NF VALIDATION

AFNOR CERTIFICATION VALIDATION STUDY

METHOD PETRIFILM AQUA COLIFORM COUNT PLATE (AQCC)
FOR THE ENUMERATION AND DETECTION OF ESCHERICHIA COLI AND COLIFORMS IN NON CHLORINATED DRINKING WATER

SUMMARY REPORT

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3M - Petrifilm AQCC - S.R.(V1) – June 2012

Summary

1. Introduction

1.1. Validation referential

The aim of this validation study is to evaluate the performance of the alternative method against the reference method ISO 9308-1 (2000). It consists in a preliminary study and a collaborative study. The studies follow the Afnor Certification referential (rév. 1 – 05/2010).

1.2. Alternative method

The 3M Petrifilm Aqua Coliform Count (AQCC) plate is a sample-ready culture medium system which contains Violet Red Bile (VRB) nutrients, a cold water soluble gelling agent and a tetrazolium indicator that facilitates colony enumeration. 3M Petrfilm Aqua AQCC plates are used for the enumeration of coliforms in the bottled water industries. ISO Standard ISO 9308-1 "Detection and enumeration of *Escherichia coli* and coliform bacteria" defines coliforms as bacteria capable of forming colonies aerobically on a selective and differential lactose culture medium with the production of acid, which are also oxidase-negative. On 3M Petrifilm Aqua AQCC Plates, confirmed coliform colonies are indicated by red colonies associated with gas, red colonies without gas are presumptive coliforms. Concerning *E. coli* and presumptive coliforms, red colonies associated or not with gas must be confirmed following the diagram below:

Confirm 5 red colonies with or without gas:
-Either by striking on 3M Petrifilm E. coli /coliforms,
-Either by following the confirmation test described in the ISO method (oxidase activity and indole test).

1.3. Scope of application

The scope includes the non chlorinated drinking waters.

1.4. Reference method (*)

The NF EN ISO 9308-1 (2000): "Water quality - Detection and enumeration of *Escherichia coli* and coliform bacteria - Part 1: Membrane filtration method" was used as reference method.

Water filtration
Filter 100 mL (or 250 mL ; bottled water)
of sample water on sterile membrane
Place the membrane on the center of the Petri dish containing TTC-tergitol media

Incubation
Incubate the Petri dish at (36± 2) °C during (21 ± 3) hours
and complementary (24 ± 2) hours if there is non characteristic colonies

Interpretation and Confirmation tests
Characteristic colonies are lactose positive bacteria.
From 10 isolated colonies, perform confirmation test: oxidase and indole production

Results expression
Coliforms number (Oxidase negative)
and *Escherichia coli* (Oxidase negative and Indole positive)
for 100 mL or 250 mL of sample

Figure 1 : alternative method protocol

2. Methods comparative study

The following characteristics are studied during the preliminary study:

- Relative accuracy,
- Linearity of the alternative method,
- Selectivity of the alternative method,
- Limit of detection and limit of quantification of the alternative method
- Practicability of the alternative method

2.1. Relative accuracy

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

2.1.1. Number and nature of samples

Two categories of water were tested (duplicate) with reference method and alternative method: bottled waters and fountain, wells and drilling waters.
Different types of analyzed samples are summarized in table 1.

Target microorganism	Water type	Number of samples analysed	Number of samples used
Coliformes	Bottled waters	22	22
	Non bottled waters	28	22
Escherichia coli	Bottled waters	20	20
	Non bottled waters	26	20
Total		96	84

Table 1: Number and nature of samples analyzed

Globally, 96 samples were analyzed and 84 results were used. No naturally contaminated sample was analyzed. The samples have been artificially contaminated. The contamination levels used cover the entire measurement range of the alternative method.

2.1.2. Results

Figure 3 presents the bidimensional graphics for the four couples matrix/strain. The y axis is reserved for the alternative method and the x axis for the reference method. The statistical calculations and graphics were established from results according to 48 hours incubation for the reference method.

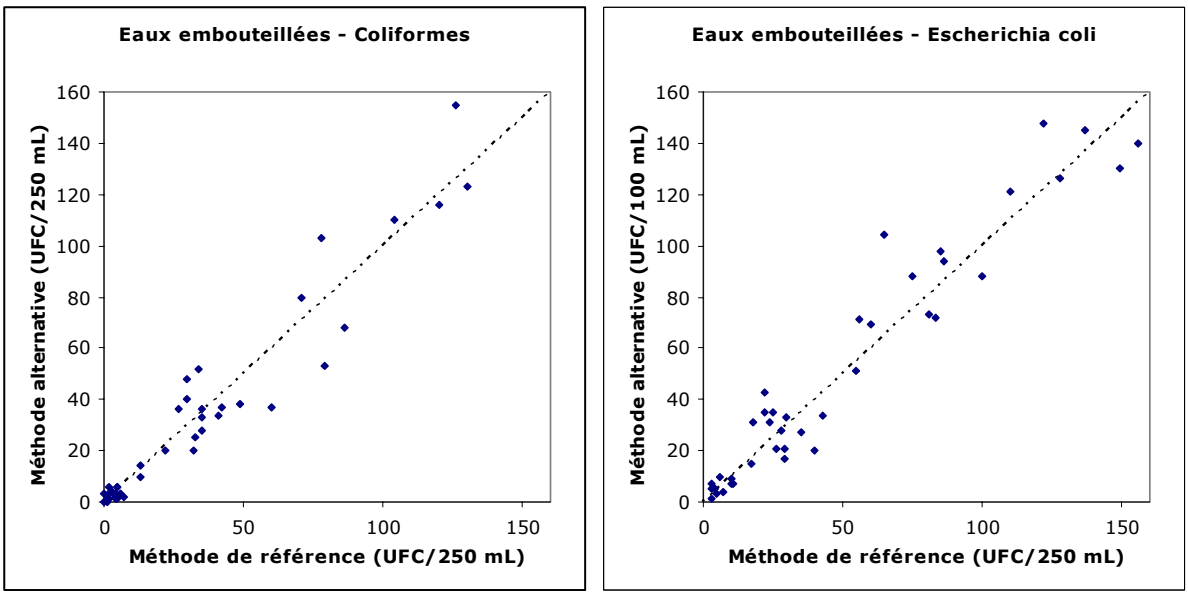


Figure 2: bidimensional graphics representing raw results and trueness

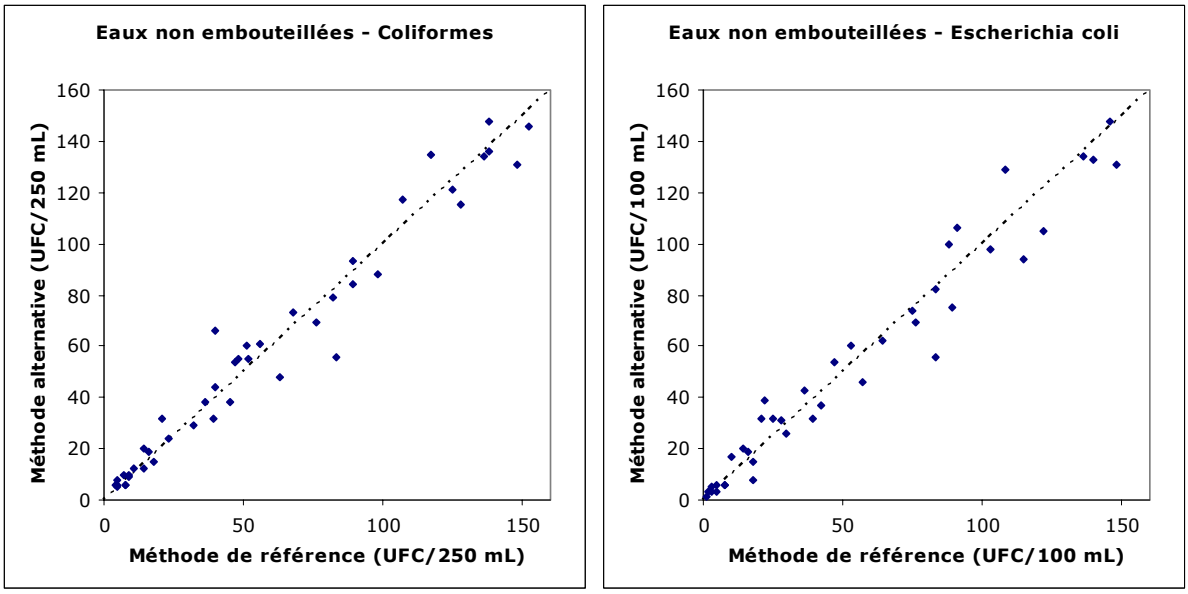


Figure 2: bidimensional graphics representing raw results and trueness

2.1.3. Statistical exploitation

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

The best accuracy between the two methods is reached if the equation y = a + bx is equal to the theoretical model y = x.
The intercept "a" is theoretically zero in this ideal model (case [a = 0]). The estimated intercept obtained with both methods was checked using p {a} = 0. If the alternative method shows a systematic bias against the reference method, the probability p {a = 0} is less than α = 0.05.
The slope "b" is theoretically equal to 1 in the ideal model (hypothesis [b = 1]). The estimated slope obtained with both methods must be verified by p = {b 1}. Statistically, if the alternative method does not give the same values as the reference method, the probability p {b} = 1 is less than α = 0.05.

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

Couple strain / matrice	**Rob.R**	**Regression used**	**T**	**a**	**t(a)**	**b**	**t(b)**	Probabilities (%)	
								Ord. at 0	Slope at 1
Coliforms / Bottled waters	1,000	GMFR	2,074	-1,936	0,840	1,048	0,998	40,6	32,4
E. coli / Bottled waters	0,550	GMFR	2,086	0,331	0,184	1,028	0,811	85,5	42,2
Coliforms / Non bottled waters	1,000	GMFR	2,074	1,541	0,864	0,975	0,762	39,3	45,0
E. coli / Non bottled waters	1,083	GMFR	2,086	1,408	0,851	0,957	1,397	40,0	17,0

Table 2: statistical data

Couple strain / matrice	Bias (D)		Repeatability			
	Average	Median	r		Rob.r	
			MR	MA	MR	MA
Coliforms / Bottled waters	-0,409	-0,500	12,699	22,142	8,806	8,806
E. coli / Bottled waters	1,725	0,500	26,045	22,144	29,354	16,125
Coliforms / Non bottled waters	0,114	0,750	21,746	19,713	17,612	17,612
E. coli / Non bottled waters	-0,950	-0,500	13,920	21,122	17,612	19,080

Table 3: bias and repeatability of the two methods

2.1.4. Conclusion

The equations for the different couples are:

Coliforms / Bottled waters : Alt. = 1,048 Ref. - 1,936
E. coli / Bottled waters : : Alt.= 1,028 Ref. + 0,331
Coliforms / Non bottled waters: Alt. = 0,975 Ref. + 1,541
E. coli / Non bottled waters: Alt. = 0,957 Ref. + 1,408

The accuracy of the alternative method is satisfactory.

2.2. Linearity

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

2.2.1. Contamination level

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

Strain	Matrix	Target level for contamination
Citrobacter freundii CIT.1.4	Mineral water	10- 30- 50- 100 (CFU/100 or 250 mL)
Escherichia coli ESC.1.112	Spring water	
Citrobacter freundii CIT.1.4	Water fountain	
Escherichia coli ESC.1.112	Well water	

Table 4: couples matrix / strain analysed

2.2.2. Results

The graphs in figure 4 show the values of each sample obtained by the alternative method and the reference method. The y axis is for the alternative method and the x axis for the reference method.

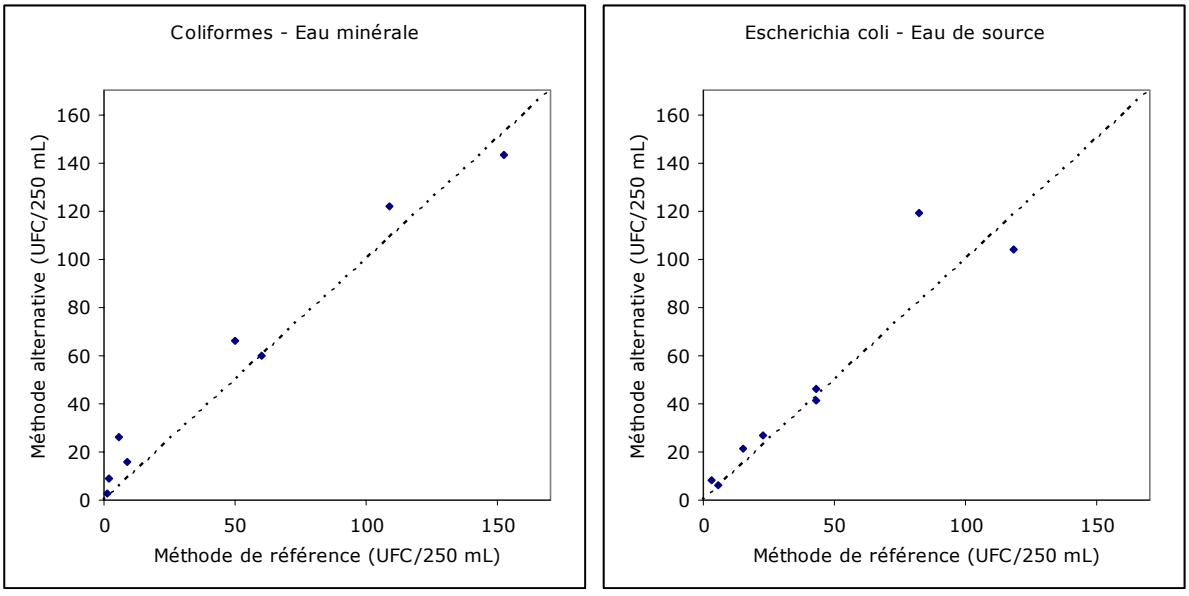


Figure 3: bidimensional graphics representing raw results and trueness

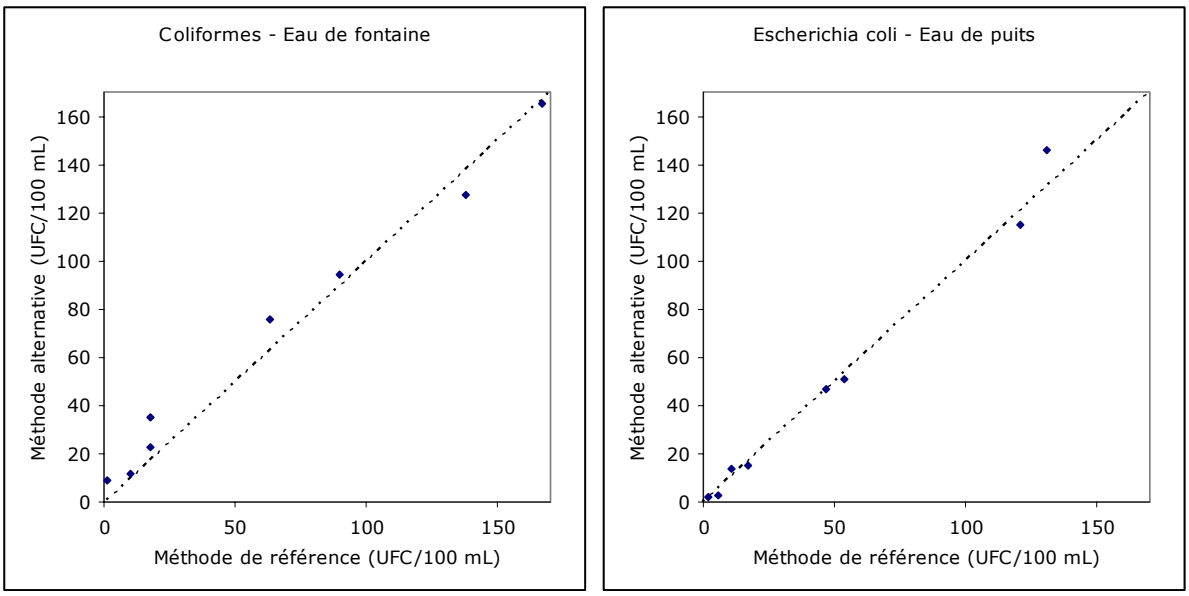


Figure 3: bidimensional graphics representing raw results and trueness

2.2.3. Statistical exploitation

Statistical interpretations are made according to requirements of standard NF ISO 16140 (see table 5).
The choice of the linear regression method is compared to the value of the robustness of the ratio R of the standard deviations of repeatability overall:
-if Rob.R> 2, a linear regression least squares (OLS 1) is used with the x-axis for the reference method,
-if Rob.R <0.5, a linear regression least squares (OLS 2) is used with the x-axis for the alternative method,
-if 0.5 <Rob.R <2, an orthogonal regression (GMFR) is used with the x-axis to the reference method.

Couple strain / matrix	Rob. R	Regression used	F critique	Rob. F	P (Rob.F)	Correlation coefficient (r)	Regression
Coliforms / Mineral water	1,231	GMFR	6,94	0,016	0,985	0,998	Alt.= 0,937 Ref. + 10,079
Escherichia coli / Spring water	1,000	GMFR	6,94	0,802	0,510	0,998	Alt.= 1,063 Ref. + 2,260
Coliforms / Water fountain	0,833	GMFR	6,94	1,217	0,386	0,997	Alt.= 0,915 Ref. + 9,826
Escherichia coli / Well water	0,385	OLS2	6,94	6,368	0,057	1,000	Alt.= 1,053 Ref. - 2,087

Table 5 : statistical data

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

Or
- if P(Rob.F) < α (=0,05)

2.2.4. Conclusion

The relationship between the two methods is linear. The correlation coefficients are satisfactory. The linearity of the alternative method is satisfactory.

2.3. Limit of detection and limit of quantification

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

measured and quantified with an accuracy and precision defined under the experimental conditions.

2.3.1. Test protocols

The limits of detection and quantification were determined by analyzing a pure culture of *Escherichia coli* strain ESC.1.120 and a pure culture of the strain of *Citrobacter freundii* CIT.1.5 by the alternative method. Six levels of contamination (including level 0), with six replications for each level, were studied in sterilized water.

2.3.2. Results

Results are shown in the following tables.

Level (CFU/100mL)	Number of positive samples	Standard deviation (So)	Bias (Xo)
0	0	0,000	0
0,4	2	0,516	0
0,7	1	0,408	0
1,1	4	0,500	0,5
2	5	1,378	1
2,1	6	1,211	2,5

Table 6: data (S_0 et X_0) of *Escherichia coli* enumeration

	Formula	Values obtained
Critical level (CL)	1,65 So +Xo	1,33
Limit of detection (LOD)	3,3 So+Xo	2,15
Limit of quantification (LOQ)	10 So + Xo	5,50

Table7: values obtained of *Escherichia coli* enumeration

Level (CFU/100mL)	Number of positive samples	Standard deviation (So)	Bias (Xo)
0	0	0,000	0
0,4	1	0,408	0
0,7	2	0,516	0
1,5	6	0,516	1
1,9	6	1,049	2,5
2,9	6	2,251	1,5

Table 8: data (S_0 et X_0) of coliforms enumeration

	Formula	Values obtained
Critical level (CL)	1,65 So +Xo	1,85
Limit of detection (LOD)	3,3 So+Xo	2,70
Limit of quantification (LOQ)	10 So + Xo	6,16

Table 9: values obtained of coliforms enumeration

2.3.3. Conclusion

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

2.4. Inclusivity / exclusivity (selectivity)

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

2.4.1. Test protocols

Fifty target strains (including twenty strains of *Escherichia coli*) and thirty non-target strains (from the collections of national, international and internal ISHA) were analyzed. The tests were performed according to the protocol of the alternative method. The contamination rate used for inclusiveness are between 30 and 100 CFU/100 mL and are

10^3 to 10^5 times the detection concentration of the alternative method (about 10^4 CFU / 100 mL) with respect to exclusivity.

2.4.2. Results

Thirty strains of coliforms and *Escherichia coli* tested are detected by the alternative method.
Of the thirty non-target strains tested, twenty-four showed no cross-reactivity with the alternative method. Alternative method was used again with the six strains which showed characteristic colonies to a target level of 100 cfu/100 mL to better identify and confirm the colonies.
A strain of *Providencia stuartii* showed uncountable micro-red colonies without gas. These colonies have nevertheless been confirmed. They have a characteristic profile on Petrifilm *E. coli* / Coliforms, although the colony size is very small. The confirmation is also positive using the confirmation protocol of the reference method. On TTC-Tergitol agar, colonies of this strain have no dark orange discoloration of the medium, so uncharacteristic.
A strain of *Pseudomonas aeruginosa* and one strain of *Aeromonas hydrophila* showed red colonies on Petrifilm AQCC without gas. However, these colonies are not confirmed nor on Petrifilm *E. coli* / Coliforms or with the confirmation protocol of the reference method.
Three strains of Enterobacteriaceae (*Shigella flexneri*, *Salmonella diarizonae* and *Proteus mirabilis*) showed characteristic colonies on Petrifilm AQCC (red without gas), confirmed with both protocols confirmation. These strains have a profile uncharacteristic of TTC-Tergitol medium (orange to red colonies without discoloration of the medium). Another strain of *Shigella* and three other *Salmonella* strains were tested using the same protocol and a similar result was obtained.

Additional tests with wild strains (ten) of *Proteus* and *Providencia* were requested. The results show that strains of *Proteus* and *Providencia* tested present characteristic colonies on the Petrifilm AQCC. Confirmation of colonies obtained by the two protocols is also provided positive. These results confirm those obtained during the preliminary study. Some strains of Enterobacteriaceae do not ferment lactose and with no β-galactosidase can be counted as coliforms.

Following the false-positive results obtained, the Technical Committee was asked to submit additional tests using coliforms bacteria with an uncharacteristic profile on TTC-Tergitol medium (colonies without discoloration of the medium under the membrane) and characteristic on Petrifilm AQCC.

Several strains of Enterobacteriaceae possessing a β-galactosidase showed this type of profile, including *Serratia marcescens* tested inclusiveness (SER.3.1). The table below shows the results obtained in parallel with both methods from artificially contaminated bottled water.

Code strain	Strain	Typical colonies on AQCC	Typical colonies on TTC-Tergitol	Presence of β-galactosidase	Lactose fermentation in 24 at 48 h
SER.3.1	*Serratia marcescens*	Yes	No	Yes	No
EWI.1.1	*Ewingella americana*	Yes	No	Yes	No
ENTB.1.4	*Enterobacter aerogenes*	Yes	No	Yes	No
ESC.2.1	*Escherichia hermanii*	Yes	No	Yes	No
/	*Enterobacter cloacae*	Yes	No	Yes	No

Bacteria with these profiles are considered as coliforms according to recent data.

2.4.3. Conclusion

Given the overall results, we can consider the selectivity of the alternative method Petrifilm AQCC as correct.

2.5. Practicability

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

1- Mode of packaging of test components
The Petrifilm AQCC are packaged in bags of 25 units.

2- Volume of reagents
None.

3- Storage conditions of components and shelf-life of unopened products
The Petrifilm AQCC should be placed in a cool place.

4- Modalities after first use
Each Petrifilm AQCC serves a unique analysis and should not be reused.

5- Equipment and specific local requirements
Usual tools for microbiology laboratory.

6- Reagents ready to use or for reconstitution
None.

7- Training period for operator with no experience with the method
Use of the method Petrifilm AQCC does not require special training.
The duration of training is estimated to be 1 hour.

8- Handling time and flexibility of the method in relation to the number of samples
The duration of a filtration according the reference method (ISO 9308-1) is approximately 1.5 min using disposable filter units and 3.5 min using non-disposable filter units. Duration of use of Petrifilm AQCC method is about 1.5 to 2 min.

9- Time required for results
The delay in obtaining results for the alternative method is 22 ± 2 hours. In case of presence of typical colonies, confirmation may take 24 hours or 48 hours of overtime based on the method selected. The delay in obtaining results for the reference method is 48 hours (count: 24 hours and confirmation: 24 hours) or 72 hours when the colonies are doubtful after 24 hours of incubation.

10- Operator qualification
Identical as necessary for the reference method

11- Steps common with the reference method
None.

12- Traceability of analysis results
None.

13- Maintenance by laboratory
None.

3. Collaborative study

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

3.1. Study organisation

3.1.1. Participating laboratories

The collaborative study was realized by the expert laboratory and fourteen participating laboratories.

3.1.2. Coliforms absence in the matrix

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

3.1.3. Strain stability in the matrix

The strain stability in mineral water matrix was evaluated for 3 days at (5±3)°C. The strain used was *E. coli* (Code ISHA: ESC.1.123) isolated from aquatic environment.
The samples were analysed at D0, D+1 and D+2 by the reference method. The results are summarized in table 10.

	Level 1	Level 2	Level 3
D0	27	80	150
D1	25	77	132
D2	20	63	123

Table 10: results (CFU/ 250 mL) of the stability study of the strain ESC 1.123 in mineral water

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

3.1.4. Samples preparation and spiking

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

-L0: 0 CFU in 250 mL
-L1: 1 to 10 CFU in 250 mL
-L2: 10 to 50 CFU in 250 mL
-L3: 50 to 150 CFU in 250 mL

The matrix was distributed at 505 mL in sterile bottles. Every bottle was individually spiked and homogenized. Eight samples per laboratory were prepared (2 samples per contamination level). Each laboratory received 8 samples to analyse, 1 sample to quantify the endogenous microflora and 1 water sample containing a temperature probe.
The results of the enumerations of the heterophilic flora, the target levels and the real levels of contamination are presented in table 11.

Contamination level	**Flora (CFU/mL)**		***Escherichia coli* ESC.1.131 (CFU/250 mL)**	
	22°C	**36°C**	**Target level**	**Real level**
0	<1	<1	0	0
1			1 to 10	16
2			10 to 50	80
3			50 to 150	130

Table 11: target level, real level and TVC of the matrix

3.1.5. Samples labelling

The labelling of the bags was realized as follows: a code to identify the laboratory: from A to N (cf. table 12) and a code to identify each sample, only known by the expert laboratory. The samples and the temperature control vials (water sample with a temperature probe) were stored at 4°C before shipping.

Contamination level (CFU/ 250 mL)	Sample code
0	4 / 6
1 – 10	3 / 5
10 – 50	2 / 7
50 – 150	1 / 8

Table 12: sample code by contamination level

3.1.6. Samples shipping

The samples were shipped in a coolbox the 9th of January 2012.

3.1.7. Samples reception and analysis

The coolboxes were received the 10th of January 2011 by all the participating laboratories. The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory. The samples were analysed on the 11st of January. The expert laboratory concurrently analysed a set of samples under the same conditions with both methods.

3.2. Results

3.2.1. Temperature and state of the samples

The temperature readings reception and the state of the samples are shown in table 13.

Laboratory	Temperature (°C)	State of the samples
A	5,0°C	Correct
B	6,2°C	Correct
C	/	/
D	2,9°C	Correct
E	10,0°C	Correct
F	5,8°C	Correct
H	7,1°C	Correct
I	5,2°C	Correct
J	4,8°C	Correct
K	12,7°C	Correct
L	5,8°C	Correct
M	/	/
N	5,8°C	Correct

Table 13: temperature and state of the samples upon reception (/: data not available)

Temperature measurements are less than 8.4 ° C for all laboratories with the exception of laboratory E (10,0 °C) and laboratory K (12,7°C). However the probes temperature indicate an average temperature by 4.0 ° C and 7.9 ° C between the sending and opening the package. The data from these laboratories are included in the analysis.
The analysis of thermal profiles of probes is presented in Table 14.

Laboratory		A	B	C	D	E	F	H	I	J	K	L	M	N
Temperature (°C)	Mean	4,9	4,6	X	4,5	4,0	4,9	/	6,2	/	7,9	5,8	X	/
	Standard deviation	0,6	0,8	X	1,4	1,0	0,8	/	0,3	/	0,5	0,7	X	/

Table 14: data of the temperature probes for the transportation time of samples (/: data not available)

The laboratory C, although it has not returned his acknowledgment or the temperature sensor, said the expert laboratory by mail that the temperature of the control flask at reception was less than 8.4 ° C.

3.2.2. Total viable counts

For the whole laboratories, the total viable counts at 22°C vary between < 1 and 1600 CFU/mL. Concerning the total viable counts at 36°C, the results were varying between < 1 and 46 CFU/mL.

3.2.3. Expert laboratory and collaborating laboratories results

The overall results are presented in Table 15. The results of the reference method are presented for a reading of the Petri dishes after 48 h incubation at (36 ± 2)°C. The results of the alternative method are presented for a reading of Petrifilm after (24 ± 2) h of incubation at (36 ± 2)°C.
For both methods, all counted coliforms were confirmed as *E. coli*. All results presented are expressed in number of *Escherichia coli* per 250 mL of water.

Data of laboratories H, I and L were excluded from final analysis of results:

-Laboratory H presented results are very different comparing target contamination level and between repetition values for the two methods. However, for each sample tested, the values were concordant between the alternative method and the reference method.

-Laboratory I, all results obtained with the reference method are between 0 and 3 UFC/250 mL while the results of the alternative method seem correct. The lead researcher in this laboratory has been contacted by the expert laboratory and it seems that the person who analyzes have encountered problems in sample handling.

-Laboratory L reported that the volume of water was less than 250 mL for two samples analyzed using the alternative method.

The laboratory M did not return his scorecard at the Expert laboratory despite several reminders.

The results of nine laboratories are presented in the following table 15.

Laboratory	Level 0				Level 1				Level 2				Level 3			
	MR		MA		MR		MA		MR		MA		MR		MA	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
A	<1	<1	<1	<1	10	6	13	13	63	75	60	60	99	103	95	97
B	<1	<1	<1	<1	15	16	15	12	67	71	62	40	82	90	108	105
C	<1	<1	<1	<1	12	17	13	15	67	67	59	68	97	103	79	86
D	<1	<1	<1	<1	12	10	15	13	68	57	68	68	82	92	80	105
E	<1	<1	<1	<1	9	18	14	17	71	68	70	70	97	126	115	94
F	<1	<1	<1	<1	23	18	14	13	82	71	80	68	105	98	95	88
J	<1	<1	<1	<1	21	8	15	12	65	62	71	79	98	90	108	105
K	<1	<1	<1	<1	18	10	19	13	41	53	50	49	73	62	71	46
N	<1	<1	<1	<1	15	20	16	18	77	84	32	71	99	93	110	104
Expert	<1	<1	<1	<1	18	17	17	11	68	60	50	57	115	78	110	86

Table 15: *E. coli* enumeration results for 250 mL sample water (MR: reference method, MA: alternative method, R1: repetition 1 and R2: repetition 2)

3.3. Interpretation

The data presented in the following paragraphs were calculated from the results in CFU/250 mL in the same way that the presentation of the results of the preliminary study.

3.3.1. Bias calculation

Table 16 shows the target value, the mean, standard deviation of fidelity, the relative bias and the bias of each level of contamination.

Contamination level	Low	Medium	High
Target value	**15,000**	**67,500**	**97,000**
Average	14,444	62,500	93,944
Standard deviation of fidelity	2,055	12,818	17,346
Intermediary coefficient	1,027	1,033	1,045
Number of degree of freedom	16,941	15,737	11,206
Relative bias f	-0,037	-0,074	-0,032
Bias	**0,963**	**0,926**	**0,968**

Table 16: Calculation of the alternative method bias

3.3.2. Accuracy profile

Table 17 shows the values of tolerance and the tolerance limits of the alternative method for a probability value of tolerance of 90% and a limit value of 50% acceptability.

Acceptability limits	Probability of tolerance	Levels	Low	Medium	High
50%	90%	Low tolerance value	10,771	39,347	61,439
		High tolerance value	18,118	85,653	126,450
		Low tolerance limit	72%	58%	63%
		High tolerance limit	121%	127%	130%
		Low acceptability limit	50%	50%	50%
		High acceptability limit	150%	150%	150%

Table 17: Values and tolerance limits of the alternative method

Figures 5 and 6 show the accuracy profiles using respectively CFU/250 mL and log CFU/250 mL.

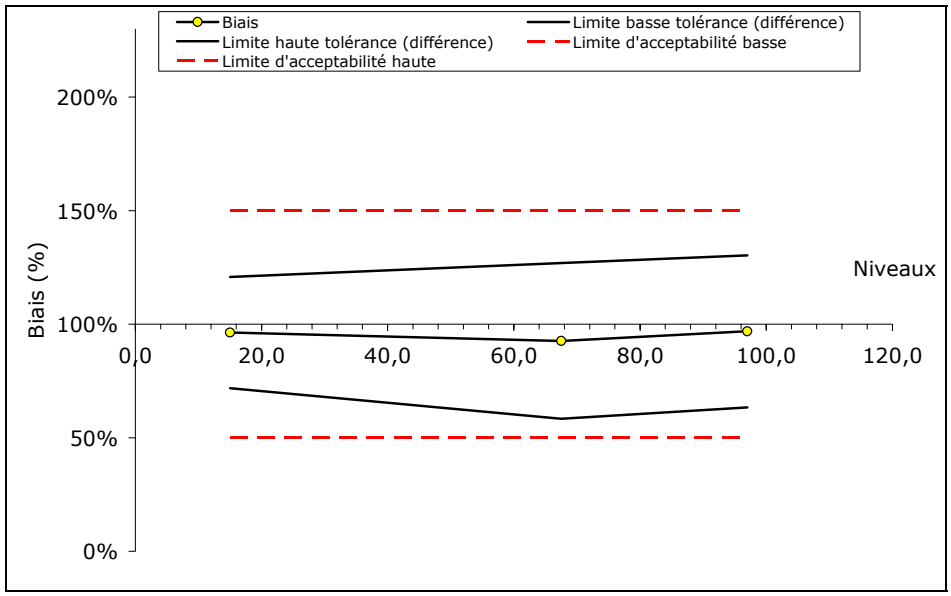


Figure 5: Accuracy profile of the alternative method with tolerance probability of 90 % and acceptability limits at 50%

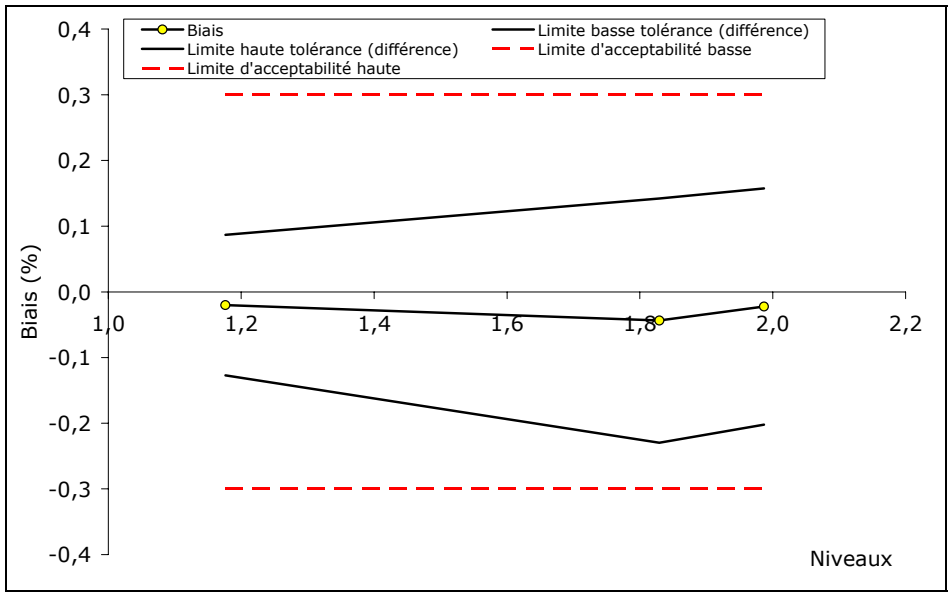


Figure 6: Accuracy profile of the alternative method with tolerance probability of 90 % and acceptability limits at 0,3 log

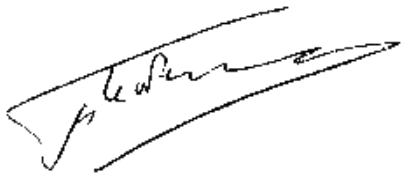
- Comments

The accuracy profile obtained from the results of the reference method and the alternative method shows that the bias of AQCC method for the enumeration of coliforms and *Escherichia coli* in non chlorinated drinking water is acceptable. The tolerance limits of the alternative method for a probability of 90% tolerance are included within the limits of acceptability of 50% or 0,3 log.

4. Conclusion

The linearity and relative accuracy of the method AQCC Petrifilm for the enumeration of *Escherichia coli* and coliforms in non chlorinated drinking water are satisfactory.
The bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.
Petrifilm AQCC method for the enumeration of coliforms and *Escherichia coli* is specific and selective.

The bias of the alternative method is relatively stable from the low level of contamination to the high level of contamination. It goes from 0.963 to 0.926 and then 0.968 UFC/250 mL. 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 50%.



Massy, the 8th of June 2012
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