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

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

Quantitative method

Colilert-18 / Quanti-Tray
(ref. Colilert-18 : 06-02027-24 / Quanti-Tray: 06-02030-17)
Attestation number: IDX 33/01-11/09
for the enumeration of Escherichia coli and coliforms
in drinking waters (except bottled waters)

Expert laboratory: Laboratoire MICROSEPT
ZA de la Sablonnière
15 rue Denis Papin
49220 LE LION D'ANGERS
FRANCE

For: IDEXX Laboratories, Inc.
IDEXX Drive, Westbrook
Maine 04 092
USA

This report contains 52 pages including 32 pages of appendices.
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March 24, 2022

Preamble

- ## Protocols of validation:

- **Validation protocol for an alternative commercial method as compared with a reference method – revision 2**

- ## Reference method:

- **NF EN ISO 9308-1 (September 2000, classification index NF T90-414):** Water quality - Detection and enumeration of Escherichia coli and coliform bacteria Part 1: Membrane filtration method

- ## Application scope:

- **Water for human consumption (drinking waters), except bottled waters**

- ## Certification body:

- **AFNOR Certification** (https://nf-validation.afnor.org/).

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APPENDICES

Appendix E : LOD-LOQ results
Appendix F : Selectivity results
Appendix G : Results of the interlaboratory study

1. Introduction

1.1. Validation history

The method Colilert-18 / QUANTI-TRAY has been validated by AFNOR Certification in November 2009 with the trademark NF Validation under the attestation number IDX 33/01 – 11/09 for the enumeration of *Escherichia coli* and coliforms.
A renewal of the method, without modifications, was conducted in October 2013 and in October 2017.
Some modifications occurred in the renewal study of September 2021: a part of the data were reinterpreted and some parameters were retested as part of the application of the ISO 819 standard.

1.2 Validation repositories

The initial validation study of the method Colilert-18 / Quanti-Tray was performed according to the validation protocol for an alternative commercial method as compared to a reference method (revision 0 – July 2008).
From 2013, the renewal studies were performed according to the version 2 of the validation protocol.

1.3. Alternative method

Colilert-18 with Quanti-Tray is a colorimetric method for detection and enumeration of β-glucuronidase positive *Escherichia coli* and coliforms in human consumption waters.

The protocol of the alternative method is as follows and in appendix A.

Figure 1 : protocol of the alternative method

Step 1 :
Add the Colilert-18 reagent to 100 mL of sample
Shake until dissolved
Pour sample/ reagent mixture into a Quanti-Tray

Step 2 :
Seal in a Quanti-Tray Sealer.

Step 3 :
Place the sealed tray in an incubator at 36±2°C for 18 to 22 hours

Step 4 :
Count the number of yellow wells and refer to the MPN table
Express results in MPN of coliforms / 100 mL of sample

Count the number of yellow and fluorescent wells with a UV lamp at 365 nm and refer to the MPN table
Express results in MPN of *Escherichia coli* / 100 mL of sample

1.3. Application scope

The application scope of the alternative method concerns waters for human consumption, except bottled waters.

1.4. Reference method (*)

The NF EN ISO 9308-1:2000 (classification index T90-414) standard: “Water quality - Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method” was used as reference method.

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

2. Method comparison study

In 2009, two categories of waters have been tested with the reference method and with the alternative method (analysis in duplicate for each method) : tap waters and fountain waters.

The two categories were grouped in the category "waters for human consumption" when the revision 2 of the protocol of validation came into effect.

The statistical exploitations in this report do not take into account samples of the reference method with a concentration inferior to 3 CFU/100 mL or superior to 80 CFU/100 ml, as stated in the standard ISO 8199.

2.1. Relative accuracy

The relative accuracy is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on the same samples.

2.1.1. Number and nature of samples

Different types of samples analyzed are summarized in table 1.

Table 1 : number and nature of samples analyzed

Targert microorganism	Water category	Water type	Number of samples analyzed	Number of samples used
Coliforms	Waters for human consumption	Tap water	25	16
		Fountain water	25	19
		Total	**50**	**35**
Escherichia coli	Waters for human consumption	Tap water	30	17
		Fountain water	28	23
		Total	**58**	**40**
Total *Escherichia coli* and other coliforms			**108**	**75**

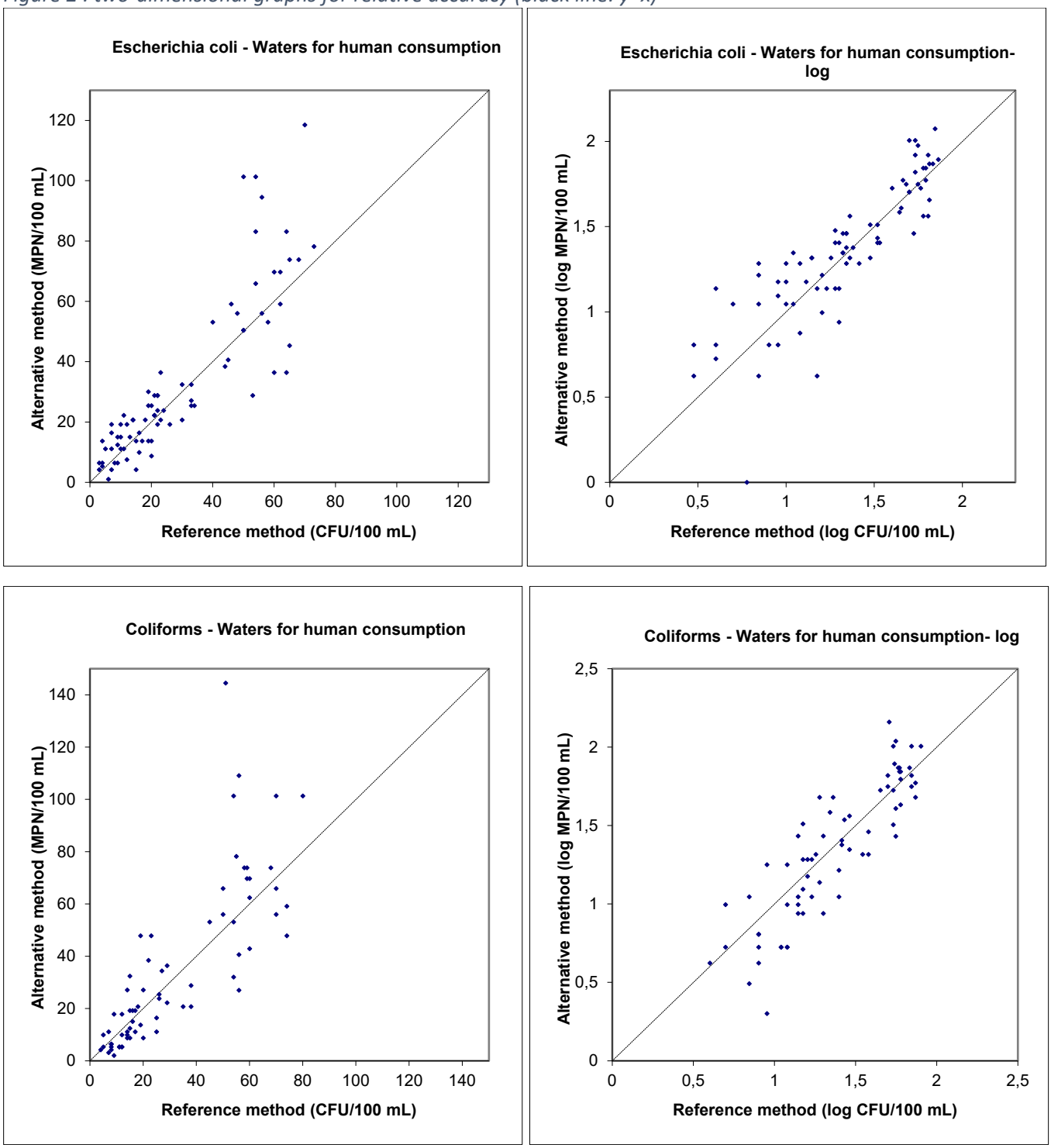
Globally, 108 samples were analyzed, and 75 results were used. No naturally contaminated sample was analyzed. The samples have been artificially contaminated. The contamination levels used cover the entire measurement range of the alternative method.

2.1.2. Raw results

Figure 2 presents the two-dimensional graphs for the two matrix/strain combinations tested and for whole category “human consumption waters”, respectively in CFU per test portion and in log CFU per test portion. The y-axis is reserved for the alternative method and the x-axis for the reference method. Raw results are presented in appendix C.
The statistical calculations and graphics were established from results obtained after 48 hours of incubation for the reference method Petri dishes.

The representation of a line of equation “y = x” figures dashed on the graphs.

Figure 2 : two-dimensional graphs for relative accuracy (black line: y=x)



2.1.3. Statistical exploitation

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

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

The 'b' slope is theoretically equal to 1 in the ideal model (hypothesis [b = 1]). The estimated slope obtained with the two methods should pass by p {b = 1}. Statistically, if the alternative method does not give the same values as the reference method, the probability p {b = 1} is less than 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) is used with the x-axis for the reference method,
- if Rob.R < 0.5, a linear regression by least-squares (OLS 2) is used with the x-axis for the alternative method,
- If 0.5 < Rob.R < 2, orthogonal regression (GMFR) is used with the x-axis to the reference method.

Table 2 : statistical data for the enumeration of E. coli and coliforms

Parameter	Data	Rob.R	Regression used	T critical	a	t(a)	b	t(b)	Probabilities (%)	
									Intercept at 0	Slope at 1
E. coli	Raw	0.970	GMFR	2.023	-4.435	1.354	1.270	2.950	18.0	0.4
	Log	1.015	GMFR	2.023	-0.022	0.233	1.043	0.631	81.6	53.0
Coliforms	Raw	2.633	OLS1	2.032	-2.020	0.271	1.158	0.821	78.7	41.4
	Log	1.938	GMFR	2.032	-0.381	2.782	1.258	2.670	0.9	0.7

Table 3 : bias and repeatability of the two parameters

Parameter	Data	Bias (D)		Repeatability			
		Mean	Median	r		Rob.r	
				RM	AM	RM	AM
E. coli	Raw	3.4	2.1	13.9	20.3	14.6	14.2
	Log	0.04	0.03	0.33	0.46	0.24	0.25
Coliforms	Raw	2.99	-0.75	11.9	22.4	8.80	23.2
	Log	-0.03	-0.02	0.23	0.30	0.17	0.33

2.1.4. Conclusion

The equations for the regression line and the correlation coefficient are as follows:

E. coli / Water for human consumption: Alt. = 1.270 Ref. – 4.435
log Alt. = 1.043 log Ref. – 0.022

Coliforms / Water for human consumption: Alt. = 1.158 Ref. – 2.020
log Alt. = 1.258 log Ref. – 0.381

For the parameter *Escherichia coli* "raw data", the hypothesis [a=0] is accepted but the hypothesis [b=1] is rejected. The hypothesis [a=0 and b= 1] is accepted for the parameter *Escherichia coli* "data log".
For the parameter Coliforms "raw data", the hypothesis [a=0 and b=1] is accepted. The hypothesis [a=0 and b= 1] is rejected for the parameter Coliform "data log".

For all parameters, results obtained for the regression line are corrects, as well as correlation coefficients:

- *Escherichia coli* "raw data" : 0.899
- *Escherichia coli* "data log" : 0.913
- Coliforms "raw data" : 0.840
- Coliforms "data log": 0.901

The bias calculated between the alternative method and the reference method are at 2.1 CFU / 100 mL and at 0.03 log CFU / 100 mL for the parameter *E.coli* and at -0.75 CFU / 100 mL and at -0.02 log CFU / 100 mL for the parameter coliforms.

The relative accuracy for the alternative method is satisfactory.

2.2. 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.

2.2.1. Contamination level

The matrix / strain combinations are presented in Table 4. For the combination *Escherichia coli* / tap water, five contamination levels were tested in duplicate by the reference method and the alternative method. For the combination *Citrobacter freundii* / fountain water, four contamination levels were tested in duplicate by the reference method and the alternative method.

The *E. coli* / tap water pair was retested during the renewal study of 2021 to meet the requirements of the ISO 8199 standard. The levels of the initial validation study were indeed over 80 CFU/100 ml for the reference method.

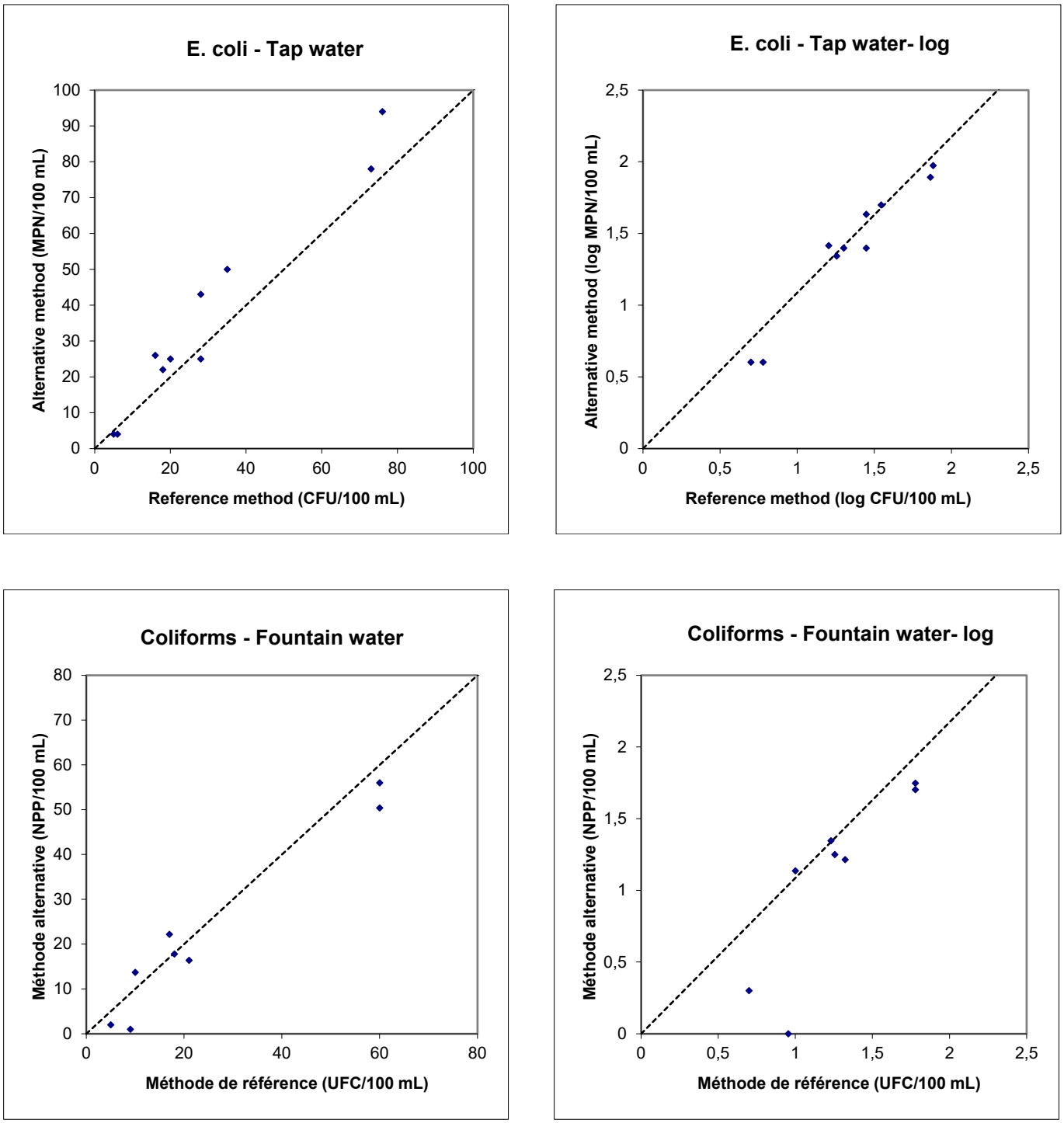
Table 4 : combinations matrix / strain analyzed

Strain	Matrix	Target level (CFU/100mL)
Escherichia coli YNQ 123	Tap water	10 - 20 - 30 - 50 - 70
Citrobacter freundii CIT.1.4	Fountain water	10 - 30 - 50 - 100

2.2.2. Raw results

Graphs of figure 3 show the values of each sample obtained by the alternative method and the reference method. The y-axis is reserved for the alternative method and the x-axis for the reference method. Raw results and statistical calculations are summarized in appendix 4. The representation of a line of equation “y = x” figures dashed on the graphs.

Figure 3 : two-dimensional graphs for the linearity of the combination E. coli / tap water and *Citrobacter freundii /* fountain water



2.2.3. Statistical exploitation

Statistical interpretations are carried out in accordance with the requirements of the validation protocol.

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

Table 5 : statistical data of combinations matrix / strain

Strain / matrix	Data	Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	Correlation coefficient (r)
E. coli / Tap water	Raw	1.333	GMFR	5.409	10.272	0.014	0.985
	Log	0.827	GMFR	5.409	12.381	0.009	0.981
Coliforms / Fountain water	Raw	1.213	GMFR	6.944	2.482	0.199	0.987
	Log	0.707	GMFR	6.944	39.275	0.002	0.913

The relationship between the 2 methods is not linear:

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

2.2.4. Conclusion

The relationship between the two methods, for matrix / strain combinations tested, is linear only for Coliforms "raw data". For the other parameters, results obtained for the regression line are corrects, as well as correlation coefficients.

The linearity of the alternative method is satisfactory.

2.3. Detection and quantification limits

The critical level is defined as the smallest amount that can be detected, but not quantified as an exact value. The detection limit is defined as the level above the critical level. The quantification limit 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. Protocol

The limits of detection and quantification were determined by analyzing a pure culture of *Escherichia coli* ESC.1.112 and a pure culture of *Citrobacter freundii* CIT.2.2 by the alternative method.

2.3.2. Results

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

Table 6 : data (s_0 and x_0) for the enumeration of Escherichia coli

Level (CFU/100 mL)	Number of positive samples	Standard deviation (s_0)	Bias (x_0)
0	0	0	0
0.25	0	0	0
0.5	2	0.516	0
1	6	0.516	1

Table 7 : LC, LOD and LOQ values of the alternative method for the enumeration of Escherichia coli

Parameter	Formula	Values obtained
Critical level (CL)	$1.65\ s_0 + x_0$	0.85
Limit of detection (LOD)	$3.30\ s_0 + x_0$	1.70
Limit of quantification (LOQ)	$10.00\ s_0 + x_0$	5.16

Table 8 : data (s_0 and x_0) for the enumeration of coliforms

Level (CFU/100 mL)	Number of positive samples	Standard deviation (s_0)	Bias (x_0)
0	0	0	0
0.25	0	0	0
0.5	3	0.548	0.5
1	6	0.516	1

Table 9 LC, LOD and LOQ values of the alternative method for the enumeration of coliforms

Parameter	Formula	Values obtained
Critical level (CL)	$1.65\ s_0 + x_0$	1.40
Limit of detection (LOD)	$3.30\ s_0 + x_0$	2.31
Limit of quantification (LOQ)	$10.00\ s_0 + x_0$	5.98

2.3.3. Conclusion

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

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. Protocols

Fifty target strains, including twenty strains of *Escherichia coli*, for inclusivity and thirty non-target strains for exclusivity (from the collections of national, international and internal of ISHA) were analyzed. The tests were performed according to the protocol of the alternative method.

The contamination rates used for inclusivity are between 30 and 100 CFU/100 mL. For exclusivity, they are 10^3 to 10^5 times the detection concentration of the alternative method (about 10^4 CFU / 100 mL).

2.4.2. Results

Raw results are in appendix F.

For thirty coliform strains tested, twenty-five were detected by the alternative method. The five undetected coliform strains by the alternative method were not detected or were not characteristic on the media of the reference method (TTC-tergitol).

Only one strain of *Escherichia coli* among twenty strains tested was not detected by the alternative method. Nevertheless, this strain was not detected by the reference method.

Identifications realized on strains giving discordant results confirmed the identity of strains tested.

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

2.4.3. Conclusion

The selectivity of the method is satisfactory.

2.5. Conclusion

The linearity and the relative accuracy of the alternative method are satisfactory.

The bias between the reference method and the alternative method is acceptable.

The alternative method is specific and selective.

3. Interlaboratory study

3.1. Study organization

3.1.1. Participating laboratories

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

3.1.2. Absence of coliforms in the matrix

Before spiking, the absence of coliforms was verified in the batch of water used, according to the reference method.

3.1.3. Strain stability in the matrix

The stability of an *Escherichia* coli strain in tap water was evaluated for 3 days at 4±2°C. The strain (code ESC.1.131) was isolated from an aquatic environment.

The numeration results on TTC-tergitol after filtration of 100 mL are summarized in table 10.

Table 10 : results of Escherichia coli by the reference method (CFU/100 mL) between D0 and D2

Day	Level 1	Level 2	Level 3
D0	2	64	100
D1	12	65	120
D2	8	66	100

3.1.4. Samples preparation and spiking

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

- level 0 : 0 CFU / 100 mL,
- level 1 : from 1 to 10 CFU / 100 mL,
- level 2 : from 10 to 50 CFU / 100 mL,
- level 3 : from 50 to 200 CFU / 100 mL

The matrix was distributed at 220 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 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 table11.

Table 11 : target level, real level and culturable microorganisms of the matrix

Level	Flora (CFU/mL)	***Escherichia coli* ESC.1.131 (CFU/100 mL)**	
		Target level	**Real level**
0	<1	0	0
1		1 to 10	12
2		10 to 50	64
3		50 to 200	120

3.1.5. Samples labeling

The labelling of the bags was realized as follows: a code to identify the laboratory: from A to M (cf. table 12) 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 4°C before shipping.

Table 12 : sample code by contamination level

Contamination level (CFU / 100 mL)	Sample code
0	3 / 4
1 – 10	1 / 8
10 – 50	2 / 5
50 – 200	6 / 7

3.1.6. Samples shipping, reception, and analysis

The samples were shipped in a coolbox on September 14th, 2009.
The coolboxes were received on March 15th, 2009 by the thirteen participating 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 March 15th. The expert laboratory concurrently analyzed a set of samples under the same conditions with both methods.

3.2. Results

3.2.1. Temperature and state of the samples

The temperature readings upon reception, the one recorded by the probe and the state of the samples are shown in table 13.

Table 13 : temperature and state of the samples upon reception and during the shipping

Laboratory	Temperature upon reception	State of the samples	Temperature (°C) recorded by the probe	
			Mean	SD
A	8.2°C	Good	3.0	0.7
B	6.5°C	Good	2.3	1.2
C	3.5°C	Good	2.9	0.6
D	3.0°C	Good	2.5	0.5
E	3.1°C	Good	3.5	0.5
F	3.3°C	Good	3.2	0.7
G	6.0°C	Good	2.8	0.7
H	4.4°C	Good	3.3	0.5
I	5.4°C	Good	2.9	0.6
J	3.9°C	Good	3.4	0.3
K	11.9°C	Good	3.0	0.5
L	3.5°C	Good	3.3	0.8
M	5.2°C	Good	2.8	0.8

Temperatures are correct for all laboratories (inferior at 8.4°C) except for the laboratory K. This laboratory showed temperature equal to 11.9°C. Nevertheless, the temperature probe indicated a mean temperature at 3.0°C. These data are included in the statistical analysis.
The analysis of thermal profiles of probes showed, for all participants, mean temperatures during the shipment between 2.3 and 3.5°C.

3.2.2. Enumeration of culturable microorganisms

For the whole laboratories, the enumeration of culturable microorganisms at 22°C was inferior to 1 CFU/mL. Concerning the enumerations at 36°C, the results were varying between <1 and 1 CFU/mL.

3.2.3. Expert and collaborative laboratories results

The overall results are presented in table 14 and in the appendix G. The results of the reference method are presented for a reading of the Petri dishes after 48 h of incubation at 37±2°C.

The reading of wells with the alternative method showed an equivalent result between the yellow wells and the fluorescent wells for each sample. Results of alternative method are presented for a reading of yellow and fluorescent wells after incubation 18 hours at 37±1°C.
Data of laboratories A and G were excluded from the final analysis of results:

- For laboratory A, reading results for fluorescent wells were not reported on the result form.
- For laboratory G, plates of TTC-tergitol superior to 100 CFU have not been counted.

Laboratories A and G have been contacted for a second reading but Petri dishes and Quanti-Tray had been already eliminated.

*Table 14 : E. coli enumeration results in CFU/100 mL (RM) and MPN/100 mL (AM) (RM: reference method, AM: alternative method, R1: replicate 1 and R2: replicate 2, *: results of the yellow well only)*

Laboratory	Level 0				Level 1				Level 2				Level 3			
	RM		AM		RM		AM		RM		AM		RM		AM	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
A*	<1	<1	0	0	8	12	12	16	32	40	41	89	130	110	165	>201
B	<1	<1	0	0	5	10	9	10	39	29	53	36	110	110	>201	130
C	<1	<1	0	0	19	10	18	14	72	52	62	53	140	140	145	118
D	<1	<1	0	0	10	8	14	11	65	64	95	78	110	120	201	145
E	<1	<1	0	0	14	12	10	14	67	66	48	78	150	120	201	201
F	<1	<1	0	0	14	11	15	11	52	56	34	50	80	78	50	50
G	<1	<1	0	0	11	8	14	11	66	65	62	59	>100	>100	144	130
H	<1	<1	0	0	9	6	8	3	30	50	62	89	70	90	130	130
I	<1	<1	0	0	9	13	12	9	55	51	56	59	130	140	145	130
J	<1	<1	0	0	4	11	15	8	33	44	89	50	89	90	130	201
K	<1	<1	0	0	9	12	18	11	53	54	66	62	130	130	118	>201
L	<1	<1	0	0	13	9	9	8	46	65	59	74	130	110	>201	130
M	<1	<1	0	0	15	8	9	12	45	52	59	48	90	74	118	165
Expert	<1	<1	0	0	5	12	11	8	69	60	74	83	130	100	201	165

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

3.3. Interpretation of the results

The data presented in the following paragraphs were calculated from the raw and logarithmic results, in the same way as for the presentation of the results of the preliminary study.

3.3.1. Bias calculation

Table 15 shows the target value, the mean, the relative bias and the bias of each level of contamination.

Table 15 : calculation of the bias of the alternative method

	Raw data			Data log		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
Target value	**10.0**	**52.0**	**112.0**	**1.000**	**1.716**	**2.049**
Mean	11.2	61.2	147.3	1.024	1.771	2.142
Relative bias	0.123	0.177	0.315	2.40%	3.20%	4.52%
Bias	**1.123**	**1.177**	**1.315**	**0.024**	**0.055**	**0.093**

The accuracy of the method is estimated by the bias which varies from 1.123 to 1.314 CFU/100 mL and from 0.024 to 0.093 log CFU/100 mL.

3.3.2. Accuracy profile

Table 16 shows the values of tolerance and the tolerance limits of the alternative method for probability of tolerance values of 80%.

Figures 4 and 5 show the accuracy profiles.

Table 16 : Values and tolerance limits of the alternative method

Values in	Probability of tolerance	Limits of acceptability	Level	Low	Medium	High
CFU/100 mL	80%	90%	Low tolerance value	6.3	38.0	84.0
			High tolerance value	16.2	84.5	210.6
			Low tolerance limit	63%	73%	75%
			High tolerance limit	162%	162%	188%
			Low acceptability limit	10%	10%	10%
			High acceptability limit	190%	190%	190%
log CFU/100 mL	80%	0,35	Low tolerance value	0.794	1.601	1.902
			High tolerance value	1.254	1.941	2.382
			Low tolerance limit	-0.206	-0.115	-0.147
			High tolerance limit	0.254	0.225	0.333
			Low acceptability limit	-0.350	-0.350	-0.350
			High acceptability limit	0.350	0.350	0.350

Figure 4: Accuracy profile of the alternative method with tolerance probability (β) at 80% and an acceptability limit at 90%

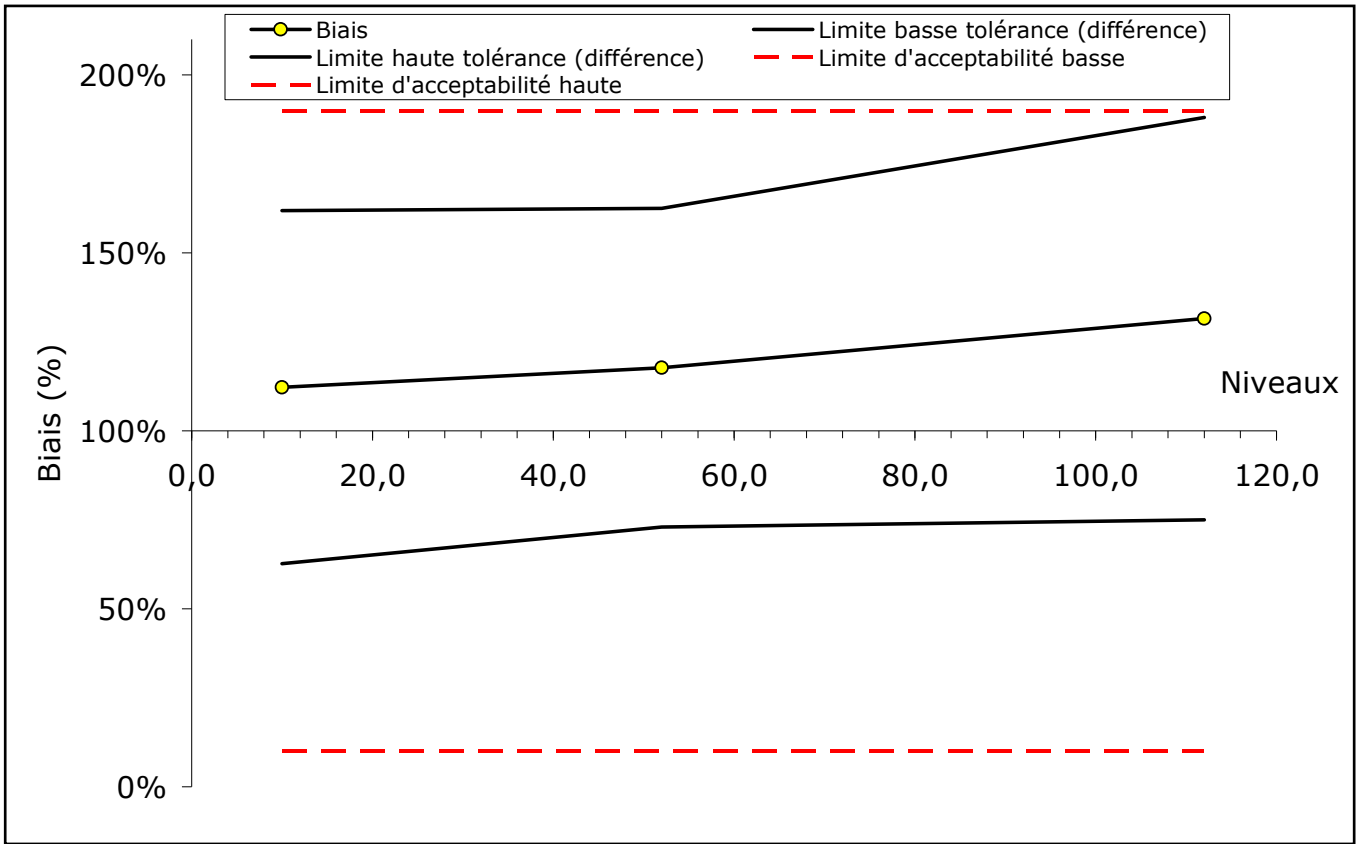
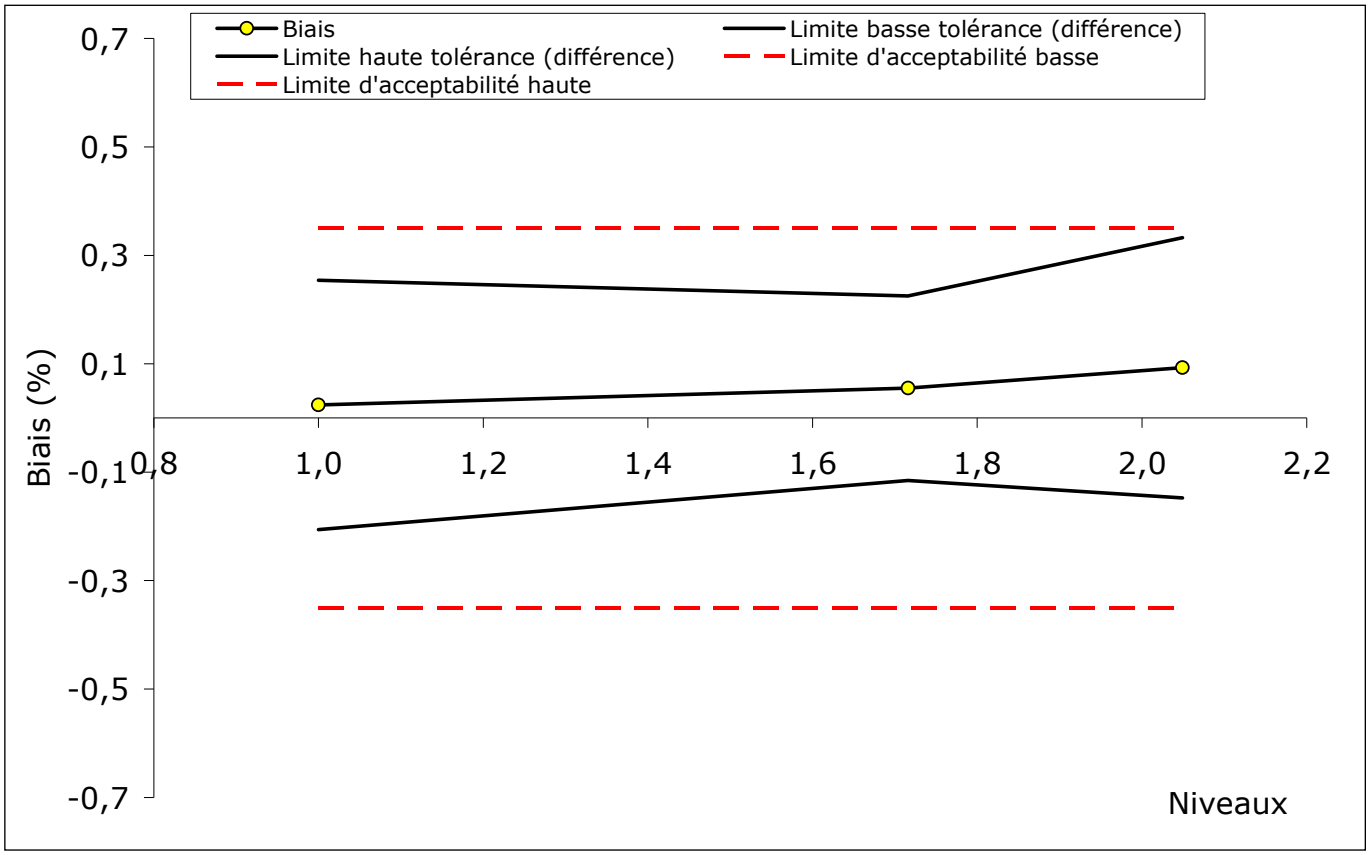


Figure 5 : Accuracy profile of the alternative method with tolerance probability (β) at 80% and an acceptability limit at 90% (with value in CFU /



The bias of the alternative method increases slightly from the low level to the high level of contamination.
For all the contamination levels, the tolerance interval is comprised between the acceptability limits.

3.4. Conclusion

The accuracy profile obtained from the results of the reference method and the alternative method shows that the bias of Colilert-18 method with Quanti-Tray for the enumeration of β-glucuronidase positive *Escherichia coli* and β-galactosidase positive coliforms in drinking water is acceptable. The tolerance limits of the alternative method for a probability of 80% are included within the limits of acceptability of 90% (data: CFU/100 mL) or of 0.35 (data: log CFU/100 mL).

4. 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 are packaged in single capsule.
The Quanti-Tray® devices are conditioned par ten in aseptic bag.

2- Volume of reagents
The Colilert®-18 are conditioned par 20 or 200 capsules.

3- Storage conditions of components and shelf-life of unopened products
The Colilert-18 reagent should be conserved between 2 and 25°C.
The Quanti-Tray devices should be conserved between 4 and 30°C.

4- Modalities after first use
Each Colilert-18 and Quanti-Tray® 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 with the reference method is more important than the duration of analysis with the alternative method.

9- Time required for results
The time-to-results for the alternative method is 22 hours.
Concerning the reference method, the time-to-results for negative samples is between 24 and 48 hours and for positive samples, the time-to-results is between 48 and 72 hours.

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.

5. Conclusion

The Colilert-18 / Quanti-Tray method for the enumeration of β-glucuronidase positive *Escherichia coli* and β-galactosidase positive coliforms in drinking water was compared to the method described in the standard ISO 9308-1 (2000).

Results obtained for the comparative study showed that :

- the linearity between the two methods is satisfactory,

- the relative accuracy of the alternative method is satisfactory: values of repeatability obtained are 0.248 log (*E. coli*) and 0.330 log (coliforms) for the alternative method and 0.244 log (*E. coli*) and 0.170 log (coliforms) for the reference method. The bias between the two methods is 0.035 log for enumeration of *E. coli* and -0.016 log for enumeration of coliforms.

- the selectivity of the alternative method is satisfactory.

Results obtained for the interlaboratory study showed that :

- the bias of the alternative method is satisfactory,

- the tolerance limits of the alternative method for a tolerance probability at 80 % are comprised in the acceptability limits of 90% (data in CFU/100 mL) or of 0.35 (data in log CFU/100 mL).

Le Lion d'Angers, 24 March 2022
François Le Nestour
Head of the Microbiology Department

APPENDICES

Appendix A

Alternative method

Alternative method

Step 1 :
Add the Colilert® 18 reagent to 100 mL of sample (at room temperature)

Close the vial and shake until dissolved

Pour sample / reagent mixture into a Quanti-Tray

Step 2 :
Seal the Quanti-Tray with a Quanti-Tray Sealer

Step 3 :
Incubate at 36±2°C for 18 to 22 h

Step 4 :
For coliforms:
Count the number of yellow wells.
Refer to the MPN table provided with the trays to obtain a Most Probable Number

For *E. coli* :
Count the number of yellow and fluorescent wells
Refer to the MPN table provided with the trays to obtain a Most Probable Number

Step 5 :
Expression of the results :
Express the results in MPN of coliforms or *E. coli* / 100 mL of water

Appendix B

Reference method

Reference method

Membrane filtration
Filter 100 mL of sample on a membrane
Place each membrane on a TTC-tergitol 7 agar plate

↓

Incubation
Incubate at 36± 2°C for 21 ± 3 h
+
(24±2) h if the colonies are not characteristic

↓

Reading and confirmation
Characteristic colonies : lactose positive

Confirm 10 isolated colonies with :
Oxydase test and production of indole

↓

Expression of the results : CFU/100 mL

Appendix C

Relative accuracy results

E.coli FOUNTAIN WATERS

	N°	Strain	Origin	Sample	Target Concentration CFU/100mL	ISO 9308-1									Colilert 18/ Quanti-tray®			
						dilution	24H				48H				Coliforms detection (yellow)		E.coli detection (fluo)	
							CFU		CFU/ 100mL		CFU		CFU/ 100mL					
							A	B	A	B	A	B	A	B	A	B	A	B
1	ESC.1.3	*Escherichia coli*	industrie laitière	FR44	15	*0*	30	33	**30**	**33**	30	33	**30**	**33**			20.7	32.4
2	ESC.1.3	*Escherichia coli*	industrie laitière	FR45	8	*0*	12	10	**12**	**10**	12	10	**12**	**10**			19.2	19.2
3	ESC.1.3	*Escherichia coli*	industrie laitière	FR46	4	*0*	4	12	**4**	**12**	4	12	**4**	**12**			13.7	7.5
4	ESC.1.3	*Escherichia coli*	industrie laitière	FR47	2	*0*	3	8	**3**	**8**	3	8	**3**	**8**			4.2	6.4
5	ESC.1.4	*Escherichia coli*	ATCC 8740	FR1	18	*0*	48	45	**48**	**45**	48	45	**48**	**45**			56	40.6
6	ESC.1.121	*Escherichia coli*	EPA QC, 031594	FB2	14	*0*	14	21	**14**	**21**	14	21	**14**	**21**			20.7	22.2
7	ESC.1.122	*Escherichia coli*	EPA QC, 082688	FB4	9	*0*	23	26	**23**	**26**	23	26	**23**	**26**			20.7	19.2
8	ESC.1.120	*Escherichia coli*	English, III-80BS	FR11	103	*1/10*	7	5	**70**	**50**	7	5	**70**	**50**			118.5	101.3
9	ESC.1.120	*Escherichia coli*	English, III-80BS	FR14	50	*0*	64	60	**64**	**60**	64	60	**64**	**60**			36.4	36.4
10	ESC.1.120	*Escherichia coli*	English, III-80BS	FR15	25	*0*	9	20	**9**	**20**	9	20	**9**	**20**			12.4	13.7
11	ESC.1.122	*Escherichia coli*	EPA QC, 082688	FR17	88	*0*	65	60	**65**	**60**	65	60	**65**	**60**			73.8	69.7
12	ESC.1.122	*Escherichia coli*	EPA QC, 082688	FR18	44	*0*	34	33	**34**	**33**	34	33	**34**	**33**			25.4	25.4
13	ESC.1.122	*Escherichia coli*	EPA QC, 082688	FR19	22	*0*	15	20	**15**	**20**	15	20	**15**	**20**			13.7	25.4
14	ESC.1.1	*Escherichia coli*	CIP 54127	FR23	24	*0*	68	73	**68**	**73**	68	73	**68**	**73**			73.8	78.2
15	ESC.1.1	*Escherichia coli*	CIP 54127	FR51 1 & 2	65	*0*	58	50	**58**	**50**	58	50	**58**	**50**			53.1	50.4
16	ESC.1.1	*Escherichia coli*	CIP 54127	FR52 1& 2	33	*0*	21	24	**21**	**24**	21	24	**21**	**24**			28.8	23.8
17	ESC.1.1	*Escherichia coli*	CIP 54127	FR53 1 & 2	17	*0*	16	22	**16**	**22**	16	22	**16**	**22**			9.9	19.2
18	ESC.1.41	*Escherichia coli*	Pâte blanche sortie mélange	FR55	53	*0*	54	62	**54**	**62**	54	62	**54**	**62**			65.9	59.1
19	ESC.1.41	*Escherichia coli*	Pâte blanche sortie mélange	FR56	18	*0*	30	21	**30**	**21**	30	21	**30**	**21**			32.4	22.2
20	ESC.1.117	*Escherichia coli*	Eau de puits	FR57	73	*0*	65	54	**65**	**54**	65	54	**65**	**54**			45.3	83.1
21	ESC.1.117	*Escherichia coli*	Eau de puits	F58	24	*0*	18	22	**18**	**22**	18	22	**18**	**22**			20.7	23.8
22	ESC.1.117	*Escherichia coli*	Eau de puits	FB8	12	*0*	17	15	**17**	**15**	17	15	**17**	**15**			13.7	4.2
23	ESC.1.117	*Escherichia coli*	Eau de puits	FB9	6	*0*	7	9	**7**	**9**	7	9	**7**	**9**			4.2	6.4
Samples not taken into account in the statistical interpretation																		
1	ESC.1.4	*Escherichia coli*	ATCC 8739	FR48	35	*0*	86	80	**86**	**80**	86	80	**86**	**80**			200.5	88.5
2	ESC.1.122	*Escherichia coli*	EPA QC, 082688	FR16	175	*0.1*	18	19	**180**	**190**	18	19	**180**	**190**			144.5	118.4
3	ESC.1.1	*Escherichia coli*	CIP 54127	FR22	48	*0*	112	123	**112**	**123**	112	123	**112**	**123**			165.2	109.1
4	ESC.1.1	*Escherichia coli*	CIP 54127	FR49	130	*1/2*	50	65	**100**	**130**	50	65	**100**	**130**			165.2	129.8
5	ESC.1.41	*Escherichia coli*	Pâte blanche sortie mélange	FR54	160	*1/2*	78	81	**156**	**162**	78	81	**156**	**162**			165.2	200.5

A: repetition 1

B: repetition 2

Estimate number

E.coli TAP WATERS

	N°	Strain	Origin	Sample	Target Concentration CFU/100mL	ISO 9308-1									Colilert 18/ Quanti-tray®			
						dilution	24H				48H				Coliforms detection (yellow)		E.coli detection (fluo)	
							CFU		CFU/ 100mL		CFU		CFU/ 100mL					
							A	B	A	B	A	B	A	B	A	B	A	B
1	ESC.1.4	*Escherichia coli*	ATCC 8741	ED1	9	*0*	23	22	**23**	**22**	23	22	**23**	**22**			**36.4**	**28.8**
2	ESC.1.4	*Escherichia coli*	ATCC 8742	ED2	4	*0*	11	7	**11**	**7**	11	7	**11**	**7**			**22.2**	**16.4**
3	ESC.1.119	*Escherichia coli*	Eau de distribution : douchette bac préparation	ED3	18	*0*	56	46	**56**	**46**	56	46	**56**	**46**			**56**	**59.1**
4	ESC.1.119	*Escherichia coli*	Eau de distribution : douchette bac préparation	ED4	9	*0*	22	33	**22**	**33**	22	33	**22**	**33**			**28.8**	**27.1**
5	ESC.1.119	*Escherichia coli*	Eau de distribution : douchette bac préparation	ED5	5	*0*	10	14	**10**	**14**	10	14	**10**	**14**			**11.1**	**20.7**
6	ESC.1.119	*Escherichia coli*	Eau de distribution : douchette bac préparation	ED6	2	*0*	4	4	**4**	**4**	4	4	**4**	**4**			**5.3**	**6.4**
7	ESC.1.37	*Escherichia coli*	Déchets de pâte recyclés	ED8	6	*0*	9	19	**9**	**19**	9	19	**9**	**19**			**15**	**13.7**
8	ESC.1.37	*Escherichia coli*	Déchets de pâte recyclés	ED9	3	*0*	6	7	**6**	**7**	6	7	**6**	**7**			**1**	**11.1**
9	ESC.1.120	*Escherichia coli*	English, III-80BS	ED11	69	*0*	13	16	**13**	**16**	13	16	**13**	**16**			**15**	**16.4**
10	ESC.1.120	*Escherichia coli*	English, III-80BS	ED12	35	*0*	11	7	**11**	**7**	11	7	**11**	**7**			**11.1**	**19.2**
11	ESC.1.121	*Escherichia coli*	EPA QC, 031593	ED17	28	*0*	53	44	**53**	**44**	53	44	**53**	**44**			**28.8**	**38.4**
12	ESC.1.117	*Escherichia coli*	Eau de puits	D26 b	8	*0*	5	3	**5**	**3**	5	3	**5**	**3**			**11.1**	**6.4**
13	ESC.1.112	*Escherichia coli*	Effluent secondaire	D17	10	*0*	19	19	**19**	**19**	19	19	**19**	**19**			**25.4**	**30**
14	ESC.1.112	*Escherichia coli*	Effluent secondaire	D17	30	*0*	62	56	**62**	**56**	62	56	**62**	**56**			**69.7**	**94.5**
15	ESC.1.39	*Escherichia coli*	Crevette crue décortiquée (isol. TBX)	D27	165	*1/10*	10	20	**10**	**20**	10	20	**10**	**20**			**15**	**8.7**
16	ESC.1.31	*Escherichia coli*	Noix de St-Jacques (isol. TBX)	D28	60	*0*	64	54	**64**	**54**	64	54	**64**	**54**			**83.1**	**101.3**
17	ESC.1.111	*Escherichia coli*	Eau de fontaine	D28	56	*1/10*	5	4	**50**	**40**	5	4	**50**	**40**			**50.4**	**53.1**
Samples not taken into account in the statistical interpretation																		
1	ESC.1.37	*Escherichia coli*	Déchets de pâte recyclés	ED7	18	*0*	3	2	**3**	**2**	3	2	**3**	**2**			**45.3**	**53.1**
2	ESC.1.37	*Escherichia coli*	Déchets de pâte recyclés	ED10	2	*0*	3	2	**3**	**2**	3	2	**3**	**2**			**5.3**	**2**
3	ESC.1.120	*Escherichia coli*	English, III-80BS	ED13	17	*0*	2	1	**2**	**1**	2	1	**2**	**1**			**3.1**	**3.1**
4	ESC.1.121	*Escherichia coli*	EPA QC, 031592	ED16	55	*0*	82	99	**82**	**99**	82	99	**82**	**99**			**109.1**	**101.3**
5	ESC.1.112	*Escherichia coli*	Effluent secondaire	D17	50	*0*	112	103	**112**	**103**	112	103	**112**	**103**			**94.5**	**129.8**
6	ESC.1.113	*Escherichia coli*	Eau de puits	D28	8	*0*	0	0	**0**	**0**	0	0	**0**	**0**			**3.1**	**5.3**
7	ESC.1.39	*Escherichia coli*	Crevette crue décortiquée (isol. TBX)	D24	124	*1/10*	4	1	**40**	**10**	4	1	**40**	**10**			**12.4**	**11.1**
8	ESC.1.39	*Escherichia coli*	Crevette crue décortiquée (isol. TBX)	D25	99	*1/10*	0	0	**0**	**0**	0	0	**0**	**0**			**5.3**	**7.5**
9	ESC.1.39	*Escherichia coli*	Crevette crue décortiquée (isol. TBX)	D28	110	*1/10*	0	0	**0**	**0**	0	0	**0**	**0**			**15**	**7.5**
10	ESC.1.31	*Escherichia coli*	Noix de St-Jacques (isol. TBX)	D24	150	*1/10*	17	22	**170**	**220**	17	22	**170**	**220**			**165.2**	**165.2**
11	ESC.1.31	*Escherichia coli*	Noix de St-Jacques (isol. TBX)	D25	100	*1/10*	12	11	**120**	**110**	12	11	**120**	**110**			**101.3**	**129.8**
12	ESC.1.31	*Escherichia coli*	Noix de St-Jacques (isol. TBX)	D27	120	*1/10*	12	10	**120**	**100**	12	10	**120**	**100**			**165.2**	**118.4**
13	ESC.1.111	*Escherichia coli*	Eau de fontaine	D27	85	*1/10*	10	10	**100**	**100**	10	10	**100**	**100**			**73.8**	**65.9**

A: repetition 1

B: repetition 2

Estimate number

Coliformes **FOUTAIN WATERS**

	N°	Strain	Origin	Sample	Target Concentration CFU/100mL	ISO 9308-1									Colilert 18/ Quanti-tray®			
						dilution	24H				48H				Coliforms detection (yellow)		E.coli detection (fluo)	
							CFU		CFU/ 100mL		CFU		CFU/ 100mL					
							A	B	A	B	A	B	A	B	A	B	A	B
1	ENTB.1.1	*Enterobacter aerogenes*	Industrie laitière	ED1	50	*0*	12	11	**12**	**11**	12	11	**12**	**11**	5.3	5.3	<1	<1
2	CIT.2.2	*Citrobacter diversus*	CIP 82.87T	F57	70	*0*	68	74	**68**	**74**	68	74	**68**	**74**	73.8	47.8	<1	<1
3	BUT.1.1	*Buttiauxella agrestis*	Eau de puits	F67	18	*0*	54	56	**54**	**56**	54	56	**54**	**56**	32	27	<1	<1
4	ENTB.3.2	*Enterobacter sakazakii*	CIP 57.33	F67	60	*0*	35	25	**35**	**25**	35	25	**35**	**25**	20.7	11.1	<1	<1
5	CIT.2.2	*Citrobacter diversus*	CIP 82.87T	F61	30	*0*	15	14	**15**	**14**	15	14	**15**	**14**	19.2	27.1	<1	<1
6	CIT.2.2	*Citrobacter diversus*	CIP 82.87T	F61	50	*0*	29	23	**29**	**23**	29	23	**29**	**23**	36.4	47.8	<1	<1
7	CIT.2.2	*Citrobacter diversus*	CIP 82.87T	F61	100	*0*	55	54	**55**	**54**	55	54	**55**	**54**	78.2	101.3	<1	<1
8	ESC.2.1	*Escherichia hermanii*	CIP 103176	F70	19	*0*	14	16	**14**	**16**	14	16	**14**	**16**	8.7	19.2	<1	<1
9	KLE.1.2	*Klebsiella oxytoca*	Effluent secondaire	F65	67	*0*	70	58	**70**	**58**	70	58	**70**	**58**	65.9	73.8	<1	<1
10	ENTB.3.3	*Enterobacter sakazakii*	CIP 103581	F67	50	*0*	38	38	**38**	**38**	38	38	**38**	**38**	28.8	20.7	<1	<1
11	ENTB.3.3	*Enterobacter sakazakii*	CIP 103581	F64	33	*0*	18	26	**18**	**26**	18	26	**18**	**26**	20.7	25.4	<1	<1
12	ENTB.3.3	*Enterobacter sakazakii*	CIP 103581	F67	20	*0*	7	15	**7**	**15**	7	15	**7**	**15**	11.1	12.4	<1	<1
13	KLE.1.1	*Klebsiella oxytoca*	Salade de soja	F72	55	*0*	54	59	**54**	**59**	54	59	**54**	**59**	53.1	69.7	<1	<1
14	KLE.1.1	*Klebsiella oxytoca*	Salade de soja	F74	22	*0*	27	29	**27**	**29**	27	29	**27**	**29**	34.4	22.2	<1	<1
15	KLE.3.1	*Klebsiella planticola*	Saucisse	F72	63	*0*	60	59	**60**	**59**	60	59	**60**	**59**	62.4	73.8	<1	<1
16	KLE.3.1	*Klebsiella planticola*	Saucisse	F73	31	*0*	26	15	**26**	**15**	26	15	**26**	**15**	23.8	32.4	<1	<1
17	KLE.3.1	*Klebsiella planticola*	Saucisse	F74	19	*0*	14	17	**14**	**17**	14	17	**14**	**17**	11.1	11.1	<1	<1
18	KLU.1.1	*Kluyvera sp.*	EPA 0115-90487	F71	133	*1/10*	5	5	**50**	**50**	5	5	**50**	**50**	56	65.9	<1	<1
19	KLU.1.1	*Kluyvera sp.*	EPA 0115-90487	F72	100	*1/10*	7	6	**70**	**60**	7	6	**70**	**60**	56	42.9	<1	<1
Samples not taken into account in the statistical interpretation																		
1	CIT.2.2	*Citrobacter diversus*	CIP 82.87T	F57	160	*0*	162	162	**162**	**162**	162	162	**162**	**162**	165.2	165.2	<1	<1
2	KLE.1.2	*Klebsiella oxytoca*	Effluent secondaire	F65	95	*1/10*	15	9	**150**	**90**	15	9	**150**	**90**	109.1	165.2	<1	<1
3	ENTB.3.3	*Enterobacter sakazakii*	CIP 103581	F64	100	*1/10*	7	9	**70**	**90**	7	9	**70**	**90**	88.5	59.1	<1	<1
4	KLE.1.1	*Klebsiella oxytoca*	Salade de soja	F71	110	*1/10*	15	8	**150**	**80**	15	8	**150**	**80**	165.2	165.2	<1	<1
5	KLE.1.1	*Klebsiella oxytoca*	Salade de soja	F73	36	*0*	7	2	**7**	**2**	7	2	**7**	**2**	42.9	40.6	<1	<1
6	KLE.3.1	*Klebsiella planticola*	Saucisse	F71	190	*1/10*	22	14	**220**	**140**	22	14	**220**	**140**	165.2	144.5	<1	<1

A: repetition 1
B: repetition 2
Estimate number

Coliformes **TAP WATERS**

	N°	Strain	Origin	Sample	Target Concentration CFU/100mL	ISO 9308-1									Colilert 18/ Quanti-tray®			
						dilution	24H				48H				Coliforms detection (yellow)		E.coli detection (fluo)	
							CFU		CFU/ 100mL		CFU		CFU/ 100mL					
							A	B	A	B	A	B	A	B	A	B	A	B
1	ENTB.1.1	*Enterobacter aerogenes*	Industrie laitière	ED1	50	*0*	12	11	**12**	**11**	12	11	**12**	**11**	**5.3**	**5.3**	<1	<1
2	ENTB.1.1	*Enterobacter aerogenes*	Industrie laitière	ED2	25	*0*	8	9	**8**	**9**	8	9	**8**	**9**	**5.3**	**2**	<1	<1
3	ENTB.1.2	*Enterobacter aerogenes*	CIP 60.86T	ED5	22	*0*	20	25	**20**	**25**	20	25	**20**	**25**	**27.1**	**16.4**	<1	<1
4	ENTB.1.2	*Enterobacter aerogenes*	CIP 60.86T	ED6	11	*0*	15	14	**15**	**14**	15	14	**15**	**14**	**8.7**	**9.9**	<1	<1
5	ENTB.1.2	*Enterobacter aerogenes*	CIP 60.86T	ED8	3	*0*	5	4	**5**	**4**	5	4	**5**	**4**	**5.3**	**4.2**	<1	<1
6	ENTB.1.3	*Enterobacter aerogenes*	4166:80 Thames isolate #358	ED9	28	*0*	17	19	**17**	**19**	17	19	**17**	**19**	**19.2**	**13.7**	<1	<1
7	ENTB.1.3	*Enterobacter aerogenes*	4166:80 Thames isolate #358	ED10	14	*0*	8	8	**8**	**8**	8	8	**8**	**8**	**6.4**	**6.4**	<1	<1
8	ENTB.2.1	*Enterobacter cloacae*	Eaux usagées	ED13	110	*0*	45	56	**45**	**56**	45	56	**45**	**56**	**53.1**	**40.6**	<1	<1
9	ENTB.2.1	*Enterobacter cloacae*	Eaux usagées	ED15	37	*0*	16	20	**16**	**20**	16	20	**16**	**20**	**15**	**8.7**	<1	<1
10	ENTB.2.1	*Enterobacter cloacae*	Eaux usagées	ED16	18	*0*	5	12	**5**	**12**	5	12	**5**	**12**	**9.9**	**9.9**	<1	<1
11	ENTB.2.1	*Enterobacter cloacae*	Eaux usagées	ED17	9	*0*	7	8	**7**	**8**	7	8	**7**	**8**	**3.1**	**4.2**	<1	<1
12	CIT.2.1	*Citrobacter koseri*	CIP 72.11	ED21	18	*0*	9	12	**9**	**12**	9	12	**9**	**12**	**17.8**	**17.8**	<1	<1
13	CIT.2.1	*Citrobacter koseri*	CIP 72.11	ED23	60	*0*	19	22	**19**	**22**	19	22	**19**	**22**	**47.8**	**38.4**	<1	<1
14	BUT.1.1	*Buttiauxella agrestis*	Eau de puits	ED23	43	*0*	56	51	**56**	**51**	56	51	**56**	**51**	**109.1**	**144.5**	<1	<1
15	CIT.1.2	*Citrobacter freundii*	ATCC 8090	ED23	58	*0*	74	60	**74**	**60**	74	60	**74**	**60**	**59.1**	**69.7**	<1	<1
16	EWI.1.1	*Ewingella americana*	JMM013, env. Sample, 4569:1	D34	67	*1/10*	8	7	**80**	**70**	8	7	**80**	**70**	**101.3**	**101.3**	<1	<1
Samples not taken into account in the statistical interpretation																		
1	ENTB.1.1	*Enterobacter aerogenes*	Industrie laitière	ED3	13	*0*	0	0	**0**	**0**	0	0	**0**	**0**	**3.1**	**4.2**	<1	<1
2	ENTB.1.2	*Enterobacter aerogenes*	CIP 60.86T	ED7	6	*0*	0	2	**0**	**2**	0	2	**0**	**2**	**9.9**	**7.5**	<1	<1
3	ENTB.1.3	*Enterobacter aerogenes*	4166:80 Thames isolate #358	ED12	4	*0*	2	1	**2**	**1**	2	1	**2**	**1**	**1**	**0**	<1	<1
4	CIT.2.1	*Citrobacter koseri*	CIP 72.11	ED19	180	*1/10*	13	14	**130**	**140**	13	14	**130**	**140**	**200.5**	**129.8**	<1	<1
5	CIT.2.1	*Citrobacter koseri*	CIP 72.11	ED22	90	*1/10*	5	10	**50**	**100**	5	10	**50**	**100**	**73.8**	**94.5**	<1	<1
6	BUT.1.1	*Buttiauxella agrestis*	Eau de puits	ED21	85	*0*	125	127	**125**	**127**	125	127	**125**	**127**	**200.5**	**200.5**	<1	<1
7	CIT.1.2	*Citrobacter freundii*	ATCC 8090	ED21	115	*1/10*	16	11	**160**	**110**	16	11	**160**	**110**	**101.3**	**165.2**	<1	<1
8	CIT.1.2	*Citrobacter freundii*	ATCC 8090	ED22	77	*0*	94	81	**94**	**81**	94	81	**94**	**81**	**65.9**	**83.1**	<1	<1
9	EWI.1.1	*Ewingella americana*	JMM013, env. Sample, 4569:1	D32	100	*1/10*	16	9	**160**	**90**	16	9	**160**	**90**	**144.5**	**101.3**	<1	<1

A: repetition 1

B: repetition 2

Estimate number

Exactitude relative - Escherichia coli - Eau de consommation humaine

Ech.	Méthode de référence (NF ISO 9308-1) Rep. 1	Rep. 2	M	SD	Méthode alternative (Quanti-tray®) Rep. 1	Rep. 2	M	SD	Différence	Est. y	Dév.
1	30	33	31.5	2.1	20.7	32.4	26.6	8.3	-5.0	35.6	-9.0
2	12	10	11.0	1.4	19.2	19.2	19.2	0.0	8.2	9.5	9.7
3	4	12	8.0	5.7	13.7	7.5	10.6	4.4	2.6	5.7	4.9
4	3	8	5.5	3.5	4.2	6.4	5.3	1.6	-0.2	2.6	2.7
5	48	45	46.5	2.1	56	40.6	48.3	10.9	1.8	54.6	-6.3
6	14	21	17.5	4.9	20.7	22.2	21.5	1.1	4.0	17.8	3.7
7	23	26	24.5	2.1	20.7	19.2	20.0	1.1	-4.6	26.7	-6.7
8	70	50	60.0	14.1	118.5	101.3	109.9	12.2	49.9	71.8	38.1
9	64	60	62.0	2.8	36.4	36.4	36.4	0.0	-25.6	74.3	-37.9
10	9	20	14.5	7.8	12.4	13.7	13.1	0.9	-1.5	14.0	-0.9
11	65	60	62.5	3.5	73.8	69.7	71.8	2.9	9.3	74.9	-3.2
12	34	33	33.5	0.7	25.4	25.4	25.4	0.0	-8.1	38.1	-12.7
13	15	20	17.5	3.5	13.7	25.4	19.6	8.3	2.1	17.8	1.8
14	68	73	70.5	3.5	73.8	78.2	76.0	3.1	5.5	85.1	-9.1
15	58	50	54.0	5.7	53.1	50.4	51.8	1.9	-2.3	64.2	-12.4
16	21	24	22.5	2.1	28.8	23.8	26.3	3.5	3.8	24.1	2.2
17	16	22	19.0	4.2	9.9	19.2	14.6	6.6	-4.5	19.7	-5.1
18	54	62	58.0	5.7	65.9	59.1	62.5	4.8	4.5	69.2	-6.7
19	30	21	25.5	6.4	32.4	22.2	27.3	7.2	1.8	28.0	-0.7
20	65	54	59.5	7.8	45.3	83.1	64.2	26.7	4.7	71.1	-6.9
21	18	22	20.0	2.8	20.7	23.8	22.3	2.2	2.3	21.0	1.3
22	17	15	16.0	1.4	13.7	4.2	9.0	6.7	-7.1	15.9	-6.9
23	7	9	8.0	1.4	4.2	6.4	5.3	1.6	-2.7	5.7	-0.4
24	23	22	22.5	0.7	36.4	28.8	32.6	5.4	10.1	24.1	8.5
25	11	7	9.0	2.8	22.2	16.4	19.3	4.1	10.3	7.0	12.3
26	56	46	51.0	7.1	56	59.1	57.6	2.2	6.6	60.3	-2.8
27	22	33	27.5	7.8	28.8	27.1	28.0	1.2	0.5	30.5	-2.5
28	10	14	12.0	2.8	11.1	20.7	15.9	6.8	3.9	10.8	5.1
29	4	4	4.0	0.0	5.3	6.4	5.9	0.8	1.9	0.6	5.2
30	9	19	14.0	7.1	15	13.7	14.4	0.9	0.4	13.3	1.0
31	6	7	6.5	0.7	1	11.1	6.1	7.1	-0.5	3.8	2.2
32	13	16	14.5	2.1	15	16.4	15.7	1.0	1.2	14.0	1.7
33	11	7	9.0	2.8	11.1	19.2	15.2	5.7	6.2	7.0	8.2
34	53	44	48.5	6.4	28.8	38.4	33.6	6.8	-14.9	57.2	-23.6
35	5	3	4.0	1.4	11.1	6.4	8.8	3.3	4.8	0.6	8.1
36	19	19	19.0	0.0	25.4	30	27.7	3.3	8.7	19.7	8.0
37	62	56	59.0	4.2	69.7	94.5	82.1	17.5	23.1	70.5	11.6
38	10	20	15.0	7.1	15	8.7	11.9	4.5	-3.2	14.6	-2.8
39	64	54	59.0	7.1	83.1	101.3	92.2	12.9	33.2	70.5	21.7
40	50	40	45.0	7.1	50.4	53.1	51.8	1.9	6.8	52.7	-1.0

q= 40
n= 2
N=qn= 80

Mx= 29.175
MEDx= 21.250
SDbx= 20.768
MEDwx = 3.536
SDwx= 4.977
rob. SDwx= 5.242

Mv= 32.621
MEDv= 23.825
SDby= 26.257
MEDwy = 3.429
SDwy= 7.239
rob. SDwy= 5.085

3.446
2.150
Biais

Choix de la méthode
GMFR

R= 1.454
rob. R= 0.970

Sx= 20.938
Sy= 26.594

r= 0.899
b= 1.270
a= -4.435

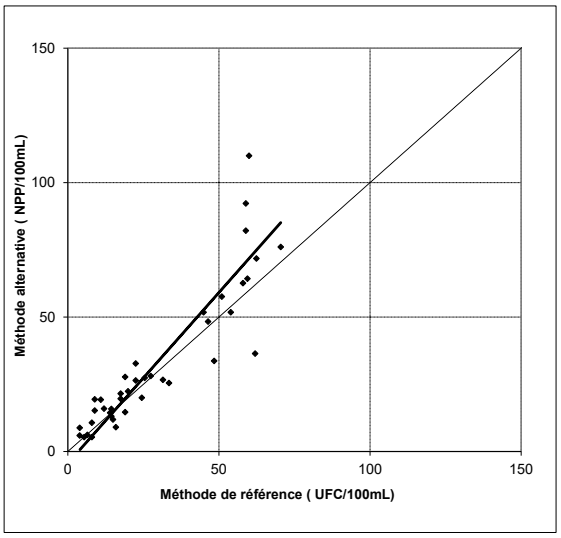
Res. SEM= 11.974
Res. SD= 16.934

S(b)= 0.092
S(a)= 3.274

p(t;b=1)= 0.004
p(t;a=0)= 0.180

t(b)= 2.950
t(a)= 1.354

Répétabilité	MR	MA
r	13.937	20.269
rob. r	14.677	14.237



Les points représentés correspondent aux moyennes des répétitions de chaque échantillon
Une courbe d'équation y = x figure en pointillés

Exactitude relative - Escherichia coli - Eau de consommation humaine

Ech.	Méthode de référence (NF ISO 9308-1) Rep. 1	Rep. 2	M	SD	Méthode alternative (Quanti-tray®) Rep. 1	Rep. 2	M	SD	Différence	Est. y	Dév.
1	1.48	1.52	1.50	0.03	1.32	1.51	1.41	0.14	-0.08	1.54	-0.13
2	1.08	1.00	1.04	0.06	1.28	1.28	1.28	0.00	0.24	1.06	0.22
3	0.60	1.08	0.84	0.34	1.14	0.88	1.01	0.19	0.17	0.85	0.15
4	0.48	0.90	0.69	0.30	0.62	0.81	0.71	0.13	0.02	0.70	0.02
5	1.68	1.65	1.67	0.02	1.75	1.61	1.68	0.10	0.01	1.72	-0.04
6	1.15	1.32	1.23	0.12	1.32	1.35	1.33	0.02	0.10	1.27	0.07
7	1.36	1.41	1.39	0.04	1.32	1.28	1.30	0.02	-0.09	1.43	-0.13
8	1.85	1.70	1.77	0.10	2.07	2.01	2.04	0.05	0.27	1.83	0.21
9	1.81	1.78	1.79	0.02	1.56	1.56	1.56	0.00	-0.23	1.85	-0.29
10	0.95	1.30	1.13	0.25	1.09	1.14	1.12	0.03	-0.01	1.15	-0.04
11	1.81	1.78	1.80	0.02	1.87	1.84	1.86	0.02	0.06	1.85	0.00
12	1.53	1.52	1.52	0.01	1.40	1.40	1.40	0.00	-0.12	1.57	-0.16
13	1.18	1.30	1.24	0.09	1.14	1.40	1.27	0.19	0.03	1.27	0.00
14	1.83	1.86	1.85	0.02	1.87	1.89	1.88	0.02	0.03	1.91	-0.03
15	1.76	1.70	1.73	0.05	1.73	1.70	1.71	0.02	-0.02	1.78	-0.07
16	1.32	1.38	1.35	0.04	1.46	1.38	1.42	0.06	0.07	1.39	0.03
17	1.20	1.34	1.27	0.10	1.00	1.28	1.14	0.20	-0.13	1.31	-0.17
18	1.73	1.79	1.76	0.04	1.82	1.77	1.80	0.03	0.03	1.82	-0.02
19	1.48	1.32	1.40	0.11	1.51	1.35	1.43	0.12	0.03	1.44	-0.01
20	1.81	1.73	1.77	0.06	1.66	1.92	1.79	0.19	0.02	1.83	-0.04
21	1.26	1.34	1.30	0.06	1.32	1.38	1.35	0.04	0.05	1.33	0.01
22	1.23	1.18	1.20	0.04	1.14	0.62	0.88	0.36	-0.32	1.23	-0.35
23	0.85	0.95	0.90	0.08	0.62	0.81	0.71	0.13	-0.18	0.92	-0.20
24	1.36	1.34	1.35	0.01	1.56	1.46	1.51	0.07	0.16	1.39	0.12
25	1.04	0.85	0.94	0.14	1.35	1.21	1.28	0.09	0.34	0.96	0.32
26	1.75	1.66	1.71	0.06	1.75	1.77	1.76	0.02	0.05	1.76	0.00
27	1.34	1.52	1.43	0.12	1.46	1.43	1.45	0.02	0.02	1.47	-0.02
28	1.00	1.15	1.07	0.10	1.05	1.32	1.18	0.19	0.11	1.10	0.08
29	0.60	0.60	0.60	0.00	0.72	0.81	0.77	0.06	0.16	0.61	0.16
30	0.95	1.28	1.12	0.23	1.18	1.14	1.16	0.03	0.04	1.14	0.01
31	0.78	0.85	0.81	0.05	0.00	1.05	0.52	0.74	-0.29	0.82	-0.30
32	1.11	1.20	1.16	0.06	1.18	1.21	1.20	0.03	0.04	1.19	0.01
33	1.04	0.85	0.94	0.14	1.05	1.28	1.16	0.17	0.22	0.96	0.20
34	1.72	1.64	1.68	0.06	1.46	1.58	1.52	0.09	-0.16	1.73	-0.21
35	0.70	0.48	0.59	0.16	1.05	0.81	0.93	0.17	0.34	0.59	0.33
36	1.28	1.28	1.28	0.00	1.40	1.48	1.44	0.05	0.16	1.31	0.13
37	1.79	1.75	1.77	0.03	1.84	1.98	1.91	0.09	0.14	1.82	0.08
38	1.00	1.30	1.15	0.21	1.18	0.94	1.06	0.17	-0.09	1.18	-0.12
39	1.81	1.73	1.77	0.05	1.92	2.01	1.96	0.06	0.19	1.82	0.14
40	1.70	1.60	1.65	0.07	1.70	1.73	1.71	0.02	0.06	1.70	0.01

Mx= 1.33
MEDx= 1.33
SDbx= 0.37
MEDwx = 0.06
SDwx= 0.12
rob. SDwx= 0.09

q= 40
n= 2
N=qn= 80

Mv= 1.36
MEDv= 1.38
SDby= 0.37
MEDwy = 0.06
SDwy= 0.16
rob. SDwy= 0.09

0.04
0.035
Biais

Choix de la méthode
GMFR

R= 1.390
rob. R= 1.015

Sx= 0.373
Sy= 0.389

r= 0.913
b= 1.043
a= -0.022

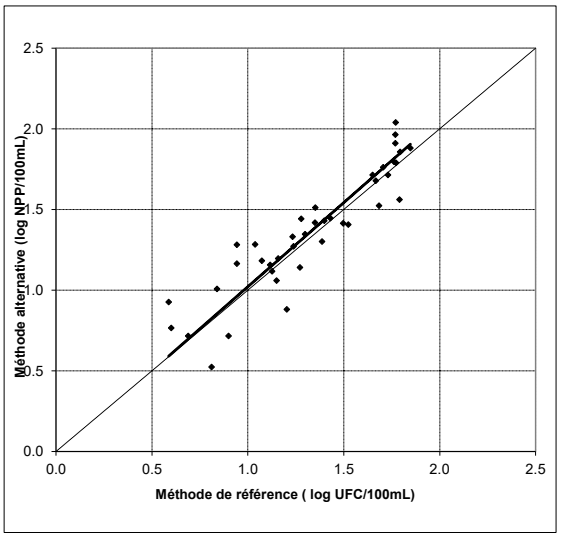
Res. SEM= 0.159
Res. SD= 0.225

S(b)= 0.068
S(a)= 0.094

p(t;b=1)= 0.530
p(t;a=0)= 0.816

t(b)= 0.631
t(a)= 0.233

Répétabilité	MR	MA
r	0.330	0.459
rob. r	0.244	0.248



Les points représentés correspondent aux moyennes des répétitions de chaque échantillon
Une courbe d'équation y = x figure en pointillés

Exactitude relative - Coliformes - Eau de consommation humaine

Ech.	Méthode de référence (NF ISO 9308-1) Rep. 1	Rep. 2	M	SD	Méthode alternative (Quanti-tray®) Rep. 1	Rep. 2	M	SD	Différence
1	12	11	11.50	0.71	5.3	5.3	5.30	0.00	-6.20
2	68	74	71.00	4.24	73.8	47.8	60.80	18.38	-10.20
3	54	56	55.00	1.41	32	27	29.50	3.54	-25.50
4	35	25	30.00	7.07	20.7	11.1	15.90	6.79	-14.10
5	15	14	14.50	0.71	19.2	27.1	23.15	5.59	8.65
6	29	23	26.00	4.24	36.4	47.8	42.10	8.06	16.10
7	55	54	54.50	0.71	78.2	101.3	89.75	16.33	35.25
8	14	16	15.00	1.41	8.7	19.2	13.95	7.42	-1.05
9	70	58	64.00	8.49	65.9	73.8	69.85	5.59	5.85
10	38	38	38.00	0.00	28.8	20.7	24.75	5.73	-13.25
11	18	26	22.00	5.66	20.7	25.4	23.05	3.32	1.05
12	7	15	11.00	5.66	11.1	12.4	11.75	0.92	0.75
13	54	59	56.50	3.54	53.1	69.7	61.40	11.74	4.90
14	27	29	28.00	1.41	34.4	22.2	28.30	8.63	0.30
15	60	59	59.50	0.71	62.4	73.8	68.10	8.06	8.60
16	26	15	20.50	7.78	23.8	32.4	28.10	6.08	7.60
17	14	17	15.50	2.12	11.1	11.1	11.10	0.00	-4.40
18	50	50	50.00	0.00	56	65.9	60.95	7.00	10.95
19	70	60	65.00	7.07	56	42.9	49.45	9.26	-15.55
20	12	11	11.50	0.71	5.3	5.3	5.30	0.00	-6.20
21	8	9	8.50	0.71	5.3	2	3.65	2.33	-4.85
22	20	25	22.50	3.54	27.1	16.4	21.75	7.57	-0.75
23	15	14	14.50	0.71	8.7	9.9	9.30	0.85	-5.20
24	5	4	4.50	0.71	5.3	4.2	4.75	0.78	0.25
25	17	19	18.00	1.41	19.2	13.7	16.45	3.89	-1.55
26	8	8	8.00	0.00	6.4	6.4	6.40	0.00	-1.60
27	45	56	50.50	7.78	53.1	40.6	46.85	8.84	-3.65
28	16	20	18.00	2.83	15	8.7	11.85	4.45	-6.15
29	5	12	8.50	4.95	9.9	9.9	9.90	0.00	1.40
30	7	8	7.50	0.71	3.1	4.2	3.65	0.78	-3.85
31	9	12	10.50	2.12	17.8	17.8	17.80	0.00	7.30
32	19	22	20.50	2.12	47.8	38.4	43.10	6.65	22.60
33	56	51	53.50	3.54	109.1	144.5	126.80	25.03	73.30
34	74	60	67.00	9.90	59.1	69.7	64.40	7.50	-2.60
35	80	70	75.00	7.07	101.3	101.3	101.30	0.00	26.30

Mx= 31.60
MEDx= 22.00
SDbx= 22.44
MEDwx = 2.12
SDwx= 4.27
rob. SDwx= 3.15

My= 34.59
MEDy= 23.15
SDby= 30.69
MEDwy = 5.59
SDwy= 8.01
rob. SDwy= 8.28

2.99
-0.75
Biais

q= 35
n= 2
N=qn= 70

Choix de la méthode
OLS1

R= 1.877
rob. R= 2.633

Sx= 22.480
Sy= 30.997

r= 0.840
b= 1.158
a= -2.020

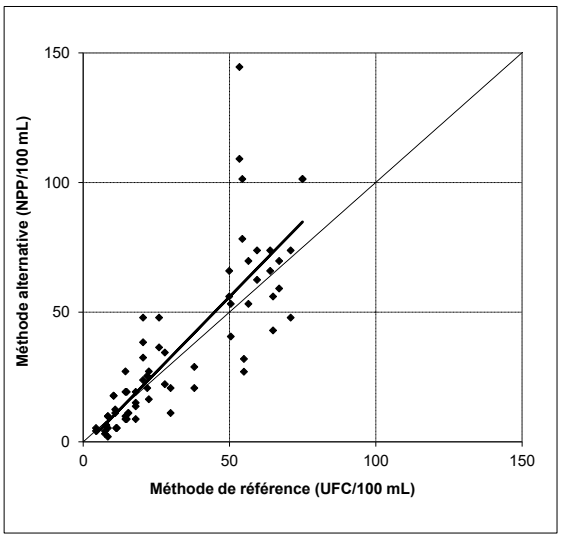
Res. SEM= 25.282
Res. SD= 35.754

S(b)= 0.193
S(a)= 7.444

p(t;b=1)= 0.414
p(t;a=0)= 0.787

t(b)= 0.821
t(a)= 0.271

Répétabilité	MR	MA
r	11.945	22.421
rob. r	8.806	23.190



Les points représentés correspondent aux moyennes des répétitions de chaque échantillon
Une courbe d'équation y = x figure en pointillés

Exactitude relative - Coliformes - Eau de consommation humaine

Ech.	Méthode de référence (NF ISO 9308-1) Rep. 1	Rep. 2	M	SD	Méthode alternative (Quanti-tray®) Rep. 1	Rep. 2	M	SD	Différence	Est. y	Dév.
1	1.08	1.04	1.06	0.03	0.72	0.72	0.72	0.00	-0.34	0.95	-0.23
2	1.83	1.87	1.85	0.03	1.87	1.68	1.77	0.13	-0.08	1.95	-0.17
3	1.73	1.75	1.74	0.01	1.51	1.43	1.47	0.05	-0.27	1.81	-0.34
4	1.54	1.40	1.47	0.10	1.32	1.05	1.18	0.19	-0.29	1.47	-0.29
5	1.18	1.15	1.16	0.02	1.28	1.43	1.36	0.11	0.20	1.08	0.28
6	1.46	1.36	1.41	0.07	1.56	1.68	1.62	0.08	0.21	1.40	0.22
7	1.74	1.73	1.74	0.01	1.89	2.01	1.95	0.08	0.21	1.80	0.15
8	1.15	1.20	1.18	0.04	0.94	1.28	1.11	0.24	-0.06	1.10	0.01
9	1.85	1.76	1.80	0.06	1.82	1.87	1.84	0.03	0.04	1.89	-0.05
10	1.58	1.58	1.58	0.00	1.46	1.32	1.39	0.10	-0.19	1.61	-0.22
11	1.26	1.41	1.34	0.11	1.32	1.40	1.36	0.06	0.03	1.30	0.06
12	0.85	1.18	1.01	0.23	1.05	1.09	1.07	0.03	0.06	0.89	0.18
13	1.73	1.77	1.75	0.03	1.73	1.84	1.78	0.08	0.03	1.82	-0.04
14	1.43	1.46	1.45	0.02	1.54	1.35	1.44	0.13	-0.01	1.44	0.00
15	1.78	1.77	1.77	0.01	1.80	1.87	1.83	0.05	0.06	1.85	-0.02
16	1.41	1.18	1.30	0.17	1.38	1.51	1.44	0.09	0.15	1.25	0.19
17	1.15	1.23	1.19	0.06	1.05	1.05	1.05	0.00	-0.14	1.11	-0.07
18	1.70	1.70	1.70	0.00	1.75	1.82	1.78	0.05	0.08	1.76	0.03
19	1.85	1.78	1.81	0.05	1.75	1.63	1.69	0.08	-0.12	1.90	-0.21
20	1.08	1.04	1.06	0.03	0.72	0.72	0.72	0.00	-0.34	0.95	-0.23
21	0.90	0.95	0.93	0.04	0.72	0.30	0.51	0.30	-0.42	0.79	-0.27
22	1.30	1.40	1.35	0.07	1.43	1.21	1.32	0.15	-0.03	1.32	0.01
23	1.18	1.15	1.16	0.02	0.94	1.00	0.97	0.04	-0.19	1.08	-0.11
24	0.70	0.60	0.65	0.07	0.72	0.62	0.67	0.07	0.02	0.44	0.24
25	1.23	1.28	1.25	0.03	1.28	1.14	1.21	0.10	-0.04	1.20	0.01
26	0.90	0.90	0.90	0.00	0.81	0.81	0.81	0.00	-0.10	0.76	0.05
27	1.65	1.75	1.70	0.07	1.73	1.61	1.67	0.08	-0.03	1.76	-0.09
28	1.20	1.30	1.25	0.07	1.18	0.94	1.06	0.17	-0.19	1.19	-0.14
29	0.70	1.08	0.89	0.27	1.00	1.00	1.00	0.00	0.11	0.74	0.26
30	0.85	0.90	0.87	0.04	0.49	0.62	0.56	0.09	-0.32	0.72	-0.16
31	0.95	1.08	1.02	0.09	1.25	1.25	1.25	0.00	0.23	0.90	0.35
32	1.28	1.34	1.31	0.05	1.68	1.58	1.63	0.07	0.32	1.27	0.36
33	1.75	1.71	1.73	0.03	2.04	2.16	2.10	0.09	0.37	1.79	0.31
34	1.87	1.78	1.82	0.06	1.77	1.84	1.81	0.05	-0.02	1.91	-0.11
35	1.90	1.85	1.87	0.04	2.01	2.01	2.01	0.00	0.13	1.98	0.03

q= 35
n= 2
N=qn= 70

Mx= 1.374
MEDx= 1.335
SDbx= 0.348
MEDwx = 0.041
SDwx= 0.083
rob. SDwx= 0.061

My= 1.347
MEDy= 1.360
SDby= 0.437
MEDwy = 0.079
SDwy= 0.106
rob. SDwy= 0.118

-0.026
-0.016
Biais

Choix de la méthode
GMFR

R= 1.282
rob. R= 1.938

Sx= 0.350
Sy= 0.441

r= 0.901
b= 1.258
a= -0.381

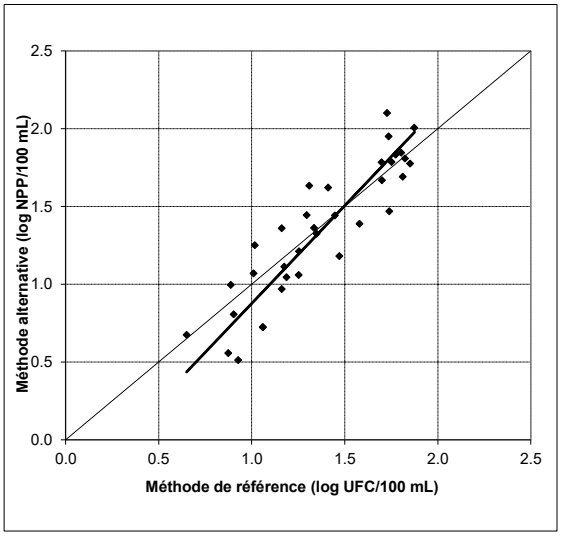
Res. SEM= 0.197
Res. SD= 0.279

S(b)= 0.097
S(a)= 0.137

p(t;b=1)= 0.009
p(t;a=0)= 0.007

t(b)= 2.670
t(a)= 2.782

Répétabilité	MR	MA
r	0.231	0.296
rob. r	0.170	0.330



Les points représentés correspondent aux moyennes des répétitions de chaque échantillon
Une courbe d'équation y = x figure en pointillés

Appendix D

Linearity results

Linéarité - Résultats bruts

Linearity Escherichia.coli

Inoculation level (CFU/ 100mL)	Matrix	Strain	Origin	NF ISO 9308-1				
				Répétition 1 24h	Répétition 2 24h	Répétition 1 24h	Répétition 2 48h	Mean
				CFU/ plate 1	CFU/ plate 2	CFU/ plate 1	CFU/ plate 2	
10	Mains water	*Escherichia coli* YNQ 123	Well water	6	5	6	5	5.5
20				18	16	18	16	17
30				28	26	20	28	24
50				38	29	28	35	31.5
70				76	73	76	73	74.5

Inoculation level (CFU/ 100mL)	Matrix	Strain	Origin	Quanti-tray®		
				Repetition 1	Repetition 2	Mean
				CFU/ 100mL	CFU/ 100mL	
10	Mains water	*Escherichia coli* YNQ 123	Well water	4	4	4
20				22	26	24
30				25	25	25
50				43	50	46.5
70				94	78	86

Linearity Coliform

Taux d'inoculation (UFC/ 100mL)	Matrice	Souche	Origine	NF ISO 9308-1				
				Répétition 1 24h	Répétition 2 24h	Répétition 1 24h	Répétition 2 48h	Moyenne
				UFC/ boîte 1	UFC/ boîte 2	UFC/ 100mL	UFC/ 100mL	
10	Eau de fontaine	*Citrobacter freundii* CIT1.4	Eau de puits	5	9	5	9	7
30				18	10	18	10	14
50				17	21	17	21	19
100				60	60	60	60	60

Taux d'inoculation (UFC/ 100mL)	Matrice	Souche	Origine	Quanti-tray®		
				Répétition 1	Répétition 2	Moyenne
				UFC/ 100mL	UFC/ 100mL	
10	Eau de fontaine	*Citrobacter freundii* CIT1.4	Eau de puits	2	1	1.5
30				17.8	13.7	15.75
50				22.2	16.4	19.3
100				50.4	56	53.2

Linéarité - E. coli - Données brutes

Niveau	Méthode de référence Rép.1	Rép.2	M	SD	Méthode alternative Rép.1	Rép.2	M	SD
1	6	5	5.5	0.707	4	4	4.0	0.000
2	18	16	17.0	1.414	22	26	24.0	2.828
3	20	28	24.0	5.657	25	25	25.0	0.000
4	28	35	31.5	4.950	43	50	46.5	4.950
5	76	73	74.5	2.121	94	78	86.0	11.314

q = 5
n = 2
N = qn = 10

Mx = 30.500
MEDx = 24.000
SDbx = 26.394

MEDwx = 2.121
SDwx = 2.520
rob. SDwx = 3.145

My = 37.100
MEDy = 25.000
SDby = 31.198

MEDwy = 2.828
SDwy = 4.006
rob. SDwy = 4.193

Choix méthode
GMFR

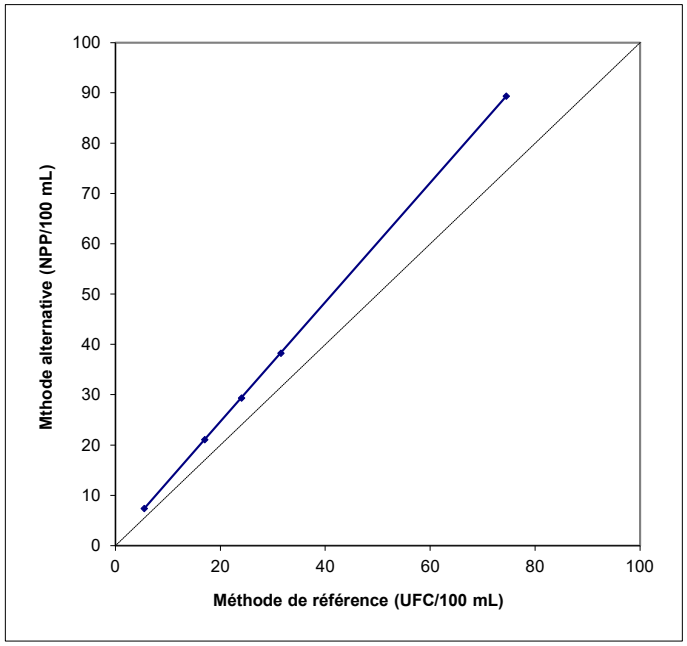
R = 1.590
rob.R = 1.333
Res.SEM = 6.274
Res.SD = 8.873

Sx = 25.026
Sy = 29.715

Est y	Déviation
7.415	-3.415
21.070	2.930
29.382	-4.382
38.287	8.213
89.345	-3.345

r = 0.985
b = 1.187
a = 0.885

Sb = 0.125 **p(t;b=1) =** 0.173 **t (b) =** 1.495
Sa = 4.742 **p(t;a=0) =** 0.857 **t (a) =** 0.024



Linéarité

F = 11.413 **p(F) =** 0.011
rob.F = 10.272 **rob.p(F) =** 0.014

Linéarité - E. coli - Données log

Niveau	Méthode de référence Rép.1	Rép.2	M	SD	Méthode alternative Rép.1	Rép.2	M	SD
1	0.778	0.699	0.7	0.056	0.602	0.602	0.6	0.000
2	1.255	1.204	1.2	0.036	1.342	1.415	1.4	0.051
3	1.301	1.447	1.4	0.103	1.398	1.398	1.4	0.000
4	1.447	1.544	1.5	0.069	1.633	1.699	1.7	0.046
5	1.881	1.863	1.9	0.012	1.973	1.892	1.9	0.057

q = 5
n = 2
N = qn = 10

Mx = 1.342
MEDx = 1.374
SDbx = 0.413

MEDwx = 0.056
SDwx = 0.045
rob. SDwx = 0.083

My = 1.396
MEDy = 1.398
SDby = 0.498

MEDwy = 0.046
SDwy = 0.028
rob. SDwy = 0.069

Choix méthode
GMFR

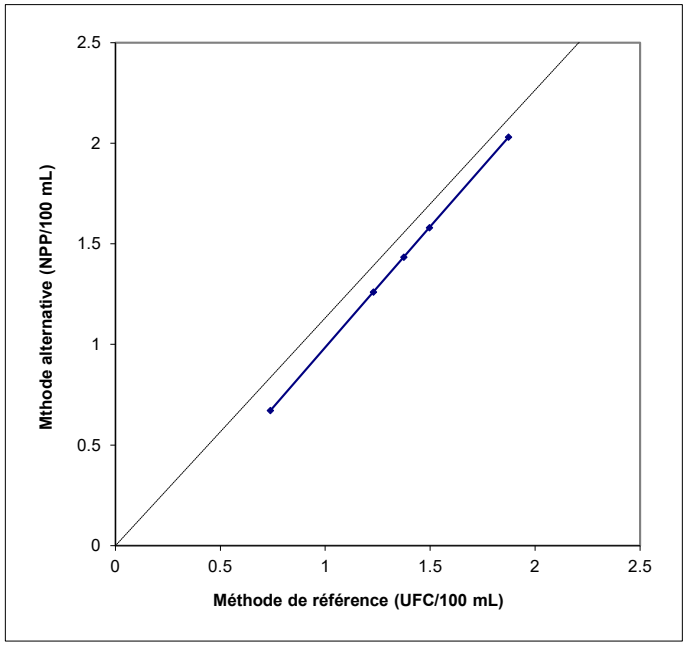
R = 0.635
rob.R = 0.827
Res.SEM = 0.111
Res.SD = 0.158

Sx = 0.392
Sy = 0.470

r = 0.981
b = 1.199
a = -0.214

Est y	Déviation
0.672	-0.070
1.261	0.118
1.434	-0.036
1.580	0.087
2.031	-0.099

Sb = 0.142 **p(t;b=1) =** 0.198 **t (b) =** 1.402
Sa = 0.197 **p(t;a=0) =** 0.309 **t (a) =** 6.160



Linéarité

F = 80.514 **p(F) =** 0.000
rob.F = 12.381 **rob.p(F) =** 0.009

Linéarité - coliformes - Données brutes

Niveau	Méthode de référence				Méthode alternative			
	Rép.1	Rép.2	M	SD	Rép.1	Rép.2	M	SD
1	5	9	7.0	2.828	2	1	1.5	0.707
2	18	10	14.0	5.657	17.8	13.7	15.8	2.899
3	17	21	19.0	2.828	22.2	16.4	19.3	4.101
4	60	60	60.0	0.000	50.4	56	53.2	3.960

q = 4
n = 2
N = qn = 8

Mx = 25.000
MEDx = 16.500
SDbx = 23.847

MEDwx = 2.828
SDwx = 2.449
rob. SDwx = 4.193

My = 22.438
MEDy = 17.525
SDby = 21.903

MEDwy = 3.429
SDwy = 2.275
rob. SDwy = 5.085

Choix méthode
GMFR

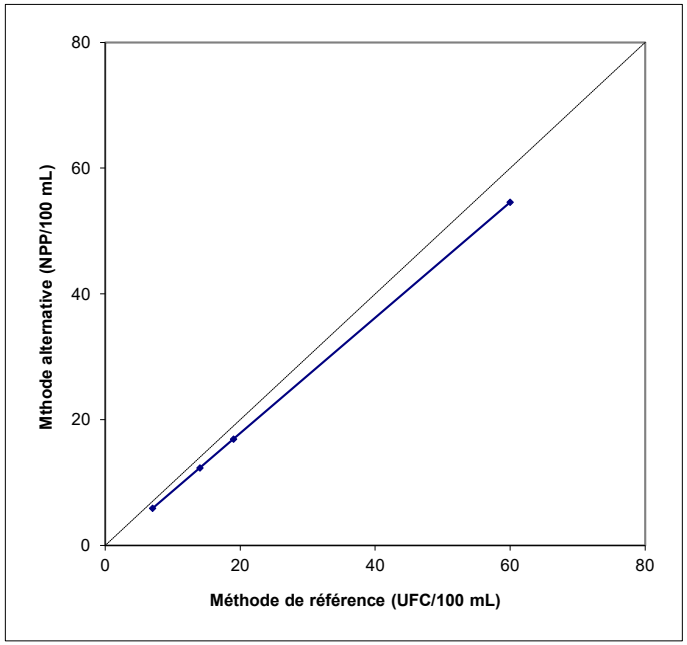
R = 0.929
rob.R = 1.213
Res.SEM = 4.395
Res.SD = 6.215

Sx = 22.233
Sy = 20.424

Est y	Déviation
5.902	-4.402
12.332	3.418
16.926	2.374
54.590	-1.390

r = 0.987
b = 0.919
a = -0.529

Sb = 0.114 **p(t;b=1) =** 0.503 **t (b) =** 0.713
Sa = 3.601 **p(t;a=0) =** 0.888 **t (a) =** 0.425



Linéarité

F = 20.390 **p(F) =** 0.008
rob.F = 2.482 **rob.p(F) =** 0.199

Linéarité - coliformes - Données log

Niveau	Méthode de référence Rép.1	Rép.2	M	SD	Méthode alternative Rép.1	Rép.2	M	SD
1	0.699	0.954	0.8	0.181	0.301	0.000	0.2	0.213
2	1.255	1.000	1.1	0.181	1.250	1.137	1.2	0.080
3	1.230	1.322	1.3	0.065	1.346	1.215	1.3	0.093
4	1.778	1.778	1.8	0.000	1.702	1.748	1.7	0.032

q = 4
n = 2
N = qn = 8

Mx = 1.252
MEDx = 1.202
SDbx = 0.397

MEDwx = 0.123
SDwx = 0.093
rob. SDwx = 0.182

My = 1.087
MEDy = 1.237
SDby = 0.667

MEDwy = 0.087
SDwy = 0.088
rob. SDwy = 0.129

Choix méthode
GMFR

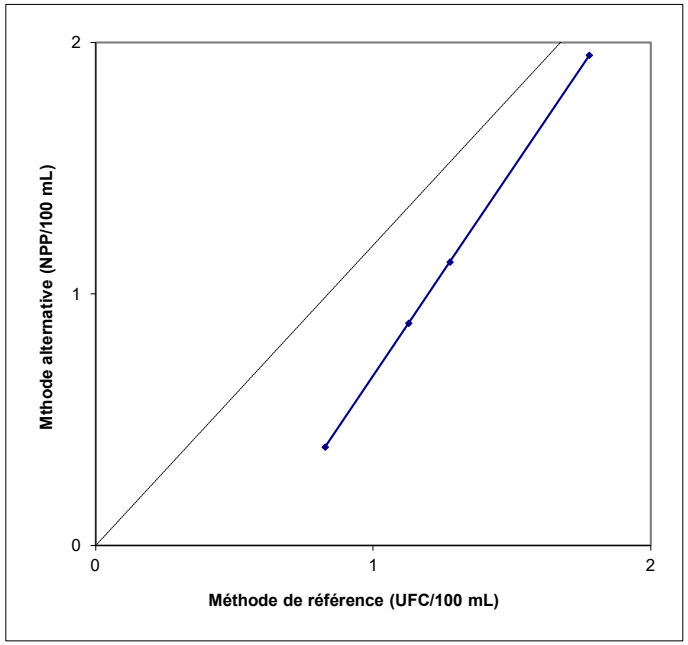
R = 0.941
rob.R = 0.707
Res.SEM = 0.337
Res.SD = 0.477

Sx = 0.381
Sy = 0.624

Est y	Déviation
0.391	-0.240
0.884	0.310
1.127	0.154
1.949	-0.224

r = 0.913
b = 1.638
a = -0.963

Sb = 0.511 **p(t;b=1) =** 0.258 **t (b) =** 1.249
Sa = 0.661 **p(t;a=0) =** 0.195 **t (a) =** 2.969



Linéarité

F = 86.748 **p(F) =** 0.001
rob.F = 39.275 **rob.p(F) =** 0.002

Appendix E

LOD-LOQ results

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

Souche : *Citrobacter koseri*

taux visé (UFC/ 100mL)	taux réel (UFC/ 100mL) (a)	réplicats					
		1	2	3	4	5	6
		Détection coliforme (UFC/ 100mL)	Détection coliforme (UFC/ 100mL)	Détection coliforme (UFC/ 100mL)	Détection coliforme (UFC/ 100mL)	Détection coliforme (UFC/ 100mL)	Détection coliforme (UFC/ 100mL)
0	0	0	0	0	0	0	0
0.25	0.13	0	0	0	0	0	0
0.5	0.4	0	1	0	1	0	1
1	0.97	1	1	2	2	1	1

Souche: *Escherichia coli*

taux visé (UFC/ 100mL)	taux réel (UFC/ 100mL) (a)	replicats					
		1	2	3	4	5	6
		Détection E.coli (UFC/ 100mL)	Détection E.coli (UFC/ 100mL)	Détection E.coli (UFC/ 100mL)	Détection E.coli (UFC/ 100mL)	Détection E.coli (UFC/ 100mL)	Détection E.coli (UFC/ 100mL)
0	0	0	0	0	0	0	0
0.25	0.13	0	0	0	0	0	0
0.5	0.4	0	1	0	1	0	0
1	0.97	1	1	2	2	1	1

(a): taux calculé à partir de 30 dénombrements

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

Escherichia coli

Niveau (UFC/100mL)	Nombre d'échantillons positifs	Ecart-type (So)	Biais (Xo)
0	0	0	0
0.25	0	0	0
0.5	2	0.516	0
1	6	0.516	1

	Formules	Valeur obtenue
Niveau critique (LC)	1,65 So +Xo	0.85
Limite de détection (LOD)	3,3 So+Xo	1.70
Limite de quantification (LOQ)	10 So + Xo	5.16

Citrobacter koseri

Niveau (UFC/100mL)	Nombre d'échantillons positifs	Ecart-type (So)	Biais (Xo)
0	0	0	0
0.25	0	0	0
0.5	3	0.548	0.5
1	6	0.516	1

	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	6.16

Appendix F

Selectivity results

Selectivity

Target strains: *Escherichia coli*

	Code	Strain	origin	Target level (CFU/100mL)	Quanti-tray®			NF EN ISO 9308-1		
					Repetitions	Results		Repetition	Results	Identification
						Coliform detection	E.coli detection			
1	ESC.1.1	*Escherichia coli*	CIP 54127	100	1	+	+			
					2	+	+			
2	ESC.1.111	*Escherichia coli*	Eau de fontaine	40	1	+	+			
					2	+	+			
3	ESC.1.112	*Escherichia coli*	Effluent secondaire	38	1	+	+			
					2	+	+			
4	ESC.1.113	*Escherichia coli*	Eau de puits	38	1	+	+			
					2	+	+			
5	ESC.1.114	*Escherichia coli*	Eau de puits	60	1	+	+			
					2	+	+			
6	ESC.1.115	*Escherichia coli*	Eau de puits	34	1	+	+			
					2	+	+			
7	ESC.1.116	*Escherichia coli*	Eau de puits	48	1	+	+			
					2	+	+			
8	ESC.1.117	*Escherichia coli*	Eau de puits	30	1	+	+			
					2	+	+			
9	ESC.1.119	*Escherichia coli*	Eau de distribution :	70	1	+	+			
					2	+	+			
10	ESC.1.120	*Escherichia coli*	English, III-80BS	33	1	+	+			
					2	+	+			
11	ESC.1.121	*Escherichia coli*	EPA QC, 031591	40	1	+	+			
					2	+	+			
12	ESC.1.122	*Escherichia coli*	EPA QC, 082688	35	1	+	+			
					2	+	+			
13	ESC.1.123	*Escherichia coli*	ERA, 4921:40	36	1	+	+			
					2	+	+			
14	ESC.1.124	*Escherichia coli*	4166:80 Thames	40	1	+	+			
					2	+	+			
15	ESC.1.3	*Escherichia coli*	industrie laitière	58	1	+	+			
					2	+	+			
16	ESC.1.31	*Escherichia coli*	Noix de St-Jacques	100	1	+	+			
					2	+	+			
17	ESC.1.37	*Escherichia coli*	Déchets de pâte recyclés	32	1	+	+			
					2	+	+			
18	ESC.1.39	*Escherichia coli*	Crevette crue décortiquée	44	1	+	+			
					2	+	+			
19	ESC.1.4	*Escherichia coli*	ATCC 8739	30	1	+	-	1	No colony	*Escherichia coli 99.9%*
					2	+	-			
20	ESC.1.41	*Escherichia coli*	Pâte blanche sortie	80	1	+	+			
					2	+	+			

Selectivity

Target strain: Coliforms

	Code	Strain	origin	Target level (CFU/100mL)	Quanti-tray®			NF EN ISO 9308-1		
					Repetitions	Results: Coliform detection	Results: E.coli detection	Repetition	Results	Identification
1	BUT.1.1	*Buttiauxella agrestis*	Eau de puits	55	1	+	-			
					2	+	-			
2	CIT.1.2	*Citrobacter freundii*	ATCC 8090	33	1	+	-			
					2	+	-			
3	CIT.1.4	*Citrobacter freundii*	Eau de puits	100	1	+	-			
					2	+	-			
4	CIT.1.5	*Citrobacter freundii*	Eau de puits	37	1	+	-			
					2	+	-			
5	CIT.2.1	*Citrobacter koseri*	CIP 72.11	43	1	+	-			
					2	+	-			
6	CIT.2.2	*Citrobacter koseri (C. diversus)*	CIP 82.87 T	33	1	+	-			
					2	+	-			
7	ENTB.1.1	*Enterobacter aerogenes*	industrie laitière	61	1	+	-			
					2	+	-			
8	ENTB.1.2	*Enterobacter aerogenes*	CIP 60.86 T	35	1	+	-			
					2	+	-			
9	ENTB.1.3	*Enterobacter aerogenes*	4166:80 Thames	33	1	+	-			
					2	+	-			
10	ENTB.2.1	*Enterobacter cloacae*	Eaux usagées	73	1	+	-			
					2	+	-			
11	ENTB.3.2	*Enterobacter sakazakii*	CIP 57.33	37.8	1	+	-			
					2	+	-			
12	ENTB.3.3	*Enterobacter sakazakii*	CIP 103581	60	1	+	-			
					2	+	-			
13	ESC.2.1	Escherichia hermanii	CIP 103176	70	1	+	-			
					2	+	-			
14	ESC.3.1	*Escherichia vulneris*	CIP 103177T	68	1	+	-			
					2	+	-			
15	EWI.1.1	*Ewingella americana*	JMM013, env. Sample,	63	1	+	-			
					2	+	-			
16	HAF.1.2	*Hafnia alvei*	CNRZ 713	42	1	+	-			
					2	+	-			
17	KLE.1.1	*Klebsiella oxytoca*	salade soja	56	1	+	-			
					2	+	-			
18	KLE.1.2	*Klebsiella oxytoca*	Effluent secondaire	35	1	+	-			
					2	+	-			
19	KLE.3.1	*Klebsiella planticola*	Saucisse	35	1	+	-			
					2	+	-			
20	KLU.1.1	*Kluyvera sp.*	EPA 0115-90487	62	1	+	-			
					2	+	-			
21	KLU.2.1	*Kluyvera intermedia*	CIP 79.27	35	1	-	-	1	No characteristic colonies	*Kluyvera intermedia 100%*
					2	-	-			
22	KLE.2.2	*Klebsiella pneumoniae*	CIP 82.91	63	1	-	-	1	No colony	*Klebsiella pneumoniae 94,7%*
					2	-	-			
23	LEC.1.1	*Leclercia adecarboxylata*	JMM001, env. Sample,	75	1	+	-			
					2	+	-			
24	PAN.1.2	*Pantoea agglomerans*	CIP 57.51T	37	1	-	-	1	No colony	*Pantoea spp 99,9%*
					2	-	-			
25	PAN.2.1	*Pantoea spp*	Robinet compteur	96	1	-	-	1	No colony	*Pantoea spp 99.7%*
					2	-	-			
26	RAH.1.1	*Rahnella aquatilis*	CIP 78.65	48	1	-	-	1	No characteristic colonies	*Rahnella aquatilis 100%*
					2	-	-			
27	SER.1.1	*Serratia ficaria*	CIP 79.23	31	1	+	-			
					2	+	-			
28	SER.2.1	*Serratia fonticola*	CIP 103580	32	1	+	-			
					2	+	-			
29	SER.3.1	*Serratia marcescens*	4166:80 Thames	65	1	+	-			
					2	+	-			
30	SHI.2.1	*Shigella sonnei*	ATCC 9290	57	1	+	-			
					2	+	-			

Selectivity

Non-target strains

	Code	Strain	origin	Target level (CFU/100mL)	Quanti-tray®			NF EN ISO 9308-1		
					Repetitions	Results		Repetition	Results	Identification
						Coliform detection	E.coli detection			
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*	Eau de fontaine	4E+04	1	0	0			
					2	0	0			
6	PSE.1.5	*Pseudomonas aeruginosa*	Eau de fontaine	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	Env. atelier (alim. humaine)	7E+04	1	0	0			
					2	0	0			
9	STA.1.5	*Staphylococcus aureus*	Eaux superficielles	7E+04	1	0	0			
					2	0	0			
10	XAN.1.1	*Xanthomonas campestris*	Evaporateur de climatisation automobile	7E+04	1	0	0			
					2	0	0			
11	AER.1.1	*Aeromonas hydrophyla*	Eau de puits	2E+04	1	0	0			
					2	0	0			
12	AER.1.2	*Aeromonas hydrophyla/sobria1*	Eau de puits	3E+04	1	0	0			
					2	0	0			
13	MIC.2.1	*Micrococcus spp*	boîte de contact in	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*	industrie laitière	6E+04	1	0	0			
					2	0	0			
16	SAL.1.99	*Salmonella enterica* Ohio	environnement atelier de production	3E+04	1	0	0			
					2	0	0			
17	STA.2.2	*Staphylococcus epidermidis 2*	boîte de contact in	4E+04	1	0	0			
					2	0	0			
18	PSE.1.6	*Pseudomonas aeruginosa*	Eau de fontaine	3E+04	1	0	0			
					2	0	0			
19	STA.4.1	*Staphylococcus piscifermentans*	Evaporateur de climatisation automobile	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*	boîte de contact in	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 G

Results of the interlaboratory study

Raw results
Level 0

Laboratory	Reference method - samples			
	3		4	
	Reading 48 h	Results	Reading 48 h	Results
B	0	<1	0	<1
C	0	<1	0	<1
D	0	<1	0	<1
E	0	<1	0	<1
F	0	<1	0	<1
H	0	<1	0	<1
I	0	<1	0	<1
J	0	<1	0	<1
L	0	<1	0	<1
K	0	<1	0	<1
M	0	<1	0	<1
Expert	0	<1	0	<1

Results CFU/100 mL

Laboratory	Alternative method - samples							
	3				4			
	Number of positive wells	Results			Number of positive wells	Results		
		LCB 95%	NPP	LCH 95%		LCB 95%	NPP	LCH 95%
B	0	0.0	<1	3.7	0	0.0	<1	3.7
C	0	0.0	<1	3.7	0	0.0	<1	3.7
D	0	0.0	<1	3.7	0	0.0	<1	3.7
E	0	0.0	<1	3.7	0	0.0	<1	3.7
F	0	0.0	<1	3.7	0	0.0	<1	3.7
H	0	0.0	<1	3.7	0	0.0	<1	3.7
I	0	0.0	<1	3.7	0	0.0	<1	3.7
J	0	0.0	<1	3.7	0	0.0	<1	3.7
L	0	0.0	<1	3.7	0	0.0	<1	3.7
K	0	0.0	<1	3.7	0	0.0	<1	3.7
M	0	0.0	<1	3.7	0	0.0	<1	3.7
Expert	0	0.0	<1	3.7	0	0.0	<1	3.7

Results MPN/100 mL

MPN: Most Probable Number
LCB 95%: limite de confiance minimum à 95%
LCH 95%: limite de confiance maximum à 95%

Raw results
Level 1

	Reference method - samples			
	1		8	
Laboratory	Reading 48 h	Results	Reading 48 h	Results
B	5	5	10	10
C	19	19	10	10
D	10	10	8	8
E	14	14	12	12
F	14	14	11	11
H	9	9	6	6
I	9	9	13	13
J	4	4	11	11
K	9	9	12	12
L	13	13	9	9
M	15	15	8	8
Expert	5	5	12	12

Results CFU/100 mL

	Alternative method - samples							
	1				8			
	Number of positive wells	Results			Number of positive wells	Results		
Laboratory		LCB 95%	NPP	LCH 95%		LCB 95%	NPP	LCH 95%
B	8	4.5	8.7	17.1	9	5.3	9.9	18.8
C	15	10.8	17.8	29.4	12	7.9	13.7	23.9
D	12	7.9	13.7	23.9	10	6.1	11.1	20.5
E	9	5.3	9.9	18.8	12	7.9	13.7	23.9
F	13	8.8	15.0	25.7	10	6.1	11.1	20.5
H	7	3.7	7.5	15.5	3	1.1	3.1	9.0
I	11	7.0	12.4	22.1	8	4.5	8.7	17.1
J	13	8.8	15.0	25.7	7	3.7	7.5	15.5
K	15	10.8	17.8	29.4	10	6.1	11.1	20.5
L	8	4.5	8.7	17.1	7	3.7	7.5	15.5
M	8	4.5	8.7	17.1	11	7.0	12.4	22.1
Expert	10	6.1	11.1	20.5	7	3.7	7.5	15.5

Results MPN/100 mL

MPN: Most Probable Number
LCB 95%: limite de confiance minimum à 95%
LCH 95%: limite de confiance maximum à 95%

Raw results
Level 2

Laboratory	Reference method - samples			
	2		5	
	Reading 48 h	Results	Reading 48 h	Results
B	39	39	29	29
C	72	72	52	52
D	65	65	64	64
E	67	67	66	66
F	52	52	56	56
H	30	30	50	50
I	55	55	51	51
J	33	33	44	44
K	53	53	54	54
L	46	46	65	65
M	45	45	52	52
Expert	69	69	60	60

Results CFU/100 mL

Laboratory	Alternative method - samples							
	2				5			
	Number of positive wells	Results			Number of positive wells	Results		
		LCB 95%	NPP	LCH 95%		LCB 95%	NPP	LCH 95%
B	33	37.5	53.1	76.2	26	24.7	36.4	53.9
C	36	44.6	62.4	88.8	33	37.5	53.1	76.2
D	43	68.2	94.5	135.4	40	56.4	78.2	111.2
E	31	33.4	47.8	69.0	40	56.4	78.2	111.2
F	25	23.3	34.4	51.2	32	35.4	50.4	72.5
H	36	44.6	62.4	88.8	42	63.9	88.5	126.2
I	34	39.7	56.0	80.1	35	42.0	59.1	84.4
J	42	63.9	88.5	126.2	32	35.4	50.4	72.5
K	37	47.2	65.9	93.7	36	44.6	62.4	88.8
L	35	42.0	59.1	84.4	39	53.1	73.8	104.8
M	35	42.0	59.1	84.4	31	33.4	47.8	69.0
Expert	39	53.1	73.8	104.8	41	59.9	83.1	118.3

Results MPN/100 mL

MPN: Most Probable Number
LCB 95%: limite de confiance minimum à 95%
LCH 95%: limite de confiance maximum à 95%

Raw results
Level 3

Laboratory	Reference method - samples			
	6		7	
	Reading 48 h	Results	Reading 48 h	Results
B	105	110	112	110
C	139	140	142	140
D	105	110	124	120
E	146	150	124	120
F	80	80	78	78
H	70	70	90	90
I	131	130	136	140
J	89	89	90	90
K	130	130	133	130
L	130	130	112	110
M	90	90	74	74
Expert	126	130	104	100

Results CFU/100 mL

Laboratory	Alternative method - samples							
	6				7			
	Number of positive wells	Results			Number of positive wells	Results		
		LCB 95%	NPP	LCH 95%		LCB 95%	NPP	LCH 95%
B	51	146.1	202.0	infinite	47	92.7	129.8	195.0
C	48	102.3	144.5	224.1	46	85.0	118.4	174.5
D	50	135.8	200.5	387.6	48	102.3	144.5	224.1
E	50	135.8	200.5	387.6	50	135.8	200.5	387.6
F	32	35.4	50.4	72.5	32	35.4	50.4	72.5
H	47	92.7	129.8	195.0	47	92.7	129.8	195.0
I	48	102.3	144.5	224.1	47	92.7	129.8	195.0
J	47	92.7	129.8	195.0	50	135.8	200.5	387.6
K	46	85.0	118.4	174.5	51	146.1	202.0	infinite
L	51	146.1	202.0	infinite	47	92.7	129.8	195.0
M	46	85.0	118.4	174.5	49	115.2	165.2	272.2
Expert	50	135.8	200.5	387.6	49	115.2	165.2	272.2

Results MPN/100 mL

MPN: Most Probable Number
LCB 95%: limite de confiance minimum à 95%
LCH 95%: limite de confiance maximum à 95%
*: une valeur de NPP de 202 a été fixée pour les résultats >200,5 UFC/100 mL