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NF VALIDATION

AFNOR Certification validation of the method

ENTEROLERT-DW / QUANTI-TRAY

For the enumeration of enterococci

Protocol for human drinking waters (except bottled waters)

SUMMARY REPORT – NOVEMBER 2013 – V1

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Only certain essays reported in this document are covered by the accreditation of the Section Laboratory of COFRAC. They are identified by the symbol (*).

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Summary

APPENDICES

1. Introduction

1.1. Validation referential

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

The validation study was realized in two successive parts : the comparative study then the interlaboratory study.

The goal of the comparative study is to evaluate the performances of the alternative method against the reference method. The following parameters were studied:

- the relative accuracy,
- the linearity,
- the limits of detection and the limits of quantification,
- the selectivity (inclusivity and exclusivity),
- the practicability.

The goal of the interlaboratory study is to determine the performances of the alternative method in several laboratories in the real conditions of the "routine" application of the method.

1.2. Alternative 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.

The protocol of the alternative method is presented in appendix 1.

1.3. Scope of application

The application scope of the method Enterolert-DW / Quanti-Tray concerns the human drinking waters except bottled waters.

1.4. Reference method

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

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

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

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

The samples analyzed are presented in table 1.

Enumeration	Type of water	Samples analyzed	Samples exploited
Enterococci	Tap water and fountain water	56	16
	Well, spring and drilling water	22	8
	Total	78	24

Table 1 : nature and number of samples analyzed

78 samples were analyzed and 24 results were treated. The samples non treated in the statistical analysis correspond to samples for which enumerations inferior to 1 CFU/100 mL or superior to 150 CFU/100 ml were found for the two replicates of the two methods. Samples for which a result inferior to 4 CFU/100 mL for at least one of the replicates was obtained are presented in raw results but were not integrated to the statistical treatment of the results.

No naturally contaminated samples were analyzed. All samples were artificially contaminated.
Contamination levels cover the whole measurement range of the alternative method.
The stress applied and the strains used are presented in appendix 3.

2.1.2. Raw results

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

Figure 1 presents the bidimensional graphics for the tested 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" appears in dotted line.

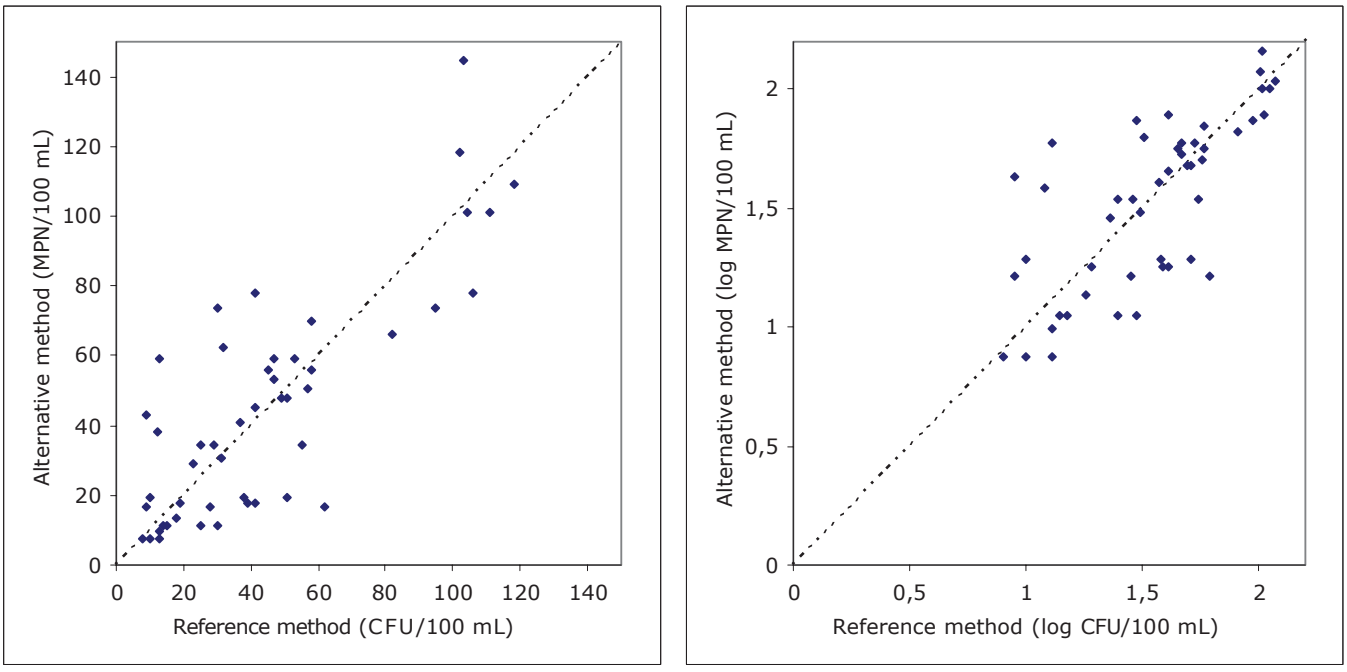


Figure 1 : bidimensional graphics presenting the raw results of the accuracy study (in CFU/100 mL and in log CFU/100 mL)

2.1.3. 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 repeatabilities 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.

Matrix	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
								Ord. at 0	Ord. at 0
Raw	0,892	GMFR	2,064	-1,770	0,717	1,059	0,650	36,5	45,7
Log	1,319	GMFR	2,064	-0,118	0,735	1,073	0,645	34,0	46,4

Table 2 : statistical data for the enumeration of enterococci in human drinking waters

Data	Bias (D)		Repeatability			
	Average	Median	R		rob. r	
			MR	MA	MR	MA
Raw	0,798	1,900	20,508	28,425	19,080	17,025
Log	-0,007	0,014	0,315	0,250	0,207	0,274

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

2.1.4. Conclusion

The equation of the regression line for the couple « enterococci – human drinking waters » is the following : Alt = 1,059 Réf - 1,770 or log Alt = 1,073 log Réf -0,118

The hypothesis [a=0 and b=1] is accepted for the tested category. The bias between the two methods is 1,900 for the raw data or 0,014 log.

The accuracy of the alternative method is satisfactory.

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

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

Strain	Matrix	Target contamination levels (CFU/100 or 250 mL)
Enterococcus faecalis ENTC.1.4 (river water)	Tap water	10- 30- 50- 100

Table 4 : couple matrix strain analyzed

2.2.2. Raw results

Raw results and statistics are summarized in in appendix 5.

Figure 2 presents the bidimensional graphics for the tested 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" appears in dotted line.

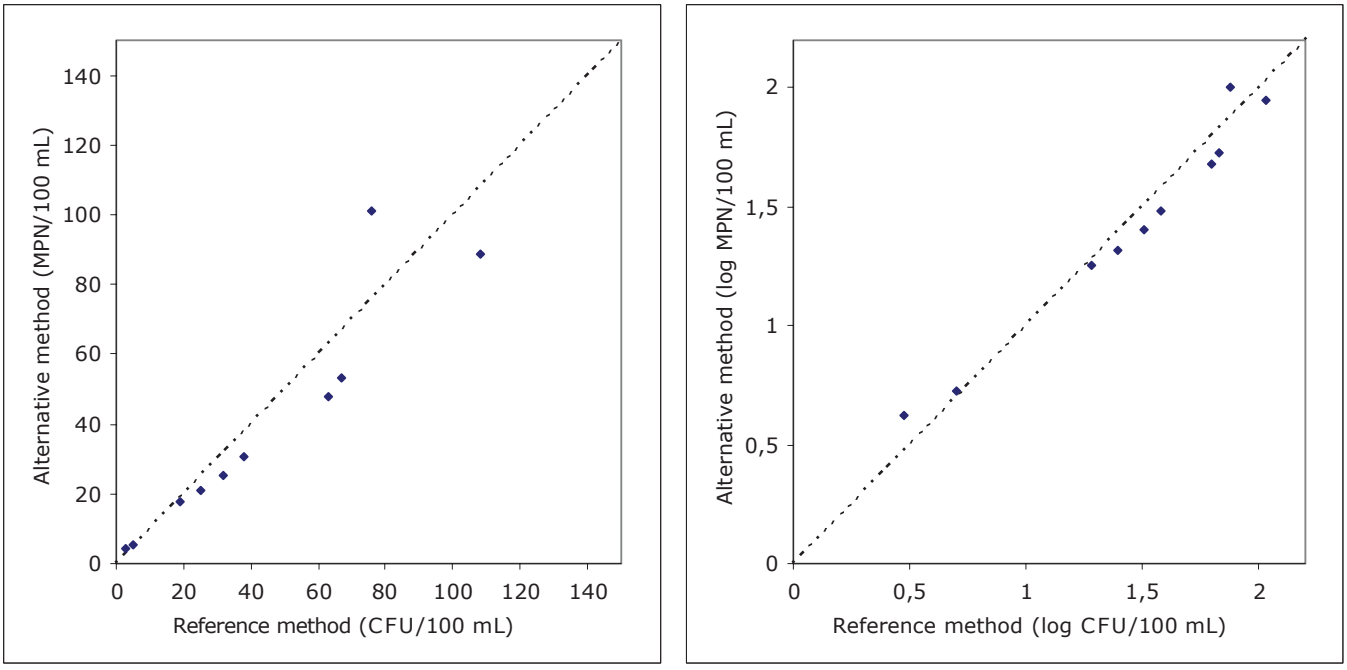


Figure 2 : bidimensional graphics presenting the raw results of the accuracy study (in CFU/100 mL and in log CFU/100 mL)

2.2.3. Statistics

Statistical interpretations are made according to requirements of standard NF ISO 16140 (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.

Data	Rob. R	Regression used	F critique	Rob. F	P (Rob.F)	Correlation coefficient (r)	Regression
Raw	0,867	GMFR	5,41	9,737	0,016	0,981	Alt = 0,981 Ref - 3,315
Log	0,550	GMFR	5,41	4,887	0,060	0,991	log Alt = 0,909 log Ref + 0,100

Table 5 : statistical data for linearity

The relationship between the two methods isn't linear:
- if Rob.F > F critique

Or - if P(Rob.F) < α (=0,05)

2.2.4. Conclusion

The relation between the two methods is not linear when the calculations are applied from the raw data. However the correlation coefficients of the couple and the equation of the regression line are satisfactory. When the data are converted in logarithm, the relation between the methods becomes linear.

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

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.

2.3.2. Results

Results are shown in the following tables and in appendix 6.

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 6 : data (S_0 et X_0) of enterococci enumeration

Parameter	Formulas	Value
LC	1,65 So +Xo	2,24
LOD	3,3 So+Xo	3,48
LOQ	10 So + Xo	8,53

Table 7 : values obtained for enterococci enumeration

2.3.3. Conclusion

The LOD and LOQ of the alternative method look satisfying.

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 à 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 7.

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 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.
Wood's lamp.

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 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.

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

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

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.

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

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

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.

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

Table 10 : sample code by contamination level

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.

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

Table 11 : temperature and state of the samples upon reception

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 were 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.

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

Table 12 : results of the interlaboratory study

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).

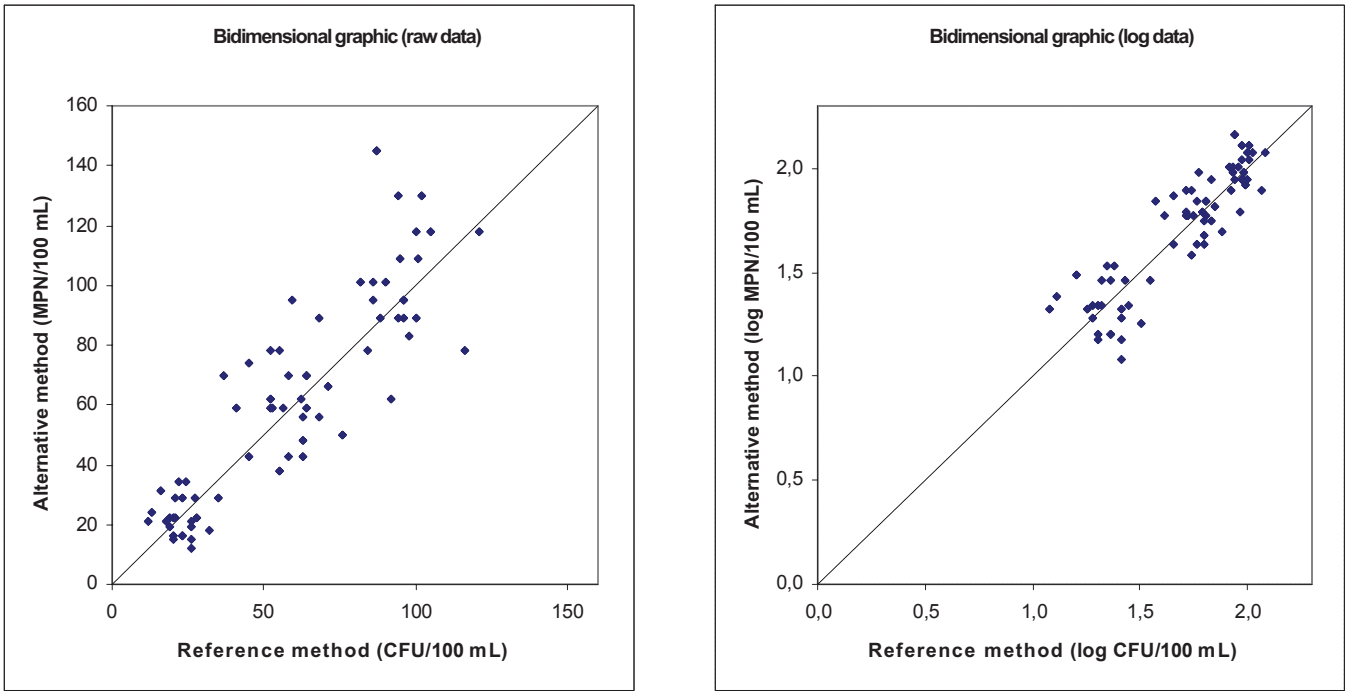


Figure 3 : bidimensional graphics

3.3. Statistical interpretation

Data presented in the following paragraphs 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.

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

Table 13 : calculations of the bias of the alternative method

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.

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

Table 14 : tolerance values for the alternative method

Figures 4 and 5 present the accuracy profiles.

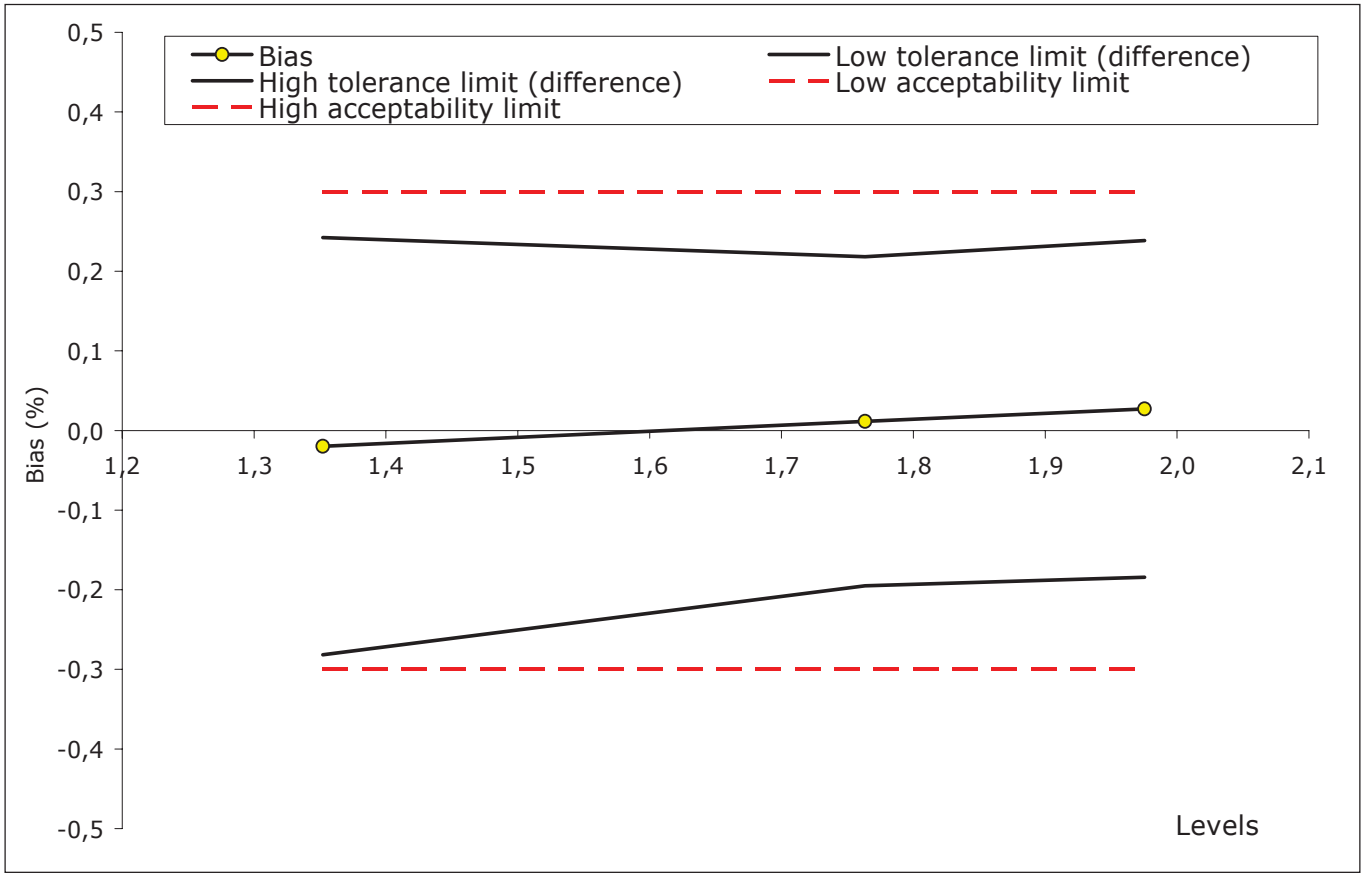


Figure 4 : accuracy profile for a tolerance probability of 95% and a tolerance limit of 0,3 log

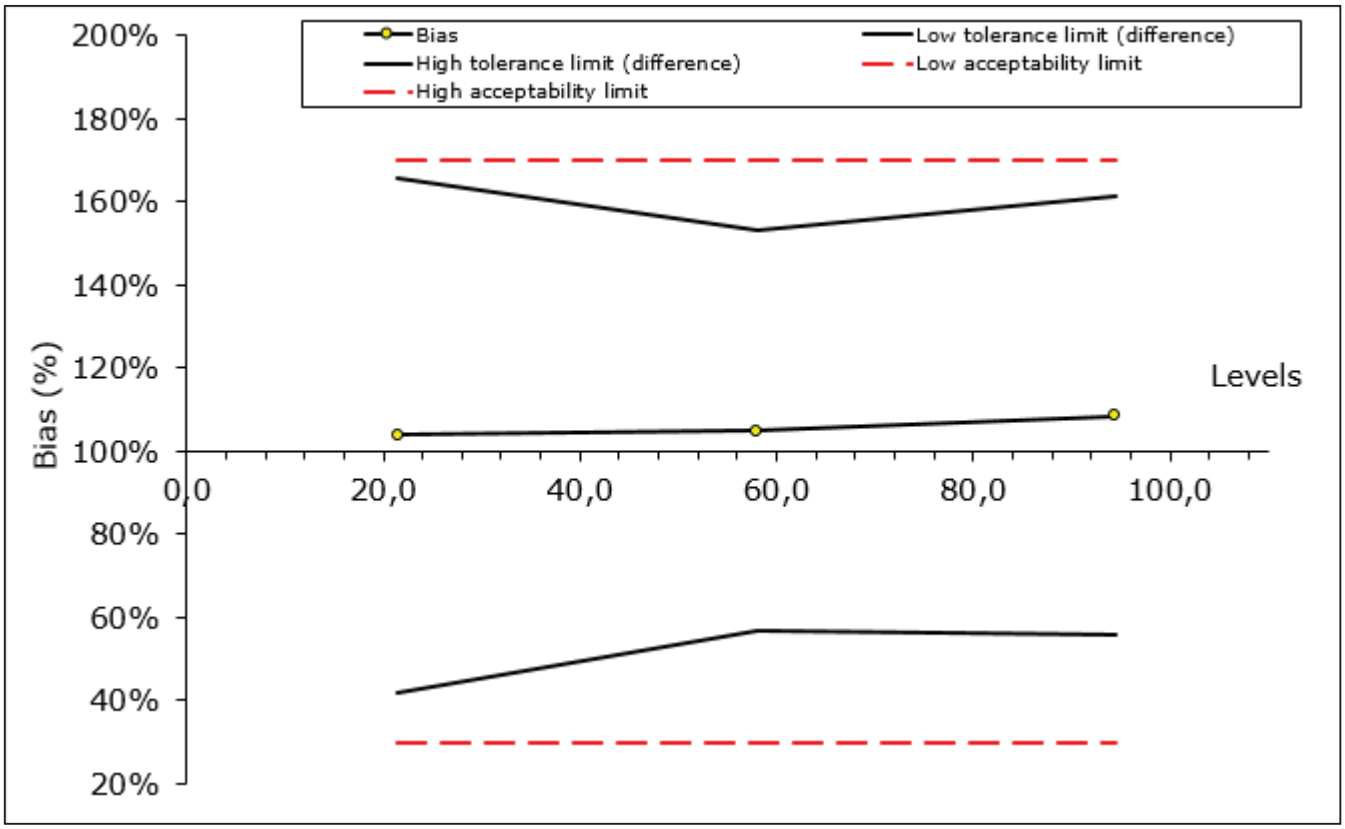


Figure 5 : accuracy profile for a tolerance probability of 95% and a tolerance limit of 70%

- 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 method for the enumeration of enterococci in human drinking waters are satisfactory.

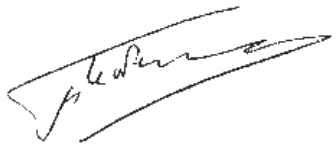
The bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.

Enterolert-DW / Quanti-Tray 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.



Massy, the 2nd of December 2013
François Le Nestour
Head of the Innovation Biology Unit

APPENDIX 1

ALTERNATIVE METHOD PROTOCOL

ALTERNATIVE METHOD 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 à 28 hours

Step 4

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

Step 5

Express the results
Number of enterococci / 100 mL of water

APPENDIX 2

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

APPENDIX 3

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

APPENDIX 4

RELATIVE ACCURACY
RAW DATA AND STATISTICAL CALCULATIONS

Relative accuracy - Raw results

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

#	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
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
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
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
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
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
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
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
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
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
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
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
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	111	102	106,5	6,364	8	101,3	118,4	109,85	12,092	3,350
9	41	39	40,0	1,414	9	17,8	17,8	17,80	0,000	-22,200
10	31	51	41,0	14,142	10	30,6	47,8	39,20	12,162	-1,800
11	104	103	103,5	0,707	11	101,1	144,5	122,80	30,688	19,300
12	10	13	11,5	2,121	12	19,2	9,9	14,55	6,576	3,050
13	58	58	58,0	0,000	13	69,7	56,0	62,85	9,687	4,850
14	47	47	47,0	0,000	14	53,1	59,1	56,10	4,243	9,100
15	55	57	56,0	1,414	15	34,4	50,4	42,40	11,314	-13,600
16	8	10	9,0	1,414	16	7,5	7,5	7,50	0,000	-1,500
17	106	118	112,0	8,485	17	78,2	109,1	93,65	21,850	-18,350
18	19	38	28,5	13,435	18	17,8	19,2	18,50	0,990	-10,000
19	30	18	24,0	8,485	19	11,1	13,7	12,40	1,838	-11,600
20	9	29	19,0	14,142	20	42,9	34,4	38,65	6,010	19,650
21	9	14	11,5	3,536	21	16,4	11,1	13,75	3,748	2,250
22	41	49	45,0	5,657	22	45,3	47,8	46,55	1,768	1,550
23	32	53	42,5	14,849	23	62,4	59,1	60,75	2,333	18,250
24	82	95	88,5	9,192	24	65,9	73,8	69,85	5,586	-18,650

q=	24	**Mx=**	43,896			**My=**	44,694		**M=**	0,798
n=	2	**MEDx=**	40,500			**MEDy=**	40,800		**MED=**	1,900
N=qn=	48	**SDbx=**	30,834			**SDby=**	32,293			Bias
			MEDwx =	4,596			**MEDwy =**	4,101		
			SDwx=	7,324			**SDwy=**	10,152		
			rob. SDwx=	6,814			**rob. SDwy=**	6,080		

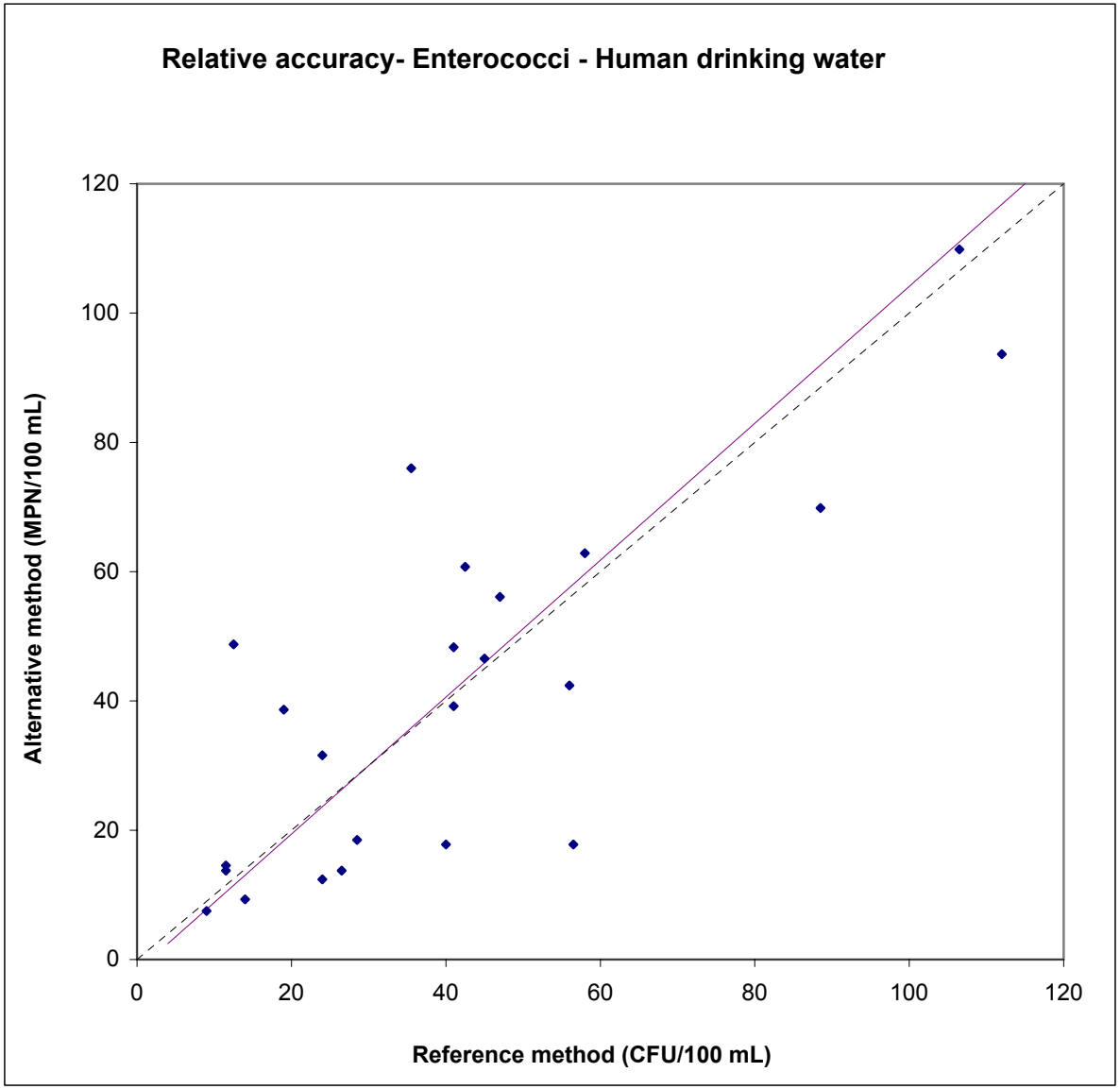
Method choice
GMFR

	R=	1,386			
	rob. R=	0,892			
			Sx=	30,950	
			Sy=	32,761	
r=	0,836				
b=	1,059		**Res. SEM=**	19,014	
a=	-1,770		**Res. SD=**	26,891	
S(b)=	0,128	**p(t;b=1)=**	0,650	**t(b)=**	0,457
S(a)=	4,847	**p(t;a=0)=**	0,717	**t(a)=**	0,365

Repeatability	Reference method	Alternative method
r	20,508	28,425
rob. r	19,080	17,025

Est. y	Dev.
11,461	37,289
58,035	-40,235
23,634	7,966
41,628	6,672
13,049	-3,749
26,280	-12,530
35,807	40,193
110,961	-1,111
40,570	-22,770
41,628	-2,428
107,785	15,015
10,402	4,148
59,623	3,227
47,980	8,120
57,506	-15,106
7,756	-0,256
116,783	-23,133
28,397	-9,897
23,634	-11,234
18,341	20,309
10,402	3,348
45,863	0,687
43,216	17,534
91,908	-22,058

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	2,045	2,009	2,027	0,026	8	2,006	2,073	2,039	0,048	0,013
9	1,613	1,591	1,602	0,015	9	1,250	1,250	1,250	0,000	-0,352
10	1,491	1,708	1,599	0,153	10	1,486	1,679	1,583	0,137	-0,017
11	2,017	2,013	2,015	0,003	11	2,005	2,160	2,082	0,110	0,067
12	1,000	1,114	1,057	0,081	12	1,283	0,996	1,139	0,203	0,082
13	1,763	1,763	1,763	0,000	13	1,843	1,748	1,796	0,067	0,032
14	1,672	1,672	1,672	0,000	14	1,725	1,772	1,748	0,033	0,076
15	1,740	1,756	1,748	0,011	15	1,537	1,702	1,619	0,117	-0,129
16	0,903	1,000	0,952	0,069	16	0,875	0,875	0,875	0,000	-0,076
17	2,025	2,072	2,049	0,033	17	1,893	2,038	1,966	0,102	-0,083
18	1,279	1,580	1,429	0,213	18	1,250	1,283	1,267	0,023	-0,162
19	1,477	1,255	1,366	0,157	19	1,045	1,137	1,091	0,065	-0,275
20	0,954	1,462	1,208	0,359	20	1,632	1,537	1,585	0,068	0,376
21	0,954	1,146	1,050	0,136	21	1,215	1,045	1,130	0,120	0,080
22	1,613	1,690	1,651	0,055	22	1,656	1,679	1,668	0,016	0,016
23	1,505	1,724	1,615	0,155	23	1,795	1,772	1,783	0,017	0,169
24	1,914	1,978	1,946	0,045	24	1,819	1,868	1,843	0,035	-0,102

q= 24 **Mx=** 1,529 **My=** 1,522 **M=** -0,007
n= 2 **MEDx=** 1,601 **MEDy=** 1,602 **MED=** 0,014
N=qn= 48 **SDbx=** 0,325 **SDby=** 0,354 Bias
MEDwx = 0,050 **MEDwy =** 0,066
SDwx= 0,113 **SDwy=** 0,089
rob. SDwx= 0,074 **rob. SDwy=** 0,098

Method choice
GMFR

R= 0,794
rob. R= 1,319
Sx= 0,332
Sy= 0,356

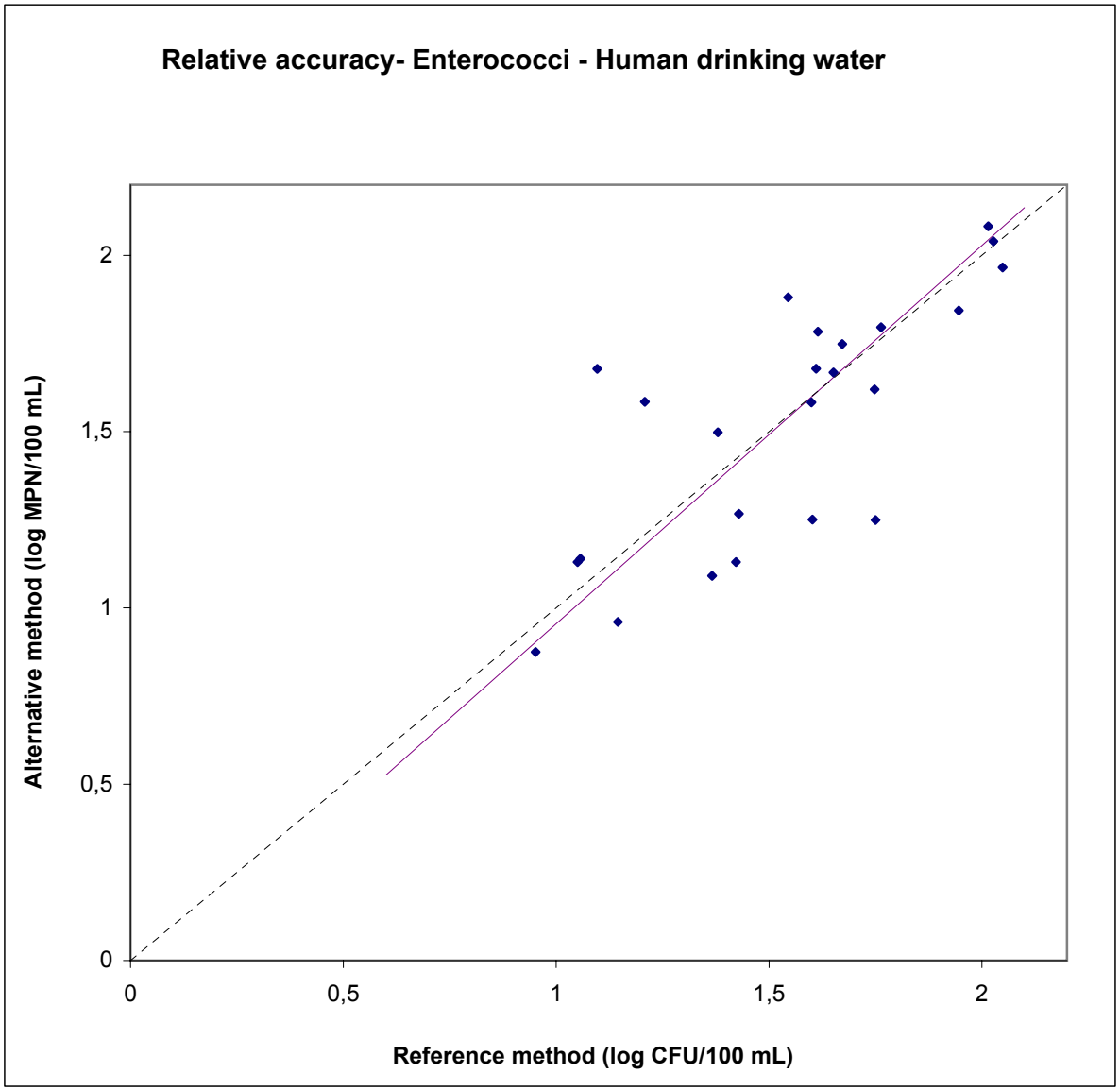
r= 0,758
b= 1,073 **Res. SEM=** 0,250
a= -0,118 **Res. SD=** 0,353

S(b)= 0,157 **p(t;b=1)=** 0,645 **t(b)=** 0,464
S(a)= 0,347 **p(t;a=0)=** 0,735 **t(a)=** 0,340

Repeatability	Reference method	Alternative method
r	0,315	0,250
rob. r	0,207	0,274

Est. y	Dev.
1,058	0,620
1,759	-0,510
1,362	0,136
1,610	0,068
1,110	-0,150
1,408	-0,278
1,539	0,341
2,057	-0,017
1,601	-0,350
1,598	-0,015
2,044	0,039
1,016	0,124
1,774	0,022
1,676	0,072
1,757	-0,138
0,903	-0,028
2,080	-0,114
1,415	-0,148
1,348	-0,257
1,178	0,406
1,009	0,121
1,654	0,014
1,614	0,169
1,969	-0,126

Points = mean of the repetitions for each sample



APPENDIX 5

LINEARITY
RAW DATA AND STATISTICAL CALCULATIONS

Linearity - Raw results

#	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

Linearity - Enterococci - Tap water - Raw data

Level	Reference method				Alternative method			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	5	3	4,0	1,414	5,3	4,2	4,8	0,778
2	25	19	22,0	4,243	20,7	17,8	19,3	2,051
3	32	38	35,0	4,243	25,4	30,6	28,0	3,677
4	63	67	65,0	2,828	47,8	53,1	50,5	3,748
5	76	108	92,0	22,627	101,3	88,5	94,9	9,051

q = 5
n = 2
N = qn = 10

Mx = 43,600
MEDx = 35,000
SDbx = 35,033

MEDwx = 4,243
SDwx = 7,470
rob. SDwx = 6,290

My = 39,470
MEDy = 28,000
SDby = 35,139

MEDwy = 3,677
SDwy = 3,381
rob. SDwy = 5,451

Method choice

GMFR

R = 0,453
rob.R = 0,867
Res.SEM = 7,972
Res.SD = 11,273

Sx = 33,955
Sy = 33,320

r = 0,981
b = 0,981
a = -3,315

Est y	Deviation
0,610	4,140
18,274	0,976
31,031	-3,031
60,470	-10,020
86,965	7,935

Sb = 0,117
Sa = 6,237

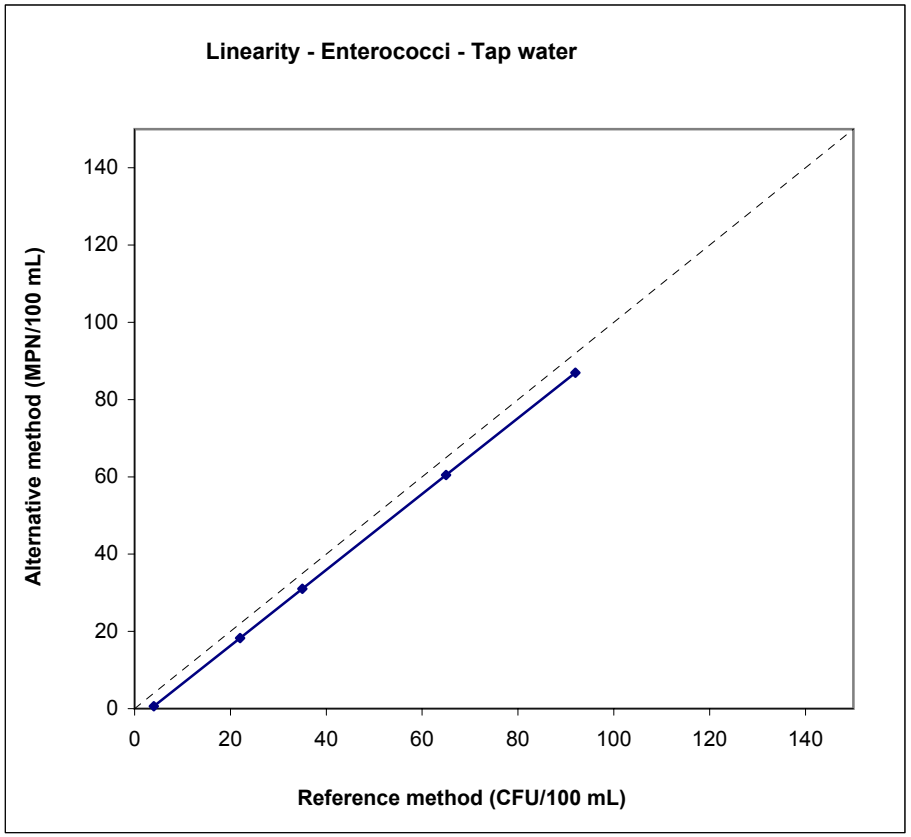
p(t;b=1) = 0,877
p(t;a=0) = 0,610

t (b) = 0,159
t (a) = 0,692

Linearity

F = 27,985
rob.F = 9,737

p(F) = 0,001
rob.p(F) = 0,016



Linearity - Enterococci - Tap water - log data

Level	Reference method			
	Rep.1	Rep.2	M	SD
1	0,699	0,477	0,6	0,157
2	1,398	1,279	1,3	0,084
3	1,505	1,580	1,5	0,053
4	1,799	1,826	1,8	0,019
5	1,881	2,033	2,0	0,108

Alternative method			
Rep.1	Rep.2	M	SD
0,724	0,623	0,7	0,071
1,316	1,250	1,3	0,046
1,405	1,486	1,4	0,057
1,679	1,725	1,7	0,032
2,006	1,947	2,0	0,041

q = 5
n = 2
N = qn = 10

Mx = 1,448
MEDx = 1,542
SDbx = 0,537

MEDwx = 0,084
SDwx = 0,068
rob. SDwx = 0,125

My = 1,416
MEDy = 1,445
SDby = 0,491

MEDwy = 0,046
SDwy = 0,036
rob. SDwy = 0,069

Method choice

GMFR

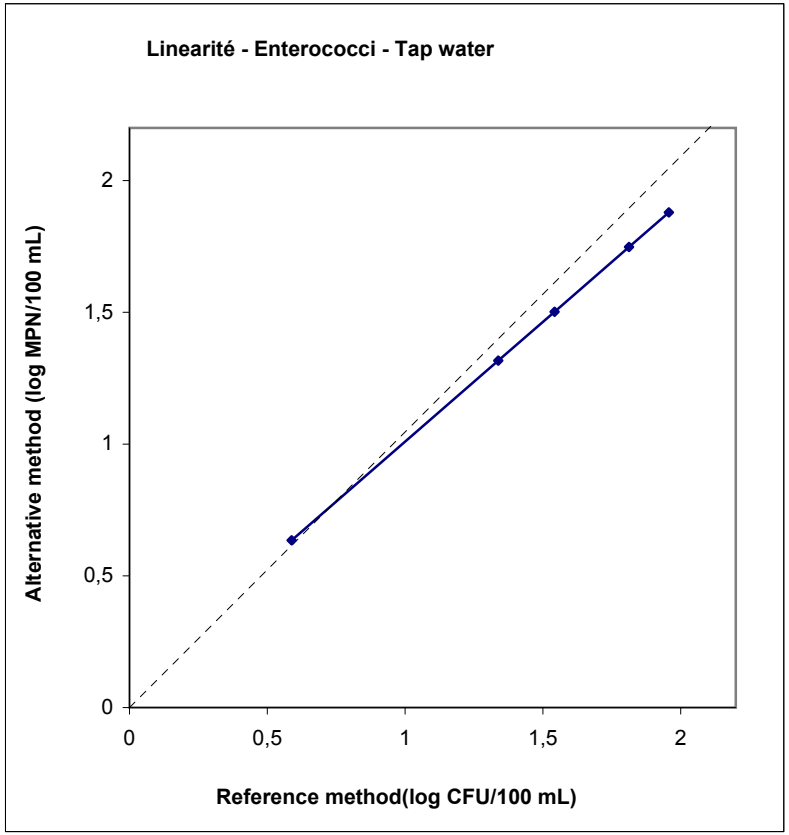
R = 0,535
rob.R = 0,550
Res.SEM = 0,076
Res.SD = 0,108

Sx = 0,511
Sy = 0,465

r = 0,991
b = 0,909
a = 0,100

Est y	Deviation
0,635	0,039
1,317	-0,034
1,502	-0,057
1,748	-0,046
1,879	0,097

Sb = 0,075 **p(t;b=1) =** 0,257 **t (b) =** 1,220
Sa = 0,113 **p(t;a=0) =** 0,403 **t (a) =** 7,956



Linearity

F = 21,628
rob.F = 4,887

p(F) = 0,003
rob.p(F) = 0,060

21,6276117

APPENDIX 6

LOD-LOQ
RAW DATA AND STATISTICAL CALCULATIONS

LOD -LOQ

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

APPENDIX 7

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 8 - 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

Appendix 9 - 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