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NF VALIDATION - Validation of alternative analysis methods
Application to the water industry

Summary report
according to the Validation protocol for an alternative commercial method as compared with a reference method – revision 2

Quantitative method

Enterolert DW / Quanti-Tray or Quanti-Tray 2000
(ref. Enterolert : 06-18085-07 / Quanti-Tray: 06-02030-18
/ Quanti-Tray 2000 : 06-02320-14)
Attestation number: IDX 33/03-10/13
for the enumeration of Enterococci in drinking waters
(except bottled waters) and bathing waters (fresh and marine)

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This report contains 59 pages including 37 pages of appendices.

The accreditation of the COFRAC (Section Laboratory) gives evidence of the expertise of the laboratory for the only tests covered by the accreditation that are specified by the symbol (■).
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APPENDICES

1. Introduction

1.1. Validation referential

This report presents the results of the extension validation study, under the NF Validation brand, of the Enterolert-DW / Quanti-Tray® method developed by IDEXX for the enumeration of enterococci in human drinking waters (except bottled waters) and in fresh and marine bathing waters.
This method was compared to the reference method: the NF EN ISO 7899-2 (August 2000) standard according to the general protocol of AFNOR Certification (rev 2 – May 2013).

This extension validation study for enumeration of enterococci includes:

- The addition of a new category: fresh and marine bathing waters using a new protocol,
- The use of Quanti-Tray® or Quanti-Tray® 2000 for this new category.

The aim of this extension study is to evaluate the performances of the alternative method against the reference method for the new category. The following parameters were studied:

- the relative accuracy,
- the linearity,
- the limits of detection and the limits of quantification,

1.2. Alternative method

1.1.1. Principe of the method

Enterolert-DW uses a Defined Substrate Technology (DST) nutrient indicator to detect enterococci. When coupled with the IDEXX Quanti-Tray® System, Enterolert-DW provides quantitative confirmed results in 24 hours. Enterolert-DW utilizes ortho-nitrophenyl-β-D-glucoside as a nutrient indicator and incorporates a specifically designed blue background color in its formulation. When the enzyme substrate is metabolized by enterococci, the sample turns from blue to green to indicate detection. Any change from the original color to green is considered a positive result. No ultraviolet light source is required.

Enterolert-DW detects enterococci in drinking water samples in 24 hours.

1.1.2. Protocols of the method

- **Current protocol (for drinking water):**

- Add the Enterolert-DW reagent to a 100 mL sample at ambient temperature. Mix to completely dissolve the reagent. Pour the mix into a Quanti-Tray®,
- Seal with a Quanti-Tray® Sealer,
- Incubate at 41±0.5 °C for 24 to 28 hours,
- Enumerate the yellow wells then look at the MPN table for the enumeration of enterococci,
- Express the results: Number of enterococci / 100 mL of water.

- **New protocol (for fresh and marine bathing water)**

- Add the Enterolert-DW reagent to a 100 mL **diluted at 1/10th** sample at ambient temperature. Mix to completely dissolve the reagent. Pour the mix into a Quanti-Tray®,
- Seal with a Quanti-Tray® Sealer,
- Incubate at 41±0.5 °C for 24 to 28 hours,
- Enumerate the yellow wells then look at the MPN table for the enumeration of enterococci,

- Express the results taking into account the dilution factor if necessary: Number of enterococci / 100 mL of water.

Note: the dilution of the sample will be applied for the alternative method and the reference method.

Protocols of the alternative method are presented in Appendix A.

1.3. Scope of application

The application scope of the method Enterolert-DW / Quanti-Tray® or Quanti-Tray® 2000 relates to the human drinking waters (except bottled waters) and the bathing (fresh and marine) waters.

1.4. Reference method

The ISO 7899-2 (August 2000) standard: « Water quality – Detection and enumeration of intestinal enterococci – Part 2 : method by membrane filtration » was used as the reference method.

A supplementary dilution at $1/10^{th}$ (or $1/100^{th}$ for high contamination samples) was realized for bathing water samples.

The protocol of the reference method is presented in Appendix B.

2. Comparative study

2.1. Relative accuracy

Relative accuracy is defined as the closeness of agreement between test result and the accepted reference value.

2.1.1. Number and nature of the samples

Two categories of waters were tested in duplicate with the reference method and the alternative method.

The samples analyzed are presented in table 1.

Table 1: nature and number of samples analyzed

Category	Type of water	Samples analyzed	Samples exploited
❶ **Water for human consumption**	Tap water and fountain water	56	14
	Well, spring and drilling water	22	6
	Total	**78**	**20**
❷ **Bathing waters**	Fresh bathing waters	18	13
	Marine bathing waters	19	13
	Total	**37**	**26**
TOTAL		**115**	**46**

115 samples were analyzed: 78 during the initial validation and 37 during the extension study.

During the past renewal studies for the category ❶, some samples were withdrawn from the statistical analysis. These samples corresponded:

- in 2017: to samples for which a result inferior to 4 CFU/100 mL for at least one of the replicates was obtained,

in 2021: to samples for which at least one of the replicates of the reference method was superior to 80 CFU/100 ml as stated in ISO 8199.The results obtained from the analysis of these samples are presented in raw results but not integrated in the statistical treatment.

During the extension study for the category ❷, 37 samples were analyzed with 26 samples exploited. The other samples not exploited are presented in raw results but are not integrated in the statistical treatment.

2.1.2. Artificial contaminations

During the initial validation study in the category ❶, no naturally contaminated samples were analyzed. All samples were artificially contaminated but contamination levels cover the whole measurement range of the alternative method.

During the extension study for the category ❷, among the samples exploited, 4 were naturally contaminated, 2 were obtained from cross-contaminations with naturally contaminated sewage treatment plant waters. The other 20 samples were obtained by artificial contamination.
The stress applied and the strains used for the two categories are presented in Appendix C.

2.1.3. Equivalence between Quanti-Tray® and Quanti-Tray® 2000

A study carried out in 2014 having demonstrated the equivalence between the Quanti-Tray® and the Quanti-Tray® 2000 for the human drinking waters.
In the current extension study, samples were analyzed by Quanti-Tray® or by Quanti-Tray® 2000. The choice was made based on the contamination rate of the target analyte and the samples analyzed with the Quanti-Tray® 2000 are identified in the raw data.

2.1.4. Raw results

Raw results and statistics are summarized in tables 2 and 3 and in Appendix D.

Figure 1 presents the two-dimensional graphics for the human drinking waters (category ❶) and the figure 2 for the bathing waters (category ❷).
For category ❶, the results are expressed in raw data and in log. For category ❷, due to the extent of the range of contamination, the results are expressed only in log MPN/100 mL.
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" appears in dotted line.

Figure 1: two-dimensional graphics presenting the raw results of the accuracy study (in MPN/100 mL and in log MPN/100 mL) in human drinking waters

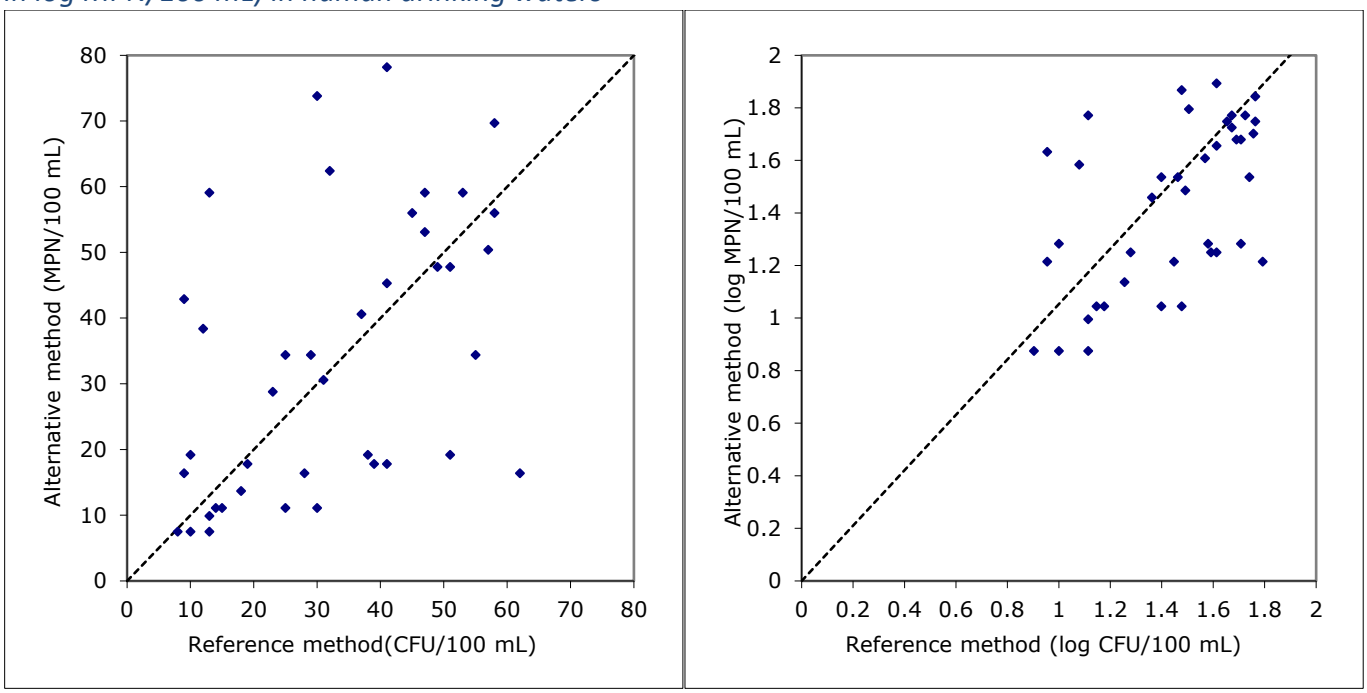
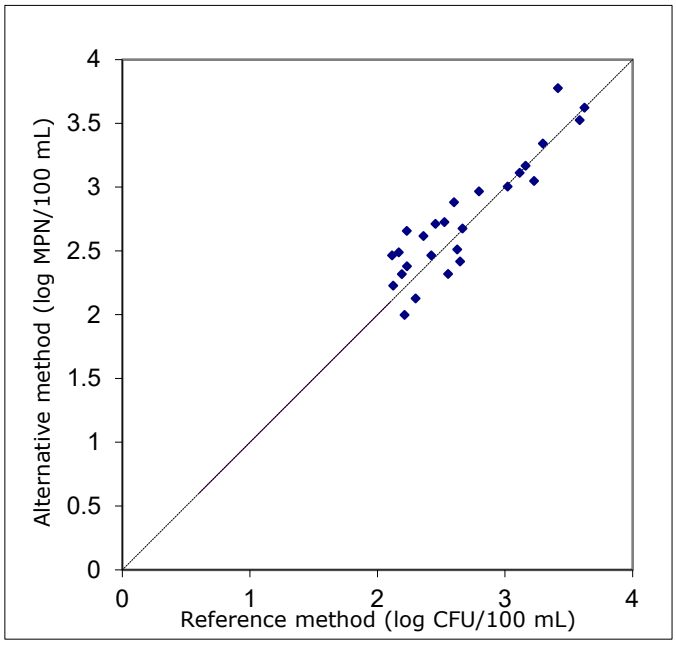


Figure 2: two-dimensional graphic presenting the raw results of the accuracy study (in log MPN/100 mL) in bathing waters



2.1.4. Statistical exploitation

The relationship between the reference method and alternative method is evaluated by linear model: y = a + bx, with y representing the alternative method and x the reference method. Statistical data, bias and repeatability of the two methods are shown in table 2 and table 3.

The best accuracy between the two methods is reached if the equation y = a + bx is equal to the theoretical model y = x.

The intercept "a" is theoretically zero in this ideal model (case [a = 0]). The estimated intercept obtained with both methods was checked using p {a} = 0. If the alternative method shows a systematic bias against the reference method, the probability p {a = 0} is less than α = 0.05.

The slope "b" is theoretically equal to 1 in the ideal model (hypothesis [b = 1]). The estimated slope obtained with both methods must be verified 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 choice of the linear regression method is compared to the value of the robustness of the ratio R of the standard deviations of repeatability overall:

-if Rob.R> 2, a linear regression least squares (OLS 1) is used with the x-axis for the reference method,

-if Rob.R <0.5, a linear regression least squares (OLS 2) is used with the x-axis for the alternative method,

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

Table 2: statistical data for the enumeration of enterococci

Category	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
									Ord. at 0	Ord. at 1
❶	Raw	1.325	GMFR	2.101	-6.384	1.086	1.251	0.899	28.4	37.4
	Log	1.098	GMFR	2.101	-0.204	0.524	1.140	0.729	60.2	47.0
❷	Log	1.836	GMFR	2.064	0.084	0.541	0.996	0.049	59.1	96.1

Table 3: bias and repeatability of the two methods (RM: reference method and AM: alternative method)

Category	Data	Bias (D)		Repeatability			
		Average	Median	R		rob. r	
				MR	MA	MR	MA
❶	Raw	1.675	1.900	20.671	18.539	11.742	15.558
	Log	-0,003	0.024	0,313	0,233	0,249	0,274
❷	Log	0.073	0.045	0.178	0.345	0.173	0.318

2.1.5. Conclusion

- **Drinking waters**

The equation of the regression line for the couple « enterococci – human drinking waters » is the following:

Alt = 1.251 Ref – 6.384 or **log Alt = 1.140 log Ref -0.204**

The hypothesis [a=0 and b=1] is accepted for the tested category. The bias between the two methods is 1.900 CFU/100 mL for the raw data or 0.024 log CFU/100 mL.

- **Bathing waters**

The equation of the regression line for the couple « enterococci – bathing waters » is the following:

log Alt = 0.966 log Ref + 0.084

The hypothesis [a=0 and b=1] is accepted for the tested category. The bias between the two methods is 0.045 log CFU/100 mL.

The accuracy of the alternative method is satisfactory.
It is possible to use the Quanti-Tray® or Quanti-Tray® 2000 test for the bathing water category.

2.2. Linearity

Linearity is defined as the ability of the method to provide results proportional to the amount of microorganisms present in the sample, an increase of the analyte is a linear increase or proportional results.

2.2.1. Contamination levels

For drinking water pairs, four contamination levels were tested in duplicate by the reference method and the alternative method.
For bathing waters pairs, five contamination levels were tested in duplicate by the reference method and the alternative method.

The two “matrix / strain” pairs analyzed are presented in table 4.

Table 4: matrix-strain pairs analyzed for drinking waters and bathing waters

Category	Strain	Matrix	Target contamination levels (CFU/100 or 250 mL)
❶	*Enterococcus faecalis* ENTC.1.4 (river water)	Tap water	10 – 30 - 50 - 100
❷	*Enterococcus faecium* TJV000 (marine water)	Marine water	120 – 200 – 500 – 1000 - 5000

2.2.2. Raw results

Raw results and statistics are summarized in Appendix E.

Figure 3 presents the two-dimensional graphics for the drinking water category and figure 4 presents the two-dimensional graphic for the bathing waters.
For category ❶, the results are expressed in raw data and in log, on the other hand for category ❷, due to the extent of the range of contamination, the results are expressed only in log MPN/100 mL.

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" appears in dotted line.

Figure 3: two-dimensional graphics presenting the raw results of the accuracy study (in MPN/100 mL and in log MPN/100 mL) for drinking waters

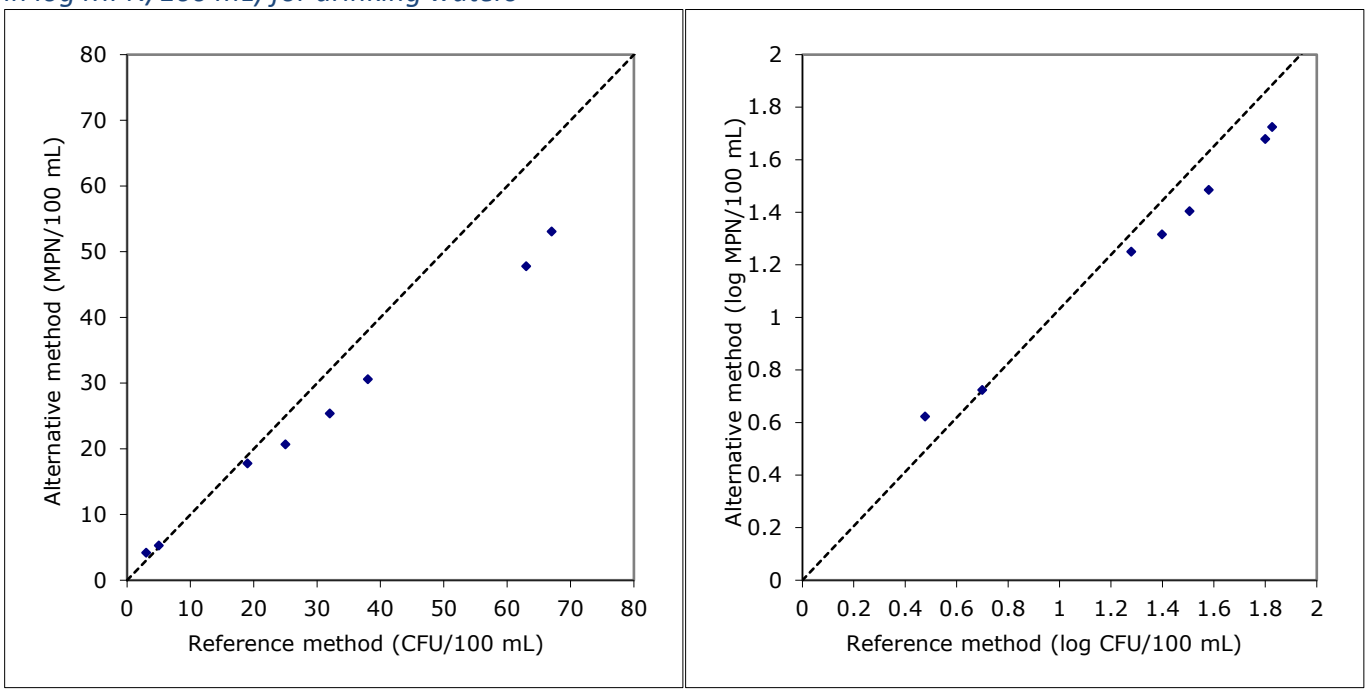
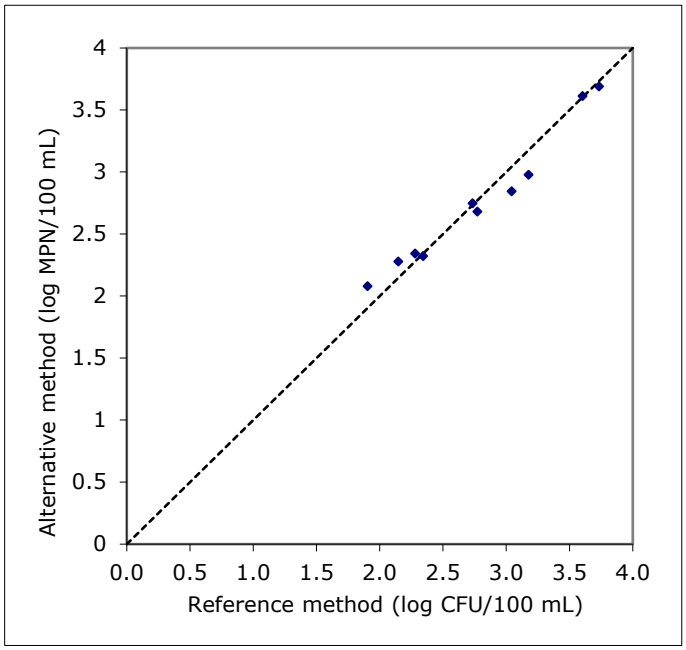


Figure 4: two-dimensional graphic presenting the raw results of the accuracy study (in log MPN/100 mL) for bathing waters



2.2.3. Statistics

Statistical interpretations are made according to the requirements of the NF ISO 16140 standard (see table 5).

The choice of the linear regression method is compared to the value of the robustness of the ratio R of the standard deviations of repeatability overall:

- if Rob.R> 2, a linear regression least squares (OLS 1) is used with the x-axis for the reference method,
- if Rob.R <0.5, a linear regression least squares (OLS 2) is used with the x-axis for the alternative method,
- if 0.5 <Rob.R <2, an orthogonal regression (GMFR) is used with the x-axis to the reference method.

Table 5 : statistical data for linearity

Category	Data	Rob. R	Regression used	F critique	Rob. F	P (Rob.F)	Correlation coefficient (r)	Regression
❶	Raw	0.810	GMFR	5.41	-1.881	0.266	1.000	Alt = 0.746 Ref + 2.121
	Log	0.550	GMFR	5.41	-1.674	0.296	0.999	log Alt = 0.827 log Ref + 0.184
❷	Log	0.594	GMFR	5.41	6.334	0.037	0.985	log Alt = 0.890 log Ref + 0.289

The relationship between the two methods isn't linear:

- if Rob.F > F critical

Or - if P(Rob.F) < α (=0,05)

2.2.4. Conclusion

The relation between the two methods is linear both when the calculations are applied from the raw data and from the data converted in logarithm.

The linearity of the alternative method is satisfactory.

2.3. Limits of detection and limits of quantification

The critical level (LC) is defined as the smallest amount that can be detected, but not quantified as an exact value.

The detection limit (LOD) is defined as the level above the critical level.

The quantification limit (LOQ) is defined as the smallest amount of analyte that can be measured and quantified with an accuracy and precision defined under the experimental conditions.

2.3.1. Test protocols

- **Protocol ❶ for human drinking water (initial validation)**

The limits of detection and quantification were determined by analyzing a pure culture of *Enterococcus faecalis* ENTC.1.5, isolated from surface water, by the alternative method. Five levels of contamination (including level 0), with six replications for each level, were studied in sterilized water.

- **Protocol ❷ for bathing water (extension study)**

The limits of detection and quantification were determined by analyzing a pure culture of *Enterococcus faecalis* BDWL25, isolated from marine bathing water, by the alternative method. Ten levels of contamination (including level 0), with six replications for each level, were studied in sterilized water.

2.3.2. Results

Results are shown in table 6 for the protocol ❶ and in table 7 for the protocol ❷.
Raw data are available in Appendix F.

Table 6: data (S0 et X0) of enterococci enumeration for the protocol ❶

Level (CFU/100mL)	Number of positive samples	Standard deviation (So)	Bias (Xo)
0.000	0	0.000	0.000
0.233	1	0.408	0.000
0.500	2	0.837	0.000
0.967	**5**	**0.753**	**1.000**
1.700	4	1.577	1.000
3.467	6	2.279	4.750

Table 7: data (S0 et X0) of enterococci enumeration for the protocol ❷

Level (CFU/100mL)	Number of positive samples	Standard deviation (So)	Bias (Xo)
0	0	0.000	0.000
5.0	1	8.981	0.000
6.3	2	5.164	0.000
7.7	**3**	**8.165**	**5.000**
8.1	4	12.111	15.000
12.0	4	17.225	10.000
18.0	5	12.554	15.000
35.0	5	22.015	42.000
52.0	6	37.538	36.500
100.0	6	22.040	81.000

Table 8 : values obtained for enterococci enumeration for the two protocols

Protocol	Parameter	Formulas	Value
❶	LC	1,65 So + Xo	2.24
	LOD	3,3 So + Xo	3.48
	LOQ	10 So + Xo	8.53
❷	LC	1,65 So + Xo	18.47
	LOD	3,3 So + Xo	31.94
	LOQ	10 So + Xo	86.65

2.3.3. Conclusion

The LOD and LOQ of the alternative method look satisfying for the two protocols applied.

2.4. Selectivity

Specificity is defined as the ability of the method to accurately measure a given analyte, or quantity in the sample without interference from non-target components. Selectivity is defined as the ability of the method to measure the analyte only.

2.4.1. Test protocols

Thirty target strains and thirty non target strains were analyzed. Assays were realized according to the protocol of the alternative method.

Contamination levels used for the inclusivity are comprised between 30 and 100 CFU/100 mL and are 10^3 to 10^5 superior to the detection level of the alternative method for exclusivity (around 10^4 CFU/mL).

2.4.2. Results

Results are presented in Appendix G.

The thirty strains of enterococci tested were detected by the alternative method.

No non-target strain showed any cross-reaction with the alternative method.

2.4.3. Conclusion

The selectivity of the alternative method is satisfactory.

2.5. Praticability

The practicability was evaluated according to the 13 criteria defined by AFNOR Technical Committee.

1- Mode of packaging of test components
The Enterolert-DW reagent is conditioned on single capsules.
The Quanti-Tray® and Quanti-Tray® 2000 devices are conditioned by ten in aseptic bags.

2- Reagents volumes
Many formats are available (20 tests, 100 tests or 200 tests).

3- Storage conditions of components and shelf-life of unopened products
Storage temperature of Enterolert-DW is 2 - 25 °C. Storage temperature of Quanti-Tray is 4 - 30°C.
The products have a shelf-life of 12 months.

4- Modalities after first use
Each Enterolert test and each Quanti-Tray® serves a unique analysis and should not be reused.

5- Equipment and specific local requirements
Quanti-Tray® Sealer model 2X or more recent model

6- Reagents ready to use or for reconstitution
None.
Reagents of the alternative method do not contain any toxic substance unlike the reference method where the confirmation step needs the use of sodium azide (toxic).

7- Training period for operator with no experience of the method

Use of the method does not require a special training. The duration of training is estimated to be 1 hour.

8- Handling time and flexibility of the method in relation to the number of samples

The duration of a filtration by the method NF EN ISO 7899-2 is around 1,5 min using disposable filtration units and around 3,5 min using non disposable units.
The duration of use of the method Enterolert-DW / Quanti-Tray® or Quanti-Tray® 2000 is around 2 min.
The alternative method does not require a confirmation step unlike the reference method.

9- Time required for results

The time-to-result for the Enterolert-DW / Quanti-Tray® is 24 hours.

The time-to-result for the method NF EN ISO 7899-2 is 48 hours (44 hours for the enumeration and 4 hours for the confirmation)

10- Operator qualification

Level inferior to the one required for the reference method due to the reading of the Quanti-tray® racks easier than the enumeration and the confirmation of the colonies for the reference method.

11- Steps common with the reference method

None.

12- Traceability of analysis results

None.

13- Maintenance by laboratory

None.

3. Interlaboratory study

The main object of the collaborative study is to determine the variability of the results obtained by different laboratories analyzing identical samples and to compare these results within the framework of the comparative study of the methods.

3.1. Study organisation

3.1.1. Participating laboratories

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

3.1.2. Matrix and strain

A dechlorinated tap water was used as test matrix. It was contaminated with a strain of *Enterococcus faecalis* (ENTC.1.10) isolated from a river water.

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

3.1.3. Stability of the strain in the 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.

Table 8 : results of the enumerations in CFU/100 mL of the strain *Enterococcus faecalis* ENTC.1.10 in dechlorinated tap water for 3 days at 5±3°C

	Level 1	Level 2	Level 3
D0	28	91	130
D1	35	76	143
D2	33	82	139

The tested strain looks stable at 5±°C in the matrix.

3.1.4. Samples preparation and spiking

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

- level 0 : 0 CFU/100 mL,
- level 1 : from 1 to 20 CFU/100 mL,
- level 2 : from 20 to 80 CFU/100 mL,
- level 3 : from 80 to 150 CFU/100 mL.

The matrix was distributed at 100 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.

Table 9 : target level, real level and endogenous flora of the matrix

Level	Culturable microorganisms (CFU/mL)		*Enterococcus faecalis* ENTC.1.10 (CFU /100 mL)	
	22°C	36°C	Target level	Real level at D0
0	15	32	0	0
1			1 to 20	21
2			20 to 80	71
3			80 to 150	113

3.1.5. Samples labelling

The labeling of the vials was realized as follows: a code to identify the laboratory: from A to M (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.

Table 10 : sample code by contamination level

Level (CFU / 100 mL)	Sample code
0	1 / 8
1 to 20	2 / 5
20 to 80	4 / 7
80 to 150	3 / 6

3.1.6. Samples shipping, reception and analysis

The samples were shipped in a coolbox the 10th of June 2013.

The coolboxes were received in 24 hours for 10 laboratories and in 48 hours for 3 laboratories. The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory. The samples were analyzed on the 12th of June. The expert laboratory concurrently analyzed a set of samples under the same conditions with both methods.

The laboratory E stopped its participation to the study after reception of the samples.

Analyses were thus realized by 12 laboratories.

3.2. Results

3.2.1. 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.

Table 11 : temperature and state of the samples upon reception

Laboratory	Temperature	State of the samples	Probe temperature	
			Mean	SD
A	5.6°C	Good	3.45°C	1.76°C
B	6.1°C	Good	3.68°C	0.47°C
C	5.1°C	Good	2.10°C	0.35°C
D	3.5°C	Good	3.43°C	0.31°C
E	/	/	5.67°C	0.78°C
F	8.8°C	Good	5.57°C	0.68°C
G	5.8°C	Good	2.69°C	0.42°C
H	2.8°C	Good	4.68°C	1.37°C
I	6.8°C	Good	3.51°C	0.53°C
J	10.5°C	Good	2.25°C	0.25°C
K	7.4°C	Good	6.51°C	0.49°C
L	10.1°C	Good	3.49°C	1.18°C
M	9.9°C	Good	3.89°C	0.60°C

Temperatures are correct for 8 laboratories. Laboratories F, J, L and M showed temperatures superior to 8°C.
The analyses of the thermal profiles of the probes showed that the shipping of the samples was realized at a correct temperature, with means comprised between 2.10°C and 6.51°C.

3.2.2. Enumerations of culturable microorganisms

For the whole laboratories, the enumerations at 22°C vary between 79 and 145 CFU/mL. Concerning the enumerations at 36°C, the results were varying between <1 and 30 CFU/mL.

3.2.3. Results from expert laboratory and participating laboratories

The overall results are presented in table 12.

Table 12 : results of the interlaboratory study

Labo-ratory	Level 1				Level 2				Level 3			
	MR (CFU/100 mL)		MA (MPN/100 mL)		MR (CFU/100 mL)		MA (MPN/100 mL)		MR (CFU/100 mL)		MA (MPN/100 mL)	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
A	26	23	12	16	55	56	38	59	100	90	89	101
B	16	23	31	16	37	41	70	59	59	88	95	89
C	20	20	16	22	52	64	62	59	86	82	101	101
D	24	19	34	19	52	64	59	70	102	121	130	118
F	27	18	29	21	76	71	50	66	116	95	78	109
G	22	13	34	24	52	45	78	74	101	105	109	118
H	32	20	18	15	62	68	62	56	92	87	62	145
I	23	21	29	22	53	55	59	78	86	96	95	95
J	26	28	15	22	64	68	70	89	94	94	89	130
K	26	12	21	21	63	63	48	43	96	87	95	145
L	19	21	22	29	45	58	43	43	84	98	78	83
M	26	35	19	29	63	58	56	70	96	100	89	118
Expert	18	17	22	22	57	55	56	59	108	96	145	95

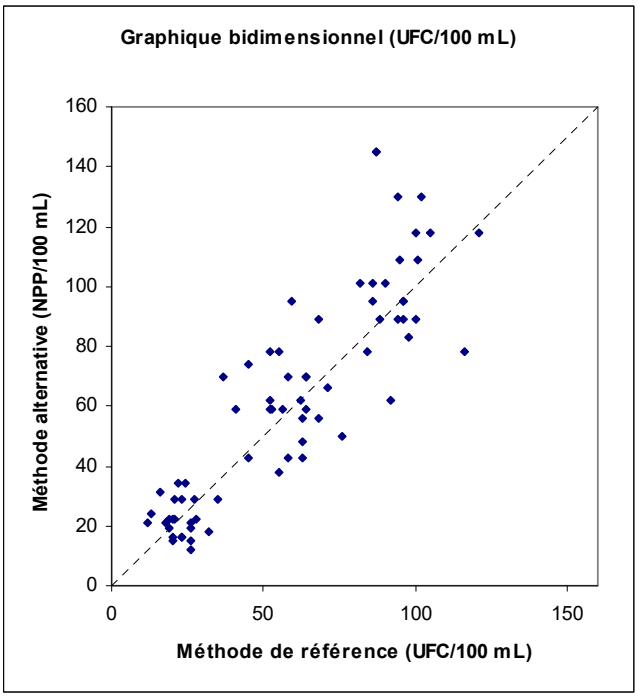
For level 0, all results were inferior to 1 CFU/100 mL for both methods.

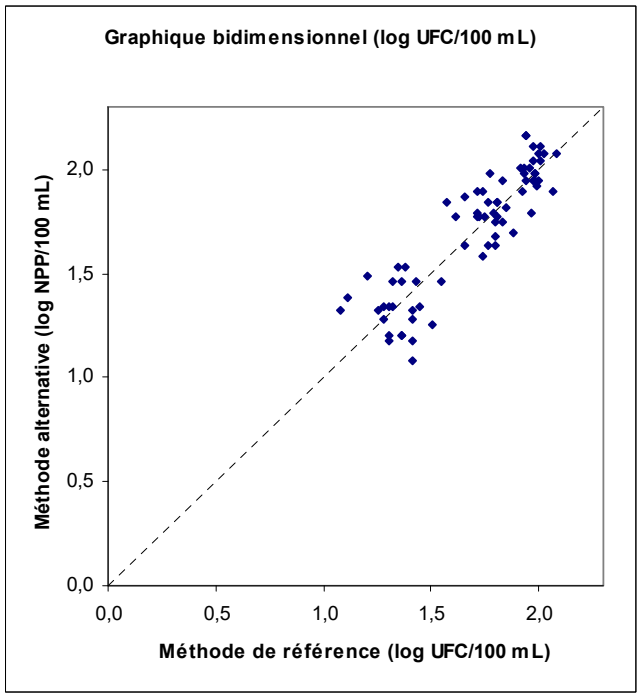
Laboratory F noticed the presence of three wells « very weakly doubtful" for one duplicate of the level 0 after 24 hours of incubation. No change of the coloration was observed after 4 hours of supplementary incubation. It seemed that these wells showed a very weak variation of colour that could not be considered as a positive result.

The data obtained by the laboratories are presented in the two bidimensional graphics of the figure 3 in CFU and MPN/100 mL and in logarithm for a better appreciation of the data (y = x in dotted line).

The highest level of contamination contains data over 80 CFU/100 mL for the reference method. However, due to the complexity of organizing an interlaboratory study and because the interpretation of the statistical tests is good for the three levels, the Expert Laboratory retained the data of all the collaborators at the highest level of contamination with the agreement of the Technical Board.

Figure 3 : two-dimensional graphics





3.3. Statistical interpretation

Data presented were calculated from raw results and from results converted in logarithm.

3.3.1. Bias

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

Table 13 : calculations of the bias of the alternative method

Values	MPN/100 mL			log MPN/100 mL		
Levels	**1 - low**	**2 - medium**	**3 - high**	**1 - low**	**2 - medium**	**3 - high**
Target value	21.5	58.0	94.5	1.352	1.763	1.975
Mean	22.3	60.9	102.6	1.332	1.775	2.002
Relative bias	0.039	0.050	0.086	-1.46%	0.65%	1.37%
Bias	1.039	1.050	1.086	-0.020	0.012	0.027

The accuracy is estimated by the bias which varies between -0.020 log MPN/100 mL (1.039 MPN/100 mL) and 0.027 log MPN/100 mL (1.086 MPN/100 mL).

3.3.2. 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 MPN/100 mL and in log MPN/100 mL.

Table 14 : tolerance values for the alternative method

Tolerance probability	Levels	MPN/100 mL			log MPN/100 mL		
		Low	**Medium**	**High**	**Low**	**Medium**	**High**
95%	Low tolerance value	42%	57%	53%	-0.282	-0.195	-0.184
	High tolerance value	166%	153%	161%	0.242	0.218	0.238
	Low tolerance limit	30%	30%	30%	-0.300	-0.300	-0.300
	High tolerance limit	170%	170%	170%	0.300	0.300	0.300

Figures 4 and 5 present the accuracy profiles.

Figure 4 : accuracy profile for a tolerance probability of 95% and a tolerance limit of 0,3 log

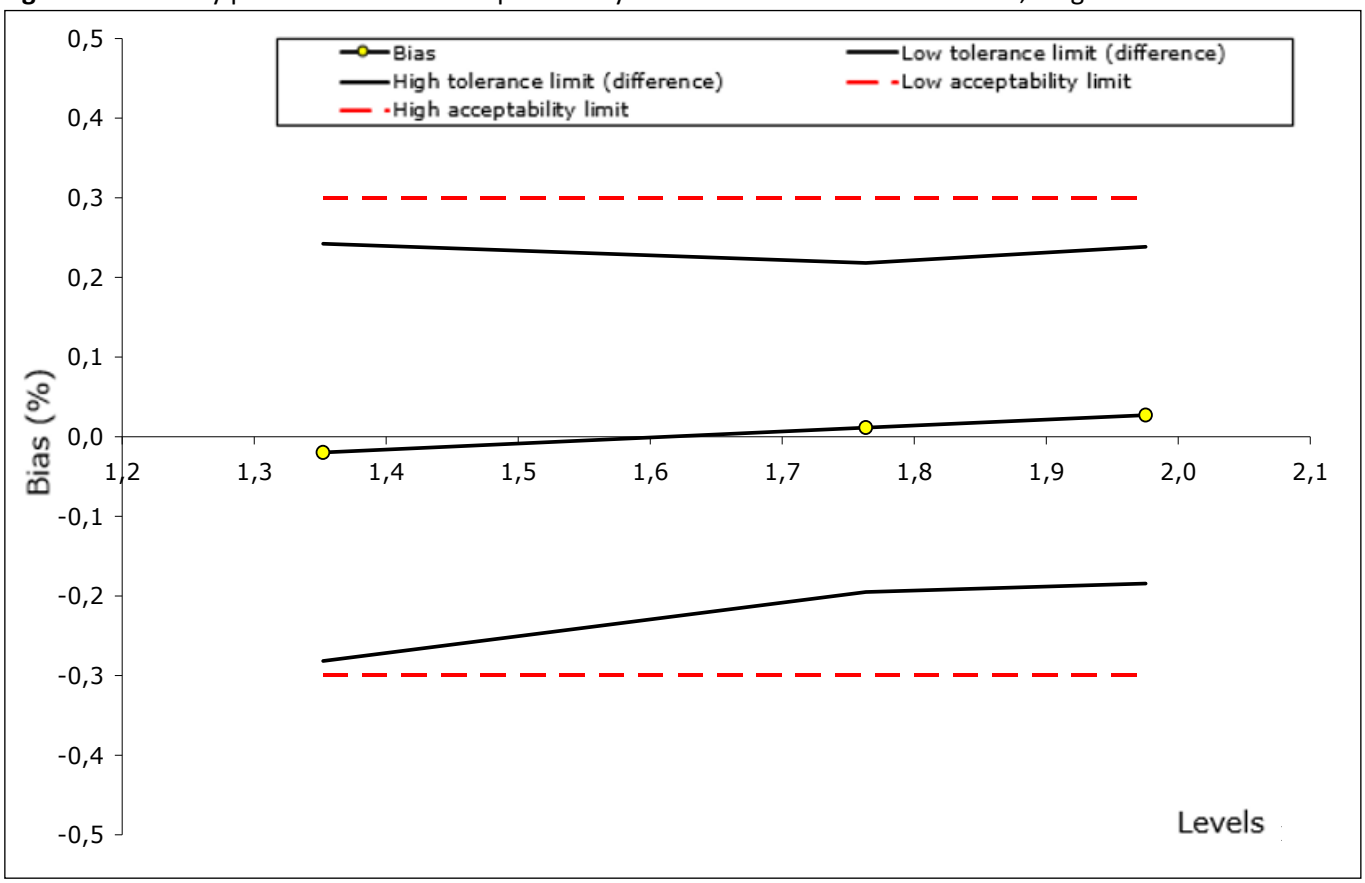
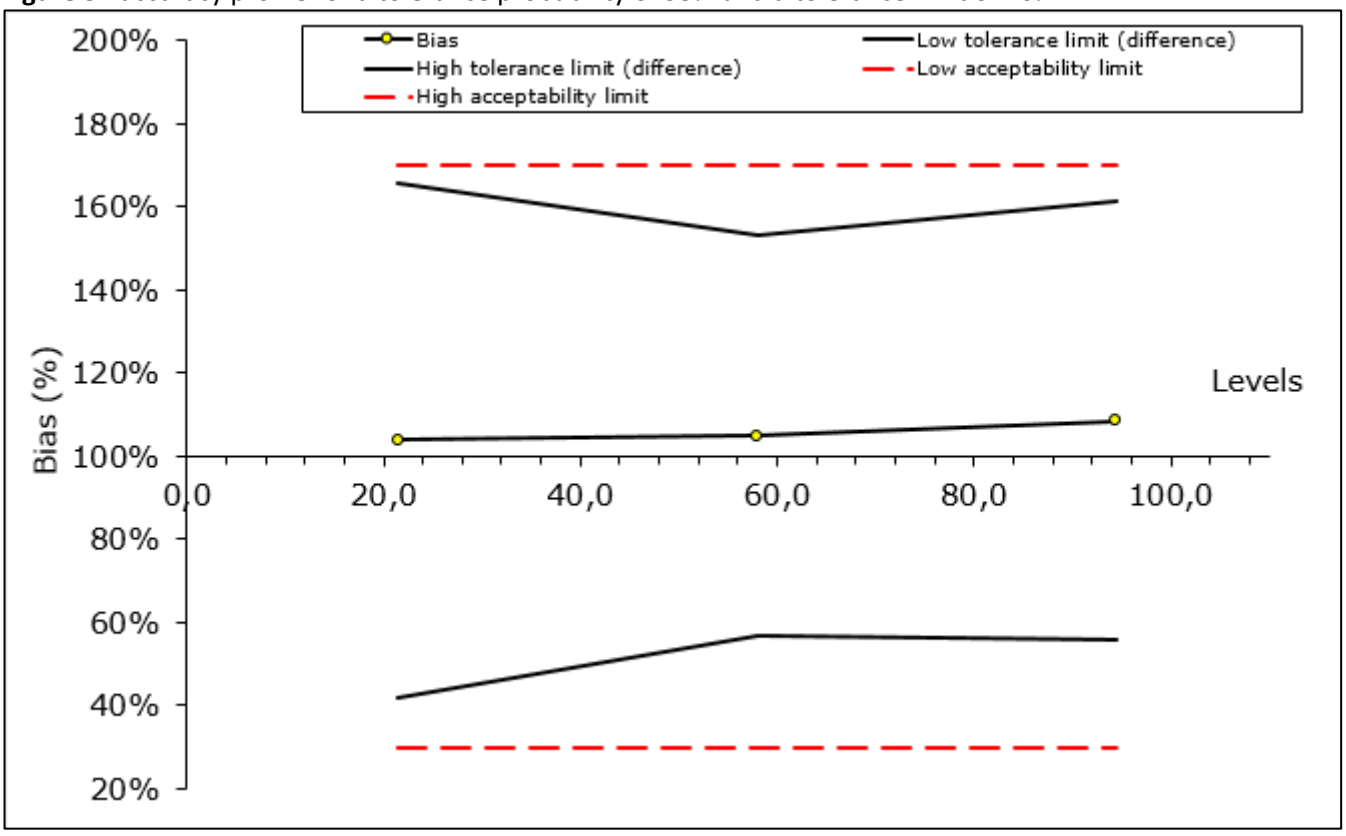


Figure 5 : accuracy profile for a tolerance probability of 95% and a tolerance limit of 70%



- Comments :

The bias of the alternative method goes up from the low level of contamination to the high level of contamination.

For all the contamination levels, the tolerance interval is comprised between the acceptability interval for a 95% tolerance probability and a limit at 0,3 log MPN/100 ml or 70% in MPN/100 mL.

3. Conclusions

- Comparative study

The linearity and relative accuracy of the Enterolert-DW / Quanti-Tray® or Quanti-Tray® 2000 method for the enumeration of enterococci in human drinking waters and bathing waters are satisfactory.

The bias between the two methods is acceptable for each category tested.

The use of Quanti-Tray® or Quanti-Tray® 2000 produces results equivalent to the reference method for bathing waters.

The limits of detection and quantification of the method are satisfactory for the two protocols.

Enterolert-DW / Quanti-Tray or Quanti-Tray 2000 method for the enumeration of enterococci is specific and selective.

- Interlaboratory study

The bias of the alternative method is 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 95% of the results will be between the limits of acceptability as defined at 0,3 log MPN/100 mL or 70% in MPN/100 mL.

Le Lion d'Angers, April 24, 2023
François Le Nestour
Head of the Microbiology Department

APPENDICES

APPENDIX A

ALTERNATIVE METHOD PROTOCOLS

ALTERNATIVE METHOD: CURRENT PROTOCOL

Step 1

Add the Enterolert-DW reagent to a 100 mL sample at ambient temperature Mix to completely dissolve the reagent

Pour the mix into a Quanti-Tray®

Step 2

Seal with a Quanti-Tray® Sealer

Step 3

Incubate at 41±0,5 °C for 24 to 28 hours

Step 4

Enumerate the green wells then look at the MPN table for the enumeration of enterococci

Step 5

Express the results

Number of enterococci / 100 mL of water

ALTERNATIVE METHOD: NEW PROTOCOL

for bathing waters

Step 1

Add the Enterolert-DW reagent to a 100 mL sample **diluted at 1/10th** at ambient temperature Mix to completely dissolve the reagent

Pour the mix into a Quanti-Tray ®

Step 2

Seal with a Quanti-Tray® Sealer

Step 3

Incubate at 41±0,5 °C for 24 to 28 hours

Step 4

Enumerate the green wells then look at the MPN table for the enumeration of enterococci

Step 5

Express the results (with dilution factor)
Number of enterococci / 100 mL of water

APPENDIX B

REFERENCE METHOD PROTOCOL

PROTOCOL STANDARD ISO 7899-2

Membrane filtration and incubation:

Filter 100 mL of sample on a sterile membrane.
Place the membrane on a Slanetz & Bartley agar
Incubate at 36±2 °C for 44 ± 4 hours

Reading

After incubation, consider as typical all colonies showing a colour red, brown or rose, either on the center or for the entire colony

Confirmation

Transfer the membrane and the colonies on a BEA agar Petri dish
pre-warmed at 44 °C.
Incubate at 44±0,5 °C for 2 h.
Read the Petri dish
Consider as typical every colony giving a black coloration in the agar medium and count them as intestinal enterococci.

Expression of the results

Number of enterococci / 100 mL of water

PROTOCOL ISO 7899-2 FOR BATHING WATERS

Membrane filtration and incubation: Filter 100 mL of **diluted at 1/10th** sample on a sterile membrane
Place the membrane on a Slanetz & Bartley agar
Incubate at 36±2 °C for 44 ± 4 hours

Reading

After incubation, consider as typical all colonies showing a colour red, brown or rose, either on the center or for the entire colony

Confirmation

Transfer the membrane and the colonies on a BEA agar Petri dish pre-warmed at 44 °C.
Incubate at 44±0,5 °C for 2 h.
Read the Petri dish
Consider as typical every colony giving a black coloration in the agar medium and count them as intestinal enterococci.

Expression of the results

Number of enterococci / 100 mL of water

APPENDIX C

STRESS OF THE BACTERIAL STRAINS

STRESS APPLIED AND STRAINS USED FOR THE ARTIFICIAL CONTAMINATIONS

Code	Strains	Origin	Stress applied	log NSM-log SM
ENTC.3.1	*E. hirae*	CIP 58.55	2 months at 5°C	0,9
ENTC.1.4	*E. faecalis*	River water	30 min at 56°C	0,6
ENTC.1.10	*E. faecalis*	River water	15 min at 60°C	1,4
ENTC.2.10	*E. faecium*	River water	15 min at 60°C	0,6
ENTC.2.9	*E. faecium*	River water	35 min at 60°C	1,6
ENTC.3.2	*E. hirae*	River water	2 months at 5°C	0,6
ENTC.1.12	*E. faecalis*	River water	15 min at 60°C	0,6
ENTC.7.4	*E. casseliflavus*	River water	15 min at 60°C + 25 min at 50°C	0,6
ENTC.5.2	*E. gallinarum*	River water	15 min at 60°C	1,0
ENTC.1.15	*E. faecalis*	River water	2 min in sodium hypochlorite diluted at 1/10000th	0,7
ENTC.7.3	*E. casseliflavus*	River water	2 cycles freezing-defrosting	0,5
ENTC.7.2	*E. casseliflavus*	River water	2 cycles freezing-defrosting	0,7
ENTC.6.1	*E. durans*	River water	2 mois at 5°C	0,9
ENTC.2.4	*E. faecium*	Surface water	15 min at 60°C	1,0
ENTC.2.5	*E. faecium*	Surface water	2 min sodium hypochlorite diluted at 1/10000th	1,3
ENTC.2.6	*E. faecium*	Surface water	3 min quaternary ammonium diluted at 1/1000th	0,8
ENTC.1.5	*E. faecalis*	Surface water	2 mois at 5°C	0,7
ENTC.1.7	*E. faecalis*	Surface water	1 min sodium hypochlorite diluted at 1/10000th	1,3
ENTC.2.8	*E. faecium*	River water	15 min at 60°C	2,1
ENTC.1.8	*E. faecalis*	River water	3 cycles freezing-defrosting	0,9
ENTC.1.9	*E. faecalis*	River water	15 min at 60°C	1,8
ENTC.1.11	*E. faecalis*	River water	3 cycles freezing-defrosting	0,8

Cat.	Type	Date	#	Matrix	Strain code	Strain	Origin	Injury protocol	Injury measurement
2	a	2022	2392479	Fresh bathing water "Villevêque"	BDWL25	*Enterococcus faecalis*	Bathing sea water	Spiking 10 min 60°C	0.5
2	a	2022	2392476	Fresh bathing water "Marigné"	QPD764	*Enterococcus mundtii*	Pond water	Spiking 20 min 60°C	0.5
2	a	2022	2392480	Fresh bathing water "La Chesnaie"	QPD764	*Enterococcus mundtii*	Pond water	Spiking 20 min 60°C	0.5
2	a	2022	2392506	Fresh bathing water "St Aubin Pouancé"	YHW642	*Enterococcus durans*	Well water	Spiking 5 min 60°C	0.5
2	a	2022	2392507	Fresh bathing water "Lac de Maine Angers"	SAK840	*Enterococcus faecium*	River water	Spiking 10 min 60°C	0.5
2	a	2022	2392508	Fresh bathing water "La Sablière"	QNA168	*Enterococcus mundtii*	River water	Spiking 20 min 60°C	0.8
2	a	2022	2392509	Fresh bathing water "Lac Cisba"	QNA169	*Enterococcus mundtii*	River water	Spiking 20 min 60°C	0.8
2	a	2022	2392513	Fresh bathing water "Krzywe Lake- main bathing area"	YHP824	*Enterococcus faecalis*	Fresh bathing water	Spiking 35 min 60°C	0.5
2	a	2022	2392514	Fresh bathing water "Krzywe Lake- swimming area for children"	QNA168	*Enterococcus mundtii*	River water	Spiking 20 min 60°C	0.8
2	a	2022	2392517	Fresh bathing water "Lac du Vioreau Joué s/Erdre"	YHW642	*Enterococcus durans*	Well water	Spiking 5 min 60°C	0.5
2	b	2022	2392457	Marine bathing water "Beach St Marguerite Dieppe"	QPD764	*Enterococcus mundtii*	Pond water	Spiking 10 min 60°C	0.5
2	b	2022	2392462	Marine bathing water "Beach Les Demoiselles St Jean de Monts"	QPD764	*Enterococcus mundtii*	Pond water	Spiking 10 min 60°C	0.5
2	b	2022	2392464	Marine bathing water "Ile de Ré"	YHW642	*Enterococcus durans*	Well water	Spiking 5 min 60°C	0.5
2	b	2022	2392466	Marine bathing water "Rochelle beach"	YHW642	*Enterococcus durans*	Well water	Spiking 5 min 60°C	0.5
2	b	2022	2392467	Marine bathing water "St Malo"	RSU238	*Enterococcus durans*	River water	Spiking 20 min 60°C	1.4
2	b	2022	2392469	Marine bathing water "Ingoldnells bathing water"	BDWL25	*Enterococcus faecalis*	Bathing sea water	Spiking 10 min 60°C	0.5
2	b	2022	2392470	Marine bathing water "Anderby creek bathing water"	SAK840	*Enterococcus faecium*	River water	Spiking 10 min 60°C	0.5
2	b	2022	2392471	Marine bathing water "Moggs"	YHP824	*Enterococcus faecalis*	Fresh bathing water	Spiking 35 min 60°C	0.5
2	b	2022	2392472	Marine bathing water "Chapel St Leonards bathing water"	YHP824	*Enterococcus faecalis*	Fresh bathing water	Spiking 35 min 60°C	0.5
2	b	2022	2392460	Marine water " Sables d'Olonne" beach - France	BDWL25	*Enterococcus faecalis*	Bathing sea water	Spiking 20 min 60°C	0.6
2	b	2022	2392458	Marine water "Dieppe Aubin sur mer" beach - France	BDWL25	*Enterococcus faecalis*	Bathing sea water	Spiking 20 min 60°C	0.6
2	b	2022	2392463	Marine water "Majorque" beach - Spain	BDWL25	*Enterococcus faecalis*	Bathing sea water	Spiking 20 min 60°C	0.6

APPENDIX D

RELATIVE ACCURACY
RAW DATA AND STATISTICAL CALCULATIONS

Relative accuracy - Raw results - Human drinking waters

at least 1 result <4 CFU/100 mL for one replicate of the reference method (presence unquantifiable)
at least 1 result >80 CFU/100 mL for one replicate of the reference method

#	Matrix	Strain	Species	Taux de contami-nation (UFC/100 mL)	Reference method						Alternative method					
					R1			R2			R1			R2		
					Number of colonies on Slanetz & Bartley	Number of colonies esculine + on BEA	log	Number of colonies on Slanetz & Bartley	Number of colonies esculine + on BEA	log	Number of yellow wells	MPN / 100 mL	log	Number of yellow wells	MPN / 100 mL	log
23	Fountain water Lisses	ENTC.1.10	*E. faecalis*	68	14	13	1,114	12	12	1,079	35	59,1	1,772	27	38,4	1,584
25	Tap water Montreuil-sous-Bois	ENTC.2.10	*E. faecium*	63	62	62	1,792	52	51	1,708	14	16,4	1,215	16	19,2	1,283
28	Tap water Paris	ENTC.1.10	*E. faecalis*	202	23	23	1,362	25	25	1,398	22	28,8	1,459	25	34,4	1,537
31	Tap water Paris 16ème	ENTC.2.9	*E. faecium*	10	37	37	1,568	47	45	1,653	28	40,6	1,609	34	56,0	1,748
33	Tap water Paris 20ème	ENTC.3.2	*E. hirae*	20	13	13	1,114	17	15	1,176	7	7,5	0,875	10	11,1	1,045
35	Tap water La Ville du Bois	ENTC.1.12	*E. faecalis*	20	29	28	1,447	27	25	1,398	14	16,4	1,215	10	11,1	1,045
36	Tap water Lozère	ENTC.7.4	*E. casseliflavus*	30	30	30	1,477	42	41	1,613	39	73,8	1,868	40	78,2	1,893
43	Tap water Clamart	ENTC.1.15	*E. faecalis*	90	42	41	1,613	39	39	1,591	15	17,8	1,250	15	17,8	1,250
46	Tap water Meudon la Forêt	ENTC.7.3	*E. casseliflavus*	120	31	31	1,491	52	51	1,708	23	30,6	1,486	31	47,8	1,679
49	Tap water Rosny sous Bois	ENTC.1.15	*E. faecalis*	30	12	10	1,000	13	13	1,114	16	19,2	1,283	9	9,9	0,996
51	Tap water Maisons Alfort	ENTC.2.5	*E. faecium*	50	58	58	1,763	58	58	1,763	38	69,7	1,843	34	56,0	1,748
52	Spring water Nantes	ENTC.2.6	*E. faecium*	60	47	47	1,672	47	47	1,672	33	53,1	1,725	35	59,1	1,772
54	Spring water Le Mont-Dore	ENTC.1.5	*E. faecalis*	80	56	55	1,740	57	57	1,756	25	34,4	1,537	32	50,4	1,702
58	Tap water Clermont-Ferrand	ENTC.1.7	*E. faecalis*	10	8	8	0,903	10	10	1,000	7	7,5	0,875	7	7,5	0,875
66	Tap water Elancourt	ENTC.2.4	*E. faecium*	20	28	19	1,279	42	38	1,580	15	17,8	1,250	16	19,2	1,283
68	Tap water Argenteuil	ENTC.2.8	*E. faecium*	10	30	30	1,477	18	18	1,255	10	11,1	1,045	12	13,7	1,137
69	Spring water Clermont-Ferrand	ENTC.1.8	*E. faecalis*	15	9	9	0,954	29	29	1,462	29	42,9	1,632	25	34,4	1,537
70	Spring water Volvic	ENTC.5.2	*E. gallinarum*	20	9	9	0,954	14	14	1,146	14	16,4	1,215	10	11,1	1,045
71	Drilling water Loire	ENTC.2.10	*E. faecium*	30	41	41	1,613	49	49	1,690	30	45,3	1,656	31	47,8	1,679
72	Drilling water Paris region 1	ENTC.1.9	*E. faecalis*	35	33	32	1,505	53	53	1,724	36	62,4	1,795	35	59,1	1,772
Samples not interpreted																
7	Tap water Rosny sous Bois	ENTC.3.1	*E. hirae*	119	2	2	0,301	3	3	0,477	2	2,0	0,301	1	1,0	0,000
8	Tap water Massy	ENTC.3.1	*E.hirae*	119	2	2	0,301	3	3	0,477	1	1,0	0,000	1	1,0	0,000
12	Fountain water Massy	ENTC.1.4	*E. faecalis*	400	2	2	0,301	8	8	0,903	2	2,0	0,301	1	1,0	0,000
13	Tap water Bordeaux	ENTC.3.1	*E. hirae*	412	4	4	0,602	3	3	0,477	7	7,5	0,875	6	6,4	0,806
50	Drilling water Mitry Mory	ENTC.2.4	*E. faecium*	10	8	5	0,699	6	4	0,602	1	1,0	0,000	5	5,3	0,724
62	Spring water Royat	ENTC.6.1	*E. durans*	80	6	5	0,699	4	4	0,602	1	1,0	0,000	3	3,1	0,491
38	Tap water Orly	ENTC.5.2	*E. gallinarum*	40	131	111	2,045	107	102	2,009	44	101,3	2,006	46	118,4	2,073
47	Tap water Saint Chéron	ENTC.7.2	*E. casseliflavus*	10	104	104	2,017	108	103	2,013	44	101,1	2,005	48	144,5	2,160
65	Tap water Nantes	ENTC.2.4	*E. faecium*	110	106	106	2,025	118	118	2,072	40	78,2	1,893	45	109,1	2,038
76	Drilling water Paris region 1	ENTC.1.11	*E. faecalis*	100	84	82	1,914	98	95	1,978	37	65,9	1,819	39	73,8	1,868

Relative accuracy - Enterococci - Human drinking water - Raw data

Reference method					Alternative method					Difference
Sample	Rep 1	Rep 2	M	SD	Sample	Rep 1	Rep 2	M	SD	
1	13	12	12,5	0,707	1	59,1	38,4	48,75	14,637	36,250
2	62	51	56,5	7,778	2	16,4	19,2	17,80	1,980	-38,700
3	23	25	24,0	1,414	3	28,8	34,4	31,60	3,960	7,600
4	37	45	41,0	5,657	4	40,6	56,0	48,30	10,889	7,300
5	13	15	14,0	1,414	5	7,5	11,1	9,30	2,546	-4,700
6	28	25	26,5	2,121	6	16,4	11,1	13,75	3,748	-12,750
7	30	41	35,5	7,778	7	73,8	78,2	76,00	3,111	40,500
8	41	39	40,0	1,414	9	17,8	17,8	17,80	0,000	-22,200
9	31	51	41,0	14,142	10	30,6	47,8	39,20	12,162	-1,800
10	10	13	11,5	2,121	12	19,2	9,9	14,55	6,576	3,050
11	58	58	58,0	0,000	13	69,7	56,0	62,85	9,687	4,850
12	47	47	47,0	0,000	14	53,1	59,1	56,10	4,243	9,100
13	55	57	56,0	1,414	15	34,4	50,4	42,40	11,314	-13,600
14	8	10	9,0	1,414	16	7,5	7,5	7,50	0,000	-1,500
15	19	38	28,5	13,435	18	17,8	19,2	18,50	0,990	-10,000
16	30	18	24,0	8,485	19	11,1	13,7	12,40	1,838	-11,600
17	9	29	19,0	14,142	20	42,9	34,4	38,65	6,010	19,650
18	9	14	11,5	3,536	21	16,4	11,1	13,75	3,748	2,250
19	41	49	45,0	5,657	22	45,3	47,8	46,55	1,768	1,550
20	32	53	42,5	14,849	23	62,4	59,1	60,75	2,333	18,250

q= 20 **Mx=** 32,150 **My=** 33,825 **M=** 1,675
n= 2 **MEDx=** 32,000 **MEDy=** 35,125 **MED=** 1,900
N=qn= 40 **SDbx=** 16,234 **SDby=** 20,834 Bias
MEDwx = 2,828 **MEDwy =** 3,748
SDwx= 7,382 **SDwy=** 6,621
rob. SDwx= 4,193 **rob. SDwy=** 5,556

Method choice
GMFR

R= 0,897
rob. R= 1,325
Sx= 16,874
Sy= 21,104

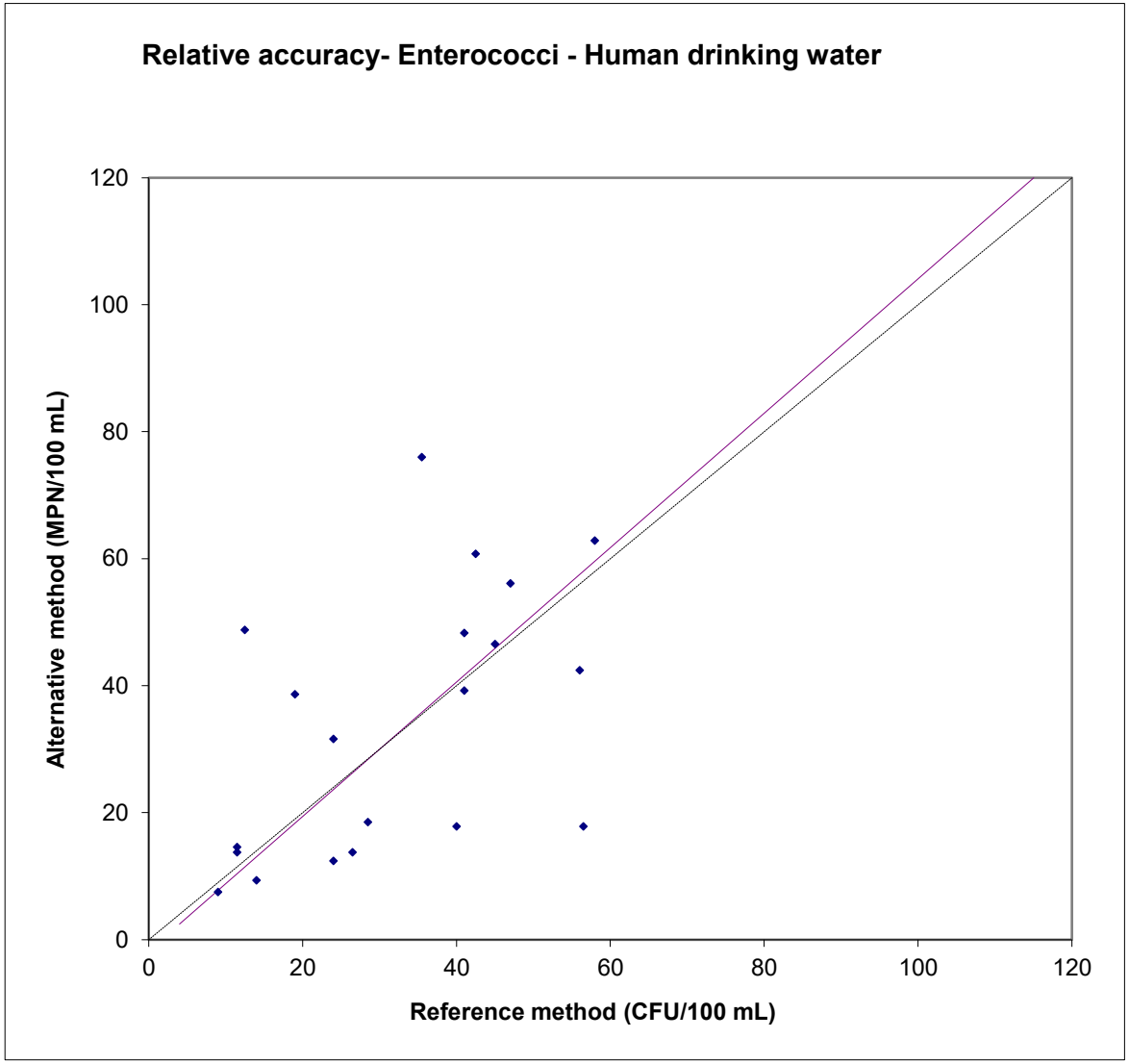
r= 0,529
b= 1,251 **Res. SEM=** 20,510
a= -6,384 **Res. SD=** 29,005

S(b)= 0,279 **p(t;b=1)=** 0,374 **t(b)=** 0,899
S(a)= 5,877 **p(t;a=0)=** 0,284 **t(a)=** 1,086

Repeatability	Reference method	Alternative method
r	20,671	18,539
rob. r	11,742	15,558

Est. y	Dev.
9,249	39,501
64,279	-46,479
23,632	7,968
44,893	3,407
11,125	-1,825
26,759	-13,009
38,015	37,985
43,643	-25,843
44,893	-5,693
7,999	6,551
66,155	-3,305
52,397	3,703
63,653	-21,253
4,872	2,628
29,260	-10,760
23,632	-11,232
17,379	21,271
7,999	5,751
49,896	-3,346
46,769	13,981

Points = mean of the repetitions for each sample



Relative accuracy - Enterococci - Human drinking water - Log data

Reference method					Alternative method					Difference
Sample	Rep 1	Rep 2	M	SD	Sample	Rep 1	Rep 2	M	SD	
1	1,114	1,079	1,097	0,025	1	1,772	1,584	1,678	0,132	0,581
2	1,792	1,708	1,750	0,060	2	1,215	1,283	1,249	0,048	-0,501
3	1,362	1,398	1,380	0,026	3	1,459	1,537	1,498	0,055	0,118
4	1,568	1,653	1,611	0,060	4	1,609	1,748	1,678	0,099	0,068
5	1,114	1,176	1,145	0,044	5	0,875	1,045	0,960	0,120	-0,185
6	1,447	1,398	1,423	0,035	6	1,215	1,045	1,130	0,120	-0,292
7	1,477	1,613	1,545	0,096	7	1,868	1,893	1,881	0,018	0,336
8	1,613	1,591	1,602	0,015	8	1,250	1,250	1,250	0,000	-0,352
9	1,491	1,708	1,599	0,153	9	1,486	1,679	1,583	0,137	-0,017
10	1,000	1,114	1,057	0,081	10	1,283	0,996	1,139	0,203	0,082
11	1,763	1,763	1,763	0,000	11	1,843	1,748	1,796	0,067	0,032
12	1,672	1,672	1,672	0,000	12	1,725	1,772	1,748	0,033	0,076
13	1,740	1,756	1,748	0,011	13	1,537	1,702	1,619	0,117	-0,129
14	0,903	1,000	0,952	0,069	14	0,875	0,875	0,875	0,000	-0,076
15	1,279	1,580	1,429	0,213	15	1,250	1,283	1,267	0,023	-0,162
16	1,477	1,255	1,366	0,157	16	1,045	1,137	1,091	0,065	-0,275
17	0,954	1,462	1,208	0,359	17	1,632	1,537	1,585	0,068	0,376
18	0,954	1,146	1,050	0,136	18	1,215	1,045	1,130	0,120	0,080
19	1,613	1,690	1,651	0,055	19	1,656	1,679	1,668	0,016	0,016
20	1,505	1,724	1,615	0,155	20	1,795	1,772	1,783	0,017	0,169

q= 24 **Mx=** 1,433 **My=** 1,430 **M=** -0,003
n= 2 **MEDx=** 1,487 **MEDy=** 1,540 **MED=** 0,024
N=qn= 48 **SDbx=** 0,264 **SDby=** 0,310 Bias
MEDwx = 0,060 **MEDwy =** 0,066
SDwx= 0,112 **SDwy=** 0,083
rob. SDwx= 0,089 **rob. SDwy=** 0,098

Method choice
GMFR

R= 0,743
rob. R= 1,098
Sx= 0,275
Sy= 0,313

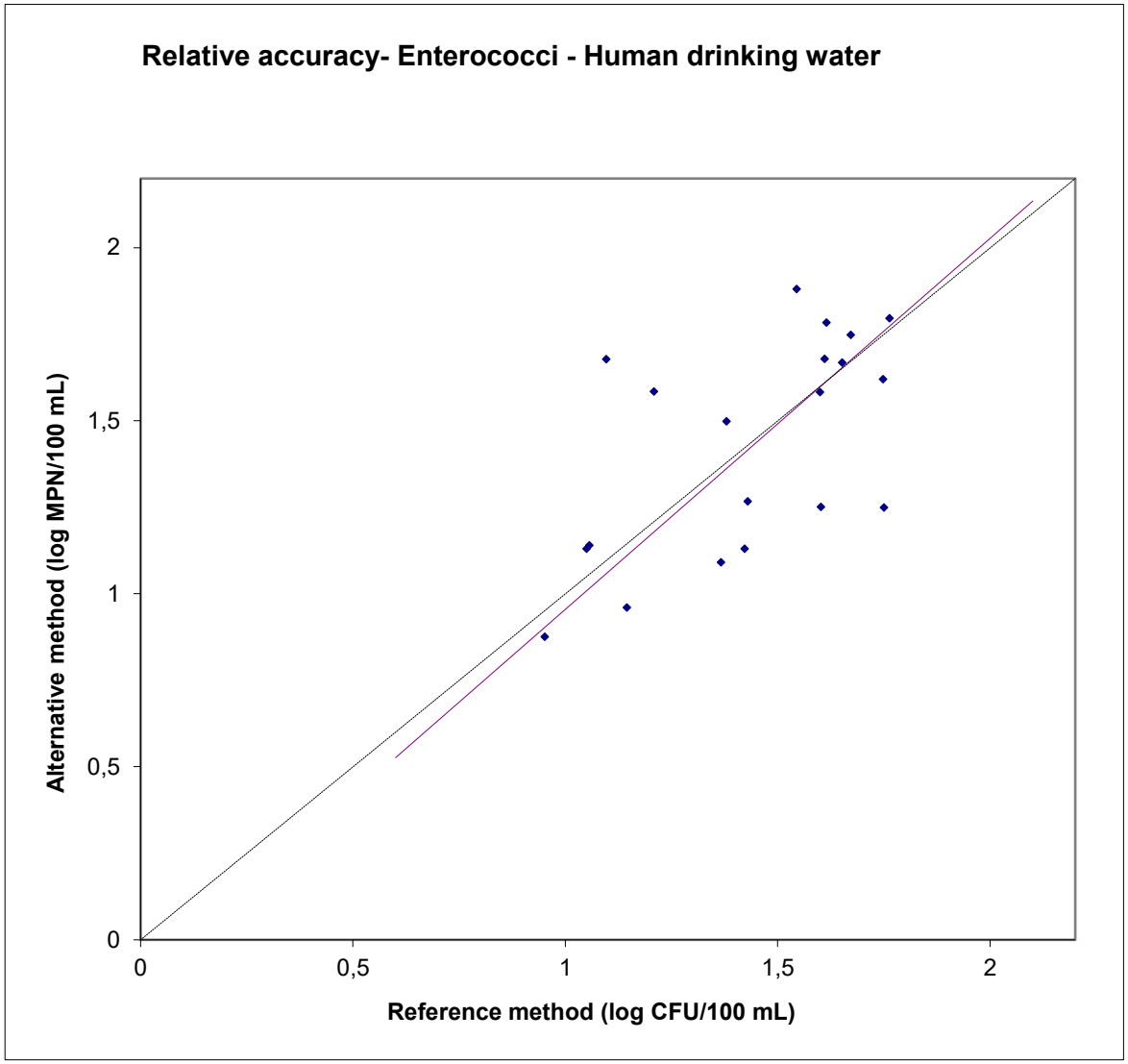
r= 0,602
b= 1,140 **Res. SEM=** 0,253
a= -0,204 **Res. SD=** 0,358

S(b)= 0,192 **p(t;b=1)=** 0,470 **t(b)=** 0,729
S(a)= 0,388 **p(t;a=0)=** 0,602 **t(a)=** 0,524

Repeatability	Reference method	Alternative method
r	0,313	0,233
rob. r	0,249	0,274

Est. y	Dev.
1,047	0,631
1,792	-0,543
1,370	0,128
1,633	0,045
1,102	-0,142
1,418	-0,288
1,558	0,323
1,623	-0,372
1,620	-0,037
1,001	0,138
1,807	-0,011
1,703	0,045
1,790	-0,170
0,881	-0,006
1,426	-0,159
1,354	-0,263
1,174	0,410
0,994	0,136
1,679	-0,012
1,637	0,146

Points = mean of the repetitions for each



Relative accuracy - Raw results

Quantitray 2000

#	Matrix	Type of contamination	Strain		Contamination level (UFC/100 mL)	Reference method - ISO 7899-2										Alternative method									
							R1				R2					R1				R2					
			Species	#		Dilution	Number of colonies on Slanetz & Bartley	Number of colonies esculine + on BEA	Result R1	log	Number of colonies on Slanetz & Bartley	Number of colonies esculine + on BEA	Result R2	log	Dilution	Number of yellow wells	MPN / 100 mL	Result R1	log	Number of yellow wells	MPN / 100 mL	Result R2	log
2392476	Fresh water "Marigné" - France	ac	*Enterococcus mundtii*	*QPD764*	6600	100	39	39	3900	3.6	38	38	3800	3.6	10	49/19	325.5	3300	3.5	48/25	344.1	3400	3.5
2392479	Fresh water "Villevêque" - France	ac	*Enterococcus faecalis*	BDWL25	1200	100	15	15	1500	3.2	21	19	1900	3.3	10	45/8	127.4	1300	3.1	42/5	96.0	960	3.0
2392480	Fresh water "La Chesnaie" - France	ac	*Enterococcus mundtii*	QPD764	3300	100	14	14	1400	3.1	15	15	1500	3.2	10	46/17	184.2	1800	3.3	45/6	119.8	1200	3.1
2392506	Fresh water "Pouancé" - France	ac	*Enterococcus durans*	YHW642	190	10	22	22	220	2.3	18	18	180	2.3	10	11	12.4	120	2.1	13	15.0	150	2.2
2392507	Fresh water "Angers" - France	ac	*Enterococcus faecium*	SAK840	930	10	43	43	430	2.6	46	46	460	2.7	10	31	47.8	180	2.3	27	38.4	380	2.6
2392508	Fresh water "La Sablière" - France	ac	*Enterococcus mundtii*	QNA168	1800	100	13	13	1300	3.1	13	13	1300	3.1	10	48	144.5	1400	3.1	46	118.4	1200	3.1
2392509	Fresh water" Séverac-le-Château" - France	ac	*Enterococcus mundtii*	QNA168	360	10	24	24	240	2.4	29	29	290	2.5	10	25	34.4	340	2.5	20	25.4	250	2.4
2392510	Fresh water "Millau" Beach - France	nc	/	/	/	10	14	14	140	2.1	19	19	190	2.3	10	10	11.1	110	2.0	8	8.7	90	2.0
2392513	Fresh water "Krzywe Lake Olsztyn" - Poland	ac	*Enterococcus faecalis*	YHP824	150	10	16	16	160	2.2	22	18	180	2.3	10	31	47.8	480	2.7	29	42.9	430	2.6
2392514	Fresh water "Krzywe Lake Olsztyn" - Poland	ac	*Enterococcus mundtii*	QNA168	270	10	17	17	170	2.2	17	17	170	2.2	10	26	36.4	360	2.6	14	16.4	160	2.2
2392515	Fresh water "Krzywe Lake Sielska" - Poland	nc	/	/	/	10	39	28	280	2.4	32	29	290	2.5	10	33	53.1	530	2.7	32	50.4	500	2.7
2392516	Fresh water "Lake Bartążek Warmiński" - Poland	cc	cross-conta with STEP water		280	10	37	35	350	2.5	33	32	320	2.5	10	35	59.1	590	2.8	31	47.8	480	2.7
2392517	Fresh water "Lac du Vioreau" - France	ac	*Enterococcus durans*	YHW642	620	10	55	55	550	2.7	39	39	390	2.6	10	31/13	51.2	510	2.7	28/3	44.1	440	2.6
2392457	Marine water "Dieppe" beach - France	ac	*Enterococcus mundtii*	QPD764	660	10	48	48	480	2.7	37	37	370	2.6	10	23	30.6	310	2.5	25	34.4	340	2.5
2392460	Marine water " Sables d'Olonne" beach - France	ac	*Enterococcus faecalis*	BDWL25	7000	100	28	28	2800	3.4	24	24	2400	3.4	10	49/28	547.5	5500	3.7	49/31	648.8	6500	3.8
2392462	Marine water "St Jean de Monts" beach - France	ac	*Enterococcus mundtii*	QPD764	3300	100	17	17	1700	3.2	23	23	2300	3.4	10	49/11	214.3	2100	3.3	48/16	228.2	2300	3.4
2392464	Marine water "Ile de Ré" beach - France	ac	*Enterococcus durans*	YHW642	5270	100	44	44	4400	3.6	40	40	4000	3.6	10	46/40	410	4100	3.6	49/24	430	4300	3.6
2392465	Marine water "Pornichet" beach - France	cc	cross-conta with STEP water		280	10	48	48	480	2.7	37	33	330	2.5	10	41	83.1	830	2.9	38	69.7	700	2.8
2392466	Marine water "La Rochelle" beach - France	ac	*Enterococcus durans*	YHW642	310	10	32	32	320	2.5	40	40	400	2.6	10	13	15.0	150	2.2	22	28.8	290	2.5
2392467	Marine water " St Malo" beach - France	ac	*Enterococcus durans*	RSU238	350	10	21	21	210	2.3	27	25	250	2.4	10	35	59.1	590	2.8	22	28.8	290	2.5
2392468	Marine water "Bastia" beach - Corse	nc	/	/	/	10	23	11	110	2.0	20	16	160	2.2	10	20/1	26.2	260	2.4	8/3	10.8	110	2.0
2392469	Marine water "Ingoldmells" beach - UK	ac	*Enterococcus faecalis*	BDWL25	170	10	18	15	150	2.2	25	16	160	2.2	10	15	17.8	180	2.3	19	23.8	240	2.4
2392470	Marine water "Anderby creek" beach - UK	ac	*Enterococcus faecium*	SAK840	3100	100	10	10	1000	3.0	16	11	1100	3.0	10	41/4	88.0	880	2.9	45/5	116.2	1160	3.1
2392471	Marine water "Moggs eye" beach - UK	ac	*Enterococcus faecalis*	YHP824	150	10	18	12	120	2.1	18	18	180	2.3	10	27	38.4	380	2.6	20	25.4	250	2.4
2392472	Marine water "Chapel St Leonards" beach - UK	ac	*Enterococcus faecalis*	YHP824	870	10	66	60	600	2.8	66	65	650	2.8	10	45	109.1	1100	3.0	40	78.2	780	2.9
2392474	Marine water "Fouras" beach - France	nc	/	/	/	10	27	13	130	2.1	18	13	130	2.1	10	23/5	36.8	370	2.6	16/3	22.6	230	2.4
	Results not exploitable for statictic calculations																						
2392456	Marine water "Dieppe Sotteville " beach - France	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392458	Marine water "Dieppe Aubin sur mer" beach - France	ac	*Enterococcus faecalis*	BDWL25	1400	100	8	8	800	2.9	4	4	400	2.6	10	44	101.3	1000	3.0	41	83.1	830	2.9
2392459	Marine water "Dieppe Quibenville" beach - France	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392461	Marine water "St Gilles Croix de Vie" beach - France	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392463	Marine water "Majorque" beach - Spain	ac	*Enterococcus faecalis*	BDWL25	2800	100	8	8	800	2.9	13	13	1300	3.1	10	47/13	178.5	1800	3.3	48/10	180	1800	3.3
2392473	Marine water - "Skegness" beach - UK	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392475	Fresh water "Argentré" - France	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392477	Fresh water "La Rincerie Craon" - France	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392478	Fresh water "Combrée" - France	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392511	Fresh water "Skanda Lake Olsztyn" - Poland	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/
2392512	Fresh water "Lake Dlugie Olsztyn" - Poland	nc	/	/	/	10	0	/	<10	/	0	/	<10	/	10	0	/	<10	/	0	/	<10	/

Relative accuracy - Enterococci - Bathing water - Raw data

Reference method					Alternative method					Difference
Sample	Rep 1	Rep 2	M	SD	Sample	Rep 1	Rep 2	M	SD	
2392476	3900	3800	3850.0	70.711	**2392476**	3300.0	3400.0	3350.00	70.711	-500.000
2392479	1500	1900	1700.0	282.843	**2392479**	1300.0	960.0	1130.00	240.416	-570.000
2392480	1400	1500	1450.0	70.711	**2392480**	1800.0	1200.0	1500.00	424.264	50.000
2392506	220	180	200.0	28.284	**2392506**	120.0	150.0	135.00	21.213	-65.000
2392507	430	460	445.0	21.213	**2392507**	180.0	380.0	280.00	141.421	-165.000
2392508	1300	1300	1300.0	0.000	**2392508**	1400.0	1200.0	1300.00	141.421	0.000
2392509	240	290	265.0	35.355	**2392509**	340.0	250.0	295.00	63.640	30.000
2392510	140	190	165.0	35.355	**2392510**	110.0	90.0	100.00	14.142	-65.000
2392513	160	180	170.0	14.142	**2392513**	480.0	430.0	455.00	35.355	285.000
2392514	170	170	170.0	0.000	**2392514**	360.0	160.0	260.00	141.421	90.000
2392515	280	290	285.0	7.071	**2392515**	530.0	500.0	515.00	21.213	230.000
2392516	350	320	335.0	21.213	**2392516**	590.0	480.0	535.00	77.782	200.000
2392517	550	390	470.0	113.137	**2392517**	510.0	440.0	475.00	49.497	5.000
2392457	480	370	425.0	77.782	**2392457**	310	340	325.00	21.213	-100.000
2392460	2800	2400	2600.0	282.843	**2392460**	5500	6500	6000.00	707.107	3400.000
2392462	1700	2300	2000.0	424.264	**2392462**	2100	2300	2200.00	141.421	200.000
2392464	4400	4000	4200.0	282.843	**2392464**	4100	4300	4200.00	141.421	0.000
2392465	480	330	405.0	106.066	**2392465**	830	700	765.00	91.924	360.000
2392466	320	400	360.0	56.569	**2392466**	150	290	220.00	98.995	-140.000
2392467	210	250	230.0	28.284	**2392467**	590	290	440.00	212.132	210.000
2392468	110	160	135.0	35.355	**2392468**	260	110	185.00	106.066	50.000
2392469	150	160	155.0	7.071	**2392469**	180	240	210.00	42.426	55.000
2392470	1000	1100	1050.0	70.711	**2392470**	880	1160	1020.00	197.990	-30.000
2392471	120	180	150.0	42.426	**2392471**	380	250	315.00	91.924	165.000
2392472	600	650	625.0	35.355	**2392472**	1100.0	780.0	940.00	226.274	315.000
2392474	130	130	130.0	0.000	**2392474**	370.0	230.0	300.00	98.995	170.000

q=	26	**Mx=**	895.000		**My=**	1055.769	**M=**	160.769
n=	2	**MEDx=**	382.500		**MEDy=**	465.000	**MED=**	50.000
N=qn=	52	**SDbx=**	1131.157		**SDby=**	1418.567		Bias
			MEDwx =	35.355		**MEDwy =**	98.995	
			SDwx=	135.576		**SDwy=**	200.298	
			rob. SDwx=	52.418		**rob. SDwy=**	146.770	

Relative accuracy - Enterococci - Bathing water - Log data

Reference method					Alternative method					Difference
Sample	Rep 1	Rep 2	M	SD	Sample	Rep 1	Rep 2	M	SD	
2392476	3.591	3.580	3.585	0.008	**2392476**	3.519	3.531	3.525	0.009	-0.060
2392479	3.176	3.279	3.227	0.073	**2392479**	3.114	2.982	3.048	0.093	-0.179
2392480	3.146	3.176	3.161	0.021	**2392480**	3.255	3.079	3.167	0.125	0.006
2392506	2.342	2.255	2.299	0.062	**2392506**	2.079	2.176	2.128	0.069	-0.171
2392507	2.633	2.663	2.648	0.021	**2392507**	2.255	2.580	2.418	0.229	-0.231
2392508	3.114	3.114	3.114	0.000	**2392508**	3.146	3.079	3.113	0.047	-0.001
2392509	2.380	2.462	2.421	0.058	**2392509**	2.531	2.398	2.465	0.094	0.043
2392510	2.146	2.279	2.212	0.094	**2392510**	2.041	1.954	1.998	0.062	-0.215
2392513	2.204	2.255	2.230	0.036	**2392513**	2.681	2.633	2.657	0.034	0.428
2392514	2.230	2.230	2.230	0.000	**2392514**	2.556	2.204	2.380	0.249	0.150
2392515	2.447	2.462	2.455	0.011	**2392515**	2.724	2.699	2.712	0.018	0.257
2392516	2.544	2.505	2.525	0.028	**2392516**	2.771	2.681	2.726	0.063	0.201
2392517	2.740	2.591	2.666	0.106	**2392517**	2.708	2.643	2.676	0.045	0.010
2392457	2.681	2.568	2.625	0.080	**2392457**	2.491	2.531	2.511	0.028	-0.113
2392460	3.447	3.380	3.414	0.047	**2392460**	3.740	3.813	3.777	0.051	0.363
2392462	3.230	3.362	3.296	0.093	**2392462**	3.322	3.362	3.342	0.028	0.046
2392464	3.643	3.602	3.623	0.029	**2392464**	3.613	3.633	3.623	0.015	0.000
2392465	2.681	2.519	2.600	0.115	**2392465**	2.919	2.845	2.882	0.052	0.282
2392466	2.505	2.602	2.554	0.069	**2392466**	2.176	2.462	2.319	0.202	-0.234
2392467	2.322	2.398	2.360	0.054	**2392467**	2.771	2.462	2.617	0.218	0.257
2392468	2.041	2.204	2.123	0.115	**2392468**	2.415	2.041	2.228	0.264	0.105
2392469	2.176	2.204	2.190	0.020	**2392469**	2.255	2.380	2.318	0.088	0.128
2392470	3.000	3.041	3.021	0.029	**2392470**	2.944	3.064	3.004	0.085	-0.016
2392471	2.079	2.255	2.167	0.125	**2392471**	2.580	2.398	2.489	0.129	0.322
2392472	2.778	2.813	2.796	0.025	**2392472**	3.041	2.892	2.967	0.106	0.171
2392474	2.114	2.114	2.114	0.000	**2392474**	2.568	2.362	2.465	0.146	0.351

q= 26 **Mx=** 2.679 **My=** 2.752 **M=** 0.073
n= 2 **MEDx=** 2.577 **MEDy=** 2.666 **MED=** 0.045
N=qn= 52 **SDbx=** 0.475 **SDby=** 0.467 Bias
MEDwx = 0.042 **MEDwy =** 0.077
SDwx= 0.064 **SDwy=** 0.123
rob. SDwx= 0.062 **rob. SDwy=** 0.114

Method choice
GMFR

R= 1.941
rob. R= 1.836
Sx= 0.473
Sy= 0.471

r= 0.913
b= 0.996 **Res. SEM=** 0.201
a= 0.084 **Res. SD=** 0.284

S(b)= 0.085 **p(t;b=1)=** 0.962 **t(b)=** 0.047
S(a)= 0.160 **p(t;a=0)=** 0.602 **t(a)=** 0.525

Repeatability	Reference method	Alternative method
r	0.178	0.345
rob. r	0.173	0.318

Est. y	Dev.
3.655	-0.130
3.298	-0.250
3.232	-0.065
2.373	-0.246
2.721	-0.304
3.185	-0.073
2.495	-0.031
2.287	-0.290
2.305	0.353
2.305	0.075
2.529	0.183
2.598	0.128
2.739	-0.063
2.698	-0.187
3.484	0.293
3.367	-0.025
3.692	-0.069
2.673	0.209
2.627	-0.308
2.434	0.182
2.198	0.030
2.265	0.053
3.092	-0.088
2.242	0.247
2.868	0.099
2.189	0.276

APPENDIX E

LINEARITY
RAW DATA AND STATISTICAL CALCULATIONS

Linearity - Raw results - Protocol for human drinking waters

#	Matrix	Reference method						Alternative method					
		R1			R2			R1			R2		
		Number of colonies on Slanetz & Bartley	Number of colonies esculine + on BEA	log	Number of colonies on Slanetz & Bartley	Number of colonies esculine + on BEA	log	Number of yellow wells	MPN / 100 mL	log	Number of yellow wells	MPN / 100 mL	log
ENTC.1.2	Tap water	5	5	0,699	3	3	0,477	5	5,3	0,724	4	4,2	0,623
		25	25	1,398	19	19	1,279	17	20,7	1,316	15	17,8	1,250
		32	32	1,505	38	38	1,580	20	25,4	1,405	23	30,6	1,486
		63	63	1,799	67	67	1,826	31	47,8	1,679	33	53,1	1,725
		76	76	1,881	108	108	2,033	44	101,3	2,006	42	88,5	1,947

Value superior to 80 CFU/100 l: data of the level not interpreted

Linearity - Enterococci - Tap water - Raw data

Level	Reference method			
	Rep.1	Rep.2	M	SD
1	5	3	4,0	1,414
2	25	19	22,0	4,243
3	32	38	35,0	4,243
4	63	67	65,0	2,828

Alternative method			
Rep.1	Rep.2	M	SD
5,3	4,2	4,8	0,778
20,7	17,8	19,3	2,051
25,4	30,6	28,0	3,677
47,8	53,1	50,5	3,748

q = 4
n = 2
N = qn = 8

Mx = 31,500
MEDx = 28,500
SDbx = 25,697

MEDwx = 3,536
SDwx = 2,398
rob. SDwx = 5,242

My = 25,613
MEDy = 23,625
SDby = 19,134

MEDwy = 2,864
SDwy = 2,012
rob. SDwy = 4,246

Method choice

GMFR

R = 0,839
rob.R = 0,810
Res.SEM = 0,599
Res.SD = 0,847

Sx = 23,928
Sy = 17,845

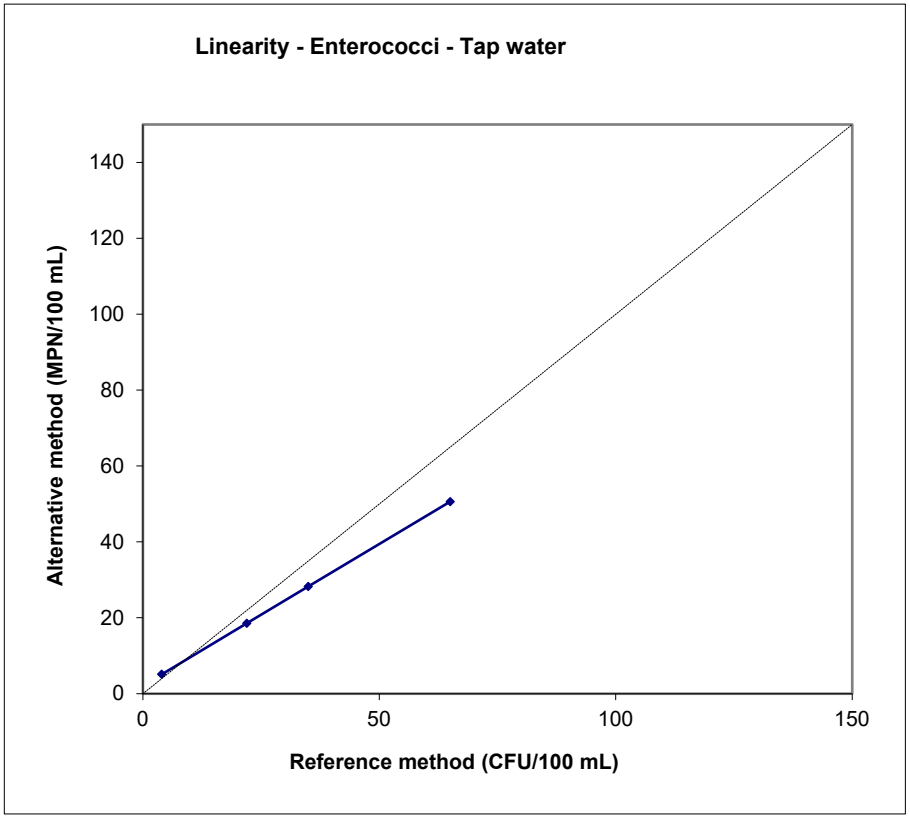
r = 1,000
b = 0,746
a = 2,121

Est y	Deviation
5,104	-0,354
18,528	0,722
28,223	-0,223
50,595	-0,145

Sb = 0,014 **p(t;b=1) =** 0,000 **t (b) =** 17,591
Sa = 0,545 **p(t;a=0) =** 0,008 **t (a) =** 2,058

Linearity

F = -1,468 **p(F) =** 0,333
rob.F = -1,881 **rob.p(F) =** 0,266



Linearity - Enterococci - Tap water - log data

Level	Reference method				Alternative method			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	0,699	0,477	0,6	0,157	0,724	0,623	0,7	0,071
2	1,398	1,279	1,3	0,084	1,316	1,250	1,3	0,046
3	1,505	1,580	1,5	0,053	1,405	1,486	1,4	0,057
4	1,799	1,826	1,8	0,019	1,679	1,725	1,7	0,032

q = 4
n = 2
N = qn = 8

Mx = 1,320
MEDx = 1,440
SDbx = 0,525

MEDwx = 0,069
SDwx = 0,066
rob. SDwx = 0,102

My = 1,276
MEDy = 1,364
SDby = 0,437

MEDwy = 0,052
SDwy = 0,038
rob. SDwy = 0,077

Method choice

GMFR

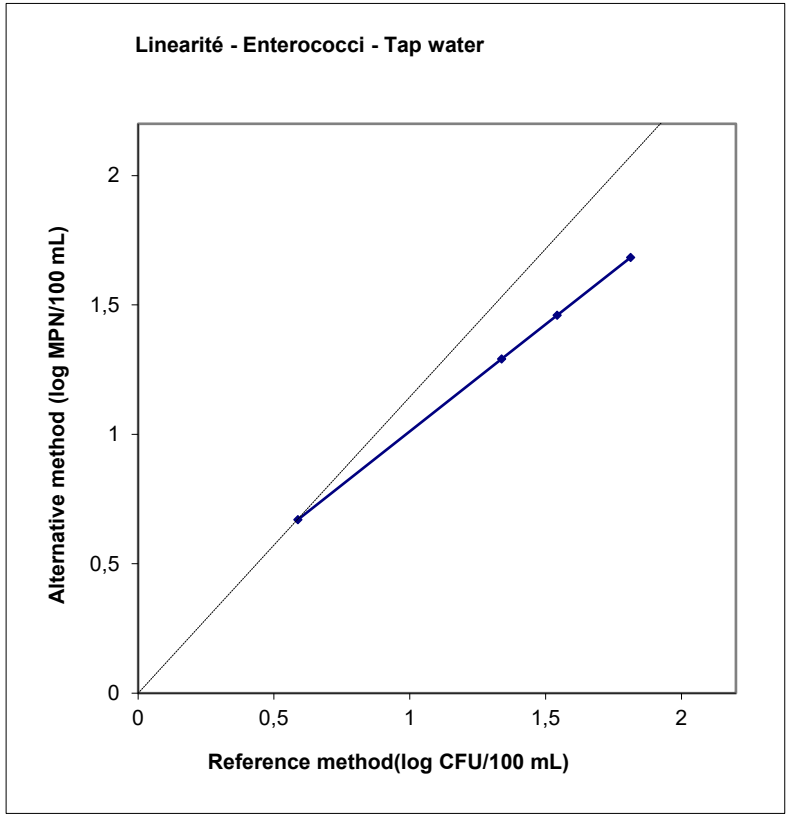
R = 0,576
rob.R = 0,756
Res.SEM = 0,018
Res.SD = 0,025

Sx = 0,492
Sy = 0,407

r = 0,999
b = 0,827
a = 0,184

Est y	Deviation
0,670	0,004
1,291	-0,008
1,460	-0,015
1,683	0,019

Sb = 0,021 **p(t;b=1) =** 0,000 **t (b) =** 8,218
Sa = 0,029 **p(t;a=0) =** 0,001 **t (a) =** 28,000



Linearity

F = -0,672
rob.F = -1,674

p(F) = 0,560
rob.p(F) = 0,296

Inoculation level (CFU/ 100mL)	Matrix	Strain	Origin	NF ISO 7899-2							
				Dilution	Répétition 1 24h CFU/ plate 1	Répétition 1 BEA CFU/ plate 1	Result 1	Répétition 2 24h CFU/ plate 2	Répétition 2 BEA CFU/ plate 2	Result 2	Mean
120	Marine bathing water "St Gilles Croix de Vie"	*Enterococcus faecium TJV000*	Marine bathing water	10	14	14	140	8	8	80	110
200				10	22	22	220	19	19	190	205
500				10	59	59	590	54	54	540	565
1000				100	15	15	1500	11	11	1100	1300
5000				100	40	40	4000	54	54	5400	4700

Inoculation level (CFU/ 100mL)	Matrix	Strain	Origin	Quanti-tray® / Quanti-tray®2000					
				Dilution	Repetition 1 CFU/ 100mL	Result 1	Repetition 2 CFU/ 100mL	Result 2	Mean
120	Marine bathing water "St Gilles Croix de Vie"	*Enterococcus faecium TJV000*	Marine bathing water	10	19.2	190	12.4	120	155
200				10	20.7	210	22.2	220	215
500				10	47.8	480	56	560	520
1000				10	94.5	950	69.7	700	825
5000				10	410.5	4100	488.4	4900	4500

Linearity - Enterococcus - raw data

Niveau	Reference method Rép.1	Rép.2	M	SD	Alternative method Rép.1	Rép.2	M	SD
1	140	80	110.0	42.426	190	120	155.0	49.497
2	220	190	205.0	21.213	210	220	215.0	7.071
3	590	540	565.0	35.355	480	560	520.0	56.569
4	1500	1100	1300.0	282.843	950	700	825.0	176.777
5	4000	5400	4700.0	989.949	4100	4900	4500.0	565.685

Mx = 1376.000
MEDx = 565.000
SDbx = 1916.147

My = 1243.000
MEDy = 520.000
SDby = 1840.247

q = 5
n = 2
N = qn = 10

MEDwx = 42.426
SDwx = 461.194
rob. SDwx = 62.901

MEDwy = 56.569
SDwy = 267.189
rob. SDwy = 83.869

Choix méthode
GMFR

R = 0.579
rob.R = 1.333
Res.SEM = 217.517
Res.SD = 307.616

Est y	Déviation
40.735	114.265
130.953	84.047
472.829	47.171
1170.826	-345.826
4399.657	100.343

Sx = 1838.974
Sy = 1746.393

r = 0.994
b = 0.950
a = -63.727

t (b) = 0.851
t (a) = 0.510

Sb = 0.059
Sa = 126.827

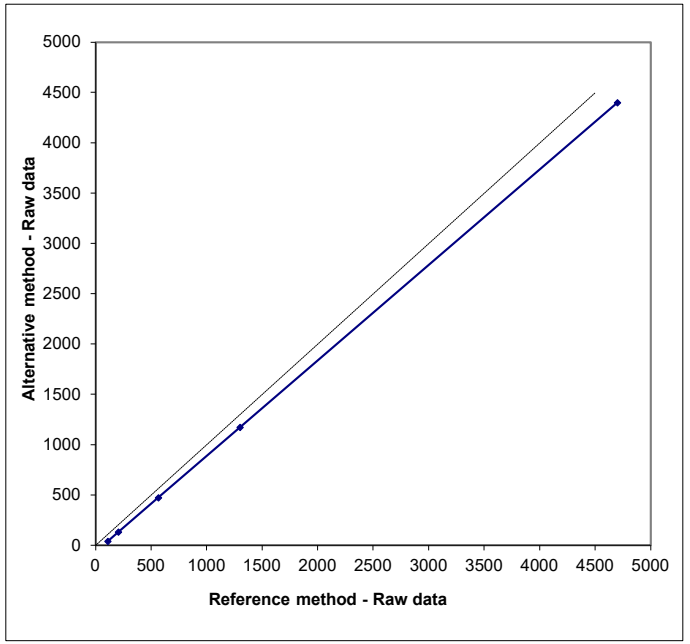
p(t;b=1) = 0.419
p(t;a=0) = 0.629

0.253
0.001

Linéarité

F = 1.868
rob.F = 34.208

p(F) = 0.253
rob.p(F) = 0.001



Linearity - Enterococcus - log data

Niveau	Reference method Rép.1	Rép.2	M	SD	Alternative method Rép.1	Rép.2	M	SD
1	2.146	1.903	2.0	0.172	2.279	2.079	2.2	0.141
2	2.342	2.279	2.3	0.045	2.322	2.342	2.3	0.014
3	2.771	2.732	2.8	0.027	2.681	2.748	2.7	0.047
4	3.176	3.041	3.1	0.095	2.978	2.845	2.9	0.094
5	3.602	3.732	3.7	0.092	3.613	3.690	3.7	0.055

q = 5
n = 2
N = qn = 10

Mx = 2.773
MEDx = 2.752
SDbx = 0.649

MEDwx = 0.092
SDwx = 0.100
rob. SDwx = 0.137

My = 2.758
MEDy = 2.715
SDby = 0.579

MEDwy = 0.055
SDwy = 0.083
rob. SDwy = 0.081

Choix méthode
GMFR

R = 0.828
rob.R = 0.594
Res.SEM = 0.099
Res.SD = 0.141

Est y	Déviation
2.092	0.087
2.346	-0.014
2.739	-0.024
3.057	-0.146
3.554	0.097

Sx = 0.617
Sy = 0.549

r = 0.985
b = 0.890
a = 0.289

t (b) = 1.359
t (a) = 3.122

Sb = 0.081
Sa = 0.228

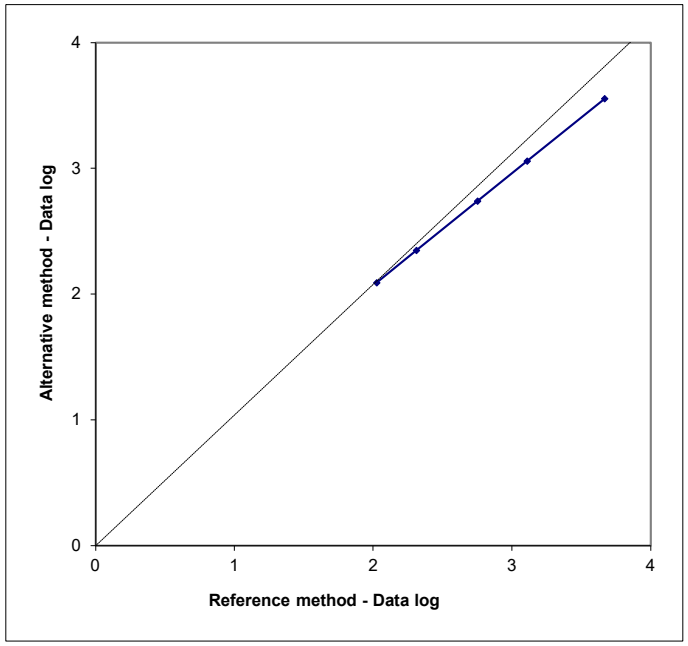
p(t;b=1) = 0.211
p(t;a=0) = 0.240

0.041
0.037

Linéarité

F = 6.049
rob.F = 6.334

p(F) = 0.041
rob.p(F) = 0.037



APPENDIX F

LOD-LOQ
RAW DATA AND STATISTICAL CALCULATIONS

LOD -LOQ - Protocol for human drinking water

Raw results

Strain : *Enterococcus faecalis*

Target level (CFU/ 100mL)	Real level (CFU/ 100mL) (a)	Replicates					
		1	2	3	4	5	6
		Enterococci detection (CFU/100 mL)	Enterococci detection (CFU/100 mL)	Enterococci detection (CFU/100 mL)	Enterococci detection (CFU/100 mL)	Enterococci detection (CFU/100 mL)	Enterococci detection (CFU/100 mL)
0	0,000	0,0	0,0	0,0	0,0	0,0	0,0
0,3	0,233	1,0	0,0	0,0	0,0	0,0	0,0
0,6	0,500	1,0	0,0	0,0	0,0	2,0	0,0
1	0,967	1,0	1,0	1,0	2,0	0,0	2,0
2	1,700	0,0	0,0	1,0	4,2	1,0	2,0
3	3,467	4,2	6,4	5,3	2,0	6,4	1,0

(a): level calculated from 30 enumerations

Detection limit (LOD) and Quantification limit (LOQ)

Strain : *Enterococcus faecalis* BDWL25 - Sterile tap water

Target level (CFU/100 mL)	Real level (CFU/100 mL) (a)	Repetition						Ecart-type (So)	Bias (Xo)	Number positive sample
		1	2	3	4	5	6			
		Enterococcus (CFU/100 mL)	*Enterococcus* (CFU/100 mL)	*Enterococcus* (CFU/100 mL)	*Enterococcus* (CFU/100 mL)	*Enterococcus* (CFU/100 mL)	*Enterococcus* (CFU/100 mL)			
0.0	0	0	0	0	0	0	0	0.000	0.000	0
5.0	5.0	0	0	0	0	22	0	8.981	0.000	1
7.0	6.3	0	0	10	10	0	0	5.164	0.000	2
8.0	7.7	20	0	0	10	10	0	8.165	5.000	3
9.0	8.1	0	10	0	20	30	20	12.111	15.000	4
10.0	12.0	10	0	42	0	10	31	17.225	10.000	4
20.0	18.0	0	31	31	20	10	10	12.554	15.000	5
30.0	35.0	0	64	31	53	42	42	22.015	42.000	5
50.0	52.0	22	22	42	111	87	31	37.538	36.500	6
100.0	100.0	99	53	87	87	75	42	22.040	81.000	6

(a): level calculated from 30 enumerations

Appendix F - LOD-LOQ raw results and statistical calculations - Protocol for bathing water

Detection limit (LOD) and quantification limit **(LOQ)**

Level (CFU/mL)	Number of positive sample	Ecart-type (So)	Bias (Xo)
0	0	0.000	0.000
5.0	1	8.981	0.000
6.3	2	5.164	0.000
7.7	3	8.165	5.000
8.1	4	12.111	15.000
12.0	4	17.225	10.000
18.0	5	12.554	15.000
35.0	5	22.015	42.000
52.0	6	37.538	36.500
100.0	6	22.040	81.000

	Formules	Valeur obtenue
Niveau critique (LC)	1,65 So +Xo	18.47
Limite de détection (LOD)	3,3 So+Xo	31.94
Limite de quantification (LOQ)	10 So + Xo	86.65

Detection limit (LOD) and Quantification limit (LOQ)

Strain :

Target level (CFU/mL)	Real level (CFU/mL) (a)	Repetition																													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5.0	5.0	4	5	8	4	5	4	5	4	4	5	8	4	5	8	5	5	5	5	5	8	4	5	8	8	5	4	8	5	5	8
7.0	6.3	2	5	6	3	6	7	4	5	4	6	8	4	8	5	6	7	5	7	7	5	7	5	8	5	7	8	9	9	7	8
8.0	7.7	2	10	6	5	7	12	9	4	6	14	9	6	3	8	3	15	4	6	8	6	3	11	9	4	11	9	14	4	8	15
9.0	8.1	16	7	4	14	5	7	10	3	8	7	6	9	10	5	7	6	10	14	8	6	11	4	10	6	8	14	5	8	6	9
10.0	12.0	10	11	10	10	15	9	10	15	10	10	14	14	11	15	14	9	10	15	10	15	10	14	14	10	10	15	10	10	14	10
20.0	18.0	16	20	16	20	14	20	15	16	20	14	20	16	16	20	14	20	16	20	14	16	20	14	15	16	20	20	20	20	20	15
30.0	35.0	30	35	35	35	35	37	36	35	35	37	36	35	36	35	35	35	30	36	35	35	37	36	35	35	37	35	35	30	37	35
50.0	52.0	50	53	53	55	50	53	50	50	53	50	53	55	50	55	50	53	50	55	50	53	50	54	50	55	50	50	50	50	55	55
100.0	100.0	101	100	100	99	98	100	103	100	99	100	100	103	100	100	100	98	100	100	99	101	100	100	99	100	103	100	100	99	100	100

(a): rate calculated from 30 counts

APPENDIX G

SELECTIVITY RAW DATA

Inclusivity

#	Code	Species	Origin	Inoculation level (CFU/100 mL)	Number of yellow wells	Result (MPN/100 mL)
1	ENTC.1.2	*Enterococcus faecalis*	ATCC 33186	25	31	47,8
2	ENTC.1.3	*Enterococcus faecalis*	CIP 103214	20	13	15
3	ENTC.1.4	*Enterococcus faecalis*	River water	30	25	34,4
4	ENTC.1.5	*Enterococcus faecalis*	Surface water	27	31	47,8
5	ENTC.1.6	*Enterococcus faecalis*	Surface water	31	27	38,4
6	ENTC.1.7	*Enterococcus faecalis*	Surface water	26	17	20,7
7	ENTC.1.8	*Enterococcus faecalis*	River water	23	12	13,7
8	ENTC.1.9	*Enterococcus faecalis*	River water	25	19	23,8
9	ENTC.1.10	*Enterococcus faecalis*	River water	35	21	27,1
10	ENTC.1.11	*Enterococcus faecalis*	River water	24	24	32,4
11	ENTC.1.12	*Enterococcus faecalis*	River water	26	35	59,1
12	ENTC.2.1	*Enterococcus faecium*	Dairy industry	19	7	7,5
13	ENTC.2.2	*Enterococcus faecium*	Water environment	15	9	9,9
14	ENTC.2.4	*Enterococcus faecium*	Surface water	10	16	19,2
15	ENTC.2.5	*Enterococcus faecium*	Surface water	6	12	13,7
16	ENTC.2.6	*Enterococcus faecium*	Surface water	11	8	8,7
17	ENTC.2.7	*Enterococcus faecium*	River water	19	11	12,4
18	ENTC.2.8	*Enterococcus faecium*	River water	9	8	8,7
19	ENTC.2.9	*Enterococcus faecium*	River water	20	10	11,1
20	ENTC.6.1	*Enterococcus durans*	Surface water	8	35	59,1
21	ENTC.6.2	*Enterococcus durans*	River water	31	15	17,8
22	ENTC.4.1	*Enterococcus avium*	Water (Germany)	31	24	32,4
23	ENTC.5.1	*Enterococcus gallinarum*	River water	16	10	11,1
24	ENTC.5.2	*Enterococcus gallinarum*	River water	9	10	11,1
25	ENTC.5.3	*Enterococcus gallinarum*	Effluent water	12	3	3,1
26	ENTC.3.1	*Enterococcus hirae*	CIP 58.55	35	32	50,4
27	ENTC.3.2	*Enterococcus hirae*	River water	21	22	28,8
28	ENTC.7.1	*Enterococcus casselifavus*	River water	16	15	17,8
29	ENTC.7.2	*Enterococcus casseliflavus*	River water	6	3	3,1
30	ENTC.7.3	*Enterococcus casselifavus*	River water	18	15	17,8

Exclusivity

#	Code	Species	Origin	Inoculation level (CFU/100 mL)	Number of yellow wells	Result (MPN/100 mL)
1	AERC.1.1	*Aerococcus viridans*	CIP 54.145	1,1E+04	0	<1
2	AUR.1.1	*Aureobacterium saperdae*	Evaporator	3,4E+04	0	<1
3	LACC.1.1	*Lactococcus lactis*	Collection strain	2,6E+04	0	<1
4	MIC.1.2	*Micrococcus luteus*	ATCC 4698	1,0E+04	0	<1
5	MIC.2.2	*Micrococcus spp*	Surface water	2,3E+04	0	<1
6	MIC.2.3	*Micrococcus spp*	Surface water	3,8E+04	0	<1
7	STA.1.6	*Staphylococcus aureus*	Surface water	2,3E+06	0	<1
8	STA.5.1	*Staphylococcus xylosus*	Surface water	5,6E+04	0	<1
9	STA.6.1	*Staphylococcus capitis*	Surface water	3,3E+04	0	<1
10	STA.2.3	*Staphylococcus epidermidis*	Surface water	2,5E+04	0	<1
11	STA.7.1	*Staphylococcus sciuri*	Surface water	1,1E+04	0	<1
12	STA.2.1	*Staphylococcus epidermidis*	Dairy product	1,0E+05	0	<1
13	STA.3.2	*Staphylococcus haemolyticus*	Surface water	1,0E+04	0	<1
14	STA.4.1	*Staphylococcus piscifermentans*	Evaporator	2,3E+04	0	<1
15	PED.1.1	*Pediococcus acidilactici*	Collection strain	1,9E+04	0	<1
16	PED.1.2	*Pediococcus spp*	Surface water	2,0E+04	0	<1
17	RHO.1.1	Rhodococcus equi	Collection strain	1,3E+04	0	<1
18	BAC.2.1	*Bacillus circulans*	Dairy industry	1,7E+05	0	<1
19	BAC.4.2	*Bacillus subtilis*	CIP 52.65 T	1,2E+04	0	<1
20	BAC.1.4	*Bacillus cereus*	Collection strain	1,0E+05	0	<1
21	STE.1.1	*Stenotrophomonas maltophilia*	Fountain water	1,0E+05	0	<1
22	AER.1.1	*Aeromonas hydrophila*	Well water	1,8E+04	0	<1
23	PSE.1.4	*Pseudomonas aeruginosa*	Fountain water	1,5E+04	0	<1
24	ACI.2.1	*Acinetobacter cloacae*	Eau de la Seine	1,2E+04	0	<1
25	RAH.1.2	*Rahnella aquatilis*	Water (Seine)	1,0E+06	0	<1
26	ESC.1.120	*Escherichia coli*	Water (UK)	1,0E+06	0	<1
27	PRO.1.2	*Proteus mirabilis*	Water	1,0E+04	0	<1
28	ENTB.2.2	*Enterobacter cloacae*	Well water	1,0E+04	0	<1
29	PROV.1.1	*Providencia stuartii*	HPA RM	1,0E+04	0	<1
30	XAN.1.1	*Xanthomonas campestris*	Evaporator	5,3E+04	0	<1

Appendix H - Culturable microorganisms

Laboratory	Result (CFU/mL) at 22°C	Result (CFU/mL) at 36°C
A	124	<1
B	79	4
C	142	30
D	145	8
F	102	11
G	142	7
H	100	7
I	108	5
J	112	3
K	99	6
L	99	9
M	105	2
Expert	124	17

Appendix I - Interlaboratory study raw results

Results inCFU/100 mL for the reference method and in MPN/100 mL for the alternative method

Level 0

Laboratory	Reference method - Samples						Alternative method - Samples					
	1			8			1			8		
	Number of typical colonies	Result after confirmation	Result log	Number of typical colonies	Result after confirmation	Result log	Number of +ve wells	Result	Result log	Number of +ve wells	Result	Result log
A	0	<1	0	0	<1	0	0	0	0	0	0	0
B	0	<1	0	0	<1	0	0	0	0	0	0	0
C	0	<1	0	0	<1	0	0	0	0	0	0	0
D	0	<1	0	0	<1	0	0	0	0	0	0	0
F	0	<1	0	0	<1	0	0	0	0	0	0	0
G	0	<1	0	0	<1	0	0	0	0	0	0	0
H	0	<1	0	0	<1	0	0	0	0	0	0	0
I	0	<1	0	2	<1	0	0	0	0	0	0	0
J	0	<1	0	0	<1	0	0	0	0	0	0	0
K	0	<1	0	0	<1	0	0	0	0	0	0	0
L	77	<1	0	4	<1	0	0	0	0	0	0	0
M	0	<1	0	0	<1	0	0	0	0	0	0	0
Expert	0	<1	0	0	<1	0	0	0	0	0	0	0

Level 1

Laboratory	Reference method - Samples						Alternative method - Samples					
	2			5			2			5		
	Number of typical colonies	Result after confirmation	Result log	Number of typical colonies	Result after confirmation	Result log	Number of +ve wells	Result	Result log	Number of +ve wells	Result	Result log
A	26	26	1,415	23	23	1,362	11	12	1,079	14	16	1,204
B	16	16	1,204	23	23	1,362	23	31	1,491	14	16	1,204
C	20	20	1,301	20	20	1,301	14	16	1,204	18	22	1,342
D	24	24	1,380	19	19	1,279	25	34	1,531	16	19	1,279
F	27	27	1,431	18	18	1,255	22	29	1,462	17	21	1,322
G	22	22	1,342	13	13	1,114	25	34	1,531	19	24	1,380
H	32	32	1,505	20	20	1,301	15	18	1,255	13	15	1,176
I	23	23	1,362	21	21	1,322	22	29	1,462	18	22	1,342
J	26	26	1,415	28	28	1,447	13	15	1,176	18	22	1,342
K	26	26	1,415	12	12	1,079	17	21	1,322	17	21	1,322
L	19	19	1,279	25	21	1,322	18	22	1,342	22	29	1,462
M	26	26	1,415	35	35	1,544	16	19	1,279	22	29	1,462
Expert	18	18	1,255	17	17	1,230	18	22	1,342	18	22	1,342

Level 2

Laboratory	Reference method - Samples						Alternative method - Samples					
	4			7			4			7		
	Number of typical colonies	Result after confirmation	Result log	Number of typical colonies	Result after confirmation	Result log	Number of +ve wells	Result	Result log	Number of +ve wells	Result	Result log
A	55	55	1,740	56	56	1,748	27	38	1,580	35	59	1,771
B	37	37	1,568	41	41	1,613	38	70	1,845	35	59	1,771
C	52	52	1,716	64	64	1,806	36	62	1,792	35	59	1,771
D	52	52	1,716	64	64	1,806	35	59	1,771	38	70	1,845
F	76	76	1,881	71	71	1,851	32	50	1,699	37	66	1,820
G	52	52	1,716	45	45	1,653	40	78	1,892	39	74	1,869
H	62	62	1,792	68	68	1,833	36	62	1,792	34	56	1,748
I	53	53	1,724	55	55	1,740	35	59	1,771	40	78	1,892
J	64	64	1,806	68	68	1,833	38	70	1,845	42	89	1,949
K	63	63	1,799	63	63	1,799	31	48	1,681	29	43	1,633
L	69	45	1,653	67	58	1,763	29	43	1,633	29	43	1,633
M	63	63	1,799	58	58	1,763	34	56	1,748	38	70	1,845
Expert	57	57	1,756	55	55	1,740	34	56	1,748	35	59	1,771

Level 3

Laboratory	Reference method - Samples						Alternative method - Samples					
	3			6			3			6		
	Number of typical colonies	Result after confirmation	Result log	Number of typical colonies	Result after confirmation	Result log	Number of +ve wells	Result	Result log	Number of +ve wells	Result	Result log
A	100	100	2,000	90	90	1,954	42	89	1,949	44	101	2,004
B	59	59	1,771	88	88	1,944	43	95	1,978	42	89	1,949
C	86	86	1,934	82	82	1,914	44	101	2,004	44	101	2,004
D	102	102	2,009	121	121	2,083	47	130	2,114	46	118	2,072
F	116	116	2,064	95	95	1,978	40	78	1,892	45	109	2,037
G	101	101	2,004	105	105	2,021	45	109	2,037	46	118	2,072
H	92	92	1,964	87	87	1,940	36	62	1,792	48	145	2,161
I	86	86	1,934	96	96	1,982	43	95	1,978	43	95	1,978
J	94	94	1,973	94	94	1,973	42	89	1,949	47	130	2,114
K	96	96	1,982	87	87	1,940	43	95	1,978	48	145	2,161
L	95	84	1,924	98	98	1,991	40	78	1,892	41	83	1,919
M	96	96	1,982	100	100	2,000	42	89	1,949	46	118	2,072
Expert	108	108	2,033	96	96	1,982	48	145	2,161	43	95	1,978