COLILERT-18®/QUANTI-TRAY® or QUANTI-TRAY® 2000 for the enumeration of Escherichia coli in bathing waters

Summary report
February 2024
Quantitative method

Certificat n°IDX 33/02-06/12

IDEXX Laboratories,INC
Idexx Drive, Westbrook
Maine 04 092
USA

This report includes 56 pages, including 8 appendices.
Reproduction of this report is only permitted in its full form

NORMEC ABIOLAB ADGÈNE
1 rue des conquérants -14 220 THURY-HARCOURT
www.abiolab.fr – 02 31 15 62 80

Preamble

Studied method:
COLILERT-18®/QUANTI-TRAY® or QUANTI-TRAY®2000 for the enumeration of *Escherichia coli*

Validation standard:
Validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013)

Reference method*:
NF EN ISO 9308-3 (1999): Detection and enumeration of *Escherichia coli* and coliform bacteria
Part 3: Miniaturized method (Most Probable Number) for the detection and enumeration of *E. coli* in surface and waste water

Scope:
Bathing waters which groups two types of waters:

- fresh waters
- sea waters

Certification body:
AFNOR Certification (http://nf-validation.afnor.org/)

*Analyses performed according to the COFRAC accreditation

1. Introduction

The method was initially validated in 2012 and renewed in 2016 and 2020 (certificate number IDX 33/02-06/12). The certificate shall expire the 19 June 2024. IDEXX Laboratories would like to renew the validation of the method according to the validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013).

2. Modifications since the previous validation

2.1. History of validation

The history of validation was summarized in the table below:

Method	Approval	Type of certification	Comments	Expert laboratory	Protocol of validation
COLILERT-18® /QUANTI-TRAY® or QUANTI-TRAY® 2000	2012	Initial Validation	/	ISHA	Rev. 1 (2010)
	2014	Extension	Use of Quanti-Tray 2000	ISHA	Rev. 2 (2013)
	2016	Renewal 1	No change	ISHA	Rev. 2 (2013)
	2020	Renewal 2	No change	AdGène laboratoire	Rev. 2 (2013)
	2024	Renewal 3	No change	Normec Abiolab AdGène	Rev. 2 (2013)

2.2. Summary of modifications in the alternative method

The protocol of validation is the same as the previous validation.

Modification of the alternative method
There were no modifications of the alternative method since the initial validation.

2.3. Users' complaints

No claim concerning the alternative method was recorded by AFNOR CERTIFICATION.

3. Method Protocol

3.1. Alternative method

Colilert-18 detects *E. coli* in bathing waters. It is based on IDEXX's patented Defined Substrate Technology (DST):

-when total or fecal coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow,

-when *E. coli* metabolize Colilert-18's nutrient-indicator, MUG, the sample also fluoresces.

Colilert-18 can simultaneously detect these bacteria at 1 CFU/100 mL within 18 hours even in the presence of as many as 2 million heterotrophic bacteria per 100 mL.
The protocol of the alternative method is presented in figure 1.

Figure 1 : protocol of the alternative method

1\.
Add contents of one pack to a 100 mL room temperature water sample in a sterile vessel. When Colilert-18 is used for *E. coli* detection in marine water, samples must be diluted at least tenfold.
Multiply the MPN value by the dilution factor to obtain the correct quantitative result.

2\.
Cap vessel and shake until dissolved.

3\.
Pour sample/reagent mixture into a Quanti-Tray or a Quanti-Tray 2000 and seal in an IDEXX Quanti-Tray Sealer.

4\.
Place the sealed tray in a 36±2°C incubator for 18 hours to 22 hours (pre-warming to 36°C is not required). For incubation in a water bath, submerge the Tray below the water level using a weighted ring.

5\.
Read results according to the Result Interpretation table. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

3.2. Reference method

The standard NF EN ISO 9308-3 (1999): Detection and enumeration of *E. coli* and coliforms – part 3: miniaturized method (MPN) for detection and enumeration of *E. coli* in surface and waste water, was used as the reference method.
The protocol of the reference method is presented in figure 2.

Figure 2: protocol of the reference method

1. Dilutions preparation
- Dilute 9 mL of sample in 9 mL of special diluent (1/2) ❶
- Transfer 1 mL of ❶ in 9 mL of special diluent (1/20)

2. Inoculation
- Inoculate 200 µL of the 1/2 dilution in each of the first 64 wells of the microplate
- Inoculate 200 µL of the 1/20 dilution in each of the 32 wells of the microplate

3. Incubation
- Cover the microplate with sterile adhesive
- Incubate the microplate at 44 ± 1° C for 36 h to 72 h

4. Reading and interpretation
Read results according to the Result Interpretation table. Count the number of positive wells using Wood lamp and refer to the MPN table provided with the trays to obtain a Most Probable Number. Express the result in MPN E. coli / 100 mL

4. Summary of the results obtained during the initial validation and any renewals and extensions

4.1. Methods comparative study

The data come from of the initial validation (2012 - ISHA Laboratory)

The following characteristics were studied during the comparative study of the methods: the relative accuracy, the linearity of the alternative method, the selectivity of the alternative method, the limit of detection and the limit of quantification of the alternative method, the practicability of the alternative method.

4.1.1. Relative accuracy

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

Number and nature of samples

Two types of water were tested (duplicate) with reference method and alternative method: freshwater and seawater.
Different types of analyzed samples are summarized in table 1.

Table 2 : Number and nature of samples analyzed

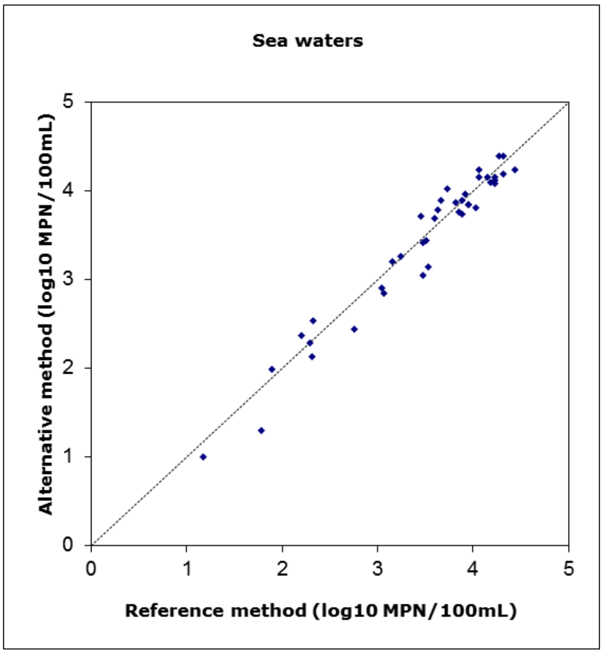
Water type	Number of samples analyzed	Number of samples used
Sea waters	53	**20**
Fresh waters	41	**22**
Total	**94**	**42**

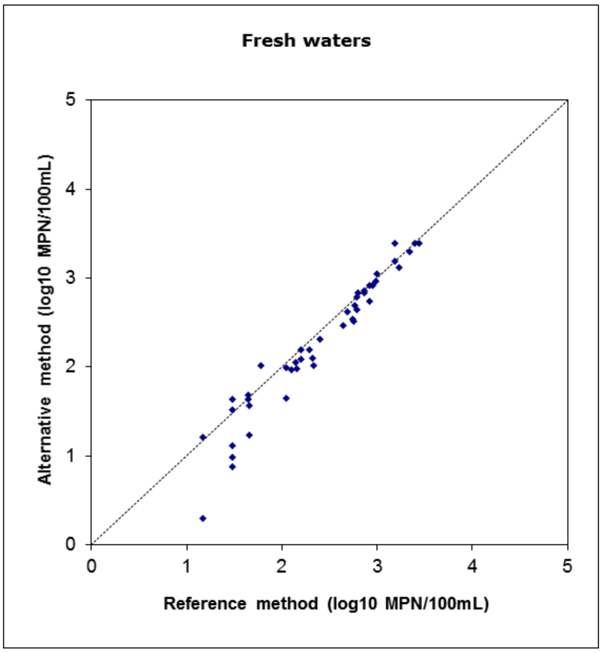
Globally, 94 samples were analyzed and 42 results were used. 16 naturally contaminated samples were analyzed. Others samples were artificially contaminated (cf. appendix 1).
The contamination levels used cover the entire measurement range of the alternative method.

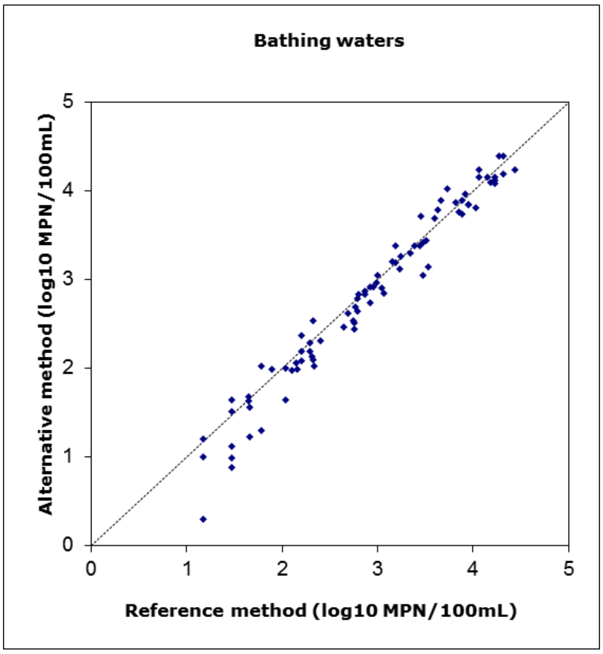
Results

Figure 3 presents the two-dimensional graphs for the two matrices. The y-axis is reserved for the alternative method and the x-axis for the reference method. . The representation of a line of equation “y = x“ figures dashed on the graphs. Raw results are in appendix 2.

Figure 3: two-dimensional graphs for relative accuracy in log CFU and log MPN / test portion (black line: y=x)







- **Statistical analysis**

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

There is a perfect accuracy (or there is no systematic bias) between the two methods if this equation is equal to the theoretical 'y = x' equation, which applies in the ideal model where the two methods behave similarly.
The intercept is theoretically zero in this ideal model (hypothesis [a = 0]). The estimated intercept obtained with the two methods is checked using p {a = 0}. If the alternative method is a systematic bias against the reference method, the probability p {a = 0} is less than α = 0.05.

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

The linear regression method is chosen over the value of the robustness of the ratio R of overall repeatability standard deviation:
- If Rob.R > 2, linear regression by least-squares (OLS 1) with the x-axis for the reference method,
- if Rob.R < 0.5, a linear regression by least-squares (OLS 2) with the x-axis for the alternative method,
- If 0.5 < Rob.R < 2, orthogonal regression (GMFR) with the x-axis to the reference method.

Table 2: statistical data (log MPN / test portion) for the enumeration of E. coli in bathing waters

Matrix	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
								Ord. at 0	Slope at 1
Sea waters	1.078	GMFR	2.086	-0.232	2.221	1.053	1.212	3.2	23.3
Fresh waters	0.926	GMFR	2.074	-0.494	3.526	1.157	2.938	0.1	0.5
Bathing waters	1.078	GMFR	2.016	-0.337	2.894	1.087	3.226	0.5	0.2

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

Matrix	Bias (D) in log		Repeatability in log			
	Mean	Median	r		Rob.r	
			MR	MA	MR	MA
Sea waters	-0.046	-0.047	0.476	0.248	0.273	0.294
Fresh waters	-0.120	-0.066	0.447	0.363	0.272	0.252
Bathing waters	-0.085	-0.058	0.461	0.313	0.273	0.294

- Sea waters

The hypothesis [b = 1] is accepted but the hypothesis [a = 0] isn't accepted. However, the correlation coefficients and equation are satisfactory as shown below:
- r = 0.984,
- log Alt. = 1.053 log Ref. – 0.232

- Fresh waters

The two hypotheses [b = 1 and a = 0] aren't accepted. However, the correlation coefficients and equation are satisfactory as shown below:

- r = 0.979,
- log Alt. = 1.157 log Ref. – 0.494

- Bathing waters (seawaters + freshwaters)

The two hypotheses [b = 1 and a = 0] aren't accepted. However, the correlation coefficients and equation are satisfactory as shown below:

- r = 0.988,
- log Alt. = 1.087 log Ref. – 0.337

- Remark:

The limits of detection of the two protocols of the alternative method and of the reference method are different, based on different dilution factors and MPN tables:

- 1 MPN/100 mL for the alternative method in fresh waters,
- 10 MPN/100 mL for the alternative method in sea waters,
- 15 MPN/100 mL for the reference method.

That's why, for fresh waters and bathing waters, if the data of the alternative method inferior to the limit of detection of the reference method are not taken into account (2 samples involved), the following values are obtained (data and calculations in appendix 3):

Fresh waters:
- r = 0.989,
- log Alt. = 1.034 log Ref. – 0.158

Bathing waters:
- r = 0.991
- log Alt. = 1.040 log Ref. – 0.180

With these values, the statistical exploitation shows that the two hypotheses [b = 1 and a = 0] are accepted with α = 5%.

- **Conclusion**

The bias of the alternative method is slightly negative. **The relative accuracy of the alternative method is satisfactory.**

4.1.2. Linearity

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

- **Contamination levels**

The couples matrix / strain are presented in Table 4. For each couple, four contamination levels were tested in duplicate by the reference method and the alternative method.

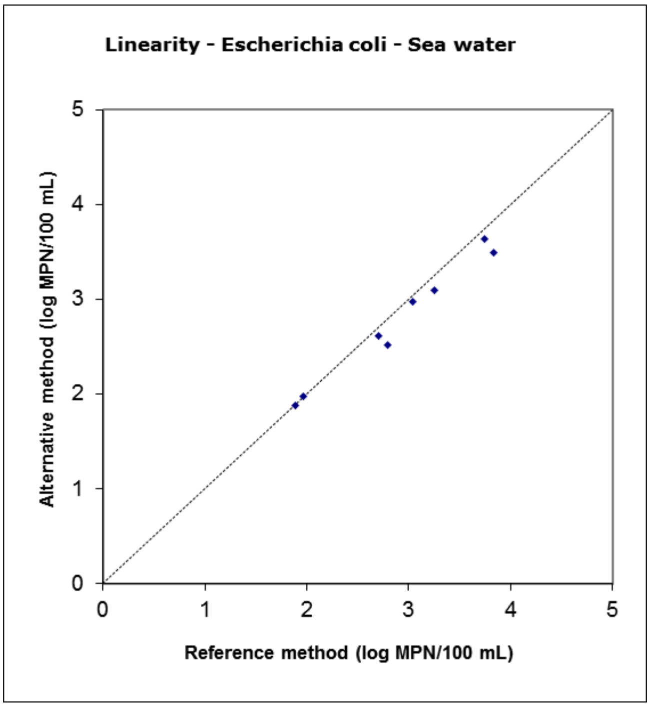
Table 4 : couples matrix – strain analyzed

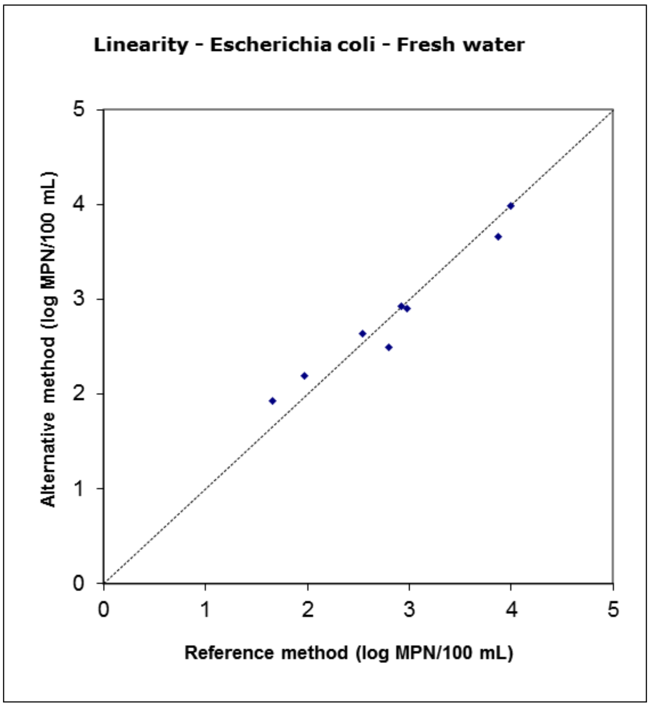
Strain	Matrix	Target contamination level (CFU/100 mL)
Escherichia coli ESC.1.112	Fresh water	50 – 500 – 1 000 – 5 000
Escherichia coli ESC.1.119	Sea water	

- **Results**

Figure 4 presents the two-dimensional graphs for the two couples matrix-strain. The y-axis is reserved for the alternative method and the x-axis for the reference method. The representation of a line of equation "y = x" figures dashed on the graphs. Raw results are in appendix 4.

Figure 4: two-dimensional graphs for linearity in log CFU and log MPN / test portion (black line: y=x)





- **Statistical analysis**

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.

Table 5: statistical data for the linearity

Strain / matrix	Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	r	Regression
***E. coli* / sea water**	1.111	GMFR	6.94	0.305	0.753	0.992	log Alt.= 0.859 log Ref + 0.404
***E. coli* / fresh water**	1.222	GMFR	6.94	0.185	0.838	0.997	log Alt.=0.891 log Ref + 0.189

The relationship between the 2 methods is not linear:

- if Rob.F > critical F or,
- if P (Rob.F) < α (= 0.05).

- **<u>Conclusion</u>**

The relationship between the two methods is linear for the two couples (*E. coli* / sea water and *E. coli* / fresh water). The correlation coefficients are satisfactory. So, the linearity of the alternative method is satisfactory.

4.1.3. Limit of detection and limit of quantification

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

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

- **<u>Test protocols</u>**

The limits of detection and quantification were determined by analysing a pure culture of *E. coli* by the alternative method. Five levels of contamination (including level 0), with six replications for each level, were studied in sterilized water.

- **Results**

Results are shown in the following tables and in appendix 5.

Table 6 : data (s_0 and x_0) of E. coli enumeration (underlined: the reference level)

Level (CFU/100mL)	Number of positive samples	Standard deviation (s_0)	Bias (x_0)
0	0	0.000	0
0.2	1	0.408	0
0.4	2	0.516	0
<u>1.5</u>	<u>3</u>	<u>0.548</u>	<u>0.5</u>
3	6	1.627	1.5

Table 7: LC, LOD and LOQ values of the alternative method

Parameter	Formula	Values obtained
Critical level (LC)	$1.65\ s_0 + x_0$	1.40
Limit of detection (LOD)	$3.3\ s_0 + x_0$	2.31
Limit of quantification (LOQ)	$10\ s_0 + x_0$	5.98

- **Conclusion**

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

4.1.4. Selectivity

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

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

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

- **Test protocols**

Twenty *E. coli* strains and thirty non-target strains (from the national, international and ISHA internal collections) were analyzed. The assays were performed by the alternative method protocol.

- **Results**

Raw results are in appendix 6.

All target strains tested are detected by the alternative method except for one strain (which is not detected by the reference method either).

For the thirty non-target strains tested, no positive result was observed. See tables below.

- **Conclusion**

The selectivity of the alternative method can be considered as satisfactory.

4.1.5. Practicability

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

1- Mode of packaging of test components The Colilert-18 reagent is conditioned on single capsules. The Quanti-Tray devices are conditioned by ten in aseptic bag. **2- Volume of reagents** Unknown. **3- Storage conditions of components and shelf-life of unopened products** The Colilert-18 reagent should be conserved at 2 – 8°C. The Quanti-Tray devices should be conserved at 4 – 30°C. **4- Modalities after first use** Each Colilert-18 test serves a unique analysis and should not be reused. **5- Equipment and specific local requirements** Quanti-Tray® Sealer model 2X. Wood lamp. **6- Reagents ready to use or for reconstitution** None. **7- Training period for operator with no experience with the method** 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 analysis according the reference method is more important than the duration of use of alternative method. **9- Time required for results** The time to obtain results for the alternative method is 18 hours for negative samples and positive samples. Concerning the reference method, the delay for negative samples is between 24 and 48 hours and for positive samples, the delay is between 48 and 72 days **10- Operator qualification** Identical as necessary for the reference method **11- Steps common with the reference method** None. **12- Traceability of analysis results** None. **13- Maintenance by laboratory** None.

4.2. Interlaboratory study

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

4.2.1. Study organisation

- **Participating laboratories**

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

- ***E. coli* absence in the matrix**

Before spiking, the absence of *E. coli* was verified in the batch of seawater used according to the reference method.

- **Strain stability in the matrix**

The strain stability in seawater matrix was evaluated for 3 days at (5±3)°C. The strain used was *E. coli* (ISHA code: ESC.1.119).
The samples were analysed at D0, D+1 and D+2 by the reference method. The results are summarized in table 10.

Table 8 : results (E. coli / 100 mL) of the stability study of the strain ESC.1.119 in seawater matrix

Day	Level 1	Level 2	Level 3
D0	60	534	1049
D1	75	563	882
D2	30	504	861

The results show that the *E. coli* strain used is stable for 2 days at (5±3)°C in sea water matrix.

- **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 50 to 100 CFU/100 mL,
-level 2: from 250 to 500 CFU/100 mL,
-level 3: from 1000 to 1500 FCU/100 mL

The matrix was distributed at 50 mL in sterile bottles. Every bottle was individually spiked and homogenized. Eight samples per laboratory were prepared (2 samples per contamination level). Each laboratory received 8 samples to analyse, 1 sample to quantify the endogenous microflora and 1 water sample containing a temperature probe.
The results of the enumerations of the heterophilic flora, the target levels and the real levels of contamination are presented in table 9.

Table 9: target level, real level and TVC of the matrix

Contamination level	Flora (CFU/mL)		*Escherichia coli* ESC.1.119 (MPN /100 mL)	
	22°C	36°C	Target level	Real level
0	10	5	0	0
1			50 to 100	147
2			250 to 500	758
3			1 000 to 1 500	1 580

- **Samples labelling**

The labelling of the bags was realized as follows: a code to identify the laboratory: from A to O (cf. table 10) and a code to identify each sample, only known by the expert laboratory. The samples and the temperature control vial (water sample with a temperature probe) were stored at 4°C before shipping.

Table 10: sample code by contamination level

Contamination level (MNP *E. coli* / 100 mL)	Sample code
0	4 / 8
50 to 100	6 / 7
250 to 500	1 / 3
1 000 to 1 500	2 / 5

- **Samples shipping**

The samples were shipped in a coolbox April 16th, 2012.

- **Samples reception and analysis**

The coolboxes were received April 17th, 2012 by all the participating laboratories. The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory. The samples were analysed on April 17th, 2012. The expert laboratory concurrently analysed a set of samples under the same conditions with both methods.

4.2.2. Results

- **Temperature and state of the samples**

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

Table 11: temperature and state of the samples upon reception and data of the temperature probes for the transportation time of samples (/: data not available)

Laboratory	Temperature (°C)	State of the samples	Temperature recorded by the probe	
			Mean	SD
A	4.1°C	Ok	2.9	1.0
B	5.2°C	Ok	3.4	0.5
C	6.7°C	Ok	3.7	0.3
D	6.8°C	Ok	2.5	0.4
E	6.4°C	Ok	2.4	1.0
F	3.8°C	Ok	/	/
G	2.0°C	Ok	2.4	0.5
H	3.0°C	Ok	2.9	0.3
I	5.2°C	Ok	2.5	0.3
J	6.0°C	Ok	/	/
K	2.1°C	Ok	2.2	0.5
L	4.8°C	Ok	2.6	0.4
M	1.6°C	Ok	2.4	0.6
N	6.1°C	Ok	2.3	0.7
O	4.8°C	Ok	1.6	0.8

The analysis of thermal profiles of probes showed for all participants that the average of temperature during the shipment is comprise between 1.6 and 3.7°C.

- **Total viable counts**

Raw results are in appendix 7.
For the whole laboratories, the total viable counts at 22°C vary between <1 and 240 CFU/mL.
Concerning the total viable counts at 36°C, the results were varying between <1 and 7 CFU/mL.

- **Expert laboratory and collaborating laboratories results**

The overall results are presented in Table 12 and in appendix 8.

The results of the reference method are presented for a reading of the microplates after 36 at 72 hours of incubation at 44 ± 1°C.

For alternative method, reading of Quanti-Tray devices was performed between 18 and 22 hours.

The results of all laboratories are presented in the following tables.

Table 12: E. coli MPN enumeration results per 100 mL seawater samples (MR: reference method, MA: alternative method, R1: repetition 1 and R2: repetition 2)

Laboratory	Level 0							
	MR						MA	
	R1			R2			R1	R2
	MPN/ 100 mL	Low limit	High limit	MPN/ 100 mL	Low limit	High limit	MPN/100 mL	MPN/100 mL
A	<15	/	/	<15	/	/	<10	<10
B	<15	/	/	<15	/	/	<10	<10
C	<15	/	/	<15	/	/	<10	<10
D	<15	/	/	<15	/	/	<10	<10
E	<15	/	/	<15	/	/	<10	<10
F	<15	/	/	<15	/	/	<10	<10
G	<15	/	/	<15	/	/	<10	<10
H	<15	/	/	<15	/	/	<10	<10
I	<15	/	/	<15	/	/	<10	<10
J	<15	/	/	<15	/	/	<10	<10
K	<15	/	/	<15	/	/	<10	<10
L	<15	/	/	<15	/	/	<10	<10
M	<15	/	/	<15	/	/	<10	<10
N	<15	/	/	<15	/	/	<10	<10
O	<15	/	/	<15	/	/	<10	<10
Expert	<15	/	/	<15	/	/	<10	<10

Laboratory	Level 1							
	MR						MA	
	R1			R2			R1	R2
	MPN/100 mL	Low limit	High limit	MPN/100 mL	Low limit	High limit	MPN/100 mL	MPN/100 mL
A	93	41	206	93	42	207	86	108
B	127	63	253	109	52	230	10	41
C	94	42	208	94	42	208	75	63
D	127	63	253	<15	/	/	41	63
E	110	52	231	15	2	106	52	63
F	46	15	142	61	23	163	63	41
G	77	32	186	160	86	298	98	135
H	15	2	106	46	15	142	51	52
I	125	62	251	61	23	163	97	30
J	61	23	163	61	23	163	52	52
K	94	42	208	93	42	207	40	41
L	94	42	208	144	75	276	52	122
M	197	63	253	46	15	142	95	109
N	94	42	208	46	15	142	62	74
O	127	63	253	126	63	252	119	109
Expert	126	63	252	30	8	121	40	84

Laboratory	Level 2							
	MR						MA	
	R1			R2			R1	R2
	MPN/ 100 mL	Low limit	High limit	MPN/ 100 mL	Low limit	High limit	MPN/ 100 mL	MPN/ 100 mL
A	697	486	981	332	212	521	496	487
B	529	363	769	434	290	650	331	404
C	332	212	521	438	293	655	389	457
D	177	98	321	465	314	689	408	374
E	234	138	394	434	290	650	425	369
F	195	111	344	393	258	598	259	238
G	415	275	626	393	258	598	292	482
H	585	408	840	465	314	689	387	331
I	654	462	927	500	341	733	393	269
J	412	272	622	375	244	575	393	309
K	344	221	537	504	344	738	754	530
L	606	424	866	640	451	909	350	529
M	476	322	703	580	403	833	231	512
N	559	387	808	640	451	909	305	231
O	585	408	840	668	473	944	496	437
Expert	697	479	953	559	387	808	616	459

Laboratory	Level 3							
	MR						MA	
	R1			R2			R1	R2
	MPN/ 100 mL	Low limit	High limit	MPN/ 100 mL	Low limit	High limit	MPN/ 100 mL	MPN/ 100 mL
A	1049	773	1423	882	642	1213	591	712
B	858	622	1182	489	333	720	809	771
C	773	555	1075	851	617	1174	733	512
D	647	456	917	838	606	1157	581	738
E	514	352	751	1007	740	1371	581	847
F	690	490	972	805	580	1116	556	594
G	580	403	833	943	690	1290	754	573
H	759	544	1058	759	544	1058	733	581
I	1305	973	1751	742	531	1037	727	663
J	918	670	1258	543	375	783	906	884
K	1136	841	1535	838	606	1157	909	1017
L	1007	740	1371	968	709	1321	776	933
M	882	642	1213	872	633	1200	988	1334
N	882	642	1213	968	709	1321	733	622
O	1567	1174	2092	893	650	1227	836	794
Expert	633	445	901	1034	761	1405	1010	833

4.2.3. Interpretation

The data presented in the following paragraphs were calculated from the results in log_{10} MPN/100 mL in the same way that the presentation of the results of the preliminary study.

- **Bias calculation**

Table 13 shows the target value, the mean, standard deviation of fidelity, the relative bias and the bias of each level of contamination for the alternative method.

Table 13: Calculation of the alternative method bias

Values	log (MPN /mL)		
Contamination level	Low	Medium	High
Target value	1.971	2.667	2.937
Average	1.795	2.580	2.870
Relative bias	-8.93%	-3.26%	-2.26%
Bias	-0.176	-0.087	-0.067

- **Accuracy profile**

Tables 14 and 15 show the values of tolerance and the tolerance limits of the alternative method for a probability value of tolerance of 80% (table 14) and of 90% (table 15).

Table 14: Values and tolerance limits of the alternative method with ß = 80 %

Probability of tolerance	Levels	log (MPN /mL)		
		Low	Medium	High
80%	Low tolerance value	1.482	2.415	2.742
	High tolerance value	2.107	2.746	2.999
	Low tolerance limit	-0.489	-0.253	-0.067
	High tolerance limit	0.137	0.079	0.062

Table 15: Values and tolerance limits of the alternative method with ß = 90 %

Probability of tolerance	Levels	log (MPN /mL)		
		Low	Medium	High
90%	Low tolerance value	1.389	2.366	2.704
	High tolerance value	2.201	2.795	3.037
	Low tolerance limit	-0.582	-0.302	-0.233
	High tolerance limit	0.230	0.128	0.100

Figures 5 and 6 show the accuracy profiles using respectively ß = 80% and ß = 90%.

Figure 5: Accuracy profile of the alternative method with tolerance probability of 80 % and acceptability limits at 0,5 log

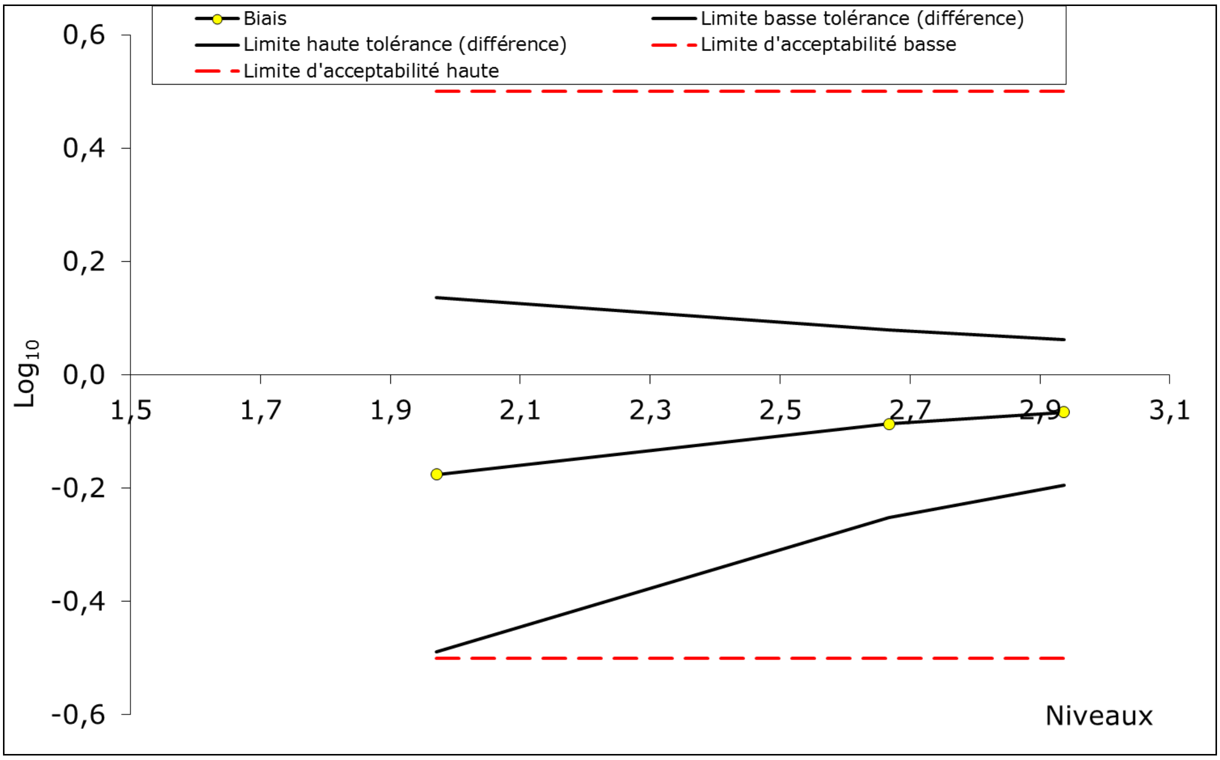
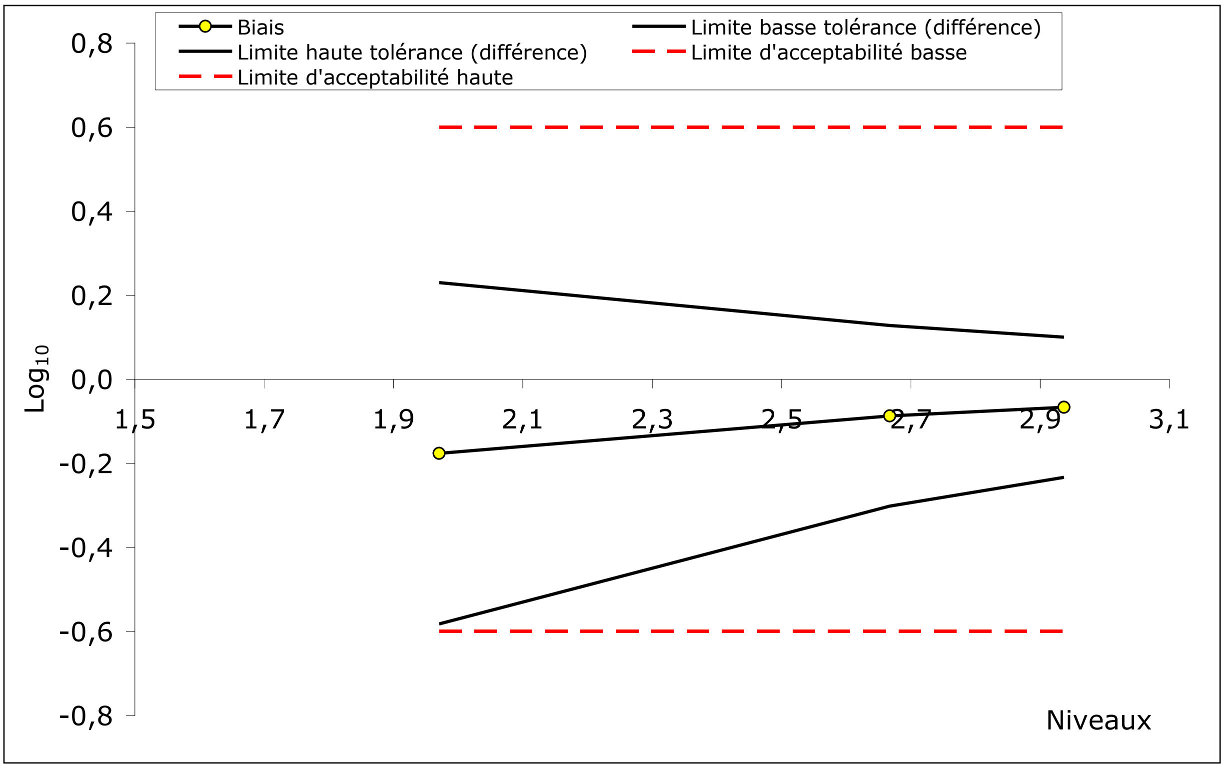


Figure 6: Accuracy profile of the alternative method with tolerance probability of 90 % and acceptability limits at 0,6 log



- Comments

The accuracy profile obtained from the results of the reference method and the alternative method shows that the bias of Colilert method for the enumeration of *E. coli* in bathing waters is acceptable. The tolerance limits of the alternative method for a probability of 90% tolerance are included within the limits of acceptability of 0,6 log.

4.3. Extension study

The aim of the extension study was compared the use of a Quanti-Tray 2000 or the use of a Quanti-Tray with an IDEXX's enumeration method. For this study, data obtained with Colilert-18 were used. Additionally, in order to increase data, other sets of results providing by comparison between Quanti-Tray and Quanti-Tray 2000 were also used. But these data providing of the alternative method Enterolert-E.

4.3.1. Results and interpretation

Two sets of results are available:

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

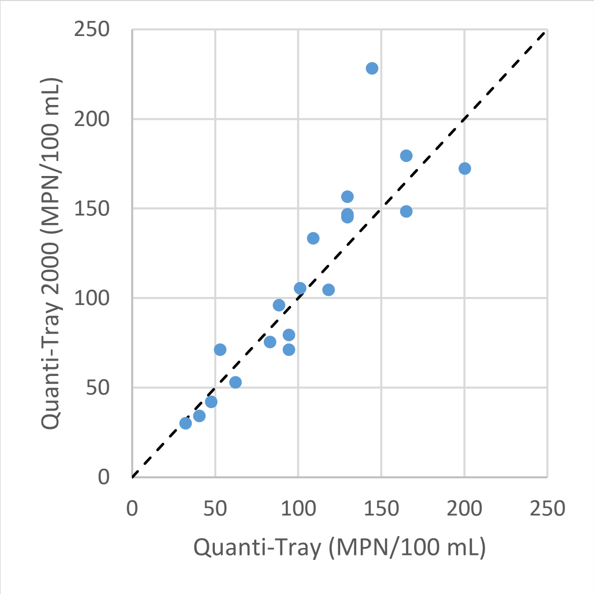
- **Results from Colilert-18 / Quanti-Tray study**

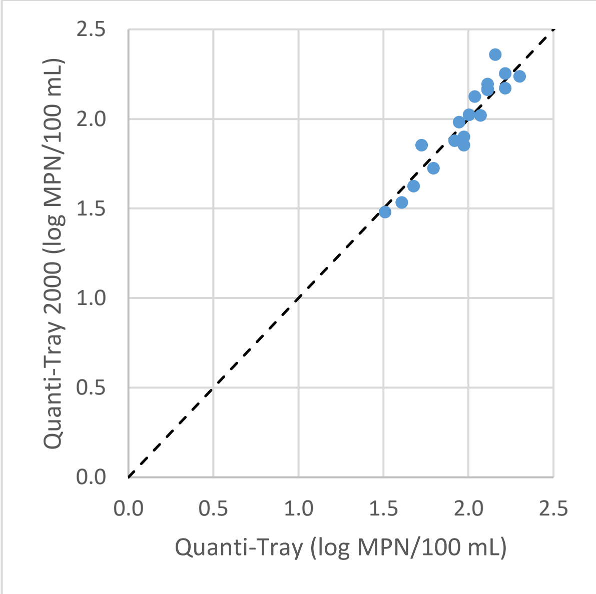
Raw results

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

Results are available in the summary report of the AFNOR Certification validation of the Enterolert-E method. Two two-dimensional graphs are shown in figure 8, presenting the results obtained with the Quanti-Tray (the "validated" Quanti-Tray for the Colilert-18 method in drinking waters) as the reference method.

Figure 8: Comparison of results obtained with Quanti-Tray 2000 and with Quanti-Tray for the enumeration of Escherichia coli in tap water





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

t-Test: Paired Two Sample for Means		
Parameter	Quanti-Tray	Quanti-Tray 2000
Mean	104.8	109.1
Variance	2119.6	3043.9
Observations	19	19
Pearson Correlation	0.892	
Hypothesized Mean Difference	0	
df	18	
t Stat	-0.745	
P(T<=t) one-tail	0.233	
t Critical one-tail	1.734	
P(T<=t) two-tail	0.466	
t Critical two-tail	2.101	

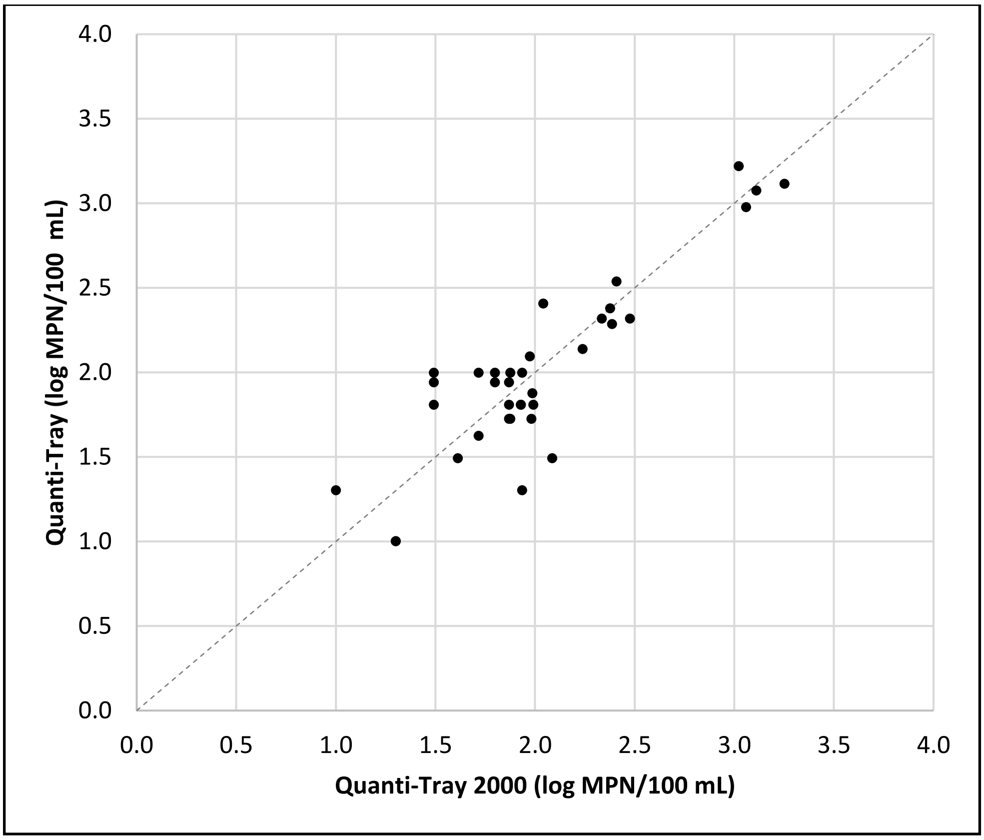
Both one-tailed and two-tailed tests conclude that there is no statistically significant difference between the enumeration of *Escherichia coli* with Quanti-Tray or with Quanti-Tray 2000 at α=0.05.

- **Results from Enterolert-E / Quanti-Tray 2000 comparative study**

Raw results

Results have been collected from samples used in the comparative study for the validation of the method Enterolert-E in the common enumeration range of the two devices, namely from 10 to 2000 MPN/100 mL. A minimum of 10 results was asked by the Technical Board: it's a total of 18 samples that have been taken into account.
Results are available in the summary report of the AFNOR Certification validation of the Enterolert-E method. A two-dimensional graph is shown in figure 7, presenting the results obtained with the Quanti-Tray 2000 (the "validated" Quanti-Tray for the Enterolert-E method) as the reference method.

Figure 7: Comparison of results obtained with Quanti-Tray 2000 and with Quanti-Tray for the validation of the Enterolert-E method



<u>**Statistical interpretation**</u>

Validation protocol for an alternative commercial method as compared with a reference method:

A statistical interpretation has been performed according to the requirements of the Validation protocol for an alternative commercial method as compared with a reference method, considering the Quanti-Tray 2000 as the reference device and using the tests for the relative accuracy. Results are available in the summary report of the AFNOR Certification validation of the Enterolert-E method.
According to this protocol, the relationship of relative accuracy between QT-2000 and QT is evaluated with the linear model: 'y = a + bx'. This formula corresponds to the equation of the linear regression

drawn from raw results obtained by experimentation, y representing the QT-2000 devices and x the QT-devices.

There is a perfect accuracy (or there is no systematic bias) between the two methods if this equation is equal to the theoretical 'y = x' equation, which applies in the ideal model where the two methods behave similarly.
The intercept is theoretically zero in this ideal model (hypothesis [a = 0]). The estimated intercept obtained with the two methods is checked using p {a = 0}. If the alternative method is a systematic bias against the reference method, the probability p {a = 0} is less than α = 0.05.
The 'b' slope is theoretically equal to 1 in the ideal model (hypothesis [b = 1]). The estimated slope obtained with the two methods should pass by p {b = 1}. Statistically, if the alternative method does not give the same values as the reference method, the probability p {b = 1} is less than α = 0.05.

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

Rob.R	Regression used	T critical	a	t(a)	b	t(b)	Probabilities (%)	
							Intercept at 0	Slope at 1
1.416	GMFR	2.101	-0.097	0.460	1.040	0.523	64.8	60.4

The equation for the regression line is as follows: log Alt = 1.040 log Ref – 0.097.
Hypothesis [a = 0 and b = 1] is accepted for the comparison of the enumeration of *enterococci* with the Enterolert-E method using a Quanti-Tray versus a Quanti-Tray 2000.

- **Student-Fisher test**

A Student-Fisher test has been also performed from the data obtained during the validation of the Enterolert-E method. The results of the test are shown in the table below:

t-Test: Paired Two Sample for Means		
Parameter	Quanti-Tray	Quanti-Tray 2000
Mean	1.998	2.015
Variance	0.280	0.259
Observations	36	36
Pearson Correlation	0.883	
Hypothesized Mean Difference	0	
df	35	
t Stat	-0.398	
P(T<=t) one-tail	0.346	
t Critical one-tail	1.690	
P(T<=t) two-tail	0.693	
t Critical two-tail	2.030	

Both one-tailed and two-tailed tests conclude that there is no statistically significant difference between the enumeration of *enterococci* with Quanti-Tray or with Quanti-Tray 2000 at α=0.05.

4.3.2. Conclusion

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

5. Conclusion

- Comparative study

The linearity and relative accuracy of the Colilert-18 / Quanti-Tray or Quanti-Tray2000 method for the enumeration of *E. coli* in bathing waters are satisfactory.
The bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.
Colilert-18 / Quanti-Tray or Quanti-Tray2000 method for the enumeration of *E. coli* is specific and selective.

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

- 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 90% of the results will be between the limits of acceptability as defined at 0,6 log.

6. BIBLIOGRAPHY

Study published since 2016 :

- Tiwari A., Niemela S., et al., Comparison of Colilert-18 with miniaturized most probable number method for monitoring of *Escherichia coli* in bathing water, Journal of Water and Health, 2016, 14(1):121-31

Method including in:

- International Organization for Standards (ISO). ISO 9308-2:2012 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 2: Most probable number method
- UK Standing Committee of Analysts (SCA) Blue Books: The Microbiology of Recreational and Environmental Waters (2016) – Part 3 - Methods for the isolation and enumeration of *Escherichia coli*
- World Health Organization (WHO). WHO recommendation on scientific, analytical, and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC). 2018

Done at Thury-Harcourt, February 07, 2024
Mickaël MORVAN
Research & Development Engineer

Appendix 1: Bacterial stress

Code	Souche	Origine	Stress appliqué	Intensité du stress	Numéro	Eau
ESC.1.116	*Escherichia coli*	Eau de puits	4 j à 4°C + 10 min à -80°C + 5 min à 51°C	1,88	52	Plage de la Roquille
ESC.1.116	*Escherichia coli*	Eau de puits	4 j à 4°C + 10 min à -80°C + 5 min à 51°C	1,88	90	La somme
ESC.1.116	*Escherichia coli*	Eau de puits	4 j à 4°C + 10 min à -80°C + 5 min à 51°C	1,88	94	Troyes
ESC.1.117	*Escherichia coli*	Eau de puits	4 j à 4°C + 10 min à -20°C + 5 min à 51°C	1,3	53	Plage de Carnon
ESC.1.117	*Escherichia coli*	Eau de puits	4 j à 4°C + 10 min à -20°C + 5 min à 51°C	1,3	91	Saint Quentin en Yvelines
ESC.1.117	*Escherichia coli*	Eau de puits	4 j à 4°C + 10 min à -20°C + 5 min à 51°C	1,3	95	Etampes
ESC.1.119	*Escherichia coli*	Eau de distribution	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,9	54	Plage du Couchant
ESC.1.119	*Escherichia coli*	Eau de distribution	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,9	92	Villennes sur Seine
ESC.1.123	*Escherichia coli*	Eau	4 j à 4°C + (5 min à -20°C + 5 min à 36°C) x2	1,1	55	Plage du Point Zero
ESC.1.123	*Escherichia coli*	Eau	4 j à 4°C + (5 min à -20°C + 5 min à 36°C) x2	1,1	93	Saint Leger en Yvelines
ESC.1.111	*Escherichia coli*	Eau de fontaine	4 j à 4°C + 30 min à -80°C + 10 min à 36°C + 10 min à 51°C	1,1	47	Saint Roch
ESC.1.111	*Escherichia coli*	Eau de fontaine	4 j à 4°C + 30 min à -80°C + 10 min à 36°C + 10 min à 51°C	1,1	20	Fécamp
ESC.1.111	*Escherichia coli*	Eau de fontaine	4 j à 4°C + 30 min à -80°C + 10 min à 36°C + 10 min à 51°C	1,1	21	Mesnil Val plage
ESC.1.111	*Escherichia coli*	Eau de fontaine	4 j à 4°C + 30 min à -80°C + 10 min à 36°C + 10 min à 51°C	1,1	22	Dieppe
ESC.1.113	*Escherichia coli*	Eau de puits	4 j à 4°C + 30 min à -20°C + 10 min à 36°C + 10 min à 51°C	1,6	48	Plage Saint Maurice (Palavas les flots)
ESC.1.114	*Escherichia coli*	Eau de puits	4j à 4°C + 10 min à -80°C + 60 min à 36°C	1,1	50	Plage des dunes (Carnon-Plage)
ESC.1.114	*Escherichia coli*	Eau de puits	4j à 4°C + 10 min à -80°C + 60 min à 36°C	1,1	51	Plage du grand travers
ESC.1.120	*Escherichia coli*	Eau	30 min à 56°C	1,7	23	Quend
ESC.1.120	*Escherichia coli*	Eau	30 min à 56°C	1,7	24	Saint Marguerite
ESC.1.122	*Escherichia coli*	Eau	10 min à -20°C + 7 min à 51°C	1,7	25	Saint Pierre en Port
ESC.1.122	*Escherichia coli*	Eau	10 min à -20°C + 7 min à 51°C	1,7	26	Veulette sur mer
ESC.1.123	*Escherichia coli*	Eau	10 min à -20°C + 5 min à 51°C	0,5	27	Charron
ESC.1.123	*Escherichia coli*	Eau	10 min à -20°C + 5 min à 51°C	0,5	28	La Rochelle
ESC.1.112	*Escherichia coli*	Effluent secondaire	(30 min à -80°C + 15 min à 55°C) x2	0,9	1	Saint Brevin
ESC.1.112	*Escherichia coli*	Effluent secondaire	(30 min à -80°C + 15 min à 55°C) x2	0,9	2	Berck
ESC.1.124	*Escherichia coli*	Eau de rivière	7 min à 51°C	0,6	15	Cayeux sur mer

Appendix 2: Relative accuracy results

Exactitude Relative- Eau de mer

N° éch.	eau	Souche	**MA: IDEXX Colilert 18**				**MR:NF ISO 9308-3 (2000)**							
			R1		**R2**		**R1**				**R2**			
							NPP / 100 mL			Log 10 (NPP/100 mL)	NPP / 100 mL			Log 10 (NPP/100 mL)
			NPP/100 mL	log 10 (NPP/100mL)	NPP/100 mL	log 10 (NPP/100mL)	NPP	limite inf.	limite sup.		NPP	limite inf.	limite sup.	
1	Saint Brevin	ESC.1.112	**235,9**	2,373	**344,8**	2,538	**160**	86	298	2,204	**212**	123	366	2,326
2	Berck	ESC.1.112	**193,5**	2,287	**272,3**	2,435	**195**	111	344	2,290	**577**	401	830	2,761
15	Cayeux sur mer	ESC.1.124	**14136**	4,150	**14136**	4,150	**11454**	7151	18344	4,059	**14171**	8995	22327	4,151
20	Fécamp	ESC.1.111	**135**	2,130	**96**	1,982	**208**	87	498	2,318	**78**	20	311	1,892
21	Mesnil Val plage	ESC.1.111	**7701**	3,887	**5794**	3,763	**7683**	4845	12182	3,886	**7101**	4489	11233	3,851
22	Dieppe	ESC.1.111	**7701**	3,887	**6131**	3,788	**4628**	3132	6841	3,665	**4267**	2937	6200	3,630
23	Quend	ESC.1.120	**796**	2,901	**706**	2,849	**1104**	816	1494	3,043	**1177**	873	1587	3,071
24	Saint Marguerite	ESC.1.120	**1396**	3,145	**1106**	3,044	**3422**	2451	4778	3,534	**3020**	2199	4146	3,480
25	Saint Pierre en Port	ESC.1.122	**5172**	3,714	**4884**	3,689	**2823**	2071	3848	3,451	**3951**	2761	5653	3,597
26	Veulette sur mer	ESC.1.122	**1842**	3,265	**1565**	3,195	**1754**	1315	2339	3,244	**1415**	1057	1893	3,151
27	Charron	ESC.1.123	**12033**	4,080	**10462**	4,020	**16740**	10880	25756	4,224	**5352**	3513	8154	3,729
28	La Rochelle	ESC.1.123	**5475**	3,738	**7270**	3,862	**7683**	4845	12182	3,886	**6581**	4184	10350	3,818
47	Plage Saint Roch (Palavas les flots)	ESC.1.111	**20**	1,301	**10**	1,000	**61**	23	163	1,785	**15**	2	106	1,176
48	Plage Saint Maurice (Palavas les flots)	ESC.1.113	**2755**	3,440	**2613**	3,417	**3225**	2329	4465	3,509	**2956**	2158	4049	3,471
50	Plage des dunes (Carnon-Plage)	ESC.1.114	**17329**	4,239	**14136**	4,150	**11636**	7487	18084	4,066	**16740**	10880	25756	4,224
51	Plage du grand travers	ESC.1.114	**6867**	3,837	**6867**	3,837	**9043**	5727	14277	3,956	**9043**	5727	14277	3,956
52	Plage de la Roquille (Agde)	ESC.1.116	**12997**	4,114	**12303**	4,090	**16740**	10880	25756	4,224	**15199**	9879	23383	4,182
53	Plage de carnon (Carnon-Plage)	ESC.1.117	**24196**	4,384	**17329**	4,239	**18563**	12030	28643	4,269	**27726**	17088	44987	4,443
54	Plage du Couchant	ESC.1.119	**15531**	4,191	**24196**	4,384	**20795**	13381	32315	4,318	**20795**	13381	32315	4,318
55	Plage du Point Zero	ESC.1.123	**9208**	3,964	**6488**	3,812	**8329**	5258	13195	3,921	**10687**	6840	16699	4,029

Exactitude relative - Escherichia coli - Eaux de mer

Méthode de référence					Méthode alternative				
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD
1	2,204	2,326	2,265	0,086	1	2,373	2,538	2,455	0,117
2	2,290	2,761	2,526	0,333	2	2,287	2,435	2,361	0,105
3	4,059	4,151	4,105	0,065	3	4,150	4,150	4,150	0,000
4	2,318	1,892	2,105	0,301	4	2,130	1,982	2,056	0,105
5	3,886	3,851	3,868	0,024	5	3,887	3,763	3,825	0,087
6	3,665	3,630	3,648	0,025	6	3,887	3,788	3,837	0,070
7	3,043	3,071	3,057	0,020	7	2,901	2,849	2,875	0,037
8	3,534	3,480	3,507	0,038	8	3,145	3,044	3,094	0,072
9	3,451	3,597	3,524	0,103	9	3,714	3,689	3,701	0,018
10	3,244	3,151	3,197	0,066	10	3,265	3,195	3,230	0,050
11	4,224	3,729	3,976	0,350	11	4,080	4,020	4,050	0,043
12	3,886	3,818	3,852	0,048	12	3,738	3,862	3,800	0,087
13	1,785	1,176	1,481	0,431	13	1,301	1,000	1,151	0,213
14	3,509	3,471	3,490	0,027	14	3,440	3,417	3,429	0,016
15	4,066	4,224	4,145	0,112	15	4,239	4,150	4,195	0,063
16	3,956	3,956	3,956	0,000	16	3,837	3,837	3,837	0,000
17	4,224	4,182	4,203	0,030	17	4,114	4,090	4,102	0,017
18	4,269	4,443	4,356	0,123	18	4,384	4,239	4,311	0,103
19	4,318	4,318	4,318	0,000	19	4,191	4,384	4,287	0,136
20	3,921	4,029	3,975	0,077	20	3,964	3,812	3,888	0,108

Différence
0,190
-0,165
0,045
-0,049
-0,044
0,189
-0,182
-0,413
0,178
0,033
0,074
-0,052
-0,330
-0,061
0,050
-0,120
-0,101
-0,045
-0,030
-0,087

q= 20
n= 2
N=qn= 40

Mx= 3,478
MEDx= 3,750
SDbx= 0,808
MEDwx = 0,066
SDwx= 0,170
rob. SDwx= 0,097

My= 3,432
MEDy= 3,812
SDby= 0,858
MEDwy = 0,071
SDwy= 0,089
rob. SDwy= 0,105

M= -0,046
MED= -0,047
Biais

Choix de la méthode
GMFR

R= 0,522
rob. R= 1,078

Sx= 0,807
Sy= 0,850

r= 0,984
b= 1,053
a= -0,232

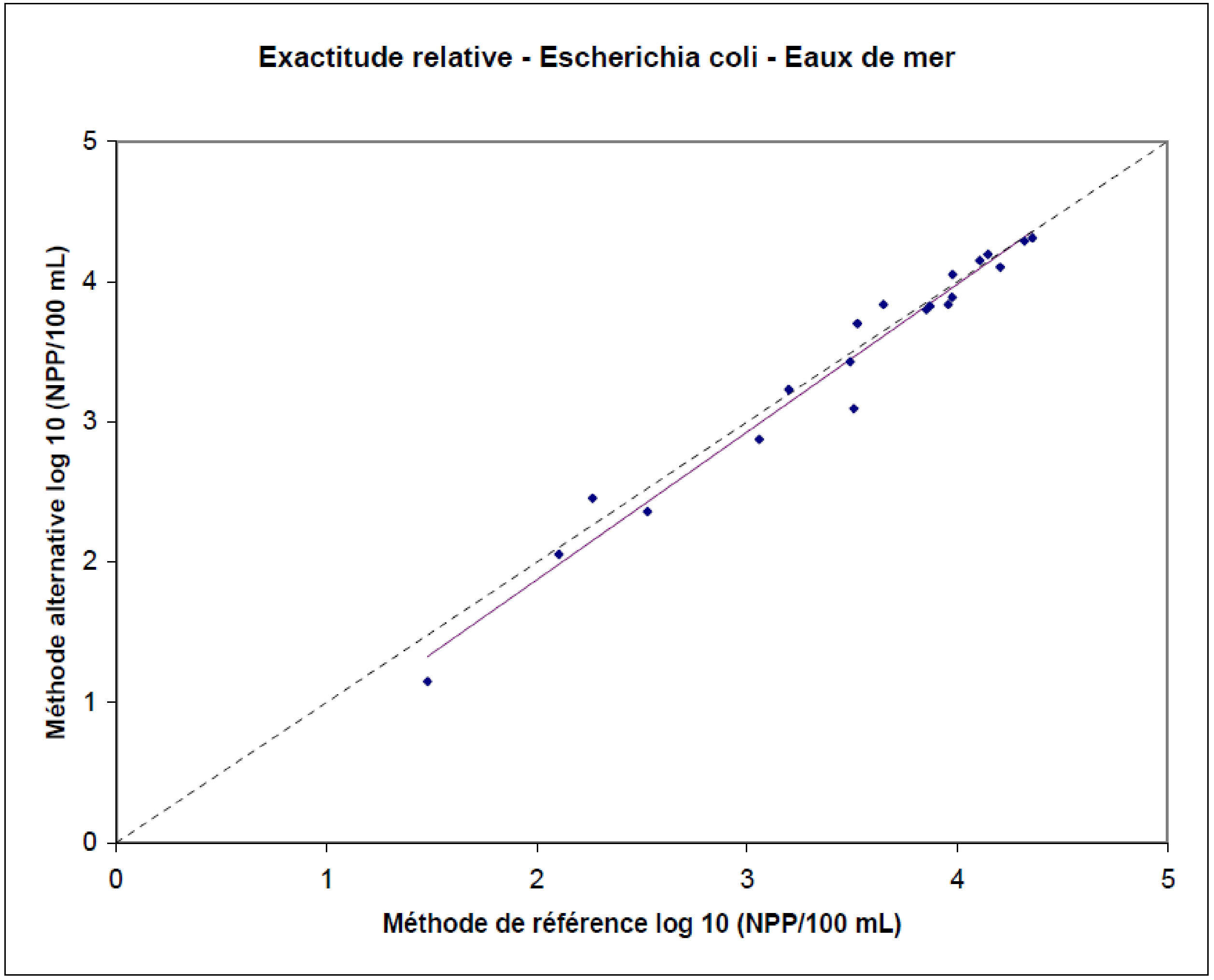
Res. SEM= 0,155
Res. SD= 0,219

S(b)= 0,044 **p(t;b=1)=** 0,233 **t(b)=** 1,212
S(a)= 0,104 **p(t;a=0)=** 0,032 **t(a)=** 2,221

Est. y	Dév.
2,155	0,301
2,429	-0,068
4,093	0,058
1,986	0,070
3,843	-0,019
3,611	0,226
2,988	-0,114
3,463	-0,368
3,480	0,221
3,136	0,093
3,957	0,093
3,826	-0,026
1,328	-0,178
3,444	-0,016
4,134	0,060
3,936	-0,099
4,196	-0,094
4,357	-0,045
4,317	-0,029
3,955	-0,067

Répétabilité	Méthode de référence	Méthode alternative
r	0,476	0,248
rob. r	0,273	0,294

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Exactitude Relative - Eau douce

N° éch.	eau	Souche	MA: IDEXX Colilert 18				MR:NF ISO 9308-3 (2000)							
			R1		R2		R1				R2			
							NPP / 100 mL			Log 10 (NPP/100 mL)	NPP / 100 mL			Log 10 (NPP/100 mL)
			NPP/100 mL	log 10 (NPP/100mL)	NPP/100 mL	log 10 (NPP/100mL)	NPP	limite inf.	limite sup.		NPP	limite inf.	limite sup.	
57	Plage bleue Valenton	NC	**9,7**	0,987	**13,1**	1,117	**30**	8	121	1,477	**30**	8	121	1,477
59	Seine Villeneuve st Georges	NC	**203,5**	2,309	**435,2**	2,639	**251**	151	416	2,400	**612**	429	874	2,787
60	L'Yerres	NC	**104,6**	2,020	**93,4**	1,970	**61**	23	163	1,785	**127**	63	253	2,104
61	Lac d'Aydat	NC	**2419,6**	3,384	**2419,6**	3,384	**2759**	2029	3752	3,441	**2469**	1831	3329	3,393
62	Annet sur marne	NC	**32,7**	1,515	**43,9**	1,642	**30**	8	121	1,477	**110**	52	231	2,041
64	Noisiel	NC	**104,6**	2,020	**95,9**	1,982	**215**	125	370	2,332	**144**	75	276	2,158
65	Seine Les Mureaux	NC	**16,9**	1,228	**16,1**	1,207	**46**	15	142	1,663	**15**	2	106	1,176
66	Etampes	NC	**2**	0,301	**7,5**	0,875	**15**	2	106	1,176	**30**	8	121	1,477
67	Orge St Chéron	NC	**344,8**	2,538	**325,5**	2,513	**559**	387	808	2,747	**574**	399	829	2,759
68	Rivière la Rémarde	NC	**1299,7**	3,114	**2419,6**	3,384	**1712**	1284	2285	3,234	**1537**	1151	2053	3,187
72	Allier	NC	**42,8**	1,631	**36,4**	1,561	**45**	14	140	1,653	**46**	15	142	1,663
73	Longarisse	NC: Dilué	**1119,9**	3,049	**547,5**	2,738	**1007**	740	1371	3,003	**838**	606	1157	2,923
74	La Somme	NC: Dilué	**410,6**	2,613	**290,9**	2,464	**489**	333	720	2,689	**442**	311	684	2,645
75	Aix les bains	NC	**155,3**	2,191	**156,5**	2,195	**161**	87	299	2,207	**197**	112	346	2,294
76	Meyrieu les étangs	NC	**98,7**	1,994	**124,6**	2,096	**110**	52	231	2,041	**213**	124	368	2,328
90	La somme	ESC.1.116	**816,4**	2,912	**613**	2,787	**838**	606	1157	2,923	**612**	429	874	2,787
91	Saint Quentin en Yvelines	ESC.1.117	**488,4**	2,689	**686,7**	2,837	**591**	412	848	2,772	**740**	529	1034	2,869
92	Villennes sur Seine	ESC.1.119	**829,7**	2,919	**913,9**	2,961	**901**	656	1236	2,955	**968**	709	1321	2,986
93	Saint Leger en Yvelines	ESC.1.123	**113,7**	2,056	**122,3**	2,087	**141**	73	272	2,149	**158**	84	295	2,199
94	Troyes	ESC.1.116	**727**	2,862	**686,7**	2,837	**732**	553	1024	2,865	**633**	445	901	2,801
95	Etampes	ESC.1.117	**1553,1**	3,191	**1986,3**	3,298	**1554**	1164	2075	3,191	**2211**	1650	2963	3,345
96	La Sioul	NC	**43,5**	1,638	**48**	1,681	**30**	15	141	1,477	**45**	23	163	1,653

Exactitude relative - Escherichia coli - Eaux douces

Méthode de référence					Méthode alternative					Différence
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	
1	1,477	1,477	1,477	0,000	1	0,987	1,117	1,052	0,092	-0,425
2	2,400	2,787	2,593	0,274	2	2,309	2,639	2,474	0,233	-0,120
3	1,785	2,104	1,945	0,225	3	2,020	1,970	1,995	0,035	0,050
4	3,441	3,393	3,417	0,034	4	3,384	3,384	3,384	0,000	-0,033
5	1,477	2,041	1,759	0,399	5	1,515	1,642	1,579	0,090	-0,181
6	2,332	2,158	2,245	0,123	6	2,020	1,982	2,001	0,027	-0,245
7	1,663	1,176	1,419	0,344	7	1,228	1,207	1,217	0,015	-0,202
8	1,176	1,477	1,327	0,213	8	0,301	0,875	0,588	0,406	-0,739
9	2,747	2,759	2,753	0,008	9	2,538	2,513	2,525	0,018	-0,228
10	3,234	3,187	3,210	0,033	10	3,114	3,384	3,249	0,191	0,039
11	1,653	1,663	1,658	0,007	11	1,631	1,561	1,596	0,050	-0,062
12	3,003	2,923	2,963	0,056	12	3,049	2,738	2,894	0,220	-0,069
13	2,689	2,645	2,667	0,031	13	2,613	2,464	2,539	0,106	-0,129
14	2,207	2,294	2,251	0,062	14	2,191	2,195	2,193	0,002	-0,058
15	2,041	2,328	2,185	0,203	15	1,994	2,096	2,045	0,072	-0,140
16	2,923	2,787	2,855	0,097	16	2,912	2,787	2,850	0,088	-0,005
17	2,772	2,869	2,820	0,069	17	2,689	2,837	2,763	0,105	-0,058
18	2,955	2,986	2,970	0,022	18	2,919	2,961	2,940	0,030	-0,030
19	2,149	2,199	2,174	0,035	19	2,056	2,087	2,072	0,022	-0,102
20	2,865	2,801	2,833	0,045	20	2,862	2,837	2,849	0,018	0,016
21	3,191	3,345	3,268	0,108	21	3,191	3,298	3,245	0,076	-0,023
22	1,477	1,653	1,565	0,125	22	1,638	1,681	1,660	0,030	0,095

q= 22
n= 2
N=qn= 44

Mx= 2,380
MEDx= 2,422
SDbx= 0,649
MEDwx = 0,066
SDwx= 0,160
rob. SDwx= 0,097

My= 2,259
MEDy= 2,333
SDby= 0,757
MEDwy = 0,061
SDwy= 0,129
rob. SDwy= 0,090

M= -0,120
MED= -0,066
Biais

Choix de la méthode
GMFR

R= 0,811
rob. R= 0,926

Sx= 0,652
Sy= 0,754

r= 0,979
b= 1,157
a= -0,494

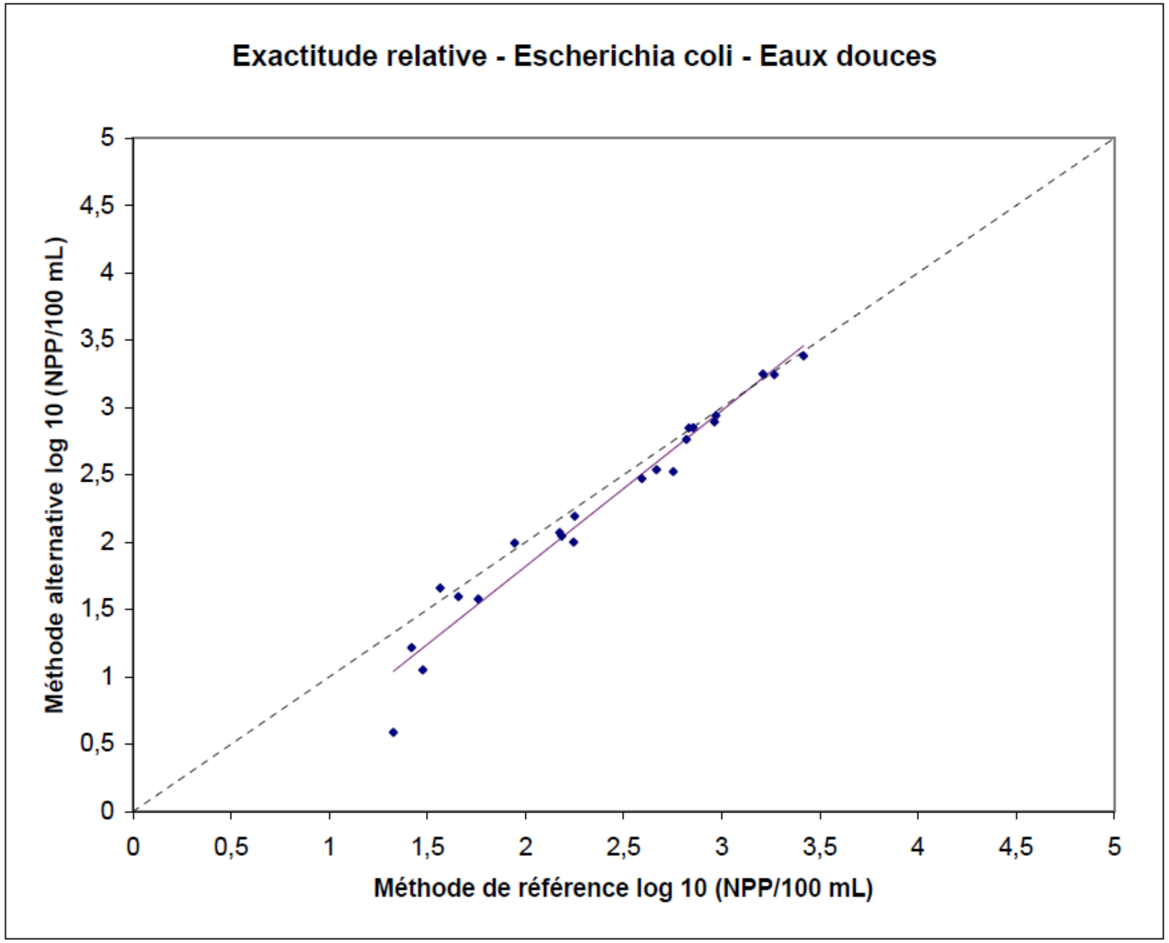
Res. SEM= 0,160
Res. SD= 0,226

S(b)= 0,053 **p(t;b=1)=** 0,005 **t(b)=** 2,938
S(a)= 0,140 **p(t;a=0)=** 0,001 **t(a)=** 3,526

Répétabilité	Méthode de référence	Méthode alternative
r	0,447	0,363
rob. r	0,272	0,252

Est. y	Dév.
1,215	-0,163
2,506	-0,033
1,756	0,239
3,459	-0,075
1,541	0,037
2,104	-0,103
1,148	0,069
1,041	-0,453
2,691	-0,166
3,220	0,029
1,424	0,172
2,934	-0,041
2,592	-0,054
2,110	0,083
2,034	0,011
2,809	0,040
2,769	-0,006
2,943	-0,003
2,021	0,050
2,784	0,065
3,287	-0,042
1,317	0,343

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Exactitude relative - Escherichia coli - Eaux de baignade (eaux douces + eaux de mer)

Méthode de référence					Méthode alternative					Différence
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	
1	2,204	2,326	2,265	0,086	1	2,373	2,538	2,455	0,117	0,190
2	2,290	2,761	2,526	0,333	2	2,287	2,435	2,361	0,105	-0,165
3	4,059	4,151	4,105	0,065	3	4,150	4,150	4,150	0,000	0,045
4	2,318	1,892	2,105	0,301	4	2,130	1,982	2,056	0,105	-0,049
5	3,886	3,851	3,868	0,024	5	3,887	3,763	3,825	0,087	-0,044
6	3,665	3,630	3,648	0,025	6	3,887	3,788	3,837	0,070	0,189
7	3,043	3,071	3,057	0,020	7	2,901	2,849	2,875	0,037	-0,182
8	3,534	3,480	3,507	0,038	8	3,145	3,044	3,094	0,072	-0,413
9	3,451	3,597	3,524	0,103	9	3,714	3,689	3,701	0,018	0,178
10	3,244	3,151	3,197	0,066	10	3,265	3,195	3,230	0,050	0,033
11	4,224	3,729	3,976	0,350	11	4,080	4,020	4,050	0,043	0,074
12	3,886	3,818	3,852	0,048	12	3,738	3,862	3,800	0,087	-0,052
13	1,785	1,176	1,481	0,431	13	1,301	1,000	1,151	0,213	-0,330
14	3,509	3,471	3,490	0,027	14	3,440	3,417	3,429	0,016	-0,061
15	4,066	4,224	4,145	0,112	15	4,239	4,150	4,195	0,063	0,050
16	3,956	3,956	3,956	0,000	16	3,837	3,837	3,837	0,000	-0,120
17	4,224	4,182	4,203	0,030	17	4,114	4,090	4,102	0,017	-0,101
18	4,269	4,443	4,356	0,123	18	4,384	4,239	4,311	0,103	-0,045
19	4,318	4,318	4,318	0,000	19	4,191	4,384	4,287	0,136	-0,030
20	3,921	4,029	3,975	0,077	20	3,964	3,812	3,888	0,108	-0,087
21	1,477	1,477	1,477	0,000	21	0,987	1,117	1,052	0,092	-0,425
22	2,400	2,787	2,593	0,274	22	2,309	2,639	2,474	0,233	-0,120
23	1,785	2,104	1,945	0,225	23	2,020	1,970	1,995	0,035	0,050
24	3,441	3,393	3,417	0,034	24	3,384	3,384	3,384	0,000	-0,033
25	1,477	2,041	1,759	0,399	25	1,515	1,642	1,579	0,090	-0,181
26	2,332	2,158	2,245	0,123	26	2,020	1,982	2,001	0,027	-0,245
27	1,663	1,176	1,419	0,344	27	1,228	1,207	1,217	0,015	-0,202
28	1,176	1,477	1,327	0,213	28	0,301	0,875	0,588	0,406	-0,739
29	2,747	2,759	2,753	0,008	29	2,538	2,513	2,525	0,018	-0,228
30	3,234	3,187	3,210	0,033	30	3,114	3,384	3,249	0,191	0,039
31	1,653	1,663	1,658	0,007	31	1,631	1,561	1,596	0,050	-0,062
32	3,003	2,923	2,963	0,056	32	3,049	2,738	2,894	0,220	-0,069
33	2,689	2,645	2,667	0,031	33	2,613	2,464	2,539	0,106	-0,129
34	2,207	2,294	2,251	0,062	34	2,191	2,195	2,193	0,002	-0,058
35	2,041	2,328	2,185	0,203	35	1,994	2,096	2,045	0,072	-0,140
36	2,923	2,787	2,855	0,097	36	2,912	2,787	2,850	0,088	-0,005
37	2,772	2,869	2,820	0,069	37	2,689	2,837	2,763	0,105	-0,058
38	2,955	2,986	2,970	0,022	38	2,919	2,961	2,940	0,030	-0,030
39	2,149	2,199	2,174	0,035	39	2,056	2,087	2,072	0,022	-0,102
40	2,865	2,801	2,833	0,045	40	2,862	2,837	2,849	0,018	0,016
41	3,191	3,345	3,268	0,108	41	3,191	3,298	3,245	0,076	-0,023
42	1,477	1,653	1,565	0,125	42	1,638	1,681	1,660	0,030	0,095

q= 42	**Mx=** 2,903		**My=** 2,818		**M=** -0,085
n= 2	**MEDx=** 2,909		**MEDy=** 2,862		**MED=** -0,058
N=qn= 84	**SDbx=** 0,909		**SDby=** 0,993		Biais
		MEDwx = 0,066		**MEDwy =** 0,071	
		SDwx= 0,165		**SDwy=** 0,112	
		rob. SDwx= 0,097		**rob. SDwy=** 0,105	

Choix de la méthode
GMFR

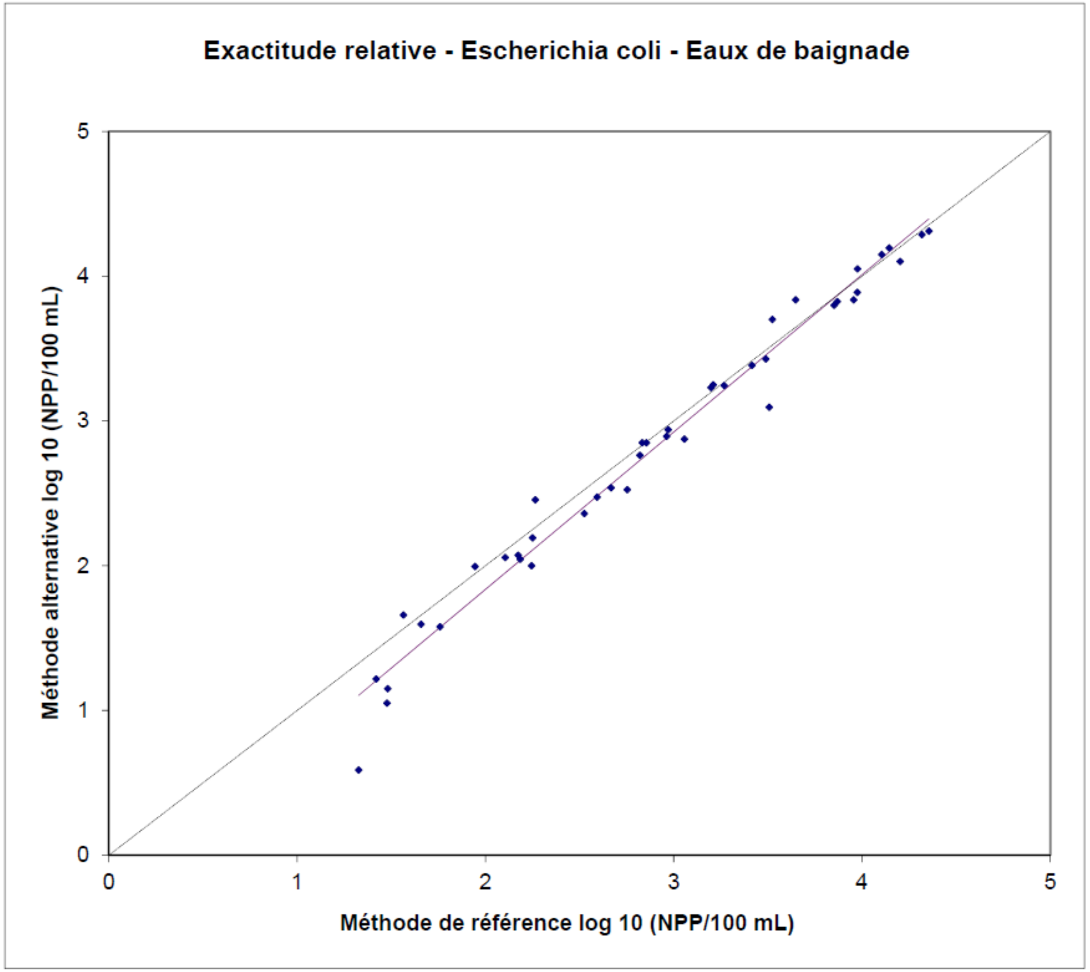
		R=	0,680		
		rob. R=	1,078		
				Sx=	0,911
				Sy=	0,990
r=	0,988				
b=	1,087			**Res. SEM=**	0,157
a=	-0,337			**Res. SD=**	0,223

S(b)=	0,027	**p(t;b=1)=**	0,002	**t(b)=**	3,226
S(a)=	0,117	**p(t;a=0)=**	0,005	**t(a)=**	2,894

Répétabilité	Méthode de référence	Méthode alternative
r	0,461	0,313
rob. r	0,273	0,294

Est. y	Dév.
2,125	0,330
2,408	-0,047
4,125	0,025
1,951	0,106
3,868	-0,043
3,628	0,209
2,985	-0,110
3,475	-0,380
3,493	0,208
3,138	0,092
3,985	0,065
3,850	-0,050
1,272	-0,122
3,456	-0,027
4,168	0,027
3,963	-0,126
4,231	-0,129
4,397	-0,086
4,356	-0,069
3,983	-0,095
1,268	-0,216
2,481	-0,008
1,776	0,219
3,376	0,007
1,575	0,004
2,103	-0,103
1,205	0,012
1,105	-0,516
2,655	-0,130
3,152	0,097
1,465	0,132
2,883	0,010
2,562	-0,023
2,109	0,084
2,037	0,007
2,766	0,084
2,728	0,034
2,891	0,049
2,026	0,046
2,742	0,107
3,215	0,030
1,364	0,296

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Appendix 3: Raw results of relative accuracy on 20 used results for fresh waters and on 20 used results for sea waters

Exactitude Relative - Eau douce

N° éch.	eau	Souche	MA: IDEXX Colilert 18				MR:NF ISO 9308-3 (2000)							
			R1		R2		R1				R2			
							NPP / 100 mL			Log 10 (NPP /100 mL)	UFC / 100 mL			Log 10 (NPP /100 mL)
			NPP/100 mL	log 10 (NPP/100mL)	NPP/100 mL	log 10 (NPP/100mL)	NPP	limite inf.	limite sup.		NPP	limite inf.	limite sup.	
59	Seine Villeneuve st Georges	NC	**203,5**	2,309	**435,2**	2,639	**251**	151	416	2,400	**612**	429	874	2,787
60	L'Yerres	NC	**104,6**	2,020	**93,4**	1,970	**61**	23	163	1,785	**127**	63	253	2,104
61	Lac d'Aydat	NC	**2419,6**	3,384	**2419,6**	3,384	**2759**	2029	3752	3,441	**2469**	1831	3329	3,393
62	Annet sur marne	NC	**32,7**	1,515	**43,9**	1,642	**30**	8	121	1,477	**110**	52	231	2,041
64	Noisiel	NC	**104,6**	2,020	**95,9**	1,982	**215**	125	370	2,332	**144**	75	276	2,158
65	Seine Les Mureaux	NC	**16,9**	1,228	**16,1**	1,207	**46**	15	142	1,663	**15**	2	106	1,176
67	Orge St Chéron	NC	**344,8**	2,538	**325,5**	2,513	**559**	387	808	2,747	**574**	399	829	2,759
68	Rivière la Rémarde	NC	**1299,7**	3,114	**2419,6**	3,384	**1712**	1284	2285	3,234	**1537**	1151	2053	3,187
72	Allier	NC	**42,8**	1,631	**36,4**	1,561	**45**	14	140	1,653	**46**	15	142	1,663
73	Longarisse	NC: Dilué	**1119,9**	3,049	**547,5**	2,738	**1007**	740	1371	3,003	**838**	606	1157	2,923
74	La Somme	NC: Dilué	**410,6**	2,613	**290,9**	2,464	**489**	333	720	2,689	**442**	311	684	2,645
75	Aix les bains	NC	**155,3**	2,191	**156,5**	2,195	**161**	87	299	2,207	**197**	112	346	2,294
76	Meyrieu les étangs	NC	**98,7**	1,994	**124,6**	2,096	**110**	52	231	2,041	**213**	124	368	2,328
90	La somme	ESC.1.116	**816,4**	2,912	**613**	2,787	**838**	606	1157	2,923	**612**	429	874	2,787
91	Saint Quentin en Yvelines	ESC.1.117	**488,4**	2,689	**686,7**	2,837	**591**	412	848	2,772	**740**	529	1034	2,869
92	Villennes sur Seine	ESC.1.119	**829,7**	2,919	**913,9**	2,961	**901**	656	1236	2,955	**968**	709	1321	2,986
93	Saint Leger en Yvelines	ESC.1.123	**113,7**	2,056	**122,3**	2,087	**141**	73	272	2,149	**158**	84	295	2,199
94	Troyes	ESC.1.116	**727**	2,862	**686,7**	2,837	**732**	553	1024	2,865	**633**	445	901	2,801
95	Etampes	ESC.1.117	**1553,1**	3,191	**1986,3**	3,298	**1554**	1164	2075	3,191	**2211**	1650	2963	3,345
96	Sioule	NC	**43,5**	1,638	**48**	1,681	**30**	15	141	1,477	**45**	23	163	1,653

Exactitude relative - Escherichia coli - Eaux douces

Méthode de référence					Méthode alternative				
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD
1	2,400	2,787	2,593	0,274	1	2,309	2,639	2,474	0,233
2	1,785	2,104	1,945	0,225	2	2,020	1,970	1,995	0,035
3	3,441	3,393	3,417	0,034	3	3,384	3,384	3,384	0,000
4	1,477	2,041	1,759	0,399	4	1,515	1,642	1,579	0,090
5	2,332	2,158	2,245	0,123	5	2,020	1,982	2,001	0,027
6	1,663	1,176	1,419	0,344	6	1,228	1,207	1,217	0,015
7	2,747	2,759	2,753	0,008	7	2,538	2,513	2,525	0,018
8	3,234	3,187	3,210	0,033	8	3,114	3,384	3,249	0,191
9	1,653	1,663	1,658	0,007	9	1,631	1,561	1,596	0,050
10	3,003	2,923	2,963	0,056	10	3,049	2,738	2,894	0,220
11	2,689	2,645	2,667	0,031	11	2,613	2,464	2,539	0,106
12	2,207	2,294	2,251	0,062	12	2,191	2,195	2,193	0,002
13	2,041	2,328	2,185	0,203	13	1,994	2,096	2,045	0,072
14	2,923	2,787	2,855	0,097	14	2,912	2,787	2,850	0,088
15	2,772	2,869	2,820	0,069	15	2,689	2,837	2,763	0,105
16	2,955	2,986	2,970	0,022	16	2,919	2,961	2,940	0,030
17	2,149	2,199	2,174	0,035	17	2,056	2,087	2,072	0,022
18	2,865	2,801	2,833	0,045	18	2,862	2,837	2,849	0,018
19	3,191	3,345	3,268	0,108	19	3,191	3,298	3,245	0,076
20	1,477	1,653	1,565	0,125	20	1,638	1,681	1,660	0,030

Différence
-0,120
0,050
-0,033
-0,181
-0,245
-0,202
-0,228
0,039
-0,062
-0,069
-0,129
-0,058
-0,140
-0,005
-0,058
-0,030
-0,102
0,016
-0,023
0,095

q= 20
n= 2
N=qn= 40

Mx= 2,478
MEDx= 2,630
SDbx= 0,596
MEDwx = 0,066
SDwx= 0,160
rob. SDwx= 0,097

My= 2,403
MEDy= 2,499
SDby= 0,623
MEDwy = 0,042
SDwy= 0,099
rob. SDwy= 0,063

M= -0,074
MED= -0,060
Biais

Choix de la méthode

GMFR

R= 0,616
rob. R= 0,645

Sx= 0,599
Sy= 0,619

r= 0,989
b= 1,034
a= -0,158

Res. SEM= 0,094
Res. SD= 0,133

S(b)= 0,036
S(a)= 0,080

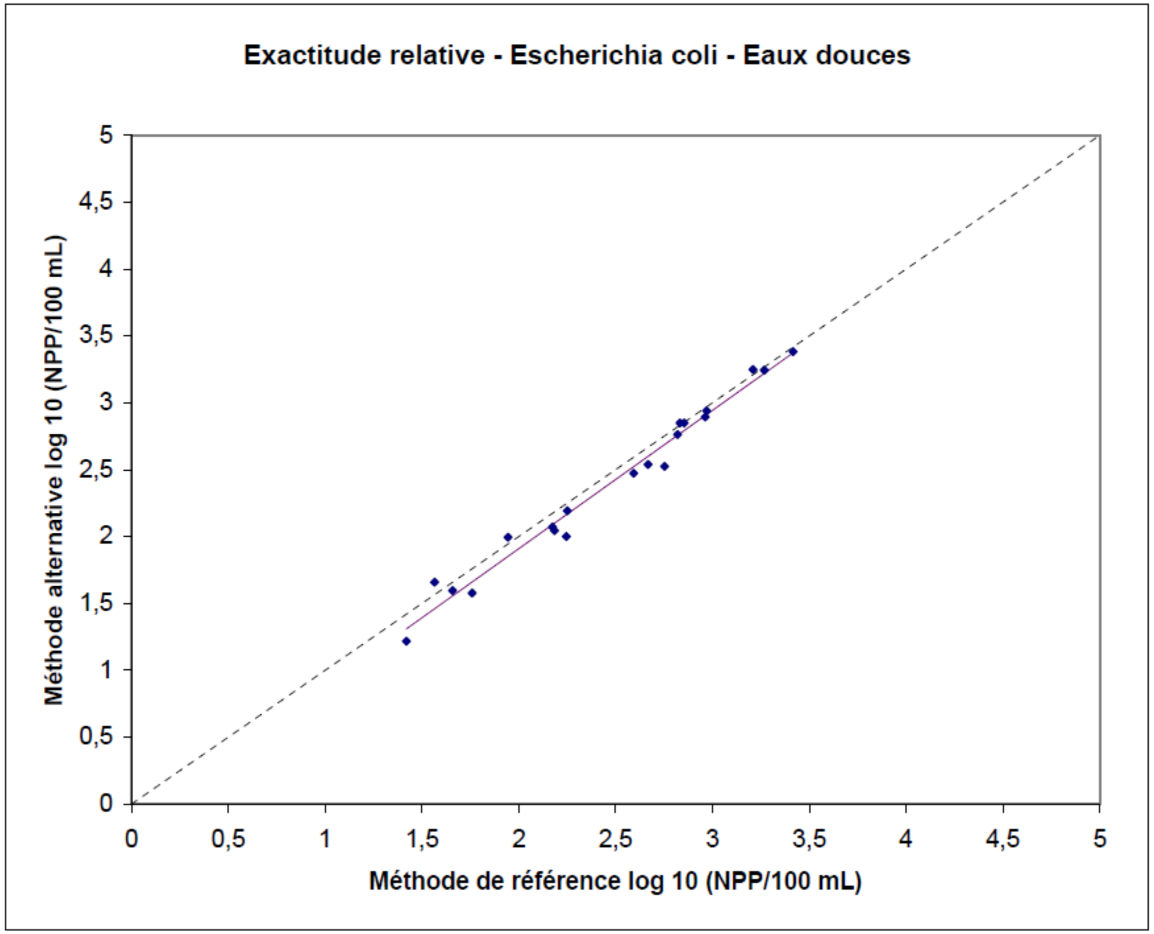
p(t;b=1)= 0,355
p(t;a=0)= 0,056

t(b)= 0,937
t(a)= 1,969

Répétabilité	Méthode de référence	Méthode alternative
r	0,449	0,277
rob. r	0,272	0,175

Est. y	Dév.
2,523	-0,049
1,852	0,143
3,374	0,010
1,661	-0,082
2,163	-0,163
1,310	-0,092
2,688	-0,163
3,161	0,088
1,556	0,040
2,905	-0,011
2,600	-0,061
2,169	0,024
2,101	-0,056
2,793	0,056
2,758	0,005
2,913	0,027
2,089	-0,018
2,771	0,078
3,220	0,024
1,460	0,200

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Exactitude relative - Escherichia coli - Eaux de baignade (eaux douces + eaux de mer)

Suppression of 2 results under the LOD of the reference method

Méthode de référence					Méthode alternative					Différence
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	
1	2,204	2,326	2,265	0,086	1	2,373	2,538	2,455	0,117	0,190
2	2,290	2,761	2,526	0,333	2	2,287	2,435	2,361	0,105	-0,165
3	4,059	4,151	4,105	0,065	3	4,150	4,150	4,150	0,000	0,045
4	2,318	1,892	2,105	0,301	4	2,130	1,982	2,056	0,105	-0,049
5	3,886	3,851	3,868	0,024	5	3,887	3,763	3,825	0,087	-0,044
6	3,665	3,630	3,648	0,025	6	3,887	3,788	3,837	0,070	0,189
7	3,043	3,071	3,057	0,020	7	2,901	2,849	2,875	0,037	-0,182
8	3,534	3,480	3,507	0,038	8	3,145	3,044	3,094	0,072	-0,413
9	3,451	3,597	3,524	0,103	9	3,714	3,689	3,701	0,018	0,178
10	3,244	3,151	3,197	0,066	10	3,265	3,195	3,230	0,050	0,033
11	4,224	3,729	3,976	0,350	11	4,080	4,020	4,050	0,043	0,074
12	3,886	3,818	3,852	0,048	12	3,738	3,862	3,800	0,087	-0,052
13	1,785	1,176	1,481	0,431	13	1,301	1,000	1,151	0,213	-0,330
14	3,509	3,471	3,490	0,027	14	3,440	3,417	3,429	0,016	-0,061
15	4,066	4,224	4,145	0,112	15	4,239	4,150	4,195	0,063	0,050
16	3,956	3,956	3,956	0,000	16	3,837	3,837	3,837	0,000	-0,120
17	4,224	4,182	4,203	0,030	17	4,114	4,090	4,102	0,017	-0,101
18	4,269	4,443	4,356	0,123	18	4,384	4,239	4,311	0,103	-0,045
19	4,318	4,318	4,318	0,000	19	4,191	4,384	4,287	0,136	-0,030
20	3,921	4,029	3,975	0,077	20	3,964	3,812	3,888	0,108	-0,087
21	2,400	2,787	2,593	0,274	22	2,309	2,639	2,474	0,233	-0,120
22	1,785	2,104	1,945	0,225	23	2,020	1,970	1,995	0,035	0,050
23	3,441	3,393	3,417	0,034	24	3,384	3,384	3,384	0,000	-0,033
24	1,477	2,041	1,759	0,399	25	1,515	1,642	1,579	0,090	-0,181
25	2,332	2,158	2,245	0,123	26	2,020	1,982	2,001	0,027	-0,245
26	1,663	1,176	1,419	0,344	27	1,228	1,207	1,217	0,015	-0,202
27	2,747	2,759	2,753	0,008	29	2,538	2,513	2,525	0,018	-0,228
28	3,234	3,187	3,210	0,033	30	3,114	3,384	3,249	0,191	0,039
29	1,653	1,663	1,658	0,007	31	1,631	1,561	1,596	0,050	-0,062
30	3,003	2,923	2,963	0,056	32	3,049	2,738	2,894	0,220	-0,069
31	2,689	2,645	2,667	0,031	33	2,613	2,464	2,539	0,106	-0,129
32	2,207	2,294	2,251	0,062	34	2,191	2,195	2,193	0,002	-0,058
33	2,041	2,328	2,185	0,203	35	1,994	2,096	2,045	0,072	-0,140
34	2,923	2,787	2,855	0,097	36	2,912	2,787	2,850	0,088	-0,005
35	2,772	2,869	2,820	0,069	37	2,689	2,837	2,763	0,105	-0,058
36	2,955	2,986	2,970	0,022	38	2,919	2,961	2,940	0,030	-0,030
37	2,149	2,199	2,174	0,035	39	2,056	2,087	2,072	0,022	-0,102
38	2,865	2,801	2,833	0,045	40	2,862	2,837	2,849	0,018	0,016
39	3,191	3,345	3,268	0,108	41	3,191	3,298	3,245	0,076	-0,023
40	1,477	1,653	1,565	0,125	42	1,638	1,681	1,660	0,030	0,095

q= 40
n= 2
N=qn= 80

Mx= 2,978
MEDx= 2,967
SDbx= 0,864
MEDwx = 0,066
SDwx= 0,165
rob. SDwx= 0,097

My= 2,918
MEDy= 2,884
SDby= 0,905
MEDwy = 0,066
SDwy= 0,094
rob. SDwy= 0,098

M= -0,060
MED= -0,055
Biais

Choix de la méthode
GMFR

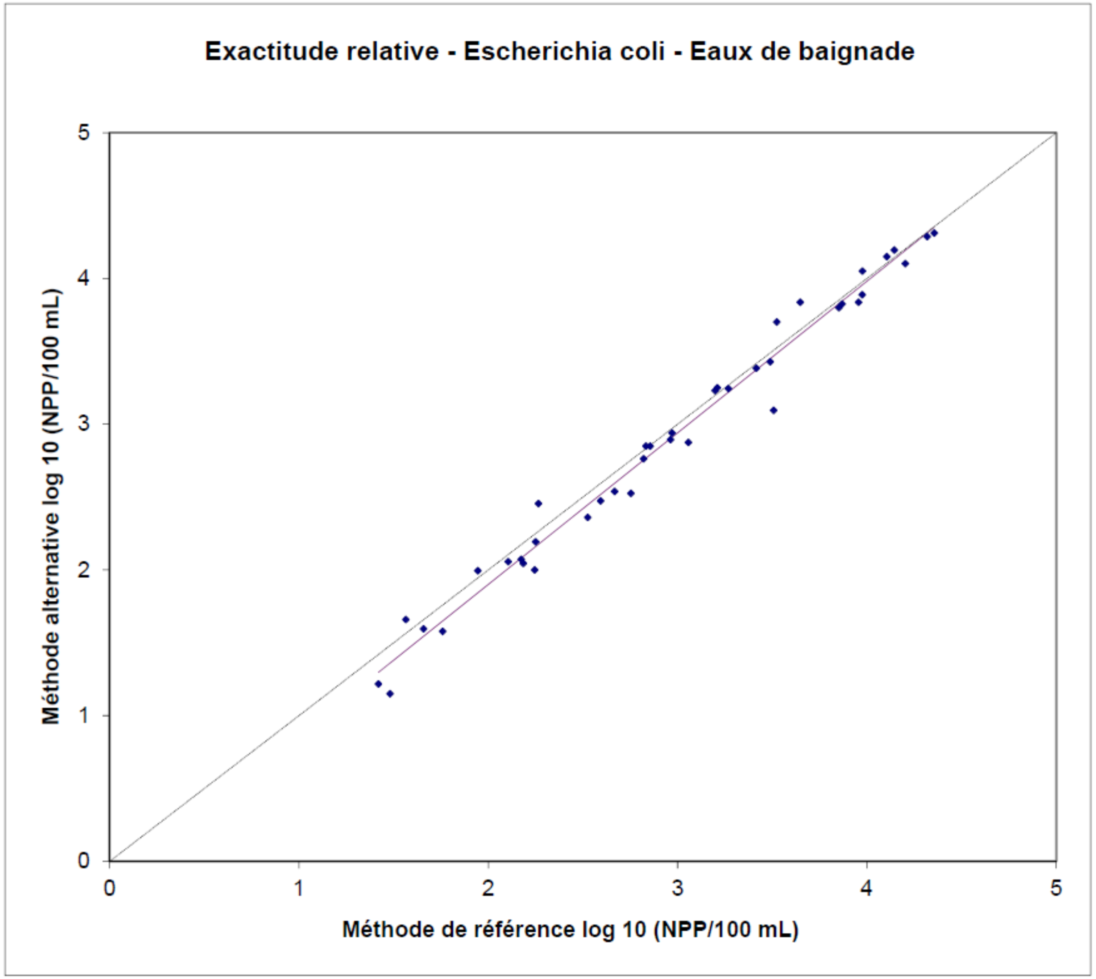
		R=	0,568		
		rob. R=	1,009		
				Sx=	0,867
				Sy=	0,902
r=	0,991				
b=	1,040			**Res. SEM=**	0,125
a=	-0,180			**Res. SD=**	0,177

S(b)=	0,023	**p(t;b=1)=**	0,084	**t(b)=**	1,750
S(a)=	0,091	**p(t;a=0)=**	0,051	**t(a)=**	1,982

Répétabilité	Méthode de référence	Méthode alternative
r	0,463	0,263
rob. r	0,273	0,275

Est. y	Dév.
2,176	0,279
2,447	-0,086
4,091	0,060
2,010	0,047
3,844	-0,020
3,615	0,222
3,000	-0,125
3,468	-0,374
3,486	0,216
3,146	0,084
3,956	0,094
3,827	-0,027
1,360	-0,210
3,450	-0,022
4,132	0,063
3,936	-0,099
4,192	-0,090
4,351	-0,040
4,312	-0,024
3,955	-0,067
2,518	-0,044
1,843	0,152
3,374	0,009
1,650	-0,071
2,156	-0,155
1,296	-0,079
2,684	-0,159
3,159	0,089
1,545	0,052
2,902	-0,009
2,595	-0,056
2,161	0,032
2,093	-0,048
2,790	0,060
2,754	0,009
2,910	0,030
2,081	-0,010
2,767	0,082
3,220	0,025
1,448	0,212

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Appendix 4: Linearity results

Linéarité- Résultats bruts

Souche	Matrice	IDEXX Colilert 18-Quanti-tray 2000 (NPP/100mL)				NF ISO 9308-3 (2000) (NPP/100mL)							
		R1		R2		R1				R2			
		NPP /100mL	log10 (NPP/100mL)	NPP /100mL	log10 (NPP/100mL)	NPP /100mL	log10 (NPP/100mL)	limite inf.	limite sup.	NPP /100mL	log10 (NPP/100mL)	limite inf.	limite sup.
ESC.1.119	Eau de mer	**84**	*1,924*	**158**	*2,199*	**46**	*1,663*	15	142	**94**	*1,973*	42	208
		441	*2,644*	**313**	*2,496*	**347**	*2,540*	223	540	**627**	*2,797*	440	892
		813	*2,910*	**842**	*2,925*	**955**	*2,980*	699	1305	**828**	*2,918*	599	1145
		4611	*3,664*	**9804**	*3,991*	**7383**	*3,868*	4845	12182	**9826**	*3,992*	6254	15439

Souche	Matrice	IDEXX Colilert 18-Quanti-tray 2000 (NPP/100mL)				NF ISO 9308-3 (2000) (NPP/100mL)							
		R1		R2		R1				R2			
		NPP /100mL	log10 (NPP/100mL)	NPP /100mL	log10 (NPP/100mL)	NPP /100mL	log10 (NPP/100mL)	limite inf.	limite sup.	NPP /100mL	log10 (NPP/100mL)	limite inf.	limite sup.
ESC.1.112	Eau douce	**77**	*1,887*	**95,9**	*1,982*	**77**	*1,886*	32	186	**94**	*1,973*	42	208
		328	*2,516*	**416**	*2,619*	**619**	*2,792*	434	882	**509**	*2,707*	348	744
		960	*2,982*	**1250**	*3,097*	**1100**	*3,041*	520	2310	**1790**	*3,253*	990	3230
		4360	*3,639*	**3130**	*3,496*	**5590**	*3,747*	3870	8080	**6900**	*3,839*	4900	9720

Linéarité - Escherichia coli - Eau de mer

Niveau
1
2
3
4

Méthode de référence (NF ISO 9308-1)			
Rep.1	Rep.2	M	SD
1,663	1,973	1,8	0,219
2,540	2,797	2,7	0,182
2,980	2,918	2,9	0,044
3,868	3,992	3,9	0,088

Méthode alternative Colilert® -18 / Quanti-Tray®			
Rep.1	Rep.2	M	SD
1,924	2,199	2,1	0,194
2,644	2,496	2,6	0,105
2,910	2,925	2,9	0,011
3,664	3,991	3,8	0,232

q = 4
n = 2
N = qn = 8

Mx = 2,842
MEDx = 2,809
SDbx = 0,871

MEDwx = 0,135
SDwx = 0,107
rob. SDwx = 0,200

My = 2,844
MEDy = 2,744
SDby = 0,744

MEDwy = 0,150
SDwy = 0,113
rob. SDwy = 0,222

Choix méthode

GMFR

R = 1,063
rob.R = 1,111
Res.SEM = 0,118
Res.SD = 0,167

Sx = 0,814
Sy = 0,699

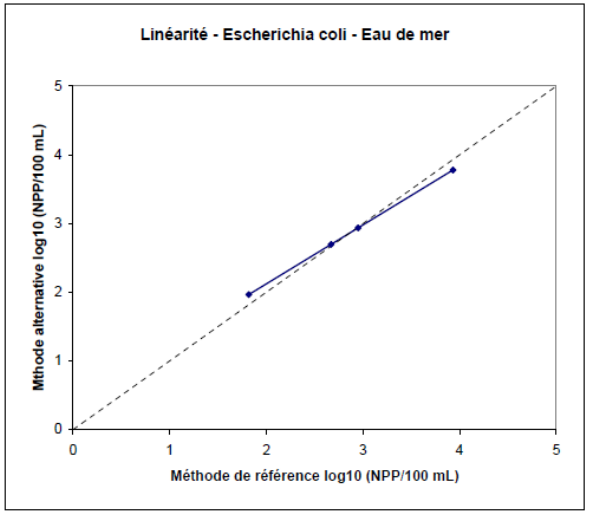
r = 0,992
b = 0,859
a = 0,404

Est y	Déviation
1,965	0,096
2,696	-0,126
2,937	-0,019
3,779	0,048

Sb = 0,084 **p(t;b=1) =** 0,143 **t (b) =** 1,687
Sa = 0,245 **p(t;a=0) =** 0,150 **t (a) =** 2,436

Linéarité

F = 4,514 **p(F) =** 0,094
rob.F = 0,305 **rob.p(F) =** 0,753



Appendix 5: LOD / LOQ results

Limite de détection (LOD) et Limite de quantification (LOQ)

Résultats bruts

N°	Contamination en UFC/100 mL (taux réel)		Jaune	Fluo	NPP
0A	0	grd puits	0	0	0
		petits puits	0	0	
0B		grd puits	0	0	0
		petits puits	0	0	
0C		grd puits	0	0	0
		petits puits	0	0	
0D		grd puits	0	0	0
		petits puits	0	0	
0E		grd puits	0	0	0
		petits puits	0	0	
0F		grd puits	0	0	0
		petits puits	0	0	
0,2A	0,2	grd puits	0	0	0
		petits puits	0	0	
0,2B		grd puits	1	1	1
		petits puits	0	0	
0,2C		grd puits	0	0	0
		petits puits	0	0	
0,2D		grd puits	0	0	0
		petits puits	0	0	
0,2E		grd puits	0	0	0
		petits puits	0	0	
0,2F		grd puits	0	0	0
		petits puits	0	0	
0,5A	0,4	grd puits	1	1	1
		petits puits	0	0	
0,5B		grd puits	0	0	0
		petits puits	0	0	
0,5C		grd puits	1	1	1
		petits puits	0	0	
0,5D		grd puits	0	0	0
		petits puits	0	0	
0,5E		grd puits	0	0	0
		petits puits	0	0	
0,5F		grd puits	0	0	0
		petits puits	0	0	
1A	1,5	grd puits	0	0	0
		petits puits	0	0	
1B		grd puits	1	1	1
		petits puits	0	0	
1C		grd puits	0	0	1
		petits puits	1	1	
1D		grd puits	0	0	0
		petits puits	0	0	
1E		grd puits	0	0	0
		petits puits	0	0	
1F		grd puits	0	0	1
		petits puits	1	1	
3A	3	grd puits	1	1	1
		petits puits	0	0	
3B		grd puits	5	5	5,2
		petits puits	0	0	
3C		grd puits	2	2	2
		petits puits	0	0	
3D		grd puits	2	2	2
		petits puits	0	0	
3E		grd puits	1	1	1
		petits puits	0	0	
3F		grd puits	1	1	1
		petits puits	0	0	

Limite de détection (LOD) et Limite de quantification (LOQ)

Calculs statistiques

Niveau (UFC/100mL)	Nombre d'échantillons positifs	Ecart-type (So)	Biais (Xo)
0	0	0,000	0
0,2	1	0,408	0
0,4	2	0,516	0
1,5	3	0,548	0,5
3	6	1,627	1,5

	Formules	Valeur obtenue
Niveau critique (LC)	1,65 So +Xo	1,40
Limite de détection (LOD)	3,3 So+Xo	2,31
Limite de quantification (LOQ)	10 So + Xo	5,98

Appendix 6: Selectivity results

Inclusivity

Nº	Code	Origin	Level (CFU/100mL)	Quanti-tray®		
				Rep.	Results	
					Coliforms detection	*E. coli* detection
1	ESC.1.1	CIP 54127	100	1	+	+
				2	+	+
2	ESC.1.111	Fountain water	40	1	+	+
				2	+	+
3	ESC.1.112	Secondary effluent	38	1	+	+
				2	+	+
4	ESC.1.113	Well water	38	1	+	+
				2	+	+
5	ESC.1.114	Well water	60	1	+	+
				2	+	+
6	ESC.1.115	Well water	34	1	+	+
				2	+	+
7	ESC.1.116	Well water	48	1	+	+
				2	+	+
8	ESC.1.117	Well water	30	1	+	+
				2	+	+
9	ESC.1.119	Tap water	70	1	+	+
				2	+	+
10	ESC.1.120	English, III-80BS	33	1	+	+
				2	+	+
11	ESC.1.121	EPA QC, 031591	40	1	+	+
				2	+	+
12	ESC.1.122	EPA QC, 082688	35	1	+	+
				2	+	+
13	ESC.1.123	ERA, 4921:40	36	1	+	+
				2	+	+
14	ESC.1.124	4166:80 Thames Isolate #216	40	1	+	+
				2	+	+
15	ESC.1.3	Dairy industry	58	1	+	+
				2	+	+
16	ESC.1.31	Scallop	100	1	+	+
				2	+	+
17	ESC.1.37	Pulp waste recycled	32	1	+	+
				2	+	+
18	ESC.1.39	Raw shrimp	44	1	+	+
				2	+	+
19	ESC.1.4	ATCC 8739	30	1	+	-
				2	+	-
20	ESC.1.41	Bakery industry	80	1	+	+
				2	+	+

Exclusivity

N°	Code	Micro-organism	Origin	Level (CFU/ 100mL)	Quanti-Tray® Rep	Results Coli-forms	Results E.coli
1	SHI.1.1	*Shigella flexneri*	CIP 82.48T	7E+04	1	0	0
					2	0	0
2	ENTC.1.3	*Enterococcus faecalis*	CIP 103214	7E+04	1	0	0
					2	0	0
3	ENTC.3.1	*Enterococcus hirae*	CIP 58.55	3E+04	1	0	0
					2	0	0
4	PRO.1.1	*Proteus mirabilis*	CIP 103181	7E+04	1	0	0
					2	0	0
5	PSE1.4	*Pseudomonas aeruginosa*	Fountain water	4E+04	1	0	0
					2	0	0
6	PSE.1.5	*Pseudomonas aeruginosa*	Fountain water	8E+04	1	0	0
					2	0	0
7	PSE.2.1	*Pseudomonas fluorescens*	CIP 69.13T	7E+04	1	0	0
					2	0	0
8	SAL.1.9	*Salmonella enterica* Braenderup	Food workshop env.	7E+04	1	0	0
					2	0	0
9	STA.1.5	*Staphylococcus aureus*	Surface water	7E+04	1	0	0
					2	0	0
10	XAN.1.1	*Xanthomonas campestris*	Air conditioning evaporator	7E+04	1	0	0
					2	0	0
11	AER.1.1	*Aeromonas hydrophyla*	Well water	2E+04	1	0	0
					2	0	0
12	AER.1.2	*Aeromonas hydrophyla/sobria1*	Well water	3E+04	1	0	0
					2	0	0
13	MIC.2.1	*Micrococcus spp*	Contact Petri dish	4E+04	1	0	0
					2	0	0
14	PROV.1.1	*Providencia stuartii*	HPA RM	4E+04	1	0	0
					2	0	0
15	ALC.1.1	*Alcaligenes xylosoxydans*	Dairy industry	6E+04	1	0	0
					2	0	0
16	SAL.1.99	*Salmonella enterica* Ohio	Food workshop env.	3E+04	1	0	0
					2	0	0
17	STA.2.2	*Staphylococcus epidermidis 2*	Contact Petri dish	4E+04	1	0	0
					2	0	0
18	PSE.1.6	*Pseudomonas aeruginosa*	Fountain water	3E+04	1	0	0
					2	0	0
19	STA.4.1	*Staphylococcus piscifermentans*	Air conditioning evaporator	1E+04	1	0	0
					2	0	0
20	PSE.1.1	*Pseudomonas aeruginosa*	ATCC 19429	4E+04	1	0	0
					2	0	0
21	ENTC.1.2	*Enterococcus faecalis*	ATCC 33186	2E+04	1	0	0
					2	0	0
22	STA.3.1	*Staphylococcus haemolyticus*	Contact Petri dish	5E+04	1	0	0
					2	0	0
23	AER.1.4	*Aeromonas hydrophila*	Japan 146	4E+04	1	0	0
					2	0	0
24	ENTC.4.1	*Enterococcus avium*	4416:88 German Entercocci E156	3E+04	1	0	0
					2	0	0
25	ENTC.1.4	*Enterococcus faecalis*	10B Thames Water, UK	1E+04	1	0	0
					2	0	0
26	ENTC.2.2	*Enterococcus faecium*	2A:48-1 Environmental	2E+04	1	0	0
					2	0	0
27	ENTC.5.1	*Enterococcus gallinarum*	EMP060, 4569:6	1E+04	1	0	0
					2	0	0
28	PRO.1.2	*Proteus mirabilis*	292-2 (Chen Vet Micro)	5E+04	1	0	0
					2	0	0
29	STA.1.6	*Staphylococcus aureus*	7612503004	7E+04	1	0	0
					2	0	0
30	PSE.1.7	*Pseudomonas aeruginosa*	C6, NH effluent, Suppl. LNB 4609	1E+04	1	0	0
					2	0	0

Appendix 7: Enumeration of culturable microorganisms

Laboratoire	Résultat (UFC/mL) à 22°C	Résultat (UFC/mL) à 36°C
A	12	7
B	88	2
C	24	4
D	3	2
E	<1	2
F	5	2
G	26	2
H	11	2
I	46	3
J	120	1
K	63	6
L	244	4
M	18	4
N	94	<1 *
O	<1	<1

Appendix 8: Interlaboratory study results

Results in MPN/100 mL

Niveau 0

Laboratoire	Méthode de référence - Echantillons						Méthode alternative - Echantillons			
	4			8			4		8	
	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP	NPP /100ml (NPP x facteur de dilution)	NPP	NPP /100ml (NPP x facteur de dilution)
A	<15	/	/	<15	/	/	<1	<10	<1	<10
B	<15	/	/	<15	/	/	<1	<10	<1	<10
C	<15	/	/	<15	/	/	<1	<10	<1	<10
D	<15	/	/	<15	/	/	<1	<10	<1	<10
E	<15	/	/	<15	/	/	<1	<10	<1	<10
F	<15	/	/	<15	/	/	<1	<10	<1	<10
G	<15	/	/	<15	/	/	<1	<10	<1	<10
H	<15	/	/	<15	/	/	<1	<10	<1	<10
I	<15	/	/	<15	/	/	<1	<10	<1	<10
J	<15	/	/	<15	/	/	<1	<10	<1	<10
K	<15	/	/	<15	/	/	<1	<10	<1	<10
L	<15	/	/	<15	/	/	<1	<10	<1	<10
M	<15	/	/	<15	/	/	<1	<10	<1	<10
N	<15	/	/	<15	/	/	<1	<10	<1	<10
O	<15	/	/	<15	/	/	<1	<10	<1	<10
Expert	<15	/	/	<15	/	/	<1	<10	<1	<10

Niveau 1

Laboratoire	Méthode de référence - Echantillons						Méthode alternative - Echantillons			
	6			7			6		7	
	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP	NPP /100ml (NPP x facteur de dilution)	NPP	NPP /100ml (NPP x facteur de dilution)
A	92	41	206	93	42	207	8,6	86	10,8	108
B	127	63	253	109	52	230	1	10	4,1	41
C	94	42	208	94	42	208	7,5	75	6,3	63
D	127	63	253	<15	/	/	4,1	41	6,3	63
E	110	52	231	15	2	106	5,2	52	6,3	63
F	46	15	142	61	23	163	6,3	63	4,1	41
G	77	32	186	160	86	298	9,8	98	13,5	135
H	15	2	106	46	15	142	5,1	51	5,2	52
I	125	62	251	61	23	163	9,7	97	3,0	30
J	61	23	163	61	23	163	5,2	52	5,2	52
K	94	42	208	93	42	207	4,0	40	4,1	41
L	94	42	208	144	75	276	5,2	52	12,2	122
M	197	63	253	46	15	142	9,5	95	10,9	109
N	94	42	208	46	15	142	6,2	62	7,4	74
O	127	63	253	126	63	252	11,9	119	10,9	109
Expert	126	63	252	30	8,0	121	4,0	40	8,4	84

Niveau 2

Laboratoire	Méthode de référence - Echantillons						Méthode alternative - Echantillons			
	1			3			1		3	
	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP	NPP /100ml (NPP x facteur de dilution)	NPP	NPP /100ml (NPP x facteur de dilution)
A	697	486	981	332	212	521	49,6	496	48,7	487
B	529	363	769	434	290	650	33,1	331	40,4	404
C	332	212	521	438	293	655	38,9	389	45,7	457
D	177	98	321	465	314	689	40,8	408	37,4	374
E	234	138	394	434	290	650	42,5	425	36,9	369
F	195	111	344	393	258	598	25,9	259	23,8	238
G	415	275	626	393	258	598	29,2	292	48,2	482
H	585	408	840	465	314	689	38,7	387	33,1	331
I	654	462	927	500	341	733	39,3	393	26,9	269
J	412	272	622	375	244	575	39,3	393	30,9	309
K	344	221	537	504	344	738	75,4	754	53,0	530
L	606	424	866	640	451	909	35	350	52,9	529
M	476	322	703	580	403	833	23,1	231	51,2	512
N	559	387	808	640	451	909	30,5	305	23,1	231
O	585	408	840	668	473	944	49,6	496	43,7	437
Expert	697	479	953	559	387	808	61,6	616	45,9	459

Niveau 3

Laboratoire	Méthode de référence - Echantillons						Méthode alternative - Echantillons			
	2			5			2		5	
	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP / 100 mL	Limite inférieur	Limite supérieure	NPP	NPP /100ml (NPP x facteur de dilution)	NPP	NPP /100ml (NPP x facteur de dilution)
A	1049	773	1423	882	642	1213	59,1	591	71,2	712
B	858	622	1182	489	333	720	80,9	809	77,1	771
C	773	555	1075	851	617	1174	73,3	733	51,2	512
D	647	456	917	838	606	1157	58,1	581	73,8	738
E	514	352	751	1007	740	1371	58,1	581	84,7	847
F	690	490	972	805	580	1116	55,6	556	59,4	594
G	580	403	833	943	690	1290	75,4	754	57,3	573
H	759	544	1058	759	544	1058	73,3	733	58,1	581
I	1305	973	1751	742	531	1037	72,7	727	66,3	663
J	918	670	1258	543	375	783	90,6	906	88,4	884
K	1136	841	1535	838	606	1157	90,9	909	101,7	1017
L	1007	740	1371	968	709	1321	77,6	776	93,3	933
M	882	642	1213	872	633	1200	98,8	988	133,4	1334
N	882	642	1213	968	709	1321	73,3	733	62,2	622
O	1567	1174	2092	893	650	1227	83,6	836	79,4	794
Expert	633	445	901	1034	761	1405	101,0	1010	83,3	833