RAPID'E.coli 2 for water testing for the enumeration of Escherichia coli and coliforms in drinking water for human consumption

Summary report

March 2023

Quantitative method

Attestation n° BRD 07/20-03/11

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Preamble

***Studied method**:*
Rapid'*E.coli 2*

***Validation standard**:*
Protocol for the validation of alternative (proprietary) methods against a reference method
Revision 2 – May 2013

***Reference method**:*
NF EN ISO 9308-1:2000 standard "Water quality – Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method".

Scope:
Waters for human consumption, with three types of matrices:

- Water from wells, springs and boreholes
- Bottled water : spring water, mineral and other water
- Mains and drinking fountain water

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

1. Definitions

Comparative study:

The aim of the study is to measure the performance of the alternative method and, if necessary, the reference method for the following parameters:

- Relative accuracy,
- Linearity,
- Detection and quantification limits,
- Inclusivity and exclusivity.

Relative Detection Level (RLOD) study:

The detection level (LOD) is defined as the minimum concentration of organisms that gives evidence of growth in a liquid medium, with a probability of P = 0.95 when they are inoculated in a defined culture medium and incubated under defined conditions. The relative detection level (RLOD) is defined as the ratio of the LOD of the alternative method to that of the reference method.

Inclusivity and exclusivity study:

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. Exclusivity is the absence of interference by an appropriate range of non-target strains of the alternative method.

Relative accuracy:

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

Practicability:

Practicability is the ability to use a method, defined by a set of criteria instructed by the user's name.

2. Introduction

The report present the renewal study of the RAPID'*E.coli 2* method for water testing for the enumeration of *Escherichia coli* and coliforms

The RAPID'*E.coli* 2 for water method has been validated in March 2011 with certificate number BRD 07/20 – 03/11 for the detection and enumeration of *E. coli* and coliforms bacteria in waters for human consumption with low levels of suspended solids, treated or not, according to the validation protocol for an alternative commercial method as compared with a reference method.
The method was compared to the method described in the standard NF EN ISO 9308-1:2000.

The method has been renewed in 2015 and 2019. All raw results were reinterpreted according to the version in force of the validation protocol, dated May 2013.

In 2023, Bio-Rad plans to renew the certification with no change in method or extension. Due to the uptade of the NF EN ISO 8199 in 2018, raw results obtained for the relative accuracy have been reinterpreded by excluding the results higher than 80 colonies.

3. Modifications changes since the previous validation

3.1. History of validation

Table 1: History of validation

Date	Expert lab.	Comments
March 2011	**I.P.L**	**Initial study**
		Validation on ○ Water from wells, springs and boreholes ○ Bottled water: spring water, mineral and other water ○ Mains and drinking fountain water
June 2015	**I.S.H.A**	**Renewal**
		New validation following AFNOR validation protocol V2.0 (10/2013) with data from I.P.L
March 2019	**AdGène**	**Renewal**
		Reconduction with a minor modification The conditionnement of supplement is now possible in 6 vials, for 200 ml bottles
March 2023	**Upscience**	**Renewal**
		Reinterpretation of the relative accuracy results due to the uptade of the NF EN ISO 8199:2018

3.2. Modifications or changes

The protocol of validation (AFNOR Validation Revision 2.0 from 10/2013) is the same as during the previous renewal.

Commercial analysis method modification:
No modification

No additional test was done.

3.3. Reclamation

No complaint reached AFNOR since the last renewal validation.

4. Method protocols

4.1. Reference method

The reference method corresponds to NF EN ISO 9308-1:2000 standard “Water quality –detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method”. (Appendix 1)
The standard test is based on membrane filtration, subsequent culture on a differential agar medium and calculation of the number of target organisms in the sample.

4.2. Alternative method

4.2.1. Principe of alternative method

RAPID’*E.coli* 2 agar for water testing allows the direct and simultaneous enumeration (without confirmation) of *Escherichia coli* and the total coliforms, by membrane filtration method.

The principle of the complete medium (supplemented RAPID’*E.coli* 2) relies on the simultaneous detection of two enzymatic activities: β-D-Galactosidase (GAL) and β-D-Glucuronidase (GLUC) by two chromogenic substrates:

- Cleavage of the GAL specific substrate leads to the formation of a precipitate giving a green coloration of the positive colonies for this enzyme (coliforms),
- Cleavage of the GLUC specific substrate leads to the formation of a precipitate giving a pink coloration of the positive colonies for this enzyme (*E. coli*).

Coliforms (GAL+/GLUC-) form green colonies, and *E. coli* (GAL+/GLUC+) form blue to violet colonies due to the superposition of both colorations.

4.2.2. Protocol of alternative method

The protocol of the alternative method is the following (Appendix 2):

- Filtration of 100 mL of drinking tap water or 250 mL of bottled water on sterile filter membrane (Ø 47 mm, 0.45 µm Millipore HAWG 047 Type HA),
- Deposit of the membrane on the surface of the agar RAPID’*E. coli* 2 medium (square side upper most),
- Incubation of dishes at 36± 2°C for 21 ± 3 h,
- Reading and interpretation: After incubation, read the dishes membranes upside and proceed to count characteristic colonies:
  - green colonies = coliforms (GAL+) other than *E. coli*,
  - dark blue to violet/grey-blue colonies with possible violet halo surrounding the typical colonies = E. coli (GAL+/GLUC+)

Note: Total coliforms are enumerated by adding together blue and dark blue to violet colonies and green colonies.
Within the framework of the study, the minimum time of incubation of 18 hours was followed.

5. Summary of results obtained during initial validation and renewal

5.1. Comparative study

5.1.1. Relative accuracy

The accuracy is the closeness of agreement between a test result and the accepted reference value.
The relative accuracy is the level of connection between the answer obtained with the reference method and the answer obtained with the alternative method on the same samples. The value of the accepted reference is chosen as being the value obtained by the reference method.

- **Number and nature of samples**

In 2011, according to the reference document in force, two categories of water (naturally contaminated or spiked samples with *E. coli* or coliform bacteria) have been analyzed in duplicate according to the two methods:

- Reference method: NF EN ISO 9308-1 :2000,
- Alternative method: RAPID'*E.coli* 2 for Water method.

The categories and the types of samples studied were the following:

- Treated waters with low levels of suspended solids (mains water),
- Not treated waters with low levels of suspended solids (mineral water, source water, well water/groundwater).

In total, 66 samples were analyzed so as to obtain at least 20 usable results per method in each water type and per tested parameter.

According to the updated validation repository, these two categories merged in only one: waters for human consumption. The samples are distributed as following:

Table 2: Samples distribution for coliform bacteria and Escherichia coli

Parameter	Analyzed samples	Exploited samples
Total coliforms	66	40
Escherichia coli	66	34

The samples for which the results were uninterpretable, presented:

- Uninterpretable enumerations on the standard agar, due to an important interfering flora on 3 samples (B07, B09 and B10),
- An uninterpretable enumeration on the standard agar and <1 CFU/100 mL for a replicate of the alternative method on 1 sample (B08),
- Enumerations higher than 80 CFU/100 mL or 250 mL with both methods on 6 samples (B01, B34, B44, B45, B112, B113, B117, B118 and B151),
- Enumerations higher than 80 CFU/100 mL with the standard agar and uninterpretable with alternative method on 3 samples (B114, B115 and B119),
- Enumerations <1 CFU/100 mL with both methods on 5 samples (B35, B122, B123, B126 and B132).
- Enumerations inferior to 1 CFU/100 mL or 250 mL with the reference method on 5 samples (B11, B12, B13, B14 and B43),
- Interpretable enumerations with the reference method and <1 CFU/100 mL for one replicate of the alternative method on 1 sample (B161).
- Results inferior to 4 CFU/100 or 250 mL (presence of the bacteria but not quantifiable) by any method for the other non-exploited results.

- **Artificial contamination of the samples**

In a mandatory point of view, according to the 2009/54/CE Directive on June 18th 2009, bottled waters have to be free of *E. coli* and coliforms in a 250 mL water sample (threshold <1 UFC in 250 mL).
In the context of the validation study, artificial spikings were thus necessary to obtain a sufficient number of positive water samples.
Artificial contaminations were achieved by using:

- Stressed contaminating suspensions of *E. coli* or coliform bacteria, whose stress treatment and efficiency were determined,
- Naturally contaminated waters (contamination by mix).

For coliforms parameter, 48 out of 50 results were processed with artificial spikings, represented a percentage of artificial spiking of 96%.
For *E. coli* parameter, all results were processed with artificial spikings (100% of artificial spiking).

The detail of the artificial contaminations is in Appendix 3.

- **Raw data**

Each sample was analyzed in duplicate by the alternative method and the reference method.

Following the EN ISO 16140 standard, the values for each sample were plotted on a two-dimensional graph. The vertical axis (y) is used for the alternative method and the horizontal axis (x) for the reference method.

The data were then tested by a linear regression program in order to determine the intercept value (a) and the slope value (b).
The relative accuracy relationship is evaluated according to the model: y = bx + a.
For each of the two methods, robust repeatability standard deviations were calculated (sr(x) and Rob.sr(x) & sr(y) and Rob.sr(y)).
As a function of the ratio of these standard deviations, R = sr(y) / sr(x) and Rob.R = Rob.sr(y) / Rob.sr(x), the linear regression to be used for the interpretation is defined in the EN ISO 16140 standard.

Synthesis of results is presented in Appendix 4.

The following two-dimensional graphs represent the raw values in CFU/100 or 250 mL and in log CFU/100 or 250 mL, obtained for the samples analyzed (incubation time: 18 hours).

The straight line represented is the first bisector (y = x).

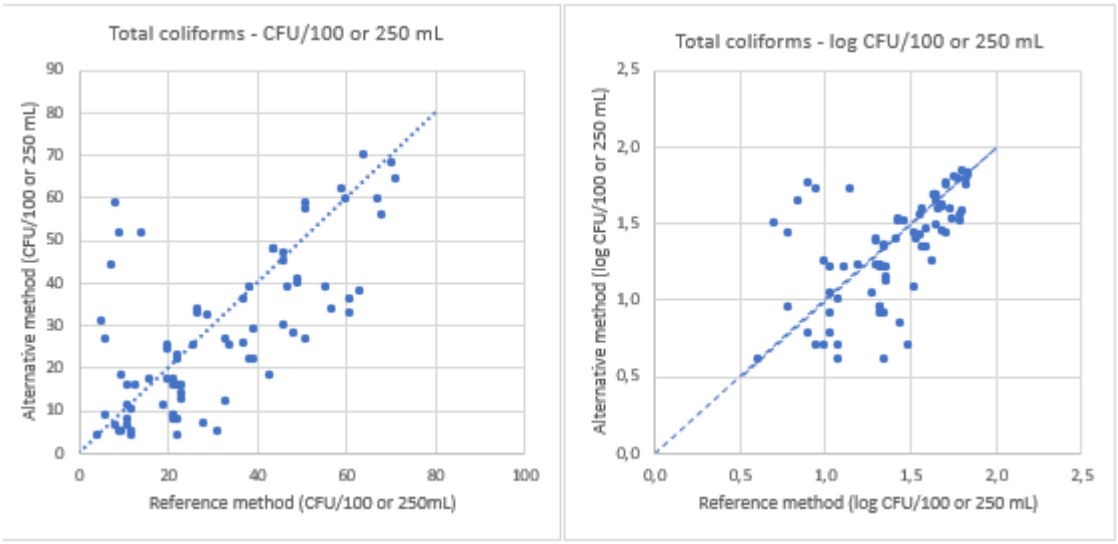


Figure 1: two-dimensional graphs for relative accuracy (total coliforms)

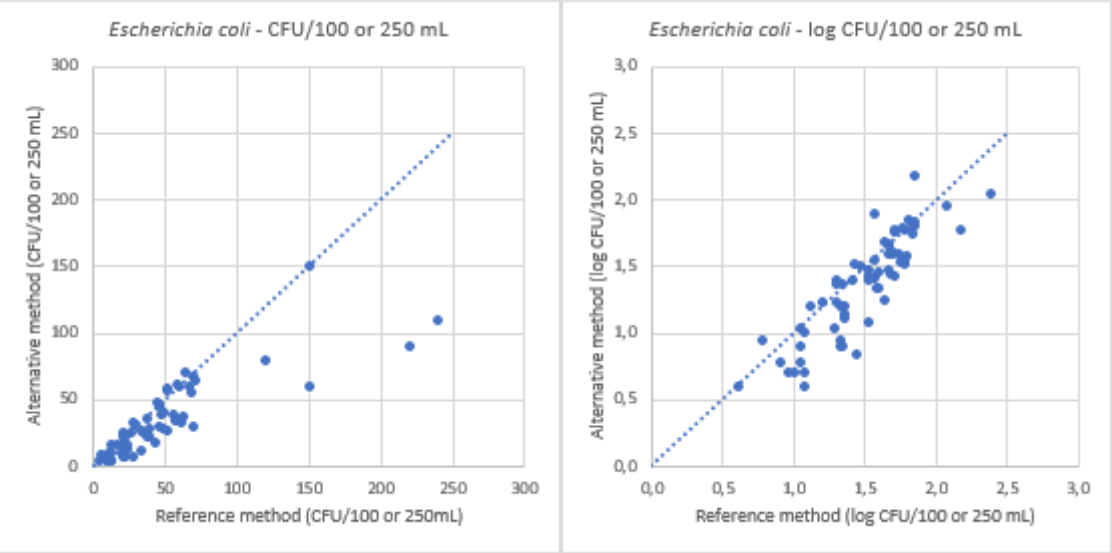


Figure 2: two-dimensional graphs for relative accuracy (Escherichia coli)

- **Statistical interpretation**

Interpretations were managed according to the EN ISO 16140 (2003) standard. 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 "a" is theoretically zero in this ideal model (Hypothesis [a=0]). We check that the value 0 is included in the confidence interval of the intercept constructed around the estimated a value. The test is two-tailed for a risk α = 0.05. If the inequality $t_{(\alpha/2\ ;\nu)} \leq t_{test} \leq t_{(\alpha/2\ ;\nu)}$ holds, we accept the hypothesis that a=0.

The slope "b" is theoretically equal to 1 in the ideal model (Hypothesis [b=1]). We check that the value of 1 is within the confidence interval of the steering coefficient constructed around the estimated b value. The test is two-tailed for a risk α = 0.05. If the inequality $t_{(\alpha/2\ ;\nu)} \leq t_{test} \leq t_{(\alpha/2\ ;\nu)}$ holds, we accept the hypothesis that b=1.

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 3: Coliforms and E.coli relative accuracy parameters per 100 or 250 ml of water (R: regression used)

Parameter	Rob.R	Regression used	a	t(a)	b	t(b)	Conclusion
Coliforms (CFU)	1,333	GMFR	-2,228	0,279	0,966	0,279	{a=0} accepted {b=1} accepted
Coliforms (log CFU)	1,490	GMFR	-0,211	0,918	1,03	0,641	{a=0} accepted {b=1} accepted
***E. coli* (CFU)**	1,331	GMFR	4,388	0,573	0,622	3,075	{a=0} accepted {b=1} rejected
***E. coli* (log CFU)**	1,785	GMFR	-0,370	0,668	1,164	0,450	{a=0} accepted {b=1} accepted

Other parameters were presented in the following tables:

- the bias between the two methods (alternative method –reference method)
- the standard deviation of repeatability robust
- the contamination area

They are presented in the tables below for the two parameters: coliform bacteria and *Escherichia coli*.

Table 4: Biais and repetability for both methods

Parameters	Bias (D)		Standard deviation of repeatability robust		Contamination area
	Mean	Median	Reference method	Alternative method	
Total coliforms UFC/ 100 or 250 mL	-3.3	-4.5	3.0	3.4	[4 ; 71]
Total coliforms Log UFC/ 100 or 250 mL	-0.067	-0.069	0.069	0.063	[0.602 ; 1.851]
E. coli UFC/ 100 or 250 mL	-12.8	-6.0	14.2	10.5	[4 ; 71]
Log UFC/ 100 or 250 mL	-0.125	-0.102	0.072	0.070	[0.602 ; 1.851]

The bias between the alternative method and the reference method is low and is respectively:

- -4.5CFU/100 or 250mL for coliform bacteria
- -0.069log CFU/100 or 250mL for coliform bacteria
- -6.0CFU/100 or 250mL for *E. coli*
- -0.102log CFU/100 or 250mL for *E. coli*

The equations of regression lines obtained are the following:

- coliform bacteria: Alt = 0,966 Ref – 2,228
  log(Alt) = 1,030 log(Ref) – 0,211
- *Escherichia coli*: Alt = 0,622 Ref + 4,388
  log(Alt) = 1,164 log(Ref) – 0,370

The standard deviations of repeatability between the alternative and reference methods are very close.

Statistical tests validate the accuracy of the alternative method by validating the hyptothesis that a is equal to 0 and b is equal to 1 for a risk α=0.05 for coliform bacteria. For *E. coli*, statistical tests validate the accuracy of the alternative method by validating the hyptothesis that a is equal to 0 and b is equal to 1 for a risk α=0.05 when the results are expressed in log UFC/100 or 250 mL.

Despite a slight negative bias corresponding to a slight underestimation of the results of the method compared to the reference method, the relative accuracy of the RAPID'*E. coli 2* Agar method is **satisfactory**.

5.1.2. 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.

- **Nature of the tests**

In 2011, two types of water were chosen in both categories of waters with a low level of suspended solids, in order to determine two levels of contamination by category, homogeneously distributed in the whole range of contaminations usually found in these waters for human consumption and including mandatory limits if existing and the maximum of the scope of application.
No statistical interpretation was realized from the data obtained. The following waters were contaminated by coliform bacteria:

Table 5: Matrix and strains for linearity

Water	Origin	Strain	Origin
Tap water	Laboratory, Lille	*E. coli*	Mains water, Bruille St Amand (59)
Mineral water	Mineral water	*E. coli*	Well water, Lille (59)

The contamination levels were distributed between 5 and 200 in 100 or 250 mL according to the type of water:

- Level 1: 5 to 10 bacteria/100 mL and bacteria/250 mL
- Level 2: 20 bacteria /100 mL and bacteria/250 mL
- Level 3: 200 bacteria /100 mL and bacteria/250 mL

For each category of water and each contamination level, the alternative and the reference methods were performed with two repetitions.
For the renewal study of 2015, the two kinds of water were kept to realize a statistical interpretation.

- **Raw data**

Raw results are presented in Appendix 5.
The values for each sample were plotted on a two-dimensional graph. The vertical axis (y) is used for the alternative method and the horizontal axis (x) for the reference method. The data were then tested by a linear regression program in order to determine the intercept value (a) and the slope value (b), like in the relative accuracy part.
The following graphs represent the raw values obtained for each water testing.

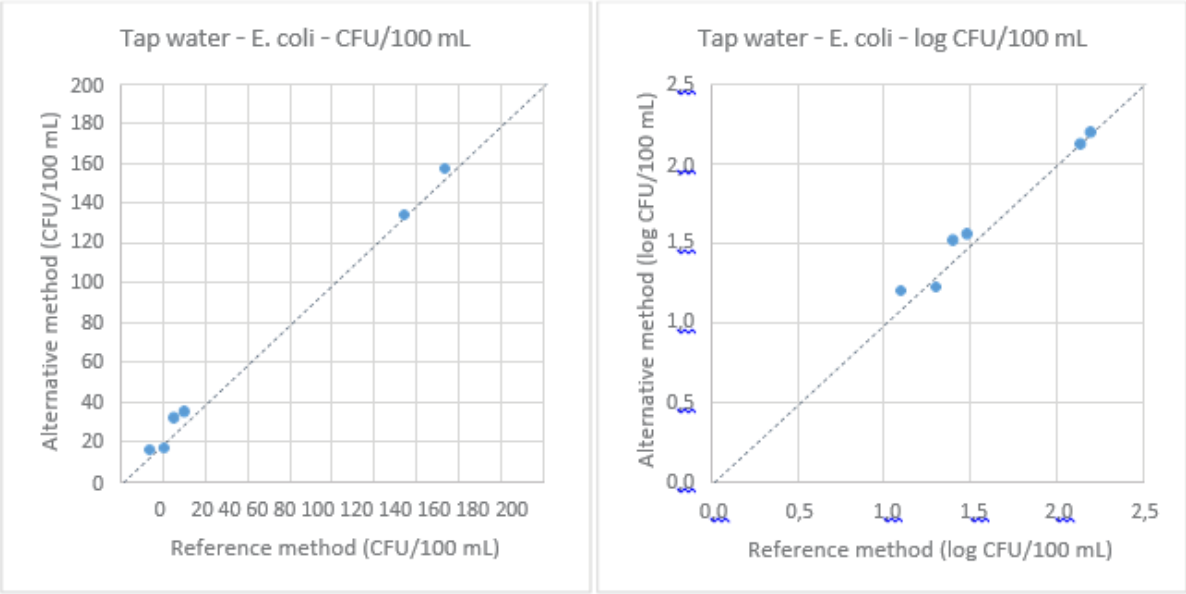


Figure 3: two-dimensional graphs for linearity (Escherichia coli in mineral water)

- **Statistical interpretation**

Statistical analysis is presented in Appendix 6.

Table 6: Linearity statistical data of the couple matrix-strains analyzes

Type	Rob. R	Re-gres-sion used	F criti-cal	Rob. F	P (Rob.F)	Correla-tion coefficient (r)	Regression line
Tap water – CFU/100 mL	0,429	OLS2	4,53	66,1	0,004	0,999	0,998 Ref + 4,313
Tap water – log CFU/100 mL	0,460	OLS2	4,53	69,9	0,004	0,995	0,935 log Ref + 0,158
Mineral water - CFU/250 mL	0,333	OLS2	4,53	256,1	0,001	1,000	1,054 Ref – 4,347
Mineral water – log CFU/250 mL	0,719	GMFR	4,53	30,4	0,012	0,994	1,030 log Ref – 0,083

The relationship between the 2 methods is not linear:

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

In conclusion, the relationships between the two methods are not linear for the two kinds of matrices tested. However, the correlation coefficient between the two sets of results and the equation for the regression line are satisfactory.
The linearity of the alternative method is satisfactory.

5.1.3. Detection and quantification limits

Detection and quantification limits were studied according to EN ISO 16140 standard on Technical Committee request.
Detection (LOD) and quantification (LOQ) limits were determined by analysis of pure culture of *E. coli* with alternative method.
Overall, the critical level (LC) and the detection limit (LOD) involved two types of statistical errors: α (to detect a difference which doesn't exist (false positive) and β (to not detect a true difference (false negative)).

The power 1-β is the probability to detect a value higher than LC.
The critical level is LC: $1{,}65.s_0$ (+ x_0 for the alternative method), with α= 5% (and 1-β= 50%).
The detection limit is LOD: $3{,}3.s_0$ (+ x_0 for the alternative method), with α= 5 % and 1-β= 95 %.
The determination (or quantification) limit is LOQ = $10.s_0$ (+ x_0 for the alternative method), with s_0 corresponding to the standard deviation of determinations and x_0 to the bias.
Five levels of inoculation were tested, with six replicates by level. Results are synthesized in tables below and raw results are in Appendix 7.

- **Results**

Results for tap water

Table 7: Detection results for the enumeration of E.coli in tap water

Level / 100 mL	Positive samples number	Standard deviation s_0	Bias x_0 (x_{0i} median)
0,00	0/6	/	/
0,18	0/6	0,00	0,00
0,22	1/6	0,41	0,00
0,50	4/6	1,38	1,50
1,44	6/6	1,05	2,50

From s_0 and x_0 values obtained for the first level, the critical limit (LC), the detection limit (LOD) and the quantification limit (LOQ) were determined:

Table 8: Results of LC, LOD and LOQ for the detection of E.coli in tap water

	Formula	Value obtained (CFU/100 mL)
LC	1,65 **s0 + x0**	4
LOD	3,3 **s0 + x0**	6
LOQ	10 **s0 + x0**	15

Results for mineral water

Table 9: Detection results for the enumeration of E.coli in mineral water

Level / 250 mL	Positive samples number	Standard deviation s_0	Bias x_0 (x_{0i} median)
0,00	0/6	/	/
0,05	2/6	0,52	0,00
0,52	2/6	0,52	0,00
0,71	4/6	0,75	1,00
1,03	6/6	1,21	2,50

From s0 and x0 values obtained for the first level, the critical limit (LC), the detection limit (LOD) and the quantification limit (LOQ) were determined:

Table 10: Results of LC, LOD and LOQ for the detection of E.coli in mineral water

	Formula	Value obtained (CFU/250 ml)
LC	1,65 **s0 + x0**	2
LOD	3,3 **s0 + x0**	3
LOQ	10 **s0 + x0**	9

The criticial limit obtained is 2 CFU/250mL with a detection limit of 3 CFU/250mL and a quantification limit of 9 CFU/250mL.

5.1.4. Inclusivity and exclusivity

Inclusivity and exclusivity of the alternative method were studied by analysis of 20 strains of *E. coli* and 23 strains of coliform bacteria other than *E. coli* (target microorganisms) and 32 strains known to cause interference or naturally present in test material (non-target microorganisms).

- **Protocol**

Protocol for inclusivity

Each strain of *E. coli* or coliform has been cultivated in TCS broth for 20 hours at 37°C.
Different dilutions were realized in tryptone-salt, to obtain between 10 and 100 cells in 100 mL before application of RAPID'*E.coli* 2 for Water method and reference method.

Note: When the alternative method gives false negative or doubtful results, the strain shall be tested once more with the reference method.

Protocol for exclusivity

The different strains were cultivated and diluted in TCS broth (20 h at 37°C), to obtain 10^3-10^5 cells in 100 mL before analysis according to RAPID'*E.coli* 2 for Water method.

Note: When the alternative method gives positive or doubtful results with non-target microorganisms, the test shall be repeated using the complete protocol of RAPID'*E.coli* 2 for Water method. The reference method shall be performed only once.

- **Results and interpretation**

Results are listed in Appendix 7.

The 20 *E. coli* strains tested were detected by the RAPID'*E.coli* 2 for Water method.
Among the 23 coliforms strains other than *E. coli*, one strain was not detected. It was a *Hafnia alvei* strain, isolated from food, which had already given similar results with non-supplemented RAPID'*E.coli* 2 in food microbiology (renewal of alternative method in 2008, reference document REC2 Coliforms-renewal 2008-11 v01).
This strain presented non characteristic white colonies (GAL-/GLUC-). On TTC-tergitol agar, colonies were characteristic (lactose +).
Two *Hafnia alvei* strains (isolated from parsley) presented characteristic colonies colored in pale turquoise.
An *Escherichia hermanii* strain presented pale blue colonies.

Out of the 32 non target strains tested, 3 strains were detected by the RAPID'*E.coli* 2 for Water method. The strains were the following:

- One *Erwinia* spp strain from food origin (green colonies GAL+/GLUC- on RAPID'*E.coli* 2 and non characteristic on TTC-tergitol),
- One *Salmonella enterica* subsp.*diarizonae* strain (characteristic violet colonies GAL+/GLUC+, and non characteristic on TTC-tergitol),
- One *Shigella sonnei* strain from food origin (dark blue colonies GAL+/GLUC+, non characteristic on TTC-tergitol).

The 2 *Salmonella* and *Shigella* strains gave similar results on non-supplemented RAPID'*E.coli* 2 (renewal of alternative method 2008 – food testing scope). The *Erwinia* strain presented grey-white colonies on non-supplemented RAPID'*E.coli* 2.

All the 29 other strains did not present any characteristic aspect on RAPID'*E.coli* 2 for Water Testing method, either there was no growth (18 strains), or they presented very small non characteristic colonies (11 strains).

In conclusion , the Inclusivity is then satisfactory and In exclusivity, cross-reactions were observed with a Salmonella strain and a Shigella sonnei strain owning β-D-Galactosidase and β-D-Glucuronidase activities (GAL+/GLUC+), and an Erwinia spp strain

5.1.5. Practicability

Practicability is assessed according to criteria which are defined by the AFNOR Technical Committee. The RAPID'*E.coli* 2 for water method is compared to the reference method NF EN ISO 9308-1 in terms of 13 criteria. They are informed below:

Criterion	Communication on the criterion
Packaging **Reagents volumes**	RAPID'*E.coli* 2 Agar Ø 55 mm × 20 Petri dishes (code 3563982) - 100 ml x 6 vials (code 3555299) - 200 ml x 6 vials (code 3555297) - 500 g (code 3564024) 6 vials of supplement x 1 box (code 12008041) RAPID'E.coli 2 kit for water testing (code 17005373) 6 vials x 200 ml RAPID'E.coli 2 medium 6 vials of supplement x 1 box (1 vial contains lyophilized in sufficient quantity for 200 ml of RAPID'E.coli 2 medium)
3.Storage conditions – Expiration date of unopened tests*)*	Ready-to-use plates and lyophilized must be kept at +2-8°C in a dark place. Expiration date and batch number are shown on each plate or vial.
4.Utilization procedure after first utilization	Re-hydrated vial must be kept 7 days at +2 - 8°C in a dark place or frozen 3 months at -20°C. Petri dishes prepared by the user must be kept 15 days at +2-8°C in a dark place.
5. Specific necessary Equipment and premises	Normal configuration of and common material of a laboratory of microbiology.
6. Ready for use reagents or to restore	Reconstitution of supplement RAPID'*E.coli* 2: addition of 2.2 mL of distilled water to the bottle under aseptic conditions, shaking until the lyophilisat is completely dissolved. Preparation of complete medium (RAPID'*E.coli* 2 + supplement): under aseptic conditions, addition of 2.0 mL of rehydrated (or unfrozen) RAPID'*E.coli* 2 supplement to 200 mL of sterile RAPID'*E.coli* 2 medium cooled and maintained at 47±2 °C. Note: 0.2 mL remains in the vial. Shake as to homogenize thoroughly. Pour approximatively 9 mL of the complete medium per sterile Petri dish (Ø 55 mm). Leave to solidify on a cool, level surface.
7. Duration of training for a non-initiated operator	For an operator trained in standard techniques of water microbiology,training in the technique requires less than 1 day.

Criterion				
8.Real time handling and technique flexibility in comparison with number of samples to analyze				
Steps	**Average time for a sample (min)**		**Average time for 30 samples (min)**	
	Reference	Alternative	Reference	Alternative
Preparation, homogeneisation, sampling **Filtration Inoculation** **Reading and confirmation** **Interpretation, and calculation**	1.0 1.0 0.5 1.0 1.0	1.0 1.0 0.5 0.5 0.5	20 30 10 25 30	20 30 10 13 12
Total per sample	**4.5**	**3.5**	**3.8**	**2.8**

These times correspond to negative and positive samples for which no confirmation is necessary for the alternative method (chromogenic medium).

The interest of the alternative method is to reduce the manipulations regarding the reference method (number of confirmations). With reference method, it's necessary to confirm all typical colonies (presumptive coliform bacteria or *E. coli*) or a representative number (at least 10) from Lactose TTC agar plate. The average time for the confirmation of a typical colony by reference method tests can be evaluated to approximately 5 minutes.

The interest of the alternative method also lies in the ease of agar readings (few flora associated) because RAPID'*E.coli* 2 for water method is a selective chromogenic medium.

Criterion		
9.Time to Result		
Step	**Time required (day) RAPID'E.coli 2 WT method**	**Time required (day) NF EN ISO 9308-1**
Sample preparation	D0	D0
Filtration	D0	D0
Media inoculation	D0	D0
Reading, interpretation and calculations	D1	D1 and D2
Obtaining negative results (if no typical colony)	D1	D2
Oxidase test	/	D2 to D3
Indole test	/	D2 to D3
Obtaining negative results (after negative confirmation if necessary)	D1	D2 to D3
Obtaining positive results (confirmation of typical colonies)	D1	D2 to D3

Criterion	Communication on the criterion
10. Type of qualification of the operator	Same as for the reference method.
11. Steps common to the reference method	/
12. Traceability of the analysis results	/
13. Maintenance by the laboratory	/

5.1.6. Conclusion of the comparative study

The renewal study for the AFNOR Certification validation of the method RAPID'*E.coli* 2 for water testing was performed according to the reference document "validation protocol for an alternative commercial method as compared to a reference method - revision 2 - May 2013" joined to the standard EN ISO 16140:2003.

The comparison of the RAPID'*E.coli* 2 for water method with the NF EN ISO 9308-1: 2000 standard allows to conclude that the alternative method gives accurate results compared to the standard method for the scope of validation (waters for human consumption) and whatever the tested parameter (coliform bacteria or *E. coli*):
the linearity of alternative method is satisfactory,the relative accuracy regarding the reference method is satisfactory even if a low but systematic bias is observed.
Repeatability values for all scope application are similar for reference method (0,15 log CFU/100 or 250 mL for coliforms and 0,14 for *E. coli*) and for alternative method (0,21 log CFU/100 or 250 mL for coliforms and 0,23 for *E. coli*).
The average bias between the two methods (alternative method-reference method) is negative but quite low (-0,05 log CFU/100 or 250 mL for coliforms and -0,13 log CFU/100 or 250 mL for *E. coli*).

Twenty strains of *E. coli* and 22 coliforms strains (target strains) were detected, except one strain of *Hafnia alvei* (foodborne origin).
In exclusivity, among 32 strains of non-target microorganisms, three cross reactions were observed with one strain of *Salmonella enterica* subsp.*diarizonae* (IIIb), one strain of *Shigella sonnei*, and one strain of *Erwinia* spp.

5.2. Interlaboratory study

The aim of the interlaboratory study was to determine the variability of the results obtained in different laboratories using identical samples and to compare these results with those obtained during the methods comparison study.

The interlaboratory study was conducted in 2011 according to the validation protocol applied to microbiological water analysis – revision 1 – May 2010.

5.2.1. Study organization

Sixteen (16) laboratories took part in the interlaboratory study.

Spring water has been inoculated by an *E. coli* strain, isolated from a non-bottled spring water.

Sixteen samples were prepared per laboratory (4 levels of contamination, two samples per inoculation level and for each method).

Interlaboratory study laboratories and the expert laboratory have carried out the analyses with the alternative and reference methods.

The analyses have been performed one day after sending the samples.According to shipping and temperature conditions, the results of 13 laboratories have been included in the statistical interpretations.

5.2.2. Control of experimental parameters

- **Strain stability during transport**

In order to evaluate the *E. coli* strain variability during transport, bacterial counts of inoculated water have been checked at different time, during storage at 4°C.
Results (CFU/100 mL) on Lactose TTC agar (TTC) and RAPID'*E.coli* 2 for water (REC2 supp), are reported in CFU/100 mL in the following table:

*Table 11: Stability of E.coli strain à 4°C (*average of 2 replicates, ** beginning of interlaboratory study)*

Level	D0		D+1**		D+2	
	TTC*	REC2supp*	TTC	REC2supp	TTC	REC2supp
Level 1	11	7	9	7	6	3
Level 2	26	23	16	9	15	11
Level 3	81	67	72	44	63	19

Contamination level decreases in the course of time, with a loss in the order of 0,2 to 0,4 unit log CFU/100 mL in prepared samples, according to the level of contamination, and used enumeration medium. The strain is not stable in the matrix between D0 and D+2.

- **Contamination levels obtained after artificial inoculation**

The four contamination levels are presented in the following table:

Table 12: Contamination levels of inoculated samples

Level	Sample	Targeted level (CFU/100 mL)	Real level (CFU/100 mL)
Level 0	7 / 8 / 9 / 10	0	0
Level 1	1 / 3 / 12 / 14	5 to 10	8
Level 2	2 / 5 / 11 / 16	30	24
Level 3	4 / 6 / 13 / 15	100	60

- **Problems of temperature recorded during transport, temperature on reception an reception times**

Measured temperatures on receipt are listed in following table:

Table 13: Temperatures at receipt

Laboratory code	Receipt temperatures (°C)		Comments
	Measured by the laboratory	Temperature probe record	
A	2,1	1,0	
B	4,8	3,8	
C	1,6	1,5	
D	3,0	1,0	
E	3,6	1,1	
F	/	/	Receipt at D+2 – analyses not realized
G	NC	5,0	
H	5,4	3,7	
I	2,0	1,6	
J	3,3	1,6	Receipt at D+2 – analyses realized
K	3,5	0,6	
L	6,5	0,1	
M	3,4	1,0	
N	2,7	1,5	
O	NC	2,0	
P	7,0	1,8	Receipt at D+2 – analyses realized

In conclusion according to receipt conditions, the results of 13 laboratories (exclusion of laboratories J, P and F which received the samples at D+2) have been included to the statistical interpretations.

5.2.3. Results

- **Expert laboratory**

Results obtained by the expert laboratory with NF EN ISO 9308-1 method and RAPID'*E.coli* 2 for water method are presented in the following table.

Table 14: Resultst of expert laboratory

Level	Reference method NF EN ISO 9308-1		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate1	Duplicate 2
Level 0	<1	<1	<1	**<1**
Level 1	10	9	5	**8**
Level 2	30	21	24	**22**
Level 3	91	71	62	**71**

Results according to standard NF EN ISO 9308-1 and alternative method were in agreement.

- **Results obtained by collaborative laboratories**

Results of the 15 laboratories which realized the analyses are presented in the tables below and in Appendix 8 and 9. Overall, enumeration in *E. coli* and total coliforms reported by laboratories are of the same range.

Note: Two laboratories (K and M) detected some green colonies on RAPID'*E.coli* 2 medium, which were identified as *E. coli*.

For level 0, all results for both methods were inferior to 1 CFU/100 mL. The results for the three other levels are presented in the table 15.

Table 15: Results of the interlaboratory study (CFU/100 ml, RM: reference method,AM: alternative method, a: error of the laboratory b: empty flask, numbers between brackets : number of green colonies in surface, violet under the membrane, identified as E.coli, included in the total number of typical colonies)

Labo-ratory	Level 1				Level 2				Level 3			
	RM		AM		RM		AM		RM		AM	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
A	7	13	1	9	24	21	13	14	64	62	40	48
B	3	9	10	1	17	21	15	10	82	71	59	39
C	2	6	5	6	23	19	11	16	46	48	41	41
D	6	9	4	2	28	14	11	12	68	83	/[b]	50
F	8	7	6	9	25	13	7	17	88	71	44	60
G	8	4	5	8	17	26	6	21	65	64	44	61
H	3	7	9	9	28	18	14	16	79	69	66	20
I	9	14	7	7	18	17	11	16	50	78	47	45
J	2	10	3	4	18	10	11	11	58	73	35	30
K	11	9	7	21 (14)	30	/[a]	11	12 (3)	76	72	45 (17)	50 (28)
L	5	11	2	2	12	18	8	4	68	76	35	22
M	8	8	3	6	13	28	13	5	68	74	51	61 (1)
N	8	7	5	4	32	17	11	16	65	76	42	43
O	14	7	2	6	28	22	13	9	77	63	19	38
P	2	7	2	4	19	20	2	2	77	64	43	35

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

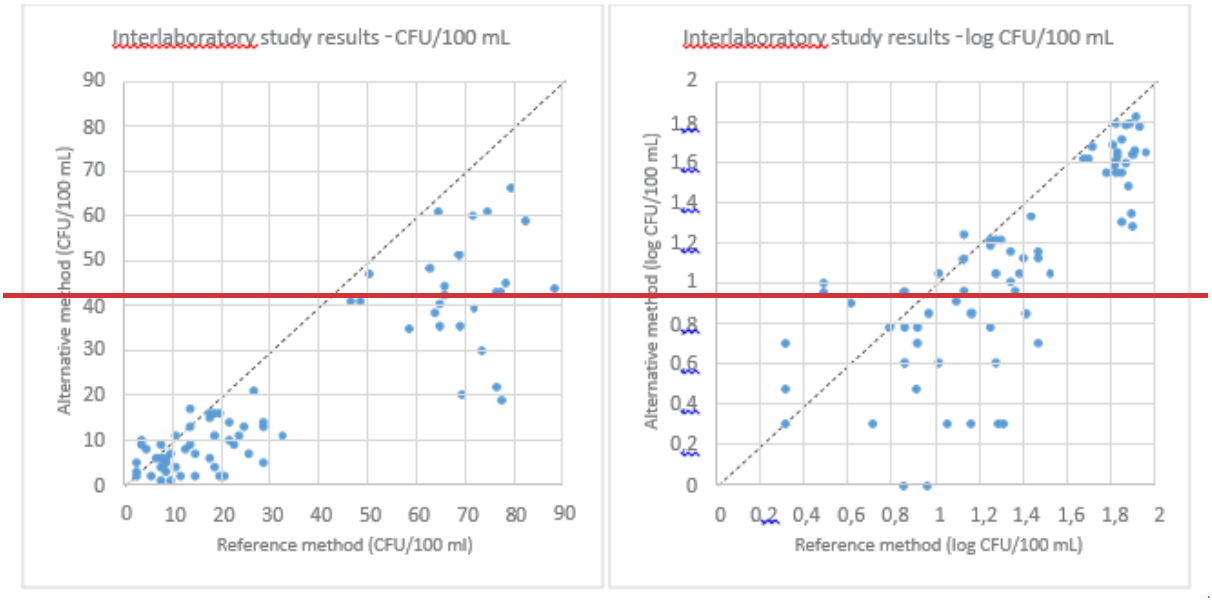


Figure 4: two-dimensional graphs in CFU/100 mL and log CFU/100 mL for interlaboratory study results

The graph representation allows viewing a weaker estimation of the number of bacteria with the alternative method, noting a dispersion of the replicates per sample obtained higher for low levels of contamination.

In conclusion, the enumerations indicated by the laboratories are in the same range than the ones obtained by the expert laboratory for each of contamination level.
The graph observation highlights a light under-estimation of the results of the alternative method versus the reference method, more visible for the weak levels of contamination for which the dispersion is maximum.

5.2.4. Calculations

Statistical calculations have been calculated according to the validation protocol of an alternative commercial method as compared to a reference method (revision 2, May 2013), per level of contamination.
Results of 13 laboratories which received samples at D+1 were processed.

- **Calculation of reference target values, fidelity and precision criteria by contamination level**

Results are expressed in CFU/100 mL and in log CFU/100 mL. A summary of the results is presented in Appendix 9.

The values of bias of the alternative method according to the levels (level 1 to 3) are presented in the table 16.

Table 16: target and mean values and biais for the three levels of the interlaboratory study

Values	CFU/100 mL			log CFU/100 mL		
Levels	**1 - Low**	**2 - Medium**	**3 - High**	**1 - Low**	**2 - Medium**	**3 - High**
Target value	7,5	19,0	68,5	0,845	1,279	1,836
Mean level	5,2	11,2	42,7	0,638	0,991	1,610
Relative bias	-0,3	-0,4	-0,4	-24,5%	-22,5%	-12,3%
Bias	0,7	0,6	0,6	-0,207	-0,288	-0,226

The bias varies from -0,288 log CFU/100 mL to -0,207 log CFU/100 mL. In the comparative study, the median bias was -0,131 log CFU/100 or 250 mL for the parameter *Escherichia coli*.

- **Accuracy profile**

Table 17 shows the tolerance values and limits of the alternative method for the different values of probability of tolerance (β) and the limits of acceptability (λ).

Table 17: Tolerance values for the alternative method

Tolerance probability and acceptability limit	Levels	CFU/100 mL			log CFU/100 mL		
		Low	**Medium**	**High**	**Low**	**Medium**	**High**
β = 80% λ= 80% in CFU/100 mL or 0,7 log CFU/100 mL	Low tolerance value	20%	25%	38%	-0,617	-0,654	-0,414
	High tolerance value	118 %	93%	86%	0,202	0,079	-0,037
	Low tolerance limit	20%	20%	20%	-0,700	-0,700	-0,700
	High tolerance limit	180 %	180%	180 %	0,700	0,700	0,700
β= 90% λ= 95% in CFU/100 mL or 0,8 log CFU/100 mL	Low tolerance value	6%	15%	31%	-0,739	-0,765	-0,470
	High tolerance value	133 %	104%	94%	0,324	0,190	0,019
	Low tolerance limit	5%	5%	5%	-0,800	-0,800	-0,800
	High tolerance limit	195 %	195%	195 %	0,800	0,800	0,800

Figures 5 to 8 present the accuracy profiles.

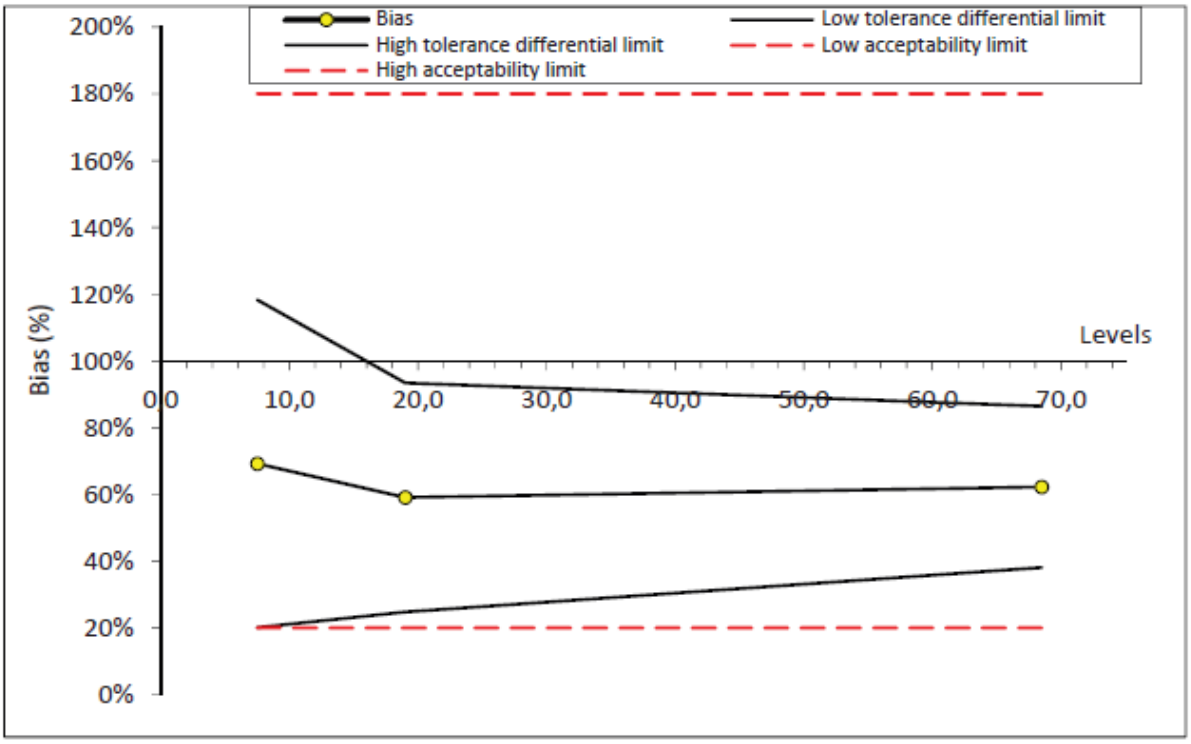


Figure 5: accuracy profile in CFU/100 mL for a tolerance probability of 80% and a tolerance limit of 80%

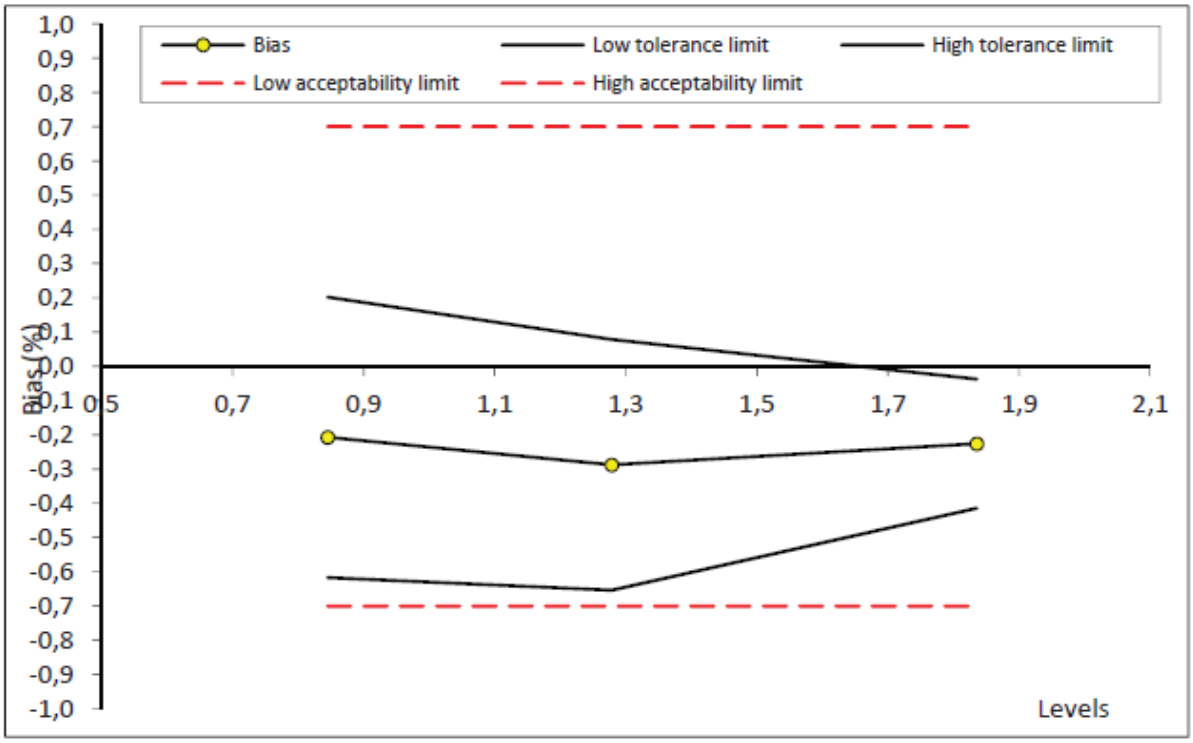


Figure 6: accuracy profile in log CFU/100 mL for a tolerance probability of 80% and a tolerance limit of 0,7

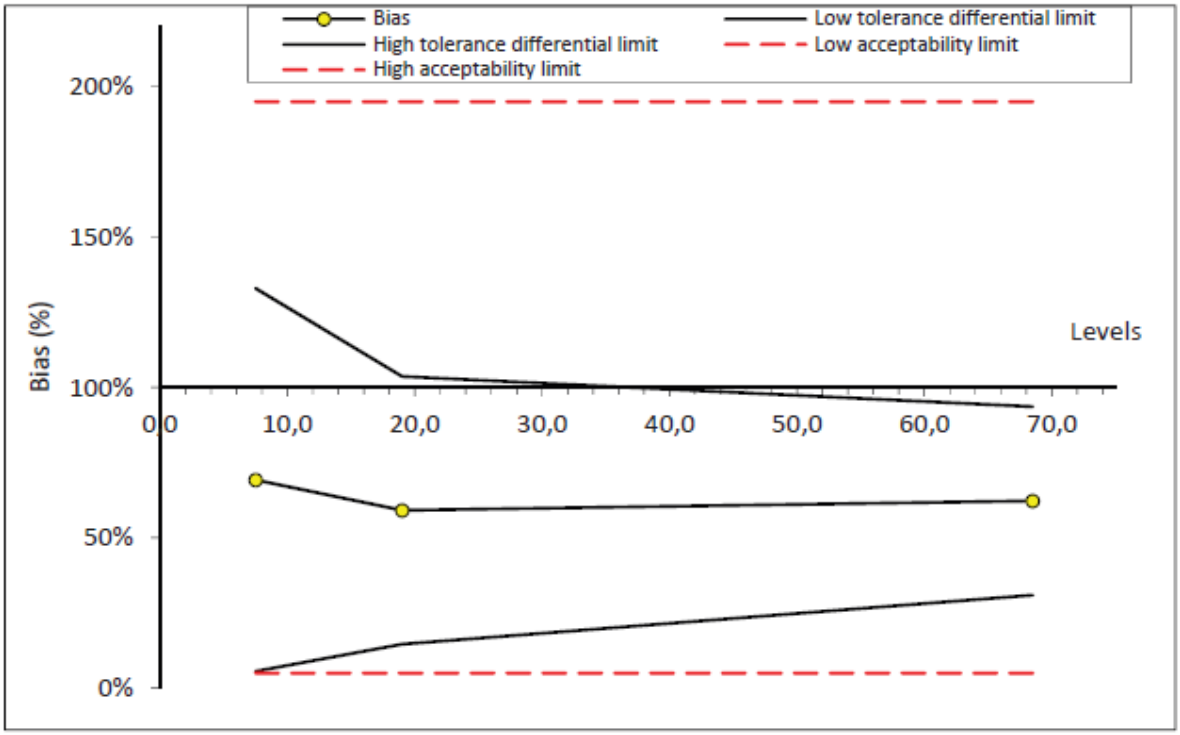


Figure 7: accuracy profile in CFU/100 mL for a tolerance probability of 90% and a tolerance limit of 95%

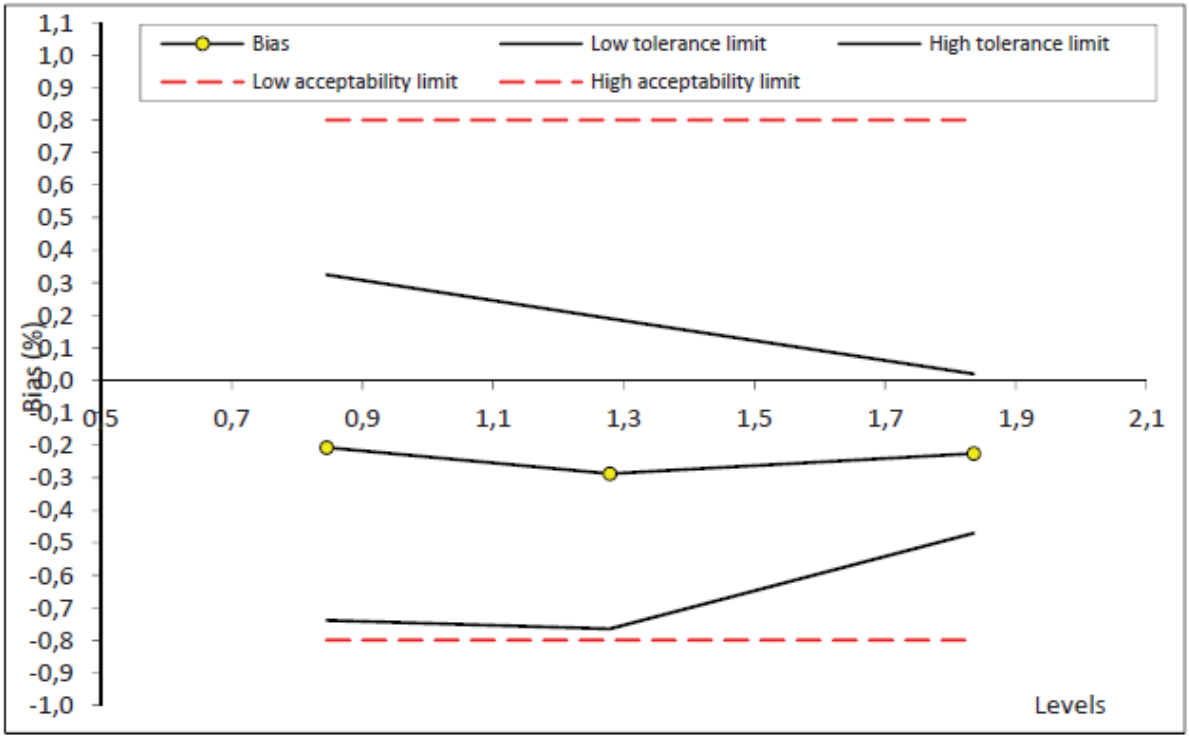


Figure 8: accuracy profile in log CFU/100 mL for a tolerance probability of 90% and a tolerance limit of 0,8

In conclusion, the bias of the alternative method is negative but stable from the low to the high level of contamination.
For all the contamination levels, the tolerance interval is comprised between the acceptability interval for a 80% tolerance probability and a limit at 0,7 log CFU/100 ml or 80% in CFU/100 mL.

5.2.5. Conclusion of the interlaboratory study

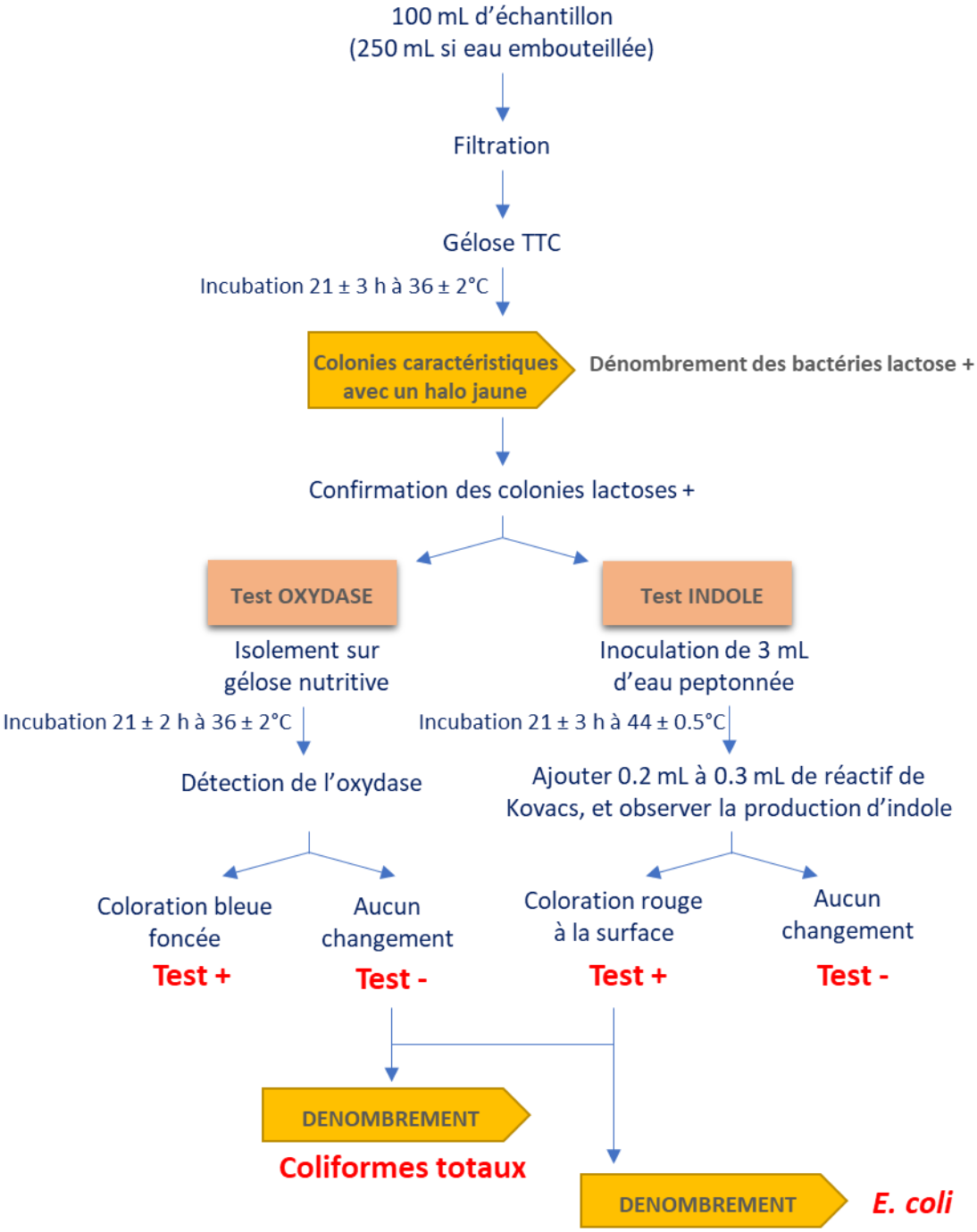
The interlaboratory study showed that the bias of the alternative method is negative but relatively stable from the low level of contamination to the high level of contamination.
For all levels of contamination, the tolerance limits are between the limits of acceptability, meaning that:

- At least 90% of the results will be between the limits of acceptability as defined at 0,8 log CFU/100 mL,
- Or at least 80% of the results will be between the limits of acceptability as defined at 0,7 log CFU/100 mL.

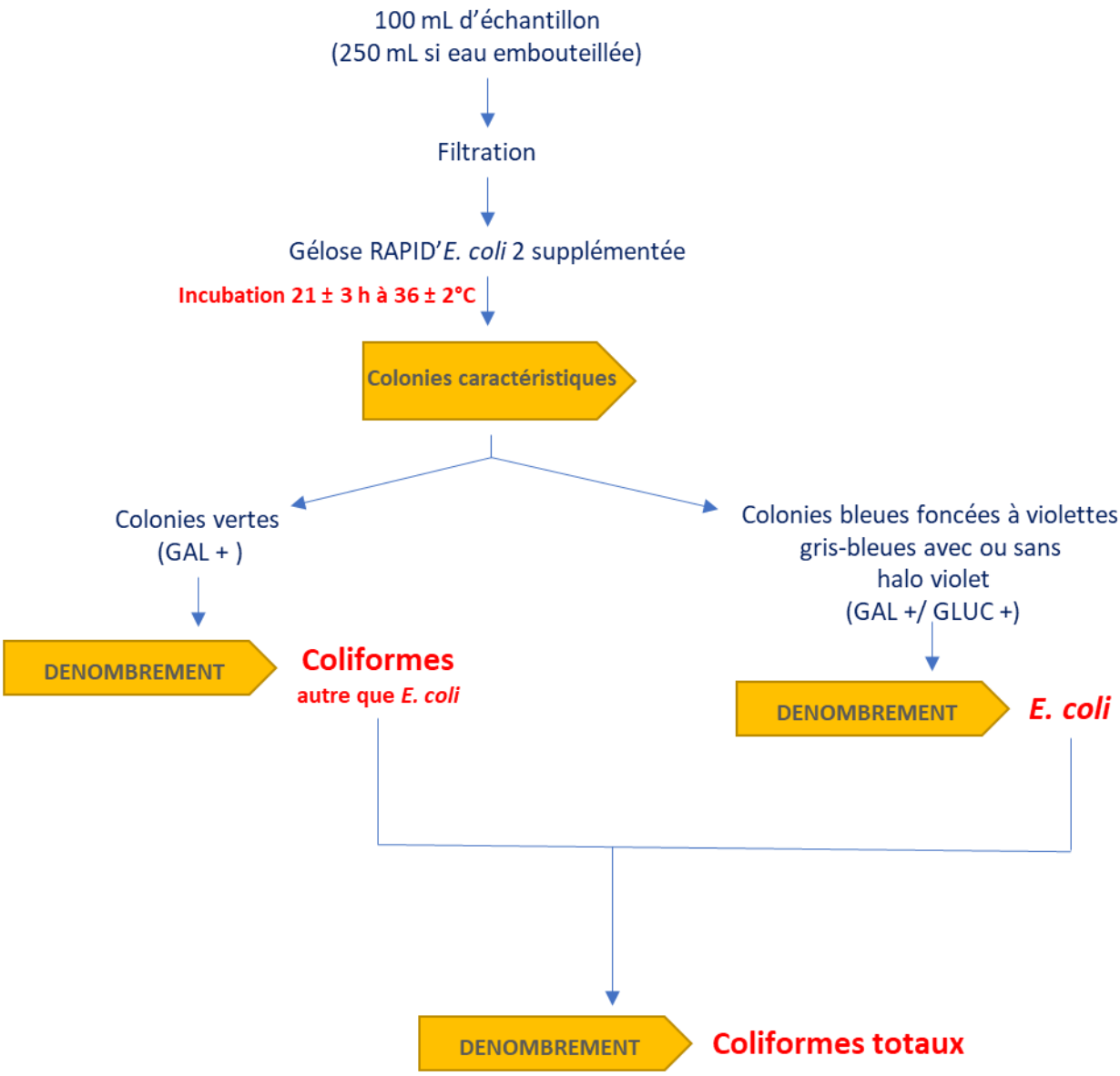
Done at Thury-Harcourt, March 17, 2023
Mickaël MORVAN
Research & Development Engineer

Appendix 1 – NF EN ISO 9308-1:2000 standard "Water quality

Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method"



Appendix 2- Flow diagram RAPID'*E.coli* 2



GAL : activité β-D-Galactosidase GLUC : activité β-D-Glucuronidase

Appendix 3 – Relative accuracy : artificial contaminations

Echantillon d'eau			Souche			Type de stress	Evaluation du stress	UFC/100mL	Détection Coliformes	Détection *E. coli*
Code	Type d'eau	Catégorie	Référence	Nom	Origine					
B07	Eau de réseau (Evin Malmaison)	1a	/	/	/	Contamination par mélange + 750 l Eau réf B01	/	/	+	+
B08	Eau de réseau (Montigny en Go-helle)	1a	/	/	/	Contamination par mélange + 500 l Eau réf B01	/	/	+/-	+/-
B09	Eau de réseau (Onnaing)	1a	/	/	/	Contamination par mélange + 250 l Eau réf B01	/	/	+	+
B10	Eau de réseau (Raismes)	1a	/	/	/	Contamination par mélange + 1 ml Eau réf B01	/	/	+	+
B12	Eau de réseau (Fresnes)	1a	/	/	/	Contamination par mélange : 20 ml d'eau de source de Bruille St Amand dans 100 ml	/	/	+	+
B13	Eau minérale (R30)	2a	/	/	/	Contamination par mélange : 40 ml d'eau de source de Bruille St Amand dans 250 ml	/	/	+	+
B34	Eau de forage (Baives)	2a	/	/	/	Contamination par mélange : 10 ml d'eau de source	/	/		
B35	Eau de réseau (Renty)	1a	Cit3e	*C. youngae*	Eau de cressonnière (Lillers)	30 min. à 55°C - 30 mi n. à -80°C	0.48	0.3	+	-
B43	Eau de surface (Renty)	2a	/	/	/	Contamination par mélange : 1 ml d'eau de source	/	/	+	-
B44	Eau de réseau (Baisieux)	1a	/	/	/	Contamination par mélange	/	/	>100	>100
B45	Eau de réseau (Annezin)	1a	/	/	/	Contamination par mélange	/	/	>100	>100
B64	Eau minérale (R12) 1,5 L	2a	EC18e	*E. coli*	Eau d'alimentation	35 min. à 55°C - 30 min. à -80°C	0.97	10.5	+	+
B65	Eau de source (R20) 1,5 L	2a	EC16e	*E. coli*	Eau de forage (Noyelles-S-Selles)	35 min. à 55°C - 30 min. à -80°C	0.83	14.5	+	+
B66	Eau de source (R16) 1,5 L	2a	EC18e	*E. coli*	Eau d'alimentation	35 min. à 55°C - 30 min. à -80°C	0.97	21.0	+	+
B67	Eau minérale (R6) 1,5 L	2a	EC16e	*E. coli*	Eau de forage (Noyelles-S-Selles)	35 min. à 55°C - 30 min. à -80°C	0.83	29.0	+	+
B68	Eau de réseau (Hallines)	1a	EC18e	*E. coli*	Eau d'alimentation	35 min. à 55°C - 30 min. à -80°C	0.97	10.5	+	+
B69	Eau de réseau Aire sur la Lys	1a	EC16e	*E. coli*	Eau de forage (Noyelles-S-Selles)	35 min. à 55°C - 30 min. à -80°C	0.83	29.0	+	+
B70	Eau de forage (Caullery)	2a	EC18e	*E. coli*	Eau d'alimentation	35 min. à 55°C - 30 min. à -80°C	0.97	16.8	+	+
Echantillon d'eau			Souche			Type de stress	Evaluation du stress	UFC/100mL	Détection Coliformes	Détection *E. coli*
Code	Type d'eau	Catégorie	Référence	Nom	Origine					

B71	Eau de forage (Preures)	2a	EC16e	*E. coli*	Eau de forage (Noyelles-S-Selles)	35 min. à 55°C - 30 min. à -80°C	0.83	20.3	+	+
B72	Eau minérale (R7) 1,5 L	2a	EC19e	*E. coli*	Eau d'alimentation (Mouveaux)	35 min. à 55°C - 30 m in. à -80°C	0.81	36.0	+	+
B73	Eau minérale (R25) 1,5 L	2a	EC19e	*E. coli*	Eau d'alimentation (Mouveaux)	35 min. à 55°C - 30 m in. à -80°C	0.81	30.0	+	+
B74	Eau de réseau (Béthune)	1a	EC19e	*E. coli*	Eau d'alimentation (Mouveaux)	35 min. à 55°C - 30 m in. à -80°C	0.81	31.5	+	+
B75	Eau de forage (Noyelles)	2a	EC19e	*E. coli*	Eau d'alimentation (Mouveaux)	35 min. à 55°C - 30 m in. à -80°C	0.81	45.0	+	+
B76	Eau de forage (Halluin)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	35 min. à 55°C - 3 0 min. à -80°C	0.50	16.0	+	+
B77	Eau de réseau (Baines)	1a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	35 min. à 55°C - 30 min. à -80°C	0.47	6.3	+	+
B78	Eau de forage (Fruges)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	35 min. à 55°C - 3 0 min. à -80°C	0.50	12.0	+	+
B79	Eau de réseau (Renty)	1a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	35 min. à 55°C - 30 min. à -80°C	0.47	4.7	+	+
B80	Eau de forage (Beuvry)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	35 min. à 55°C - 3 0 min. à -80°C	0.50	8.0	+	+
B81	Eau de forage (Camblain)	2a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	35 min. à 55°C - 30 min. à -80°C	0.47	12.6	+	+
B82	Eau de réseau (La loge)	1a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	35 min. à 55°C - 3 0 min. à -80°C	0.50	48.0	+	+
B83	Eau de forage (Malincourt)	2a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	35 min. à 55°C - 30 min. à -80°C	0.47	37.8	+	+
B84	Eau de source (R1)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	48h à -20°C - 20 mi n. à 55°C	0.54	4.3	+	+
B85	Eau de source (R8)	2a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	48h à -20°C - 20 min. à 55°C	0.63	33.0	+	+
B86	Eau minérale (R9)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	48h à -20°C - 20 mi n. à 55°C	0.54	12.9	+	+
B87	Eau minérale (R14)	2a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	48h à -20°C - 20 min. à 55°C	0.63	22.0	+	+
B88	Eau minérale (R30)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	48h à -20°C - 20 mi n. à 55°C	0.54	21.5	+	+
B89	Eau minérale (R21)	2a	EC26e	*E. coli*	Eau d'alimentation (Liencourt	48h à -20°C - 20 min. à 55°C	0.63	11.0	+	+
B90	Eau minérale (R29)	2a	EC3e	*E. coli*	Eau de réseau (Bruille St Amand)	48h à -20°C - 20 min. à 55°C	0.54	30.1	+	+
B91	Eau minérale (R31)	2a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	48h à -20°C - 20 min. à 55°C	0.63	2.8	+	+
B112	Eau de réseau (Baives)	1a	EC2e	*E. coli*	Eau de puits - Lille (59)	40 min. à 55°C - 30 min. à -80°C	1.31	1183	>100	>100
B113	Eau de réseau (Evin Malmaison)	1a	EC2e	*E. coli*	Eau de puits - Lille (59)	40 min. à 55°C - 30 min. à -80°C	1.31	845	>100	>100
B114	Eau de réseau (Montigny en Gohelle)	1a	EC2e	*E. coli*	Eau de puits - Lille (59)	40 min. à 55°C - 30 min. à -80°C	1.31	507	+	+
B115	Eau de réseau (Raismes)	1a	EC2e	*E. coli*	Eau de puits - Lille (59)	40 min. à 55°C - 30 min. à -80°C	1.31	254	+	+
B116	Eau de réseau (Fresnes)	1a	EC4e	*E. coli*	Eau de lac (Villeneuve d'Ascq)	7 jours à 4°C - 15 mi n. à -80°C	0.47	50.0	+	+
B117	Eau de réseau (Loison-sous-Lens)	1a	EC4e	*E. coli*	Eau de lac (Villeneuve d'Ascq)	7 jours à 4°C - 15 mi n. à -80°C	0.47	75.0	+	+
B118	Eau de réseau (Marcq-en-Baroeul)	1a	EC4e	*E. coli*	Eau de lac (Villeneuve d'Ascq)	7 jours à 4°C - 15 mi n. à -80°C	0.47	150	+	+
B119	Eau de réseau (Annoeullin)	1a	EC4e	*E. coli*	Eau de lac (Villeneuve d'Ascq)	7 jours à 4°C - 15 mi n. à -80°C	0.47	250	+	+

B122	Eau de réseau (Fresnes F3)	1a	Ser 3e	*Serratia marcescens*	Eau	28 h à 4°C - 35 min. à 55°C - 20 min. à -80°C	0.46	<1	-	-
B123	Eau de réseau (Baisieux)	1a	Ser 3e	*Serratia marcescens*	Eau	28 h à 4°C - 35 min. à 55°C - 20 min. à -80°C	0.46	<1	-	-
B124	Eau de source (R18)	2a	Ser 3e	*Serratia marcescens*	Eau	28 h à 4°C - 35 min. à 55°C - 20 min. à -80°C	0.46	3.4		
B125	Eau de source (R11)	2a	Ser 3e	*Serratia marcescens*	Eau	28 h à 4°C - 35 min. à 55°C - 20 min. à -80°C	0.46	13.2		
B126	Eau de réseau (Tourcoing)	1a	Ser 3e	*Serratia marcescens*	Eau	28 h à 4°C - 35 min. à 55°C - 20 min. à -80°C	0.46	6.8	+	-
B127	Eau de fontaine (Bray-Dunes)	2a	Ser 3e	*Serratia marcescens*	Eau	28 h à 4°C - 35 min. à 55°C - 20 min. à -80°C	0.46	13.6		
B128	Eau de réseau (Loison-sous-Lens)	1a	EC21e	*E. coli*	Eau d'alimentation (Megève)	24 h à 4°C - 30 min. à 5 5°C - 30 min. à -80°C	0.73	13.5	+	+
B129	Eau de réseau (Roncq)	1a	EC21e	*E. coli*	Eau d'alimentation (Megève)	24 h à 4°C - 30 min. à 5 5°C - 30 min. à -80°C	0.73	8.1	+	+
B130	Eau de réseau (La Loge)	1a	EC21e	*E. coli*	Eau d'alimentation (Megève)	24 h à 4°C - 30 min. à 5 5°C - 30 min. à -80°C	0.73	16.2	+	+
B131	Eau de réseau (Fresnes F2)	1a	EC21e	*E. coli*	Eau d'alimentation (Megève)	24 h à 4°C - 30 min. à 5 5°C - 30 min. à -80°C	0.73	21.6	+	+
B150	Eau de réseau (Bois Grenier)	1a	/	/	/	Contamination par mélange avec de l'eau de source de Bruille St Amand (dilution 1/2)	/	/	+	1+ /1-
B151	Eau de réseau (Roubaix)	1a	/	/	/	Contamination par mélange avec de l'eau de source de Bruille St Amand (dilution 1/2)	/	/	+	+
B154	Eau de réseau (Tourcoing)	1a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	18h à -20°C-4h à +20° C-18h à -20°C-4h à +20°C	0.61	200.0	+	+
B155	Eau de réseau (Pecquencourt)	1a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	18h à -20°C-4h à +20° C-18h à -20°C-4h à +20°C	0.61	150.0	+	+
B156	Eau de réseau (Liessies)	1a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	18h à -20°C-4h à +20° C-18h à -20°C-4h à +20°C	0.61	100.0	+	+
B157	Eau de réseau (Marcq en Baroeul)	1a	EC26e	*E. coli*	Eau d'alimentation (Liencourt)	18h à -20°C-4h à +20° C-18h à -20°C-4h à +20°C	0.61	50.0	+	+
B160	Eau de réseau (Roubaix)	1a	/	/	/	Contamination par mélange avec de l'eau de source de Bruille St Amand - Chauffage 10 min. à 55°C	/	/	+	+
B161	Eau de réseau (Marcq en Baroeul)	1a	/	/	/	Contamination par mélange avec de l'eau de source de Bruille St Amand - Chauffage 10 min. à 55°C	/	/	+	+

Rx : référence de l'eau embouteillée testée (marque commerciale)

Résultat non exploité

Appendix 4 – Relative accuracy : results et statistics

Legend

cat : catégorie du domaine d'application
CA : contamination artificielle
o : oui (réalisation d'une CA)
n : non (échantillon naturellement contaminé)
lac+ : fermentation du lactose positive
ox+ : test oxydase positif
indole + : test indole positif
Gal+ : β-D-Galactosidase (Gal) positive
Gluc+ : β-D-Glucuronidase (Gluc) positive
CT : coliformes totaux
EC : *E. coli*
INC : incomptable (flore interférente importante)
∑C: somme des colonies comptées

Eau minérale ou eau de source (R1 à R36) = Eau embouteillée (analyse de 250 mL)
Eau de source (nom de la localité de prélèvement) = eau non embouteillée (analyse de 100 mL)

In red: results excluded from statistical analysis in accordance with NF EN ISO 8199 (>80 colonies)

#	Code	Sample	CA	Filtered volume in ml	NF EN ISO 9308-1								RAPID'*E.coli* 2 for Water method									
					Typical colonies on TTC agar(24 and 48 h 36°C)				Result in CFU/100 mL or 250 mL				Typical colonies on supplemented RAPID'*E.coli* 2									
					CT (lac+, ox -)		*E. coli* (lac+, ox-, indole +)		CT		*E. coli*		Replicate 1					Replicate 2				
					R1	R2	R1	R2	R1	R2	R1	R2	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL
1	B07	Tap drinking water	y	100	INC	INC	INC	INC	INC	INC	INC	INC	0	6	6	6,0E+00	6,0E+00	0	1	1	1,0E+00	1,0E+00
2	B08	Tap drinking water	y	100	INC	INC	INC	INC	INC	INC	INC	INC	0	4	4	4,0E+00	4,0E+00	0	0	0	<1	<1
3	B09	Tap drinking water	y	100	INC	INC	INC	INC	INC	INC	INC	INC	0	1	1	1,0E+00	1,0E+00	0	1	1	1,0E+00	1,0E+00
4	B10	Tap drinking water	y	100	INC	INC	INC	INC	INC	INC	INC	INC	0	3	3	3,0E+00	3,0E+00	0	8	8	8,0E+00	8,0E+00
5	B12	Tap drinking water	y	100	1	2	0	0	1,0E+00	2,0E+00	<1	<1	7	0	7	7,0E+00	7,0E+00	7	0	7	7,0E+00	7,0E+00
6	B35	Tap drinking water (Renty)	y	100	38	44	0	0	3,8E+01	4,4E+01	<1	<1	39	0	39	3,9E+01	<1	48	0	48	4,8E+01	<1
7	B44	Tap drinking water (Baisieux)	y	100	>100	>100	>100	>100	>100	>100	>100	>100	0	135	>100	>100	>100	0	136	>100	>100	>100
8	B45	Tap drinking water (Annezin)	y	100	>100	>100	>100	>100	>100	>100	>100	>100	0	229	>100	>100	>100	0	227	>100	>100	>100
9	B68	Tap drinking water (Hallines)	y	100	16	13	16	13	1,6E+01	1,3E+01	1,6E+01	1,3E+01	0	17	17	1,7E+01	1,7E+01	0	16	16	1,6E+01	1,6E+01
10	B69	Tap drinking water (Aire sur la Lys)	y	100	63	61	63	61	6,3E+01	6,1E+01	6,3E+01	6,1E+01	0	38	38	3,8E+01	3,8E+01	0	33	33	3,3E+01	3,3E+01
11	B74	Tap drinking water (Béthune)	y	100	46	68	46	68	4,6E+01	6,8E+01	4,6E+01	6,8E+01	0	47	47	4,7E+01	4,7E+01	0	56	56	5,6E+01	5,6E+01
12	B77	Tap drinking water (Baines)	y	100	21	22	21	22	2,1E+01	2,2E+01	2,1E+01	2,2E+01	6	3	9	9,0E+00	3,0E+00	11	11	22	2,2E+01	1,1E+01
13	B79	Tap drinking water (Renty)	y	100	9	11	9	11	9,0E+00	1,1E+01	9,0E+00	1,1E+01	0	5	5	5,0E+00	5,0E+00	0	6	6	6,0E+00	6,0E+00
14	B82	Tap drinking water (La loge)	y	100	71	70	71	70	7,1E+01	7,0E+01	7,1E+01	7,0E+01	0	64	64	6,4E+01	6,4E+01	0	68	68	6,8E+01	6,8E+01
15	B112	Tap drinking water (Baives)	y	100	>100	>100	>100	>100	>100	>100	>100	>100	0	>200	>200	>100	>100	0	>200	>200	>100	>100

#	Code	Sample	CA	Filtered volume in ml	NF EN ISO 9308-1								RAPID'*E.coli* 2 for Water method									
					Typical colonies on TTC agar(24 and 48 h 36°C)				Result in CFU/100 mL or 250 mL				Typical colonies on supplemented RAPID'*E.coli* 2									
					CT (lac+, ox -)		*E. coli* (lac+, ox-, indole +)		CT		*E. coli*		Replicate 1					Replicate 2				
					R1	R2	R1	R2	R1	R2	R1	R2	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL
16	B113	Tap drinking water (Evin Malmaison)	y	100	>100	>100	>100	>100	>100	>100	>100	>100	0	231	>200	>100	>100	0	253	>200	>100	>100
17	B114	Tap drinking water (Montigny en Gohelle)	y	100	237	233	237	233	>100	>100	>100	>100	0	163	163	1,6E+02	1,6E+02	0	144	144	1,4E+02	1,4E+02
18	B115	Tap drinking water (Raismes)	y	100	172	180	172	180	>100	>100	>100	>100	0	87	87	8,7E+01	8,7E+01	0	84	84	8,4E+01	8,4E+01
19	B116	Tap drinking water (Fresnes)	y	100	60	59	60	59	6,0E+01	5,9E+01	6,0E+01	5,9E+01	0	60	60	6,0E+01	6,0E+01	0	62	62	6,2E+01	6,2E+01
20	B117	Tap drinking water (Loison-sous-Lens)	y	100	83	82	83	82	8,3E+01	8,2E+01	8,3E+01	8,2E+01	0	86	86	8,6E+01	8,6E+01	0	80	80	8,0E+01	8,0E+01
21	B118	Tap drinking water (Marcq-en- Baroeul)	y	100	173	163	173	163	1,7E+02	1,6E+02	1,7E+02	1,6E+02	0	147	147	1,5E+02	1,5E+02	0	151	151	1,5E+02	1,5E+02
22	B119	Tap drinking water (Annoeullin)	y	100	199	222	199	222	>100	>100	>100	>100	0	193	193	1,9E+02	1,9E+02	0	217	214	2,1E+02	2,1E+02
23	B122	Tap drinking water (Fresnes F3)	y	100	0	0	0	0	<1	<1	<1	<1	0	0	0	<1	<1	0	0	0	<1	<1
24	B123	Tap drinking water (Baisieux)	y	100	0	0	0	0	<1	<1	<1	<1	0	0	0	<1	<1	0	0	0	<1	<1
25	B126	Tap drinking water (Tourcoing)	y	100	12	9	0	0	1,2E+01	9,0E+00	<1	<1	3	0	3	3,0E+00	<1	6	0	6	6,0E+00	<1
26	B128	Tap drinking water (Loison-sous-Lens)	y	100	12	10	12	10	1,2E+01	1,0E+01	1,2E+01	1,0E+01	0	4	4	4,0E+00	4,0E+00	0	5	5	5,0E+00	5,0E+00
27	B129	Tap drinking water (Roncq)	y	100	8	5	8	5	8,0E+00	5,0E+00	8,0E+00	5,0E+00	0	9	9	9,0E+00	9,0E+00	0	2	2	2,0E+00	2,0E+00
28	B130	Tap drinking water (La Loge)	y	100	21	28	21	28	2,1E+01	2,8E+01	2,1E+01	2,8E+01	0	8	8	8,0E+00	8,0E+00	0	7	7	7,0E+00	7,0E+00
29	B131	Tap drinking water (Fresnes F2)	y	100	22	23	22	23	2,2E+01	2,3E+01	2,2E+01	2,3E+01	0	8	8	8,0E+00	8,0E+00	0	14	14	1,4E+01	1,4E+01
30	B132	Tap drinking water (Lourches)	n	100	0	0	0	0	<1	<1	<1	<1	0	0	0	<1	<1	0	0	0	<1	<1
31	B150	Tap drinking water (Bois Grenier)	y	100	17	23	9	2	1,7E+01	2,3E+01	9,0E+00	2,0E+00	100	1	101	1,0E+02	1,0E+00	91	2	93	9,3E+01	2,0E+00

#	Code	Sample	CA	Filtered volume in ml	NF EN ISO 9308-1								RAPID'*E.coli* 2 for Water method									
					Typical colonies on TTC agar(24 and 48 h 36°C)				Result in CFU/100 mL or 250 mL				Typical colonies on supplemented RAPID'*E.coli* 2									
					CT (lac+, ox -)		*E. coli* (lac+, ox-, indole +)		CT		*E. coli*		Replicate 1					Replicate 2				
					R1	R2	R1	R2	R1	R2	R1	R2	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL
32	B151	Tap drinking water (Roubaix)	y	100	15	17	3	8	1,5E+01	1,7E+01	3,0E+00	8,0E+00	93	1	94	9,4E+01	1,0E+00	101	2	103	1,0E+02	2,0E+00
33	B154	Tap drinking water (Tourcoing)	y	10	24	15	24	15	2,4E+02	1,5E+02	2,4E+02	1,5E+02	0	11	11	1,1E+02	1,1E+02	0	6	6	6,0E+01	6,0E+01
34	B155	Tap drinking water (Pecquencourt)	y	10	22	15	22	15	2,2E+02	1,5E+02	2,2E+02	1,5E+02	0	9	9	9,0E+01	9,0E+01	0	15	15	1,5E+02	1,5E+02
35	B156	Tap drinking water (Liessies)	y	10	12	7	12	7	1,2E+02	7,0E+01	1,2E+02	7,0E+01	0	8	8	8,0E+01	8,0E+01	0	3	3	3,0E+01	3,0E+01
36	B157	Tap drinking water (Marcq en Baroeul)	y	100	37	34	37	34	3,7E+01	3,4E+01	3,7E+01	3,4E+01	0	26	26	2,6E+01	2,6E+01	0	25	25	2,5E+01	2,5E+01
37	B160	Tap drinking water (Roubaix)	y	100	8	9	2	2	8,0E+00	9,0E+00	2,0E+00	2,0E+00	57	2	59	5,9E+01	2,0E+00	51	1	52	5,2E+01	1,0E+00
38	B161	Tap drinking water (Marcq en Baroeul)	y	100	7	14	1	7	7,0E+00	1,4E+01	1,0E+00	7,0E+00	44	0	44	4,4E+01	<1	51	1	52	5,2E+01	1,0E+00
39	B01	Well water (Liessies)	n	250	INC	INC	INC	INC	>100	>100	>100	>100	INC	INC	/	>100	>100	INC	INC	/	>100	>100
40	B11	Spring water (R21)	n	250	22	31	0	0	2,2E+01	3,1E+01	<1	<1	0	4	4	4,0E+00	4,0E+00	0	5	5	5,0E+00	5,0E+00
41	B13	Mineral water (R30)	y	250	11	10	0	0	1,1E+01	1,0E+01	<1	<1	15	1	16	1,6E+01	1,0E+00	17	1	18	1,8E+01	1,0E+00
42	B14	Spring water (R21)	n	250	5	6	0	0	5,0E+00	6,0E+00	<1	<1	7	25	31	3,1E+01	2,5E+01	7	20	27	2,7E+01	2,0E+01
43	B34	Raw water (Baives)	y	100	INC	INC	INC	INC	>100	>100	>100	>100	>100	61	>100	>100	6,1E+01	>100	73	>100	>100	7,3E+01

#	Code	Sample	CA	Filtered volume in ml	NF EN ISO 9308-1							RAPID'*E.coli* 2 for Water method										
					Typical colonies on TTC agar(24 and 48 h 36°C)				Result in CFU/100 mL or 250 mL			Typical colonies on supplemented RAPID'*E.coli* 2										
					CT (lac+, ox -)		*E. coli* (lac+, ox-, indole +)		CT	*E. coli*		Replicate 1						Replicate 2				
					R1	R2	R1	R2	R1	R2	R1	R2	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	LC	TC in 100 or 250 mL	*E. coli* in 100 or 250 mL
44	B43	Raw water (Renty)	y	100	27	21	0	0	2,7E+01	2,1E+01	<1	<1	26	7	33	3,3E+01	7,0E+00	14	3	17	1,7E+01	3,0E+00
45	B64	Mineral water - 1,5 L (R12)	y	250	11	11	11	11	1,1E+01	1,1E+01	1,1E+01	1,1E+01	0	11	11	1,1E+01	1,1E+01	0	8	8	8,0E+00	8,0E+00
46	B65	Spring water - 1,5 L (R20)	y	250	20	33	20	33	2,0E+01	3,3E+01	2,0E+01	3,3E+01	0	17	17	1,7E+01	1,7E+01	0	12	12	1,2E+01	1,2E+01
47	B66	Spring water - 1,5 L (R16)	y	250	43	37	43	37	4,3E+01	3,7E+01	4,3E+01	3,7E+01	0	18	18	1,8E+01	1,8E+01	0	36	36	3,6E+01	3,6E+01
48	B67	Mineral water - 1,5 L (R6)	y	250	51	57	51	57	5,1E+01	5,7E+01	5,1E+01	5,7E+01	0	27	27	2,7E+01	2,7E+01	0	34	34	3,4E+01	3,4E+01
49	B70	Raw water (Caullery)	y	100	12	4	12	4	1,2E+01	4,0E+00	1,2E+01	4,0E+00	0	5	5	5,0E+00	5,0E+00	0	4	4	4,0E+00	4,0E+00
50	B71	Raw water (Preures)	y	100	38	39	38	39	3,8E+01	3,9E+01	3,8E+01	3,9E+01	0	22	22	2,2E+01	2,2E+01	0	22	22	2,2E+01	2,2E+01
51	B72	Mineral water - 1,5 L (R7)	y	250	51	51	51	51	5,1E+01	5,1E+01	5,1E+01	5,1E+01	0	59	59	5,9E+01	5,9E+01	0	57	57	5,7E+01	5,7E+01
52	B73	Mineral water - 1,5 L (R25)	y	250	39	44	39	44	3,9E+01	4,4E+01	3,9E+01	4,4E+01	0	29	29	2,9E+01	2,9E+01	0	48	48	4,8E+01	4,8E+01
53	B75	Raw water (Noyelles)	y	100	67	64	67	64	6,7E+01	6,4E+01	6,7E+01	6,4E+01	0	60	60	6,0E+01	6,0E+01	0	70	70	7,0E+01	7,0E+01
54	B76	Raw water (Halluin)	y	100	20	27	20	27	2,0E+01	2,7E+01	2,0E+01	2,7E+01	1	23	24	2,4E+01	2,3E+01	1	33	34	3,4E+01	3,3E+01
55	B78	Raw water (Fruges)	y	100	20	26	20	26	2,0E+01	2,6E+01	2,0E+01	2,6E+01	0	25	25	2,5E+01	2,5E+01	0	25	25	2,5E+01	2,5E+01
56	B80	Raw water (Beuvry)	y	100	19	21	19	21	1,9E+01	2,1E+01	1,9E+01	2,1E+01	0	11	11	1,1E+01	1,1E+01	0	9	9	9,0E+00	9,0E+00
57	B81	Raw water (Camblain)	y	100	21	23	21	23	2,1E+01	2,3E+01	2,1E+01	2,3E+01	0	16	16	1,6E+01	1,6E+01	0	13	13	1,3E+01	1,3E+01
58	B83	Raw water (Malincourt)	y	100	61	55	61	55	6,1E+01	5,5E+01	6,1E+01	5,5E+01	0	36	36	3,6E+01	3,6E+01	0	39	39	3,9E+01	3,9E+01
59	B84	Spring water (R1)	y	250	8	6	8	6	8,0E+00	6,0E+00	8,0E+00	6,0E+00	0	6	6	6,0E+00	6,0E+00	0	9	9	9,0E+00	9,0E+00
60	B85	Spring water (R8)	y	250	47	49	47	49	4,7E+01	4,9E+01	4,7E+01	4,9E+01	0	39	39	3,9E+01	3,9E+01	0	40	40	4,0E+01	4,0E+01
61	B86	Mineral water (R9)	y	250	22	22	22	22	2,2E+01	2,2E+01	2,2E+01	2,2E+01	0	16	16	1,6E+01	1,6E+01	0	23	23	2,3E+01	2,3E+01
62	B87	Mineral water (R14)	y	250	33	29	33	29	3,3E+01	2,9E+01	3,3E+01	2,9E+01	0	27	27	2,7E+01	2,7E+01	0	32	32	3,2E+01	3,2E+01
63	B88	Mineral water (R30)	y	250	46	48	46	48	4,6E+01	4,8E+01	4,6E+01	4,8E+01	0	30	30	3,0E+01	3,0E+01	0	28	28	2,8E+01	2,8E+01
64	B89	Mineral water (R21)	y	250	12	23	12	23	1,2E+01	2,3E+01	1,2E+01	2,3E+01	0	10	10	1,0E+01	1,0E+01	0	16	16	1,6E+01	1,6E+01
65	B90	Mineral water (R29)	y	250	46	49	46	49	4,6E+01	4,9E+01	4,6E+01	4,9E+01	0	45	45	4,5E+01	4,5E+01	0	41	41	4,1E+01	4,1E+01
66	B91	Mineral water (R31)	y	250	1	5	1	5	1,0E+00	5,0E+00	1,0E+00	5,0E+00	0	1	1	1,0E+00	1,0E+00	0	4	4	4,0E+00	4,0E+00

Relative accuracy - Total coliforms

Reference method					Alternative method					Difference
Sample	Replicate 1	Replicate 2	M	SD	Sample	Replicate 1	Replicate 2	M	SD	
1	38	44	41,0	4,2	1	39	48	43,5	6,4	2,5
2	16	13	14,5	2,1	2	17	16	16,5	0,7	2,0
3	63	61	62,0	1,4	3	38	33	35,5	3,5	-26,5
4	46	68	57,0	15,6	4	47	56	51,5	6,4	-5,5
5	21	22	21,5	0,7	5	9	22	15,5	9,2	-6,0
6	9	11	10,0	1,4	6	5	6	5,5	0,7	-4,5
7	71	70	70,5	0,7	7	64	68	66,0	2,8	-4,5
8	60	59	59,5	0,7	8	60	62	61,0	1,4	1,5
9	12	10	11,0	1,4	11	4	5	4,5	0,7	-6,5
10	21	28	24,5	4,9	12	8	7	7,5	0,7	-17,0
11	22	23	22,5	0,7	13	8	14	11,0	4,2	-11,5
12	37	34	35,5	2,1	16	26	25	25,5	0,7	-10,0
13	8	9	8,5	0,7	17	59	52	55,5	4,9	47,0
14	7	14	10,5	4,9	18	44	52	48,0	5,7	37,5
15	22	31	26,5	6,4	19	4	5	4,5	0,7	-22,0
16	11	10	10,5	0,7	20	16	18	17,0	1,4	6,5
17	5	6	5,5	0,7	21	31	27	29,0	2,8	23,5
18	27	21	24,0	4,2	22	33	17	25,0	11,3	1,0
19	11	11	11,0	0,0	23	11	8	9,5	2,1	-1,5
20	20	33	26,5	9,2	24	17	12	14,5	3,5	-12,0
21	43	37	40,0	4,2	25	18	36	27,0	12,7	-13,0
22	51	57	54,0	4,2	26	27	34	30,5	4,9	-23,5
23	12	4	8,0	5,7	27	5	4	4,5	0,7	-3,5
24	38	39	38,5	0,7	28	22	22	22,0	0,0	-16,5
25	51	51	51,0	0,0	29	59	57	58,0	1,4	7,0
26	39	44	41,5	3,5	30	29	48	38,5	13,4	-3,0
27	67	64	65,5	2,1	31	60	70	65,0	7,1	-0,5
28	20	27	23,5	4,9	32	24	34	29,0	7,1	5,5
29	20	26	23,0	4,2	33	25	25	25,0	0,0	2,0
30	19	21	20,0	1,4	34	11	9	10,0	1,4	-10,0
31	21	23	22,0	1,4	35	16	13	14,5	2,1	-7,5
32	61	55	58,0	4,2	36	36	39	37,5	2,1	-20,5
33	8	6	7,0	1,4	37	6	9	7,5	2,1	0,5
34	47	49	48,0	1,4	38	39	40	39,5	0,7	-8,5
35	22	22	22,0	0,0	39	16	23	19,5	4,9	-2,5
36	33	29	31,0	2,8	40	27	32	29,5	3,5	-1,5
37	46	48	47,0	1,4	41	30	28	29,0	1,4	-18,0
38	12	23	17,5	7,8	42	10	16	13,0	4,2	-4,5
39	46	49	47,5	2,1	43	45	41	43,0	2,8	-4,5

q= 39
n= 2
N=qn= 78

Mx= 31,2
MEDx= 24,5
SDbx= 18,9
MEDwx = 2,1
SDwx= 3,0
rob. SDwx= 3,1

My= 28,0
MEDy= 26,0
SDby= 18,0
MEDwy = 2,8
SDwy= 3,4
rob. SDwy= 4,2

M= -3,3
MED= -4,5
Bias

Method choice

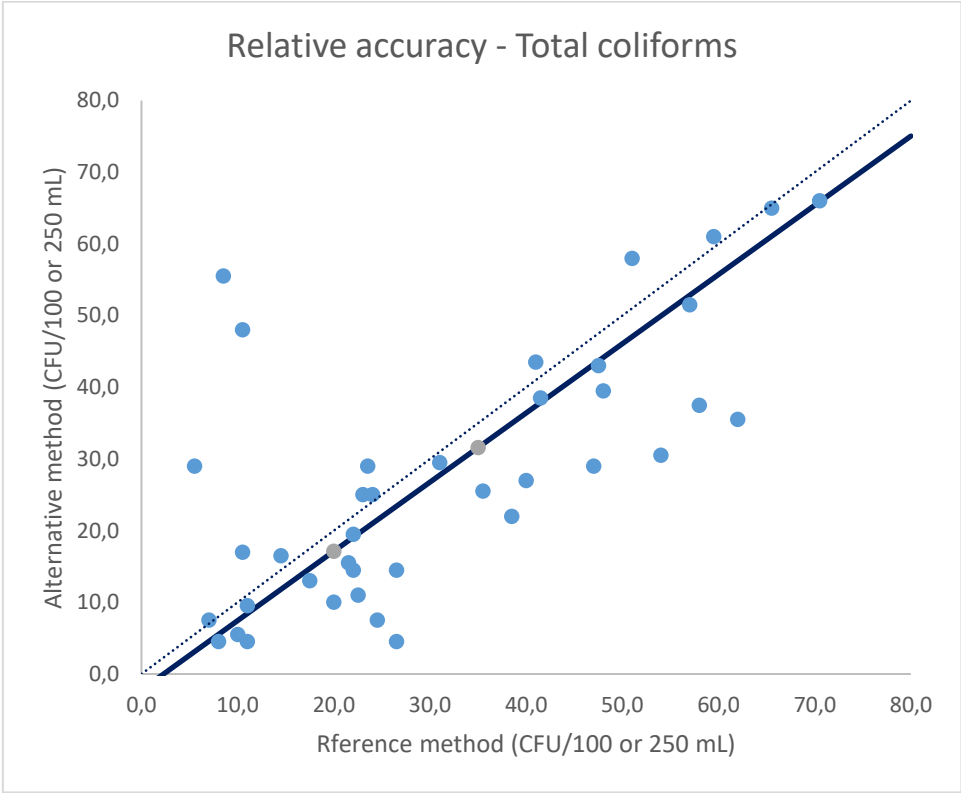
GMFR

		R=	1,130		
		rob. R=	1,333		
				Sx=	18,991
				Sy=	18,342
r=	0,683				
b=	0.966			**Res. SEM=**	3,327
a=	-2.228			**Res. SD=**	4.705

S(b)=	0,123	**t(b)=**	0.279	-2.021<**0.279**<2.021 Hypothèse b=1 validé
S(a)=	4.467	**t(a)=**	0.499	-2.021<**0.499**<2.021 Hypothèse a = 0 validé

Points correspond to the mean of the repetitions for each sample

Est. y	Deviation
37,4	6,1
11,8	4,7
57,7	-22,2
52,8	-1,3
18,5	-3,0
7,4	-1,9
65,9	0,1
55,2	5,8
8,4	-3,9
21,4	-13,9
19,5	-8,5
32,1	-6,6
6,0	49,5
7,9	40,1
23,4	-18,9
7,9	9,1
3,1	25,9
21,0	4,0
8,4	1,1
23,4	-8,9
36,4	-9,4
49,9	-19,4
5,5	-1,0
35,0	-13,0
47,0	11,0
37,9	0,6
61,0	4,0
20,5	8,5
20,0	5,0
17,1	-7,1
19,0	-4,5
53,8	-16,3
4,5	3,0
44,1	-4,6
19,0	0,5
27,7	1,8
43,2	-14,2
14,7	-1,7
43,6	-0,6


Relative accuracy - Total coliforms
Alternative method (CFU/100 or 250 mL)
Rference method (CFU/100 or 250 mL)
0,0
10,0
20,0
30,0
40,0
50,0
60,0
70,0
80,0

Relative accuracy - Total coliforms - Logarithmic data

Reference method					Alternative method					Difference
Sample	Replicate 1	Replicate 2	M	SD	Sample	Replicate 1	Replicate 2	M	SD	
1	1,580	1,643	1,612	0,045	1	1,591	1,681	1,636	0,064	0,025
2	1,204	1,114	1,159	0,064	2	1,230	1,204	1,217	0,019	0,058
3	1,799	1,785	1,792	0,010	3	1,580	1,519	1,549	0,043	-0,243
4	1,663	1,833	1,748	0,120	4	1,672	1,748	1,710	0,054	-0,037
5	1,322	1,342	1,332	0,014	5	0,954	1,342	1,148	0,274	-0,184
6	0,954	1,041	0,998	0,062	6	0,699	0,778	0,739	0,056	-0,259
7	1,851	1,845	1,848	0,004	7	1,806	1,833	1,819	0,019	-0,029
8	1,778	1,771	1,775	0,005	8	1,778	1,792	1,785	0,010	0,011
11	1,079	1,000	1,040	0,056	11	0,602	0,699	0,651	0,069	-0,389
12	1,322	1,447	1,385	0,088	12	0,903	0,845	0,874	0,041	-0,511
13	1,342	1,362	1,352	0,014	13	0,903	1,146	1,025	0,172	-0,327
16	1,568	1,531	1,550	0,026	16	1,415	1,398	1,406	0,012	-0,143
17	0,903	0,954	0,929	0,036	17	1,771	1,716	1,743	0,039	0,815
18	0,845	1,146	0,996	0,213	18	1,643	1,716	1,680	0,051	0,684
19	1,342	1,491	1,417	0,105	19	0,602	0,699	0,651	0,069	-0,766
20	1,041	1,000	1,021	0,029	20	1,204	1,255	1,230	0,036	0,209
21	0,699	0,778	0,739	0,056	21	1,491	1,431	1,461	0,042	0,723
22	1,431	1,322	1,377	0,077	22	1,519	1,230	1,374	0,204	-0,002
23	1,041	1,041	1,041	0,000	23	1,041	0,903	0,972	0,098	-0,069
24	1,301	1,519	1,410	0,154	24	1,230	1,079	1,155	0,107	-0,255
25	1,633	1,568	1,601	0,046	25	1,255	1,556	1,406	0,213	-0,195
26	1,708	1,756	1,732	0,034	26	1,431	1,531	1,481	0,071	-0,250
27	1,079	0,602	0,841	0,337	27	0,699	0,602	0,651	0,069	-0,190
28	1,580	1,591	1,585	0,008	28	1,342	1,342	1,342	0,000	-0,243
29	1,708	1,708	1,708	0,000	29	1,771	1,756	1,763	0,011	0,056
30	1,591	1,643	1,617	0,037	30	1,462	1,681	1,572	0,155	-0,045
31	1,826	1,806	1,816	0,014	31	1,778	1,845	1,812	0,047	-0,005
32	1,301	1,431	1,366	0,092	32	1,380	1,531	1,456	0,107	0,090
33	1,301	1,415	1,358	0,081	33	1,398	1,398	1,398	0,000	0,040
34	1,279	1,322	1,300	0,031	34	1,041	0,954	0,998	0,062	-0,303
35	1,322	1,362	1,342	0,028	35	1,204	1,114	1,159	0,064	-0,183
36	1,785	1,740	1,763	0,032	36	1,556	1,591	1,574	0,025	-0,189
37	0,903	0,778	0,841	0,088	37	0,778	0,954	0,866	0,125	0,026
38	1,672	1,690	1,681	0,013	38	1,591	1,602	1,597	0,008	-0,085
39	1,342	1,342	1,342	0,000	39	1,204	1,362	1,283	0,111	-0,059
40	1,519	1,462	1,490	0,040	40	1,431	1,505	1,468	0,052	-0,022
41	1,663	1,681	1,672	0,013	41	1,477	1,447	1,462	0,021	-0,210
42	1,079	1,362	1,220	0,200	42	1,000	1,204	1,102	0,144	-0,118
43	1,663	1,690	1,676	0,019	43	1,653	1,613	1,633	0,029	-0,043

q= 39
n= 2
N=qn= 78

Mx= 1,397
MEDx= 1,385
SDbx= 0,311
MEDwx = 0,036
SDwx= 0,069
rob. SDwx= 0,053

My= 1,329
MEDy= 1,406
SDby= 0,344
MEDwy = 0,054
SDwy= 0,063
rob. SDwy= 0,080

M= -0,067
MED= -0,069
Bias

Method choice

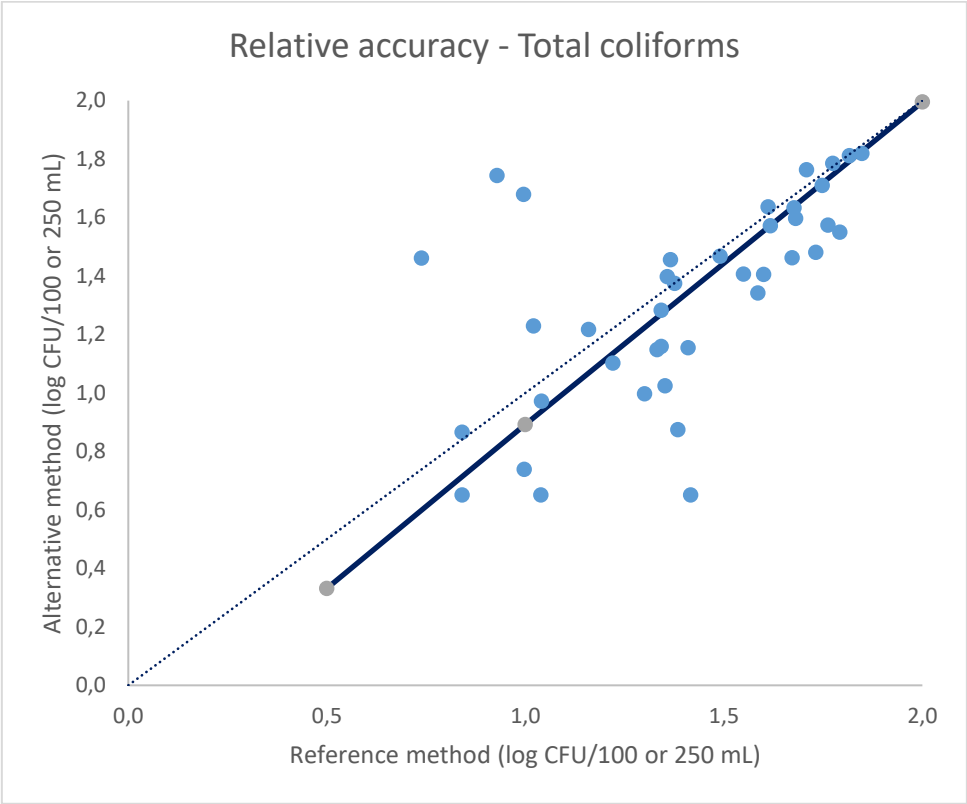
GMFR

		R=	0,923		
		rob. R=	1,490		
				Sx=	0,316
				Sy=	0,348
r=	0,580				
b=	1,103			**Res. SEM=**	0,313
a=	-0,211			**Res. SD=**	0,443

S(b)=	0,161	**t(b)=**	0,641	-2.021<**0.641**<2.021 Hypothèse b=1 validé
S(a)=	0,230	**t(a)=**	0,918	-2.021<**0.918**<2.021 Hypothèse a = 0 validé

Points correspond to the mean of the repetitions for each sample

Est. y	Deviation
1,566	0,070
1,067	0,150
1,766	-0,216
1,717	-0,007
1,258	-0,110
0,889	-0,151
1,827	-0,008
1,746	0,039
0,935	-0,285
1,316	-0,442
1,280	-0,256
1,498	-0,092
0,813	0,931
0,887	0,793
1,351	-0,701
0,914	0,315
0,603	0,858
1,307	0,067
0,937	0,035
1,344	-0,190
1,554	-0,149
1,699	-0,218
0,716	-0,065
1,538	-0,196
1,673	0,091
1,572	-0,001
1,792	0,020
1,296	0,160
1,287	0,111
1,223	-0,226
1,269	-0,110
1,733	-0,159
0,716	0,150
1,643	-0,047
1,269	0,014
1,433	0,035
1,633	-0,171
1,135	-0,033
1,638	-0,005


Relative accuracy - Total coliforms
Alternative method (log CFU/100 or 250 mL)
Reference method (log CFU/100 or 250 mL)
2,0
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
0,0
0,5
1,0
1,5
2,0

Relative accuracy - *E. coli*

Reference method					Alternative method					Difference
Sample	Replicate 1	Replicate 2	M	SD	Sample	Replicate 1	Replicate 2	M	SD	
1	16	13	14,500	2,121	1	17	16	16,500	0,707	2,0
2	63	61	62,000	1,414	2	38	33	35,500	3,536	-26,5
3	46	68	57,000	15,556	3	47	56	51,500	6,364	-5,5
4	9	11	10,000	1,414	4	5	6	5,500	0,707	-4,5
5	71	70	70,500	0,707	5	64	68	66,000	2,828	-4,5
6	60	59	59,500	0,707	6	60	62	61,000	1,414	1,5
7	12	10	11,000	1,414	7	4	5	4,500	0,707	-6,5
8	21	28	24,500	4,950	8	8	7	7,500	0,707	-17,0
9	22	23	22,500	0,707	9	8	14	11,000	4,243	-11,5
10	37	34	35,500	2,121	10	26	25	25,500	0,707	-10,0
11	11	11	11,000	0,000	11	11	8	9,500	2,121	-1,5
12	20	33	26,500	9,192	12	17	12	14,500	3,536	-12,0
13	43	37	40,000	4,243	13	18	36	27,000	12,728	-13,0
14	51	57	54,000	4,243	14	27	34	30,500	4,950	-23,5
15	12	4	8,000	5,657	15	5	4	4,500	0,707	-3,5
16	38	39	38,500	0,707	16	22	22	22,000	0,000	-16,5
17	51	51	51,000	0,000	17	59	57	58,000	1,414	7,0
18	39	44	41,500	3,536	18	29	48	38,500	13,435	-3,0
19	67	64	65,500	2,121	19	60	70	65,000	7,071	-0,5
20	20	27	23,500	4,950	20	23	33	28,000	7,071	4,5
21	20	26	23,000	4,243	21	25	25	25,000	0,000	2,0
22	19	21	20,000	1,414	22	11	9	10,000	1,414	-10,0
23	21	23	22,000	1,414	23	16	13	14,500	2,121	-7,5
24	61	55	58,000	4,243	24	36	39	37,500	2,121	-20,5
25	8	6	7,000	1,414	25	6	9	7,500	2,121	0,5
26	47	49	48,000	1,414	26	39	40	39,500	0,707	-8,5
27	22	22	22,000	0,000	27	16	23	19,500	4,950	-2,5
28	33	29	31,000	2,828	28	27	32	29,500	3,536	-1,5
29	46	48	47,000	1,414	29	30	28	29,000	1,414	-18,0
30	12	23	17,500	7,778	30	10	16	13,000	4,243	-4,5
31	46	49	47,500	2,121	31	45	41	43,000	2,828	-4,5
32	240	150	195,000	63,640	32	110	60	85,000	35,355	-110,0
33	220	150	185,000	49,497	33	90	150	120,000	42,426	-65,0
34	120	70	95,000	35,355	34	80	30	55,000	35,355	-40,0

q= 34
n= 2
N=qn= 68

Mx= 45,4
MEDx= 37,0
SDbx= 42,3
MEDwx = 2,1
SDwx= 14,2
rob. SDwx= 2,8

My= 33,0
MEDy= 28,0
SDby= 26,0
MEDwy = 2,5
SDwy= 10,5
rob. SDwy= 3,7

M= -12,8
MED= -6,0
Bias

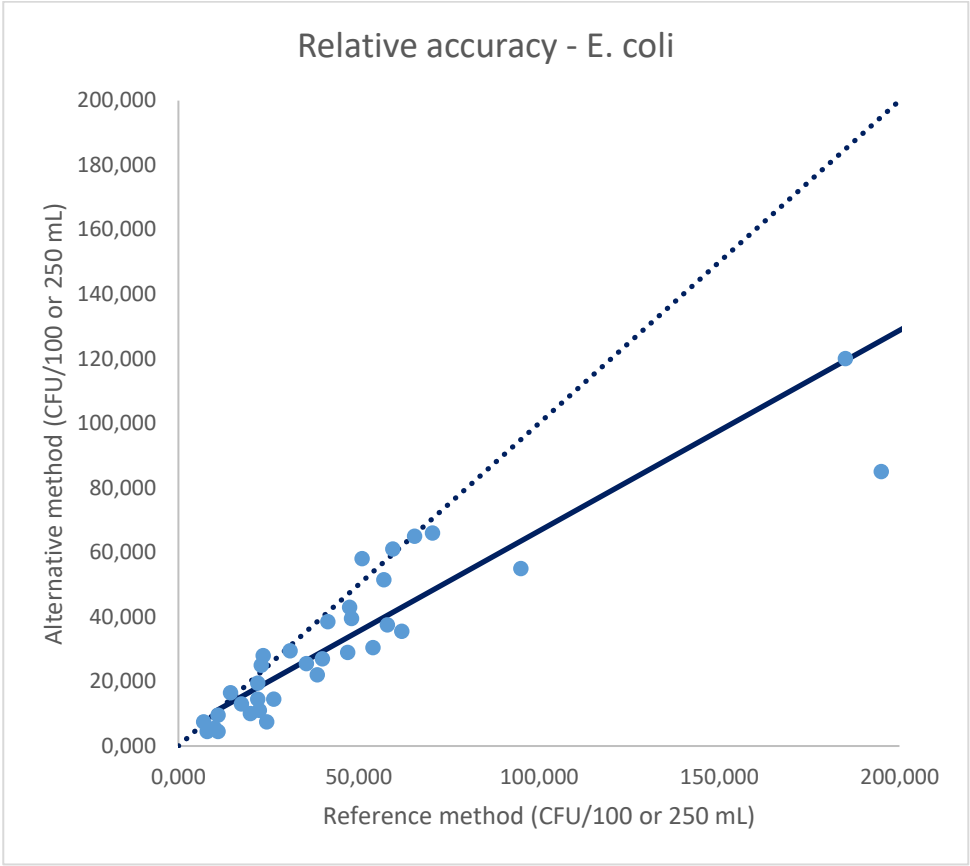
Method	choice
GMFR	

R=	0,739		
rob. R=	1,331		
		Sx=	43,396
		Sy=	26,996
r=	0,902		
b=	0,622	Res. SEM=	29,207
a=	4,388	Res. SD=	41,305

S(b)=	0,123	t(b)=	3,075	**3.075**>2.021 Hypothèse b=1 non validée
S(a)=	7,660	t(a)=	0,573	-2.021<**0.573**<2.021 Hypothèse a = 0 validée

Est. y	Deviation
13,4	3,1
43,0	-7,5
39,8	11,7
10,6	-5,1
48,2	17,8
41,4	19,6
11,2	-6,7
19,6	-12,1
18,4	-7,4
26,5	-1,0
11,2	-1,7
20,9	-6,4
29,3	-2,3
38,0	-7,5
9,4	-4,9
28,3	-6,3
36,1	21,9
30,2	8,3
45,1	19,9
19,0	9,0
18,7	6,3
16,8	-6,8
18,1	-3,6
40,5	-3,0
8,7	-1,2
34,2	5,3
18,1	1,4
23,7	5,8
33,6	-4,6
15,3	-2,3
33,9	9,1

Points correspond to the mean of the repetitions for each sample


Relative accuracy - E. coli
Alternative method (CFU/100 or 250 mL)
200,000
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0,000
0,000
50,000
100,000
150,000
200,000
Reference method (CFU/100 or 250 mL)

Relative accuracy - *E. coli* - Logarithmic data

Reference method					Alternative method					Difference
Sample	Replicate 1	Replicate 2	M	SD	Sample	Replicate 1	Replicate 2	M	SD	
1	1,204	1,114	1,159	0,064	1	1,230	1,204	1,217	0,018	0,058
2	1,799	1,785	1,792	0,010	2	1,580	1,519	1,550	0,043	-0,243
3	1,663	1,833	1,748	0,120	3	1,672	1,748	1,710	0,054	-0,038
4	0,954	1,041	0,998	0,062	4	0,699	0,778	0,739	0,056	-0,259
5	1,851	1,845	1,848	0,004	5	1,806	1,833	1,820	0,019	-0,028
6	1,778	1,771	1,775	0,005	6	1,778	1,792	1,785	0,010	0,011
7	1,079	1,000	1,040	0,056	7	0,602	0,699	0,651	0,069	-0,389
8	1,322	1,447	1,385	0,088	8	0,903	0,845	0,874	0,041	-0,511
9	1,342	1,362	1,352	0,014	9	0,903	1,146	1,025	0,172	-0,328
10	1,568	1,531	1,550	0,026	10	1,415	1,398	1,407	0,012	-0,143
11	1,041	1,041	1,041	0,000	11	1,041	0,903	0,972	0,098	-0,069
12	1,301	1,519	1,410	0,154	12	1,230	1,079	1,155	0,107	-0,256
13	1,633	1,568	1,601	0,046	13	1,255	1,556	1,406	0,213	-0,195
14	1,708	1,756	1,732	0,034	14	1,431	1,531	1,481	0,071	-0,251
15	1,079	0,602	0,841	0,337	15	0,699	0,602	0,651	0,069	-0,190
16	1,580	1,591	1,586	0,008	16	1,342	1,342	1,342	0,000	-0,244
17	1,708	1,708	1,708	0,000	17	1,771	1,756	1,764	0,011	0,056
18	1,591	1,643	1,617	0,037	18	1,462	1,681	1,572	0,155	-0,046
19	1,826	1,806	1,816	0,014	19	1,778	1,845	1,812	0,047	-0,004
20	1,301	1,431	1,366	0,092	20	1,362	1,519	1,441	0,111	0,075
21	1,301	1,415	1,358	0,081	21	1,398	1,398	1,398	0,000	0,040
22	1,279	1,322	1,301	0,030	22	1,041	0,954	0,998	0,062	-0,303
23	1,322	1,362	1,342	0,028	23	1,204	1,114	1,159	0,064	-0,183
24	1,785	1,740	1,763	0,032	24	1,556	1,591	1,574	0,025	-0,189
25	0,903	0,778	0,841	0,088	25	0,778	0,954	0,866	0,124	0,026
26	1,672	1,690	1,681	0,013	26	1,591	1,602	1,597	0,008	-0,085
27	1,342	1,342	1,342	0,000	27	1,204	1,362	1,283	0,112	-0,059
28	1,519	1,462	1,491	0,040	28	1,431	1,505	1,468	0,052	-0,023
29	1,663	1,681	1,672	0,013	29	1,477	1,447	1,462	0,021	-0,210
30	1,079	1,362	1,221	0,200	30	1,000	1,204	1,102	0,144	-0,119
31	1,663	1,690	1,677	0,019	31	1,653	1,613	1,633	0,028	-0,043
32	2,380	2,176	2,278	0,144	32	2,041	1,778	1,910	0,186	-0,369
33	2,079	1,845	1,962	0,165	33	1,954	2,176	2,065	0,157	0,103
34	1,568	1,531	1,550	0,026	34	1,903	1,477	1,690	0,301	0,141

q= 34
n= 2
N=qn= 68

Mx= 1,495
MEDx= 1,550
SDbx= 0,324
MEDwx = 0,033
SDwx= 0,072
rob. SDwx= 0,049

My= 1,370
MEDy= 1,424
SDby= 0,371
MEDwy = 0,059
SDwy= 0,070
rob. SDwy= 0,087

M= -0,125
MED= -0,102
Bias

Method choice

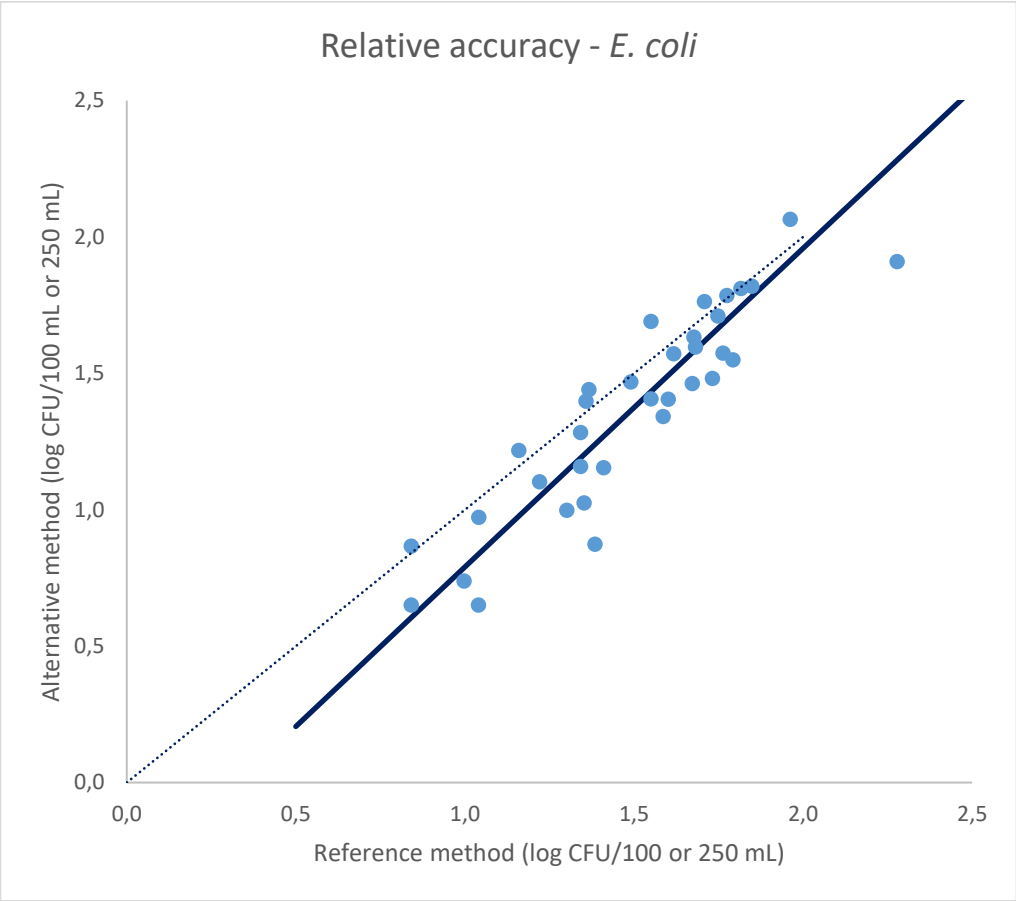
GMFR

		R=	0.980		
		rob. R=	1,785		
				Sx=	0,300
				Sy=	0,349
r=	0,919				
b=	1,164			**Res. SEM=**	0,597
a=	-0,370			**Res. SD=**	0,844

S(b)=	0,364	**t(b)=**	0.450	-2.021<**0.450**<2.021 Hypothèse b = 1 validée
S(a)=	0,554	**t(a)=**	0.668	-2.021<**0.668**<2.021 Hypothèse a = 0 validée

Est. y	Deviation
0,978	0,239
1,715	-0,166
1,664	0,046
0,791	-0,052
1,780	0,039
1,695	0,090
0,839	-0,189
1,241	-0,367
1,203	-0,179
1,433	-0,026
0,841	0,131
1,271	-0,116
1,492	-0,087
1,645	-0,164
0,608	0,043
1,475	-0,133
1,617	0,146
1,511	0,060
1,743	0,068
1,219	0,221
1,210	0,188
1,143	-0,146
1,191	-0,032
1,681	-0,107
0,608	0,258
1,586	0,011
1,191	0,092
1,364	0,104
1,575	-0,113
1,050	0,052
1,581	0,052
0,978	0,239
1,715	-0,166
1,664	0,046

Points correspond to the mean of the repetitions for each sample


Relative accuracy - E. coli
Alternative method (log CFU/100 mL or 250 mL)
Reference method (log CFU/100 or 250 mL)
0,0
0,5
1,0
1,5
2,0
2,5

Appendix 5– Linearity raw data

Legend

cat : catégorie du domaine d'application
CA : contamination artificielle
lac+ : fermentation du lactose positive
ox+ : test oxydase positif
indole + : test indole positif
GAL : souche ayant (+) ou non (-) une activité β-D-Galactosidase
GLUC : souche ayant (+) ou non (-) une activité β-D-Glucuronidase
R1 : réplicat 1
R2 : réplicat 2
ΣC : somme des colonies sur la boîte comptée
Ctot : coliformes totaux
EC : *E. coli*

Raw data

Matrice / souche	Taux visé en UFC/100 ou 250 mL	Taux réel en UFC/100 ou 250 mL	Cat	Quantité filtrée en mL	NF EN ISO 9308-1								Méthode alternative RAPID'E.coli 2 + suppément Water Testing									
					Colonies typiques sur gélose TTC				Résultat en UFC /100 mL				Colonies caractéristiques sur gélose RAPID'E.coli 2 supplémenté									
					Coliformes totaux		*E. coli*		Coliformes totaux		*E. coli*		R1					R2				
					R1	R2	R1	R2	R1	R2	R1	R2	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	□C	Ctot dans 100 mL	*E. coli* dans 100 mL	Colonies Gal+/ Gluc-	Colonies Gal+/ Gluc+	□C	Ctot dans 100 mL	*E. coli* dans 100 mL
Eau de réseau contaminée par ***Escherichia coli*** (Ec3e)	5 à 10	9.20	1a	100	12	19	12	19	1.20E+01	1.90E+01	1.20E+01	1.90E+01	0	16	**16**	1.60E+01	1.60E+01	0	17	**17**	1.70E+01	1.70E+01
	20	18.40	1a	100	29	24	29	24	2.90E+01	2.40E+01	2.90E+01	2.40E+01	0	36	**36**	3.60E+01	3.60E+01	0	33	**33**	3.30E+01	3.30E+01
	200	184.00	1a	100	152	133	152	133	1.52E+02	1.33E+02	1.52E+02	1.33E+02	0	158	**158**	1.58E+02	1.58E+02	0	134	**134**	1.34E+02	1.34E+02
Eau minérale contaminée par ***Escherichia coli*** (Ec2e)	5 à 10	8.17	2a	250	8	11	8	11	3.20E+00	4.40E+00	3.20E+00	4.40E+00	0	10	**10**	4.00E+00	4.00E+00	0	9	**9**	3.60E+00	3.60E+00
	20	16.30	2a	250	19	22	19	22	7.60E+00	8.80E+00	7.60E+00	8.80E+00	0	15	**15**	6.00E+00	6.00E+00	0	16	**16**	6.40E+00	6.40E+00
	200	163.00	2a	250	154	161	154	161	6.16E+01	6.44E+01	6.16E+01	6.44E+01	0	133	**133**	5.32E+01	5.32E+01	0	186	**186**	7.44E+01	7.44E+01

Synthesis of the results (log(UFC/100 mL))

Matrice / souche	Taux visé en UFC/100 ou 250 mL	Taux réel en UFC/100 ou 250 mL	NF EN ISO 9308-1				RAPID'E.coli 2 + suppément Water Testing			
			Résultat en UFC dans 100 mL				Résultat en UFC dans 100 mL			
			Coliformes totaux		*E. coli*		Coliformes totaux		*E. coli*	
			R1	R2	R1	R2	R1	R2	R1	R2
Eau de réseau contaminée par ***Escherichia coli*** (Ec3e)	5 à 10	9.20	1.08	1.28	1.08	1.28	1.20	1.23	1.20	1.23
	20	18.40	1.46	1.38	1.46	1.38	1.56	1.52	1.56	1.52
	200	184.00	2.18	2.12	2.18	2.12	2.20	2.13	2.20	2.13
Eau minérale contaminée par ***Escherichia coli*** (Ec2e)	5 à 10	8.17	0.51	0.64	0.51	0.64	0.60	0.56	0.60	0.56
	20	16.30	0.88	0.94	0.88	0.94	0.78	0.81	0.78	0.81
	200	163.00	1.79	1.81	1.79	1.81	1.73	1.87	1.73	1.87

In red: Not in agreement with the standard NF EN ISO 8199 of 2018 but validation of 2011 realized according to the version of 2005 of the standard.

Linearity - *E. coli* - Coliforms - Tap water - Data in CFU/100 mL

Level
1
2
3

Reference method			
Rep.1	Rep.2	M	SD
12	19	15,5	4,9
29	24	26,5	3,5
152	133	142,5	13,4

Alternative method			
Rep.1	Rep.2	M	SD
16	17	16,5	0,7
36	33	34,5	2,1
158	134	146,0	17,0

q = 3
n = 2
N = qn = 6

Mx = 61,5
MEDx = 26,5
SDbx = 70,4

MEDwx = 4,9
SDwx = 6,0
rob. SDwx = 7,3

My = 65,7
MEDy = 34,5
SDby = 70,2

MEDwy = 2,1
SDwy = 7,0
rob. SDwy = 3,1

Choix méthode OLS2;

y=reference

R = 1,161
rob.R = 0,429

Res.SD = 13,075

M(Alt.)	Ref.	Est. y	Deviation
16,5	12	20,8	-8,8
34,5	29	38,7	-9,7
146,0	152	150,0	2,0
16,5	19	20,8	-1,8
34,5	24	38,7	-14,7
146,0	133	150,0	-17,0

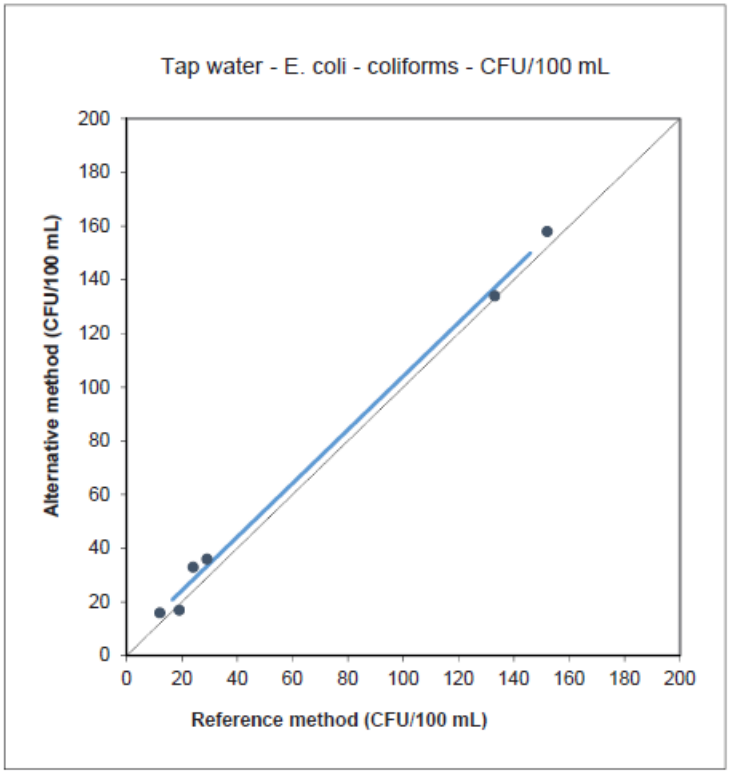
Sx = 63,280
Sy = 63,210

r = 0,999
b = 0,998
a = 4,313

Sb = 0,146
Sa = 11,735

p(t;b=1) = 0,988
p(t;a=0) = 0,732

t (b) = 0,016
t (a) = 0,367



Linearity

F = 11,002
rob.F = 66,129

p(F) = 0,045
rob.p(F) = 0,004

Linearity - *E. coli* - Coliforms - Tap water - Data in log CFU/100 mL

Level
1
2
3

Reference method			
Rep.1	Rep.2	M	SD
1,079	1,279	1,179	0,141
1,462	1,380	1,421	0,058
2,182	2,124	2,153	0,041

Alternative method			
Rep.1	Rep.2	M	SD
1,204	1,230	1,217	0,019
1,556	1,519	1,537	0,027
2,199	2,127	2,163	0,051

q = 3
n = 2
N = qn = 6

Mx = 1,584
MEDx = 1,421
SDbx = 0,507

MEDwx = 0,058
SDwx = 0,065
rob. SDwx = 0,086

My = 1,639
MEDy = 1,537
SDby = 0,481

MEDwy = 0,027
SDwy = 0,025
rob. SDwy = 0,040

Choix méthode OLS2; y=reference

R = 0,381
rob.R = 0,460

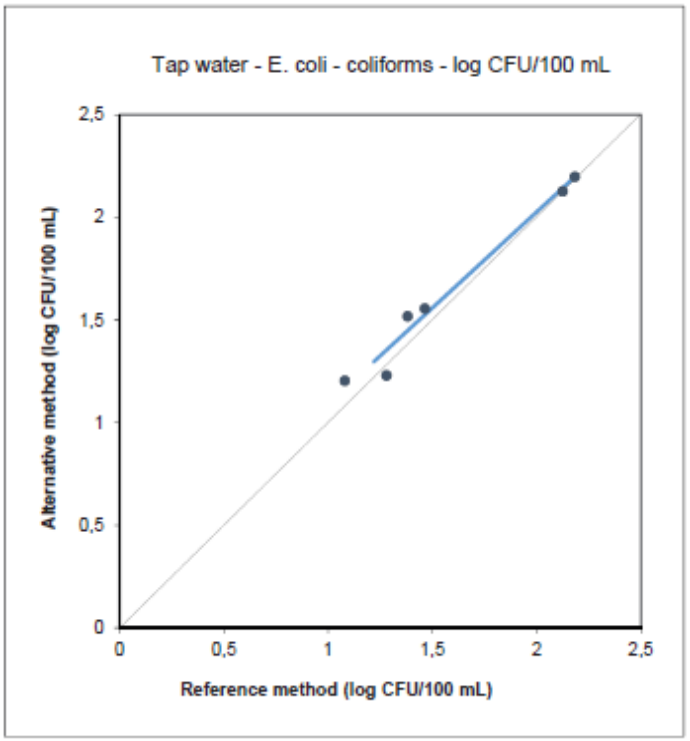
Res.SD = 0,169

M(Alt.)	Ref.	Est. y	Deviation
1,217	1,079	1,296	-0,217
1,537	1,462	1,595	-0,133
2,163	2,182	2,180	0,002
1,217	1,279	1,296	-0,017
1,537	1,380	1,595	-0,215
2,163	2,124	2,180	-0,056

Sx = 0,459
Sy = 0,431

r = 0,995
b = 0,935
a = 0,158

Sb = 0,261 **p(t;b=1) =** 0,814 **t (b) =** 0,251
Sa = 0,424 **p(t;a=0) =** 0,728 **t (a) =** 0,373



Linearity

F = 186,627 **p(F) =** 0,001
rob.F = 69,908 **rob.p(F) =** 0,004

Linearity - *E. coli* - Coliforms - Mineral water - Data in CFU/250 mL

Level
1
2
3

Reference method			
Rep.1	Rep.2	M	SD
8	11	9,5	2,1
19	22	20,5	2,1
154	161	157,5	4,9

Alternative method			
Rep.1	Rep.2	M	SD
10	9	9,5	0,7
15	16	15,5	0,7
133	186	159,5	37,5

q = 3
n = 2
N = qn = 6

Mx = 62,5
MEDx = 20,5
SDbx = 82,5

MEDwx = 2,1
SDwx = 2,4
rob. SDwx = 3,1

My = 61,5
MEDy = 15,5
SDby = 84,9

MEDwy = 0,7
SDwy = 15,3
rob. SDwy = 1,0

Choix méthode OLS2;
y=reference

R = 6,477
rob.R = 0,333

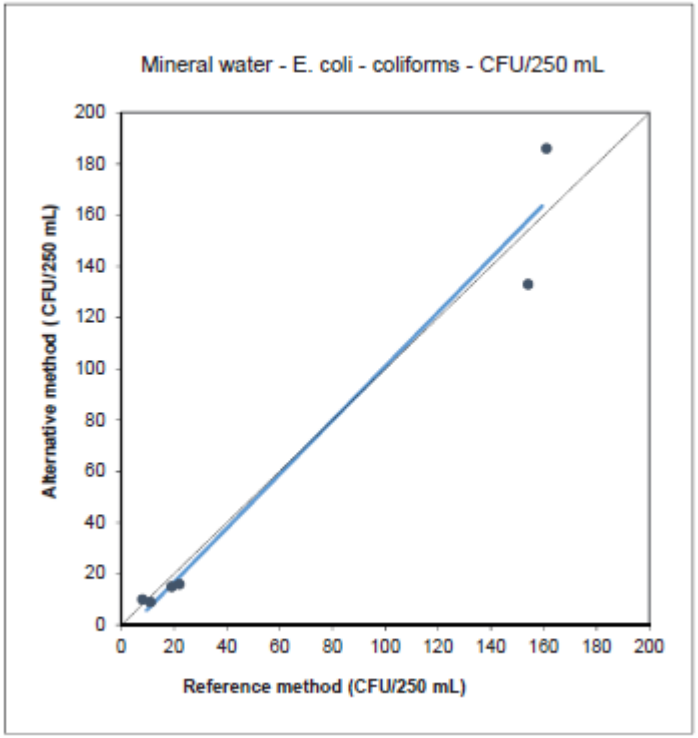
Res.SD = 8,437

M(Alt.)	Ref.	Est. y	Deviation
9,5	8,0	5,7	2,338
15,5	19,0	12,0	7,017
159,5	154,0	163,7	-9,694
9,5	11,0	5,7	5,338
15,5	22,0	12,0	10,017
159,5	161,0	163,7	-2,694

Sx = 73,796
Sy = 77,786

r = 1,000
b = 1,054
a = -4,347

Sb = 0,081 **p(t;b=1) =** 0,544 **t (b) =** 0,662
Sa = 7,019 **p(t;a=0) =** 0,569 **t (a) =** 0,619



Linearity

F = -1,784 **p(F) =** 0,274
rob.F = 256,094 **rob.p(F) =** 0,001

Linearity - *E. coli* - Coliforms - Mineral water - Data in log CFU/250 mL

Level
1
2
3

Reference method			
Rep.1	Rep.2	M	SD
0,903	1,041	1,0	0,098
1,279	1,342	1,3	0,045
2,188	2,207	2,2	0,014

Alternative method			
Rep.1	Rep.2	M	SD
1,000	0,954	1,0	0,032
1,176	1,204	1,2	0,020
2,124	2,270	2,2	0,103

q = 3
n = 2
N = qn = 6

Mx = 1,493
MEDx = 1,311
SDbx = 0,633

MEDwx = 0,045
SDwx = 0,044
rob. SDwx = 0,067

My = 1,455
MEDy = 1,190
SDby = 0,651

MEDwy = 0,032
SDwy = 0,045
rob. SDwy = 0,048

Choix méthode GMFR

R = 1,011
rob.R = 0,719

Res.SEM = 0,098
Res.SD = 0,139

Est y	Déviation
0,918	0,059
1,266	-0,076
2,179	0,017

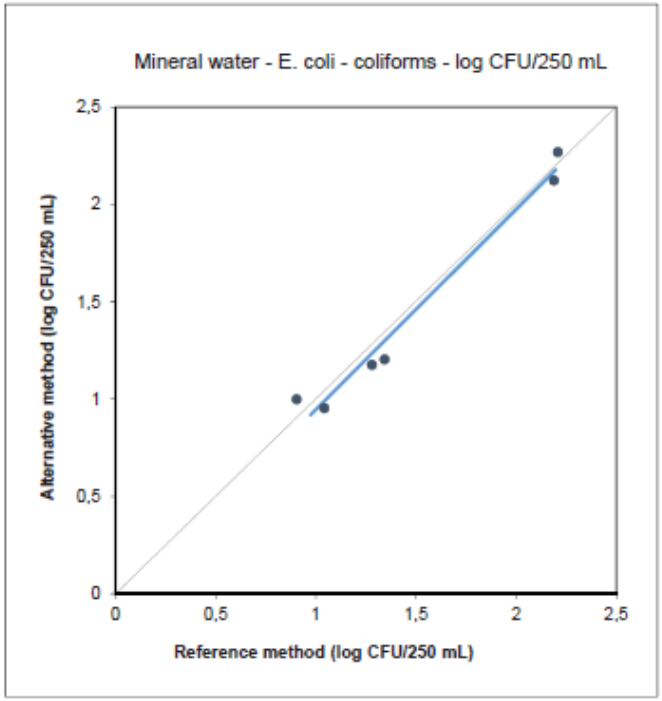
Sx = 0,568
Sy = 0,585

r = 0,994
b = 1,030
a = -0,083

Sb = 0,122 **p(t;b=1) =** 0,820 **t (b) =** 0,242
Sa = 0,191 **p(t;a=0) =** 0,687 **t (a) =** 0,434

Linearity

F = 35,311 **p(F) =** 0,010
rob.F = 30,432 **rob.p(F) =** 0,012



Appendix 6 – Relative level of detection study raw data: LOD-LOQ results

RESULTATS DETAILLES OBTENUS POUR LA DETERMINATION DES LIMITES DE DETECTION (LOD) ET DE QUANTIFICATION (LOQ)

Limite de détection Eau de réseau (catégorie 1a)

Souche utilisée : *E. coli* (eau de réseau, Bruille St Amand, référence : Ec3e)

Résultats de la méthode alternative Rapid'*E.coli* 2 supplémenté

		Réplicats (nombre de colonies par gélose REC2 supp)					
Niveau d'inoculation (UFC/100 mL)	IC*	1	2	3	4	5	6
0,180	0,156-0,207	Ø	Ø	Ø	Ø	Ø	Ø
0,224	0,194-0,257	Ø	Ø	1	Ø	Ø	Ø
0,500	0,455-0,624	Ø	1	Ø	2	3	3
1,440	1,500-1,660	4	1	3	3	2	2

Limite de détection Eau minérale (catégorie 2a)

Souche utilisée : *E. coli* (eau douce (puits), Lille, référence : Ec2e)

Résultats de la méthode alternative Rapid'*E.coli* 2 supplémenté

		Réplicats (nombre de colonies par gélose REC2 supp)					
Niveau d'inoculation (UFC/250 mL)	IC*	1	2	3	4	5	6
0,050	0,044-0,060	Ø	Ø	Ø	1	Ø	1
0,520	0,440-0,605	Ø	Ø	Ø	1	1	Ø
0,705	0,595-0,834	Ø	1	1	Ø	1	2
1,030	0,880-1,210	3	2	4	2	1	4

* IC : intervalle de confiance selon la loi de Poisson

Appendix 7 – Relative level of detection study raw data: selectivity (inclusivity/exclusivity)

Sélectivité
Souches cibles : *Escherichia coli*

	Référence	Souche	Origine	Taux cible inoculum (UFC/100 mL) Résultat sur gélose		Méthode alternative : Rapid'E.coli 2 + supplément Water Testing		
						Croissance et comptage (UFC/100 mL)		
				PCA	TTC	Aspect caractéristique	Résultat	Détection *E. coli*
1	EC1e	*E. coli*	CIP 106878	25	18	+	25	+
2	EC2e	*E. coli*	Eau douce (puits) - Lille (59)	22	19	+	20	+
3	EC3e	*E. coli*	Eau de réseau - Bruille St Amand (59)	26	27	+	25	+
4	EC4e	*E. coli*	Eau de surface - Lac du Héron, Villeneuve d'Ascq (59)	37	35	+	30	+
5	EC5e	*E. coli*	Eau de surface - Etang du parc Barbieux, Croix (59)	18	28	+	26	+
6	EC6e	*E. coli*	Eau de surface - Etang Les Parcs, Wingles (62)	29	37	+	35	+
7	EC7e	*E. coli*	Eau de surface - Etang Parc d'Immercourt, Athies (62)	40	39	+	52	+
8	EC8e	*E. coli*	Eau de surface - Base de loisirs, Biache- St-Vaast (62)	37	28	+	23	+
9	EC9e	*E. coli*	Eau de surface - Etang Loisiparc, Aubigny-au-Bac (59)	45	48	+	50	+
10	EC10e	*E. coli*	Eau de surface - Canal de la Sensée, Arleux (59)	26	27	+	23	+
11	EC11e	*E. coli*	Eau de surface - Lac de Waziers (59)	31	31	+	27	+
12	EC12e	*E. coli*	ATCC 8739	12	14	+	12	+
13	EC13e	*E. coli*	Eau d'alimentation (colonne descendante, traitement javel)	33	39	+	32	+
14	EC14e	*E. coli*	Eau de source - Bruille St Amand	24	27	+	34	+
15	EC15e	*E. coli*	Eau de source - Bruille St Amand	25	29	+	28	+
16	EC16e	*E. coli*	Eau de forage - Noyelle sur Selle	32	31	+	25	+
17	EC17e	*E. coli*	Eau de forage - Croix Fonsonnes	19	25	+	22	+
18	EC18e	*E. coli*	Eau d'alimentation - Verchocq	20	39	+	32	+
19	EC19e	*E. coli*	Eaux d'alimentation - Mouvaux	27	26	+	31	+
20	EC20e	*E. coli*	Eau de source - Bruille St Amand	21	22	+	17	+

Sélectivité : Souches cibles : Coliformes

	Référence	Souche	Origine	Taux cible inoculum (UFC/100 mL) Résultat sur gélose		Méthode alternative : Rapid'E.coli 2 + supplément Water Testing			Commentaires
						Croissance et comptage (UFC/100 mL)			
				PCA	TTC	Aspect caractéristique	Résultat	Détection Coliformes	
1	Cit1e	*Citrobacter braakii*	Eau de forage (Steenwerck)	34	27	+	30	+	
2	Cit2e	*Citrobacter freundii*	ATCC 8090	11	21	+	18	+	
3	Cit3e	*Citrobacter youngae*	Eau de cressonnière (Lillers)	50	65	+	36	+	
4	Cit4e	*Citrobacter youngae*	Eau de forage (Paillencourt)	76	55	+	45	+	
5	Ent1e	*Enterobacter cloacae*	NCTC 13168	36	32	+	35	+	
6	Ent2e	*Enterobacter cloacae*	Eau de piscine	8	11	+	9	+	
7	EN16	*Enterobacter cloacae*	Prélèvement de surface	39	31	+	32	+	
8	Ent3e	*Enterobacter sakazakii*	CIP 5733	22	23	+	26	+	
9	ESC15	*Escherichia hermanii*	Alimentaire	22	28	+	33	+	Colonies bleu clair
10	ESC50	*Escherichia vulneris*	Alimentaire	10	12	+	9	+	
11	Ha67	*Hafnia alvei*	Filet de flétan	32	38	-	38	-	Colonies non caractéristiques (UFC blanches). Souche GAL-/GLUC-
12	Ha36	*Hafnia alvei*	Persil	26	34	+	34	+	Colonies turquoises très pâles
13	Ha37	*Hafnia alvei*	Persil	36	36	+	36	+	Colonies turquoises très pâles
14	Kle1e	*Klebsiella oxytoca*	ATCC 49473	62	55	+	44	+	
15	Kle2e	*Klebsiella planticola*	ATCC 33531	12	19	+	17	+	
16	Kle3e	*Klebsiella terrigena*	ATCC 33257	55	54	+	16	+	
17	Kle4e	*Klebsiella pneumoniae*	ATCC 13883	9	5	+	7	+	
18	Lec1e	*Leclercia adecarboxylata*	Eau de puits	33	20	+	26	+	
19	EN70	*Moellerella wisconsensis*	Andouillette	21	22	+	23	+	
20	Pan1e	*Pantoea spp*	Eau de source (Bruille-les-St-Amand)	43	28	+	33	+	
21	Rah1e	*Rahnella aqualitilis*	Eau de source (Bruille-les-St-Amand)	2	3	+	5	+	
22	Ser1e	*Serratia fonticola*	ATCC 29845	27	26	+	19	+	
23	Ser2e	*Serratia marcescens*	ATCC 8100	30	41	+	33	+	

GAL : souche ayant (+) ou non (-) une activité β-D-Galactosidase

GLUC : souche ayant (+) ou non (-) une activité β-D-Glucuronidase

Sélectivité : Souches non cibles

	Référence	Souche	Origine	Taux cible inoculum (UFC/100 mL) sur PCA	Méthode alternative Rapid'E.coli 2 + supplément Water Testing		Commentaires
					Colonies sur gélose REC2 supp	Détection *E.coli* /Coliformes	
1	Aci1e	*Acinetobacter johnsonii*	CIP 64.6T	5.00E+03	pas de croissance	-	
2	Aer1e	*Aeromonas hydro/caviae*	Eau de forage (Wattrelos)	2.50E+04	colonies non caractéristiques	-	
3	Aer2e	*Aeromonas sp*	ATCC 7966	2.40E+06	pas de croissance	-	
4	Ba 47	*Bacillus badius*	Environnement	1.80E+04	pas de croissance	-	
5	Ba1e	*Bacillus cereus*	CIP 64.52	2.80E+03	pas de croissance	-	
6	Ba 26	*Bacillus circulans*	Environnement	3.20E+05	pas de croissance	-	
7	Ba 24	*Bacillus mycoides*	Environnement (sol)	7.00E+04	pas de croissance	-	
8	Ba2e	*Bacillus subtilis*	ATCC 6633	1.00E+03	pas de croissance	-	
9	Cor1e	*Corynebacterium propinquum*	IPL, eau douce (réseau)	7.80E+04	pas de croissance	-	
10	17	*Erwinia spp*	Alimentaire	9.10E+02	colonies vertes	+	Colonies non caractéristiques sur TTC. GAL+/GLUC-
11	Prot1e	*Proteus mirabilis*	Eau de rivière	7.80E+03	petites colonies non caractéristiques	-	
12	Prot2e	*Proteus vulgaris*	Eau de rivière	5.60E+03	petites colonies non caractéristiques	-	
13	Prov1e	*Providencia stuartii*	Eau de rivière	5.90E+03	petites colonies non caractéristiques	-	
14	Ps1e	*Pseudomonas aeruginosa*	ATCC 9027	3.90E+03	pas de croissance	-	
15	Ps2e	*Pseudomonas aeruginosa*	Eau de thermes	4.70E+03	pas de croissance	-	
16	Ps4e	*Pseudomonas fluorescens*	Eau douce (La Chapelle St Hersin)	1.20E+05	pas de croissance	-	
17	PS12	*Pseudomonas fluorescens*	Eau minérale	5.80E+05	pas de croissance	-	
18	Ps7e	*Pseudomonas mendocina*	/	1.00E+02	pas de croissance	-	
19	Ps5e	*Pseudomonas putida*	CIP 103281	1.50E+05	pas de croissance	-	
20	Ps3e	*Pseudomonas stutzeri*	Effluent (Harnes)	6.30E+03	pas de croissance	-	
21	S162	*Salmonella* IIIb 16:z10:e,n,x,z15	Boue station d'épuration	4.10E+03	présence de colonies violettes	+	Souche GAL+/GLUC+. Colonies non caractéristiques sur TTC
22	Sal1e	*Salmonella* enterica	Eaude forage	5.50E+03	petites colonies non caractéristiques	-	
23	Sal2e	*Salmonella* enterica	ATCC 13311	6.70E+03	petites colonies non caractéristiques	-	
24	Sal3e	*Salmonella* enterica	Eau de forage	4.80E+03	petites colonies non caractéristiques	-	
25	Sal4e	*Salmonella* Enteritidis	Collection	2.20E+03	petites colonies non caractéristiques	-	
26	Sal5e	*Salmonella* Typhimurium	ATCC 14028	6.10E+03	petites colonies non caractéristiques	-	
27	S53	*Salmonella* umbilo	Eau (flaque)	8.40E+03	petites colonies non caractéristiques	-	
28	EN72	*Shigella flexneri*	Alimentaire	1.70E+04	colonies blanches non caractéristiques	-	

	Référence	Souche	Origine	Taux cible inoculum (UFC/100 mL) sur PCA	Méthode alternative Rapid'E.coli 2 + supplément Water Testing		Commentaires
					Colonies sur gélose REC2 supp	Détection *E.coli* /Coliformes	
29	EN73	*Shigella sonnei*	Alimentaire	1.90E+04	colonies bleu marine	+	Souche GAL+/GLUC+. Colonies non caractéristiques sur TTC
30	Sta1e	*Staphylococcus aureus*	ATCC 9144	1.05E+04	pas de croissance	-	
31	Vib1e	*Vibrio fluvialis*	Eau de rivière	8.00E+03	pas de croissance	-	
32	Vib2e	*Vibrio parahaemolyticus*	Eau de réseau	1.00E+03	pas de croissance	-	

Appendix 8 – enumerations of culturable micro-organisms (at 22°C and 36°C)

Laboratoire	Germes revivifiables à 22°C en UFC/mL	Germes revivifiables à 36°C en UFC/mL
A	<1	<1
B	1 Ne (présence <40)	1 Ne (présence <40)
C	<1	<1
D	<1	<1
E	<1	<1
F	/	/
G	<1	<1
H	4 Ne (présence <40)	<1
I	6 Ne (présence <40)	2 Ne (présence <40)
J	5 Ne (présence <40)	1 Ne (présence <40)
K	2 Ne (présence <40)	<1
L	3 Ne (présence <40)	<1
M	<1	<1
N	<1	<1
O	1 Ne (présence <40)	<1
P	1 Ne (présence <40)	<1
Laboratoire expert	<1	<1

Appendix 9 – collaborative laboratories results and synthesis

LEVEL 0

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	0	0	<1	<1	0	0	<1	<1
B	0	0	<1	<1	0	0	<1	<1
C	0	0	<1	<1	0	0	<1	<1
D	0	0	<1	<1	0	0	<1	<1
E	0	0	<1	<1	0	0	<1	<1
G	0	0	<1	<1	0	0	<1	<1
H	0	0	<1	<1	0	0	<1	<1
I	0	0	<1	<1	0	0	<1	<1
J	0	0	<1	<1	0	0	<1	<1
K	0	0	<1	<1	0	0	<1	<1
L	0	0	<1	<1	0	0	<1	<1
M	0	0	<1	<1	0	0	<1	<1
N	0	0	<1	<1	0	0	<1	<1
O	0	0	<1	<1	0	0	<1	<1
P	0	0	<1	<1	0	0	<1	<1
Laboratoire expert	0	0	<1	<1	0	0	<1	<1

Laboratoire	**Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL**							
	44 ± 4 heures d'incubation							
	Réplicat 1				**Réplicat 2**			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	0	0	<1	<1	0	0	<1	<1
B	0	0	<1	<1	0	0	<1	<1
C	0	0	<1	<1	0	0	<1	<1
D	0	0	<1	<1	0	0	<1	<1
E	0	0	<1	<1	0	0	<1	<1
G	0	0	<1	<1	0	0	<1	<1
H	0	0	<1	<1	0	0	<1	<1
I	0	0	<1	<1	0	0	<1	<1
J	0	0	<1	<1	0	0	<1	<1
K	0	0	<1	<1	0	0	<1	<1
L	0	0	<1	<1	0	0	<1	<1
M	0	0	<1	<1	0	0	<1	<1
N	0	0	<1	<1	0	0	<1	<1
O	0	0	<1	<1	0	0	<1	<1
P	0	0	<1	<1	0	0	<1	<1
Laboratoire expert	0	0	<1	<1	0	0	<1	<1

Ct : coliformes totaux

LEVEL 1

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	7	7	7	7	13	10	13	13
B	3	3	3	3	9	9	9	9
C	2	2	2	2	6	5	6	6
D	6	6	6	6	9	9	9	9
E	8	8	8	8	7	7	7	7
G	8	8	8	8	4	4	4	4
H	3	3	3	3	7	7	7	7
I	9	9	9	9	14	10	14	14
J	2	/	/	/	10	/	/	/
K	11	/	/	/	9	/	/	/
L	4	4	4	4	11	11	11	11
M	8	/	/	/	8	/	/	/
N	8	8	8	8	7	7	7	7
O	14	14	14	14	7	7	7	7
P	2	/	/	/	7	/	/	/
Laboratoire expert	10	/	/	/	9	/	/	/

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	44 ± 4 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	7	/	7	7	13	/	13	13
B	3	3	3	3	9	9	9	9
C	2	2	2	2	6	5	6	6
D	6	6	6	6	9	9	9	9
E	8	8	8	8	7	7	7	7
G	8	/	8	8	4	/	4	4
H	3	/	3	3	7	/	7	7
I	9	/	9	9	14	/	14	14
J	2	2	2	2	10	10	10	10
K	11	10	11	11	9	9	9	9
L	5	/	5	5	11	/	11	11
M	8	8	8	8	8	8	8	8
N	8	8	8	8	7	7	7	7
O	14	/	14	14	7	/	7	7
P	3	3	2	2	7	7	7	7
Laboratoire expert	10	10	10	10	9	9	9	9

Ct : coliformes totaux

LEVEL 2

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	24	10	24	24	21	10	21	21
B	17	17	17	17	21	21	21	21
C	23	23	23	23	19	19	19	19
D	28	10	28	28	14	14	14	14
E	25	25	25	25	13	13	13	13
G	17	10	17	17	26	10	26	26
H	21	10	21	21	18	10	18	18
I	18	10	18	18	17	10	17	17
J	18	/	/	/	10	/	/	/
K	30	/	/	/	/	/	/*	/*
L	12	12	12	12	16	16	16	16
M	13	/	/	/	28	/	/	/
N	32	10	32	32	17	17	17	17
O	28	10	28	28	22	10	22	22
P	19	/	/	/	20	/	/	/
Laboratoire expert	30	/	/	/	21	/	/	/

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	44 ± 4 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	24	/	24	24	21	/	21	21
B	17	/	17	17	21	/	21	21
C	23	/	23	23	19	/	19	19
D	28	/	28	28	14	/	14	14
E	25	/	25	25	13	/	13	13
G	17	/	17	17	26	/	26	26
H	28	10	28	28	18	/	18	18
I	18	/	18	18	17	/	17	17
J	18	18	18	18	10	10	10	10
K	30	10	30	30	/	/	/*	/*
L	12	/	12	12	18	/	18	18
M	13	13	13	13	28	28	28	28
N	32	/	32	32	17	/	17	17
O	28	10	28	28	22	10	22	22
P	19	10	19	19	20	10	20	20
Laboratoire expert	30	10	30	30	21	10	21	21

Ct : coliformes totaux

LEVEL 3

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	64	10	64	64	62	10	62	62
B	82	10	82	82	71	10	71	71
C	46	46	46	46	48	48	48	48
D	68	10	68	68	83	10	83	83
E	88	10	88	88	71	10	71	71
G	65	10	65	65	64	10	64	64
H	77	10	77	77	69	10	69	69
I	50	10	50	50	78	10	78	78
J	58	/	/	/	73	/	/	/
K	76	/	/	/	72	/	/	/
L	68	/	/	/	76	/	/	/
M	68	/	/	/	74	/	/	/
N	65	10	65	65	76	10	76	76
O	77	10	77	77	63	10	63	63
P	77	/	/	/	64	/	/	/
Laboratoire expert	91	/	/	/	71	/	/	/

Laboratoire	Méthode de référence NF EN ISO 9308-1 - Filtration 100 mL							
	44 ± 4 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL		Nombre de colonies typiques sur TTC		Résultats en UFC / 100 mL	
	Comptées	Confirmées	Ct	*E. coli*	Comptées	Confirmées	Ct	*E. coli*
A	64	/	64	64	62	/	62	62
B	82	/	82	82	71	/	71	71
C	46	/	46	46	48	/	48	48
D	68	/	68	68	83	/	83	83
E	88	/	88	88	71	/	71	71
G	65	/	65	65	64	/	64	64
H	79	10	79	79	69	10	69	69
I	50	/	50	50	78	/	78	78
J	58	10	58	58	73	10	73	73
K	76	10	76	76	72	10	72	72
L	68	10	68	68	76	10	76	76
M	68	10	68	68	74	10	74	74
N	65	/	65	65	76	/	76	76
O	77	/	77	77	63	/	63	63
P	77	10	77	77	64	10	64	64
Laboratoire expert	91	10	91	91	71	10	71	71

Ct : coliformes totaux

LEVEL 0

Laboratoire	Méthode alternative RAPID'*E. coli* 2 supp - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL		Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL	
	C*	*E. coli*	Ct	*E. coli*	C*	*E. coli*	Ct	*E. coli*
A	0	0	<1	<1	0	0	<1	<1
B	0	0	<1	<1	0	0	<1	<1
C	0	0	<1	<1	0	0	<1	<1
D	0	0	<1	<1	0	0	<1	<1
E	0	0	<1	<1	0	0	<1	<1
G	0	0	<1	<1	0	0	<1	<1
H	0	0	<1	<1	0	0	<1	<1
I	0	0	<1	<1	0	0	<1	<1
J	0	0	<1	<1	0	0	<1	<1
K	0	0	<1	<1	0	0	<1	<1
L	0	0	<1	<1	0	0	<1	<1
M	0	0	<1	<1	0	0	<1	<1
N	0	0	<1	<1	0	0	<1	<1
O	0	0	<1	<1	0	0	<1	<1
P	0	0	<1	<1	0	0	<1	<1
Laboratoire expert	0	0	<1	<1	0	0	<1	<1

C : coliformes autres que *E. coli*
Ct : coliformes totaux

LEVEL 1

Laboratoire	Méthode alternative RAPID'*E. coli* 2 supp - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL		Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL	
	C*	*E. coli*	Ct	*E. coli*	C*	*E. coli*	Ct	*E. coli*
A	0	1	1	1	0	9	9	9
B	0	10	10	10	0	1	1	1
C	0	5	5	5	0	6	6	6
D	0	4	4	4	0	2	2	2
E	0	6	6	6	0	9	9	9
G	0	5	5	5	0	8	8	8
H	0	9	9	9	0	9	9	9
I	0	7	7	7	0	7	7	7
J	0	3	3	3	0	4	4	4
K	0	7	7	7	14*	7	21	21
L	0	2	2	2	0	2	2	2
M	0	3	3	3	0	6	6	6
N	0	5	5	5	0	4	4	4
O	0	2	2	2	0	6	6	6
P	0	2	2	2	0	4	4	4
Laboratoire expert	0	5	5	5	0	8	8	8

C : coliformes autres que *E. coli* (colonies vertes)
Ct : coliformes totaux

LEVEL 2

Laboratoire	Méthode alternative RAPID'*E. coli* 2 supp - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL		Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL	
	C*	*E. coli*	Ct	*E. coli*	C*	*E. coli*	Ct	*E. coli*
A	0	13	13	13	0	14	14	14
B	0	15	15	15	0	10	10	10
C	0	11	11	11	0	16	16	16
D	0	11	11	11	0	12	12	12
E	0	7	7	7	0	17	17	17
G	0	6	6	6	0	21	21	21
H	0	14	14	14	0	16	16	16
I	0	11	11	11	0	16	16	16
J	0	11	11	11	0	11	11	11
K	0	11	11	11	3**	9	12	12
L	0	8	8	8	0	4	4	4
M	0	13	13	13	0	5	5	5
N	0	11	11	11	0	16	16	16
O	0	13	13	13	0	9	9	9
P	0	2	2	2	0	2	2	2
Laboratoire expert	0	24	24	24	0	22	22	22

C : coliformes autres que *E. coli* (colonies vertes)
Ct : coliformes totaux

LEVEL 3

Laboratoire	Méthode alternative RAPID'*E. coli* 2 supp - Filtration 100 mL							
	21 ± 3 heures d'incubation							
	Réplicat 1				Réplicat 2			
	Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL		Nombre de colonies typiques sur REC2 Supp		Résultats en UFC / 100 mL	
	C*	*E. coli*	Ct	*E. coli*	C*	*E. coli*	Ct	*E. coli*
A	0	40	40	40	0	48	48	48
B	0	59	59	59	0	39	39	39
C	0	41	41	41	0	41	41	41
D	/	/	/*	/*	0	50	50	50
E	0	44	44	44	0	60	60	60
G	0	44	44	44	0	61	61	61
H	0	66	66	66	0	20	20	20
I	0	47	47	47	0	45	45	45
J	0	35	35	35	0	30	30	30
K	17**	28	45	45	28**	22	50	50
L	0	35	35	35	0	22	22	22
M	0	51	51	51	1***	60	61	61
N	0	42	42	42	0	43	43	43
O	0	19	19	19	0	38	38	38
P	0	43	43	43	0	35	35	35
Laboratoire expert	0	62	62	62	0	71	71	71

C : coliformes autres que *E. coli* (colonies vertes)
Ct : coliformes totaux
* flacon vide
** colonie verte en surface mais violette sous la membrane identifiée à *E. coli*

Synthesis of collaborative laboratories results (N en UFC/100 mL et log(N))

	Niveau 1				Niveau 2				Niveau 3			
	Méthode de référence		Méthode alternative		Méthode de référence		Méthode alternative		Méthode de référence		Méthode alternative	
Laboratoire	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2
A	7	13	1	9	24	21	13	14	64	62	40	48
B	3	9	10	1	17	21	15	10	82	71	59	39
C	2	6	5	6	23	19	11	16	46	48	41	41
D	6	9	4	2	28	14	11	12	68	83	/*	50
E	8	7	6	9	25	13	7	17	88	71	44	60
G	8	4	5	8	17	26	6	21	65	64	44	61
H	3	7	9	9	28	18	14	16	79	69	66	20
I	9	14	7	7	18	17	11	16	50	78	47	45
J	2	10	3	4	18	10	11	11	58	73	35	30
K	11	9	7	21	30	/*	11	12	76	72	45	50
L	5	11	2	2	12	18	8	4	68	76	35	22
M	8	8	3	6	13	28	13	5	68	74	51	61
N	8	7	5	4	32	17	11	16	65	76	42	43
O	14	7	2	6	28	22	13	9	77	63	19	38
P	2	7	2	4	19	20	2	2	77	64	43	35

log

	Niveau 1				Niveau 2				Niveau 3			
	Méthode de référence		Méthode alternative		Méthode de référence		Méthode alternative		Méthode de référence		Méthode alternative	
Laboratoire	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2	Résultat 1	Résultat 2
A	0.85	1.11	0.00	0.95	1.38	1.32	1.11	1.15	1.81	1.79	1.60	1.68
B	0.48	0.95	1.00	0.00	1.23	1.32	1.18	1.00	1.91	1.85	1.77	1.59
C	0.30	0.78	0.70	0.78	1.36	1.28	1.04	1.20	1.66	1.68	1.61	1.61
D	0.78	0.95	0.60	0.30	1.45	1.15	1.04	1.08	1.83	1.92		1.70
E	0.90	0.85	0.78	0.95	1.40	1.11	0.85	1.23	1.94	1.85	1.64	1.78
G	0.90	0.60	0.70	0.90	1.23	1.41	0.78	1.32	1.81	1.81	1.64	1.79
H	0.48	0.85	0.95	0.95	1.45	1.26	1.15	1.20	1.90	1.84	1.82	1.30
I	0.95	1.15	0.85	0.85	1.26	1.23	1.04	1.20	1.70	1.89	1.67	1.65
J	0.30	1.00	0.48	0.60	1.26	1.00	1.04	1.04	1.76	1.86	1.54	1.48
K	1.04	0.95	0.85	1.32	1.48		1.04	1.08	1.88	1.86	1.65	1.70
L	0.70	1.04	0.30	0.30	1.08	1.26	0.90	0.60	1.83	1.88	1.54	1.34
M	0.90	0.90	0.48	0.78	1.11	1.45	1.11	0.70	1.83	1.87	1.71	1.79
N	0.90	0.85	0.70	0.60	1.51	1.23	1.04	1.20	1.81	1.88	1.62	1.63
O	1.15	0.85	0.30	0.78	1.45	1.34	1.11	0.95	1.89	1.80	1.28	1.58
P	0.30	0.85	0.30	0.60	1.28	1.30	0.30	0.30	1.89	1.81	1.63	1.54