. Forty of these samples were chlorinated using filtered sewage effluent that had been subjected to controlled chlorination and dechlorination in the laboratory prior to being inoculated into samples of drinking water. The resulting MPN values were recorded as whole integers. The counting was undertaken after 24 hours of incubation at 41°C ±0.5°C. The data generated from these samples and analyzed according to ISO 13843 is presented in Appendix D. The calculated method uncertainties are shown in table 9.

Table 9. Calculated method uncertainties

Counting uncertainty	Sample type	u_{rel}
Repeatability		15.4%
Reproducibility	Chlorinated samples	17.5%
	Unchlorinated samples	33.8%

There are no guideline values for method repeatability and reproducibility given in ISO 13843.

V. Robustness of incubation time (ISO 13843 clause 6.5)

The aspect of robustness that is relevant to the Enterolert-DW/Quanti-Tray method is the incubation period of 24 hours with a valid result up to 28 hours as described in the method. The results of triplicate counts of suspensions of reference cultures of *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus durans*, *Enterococcus hirae*, *Enterococcus mundtii*, and *Enterococcus gallinarum* analyzed using the same batch of Enterolert-DW at both the 24-hour and 28-hour incubation periods are presented in Appendix E.

There was no change in the counts observed for six of the seven type strains validated. The *Enterococcus gallinarum* ATCC 49573 counts increased by one positive well during the 24- to 28-hour read window. This strain of *Enterococcus gallinarum* ATCC 49573 appears to show a relatively slower growth rate or lower enzyme activity compared to the other bacterial strains tested.

The data suggests that the recommended incubation period of a minimum of 24 hours and up to 28 hours is robust. However, for some strains of *Enterococcus* that either grow slowly or have reduced β-glucosidase activity, there may be a marginal increase in counts at 28 hours of incubation compared to those at 24 hours of incubation.

An increase in counts over a prescribed incubation period (e.g., 40–48 hours for typical membrane filtration methods such as ISO 7899-2 or MoDW 2012 Part 5) is a well-recognized phenomenon, particularly with environmental *Enterococcus* spp. However, provided that the increase in counts after an extended incubation is not excessive, for practical and operational considerations counts from Enterolert-DW/Quanti-Tray at either 24 or 28 hours of incubation can be considered acceptable.

VI. Upper working limit

The Enterolert-DW/Quanti-Tray system is an MPN-based method with an arbitrary counting range of 0 to 201 for Quanti-Tray and 0 to 2,420 for Quanti-Tray/2000, determined by the number of wells in the tray. For practical and statistical reasons, an upper limit cannot be set for MPN methods because precision does not depend in a simple way on the number of particles introduced in the detection set. According to ISO 13843, "For MPN-based methods, the working range will be determined by the sample size used and the number of reaction vessels used. The practical upper limit has been exceeded when all tubes of all dilutions are found positive."

VII. Precision

The precision of an MPN method is described by the 95% confidence intervals calculated for each MPN value. The confidence intervals for MPN counts from Enterolert-DW/Quanti-Tray and Enterolert-DW/Quanti-Tray/2000 are available using the MPN Generator software available for download from the IDEXX website (idexx.com/water/mpn-generator.html).

VIII. Relative recoveries (ISO 13843 clause 6.6 and ISO 17994)

The relative recovery of *Enterococcus* spp. by the Enterolert-DW/Quanti-Tray system was compared to that of the reference methods of ISO 7899-2 and MoDW 2012 Part 5 following the requirements of *ISO 17994:2017 Water Quality – Criteria for establishing equivalence between microbiological methods* (ISO 17994).

Four hundred and two (402) drinking water samples from geographically diverse locations within the United Kingdom, Italy, Spain, Belgium, and the United States were used in this validation. Split samples were used in each of the three methods under comparison. Among those, 197 samples were chlorinated and 205 were unchlorinated. The relative recoveries for each method are summarized in Appendix F.

Paired confirmed count data for the two methods (either Enterolert-DW and ISO 7899-2 or Enterolert-DW and MoDW Part 5) were transformed using natural logarithms (ln), and the relative difference in counts for each pair (x_i) was calculated using the formula $[\ln(a_i) - \ln(b_i)] \times 100\%$, where a_i and b_i are the paired confirmed counts for the trial and reference methods, respectively). MPN values were rounded to the nearest whole integer. If one of the counts was 0 (zero), then 1 was added to both counts prior to log-transformation. From the data, the mean relative differences and standard deviations were calculated. Evaluation of equivalence is based on the mean relative difference and the expanded uncertainty (W) based on the standard deviation of the mean ($W = 2s/\sqrt{n}$), from which the lower (X_L) and higher (X_U) limits of the confidence interval are calculated. Based on this statistical approach, the outcomes of these analyses are summarized in tables 10 and 11.

Table 10. Outcomes of ISO 17994 analysis of paired-count data for Enterolert-DW vs. ISO 7899-2 for chlorinated and unchlorinated drinking water

Comparison	MRD%	SD	W	X_L	X_U	Conclusion
Chlorinated waters	28.6	81.0	11.5	17.0	40.1	Enterolert-DW higher
Unchlorinated waters	4.5	53.8	7.5	-3.0	12.0	Methods not different
All data	16.3	69.5	6.9	9.4	23.2	Enterolert-DW higher

MRD% = Mean relative difference expressed as a percentage
SD = Standard deviation

Table 11. Outcomes of ISO 17994 analysis of paired-count data for Enterolert-DW MoDW Part 5 for chlorinated and unchlorinated drinking water

Comparison	MRD%	SD	*W*	X_L	X_U	Conclusion
Chlorinated waters	14.4	81.1	11.6	2.9	26.0	Enterolert-DW higher
Unchlorinated waters	-9.0	53.0	7.4	-16.4	-1.6	MoDW Part 5 higher
All data	2.5	69.1	6.9	-4.4	9.4	Methods not different

MRD% = Mean relative difference expressed as a percentage
SD = Standard deviation

The analysis showed the Enterolert-DW/Quanti-Tray method gives overall higher recoveries in comparison with ISO 7899-2. This result was largely driven by the relative differences obtained using chlorinated sample matrices. For the unchlorinated samples, the validation indicated “Methods not different” between the two methods.

The overall conclusion was “Methods not different” when comparing Enterolert-DW to MoDW 2012 Part 5 with results from Enterolert-DW being higher for the chlorinated samples but lower for the unchlorinated samples.

The analyses show that the Enterolert-DW/Quanti-Tray method gives significantly higher recoveries of *Enterococcus* spp. in comparison with the ISO 7899-2 method as the confidence limits (X_L and X_U) are above the +10% upper criterion proposed by ISO 17994 for drinking water samples. Overall, the relative recoveries of *Enterococcus* spp. by Enterolert-DW/Quanti-Tray are not different from those of MoDW Part 5 and are superior for chlorinated drinking waters samples. However, it appears that performance may be less efficient for unchlorinated drinking waters.

IX. References

ISO 7899-2:2000 Water Quality – Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method. Geneva: International Organization for Standardization.

ISO 17994:2014 Water Quality – Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods. Geneva: International Organization for Standardization.

ISO 13843:2017 Water Quality – Requirements for establishing performance characteristics of quantitative microbiological methods. Geneva: International Organization for Standardization.

World Health Organization Environment Agency. *The Microbiology of Drinking Water* – Part 5 – Methods for the isolation and enumeration of enterococci. gov.uk website. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/755548/geho0812bwwj-e-e.pdf. Published 2012. Accessed December 3, 2019.

X. Appendices

Appendix A. Identification of isolates from positive Enterolert-DW/Quanti-Tray wells

Sample ID	Enterolert-DW reactions	VITEK II ID	ID confidence
1	+	*E. gallinarum*	99%
2	+	*E. faecium*	97%
3	+	*E. faecium*	99%
4	+	*E. gallinarum*	99%
5	+	*E. gallinarum*	99%
6	+	*E. faecium*	99%
7	+	*E. faecalis*	99%
8	+	*E. faecium*	99%
9	+	*E. faecium*	96%
10	+	*E. gallinarum*	99%
11	+	*E. gallinarum*	99%
12	+	*E. faecium*	97%
13	+	*E. hirae*	99%
14	+	*E. faecalis*	99%
15	+	*E. faecium*	98%
16	+	*E. gallinarum*	99%
17	+	*E. faecium*	98%
18	+	*E. gallinarum*	93%
19	+	*E. faecalis*	99%
20	+	*E. avium*	92%
21	+	*E. faecalis*	99%
22	+	*E. durans/hirae*	Low Dis
23	+	*E. faecalis*	99%
24	+	*Lactococcus garvieae*†	99%
25	+	*E. faecalis*	99%
26	+	*Lactococcus garvieae*†	99%
27	+	*Lactococcus garvieae*†	95%
28	+	*E. gallinarum*	98%
29	+	*Lactococcus garvieae*†*/E. lactis*	Low Dis
30	+	*E. faecium*	99%
31	+	*Lactococcus raffinolactis*	90%
32	+	*E. gallinarum*	99%
33	+	*Lactococcus garvieae*†	99%
34	+	*E. faecium*	99%
35	+	*E. faecium*	99%
36	+	*E. faecium*	99%
37	+	*E. gallinarum*	99%
38	+	*E. faecium*	99%

Sample ID	Enterolert-DW reactions	VITEK II ID	ID confidence
39	+	*E. faecium*	99%
40	+	*E. faecium*	98%
41	+	*E. hirae*	95%
42	+	*E. hirae*	99%
43	+	*E. faecium*	98%
44	+	*E. hirae*	95%
45	+	*E. hirae*	99%
46	+	*E. gallinarum*	99%
47	+	*E. faecium*	99%
48	+	*E. faecium*	99%
49	+	*E. faecium*	99%
50	+	*E. hirae*	99%
51	+	*E. durans/E. faecium*	Low Dis
52	+	*E. faecalis*	99%
53	+	*E. gallinarum*	93%
54	+	*E. durans*	98%
55	+	*E. faecium*	96%
56	+	*E. faecium*	99%
57	+	*E. faecalis*	99%
58	+	*E. hirae*	99%
59	+	*E. faecium*	92%
60	+	*E. hirae*	99%
61	+	*E. faecium*	99%
62	+	*E. faecium*	99%
63	+	*E. faecium*	94%
64	+	*E. faecium*	99%
65	+	*E. durans*	97%
66	+	*E. faecium*	98%
67	+	*E. faecalis*	99%
68	+	*E. hirae*	95%
69	+	*E. faecium*	99%
70	+	*E. faecalis*	99%
71	+	*E. faecium*	96%
72	+	*E. casseliflavus*	89%
73	+	*E. faecalis*	99%
74	+	*E. casseliflavus*	99%
75	+	*Lactococcus garvieae*†	99%
76	+	*Lactococcus garvieae*†*/E. lactis*	Low Dis
77	+	*E. durans/E. faecium/E. hirae*	Low Dis
78	+	*E. casseliflavus*	97%
79	+	*E. gallinarum*	99%
80	+	*E. casseliflavus*	89%

Sample ID	Enterolert-DW reactions	VITEK II ID	ID confidence
81	+	*E. durans/E. hirae*	Low Dis
82	+	*E. gallinarum*	98%
83	+	*E. gallinarum*	99%
84	+	*E. hirae*	95%
85	+	*E. faecalis*	99%
86	+	*Lactococcus garvieae*†*/E. lactis*	Low Dis
87	+	*E. faecium*	95%
88	+	*Lactococcus garvieae*†*/E. lactis*	Low Dis
89	+	*E. faecium*	98%
90	+	*E. avium*	95%
91	+	*E. gallinarum*	99%
92	+	*E. faecium*	93%
93	+	*E. gallinarum*	99%
94	+	*E. gallinarum*	99%
95	+	*E. gallinarum*	99%
96	+	*E. faecium*	98%
97	+	*E. faecium*	99%
98	+	*E. faecium*	99%
99	+	*E. faecalis*	99%
100	+	*E. gallinarum*	99%
101	+	*Lactococcus garvieae*†*/E. lactis*	Low Dis
102	+	*E. faecium*	96%
103	+	*E. faecium*	99%
104	+	*E. faecium*	92%
105	+	*E. gallinarum*	99%
106	+	*E. faecium*	99%
107	+	*E. durans/E. gallinarum/E. hirae*	Low Dis
108	+	*E. gallinarum*	99%
109	+	*E. faecalis*	99%
110	+	*E. faecium*	98%
111	+	*Elizabethkingia meningoseptica*	95%
112	+	*E. gallinarum*	99%
113	+	*Elizabethkingia meningoseptica*	99%
114	+	*E. faecium*	98%
115	+	*E. casseliflavus*	99%
116	+	*E. faecium*	99%
117	+	*E. casseliflavus/E. gallinarum*	Low Dis
118	+	*E. faecium*	99%
119	+	*E. casseliflavus*	99%
120	+	*E. gallinarum*	99%
121	+	*E. faecium/E. gallinarum*	Low Dis
122	+	*E. gallinarum*	98%

Sample ID	Enterolert-DW reactions	VITEK II ID	ID confidence
123	+	*E. hirae*	99%
124	+	*E. raffinosus*	91%
125	+	*E. faecium*	99%
126	+	*E. faecalis*	85%
127	+	*E. faecalis*	99%
128	+	*Elizabethkingia meningoseptica*	99%
129	+	*E. hirae*	99%
130	+	*E. durans*	95%
131	+	*E. casseliflavus*	93%
132	+	*Lactococcus garvieae*† */E. lactis*	Low Dis
133	+	*E. faecium*	86%
134	+	*E. casseliflavus/E. gallinarum*	Low Dis
135	+	*E. gallinarum*	98%
136	+	*E. casseliflavus/E. gallinarum*	Low Dis
137	+	*E. faecium*	99%
138	+	*E. hirae*	99%
139	+	*E. faecium*	98%
140	+	*E. gallinarum*	99%
141	+	*E. faecium*	98%
142	+	*E. gallinarum*	99%
143	+	*E. faecium*	99%
144	+	*E. faecium*	99%
145	+	*E. gallinarum*	98%
146	+	*E. durans/faecium*	Low Dis
147	+	*E. faecium*	99%
148	+	*E. faecium*	99%
149	+	*E. durans*	85%
150	+	*E. gallinarum*	99%
151	+	*E. faecium/E. gallinarum*	Low Dis
152	+	*E. faecium*	99%
153	+	*E. faecium*	94%
154	+	*E. faecium*	99%
155	+	*E. faecalis*	99%
156	+	*E. hirae*	91%
157	+	*E. durans*	94%
158	+	*E. faecium*	99%
159	+	*E. faecium*	98%
160	+	*E. durans*	98%
161	+	*E. faecalis*	99%
162	+	*E. durans*	92%
163	+	*E. faecalis*	99%
164	+	*E. durans/E. faecium*	Low Dis

Sample ID	Enterolert-DW reactions	VITEK II ID	ID confidence
165	+	*E. faecium*	99%
166	+	*E. faecium*	99%
167	+	*E. faecium*	98%
168	+	*E. gallinarum*	99%
169	+	*E. faecalis*	99%
170	+	*E. casseliflavus/E. gallinarum*	Low Dis
171	+	*E. durans*	98%
172	+	*E. faecium*	99%
173	+	*E. hirae*	99%
174	+	*E. faecium*	99%
175	+	*E. gallinarum*	99%
176	+	*E. faecium*	98%
177	+	*E. hirae*	95%
178	+	*E. avium*	90%
179	+	*E. gallinarum*	98%
180	+	*E. faecalis*	99%
181	+	*E. gallinarum*	99%
182	+	*E. faecium*	99%
183	+	*E. hirae*	99%
184	+	*E. faecium*	95%
185	+	*E. gallinarum*	98%
186	+	*E. gallinarum*	99%
187	+	*E. gallinarum*	99%
188	+	*E. faecium*	93%
189	+	*E. faecium*	99%
190	+	*E. faecium*	93%
191	+	*E. faecium*	99%
192	+	*E. gallinarum*	99%
193	+	*E. gallinarum*	99%
194	+	*E. faecium*	99%
195	+	*E. faecalis*	99%
196	+	*E. faecium*	99%
197	+	*E. gallinarum*	99%
198	+	*E. faecium*	98%
199	+	*E. faecium*	98%
200	+	*E. durans/E. faecium*	Low Dis
201	+	*E. faecium*	91%
202	+	*E. faecium*	99%
203	+	*E. faecalis*	99%
204	+	*E. faecalis*	99%
205	+	*E. faecium*	97%
206	+	*E. gallinarum*	99%

Sample ID	Enterolert-DW reactions	VITEK II ID	ID confidence
207	+	*E. faecium*	99%
208	+	*E. faecium*	98%
209	+	*E. gallinarum*	99%
210	+	*E. faecalis*	99%
211	+	*E. faecium*	99%
212	+	*E. casseliflavus/E. gallinarum*	Low Dis
213	+	*E. faecalis*	95%
214	+	*E. faecium*	99%
215	+	*E. durans*	93%
216	+	*E. faecium*	98%
217	+	*E. durans*	99%
218	+	*E. faecalis*	99%
219	+	*E. faecium*	97%
220	+	*Lactococcus garvieae*[†]*/E. lactis*	Low Dis
221	+	*E. faecium*	98%
222	+	*E. faecium*	99%
223	+	*E. faecalis*	99%
224	+	*E. faecium*	97%
225	+	*E. faecalis*	99%
226	+	*E. faecalis*	99%
227	+	*E. faecalis*	99%
228	+	*E. faecium*	99%
229	+	*E. faecium*	98%

[†] Identified by VITEK II as *Enterococcus seriolicida* which has subsequently been reclassified.

Appendix B. Identification of isolates from negative Enterolert-DW/Quanti-Tray wells

Sample ID	Enterolert-DW reaction	Aesculin hydrolysis	VITEK II ID	ID confidence
1	-	-		
2	-	-		
3	-	+	*E. hirae*	99%
4	-	+	*E. hirae*	95%
5	-	-		
6	-	-		
7	-	-		
8	-	-		
9	-	-		
10	-	-		
11	-	-		
12	-	-		
13	-	-		
14	-	-		

Sample ID	Enterolert-DW reaction	Aesculin hydrolysis	VITEK II ID	ID confidence
15	-	+	*E. faecium*	99%
16	-	+	*E. faecium*	98%
17	-	-		
18	-	-		
19	-	-		
20	-	-		
21	-	-		
22	-	-		
23	-	-		
24	-	-		
25	-	-		
26	-	-		
27	-	-		
28	-	-		
29	-	-		
30	-	-		
31	-	-		
32	-	+	*E. faecium*	99%
33	-	-		
34	-	-		
35	-	+	*E. avium*	99%
36	-	+	*E. avium*	99%
37	-	-		
38	-	-		
39	-	-		
40	-	-		
41	-	-		
42	-	-		
43	-	-		
44	-	-		
45	-	-		
46	-	-		
47	-	-		
48	-	-		
49	-	-		
50	-	-		
51	-	-		
52	-	-		
53	-	-		
54	-	-		
55	-	-		

Sample ID	Enterolert-DW reaction	Aesculin hydrolysis	VITEK II ID	ID confidence
56	-	-		
57	-	-		
58	-	+	*E. avium*	99%
59	-	-		
60	-	-		
61	-	-		
62	-	-		
63	-	-		
64	-	-		
65	-	-		
66	-	-		
67	-	-		
68	-	-		
69	-	-		
70	-	-		
71	-	-		
72	-	-		
73	-	-		
74	-	-		
75	-	-		
76	-	-		
77	-	-		
78	-	-		
79	-	-		
80	-	-		
81	-	-		
82	-	-		
83	-	-		
84	-	-		
85	-	-		
86	-	-		
87	-	-		
88	-	-		
89	-	+	*E. avium*	99%
90	-	-		
91	-	-		
92	-	-		
93	-	-		
94	-	-		
95	-	+	*E. faecalis*	99%
96	-	-		

Sample ID	Enterolert-DW reaction	Aesculin hydrolysis	VITEK II ID	ID confidence
97	-	-		
98	-	-		
99	-	-		
100	-	-		
101	-	-		
102	-	-		
103	-	-		
104	-	-		
105	-	+	*E. casseliflavus*	99%
106	-	-		
107	-	-		
108	-	-		
109	-	-		
110	-	-		
111	-	-		
112	-	-		
113	-	-		
114	-	-		
115	-	-		
116	-	-		
117	-	-		
118	-	-		
119	-	-		
120	-	-		
121	-	-		
122	-	-		
123	-	+	*E. avium*	99%
124	-	-		
125	-	-		
126	-	-		
127	-	-		
128	-	-		
129	-	-		
130	-	-		
131	-	+	*E. faecium*	98%
132	-	-		
133	-	-		
134	-	-		
135	-	-		
136	-	-		
137	-	-		

Sample ID	Enterolert-DW reaction	Aesculin hydrolysis	VITEK II ID	ID confidence
138	-	-		
139	-	-		
140	-	-		
141	-	-		
142	-	-		
143	-	-		
144	-	-		
145	-	-		
146	-	-		
147	-	-		
148	-	-		
149	-	-		
150	-	-		
151	-	-		
152	-	-		
153	-	-		
154	-	-		
155	-	-		
156	-	-		
157	-	-		
158	-	-		
159	-	-		
160	-	-		
161	-	-		
162	-	-		
163	-	-		
164	-	-		
165	-	-		
166	-	-		
167	-	-		
168	-	-		
169	-	-		
170	-	-		
171	-	-		
172	-	-		
173	-	-		
174	-	-		
175	-	-		
176	-	-		
177	-	-		
178	-	-		

Sample ID	Enterolert-DW reaction	Aesculin hydrolysis	VITEK II ID	ID confidence
179	-	-		
180	-	-		
181	-	-		
182	-	-		
183	-	-		
184	-	-		
185	-	-		
186	-	-		

Appendix C. Counting uncertainty for Enterolert-DW/Quanti-Tray (see Uncertainty of measurement)

Table C1. Repeatability data for MPN counts

Sample	Analyst A		MPN 1 – MPN 2	MPN 1 + MPN 2	Relative variance
	MPN 1	MPN 2			
1	25	25	0	50	0.0000
2	12	14	-2	26	0.0118
3	16	15	1	31	0.0021
4	22	22	0	44	0.0000
5	24	24	0	48	0.0000
6	21	21	0	42	0.0000
7	34	34	0	68	0.0000
8	16	16	0	32	0.0000
9	21	21	0	42	0.0000
10	16	16	0	32	0.0000
11	62	62	0	124	0.0000
12	19	18	1	37	0.0015
13	48	48	0	96	0.0000
14	34	34	0	68	0.0000
15	12	12	0	24	0.0000
16	53	50	3	103	0.0017
17	45	45	0	90	0.0000
18	19	19	0	38	0.0000
19	66	74	-8	140	0.0065
20	36	41	-5	77	0.0084
21	59	59	0	118	0.0000

Sample	Analyst A		MPN 1 – MPN 2	MPN 1 + MPN 2	Relative variance
	MPN 1	MPN 2			
22	89	89	0	178	0.0000
23	78	78	0	156	0.0000
24	95	95	0	190	0.0000
25	59	59	0	118	0.0000
26	70	74	-4	144	0.0015
27	66	66	0	132	0.0000
28	62	62	0	124	0.0000
29	74	74	0	148	0.0000
30	62	66	-4	128	0.0020
				Mean	0.0012
			Counting repeatability (r)		3.44%

Table C2. Reproducibility data for MPN counts

Sample	MPN		MPN A – MPN B	MPN A + MPN B	Relative variance
	Analyst A	Analyst B			
1	5	5	0	10	0.0000
2	19	19	0	38	0.0000
3	9	9	0	18	0.0000
4	15	15	0	30	0.0000
5	6	6	0	12	0.0000
6	11	11	0	22	0.0000
7	4	4	0	8	0.0000
8	11	11	0	22	0.0000
9	11	11	0	22	0.0000
10	21	21	0	42	0.0000
11	43	50	-7	93	0.0113
12	16	18	-2	34	0.0069
13	32	32	0	64	0.0000
14	43	43	0	86	0.0000
15	12	12	0	24	0.0000
16	53	56	-3	109	0.0015
17	43	43	0	86	0.0000
18	5	5	0	10	0.0000
19	48	48	0	96	0.0000
20	36	41	-5	77	0.0084

Sample	MPN		MPN A – MPN B	MPN A + MPN B	Relative variance
	Analyst A	Analyst B			
21	74	74	0	148	0.0000
22	83	83	0	166	0.0000
23	66	66	0	132	0.0000
24	74	74	0	148	0.0000
25	74	74	0	148	0.0000
26	70	70	0	140	0.0000
27	78	78	0	156	0.0000
28	66	66	0	132	0.0000
29	66	66	0	132	0.0000
30	95	95	0	190	0.0000
				Mean	0.0009
			Counting reproducibility (R)		3.07%

Appendix D. Method uncertainty for Enterolert-DW inoculated with either naturally contaminated samples (see Uncertainty of measurement)

Table D1. Repeatability data

	Sample		
Replicate	Tap 1	Tap 2	Tap 3
1	18	1	18
2	24	16	15
3	21	22	27
4	24	16	19
5	22	16	19
6	27	14	25
7	27	19	21
8	15	21	12
9	27	21	15
10	25	18	24
Relative operational variance	-0.012	0.074	0.010
Method repeatability RSD		15.40%	

Table D2. Reproducibility data for chlorinated samples

Sample	Analyst 1 MPN count	Analyst 2 MPN count	Overlap of confidence intervals	Reproducibility (R)	Intrinsic variability Analyst 1	Intrinsic variability Analyst 2	Average intrinsic variability	Relative operational variance
1	62	43	Yes	0.0670	0.0303	0.0358	0.0330	0.0339
2	19	16	Yes	0.0148	0.0586	0.0690	0.0638	-0.0490
3	48	32	Yes	0.0822	0.0354	0.0417	0.0386	0.0436
4	34	43	Yes	0.0276	0.0413	0.0358	0.0385	-0.0110
5	12	12	Yes	0.0000	0.0853	0.0853	0.0853	-0.0853
6	53	53	Yes	0.0000	0.0313	0.0313	0.0313	-0.0313
7	45	43	Yes	0.0010	0.0341	0.0358	0.0350	-0.0339
8	19	5	Yes	0.8911	0.0586	0.2089	0.1338	0.7573
9	74	48	Yes	0.0937	0.0304	0.0354	0.0329	0.0608
10	41	36	Yes	0.0085	0.0378	0.0386	0.0382	-0.0297
11	59	56	Yes	0.0014	0.0313	0.0313	0.0313	-0.0299
12	83	56	Yes	0.0774	0.0298	0.0313	0.0305	0.0469
13	95	62	Yes	0.0911	0.0306	0.0303	0.0304	0.0606
14	101	78	Yes	0.0334	0.0313	0.0305	0.0309	0.0025
15	78	89	Yes	0.0087	0.0305	0.0299	0.0302	-0.0215
16	95	83	Yes	0.0091	0.0306	0.0298	0.0302	-0.0211
17	66	59	Yes	0.0063	0.0313	0.0313	0.0313	-0.0250
18	89	70	Yes	0.0288	0.0299	0.0304	0.0301	-0.0013
19	83	74	Yes	0.0066	0.0298	0.0304	0.0301	-0.0235
20	59	89	Yes	0.0845	0.0313	0.0299	0.0306	0.0539
21	21	15	Yes	0.0566	0.0565	0.0732	0.0649	-0.0083
22	19	43	Yes	0.3335	0.0586	0.0358	0.0472	0.2863

Sample	Analyst 1 MPN count	Analyst 2 MPN count	Overlap of confidence intervals	Reproducibility (R)	Intrinsic variability Analyst 1	Intrinsic variability Analyst 2	Average intrinsic variability	Relative operational variance
23	27	12	Yes	0.3288	0.0467	0.0853	0.0660	0.2628
24	36	32	Yes	0.0069	0.0386	0.0417	0.0402	-0.0332
25	18	19	Yes	0.0015	0.0655	0.0586	0.0621	-0.0606
26	36	18	Yes	0.2402	0.0386	0.0655	0.0521	0.1882
27	25	24	Yes	0.0008	0.0449	0.0530	0.0490	-0.0481
28	25	29	Yes	0.0110	0.0449	0.0459	0.0454	-0.0344
29	15	15	Yes	0.0000	0.0732	0.0732	0.0732	-0.0732
30	19	16	Yes	0.0148	0.0586	0.0690	0.0638	-0.0490
31	78	95	Yes	0.0194	0.0305	0.0306	0.0305	-0.0111
32	118	109	Yes	0.0031	0.0339	0.0318	0.0329	-0.0297
33	89	95	Yes	0.0021	0.0299	0.0306	0.0302	-0.0281
34	130	83	Yes	0.1007	0.0357	0.0298	0.0327	0.0679
35	89	118	Yes	0.0398	0.0299	0.0339	0.0319	0.0079
36	95	74	Yes	0.0312	0.0306	0.0304	0.0305	0.0007
37	109	62	Yes	0.1592	0.0339	0.0303	0.0321	0.1271
38	101	101	Yes	0.0000	0.0313	0.0313	0.0313	-0.0313
39	118	89	Yes	0.0398	0.0339	0.0299	0.0319	0.0079
40	83	101	Yes	0.0193	0.0298	0.0313	0.0305	-0.0113
				Mean relative operational variance				0.0307
				Method reproducibility				**17.52%**

Table D3. Reproducibility data for unchlorinated samples

Sample	Analyst 1 MPN count	Analyst 2 MPN count	Overlap of confidence intervals	Reproducibility (R)	Intrinsic variability Analyst 1	Intrinsic variability Analyst 2	Average intrinsic variability	Relative operational variance
1	25	5	No	1.2951	0.0449	0.2089	0.1269	1.1682

Sample	Analyst 1 MPN count	Analyst 2 MPN count	Overlap of confidence intervals	Reproducibility (R)	Intrinsic variability Analyst 1	Intrinsic variability Analyst 2	Average intrinsic variability	Relative operational variance
2	12	19	Yes	0.1056	0.0853	0.0586	0.0720	0.0336
3	16	9	Yes	0.1655	0.0690	0.0975	0.0832	0.0823
4	22	15	Yes	0.0733	0.0546	0.0732	0.0639	0.0094
5	24	6	No	0.9609	0.0530	0.1544	0.1037	0.8572
6	21	11	Yes	0.2091	0.0565	0.1021	0.0793	0.1298
7	34	4	No	2.2899	0.0413	0.1891	0.1152	2.1747
8	16	11	Yes	0.0702	0.0690	0.1021	0.0856	-0.0154
9	21	11	Yes	0.2091	0.0565	0.1021	0.0793	0.1298
10	16	21	Yes	0.0370	0.0690	0.0565	0.0627	-0.0258
11	59	74	Yes	0.0257	0.0313	0.0304	0.0308	-0.0052
12	89	83	Yes	0.0024	0.0299	0.0298	0.0298	-0.0274
13	78	66	Yes	0.0140	0.0305	0.0313	0.0309	-0.0169
14	95	74	Yes	0.0312	0.0306	0.0304	0.0305	0.0007
15	59	74	Yes	0.0257	0.0313	0.0304	0.0308	-0.0052
16	74	70	Yes	0.0015	0.0304	0.0304	0.0304	-0.0288
17	66	78	Yes	0.0140	0.0313	0.0305	0.0309	-0.0169
18	62	66	Yes	0.0020	0.0303	0.0313	0.0308	-0.0288
19	74	66	Yes	0.0065	0.0304	0.0303	0.0303	-0.0238
20	66	94	Yes	0.0625	0.0303	0.0306	0.0304	0.0321
21	27	29	Yes	0.0026	0.0467	0.0459	0.0463	-0.0438
22	38	25	Yes	0.0877	0.0401	0.0449	0.0425	0.0452
23	32	29	Yes	0.0048	0.0417	0.0459	0.0438	-0.0390
24	41	32	Yes	0.0307	0.0378	0.0417	0.0398	-0.0091
25	27	34	Yes	0.0266	0.0467	0.0413	0.0440	-0.0174
26	27	36	Yes	0.0414	0.0467	0.0386	0.0427	-0.0013
27	25	31	Yes	0.0231	0.0449	0.0451	0.0450	-0.0219
28	38	18	Yes	0.2792	0.0401	0.0612	0.0506	0.2285
29	32	22	Yes	0.0702	0.0417	0.0546	0.0482	0.0220
30	19	34	Yes	0.1693	0.0586	0.0413	0.0499	0.1194
31	89	78	Yes	0.0087	0.0299	0.0305	0.0302	-0.0215
32	83	70	Yes	0.0145	0.0298	0.0304	0.0301	-0.0156
33	118	78	Yes	0.0857	0.0339	0.0305	0.0322	0.0535
34	118	109	Yes	0.0031	0.0339	0.0318	0.0329	-0.0297

Sample	Analyst 1 MPN count	Analyst 2 MPN count	Overlap of confidence intervals	Reproducibility (R)	Intrinsic variability Analyst 1	Intrinsic variability Analyst 2	Average intrinsic variability	Relative operational variance
35	101	109	Yes	0.0029	0.0313	0.0318	0.0316	-0.0286
36	66	66	Yes	0.0000	0.0313	0.0313	0.0313	-0.0313
37	83	62	Yes	0.0425	0.0298	0.0303	0.0300	0.0125
38	89	83	Yes	0.0024	0.0299	0.0298	0.0298	-0.0274
39	78	89	Yes	0.0087	0.0305	0.0299	0.0302	-0.0215
40	89	83	Yes	0.0024	0.0299	0.0298	0.0298	-0.0274
				Mean relative operational variance				0.1142
				Method reproducibility				**33.80%**

Appendix E. Robustness of incubation time assessment for Enterolert-DW using type strains (see Robustness of incubation time [ISO 13843 clause 6.5])

		Number of positive wells		
Isolate	**Rep**	**24 hr**	**28 hr**	**% Change**
E. faecium ATCC 35667	1	15	15	0%
	2	10	10	0%
	3	10	10	0%
E. faecalis ATCC 33186	1	23	23	0%
	2	21	21	0%
	3	21	21	0%
E. faecalis ATCC 19433	1	13	13	0%
	2	17	17	0%
	3	13	13	0%
E. durans ATCC 11576	1	19	19	0%
	2	18	18	0%
	3	30	30	0%
E. hirae ATCC 8043	1	23	23	0%
	2	19	19	0%
	3	24	24	0%
E. mundtii ATCC 43187	1	11	11	0%
	2	19	19	0%
	3	17	17	0%
E. gallinarum ATCC 49573	1	25	26	4%
	2	27	28	4%
	3	37	38	3%

Appendix F. Enterolert-DW vs. ISO 7899-2 and MoDW 2012 Part 5 relative recovery results

Table F1. Chlorinated results

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006 Part 5 (CFU/100 mL)
1	23	80	95
2	69	70	86
3	7	7	11
4	54	78	87
5	79	96	106
6	116	90	100
7	10	6	10
8	52	79	95
9	68	61	72
10	39	48	59
11	47	43	52
12	9	10	13
13	58	53	60
14	57	58	70
15	18	2	2
16	45	48	54
17	109	29	43
18	64	43	53
19	53	82	87
20	70	73	78
21	13	62	72
22	83	67	71
23	56	22	25
24	124	75	89
25	34	26	29
26	62	63	73
27	119	66	72
28	74	77	78
29	16	4	7
30	96	37	50
31	183	172	181

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006 Part 5 (CFU/100 mL)
32	101	60	68
33	123	129	153
34	56	31	38
35	201	203	208
36	173	141	153
37	62	154	167
38	41	26	33
39	12	3	8
40	95	85	116
41	13	3	7
42	68	74	95
43	9	2	3
44	201	156	161
45	142	79	114
46	13	139	149
47	5	4	6
48	21	0	0
49	74	17	18
50	165	114	130
51	74	17	29
52	118	55	72
53	45	3	6
54	201	123	124
55	159	97	103
56	39	79	103
57	40	5	6
58	24	6	7
59	25	19	25
60	51	22	25
61	35	16	18
62	35	16	18
63	59	56	57
64	49	28	31
65	14	5	5

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006 Part 5 (CFU/100 mL)
66	50	40	44
67	61	45	49
68	28	19	20
69	63	50	54
70	56	57	63
71	30	48	52
72	56	54	55
73	40	95	102
74	56	45	50
75	16	9	12
76	64	84	90
77	65	104	105
78	27	6	8
79	35	31	39
80	70	22	28
81	15	17	22
82	78	46	48
83	49	72	87
84	144	44	53
85	5	2	3
86	63	72	81
87	58	87	96
88	66	36	37
89	55	12	14
90	8	0	0
91	1	0	0
92	78	13	15
93	57	27	35
94	75	48	53
95	123	22	34
96	24	0	0
97	74	5	7
98	43	48	50
99	59	29	35

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006 Part 5 (CFU/100 mL)
100	16	16	18
101	8	1	1
102	66	43	46
103	38	45	46
104	38	52	54
105	53	38	41
106	37	22	24
107	44	39	41
108	91	42	51
109	91	48	51
110	48	19	21
111	5	3	5
112	95	41	51
113	86	34	45
114	106	41	56
115	68	39	47
116	55	30	32
117	78	40	48
118	165	123	132
119	165	103	112
120	106	109	118
121	119	105	119
122	159	121	133
123	31	24	31
124	137	110	124
125	106	103	117
126	96	90	94
127	114	79	86
128	98	76	93
129	70	73	85
130	53	73	82
131	61	71	77
132	11	9	13
133	137	70	80

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006 Part 5 (CFU/100 mL)
134	98	67	77
135	70	65	78
136	44	70	76
137	37	22	28
138	49	25	30
139	44	36	37
140	37	28	34
141	37	29	35
142	21	4	5
143	50	22	27
144	42	24	33
145	31	22	30
146	26	20	24
147	17	46	52
148	31	43	55
149	23	48	54
150	17	61	70
151	48	53	61
152	31	25	32
153	22	42	48
154	48	36	44
155	63	41	51
156	50	42	49
157	116	98	103
158	130	80	86
159	137	82	86
160	106	82	85
161	94	78	81
162	47	31	38
163	39	44	51
164	130	49	56
165	159	69	78
166	103	52	57
167	12	41	44

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006 Part 5 (CFU/100 mL)
168	28	36	43
169	19	41	45
170	21	34	40
171	27	33	34
172	5	0	0
173	5	7	8
174	11	2	2
175	37	18	22
176	22	12	17
177	58	51	58
178	48	59	68
179	6	30	36
180	9	29	36
181	7	25	31
182	10	16	22
183	13	23	29
184	5	23	32
185	2	0	0
186	32	38	49
187	29	33	40
188	26	40	49
189	26	44	49
190	33	37	39
191	29	37	39
192	1	3	4
193	159	122	127
194	155	131	138
195	64	29	40
196	65	42	48
197	22	74	91

Table F2. Unchlorinated samples

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
1	15	21	23
2	19	20	25
3	8	21	22
4	20	18	27
5	17	21	27
6	23	29	35
7	15	17	18
8	17	19	23
9	23	20	24
10	24	23	25
11	21	24	29
12	21	20	26
13	17	25	29
14	29	20	23
15	20	22	29
16	201	22	25
17	21	21	25
18	17	18	23
19	21	23	33
20	29	27	30
21	25	29	32
22	17	21	22
23	35	29	32
24	41	21	25
25	34	29	31
26	25	20	22
27	28	26	30
28	44	21	25
29	25	21	23
30	31	53	54
31	39	50	51
32	35	50	51

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
33	34	51	52
34	30	51	52
35	42	51	54
36	36	51	54
37	35	48	49
38	17	64	65
39	32	57	58
40	76	53	55
41	72	44	44
42	72	49	53
43	61	51	57
44	44	42	51
45	59	45	50
46	48	51	70
47	63	49	63
48	33	58	65
49	23	47	52
50	28	29	30
51	23	35	37
52	30	44	44
53	26	38	41
54	28	35	40
55	34	34	37
56	47	32	35
57	35	37	39
58	19	35	37
59	33	40	41
60	41	28	30
61	42	37	40
62	52	34	37
63	45	34	38
64	34	36	40
65	49	33	36
66	33	35	37

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
67	21	21	23
68	39	32	38
69	44	25	32
70	32	23	25
71	21	26	29
72	19	19	22
73	19	23	28
74	18	21	26
75	19	22	29
76	23	21	26
77	17	16	17
78	23	17	24
79	25	29	32
80	33	26	32
81	30	34	36
82	38	39	41
83	32	39	47
84	35	31	38
85	34	33	40
86	47	32	37
87	21	29	34
88	37	40	48
89	51	37	42
90	41	35	37
91	32	36	38
92	32	28	30
93	10	17	18
94	31	28	31
95	36	30	36
96	38	33	39
97	118	34	36
98	201	29	30
99	29	30	31
100	65	31	33

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
101	165	35	37
102	35	26	26
103	52	20	22
104	173	37	38
105	44	38	39
106	44	36	39
107	183	36	40
108	144	37	39
109	183	31	33
110	42	19	24
111	53	14	15
112	44	18	26
113	4	11	14
114	32	15	20
115	47	15	18
116	50	13	19
117	67	12	19
118	29	18	22
119	49	21	25
120	4	3	4
121	4	4	5
122	5	6	6
123	5	4	4
124	2	3	4
125	1	2	2
126	2	4	4
127	2	4	5
128	5	4	5
129	2	3	3
130	40	39	46
131	43	31	37
132	40	27	34
133	38	32	37
134	30	28	36

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
135	24	31	35
136	33	29	43
137	38	36	46
138	35	39	44
139	41	31	39
140	12	10	12
141	11	8	14
142	15	15	17
143	12	14	16
144	15	9	10
145	11	15	16
146	11	9	10
147	14	13	15
148	11	11	13
149	15	12	13
150	5	35	42
151	22	33	40
152	21	30	36
153	15	35	40
154	44	34	39
155	36	29	35
156	25	32	37
157	28	30	33
158	35	30	34
159	32	28	32
160	26	41	45
161	34	28	32
162	31	30	35
163	29	30	32
164	21	36	39
165	25	26	31
166	21	38	39
167	24	23	28
168	34	28	30

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
169	24	35	38
170	35	32	42
171	50	25	35
172	32	33	40
173	35	36	44
174	38	37	41
175	29	33	41
176	27	28	35
177	34	28	35
178	40	33	41
179	26	35	45
180	28	38	42
181	28	33	38
182	25	38	41
183	29	32	36
184	33	30	30
185	22	33	39
186	33	60	61
187	31	43	51
188	31	50	55
189	35	48	52
190	37	41	46
191	30	44	48
192	26	46	47
193	29	36	40
194	37	41	46
195	29	37	42
196	31	36	42
197	27	39	44
198	30	34	39
199	22	36	43
200	37	40	43
201	43	38	41
202	36	38	40

Sample number	Enterolert-DW (MPN/100 mL)	ISO 7899-2 (CFU/100 mL)	MoDW 2006, Part 5 (CFU/100 mL)
203	35	39	41
204	15	25	28
205	17	25	26

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