ENTEROLERT-E / QUANTI-TRAY OR QUANTI-TRAY 2000 FOR THE ENUMERATION OF INTESTINAL ENTEROCOCCI.

October 2022

Quantitative method

Summary report

IDEXX Laboratories, Inc

IDEXX Drive, Westbrook

Maine 04 092

USA

FOREWORD

***Alternative method**:*

Enterolert-E / Quanti-Tray or Quanti-Tray 2000

***Validation protocol**:*

Protocol for the validation of an alternative commercial method against a reference method (revision 2 – May 2013).

***Reference method**:*

ISO 7899-1: Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium (1999-03-01).

Scope:

- Bathing waters including
  - Marine waters
  - Fresh waters

Certification body :

AFNOR Certification https://nf-validation.afnor.org/

1. Introduction

The method Enterolert-E / Quanti-Tray or Quanti-Tray 2000 was validated in 2015 and renewed in 2019 by AFNOR Certification according to the Validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013) under the certification number IDX 33/04-02/15.

The validation study was divided in two parts: a comparative study followed by an interlaboratory study. Moreover, complementary assays were also realized in 2014 to also allow the use of a Quanti-Tray instead of a Quanti-Tray 2000 with the method Enterolert-E or Colilert-18 for bathing water analysis. The results of this study are presented in the present report.

In 2022, IDEXX plans to renew the certification with no change in method or extension.

2. Methods protocols

2.1 Alternative method

Enterolert-E detects enterococci, such as *E. faecium* and *E. faecalis*, in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology (DST). When enterococci utilize their ß-glucosidase enzyme to metabolize Enterolert-E's nutrient-indicator, 4-methyl-umbelliferyl ß-D-glucoside, the sample fluoresces.

Enterolert-E detects enterococci at 1 MNP per 100 mL sample within 24 hours.

The alternative method protocol is presented in appendix 1.

2.2 Application scope

The application scope of the alternative method concerns one category of waters: the bathing waters including marine waters and fresh waters.

2.3 Reference method

The alternative method was compared to the standard ISO 7899-1: Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium (1999-03-01).

The protocol of the reference method is presented in appendix 2.

3. Method comparison study

3.1 Relative accuracy

The relative accuracy is defined as the closeness of agreement between test result and the accepted reference value.The relative accuracy is the level of correspondence between the response obtained with the reference method and the response obtained with the alternative method on the same samples.

Number and nature of samples

One category of waters was tested in duplicate with the alternative method and the reference method.

Samples analyzed are presented in table 1.

Category	Water type	Samples analyzed	Samples exploited
Bathing waters	Marine water	16	13
	Fresh water	20	16
	Total	36	29

TABLE 1 : NUMBER AND NATURE OF SAMPLES ANALYZED

A total of 36 samples was analyzed and 29 were exploited. Samples that were not retained in the statistical analysis correspond to samples for which enumerations inferior to 10 CFU/100 mL or superior to the limit of detection were found for at least one of the replicates of the two methods.

One naturally contaminated sample was analyzed. The others were artificially contaminated. The contamination levels used cover all the measuring range of the alternative method. The stress applied and the strains used are presented in appendix 3.

Raw results

Raw results and statistical calculations are summarized in tables 2 and 3 and in appendix 4.

Figure 1 shows the two-dimensional graph for the test category. The y-axis is reserved for the alternative method and the x- axis for the reference method. The representation of a line of equation “y = x“ figures dashed on the figures.

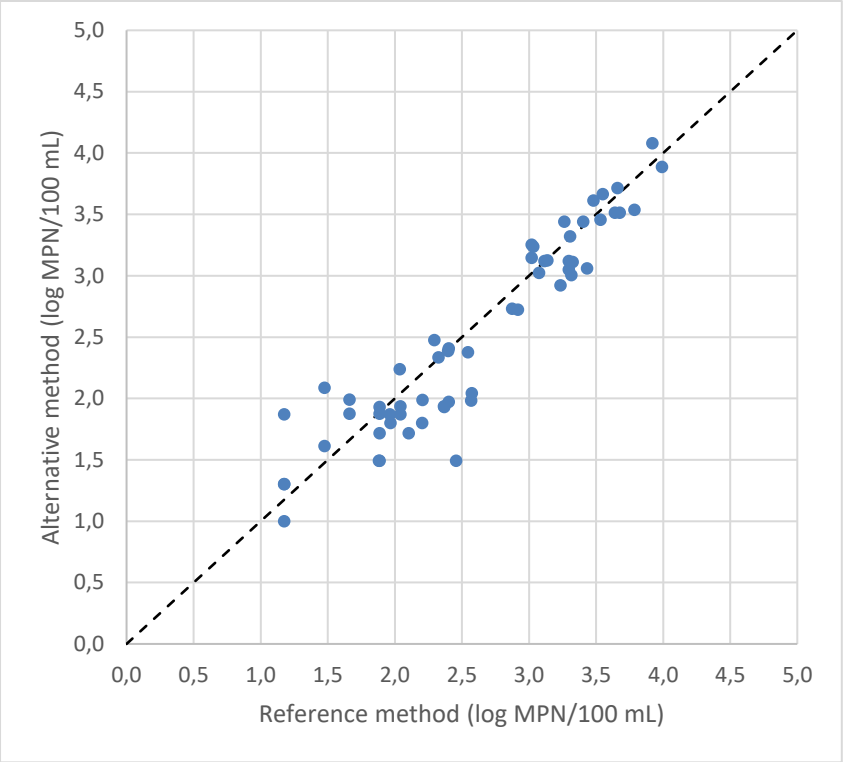


FIGURE 1 : TWO-DIMENSIONAL GRAPH FOR RELATIVE ACCURACY (BLACK LINE: Y=X)

Statistical analysis

The relationship of relative accuracy between the reference method and the alternative method is evaluated with the linear model: 'y = a + bx '. This formula corresponds to the equation of the linear regression drawn from raw results obtained by experimentation, y representing the alternative method and x the reference method.

There is a perfect accuracy (or there is no systematic bias) between the two methods if this equation is equal to the theoretical 'y = x' equation, which applies in the ideal model where the two methods behave similarly.

The intercept is theoretically zero in this ideal model (hypothesis [a = 0]). The estimated intercept obtained with the two methods is checked using p {a = 0}. If the alternative method is a systematic bias against the reference method, the probability p {a = 0} is less than $\alpha = 0.05$.

The 'b' slope is theoretically equal to 1 in the ideal model (hypothesis [b = 1]). The estimated slope obtained with the two methods should pass by p {b = 1}. Statistically, if the alternative method does not give the same values as the reference method, the probability p {b = 1} is less than $\alpha = 0.05$.

The linear regression method is chosen over the value of the robustness of the ratio R of overall repeatability standard deviation:

- If Rob.R > 2, an ordinary least-squares regression (OLS 1) is used with the x-axis for the reference method,
- If Rob.R < 0.5, an ordinary least-squares regression (OLS 2) is used with the x-axis for the alternative method,

- If 0.5 < Rob.R < 2, orthogonal regression (GMFR) is used with the x-axis to the reference method.

Rob.R	Regression used	T critical	a	t(a)	b	t(b)	Probabilities (%)	
							Intercept at 0	Slope at 1
0,809	GMFR	2,045	-0,110	0,715	1,008	0,184	47,7	85,5

TABLE 2 : STATISTICAL DATA FOR THE ENUMERATION OF INTESTINAL ENTEROCOCCI IN BATHING WATERS

Bias (D)		Repeatability			
Average	Median	r		rob. r	
		RM	AM	RM	AM
-0,088	-0,055	0,437	0,430	0,302	0,245

TABLE 3 : BIAS AND REPEATABILITY OF THE TWO METHODS

Conclusion

The equation for the regression line of the couple "enterococci - bathing water" is as follows:

log Alt = 1,008 log Ref – 0,110

Hypothesis [a = 0 and b = 1] is accepted for the test category. Bias between the two methods is -0,055 log MPN/100 mL.

The relative accuracy of the alternative method is **satisfactory**.

3.2 Linearity

The linearity is the ability of the method when used with a given matrix to give results that are in proportion to the amount of analyte present in the sample, that is an increase in analyte corresponds to a linear or proportional increase in results.

Matrix used and contamination protocol

The couple matrix / strain is presented in table 4. For this couple, six levels of contamination were tested in duplicate by the reference method and the alternative method.

Strain	Matrix	Contamination level (CFU/100 mL)
Enterococcus faecalis	Marine water	50 - 200 - 500 - 1 000 - 5 000 - 20 000

TABLE 4 : COUPLE MATRIX – STRAIN ANALYZED

Raw results

The Raw results and statistical calculations are summarized in appendix 5. Graph of figure 2 show the values of each sample obtained by the alternative method and the reference method. The y-axis is reserved for the alternative method and the x-axis for the reference method.

The representation of a line of equation 'y = x' figures dashed on the figures.

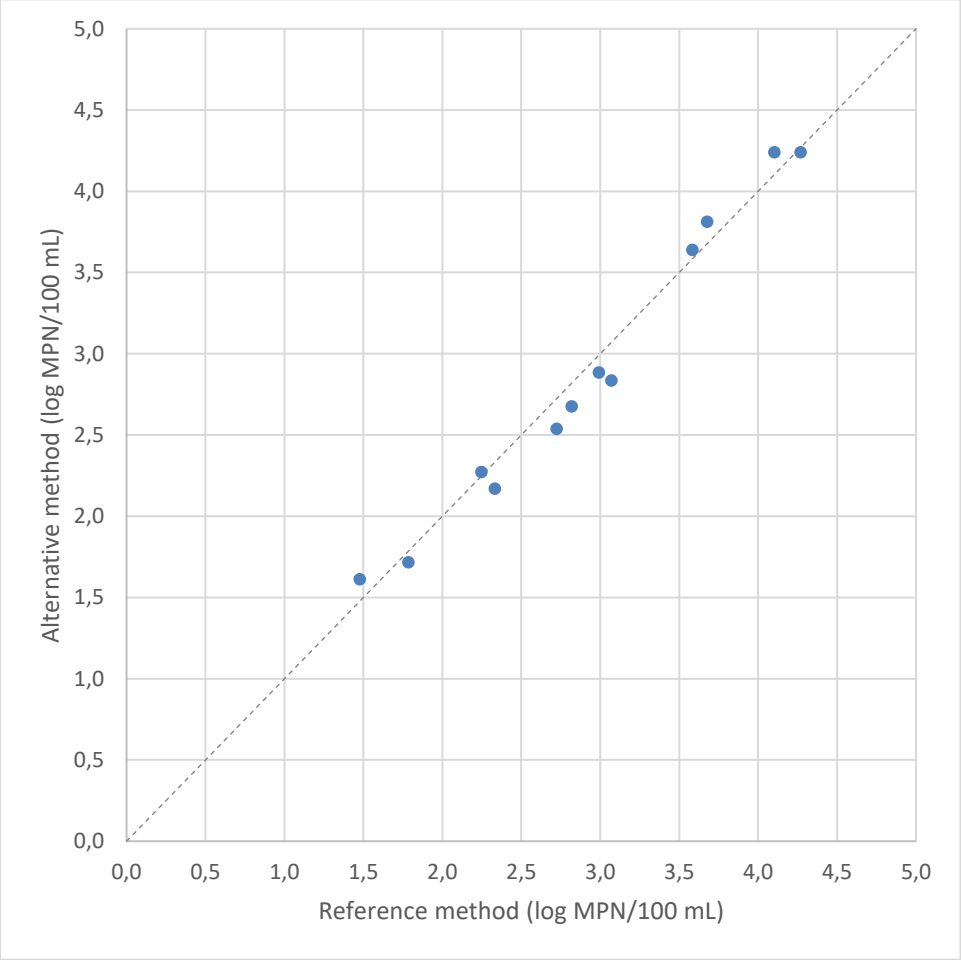


FIGURE 2 : TWO-DIMENSIONAL GRAPH FOR LINEARITY (BLACK LINE: Y=X)

Statistical exploitation

Statistical interpretations are carried out in accordance with the requirements of standard NF ISO 16140 (see table 5).

The linear regression method is chosen over the value of the robustness of the ratio R of overall repeatability standard deviation:

- If Rob.R > 2, an ordinary least-squares regression (OLS 1) is used with the x-axis for the reference method,

- If Rob.R < 0.5, an ordinary least-squares regression (OLS 2) is used with the x-axis for the alternative method,
- If 0.5 < Rob.R < 2, orthogonal regression (GMFR) is used with the x-axis to the reference method.

Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	Correlation coefficient (r)	Regression line
1,083	GMFR	4,53	4,953	0,041	0,993	1,038 log Ref – 0,149

TABLE 5 : STATISTICAL DATA OF THE COUPLE MATRIX – STRAIN ANALYZED

The relationship between the 2 methods is not linear:

- If Rob.F > critical F or,
- If P (Rob.F) < α (= 0,05)

Conclusion

The relationship between the two methods is not linear. However, the correlation coefficient of the couple and the equation for the regression line are satisfactory.

The linearity of the alternative method is **satisfactory.**

3.3 Detection and quantification limits

The detection and quantification limits are checked in accordance with the standard EN ISO 16140. Three parameters are determined.
Here are their ISO 16140 definitions:

- the critical level (LC) is the smallest amount which can be detected (not null), but not quantified as an exact value. Below this value, it cannot be sure that the true value is not null. At this level, the false negatives probability β is 50 % (β is the second type of statistical error).
- the detection limit (LOD) is higher than the critical level, because it involves a power, the probability 1 - β, which has to be well over 50 %, for example 95 %.
- the quantification limit (LOQ) is the smallest amount of analyte, (that is the lowest actual number of organisms), which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation.

Protocol

Detection and quantification limits were determined by analyzing a pure culture of an *Enterococcus faecalis* strain ENTC.1.5, isolated from a surface water, by the alternative method. Eight levels of contamination, with six repetitions for each level, have been studied in a sterilized water.

Results

Raw results are presented in appendix 6 and the summary in the following tables.

Level (CFU/100 mL)	Number of positive samples	Standard deviation (s_0)	Bias (x_0)
0	0 / 6	0	0
3	2 / 6	5,164	0
6	2 / 6	5,164	0
9	2 / 6	5,164	0
10	4 / 6	5,164	10
12	5 / 6	7,528	10
14	5 / 6	10,778	15
18	6 / 6	8,495	15

TABLE 6 : DATA (S_0 AND X_0) FOR THE ENUMERATION OF INTESTINAL ENTEROCOCCI (UNDERLINED: THE REFERENCE LEVEL)

Parameter	Formulas	Value
Critical level	1,65 So +Xo	18,5 MPN / 100 mL
Detection limit	3,3 So+Xo	27,0 MPN / 100 mL
Quantification limit	10 So + Xo	61,6 MPN / 100 mL

TABLE 7 : LC, LOD AND LOQ VALUES OF THE ALTERNATIVE METHOD FOR THE ENUMERATION OF INTESTINAL ENTEROCOCCI

Conclusion

The limit of detection and limit of quantification of the alternative method are **satisfactory**.

3.4 Selectivity

The selectivity of the alternative method is evaluated by its inclusivity and its exclusivity.

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.

Exclusivity is the lack of interference by a relevant range of non-target strains with the alternative method.

Protocol

Thirty target strains and thirty non target strains (from national, international and internal collections) were analyzed. The tests were conducted according to the protocol of the alternative method.

The contamination levels used for inclusivity were between 30 and 100 CFU / 100mL and for exclusivity 10^3 to 10^5 times higher than the level of detection of the alternative method (approximately 10^4 CFU / 100 mL).

Results

The results are presented in appendix 7.
Thirty strains of enterococci tested are detected by the alternative method. No non target strain showed cross-reaction with the alternative method.

Conclusion

The selectivity of the method is **satisfactory**.

3.5 Practicability

- Procedure for conditioning the elements of the method

Enterolert-E reagent is packaged in sealed individual capsules. TheQuanti-Tray and Quanti-Tray 2000 are conditioned by 10 in sterile plastic bags.

- Reagent volume

Several formats are available (20 tests, 100 tests or 200 tests).

- Conditions of storage of the elements (expiry date for unopened products)

Enterolert-E storage temperature is between 2 and 25 ° C. The storage temperature of the Quanti - Tray 2000 is between 4 and 30 ° C. Products have a 12 months DLC.

- Modalities of use after the first use (expiry dates for use)

Each Quanti-Tray and each Enterolert-E capsule serves a unique analysis and must not be re-used.

- Specific equipment or premises required

A Quanti-Tray Sealer model 2X is required.

- Reagents ready-to-use or to be reconstituted

There is no reagent to restore.

- Period required to train an operator not initiated into the method

The use of the method Enterolert-E / Quanti-Tray 2000 requires no specific training. The duration of training is estimated at 1 hour.

- Real-time handling and flexibility of the method depending on the number of samples to be analyzed.

The duration of an analysis by the method NF EN ISO 7899-1 is about 1.5 min using disposable filtration units of 3.5 min using non-disposable filtration units. The duration of use of the method Enterolert-E / Quanti-Tray or Quanti-Tray 2000 is about 2 min (time including: dissolution of the Enterolert-E waiting time and the time for sealing the Quanti-Tray). Neither the alternative method nor the reference method require a confirmation step.

- Time required for obtaining the results

Time-to-result for the method Enterolert-E / Quanti-Tray or Quanti-Tray 2000 is 24 - 28 hours.
Time-to-result for the method EN ISO 7899-1 is 36 – 72 hours.

❖ Operator qualification type

The qualification of the operator is similar to the qualification needed for the reference method.

❖ Phases shared with the reference method

None.

❖ Means or traceability of the analysis results for the user

No traceability procedure is proposed. The laboratory shall use its internal procedures.

❖ Obligation to maintain specific apparatus for the user

None.

3.6 Conclusion

The linearity and the relative accuracy of the method Enterolert-E / Quanti-Tray 2000 for the enumeration of intestinal enterococci in bathing waters are satisfactory.

Bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.

The method Enterolert-E / Quanti-Tray 2000 for the enumeration of intestinal enterococci is specific and selective.

Results are obtained in 24 to 28 hours with the alternative method against 36 to 72 hours with the reference method.

4. Interlaboratory study

4.1 Interlaboratory study implementation

Participating laboratories

The interlaboratory study was realized by the expert laboratory and fourteen participating laboratories.

Each laboratory received the instructions relative to the organization of the study a week before its beginning.

Matrix and strain used

A marine water was used as test matrix. It was contaminated with a strain of *Enterococcus faecalis* (ENTC.1.6) isolated from a surface water.

The absence of enterococci in this matrix before the contamination was checked using the reference method.

Stability of the strain in the test matrix

The stability of the strain in the matrix was evaluated for 3 days at 5±3°C. Results of the enumerations are presented in table 8.

	Level 1	Level 2	Level 3
D0	77	1 007	4 368
D1	46	858	2 536
D2	30	606	2 140

TABLE 8 : RESULTS OF THE ENUMERATIONS IN CFU/100 ML OF THE STRAIN ENTEROCOCCUS FAECALIS ENTC.1.6 IN MARINE WATER FOR 3 DAYS AT 5±3°C (*)

A diminution of the concentration of the tested strain is observed from day 0 to day 2 at 5±3°C in the matrix

Inoculation

The matrix was inoculated with the target strain suspension to obtain 4 contamination levels:

- level 0 : 0 CFU/100 mL,
- level 1 : from 15 to 50 CFU/100 mL,
- level 2 : from 250 to 500 CFU/100 mL,
- level 3 : from 1000 to 1500 CFU/100 mL.

The matrix was distributed at 50 mL in sterile bottles. Every bottle was individually spiked and homogenized. Eight samples per laboratory were prepared (2 samples per contamination level).

Each laboratory received 8 samples to analyze, 1 sample to quantify culturable microorganisms and 1 water sample containing a temperature probe.

The results of the enumerations of culturable microorganisms, the target levels and the real levels of contamination are presented in table 9.

Level	Culturable microorganisms (CFU/mL)		*Enterococcus faecalis* ENTC.1.10 (CFU /100 mL)	
	22°C	36°C	Target level	Real level at D0
0	142	66	/	/
1			75	70
2			800	760
3			4 000	3 400

TABLE 9 : TARGET LEVEL, REAL LEVEL AND ENDOGENOUS FLORA OF THE MATRIX

Samples labelling

The labeling of the vials was realized as follows: a code to identify the laboratory: from A to N (cf. table 10) and a code to identify each sample, only known by the expert laboratory.

The samples and the temperature control vials (water sample with a temperature probe) were stored at 5°C before shipping.

Level (CFU / 100 mL)	Sample code
0	1 / 4
15 to 50	5 / 7
250 to 500	2 / 6
1000 to 1500	3 / 8

TABLE 10 : SAMPLE CODE BY CONTAMINATION LEVEL.

Elements Samples shipping, reception and analysis

The samples were shipped in a coolbox the 8th of December 2014.

The coolboxes were received in 24 hours for twelve laboratories and in 48 hours for one laboratory. Laboratory I received the samples in 72 hours. This lab did not thus participate in the study.

The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory.

The samples were analyzed the 10th of December. The expert laboratory concurrently analyzed a set of samples under the same conditions with both methods.

Analyses were thus realized by thirteen laboratories.

4.2 Results

Temperature and state of the samples at reception

The temperature readings at reception, the state of the samples and probes data are shown in table 11.

Laboratory	Temperature	State of the samples	Probe temperature	
			Mean	SD
A	10,4°C	Correct	3,0°C	0,6°C
B	2,6°C	Correct	0,2°C	0,3°C
C	5,8°C	Correct	2,6°C	0,8°C
D	8,5°C	Correct	1,4°C	0,7°C
E	4,0°C	Correct	0,6°C	0,3°C
F	3,8°C	Correct	-1,5°C	2,1°C
G	6,1°C	Correct	2,2°C	0,8°C
H	2,4°C	Correct	2,4°C	1,0°C
J	4,8°C	Correct	3,3°C	1,0°C
K	4,6°C	Correct	1,9°C	1,4°C
L	6,3°C	Correct	1,9°C	1,0°C
M	6,6°C	Correct	1,5°C	0,6°C
N	5,4°C	Correct	1,6°C	0,9°C

TABLE 11 : TEMPERATURE AND STATE OF THE SAMPLES UPON RECEPTION

Temperatures are correct for 10 laboratories.

Laboratories A and D showed temperatures superior to 8°C. The analyses of the thermal profiles of the probes showed that the shipping of the samples were realized at a correct temperature, with means between 1,4°C and 3,0°C.

Results from expert laboratory and participating laboratories

The overall results are presented in table 12. Detailed results are provided in appendix 8.

For level 0, all results of the reference method were inferior to 15 MPN/100 mL and all results of the alternative method were inferior to 10 MPN/100 mL.

Laboratory	Level 1				Level 2				Level 3			
	RM		AM		RM		AM		RM		AM	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
A	46	61	20	52	648	495	282	341	838	2194	857	862
B	109	77	52	41	640	461	384	327	2759	2182	1354	2755
C	126	15	20	20	594	485	512	441	2604	2140	2247	2014
D	77	46	75	31	509	534	395	495	1797	1927	2098	1500
E	61	110	63	52	612	712	246	441	2873	2792	1483	1467
F	61	46	121	20	390	585	563	650	1599	2536	1553	1722
G	94	15	31	52	559	697	727	512	2023	2206	3255	2282
H	<15	15	75	31	476	606	465	605	1716	2087	2359	1850
J	77	46	41	41	718	621	644	691	1929	2079	2489	2187
K	94	30	52	41	383	485	602	345	1984	1838	1850	1145
L	61	46	63	52	489	480	480	529	2130	1567	2014	2014
M	77	46	31	20	485	485	241	278	1494	1523	1187	1010
N	46	61	52	63	600	627	399	305	2640	2444	2098	712
Expert	46	61	86	52	559	627	556	464	2263	2249	2613	1918

TABLE 12 : RESULTS OF THE INTERLABORATORY STUDY (MPN/100 ML)

Laboratory H found one replicate of the level 1 inferior to 15 MPN/100 mL with the reference method. Consequently this lab was not included in the statistical analysis of the data, because of the impossibility of the transformation of this result in logarithm.

The data obtained by the twelve remaining laboratories are presented in the two dimensional graph of the figure 3 in log MPN/100 mL for a better appreciation of the data (y = x in dotted line).

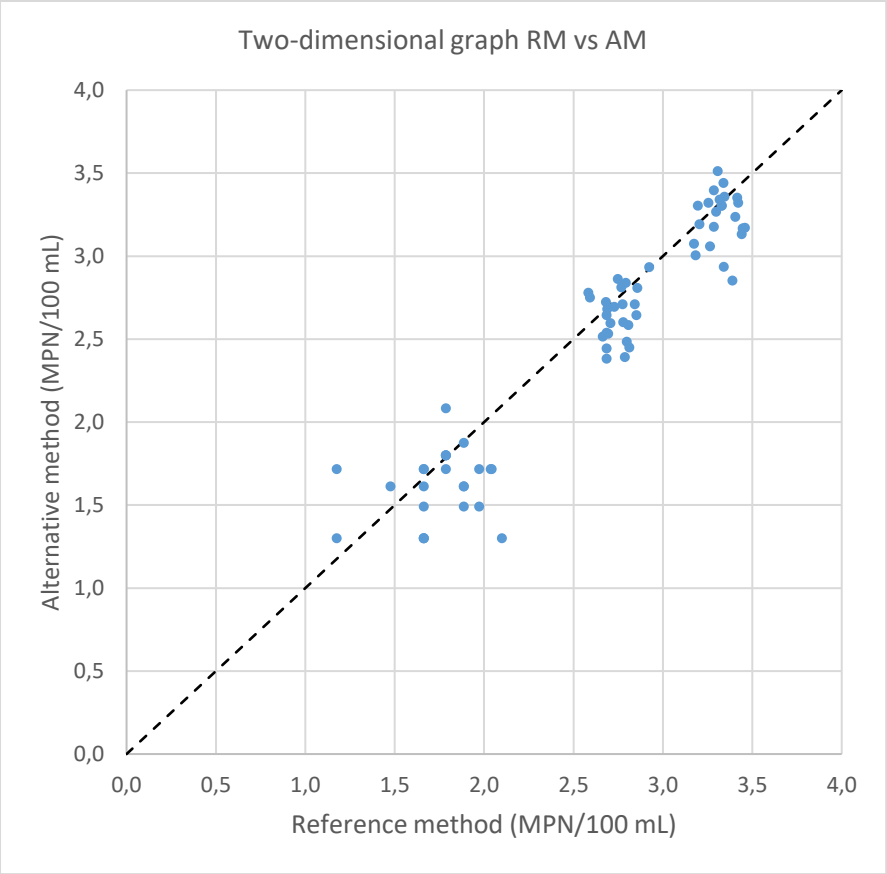


FIGURE 3 : TWO-DIMENSIONAL GRAPH (MPN/100 ML)

Enumerations of culturable microorganisms

For the whole laboratories, the enumerations at 22°C vary between 27 and 190 CFU/mL. Concerning the enumerations at 36°C, the results were varying between 24 and 180 CFU/mL.
The results of each lab are shown in appendix 9.

4.3 Statistical interpretation

Bias

Table 13 presents the target value, the mean, and the bias for each level of contamination.

Values	log MPN/100 mL		
Levels	**1 - low**	**2 - medium**	**3 - high**
Target value	1,785	2,737	3,323
Mean	1,617	2,632	3,215
Relative bias	-9,45%	-3,84%	-3,26%
Bias	-0,169	-0,105	-0,108

TABLE 13 : CALCULATIONS OF THE BIAS OF THE ALTERNATIVE METHOD

The accuracy is estimated by the bias which varies between -0,169 log MPN/100 mL and -0,105 log MPN/100 mL.

The bias obtained during the comparative study was -0,055 log MPN/100 mL.

Accuracy profile

Table 14 shows the tolerance values and limits of the alternative method for the different values of probability of tolerance and the limits of acceptability.

Data are presented in log MPN/100 mL

Tolerance probability	Parameters	log MPN/100 mL		
		Low	**Medium**	**High**
90%	Low tolerance value	-0,536	-0,366	-0,414
	High tolerance value	0,199	0,156	0,197
	Low tolerance limit	**-0,600**	**-0,600**	**-0,600**
	High tolerance limit	**0,600**	**0,600**	**0,600**
80%	Low tolerance value	-0,452	-0,305	-0,343
	High tolerance value	0,114	0,095	0,126
	Low tolerance limit	**-0,500**	**-0,500**	**-0,500**
	High tolerance limit	**0,500**	**0,500**	**0,500**

TABLE 14 : TOLERANCE VALUES FOR THE ALTERNATIVE METHOD

Figures 4 and 5 present the accuracy profiles.

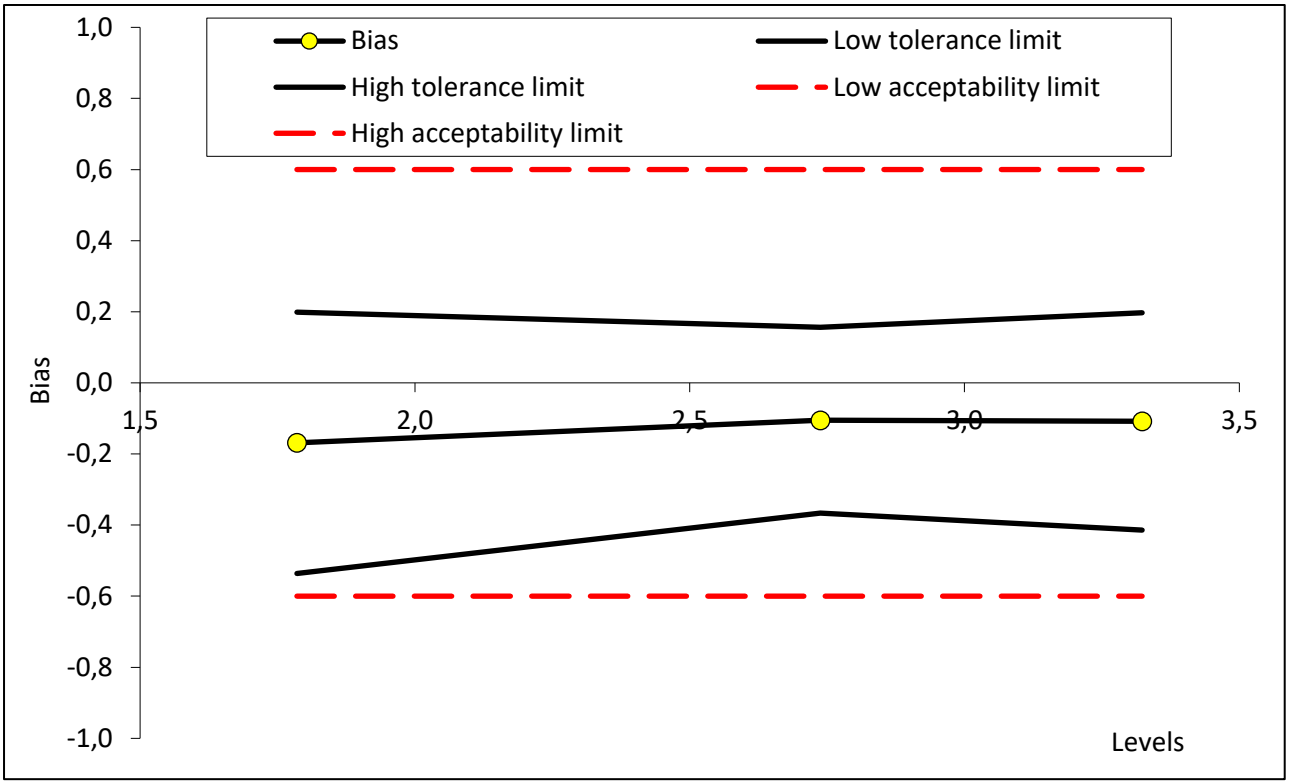


FIGURE 4 : ACCURACY PROFILE FOR A TOLERANCE PROBABILITY AT 90% AND ACCEPTABILITY LIMITS AT 0,6 LOG MPN/100 ML

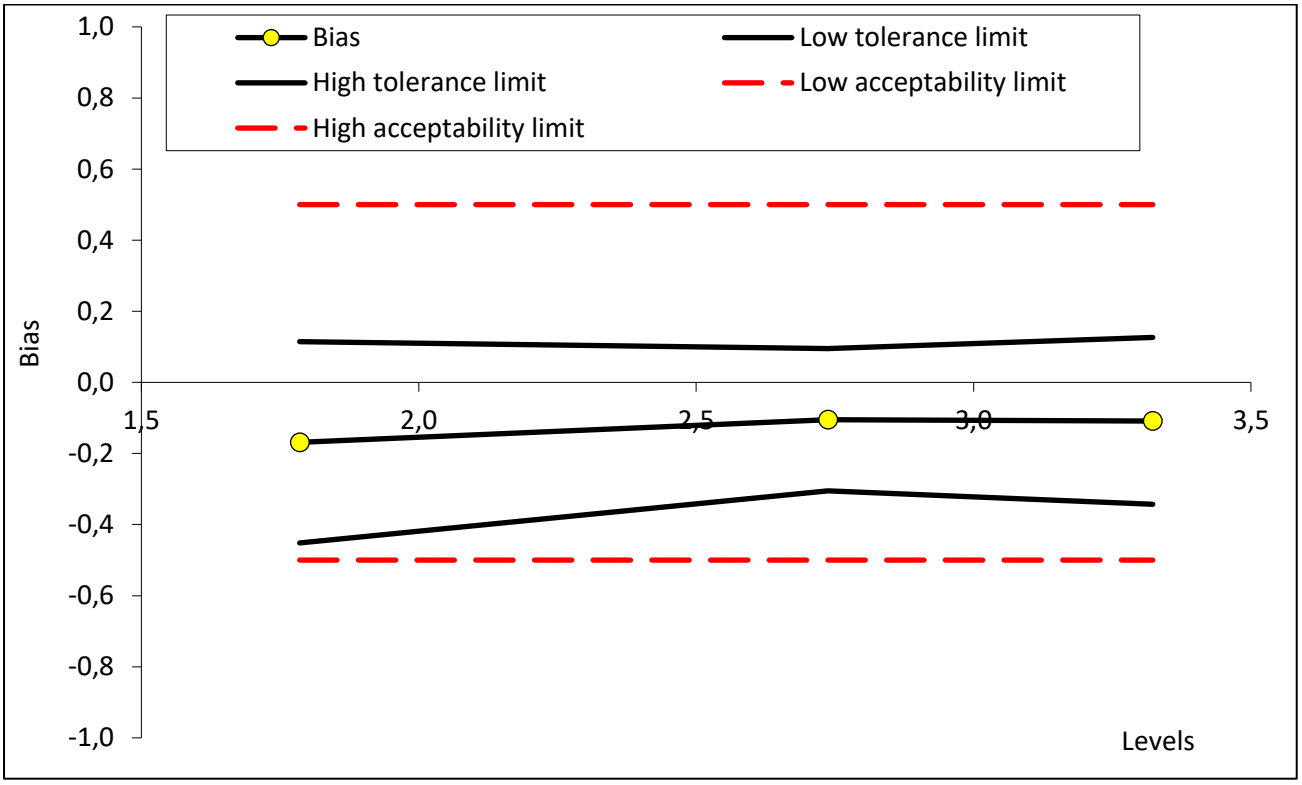


FIGURE 5 : ACCURACY PROFILE FOR A TOLERANCE PROBABILITY AT 80% AND ACCEPTABILITY LIMITS AT 0,5 LOG MPN/100 ML

Comments : For all the contamination levels, the tolerance interval is comprised between the acceptability interval for a 90% tolerance probability and a limit at 0,6 log MPN/100 mL or for a 80% tolerance probability and a limit at 0,5 log MPN/100 mL.

Conclusion

The bias of the alternative method is slightly negative but relatively stable from the low level of contamination to the high level of contamination.

For all levels of contamination, the tolerance limits are between the limits of acceptability, meaning that: at least 90% of the results will be between the limits of acceptability as defined at 0,6 log MPN/100 mL, or at least 80% of the results will be between the limits of acceptability as defined at 0,5 log MPN/100 mL.

5. Complementary assays

The aim of the complementary assays are to compare the results obtained with Enterolert-E or Colilert-18 with the use of a Quanti-Tray 2000 or the use of a Quanti-Tray, in order to allow the use of both devices in the framework of a certification NF Validation concerning each IDEXX kit using a Quanti-Tray or a Quanti-Tray 2000.

5.1 Results and interpretation

Two sets of results are available:

- ISHA data from the comparative study for the NF Validation certification of the method Enterolert-E with Quanti-Tray 2000,
- IDEXX data from an analysis of a tap water using Colilert-18 associated with Quanti-Tray 2000 and with Quanti-Tray.

Results from Enterolert-E / Quanti-Tray 2000 compartive study

Raw results

Results have been collected from samples used in the comparative study for the validation of the method Enterolert-E in the common enumeration range of the two devices, namely from 10 to 2000 MPN/100 mL. A minimum of 10 results was asked by the Technical Board: it's a total of 18 samples that have been taken into account.

Results are in appendix 10. A two-dimensional graph is shown in figure 6, presenting the results obtained with the Quanti-Tray 2000 (the "validated" Quanti-Tray for the Enterolert-E method) as the reference method.

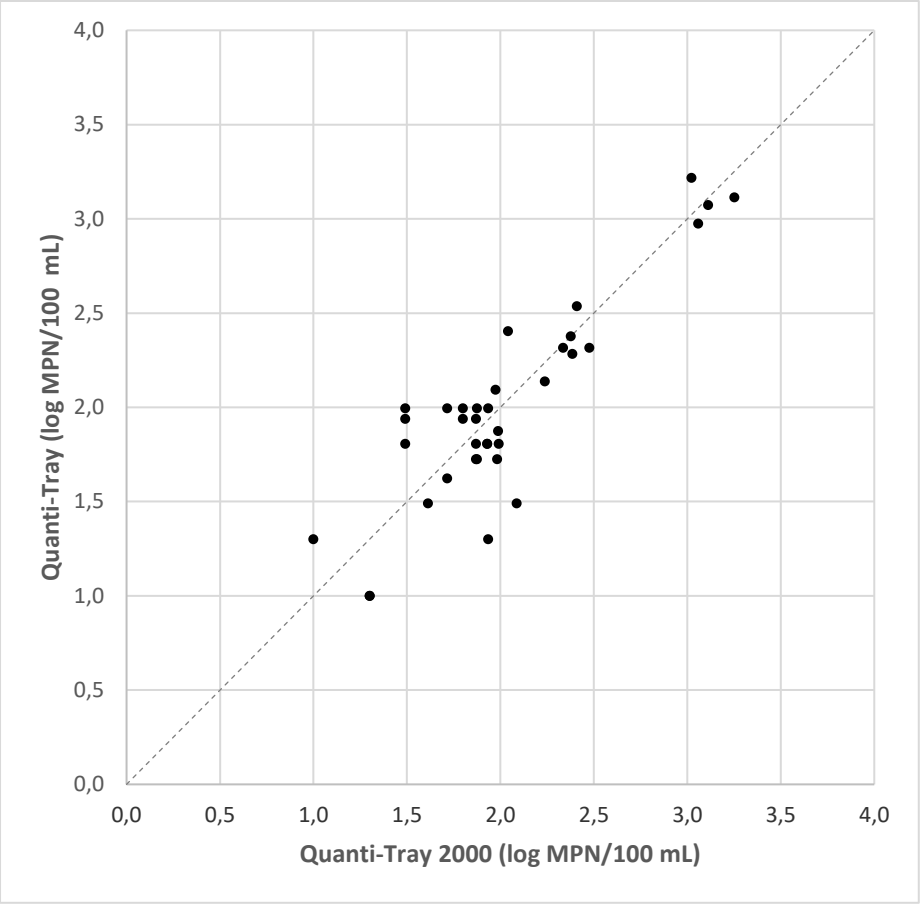


FIGURE 6 : COMPARISON OF RESULTS OBTAINED WITH QUANTI-TRAY 2000 AND WITH QUANTI-TRAY FOR THE VALIDATION OF THE ENTEROLERT-E METHOD

Statistical interpretation

A statistical interpretation has been performed according to the requirements of the Validation protocol for an alternative commercial method as compared with a reference method, considering the Quanti-Tray 2000 as the reference device and using the tests for the relative accuracy. Results are shown in appendix 10.

According to this protocol, the relationship of relative accuracy between QT-2000 and QT is evaluated with the linear model: 'y = a + bx'. This formula corresponds to the equation of the linear regression drawn from raw results obtained by experimentation, y representing the QT-2000 devices and x the QT-devices.

There is a perfect accuracy (or there is no systematic bias) between the two methods if this equation is equal to the theoretical 'y = x' equation, which applies in the ideal model where the two methods behave similarly.

The intercept is theoretically zero in this ideal model (hypothesis [a = 0]). The estimated intercept obtained with the two methods is checked using p {a = 0}. If the alternative method is a systematic bias against the reference method, the probability p {a = 0} is less than $\alpha = 0.05$.

The 'b' slope is theoretically equal to 1 in the ideal model (hypothesis [b = 1]). The estimated slope obtained with the two methods should pass by p {b = 1}. Statistically, if the alternative method does not give the same values as the reference method, the probability p {b = 1} is less than α = 0.05.

The results of the statistical tests are shown in the table 15.

Rob.R	Regression used	T critical	a	t(a)	b	t(b)	Probabilities (%)	
							Intercept at 0	Slope at 1
1,416	GMFR	2,101	-0,097	0,460	1,040	0,523	64,8	60,4

TABLE 15 : STATISTICAL DATA FOR THE COMPARISON QT 2000-QT

The equation for the regression line is as follows: log Alt = 1,040 log Ref – 0,097

Hypothesis [a = 0 and b = 1] is accepted for the comparison of the enumeration of enterococci with the Enterolert-E method using a Quanti-Tray versus a Quanti-Tray 2000.

Student-Fisher test

A Student-Fisher test has been also performed from the data obtained during the validation of the Enterolert-E method.

The results of the test are shown in the table below:

t-Test: Paired Two Sample for Means		
Parameter	***Quanti-Tray***	***Quanti-Tray 2000***
Mean	1,998	2,015
Variance	0,280	0,259
Observations	36	36
Pearson Correlation	0,883	
Hypothesized Mean Difference	0	
df	35	
t Stat	-0,398	
P(T<=t) one-tail	0,346	
t Critical one-tail	1,690	
P(T<=t) two-tail	0,693	
t Critical two-tail	2,030	

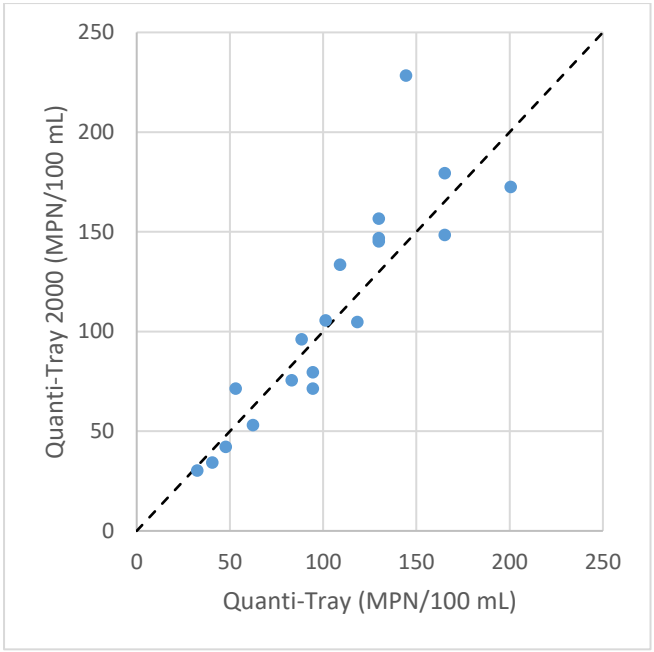
Both one-tailed and two-tailed tests conclude that there is no statistically significant difference between the enumeration of enterococci with Quanti-Tray or with Quanti-Tray 2000 at α=0,05.

Results from Colilert-18 / Quanti-Tray study

Raw results

Results were obtained from IDEXX Company. An *Escherichia coli* suspension was spiked in a neutralized tap water from 30 to 180 CFU/100 mL and then analyzed with Colilert-18 associated with Quanti-Tray and with Quanti-Tray 2000.

Results are shown in appendix 10. Two two-dimensional graphs are shown in figure 7, presenting the results obtained with the Quanti-Tray (the "validated" Quanti-Tray for the Colilert-18 method in drinking waters) as the reference method.



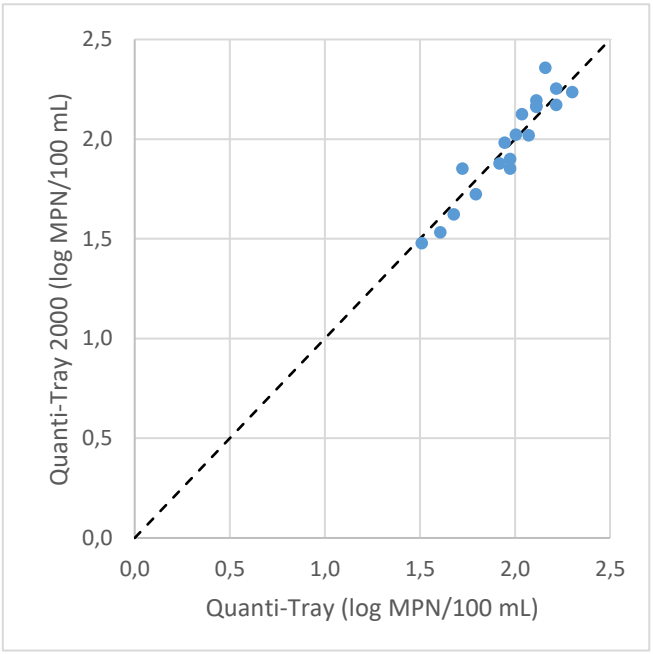


FIGURE 7 : COMPARISON OF RESULTS OBTAINED WITH QUANTI-TRAY 2000 AND WITH QUANTI-TRAY FOR THE ENUMERATION OF ESCHERICHIA COLI IN TAP WATER

Statistical interpretation

A Student-Fisher test has been performed from the data obtained. The results are shown in the table below.

t-Test: Paired Two Sample for Means		
Parameter	***Quanti-Tray***	***Quanti-Tray 2000***
Mean	104,8	109,1
Variance	2119,6	3043,9
Observations	19	19
Pearson Correlation	0,892	
Hypothesized Mean Difference	0	
df	18	
t Stat	-0,745	
P(T<=t) one-tail	0,233	
t Critical one-tail	1,734	
P(T<=t) two-tail	0,466	
t Critical two-tail	2,101	

Both one-tailed and two-tailed tests conclude that there is no statistically significant difference between the enumeration of *Escherichia coli* with Quanti-Tray or with Quanti-Tray 2000 at α=0,05.

5.2 Conclusion

The assays realized showed that the enumerations with the NF Validation certified IDEXX methods can be performed either with a Quanti-Tray device or with a Quanti-Tray 2000 device according to the expected concentration of the target analyte in the sample without introducing any bias in the measurement.

6. Bibliography study

No publication concerning the method was recorded since the last renewal.

7. Assessment of the complaints

No complaint from the users of the alternative method was received by AFNOR Certification.

8. State of the intervened modifications and the envisaged modifications

8.1 Technical repository of validation

No modification since the last renewal on the technical repository: “the protocol of validation of an alternative commercial method as compared to a reference method - May 2013”.

8.2 Reference method

No modification was brought to the reference method, the reference method NF EN ISO ISO 7899-1: Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium (1999-03-01).

8.3 Alternative method

No modification was brought to the alternative method since the initial validation.

9. Presentation of the possible modifications envisaged in the alternative method

No modification is planned.

10. Conclusion

The validation study for the AFNOR Certification validation of the method Enterolert-E for the enumeration of enterococci in bathing waters was realized in three steps in 2014 and 2015:

- a comparative study to compare the performance of the Enterolert-E method to the reference method ISO 7899-1, based on the determination of several parameters (linearity, relative accuracy, limits of detection and quantification and selectivity),
- complementary assays to validate the use of Quanti-Tray and Quanti-Tray 2000 with the method,
- an interlaboratory study to evaluate the performance of the method in several laboratories under real conditions that represent its routine application.

Concerning the comparative study, the linearity and the relative accuracy of the method Enterolert-E / Quanti-Tray 2000 for the enumeration of intestinal enterococci in bathing waters are satisfactory.

Bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.

The method Enterolert-E / Quanti-Tray 2000 for the enumeration of intestinal enterococci is specific and selective.

Results are obtained in 24 to 28 hours with the alternative method against 36 to 72 hours with the reference method.

Complementary assays showed that the enumerations with the NF Validation certified IDEXX methods can be performed either with a Quanti-Tray device or with a Quanti-Tray 2000 device according to the expected concentration of the target analyte in the sample without introducing any bias in the measurement.

The interlaboratory study showed that the bias of the alternative method is slightly negative but relatively stable from the low level of contamination to the high level of contamination.

For all levels of contamination, the tolerance limits are between the limits of acceptability, meaning that: at least 90% of the results will be between the limits of acceptability as defined at 0,6 log MPN/100 mL, or at least 80% of the results will be between the limits of acceptability as defined at 0,5 log MPN/100 mL.

Done at Thury-Harcourt, October 4, 2022

Mickaël MORVAN

Research & Development Engineer

APPENDIX 1 - PROTOCOL FOR THE ENTEROLERT-E / QUANTI-TRAY OR QUANTI-TRAY 2000

Step 1

Fill a test vial with 90 mL of sterile distilled water

Add the contents of one Enterolert-E snap pack to the test vessel, cap vessel and shake until reagent is dissolved

Mix the sample of water thoroughly and transfer 10 ml to the test vessel, cap vessel and shake well

Pour the contents of the test vessel into a Quanti-Tray or a Quanti-Tray 2000 and seal in a Quanti-Tray Sealer

Step 2

Place the sealed tray in a 41°C (+/- 0.5°C) incubator for 24–28 hours.

Step 3

Look for fluorescence with a 6-watt, 365 nm, UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.

Step 4

Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Appearance	Result
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

Step 5

To obtain the correct quantitative result, refer to the MPN table provided with the trays. Multiply this value by the dilution factor of 10.

Detection and enumeration of intestinal enterococci in surface and waste water

Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium

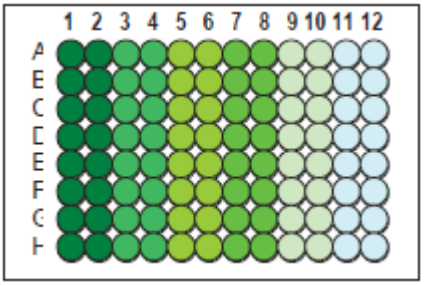
9 mL of sample

⬇

Preparation of the dilution range
6 dilutions in synthetic sea salt diluent
16 wells per dilution (from 1/2 to 1/200 000)

⬇

Inoculation
200 µL per well containing the MUD medium



⬇

Incubation 36 to 72 hours at 44±0,5°C

⬇

Enumeration of the positive wells (fluorescent) by UV reading

⬇

Expression of the results: MPN / 100 mL of intestinal enterococci

APPENDIX 3 – BACTERIAL STRESS

Code	Strain	Origin	Stress applied	Stress intensity
ENTC.1.5	*E. faecalis*	River water	3 hypochlorite treatments + 20 min at 56°C	0,6
ENTC.1.8	*E. faecalis*	River water	3 hypochlorite treatments + 20 min at 56°C	0,8
ENTC.1.11	*E. faecalis*	River water	3 hypochlorite treatments + 8 days at 5°C + 20 min at 56°C	0,7
ENTC.6.2	*E. durans*	River water	3 hypochlorite treatments + 8 days at 5°C + 20 min at 56°C	1,2
ENTC.1.6	*E. faecalis*	River water	1 cycle freezing-defrosting + 6 days at 5°C + 3 hypochlorite treatments	0,6
ENTC.1.9	*E. faecalis*	River water	1 cycle freezing-defrosting + 6 days at 5°C + 3 hypochlorite treatments	0,8
ENTC.2.5	*E. faecium*	River water	1 cycle freezing-defrosting + 6 days at 5°C + 3 hypochlorite treatments	0,5
ENTC.7.1	*E. casseliflavus*	River water	1 cycle freezing-defrosting + 6 days at 5°C + 3 hypochlorite treatments	0,9
ENTC.7.3	*E. casseliflavus*	River water	3 cycles freezing-defrosting + 6 days at 5°C + sodium hypochlorite	1,1
ENTC.2.8	*E. faecium*	River water	3 cycles freezing-defrosting	0,7
ENTC.5.3	*E. gallinarum*	Waste water	3 cycles freezing-defrosting + 6 days at 5°C	1,2
ENTC.2.9	*E. faecium*	River water	3 cycles freezing-defrosting + 6 days at 5°C	0,8
ENTC.3.2	*E. hirae*	River water	3 cycles freezing-defrosting + 6 days at 5°C	0,9
ENTC.4.1	*E. avium*	Water	3 cycles freezing-defrosting + 6 days at 5°C + sodium hypochlorite	1,0

APPENDIX 4 – RELATIVE ACCURACY RESULTS

Total raw results

N°	Water type	Sample	Strain	Contamination level (CFU/100 mL)	Enterolert-E										Reference method 7899-1					
					Quanti-tray				Quanti-tray 2000						R1			R2		
					R1		R2		R1			R2								
					Number of fluorescent wells	MPN/100 mL	Number of fluorescent wells	MPN/100 mL	Number of large fluorescent wells	Number of small fluorescent wells	MPN/100 mL	Number of large fluorescent wells	Number of small fluorescent wells	MPN/100 mL	1/2	1/20	MPN/100 mL	1/2	1/20	MPN/100 mL
ID 1	Marine	English Channel, Perros-Guirrec (France)	/	/	1	10	1	10	0	0	<10	0	0	<10	0	0	<15	0	0	<15
ID 2	Marine	Atlantic Ocean, La Rochelle (France)	/	/	0	<10	0	<10	0	0	<10	0	0	<10	0	0	<15	0	0	<15
ID 3	Marine	Atlantic Ocean, St-Jean-de-Luz (France)	/	/	1	10	2	20	2	0	20	1	0	10	0	0	<15	0	0	<15
ID 4	Fresh	Pond, Trivaux (France)	ENTC.1.5	1000	49	1 652	47	1 298	44	4	1 054	47	13	1 785	43	6	1 188	43	1	1 049
ID 5	Fresh	Pond, Villebon (France)	ENTC.1.8	1000	49	1 652	51	>2005	44	6	1 119	45	9	1 314	55	6	1 976	56	4	1 980
ID 6	Fresh	Pond, Meudon (France)	ENTC.1.11	5000	51	>2005	51	>2005	49	27	5 172	49	19	3 255	63	13	4 573	64	10	4 753
ID 7	Marine	English Channel, Perros-Guirrec (France)	ENTC.1.5	1000	50	2 005	51	>2005	39	6	836	39	13	1 010	50	10	1 722	54	10	2 065
ID 8	Marine	Atlantic Ocean, St-Jean-de-Luz (France)	ENTC.1.8	1000	43	945	46	1 184	43	9	1 145	46	6	1 291	60	7	2 715	55	9	2 121
ID 9	Marine	Atlantic Ocean, La Rochelle (France)	ENTC.1.5	1000	51	>2005	51	>2005	49	20	3 448	49	19	3 255	64	14	6 119	63	12	4 368
ID 10	Marine	North Sea, Kristiansand (Norway)	ENTC.6.2	10000	51	>2005	51	>2005	49	41	12 033	49	34	7 701	64	18	8 329	64	20	9 826
ID 11	Fresh	Pond, Vert-le-Petit (France)	ENTC.1.5	200	8	87	2	20	6	1	74	8	0	86	7	0	110	7	0	110
ID 12	Fresh	River Seine, Saintry (France)	ENTC.6.2	500	49	1 652	51	>2005	49	16	2 755	48	14	2 098	53	7	1 838	56	5	2 029
ID 13	Marine	English Channel, Grainval (France)	ENTC.1.8	500	8	87	12	137	3	0	31	14	1	173	15	2	287	6	1	109
ID 14	Marine	English Channel, Yport (France)	ENTC.1.11	500	50	2 005	51	>2005	49	16	2 755	48	21	2 851	59	7	2 536	62	9	3 421
ID 15	Marine	English Channel, Vaucottes (France)	ENTC.1.6	300	3	31	6	64	11	0	122	7	1	85	2	0	30	5	0	77
ID 16	Marine	English Channel, Fécamp (France)	ENTC.1.9	300	25	344	17	207	18	3	256	23	0	299	15	0	253	12	0	197
ID 17	Marine	English Channel, Etretat (France)	ENTC.2.5	300	11	124	6	64	5	4	94	7	1	85	15	0	253	14	0	234
ID 18	Marine	Mediterranean Sea, Vilanova I La Geltrù (Spain)	ENTC.1.6	300	9	99	8	87	4	1	52	6	0	63	5	0	77	5	1	93
ID 19	Marine	Mediterranean Sea, Sitges, Aiguadolç beach (Spain)	ENTC.1.9	300	17	207	20	254	17	1	216	10	0	110	11	2	212	21	0	375
ID 20	Marine	Mediterranean Sea, Sitges, Les Botigues beach (Spain)	ENTC.2.5	300	5	53	9	99	7	2	96	8	0	86	20	1	371	13	1	232
ID 21	Marine	Mediterranean Sea, Gavà, Gavà Mar beach (Spain)	ENTC.1.5	300	4	42	7	75	5	0	52	8	1	97	8	0	127	10	0	161
ID 22	Fresh	Watercourse, Antony (France)	/	/	16	192	19	238	18	2	243	16	4	238	13	2	249	19	1	350
ID 23	Fresh	River Bièvre, Igny (France)	/	/	0	<10	0	<10	0	0	<10	0	0	<10	0	0	<15	0	0	<15
ID 24	Fresh	La Blanchette lake, Massy (France)	ENTC.1.9	40	3	31	1	10	4	0	41	2	0	20	2	0	30	1	0	15

ID 25	Fresh	Brassens pool, Massy (France)	/	/	0	<10	0	<10	0	0	<10	0	0	<10	0	0	<15	0	0	<15
ID 26	Fresh	Bois de Briis pool, Massy (France)	/	/	0	<10	0	<10	0	0	<10	0	0	<10	0	0	<15	0	0	<15
ID 27	Fresh	Goachères pool, Massy (France)	ENTC.7.1	600	51	>2005	51	>2005	49	25	4 611	49	23	4 106	63	7	3 552	61	8	3 020
ID 28	Fresh	River Bièvre, Igny (France)	ENTC.7.3	93	5	53	5	53	7	0	75	6	1	74	3	0	46	4	2	92
ID 29	Fresh	River Marne, Nogent-sur-Marne (France)	ENTC.2.8	86	6	64	9	99	3	0	31	6	0	63	3	2	76	9	1	160
ID 30	Fresh	Ponds of Cergy, Cergy (France)	ENTC.5.3	58	9	99	6	64	7	0	75	9	0	98	5	0	77	3	0	46
ID 31	Fresh	Aydat lake, Aydat (France)	ENTC.2.9	73	9	99	6	64	3	0	31	6	1	74	5	0	77	1	0	15
ID 32	Fresh	Villefort lake, Villefort (France)	ENTC.3.2	63	2	20	2	20	0	0	<10	2	0	20	0	0	<10	0	0	<10
ID 33	Fresh	River Allier, Longues (France)	ENTC.2.8	1700	/	/	/	/	46	7	1 333	45	9	1 314	49	2	1 372	46	5	1 305
ID 34	Fresh	River Seine, Corbeil-Essonne (France)	ENTC.5.3	810	/	/	/	/	32	3	538	31	4	529	34	2	750	35	4	828
ID 35	Fresh	Brassens pool, Massy (France)	ENTC.7.3	1700	/	/	/	/	48	9	1 722	45	11	1 396	41	5	1 074	41	4	1 047
ID 36	Fresh	River Essonne, Ballancourt-sur-Essonne (France)	ENTC.4.1	30	2	20	1	10	1	0	10	2	0	20	1	0	15	1	0	15

Total exploitable results

N°	Water type	Sample	Strain	Contamination level (CFU/100 mL)	Enterolert-E														Reference method ISO 7899-1							
					Quanti-tray						Quanti-tray 2000								R1				R2			
					R1			R2			R1				R2											
					Number of fluorescent wells	MPN/100 mL	log MPN/100 mL	Number of fluorescent wells	MPN/100 mL	log MPN/100 mL	Number of large fluorescent wells	Number of small fluorescent wells	MPN/100 mL	log MPN/100 mL	Number of large fluorescent wells	Number of small fluorescent wells	MPN/100 mL	log MPN/100 mL	1/2	1/20	MPN/100 mL	log MPN/100 mL	1/2	1/20	MPN/100 mL	log MPN/100 mL
ID 4	Fresh	Pond, Trivaux (France)	ENTC.1.5	1000	49	1 652	3,218	47	1 298	3,113	44	4	1 054	3,023	47	13	1 785	3,252	43	6	1 188	3,075	43	1	1 049	3,021
ID 5	Fresh	Pond, Villebon (France)	ENTC.1.8	1000	49	1 652	3,218	51	>2005	/	44	6	1 119	3,049	45	9	1 314	3,119	55	6	1 976	3,296	56	4	1 980	3,297
ID 6	Fresh	Pond, Meudon (France)	ENTC.1.11	5000	51	>2005	/	51	>2005	/	49	27	5 172	3,714	49	19	3 255	3,513	63	13	4 573	3,660	64	10	4 753	3,677
ID 7	Marine	English Channel, Perros-Guirrec (France)	ENTC.1.5	1000	50	2 005	3,302	51	>2005	/	39	6	836	2,922	39	13	1 010	3,004	50	10	1 722	3,236	54	10	2 065	3,315
ID 8	Marine	Atlantic Ocean, St-Jean-de-Luz (France)	ENTC.1.8	1000	43	945	2,975	46	1 184	3,073	43	9	1 145	3,059	46	6	1 291	3,111	60	7	2 715	3,434	55	9	2 121	3,327
ID 9	Marine	Atlantic Ocean, La Rochelle (France)	ENTC.1.5	1000	51	>2005	/	51	>2005	/	49	20	3 448	3,538	49	19	3 255	3,513	64	14	6 119	3,787	63	12	4 368	3,640
ID 10	Marine	North Sea, Kristiansand (Norway)	ENTC.6.2	10000	51	>2005	/	51	>2005	/	49	41	12 033	4,080	49	34	7 701	3,887	64	18	8 329	3,921	64	20	9 826	3,992
ID 11	Fresh	Pond, Vert-le-Petit (France)	ENTC.1.5	200	8	87	1,940	2	20	1,301	6	1	74	1,869	8	0	86	1,934	7	0	110	2,041	7	0	110	2,041
ID 12	Fresh	River Seine, Saintry (France)	ENTC.6.2	500	49	1 652	3,218	51	>2005	/	49	16	2 755	3,440	48	14	2 098	3,322	53	7	1 838	3,264	56	5	2 029	3,307
ID 13	Marine	English Channel, Grainval (France)	ENTC.1.8	500	8	87	1,940	12	137	2,137	3	0	31	1,491	14	1	173	2,238	15	2	287	2,458	6	1	109	2,037
ID 14	Marine	English Channel, Yport (France)	ENTC.1.11	500	50	2 005	3,302	51	>2005	/	49	16	2 755	3,440	48	21	2 851	3,455	59	7	2 536	3,404	62	9	3 421	3,534
ID 15	Marine	English Channel, Vaucottes (France)	ENTC.1.6	300	3	31	1,491	6	64	1,806	11	0	122	2,086	7	1	85	1,929	2	0	30	1,477	5	0	77	1,886
ID 16	Marine	English Channel, Fécamp (France)	ENTC.1.9	300	25	344	2,537	17	207	2,316	18	3	256	2,408	23	0	299	2,476	15	0	253	2,403	12	0	197	2,294
ID 17	Marine	English Channel, Etretat (France)	ENTC.2.5	300	11	124	2,093	6	64	1,806	5	4	94	1,973	7	1	85	1,929	15	0	253	2,403	14	0	234	2,369
ID 18	Marine	Mediterranean Sea, Vilanova I La Geltrù (Spain)	ENTC.1.6	300	9	99	1,996	8	87	1,940	4	1	52	1,716	6	0	63	1,799	5	0	77	1,886	5	1	93	1,968
ID 19	Marine	Mediterranean Sea, Sitges, Aiguadolç beach (Spain)	ENTC.1.9	300	17	207	2,316	20	254	2,405	17	1	216	2,334	10	0	110	2,041	11	2	212	2,326	21	0	375	2,574
ID 20	Marine	Mediterranean Sea, Sitges, Les Botigues beach (Spain)	ENTC.2.5	300	5	53	1,724	9	99	1,996	7	2	96	1,982	8	0	86	1,934	20	1	371	2,569	13	1	232	2,365
ID 21	Marine	Mediterranean Sea, Gavà, Gavà Mar beach (Spain)	ENTC.1.5	300	4	42	1,623	7	75	1,875	5	0	52	1,716	8	1	97	1,987	8	0	127	2,104	10	0	161	2,207
ID 22	Fresh	Watercourse, Antony (France)	/	/	16	192	2,283	19	238	2,377	18	2	243	2,386	16	4	238	2,377	13	2	249	2,396	19	1	350	2,544
ID 24	Fresh	La Blanchette lake, Massy (France)	ENTC.1.9	40	3	31	1,491	1	10	1,000	4	0	41	1,613	2	0	20	1,301	2	0	30	1,477	1	0	15	1,176
ID 27	Fresh	Goachères pool, Massy (France)	ENTC.7.1	600	51	>2005	/	51	>2005	/	49	25	4 611	3,664	49	23	4 106	3,613	63	7	3 552	3,550	61	8	3 020	3,480
ID 28	Fresh	River Bièvre, Igny (France)	ENTC.7.3	93	5	53	1,724	5	53	1,724	7	0	75	1,875	6	1	74	1,869	3	0	46	1,663	4	2	92	1,964
ID 29	Fresh	River Marne, Nogent-sur-Marne (France)	ENTC.2.8	86	6	64	1,806	9	99	1,996	3	0	31	1,491	6	0	63	1,799	3	2	76	1,881	9	1	160	2,204
ID 30	Fresh	Ponds of Cergy, Cergy (France)	ENTC.5.3	58	9	99	1,996	6	64	1,806	7	0	75	1,875	9	0	98	1,991	5	0	77	1,886	3	0	46	1,663
ID 31	Fresh	Aydat lake, Aydat (France)	ENTC.2.9	73	9	99	1,996	6	64	1,806	3	0	31	1,491	6	1	74	1,869	5	0	77	1,886	1	0	15	1,176
ID 33	Fresh	River Allier, Longues (France)	ENTC.2.8	1700	/	/	/	/	/	/	46	7	1 333	3,125	45	9	1 314	3,119	49	2	1 372	3,137	46	5	1 305	3,116

ID 34	Fresh	River Seine, Corbeil-Essonne (France)	ENTC.5.3	810	/	/	/	/	/	/	32	3	538	2,731	31	4	529	2,723	34	2	750	2,875	35	4	828	2,918
ID 35	Fresh	Brassens pool, Massy (France)	ENTC.7.3	1700	/	/	/	/	/	/	48	9	1 722	3,236	45	11	1 396	3,145	41	5	1 074	3,031	41	4	1 047	3,020
ID 36	Fresh	River Essonne, Ballancourt-sur-Essonne (France)	ENTC.4.1	30	2	20	1,301	1	10	1,000	1	0	10	1,000	2	0	20	1,301	1	0	15	1,176	1	0	15	1,176

Relative accuracy - Enterococci - Bathing waters - Logarithmic data

Reference method					Alternative method					Difference
Sample	Replicate 1	Replicate 2	M	SD	Sample	Replicate 1	Replicate 2	M	SD	
1	3,075	3,021	3,048	0,038	1	3,023	3,252	3,137	0,162	0,089
2	3,296	3,297	3,296	0,001	2	3,049	3,119	3,084	0,049	-0,213
3	3,660	3,677	3,669	0,012	3	3,714	3,513	3,613	0,142	-0,055
4	3,236	3,315	3,275	0,056	4	2,922	3,004	2,963	0,058	-0,312
5	3,434	3,327	3,380	0,076	5	3,059	3,111	3,085	0,037	-0,295
6	3,787	3,640	3,713	0,104	6	3,538	3,513	3,525	0,018	-0,188
7	3,921	3,992	3,956	0,051	7	4,080	3,887	3,983	0,137	0,027
8	2,041	2,041	2,041	0,000	8	1,869	1,934	1,902	0,046	-0,140
9	3,264	3,307	3,286	0,030	9	3,440	3,322	3,381	0,084	0,095
10	2,458	2,037	2,248	0,297	10	1,491	2,238	1,865	0,528	-0,383
11	3,404	3,534	3,469	0,092	11	3,440	3,455	3,448	0,011	-0,022
12	1,477	1,886	1,682	0,289	12	2,086	1,929	2,008	0,111	0,326
13	2,403	2,294	2,349	0,077	13	2,408	2,476	2,442	0,048	0,093
14	2,403	2,369	2,386	0,024	14	1,973	1,929	1,951	0,031	-0,435
15	1,886	1,968	1,927	0,058	15	1,716	1,799	1,758	0,059	-0,170
16	2,326	2,574	2,450	0,175	16	2,334	2,041	2,188	0,207	-0,262
17	2,569	2,365	2,467	0,144	17	1,982	1,934	1,958	0,034	-0,509
18	2,104	2,207	2,155	0,073	18	1,716	1,987	1,851	0,191	-0,304
19	2,396	2,544	2,470	0,105	19	2,386	2,377	2,381	0,006	-0,089
20	1,477	1,176	1,327	0,213	20	1,613	1,301	1,457	0,220	0,130
21	3,550	3,480	3,515	0,050	21	3,664	3,613	3,639	0,036	0,123
22	1,663	1,964	1,813	0,213	22	1,875	1,869	1,872	0,004	0,059
23	1,881	2,204	2,042	0,229	23	1,491	1,799	1,645	0,218	-0,397
24	1,886	1,663	1,775	0,158	24	1,875	1,991	1,933	0,082	0,159
25	1,886	1,176	1,531	0,502	25	1,491	1,869	1,680	0,267	0,149
26	3,137	3,116	3,126	0,015	26	3,125	3,119	3,122	0,004	-0,005
27	2,875	2,918	2,897	0,030	27	2,731	2,723	2,727	0,005	-0,169
28	3,031	3,020	3,025	0,008	28	3,236	3,145	3,190	0,064	0,165
29	1,176	1,176	1,176	0,000	29	1,000	1,301	1,151	0,213	-0,026

q= 29 **Mx=** 2,603 **My=** 2,515 **M=** -0,088

n= 2 **MEDx=** 2,467 **MEDy=** 2,381 **MED=** -0,055

N=qn= 58 **SDbx=** 0,781 **SDby=** 0,788 Bias

MEDwx = 0,073 **MEDwy =** 0,059

SDwx= 0,156 **SDwy=** 0,154

rob. SDwx= 0,108 **rob. SDwy=** 0,087

Method choice GMFR

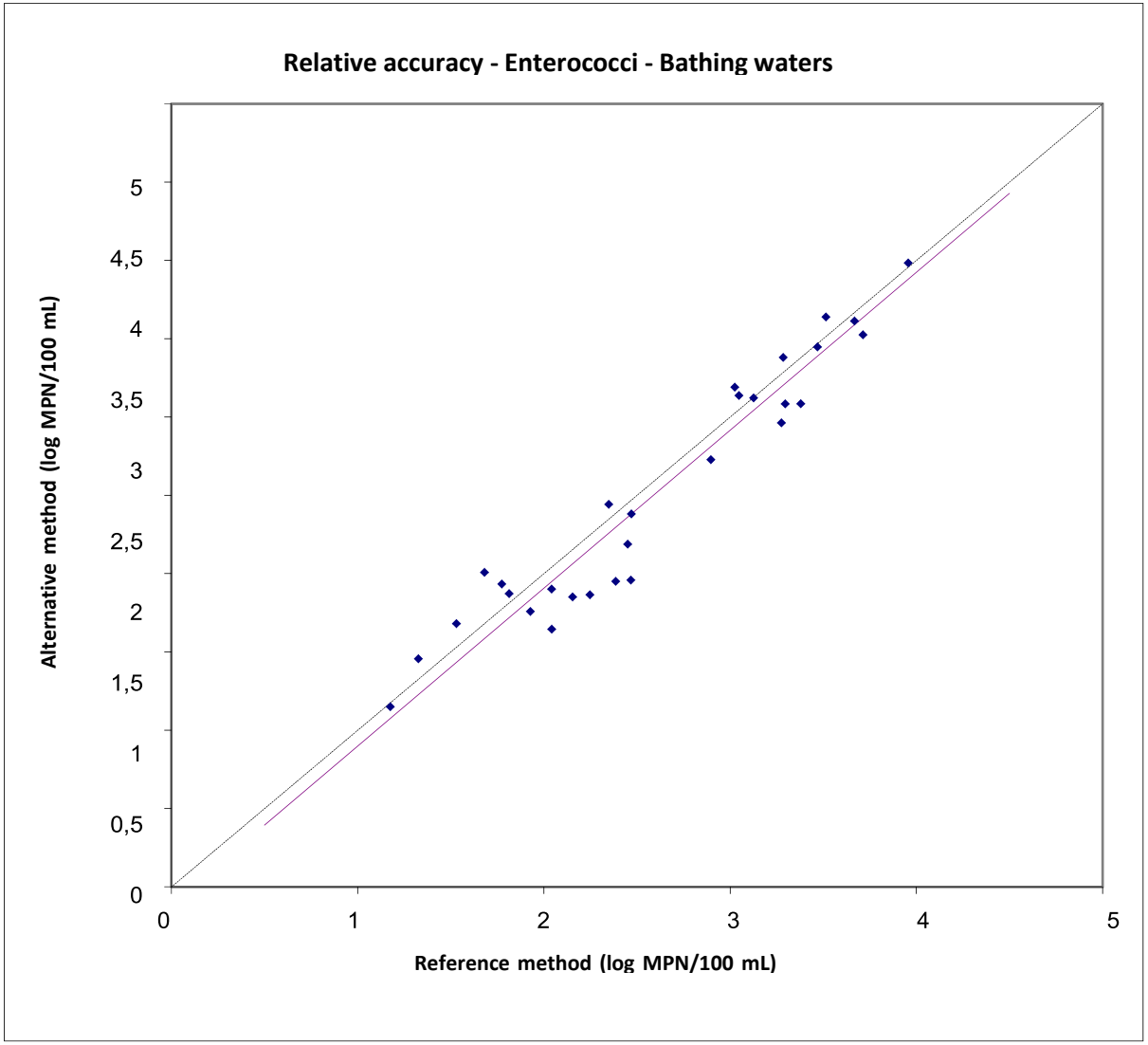
		R=	0,985		
		rob. R=	0,809		
				Sx=	0,782
				Sy=	0,789
r=	0,963				
b=	1,008			**Res. SEM=**	0,190
a=	-0,110			**Res. SD=**	0,268

S(b)=	0,046	**p(t;b=1)=**	0,855	**t(b)=**	0,184
S(a)=	0,154	**p(t;a=0)=**	0,477	**t(a)=**	0,715

Reapetability	Reference method	Alternative method
r	0,437	0,430
rob. r	0,302	0,245

Est. y	Deviation
2,963	0,174
3,214	-0,130
3,589	0,024
3,193	-0,230
3,298	-0,214
3,635	-0,110
3,880	0,104
1,948	-0,047
3,203	0,178
2,156	-0,292
3,388	0,059
1,586	0,422
2,258	0,184
2,296	-0,345
1,834	-0,076
2,361	-0,173
2,378	-0,420
2,063	-0,212
2,381	0,000
1,228	0,229
3,435	0,204
1,718	0,154
1,950	-0,304
1,679	0,254
1,434	0,246
3,043	0,079
2,811	-0,084
2,941	0,250
1,076	0,075

Points correspond to the means of the repetitions for each sample



APPENDIX 5 – LINEARITY RESULTS

N°	Echantillon	Strain	Target contamination level (CFU/100 mL)	Real contamination level (CFU/100 mL)	Alternative method								Reference method							
					R1				R2				R1				R2			
					Large wells	Small wells	MPN/100 mL	log MPN/100 mL	Large wells	Small wells	MPN/100 mL	log MPN/100 mL	1/2	1/20	MPN/100 mL	log MPN/100 mL	1/2	1/20	MPN/100 mL	log MPN/100 mL
A	Marine water	*E. faecalis*	50	45	4	0	41	1,613	5	0	52	1,716	2	0	30	1,477	3	1	61	1,785
B	Marine water	*E. faecalis*	500	560	28	5	473	2,675	24	2	345	2,538	31	2	661	2,820	26	2	529	2,723
C	Marine water	*E. faecalis*	1000	990	39	3	767	2,885	35	6	683	2,834	38	6	981	2,992	44	4	1177	3,071
D	Marine water	*E. faecalis*	5000	5400	49	24	4352	3,639	49	31	6488	3,812	63	9	3843	3,585	64	10	4753	3,677
E	Marine water	*E. faecalis*	200	270	15	1	187	2,272	13	0	148	2,170	10	1	177	2,248	13	0	215	2,332
F	Marine water	*E. faecalis*	20000	27000	49	45	17329	4,239	49	45	17329	4,239	64	23	12687	4,103	64	27	18563	4,269

Linearity - Enterococci - Marine water - Log data

Level	Reference method				Alternative method			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	1,477	1,785	1,6	0,218	1,613	1,716	1,7	0,073
2	2,820	2,723	2,8	0,068	2,675	2,538	2,6	0,097
3	2,992	3,071	3,0	0,056	2,885	2,834	2,9	0,036
4	3,585	3,677	3,6	0,065	3,639	3,812	3,7	0,123
5	2,248	2,332	2,3	0,060	2,272	2,170	2,2	0,072
6	4,103	4,269	4,2	0,117	4,239	4,239	4,2	0,000

q =	6	**Mx =**	2,924	**My =**	2,886	
n =	2	**MEDx =**	2,902	**MEDy =**	2,733	
N = qn =	12	**SDbx =**	0,916	**SDby =**	0,954	
		MEDwx =	0,067	**MEDwy =**	0,072	
		SDwx =	0,080	**SDwy =**	0,055	
		rob. SDwx =	0,099	**rob. SDwy =**	0,107	

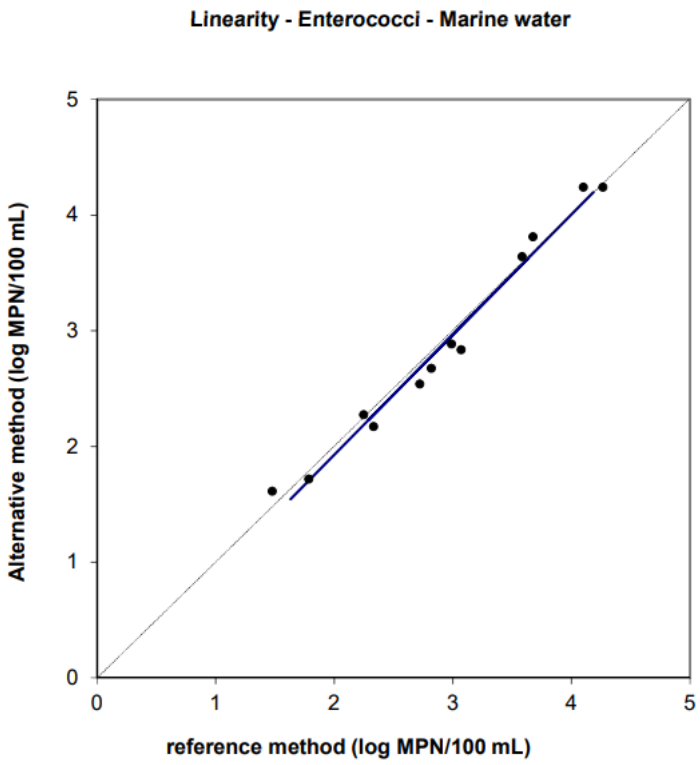
Method choiceGMFR

R =	0,686
rob.R =	1,083
Res.SEM =	0,122
Res.SD =	0,172

Sx =	0,878
Sy =	0,911
r =	0,993
b =	1,038
a =	-0,149

Est y	Deviation
1,545	0,120
2,728	-0,122
2,998	-0,138
3,620	0,105
2,229	-0,007
4,196	0,042

Sb =	0,062	**p(t;b=1) =**	0,555	**t (b) =**	0,611
Sa =	0,188	**p(t;a=0) =**	0,448	**t (a) =**	0,789



Linéarité

F =	23,164	**p(F) =**	0,001
rob.F =	4,953	**rob.p(F) =**	0,041

APPENDIX 6 – LOD-LOQ RESULTS

N°	Contamination (CFU/100 mL)	Large wells	Small wells	MPN/100 mL	Number of positive samples	Standard deviation	Median
1	0	0	0	**0**	0/6	0,000	0,000
2		0	0	**0**			
3		0	0	**0**			
4		0	0	**0**			
5		0	0	**0**			
6		0	0	**0**			
1	3	1	0	**10**	2/6	5,164	0,000
2		0	0	**0**			
3		0	0	**0**			
4		0	0	**0**			
5		1	0	**10**			
6		0	0	**0**			
1	6	1	0	**10**	2/6	5,164	0,000
2		0	0	**0**			
3		1	0	**10**			
4		0	0	**0**			
5		0	0	**0**			
6		0	0	**0**			
1	9	1	0	**10**	2/6	5,164	0,000
2		0	0	**0**			
3		0	0	**0**			
4		1	0	**10**			
5		0	0	**0**			
6		0	0	**0**			
1	10	0	0	**0**	4/6	5,164	10,000
2		1	0	**10**			
3		1	0	**10**			
4		1	0	**10**			
5		0	0	**0**			
6		1	0	**10**			
1	12	1	0	**10**	5/6	7,528	10,000
2		0	0	**0**			
3		1	1	**20**			
4		1	0	**10**			
5		2	0	**20**			
6		1	0	**10**			
1	14	2	0	**20**	5/6	10,778	15,000
2		3	0	**31**			
3		2	0	**20**			
4		1	0	**10**			
5		1	0	**10**			
6		0	0	**0**			
1	18	2	0	**20**	6/6	8,495	15,000
2		1	0	**10**			
3		3	0	**31**			
4		1	0	**10**			
5		2	0	**20**			
6		1	0	**10**			

APPENDIX 7 – SELECTIVITY RESULTS

Inclusivity

N°	Code	Species	Origin	Inoculation level (CFU/100 mL)	Number of large wells	Number of small wells	Result (MPN/100 mL)
1	ENTC.1.2	*Enterococcus faecalis*	ATCC 33186	35	4	0	41
2	ENTC.1.3	*Enterococcus faecalis*	CIP 103214	55	6	0	63
3	ENTC.1.4	*Enterococcus faecalis*	River water	62	3	0	31
4	ENTC.1.5	*Enterococcus faecalis*	Surface water	52	5	0	52
5	ENTC.1.6	*Enterococcus faecalis*	Surface water	57	5	1	63
6	ENTC.1.7	*Enterococcus faecalis*	Surface water	142	11	2	145
7	ENTC.1.8	*Enterococcus faecalis*	River water	70	1	0	10
8	ENTC.1.9	*Enterococcus faecalis*	River water	45	4	0	41
9	ENTC.1.10	*Enterococcus faecalis*	River water	120	8	2	108
10	ENTC.1.11	*Enterococcus faecalis*	River water	45	7	1	85
11	ENTC.1.12	*Enterococcus faecalis*	River water	150	12	1	146
12	ENTC.2.1	*Enterococcus faecium*	Dairy industry	258	17	1	216
13	ENTC.2.2	*Enterococcus faecium*	Aqueous environment	73	5	0	52
14	ENTC.2.4	*Enterococcus faecium*	Surface water	53	5	0	52
15	ENTC.2.5	*Enterococcus faecium*	Surface water	43	2	0	20
16	ENTC.2.6	*Enterococcus faecium*	Surface water	41	2	0	20
17	ENTC.2.7	*Enterococcus faecium*	River water	52	2	0	20
18	ENTC.2.8	*Enterococcus faecium*	River water	66	3	0	31
19	ENTC.2.9	*Enterococcus faecium*	River water	71	6	0	63
20	ENTC.6.1	*Enterococcus durans*	Surface water	52	6	0	63
21	ENTC.6.2	*Enterococcus durans*	River water	49	2	0	20
22	ENTC.4.1	*Enterococcus avium*	Water	30	1	0	10
23	ENTC.5.1	*Enterococcus gallinarum*	River water	49	4	0	41
24	ENTC.5.2	*Enterococcus gallinarum*	River water	37	2	0	20
25	ENTC.5.3	*Enterococcus gallinarum*	Effluent water	40	3	0	31
26	ENTC.3.1	*Enterococcus hirae*	CIP 58.55	124	7	0	75

27	ENTC.3.2	*Enterococcus hirae*	River water	177	7	1	85
28	ENTC.7.1	*Enterococcus casselifavus*	River water	565	10	1	121
29	ENTC.7.2	*Enterococcus casseliflavus*	River water	36	3	0	31
30	ENTC.7.3	*Enterococcus casselifavus*	River water	42	2	0	20

Exclusivity

N°	Code	Species	Origin	Inoculation level (CFU/100 mL)	Number of large wells	Number of small wells	Result (MPN/100 mL)
1	AERC.1.1	*Aerococcus viridans*	CIP 54.145	2,1E+04	0	0	<10
2	AUR.1.1	*Aureobacterium saperdae*	Evaporator	5,0E+05	0	0	<10
3	LACC.1.1	*Lactococcus lactis*	Collection strain	4,0E+04	0	0	<10
4	MIC.1.2	*Micrococcus luteus*	ATCC 4698	1,8E+04	0	0	<10
5	MIC.2.2	*Micrococcus spp*	Surface water	5,0E+04	0	0	<10
6	MIC.2.3	*Micrococcus spp*	Surface water	3,2E+04	0	0	<10
7	STA.1.6	*Staphylococcus aureus*	Surface water	1,2E+04	0	0	<10
8	STA.5.1	*Staphylococcus xylosus*	Surface water	2,1E+04	0	0	<10
9	STA.6.1	*Staphylococcus capitis*	Surface water	3,6E+04	0	0	<10
10	STA.2.3	*Staphylococcus epidermidis*	Surface water	5,0E+04	0	0	<10
11	STA.7.1	*Staphylococcus sciuri*	Surface water	1,6E+04	0	0	<10
12	STA.2.1	*Staphylococcus epidermidis*	Dairy product	5,0E+04	0	0	<10
13	STA.3.2	*Staphylococcus haemolyticus*	Surface water	5,2E+04	0	0	<10
14	STA.4.1	*Staphylococcus piscifermentans*	Evaporator	4,1E+04	0	0	<10
15	PED.1.1	*Pediococcus acidilactici*	Souche de collection	5,2E+04	0	0	<10
16	PED.1.2	*Pediococcus spp*	Surface water	2,6E+04	0	0	<10
17	RHO.1.1	Rhodococcus equi	Collection strain	1,8E+04	0	0	<10
18	BAC.2.1	*Bacillus circulans*	Dairy industry	5,0E+04	0	0	<10
19	BAC.4.2	*Bacillus subtilis*	CIP 52.65 T	4,1E+04	0	0	<10
20	BAC.1.4	*Bacillus cereus*	Collection strain	1,9E+04	0	0	<10
21	STE.1.1	*Stenotrophomonas maltophilia*	Eau de fontaine	3,2E+04	0	0	<10
22	AER.1.1	*Aeromonas hydrophila*	Well water	2,3E+04	0	0	<10
23	PSE.1.4	*Pseudomonas aeruginosa*	Fountain water	1,1E+04	0	0	<10
24	ACI.2.1	*Acinetobacter cloacae*	River water	1,9E+04	0	0	<10
25	RAH.1.2	*Rahnella aquatilis*	River water	4,4E+04	0	0	<10
26	ESC.1.120	*Escherichia coli*	River water	2,4E+04	0	0	<10
27	PRO.1.2	*Proteus mirabilis*	Water	1,2E+04	0	0	<10

28	ENTB.2.2	*Enterobacter cloacae*	Well water	2,0E+04	0	0	<10
29	PROV.1.1	*Providencia stuartii*	HPA RM	1,1E+04	0	0	<10
30	XAN.1.1	*Xanthomonas campestris*	Evaporator	3,6E+04	0	0	<10

APPENDIX 8 – INTERLABORATORY STUDY RESULTS

RAW RESULTS

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
A	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	3	0	46	1,663	2	0	20	1,301
	7	4	0	61	1,785	5	0	52	1,716
	2	29	4	648	2,812	22	0	282	2,450
	6	23	4	495	2,695	23	3	341	2,533
	3	36	3	838	2,923	40	5	857	2,933
	8	57	6	2194	3,341	38	9	862	2,936

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
B	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	6	1	109	2,037	5	0	52	1,716
	7	5	0	77	1,886	4	0	41	1,613
	2	31	1	640	2,806	26	2	384	2,584
	6	24	1	461	2,664	23	2	327	2,515
	3	61	5	2759	3,441	45	10	1354	3,132
	8	56	8	2182	3,339	49	16	2755	3,440

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
C	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	7	1	126	2,100	2	0	20	1,301
	7	1	0	15	1,176	2	0	20	1,301
	2	27	4	594	2,774	31	3	512	2,709
	6	25	1	485	2,686	28	3	441	2,644
	3	59	8	2604	3,416	49	12	2247	3,352
	8	57	5	2140	3,330	48	13	2014	3,304

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
D	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	5	0	77	1,886	7	0	75	1,875
	7	3	0	46	1,663	3	0	31	1,491
	2	26	1	509	2,707	28	0	395	2,597
	6	27	1	534	2,728	31	2	495	2,695
	3	52	8	1797	3,255	48	14	2098	3,322
	8	54	7	1927	3,285	47	8	1500	3,176

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution				Number of yellow and fluorescent wells			

		1/2	1/20	Result (MPN/100 mL)	Result (log MPN/100 mL)	Big wells	Small wells	Result (MPN/100 mL)	Result (log MPN/100 mL)
E	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	4	0	61	1,785	6	0	63	1,799
	7	7	0	110	2,041	4	1	52	1,716
	2	30	1	612	2,787	19	1	246	2,391
	6	32	3	712	2,852	28	3	441	2,644
	3	60	9	2873	3,458	45	13	1483	3,171
	8	60	8	2792	3,446	46	10	1467	3,166

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
F	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	3	1	61	1,785	10	1	121	2,083
	7	3	0	46	1,663	1	1	20	1,301
	2	20	2	390	2,591	31	6	563	2,751
	6	29	1	585	2,767	37	1	650	2,813
	3	52	3	1599	3,204	47	9	1553	3,191
	8	59	7	2536	3,404	48	9	1722	3,236

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
G	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	6	0	94	1,973	3	0	31	1,491
	7	1	0	15	1,176	4	1	52	1,716
	2	28	1	559	2,747	38	3	727	2,862
	6	33	1	697	2,843	31	3	512	2,709
	3	55	7	2023	3,306	49	19	3255	3,513
	8	58	4	2206	3,344	48	16	2282	3,358

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
H	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	0	0	<15	/	7	0	75	1,875
	7	0	1	15	1,176	3	0	31	1,491
	2	23	3	476	2,678	27	6	465	2,667
	6	29	2	606	2,782	35	2	605	2,782
	3	52	6	1716	3,235	47	13	2359	3,373
	8	57	4	2087	3,320	47	14	1850	3,267

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
J	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	5	0	77	1,886	4	0	41	1,613
	7	3	0	46	1,663	4	0	41	1,613
	2	30	6	718	2,856	35	4	644	2,809
	6	28	4	621	2,793	37	3	691	2,839
	3	55	5	1929	3,285	48	18	2489	3,396
	8	56	6	2079	3,318	48	15	2187	3,340

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
K	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	6	0	94	1,973	5	0	52	1,716
	7	2	0	30	1,477	4	0	41	1,613
	2	18	4	383	2,583	33	5	602	2,780
	6	25	1	485	2,686	24	2	345	2,538
	3	27	2	1984	3,298	47	14	1850	3,267
	8	54	5	1838	3,264	43	9	1145	3,059
Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
L	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	4	0	61	1,785	6	0	63	1,799
	7	3	0	46	1,663	5	0	52	1,716
	2	26	0	489	2,689	29	4	480	2,681
	6	24	2	480	2,681	31	4	529	2,723
	3	56	7	2130	3,328	48	13	2014	3,304
	8	51	4	1567	3,195	48	13	2014	3,304

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
M	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	4	1	77	1,886	3	0	31	1,491
	7	3	0	46	1,663	2	0	20	1,301
	2	25	1	485	2,686	17	3	241	2,382
	6	25	1	485	2,686	17	6	278	2,444
	3	51	2	1494	3,174	44	8	1187	3,074
	8	52	1	1523	3,183	39	13	1010	3,004

Laboratory	Sample number	Reference method				Alternative method			
		Number of positive samples per dilution		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and fluorescent wells		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
N	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	3	0	46	1,663	5	0	52	1,716
	7	4	0	61	1,785	5	1	63	1,799
	2	28	3	600	2,778	26	3	399	2,601
	6	29	3	627	2,797	21	3	305	2,484
	3	28	11	2640	3,422	48	14	2098	3,322
	8	58	8	2444	3,388	37	4	712	2,852

Laboratory	Sample number	Reference method (*)				Alternative method			
		Number of positive		Result (MPN/100 mL)	Result (log MPN/100 mL)	Number of yellow and		Result (MPN/100 mL)	Result (log MPN/100 mL)
		1/2	1/20			Big wells	Small wells		
Expert	1	0	0	<15	/	0	0	<10	/
	4	0	0	<15	/	0	0	<10	/
	5	3	0	46	1,663	8	0	86	1,934
	7	4	0	61	1,785	5	0	52	1,716
	2	28	1	559	2,747	32	4	556	2,745
	6	29	3	627	2,797	29	3	464	2,667
	3	58	5	2263	3,355	49	15	2613	3,417
	8	57	7	2249	3,352	47	15	1918	3,283

APPENDIX 9 – ENUMERATION OF CULTURABLE MICROORGANISMS

Laboratory	Culturable microorganisms at 22°C	Culturable microorganisms at 36°C
A	190	180
B	142	58
C	100	75
D	27	28
E	82	29
F	75	24
G	65	47
H	105	44
J	159	146
K	119	54
L	53	25
M	64	24
N	38	27
Expert	136	53

APPENDIX 10 – COMPLEMENTARY ASSAYS RESULTS AND CALCULATIONS

RESULTS FROM ENTEROLERT-E VALIDATION

N°	Enterolert-E														Reference method ISO 7899-1							
	Quanti-tray						Quanti-tray 2000															
	R1			R2			R1				R2				R1				R2			
	Number of fluorescent wells	MPN/100 mL	log MPN/100 mL	Number of fluorescent wells	MPN/100 mL	log MPN/100 mL	Number of large fluorescent wells	Number of small fluorescent wells	MPN/100 mL	log MPN/100 mL	Number of large fluorescent wells	Number of small fluorescent wells	MPN/100 mL	log MPN/100 mL	1/2	1/20	MPN/100 mL	log MPN/100 mL	1/2	1/20	MPN/100 mL	log MPN/100 mL
ID 4	49	1 652	3,218	47	1 298	3,113	44	4	1 054	3,023	47	13	1 785	3,252	43	6	1 188	3,075	43	1	1 049	3,021
ID 8	43	945	2,975	46	1 184	3,073	43	9	1 145	3,059	46	6	1 291	3,111	60	7	2 715	3,434	55	9	2 121	3,327
ID 11	8	87	1,940	2	20	1,301	6	1	74	1,869	8	0	86	1,934	7	0	110	2,041	7	0	110	2,041
ID 13	8	87	1,940	12	137	2,137	3	0	31	1,491	14	1	173	2,238	15	2	287	2,458	6	1	109	2,037
ID 15	3	31	1,491	6	64	1,806	11	0	122	2,086	7	1	85	1,929	2	0	30	1,477	5	0	77	1,886
ID 16	25	344	2,537	17	207	2,316	18	3	256	2,408	23	0	299	2,476	15	0	253	2,403	12	0	197	2,294
ID 17	11	124	2,093	6	64	1,806	5	4	94	1,973	7	1	85	1,929	15	0	253	2,403	14	0	234	2,369
ID 18	9	99	1,996	8	87	1,940	4	1	52	1,716	6	0	63	1,799	5	0	77	1,886	5	1	93	1,968
ID 19	17	207	2,316	20	254	2,405	17	1	216	2,334	10	0	110	2,041	11	2	212	2,326	21	0	375	2,574
ID 20	5	53	1,724	9	99	1,996	7	2	96	1,982	8	0	86	1,934	20	1	371	2,569	13	1	232	2,365
ID 21	4	42	1,623	7	75	1,875	5	0	52	1,716	8	1	97	1,987	8	0	127	2,104	10	0	161	2,207
ID 22	16	192	2,283	19	238	2,377	18	2	243	2,386	16	4	238	2,377	13	2	249	2,396	19	1	350	2,544
ID 24	3	31	1,491	1	10	1,000	4	0	41	1,613	2	0	20	1,301	2	0	30	1,477	1	0	15	1,176
ID 28	5	53	1,724	5	53	1,724	7	0	75	1,875	6	1	74	1,869	3	0	46	1,663	4	2	92	1,964
ID 29	6	64	1,806	9	99	1,996	3	0	31	1,491	6	0	63	1,799	3	2	76	1,881	9	1	160	2,204
ID 30	9	99	1,996	6	64	1,806	7	0	75	1,875	9	0	98	1,991	5	0	77	1,886	3	0	46	1,663
ID 31	9	99	1,996	6	64	1,806	3	0	31	1,491	6	1	74	1,869	5	0	77	1,886	1	0	15	1,176
ID 36	2	20	1,301	1	10	1,000	1	0	10	1,000	2	0	20	1,301	1	0	15	1,176	1	0	15	1,176

Enterococci - Comparison QT2000-QT - Logarithmic data

QT 2000					QT					Difference
Sample	Replicate 1	Replicate 2	M	SD	Sample	Replicate 1	Replicate 2	M	SD	
1	3,023	3,252	3,137	0,162	1	3,218	3,113	3,166	0,074	0,028
2	3,059	3,111	3,085	0,037	2	2,975	3,073	3,024	0,069	-0,060
3	1,869	1,934	1,902	0,046	3	1,940	1,301	1,620	0,451	-0,282
4	1,491	2,238	1,865	0,528	4	1,940	2,137	2,038	0,139	0,173
5	2,086	1,929	2,008	0,111	5	1,491	1,806	1,649	0,223	-0,359
6	2,408	2,476	2,442	0,048	6	2,537	2,316	2,426	0,156	-0,016
7	1,973	1,929	1,951	0,031	7	2,093	1,806	1,950	0,203	-0,001
8	1,716	1,799	1,758	0,059	8	1,996	1,940	1,968	0,040	0,210
9	2,334	2,041	2,188	0,207	9	2,316	2,405	2,360	0,063	0,172
10	1,982	1,934	1,958	0,034	10	1,724	1,996	1,860	0,192	-0,098
11	1,716	1,987	1,851	0,191	11	1,623	1,875	1,749	0,178	-0,102
12	2,386	2,377	2,381	0,006	12	2,283	2,377	2,330	0,066	-0,051
13	1,613	1,301	1,457	0,220	13	1,491	1,000	1,246	0,347	-0,211
14	1,875	1,869	1,872	0,004	14	1,724	1,724	1,724	0,000	-0,148
15	1,491	1,799	1,645	0,218	15	1,806	1,996	1,901	0,134	0,256
16	1,875	1,991	1,933	0,082	16	1,996	1,806	1,901	0,134	-0,032
17	1,491	1,869	1,680	0,267	17	1,996	1,806	1,901	0,134	0,221
18	1,000	1,301	1,151	0,213	18	1,301	1,000	1,151	0,213	0,000

q=	18	**Mx=**	2,015			**My=**	1,998		**M=**	-0,017
n=	2	**MEDx=**	1,918			**MEDy=**	1,901		**MED=**	-0,024
N=qn=	36	**SDbx=**	0,498			**SDby=**	0,519			Bias
			MEDwx =	0,097			**MEDwy =**	0,137		
			SDwx=	0,187			**SDwy=**	0,190		
			rob. SDwx=	0,143			**rob. SDwy=**	0,203		

Method choice: GMFR

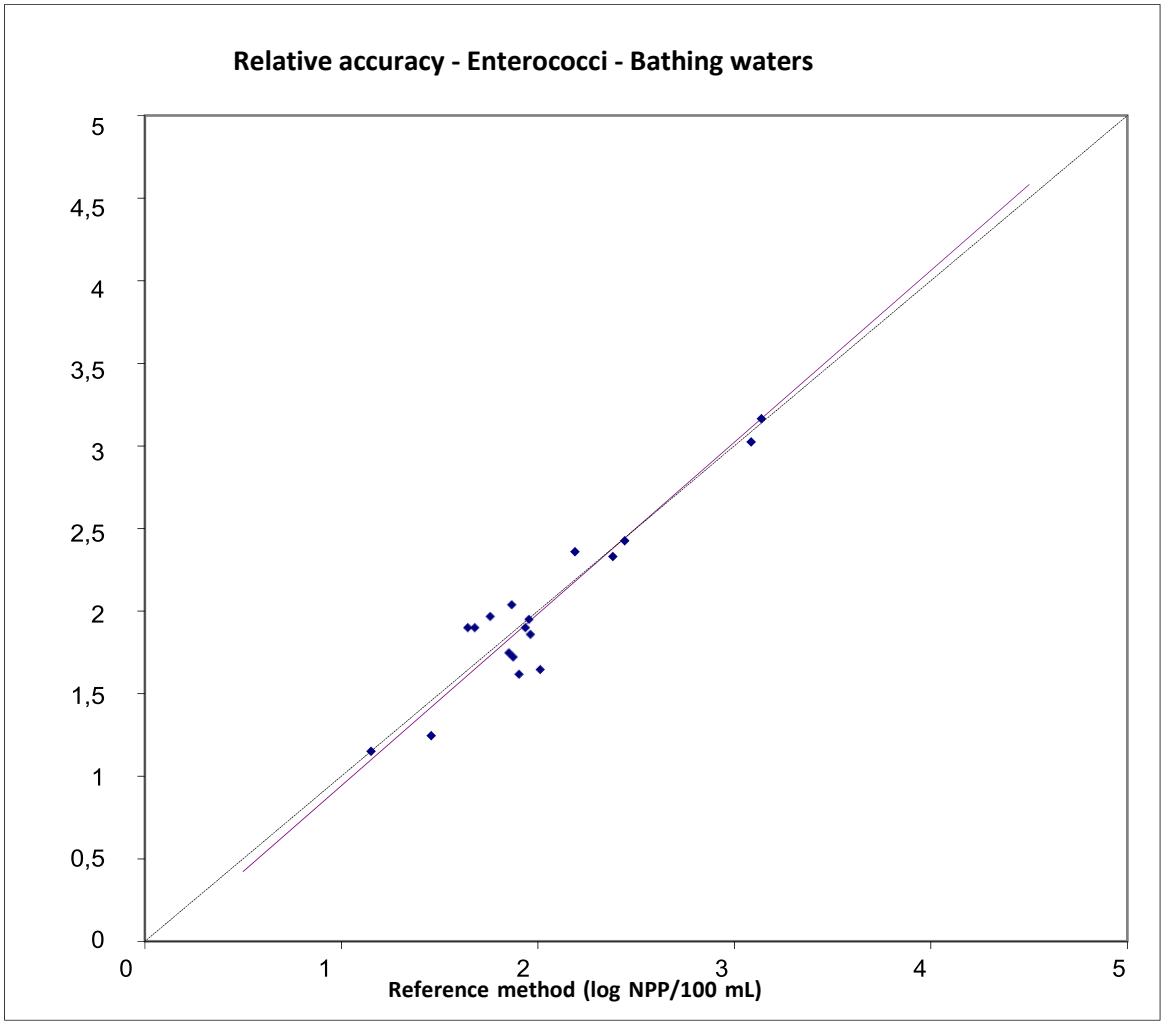
		R=	1,015		
		rob. R=	1,416		
				Sx=	0,509
				Sy=	0,529
r=	0,942				
b=	1,040			**Res. SEM=**	0,182
a=	-0,097			**Res. SD=**	0,257

S(b)=	0,087	**p(t;b=1)=**	0,648	**t(b)=**	0,460
S(a)=	0,186	**p(t;a=0)=**	0,604	**t(a)=**	0,523

Est. y	Dév.
3,165	0,000
3,111	-0,086
1,881	-0,260
1,842	0,196
1,991	-0,342
2,442	-0,016
1,932	0,018
1,731	0,237
2,178	0,182
1,939	-0,079
1,828	-0,079
2,379	-0,049
1,418	-0,172
1,850	-0,125
1,614	0,287
1,913	-0,012
1,650	0,251
1,099	0,051

Répétabilité	Méthode de référence	Méthode alternative
r	0,523	0,531
rob. r	0,401	0,567

Points correspond to the means of the two replicates for each sample



RESULTS FROM IDEXX EXPERIMENTATION

Target inoculum (cfu per 100ml volume)	Colilert-18 @ 18.5 hours						
	Quanti-Tray (51 wells)			Quanti-Tray 2000			
	Wells	MPN	log MPN	Large wells	Small wells	MPN	log MPN
30	24	32,4	1,511	20	4	30,1	1,479
40	28	40,6	1,609	23	3	34,1	1,533
50	31	47,8	1,679	27	3	42,0	1,623
50	33	53,1	1,725	37	4	71,2	1,852
60	36	62,4	1,795	33	1	53,0	1,724
70	43	94,5	1,975	37	4	71,2	1,852
80	41	83,1	1,920	37	6	75,4	1,877
90	43	94,5	1,975	38	6	79,4	1,900
100	42	88,5	1,947	42	5	96,0	1,982
110	46	118,4	2,073	42	8	104,6	2,020
110	44	101,3	2,006	44	4	105,4	2,023
120	45	109,1	2,038	46	7	133,3	2,125
130	47	129,8	2,113	47	7	145,0	2,161
130	49	129,8	2,113	46	12	156,5	2,195
140	49	129,8	2,113	46	10	146,7	2,166
150	50	200,5	2,302	47	12	172,3	2,236
160	49	165,2	2,218	45	13	148,3	2,171
170	49	165,2	2,218	49	7	179,3	2,254
180	48	144,5	2,160	48	16	228,2	2,358