« XplOrer64™ / CheckN'Safe™ Enterococci » for the real-time and continuous detection and quantification of intestinal Enterococci in Bathing Recreational waters and Waste waters

Summary report
September 2021

Attestation n° BRD 07/19-11/09

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Competencies of the laboratory are certified by COFRAC accreditation for the analyses marked with the symbol.*

Foreword

Studied method
XplOrer64™ / CheckN'Safe™ *Enterococci* Kit.

Reference method*
- NF EN ISO 7899-1: 1999 « Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: miniaturized method (Most Probable Number) by inoculation in liquid medium».

Scope
- Bathing waters
- Waste waters
- Raw and processed waste water

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

APPENDICES

1 Introduction

XplOrer64™ / CheckN'Safe™ Enteroccocci was validated in 2009. Then, it was extended in 2010 for application in waste waters. Finally, this method was renewed in 2014 and 2017.

This project concerns a new renewal without modification of the method.

2 Review of changes in the alternative method since the previous validation

2.1 History of validation

The method XplOrer64™ for the enumeration of intestinal enterococci was validated by AFNOR Certification in 2009 under the certificate number BRD 07-19 – 11/09 according to the validation protocol for an alternative commercial method as compared with a reference method (revision 1).

An extension study was realized in 2011 for application in waste waters (category 1b in 2010, new sub-category in 2013 « waste water, raw and processed waste water » of the category of industrial waters). The three protocols of the alternative method for this category were applied.

In 2011, all the results of the comparative study of the initial validation and the extension study were analysed with the XplOrer64™ V3.0 software and the calibration curve QC *Entero*. The analyses were performed by EUROFINS IPL Nord in accordance with the applicable requirements.

In 2014, for the first renewal study, exactitude further tests in samples of the category « bathing waters » were performed in duplicate, in order to be conform to the revision 2 of the « Validation protocol for an alternative commercial method as compared with a reference method (revision 2) » of May 2013. These tests were realized by the ISHA laboratory.

Since the last renewal in 2017, there were no changes of the alternative method: XplOrer64™, no changes of the reference method: NF EN ISO 7899-1: 1999 and no changes of the validation referential: « Validation protocol for an alternative commercial method as compared with a reference method (revision 2) ».

The validation history is summarized in the following table :

Method	Date of approval	Type of validation	Comments	Expert laboratory	Protocol of validation	Reference method
XplOrer64™ / CheckN'Safe™ *Enterococci*	06/11/2009	Validation	Software V 1.0	IPL SED Nord	Rev. 0 (2008)	NF EN ISO 7899-1 (1999)
	10/05/2010	Modification 1	Software V 2.0	NA	Rev. 0 (2008)	NF EN ISO 7899-1 (1999)
	10/06/2011	Extension 1	Extension for waste waters & Software 3.0	IPL SED Nord	Rev. 1 (2010)	NF EN ISO 7899-1 (1999)
	19/06/2012	Modification 2	Add of the DropStop tool in the XplOrer software	NA	Rev. 1 (2010)	NF EN ISO 7899-1 (1999)
	13/05/2014	Renewal 1	Updates according to the revision 2 of the validation protocol	ISHA	Rev. 2 (2013)	NF EN ISO 7899-1 (1999)
	10/10/2017	Renewal 2	Request of supplementary tests of exclusivity	ISHA	Rev. 2 (2013)	NF EN ISO 7899-1 (1999)
	2021	Renewal 3		AdGène	Rev. 2 (2013)	NF EN ISO 7899-1 (1999)

2.2 Review of user complaints about the method

No user customer claims have been registered by AFNOR Certification.

3 Methods protocols

3.1 Principle of alternative method

Conventional microbiological methods for intestinal *Enterococci* quantification in bathing water samples required between 2 to 3 days according to the referential used. 24 hrs additional confirmation tests are required in the membrane filtration methods, which are particularly tedious to perform.

Requiring only few minutes to handle a filtration step or a direct inoculation according to the type of water to analyse, **CheckN'Safe *Enterococci test*** is a simple method to perform, for a quantitative result in real-time, specific to intestinal *Enterococci* species such as *E. fecium*, *E. faecalis*, *E. durans*, *E. hirae*… found in **bathing water** (sea marine or fresh) and in **waste waters** (water concentrated in suspended matters, like water from treatment plants). This test uses the XplOrer64™ System (Bio-Rad), based on a continuous real-time impedance technology which can process up to 64 samples simultaneously in random access.

With XplOrer64 method, the highest the bacterial concentration is, the soonest the time to results gives a positive signal and determines the *Enterococci* concentration in the water sample, constituting a real active tool for the water sanitary survey management:

- **under the surveillance of bathing water**: for public information on water quality, prevention of public health, protection of coastal waters and freshwater pollution events,
- **for monitoring the quality of waste water from treatment plants or sewerage facilities**: the reuse of effluent water for irrigation of crops or green areas for example, monitoring of raw water during heavy rains for example.

CheckN'Safe™ technology principle:

The **XplOrer64-CheckN'Safe *Enterococci* method is an automated method for the detection and enumeration of intestinal *Enterococci*** by measuring the impedance in a liquid medium, without confirmation (*appendices 1 & 2*). CheckN'Safe *Enterococci* test is based on bacterial growth in liquid culture medium, which takes place in an impedance measurement. Each cell of measurement contains a selective culture medium which allows the specific growth of *Enterococci* strains, two electrodes measuring the variation of the impedance signal during growth.

The detection and analysis of data is optimized for use with the automated XplOrer64 (Bio-Rad). This test is particularly useful for detecting:

- **370 Enterococci/100 ml of bathing water** (freshwater or sea marine) **in 10 h 15 minutes**
- **250 Enterococci/100 ml of waste water in 10 hours 20 minutes**

3.2 Protocol references

CheckN'Safe™ Enterococci test – Code : 3554721 : V3.5
Notice XplOrer64 – V3.0

3.3 Restrictions

The kit certification of the CheckN'Safe™ *Enterococci* kit is for use with XplOrer64™ (Biorad).

3.4 Reference method

- **NF EN ISO 7899-1: 1999** « NF EN ISO 7899-1: 1999 « Water quality - Detection and enumeration of intestinal enterococci in surface and waste water - Part 1: miniaturized method (Most Probable Number) by inoculation in liquid medium».

Steps of the reference method are schematized in *appendix 1*.

4 Goal of the renewal project

This study concerns the renewal of the XplOrer64/CheckN'Safe™ *Enteroccocci* method.

During the previous renewal in 2017, the BT issued a favourable opinion to renew the validation of the method by comparison with the reference method NF EN ISO 7899-1 (1999) and according to the validation protocol for an alternative commercial method as compared with a reference method - Revision 2 (2013) of the NF VALIDATION mark, in its application to water analysis (NF148), for the following field of application: enumeration of intestinal enterococci "bathing water "and" waste water, raw and processed waste water". This opinion was issued subject to additional exclusivity studies on 8 species tested at rates below 10^4 CFU / mL. **As the exclusive data have not been submitted by the ISHA expert laboratory, the experiments will be repeated by AdGène.**

On the other hand, a **modification of the standard NF EN ISO 8199: 2018** was done by the last renewal of 2017 implying an evolution of the enumeration rules for the reference method. This concerns the quantitative methods of enumeration on solid medium by incorporation; spreading or by membrane filtration but does not involve any modification for the enumeration using a liquid medium; which is the case for the NPP reference method of the NF EN ISO 7899-1 reference method. **Therefore, these updates of NF EN ISO 8199: 2018 will not involve any further testing.**

5 Summary of results

5.1 Method comparison study

5.1.1 Relative accuracy

The relative accuracy is defined as the closeness of agreement between test result and the accepted reference value. The relative accuracy is the level of correspondence between the response obtained with the reference method and the response obtained with the alternative method on the same samples.

5.1.1.1 Number and nature of samples

- Initial validation study (2009)

The first statistical analysis involved 63 exploitable results from 170 samples (Including 160 samples naturally contaminated and 10 samples artificially contaminated), belonging to the two categories water: fresh water (40 exploited samples) and marine water (33 exploited samples). The 10 artificially contaminated samples were seawater samples obtained by contaminating seawater with wastewater treatment plant water (concentration between 10^3 and 10^4 cells / 100 mL) in order to obtain high concentrations.
The trials were performed in simples by both methods. Data exploitation in 2011 with the version 3 of the XplOrer64 software had made it possible to retain 39 results for fresh water and 15 results for marine waters (*appendix 3)*.

- Extension study (2011)

Statistical analysis focused on 68 exploitable results from 109 treated waste water (wastewater treatment plant effluents), all naturally contaminated, and analyzed in duplicate by both methods. Three protocols were tested, the general protocol (filtration of 100 mL), the protocol specific 1 (filtration of 10 mL) and specific protocol 2 (inoculation of 1 mL) (*appendix 5)*.

- Renewal study and further testing (2014)

One category of water, bathing water, was tested in duplicate with the reference method and the alternative method.
The different types of samples analyzed are presented in Table 1.

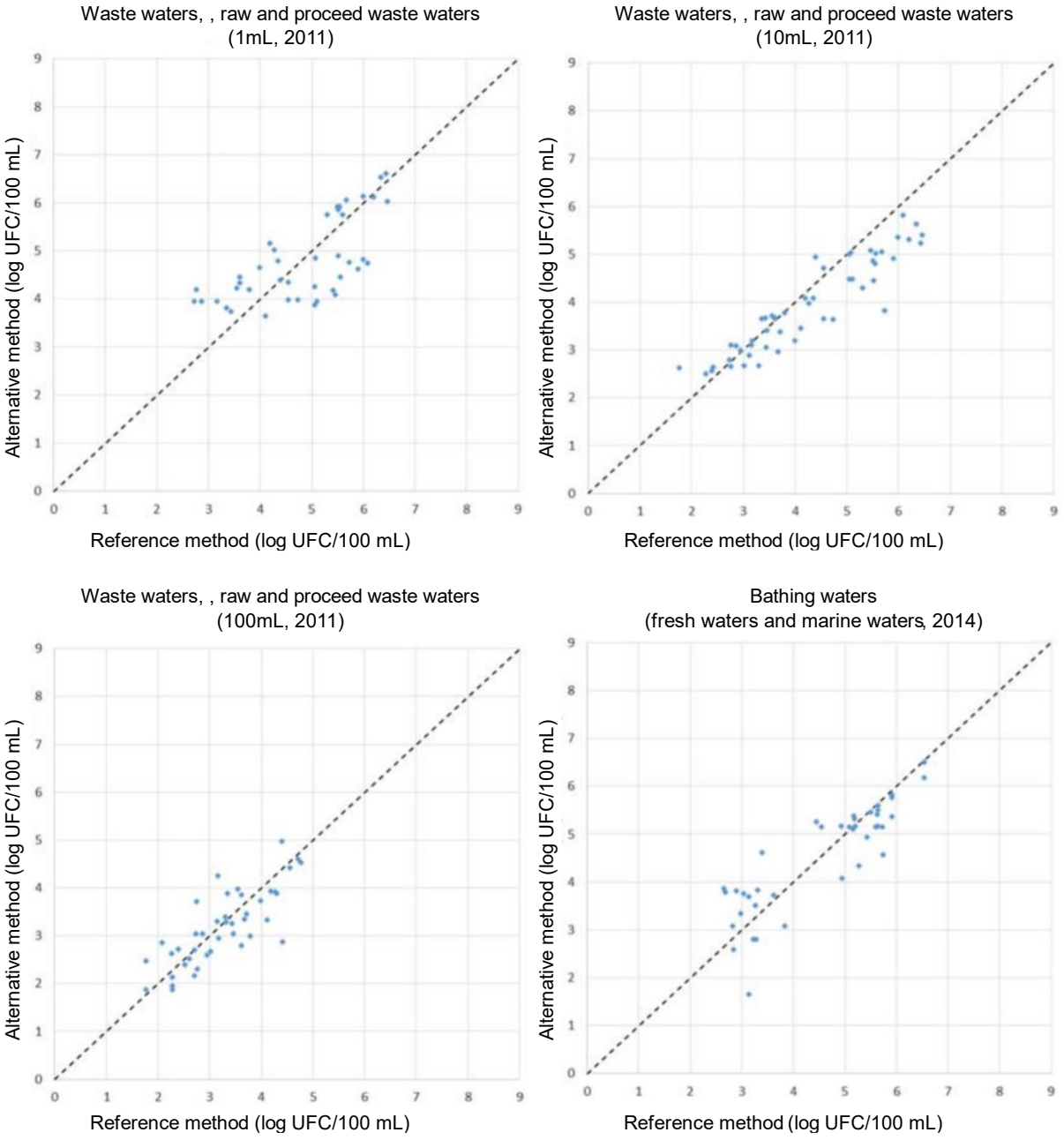
Enumeration	Water type	Samples analyzed	Samples exploited
Enterocoques	Fresh water	14	10
	Marine water	24	10
	Total	38	20

Table 1: number and nature of samples analyzed

A total of 38 samples were analysed and 20 results were used. Samples not retained in the statistical analysis correspond to samples for which lower counts or above the detection limit were found for at least one of the replicates of either method.
The contamination rates used cover the entire measurement range of the alternative method. The stresses applied and the strains used are presented in *appendix 4*.

5.1.1.2 Raw results

Raw results and statistical calculations are summarized in tables 2 and 3 and in appendix 5. Figure 1 shows the two-dimensional graph for the test category. 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 figures.



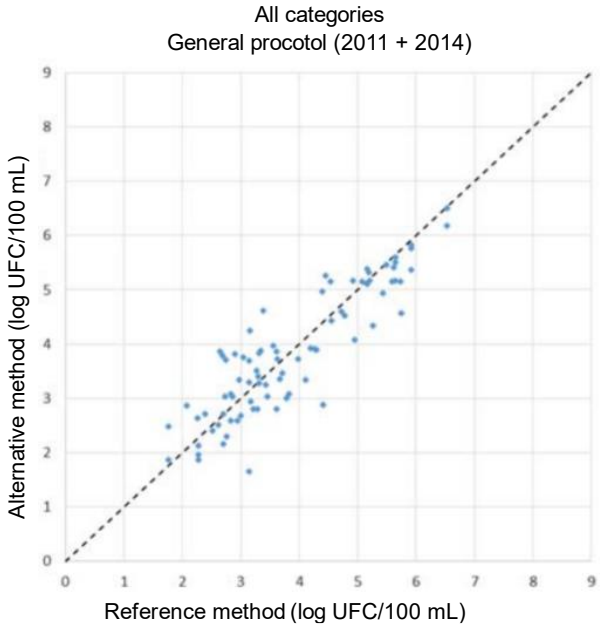


Figure 1: two-dimensional graphs for relative accuracy (black line: y=x)

5.1.1.3 Statistical exploitation

The relationship of relative accuracy between the reference method and the alternative method is evaluated with the linear model: 'y = a + bx '. This formula corresponds to the equation of the linear regression drawn from raw results obtained by experimentation, y representing the alternative method and x the reference method.
There is a perfect accuracy (or there is no systematic bias) between the two methods if this equation is equal to the theoretical 'y = x' equation, which applies in the ideal model where the two methods behave similarly.

The intercept is theoretically zero in this ideal model (hypothesis [a = 0]). The estimated intercept obtained with the two methods is checked using p {a = 0}. If the alternative method is a systematic bias against the reference method, the probability p {a = 0} is less than 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, an ordinary least-squares regression (OLS 1) is used with the x-axis for the reference method,
- If Rob.R < 0.5, an ordinary least-squares regression (OLS 2) is used with the x-axis for the alternative method,
- If 0.5 < Rob.R < 2, orthogonal regression (GMFR) is used with the x-axis to the reference method.

Category	Rob.R	Regression used	T critical	a	T(a)	b	T(b)	Probabilities (%)	
								Intercept at 0	Slope at 1
Waste waters,Raw and processed 1 mL	0.660	GMFR	2.080	0.980	1.475	0.790	1.559	15.7	13.5
Waste waters,Raw and processed 10 mL	0.900	GMFR	2.056	0.700	3.046	0.759	4.654	0.5	0.01
Waste waters,Raw and processed 100 mL	2.342	GMFR	2.080	0.606	1.428	0.792	1.637	17.0	11.8
Bathing waters	4.391	GMFR	2.086	0.458	1.303	0.895	0.306	20.1	76.2
All categories general protocol	3.553	GMFR	2.019	0.146	0.141	0.951	0.191	88.8	84.9

Table 2: statistical data for the enumeration of intestinal enterococci in all categories

Category	Bias (D)		Repeatability			
			r		rob. r	
	Average	Median	RM	AM	RM	AM
Waste waters,Raw and processed 1 mL	-0.032	0.109	0.691	0.754	0.373	0.246
Waste waters,Raw and processed 10 mL	-0.326	-0.319	0.669	0.579	0.413	0.372
Waste waters,Raw and processed 100 mL	-0.069	-0.140	0.464	1.011	0.453	1.061
Bathing waters	-0.016	-0.155	0.238	1.269	0.222	0.973
All categories general protocol	-0.043	-0.140	0.371	1.144	0.276	0.980

Table 3: bias and repeatability of the two methods

5.1.1.4 Conclusion

The equation for the regression line of the different couples are as follows:

Tested matrices	**Regression line (log enterocoque/100mL)**
Waste waters,Raw and processed 1 mL	log Alt = 0.790 log(Ref) + 0.980
Waste waters,Raw and processed 10 mL	log Alt = 0.759 log(Ref) + 0.700
Waste waters,Raw and processed 100 mL	log Alt = 0.792 log(Ref) + 0.606
Bathing waters	log Alt = 0.895 log(Ref) + 0.458
All categories general protocol	log Alt = 0.951 log(Ref) + 0.146

Hypothesis [a = 0 and b = 1] is accepted for all the categories tested, expected for the category raw and processed waste water for the 10 mL protocol. For this couple, the correlation coefficient and the equation of the regression line are corrects: respectively 0.944 and log Alt = 0.759 log(Ref) + 0.700.

Bias between the two methods is between -0.319 and 0.109 depending on protocol and category tested.

The relative accuracy of the alternative method is satisfactory.

5.1.2 Linearity

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

5.1.2.1 Contamination levels

The couple matrix / strain is presented in table 4. For this couple, three levels of contamination were tested in duplicate by the reference method and the alternative method.

Strain	Matrix	Contamination level (CFU/100 mL)
E. faecalis (souche CCM 2541, Eurofins IPL Nord collection)	Fresh surface water	50 / 500 / 5000
	Marine water	
E. faecalis (waste waters, Eurofins IPL Nord collection)	Processed waste water General protocol	2.10^2
E. faecalis (waste waters, Eurofins IPL Nord collection)	Processed waste water 10 mL protocol	2.10^4
E. faecalis (waste waters, Eurofins IPL Nord collection)	Processed waste water 1 mL protocol	2.10^6

Table 4 : couple matrix – strain analyzed

5.1.2.2 Raw results

Raw results and statistical calculations are summarized in appendix 6. Graph of figure 2 show the values of each sample obtained by the alternative method and the reference method. The y-axis is reserved for the alternative method and the x-axis for the reference method.

The representation of a line of equation 'y = x' figures dashed on the figures.

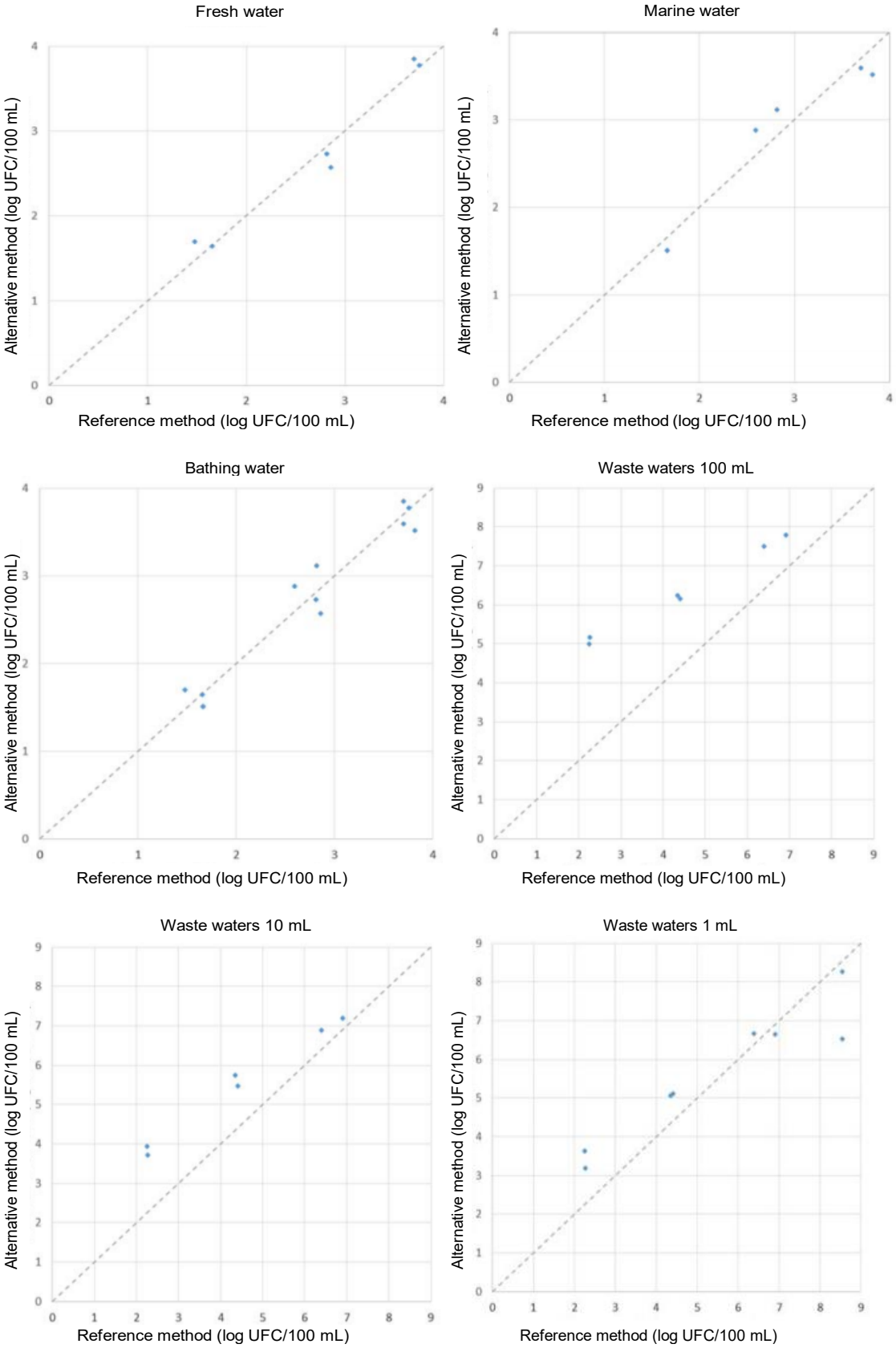


Figure 2: two-dimensional graph for linearity (black line: y=x)

5.1.2.3 Statistical exploitation

Statistical interpretations are carried out in accordance with the requirements of standard NF ISO 16140 (see table 5).

For the lowest concentration level for the “marine water” subcategory, one of the replicates of the alternative method gave a result of <1 CFU / 100 mL in the initial validation study. The interpretation statistic is therefore not presented for this subcategory due to the low number of levels available (only two). The two remaining usable levels are, however, used for the statistical interpretation of the "bathing water" category which groups together the results obtained for water fresh and marine water.

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

Category	Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	Correlation coefficient (r)	Regression line
Fresh water	1.413	GMFR	10.1	56.327	0.005	0.989	log Alt = 0.986 log(Ref) + 0.039
Bathing water	0.667	GMFR	5.41	13.092	0.008	0.973	log Alt = 0.935 log(Ref) + 0.207
Waste waters,Raw and processed 100 mL	2.524	OLS1	10.1	0.484	0.537	0.999	log Alt = 0.586 log(Ref) + 3.712
Waste waters,Raw and processed 10 mL	4.132	OLS1	10.1	0.788	0.440	0.996	log Alt = 0.734 log(Ref) + 2.245
Waste waters,Raw and processed 1 mL	6.628	OLS1	6.94	11.456	0.022	0.991	log Alt = 0.674 log(Ref) + 1.960

Table 4: statistical data of the couple matrix – strain analyzed

The relationship between the 2 methods is not linear:

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

5.1.2.4 Conclusion

The relationship between the two methods is linear for the matrices waste water and raw and processed waste waters, 100 mL and 10 mL protocol.

For the matrices fresh water and bathing water, the correlation coefficient and the equation for the regression line are satisfactory.

For the 1 mL protocol performed with treated proceed waste waters, the correlation coefficients and the equation of the regression line are correct. The result of the statistical test illustrates the dispersion of the points obtained for the highest level with these matrices.

The linearity of the alternative method is satisfactory.

5.1.3 Detection and quantification limits

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.

5.1.3.1 Protocol

Detection and quantification limits were determined by analyzing a pure culture of an *Enterococcus faecalis* isolated from a waste water, by the alternative method.

Five levels of contamination, with six repetitions for each level, have been studied.

5.1.3.2 Results

Raw results are presented in appendix 7 and the summary in the following tables.

- <u>General protocol (filtration of 100 mL)</u>

Level (CFU/100mL)	Number of positive samples	Standard deviation (S_0)	Bias (x_0)
0	0/6	0	0
0.5	3/6	634	22
1.1	6/6	211	153
2.1	6/6	675	696

From the values s_0 and x_0 obtained for the first level, the critical limit (LC) and the detection limit (LOD) were determined in 100 mL:

Parameter	Formulas	Value (*Enterococci*/100 mL)
Critical level	1.65 s_0 + x_0	$1.0.10^3$
Detection limit	3.3 s_0 + x_0	$2.1.10^3$
Quantification limit	10 s_0 + x_0	$3.6.10^3$

❖ Specific protocol 1 (filtration of 10 mL)

Level (CFU/100mL)	Number of positive samples	Standard deviation (S_0)	Bias (x_0)
0	0/6	0	0
1.0	3/6	352	90
2.1	4/6	135	10
5.2	5/6	295	355
10.4	6/6	441	220

From the values s_0 and x_0 obtained for the first level, the critical limit (LC) and the detection limit (LOD) were determined in 10 mL:

Parameter	Formulas	Value (*Enterococci*/100 mL)
Critical level	1.65 s_0 + x_0	$6.7.10^2$
Detection limit	3.3 s_0 + x_0	$1.2.10^3$
Quantification limit	10 s_0 + x_0	$3.6.10^3$

❖ Specific protocol 2 (filtration of 1 mL)

Level (CFU/100mL)	Number of positive samples	Standard deviation (S_0)	Bias (x_0)
0	0/6	0	0
0.5	0/6	0	0
0.7	3/6	289	1
1.4	6/6	632	785
2.9	6/6	349	1200

From the values s_0 and x_0 obtained for the first level, the critical limit (LC) and the detection limit (LOD) were determined in 1 mL:

Parameter	Formulas	Value (*Enterococci*/100 mL)
Critical level	1.65 s_0 + x_0	$4.8.10^2$
Detection limit	3.3 s_0 + x_0	$9.5.10^2$
Quantification limit	10 s_0 + x_0	$2.9.10^3$

5.1.3.3 Conclusion

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

5.1.4 Specificity/selectivity

The **specificity** is defined as the ability of the method to accurately measure a given analyte, or its amount in the sample without interference from non-target components.

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

5.1.4.1 Protocol

Thirty target strains and thirty non target strains (from national, international and internal collections) were analyzed. The tests were conducted according to the protocol of the alternative method.

The contamination levels used for inclusivity were between 10 and 100 CFU / 100mL and for exclusivity approximately 10^4 CFU / 100 mL.

5.1.4.2 Results

Inclusivity:

Out of 30 strains tested, 28 were detected (*appendix 8)*. Regarding the 2 strains not detected:

- one strain of *Enterococcus avium* (well water) was not detected by the alternative method and the reference method
- one strain of *Enterococcus faecalis* out of 3 tested was not detected by the alternative method

Exclusivity:

During the initial validation in 2009, 29 of the 30 interfering strains tested were not detected by the alternative method as expected (*appendix 8)*. Only the sample spiked with *Providencia stuartii* gave a result false positive.

During the 2011 expansion (new software version), the cross-reaction with *Providencia stuarti*i was not more observed.

In the initial exclusivity study, 8 species were tested at levels below 10^4 CFU / mL. The tests carried out in 2021 by the AdGene laboratory gave satisfactory results (Annex 8, further testing).

5.1.4.3 Conclusion

The selectivity of the method is satisfactory.

5.1.5 Practicability

The practicability is studied by filling in the 13 criteria defined by the Technical Board

- ❖ Procedure for conditioning the elements of the method

Each kit contains 60 disposable vials packaged in boxes of 120 or 60 units ready to use.

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- ❖ Reagent volume

Each kit contains 60 disposable vials, pre-filled with 9 ml of a selective Enterococci broth culture medium.

- ❖ Conditions of storage of the elements (expiry date for unopened products)

Once received, the kit must be stored at +2-8°C. Reagents can then be used until the expiration date indicated on the reagent vial and the package. It is recommended to keep the necessary reagents at room temperature 1 h before use.

- ❖ Modalities of use after the first use (expiry dates for use)

Each vial is for single use and must therefore be disposed of at the end of the analysis as a material potentially infectious.

- ❖ Specific equipment or premises required
  - Equipment for membrane filtration in accordance with ISO 8199 requirements
  - XplOrer64™ System (Bio-Rad)
  - CheckN'Safe Racks (Bio-Rad, code 359-3455, 4 units are provided with the XplOrer64 System)
  - CheckN'Safe Screw caps, 40 sterile units x 1 bag (Bio-Rad, code 359-3457)

- ❖ Reagents ready-to-use or to be reconstituted

CheckN'Safe Enterococci is ready-to-use

- ❖ Period required to train an operator not initiated into the method

Less than 1 day for an operator trained in classical microbiology techniques (in particular technical membrane filtration).

- ❖ Real-time handling and flexibility of the method
  - *Protocols with filtration (general and specific 1)* = For the analysis of a sample the time required for the alternative method is 2.7 minutes, while it takes 5.5 minutes to complete the reference method.

  - *Specific protocol 2 (direct inoculation)* = Specific protocol 2 using direct inoculation is faster than the other 2 protocols of the alternative method using a filtration step (general protocol and specific protocol 1).

Filtration is all the longer as the samples have reduced filterability, often linked to the rate and size of suspended matter.
The duration of inoculation of a sample by XplOrer64 ™ CheckN'Safe ™ Enterococci method does not vary depending on the number of samples (in particular for specific protocol 2, based on inoculation direct 1 mL).

Time savings are obtained in the preparation of samples and readings: the machine can accommodate 62 vials, and therefore simultaneously give 62 results.

❖ Time required for obtaining the results

The alternative method gives a negative or positive result depending on the type of water on the same day or on D + 1, then that the result is obtained between D + 2 and D + 3 by the reference method.

❖ Operator qualification type

The qualification of the operator is similar to the qualification needed for the reference method.

❖ Phases shared with the reference method

None.

❖ Traceability of the analysis results

The names of the different operators can be saved in the device and selected according to the person performing the analysis. Information relating to the sample (reference, origin, etc.) can be recorded in the device, the software. All analysis data (date, time, DT detection results-time, etc.) can be viewed at any time.

❖ Obligation to maintain specific apparatus for the user

None.

5.1.6 Conclusion

The comparative study of the methods was carried out according to the standards applied to the microbiological analysis of water "Validation protocol of an alternative commercial method compared to a method of reference" (revision 2) adopted by AFNOR Certification in May 2013 associated with the NF EN ISO reference system 16140: 2003 for parts of the validation.

A reinterpretation of the previously acquired results was carried out according to the new software version the XplOrer64 ™ V3.0, in the bathing water application area.

The XplOrer64 ™ - CheckN'Safe ™ Enterococci method has been compared to the NF EN ISO 7899-1 method.

The results allow to conclude that:

- ❖ the linearity of the alternative method is satisfactory,
- ❖ the relative accuracy of the alternative method compared to the reference method is satisfactory.

The general protocol validated during the initial study on the field of application "bathing water" appeared more suitable for samples with low SS content.
The correlation between the reference method and the alternative method according to the 3 studied protocols is appeared satisfactory.

The repeatability values of the general protocol (F100 mL) and of the specific protocol 2 (Direct 1 mL) are from same order and higher than that of the reference method.

The average biases between the two methods (alternative method - reference method) are:

- ❖ -0.155 log to -0.140 log for the general protocol,
- ❖ -0.319 log for specific protocol 1 (F10 mL),
- ❖ 0.109 log for specific protocol 2 (Direct 1 mL).

Finally, the specificity results are satisfactory

5.2 Interlaboratory study

5.2.1 Interlaboratory study implementation

5.2.1.1 Participating laboratories

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

5.2.1.2 Verification of the absence of enterococcus in the matrix

A marine water providing of Gravelines (59) was used as test matrix The absence of *enterococci* in this matrix before the contamination was checked.

5.2.1.3 Stability of the strain in the test matrix

The expert laboratory kept 3 packages of identical composition to those sent to the participants for verification of the homogeneity of batches of samples prepared by analyzes according to NF EN ISO 7899-1 in duplicate A, B, C, D, E and F samples contained in each package.
Analysis of the results did not reveal any anomalies, the samples prepared were of sufficient quality for use in this collaborative study

5.2.1.4 Samples preparation and inoculation

From this matrix, 4 batches, named I, II, III and IV, were successively created:

- Undoped Batch I consisting of 150 liters of marine water placed in a polyethylene tank equipped with a mechanical agitator arm (100 rpm). After 10 minutes of stirring, were extracted, without interruption and under shaking, 15 flasks denoted X and 15 flasks denoted Y.

- Batch II consisting of the remainder of batch I doped with approximately 10^2 enterococci / 100 ml. After 10 minutes of stirring, were extracted, without interruption and with stirring, 15 bottles rated A and 15 bottles rated B, previously mixed together and taken at random.

- Batch III consisting of the remainder of batch II doped with approximately 10^3 enterococci / 100 ml. After 10 minutes with stirring, were extracted, without interruption and with stirring, 15 flasks denoted C and 15 flasks denoted D, previously mixed together and taken at random.

- Batch IV consisting of the remainder of batch III doped with approximately 10^4 enterococci / 100 ml. After 10 minutes with stirring, were extracted, without interruption and with stirring, 15 bottles marked E and 15 bottles marked F, previously mixed together and taken at random.

The strain of enterococcus used for doping is a strain isolated from the environment (central range from Dunkirk (59)). The doping was carried out with a dilute suspension of germs grown in broth non-selective for 24 hours.
After racking, the samples remained at room temperature for 1 hour then packaged, the bottles being distributed randomly in the packages. A thermo-button was placed in each vial noted X before packing.

5.2.1.5 Samples labeling

Fifteen packages were thus made up, each containing refrigerants and 8 samples:

- samples A, B, C, D, E and F for enumeration of E. coli and intestinal enterococci in duplicate by each method,
- sample X for temperature measurement, sample Y for enumeration of revivable germs at 22 ° C and 36 ° C.

5.2.1.6 Samples shipping, reception and analysis

The samples were shipped in a cold kit on October 5, 2009.
The packages were delivered between October 6 and 7, 2009.

5.2.2 Results

5.2.2.1 Temperature and state of the samples at reception

All the packages arrived in good condition and all the participants measured a temperature in flask X at reception between 1 ° C and 7 ° C. All thermo-buttons have been returned and the readings recorded temperatures confirm that the samples remained at refrigerated temperature during the transport.

5.2.2.2 Enumeration of total flora

The average concentration observed in revivable germs at 22 ° C in the samples on Marine Agar is of about 4800 / mL, that in revivable germs at 36 ° C of about 2900 / mL.

5.2.2.3 Results from expert laboratory and participating laboratories

All the results are presented in appendix 9.

The final results obtained by the participants by the XplOrer64 ™ method were recalculated by the expert laboratory using a new optimized calibration equation, transmitted by the manufacturer, in using the detection times (DT) observed by the participants (these DT were to be completed by the participants in the results form).

The results of participants 9, 10, 11 and 12 have not been used. Indeed, the participant n ° 9 met a software problem during the analysis by alternative method, the participant n ° 10 reports results outliers by alternative method and laboratories n ° 11 and 12 of abnormal results by method standardized. The results of the 8 other laboratories could well be used.

5.2.3 Statistical interpretation

5.2.3.1 Bias

The table below represents the target value, the mean, the standard deviation of fidelity, the relative bias and the bias of each contamination level in log CFU / 100 mL.

Levels	Low	Medium	High
Target value	**1.72**	**2.89**	**3.79**
Mean	1.86	2.91	3.63
Repeatability standard deviation	0.15	0.27	0.14
Inter-assay standard deviation	0.32	0.10	0.25
Fidelity standatd deviation	0.35	0.29	0.28
Relative bias	8.04%	0.54%	-4.21%
Bias	**0.14**	**0.02**	**-0.16**

The accuracy is estimated by the bias which varies between -0.16 and 0.14 CFU / 100 mL.

5.2.3.2 Accuracy profile

The table below shows the tolerance values and tolerance limits of the alternative method for a tolerance probability value of 80% and an acceptability limit value of 0.8 log. The figure 3 shows the plot of this accuracy profile.

Tolerance probability	Limits of acceptability	Levels	Low	Medium	High
80%	**0.8 log**	Target value (x)	1.72	2.89	3.79
		Low tolerance limit (l)	1.38	2.52	3.24
		High tolerance limit (h)	2.34	3.29	4.02
		Low acceptability limit (l-x)	-0.34	-0.37	-0.55
		High acceptability limit (h-x)	0.62	0.40	0.23

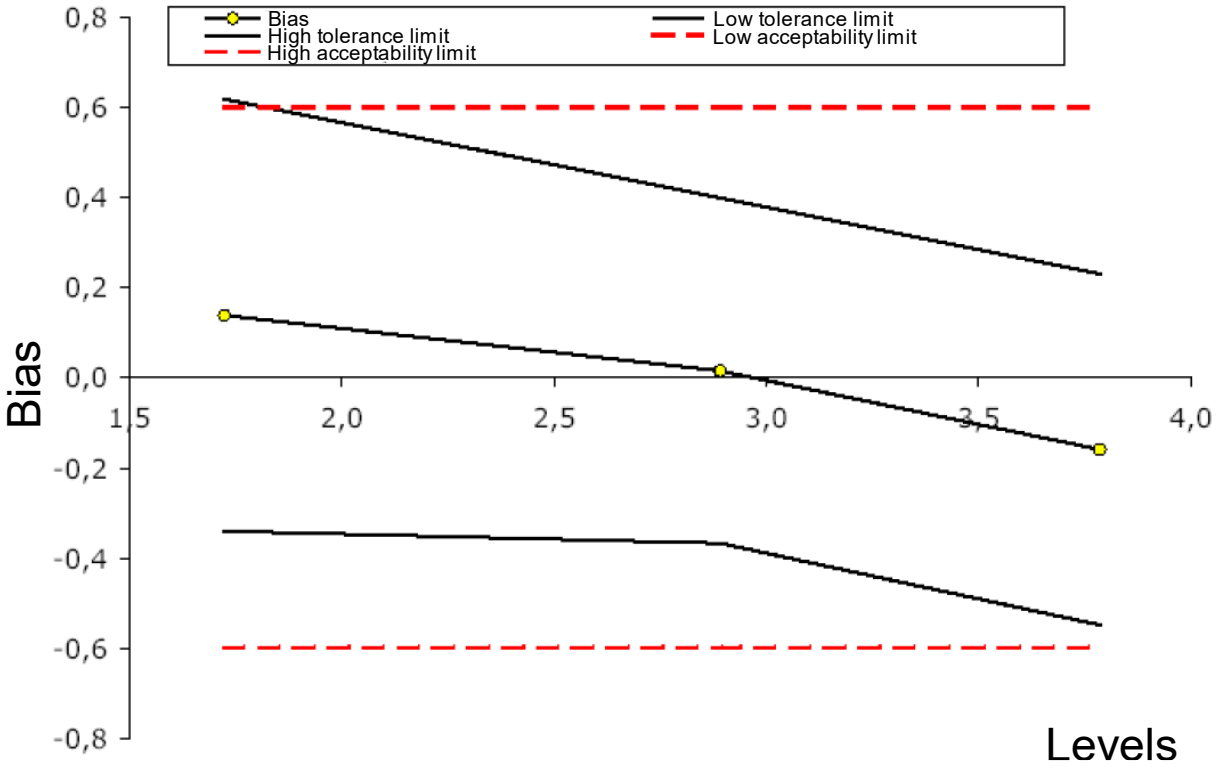


Figure 3: Accuracy profile for a tolerance probability at 80% and acceptability limits at 0,8 log

5.2.3.3 Conclusion

The results of 8 participating laboratories could be used as part of this interlaboratory study.

The study of the accuracy profile obtained with these results reveals that the alternative method XplOrer64 ™ is valid for all levels (tolerance interval between the acceptability limits) for a value acceptability criterion λ fixed at 0.6.

For lower values of λ, the upper limit of the low-level tolerance interval and the lower limit at high level are the first limits to go beyond the limits of acceptability (from λ fixed at 0.5).

5.3 Conclusion

The XplOrer64 ™ - CheckN'Safe ™ Enterococci method has been compared to the NF EN ISO 7899-1 method.

The results allow to conclude that:

- the linearity of the alternative method is satisfactory,
- the relative accuracy of the alternative method compared to the reference method is satisfactory.

The general protocol validated during the initial study on the field of application "bathing water" appeared more suitable for samples with low SS content.
The correlation between the reference method and the alternative method according to the 3 studied protocols is appeared satisfactory.
The repeatability values of the general protocol (F100 mL) and of the specific protocol 2 (Direct 1 mL) are from same order and higher than that of the reference method.

The average biases between the two methods (alternative method - reference method) are:

- -0.155 log to -0.140 log for the general protocol,
- -0.319 log for specific protocol 1 (F10 mL),
- 0,109 log pour le protocole spécifique 2 (Direct 1 mL).

The specificity results are satisfactory.

The study of the accuracy profile obtained with the results of the interlaboratory study reveals that the method alternative XplOrer64 ™ is valid for all levels (tolerance interval between the limits acceptability) for an acceptability criterion value λ set at 0.6.

For lower values of λ, the upper limit of the low level tolerance interval and the lower limit at high level are the first limits to go beyond the limits of acceptability (from λ fixed at 0.5).

Done at Thury-Harcourt, July 29th, 2021
Mickaël MORVAN
Research and Development Engineer

APPENDIX 1

ANALYTICAL PROTOCOLS

EN ISO 7899-1: 1999

Detection and enumeration of intestinal enterococci in surface and waste water

Part 1 : Miniaturized method (Most Probable Number) by inoculation in liquid medium

9 mL of sample

↓

Preparation of the dilution range

6 dilutions in synthetic sea salt diluent

16 wells per dilution (from 1/2 to 1/200 000)

↓

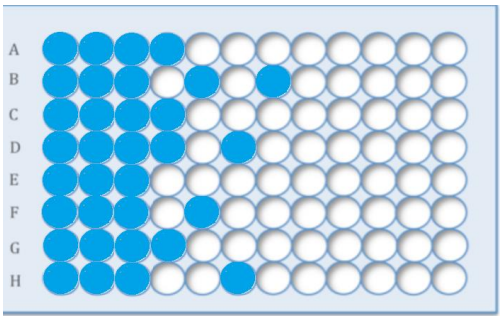
Inoculation

200µL per well containing the MUD medium

↓

Incubation 36 to 72 hours at 44°C ± 0.5°C

↓



Enumeration of the positive wells (fluorescent) by UV reading

Expression of the results : MPN per 100 mL of intestinal enterococci

Alternative method

XplOrer64™ – CheckN'Safe™ *Enterococci* test

Detection and quantification of intestinal *Enterococci* in Bathing Recreational waters and Waste wasters

100mL of sample

Filtration on a membrane

Transfer of the membrane

Inoculation of the membrane into the broth Check'NSafe™ *Enterococci*

Real-time and continuous detection and quantification with XplOrer64™

Incubation of 16 hours at 37°C ± 0.5°C

Impedance measurement

Detection time (Dt)

NO

Absence of *Enterococci* / 100mL of water tested

YES

Detection of *Enterococci* / 100mL of water tested

APPENDIX 2

MANUFACTURER NOTICE

CheckN'Safe™ *Enterococci* test

Code 3554721

Notice Version 3.5

XplOrer64 Software V3.0

Automated test for the real-time and continuous detection and quantification of intestinal *Enterococci* in Bathing Recreational waters and Waste waters

code 3554721 - 60 tests

TABLE OF CONTENT

BIO-RAD

1. INTRODUCTION

Conventional microbiological methods for intestinal *Enterococci* quantification in bathing water samples required between 2 to 3 days according to the referential used. 24 hrs additional confirmation tests are required in the membrane filtration methods, which are particularly tedious to perform.

Requiring only few minutes to handle a filtration step or a direct inoculation according to the type of water to analyse, CheckN'Safe *Enterococci* test is a simple method to perform, for a quantitative result in real-time, specific to intestinal *Enterococci* species such as *E. fecium, E. faecalis, E. durans, E. hirae*… found in **bathing water** (sea marine or fresh) and in **waste waters** (water concentrated in suspended matters, like water from treatment plants). This test uses the XplOrer64™ System (Bio-Rad), based on a continuous real-time impedance technology which can process up to 64 samples simultaneously in random access.

With XplOrer64 method, the highest the bacterial concentration is, the soonest the time to results gives a positive signal and determines the *Enterococci* concentration in the water sample, constituting a real active tool for the water sanitary survey management:

- under the surveillance of bathing water: for public information on water quality, prevention of public health, protection of coastal waters and freshwater pollution events,
- for monitoring the quality of waste water from treatment plants or sewerage facilities: the reuse of effluent water for irrigation of crops or green areas for example, monitoring of raw water during heavy rains for example.

2. CHECKN'SAFE TECHNOLOGY PRINCIPLE

The XplOrer64-CheckN'Safe *Enterococci* method is an automated method for the detection and enumeration of intestinal *Enterococci* by measuring the impedance in a liquid medium, without confirmation. CheckN'Safe *Enterococci* test is based on bacterial growth in liquid culture medium, which takes place in an impedance measurement. Each cell of measurement contains a selective culture medium which allows the specific growth of *Enterococci* strains, two electrodes measuring the variation of the impedance signal during growth.

The detection and analysis of data is optimized for use with the automated XplOrer64 (Bio-Rad). This test is particularly useful for detecting:

- **370 *Enterococci*/100 ml of bathing water** (freshwater or sea marine) **in 10 h 15 minutes***
- **250 *Enterococci*/100 ml of waste water in 10 hours 20 minutes *** *(*pre-heating hour included)*

3. NF VALIDATION

BRD 07/19 - 11/09

Alternative ANALYTICAL methods for WATER
Certified by AFNOR Certification
www.afnor-validation.com

The XplOrer64-CheckN'Safe *Enterococci* method is certified by AFNOR Certification as an alternative method to the standard EN ISO 7899-1, for **the enumeration of *Enterococci* at 37°C in bathing waters, in effluent water, and in treated and untreated waste waters**, according to the reference for validation "Protocol for the Validation of a commercial method versus a reference method in the water microbiology field (revision 2)" (adopted by AFNOR Certification on 17/05/2013), under Attestation No: **BRD 07/19 - 11/09**.

Valid until: **06/11/2021**

4. REGULATORY AND STANDARDS REFERENCES

Regulatory references

- **2006/7/EC Directive** of the Council of 15th February 2006 concerning the management of bathing water quality and repealing the Directive 76/160/EEC of 8th December 1975; EUOJ L64 March 4th, 2006.
- **French Order of 2 August 2010** relating to the use of water from the treatment of urban waste water treatment plants for irrigation of crops or green areas; Official Journal N°0201 dated August 31st, 2010.

Standards references

- **NF EN ISO 7899-1 (Nov. 1998)**: Water quality - Detection and enumeration of intestinal *Enterococci* in surface and waste water - Part 1: Miniaturized method (Most Probable Number) by inoculation in liquid medium.
- **ISO/CD 11133 (2010)**: Food Microbiology - Guidelines on preparation and production of culture media, general guide for performance testing of culture media.
- **NF T90-461/A2 (May 2007)**: Water quality - Microbiology - Quality control for culture media.

5. PRESENTATION

Each kit contains **60 disposable vials**, pre-filled with 9 ml of a selective *Enterococci* broth culture medium.

6. SHELF-LIFE AND STORAGE

Once received, the **kit must be stored at +2-8°C**. Reagents can then be used until the expiration date indicated on the reagent vial and the package.

It is recommended to **keep the necessary reagents at room temperature 1 h before use**.

7. EQUIPMENT AND MATERIAL REQUIRED (NOT SUPPLIED)

The protocol to be followed depends on the matrix to be analyzed and its filterability, as shown in table below:

Origin and nature of the sample	Suitable Protocol	Analytical volume
Untreated waste water	direct inoculation membrane filtration	1 ml 10 ml
Treated waste water	membrane filtration	100 ml
Bathing water	membrane filtration	100 ml

Protocol by membrane filtration

- Equipment for membrane filtration in accordance with ISO 8199 requirements:
  - Filtration apparatus (mounted either to a air pump or a vacuum flask) in a sterile environment, i.e. near a Bunsen burner
  - Disposable sterile funnels of 250 ml
  - Membrane filters, composed of cellulose esters, usually about 47 mm or 50 mm in diameter, with filtration characteristics equivalent to a rated nominal pore diameter of 0.45µm. (The filters shall be free from growth-inhibiting or growth-promoting properties. Every batch of membranes should be tested in accordance with ISO 7704 for its suitability to the test).
  - Two sterile metallic inox tweezers with rounded ends, for handling membranes
- Distilled or deionized sterile: **a minimum of 50 to 100 ml per sample** to analyze
- CheckN'Safe Screw caps, 40 sterile units x 1 bag (Bio-Rad, **code 359-3457**).
- CheckN'Safe Racks (Bio-Rad, **code 359-3455**, 4 units are provided with the XplOrer64 System).

Protocol by direct inoculation

- Sterile graduated pipettes 1 ml (1 pipette per sample)
- CheckN'Safe Screw caps, 40 sterile units x 1 bag (Bio-Rad, **code 359-3457**).
- CheckN'Safe Racks (Bio-Rad, **code 359-3455**, 4 units are provided with the XplOrer64 System).

8. PRECAUTIONS

- This test must be performed by adequately trained personnel.

- Water samples must be handled and eliminated as potentially infectious material.
- All potentially infectious material should be autoclaved before disposal.
- The quality of results depends on strict compliance with Good Laboratory Practices.

9. SAMPLING

Water samples are collected and deliver to the laboratory in accordance with the general water quality standards for bacteria detection and enumeration by culture method (see ISO 8199 and ISO 5667-1, ISO 5667-2 and ISO 5667-3 standards).

The samples shall be delivered to the laboratory as soon as possible. Start the examination preferably immediately after taking the samples. If the samples are kept at ambient temperature (in the dark, not exceeding 25°C), then analyses should begin within 6 h after sampling. Under exceptional circumstances, the samples may be kept at 2-8°C for up to 24 h prior to analysis.

10. PROTOCOL

It is strongly recommended to read all the protocol before starting the test. Please also refer to the complete User Manual for detailed instructions relative to the software parameter settings.

A. PRELIMINARY OPERATIONS

a. Switch-on respectively the uninterruptible power source (UPS), computer (and printer) and finally the XplOrer64 System.

b. Start the XplOrer64 Manager software by cliquing on its icon. In the Overview mode, start the focused XplOrer64 System. The spot on the top right turns green.

c. Warm-up the incubator(s) defined for *Enterococci* samples **at 37.0°C** (at least 30 minutes before to start any sample monitoring), and set the measuring cycle on **10 minutes**.

d. Prepare the necessary broth vials into rack(s) and **keep them at ambient temperature**.

e. Prepare necessary sterile caps and membrane filtration apparatus.

B. PARAMETER SETTINGS

To perform analyses, following parameters must be set into XplOrer64 incubator(s) and spots to be used.

Important remarks:

- If these parameters are not selected before the start of a measurement and until the end of the warm-up time (with the exception of measurement duration), **XplOrer64 System will proceed on the basis of the parameters used for the previous measurement for the position(s) selected.**
- With the exception of the measurement duration, changes of parameters for measuring cells already inserted in the incubator are limited to the warm-up stage.
- If a new sample vial is inserted into an empty incubator well without changing the analysis parameters, the **measurement will continue using the "old" parameters stored** for the previous measurement. In that case, it is possible to change the parameters once even if the warm-up time is already expired. **(6)**

B.1. In the main window "**XplOrer64 Manager Overview mode**", open the "**Parameter setting**" window.

B.2. Enter/Verify *Enterococci* parameters:

(1) Select the incubator **"A"** and the spot "**Position 1"**.
(NB: it is recommended to set the lowest temperature in the down incubator)

(2) Enter your **User login** using the pull-down menu.

(3) Select the "**Type of water"** in the pull-down menu (i.e.: *1 - Bathing water*)

(4) Select "***Enterococci***" in the list of germs.

(5) Select the **"Classification"** desired for automated interpretation of results (based on regulatory criteria to be followed). All parameters pre-recorded (evaluation mode, thresholds, pre-incubation and duration of cycle analysis) are then automatically implemented in the bottom of the window (shaded fields) **and confirm by clicking on the blue arrow.**

This setting will automatically implement the corresponding mandatory value (Limit 1) and guidance value (Limit 2), attributing a color to the spot when the time to detection had been detected, as described in the *Table 1* hereafter. If none classification has been selected, no limits will be displayed.

	Classification	*Enterococci* Analysis Origin of water to be tested	Limit 1 ≥ Limit 1 red	Limit 2 [Limit 1-Limit 2] yellow	Limit 2 < Limit 2 green
Bathing water	**76/160/CEE**	Sea/Fresh surface water	-	≥ 100	< 100
	2006/7/CE	Sea water	≥ 250	[100 - 250]	< 100
		Fresh surface water	≥ 400	[200 - 400]	< 200
	AFSSET 2007	Sea water	≥ 370	-	< 370
		Fresh surface water	≥ 660	-	< 660
Waste water	**None**	Traited or untraited waste waters	**≥ Detection limit**	-	**< Detection limit**

Table 1: Enterococci sample classification limits

(6) Enter the **sample identification info**. It is recommended to use the first field to the sample number (Note: This step can be carried out later until the end of the cycle of analysis, but in all cases before removing cells the incubator. Otherwise, it would be impossible to identify the sample in the database).

(7) **Confirm your new settings** by clicking on **the red arrow** (then all texts turn in black characters). Close this window and confirm by "**Yes**".

B.3. At **this step, only one spot (Position 1) is correctly parametered** in the incubator. In the main overview mode, follow the instructions below:

- Click into the **spot 1** with the mouse (the circle is surrounded with a discontinued black static line).
- Select in the menu: "**Edit**" and "**Copy position parameter**" (the discontinued line is now running around the spot; the copy is activated).
- Press simultaneously the **shift button** on the keyboard and click into the **spot 31**: circles of all selected spots turn violet. (NB: By default, the spot 32 will be dedicated to the internal temperature control. If the temperature cell is not used, select all the 32 spots).
- Select in the menu, "**Edit**" and "**Insert all parameters**": one by one, spots turn back to grey, as from the beginning. The pasting was done successfully. Then, select again "**Edit**" and "**Clear copy/paste buffer"** to clear the buffer.

B.5. Now all the selected incubator is parametered. **The parameters have to be established now also in the second incubator**, with the same procedure if it concerns also *Enterococci* testing, or according to the suitable parameters if it concerns another bacteria analysis.

C. SAMPLE PREPARATION

***Note**: as far as possible, it is recommended to respect 10 minutes maximum between the insert of the membrane into the broth vial and the transfer of the broth vial into the incubator of the system. This corresponds to a 6 sample serial.*

C.1. Prepare the *Enterococci* broth vials into their rack. Pre-open the seal of each and replace it onto the vial to avoid external or air contaminations. Prepare sterile screw caps, writing any "sample id" on.

C.2. According to the type of sample (Cf Table §7):

a/ Protocol by membrane filtration

- **Filter 100 ml** of water sample on a membrane:
  - Bathing water or treated waste water: 100 ml
  - Untreated waste water: 10 ml
- Rinse this membrane with at least **50 ml** sterile deionised or distilled water.
- Using two sterile tweezers, fold twice the membrane in a cone shape (refer to Appendix A). Open the seal of a vial, inoculate the membrane into the broth, **tip downside**.
- Put the seal back in its place and add a sterile cap on it.
- At this step, **ensure that the entire membrane is immersed**. If necessary, rock the vial gently avoiding the formation of foam.

b/ Protocol by direct inoculation

- **Inoculate 1 ml** of the **untreated waste water sample directly** into a CheckN'Safe *Enterococci* cell.
- Put the seal back in its place and add a sterile cap on it.

C.3. Check that **the temperature of incubator is stabilized at 37 ± 0.5°C** (if not, wait until warm-up will be completed). Then, introduce each serial of vials into wells of the incubator, parametered as described in part A and B.

⚠ **Verify each vial is correctly linked at bottom of the well**. Then, warming-up of the samples introduced starts for 1 hr.

D. DATA ANALYSIS

From the end of the 1 h warming-up, samples are analyzed each 10 minutes in each well of both incubators, whether there are full or empty, building the individual curves of impedance measurement in each sample. Here is the average detection time (Dt) of natural *Enterococci* population

Spot reading

a. Real-time analysis moving forward can be followed **at any times during the 12.5 h or 16 h of the cycle** of analysis, within the XplOrer64 Manager, by simple **observation of spots** into the main overview window:

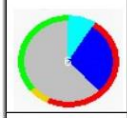
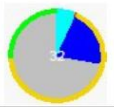
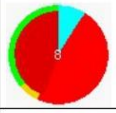
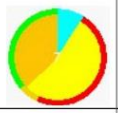
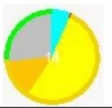
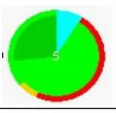
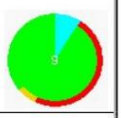
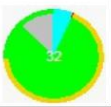
Real-time analysis results	**In progress…**	**Positive samples**			**Negative sample**
Spots appearance *(NB : the circumference color is linked to the classification choosen)*	 	 *Not applicable**	 	 	 
Automated results interpretation	*Not determined yet…*	**Polluted water sample**	**Suspicious polluted water**	**Good water quality**	**Excellent water quality**
***Enterococci* concentration (germs/100ml)**	*Not determined yet…*	≥ Limit 1	] Limit 1; Limit 2 [	≤ Limit 2	< Detection limit

Table 2: Sample results

b. To obtain the precise quantified results, click on the selected sample spot circumference. Then the corresponding graph will show the exact *Enterococci* concentration (in *Enterococci*/100 ml) in the considered sample, and its corresponding status.

c. The integrality of the registered data can be analyzed with the XplOrer64 Smart View software (Refer to the User Manual for further details).

- *Average Time to detection (Dt) observed*

Enterococci / 100 ml	Detection Time *(pre-warming up hour not included)*
100	10.07 h / **10 h 04'**
200	9.49 h / **9 h 30'**
250	9.33 h / **9 h 20'**
370	9.06 h / **9 h 04'**
400	9.003 h / **9 h 18'**
660	8.69 h / **8 h 42'**

- *Quantification intervals*

Bathing waters	**Protocol**	**Low quantification limit**	**High quantification limit**	**Cycle of analysis**
Fresh and marine sea waters	Filtration, 100 ml	< 28 *Entero* /100 ml	$3{,}9.10^7$ *Entero*/100 ml	12,5 h

See Waste water on next page …

Waste waters	Protocol	Low quantification limit	High quantification limit	Cycle of analysis
Treated	Filtration, **100 ml**	< 1 *Entero* /100 ml	$3{,}9.10^{7}$ *Entero* /100 ml	16 h
Untreated	Filtration, **10 ml**	< 10 *Entero* /100 ml	$3{,}9.10^{8}$ *Entero* /100 ml	
Untreated	Directe inoculation, **1 ml**	< 100 *Entero* /100 ml	$3{,}9.10^{9}$ *Entero* /100 ml	

11. LIMITS OF USE

A. HIGHLY CONTAMINATED SAMPLES

When the samples are highly contaminated (≥ Quantification limit for the relevant protocol) (See page 8), the system can not detect/count during the first hour of the cycle analysis: false-negative results are obtained (< Quantification limit). They can be avoided by dilutions of the sample.

B. SAMPLES TREATED WITH UV

The water samples disinfected by ultraviolet treatment may have a particular kinetics, reflecting the damage suffered by the DNA of bacterial cells and their survival by photoreactivation process. These samples may therefore lead to an underestimation.

C. INTERNAL QUALITY CONTROL (SPIKED)

The different calibrations included in **XplOrer64™ Manager software** (*Cf* §10.B.B2) have been established according to the physiological level of stress of micro-organisms:

- **« ENTERO » calibration**: for natural samples monitoring (germs stressed by their environment, presenting an extended latent phase of growth),
- **« QC ENTEROCOCCI » calibration**: for internal quality control (cultural performances), realised from spiked samples with revivified strain(s) on nutritive medium (germ(s) in exponential phase of growth).

These two calibrations do not allow to answer to inter-laboratories studies (Ring test), which present an intermediary level of physiological stress of micro-organisms.

12. QUALITY CONTROL OF MANUFACTURER

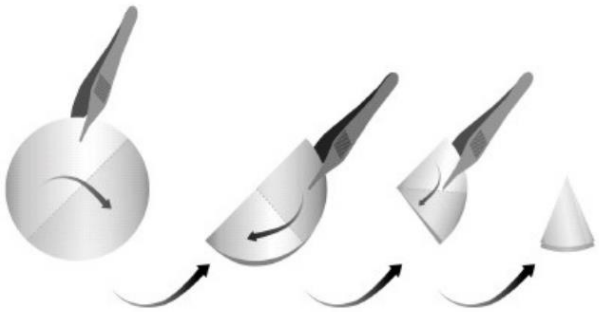
Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

13. BIBLIOGRAPHY

- **ZIMMER J. L. and SLAWSON R.M. (2002):** Potential Repair of *Escherichia coli* DNA following Exposure to UV Radiation from Both Medium and Low Pressure UV Sources Used in Drinking Water Treatment. *AEM*, **Vol. 68**, No. 7, p. 3293–3299.
- **TOSA K. and HIRATA T. (1999):** Photoreactivation of Enterohemorrhagic *Escherichia coli* following UV disinfection. *Wat. Res.* **Vol. 33**, No. 2, pp. 361-366

APPENDIX A: Membrane folding Schema



Carefully fold the membrane in 2, three times in order to obtain a cone.

Then, add the cone into the culture medium broth.

APPENDIX 3

RELATIVE ACCURACY
RESULTS OF 2009

The grey boxes correspond to the results not exploited

Scope: **Fresh water**

Code	Echantillon*	Entérocoques (bactéries/100 mL)			Entérocoques (log (bactéries/100 mL))	
		NF EN ISO 7899-1	DT (heures) V3.0	XpIOrer64 CheckN'Safe *Enterococci* V3.0	NF EN ISO 7899-1	XpIOrer64 CheckN'Safe *Enterococci* V3.0
1 à 3	Eaux douce	<15	-	<28		
4 à 6	Eaux douce	<15	-	<28		
7	Eaux douce	<15	-	<28		
8 à 10	Eaux douce	1.50E+01	-	<28		
11	Eaux douce	1.50E+01	11.31	3.47E+01	1.18	1.54
12	Eaux douce	1.50E+01	11.27	3.56E+01	1.18	1.55
13	Eaux douce	1.50E+01	10.85	4.78E+01	1.18	1.68
14	Eaux douce	3.00E+01	11.36	3.37E+01	1.48	1.53
15	Eaux douce	4.60E+01	10.71	5.36E+01	1.66	1.73
16	Eaux douce	4.60E+01	10.64	5.69E+01	1.66	1.76
17	Eaux douce	6.10E+01	11.47	3.17E+01	1.79	1.50
18	Eaux douce	6.10E+01	10.85	4.78E+01	1.79	1.68
19	Eaux douce	6.10E+01	10.28	7.99E+01	1.79	1.90
20	Eaux douce	9.40E+01	10.53	6.28E+01	1.97	1.80
21	Eaux douce	9.40E+01	10.81	4.94E+01	1.97	1.69
22	Eaux douce	1.26E+02	9.82	1.33E+02	2.10	2.12
23	Eaux douce	2.13E+02	9.34	2.46E+02	2.33	2.39
24	Eaux douce	2.15E+02	9.47	2.06E+02	2.33	2.31
25	Eaux douce	2.32E+02	8.89	4.75E+02	2.37	2.68
26	Eaux douce	2.49E+02	8.35	1.17E+03	2.40	3.07
27	Eaux douce	2.49E+02	9.20	2.99E+02	2.40	2.48
28	Eaux douce	2.89E+02	9.86	1.26E+02	2.46	2.10
29	Eaux douce	3.86E+02	9.61	1.72E+02	2.59	2.23
30	Eaux douce	4.34E+02	9.47	2.06E+02	2.64	2.31
31	Eaux douce	4.34E+02	9.07	3.62E+02	2.64	2.56
32	Eaux douce	4.65E+02	8.91	4.61E+02	2.67	2.66
33	Eaux douce	4.76E+02	8.70	6.43E+02	2.68	2.81
34	Eaux douce	5.04E+02	8.60	7.59E+02	2.70	2.88
35	Eaux douce	5.34E+02	-	<28	2.73	
36	Eaux douce	5.34E+02	8.17	1.61E+03	2.73	3.21
37	Eaux douce	5.54E+02	8.55	8.25E+02	2.74	2.92
38	Eaux douce	5.65E+02	8.59	7.71E+02	2.75	2.89
39	Eaux douce	5.74E+02	9.36	2.39E+02	2.76	2.38
40	Eaux douce	5.88E+02	7.75	3.61E+03	2.77	3.56
41	Eaux douce	6.00E+02	8.40	1.07E+03	2.78	3.03
42	Eaux douce	6.33E+02	8.46	9.62E+02	2.80	2.98
43	Eaux douce	6.54E+02	9.60	1.74E+02	2.82	2.24
44	Eaux douce	8.14E+02	8.65	6.98E+02	2.91	2.84
45	Eaux douce	8.82E+02	8.22	1.47E+03	2.95	3.17
46	Eaux douce	9.94E+02	8.87	4.90E+02	3.00	2.69
47	Eaux douce	1.45E+03	8.69	6.54E+02	3.16	2.82
48	Eaux douce	1.48E+03	8.32	1.23E+03	3.17	3.09
49	Eaux douce	2.08E+03	7.98	2.30E+03	3.32	3.36
50	Eaux douce	2.15E+03	8.40	1.07E+03	3.33	3.03
51	Eaux douce	>350000	-	non exploité		

* Echantillons naturellement contaminés

Scope: **Marine water**

Code	Echantillon*	Entérocoques (bactéries/100 mL)			Entérocoques (log (bactéries/100 mL))	
		NF EN ISO 7899-1	DT (heures) V3.0	XpIOrer64 CheckN'Safe *Enterococci* V3.0	NF EN ISO 7899-1	XpIOrer64 CheckN'Safe *Enterococci* V3.0
52 à 110	Eaux de mer	<15	-	<28		
111 à 116	Eaux de mer	<15	-	non exploité		
117 à 126	Eaux de mer	1.50E+01	-	<28	1.18	
127	Eaux de mer	1.50E+01	-	<28	1.18	
128	Eaux de mer	1.50E+01	13.02	<28	1.18	
129	Eaux de mer	1.50E+01	11.53	4.5E+01	1.18	1.65
130	Eaux de mer	1.50E+01	11.10	5.5E+01	1.18	1.74
131	Eaux de mer	1.50E+01	-	<28	1.18	
132	Eaux de mer	1.50E+01	10.76	6.5E+01	1.18	1.81
133	Eaux de mer	1.50E+01	10.78	6.4E+01	1.18	1.81
134	Eaux de mer	1.50E+01	10.89	6.1E+01	1.18	1.79
135	Eaux de mer	1.50E+01	10.55	7.3E+01	1.18	1.86
136 à 140	Eaux de mer	3.00E+01	-	<28	1.48	
141	Eaux de mer	3.0E+01	11.75	4.1E+01	1.48	1.61
142	Eaux de mer	3.0E+01	10.28	8.5E+01	1.48	1.93
143	Eaux de mer	3.0E+01	9.39	1.5E+02	1.48	2.18
144 à 147	Eaux de mer	3.0E+01	-	<28	1.48	
148	Eaux de mer	4.6E+01	-	<28	1.66	
149	Eaux de mer	4.6E+01	12.14	3.6E+01	1.66	1.56
150	Eaux de mer	4.6E+01	-	<28	1.66	
151	Eaux saumâtre	4.6E+01	11.18	5.3E+01	1.66	1.72
152	Eaux de mer	6.1E+01	-	<28	1.79	
153	Eaux de mer	6.1E+01	-	<28	1.79	
154	Eaux de mer	7.7E+01	11.26	3.6E+01	1.89	1.56
155 à 156	Eaux de mer	7.7E+01	-	<28	1.89	
157	Eaux saumâtre	9.4E+01	-	<28	1.97	
158	Eaux de mer	1.4E+02	11.43	3.2E+01	2.16	1.51
159	Eaux de mer	2.9E+02	9.61	1.3E+02	2.46	2.11
160	Eaux de mer	6.5E+02	10.59	6.0E+01	2.81	1.78

* Echantillons naturellement contaminés

APPENDIX 4

BACTERIAL STRESS

N° échantillon	Code souche	Souche	Origine	Stress appliqué	Intensité du stress
eau de mer 1	ENTC.2.1	*Enterococcus faecium*	industrie laitière	10min à -80°C + 10min à 37°C + 10min à -80°C + 10min à 37°C	0,5
eau de mer 2	ENTC.2.1	*Enterococcus faecium*	industrie laitière	10min à -80°C + 10min à 37°C + 10min à -80°C + 10min à 37°C	0,5
eau de mer 3	ENTC.2.3	*Enterococcus faecium*	Eau	10min à -80°C + 10min à 37°C + 10min à -80°C + 10min à 37°C	0,5
eau de mer 4	ENTC.2.3	*Enterococcus faecium*	Eau	10min à -80°C + 10min à 37°C + 10min à -80°C + 10min à 37°C	0,5
eau de mer 5	ENTC.1.2	*Enterococcus faecalis*	ATCC 33186	2 min hypochlorite de sodium dilué au 1/10000éme	0,6
eau de mer 6	ENTC.1.2	*Enterococcus faecalis*	ATCC 33186	2 min hypochlorite de sodium dilué au 1/10000éme	0,6
eau de mer 7	ENTC.1.2	*Enterococcus faecalis*	ATCC 33186	2 min hypochlorite de sodium dilué au 1/10000éme	0,6
eau de mer 8	ENTC.3.2	*Enterococcus hirae*	Eau de rivière	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,8
eau de mer 9	ENTC.3.2	*Enterococcus hirae*	Eau de rivière	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,8
eau de mer 10	ENTC.3.2	*Enterococcus hirae*	Eau de rivière	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,8
eau douce 1	ENTC.1.3	*Enterococcus faecalis*	CIP 103214	2 min hypochlorite de sodium dilué au 1/10000éme	0,8
eau douce 2	ENTC.1.3	*Enterococcus faecalis*	CIP 103214	2 min hypochlorite de sodium dilué au 1/10000éme	0,8
eau douce 3	ENTC.1.3	*Enterococcus faecalis*	CIP 103214	2 min hypochlorite de sodium dilué au 1/10000éme	0,8
eau douce 4	ENTC.4.1	*Enterococcus avium*	Eau (Allemagne)	3 semaines à 5°C	0,8
eau douce 5	ENTC.4.1	*Enterococcus avium*	Eau (Allemagne)	3 semaines à 5°C	0,8
eau douce 6	ENTC.4.1	*Enterococcus avium*	Eau (Allemagne)	3 semaines à 5°C	0,8
eau douce 7	ENTC.5.1	*Enterococcus gallinarum*	Eau	3 semaines à 5°C	0,7
eau douce 8	ENTC.5.1	*Enterococcus gallinarum*	Eau	3 semaines à 5°C	0,7
eau douce 9	ENTC.3.1	*Enterococcus hirae*	CIP 58.55	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,6
eau douce 10	ENTC.3.1	*Enterococcus hirae*	CIP 58.55	4 j à 4°C + (5 min à -80°C + 5 min à 36°C) x2	0,6

APPENDIX 5

RELATIVE ACCURACY RESULTS OF 2011

+

FURTHER TESTING RESULTS OF 2014

The grey boxes correspond to the results not exploited

Scope: **Wastewater**

Cat	Catégorie (Classification du BT*)
Code	Code échantillon
NF	Non filtrable
R1 : réplicat 1	
R2 : réplicat 2	
b/100 mL : bactéries dans 100 mL	
DT : temps de détection	

<1	inférieur à 10 b/100 mL (seuil méthode, protocole général)
<10	inférieur à 10 b/100 mL (seuil méthode- protocole spécifique 1)
<100	inférieur à 10 b/100 mL (seuil méthode- protocole spécifique 2)
<58	inférieur à 58 UFC/PE (seuil méthode de référence)

* Classification des catégories d'eau par type

Eaux traitées 1	Faible teneur en MES **a**	Eau de réseau de distribution Eau de dialyse Eau de bassins de piscine Eau de circuits aéroréfrigérants
	Forte teneur en MES **b**	Eau de circuits aéroréfrigérants Eau de process **Eau résiduaire traitée**
Eaux non traitées 2	Faible teneur en MES **a**	Eau souterraine Eau minérale Eau de source Eau thermale
	Forte teneur en MES **b**	Eau superficielle Eau de mer Eau résiduaire brute

Detailed results

Scope: **Wastewater**

		Code	Echantillon d'eau	MES en mg/L	NF EN ISO 7899-1								Méthode alternative XpIOrer64 CheckN'Safe *Enterococci*			
					Lecture après 36 heures				Lecture après 72 heures				Ensemencement 1 mL			
					Résultats NPP		Enterococci (b/100 ml)		Résultats NPP		Enterococci (b/100 mL)		R1		R2	
					R1	R2	R1	R2	R1	R2	R1	R2	DT (heures)	Réponse dans 100 mL	DT (heures)	Réponse dans 100 mL
110105step1	1	A1	Effluent de station d'épuration, Solesmes	2	10/2/0/0/0/0	9/2/0/0/0/0	1.01E+03	8.78E+02	10/2/0/0/0/0	9/2/0/0/0/0	1.01E+03	8.78E+02	10.45	6.76E+03	-	<100
110110step2	2	A2	Effluent de station d'épuration, Saulzoir	5	6/0/0/0/0/0	7/0/0/0/0/0	4.12E+02	5.00E+02	6/0/0/0/0/0	7/0/0/0/0/0	4.12E+02	5.00E+02	-	<100	-	<100
110110step3	3	A3	Eau usée brute, Solesmes	334	16/16/11/2/1/0	16/16/11/0/0/0	1.27E+05	9.65E+04	16/16/11/2/1/0	16/16/12/0/0/0	1.27E+05	1.12E+05	10.15	9.14E+03	10.33	7.60E+03
110111step4	4	A4	Effluent de station, Bierne	16	0/0/0/0/0/0	1/0//0/0/0/0	<58	5.80E+01	0/0/0/0/0/0	1/0/0/0/0/0	<58	5.80E+01	-	<100	-	<100
110111step5	5	A5	Eau usée brute, Cysoing	175	16/16/14/6/2/1	16/16/15/5/0/0	3.17E+05	2.96E+05	16/16/14/6/2/1	16/16/15/5/2/0	3.17E+05	3.47E+05	7.22	8.56E+05	7.25	8.26E+05
110112step6	6	A6	Effluent de station, Oxelaere	2	3/0/0/0/0/0	5/0/0/0/0/0	1.85E+02	3.30E+02	3/0/0/0/0/0	5/0/0/0/0/0	1.85E+02	3.30E+02	-	<100	-	<100
110112step7	7	A7	Eau usée brute, Merville	292	16/16/16/2/0/0	16/16/16/4/0/0	2.86E+05	3.60E+05	16/16/16/2/0/0	16/16/16/4/0/0	2.86E+05	3.60E+05	9.89	1.22E+04	9.23	2.87E+04
110114step8	8	A8	Eau usée brute, Bierne	51	16/16/3/1/0/0	16/16/7/0/0/0	3.53E+04	5.31E+04	16/16/3/1/0/0	16/16/7/0/0/0	3.53E+04	5.31E+04	10.09	9.75E+03	10.11	9.54E+03
110114step9	9	A9	Eau usée brute, Boeschepe	366	16/16/16/10/4/0	16/16/16/9/1/0	1.21E+06	7.97E+05	16/16/16/10/4/0	16/16/16/9/1/0	1.21E+06	7.97E+05	8.79	5.56E+04	8.97	4.20E+04
110118step10	10	A10	Effluent de station, Neuf Berquin	4	16/6/1/0/0/0	16/5/0/0/0/0	5.17E+03	4.06E+03	16/6/1/0/0/0	16/6/0/0/0/0	5.17E+03	4.63E+03	-	<100	-	<100
110118step11	11	A11	Effluent de station, Saulzoir	7	16/1/0/0/0/0	13/4/0/0/0/0	2.56E+03	1.85E+03	16/2/0/0/0/0	13/5/0/0/0/0	2.86E+03	2.00E+03	-	<100	-	<100
110118step12	12	A12	Effluent de station, Boeschepe	7	16/12/5/1/0/0	16/11/1/0/0/0	1.84E+04	1.07E+04	16/12/5/1/0/0	16/13/1/1/0/0	1.84E+04	1.56E+04	8.41	1.05E+05	8.23	1.45E+05
110118step13	13	B1	Effluent de station, Sommaing	2	14/6/1/0/0/0	11/3/0/0/0/0	2.77E+03	1.27E+03	14/6/1/0/0/0	11/3/0/0/0/0	2.77E+03	1.27E+03	-	<100	-	<100
110119step14	14	B2	Eau usée brute, Saulzoir	311	16/16/16/16/2/0	16/16/16/12/2/2	2.90E+06	1.58E+06	16/16/16/16/2/0	16/16/16/12/2/2	2.90E+06	1.58E+06	7.02	1.08E+06	6.86	1.31E+06
110119step15	15	B3	Eau usée brute, Sommaing	287	16/16/16/15/4/0	16/16/16/14/4/0	2.71E+06	2.21E+06	16/16/16/15/4/0	16/16/16/14/4/0	2.71E+06	2.21E+06	5.91	4.03E+06	6.06	3.38E+06
110119step16	16	B4	Eau usée brute, Villers Sire Nicolle	264	16/16/11/2/0/0	16/16/10/3/0/0	1.17E+05	1.11E+05	16/16/11/2/0/0	16/16/10/3/0/0	1.17E+05	1.11E+05	8.64	7.10E+04	9.57	1.81E+04
110119step17	17	B5	Eau usée brute, Cousolre	84	16/15/7/0/0/0	16/15/3/0/0/0	3.56E+04	2.46E+04	16/15/7/0/0/0	16/15/3/0/0/0	3.56E+04	2.46E+04	9.41	2.23E+04	9.33	2.49E+04
110125step18	18	B6	Effluent de station, Solesmes	2	1/0/0/0/0/0	2/0/0/0/0/0	5.80E+01	1.85E+02	1/0/0/0/0/0	3/0/0/0/0/0	5.80E+01	1.85E+02	-	<100	-	<100
110125step19	19	B7	Eau usée brute, Bois Grenier	222	16/16/16/11/0/0	16/16/16/6/0/0	9.71E+05	4.63E+05	16/16/16/11/0/0	16/16/16/6/0/0	9.71E+05	4.63E+05	6.82	1.37E+06	6.98	1.14E+06
110125step20	20	B8	Effluent de station, Beauvois en Cambresis	27	13/1/0/0/0/0	6/2/0/0/0/0	1.44E+03	5.56E+02	13/1/0/0/0/0	7/3/0/0/0/0	1.44E+03	5.56E+02	-	<100	10.39	7.16E+03
110125step21	21	B9	Effluent - Conserverie, St Pol ZI	348	16/15/8/1/0/0	16/16/9/1/0/0	4.24E+04	7.95E+04	16/16/16/1/0/0	16/16/15/6/0/0	2.57E+05	3.24E+05	9.72	1.50E+04	8.57	7.98E+04
110125step22	22	B10	Effluent - Eau épurée St Pol ZI	8	2/0/0/0/0/0	0/0/0/0/0/0	1.19E+02	<58	3/0/0/0/0/0	0/0/0/0/0/0	1.19E+02	<58	-	<100	-	<100
110126step23	23	C1	Effluent de station, Bierne	1	0/0/0/0/0/0	0/0/0/0/0/0	<58	<58	0/0/0/0/0/0	0/0/0/0/0/0	<58	<58	-	<100	11.25	3.60E+03
110126step24	24	C2	Effluent de station, Avesne sur Helpes	2	11/4/0/0/0/0	14/3/0/0/0/0	1.38E+03	2.02E+03	11/4/0/0/0/0	14/3/0/0/0/0	1.38E+03	2.02E+03	9.73	1.50E+04	-	<100
110126step25	25	C3	Effluent de station, Doullens	3	16/10/3/1/1/0	16/9/3/0/0/0	1.30E+04	9.68E+03	16/10/3/1/1/0	16/9/3/0/0/0	1.30E+04	9.68E+03	10.92	4.50E+03	8.90	4.60E+04
110126step26	26	C4	Effluent de station, Bavay	2	5/3/0/0/0/0	7/1/0/0/0/0	5.37E+02	5.76E+02	5/3/0/0/0/0	7/1/0/0/0/0	5.37E+02	5.76E+02	10.18	8.90E+03	9.69	1.60E+04
110126step27	27	C5	Effluent de station, La Longueville	<1	12/3/0/0/0/0	7/3/0/0/0/0	1.47E+03	7.30E+02	12/3/0/0/0/0	7/3/0/0/0/0	1.47E+03	7.30E+02	10.15	9.10E+03	10.17	8.90E+03
110131step28	28	C6	Effluent, Béthune	/	0/0/0/0/0/0	0/0/0/0/0/0	<58	<58	0/0/0/0/0/0	0/0/0/0/0/0	<58	<58	11.90	2.60E+03	-	<100
110131step29	29	C7	Effluent de station, Bierne	4	2/0/0/0/0/0	1/0/0/0/0/0	1.19E+02	5.80E+01	3/0/0/0/0/0	1/0/0/0/0/0	1.85E+02	5.80E+01	-	<100	-	<100
110131step30	30	C8	Effluent de station, Le Cateau Cambresis	2	16/2/0/0/0/0	16/4/0/0/0/0	2.86E+03	3.59E+03	16/5/0/0/0/0	16/5/0/0/0/0	4.06E+03	4.06E+03	9.26	2.80E+04	9.43	2.20E+04
110131step31	31	C9	Effluent de station, Rieux en Cambresis	3	16/7/0/0/0/0	15/6/0/0/0/0	5.31E+03	3.24E+03	16/8/0/0/0/0	15/7/0/0/0/0	6.14E+03	3.56E+03	9.65	1.60E+04	9.62	1.70E+04
110131step32	32	C10	Effluent de station, Beaudignies	9	15/4/0/0/0/0	15/1/1/0/0/0	2.70E+03	2.22E+03	15/4/0/0/0/0	15/1/1/0/0/0	2.70E+03	2.22E+03	10.69	5.50E+03	10.47	6.60E+03
110131step33	33	C11	Eau usée brute, Lecelles	312	16/16/16/4/1/0	16/16/16/9/2/1	3.99E+05	9.67E+05	16/16/16/4/1/0	16/16/16/9/2/1	3.99E+05	9.67E+05	7.58	5.60E+05	8.68	6.70E+04
110131step34	34	C12	Eau usée brute, Bierne	28	16/15/2/0/0/0	16/16/7/0/0/0	2.24E+04	5.31E+05	16/15/2/0/0/0	16/16/7/0/0/0	2.24E+04	5.31E+05	8.73	6.10E+04	8.75	5.90E+04
110131step35	35	C13	Eau usée brute, Cartignies	19	3/1/0/0/0/0	4/0/0/0/0/0	2.47E+02	2.55E+02	3/1/0/0/0/0	4/0/0/0/0/0	2.47E+02	2.55E+02	-	<100	14.30	4.50E+02
110131step36	36	C14	Eau usée brute, Etroeungt	172	16/16/14/2/1/0	16/16/15/6/0/0	2.01E+05	3.24E+05	16/16/14/2/1/0	16/16/15/6/0/0	2.01E+05	3.24E+05	7.54	5.80E+05	7.36	7.30E+05
110131step37	37	C15	Eau usée brute, Socx	38	16/7/1/0/0/0	16/9/2/0/0/0	5.94E+03	8.80E+03	16/7/1/0/0/0	16/11/2/0/0/0	5.94E+03	1.17E+04	-	<100	-	<100
110204step38	38	D1	Effluent de station, Beauvois en Cambresis	18	15/4/0/0/0/0	14/2/0/0/0/0	2.70E+03	1.86E+03	15/4/0/0/0/0	14/2/0/0/0/0	2.70E+03	1.86E+03	-	<100	-	<100
110204step39	39	D2	Effluent de station, Bierne	6	1/0/0/0/0/0	3/1/0/0/0/0	5.80E+01	2.47E+02	1/0/0/0/0/0	3/1/0/0/0/0	5.80E+01	2.47E+02	-	<100	-	<100
110204step40	40	D3	Effluent de station, Beauvois en Cambresis	14	9/2/0/0/0/0	12/4/0/0/0/0	8.78E+02	1.59E+03	9/2/0/0/0/0	12/4/0/0/0/0	8.78E+02	1.59E+03	-	<100	-	<100
110207step41	41	D4	Effluent de station, Bantouzelle	8	3/1/0/0/0/0	6/0/0/0/0/0	2.47E+02	4.12E+02	3/1/0/0/0/0	7/0/0/0/0/0	2.47E+02	5.00E+02				
110207step42	42	D5	Effluent de station, Bierne	6	2/1/0/0/0/0	2/0/0/0/0/0	1.80E+02	1.19E+02	2/1/0/0/0/0	2/0/0/0/0/0	1.80E+02	1.19E+02				
110207step43	43	D6	Effluent de station, Steene	9	16/13/4/1/0/0	16/15/1/0/0/0	1.99E+04	2.04E+04	16/13/4/1/0/0	16/16/1/0/0/0	1.99E+04	2.56E+04				
110207step44	44	D7	Effluent de station, Landrecies	8	16/16/6/1/0/0	16/16/6/1/0/0	5.17E+04	5.17E+04	16/16/6/1/0/0	16/16/7/1/0/0	5.17E+04	5.94E+04				

Detailed results

Scope: **Wastewater**

		Code	Méthode alternative XplOrer64 CheckN'Safe *Enterococci*							
			Filtration 10 mL				Filtration 100 mL			
			R1		R2		R1		R2	
			DT (heures)	Réponse dans 100 mL	DT (heures)	Réponse dans 100 mL	DT (heures)	Réponse dans 100 mL	DT (heures)	Réponse dans 100 mL
110105step1	1	A1	10.87	4.71E+02	10.07	9.96E+02	8.89	4.75E+02	9.02	3.90E+02
110110step2	2	A2	-	<10	11.04	4.15E+02	9.13	3.31E+02	9.75	1.44E+02
110110step3	3	A3	7.84	3.02E+04	7.83	3.08E+04				
110111step4	4	A4	-	<10	-	<10	-	<1	-	<1
110111step5	5	A5	7.36	7.25E+04	7.48	6.29E+04				
110112step6	6	A6	-	<10	-	<10	9.80	1.36E+02	9.32	2.52E+02
110112step7	7	A7	6.92	1.22E+05	7.04	1.06E+05				
110114step8	8	A8	8.92	4.54E+03	8.95	4.33E+03				
110114step9	9	A9	5.50	6.55E+05	7.27	8.07E+04				
110118step10	10	A10	9.36	2.39E+03	10.16	9.04E+02	7.86	2.90E+03	7.99	2.26E+03
110118step11	11	A11	9.31	2.56E+03	10.88	4.67E+02	8.39	1.09E+03	7.93	2.53E+03
110118step12	12	A12	8.47	9.46E+03	8.33	1.21E+04	7.27	8.07E+03	7.23	8.46E+03
110118step13	13	B1	9.94	1.15E+03	10.31	7.75E+02	8.93	4.47E+02	-	<1
110119step14	14	B2	6.32	2.48E+05	6.49	2.03E+05				
110119step15	15	B3	6.62	1.74E+05	5.86	4.28E+05				
110119step16	16	B4	7.03	1.07E+05	7.07	1.02E+05				
110119step17	17	B5	7.63	5.27E+04	7.21	8.66E+04	6.26	2.67E+04	5.21	9.24E+04
110125step18	18	B6	-	<10	-	<10	10.34	7.50E+01	10.34	7.50E+01
110125step19	19	B7	6.38	2.31E+05	6.99	1.12E+05				
110125step20	20	B8	9.84	1.29E+03	9.87	1.25E+03	6.59	1.80E+04	7.65	5.15E+03
110125step21	21	B9	-	<10	-	<10				
110125step22	22	B10	-	<10	-	<10	10.25	8.20E+01	10.12	9.40E+01
110126step23	23	C1	-	<10	11.43	3.20E+02	9.30	2.60E+02	11.22	3.70E+01
110126step24	24	C2	9.40	2.30E+03	-	<10	8.05	2.00E+03	8.08	1.90E+03
110126step25	25	C3	9.23	2.90E+03	9.68	1.60E+03	7.99	2.20E+03	7.62	5.40E+03
110126step26	26	C4	10.54	6.20E+02	10.91	4.60E+02	8.38	1.10E+03	9.49	2.00E+02
110126step27	27	C5	9.69	1.60E+03	9.92	1.20E+03	8.51	8.90E+02	8.36	1.10E+03
110131step28	28	C6	11.20	3.70E+02	-	<10	-	<1	-	<1
110131step29	29	C7	11.45	3.20E+02	11.00	4.30E+02	10.17	9.00E+01	9.20	3.00E+02
110131step30	30	C8	8.93	4.50E+03	8.89	4.70E+03	8.72	6.30E+02	7.37	7.20E+03
110131step31	31	C9	8.74	6.00E+03	8.82	5.30E+03	8.43	1.00E+03	7.14	9.40E+03
110131step32	32	C10	8.90	4.70E+03	8.93	4.50E+03	8.11	1.80E+03	7.33	7.50E+03
110131step33	33	C11	7.71	3.40E+04	-	<10				
110131step34	34	C12	8.34	1.20E+04	8.67	6.70E+03				
110131step35	35	C13	11.19	3.70E+02	10.97	4.40E+02				
110131step36	36	C14	8.06	2.00E+04	7.88	2.80E+04				
110131step37	37	C15	-	<10	-	<10				
110204step38	38	D1	-	<10	-	<10	-	<1	-	<1
110204step39	39	D2	-	<10	-	<10	-	<1	-	<1
110204step40	40	D3	-	<10	-	<10	13.60	8.40E+00	-	<1
110207step41	41	D4					8.83	5.20E+02	8.84	5.10E+02
110207step42	42	D5					8.96	4.30E+02	8.62	7.30E+02
110207step43	43	D6					7.30	7.80E+03	8.61	7.50E+02
110207step44	44	D7					5.93	4.00E+04	6.06	3.40E+04

Synthesis of results [log(*Enterococci*/100 mL)]

Scope: **Wastewater**

	Code	Echantillon d'eau	MES en mg/L	NF EN ISO 7899-1		Méthode alternative XpIOrer64 CheckN'Safe *Enterococci*					
				Lecture après 72 h d'incubation		Ensemencement 1 mL		Filtration 10 mL		Filtration 100 mL	
				Enterococci (log b/100 mL)		*Enterococci* (log b/100 mL)		*Enterococci* (log b/100 mL)		*Enterococci* (log UFC/100 mL)	
				R1	R2	R1	R2	R1	R2	R1	R2
1	A1	Effluent de station d'épuration, Solesmes	2	3.01	2.94	3.83		2.67	3.00	2.68	2.59
2	A2	Effluent de station d'épuration, Saulzoir	5	2.61	2.70				2.62	2.52	2.16
3	A3	Eau usée brute, Solesmes	334	5.10	5.05	3.96	3.88	4.48	4.49		
4	A4	Effluent de station, Bierne	16		1.76						
5	A5	Eau usée brute, Cysoing	175	5.50	5.54	5.93	5.92	4.86	4.80		
6	A6	Effluent de station, Oxelaere	2	2.27	2.52					2.13	2.40
7	A7	Eau usée brute, Merville	292	5.46	5.56	4.09	4.46	5.09	5.02		
8	A8	Eau usée brute, Bierne	51	4.55	4.73	3.99	3.98	3.66	3.64		
9	A9	Eau usée brute, Boeschepe	366	6.08	5.90	4.75	4.62	5.82	4.91		
10	A10	Effluent de station, Neuf Berquin	4	3.71	3.67			3.38	2.96	3.46	3.35
11	A11	Effluent de station, Saulzoir	7	3.46	3.30			3.41	2.67	3.04	3.40
12	A12	Effluent de station, Boeschepe	7	4.27	4.19	5.02	5.16	3.98	4.08	3.91	3.93
13	B1	Effluent de station, Sommaing	2	3.44	3.11			3.06	2.89	2.65	
14	B2	Eau usée brute, Saulzoir	311	6.46	6.20	6.03	6.12	5.40	5.31		
15	B3	Eau usée brute, Sommaing	287	6.43	6.34	6.61	6.53	5.24	5.63		
16	B4	Eau usée brute, Villers Sire Nicolle	264	5.07	5.05	4.85	4.26	5.03	5.01		
17	B5	Eau usée brute, Cousolre	84	4.55	4.39	4.35	4.40	4.72	4.94	4.43	4.97
18	B6	Effluent de station, Solesmes	2	1.76	2.27					1.88	1.88
19	B7	Eau usée brute, Bois Grenier	222	5.99	5.67	6.14	6.06	5.36	5.05		
20	B8	Effluent de station, Beauvois en Cambresis	27	3.16	2.75		3.85	3.11	3.10	4.26	3.71
21	B9	Effluent - Conserverie, St Pol ZI	348	5.41	5.51	4.18	4.90				
22	B10	Effluent - Eau épurée St Pol ZI	8	2.08						1.91	1.97
23	C1	Effluent de station, Bierne	1				3.56		2.51	2.41	1.57
24	C2	Effluent de station, Avesne sur Helpes	2	3.14	3.31	4.18		3.36		3.30	3.28
25	C3	Effluent de station, Doullens	3	4.11	3.99	3.65	4.66	3.46	3.20	3.34	3.73
26	C4	Effluent de station, Bavay	2	2.73	2.76	3.95	4.20	2.79	2.66	3.04	2.30
27	C5	Effluent de station, La Longueville	<1	3.17	2.86	3.96	3.95	3.20	3.08	2.95	3.04
28	C6	Effluent, Béthune	/			3.41		2.57			
29	C7	Effluent de station, Bierne	4	2.27	1.76			2.51	2.63	1.95	2.48
30	C8	Effluent de station, Le Cateau Cambresis	2	3.61	3.61	4.45	4.34	3.65	3.67	2.80	3.86
31	C9	Effluent de station, Rieux en Cambresis	3	3.79	3.55	4.20	4.23	3.78	3.72	3.00	3.97
32	C10	Effluent de station, Beaudignies	9	3.43	3.35	3.74	3.82	3.67	3.65	3.26	3.88
33	C11	Eau usée brute, Lecelles	312	5.60	5.99	5.75	4.83	4.53			
34	C12	Eau usée brute, Bierne	28	4.35	5.73	4.79	4.77	4.08	3.83		
35	C13	Eau usée brute, Cartignies	19	2.39	2.41		2.65	2.57	2.64		
36	C14	Eau usée brute, Etroeungt	172	5.30	5.51	5.76	5.86	4.30	4.45		
37	C15	Eau usée brute, Socx	38	3.77	4.07						
38	D1	Effluent de station, Beauvois en Cambresis	18	3.43	3.27						
39	D2	Effluent de station, Bierne	6	1.76	2.39						
40	D3	Effluent de station, Beauvois en Cambresis	14	2.94	3.20					0.92	
41	D4	Effluent de station, Bantouzelle	8	2.39	2.70					2.72	2.71
42	D5	Effluent de station, Bierne	6	2.26	2.08					2.63	2.86
43	D6	Effluent de station, Steene	9	4.30	4.41					3.89	2.88
44	D7	Effluent de station, Landrecies	8	4.71	4.77					4.60	4.53

Prise d'essai : 1 mL
21 eaux usées

		Méthode de référence				Méthode alternative			
Ech	Rang	Rep.1	Rep.2	Mxi	sxi interne	Rep.1	Rep.2	Myi	syi interne
A3	1	5.10	5.05	5.07716	0.03709	3.96	3.88	3.92088	0.05666
A5	2	5.50	5.54	5.52077	0.02741	5.93	5.92	5.92473	0.01096
A7	3	5.46	5.56	5.50652	0.07031	4.09	4.46	4.27212	0.26271
A8	4	4.55	4.73	4.63676	0.12529	3.99	3.98	3.98428	0.00669
A9	5	6.08	5.90	5.99226	0.12851	4.75	4.62	4.68416	0.08614
A12	6	4.27	4.19	4.22979	0.05040	5.02	5.16	5.09128	0.09912
B2	7	6.46	6.20	6.33066	0.18680	6.03	6.12	6.07535	0.05929
B3	8	6.43	6.34	6.38842	0.06331	6.61	6.53	6.56711	0.05401
B4	9	5.07	5.05	5.05651	0.01578	4.85	4.26	4.55447	0.41972
B5	10	4.55	4.39	4.47111	0.11343	4.35	4.40	4.37225	0.03387
B7	11	5.99	5.67	5.82644	0.22709	6.14	6.06	6.09681	0.05644
B9	12	5.41	5.51	5.46026	0.07173	4.18	4.90	4.53905	0.51330
C3	13	4.11	3.99	4.04931	0.08990	3.65	4.66	4.15799	0.71386
C4	14	2.73	2.76	2.74520	0.02153	3.95	4.20	4.07675	0.18012
C5	15	3.17	2.86	3.01532	0.21496	3.96	3.95	3.95422	0.00682
C8	16	3.61	3.61	3.60885	0.00000	4.45	4.34	4.39479	0.07406
C9	17	3.79	3.55	3.66976	0.16766	4.20	4.23	4.21728	0.01862
C10	18	3.43	3.35	3.38883	0.05924	3.74	3.82	3.77995	0.05599
C11	19	5.60	5.99	5.79295	0.27201	5.75	4.83	5.28713	0.65203
C12	20	4.35	5.73	5.03798	0.97188	4.79	4.77	4.77809	0.01024
C14	21	5.30	5.51	5.40679	0.14735	5.76	5.86	5.81338	0.07064

Médiane x	5.05651	0.08990
Moyenne x	4.81960	

Médiane y	4.53905	0.05929
Moyenne y	4.78772	

écart-type de répétabilité global	Srx	0.24661
écart-type de répétabilité robuste	Rob Swx	0.13328

Sry	0.26915
Rob Swy	0.08790

R	1.09140
Rob.R	0.65953

n = 2
q = 21
nq = 42

0,5 < R < 2	GMFR
R > 2	OLS
R < 0,5	OLS chgt

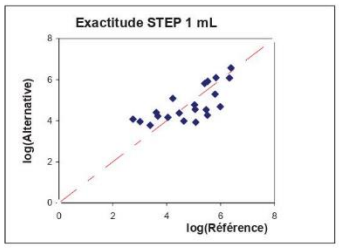
Calcul sur les moyennes des deux méthodes

Régression GMFR
Ecart-types globaux

Vxi	Vyi
0.13404	1.50603
0.98402	2.58570
0.94867	0.60069
0.08256	1.29108
2.76679	0.02887
0.69829	0.19412
4.60148	3.31950
4.92641	6.33540
0.11250	0.28498
0.25576	0.34637
2.07900	3.43065
0.82603	0.38715
1.19479	1.30272
8.60876	1.04338
6.55707	1.38950
2.93186	0.31427
2.67239	0.65113
4.09773	2.03431
1.96879	0.92398
1.03993	0.00029
0.71129	2.10894

Vx	Vy	Vxy
1.17552	0.73364	0.62739

Sx	Sy
1.08421	0.85653



Estimation des paramètres

sur les moyennes	
r =	0.71987
b =	0.79000
a =	0.98024

Ecart-type résiduel par rapport aux points estimés de la régression

Sy.x =	0.923549887

yi estimés	résidus	Smy.x
4.99118	-1.07030	0.65305
5.34164	0.58309	
5.33038	-1.05826	
4.64327	-0.65900	
5.71412	-1.02996	
4.32177	0.76951	
5.98145	0.09390	
6.02708	0.54003	
4.97488	-0.42041	
4.51241	-0.14016	
5.58311	0.51370	
5.29384	-0.75479	
4.17919	-0.02120	
3.14894	0.92781	
3.36234	0.59188	
3.83122	0.56357	
3.87934	0.33794	
3.65741	0.12254	
5.55666	-0.26952	
4.96023	-0.18214	
5.25159	0.56178	

Ecart-types des paramètres

S(a)	0.66458	t(a)	1.47498	p(a=0)	0.15660
S(b)	0.13468	t(b)	1.55922	p(b=1)	0.13545

Répétabilité
= 2,8 Sr

	Méthode de référence	Méthode alternative
Sr	0.24661	0.26915
r	0.69052	0.75363
Rob.Sr	0.13328	0.08790
Rob.r	0.37318	0.24613

Biais

Différences
-1.15628
0.40396
-1.23440
-0.65249
-1.30810
0.86149
-0.25531
0.17869
-0.50204
-0.09886
0.27038
-0.92121
0.10868
1.33156
0.93890
0.78594
0.54753
0.39112
-0.50581
-0.25989
0.40659

D =	-0.03188	moyenne
D =	0.10868	médiane

Prise d'essai : 10 mL
26 eaux usées

		Méthode de référence				Méthode alternative			
Ech	Rang	Rep.1	Rep.2	Mxi	sxi interne	Rep.1	Rep.2	Myi	syi interne
A1	1	3.01	2.94	2.97455	0.04392	2.67	3.00	2.83564	0.22998
A3	2	5.10	5.05	5.07716	0.03709	4.48	4.49	4.48436	0.00604
A5	3	5.50	5.54	5.52077	0.02741	4.86	4.80	4.82962	0.04362
A7	4	5.46	5.56	5.50652	0.07031	5.09	5.02	5.05578	0.04362
A8	5	4.55	4.73	4.63676	0.12529	3.66	3.64	3.64673	0.01420
A9	6	6.08	5.90	5.99226	0.12851	5.82	4.91	5.36161	0.64331
A10	7	3.71	3.67	3.68923	0.03384	3.38	2.96	3.16719	0.29843
A11	8	3.46	3.30	3.37906	0.10912	3.41	2.67	3.03878	0.52250
A12	9	4.27	4.19	4.22979	0.05040	3.98	4.08	4.02882	0.07511
B1	10	3.44	3.11	3.27398	0.23874	3.06	2.89	2.97538	0.12173
B2	11	6.46	6.20	6.33066	0.18680	5.40	5.31	5.35133	0.06179
B3	12	6.43	6.34	6.38842	0.06331	5.24	5.63	5.43614	0.27622
B4	13	5.07	5.05	5.05651	0.01578	5.03	5.01	5.01980	0.01454
B5	14	4.55	4.39	4.47111	0.11343	4.72	4.94	4.82962	0.15265
B7	15	5.99	5.67	5.82644	0.22709	5.36	5.05	5.20741	0.22171
B8	16	3.16	2.75	2.95187	0.29245	3.11	3.10	3.10425	0.01087
C3	17	4.11	3.99	4.04931	0.08990	3.46	3.20	3.33326	0.18263
C4	18	2.73	2.76	2.74520	0.02153	2.79	2.66	2.72757	0.09166
C5	19	3.17	2.86	3.01532	0.21496	3.20	3.08	3.14165	0.08835
C7	20	2.27	1.76	2.01530	0.35620	2.51	2.83	2.58931	0.09073
C8	21	3.61	3.61	3.60885	0.00000	3.65	3.67	3.66266	0.01335
C9	22	3.79	3.55	3.66976	0.16766	3.78	3.72	3.75121	0.03810
C10	23	3.43	3.35	3.38883	0.05924	3.67	3.65	3.66266	0.01335
C12	24	4.35	5.73	5.03798	0.97188	4.08	3.83	3.95263	0.17897
C13	25	2.39	2.41	2.39962	0.00979	2.57	2.64	2.60583	0.05321
C14	26	5.30	5.51	5.40679	0.14735	4.30	4.45	4.37409	0.10333

	Médiane x	4.13955	0.09951	Médiane y	3.70693	0.08954
	Moyenne x	4.25546		Moyenne y	3.92897	

écart-type de répétabilité global	Srx	0.23879		Sry	0.20675
écart-type de répétabilité robuste	Rob Swx	0.14753		Rob Swy	0.13275

R	0.86581
Rob.R	0.89983

n =	2
q =	26
nq =	52

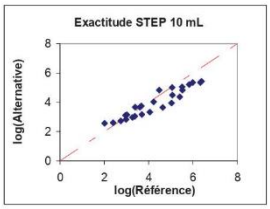
0,5 < R < 2	GMFR
R > 2	OLS
R < 0,5	OLS chgt

Calcul sur les moyennes des deux méthodes

Régression GMFR
Ecart-types globaux

Vxi	Vyi
3.26339	2.44365
1.35173	0.61695
3.20275	1.62422
3.13525	2.54128
0.30648	0.15953
6.04947	4.51874
0.64239	1.24968
1.54809	1.85790
0.00386	0.02558
1.98361	1.83351
8.64777	4.05001
9.10304	4.61940
1.28361	2.38001
0.10587	1.64562
4.98748	3.31795
3.48424	1.36046
0.00308	0.74311
4.56226	2.89512
3.12211	1.24756
10.16354	3.70561
0.83622	0.14203
0.71421	0.06465
1.50562	0.14203
2.16921	0.03315
6.88841	3.50426
2.67282	0.40694

Vx	Vy	Vxy
1.60483	0.92410	1.10385
Sx	Sy	
1.26682	0.96130	



Estimation des paramètres

sur les moyennes	
r =	0.94397
b =	0.75883
a =	0.69081

Ecart-type résiduel par rapport aux points estimés de la régression

Syx =	0.464185323

yi estimés	résidus	Smy.x
2.99698	-0.12134	0.32823
4.55250	-0.06813	
4.88912	-0.05951	
4.87831	0.17746	
4.21831	-0.57159	
5.24691	0.11470	
3.49930	-0.33211	
3.26393	-0.22515	
3.90949	0.11932	
3.18420	-0.20882	
5.50369	-0.15236	
5.54752	-0.11139	
4.53683	0.48297	
4.09261	0.73701	
5.12107	0.08634	
2.93677	0.16448	
3.77254	-0.43928	
2.78294	-0.05537	
2.98792	0.15373	
2.22907	0.34024	
3.43830	0.22435	
3.48452	0.26869	
3.27135	0.38131	
4.52277	-0.57014	
2.52071	0.08512	
4.80263	-0.42854	

Ecart-types des paramètres

S(a)	0.22972	t(a)	3.04637	p(a=0)	0.00556
S(b)	0.05182	t(b)	4.65409	p(b=1)	0.00010

Répétabilité
= 2,8 Sr

	Méthode de référence	Méthode alternative
Sr	0.23879	0.20675
r	0.66861	0.57889
Rob.Sr	0.14753	0.13275
Rob.r	0.41308	0.37171

Biais

Différences
-0.13891
-0.59279
-0.69115
-0.45075
-0.99003
-0.63066
-0.52203
-0.34028
-0.20098
-0.29860
-0.97933
-0.95228
-0.03671
0.35851
-0.61903
0.15238
-0.71605
-0.01762
0.12633
0.55401
0.05381
0.08146
0.27383
-1.08535
0.20621
-1.03270

D =	-0.32649	moyenne
D =	-0.31944	médiane

Prise d'essai : 100 mL
21 eaux usées

		Méthode de référence				Méthode alternative			
Ech	Rang	Rep.1	Rep.2	Mxi	sxi interne	Rep.1	Rep.2	Myi	syi interne
A1	1	3.01	2.94	2.97455	0.04392	2.68	2.59	2.63388	0.06055
A2	2	2.61	2.70	2.65693	0.05945	2.52	2.16	2.33910	0.25559
A6	3	2.27	2.52	2.39284	0.17773	2.13	2.40	2.26747	0.18941
A10	4	3.71	3.67	3.68923	0.03384	3.46	3.35	3.40836	0.07727
A11	5	3.46	3.30	3.37906	0.10912	3.04	3.40	3.21982	0.26020
A12	6	4.27	4.19	4.22979	0.05040	3.91	3.93	3.91890	0.01454
B5	7	4.55	4.39	4.47111	0.11343	4.43	4.97	4.69570	0.38164
B6	8	1.76	2.27	2.01530	0.35620	1.88	1.88	1.87506	0.00000
B8	9	3.16	2.75	2.95187	0.29245	4.26	3.71	3.98381	0.38527
C2	10	3.14	3.31	3.22319	0.11649	3.30	3.28	3.28989	0.01575
C3	11	4.11	3.99	4.04931	0.08990	3.34	3.73	3.53741	0.27575
C4	12	2.73	2.76	2.74520	0.02153	3.04	2.30	2.67121	0.52352
C5	13	3.17	2.86	3.01532	0.21496	2.95	3.04	2.99539	0.06506
C7	14	2.27	1.76	2.01530	0.35620	1.95	2.48	2.21568	0.36973
C8	15	3.61	3.61	3.60885	0.00000	2.80	3.86	3.32834	0.74811
C9	16	3.79	3.55	3.66976	0.16766	3.00	3.97	3.48656	0.68811
C10	17	3.43	3.35	3.38883	0.05924	3.26	3.88	3.56517	0.43826
D4	18	2.39	2.70	2.54583	0.21657	2.72	2.71	2.71179	0.00596
D5	19	2.26	2.08	2.16541	0.12709	2.63	2.86	2.74840	0.16253
D6	20	4.30	4.41	4.35402	0.07755	3.89	2.88	3.38358	0.71915
D7	21	4.71	4.77	4.74363	0.04303	4.60	4.53	4.56677	0.04991

Médiane x	3.22319	0.10912	Médiane y	3.28989	0.25559
Moyenne x	3.25168		Moyenne y	3.18287	

écart-type de répétabilité global	Srx	0.16557	Sry	0.36092
écart-type de répétabilité robuste	Rob Swx	0.16178	Rob Swy	0.37894

R	2.17990
Rob R	2.34237

n = 2
q = 21
nq = 42

0,5 < R < 2	GMFR
R > 2	**OLS**
R < 0,5	OLS chgt

Calcul sur les données individuelles alternative et les moyennes référence

Régression OLS

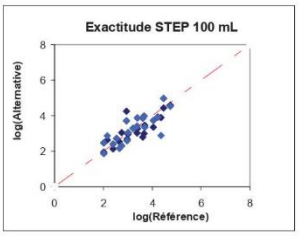
y = a + bx

Ecart-types globaux

Vxi	Vyi	"SPE"
0.07680	0.60646	0.30429
0.35373	1.48926	1.00367
0.73761	1.71181	1.57237
0.19145	0.10766	0.19732
0.01622	0.07043	0.00941
0.95670	1.07806	1.43609
1.48700	4.72294	3.68956
1.52864	3.42075	3.23391
0.08989	1.43143	-0.48026
0.00081	0.02315	-0.00610
0.63621	0.32743	0.56557
0.25653	0.79767	0.51830
0.05587	0.07453	0.08863
1.52864	2.00762	2.39164
0.12757	0.60199	0.10391
0.17479	0.65794	0.25393
0.01881	0.48436	0.10486
0.49822	0.44388	0.66503
1.17999	0.40396	0.94393
1.21514	0.59774	0.44248
2.22592	3.83282	4.12941

Vx	Vy	Vxy
0.65154	0.60712	0.51629

Sx	Sy
0.80718	0.77918



Estimation des paramètres

sur les données individuelles	
r =	0.82090
b =	0.79242
a =	0.60617

Ecart-type résiduel par rapport aux points estimés de la régression

Sy.x = 0.65536293

Yk estimés		résidus : yk - Yk estimés	
2.98788	2.93866	-0.31119	-0.34760
2.67827	2.74489	-0.15844	-0.58853
2.40273	2.60190	-0.26919	-0.20050
3.54855	3.51063	-0.08556	-0.15691
3.34495	3.22267	-0.30912	0.18114
3.98619	3.92971	-0.07948	-0.00244
4.21273	4.08561	0.21311	0.87994
2.00355	2.40273	-0.12849	-0.52767
3.10917	2.78143	1.14707	0.92996
3.09503	3.22557	0.20600	0.05318
3.86530	3.76456	-0.52288	-0.03216
2.76946	2.79359	0.27193	-0.49256
3.11602	2.87513	-0.16663	0.16626
2.40273	2.00355	-0.44849	0.47357
3.46590	3.46590	-0.66656	0.39143
3.60811	3.42022	-0.60811	0.55290
3.32475	3.25836	-0.06948	0.61670
2.50220	2.74489	0.21381	-0.03732
2.39330	2.25088	0.24017	0.61244
4.01293	4.09684	-0.12084	-1.22478
4.34102	4.38924	0.26104	0.14224

Ecart-types des paramètres

S(a)	0.42453	t(a)	1.42786	p(t;a=0)	0.16956
S(b)	0.12680	t(b)	1.63706	p(t;b=1)	0.11807

Répétabilité
= 2,8 Sr

	Méthode de référence	Méthode alternative
Sr	0.16557	0.36092
r	0.46358	1.01057
Rob Sr	0.16178	0.37894
Rob r	0.45298	1.06105

Biais

Différences	
-0.34067	
-0.31784	
-0.12537	
-0.28087	
-0.15924	
-0.31280	
0.22459	
-0.14024	
1.03194	
0.06670	
-0.51190	
-0.07399	
-0.01993	
0.20038	
-0.28051	
-0.18319	
0.17634	
0.16595	
0.58299	
-0.97044	
-0.17687	
D = -0.06881	moyenne
D = -0.14024	médiane

Further testing

Scope: **Bathing water**

N°	N° Echantillon	Xplorer64 Enterococci						Méthode NPP E. coli (*)			
		R1			R2			R1		R2	
		s dét. d'origi	Résultat	log	s dét. d'origi	Résultat	log	Résultat	log	Résultat	log
1	Eau douce 1	8,33	1,20E+03	3,079	7,6	5,40E+03	3,732	6,70E+03	3,826	4,20E+03	3,623
2	Eau douce 2	4,88	1,40E+05	5,146	4,24	2,90E+05	5,462	3,90E+05	5,591	3,10E+05	5,491
3	Eau douce 3	5,99	3,70E+04	4,568	5,25	8,80E+04	4,944	5,50E+05	5,74	2,70E+05	5,431
4	Eau douce 4	4,33	2,60E+05	5,415	4,84	1,40E+05	5,146	4,20E+05	5,623	5,40E+05	5,732
5	Eau de mer 1	4,87	1,40E+05	5,146	4,64	1,80E+05	5,255	3,50E+04	4,54	2,80E+04	4,443
6	Eau de mer 2	3,96	4,00E+05	5,602	4,79	1,50E+05	5,176	4,40E+05	5,641	4,40E+05	5,641
7	Eau de mer 3	4,16	3,20E+05	5,505	4,42	2,30E+05	5,362	4,40E+05	5,641	8,20E+05	5,914
8	Eau de mer 4	3,66	5,80E+05	5,763	3,54	6,70E+05	5,826	8,20E+05	5,914	8,20E+05	5,914
9	Eau de mer 5	2,21	3,20E+06	6,505	2,84	1,50E+06	6,176	3,40E+06	6,531	3,40E+06	6,531
10	Eau douce 5	4,82	1,50E+05	5,176	6,43	2,20E+04	4,342	1,60E+05	5,203	1,90E+05	5,267
11	Eau douce 6	4,94	1,30E+05	5,114	4,85	1,40E+05	5,146	1,40E+05	5,16	1,20E+05	5,078
12	Eau douce 7	6,91	1,20E+04	4,079	4,83	1,50E+05	5,176	8,80E+04	4,944	8,40E+04	4,926
13	Eau de mer 6	4,41	2,40E+05	5,38	4,53	2,10E+05	5,322	1,50E+05	5,164	1,50E+05	5,183
14	Eau de mer 7	7,4	6,90E+03	3,839	7,82	3,20E+03	3,505	2,00E+03	3,305	1,80E+03	3,264
15	Eau de mer 8	9,01	3,90E+02	2,591	8,33	1,20E+03	3,079	6,80E+02	2,834	6,70E+02	2,825
16	Eau de mer 9	7,55	5,80E+03	3,763	7,69	4,90E+03	3,69	1,10E+03	3,043	1,40E+03	3,137
17	Eau douce 8	7,37	7,20E+03	3,857	7,5	6,10E+03	3,785	4,40E+02	2,645	4,90E+02	2,686
18	Eau de mer 10	8,7	6,40E+02	2,806	10,92	4,50E+01	1,653	1,60E+03	3,215	1,40E+03	3,141
19	Eau douce 9	5,87	4,20E+04	4,623	8,71	6,30E+02	2,799	2,40E+03	3,388	1,90E+03	3,285
20	Eau douce 10	8	2,20E+03	3,342	7,45	6,60E+03	3,82	9,40E+02	2,975	7,90E+02	2,898

Relative accuracy – *Enterococci* – Bathing water – Results in log

Scope: **Bathing water**

Méthode de référence					Méthode alternative					Différence
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	
1	3,826	3,623	3,725	0,144	1	3,079	3,732	3,406	0,462	-0,319
2	5,591	5,491	5,541	0,071	2	5,146	5,462	5,304	0,223	-0,237
3	5,740	5,431	5,586	0,218	3	4,568	4,944	4,756	0,266	-0,829
4	5,623	5,732	5,678	0,077	4	5,415	5,146	5,281	0,190	-0,397
5	4,540	4,443	4,492	0,069	5	5,146	5,255	5,201	0,077	0,709
6	5,641	5,641	5,641	0,000	6	5,602	5,176	5,389	0,301	-0,252
7	5,641	5,914	5,778	0,193	7	5,505	5,362	5,434	0,101	-0,344
8	5,914	5,914	5,914	0,000	8	5,763	5,826	5,795	0,045	-0,120
9	6,531	6,531	6,531	0,000	9	6,505	6,176	6,341	0,233	-0,190
10	5,203	5,267	5,235	0,045	10	5,176	4,342	4,759	0,590	-0,476
11	5,160	5,078	5,119	0,058	11	5,114	5,146	5,130	0,023	0,011
12	4,944	4,926	4,935	0,013	12	4,079	5,176	4,628	0,776	-0,308
13	5,164	5,183	5,174	0,013	13	5,380	5,322	5,351	0,041	0,178
14	3,305	3,264	3,285	0,029	14	3,839	3,505	3,672	0,236	0,388
15	2,834	2,825	2,830	0,006	15	2,591	3,079	2,835	0,345	0,005
16	3,043	3,137	3,090	0,066	16	3,763	3,690	3,727	0,052	0,637
17	2,645	2,686	2,666	0,029	17	3,857	3,785	3,821	0,051	1,156
18	3,215	3,141	3,178	0,052	18	2,806	1,653	2,230	0,815	-0,949
19	3,388	3,285	3,337	0,073	19	4,623	2,799	3,711	1,290	0,375
20	2,975	2,898	2,937	0,054	20	3,342	3,820	3,581	0,338	0,645

q=	20	**Mx=**	4,533		**My=**	4,517		**M=**	-0,016
n=	2	**MEDx=**	5,027		**MEDy=**	4,758		**MED=**	-0,155
N=qn=	40	**SDbx=**	1,257		**SDby=**	1,078		Biais	
			MEDwx =	0,053		**MEDwy =**	0,234		
			SDwx=	0,085		**SDwy=**	0,453		
			rob. SDwx=	0,079		**rob. SDwy=**	0,348		

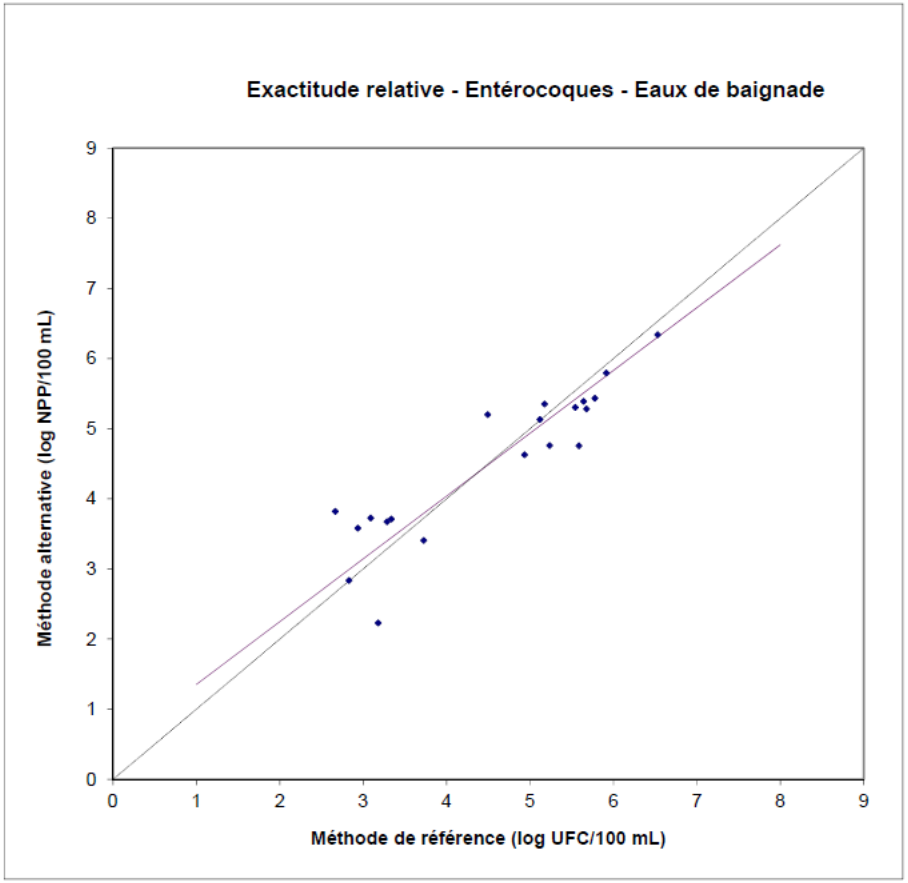
Choix de la méthode
OLS1; x=réf

	R=	5,338			
	rob. R=	4,391			
			Sx=	1,242	
			Sy=	1,112	
r=	0,906				
b=	0,895				
a=	0,458		**Res. SD=**	0,581	
S(b)=	0,342	**p(t;b=1)=**	0,762	**t(b)=**	0,306
S(a)=	0,352	**p(t;a=0)=**	0,201	**t(a)=**	1,303

Répétabilité	Méthode de référence	Méthode alternative
r	0,238	1,269
rob. r	0,222	0,973

M. réf	Alt	Est.Y	Déviation
3,725	3,079	3,793	-0,714
5,541	5,146	5,420	-0,274
5,586	4,568	5,459	-0,891
5,678	5,415	5,542	-0,127
4,492	5,146	4,480	0,666
5,641	5,602	5,509	0,093
5,778	5,505	5,631	-0,126
5,914	5,763	5,754	0,009
6,531	6,505	6,306	0,199
5,235	5,176	5,146	0,030
5,119	5,114	5,042	0,072
4,935	4,079	4,877	-0,798
5,174	5,380	5,091	0,289
3,285	3,839	3,399	0,440
2,830	2,591	2,992	-0,401
3,090	3,763	3,225	0,538
2,666	3,857	2,845	1,012
3,178	2,806	3,304	-0,498
3,337	4,623	3,446	1,177
2,937	3,342	3,088	0,254
3,725	3,732	3,793	-0,061
5,541	5,462	5,420	0,042
5,586	4,944	5,459	-0,515
5,678	5,146	5,542	-0,396
4,492	5,255	4,480	0,775
5,641	5,176	5,509	-0,333
5,778	5,362	5,631	-0,269
5,914	5,826	5,754	0,072
6,531	6,176	6,306	-0,130
5,235	4,342	5,146	-0,804
5,119	5,146	5,042	0,104
4,935	5,176	4,877	0,299
5,174	5,322	5,091	0,231
3,285	3,505	3,399	0,106
2,830	3,079	2,992	0,087
3,090	3,690	3,225	0,465
2,666	3,785	2,845	0,940
3,178	1,653	3,304	-1,651
3,337	2,799	3,446	-0,647
2,937	3,820	3,088	0,732

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Relative accuracy – *Enterococci* – All categories – Results in log

Méthode de référence					Méthode alternative					Différence
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	
EB1	3,826	3,623	3,725	0,144	EB1	3,079	3,732	3,406	0,462	-0,319
EB2	5,591	5,491	5,541	0,071	EB2	5,146	5,462	5,304	0,223	-0,237
EB3	5,740	5,431	5,586	0,218	EB3	4,568	4,944	4,756	0,266	-0,829
EB4	5,623	5,732	5,678	0,077	EB4	5,415	5,146	5,281	0,190	-0,397
EB5	4,540	4,443	4,492	0,069	EB5	5,146	5,255	5,201	0,077	0,709
EB6	5,641	5,641	5,641	0,000	EB6	5,602	5,176	5,389	0,301	-0,252
EB7	5,641	5,914	5,778	0,193	EB7	5,505	5,362	5,434	0,101	-0,344
EB8	5,914	5,914	5,914	0,000	EB8	5,763	5,826	5,795	0,045	-0,120
EB9	6,531	6,531	6,531	0,000	EB9	6,505	6,176	6,341	0,233	-0,190
EB10	5,203	5,267	5,235	0,045	EB10	5,176	4,342	4,759	0,590	-0,476
EB11	5,160	5,078	5,119	0,058	EB11	5,114	5,146	5,130	0,023	0,011
EB12	4,944	4,926	4,935	0,013	EB12	4,079	5,176	4,628	0,776	-0,308
EB13	5,164	5,183	5,174	0,013	EB13	5,380	5,322	5,351	0,041	0,178
EB14	3,305	3,264	3,285	0,029	EB14	3,839	3,505	3,672	0,236	0,388
EB15	2,834	2,825	2,830	0,006	EB15	2,591	3,079	2,835	0,345	0,005
EB16	3,043	3,137	3,090	0,066	EB16	3,763	3,690	3,727	0,052	0,637
EB17	2,645	2,686	2,666	0,029	EB17	3,857	3,785	3,821	0,051	1,156
EB18	3,215	3,141	3,178	0,052	EB18	2,806	1,653	2,230	0,815	-0,949
EB19	3,388	3,285	3,337	0,073	EB19	4,623	2,799	3,711	1,290	0,375
EB20	2,975	2,898	2,937	0,054	EB20	3,342	3,820	3,581	0,338	0,645
ERSTEP1	3,004	2,943	2,974	0,043	ERSTEP1	2,677	2,591	2,634	0,061	-0,340
ERSTEP2	2,615	2,699	2,657	0,059	ERSTEP2	2,520	2,158	2,339	0,256	-0,318
ERSTEP3	2,267	2,519	2,393	0,178	ERSTEP3	2,134	2,401	2,267	0,189	-0,125
ERSTEP4	3,713	3,666	3,690	0,034	ERSTEP4	3,462	3,354	3,408	0,077	-0,281
ERSTEP5	3,456	3,301	3,379	0,110	ERSTEP5	3,037	3,403	3,220	0,259	-0,158
ERSTEP6	4,265	4,193	4,229	0,051	ERSTEP6	3,907	3,927	3,917	0,014	-0,312
ERSTEP7	4,551	4,391	4,471	0,114	ERSTEP7	4,427	4,966	4,696	0,381	0,225
ERSTEP8	1,763	2,267	2,015	0,356	ERSTEP8	1,875	1,875	1,875	0,000	-0,140
ERSTEP9	3,158	2,745	2,952	0,292	ERSTEP9	4,255	3,712	3,984	0,384	1,032
ERSTEP10	3,140	3,305	3,223	0,117	ERSTEP10	3,301	3,279	3,290	0,016	0,067
ERSTEP11	4,114	3,986	4,050	0,091	ERSTEP11	3,342	3,732	3,537	0,276	-0,513
ERSTEP12	2,730	2,760	2,745	0,022	ERSTEP12	3,041	2,301	2,671	0,524	-0,074
ERSTEP13	3,167	2,863	3,015	0,215	ERSTEP13	2,949	3,041	2,995	0,065	-0,020
ERSTEP14	2,267	1,763	2,015	0,356	ERSTEP14	1,954	2,477	2,216	0,370	0,200
ERSTEP15	3,609	3,609	3,609	0,000	ERSTEP15	2,799	3,857	3,328	0,748	-0,280
ERSTEP16	3,788	3,551	3,670	0,167	ERSTEP16	3,000	3,973	3,487	0,688	-0,183
ERSTEP17	3,431	3,346	3,389	0,060	ERSTEP17	3,255	3,875	3,565	0,438	0,176
ERSTEP18	2,393	2,699	2,546	0,217	ERSTEP18	2,716	2,708	2,712	0,006	0,166
ERSTEP19	2,255	2,076	2,165	0,127	ERSTEP19	2,633	2,863	2,748	0,163	0,583
ERSTEP20	4,299	4,408	4,354	0,077	ERSTEP20	3,892	2,875	3,384	0,719	-0,970
ERSTEP21	4,713	4,774	4,744	0,043	ERSTEP21	4,602	4,531	4,567	0,050	-0,177

q= 41
n= 2
N=qn= 82

Mx= 3,877
MEDx= 3,609
SDbx= 1,227
MEDwx = 0,066
SDwx= 0,133
rob. SDwx= 0,099

My= 3,834
MEDy= 3,581
SDby= 1,134
MEDwy = 0,236
SDwy= 0,408
rob. SDwy= 0,350

M= -0,043
MED= -0,140
Biais

Choix de la méthode
OLS1; x=réf

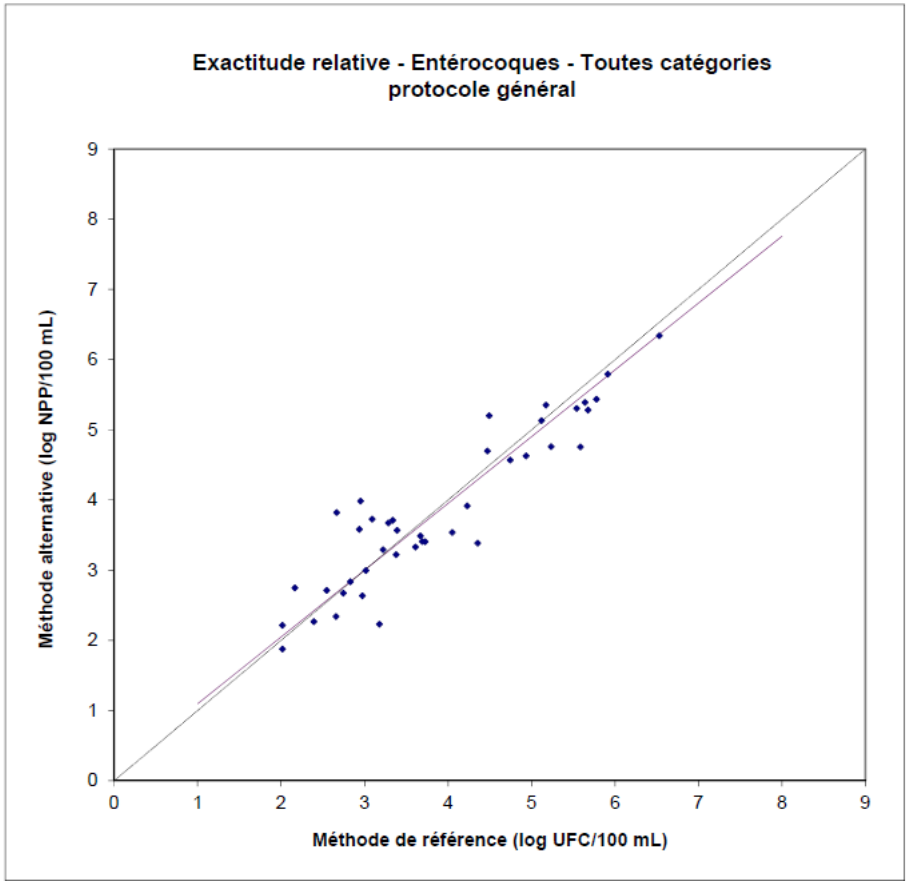
R=	3,082				
rob. R=	3,553				
			Sx=	1,223	
			Sy=	1,163	
r=	0,925				
b=	0,951				
a=	0,146		**Res. SD=**	0,535	
S(b)=	0,254	**p(t;b=1)=**	0,849	**t(b)=**	0,191
S(a)=	1,031	**p(t;a=0)=**	0,888	**t(a)=**	0,141

Répétabilité	Méthode de référence	Méthode alternative
r	0,371	1,144
rob. r	0,276	0,980

	1,000	1,097
	8,000	7,756

M. réf	Alt	Est.Y	Déviation
3,725	3,079	3,689	-0,610
5,541	5,146	5,417	-0,271
5,586	4,568	5,459	-0,891
5,678	5,415	5,547	-0,132
4,492	5,146	4,419	0,727
5,641	5,602	5,512	0,090
5,778	5,505	5,642	-0,137
5,914	5,763	5,772	-0,009
6,531	6,505	6,359	0,146
5,235	5,176	5,126	0,050
5,119	5,114	5,016	0,098
4,935	4,079	4,841	-0,762
5,174	5,380	5,067	0,313
3,285	3,839	3,270	0,569
2,830	2,591	2,838	-0,247
3,090	3,763	3,085	0,678
2,666	3,857	2,681	1,176
3,178	2,806	3,169	-0,363
3,337	4,623	3,320	1,303
2,937	3,342	2,939	0,403
2,974	2,677	2,975	-0,298
2,657	2,520	2,673	-0,154
2,393	2,134	2,422	-0,289
3,690	3,462	3,656	-0,193
3,379	3,037	3,360	-0,323
4,229	3,907	4,169	-0,262
4,471	4,427	4,399	0,027
2,015	1,875	2,063	-0,188
2,952	4,255	2,954	1,301
3,223	3,301	3,211	0,090
4,050	3,342	3,999	-0,656
2,745	3,041	2,757	0,284
3,015	2,949	3,014	-0,065
2,015	1,954	2,063	-0,109
3,609	2,799	3,579	-0,779
3,670	3,000	3,637	-0,637
3,389	3,255	3,370	-0,114
2,546	2,716	2,568	0,148
2,165	2,633	2,206	0,428
4,354	3,892	4,287	-0,395
4,744	4,602	4,659	-0,056

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



3,725	3,732	3,689	0,043
5,541	5,462	5,417	0,045
5,586	4,944	5,459	-0,515
5,678	5,146	5,547	-0,401
4,492	5,255	4,419	0,836
5,641	5,176	5,512	-0,336
5,778	5,362	5,642	-0,280
5,914	5,826	5,772	0,054
6,531	6,176	6,359	-0,183
5,235	4,342	5,126	-0,784
5,119	5,146	5,016	0,130
4,935	5,176	4,841	0,335
5,174	5,322	5,067	0,255
3,285	3,505	3,270	0,235
2,830	3,079	2,838	0,241
3,090	3,690	3,085	0,605
2,666	3,785	2,681	1,104
3,178	1,653	3,169	-1,516
3,337	2,799	3,320	-0,521
2,937	3,820	2,939	0,881
2,974	2,591	2,975	-0,384
2,657	2,158	2,673	-0,515
2,393	2,401	2,422	-0,021
3,690	3,354	3,656	-0,302
3,379	3,403	3,360	0,043
4,229	3,927	4,169	-0,242
4,471	4,966	4,399	0,566
2,015	1,875	2,063	-0,188
2,952	3,712	2,954	0,758
3,223	3,279	3,211	0,067
4,050	3,732	3,999	-0,266
2,745	2,301	2,757	-0,456
3,015	3,041	3,014	0,027
2,015	2,477	2,063	0,414
3,609	3,857	3,579	0,279
3,670	3,973	3,637	0,336
3,389	3,875	3,370	0,505
2,546	2,708	2,568	0,140
2,165	2,863	2,206	0,658
4,354	2,875	4,287	-1,412
4,744	4,531	4,659	-0,127

APPENDIX 6

LINEARITY

Scope: **Bathing water**

		Résultats bruts / 100 ml		Résultats en LOG		Exactitude relative	
Niveaux (j)	Répétition (k)	NF EN 7899-1	XplOrer64™	NF EN 7899-1 (x)	XplOrer64™ (y)	Différence (d)	Moyenne des différences
Environ 5.10^1 ufc / 100 ml	répétion 1	3,0E+01	5,0E+01	1,48	1,70	0,22	0,11
	répétion 2	4,5E+01	4,4E+01	1,65	1,64	-0,01	
Environ 5.10^2 ufc / 100 ml	répétion 1	6,5E+02	5,3E+02	2,82	2,73	-0,09	-0,19
	répétion 2	7,2E+02	3,7E+02	2,86	2,57	-0,29	
Environ 5.10^3 ufc / 100 ml	répétion 1	5,7E+03	5,9E+03	3,76	3,77	0,01	0,08
	répétion 2	5,0E+03	7,1E+03	3,70	3,85	0,15	

avec $d_{jk} = y_{jk} - x_{jk}$

Caractéristiques physico-chimiques de l'eau douce superficielle utilisée (base canoë-kayak du canal de la Deûle, Lille) :

- conductivité : 1030 µS/cm
- turbidité : 1,4 NFU
- MEST : 1,6 mg/l

Scope: **Marine water**

		Résultats bruts / 100 ml		Résultats en LOG		Exactitude relative	
Niveaux (j)	Répétition (k)	NF EN 7899-1	XplOrer64™	NF EN 7899-1 (x)	XplOrer64™ (y)	Différence (d)	Moyenne des différences
Environ 5.10^1 ufc / 100 ml	répétion 1	4,6E+01	3,2E+01	1,66	1,51	-0,16	-0,16
	répétion 2	4,6E+01	<1	1,66			
Environ 5.10^2 ufc / 100 ml	répétion 1	6,6E+02	1,3E+03	2,82	3,10	0,28	0,28
	répétion 2	3,9E+02	7,6E+02	2,59	2,88	0,29	
Environ 5.10^3 ufc / 100 ml	répétion 1	5,0E+03	3,9E+03	3,70	3,59	-0,11	-0,21
	répétion 2	6,6E+03	3,3E+03	3,82	3,52	-0,30	

avec $d_{jk} = y_{jk} - x_{jk}$

Caractéristiques physico-chimiques de l'eau de mer utilisée (littoral, Gravelines) :

- conductivité : 49000 µS/cm
- turbidité : 0,92 NFU
- MEST : 13,2 mg/l

Remarque :
Le nombre de données proposées est conforme au référentiel en vigueur mais cependant insuffisant pour exploiter les résultats par exemple selon NF EN ISO 16140 « Protocole pour la validation des méthodes alternatives en microbiologie des aliments » (5 niveaux et 2 répétitions par niveau requis au minimum) ou encore selon XP T 90-210 « Protocole d'évaluation d'une méthode alternative d'analyse physico-chimique quantitative par rapport à une méthode de référence » (5 niveaux et 5 répétitions par niveau requis au minimum). C'est pourquoi aucune exploitation statistique n'est proposée pour cette partie, le but de ces essais sur échantillons dopés étant avant tout d'avoir une aperçu descriptif de la relation entre les 2 méthodes.

Detailed results – Scope: Wastewater

Version logiciel XplOrer64™ Manager : V3.0
Souche utilisée : *Enterococcus faecalis* (Eau de STEP, Solesmes 2011, A1)
Matrice utilisée : Effluent de station (Douvrin, MES : 4 mg/L)

Protocole général pour des échantillons filtrables (filtration de 100 ml), protocole validé en 2009 sur eaux de baignade

Taux visé (UFC/100 mL)	Taux réel (UFC/100 mL)	NF EN ISO 7899-1				Méthode alternative XplOrer64 CheckN'Safe *Enterococci*			
		Résultat NPP		*Enterococci* / 100 mL		R1		R2	
		R1	R2	R1	R2	DT (heures)	Réponse (b/100 mL)	DT (heures)	Réponse (b/100 mL)
2.00E+02	2.25E+02	2/1/0/0/0/0	3/0/0/0/0/0	1.80E+02	1.85E+02	6.67	9.96E+04	6.43	1.46E+05
2.00E+04	1.89E+04	16/14/5/1/0/0	16/14/4/0/0/0	2.56E+04	2.20E+04	5.02	1.41E+06	4.88	1.76E+06
2.00E+06	1.95E+06	16/16/16/15/2/1	16/16/16/16/9/1	2.46E+06	8.12E+06	3.07	3.21E+07	2.67	6.10E+07

Protocole spécifique 1 pour des échantillons filtrables (filtration de 10 mL)

Taux visé (UFC/100 mL)	Taux réel (UFC/100 mL)	NF EN ISO 7899-1				Méthode alternative XplOrer64 CheckN'Safe *Enterococci*			
		Résultat NPP		*Enterococci* / 100 mL		R1		R2	
		R1	R2	R1	R2	DT (heures)	Réponse (b/100 mL)	DT (heures)	Réponse (b/100 mL)
2.00E+02	2.25E+02	2/1/0/0/0/0	3/0/0/0/0/0	1.80E+02	1.85E+02	8.19	8.70E+03	8.50	5.29E+03
2.00E+04	1.89E+04	16/14/5/1/0/0	16/14/4/0/0/0	2.56E+04	2.20E+04	5.98	3.01E+05	5.59	5.63E+05
2.00E+06	1.95E+06	16/16/16/15/2/1	16/16/16/16/9/1	2.46E+06	8.12E+06	3.95	7.82E+06	3.52	1.56E+07

Protocole spécifique 2 pour des échantillons non filtrables (ensemencement direct de 1 mL)

Taux visé (UFC/100 mL)	Taux réel (UFC/100 mL)	NF EN ISO 7899-1				Méthode alternative XplOrer64 CheckN'Safe *Enterococci*			
		Résultat NPP		*Enterococci* / 100 mL		R1		R2	
		R1	R2	R1	R2	DT (heures)	Réponse (b/100 mL)	DT (heures)	Réponse (b/100 mL)
2.00E+02	2.25E+02	2/1/0/0/0/0	3/0/0/0/0/0	1.80E+02	1.85E+02	8.62	4.36E+03	9.27	1.54E+03
2.00E+04	1.89E+04	16/14/5/1/0/0	16/14/4/0/0/0	2.56E+04	2.20E+04	6.49	1.33E+05	6.58	1.15E+05
2.00E+06	1.95E+06	16/16/16/15/2/1	16/16/16/16/9/1	2.46E+06	8.12E+06	4.28	4.61E+06	4.31	4.39E+06
2.00E+08	1.91E+08	16/16/16/16/16/16	16/16/16/16/16/15	3.50E+08	3.46E+08	4.46	3.45E+06	1.98	1.84E+08

MES : taux de matières en suspension
DT : temp de détection
b : bactéries
R1, R2 : réplicats

Synthesis of results [log(*Enterococci*/100 mL)]

According to the XplOrer64 Manager V3.0 software

Scope: Wastewater

Protocole général (Filtration100 mL)

Taux réel (UFC/100 mL)	NF EN ISO 7899-1		XplOrer64 CheckN'Safe *Enterococcus*	
	R1 (b / 100 mL)	R2 (b / 100 mL)	R1 (b / 100 mL)	R2 (b / 100 mL)
2.35	2.26	2.27	5.00	5.16
4.28	4.41	4.34	6.15	6.25
6.29	6.39	6.91	7.51	7.79

Protocole spécifique 1 (Filtration 10 mL)

Taux réel (UFC/100 mL)	NF EN ISO 7899-1		XplOrer64 CheckN'Safe *Enterococcus*	
	R1 (b / 100 mL)	R2 (b / 100 mL)	R1 (b / 100 mL)	R2 (b / 100 mL)
2.35	2.26	2.27	3.94	3.72
4.28	4.41	4.34	5.48	5.75
6.29	6.39	6.91	6.89	7.19

Protocole spécifique 2 (ensemencement direct de 1 mL)

Taux réel (UFC/100 mL)	NF EN ISO 7899-1		XplOrer64 CheckN'Safe *Enterococcus*	
	R1 (b / 100 mL)	R2 (b / 100 mL)	R1 (b / 100 mL)	R2 (b / 100 mL)
2.35	2.26	2.27	3.64	3.19
4.28	4.41	4.34	5.12	5.06
6.29	6.39	6.91	6.66	6.64
8.28	8.54	8.54	6.54	8.26

b : bactéries
R1, R2 : réplicats

Linearity – *Enterococci* – Fresh water – Results in log

Niveau	Méthode de référence				Méthode alternative			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	1,477	1,653	1,6	0,125	1,699	1,643	1,7	0,039
2	2,813	2,857	2,8	0,031	2,724	2,568	2,6	0,110
3	3,756	3,699	3,7	0,040	3,771	3,851	3,8	0,057

q = 3
n = 2
N = qn = 6

Mx = 2,709
MEDx = 2,835
SDbx = 1,087

MEDwx = 0,040
SDwx = 0,055
rob. SDwx = 0,060

My = 2,710
MEDy = 2,646
SDby = 1,071

MEDwy = 0,057
SDwy = 0,053
rob. SDwy = 0,084

Choix méthode

GMFR

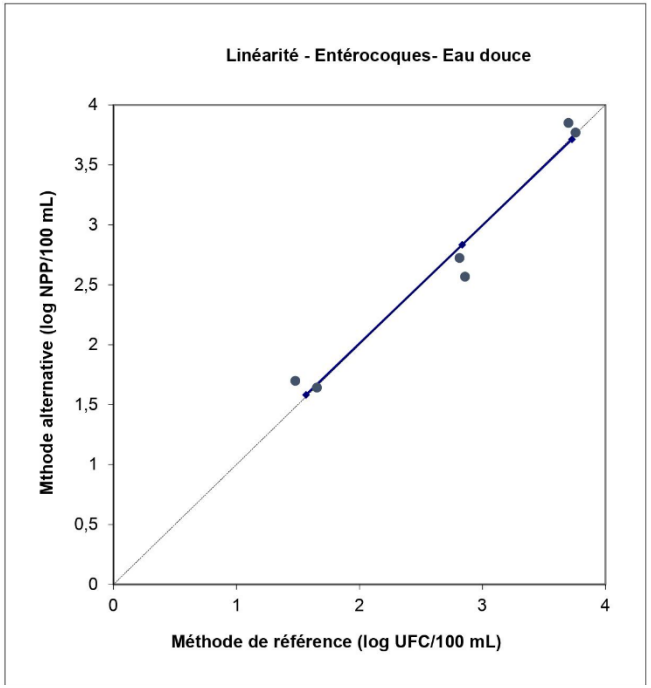
R = 0,968
rob.R = 1,413
Res.SEM = 0,230
Res.SD = 0,325

Sx = 0,974
Sy = 0,960

Est y	Déviation
1,582	0,090
2,834	-0,187
3,713	0,098

r = 0,989
b = 0,986
a = 0,039

Sb = 0,167 **p(t;b=1) =** 0,936 **t (b) =** 0,085
Sa = 0,471 **p(t;a=0) =** 0,939 **t (a) =** 2,043



Linéarité

F = 146,193 **p(F) =** 0,001
rob.F = 56,327 **rob.p(F) =** 0,005

146,19291

Linearity – *Enterococci* – Bathing water – Results in log

Niveau	Méthode de référence				Méthode alternative			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	1,477	1,653	1,6	0,125	1,699	1,643	1,7	0,039
2	2,813	2,857	2,8	0,031	2,724	2,568	2,6	0,110
3	3,756	3,699	3,7	0,040	3,771	3,851	3,8	0,057
4	2,820	2,591	2,7	0,162	3,114	2,881	3,0	0,165
5	3,699	3,820	3,8	0,085	3,591	3,519	3,6	0,051

q = 5
n = 2
N = qn = 10

Mx = 2,918
MEDx = 2,835
SDbx = 0,901

MEDwx = 0,085
SDwx = 0,072
rob. SDwx = 0,126

My = 2,936
MEDy = 2,997
SDby = 0,842

MEDwy = 0,057
SDwy = 0,068
rob. SDwy = 0,084

Choix méthode
GMFR

R = 0,953
rob.R = 0,667
Res.SEM = 0,140
Res.SD = 0,198

Sx = 0,853
Sy = 0,797

r = 0,973
b = 0,935
a = 0,207

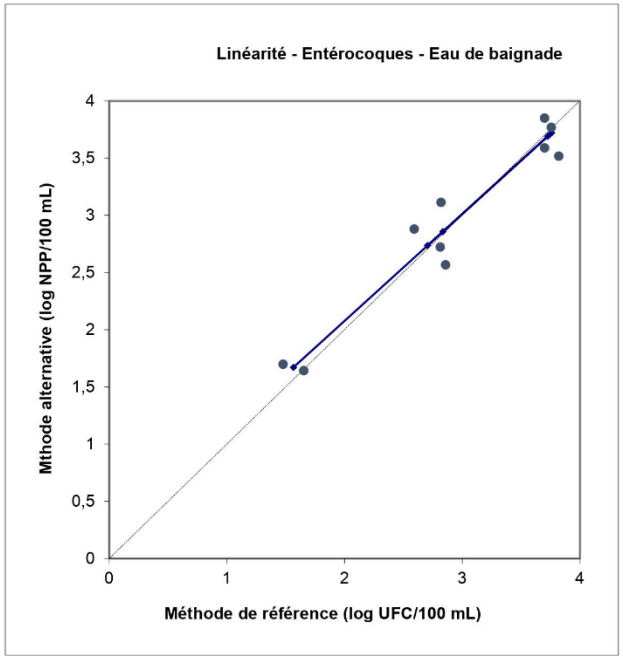
Est y	Déviation
1,671	0,000
2,858	-0,212
3,693	0,119
2,737	0,261
3,722	-0,167

Sb = 0,082 **p(t;b=1) =** 0,452 **t (b) =**
Sa = 0,248 **p(t;a=0) =** 0,427 **t (a) =** 0,791
3,195

Linéarité

F = 20,761
rob.F = 13,092

p(F) = 0,003
rob.p(F) = 0,008



Linearity – *Enterococci* – Wastewater (100 mL) – Results in log

Niveau
1
2
3

Méthode de référence			
Rep.1	Rep.2	M	SD
2,255	2,267	2,3	0,008
4,408	4,342	4,4	0,047
6,391	6,910	6,7	0,367

Méthode alternative			
Rep.1	Rep.2	M	SD
4,998	5,164	5,1	0,117
6,149	6,246	6,2	0,068
7,507	7,785	7,6	0,197

q = 3 **Mx =** 4,429 **My =** 6,308
n = 2 **MEDx =** 4,375 **MEDy =** 6,197
N = qn = 6 **SDbx =** 2,195 **SDby =** 1,286

MEDwx = 0,047 **MEDwy =** 0,117
SDwx = 0,151 **SDwy =** 0,098
rob. SDwx = 0,069 **rob. SDwy =** 0,174

Choix méthode
OLS1; x=réf

R = 0,647
rob.R = 2,524

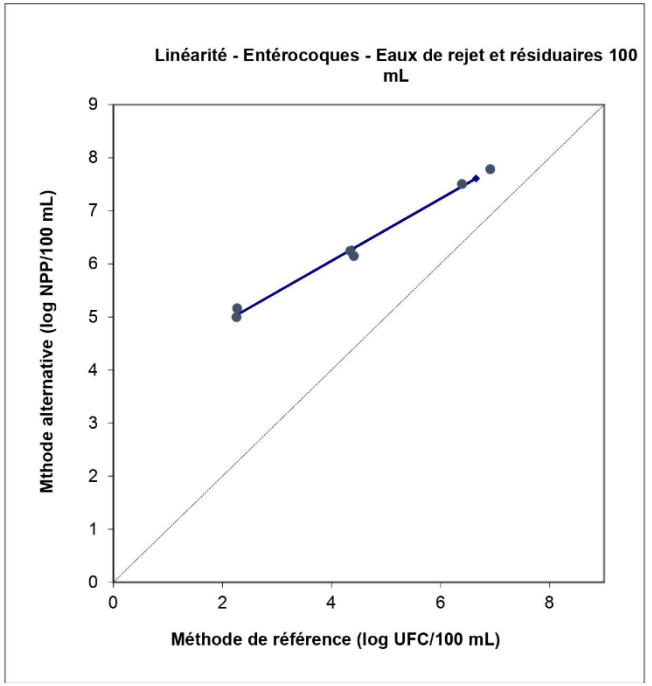
Res.SD = 0,138

M. (réf)	Alt.	Est. Y	Déviation
2,261	4,998	5,037	-0,039
4,375	6,149	6,277	-0,128
6,650	7,507	7,611	-0,104
2,261	5,164	5,037	0,127
4,375	6,246	6,277	-0,031
6,650	7,785	7,611	0,175

Sx = 1,970
Sy = 1,155

r = 0,999
b = 0,586
a = 3,712

Sb = 6,380 **p(t;b=1) =** 0,951 **t (b) =** 0,065
Sa = 0,150 **p(t;a=0) =** 0,000 **t (a) =** 18,096



Linéarité

F = 4,988 **p(F) =** 0,112
rob.F = 0,484 **rob.p(F) =** 0,537

4,98836565

Linearity – *Enterococci* – Wastewater (10 mL) – Results in log

Niveau	Méthode de référence Rep.1	Rep.2	M	SD	Méthode alternative Rep.1	Rep.2	M	SD
1	2,255	2,267	2,3	0,008	3,940	3,723	3,8	0,153
2	4,408	4,342	4,4	0,047	5,479	5,751	5,6	0,192
3	6,391	6,910	6,7	0,367	6,893	7,193	7,0	0,212

q = 3 **Mx =** 4,429 **My =** 5,496
n = 2 **MEDx =** 4,375 **MEDy =** 5,615
N = qn = 6 **SDbx =** 2,195 **SDby =** 1,609

MEDwx = 0,047 **MEDwy =** 0,192
SDwx = 0,151 **SDwy =** 0,132
rob. SDwx = 0,069 **rob. SDwy =** 0,285

Choix méthode
OLS1; x=réf

R = 0,878
rob.R = 4,132

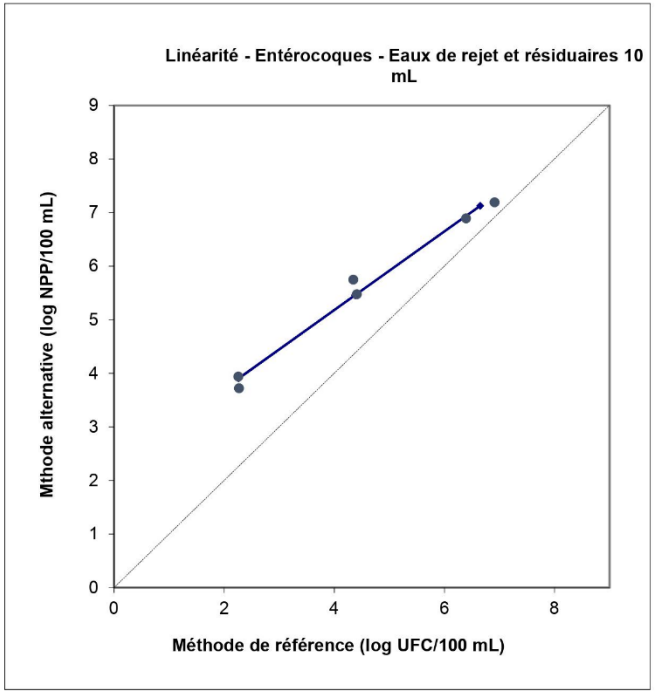
Res.SD = 0,212

M. (réf)	Alt.	Est. Y	Déviation
2,261	3,940	3,905	0,035
4,375	5,479	5,457	0,022
6,650	6,893	7,127	-0,234
2,261	3,723	3,905	-0,181
4,375	5,751	5,457	0,293
6,650	7,193	7,127	0,066

Sx = 1,970
Sy = 1,447

r = 0,996
b = 0,734
a = 2,245

Sb = 4,156 **p(t;b=1) =** 0,952 **t (b) =** 0,064
Sa = 0,230 **p(t;a=0) =** 0,001 **t (a) =** 5,410



Linéarité

F = 7,247 **p(F) =** 0,074
rob.F = 0,788 **rob.p(F) =** 0,440

7,24687148

Linearity – *Enterococci* – Wastewater (1 mL) – Results in log

Niveau	Méthode de référence				Méthode alternative			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	2,255	2,267	2,3	0,008	3,639	3,188	3,4	0,320
2	4,408	4,342	4,4	0,047	5,124	5,061	5,1	0,045
3	6,391	6,910	6,7	0,367	6,664	6,642	6,7	0,015
4	8,544	8,539	8,5	0,004	6,538	8,265	7,4	1,221

q = 4 **n =** 2 **N = qn =** 8

Mx = 5,457 **MEDx =** 5,513 **SDbx =** 2,728

MEDwx = 0,027 **SDwx =** 0,131 **rob. SDwx =** 0,041

My = 5,640 **MEDy =** 5,873 **SDby =** 1,769

MEDwy = 0,182 **SDwy =** 0,447 **rob. SDwy =** 0,270

Choix méthode
OLS1

R = 3,416
rob.R = 6,628

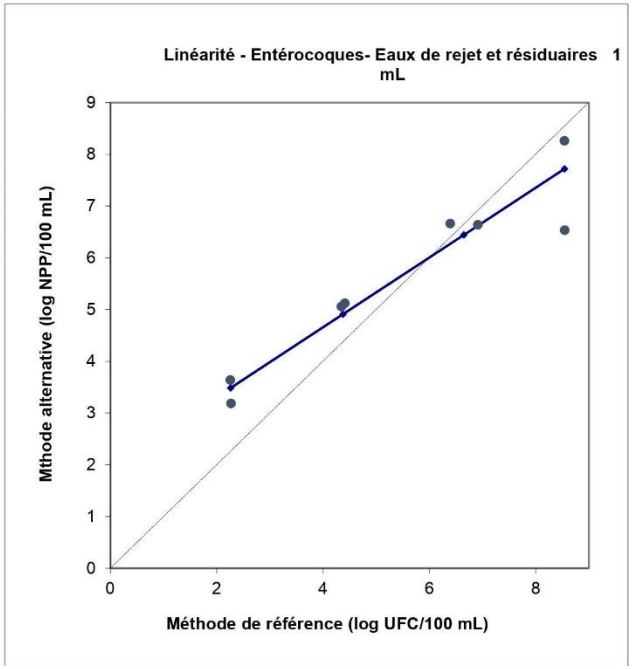
Res.SD = 0,572

M. (réf)	Alt.	Est. Y	Déviation
2,261	3,639	3,485	0,155
4,375	5,124	4,910	0,213
6,650	6,664	6,445	0,219
8,542	6,538	7,720	-1,182
2,261	3,188	3,485	-0,297
4,375	5,061	4,910	0,150
6,650	6,642	6,445	0,198
8,542	8,265	7,720	0,545

Sx = 2,529
Sy = 1,706

r = 0,991
b = 0,674
a = 1,960

Sb = 1,672 **p(t;b=1) =** 0,852 **t (b) =** 0,195
Sa = 0,508 **p(t;a=0) =** 0,008 **t (a) =** 1,888



Linéarité

F = 2,919 **p(F) =** 0,165
rob.F = 11,456 **rob.p(F) =** 0,022

2,91878963

APPENDIX 7

LOD-LOQ RESULTS

Scope: Wastewater

Souche utilisée : *E. faecalis* (Effluent de station, Solesme)
Matrice utilisée : Effluent de station (Douvrin, MES : 4 mg/L)

↘ Résultats de la méthode alternative XplOrer64™ CheckN'Safe *E. coli* en Germes dans 100 mL

Protocole général : filtration de 100 mL

Niveau d'inoculation (Germes/100mL)	IC* (Germes/100mL)	Réplicats								
		1			2			3		
		DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection
0,5	0,4 - 0,7	11,49	44	+	/	<seuil	-	/	<seuil	-
1,1	0,9 – 1,3	12,47	9	+	10,07	426	+	10,00	477	+
2,1	1,7 – 2,6	11,54	40	+	9,50	1063	+	9,80	657	+

Niveau d'inoculation (Germes/100mL)	IC* (Germes/100mL)	Réplicats									
		4			5			6			
		DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	Taux
0,5	0,4 - 0,7	9,27	1537	+	/	<seuil	-	9,73	735	+	3/6
1,1	0,9 – 1,3	11,60	37	+	10,36	268	+	11,62	35	+	6/6
2,1	1,7 – 2,6	9,17	1805	+	13,36	2	+	9,73	735	+	6/6

DT : Temps de détection / : non détecté - : négatif + : positif
* IC : Indice de confiance (loi de Poisson)

Protocole spécifique 1 : filtration de 10 mL

Niveau d'inoculation (Germes/100mL)	IC* (Germes/100mL)	Réplicats								
		1			2			3		
		DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection
1,0	0,9 – 1,3	10,59	180	+	9,63	870	+	/	<seuil	-
2,1	1,7 – 2,5	/	<seuil	-	10,21	340	+	11,55	39	+
5,2	4,3 – 6,3	10,03	450	+	9,98	490	+	12,88	4,7	+
10,4	8,5 – 12,7	9,61	890	+	11,11	80	+	9,47	1100	+

Niveau d'inoculation (Germes/100mL)	IC* (Germes/100mL)	Réplicats									
		4			5			6			
		DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	Taux
1,0	0,9 – 1,3	10,03	460	+	/	<seuil	-	/	<seuil	-	3/6
2,1	1,7 – 2,5	12,74	5,9	+	12,18	15	+	/	<seuil	-	4/6
5,2	4,3 – 6,3	/	<seuil	-	10,38	260	+	9,72	750	+	5/6
10,4	8,5 – 12,7	10,51	210	+	10,46	230	+	10,87	112	+	6/6

DT : Temps de détection / : non détecté - : négatif + : positif
* IC : Indice de confiance (loi de Poisson)

Scope: Wastewater

Souche utilisée : *E. faecalis* (Effluent de station, Solesme)
Matrice utilisée : Effluent de station (Douvrin, MES : 4 mg/L)

⇘ Résultats de la méthode alternative XplOrer64™ CheckN'Safe *E. coli* en Germes dans 100 mL

Protocole spécifique : ensemencement direct de 1 mL

Niveau d'inoculation (Germes/100mL)	IC* (Germes/100mL)	Réplicats								
		1			2			3		
		DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection
0,5	0,4 – 0,6	/	<seuil	-	/	<seuil	-	/	<seuil	-
0,7	0,6 – 0,8	13,45	1,9	+	9,79	670	+	/	<seuil	-
1,4	1,2 – 1,6	10,76	140	+	9,17	1800	+	9,65	830	+
2,9	2,5 – 3,3	9,51	1100	+	9,60	910	+	9,17	1800	+

Niveau d'inoculation (Germes/100mL)	IC* (Germes/100mL)	Réplicats									
		4			5			6			
		DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	DT	Réponse dans 100 mL	Détection	Taux
0,5	0,4 – 0,6	/	<seuil	-	/	<seuil	-	/	<seuil	-	0/6
0,7	0,6 – 0,8	10,11	400	+	/	<seuil	-	/	<seuil	-	3/6
1,4	1,2 – 1,6	9,64	850	+	9,25	1600	+	9,73	740	+	6/6
2,9	2,5 – 3,3	9,37	1300	+	9,28	1500	+	9,58	930	+	6/6

DT : Temps de détection / : non détecté - : négatif + : positif
* IC : Indice de confiance (loi de Poisson)

APPENDIX 8

SELECTIVITY
(Inclusivity/Exclusivity)

Results of the initial study according to XplOrer64 Software Version 3.0
Inclusivity (2009) V3.0

N°	Species	Origin	Inoculation level (CFU/100mL)	Alternative method XplOrer64™ CheckN'Safe™ Enterococci		
				DT (hours)	Result (b/100 mL)	Final result Enterococci detection
1	*Enterococcus avium*	Well water – Lille (59)	2.5+01	/	ND	-
2	*Enterococcus durans*	Well water – Lille (59)	2.4E+01	12.50	8.6E+00	+
3	*Enterococcus durans*	Surface water – Etang du Parc Barbieux, Croix (59)	8.1E+01	9.70	7.7E+02	+
4	*Enterococcus durans*	Marine water – Plage sud, Oyes-Plage (62)	7.7E+01	8.73	3.7E+03	+
5	*Enterococcus durans*	Marine water – Plage de Wissant (62)	4.8E+01	8.72	3.7E+03	+
6	*Enterococcus durans*	Marine water – Plage du Chatelet, Tardinghen (62)	5.9E+01	9.00	2.4E+03	+
7	*Enterococcus faecalis*	Collection CCM 2541	2.6E+01	10.09	4.1E+02	+
8	*Enterococcus faecalis*	Marine water – Plage sud, Audresselles (62)	7.7E+01	/	ND	-
9	*Enterococcus faecalis*	Marine water – Plage des Dunes de la Slack, Ambleuteuse (62)	1.1E+02	10.07	4.3E+02	+
10	*Enterococcus faecium*	Collection RIVM WR63	1.7E+01	10.02	4.6E+02	+
11	*Enterococcus faecium*	Surface water – Port de plaisance, Fort-Philippe (59)	4.6E+01	11.83	2.5E+01	+
12	*Enterococcus faecium*	Surface water – Etang du Parc d'Immercourt, Athies (62)	5.9E+01	11.76	2.8E+01	+
13	*Enterococcus faecium*	Surface water – Rivière, Roeux (62)	4.5E+01	11.03	9.1E+01	+
14	*Enterococcus faecium*	Surface water – Lac de Waziers (59)	2.1E+01	10.26	3.1E+02	+
15	*Enterococcus faecium*	Surface water – Rivière, Lambres les Douai (59)	3.5E+01	12.33	1.1E+01	+
16	*Enterococcus faecium*	Surface water – Lac du Brunemont (59)	8.1E+01	11.50	4.3E+01	+
17	*Enterococcus faecium*	Surface water – Canal de la Sensée, Arieux (59)	4.2E+01	10.26	3.1E+02	+
18	*Enterococcus faecium*	Brackish water – Estuaire de l'Aa, Gravelines (59)	5.2E+01	12.99	3.9E+00	+
19	*Enterococcus faecium*	Brackish water – Canal de Bourbourg, Dunkerque (59)	5.2E+01	9.01	2.3E+03	+
20	*Enterococcus faecium*	Marine water – Plage Centrale, Dunkerque (59)	3.6E+01	9.69	7.8E+02	+
21	*Enterococcus faecium*	Marine water – Plage Centrale, Gravelines (59)	6.4E+01	8.28	7.5E+03	+
22	*Enterococcus faecium*	Marine water – Plage de Fort-Vert, Hemmes de Marck (62)	3.2E+01	10.32	2.9E+02	+
23	*Enterococcus faecium*	Marine water – Plage Centrale, Sangatte (62)	6.3E+01	9.25	1.6E+03	+
24	*Enterococcus faecium*	Marine water – Plage du Cap Gris, Nez (62)	4.6E+01	9.76	7.0E+02	+
25	*Enterococcus gallinarum*	Surface water – Douves, Gravelines (59)	5.3E+01	11.28	6.1E+01	+
26	*Enterococcus gallinarum*	Surface water – Lac du héron, Villeneuve d'Ascq (59)	5.0E+01	11.37	5.3E+01	+
27	*Enterococcus gallinarum*	Surface water – Rivière, Armentières (59)	3.3E+01	11.61	3.6E+01	+
28	*Enterococcus gallinarum*	Surface water – Etang Loisirparc, Aubigny-au-Bac (59)	4.8E+01	11.09	8.3E+01	+
29	*Enterococcus gallinarum*	Marine water – Plage du Cap Blanc-Nez (62)	2.0E+01	13.57	1.6E+00	+
30	*Enterococcus hirae*	Collection CCM 2423	1.2E+02	11.83	2.5E+01	+

b/100 mL : bacteria in 100 mL
-: negative test
+: positive test
ND: undetected

Results of the initial study according to XplOrer64 Software Version 3.0
Exclusivity (2009) V3.0

N°	Species	Origin	Inoculation level (CFU/100mL)	Alternative method XplOrer64™ CheckN'Safe™ Enterococci			NF EN ISO 7899-1 (CFU/100 mL)
				DT (hours)	Result (b/100 mL)	Final result Enterococci detection	
1	*Aerococcus viridans*	Collection CIP 54.145T	4.4E+03	/	ND	-	/
2	*Aerococcus viridans*	Tap water	3.4E+04	/	ND	-	/
3	*Lactococcus cremoris*	Tap water	2.6E+04	/	ND	-	/
4	*Lactococcus lactis lactis*	Collection CIP 70.56T	6.0E+04	/	ND	-	/
5	*Micrococcus luteus*	Collection CIP 53.45	8.0E+04	/	ND	-	/
6	*Micrococcus (Kocuria) varians*	Collection CIP 81.73T	8.0E+04	/	ND	-	/
7	*Pediococcus damnonus*	Collection CIP 102264T	1.8E+04	/	ND	-	/
8	*Pediococcus (Tertragenococcus) halophilus*	Collection CIP 102263T	2.0E+04	/	ND	-	/
9	*Pediococcus inopinatus*	Collection CIP 102406T	5.4E+04	/	ND	-	/
10	*Pediococcus pentosaceus*	Collection CIP 10260T	8.0E+04	/	ND	-	/
11	*Planococcus citreus*	Collection CIP 81.74T	1.5E+05	/	ND	-	/
12	*Staphylococcus aureus*	Collection CIP 53.154	1.2E+04	/	ND	-	/
13	*Staphylococcus capitis*	Swimming pool water	3.4E+03	/	ND	-	/
14	*Staphylococcus chromogenes*	Swimming pool water	2.5E+04	/	ND	-	/
15	*Staphylococcus epidermidis*	Collection CIP 68.21	2.4E+05	/	ND	-	/
16	*Satphylococcus saprophyticus*	Tap water	1.9E+04	/	ND	-	/
17	*Satphylococcus saprophyticus*	Thermal baths	2.5E+04	/	ND	-	/
18	*Satphylococcus xylosus*	Tap water	2.3E+04	/	ND	-	/
19	*Acinetobacter johnsonii*	Collection CIP 64.6T	3.1E+04	/	ND	-	/
20	*Aeromonas hydrophila*	Drill water	2.5E+05	/	ND	-	/
21	*Bacillus cereus*	Collection CIP 64.42	3.0E+05	/	ND	-	/
22	*Bacillus subtilis*	Collection CIP 52.62	2.5E+05	/	ND	-	/
23	*Corynebacterium propinquum*	Tap water	1.9E+03	/	ND	-	/
24	*Enterobacter cloacae*	Swimming pool water	1.2E+04	/	ND	-	/
25	*Proteus mirabilis*	River water	8.8E+03	/	ND	-	/
26	*Proteus vulgaris*	River water	6.8E+03	/	ND	-	<15
27	*Providencia stuartii*	River water	6.5E+03	/	ND	-	/
28	*Pseudomonas aeruginosa*	Thermal baths	4.6E+03	/	ND	-	/
29	*Vibrio fluvialis*	River water	9.0E+03	/	ND	-	/
30	*Vibrio parahaemolyticus*	Tap water	9.6E+04	/	ND	-	/

b/100 mL : bacteria in 100 mL
-: negative test
+: positive test
ND: undetected

Further testing for bacterial species inoculating tested at rates below 10^4 CFU / mL
Exclusivity (2021 by Ad.Gène)

N°	Species	Origin	Inoculation level (CFU/100mL)	Alternative method XplOrer64™ CheckN'Safe™ Enterococci			NF EN ISO 7899-1 (CFU/100 mL)
				DT (hours)	Result (b/100 mL)	Final result Enterococci detection	
1	*Aerococcus viridans*	Référence WDCM 00061	1.5E+05	/	ND	-	/
2	*Staphylococcus capitis*	Référence ATCC 35661	3.0E+04	/	ND	-	/
3	*Corynebacterium striatum*	Référence ATCC BAA-1293 TM	1.3E+05	/	ND	-	/
4	*Proteus mirabilis*	Isolée d'une lingette	3.0E+06	/	ND	-	/
5	*Proteus vulgaris*	Référence ATCC 8427	1.8E+06	/	ND	-	/
6	*Providencia struartii*	Référence ATCC 33672	3.0E+06	/	ND	-	/
7	*Pseudomonas aeruginosa*	Référence WDCM 00025	1.3E+06	/	ND	-	/
8	*Vibrio furnissii*	Référence NCTC 11218	6.6E+05	/	ND	-	/

b/100 mL : bacteria in 100 mL
-: negative test
+: positive test
ND: undetected

APPENDIX 9

RESULTS IN *ENTEROCOCCI* OBSERVED BY PARTCIPANTS (Interlaboratory study)

Niveau bas (flacons A et B)

LE : laboratoire expert (seul les échantilons du colis 2 ont été analysés par méthode alternative)

	Résultats bruts en entérocoques / 100 ml								Résultats LOG									
	NF EN ISO 7899-1				XplOrer64 [1]				NF EN ISO 7899-1				XplOrer64				Moyennes	
	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	A1	A2	B1	B2	NF	XplOrer
1	61	30	61	30	54	67	23	21	1,79	1,48	1,79	1,48	1,73	1,83	1,36	1,32	**1,63**	**1,56**
2	109	61	61	46	91	115	99	89	2,04	1,79	1,79	1,66	1,96	2,06	2,00	1,95	**1,82**	**1,99**
3	46	30	46	61	83	33	72	149	1,66	1,48	1,66	1,79	1,92	1,52	1,86	2,17	**1,65**	**1,87**
4	93	30	94	<15	34	49	49	48	1,97	1,48	1,97	-	1,53	1,69	1,69	1,68	**1,81**	**1,65**
5	94	46	94	61	91	114	277	162	1,97	1,66	1,97	1,79	1,96	2,06	2,44	2,21	**1,85**	**2,17**
6	15	30	46	30	19	21	37	43	1,18	1,48	1,66	1,48	1,28	1,32	1,57	1,63	**1,45**	**1,45**
7	61	77	15	110	338	162	281	179	1,79	1,89	1,18	2,04	2,53	2,21	2,45	2,25	**1,72**	**2,36**
8	61	44	77	45	66	59	37	82	1,79	1,64	1,89	1,65	1,82	1,77	1,57	1,91	**1,74**	**1,77**
9	61	61	110	46	25	83	114	34	1,79	1,79	2,04	1,66	1,40	1,92	2,06	1,53	**1,82**	**1,73**
10	61	94	61	110	<40	<40	<40	<40	1,79	1,97	1,79	2,04	-	-	-	-	**1,90**	**-**
11	<15	15	30	15	76	59	65	119	-	1,18	1,48	1,18	1,88	1,77	1,81	2,08	**1,28**	**1,89**
12	<15	<15	<15	<15	164	273	200	174	-	-	-	-	2,21	2,44	2,30	2,24	**-**	**2,30**
LE	61	110	77	30	237	102	77	102	1,79	2,04	1,89	1,48	2,37	2,01	1,89	2,01	**1,80**	**2,07**

Niveau moyen (flacons C et D)

	Résultats bruts en entérocoques / 100 ml								Résultats LOG									
	NF EN ISO 7899-1				XplOrer64 [1]				NF EN ISO 7899-1				XplOrer64				Moyennes	
	C1	C2	D1	D2	C1	C2	D1	D2	C1	C2	D1	D2	C1	C2	D1	D2	NF	XplOrer
1	956	640	705	1 020	153	612	3 434	324	2,98	2,81	2,85	3,01	2,18	2,79	3,54	2,51	**2,91**	**2,75**
2	1 089	690	828	828	657	475	1 224	1 390	3,04	2,84	2,92	2,92	2,82	2,68	3,09	3,14	**2,93**	**2,93**
3	565	690	861	690	812	847	812	757	2,75	2,84	2,94	2,84	2,91	2,93	2,91	2,88	**2,84**	**2,91**
4	814	848	865	931	395	539	603	639	2,91	2,93	2,94	2,97	2,60	2,73	2,78	2,81	**2,94**	**2,73**
5	872	585	767	514	896	621	1 062	1 062	2,94	2,77	2,88	2,71	2,95	2,79	3,03	3,03	**2,83**	**2,95**
6	565	559	750	697	705	418	1 313	400	2,75	2,75	2,88	2,84	2,85	2,62	3,12	2,60	**2,80**	**2,80**
7	683	585	838	647	2 663	1 351	1 870	2 279	2,83	2,77	2,92	2,81	3,43	3,13	3,27	3,36	**2,83**	**3,30**
8	1 007	872	791	828	1 295	735	319	1 224	3,00	2,94	2,90	2,92	3,11	2,87	2,50	3,09	**2,94**	**2,89**
9	896	734	824	791	1 767	1 259	603	835	2,95	2,87	2,92	2,90	3,25	3,10	2,78	2,92	**2,91**	**3,01**
10	742	759	524	683	578	<40	20 978	<40	2,87	2,88	2,72	2,83	2,76	-	4,32	-	**2,83**	**3,54**
11	690	110	61	61	746	812	871	847	2,84	2,04	1,79	1,79	2,87	2,91	2,94	2,93	**2,11**	**2,91**
12	<15	<15	<15	<15	373	1 897	517	2 588	-	-	-	-	2,57	3,28	2,71	3,41	**-**	**2,99**
LE	612	683	509	600	657	725	621	695	2,79	2,83	2,71	2,78	2,82	2,86	2,79	2,84	**2,78**	**2,83**

Niveau haut (flacons E et F)

	Résultats bruts en entérocoques / 100 ml								Résultats LOG									
	NF EN ISO 7899-1				XplOrer64 [1]				NF EN ISO 7899-1				XplOrer64				Moyennes	
	E1	E2	F1	F2	E1	E2	F1	F2	E1	E2	F1	F2	E1	E2	F1	F2	NF	XplOrer
1	7 101	6 581	5 712	7 683	1 033	1 295	2 094	2 625	3,85	3,82	3,76	3,89	3,01	3,11	3,32	3,42	**3,83**	**3,22**
2	6 119	6 119	9 043	6 119	3 533	3 583	2 940	10 346	3,79	3,79	3,96	3,79	3,55	3,55	3,47	4,01	**3,83**	**3,65**
3	4 267	6 581	9 826	4 005	4 822	4 960	4 960	4 246	3,63	3,82	3,99	3,60	3,68	3,70	3,70	3,63	**3,76**	**3,68**
4	4 753	4 573	6 119	6 581	2 035	2 858	1 897	3 434	3,68	3,66	3,79	3,82	3,31	3,46	3,28	3,54	**3,74**	**3,39**
5	8 329	5 712	4 753	6 581	4 822	5 795	4 557	6 964	3,92	3,76	3,68	3,82	3,68	3,76	3,66	3,84	**3,79**	**3,74**
6	5 712	4 502	4 368	5 352	2 940	2 588	pa	8 609	3,76	3,65	3,64	3,73	3,47	3,41	-	3,93	**3,69**	**3,61**
7	4 573	7 101	7 101	5 712	7 909	11 917	9 504	14 120	3,66	3,85	3,85	3,76	3,90	4,08	3,98	4,15	**3,78**	**4,03**
8	5 918	5 712	4 902	8 329	5 102	3 483	7 688	10 346	3,77	3,76	3,69	3,92	3,71	3,54	3,89	4,01	**3,78**	**3,79**
9	5 035	7 101	9 043	6 581	4 557	5 961	4 069	13 156	3,70	3,85	3,96	3,82	3,66	3,78	3,61	4,12	**3,83**	**3,79**
10	6 581	5 352	5 306	5 352	6 046	4 367	274 941	3 738	3,82	3,73	3,72	3,73	3,78	3,64	5,44	3,57	**3,75**	**4,11**
11	75	7 700	30	2 200	5 031	6 308	2 154	5 476	1,88	3,89	1,48	3,34	3,70	3,80	3,33	3,74	**2,65**	**3,64**
12	<15	<15	<15	<15	3 634	16 036	5 175	7 265	-	-	-	-	3,56	4,21	3,71	3,86	**-**	**3,84**
LE	5 712	5 352	4 368	5 035	4493	4687	5323	5476	3,76	3,73	3,64	3,70	3,65	3,67	3,73	3,74	**3,71**	**3,70**

[1] Résultats recalculés à l'aide de la nouvelle équation de calibration fournie par le fabricant

pa : problème analytique (labo n°6 échantillon F1 : membrane déchirée)

Participants écartés de l'exploitation statistique :

n°9 : problème de logiciel ayant entraîné le reposi tionnement de cellules dans l'automate XplOrer64 et recalcul des résultats

n°10 : résultats obtenus par méthode XplOrer64 aber rants

n°11 : résultats obtenus par méthode de référence a normaux (réplicats E et F)

n°12 : résultats obtenus par méthode de référence a normaux + analyse le 08/10

Temps de détection (DT) en h observés par les participants par la méthode Xplorer64

	Niveau bas (A et B)				Niveau moyen (C et D)				Niveau haut (E et F)			
	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2	F1	F2
1	9,63	9,47	10,23	10,29	8,89	7,91	6,69	8,36	7,54	7,38	7,04	6,88
2	9,26	9,09	9,20	9,27	7,86	8,09	7,42	7,33	6,67	6,66	6,80	5,91
3	9,32	9,97	9,42	8,91	7,71	7,68	7,71	7,76	6,45	6,43	6,43	6,54
4	9,95	9,70	9,70	9,71	8,22	8,00	7,92	7,88	7,06	6,82	7,11	6,69
5	9,26	9,10	8,47	8,85	7,64	7,90	7,52	7,52	6,45	6,32	6,49	6,19
6	10,36	10,29	9,90	9,79	7,81	8,18	7,37	8,21	6,80	6,89	pa	6,04
7	8,33	8,85	8,46	8,78	6,87	7,35	7,12	6,98	6,10	5,81	5,97	5,69
8	9,48	9,56	9,89	9,33	7,38	7,78	8,37	7,42	6,41	6,68	6,12	5,91
9	10,16	9,32	9,10	9,95	7,16	7,40	7,92	7,69	6,49	6,30	6,57	5,74
10	-	-	-	-	7,95	-	5,41	-	6,29	6,52	3,59	6,63
11	9,39	9,57	9,50	9,07	7,77	7,71	7,66	7,68	6,42	6,26	7,02	6,36
12	8,84	8,48	8,70	8,80	8,26	7,11	8,03	6,89	6,65	5,60	6,40	6,16
LE	8,58	9,18	9,38	9,18	7,86	7,79	7,90	7,82	6,50	6,47	6,38	6,36