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

Summary report – V0
June 2020
Quantitative method

Certificat n°IDX 33/02-06/12

Renewal validation study

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This report includes 55 pages, including 8 appendices.
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Preamble

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

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

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

Scope:
Bathing waters which groups two types of waters:

- fresh waters
- sea waters

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

*Analyses performed according to the COFRAC accreditation

1. Introduction

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

2. Modifications since the previous validation

2.1. History of validation

The history of validation was summarized in the table below:

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

2.2. Summary of modifications in the alternative method

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

Modification of the alternative method

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

2.3. Users' complaints

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

3. Method Protocol

3.1. Alternative method

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

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

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

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

Figure 1 : protocol of the alternative method

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

↓

2.
Cap vessel and shake until dissolved.

↓

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

↓

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

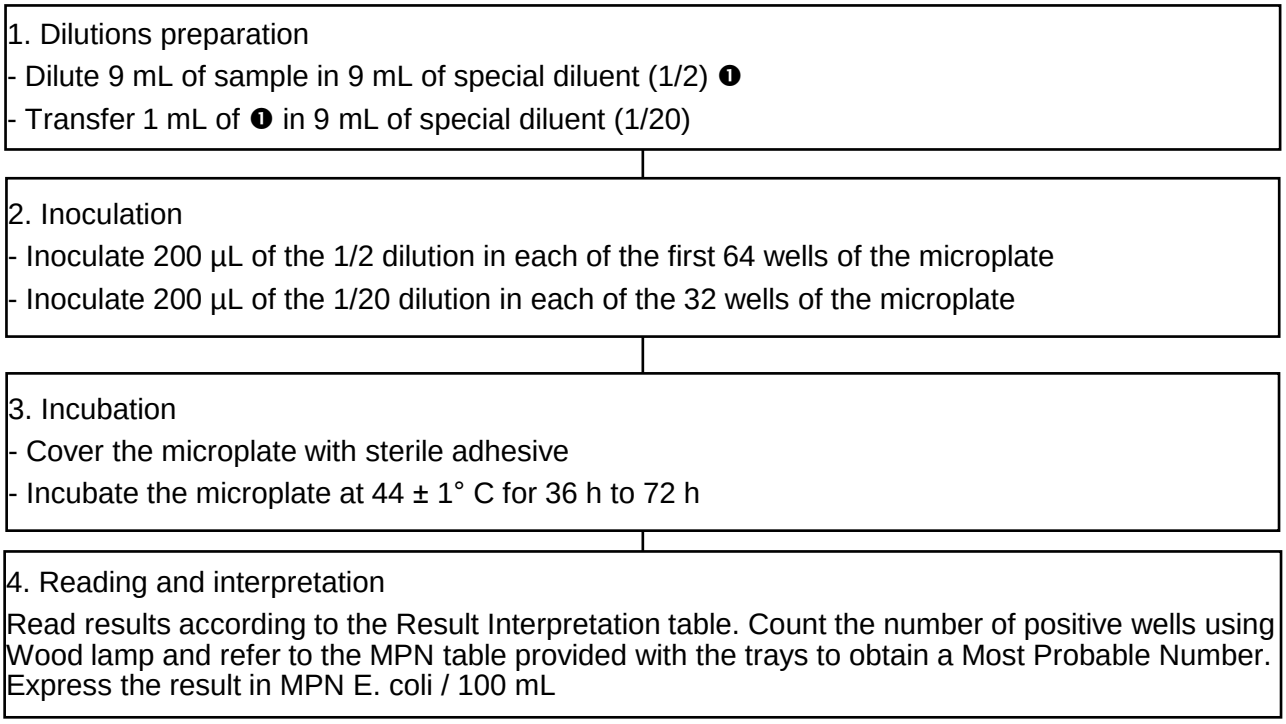
↓

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

3.2. Reference method

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

Figure 2: protocol of the reference method



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

4.1. Methods comparative study

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

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

4.1.1. Relative accuracy

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

- **Number and nature of samples**

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

Table 2 : Number and nature of samples analyzed

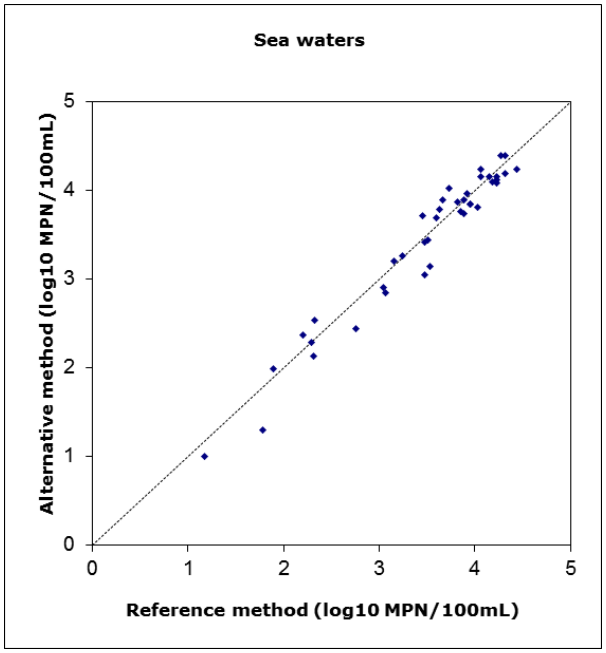
Water type	Number of samples analyzed	Number of samples used
Sea waters	53	**20**
Fresh waters	41	**22**
Total	**94**	**42**

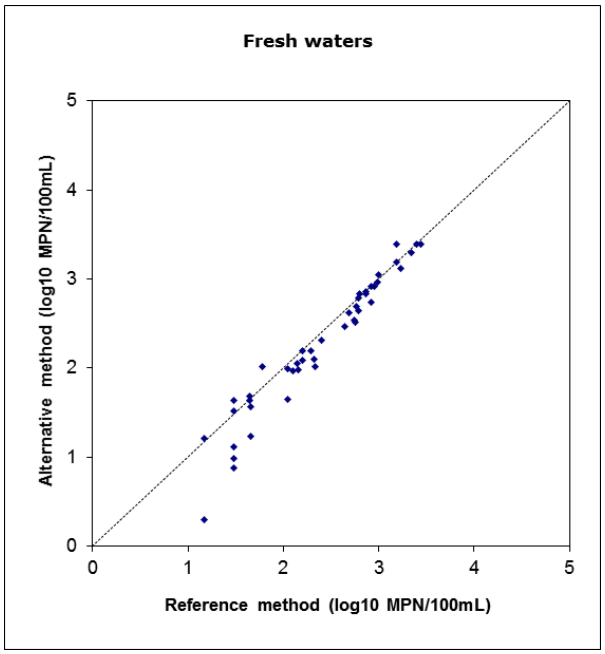
Globally, 94 samples were analyzed and 42 results were used. 16 naturally contaminated samples were analyzed. Others samples were artificially contaminated (cf. appendix 1).
The contamination levels used cover the entire measurement range of the alternative method.

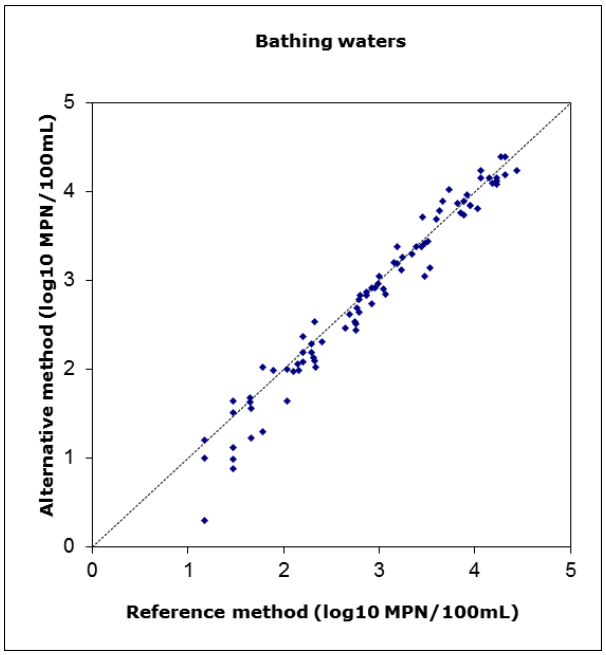
- **Results**

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

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







- **Statistical analysis**

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

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

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

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

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

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

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

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

- Sea waters

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

- Fresh waters

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

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

- Bathing waters (seawaters + freshwaters)

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

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

- Remark:

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

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

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

Fresh waters:	Bathing waters:
- r = 0.989,	- r = 0.991
- log Alt. = 1.034 log Ref. – 0.158	- log Alt. = 1.040 log Ref. – 0.180

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

- **Conclusion**

The relative accuracy of the alternative method is satisfactory.

4.1.2. Linearity

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

- **Contamination levels**

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

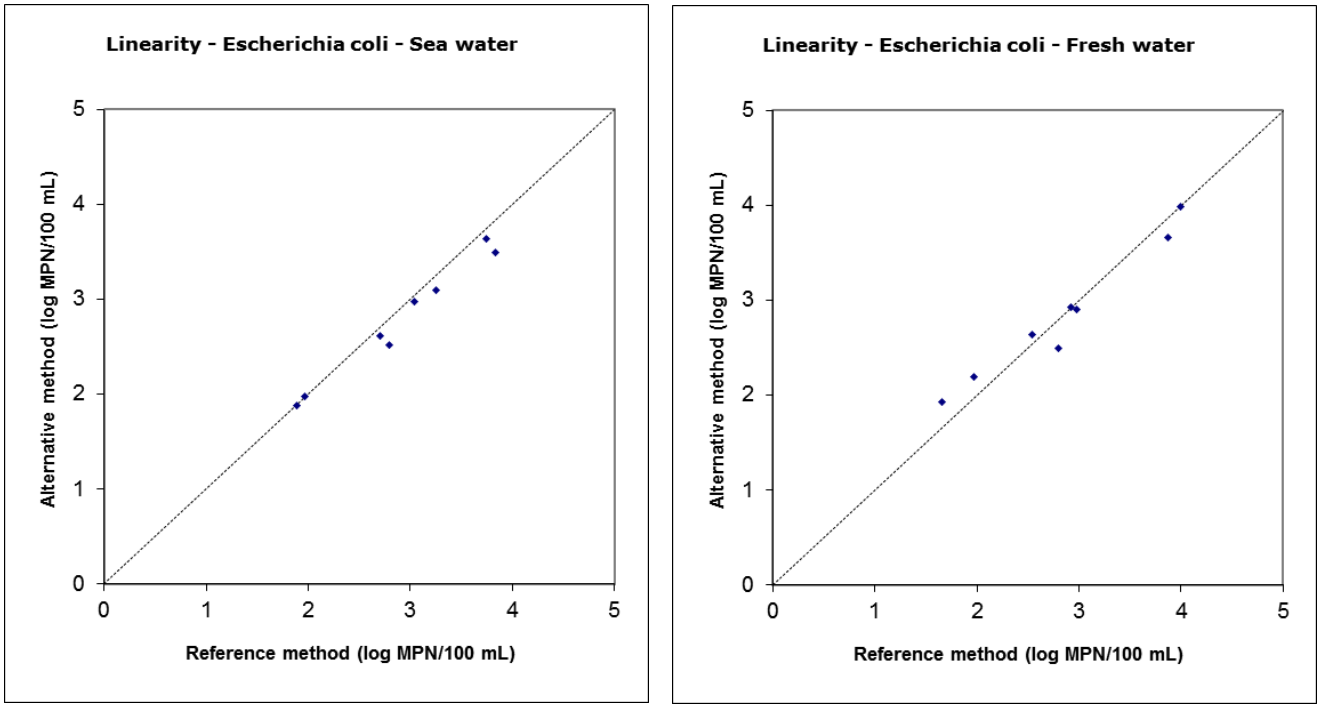
Table 4 : couples matrix – strain analyzed

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

- **Results**

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

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



- **Statistical analysis**

Statistical interpretations are made according to requirements of standard NF ISO 16140 (see table 5).

The choice of the linear regression method is compared to the value of the robustness of the ratio R of the standard deviations of repeatability overall:

- if Rob.R> 2, a linear regression least squares (OLS 1) is used with the x-axis for the reference method,
- if Rob.R <0.5, a linear regression least squares (OLS 2) is used with the x-axis for the alternative method,
- if 0.5 <Rob.R <2, an orthogonal regression (GMFR) is used with the x-axis to the reference method.

Table 5: statistical data for the linearity

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

The relationship between the 2 methods is not linear:

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

- **Conclusion**

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

4.1.3. Limit of detection and limit of quantification

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

Here are their ISO 16140 definitions:

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

- **Test protocols**

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

- **Results**

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

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

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

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

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

- **Conclusion**

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

4.1.4. Selectivity

The selectivity of the alternative method is evaluated by its inclusivity and its exclusivity.
Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.
Exclusivity is the lack of interference by a relevant range of non-target strains with the alternative method.

- **Test protocols**

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

- **Results**

Raw results are in appendix 6.
All target strains tested are detected by the alternative method except for one strain (which is not detected by the reference method either).
For the thirty non-target strains tested, no positive result was observed. See tables below.

- **Conclusion**

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

4.1.5. Practicability

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

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

4.2. Interlaboratory study

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

4.2.1. Study organisation

- **Participating laboratories**

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

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

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

- **Strain stability in the matrix**

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

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

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

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

- **Samples preparation and spiking**

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

-level 0: 0 CFU/100 mL,
-level 1: from 50 to 100 CFU/100 mL,
-level 2: from 250 to 500 CFU/100 mL,
-level 3: from 1000 to 1500 FCU/100 mL

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

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

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

- **Samples labelling**

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

Table 10: sample code by contamination level

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

- **Samples shipping**

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

- **Samples reception and analysis**

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

4.2.2. Results

- **Temperature and state of the samples**

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

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

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

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

- **Total viable counts**

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

- **Expert laboratory and collaborating laboratories results**

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

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

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

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

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

Laboratory	Level 0							
	MR						MA	
	R1			R2			R1	R2
	MPN/ 100 mL	Low limit	High limit	MPN/ 100 mL	Low limit	High limit	MPN/100 mL	MPN/100 mL
A	<15	/	/	<15	/	/	<10	<10
B	<15	/	/	<15	/	/	<10	<10
C	<15	/	/	<15	/	/	<10	<10
D	<15	/	/	<15	/	/	<10	<10
E	<15	/	/	<15	/	/	<10	<10
F	<15	/	/	<15	/	/	<10	<10
G	<15	/	/	<15	/	/	<10	<10
H	<15	/	/	<15	/	/	<10	<10
I	<15	/	/	<15	/	/	<10	<10
J	<15	/	/	<15	/	/	<10	<10
K	<15	/	/	<15	/	/	<10	<10
L	<15	/	/	<15	/	/	<10	<10
M	<15	/	/	<15	/	/	<10	<10
N	<15	/	/	<15	/	/	<10	<10
O	<15	/	/	<15	/	/	<10	<10
Expert	<15	/	/	<15	/	/	<10	<10
Laboratory	Level 1							
	MR						MA	
	R1			R2			R1	R2
	MPN/100 mL	Low limit	High limit	MPN/100 mL	Low limit	High limit	MPN/100 mL	MPN/100 mL
A	93	41	206	93	42	207	86	108
B	127	63	253	109	52	230	10	41
C	94	42	208	94	42	208	75	63
D	127	63	253	<15	/	/	41	63
E	110	52	231	15	2	106	52	63
F	46	15	142	61	23	163	63	41
G	77	32	186	160	86	298	98	135
H	15	2	106	46	15	142	51	52
I	125	62	251	61	23	163	97	30
J	61	23	163	61	23	163	52	52
K	94	42	208	93	42	207	40	41
L	94	42	208	144	75	276	52	122
M	197	63	253	46	15	142	95	109
N	94	42	208	46	15	142	62	74
O	127	63	253	126	63	252	119	109
Expert	126	63	252	30	8	121	40	84

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

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

4.2.3. Interpretation

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

- **Bias calculation**

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

Table 13: Calculation of the alternative method bias

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

- **Accuracy profile**

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

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

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

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

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

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

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

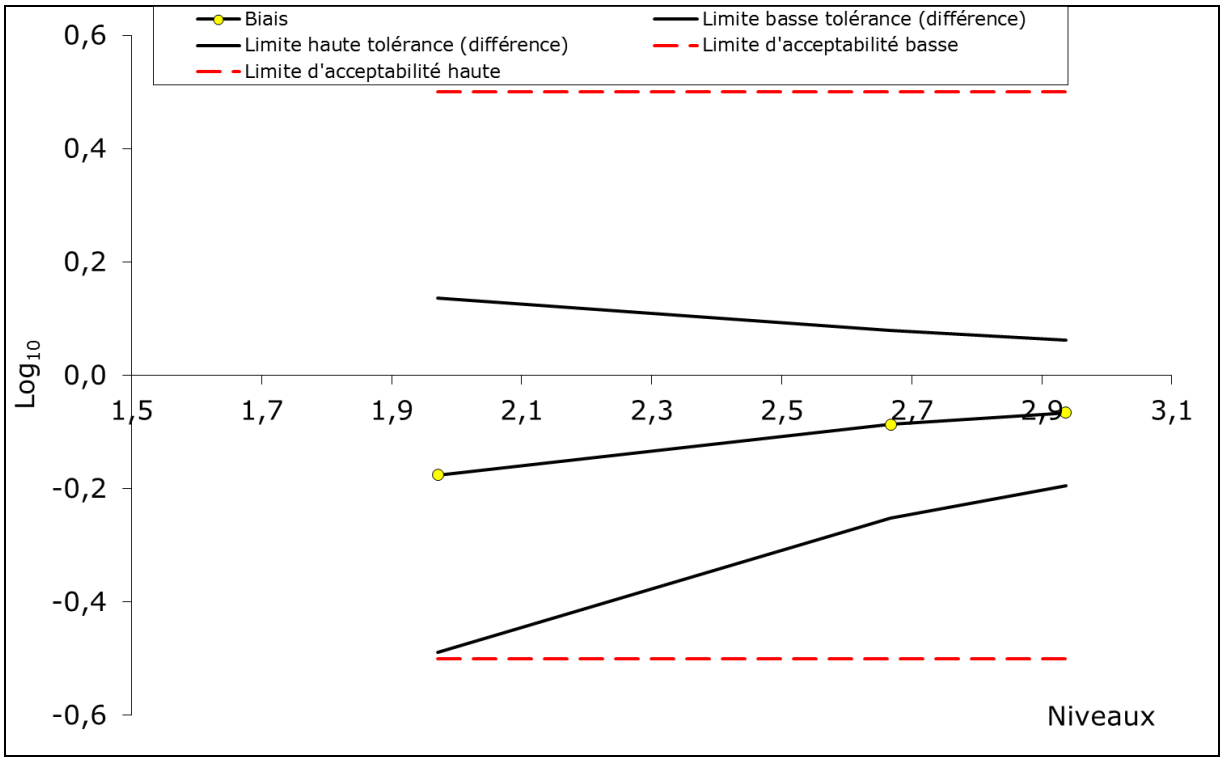
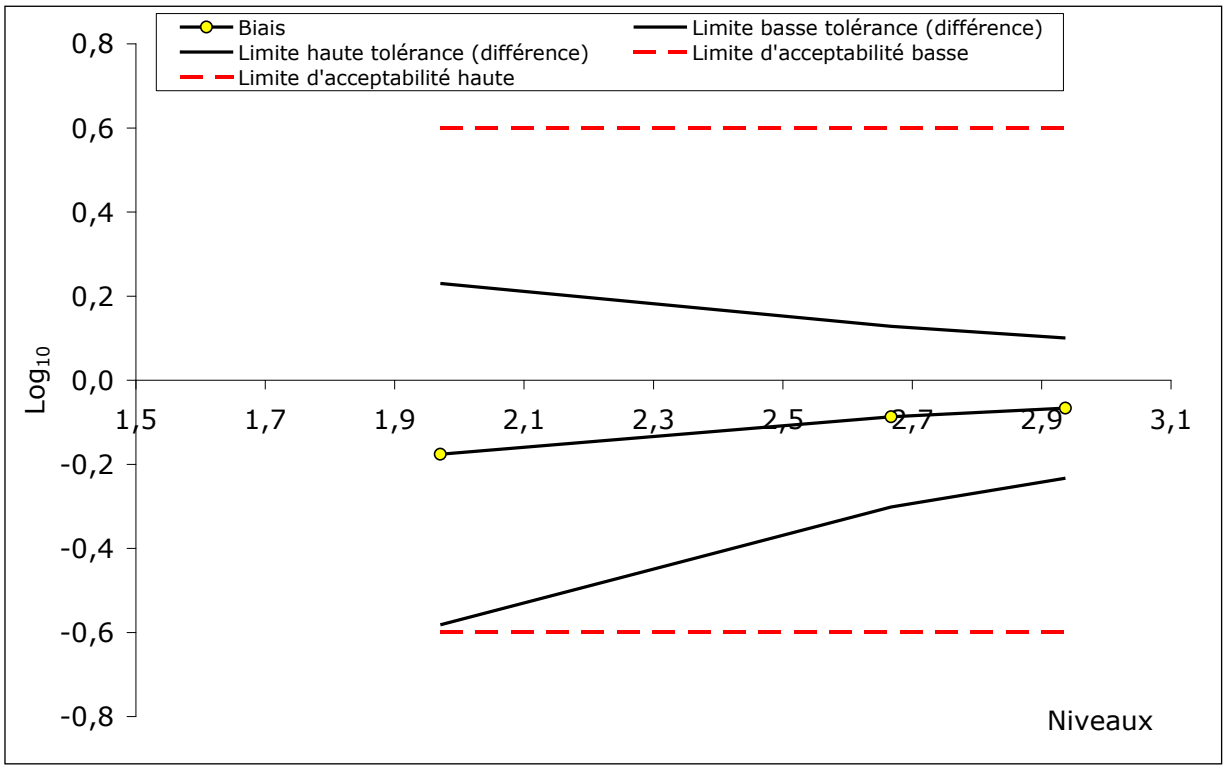


Figure 6: Accuracy profile of the alternative method with tolerance probability of 90 % and acceptability limits at 0,6 log



- Comments

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

4.3. Extension study

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

4.3.1. Results and interpretation

Two sets of results are available:

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

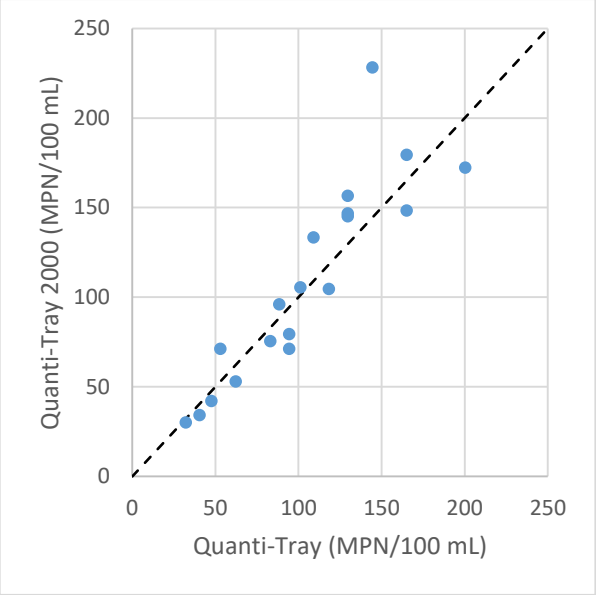
- **Results from Colilert-18 / Quanti-Tray study**

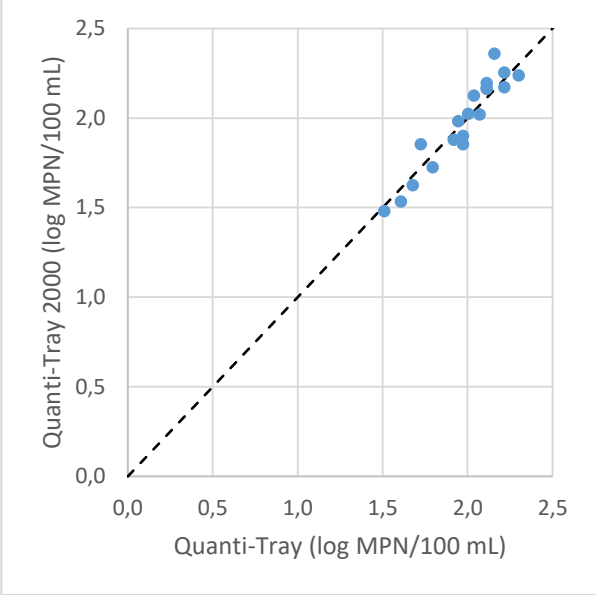
Raw results

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

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

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





Statistical interpretation

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

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

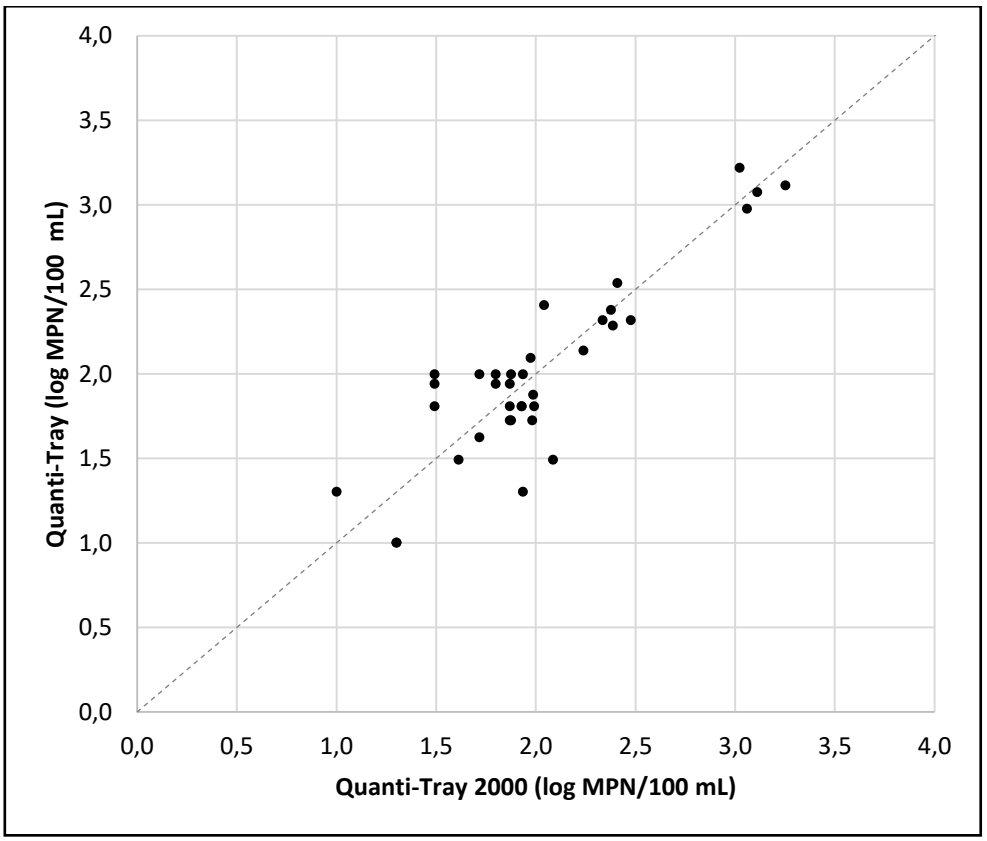
Both one-tailed and two-tailed tests conclude that there is no statistically significant difference between the enumeration of *Escherichia coli* with Quanti-Tray or with Quanti-Tray 2000 at α=0.05.

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

Raw results

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

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



Statistical interpretation

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

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

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

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

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

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

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

- **Student-Fisher test**

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

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

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

4.3.2. Conclusion

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

5. Conclusion

- Comparative study

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

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

- Interlaboratory study

The bias of the alternative method is relatively stable from the low level of contamination to the high level of contamination. For all levels of contamination, the tolerance limits are between the limits of acceptability, meaning that at least 90% of the results will be between the limits of acceptability as defined at 0,6 log.

6. BIBLIOGRAPHY~~ICAL STUDIES~~

- Study published since 2016 :

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

- ~~External validations by another certification body.~~

~~- Finland, 2016, 5CT-v2, Approval of Colilert-18®/Quanti-Tray® Method for Bathing Waters~~
~~- Ireland, 2011, 5DG, Approval of Colilert-18®/Quanti-Tray® Method for Bathing Waters~~
~~- Sweden, 2008, 5BZ, Approval of Colilert-18®/Quanti-Tray® Method for Bathing Waters~~

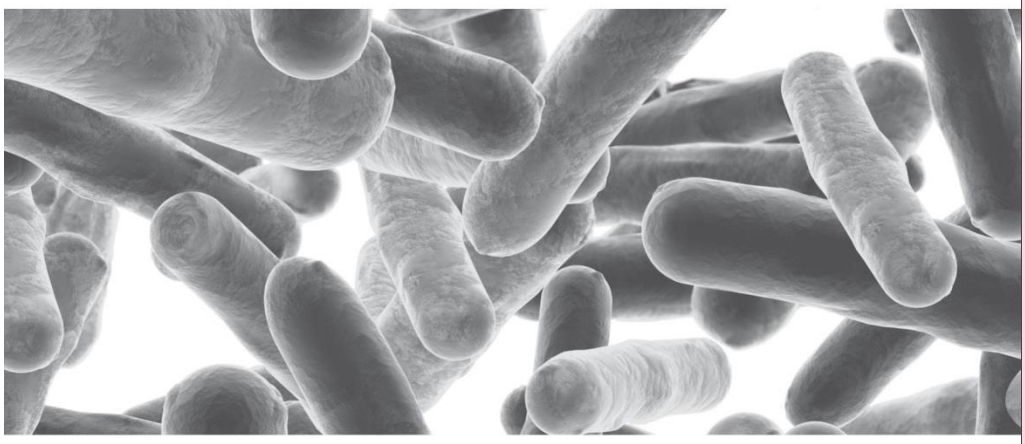
- Method including in:

- International Organization for Standards (ISO). ISO 9308-2:2012 Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 2: Most probable number method

- UK Standing Committee of Analysts (SCA) Blue Books: The Microbiology of Recreational and Environmental Waters (2016) – Part 3 - Methods for the isolation and enumeration of *Escherichia coli*.

- World Health Organization (WHO). WHO recommendation on scientific, analytical, and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC). 2018.

Colilert®-18



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IDEXX

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IDEXX

IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, Maine 04092 USA

idexx.com/water

[RP1]

Colilert-18® /Quanti-Tray®
or Quanti-Tray® 2000

Ad.Gène by UPscience

Colilert*-18 Test Kit

Introduction

Colilert*-18 either simultaneously detects total coliforms and *E. coli*; or fecal coliforms in water. It is based on IDEXX's patented Defined Substrate Technology* (DST*). When total or fecal coliforms metabolize Colilert-18's nutrient-indicator, ONPG, the sample turns yellow. When *E. coli* metabolize Colilert-18's nutrient-indicator, MUG, the sample also fluoresces. Colilert-18 can simultaneously detect these bacteria at 1 cfu/100 mL within 18 hours even with as many as 2 million heterotrophic bacteria per 100 mL present.

Storage

Store at 2–25°C away from light.

Presence/Absence (P/A) Procedure

1. Add contents of one pack to a 100 mL sample in a sterile, transparent, nonfluorescing vessel.
2. Cap vessel and shake.
3. If sample is not already at 33–38°C, then place vessel in a 35°C waterbath for 20 minutes or, alternatively, a 44.5°C waterbath for 7–10 minutes.
4. Incubate at 35±0.5°C for the remainder of the 18 hours.
5. Read results according to Result Interpretation table below.

Quanti-Tray* Enumeration Procedure

1. Add contents of one pack to a 100 mL room temperature water sample in a sterile vessel.
2. Cap vessel and shake until dissolved.
3. Pour sample/reagent mixture into a Quanti-Tray* or Quanti-Tray*/2000 and seal in an IDEXX Quanti-Tray* Sealer.
4. Place the sealed tray in a 35±0.5°C (or 44.5±0.2°C for fecal coliforms) incubator for 18 hours (prewarming to 35°C is not required). For incubation in a water bath, submerge the Quanti-Tray, as is, below the water level using a weighted ring.
5. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the MPN table provided with the trays to obtain a Most Probable Number.

Result Interpretation

Appearance	Result
Less yellow than the comparator[1] when incubated at 35±0.5°C or 44.5±0.2°C	Negative for total coliforms and *E. coli*; Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 35±0.5°C	Positive for total coliforms
Yellow equal to or greater than the comparator when incubated at 44.5±0.2°C	Positive for fecal coliforms
Yellow and fluorescence equal to or greater than the comparator when incubated at 35±0.5°C	Positive for *E. coli*

- Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment. Face light away from your eyes and towards the sample.
- Colilert-18 results are to be read after 18 hours of incubation.
- However, if the results are ambiguous to the analyst based on the initial reading, incubate up to an additional four hours (but not to exceed 22 hours total) to allow the color and/or fluorescence to intensify.
- Positives for both total coliforms and *E. coli* observed before 18 hours and negatives observed after 22 hours are also valid.
- In addition, laboratories may incubate samples for additional time (up to 22 hours total) for their convenience.

Procedural Notes

- A slight tinge may be observed when Colilert-18 is added to the sample.
- If excess foam causes problems while using Quanti-Tray, you may choose to use IDEXX Antifoam Solution (Catalog# WAFDB) or IDEXX 120 ml vessels with Antifoam (Catalog# WV120SBAF-200).
- This insert may not reflect your local regulations. For compliance testing, be sure to follow appropriate regulatory procedures. For example, samples run in other countries should be incubated at 36±2°C for 18-22 hours.
- When following AFNOR validated method for drinking water or bathing water testing, place the sealed tray in a 36±2°C incubator for 18 hours (prewarming to 36°C is not required).
- Colilert-18 can be run in any multiple tube format. *Standard Methods for the Examination of Water and Wastewater*[2] MPN tables should be used to find Most Probable Numbers (MPNs).
- If a water sample has some background color, compare inoculated Colilert-18 sample to a control blank of the same water sample.
- Colilert-18 can be used for *E. coli* detection (but not coliforms) in marine water. Samples must be diluted at least tenfold. Multiply the MPN value by the dilution factor to obtain the proper quantitative result.
- Use only sterile, nonbuffered, oxidant-free water for dilutions.
- Colilert-18 is a primary water test. Colilert-18 performance characteristics do not apply to samples altered by any pre-enrichment or concentration.
- In samples with excessive chlorine, a blue flash may be seen when adding Colilert-18. If this is seen, consider sample invalid and discontinue testing.
- Aseptic technique should always be followed when using Colilert-18. Dispose of in accordance with Good Laboratory Practices.

Quality Control Procedures—Total Coliform and *E. coli*

1. One of the following quality control procedures is recommended for each lot of Colilert-18:
   A. IDEXX-QC Coliform and *E. coli*[3]: *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.
   B. Quanti-Cult*[4] *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.
   C. Fill three sterile vessels with 100 mL of sterile non-buffered oxidant-free water and inoculate with a sterile loop of ATCC[5] strains, *Escherichia coli* ATCC 25922/WDCM 00013 or ATCC 11775/WDCM 00090, *Klebsiella pneumoniae* ATCC 31488/WDCM 00206 and *Pseudomonas aeruginosa* ATCC 10145/WDCM 00024 or ATCC 27853.
2. Follow the P/A Procedure or Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

NOTE: IDEXX internal quality control testing is performed in accordance with ISO 11133:2014. Quality Control Certificates are available at idexx.com/water.

Quality Control Procedures—Fecal Coliform

1. One of the following quality control procedures is recommended for each lot of Colilert-18:
   A. IDEXX-QC Fecal Coliform[6]: *Escherichia coli* and *Pseudomonas aeruginosa*.
   B. Quanti-Cult[4] *Escherichia coli* (fecal coliform), *Klebsiella pneumoniae* (fecal coliform) and *Pseudomonas aeruginosa* (non-fecal).
   C. Fill two sterile vessels with 100 mL of sterile non-buffered oxidant-free water and inoculate with a sterile loop of ATCC[5] strains, *Escherichia coli* ATCC 11775 (fecal coliform) and *Pseudomonas aeruginosa* ATCC 10145 or 27853 (non-fecal coliform).
2. Follow the Quanti-Tray Enumeration Procedure above.
3. Results should match the Result Interpretation table above.

1. IDEXX P/A Comparator, catalog # WP104; Quanti-Tray Comparator #WQTC, or Quanti-Tray/2000 Comparator #WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
3. IDEXX-QC Coliform and *E coli*, IDEXX Catalog #UN3373-WQC-TCEC
4. Quanti-Cult™ cultures—IDEXX catalog # WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org
6. IDEXX-QC Fecal Coliform—IDEXX Catalog #UN3373-WQC-FC

[RP1]

Kit d'analyse Colilert*-18

Introduction

Colilert*-18 permet de détecter dans l'eau soit la présence de coliformes totaux et de bactéries *E. coli*, soit la présence de coliformes fécaux. Ce test est basé sur la technologie brevetée Defined Substrate Technology* (DST*) d'IDEXX. Lorsque les coliformes totaux ou fécaux métabolisent ONPG, le substrat chromogène-indicateur de Colilert-18, l'échantillon vire au jaune. Lorsque l'échantillon est positif, le réactif MUG contenu dans Colilert-18 est métabolisé par les *E. coli* et génère une fluorescence. Colilert-18 peut détecter simultanément ces bactéries à 1 cfu/100 ml en 18 heures, même en présence de bactéries hétérotrophes à une concentration de 2 millions par 100 ml.

Conditions de conservation

Conserver entre 2–25°C à l'abri de la lumière.

Procédure de Présence/Absence (P/A)

1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml placé dans un récipient stérile, transparent et non fluorescent.
2. Fermer le récipient et agiter.
3. Si le prélèvement n'est pas déjà à 33-38°C, placer le récipient dans un bain-marie à 35°C pendant 20 minutes ou dans un bain-marie à 44,5°C pendant 7 à 10 minutes.
4. Incuber à 35±0,5°C pendant les 18 heures qui suivent.
5. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous.

Procédure de numération Quanti-Tray*

1. Ajouter le contenu d'un sachet dans un prélèvement de 100 ml d'eau à température ambiante placé dans un récipient stérile.
2. Fermer le récipient et agiter jusqu'à dissolution.
3. Verser le mélange prélèvement/réactif dans un Quanti-Tray* ou un Quanti-Tray*/2000 et fermer hermétiquement dans un IDEXX Quanti-Tray* Sealer.
4. Placer le plateau hermétiquement fermé dans un incubateur à 35±0,5°C (ou à 44,5±0,2°C pour les coliformes fécaux) pendant 18 heures (aucun réchauffage préalable à 35°C n'est requis). Si le plateau est incubé dans un bain-marie, immerger le Quanti-Tray à l'aide d'un anneau lesté.
5. Interpréter les résultats en se référant au tableau d'interprétation des résultats ci-dessous. Compter le nombre de puits positifs et se référer au tableau NPP fourni avec les plateaux Quanti-Tray pour obtenir le Nombre le plus probable (NPP).

Interprétation des résultats

Aspect	Résultat
Moins jaune que le comparateur[1] après une incubation à 35±0,5°C ou à 44,5±0,2°C	Négatif pour les coliformes totaux et *E. coli*; négatif pour les coliformes fécaux
Aussi jaune ou plus jaune que le comparateur après une incubation à 35±0,5°C	Positif pour les coliformes totaux
Aussi jaune ou plus jaune que le comparateur après une incubation à 44,5±0,2°C	*Positif pour les coliformes fécaux*
Couleur jaune et fluorescence égales ou supérieures au comparateur après une incubation à 35±0,5°C	Positif pour *E. coli*

- Évaluer la fluorescence avec une lampe UV de 6 watts et 365 nm placée à 13 cm de l'échantillon, dans un endroit obscur. Orienter la lumière vers l'échantillon en l'éloignant des yeux de l'opérateur.
- Les résultats du test Colilert-18 doivent être lus après 18 heures d'incubation.
- Toutefois, si les résultats de la première lecture sont ambigus pour l'analyste, incuber jusqu'à quatre heures supplémentaires (sans dépasser 22 heures au total) pour laisser la couleur et/ou la fluorescence s'intensifier.
- Les résultats positifs en coliformes et *E. coli* observés avant 18 heures et les résultats négatifs observés après 22 heures sont également valables.
- En outre, les laboratoires peuvent incuber des échantillons pendant une durée plus longue (jusqu'à 22 heures en tout) par souci de commodité.

Remarques concernant la procédure

- Il est possible d'observer une légère coloration lorsque Colilert-18 est ajouté au prélèvement.
- Si l'excès de mousse pose des problèmes avec le Quanti-Tray, il est possible d'utiliser la solution antimousse d'IDEXX (réf. n° WAFDB) ou les récipients IDEXX de 120 ml (réf. n° WV120SBAF-200).
- Cette notice d'utilisation peut ne pas refléter les réglementations locales en vigueur dans votre pays. Pour les tests de conformité, assurez-vous de suivre les procédures réglementaires appropriées. Par exemple l'incubation des échantillons dans certains pays doit être réalisée à 36±2 °C pendant 18 à 22 heures.
- Lors de l'utilisation de la méthode telle que validée par AFNOR certification pour l'analyse de l'eau potable ou l'eau de baignade =, placer le plateau hermétiquement fermé dans un incubateur à 36±2°C pendant 18 heures (aucun réchauffage préalable à 36°C n'est requis).
- Colilert-18 peut être effectué en tubes multiples. Utiliser les tableaux NPP des *Méthodes de référence pour l'analyse de l'eau et des eaux usées*[2] afin de déterminer les Nombres les plus probables (NPP).
- Si un prélèvement d'eau présente une couleur de fond, comparer le prélèvement inoculé avec Colilert-18 à un échantillon non inoculé du même prélèvement d'eau.
- Colilert-18 peut être utilisé pour la quantification des *E. coli* (pas les coliformes) dans les eaux de mer. Les prélèvements doivent être dilués au moins au dixième. Multiplier la valeur NPP par le facteur de dilution pour obtenir le résultat quantitatif correspondant.
- Utiliser uniquement de l'eau stérile, non tamponnée et sans oxydant pour les dilutions.
- Colilert-18 est avant tout un test pour l'analyse des eaux. Les caractéristiques de performance de Colilert-18 ne s'appliquent pas aux prélèvements modifiés par tout enrichissement préalable ou toute concentration.
- Avec les prélèvements présentant un excédent de chlore, il peut se produire une rapide lueur bleuâtre lors de l'ajout de Colilert-18. Si tel est le cas, le prélèvement n'est pas valide et il faut cesser le test.
- Il est recommandé de toujours utiliser des techniques aseptiques avec Colilert-18. À éliminer conformément aux Bonnes pratiques de laboratoire.

Test de contrôle qualité — Coliformes et *E. coli*

1. L'une des procédures de contrôle qualité suivantes est recommandée pour chaque lot de Colilert 18:
   A. IDEXX-QC pour les Coliformes et *E. coli*[3]: *Escherichia coli*, *Klebsiella pneumoniae* et *Pseudomonas aeruginosa*.
   B. Quanti-Cult*[4] *Escherichia coli*, *Klebsiella pneumoniae et Pseudomonas aeruginosa*.
   C. Remplir trois récipients stériles avec 100 ml d'eau stérile, non tamponnée et sans oxydant puis inoculer les récipients avec une anse stérile avec des souches ATCC[5], *Escherichia coli* ATCC 25922/WDCM 00013 ou ATCC 11775/WDCM 00090, *Klebsiella pneumoniae* ATCC 31488/WDCM 00206 et *Pseudomonas aeruginosa* ATCC 10145/ WDCM 00024 ou ATCC 27853.
2. Suivre la procédure P/A ou la procédure de numération Quanti-Tray ci-dessus.
3. Les résultats doivent correspondre aux résultats du tableau d'interprétation ci-dessus.

REMARQUE: les tests de contrôle qualité internes d'IDEXX sont effectués conformément à la norme ISO 11133:2014. Les certificats de contrôle qualité sont disponible à l'adresse idexx.fr/water.

Procédures de contrôle qualité — Coliformes fécaux

1. L'une des procédures de contrôle qualité suivantes est recommandée pour chaque lot de Colilert 18:
   A. IDEXX-QC pour les coliformes fecaux[6]: *Escherichia coli* et *Pseudomonas aeruginosa*.
   B. Quanti-Cult *Escherichia coli* (coliforme fécal), *Klebsiella pneumoniae* (coliforme fécal) et *Pseudomonas aeruginosa* (non fécal).
   C. Remplir deux récipients stériles avec 100 ml d'eau stérile non tamponnée et sans oxydant puis les inoculer avec une anse stérile de souches ATCC, *Escherichia coli* ATCC 11775 (coliforme fécal) et *Pseudomonas aeruginosa* ATCC 10145 ou 27853 (non fécal).
2. Suivre la procédure de numération Quanti-Tray.
3. Les résultats doivent correspondre aux résultats du tableau d'interprétation ci-dessus.

1. Comparateur P/A IDEXX, réf. n° WP104; Comparateur Quanti-Tray n° WQTC ou Quanti-Tray/2000 Comparateur n° WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
3. IDEXX-QC pour les Coliformes et *E. coli*, IDEXX Catalog #UN3373-WQC-TCEC
4. Cultures Quanti-Cult* IDEXX réf. n° WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org
6. IDEXX-QC pour les coliformes fecaux — IDEXX Catalog #UN3373-WQC-FC

Kit di analisi Colilert*-18

Introduzione

Colilert*-18 rileva simultaneamente i coliformi totali e *E. coli* o i coliformi fecali in acqua. Si basa su una tecnologia di substrato definito (Defined Substrate Technology) brevettata IDEXX* (DST*). Quando i coliformi totali o fecali metabolizzano il nutriente indicatore ONPG di Colilert-18, il campione diventa giallo. Quando l'*E. coli* metabolizza il nutriente-indicatore MUG, il campione presenta anche fluorescenza. Il Colilert-18 è in grado di rilevare simultaneamente questi batteri in concentrazioni di 1 UFC/100 ml entro 18 ore anche se sono presenti addirittura 2 milioni di batteri eterotrofici per 100 ml.

Conservazione

Conservare a 2–25°C lontano dalla luce.

Procedura relativa a Presenza/Assenza (P/A)

1. Unire il contenuto di un pacchetto ad un campione da 100 ml in un a provetta sterile, trasparente e non fluorescente.
2. Chiudere la provetta ed agitarla.
3. Se il campione non è già a 33–38°C, mettere la provetta a bagno maria a 35°C per 20 minuti oppure, alternativamente, a bagno maria a 44,5°C per 7–10 minuti.
4. Incubare a 35±0,5°C per il resto delle 18 ore.
5. Leggere i risultati secondo la tabella di Interpretazione dei risultati qui sotto.

Procedura di enumerazione Quanti-Tray*

1. Unire il contenuto di un pacchetto ad un campione di acqua da 100 ml a temperatura ambiente in una provetta sterile.
2. Chiudere la provetta e agitarla fino a dissoluzione.
3. Versare la miscela campione/reagente in un vassoietto Quanti-Tray* o Quanti-Tray*/2000 e sigillarlo in un IDEXX Quanti-Tray* Sealer.
4. Posizionare il vassoietto sigillato in un'incubatrice a 35±0,5°C (o 44,5±0,2°C per i coliformi fecali) per 18 ore (non si richiede il pre-riscaldamento a 35°C). Per l'incubazione in un bagno termostatato, immergere completamente il Quanti-Tray nell'acqua utilizzando un anello appesantito.
5. Leggere i risultati secondo la tabella di Interpretazione dei risultati qui sotto. Contare il numero di gialli positivi e consultare la tabella MPN fornita insieme ai vassoietti per ottenere il numero più probabile.

Interpretazione dei risultati

Aspetto	Risultato
Colore giallo meno intenso del comparatore[1] quando viene incubato a 35±0,5°C o a 44,5±0,2°C	Negativo per coliformi totali ed *E. coli*; negativo per coliformi fecali
Colore giallo uguale o più intenso del comparatore quando viene incubato a 35±0,5°C	Positivo per coliformi totali
Colore giallo uguale o più intenso del comparatore quando viene incubato a 44,5±0,2°C	Positivo per coliformi fecali
Colore giallo e fluorescenza uguali o più intensi del comparatore quando viene incubato a 35±0,5°C	Positivo per *E. coli*

- Individuare la fluorescenza con una luce a raggi ultravioletti da 6 watt, 365 nm, entro circa 13 cm dal campione in un ambiente non illuminato. Dirigere la luce verso il campione, in direzione opposta ai propri occhi.
- I risultati di Colilert-18 devono essere letti dopo 18 ore di incubazione.
- Tuttavia, se i risultati sono ambigui per l'analista sulla base della lettura iniziale, incubare fino a quattro ore in più (non superando tuttavia 22 ore in totale) in modo da consentire l'intensificarsi del colore e/o della fluorescenza.
- Sono validi anche i positivi sia per i coliformi totali sia per *E. coli* osservati prima di 18 ore e i negativi osservati dopo 22 ore.
- Inoltre, i laboratori possono incubare i campioni per un periodo aggiuntivo (fino a 22 ore in totale) per loro comodità.

Note sulla procedura

- Una leggera colorazione si può osservare quando il Colilert-18 viene aggiunto al campione.
- Se la schiuma in eccesso causa problemi mentre si usa il Quanti-Tray, si può scegliere di usare la Soluzione antischiuma IDEXX (Codice catalogo WAFDB) o provetta IDEXX da 120 ml con antischiuma (Codice catalogo WV120SBAF-200).
- Quest'inserzione potrebbe non rispondere ai regolamenti locali. Per eseguire un test di conformità, assicurarsi di seguire le procedure normative opportune. Ad esempio, i campioni trattati in altri Paesi vengono incubati a 36±2°C per 18-22 ore.
- Nel seguire la validazione di metodo AFNOR per acque potabili o di balneazione, mettere la piastra a pozzetti sigillata nell'incubatore a 36±2° per 18 ore (non e' necessario il preriscaldamento a 36°).
- Il Colilert-18 si può eseguire in qualsiasi formato a provetta multipla. I metodi standard[2] per l'esame delle tabelle MPN dell'acqua e delle acque di scarico vanno usati per ottenere i numeri più probabili (MPN).
- Se un campione di acqua dovesse presentare della colorazione di sfondo, confrontare il campione Colilert-18 inoculato con controllo negativo dello stesso campione di acqua.
- Nelle acque marine, Colilert 18 puo' essere utilizzato per determinare la presenza di E. coli (ma NON per i coliformi). I campioni devono essere diluiti almeno con fattore 1/10. Moltiplicare il valore MPN per il fattore di diluizione per ottenere il risultato quantitativo adeguato.
- Per le diluizioni usare solo acqua sterile, non tamponata, priva di ossidanti.
- Il Colilert-18 è un test primario per l'acqua. Le caratteristiche di prestazione del Colilert-18 non sono applicabili a campioni alterati da qualsiasi pre-arricchimento o da concentrazione.
- In campioni con cloro eccessivo, quando si aggiunge il Colilert-18 si potrebbe vedere un lampo azzurro. In questo caso, considerare il campione non valido e interrompere l'analisi.
- Quando si usa il Colilert-18 va sempre seguita la tecnica asettica. Eliminare secondo le buone pratiche di laboratorio.

Procedura Controllo Qualità—coliformi totali ed *E. coli*

1. Per ciascun lotto di Colilert-18 si consiglia una delle seguenti procedure di controllo della qualità:
   A. Coliformi ed *E. coli*[3] IDEXX-QC: *Escherichia coli*, *Klebsiella pneumoniae* e *Pseudomonas aeruginosa*.
   B. Quanti-Cult*[4] *Escherichia coli* (coliforme fecale), *Klebsiella pneumoniae* (coliforme fecale) e *Pseudomonas aeruginosa* (non fecale).
   C. Riempire tre contenitori sterili con 100 ml di acqua sterile non tamponata e senza ossidanti e inoculare con un'ansa sterile di ceppi ATCC[5], *Escherichia coli* ATCC 25922/ WDCM 00013 o ATCC 11775/ WDCM 00090, *Klebsiella pneumoniae* ATCC 31488/ WDCM 00206 e *Pseudomonas aeruginosa* ATCC 10145/ WDCM 00024 o ATCC 27853.
2. Seguire la procedura P/A o la procedura di enumerazione Quanti-Tray di cui sopra.
3. I risultati devono corrispondere a quelli inseriti nella tabella di interpretazione di cui sopra.

NOTA: i test di controllo di qualità interni IDEXX sono condotti in conformità con ISO 11133:2014. I certificati di controllo qualità sono disponibili sul sito idexx.it/water.

Procedura Controllo Qualità—coliformi fecali

1. Per ciascun lotto di Colilert-18 si consiglia una delle seguenti procedure di controllo della qualità:
   A. Coliformi fecali IDEXX-QC[6]: *Escherichia coli* e *Pseudomonas aeruginosa*.
   B. Quanti-Cult *Escherichia coli* (coliforme fecale), *Klebsiella pneumoniae* (coliforme fecale) e *Pseudomonas aeruginosa* (non fecale).
   C. Riempire due contenitori sterili con 100 ml di acqua sterile non tamponata e senza ossidanti e inoculare con un'ansa sterile di ceppi ATCC[5], *Escherichia coli* ATCC 25922/WDCM 00013 o ATCC 11775/WDCM 00090, Klebsiella pneumoniae ATCC 31488/WDCM 00206 e Pseudomonas aeruginosa ATCC 10145/ WDCM 00024 o 27853.
2. Seguire la procedura di enumerazione Quanti-Tray sopra riportata.
3. I risultati devono corrispondere alla tabella di interpretazione dei risultati indicata sopra.

1. Comparatore P/A IDEXX, réf. n° WP104; Comparatore Quanti-Tray n° WQTC o Quanti-Tray/2000 Comparatore n° WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Metodo standard per l'esame dell'acqua e delle acque di scarico*. American Public Health Association, 2005. Washington, DC.
3. Coliformi ed *E. coli* IDEXX-QC, Catalogo IDEXX N. UN3373-WQC-TCEC
4. Colture Quanti-Cult* N. di catalogo IDEXX WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org
6. Coliformi fecali IDEXX-QC—Catalogo IDEXX N. UN3373-WQC-FC

Colilert*-18 Testkit

Einführung

Colilert*-18 ist zum gleichzeitigen Nachweis von Gesamtcoliformen und *E. coli* oder Fäkalcoliformen in Wasser bestimmt. Es basiert auf der patentierten Defined Substrate Technology* (DST*) von IDEXX. Bei der Metabolisierung von ONPG, des Nährstoffindikators von Colilert-18, durch die Gesamtcoliformen oder Fäkalcoliformen verfärbt sich die Probe gelb. Wenn *E. coli* den Nährstoffindikator MUG verstoffwechselt, fluoresziert die Probe. Colilert-18 kann diese Bakterien gleichzeitig im Bereich von 1 CFU/100 ml innerhalb von 18 Stunden nachweisen, selbst wenn 2 Mio. heterotrophe Bakterien pro 100 ml vorhanden sind.

Lagerung

Bei 2–25°C und nicht im Licht lagern.

Presence/Absence (P/A)-Test

1. Den Inhalt einer Packung zu einer 100 ml Probe in einem sterilen, transparenten, nicht fluoreszierenden Gefäß hinzugeben.
2. Das Gefäß verschließen und schütteln.
3. Wenn die Probe noch nicht im Temperaturbereich von 33–38°C ist, das Gefäß 20 Minuten in ein Wasserbad von 35°C oder alternativ 7–10 Minuten lang in ein Wasserbad von 44,5°C stellen.
4. Für den verbleibenden 18-Stunden-Zeitraum bei 35±0,5°C inkubieren.
5. Die Ergebnisse gemäß der nachstehenden Ergebnisauswerte-Tabelle ablesen.

Quanti-Tray* Auszähl-Methode

1. Den Inhalt einer Packung zu einer 100 ml Wasserprobe mit Zimmertemperatur in einem sterilen Gefäß hinzugeben.
2. Das Gefäß verschließen und so lange schütteln, bis der Inhalt aufgelöst ist.
3. Die aus Probe und Reagenz bestehende Mischung in ein Quanti-Tray* oder Quanti-Tray/2000 gießen und in einem IDEXX Quanti-Tray Sealer fest verschließen.
4. Den versiegelten Quanti-Tray bei 35±0,5°C (oder bei -Fäkalcoliformen bei 44,5±0,2°C) 18 Stunden inkubieren (ein Vorwärmen auf 35°C ist nicht erforderlich). Sollte die Inkubation des Quanti-Tray im Wasserbad erfolgen, den Tray so beschweren, dass er vollständig von Wasser bedeckt ist.
5. Die Ergebnisse anhand der nachstehenden Ergebnisauswerte-Tabelle ablesen. Die Anzahl der positiven Vertiefungen zählen und die wahrscheinlichste Zahl (MPN; Most Probable Number) anhand der MPN-Tabelle, die den Trays beiliegt, ermitteln.

Ergebnisauswertung

Aussehen der Probe	Mögliche Ergebnisse
Geringere Gelbfärbung als der Comparator[1] bei Inkubation bei 35±0,5°C oder bei 44,5±0,2°C	Negativ für Gesamtcoliforme und *E. coli*; negativ für fäkale Coliforme
Gleiche oder stärkere Gelbfärbung als der Comparator[1] bei Inkubation bei 35±0,5°C	Positiv für Gesamtcoliforme
Gleiche oder stärkere Gelbfärbung als der Comparator[1] bei Inkubation bei 44,5±0,2°C	Positiv für Fäkalcoliforme
Gleiche oder stärkere Gelbfärbung und Fluoreszenz als der Comparator[1] bei Inkubation bei 35±0,5°C	Positiv für *E. coli*

- Die Anwesenheit von Fluoreszenz wird mit einer UV-Lampe von 6 Watt bei einer Wellenlänge von 365 nm im Dunkeln in einem Höchstabstand von 12 cm von der Probe geprüft. Dabei muß das UV-Licht von den Augen des Anwenders weg- und zur Probe gerichtet sein.
- Colilert-18-Ergebnisse sollten nach einer Inkubationszeit von 18 Stunden abgelesen werden.
- Wenn die Ergebnisse jedoch nach der ersten Ablesung nicht eindeutig sind, nochmals bis zu vier Stunden (insgesamt jedoch nicht länger als 18 Stunden) inkubieren, um die Intensivierung der Farbe und/oder Fluoreszenz zu ermöglichen.
- Positive Ergebnisse für Gesamtcoliforme und *E. coli*, die vor Ablauf von 18 Stunden und negative Ergebnisse, die nach Ablauf von 22 Stunden beobachtet werden, sind ebenfalls gültig.
- Darüber hinaus können Labors die Proben aus praktischen Gründen auch länger (insgesamt bis zu 22 Stunden) inkubieren.

Verfahrenshinweise

- Beim Hinzugeben von Colilert-18 zur Probe kann eine leichte Färbung beobachtet werden.
- Wenn die Verwendung des Quanti-Tray durch übermäßige Schaumentwicklung erschwert wird, kann die IDEXX Antifoam Solution (Antischaum-Lösung; Best.-Nr. WAFDB) oder IDEXX 120 ml Gefäße mit Antifoam (Best.-Nr. WV120SBAF-200) verwendet werden.
- Diese Packungsbeilage entspricht unter Umständen nicht Ihren örtlichen Bestimmungen. Bei Konformitätsprüfungen unbedingt die entsprechenden aufsichtsbehördlichen Verfahren anwenden. In anderen Ländern werden zum Beispiel zu untersuchende Proben 18-22 Stunden bei 36±2°C inkubiert.
- Falls das Colilert-18/Quanti-Tray Verfahren entsprechend der AFNOR Validierung für Trink- oder Badegewässer durchgeführt wird, das verschlossene Tray für 18 Stunden bei 36+/-2°C inkubieren. (Vorwärmen auf 36°C ist nicht erforderlich).
- Das Colilert-18 Verfahren kann in jedem Multiple-Tube-Format durchgeführt werden. Zur Ermittlung der MPNs (wahrscheinlichste Zahlen) sollten MPN-Tabellen für Standardverfahren[2] zur Untersuchung von Wasser und Abwasser verwendet werden.
- Wenn eine Wasserprobe etwas Hintergrundfarbe aufweist, ist die inokulierte Colilert-18 Probe mit einer Kontrollprobe derselben Wasserprobe zu vergleichen.
- Colilert-18 kann zum Nachweis von *E. coli* (aber nicht für Coliforme) in Meerwasser verwendet werden. Die Proben müssen mindestens 10fach verdünnt werden. Multiplizieren Sie den ermittelten MPN Wert mit dem Verdünnungsfaktor um das quantitative Ergebnis zu erhalten.
- Nur steriles, nicht gepuffertes, keine Oxidantien enthaltendes Wasser zur Verdünnung verwenden.
- Colilert-18 ist ein primärer Wassertest. Die Leistungsmerkmale von Colilert-18 gelten nicht für Proben, die durch Voranreicherung oder Konzentration modifiziert wurden.
- In Proben mit übermäßigem Chlorgehalt wird bei der Zugabe von Colilert-18 u.U. ein blaues Aufleuchten beobachtet. In diesem Fall ist die Probe als ungültig zu betrachten und der Test abzubrechen.
- Bei der Verwendung von Colilert-18 ist ein aseptisches Vorgehen vorgeschrieben. Entsorgung gemäß Standard-Laborpraktiken.

Qualitätskontrollverfahren—Coliforme und *E. coli*

1. Eines der folgenden Qualitätskontrollverfahren wird für jede Colilert-18-Charge empfohlen:
   A. IDEXX-QC Coliform und *E. coli*[3]: *Escherichia coli*, *Klebsiella pneumoniae* und *Pseudomonas aeruginosa*.
   B. Quanti-Cult*[4] *E. coli*, *Klebsiella pneumoniae* und *Pseudomonas aeruginosa*.
   C. Drei sterile Gefäße mit 100 ml sterilem, ungepuffertem, oxidansfreiem Wasser füllen und mit einer sterilen Öse ATCC[5]-Stämme, *Escherichia coli* ATCC 25922/WDCM 00013 oder ATCC 11775/WDCM 00090, *Klebsiella pneumoniae* ATCC 31488/WDCM 00206 und *Pseudomonas aeruginosa* ATCC 10145/WDCM 00024 oder ATCC 27853 inokulieren.
2. Das oben beschriebene P/A-Verfahren oder das Quanti-Tray-Auszählverfahren befolgen.
3. Die Ergebnisse sollten mit der Tabelle zur Ergebnisauswertung (siehe oben) übereinstimmen.

HINWEIS: Die internen Qualitätskontrollprüfungen von IDEXX werden im Einklang mit ISO 11133:2014 durchgeführt. Qualitätskontrollzertifikate sind unter idexx.de/water erhältlich.

Qualitätskontrollverfahren—Fäkalcoliforme

1. Eines der folgenden Qualitätskontrollverfahren wird für jede Colilert-18-Charge empfohlen:
   A. IDEXX-QC Fecal Coliform[6]: *Escherichia coli* und *Pseudomonas aeruginosa*.
   B. Quanti-Cult *Escherichia coli* (Fäkalcoliform), *Klebsiella pneumoniae* (Fäkalcoliform) und *Pseudomonas aeruginosa* (nicht fäkal).
   C. Zwei sterile Gefäße mit 100 ml sterilem, ungepuffertem, oxidansfreiem Wasser füllen und mit einer sterilen Öse ATCC-Stämme, *Escherichia coli* ATCC 11775 (Fäkalcoliform) und *Pseudomonas aeruginosa* ATCC 10145 oder 27853 (nicht fäkal) inokulieren.
2. Das oben geschilderte Quanti-Tray Auszählverfahren befolgen.
3. Die Ergebnisse müssen mit der Auswertungstabelle oben übereinstimmen.

1. Comparateur P/A IDEXX, Art. Nr. WP104; Comparateur Quanti-Tray Nr. WQTC oder Quanti-Tray/2000 Comparateur Nr. WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater (Standardverfahren für die Wasser- und Abwasseruntersuchung)*. American Public Health Association, 2005. Washington, DC, USA.
3. IDEXX-QC Coliform und *E. coli*, IDEXX Bestellnr. UN3373-WQC-TCEC
4. Quanti-Cult* Kulturen—IDEXX Best.-Nr. WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org
6. IDEXX-QC Fecal Coliform—IDEXX Bestellnr. UN3373-WQC-FC

Kit de análisis Colilert*-18

Introducción

Colilert*-18 detecta de manera simultánea los coliformes totales y *E. coli* o coliformes fecales en el agua. Se basa en la Defined Substrate Technology* (Tecnología de substrato definido [DST*]), patentada por IDEXX. Cuando los coliformes totales o los coliformes fecales metabolizan el indicador de nutrientes de Colilert-18, la muestra se vuelve de color amarillo. Cuando *E. coli* metaboliza el indicador MUG de nutrientes de Colilert-18, la muestra además fluoresce. Colilert-18 puede detectar simultáneamente estas bacterias a una concentración de 1 ufc/100 ml dentro de las 18 horas, hasta en presencia de 2 millones de bacterias heterotróficas por cada 100 ml.

Almacenamiento

Almacenar a temperatura de 2–25°C, alejado de la luz.

Procedimiento de presencia/ausencia (P/A)

1. Añadir el contenido de un paquete a una muestra de 100 ml, en un recipiente estéril transparente, no fluorescente.
2. Tapar y agitar el recipiente.
3. Si la muestra no está ya a temperatura de 33–38°C, colocar el recipiente en un baño baño termostático a 35°C durante 20 minutos.
   o, como alternativa, en un baño baño termostático a 44,5°C entre 7 a 10 minutos.
4. Incubar a 35±0,5°C durante el resto de las 18 horas.
5. Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo.

Procedimiento de enumeración Quanti-Tray*

1. Añadir el contenido de un paquete a una muestra de 100 ml de agua a temperatura ambiente, en un recipiente estéril.
2. Tapar y agitar el recipiente hasta disolver.
3. Verter la mezcla de muestra/reactivo en una Quanti-Tray* o una Quanti-Tray*/2000 y sellar en un IDEXX Quanti-Tray* Sealer.
4. Colocar la bandeja sellada en una estufa de incubación a 35±0,5°C (o a 44,5±0,2°C para los coliformes fecales) durante 18 horas (no se necesita precalentar a 35ºC).Si se incuba en un baño termostático, sumergir la Quanti-Tray con la ayuda de un anillo pesado, hasta situarla por debajo del nivel del agua.
5. Leer los resultados de acuerdo con el cuadro de interpretación de resultados, más abajo. Contar el número de pocillos positivos y referirse al cuadro NMP proporcionado con las bandejas para obtener el número más probable.

Interpretación de resultados

Aspecto	Resultado
Menos amarillo que el comparador[1] cuando se incuba a 35±0,5°C o a 44,5±0,2°C	Negativo para coliformes totales y *E. coli*; negativo para coliformes fecales
Amarillo igual o mayor que el del comparador[1] cuando se incuba a 35±0,5°C	Positivo para coliformes totales
Amarillo igual o mayor que el del comparador[1] cuando se incuba a 44,5±0,2°C	Positivo para coliformes fecales
Amarillo y fluorescencia iguales o mayores que los del comparador[1] cuando se incuba a 35±0,5°C	Positivo para *E. coli*

- Buscar fluorescencia usando una luz UV de 6 vatios, 365 nm a distancia de unos 13 cm de la muestra en un ambiente oscuro. Apuntar el haz de luz en dirección contraria a los ojos y hacia la muestra.
- Los resultados de Colilert-18 se deben leer a las 18 horas de incubación.
- Es possible prolonger el tiempo de lectura 4 horas mas, hasta las 22 horas, para que en raro pero posible caso de duda el color o la fluorescencia se intensifiquen.
- Los resultados positivos para coliformes totales y *E. coli* antes de las 18 horas y negativos tras 22 horas también son válidos.
- Asimismo, los laboratorios pueden incubar muestras (hasta 22 horas en total) si lo desean, para mayor comodidad.

Notas sobre el procedimiento

- Cuando se agrega Colilert-18 a la muestra es posible que se observe una tinción leve.
- Si el exceso de espuma causa problemas mientras se usa Quanti-Tray, se puede decidir utilizar solución antiespumante IDEXX (Nº de catálogo WAFDB) o recipientes de 120 ml IDEXX con antiespumante (Nº de catálogo WV120SBAF-200).
- Es posible que este prospecto no refleje sus regulaciones locales. Para las pruebas de conformidad, asegúrese de seguir los procedimientos reglamentarios apropiados. Por ejemplo, las pruebas realizadas en otros países deben incubarse a 36±2°C durante 18 a 22 horas.
- Cuando se siga el método AFNOR para agua destinada al consumo humano o aguas de baño, marinas o recreativas, coloque la bandeja sellada en una estufa a 36+/-2 durante 18 horas (no es necesario un precalentamiento a 36°C).
- Colilert-18 puede procesarse en cualquier formato de múltiples tubos. Deben usarse los Métodos estándares[2] para examen del agua y las tablas NMP de aguas residuales para encontrar.
- Si la muestra de agua tiene un cierto color de fondo, comparar la muestra inoculada de Colilert-18 con un blanco testigo de la misma muestra de agua.
- Colilert-18 puede usarse para la recuento de *E. coli* (pero no para coliformes) en aguas marinas. Las muestras deben diluirse al menos diez veces. Multiplicar el valor NMP por el factor de dilución para obtener el resultado cuantitativo apropiado.
- Usar solamente agua estéril, no tamponada, libre de oxidantes, para efectuar las diluciones.
- Colilert-18 es una prueba principalmente para agua. Las características de rendimiento de Colilert-18 no se aplican a muestras alteradas por enriquecimiento o concentración previos.
- En el caso de muestras con un exceso de cloro, tal vez se observe un destello azul al añadir Colilert-18. Si se observa, considerar que la muestra no es válida y suspender la prueba.
- Siempre debe utilizarse una técnica aséptica cuando se use Colilert-18. Desechar en cumplimiento con las Buenas Prácticas de Laboratorio.

Procedimientos para el Control de Calidad—Coliformes Totales y *E. coli*

1. Se recomienda uno de los siguientes procedimientos de control de calidad para cada lote de Colilert-18:
   A. IDEXX-QC Coliform and *E. coli*[3]: *Escherichia coli*, *Klebsiella pneumoniae* y *Pseudomonas aeruginosa*.
   B. Quanti-Cult*[4] *E. coli* (coliforme fecal), *Klebsiella pneumoniae* (coliforme fecal) y *Pseudomonas aeruginosa* (no fecal)
   C. Llene tres recipientes estériles con 100 ml de agua estéril, libre de oxidantes, no tamponada e inocule con un asa estéril de cepas ATCC[5], *Escherichia coli* ATCC 25922/WDCM 00013 o ATCC 11775/WDCM 00090, *Klebsiella pneumoniae* ATCC 31488/WDCM 00206 y *Pseudomonas aeruginosa* ATCC 10145/ WDCM 00024 o ATCC 27853.
2. Seguir el procedimiento P/A o el procedimiento de enumeración Quanti-Tray mencionado anteriormente.
3. Los resultados deben corresponder a los del Cuadro de Interpretación de resultados, más arriba.

NOTA: Las pruebas de control de calidad interna de IDEXX se realizan según ISO 11133:2014. Los certificados de control de calidad se encuentran disponibles en idexx.es/water.

Procedimientos de control de calidad: Coliforme fecal

1. Se recomienda uno de los siguientes procedimientos de control de calidad para cada lote de Colilert-18:
   A. IDEXX-QC de coliforme fecal[6]: *Escherichia coli* y *Pseudomonas aeruginosa*.
   B. Quanti-Cult de *Escherichia coli* (coliforme fecal), *Klebsiella pneumoniae* (coliforme fecal) y *Pseudomonas aeruginosa* (no fecal).
   C. Llenar dos recipientes estériles con 100 ml de agua estéril, no tamponada, libre de oxidantes para inocular con un asa estéril de cepas ATCC, *Escherichia coli* ATCC 11775 (coliforme fecal) y *Pseudomonas aeruginosa* ATCC 10145 o 27853 (no fecal).
2. Seguir el procedimiento de enumeración Quanti-Tray mencionado anteriormente.
3. Los resultados deben corresponder a los del cuadro de interpretación de resultados, más arriba.

1. Comparateur P/A IDEXX, réf. n° WP104: Comparateur Quanti-Tray n° WQTC ou Quanti-Tray/2000 Comparateur n° WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water & Wastewater (Métodos estándar para el análisis de agua y aguas residuales)*. American Public Health Association; 2005. Washington, DC.
3. IDEXX-QC de coliforme y *E. coli*, N.° de catálogo de IDEXX: UN3373-WQC-TCEC
4. Cultivos Quanti-Cult*—Nº de catálogo IDEXX WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org
6. IDEXX-QC de coliforme fecal. N.° de catálogo de IDEXX: UN3373-WQC-FC

Colilert*-18 コリラート１８ テストキット

はじめに
コリラート18は水中の大腸菌群と大腸菌または糞便性大腸菌群を同時に検出します。検出方法はIDEXXが特許を取得したDefined Substrate Technology* (DST*) (特定酵素基質法)に基づいています。大腸菌群や糞便性大腸菌群がコリラート18の発色酵素基質OPNGを代謝すると、検体は黄変します。大腸菌が、コリラート18に含まれた栄養指標であるMUGを代謝すると、検水は蛍光も呈します。コリラート18は100mlあたり最大200万の従属栄養細菌が存在したとしても、18時間以内に、1cfu/100mlの感度で大腸菌群および大腸菌を同時に検出することができます。

内容
- WP020I-18 は、検体100ml用のスナップパック20個入りです
- WP200I-18 は、検体100ml用のスナップパック200個入りです

保管
直射日光を避け、2～25℃で保管してください。

定性法(P/A)の手順

1. 1パックの中身を、滅菌済みの透明な蛍光を発しない容器の中に入った100mlの検体に加えてください。
2. 容器の蓋を締め、振ってください。
3. 検体がこの時点で 33～38℃ でない場合、35℃の恒温漕に20分、または、44.5℃の恒温漕に7～10分間置いてください。
4. 36℃で、18時間培養してください。
5. 以下の結果判定表に従って、結果判定してください。

Quanti-Tray* QTトレイの計算手順

1. 1パックの中身を、滅菌容器の中に入った室温の検水100mlに加えてください。
2. 容器の蓋を締め溶けるまで静かに振ってください。
3. QTトレイ/2000 に検水/コリラート18混合を注ぎ、シーラーで密封してください。
4. 密封されたトレイを36℃（糞便性大腸菌群の場合は44.5±0.2℃）の培養器の中に18時間置いてください（前もって35℃にする必要はありません）。
5. 以下の結果判定表に従って、結果を判定してください。陽性ウェルの数を数え、専用MPN表を参照して、最確数を求めてください。

結果判定

培養液の状態	結果
36℃または44.5±0.2℃で培養した場合、比色管[1]より薄い黄色	大腸菌群と大腸菌共に陰性
36℃で培養した場合、比色管と同等かそれより濃い黄色	大腸菌群陽性
44.5±0.2℃で培養した場合、比色管と同等かそれより濃い黄色	糞便性大腸菌群陽性
36℃で培養した場合、比色管と同等かそれより濃い黄色および蛍光	大腸菌陽性

- 暗所で、検体の5インチ（12.7cm）以内で6W・365-366 nmのUVランプを使用して、判定してください。光は、目に向けないようにし、検体に向けてください。
- Colilert-18 の結果は培養18時間後に判定してください。
- 但し、初回の判定において分析結果があいまいな場合には、さらに最長4時間（総時間数が22時間を超えないように）培養を続け、色および/または蛍光度を増強させます。
- 18時間以内に観察された大腸菌群と大腸菌両者の陽性、および22時間後に観察された陰性は有効です。
- また、検査の便宜上、検体の培養時間（総時間数22時間以内）を延長することも可能です。

操作上の注意
- コリラート18を検体に加えた時、かすかな色が見られる場合があります。
- ＱＴトレイを使用中に、泡が問題になる場合は、IDEXX 消泡液(カタログ# WAFDB)、または消泡剤入りのIDEXX 120 ml容器(カタログ# WV120SBAF-200)の使用を選択できます。
- 本説明書の内容は該当する地域の法律・条例に適合していない場合があります。法律・条例に準拠した検査を行うために、必ず適切な規制手順に従ってください。例えば、他の国で検査を行う際は、36±2°Cで18～22時間培養する必要があります。
- AFNORが認証した飲料水もしくは浴槽水の検査に従う際には、密封されたQTトレイを36±2℃の培養器に18時間静置して下さい。（前もって36℃に予熱する必要はありません。）
- コリラート18は、5本法などの最確数法でも実施できます。MPN表は、最確数 (MPN)を求めるために使用してください。
- 検水に何らかの着色がある場合、同じ検水を用いたブランクと比較してください。[2]
- コリラート18は、海水中の大腸菌に使用可能です(大腸菌群を除く)。検体を１０倍以上に希釈してください。MPN値に希釈倍数を掛けて、適切な定量結果を求めてください。
- 希釈には、緩衝液や酸化物質の入っていない、滅菌された水だけを使用してください。
- コリラート18は、水の一次検査です。コリラート18の性能特性として、増菌培地で培養または濃縮によって変質した検体に適用できません。
- コリラート18を加えるとき、過剰の塩素がある検体で、青色を呈する場合があります。 これが見られる場合、検体はテストに適さないので、テストを中止してください。
- コリラート18を使用する際は、常に無菌操作を行ってください。ＧＬＰに従って、廃棄してください。

品質管理手順 – 大腸菌群および大腸菌
1. Colilert-18を使用する場合、ロット毎に次の品質管理手順のいずれかを行うことをお薦めします。:
   A. IDEXX-QC大腸菌群および大腸菌[3]：大腸菌、*Klebsiella pneumoniae*（肺炎桿菌）、*Pseudomonas aeruginosa*（緑膿菌）。
   B. Quanti-Cult*[4] 大腸菌、肺炎桿菌、緑膿菌.
   C. 滅菌容器3本に、それぞれ緩衝剤や酸化剤の入っていない滅菌水100 mLを入れ、大腸菌ATCC 25922/WDCM 00013またはATCC 11775/WDCM 00090、*Klebsiella pneumoniae* ATCC 31488/WDCM 00206、および *Pseudomonas aeruginosa* ATCC 10145/ WDCM 00024または27853のATCC[5]菌株を、滅菌ループを用いて接種してください 。
2. 上記の定性法（P/A）手順、またはQTトレイ定量法操作手順に従ってください。
3. 結果は上記の結果解釈表と一致するはずです。

注: IDEXXの社内品質管理検査は、ISO 11133:2014に準拠して行われます。成績証明証（品質管理認証）は idexx.co.jp/waterにて利用可能です。

品質管理手順 – 糞便性大腸菌
1. Colilert-18を使用する場合、ロット毎に次の品質管理手順のいずれかを行うことをお薦めします。:
   A. IDEXX-QC糞便性大腸菌群[6]：大腸菌および *Pseudomonas aeruginosa*（緑膿菌）。
   B. Quanti-Cult：大腸菌（糞便性大腸菌）、*Klebsiella pneumoniae*（肺炎桿菌）（糞便性大腸菌）、*Pseudomonas aeruginosa*（非糞便性）。
   C. 滅菌容器2本に、それぞれ緩衝剤や酸化剤の入っていない滅菌水100 mLを入れ、大腸菌ATCC 11775（糞便性大腸菌）、*Pseudomonas aeruginosa* ATCC 10145または27853（非糞便性）のATCC菌株を、滅菌ループを用いて接種してください。
2. 上記のQTトレイ定量法手順に従ってください。
3. 結果は上の結果解釈表と一致するはずです。

1. IDEXX P/A用 比色管　カタログ番号 WP104、QTトレイ用比色トレイ　カタログ番号 WQT2KC
2. Eaton, AD, Clesceri, LS, Greenberg, AE, Rice, EN. *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, 2005. Washington, DC.
3. IDEXX-QC大腸菌群および大腸菌－IDEXX カタログ番号UN3373-WQC-TCEC
4. Quanti-Cult*—IDEXX カタログ # WKIT-1001
5. American Type Culture Collection 1-800-638-6597 atcc.org
6. IDEXX-QC糞便性大腸菌群－IDEXX カタログ番号UN3373-WQC-FC

IDX 33/02 - 06/12
IDX 33/01 - 11/09
WATER ANALYSIS METHODS
http://nf-validation.afnor.org

The method Colilert*-18/Quanti-Tray* or Quanti-Tray*/2000 for water analysis is granted NF Validation by AFNOR Certification as an alternative method to the standard ISO 9308-3 for enumeration of *Escherichia coli* ß-glucuronidase positive in bathing water, under the Certificate number: IDX 33/02–06/12.

The method Colilert*-18/Quanti-Tray* for water analysis is granted NF Validation by AFNOR Certification as an alternative method to the standard ISO 9308-1 for enumeration of *Escherichia coli* ß-glucuronidase positive and coliform bacteria ß-galactosidase positive in drinking water, under the Certificate number: IDX 33/01–11/09.

For more information about end of validity, please refer to the certificate NF Validation available on website mentioned above.

La méthode Colilert*-18/Quanti-Tray* or Quanti-Tray*/2000 pour le contrôle des eaux est certifiée NF Validation par AFNOR Certification comme méthode alternative à la norme NF EN ISO 9308-3 pour le dénombrement des *Escherichia coli* ß-glucuronidase positive dans les eaux de baignades sous le n° d'attestation: IDX 33/02 – 06/12.

La méthode Colilert*-18/Quanti-Tray* pour le contrôle des eaux est certifiée NF Validation par AFNOR Certification comme méthode alternative à la norme NF EN ISO 9308-1 pour le dénombrement des bactéries coliformes ß-galactosidase positive et des *Escherichia coli* ß-glucuronidase positive dans les eaux de consommation humaine, sous le n° d'attestation: IDX 33/01 – 11/09.

La date de fin de validité de la certification NF Validation est précisée sur l'attestation, disponible auprès d'IDEXX ou d'AFNOR Certification.

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY Accreditation #: AT-1931

IDEXX Water Quality Control Laboratory is accredited to ISO/IEC 17025:2005

Appendix 1: Bacterial stress

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

Appendix 2: Relative accuracy results

Exactitude Relative- Eau de mer

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

Exactitude relative - Escherichia coli - Eaux de mer

Méthode de référence					Méthode alternative					Différence
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	
1	2,204	2,326	2,265	0,086	1	2,373	2,538	2,455	0,117	0,190
2	2,290	2,761	2,526	0,333	2	2,287	2,435	2,361	0,105	-0,165
3	4,059	4,151	4,105	0,065	3	4,150	4,150	4,150	0,000	0,045
4	2,318	1,892	2,105	0,301	4	2,130	1,982	2,056	0,105	-0,049
5	3,886	3,851	3,868	0,024	5	3,887	3,763	3,825	0,087	-0,044
6	3,665	3,630	3,648	0,025	6	3,887	3,788	3,837	0,070	0,189
7	3,043	3,071	3,057	0,020	7	2,901	2,849	2,875	0,037	-0,182
8	3,534	3,480	3,507	0,038	8	3,145	3,044	3,094	0,072	-0,413
9	3,451	3,597	3,524	0,103	9	3,714	3,689	3,701	0,018	0,178
10	3,244	3,151	3,197	0,066	10	3,265	3,195	3,230	0,050	0,033
11	4,224	3,729	3,976	0,350	11	4,080	4,020	4,050	0,043	0,074
12	3,886	3,818	3,852	0,048	12	3,738	3,862	3,800	0,087	-0,052
13	1,785	1,176	1,481	0,431	13	1,301	1,000	1,151	0,213	-0,330
14	3,509	3,471	3,490	0,027	14	3,440	3,417	3,429	0,016	-0,061
15	4,066	4,224	4,145	0,112	15	4,239	4,150	4,195	0,063	0,050
16	3,956	3,956	3,956	0,000	16	3,837	3,837	3,837	0,000	-0,120
17	4,224	4,182	4,203	0,030	17	4,114	4,090	4,102	0,017	-0,101
18	4,269	4,443	4,356	0,123	18	4,384	4,239	4,311	0,103	-0,045
19	4,318	4,318	4,318	0,000	19	4,191	4,384	4,287	0,136	-0,030
20	3,921	4,029	3,975	0,077	20	3,964	3,812	3,888	0,108	-0,087

q= 20 **Mx=** 3,478 **My=** 3,432 **M=** -0,046
n= 2 **MEDx=** 3,750 **MEDy=** 3,812 **MED=** -0,047
N=qn= 40 **SDbx=** 0,808 **SDby=** 0,858 Biais
MEDwx = 0,066 **MEDwy =** 0,071
SDwx= 0,170 **SDwy=** 0,089
rob. SDwx= 0,097 **rob. SDwy=** 0,105

Choix de la méthode
GMFR

R= 0,522
rob. R= 1,078
Sx= 0,807
Sy= 0,850

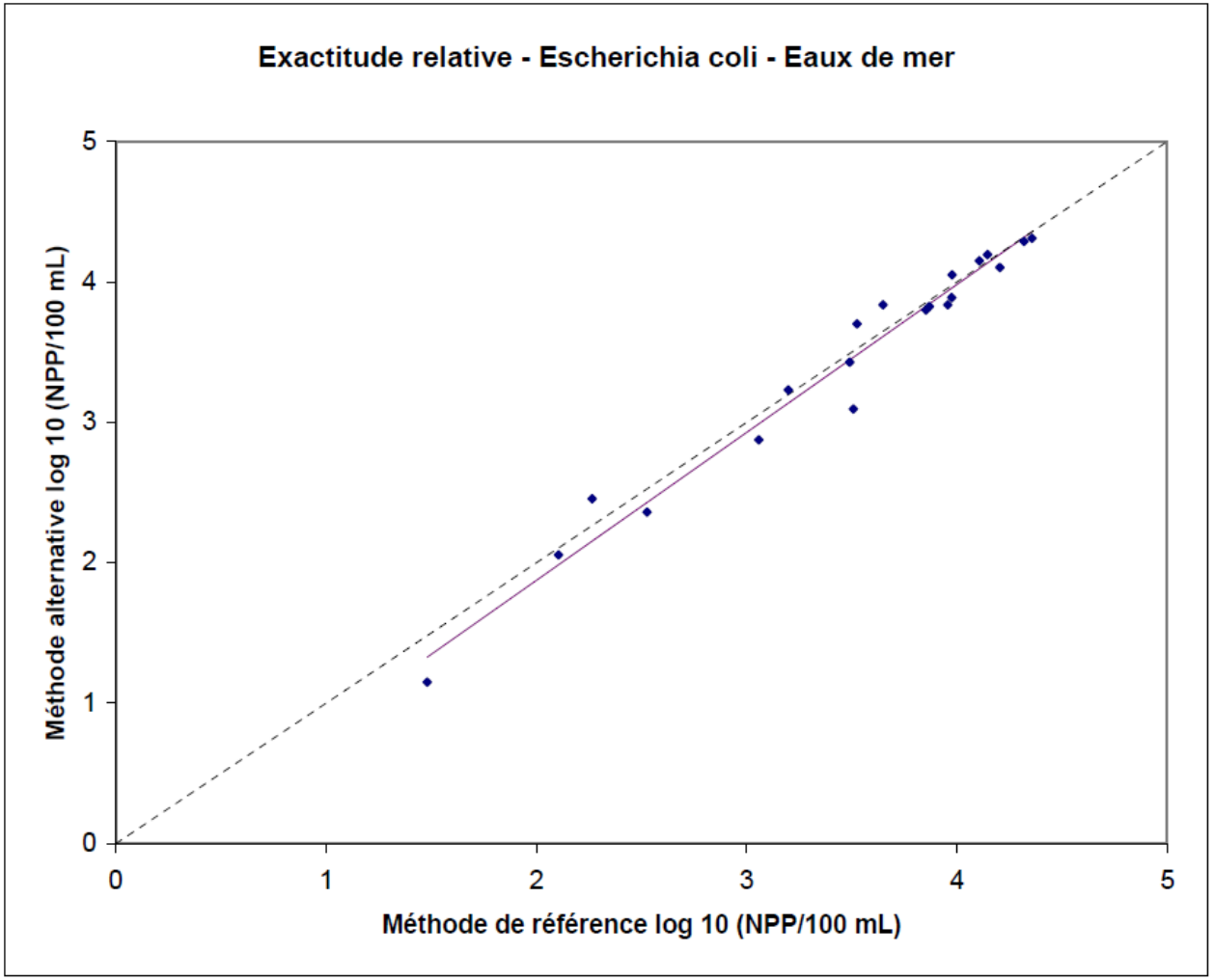
r= 0,984
b= 1,053
a= -0,232
Res. SEM= 0,155
Res. SD= 0,219

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

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

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

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Exactitude Relative - Eau douce

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

Exactitude relative - Escherichia coli - Eaux douces

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

q= 22 **Mx=** 2,380 **My=** 2,259 **M=** -0,120
n= 2 **MEDx=** 2,422 **MEDy=** 2,333 **MED=** -0,066
N=qn= 44 **SDbx=** 0,649 **SDby=** 0,757 Biais
MEDwx = 0,066 **MEDwy =** 0,061
SDwx= 0,160 **SDwy=** 0,129
rob. SDwx= 0,097 **rob. SDwy=** 0,090

Choix de la méthode

GMFR

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

Sx= 0,652
Sy= 0,754

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

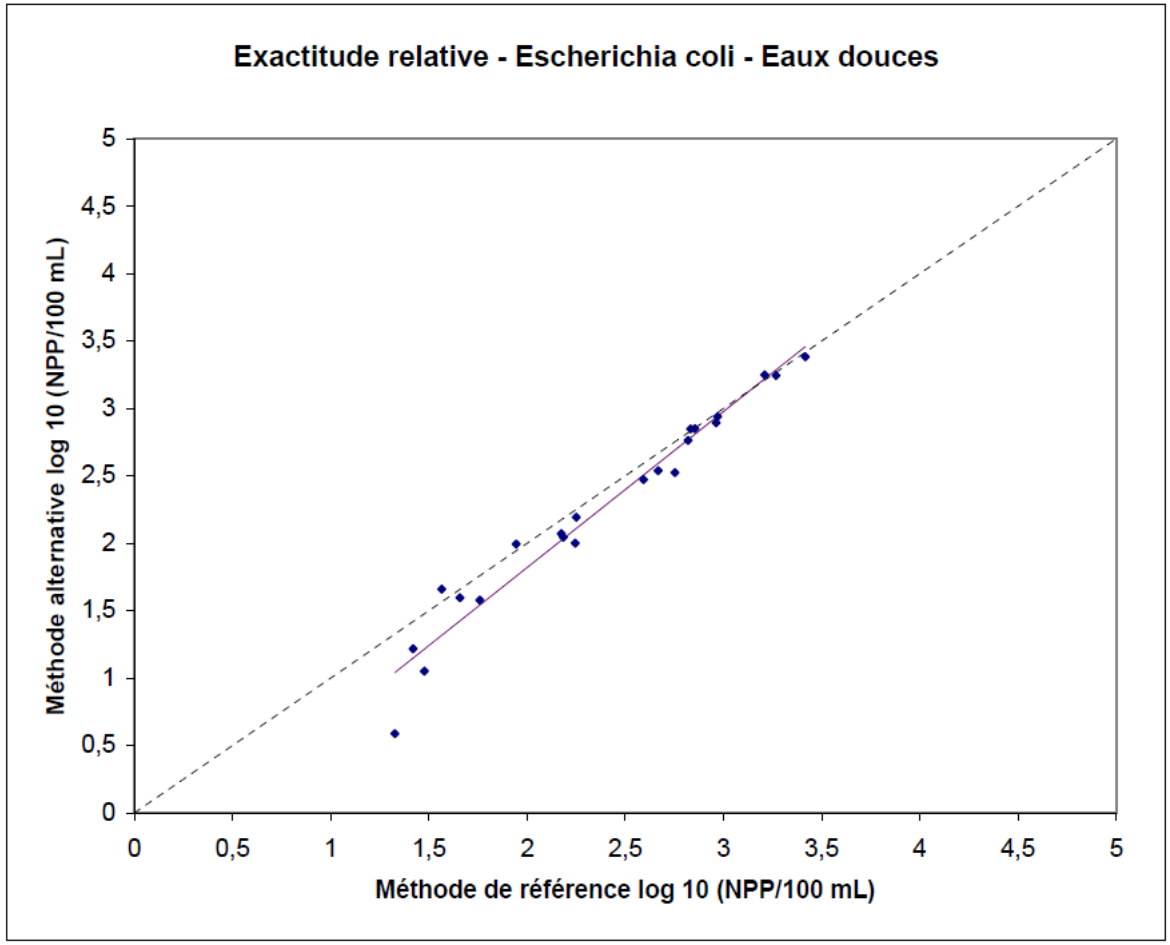
Res. SEM= 0,160
Res. SD= 0,226

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

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

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

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



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

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

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

Choix de la méthode
GMFR

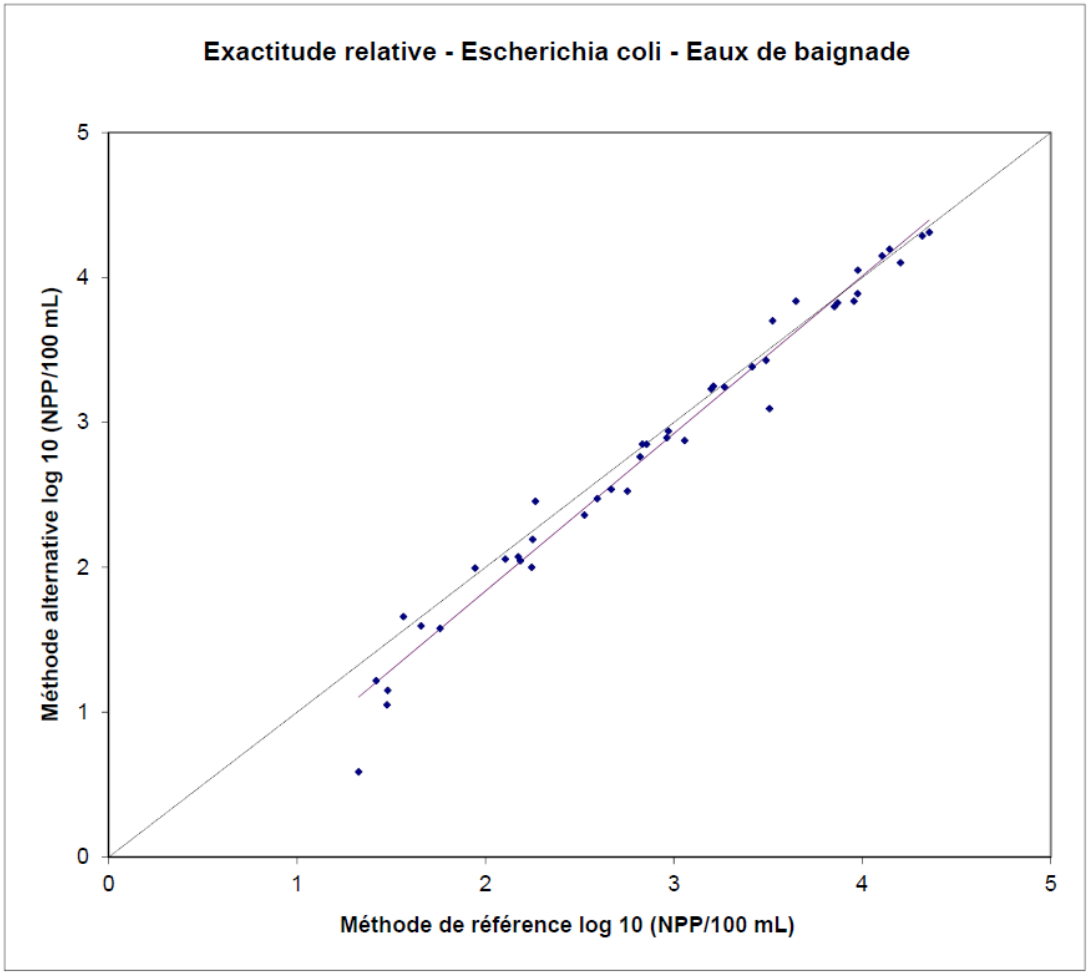
R=	0,680		
rob. R=	1,078		
		Sx=	0,911
		Sy=	0,990
r=	0,988		
b=	1,087	**Res. SEM=**	0,157
a=	-0,337	**Res. SD=**	0,223

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

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

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

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



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

Exactitude Relative - Eau douce

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

Exactitude relative - Escherichia coli - Eaux douces

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

q= 20 **n=** 2 **N=qn=** 40

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

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

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

Choix de la méthode
GMFR

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

Sx= 0,599 **Sy=** 0,619

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

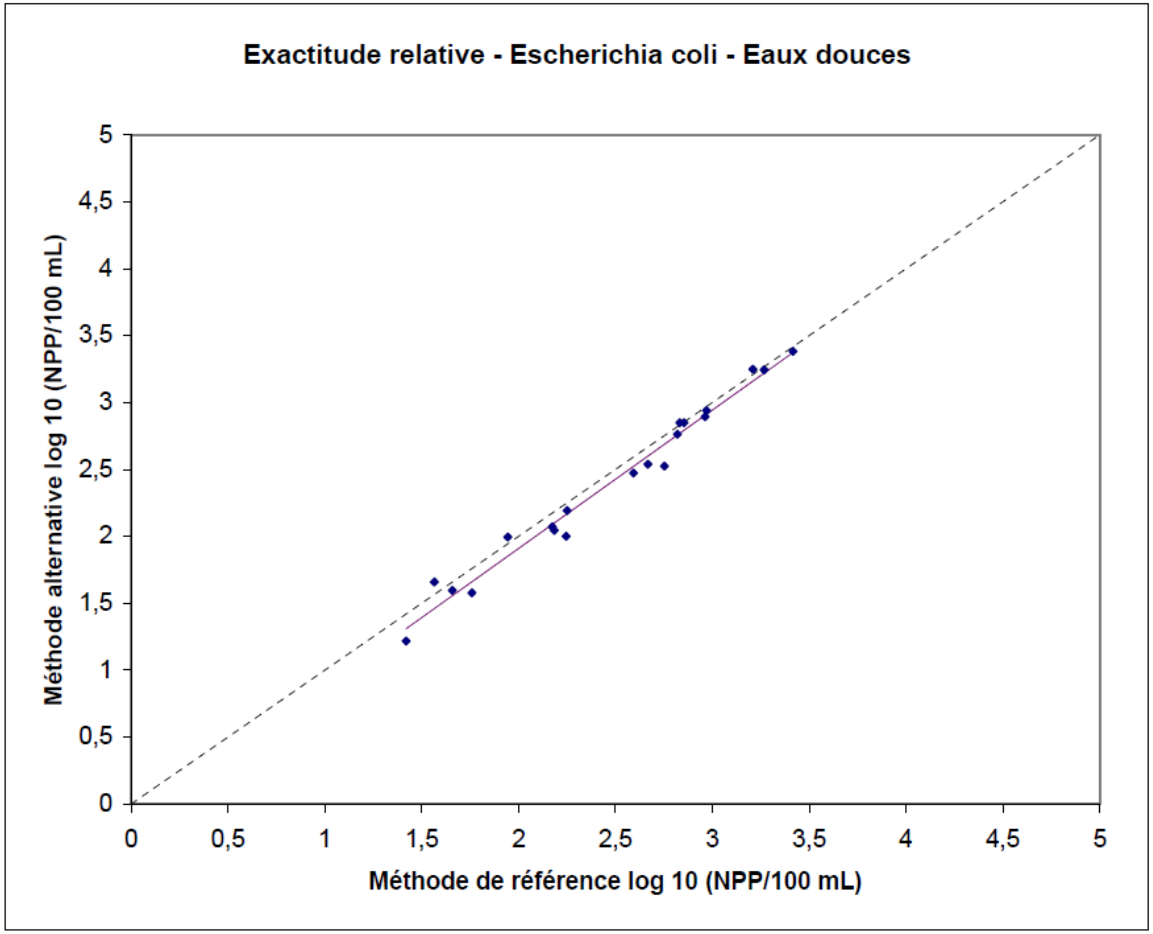
Res. SEM= 0,094 **Res. SD=** 0,133

S(b)= 0,036 **S(a)=** 0,080 **p(t;b=1)=** 0,355 **p(t;a=0)=** 0,056 **t(b)=** 0,937 **t(a)=** 1,969

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

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

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



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

Suppression of 2 results under the LOD of the reference method

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

q=	40	**Mx=**	2,978		**My=**	2,918	**M=**	-0,060
n=	2	**MEDx=**	2,967		**MEDy=**	2,884	**MED=**	-0,055
N=qn=	80	**SDbx=**	0,864		**SDby=**	0,905		Biais
			MEDwx =	0,066		**MEDwy =**	0,066	
			SDwx=	0,165		**SDwy=**	0,094	
			rob. SDwx=	0,097		**rob. SDwy=**	0,098	

Choix de la méthode

GMFR

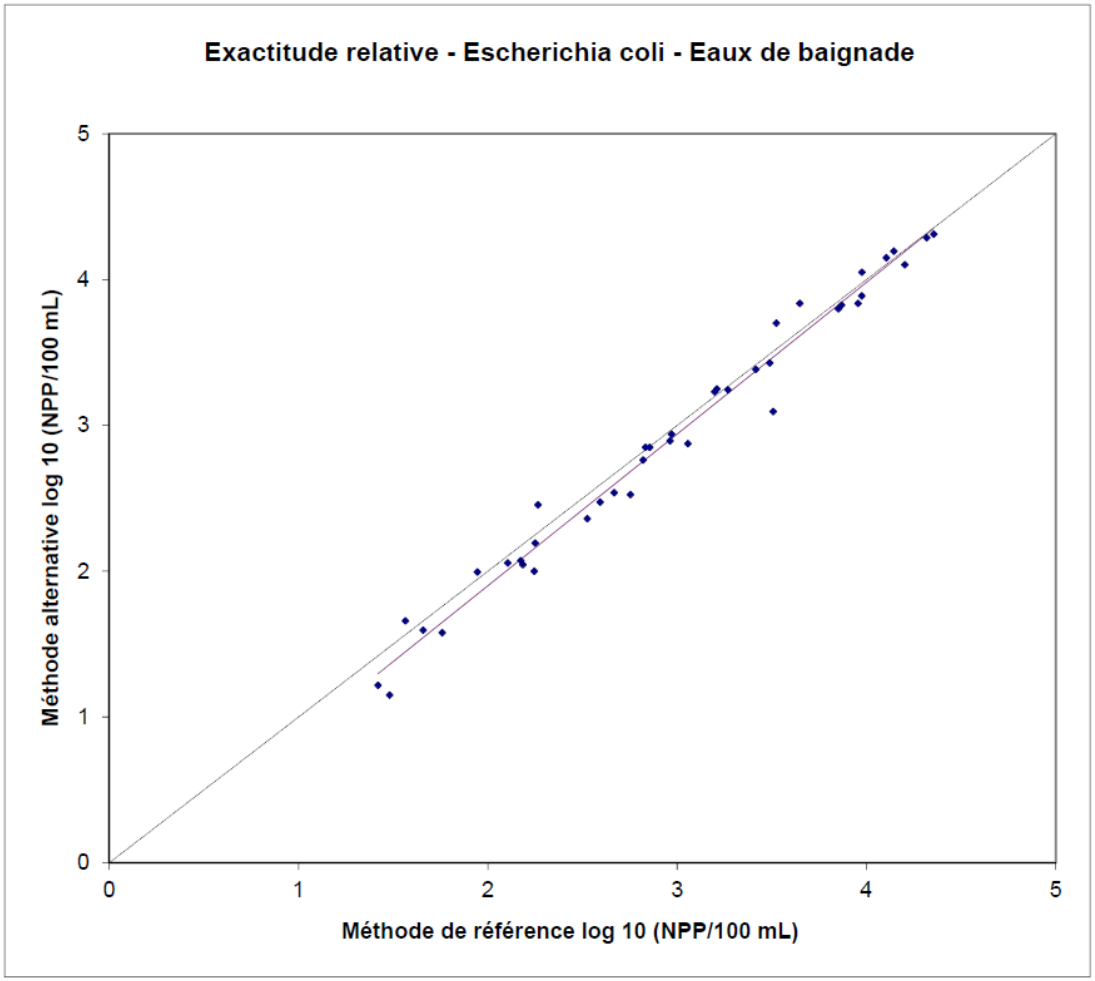
R=	0,568		
rob. R=	1,009		
		Sx=	0,867
		Sy=	0,902
r=	0,991		
b=	1,040	**Res. SEM=**	0,125
a=	-0,180	**Res. SD=**	0,177

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

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

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

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Appendix 4: Linearity results

Linéarité- Résultats bruts

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

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

Linéarité - Escherichia coli - Eau de mer

Niveau	Méthode de référence (NF ISO 9308-1)				Méthode alternative Colilert® -18 / Quanti-Tray®			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	1,663	1,973	1,8	0,219	1,924	2,199	2,1	0,194
2	2,540	2,797	2,7	0,182	2,644	2,496	2,6	0,105
3	2,980	2,918	2,9	0,044	2,910	2,925	2,9	0,011
4	3,868	3,992	3,9	0,088	3,664	3,991	3,8	0,232

q = 4
n = 2
N = qn = 8

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

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

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

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

Choix méthode

GMFR

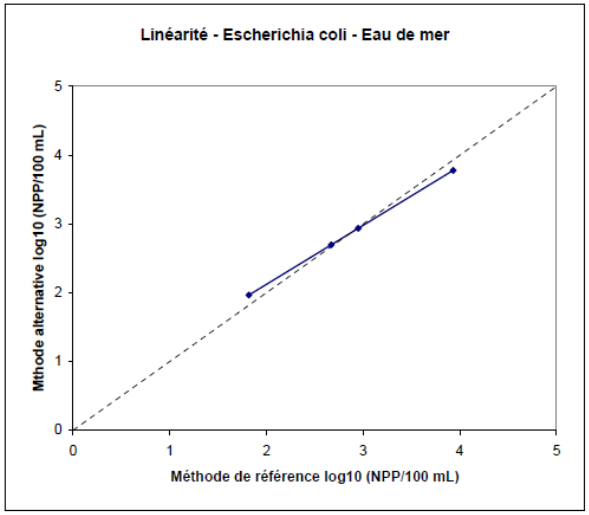
R = 1,063
rob.R = 1,111
Res.SEM = 0,118
Res.SD = 0,167

Sx = 0,814
Sy = 0,699

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

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

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



Linéarité

F = 4,514
rob.F = 0,305

p(F) = 0,094
rob.p(F) = 0,753

Appendix 5: LOD / LOQ results

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

Résultats bruts

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

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

Calculs statistiques

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

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

Appendix 6: Selectivity results

Inclusivity

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

Exclusivity

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

Appendix 7: Enumeration of culturable microorganisms

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

Appendix 8: Interlaboratory study results

Results in NPP/100 mL

Niveau 0

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

Niveau 1

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

Niveau 2

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

Niveau 3

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