LEGIOLERT/QUANTI-TRAY LEGIOLERT for the enumeration of *Legionella pneumophila* in water for human consumption and industrial waters

March 2023

Summary report

Quantitative method

Attestation n° IDX 33/06-06/19

IDEXX Laboratories, INC.
Idexx Drive , Westbrook
Maine 04092
USA

This report includes 181 pages, including 11 appendices. Reproduction of this report is only permitted in its full form

Preamble

***Studied method**:*
Legiolert for the enumeration of *Legionella pneumophila*

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

***Reference method**♦ :*

- NF T90-431 (2017) : "Detection and enumeration of *Legionella* spp and *Legionella pneumophila*",

and

- NF EN ISO 11731 (2017): "Enumeration of *Legionella*".

Scope:

- Human consumption water including domestic hot water
- Industrial waters

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

♦ Analyses performed according to the COFRAC accreditation

1- INTRODUCTION 5
1.1. ALTERNATIVE METHOD 5
1.2. REFERENCE METHOD 5
1.3. APPLICATION SCOPE 6
2- COMPARATIVE STUDY 7
2.1. RELATIVE ACCURACY 7
2.1.1. NUMBER AND NATURE OF SAMPLES 7
2.1.2. ARTIFICIAL CONTAMINATION OF SAMPLES 8
2.1.3. RESULTS AND STATISTICAL ANALYSIS FOR THE CATEGORY «WATER FOR HUMAN CONSUMPTION» 9
2.1.4. RESULTS AND STATISTICAL ANALYSIS FOR THE CATEGORY «INDUSTRIAL WATERS» 17
2.2. LINEARITY 23
2.2.1. CONTAMINATION LEVEL 23
2.2.2. RESULTS 24
2.3. DETECTION AND QUANTIFICATION LIMITS 27
2.3.1. PROTOCOL 28
2.3.2. RESULTS FOR POTABLE PROTOCOL OF LEGIOLERT 28
2.3.3. RESULTS FOR NON-POTABLE PROTOCOL OF LEGIOLERT 30
2.3.4. CONCLUSION 30
2.4. SELECTIVITY 30
2.4.1. PROTOCOLS 31
2.4.2. RESULTS FOR POTABLE PROTOCOL 31
2.4.3. RESULTS FOR NON-POTABLE PROTOCOL 32
2.4.4. CONCLUSION 32
2.5. PRACTICABILITY 33
2.6. CONCLUSION OF THE COMPARATIVE STUDY 35
3- INTERLABORATORY STUDY 35
3.1. STUDY ORGANISATION 36
3.1.1. PARTICIPATING LABORATORIES 36
3.1.2. MATRICES AND STRAIN 36
3.1.3. SAMPLES PREPARATION AND SPIKING 36
3.1.4. TRANSPORT 36
3.1.5. ANALYSIS 36
3.2. INTERLABORATORY STUDY 1 (ILS 1) 37
3.2.1. CONTROL OF EXPERIMENTAL PARAMETERS 37
3.2.2. TESTS ANALYSIS 39
3.2.3. RESULTS SELECTED FOR STUDY AND CONCLUSION OF ILS1 41

3.3. INTERLABORATORY STUDY 2 (ILS 2) 42
3.3.1. CONTROL OF EXPERIMENTAL PARAMETERS 42
3.3.2. TEST RESULTS 45
3.3.3. RESULTS SELECTED FOR THE STUDY AND CONCLUSION OF ILS2 48
3.4. COMBINATION OF DATA FROM THE TWO ILS TRIALS 49
3.4.1. COMBINED CONTAMINATION RATE 49
3.4.2. CODE MATCHING BETWEEN THE TWO ILS TRIALS 49
3.4.3. RESULTS OF THE 2 COMBINED STUDIES 50
3.4.4. CALCUL AND INTERPRETATION 52
3.5. CONCLUSION OF THE INTERLABORATORY STUDY 55
4- BIBLIOGRAPHICAL STUDIES 55
5- ASSESSMENT OF THE COMPLAINTS 57
6- STATE OF THE INTERVENED MODIFICATIONS AND THE ENVISAGED MODIFICATIONS 57
6.1.TECHNICAL REPOSITORY OF VALIDATION 57
6.2.REFERENCE METHOD 57
6.3.ALTERNATIVE METHOD 57
7- PRESENTATION OF THE POSSIBLE MODIFICATIONS ENVISAGED IN THE ALTERNATIVE METHOD 57
8- CONCLUSION 58
APPENDIX 1: ALTERNATIVE METHOD PROTOCOL 59
APPENDIX 2: REFERENCE METHOD PROTOCOL 60
APPENDIX 3: ARTIFICIAL CONTAMINATION 64
APPENDIX 4: RELATIVE ACCURACY RESULTS 66
APPENDIX 5: LINEARITY RESULTS 92
APPENDIX 6: LOD-LOQ RESULTS 100
APPENDIX 7: SELECTIVITY RESULTS 102
APPENDIX 8 : TEMPERATURE CURVES ILS1 THEN ILS2 106
APPENDIX 9: RESULTS - CULTURABLE MICROORGANISMS 122
APPENDIX 10: INTERLABORATORY STUDY RAW DATA ILS1 & ILS2 124
APPENDIX 11 : EXPLANATIONS OF THE TWO CALCULATION METHODS FOR DETERMINING COUNTS FOR THE REFERENCE METHOD AND FOR THE ALTERNATIVE METHOD 179

1- Introduction

The method Legiolert was validated in 2019 by AFNOR Certification according to the Validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013) under the certification number IDX 33/06-06/19.

In 2023, IDEXX plans to renew the certification with no change in method or extension.

1.1. Alternative method

The Legiolert test detects *Legionella pneumophila* in water for human consumption (including domestic hot water) and industrial waters. The test is based on a bacterial enzyme detection technology that signals the presence of *Legionella pneumophila* through utilization of a substrate present in the Legiolert reagent. *Legionella pneumophila* cells rapidly grow and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the Legiolert reagent. Actively growing strains of *Legionella pneumophila* use the substrate to produce a brown color or turbidity.

The Legiolert method includes two protocols:

- The potable water protocol quantifies *Legionella pneumophila* in 100 mL within 7 days using Most Probable Number (MPN). The detection threshold of this protocol is 10 MPN/L. This protocol was used for the category “Water for human consumption”.
- The non-potable protocol quantifies *Legionella pneumophila* in 1 mL within 7 days using MPN. The detection threshold of this protocol is 1000 MPN/L. This protocol was used for the category “Industrial water”.

The two alternative method protocols are presented in appendix 1.

1.2. Reference method

The alternative method was compared to the two-reference method:

- French standard NF T90-431 (2017): “Detection and enumeration of *Legionella* spp and *Legionella pneumophila*”,
- International standard ISO 11731 (2017): “Enumeration of *Legionella*”.

The protocols for each of the reference methods are presented in appendix 2.

1.3. Application scope

The application scope of the alternative method extends to two categories of waters: water for human consumption (including domestic hot water) and industrial waters.

2- Comparative study

This study was made by ISHA.

The following characteristics were studied during the comparative study:

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

2.1. Relative accuracy

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

2.1.1. Number and nature of samples

Two categories of waters were tested in duplicate with the alternative method and the two reference methods.
Samples analyzed are presented in table 1.

Table 1 : number and nature of samples analyzed

Category	Protocol Legiolert	Water type	Samples analyzed	Samples exploited (NF T90-431)	Samples exploited (ISO 11731)
Water for human consumption	**1. Potable**	Hot water for sanitary use	17	7	8
		Tap water and fountain water	25	9	9
		Spring water	18	6	6
		Total	**60**	**22**	**23**
Industrial water	**2. Non-potable**	Cooling water	23	12	12
		Water spray	13	8	8
		Purification plant water	4	4	4
		Total	**40**	**24**	**24**
		TOTAL	100	46	**47**

For protocol 1 (potable water samples), a total of 60 samples was analyzed. Twenty-two and 23 results respectively obtained with the reference method NF T90-431 and ISO 11731, were exploited for data analysis. For the protocol 2 (non-potable water samples) a total of 40 samples was analyzed and 24 were exploited for data analysis.

Samples were excluded from the statistical analysis when counts were inferior or superior to the detection limit for at least one of the replicates of the reference methods or the alternative method.

All samples were artificially contaminated.

2.1.2. Artificial contamination of samples

All samples (for the two categories) were artificially contaminated by spiking. The contamination levels used cover the measuring range of the alternative method.
The bacterial strains used were stressed using different treatments. The stress intensity was evaluated (logarithmic difference between enumeration on BCYE agar plates containing cysteine and

GVPC agar plates, cf. appendix 3). The contamination levels used cover the entire measurement range of the alternative method. The stress applied and the strains used are presented in appendix 3.

It is important to note that the Legiolert non-potable protocol (used for the category “industrial water”) employs a pretreatment step to increase selectivity and reduce interference from non-target organisms. Natural isolates of *L. pneumophila*, as found in environmental samples, are resistant to the pretreatment step and are detected by Legiolert. In contrast, *L. pneumophila* cultures obtained by culture in the laboratory using typical agar plate methods are sensitive to the pretreatment and are not detected using the Legiolert non-potable protocol. The employment of a “Resistance protocol” enables strains cultured in the laboratory to be detected using the Legiolert non-potable protocol. Consequently, a specific protocol was applied for the spiking of the industrial waters sample. The protocol and buffer composition are presented in the appendix 3.

2.1.3. Results and statistical analysis for the category «water for human consumption»

- **Results for the category «water for human consumption»**

Figures 1 and 2 present the two-dimensional graphs for the category “water for human consumption”, Legiolert versus NF T90-431 and Legiolert versus ISO 11731, respectively. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method.

The line of equivalence “y=x” is included in the different graphs. Raw results are summarized in appendix 4

Figure 1 : Two-dimensional graph for relative accuracy Legiolert vs NF T90-431 for water for human consumption

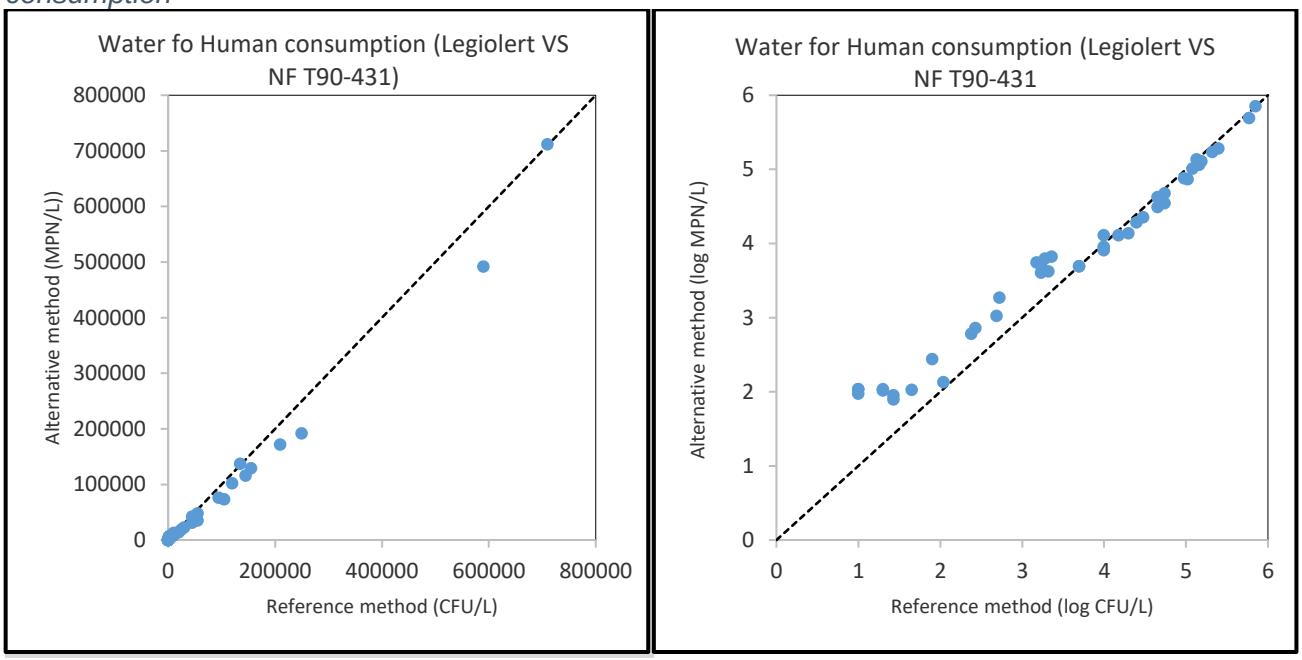
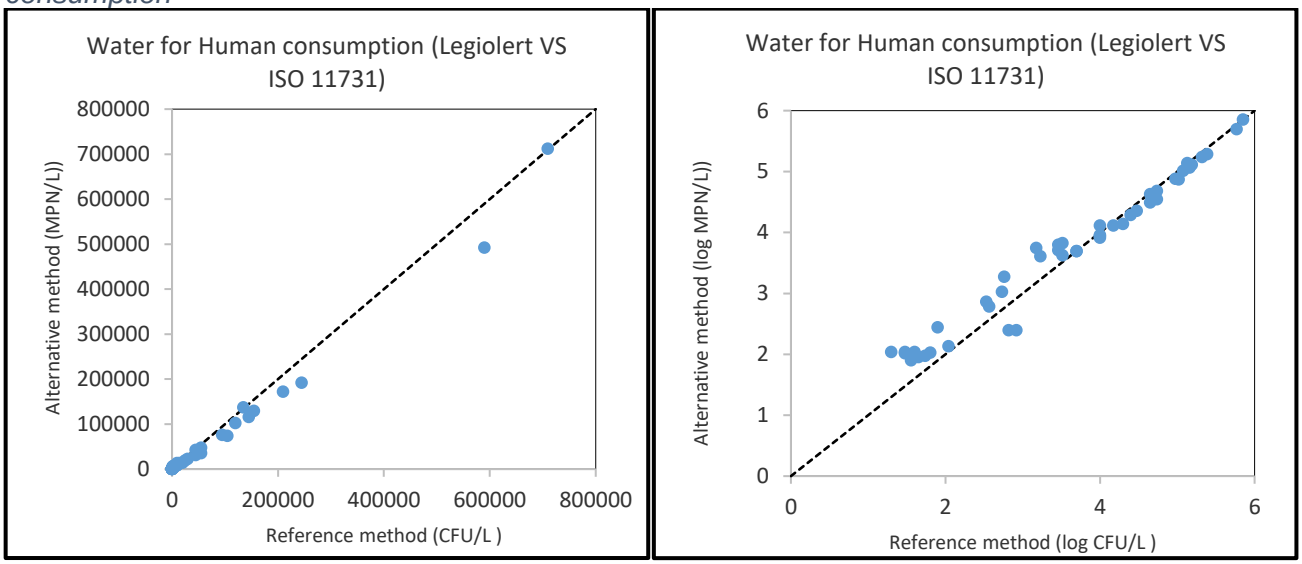


Figure 2: Two-dimensional graph for relative accuracy Legiolert vs ISO 11731 for water for human consumption



- **<u>Statistical analysis for the category «water for human consumption»</u>**

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 where y represents the alternative method and x the reference method.

According to the ISO 16140: 2003 standard: “Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods”, used for the statistical analysis of the data, there is 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 has a systematic bias against the reference method, the probability p {a = 0} is less than α = 0.05.

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

The linear regression method is chosen over the value of the robustness of the ratio R of overall repeatability standard deviation:

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

Table 2: Statistical data for the enumeration in water for human consumption, Legiolert vs NF T90-431 and Legiolert VS ISO 11731

Matrix	Reference method	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
										Ord. at 0	Slope at 1
Human consumption waters	NF T90-431	Raw data	2.97	OLS1	2.07	-3022.97	0.64	0.92	2.66	52.84	1.10
		Log	0.68	GMFR	2.07	0.94	9.37	0.80	9.10	0.00	0.00
	ISO 11731	Raw data	4.30	OLS1	2.07	-3193.48	071	0.92	2.78	52.48	1.07
		Log	0.79	GMFR	2.07	0.55	6.20	0.87	5.87	0.00	0.00

Table 3: Bias and repeatability of Legiolert VS NF T90-431 and Legiolert vs ISO 11731

Matrix	Reference method	Data	Bias (D)		Repeatability			
			Average	Median	r		Rob.r	
					RM	AM	RM	AM
Human consumption waters	NF T90-431	Raw data	-8323.71	-11.25	54997.29	94653.34	880.62	2612.50
		Log	0.21	0.01	0.36	0.23	0.23	0.16
	ISO 11731	Raw data	-8305.72	-80.00	53788.45	92572.79	587.08	2524.44
		Log	0.01	-0.01	0.17	0.22	0.19	0.16

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

Matrix	Reference method	Data	Hypothesis [a=0]	Hypothesis [b=1]	Regression line	Correlation coefficient (r)
Human consumption waters	NF T90-431	Raw data	Accepted	Rejected	Alt.=0.92 Ref. - 3022.97	1.00
		Log	Rejected	Rejected	Log Alt.= 0.80 log Ref. + 0.94	0.99
	ISO 11731	Raw data	Accepted	Rejected	Alt.=0.92 Ref. – 3193.48	1.00
		Log	Rejected	Rejected	Log Alt.= 0.87 log Ref. + 0.55	0.99

For both comparisons performed (Legiolert versus NF T90-431 and Legiolert versus ISO 11731), using an expression of the results in CFU or MPN per liter, the first hypothesis {a = 0} is accepted while the hypothesis {b = 1} is rejected (< 5%). With the log statistical analysis, the two hypotheses {a = 0 and b = 1} are rejected.

However, the correlation coefficients and equations are acceptable.

The relative accuracy of the alternative method is acceptable.

Important note:

A large dispersion of the points is observed with the samples presenting low concentrations of *Legionella pneumophila* (<5000 CFU/L). This dispersion phenomenon for low levels of *Legionella pneumophila* has also been observed in several studies and in particular by Rech *et al.*, 2018 ; Barette,2019 & Boczek *et al.,* 2021.

For these samples, the results of the reference methods are obtained with the plates inoculated after concentration (filtration of 10 mL or 100 mL).

In contrast, a low dispersion is observed for the samples containing concentrations of *Legionella pneumophila* greater than or equal to 5000 CFU/L. These results of the reference methods are obtained with the plates inoculated by direct plating.

To better understand the relationship of relative accuracy between the two reference methods and the alternative method, raw data for the reference methods were split in order to make two comparisons:

- Comparison 1: results obtained using concentration by filtration
- Comparison 2: results obtained by direct plating

These two groups of results were compared independently with the corresponding results of the alternative method. New statistical analysis was performed.

- **Comparison 1: results obtained with concentration by filtration for the category «water for human consumption»**

Ten results were obtained with concentration by filtration for a total of 22 results for the category «water for human consumption».

Figures 3 and 4 present the two-dimensional graphs for the category “water for human consumption”, Legiolert versus NF T90-431 and Legiolert versus ISO 11731, respectively. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method. The line of equivalence “y=x” is included in the different graphs. Raw results are included in appendix 4.

Figure 3: Two-dimensional graph for relative accuracy Legiolert vs NF T90-431 for water for human consumption

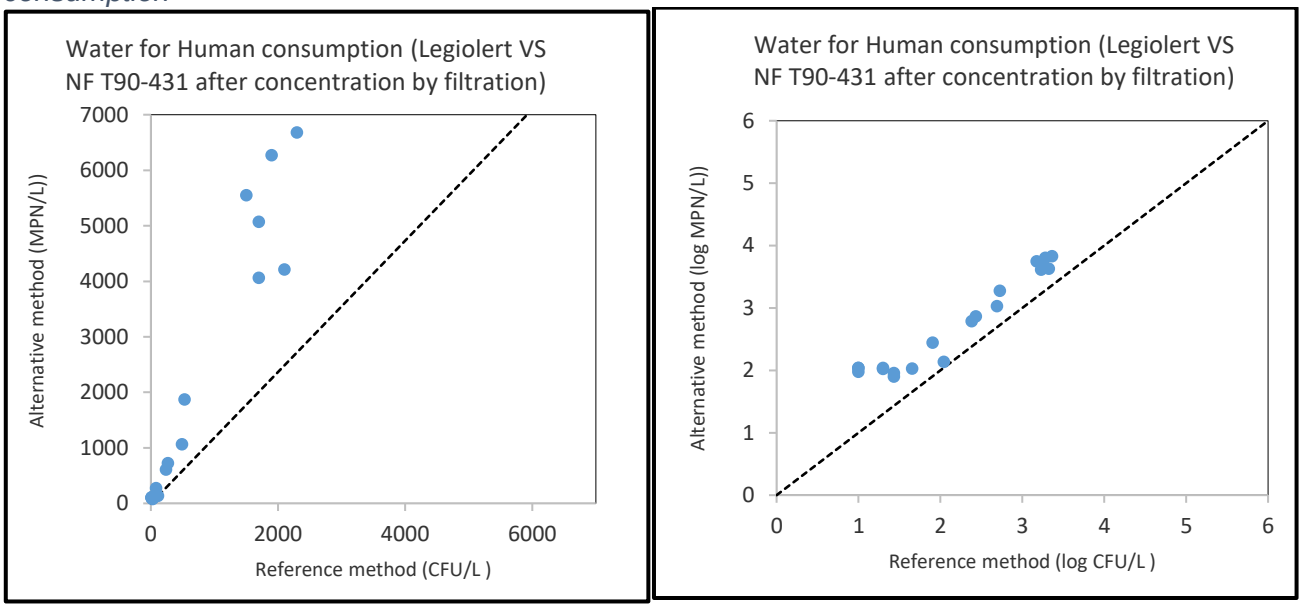
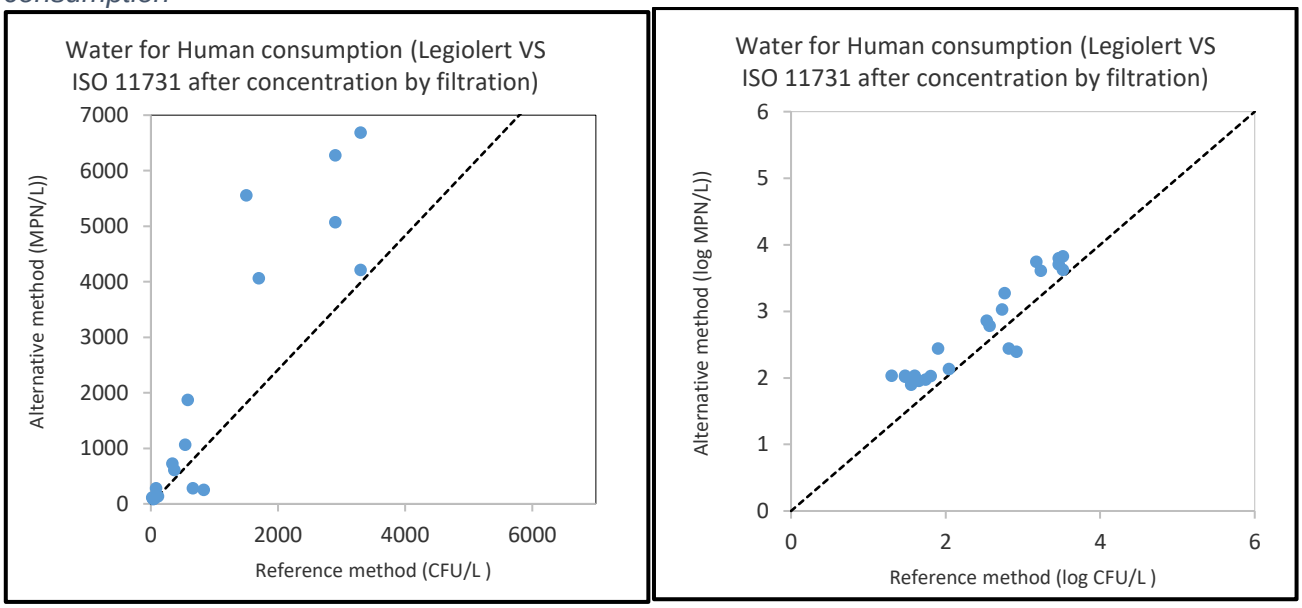


Figure 4: Two-dimensional graph for relative accuracy Legiolert vs ISO 11731 for water for human consumption



In comparison with the two reference methods (using concentration by filtration), Legiolert seems more sensitive to the concentration of *Legionella pneumophila*.

- **<u>Statistical analysis 1 of the results obtained with concentration by filtration for the category «water for human consumption»</u>**

Statistical analysis is presented in the following tables.

Table 4: Statistical data for the enumeration in human consumption waters, Legiolert vs NF T90-431 and Legiolert VS ISO 11731

Matrix	Reference method	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
										Ord. at 0	Slope at 1
Human consumption waters	NF T90-431	Raw data	3.95	OLS1	2.23	3.14	0.01	2.85	4.17	99.47	0.06
		Log	0.76	GMFR	2.23	0.85	5.70	0.86	2.43	0.00	2.60
	ISO 11731	Raw data	3.90	OLS1	2.20	50.24	0.21	1.89	5.42	83.42	0.00
		Log	0.86	GMFR	2.20	0.44	1.73	0.94	0.53	9.75	59.99

Table 5: Bias and repeatability of Legiolert VS NF T90-431 and Legiolert vs ISO 11731

Matrix	Reference method	Data	Bias (D)		Repeatability			
			Average	Median	r		Rob.r	
					RM	AM	RM	AM
Human consumption waters	NF T90-431	Raw data	1210.85	259.75	378.17	1223.29	95.40	377.20
		Log	0.55	0.48	0.50	0.28	0.26	0.20
	ISO 11731	Raw data	836.23	110.00	374.08	1166.49	88.06	343.44
		Log	0.29	0.32	0.19	0.27	0.197	0.17

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

Matrix	Reference method	Data	Hypothesis [a=0]	Hypothesis [b=1]	Regression line	Correlation coefficient (r)
Human consumption waters	NF T90-431	Raw data	Accepted	Rejected	Alt.= 2.85 Ref. + 3.141	0.99
		Log	Rejected	Rejected	Log Alt.= 0.86 log Ref. + 0.85	0.98
	ISO 11731	Raw data	Accepted	Rejected	Alt.= 1.89 Ref. + 50.24	0.94
		Log	Accepted	Accepted	Log Alt.= 0.94 log Ref. – 0.44	0.94

For both comparisons performed (Legiolert versus NF T90-431 and Legiolert versus ISO 11731), using an expression of the results in CFU or MPN per liter, the first hypothesis {a = 0} is accepted while the hypothesis {b = 1} is rejected (< 5%).

With the NF T90-431, the hypothesis [b=1] is accepted while the hypothesis [a=0] is rejected for an expression of the results in log CFU/L or MPN /L.

With the ISO 11731, the two hypotheses [b=1 and a=0] are accepted for an expression of the results in Log CFU or MPN per liter.

The median bias of the results in log MPN/L is low (0.48 for the NF T90-431 and 0.32 for the ISO 11731).

The correlation coefficients and equations calculated with the results in log CFU or MPN /L are acceptable.

The relative accuracy of the alternative method for the results obtained with the plates inoculated after concentration is acceptable.

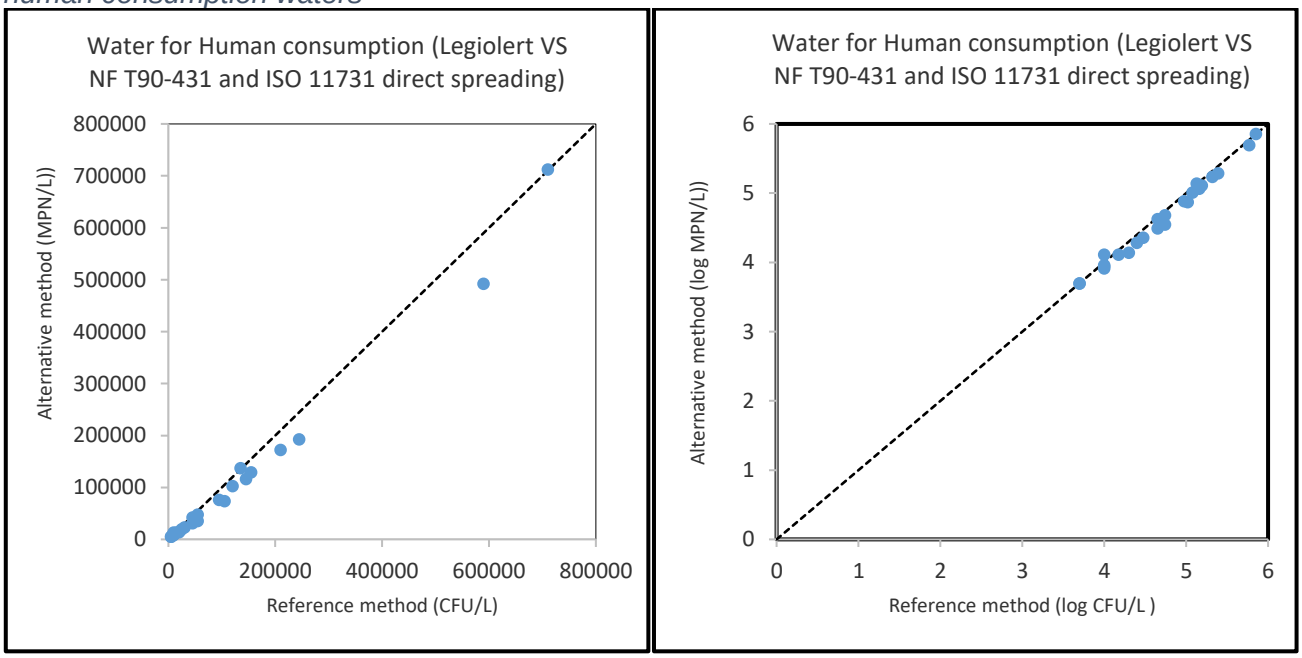
- **Comparison 2: results obtained after direct plating for the category «water for human consumption»**

Twelve results were obtained by direct plating for a total of 22 results for the category «water for human consumption».

The same direct plating was performed for the two reference methods. Results are the same for the two methods therefore only one figure is presented.

Figure 5 presents the two-dimensional graphs for the water for human consumption category, Legiolert versus NF T90-431 and ISO 11731. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method. The line of equivalence “y=x” is included in the different graphs. Raw results are included in appendix 4.

Figure 5: Two-dimensional graph for relative accuracy Legiolert vs NF T90-431 and ISO 11731 for human consumption waters



- **Statistical analysis of the results obtained by direct spreading for the category «water for human consumption»**

Statistical analysis is presented in the following tables.

Table 6: Statistical data for the enumeration in human consumption waters, Legiolert vs NF T90-431 and Legiolert vs ISO 11731

Matrix	Reference method	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
										Ord. at 0	Slope at 1
Human consumption waters	NF T90-431 and NF ISO 1731	Raw data	0.41	OLS2	2.18	9350.52	1.34	1.07	2.04	19.27	5.37
		Log	0.79	GMFR	2.18	0.01	0.27	0.98	0.63	79.00	53.42

Table 7: Bias and repeatability of Legiolert vs NF T90-431 and Legiolert vs ISO 11731

Matrix	Reference method	Data	Bias (D)		Repeatability			
			Average	Median	r		Rob.r	
					RM	AM	RM	AM
Human consumption waters	NF T90-431 and ISO 11731	Raw data	-16269.17	-7275.00	73804.92	128156.36	0.00	12005.78
		Log	-0.08	-0.08	0.13	0.19	0.17	0.15

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

Matrix	Reference method	Data	Hypothesis [a=0]	Hypothesis [b=1]	Regression line	Correlation coefficient (r)
Human consumption waters	NF T90-431 and ISO 11731	Raw data	Accepted	Accepted	Ref.=1.07 Alt. + 9350.52	1.00
		Log	Accepted	Accepted	Log Alt.= 0.98 log Ref.+0.01	1.00

The hypothesis {a = 0 and b = 1} is accepted for the raw data and the log statistical analysis.
The correlation coefficients and equations calculated with the results in log CFU or MPN /L are acceptable.

The relative accuracy of the alternative method for the results obtained with the direct plating is acceptable.

- **Conclusion for the category «water for human consumption»**

The relative accuracy of the alternative method in Water for Human consumption is acceptable.

2.1.4. Results and statistical analysis for the category «industrial waters»

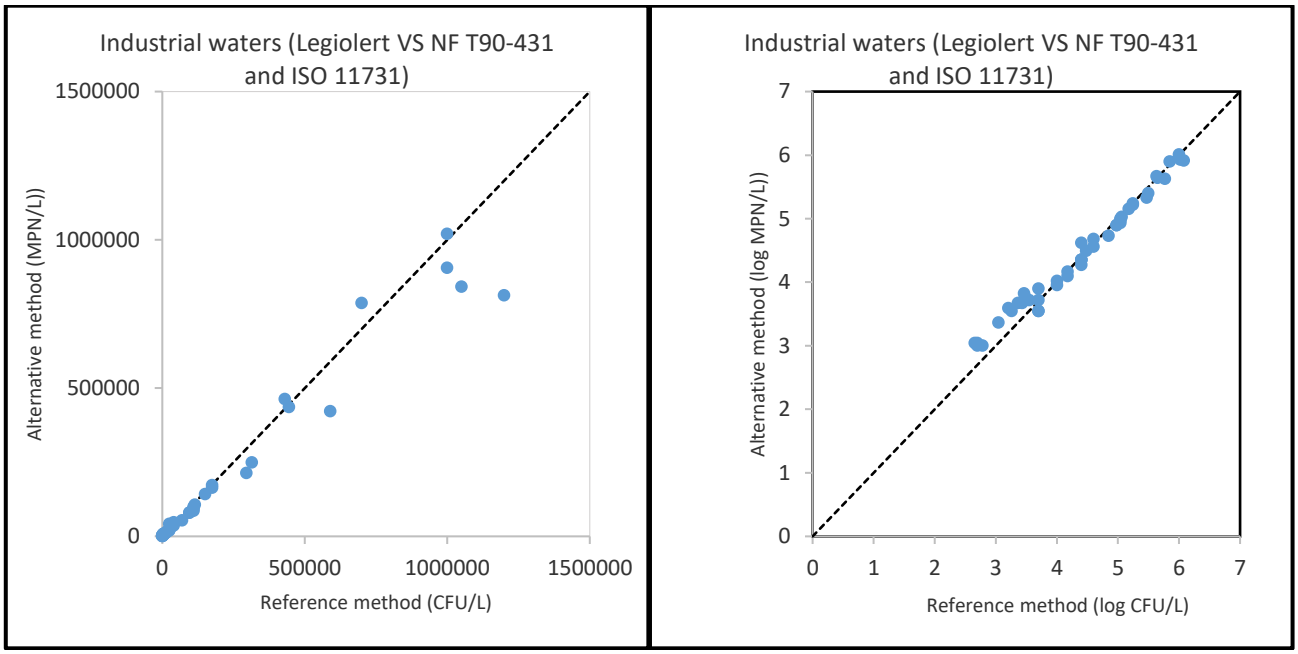
- **Results for the category «industrial waters»**

For this category, results obtained with the two reference methods used are identical.

Figure 6 presents the two-dimensional graphs for the industrial waters category, Legiolert versus NF T90-431 and ISO 11731. For each graph, the y-axis is reserved for the alternative method and the x-

axis for the reference method. The line of equivalence “y=x” is included in the different graphs. Raw results are included in appendix 4.

Figure 6: Two-dimensional graph for relative accuracy Legiolert vs NF T90-431 and ISO 11731 for industrial waters



- **Statistical analysis for the category «industrial waters»**

Statistical analysis is presented in the following tables.

Table 8: Statistical data for the enumeration in industrial waters, Legiolert vs NF T90-431 and ISO 11731

Matrix	Reference method	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
										Ord. at 0	Slope at 1
Industrial waters	NF T90-431 and ISO 11731	Raw data	0.86	GMFR	2.06	2458.82	0.56	0.87	8.96	58.0	0.0
		Log	0.98	GMFR	2.06	0.56	9.13	0.88	5.26	0.0	0.0

Table 9: Bias and repeatability of Legiolert vs NF T90-431 and ISO 11731

Matrix	Reference method	Data	Bias (D)		Repeatability			
			Average	Median	r		Rob.r	
					RM	AM	RM	AM
Industrial waters	NF T90-431 and ISO 11731	Raw data	-20571.00	-1525.00	97521.84	174365.25	22015.49	18933.32
		Log	0.05	-0.02	0.24	0.26	0.20	0.20

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

Matrix	Reference method	Data	Hypothesis [a=0]	Hypothesis [b=1]	Regression line	Correlation coefficient (r)
Industrial waters	NF T90-431 and ISO 11731	Raw data	Accepted	Rejected	Alt.=0.87 Ref. + 2458.82	1.00
		Log	Rejected	Rejected	Log Alt.= 0.88 log Ref. + 0.56	0.99

The hypothesis [a=0] is accepted while the hypothesis [b=1] is rejected for an expression of the results in CFU/L or MPN /L.
The two hypotheses [b=1 and a=0] are rejected for an expression of the results in Log CFU or MPN per liter.

The median bias of the results in log MPN/L is low (-0.02 for both methods).
The correlation coefficients and equations calculated with the results in log CFU or MPN /L are acceptable.

The relative accuracy of the alternative method is acceptable.

Important remarks:

A large dispersion of the points is observed with the samples containing low concentrations of *Legionella pneumophila* (<5000 CFU/L).
For these samples, the results of the reference methods are obtained with the plates inoculated after concentration (filtration of 10 mL or 100 mL).
In contrast, a low dispersion is observed for the samples containing concentrations of *Legionella pneumophila* greater than or equal to 5000 CFU/L. These results of the reference methods are obtained with the plates inoculated with direct plating.

Unlike the two reference methods, it is interesting to note that the Legiolert protocol used is the same for all samples analyzed (low and high concentration of *Legionella pneumophila*). No concentration of the sample is performed.

To better understand the relationship of relative accuracy between the two reference methods and the alternative method, raw data of the reference methods were split in order to make two comparisons:

- ❖ Comparison 3: results obtained by concentration by filtration
- ❖ Comparison 4: results obtained by direct plating

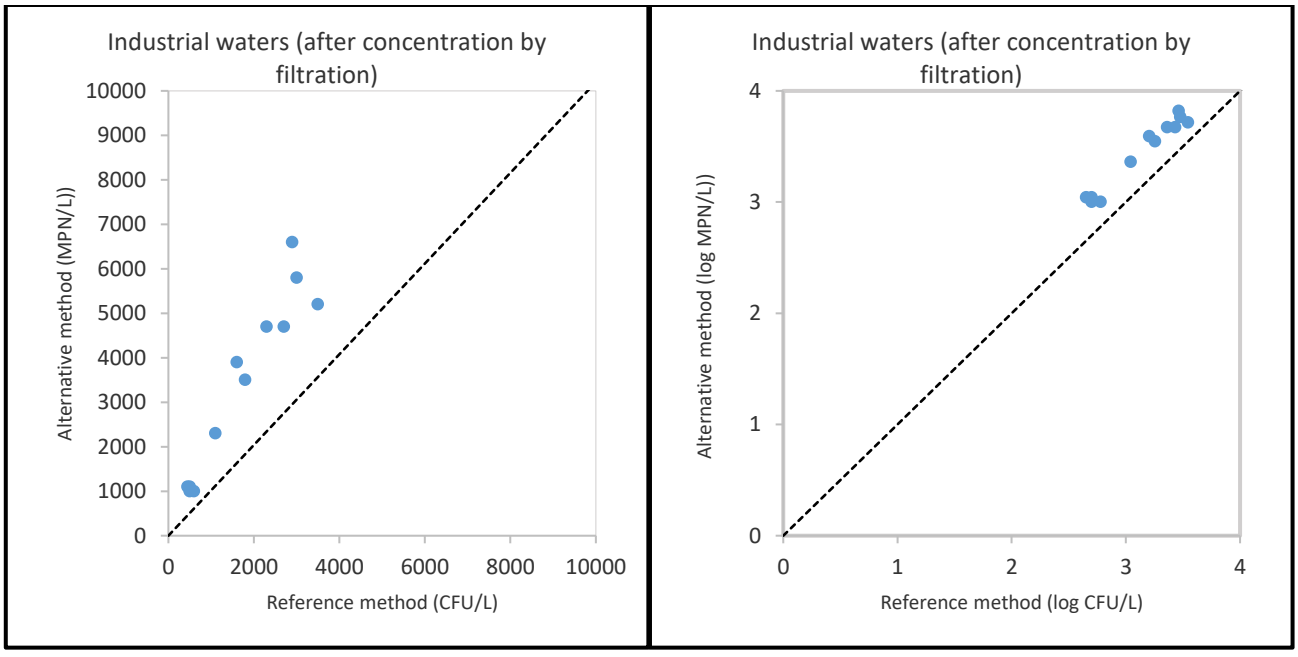
These two groups of results were compared independently with the corresponding results of the alternative method. New statistical analysis was performed.

- **<u>Comparison 3: results obtained after concentration by filtration for the category «industrial waters»</u>**

Six results were obtained with concentration by filtration for a total of 24 results for the category «industrial water».

Figure 7 present the two-dimensional graphs for the industrial water's category, Legiolert versus NF T90-431 and ISO 11731, respectively. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method. The line of equivalence "y=x" is included in the different graphs. Raw results are included in appendix 4.

Figure 7: Two-dimensional graph for relative accuracy Legiolert vs NF T90-431 and ISO 11731 for industrial waters



In comparison with the two reference methods (using the concentration by filtration), Legiolert seems to detect more the *Legionella pneumophila*.

- **Statistical analysis 3 of the results obtained after concentration by filtration for the category «industrial waters»**

Statistical analysis is presented in the following tables.

Table 10: Statistical data for the enumeration in industrial waters, Legiolert vs NF T90-431 and ISO 11731

Matrix	Reference method	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
										Ord. at 0	Slope at 1
Industrial waters	NF T90-431 and NF ISO 11731	Raw data	0.78	GMFR	2.45	207.06	0.880	1.83	3.62	39.93	0.47
		Log	0.60	GMFR	2.45	0.45	10.576	0.95	0.69	0.00	50.76

Table 11: Bias and repeatability of Legiolert vs NF T90-431 and ISO 11731

Matrix	Reference method	Data	Bias (D)		Repeatability			
			Average	Median	r		Rob.r	
					RM	AM	RM	AM
Industrial waters	NF T90-431 and ISO 11731	Raw data	1662.50	1825.00	1303.96	2439.65	1320.93	1027.39
		Log	0.30	0.29	0.29	0.24	0.22	0.13

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

Matrix	Reference method	Data	Hypothesis [a=0]	Hypothesis [b=1]	Regression line	Correlation coefficient (r)
Industrial waters	NF T90-431 and ISO 11731	Raw data	Accepted	Rejected	Alt.= 1.83 Ref. + 207.06	0.97
		Log	Rejected	Accepted	Log Alt.= 0.95 log Ref. + 0.45	0.99

For an expression of the results in CFU or MPN per Liter, the hypothesis [a=0] is accepted while the hypothesis [b=1] is rejected.

For an expression of the results in log CFU/L or MPN/L., the hypothesis [a=0] is rejected while the hypothesis [b=1] is accepted.

The median bias of the results in log MPN/L is acceptable. (D median =0.29).

The correlation coefficients and equations calculated with the results in log CFU or MPN/L are acceptable.

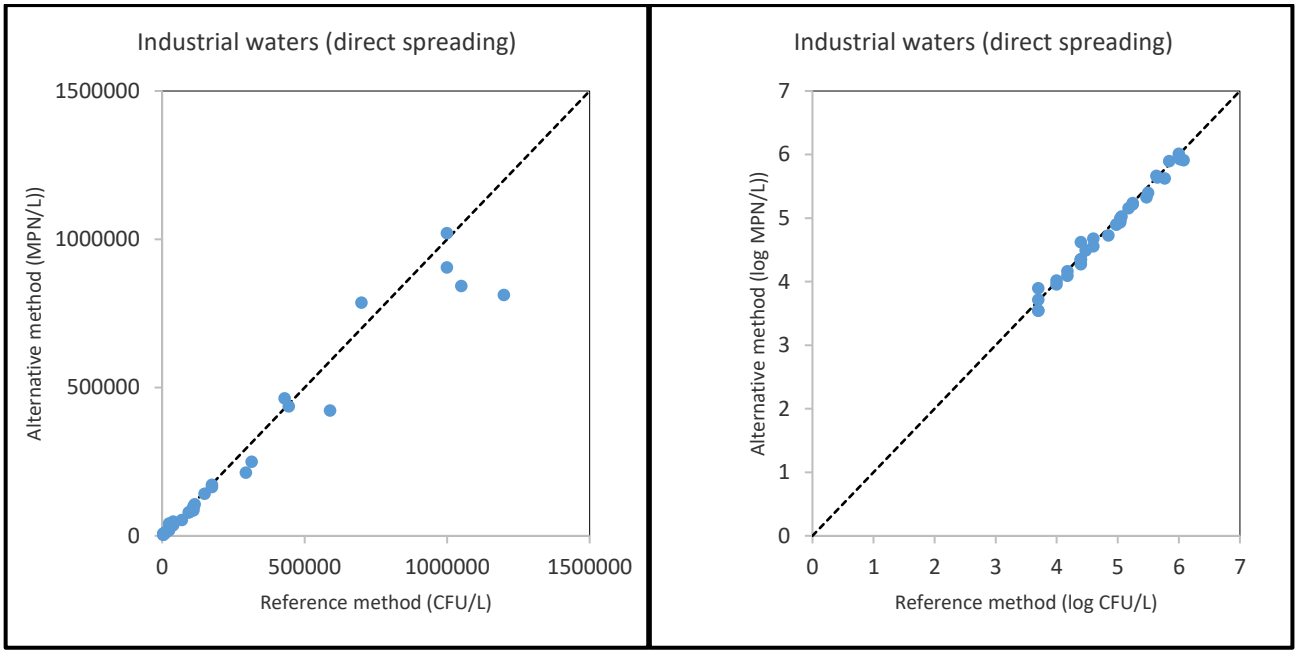
The relative accuracy of the alternative method for the results obtained with the plates inoculated after concentration is acceptable.

- **Comparison 4: results obtained with direct spreading for the category «industrial waters»**

Eighteen results were obtained by direct plating for a total of 24 results for the category «industrial water».

Figure 7 present the two-dimensional graphs for the industrial water's category, Legiolert versus NF T90-431 and ISO 11731, respectively. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method. The line of equivalence "y=x" figures is included in the different graphs. Raw results are included in appendix 4.

Figure 8: Two-dimensional graph for relative accuracy Legiolert vs NF T90-431 and ISO 11731 for industrial waters



- **Statistical analysis 4 of the results obtained after direct spreading for the category «industrial waters»**

Statistical analysis is presented in the following tables.

Table 12: Statistical data for the enumeration in industrial waters, Legiolert vs NF T90-431 and ISO 11731

Matrix	Reference method	Data	Rob.R	Regression used	T	a	t(a)	b	t(b)	Probabilities (%)	
										Ord. at 0	Slope at 1
Industrial waters	NF T90-431 and NF ISO 11731	Raw data	0.72	GMFR	2.10	2743.87	0.46	0.87	7.22	64.84	0.00
		Log	1.47	GMFR	2.10	0.04	0.91	0.98	0.65	37.06	51.75

Table 13: Bias and repeatability of Legiolert vs NF T90-431 and ISO 11731

Matrix	Reference method	Data	Bias (D)		Repeatability			
			Average	Median	r		Rob.r	
					RM	AM	RM	AM
Industrial waters	NF T90-431 and ISO 11731	Raw data	-27983.33	-5750.00	112606.00	201334.72	36692.48	26271.81
		Log	-0.04	-0.06	0.21	0.27	0.14	0.20

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

Matrix	Reference method	Data	Hypothesis [a=0]	Hypothesis [b=1]	Regression line	Correlation coefficient (r)
Industrial waters	NF T90-431 and ISO 11731	Raw data	Accepted	Rejected	Alt.=0.87 Ref. + 2743.87	1.00
		Log	Accepted	Accepted	Log Alt.= 0.98 log Ref. + 0.04	1.00

For an expression of the results in CFU or MPN per Liter, the hypothesis [a=0] is accepted while the hypothesis [b=1] is rejected.

For an expression of the results in log CFU or MPN per Liter, the two hypotheses [b=1 and a=0] are accepted.

For both comparisons performed (Legiolert versus NF T90-431 and Legiolert versus ISO 11731), using an expression of the results in CFU or MPN per liter, the first hypothesis {a = 0} is accepted while the hypothesis {b = 1} is rejected (< 5%).

The median bias of the results in log MPN/L is low (D median =-0.06).

The correlation coefficients and equations calculated with the results in log CFU or MPN /L are acceptable.

The relative accuracy of the alternative method for the results obtained with plates inoculated direct plating is acceptable.

- **Conclusion for the category « Industrial waters »**

The relative accuracy of the alternative method in industrial waters is acceptable.

2.2. Linearity

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

2.2.1. Contamination level

The combinations of matrix / strain are presented in table 14. For the first combination, eight contamination levels were tested while for the second combination seven contamination levels were

tested. All samples were analyzed in duplicate by the two reference methods and the alternative method.

Table 14: Couples matrix / strain analyzed

Strain	Code	Matrix	Target contamination level (CFU/L)
Legionella pneumophila	LEG 1.21	Tap water	100-500-1000-5000-10000-50000-100000-500000
Legionella pneumophila	LEG 1.18	Cooling tower water	1000-5000-10000-50000-100000-500000-1000000

2.2.2. Results

- **Results for the category «water for human consumption»**

Figure 9 and 10 present the two-dimensional graphs for the water for Human consumption category, Legiolert versus NF T90-431 and Legiolert versus ISO 11731, respectively. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method. The line of equivalence “y=x” is included in the different graphs. Raw results are included in appendix 5.

Figure 9: Two-dimensional graph for linearity, Legiolert vs NF T90-431 for water for human consumption

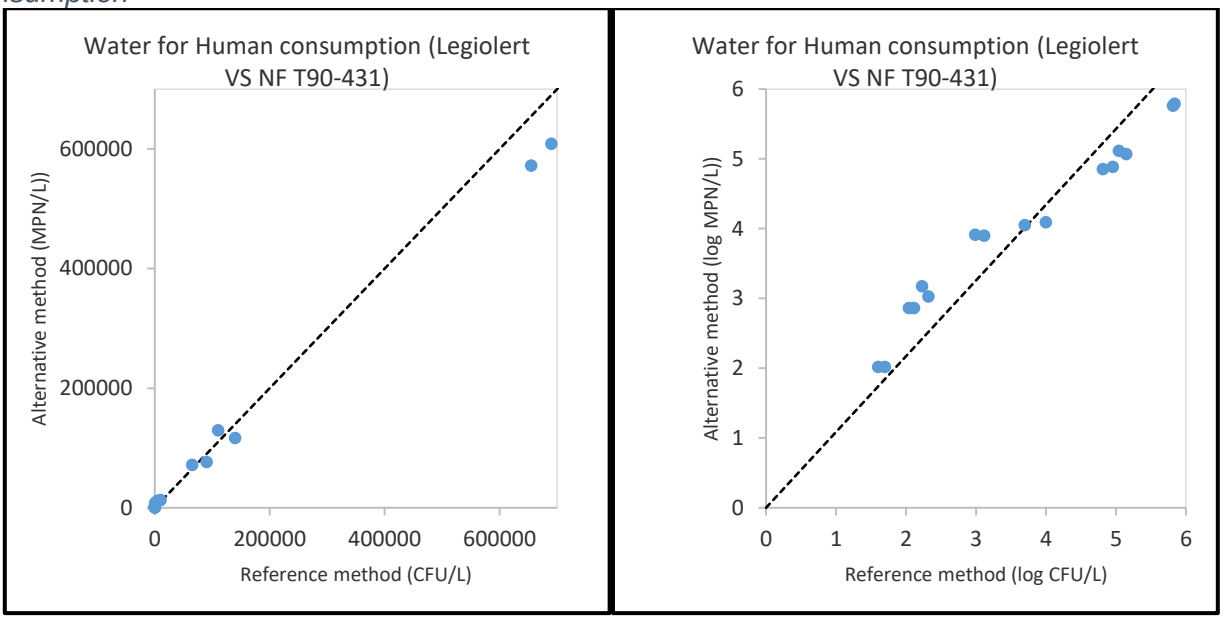
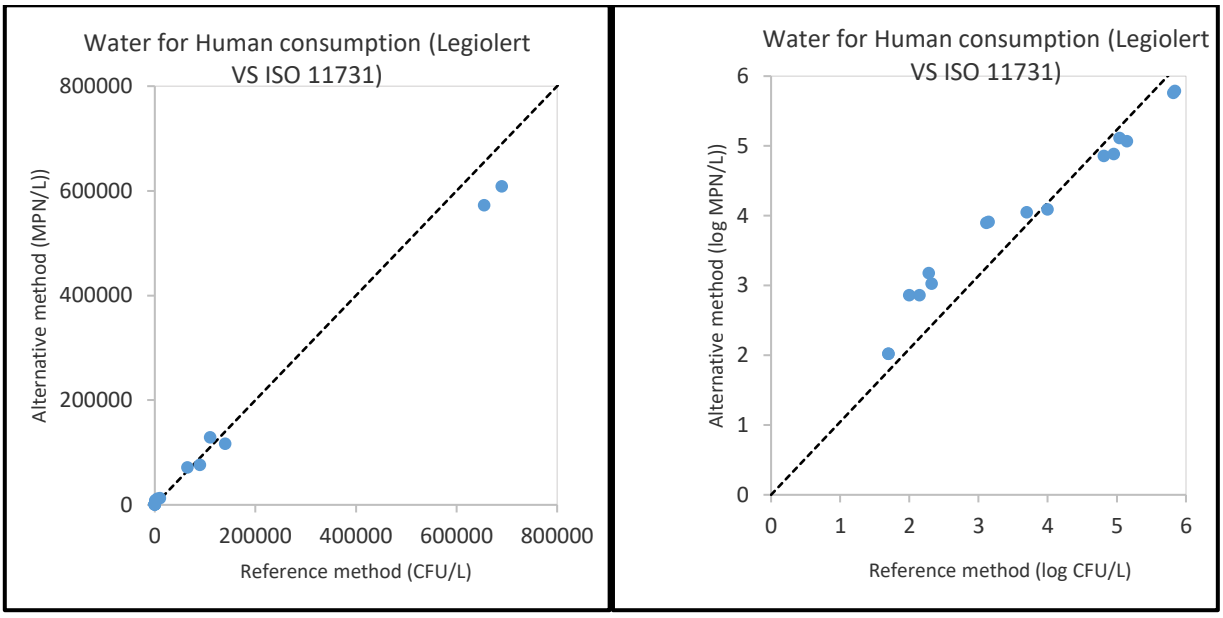


Figure 10: Two-dimensional graph for linearity, Legiolert vs ISO 11731 for water for human consumption



- **Statistical analysis for the category «water for human consumption»**

Statistical interpretations are made according to requirements of standard NF ISO 16140 (see table 6).

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

- ❖ if Rob.R> 2, 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, an orthogonal regression (GMFR) is used with the x-axis to the reference method.

Table 15: Statistical data (r: correlation coefficient, data grayed: non-linearity according to the test)

Couple strain / matrix	Reference method	Data	Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	r	Regression
Legionella pneumophila / Tap water	***T90-431***	*Raw data*	0.29	OLS2	3.58	2090.19	0.00	1.00	Ref.= 1,15 Alt. - 25481.34
		Log	0.27	OLS2	3.58	2408.40	0.00	0.98	Log Ref.= 1,22 log Alt. - 0.418
	ISO 11731	*Raw data*	0.31	OLS2	3.58	2095.42	0.00	1.00	Ref.= 1,15 Alt. - 25505.99
		Log	0.37	OLS2	3.58	2273.08	0.00	0.98	Log Ref.= 1,21 log Alt. - 0.403

The relationship between the two methods isn't linear:

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

- **Conclusion**

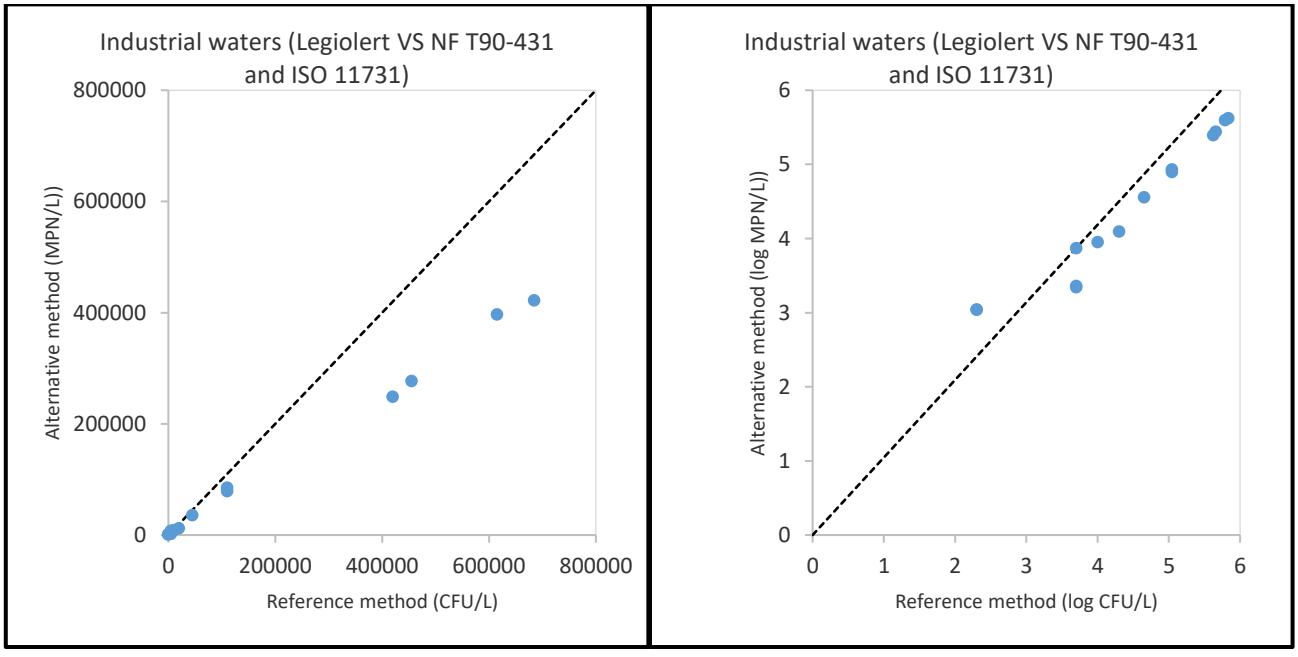
For this combination (*Legionella pneumophila* / Tap water), the relationship between the two reference methods and the alternative method is not linear with the raw data and the log data.
However, the correlation coefficients and the regression lines are acceptable for an expression of the results in log CFU or MPN per Liter.

The linearity of the alternative method is acceptable.

- **Results for the category «industrial waters»**

Figure 11 and 12 present the two-dimensional graphs for the industrial waters category, Legiolert versus NF T90-431 and Legiolert versus ISO 11731, respectively. For each graph, the y-axis is reserved for the alternative method and the x-axis for the reference method. The line of equivalence "y=x" is included in the different graphs. Raw results are included in appendix 5.

Figure 11: Two-dimensional graph for linearity, Legiolert vs NF T90-431 and ISO 11731 for industrial waters



- **Statistical analysis for the category «industrial waters»**

Statistical interpretations are made according to requirements of standard NF ISO 16140 (see table 6).

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

- ❖ if Rob.R> 2, 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, an orthogonal regression (GMFR) is used with the x-axis to the reference method.

Table 16: Statistical data (r: correlation coefficient, data grayed: non-linearity according to the test)

Couple strain / matrix	Reference method	Data	Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	r	Regression
***Legionella pneumophila* / Cooling water**	*T90-431 and ISO 11731*	*Raw data*	1.32	GMFR	3.97	4.10	0.05	1.00	Alt.= 0.617 Ref. - 3339.67
		Log	1.01	GMFR	3.97	258.67	0.00	0.97	Log Alt.= 0,808 log Ref. - 0.810

The relationship between the two methods is not linear:

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

For this combination (*Legionella pneumophila* / Cooling tower water), the relationship between the two reference methods and the alternative method is not linear with the raw data and the log data.

However, the correlation coefficients and the regression lines are acceptable for an expression of the results in log CFU or MPN per Liter.

The linearity of the alternative method is acceptable.

2.3. Detection and quantification limits

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

The ISO 16140 definitions are:

- ❖ 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.

2.3.1. Protocol

Detection and quantification limits were determined by analyzing a pure culture of a strain of *Legionella pneumophila* by the alternative method. The strains LEG 1.21 isolated from a "potable water sample", and the strain LEG 1.18 isolated from a "cooling tower water", were used to determine the detection and quantification limits of the potable and non-potable protocols of Legiolert, respectively.

Four levels of contamination, with six repetitions for each level, have been studied in sterilized water.

2.3.2. Results for potable protocol of Legiolert

Raw results are presented in appendix 6 and the summary in the following tables.

Table 17: Data assay 1 (s0 and x0) for the enumeration of Legionella pneumophila (underlined: the reference level)

Target level (CFU/L)	Real level (CFU/L)	Number of positive samples	Standard deviation (s0)	Bias (Xo)
0	0	0	0.0	0
10	6	2	21.4	0
30	20	5	35.3	45.5
50	36	6	20.2	55.0

Table 18: LC, LOD and LOQ values for the assay 1 of the alternative method for the enumeration of Legionella pneumophila (MPN/L)

Parameter	Formulas	Value obtained (MPN/L)
Critical level (LC)	$1.65\ s_0 + x_0$	103.78
Detection limit (LOD)	$3.3\ s_0 + x_0$	162.06
Quantification limit (LOQ)	$10\ s_0 + x_0$	398.70

In this first assay, the calculated LOD (162 MPN/L) is abnormally high compared to the theoretical LOD (10 MPN/L). This high difference could be due to a problem of stability and homogeneity of the *Legionella pneumophila* stock solution used to perform the contamination of the samples during the LOD/LOQ assay.

That is why the same experiment was performed two times with two different sterilized water contaminated with two different stock solutions of the strain LEG 1.21. The stability and homogeneity of the two *Legionella pneumophila* stock solutions were checked. The results are presented in the following tables.

Table 18: Data assay 2 (s0 and x0) for the enumeration of Legionella pneumophila (underlined: the reference level)

Target level (CFU/L)	Real level (CFU/L)	Number of positive samples	Standard deviation (s0)	Bias (Xo)
0	0	0	0.0	0.0
10	8	2	9.6	0.0
20	16	4	10.4	15.0
40	34	6	17.6	61.5

Table 19: LC, LOD and LOQ values for the assay 2 of the alternative method for the enumeration of Legionella pneumophila (MPN/L)

Parameter	Formulas	Value obtained (MPN/L)
Critical level (LC)	$1.65\ s_0 + x_0$	32.16
Detection limit (LOD)	$3.3\ s_0 + x_0$	49.32
Quantification limit (LOQ)	$10\ s_0 + x_0$	119.00

Table 20: Data assay 3 (s0 and x0) for the enumeration of Legionella pneumophila (underlined: the reference level)

Target level (CFU/L)	Real level (CFU/L)	Number of positive samples	Standard deviation (s0)	Bias (Xo)
0	0	0	0.0	0.0
10	8	2	8.5	0.0
20	16	4	9.7	10.5
40	32	6	9.8	49.5

Table 21: LC, LOD and LOQ values for the assay 3 of the alternative method for the enumeration of Legionella pneumophila (MPN/L)

Parameter	Formulas	Value obtained (MPN/L)
Critical level (LC)	1.65 $s_0 + x_0$	26.45
Detection limit (LOD)	3.3 $s_0 + x_0$	42.40
Quantification limit (LOQ)	10 $s_0 + x_0$	107.18

This new results of LOD (assay 2: 49.32 MPN/L and assay 3: 42.40 MPN/L) are more concordant with the theoretical LOD (10 MLPN/L) indicated by the manufacturer.

2.3.3. Results for non-potable protocol of Legiolert

Raw results are presented in appendix 6 and the summary in the following tables.

Table 22: Data (s0 and x0) for the enumeration of Legionella pneumophila (underlined: the reference level)

Level (MPN/L)	Number of positive samples	Standard deviation (s0)	Bias (Xo)
0	0	0.00	0
780	1	408.25	0
1150	4	1006.81	1100
3110	6	2750.00	2750

Table 23: LC, LOD and LOQ values of the alternative method for the enumeration of Legionella pneumophila (MPN/L)

Parameter	Formulas	Value obtained
Critical level	1.65 $s_0 + x_0$	2761.24
Detection limit	3.3 $s_0 + x_0$	4422.47
Quantification limit	10 $s_0 + x_0$	11168.10

2.3.4. Conclusion

The limits of detection and the limits of quantification of the Legiolert potable protocol and non-potable protocol are acceptable.

2.4. Selectivity

The selectivity of the alternative method is evaluated by its inclusivity and its exclusivity.

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.

Exclusivity is the lack of interference by a relevant range of non-target strains with the alternative method.

Twenty target strains and twenty non-target strains (from national, international and internal collections) were analyzed according to the “potable water” and “non-potable water” protocols.

As requested by the AFNOR technical board because of non-characteristic color observed (orange) with *a Proteus mirabilis* strain, 9 others non-target strains (including three strains of the genus *Proteus*) were tested to complete the exclusivity study for the “potable protocol”. Finally, a total of 29 non-target strains were tested for the “potable protocol”.

All target and non-target strains tested for the two protocols are presented in the appendix 7.

2.4.1. Protocols

For the potable protocol, the contamination levels used for inclusivity were between 100 and 1000 CFU/L (10 to 100 times higher than the theoretical threshold of detection of the Legiolert potable protocol) and for exclusivity about 10^5 CFU/L (between 10^3 to 10^5 times higher than the theoretical threshold of detection of the Legiolert potable protocol).

For the non-potable protocol, the contamination levels used for inclusivity were between 10^4 and 10^5 CFU/L (10 to 100 times higher than the theoretical threshold of detection of the Legiolert potable protocol) and for exclusivity about 10^7 CFU/L (between 10^3 to 10^5 times higher than the theoretical threshold of detection of the Legiolert potable protocol).

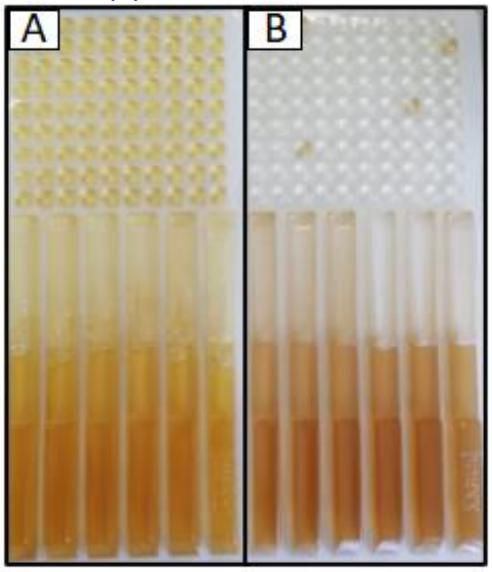
2.4.2. Results for potable protocol

The results are presented in appendix 7.

The twenty strains of *Legionella pneumophila* are detected by the alternative method.

With the potable protocol, four non-target strain in twenty-nine analyzed showed a cross-reaction with the alternative method. This cross reaction was observed with the four strains of the genus *Proteus* tested (*Proteus mirabilis* PRO 1.2 and PRO 1.4 ; *Proteus vulgaris* PRO.2.1 ; *Proteus penneri* PRO.3.1).
After seven days of incubation, the solution in the Quanti-Tray was orange with a precipitate. Figure 12 presents the picture of a positive Quanti-Tray with a strain of *Legionella pneumophila* and a Quanti-Tray with the strain of *Proteus mirabilis*. The orange color observed was very different to a *Legionella pneumophila* positive Quanti-Tray with typical brown coloration. Moreover, a precipitate is present only in the Quanti-Tray containing the *Proteus* strain. It is important to notice for all other positives Quanti-Tray obtained, no precipitate was observed.

Figure 12: Quanti-Trays after 7 days at 39 °C incubated with Proteus mirabilis *strain PRO.1.2 (A) and with Legionella pneumophila LEG.1.21 (B).*



2.4.3. Results for non-potable protocol

The results are presented in appendix 7.

The twenty strains of *Legionella pneumophila* are detected by the alternative method.

None of the non-target strains showed cross-reaction with the alternative method. The cross reaction observed with *Proteus mirabilis* using the potable protocol was not observed with the non-potable protocol.

2.4.4. Conclusion

The selectivity of the method is acceptable.

2.5. Practicability

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

❖ Procedure for conditioning the elements of the method

Legiolert reagent is packaged in sealed individual capsules. The Quanti-Tray Legiolert is provided in sterile plastic bags containing 10 trays each.

❖ Reagent volume

Two formats are available for the Legiolert reagent and for the Quanti-Tray Legiolert (20 tests, 100 tests).

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

Legiolert reagent storage temperature is between 2-25°C away from direct light and humidity.

The storage temperature of the Quanti - Tray Legiolert is between 4-30°C away from light and humidity.

Products have a 15 months DLC.

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

Each Quanti-Tray and each Legiolert reagent capsule serves a unique analysis and must not be re-used.

❖ Specific equipment or premises required

A Quanti-Tray Sealer PLUS is required.

❖ Reagents ready-to-use or to be reconstituted

Supplement kit (to be reconstituted for the potable protocol).

Pretreatment kit (to be reconstituted for the non-potable protocol)

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

The use of the method Legiolert reagent / Quanti-Tray Legiolert requires no specific training. The duration of training is estimated at 1 hour.

❖ Real-time handling and flexibility of the method depending on the number of samples to be analyzed.

The duration of an analysis of by the method NF T90-431 using the “clean water” protocol (without the confirmation step) is approximately 10 min

The duration of an analysis by the method NF T90-431 using the “dirty water” protocol ((without the confirmation step) is approximately 40 min.

The duration of an analysis with the ISO 11731 is quite difficult to estimate. However, in this study the durations were the same as the method NF T90-431 for the two protocols tested (“clean water” and “dirty water”).

The duration of an analysis with the method Legiolert is approximately 5 min (time including: dissolution of the Legiolert reagent, waiting time, and the time for sealing the Quanti-Tray) for the two protocols, potable and non-potable.

The alternative method doesn’t require any confirmation step.

- ❖ Time required for obtaining the results

Time-to-result with negative and positive sample is 7 days with the Legiolert method.

Time-to-result for the reference method NF T90-431:
- Negative result with absence of *Legionella spp* and *Legionella pneumophila*: 11 days
- Negative result with presence of *Legionella spp* and absence of *Legionella pneumophila*: 13 to 15 days
- Positive result with the presence of *Legionella pneumophila*: 13 to 15 days

Time-to-result for the reference method ISO 11731:
- Negative result with absence of *Legionella spp* and *Legionella pneumophila*: 10 days
- Negative result with presence of *Legionella spp* and absence of *Legionella pneumophila*: 12 to 15 days
- Positive result with the presence of *Legionella pneumophila*: 12 to 15 days

- ❖ Operator qualification type

The qualification of the operator is lower than required for the reference method as to Quanti-Tray reading is easier than colony counting.

- ❖ Phases shared with the reference method

None.

- ❖ Means or traceability of the analysis results for the user

No traceability procedure is proposed. The laboratory shall use its internal procedures.

- ❖ Obligation to maintain specific apparatus for the user

2.6. None.Conclusion of the comparative study

The linearity and the relative accuracy of the Legiolert method for the enumeration of *Legionella pneumophila* in water for human consumption and industrial waters are acceptable.

Bias between the two reference methods and the alternative method are acceptable for the two categories tested. The limits of detection and quantification of the two protocols of the alternative method are acceptable.

The method Legiolert for the enumeration of *Legionella pneumophila* is specific and selective. Except cross reactions were observed with four strains of *Proteus tested* using the potable protocol.

Results are obtained in 7 days with the alternative method against 12 to 15 days with the two reference methods.

The comparison of the Legiolert test with the French standard NF T90-431 (2017) and the international standard ISO 11731 (2017) allows to conclude that the alternative method give similar results compared to the reference methods for this scope of validation.

3- Interlaboratory study

The aim of the interlaboratory study was to determine the variability of the results obtained in different laboratories using identical samples and to compare these results with those obtained during the methods comparison study.
The interlaboratory study was conducted according to « Validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013)."

The preliminary study did not show a major difference between the two reference methods, therefore, for the inter-laboratory study, only the REFERENCE method NF T90-431 was carried out.

The 2 ILS tests were performed under the same conditions with the same collaborating laboratories. The results were combined given the two non-usable levels. It was decided to perform a second ILS with the same laboratories and the same matrix/strain combination.
A combination of the results of the 2 ILS was required to obtain the three contamination levels as envisaged in the protocol and to interpret the results of the inter-laboratory study. Level 1 of ILS1 and Levels 2 and 3 of ILS2 were used to interpret the results. This combination is permissible as long as:

- ❖ the same laboratories were requested in both cases
- ❖ the same matrix/strain combination was used
- ❖ the analysis protocols are exactly the same between the two studies
- ❖ after exclusion from laboratories, there are still 10 datasets that can be exploited

3.1. Study Organisation

3.1.1. Participating laboratories

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

3.1.2. Matrices and strain

The matrix selected for this study was a network water. It was artificially contaminated with a strain of *Legionella pneumophila* serogroup 4, isolated from the environment.

To verify the absence of initial contamination of *Legionella pneumophila* from the matrix, a search was conducted on 10 samples before inoculation. All results were negative.

3.1.3. Samples preparation and spiking

The target levels of contamination were:

- Level 0 : 0 UFC/ L,
- Level 1 : 10^2 UFC/ L,
- Level 2 : 10^4 UFC/ L,
- Level 3 : 10^6 UFC/ L.

Samples were individually inoculated.

3.1.4. Transport

The anonymized and codified samples were prepared and packaged in insulated boxes containing refrigerated blocks and were sent on 28 January 2019 ; for a theoretical reception at D+1.

Temperature changes during transport were recorded by probes contained within each package.

The temperature was also taken systematically when the samples arrived.

This temperature should be between 0 and 10°C. This was noted on the acknowledgment, which was referred by each collaborating laboratory to the expert laboratory. The date and time of receipt, as well as the status of the samples were indicated.

3.1.5. Analysis

The collaborating laboratories had to analyze the samples at J+2 .

The samples were all analyzed on the same day using both methods. To allow quantification over an extended range, the samples were analyzed with and without dilution. Two calculation methods were made to determine the values of the counts. They are detailed in appendix 11. No differences were observed in the results between the two calculation modes. In the report, the results obtained with the calculation mode 1 are presented.

3.2. Interlaboratory study 1 (ILS 1)

3.2.1. Control of experimental parameters

- **Stability of the strain and ancillary flora**

The stability of the strain was assessed for 3 days at 5°C -/+ 3 °C. The strain used was *Legionella pneumophila* 4 (code AdGène Lp4).

The samples were analyzed at D0, D+1 and D+2 using the reference method. The results are summarized in the table below.

	Level 0	Level 1	Level 2	Level 3
D0	0	$4{,}8.10^2$	1.10^4	3.10^6
D+1	0	$5{,}10^2$	$3{,}10^4$	$4{,}2.10^6$
D+2	0	$5{,}2.10^2$	$5{,}10^4$	$4{,}5.10^6$

The results show that the *Legionella* strain used is stable at 5°C -/+ 3 °C in the matrix use

- **Contamination rate and sample coding**

Samples were distributed at a rate of 300ml per sterile vial. Each vial was individually contaminated and then homogenized. Eight samples per laboratory were prepared (2 samples per level of contamination).

Each laboratory also received an additional sample for the total bacterial count according to NF EN ISO 6222.

The results of the ancillary flora as well as the targeted and actual levels of contamination levels are presented in the table below :

Level of contamination	Sample reference	Annex Flora (UFC/ml)	

		22°C	36°C	Target Level (UFC/L)	Actual Level (UFC/L)
0	1-8	0	0	0	0
1	2-7			2.10^2	$1,5.10^4$
2	3-6			2.10^4	7.10^5
3	4-5			2.10^6	$3,2.10^6$

- **Temperature control**

The table below includes key information on the receipt of the package and samples from the collaborating laboratories.

Temperature raised	Temperature recorder	Day and hour reception	Package status	Analysis day	Temperature raised
A1	6.5	7,2	29/01/2019 14 :00	Compliant	30/01/2019
B1	not determined	5,4	29/01/2019 10 :55	Compliant	30/01/2019
C1	/	/	/	/	/
D1	9.1	6,5	29/01/2019 11 :35	Compliant	30/01/2019
E1	5.8	5,5	29/01/2019 14 :25	Compliant	30/01/2019
F1	5.0	6,9	29/01/2019 13 :00	Compliant	30/01/2019
G1	4.5	4,7	29/01/2019 11 :20	Compliant	30/01/2019
H1	5.2	6,4	29/01/2019 10 :00	Compliant	30/01/2019
I1	5.5	5,7	29/01/2019 9 :05	Compliant	30/01/2019
J1	6.5	5,7	29/01/2019 11 :00	Compliant	30/01/2019
K1	9.6	6,6	29/01/2019 15 :00	Compliant	30/01/2019
L1	6.1	4,9	29/01/2019 11 :00	Compliant	30/01/2019
M1	5.0	5,9	29/01/2019 10 :30	Compliant	30/01/2019
N1	9.8	5.2	29/01/2019 8 :30	Compliant	30/01/2019

All temperature record curves during transport are listed in Appendix 8. The C1 laboratory ultimately did not participate in the study and therefore did not return a result. Laboratories D1, K1 and N1 have measured temperatures above 8°C, but the temperatures recorded by the probe during delivery were always below 8°C.

3.2.2. Tests analysis

- **Results from the expert laboratory**

Bacterial counts at 36°C and 22°C are 0 UFC/ml.

The results obtained for both methods are summarised below :

Level of contamination	Réplicates	Reference Method	Alternative Method
L0	1	<10	<10
	2	<10	<10
L1	1	20 000	59 000
	2	15 000	72 000
L2	1	710 000	>2 400 000
	2	680 000	>2 400 000
L3	1	>750 000	>2 400 000
	2	>750 000	>2 400 000

- **Results obtained by collaborating laboratories**

Bacterial Counts at 22°C and 36°C, for all collaborating laboratories, are all less than 1 UFC/ml (cf appendix 9).

The raw results of the laboratories are presented in appendix 10.

The C1 laboratory did not report any results.

B1 and G1 achieved positive results for Level 0.

Laboratory	Level 0 ILS1			
	Reference		Alternative	
	1	2	1	2
A1	<10	<10	<10	<10
B1	<10	345	<10	<10
D1	<10	<10	<10	<10
E1	<10	<10	<10	<10
F1	<10	<10	<10	<10
G1	100	<10	73	<10
H1	<10	<10	<10	<10
I1	<10	<10	<10	<10

J1	<10	<10	<10	<10
K1	<10	<10	<10	<10
L1	<10	<10	<10	<10
M1	<10	<10	<10	<10
N1	<10	<10	<10	<10

Laboratory	Level 1 ILS1			
	Reference		Alternative	
	1	2	1	2
A1	77273	60000	100273	73564
B1	136363	95454	109873	87673
D1	200000	85000	65 975	83 140
E1	136363	63636	102382	128145
F1	131818	136364	91873	97636
G1	100000	63636	115827	73045
H1	2700	86362	57418	48473
I1	26000	55000	46227	47509
J1	22727	60000	22327	65000
K1	65000	45454	70100	54755
L1	77273	63636	61373	61982
M1	50000	70000	15917	15578
N1	31818	59090	53791	55282

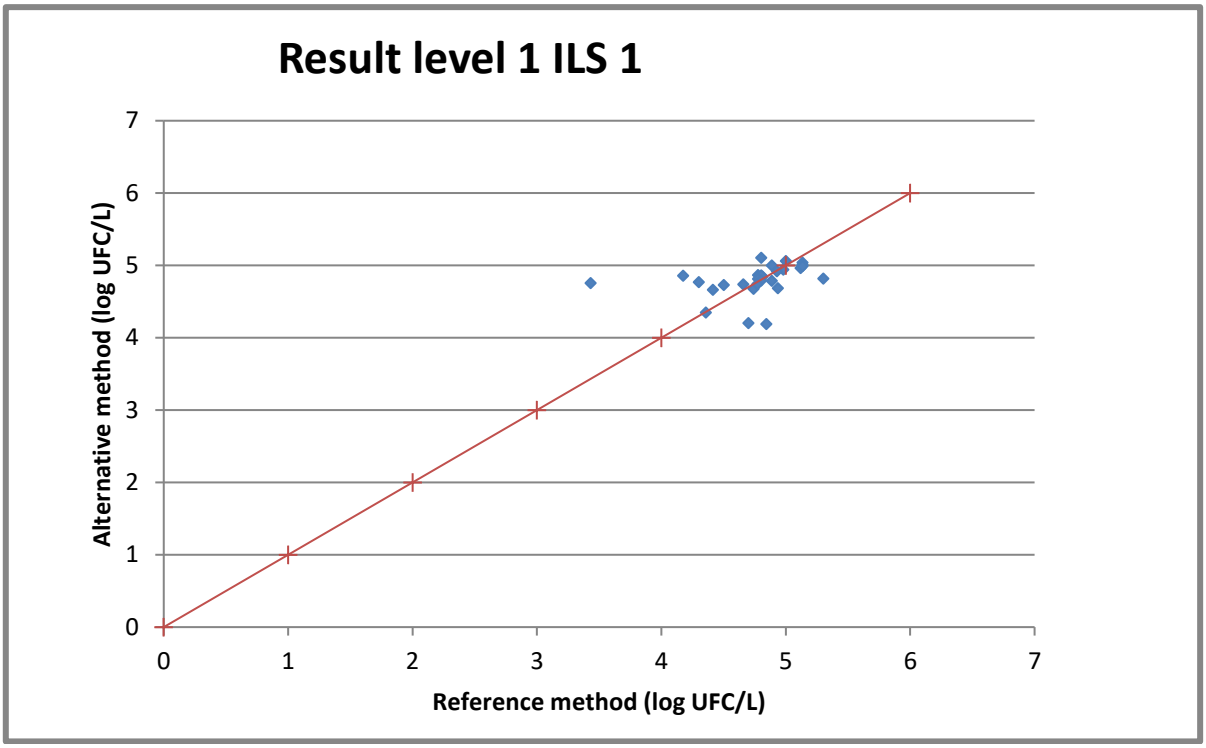
Laboratoire	Level 2 ILS1			
	Reference		Alternative	
	1	2	1	2
A1	6 800 000	5 500 000	>2 300 000	>2 300 000
B1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
D1	5 100 000	6 100 000	>2 300 000	>2 300 000
E1	7200000	>7 500 000	>2 300 000	>2 300 000
F1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
G1	>750 000	>750 000	>2 300 000	>2 300 000
H1	4 800 000	>750 000	>2 300 000	>2 300 000
I1	7 300 000	2 500 000	>2 300 000	>2 300 000

J1	17 000 000	1 900 000	>2 300 000	>2 300 000
K1	5 700 000	4 600 000	>2 300 000	>2 300 000
L1	6 700 000	5 800 000	>2 300 000	>2 300 000
M1	>750 000	>750 000	1 162 900	1 162 900
N1	5 400 000	5 200 000	>2 300 000	>2 300 000

Laboratory	**Level 3 ILS1**			
	Reference		Alternative	
	1	2	1	2
A1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
B1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
D1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
E1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
F1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
G1	>750 000	>750 000	>2 300 000	>2 300 000
H1	<10	>7 500 000	<10	>2 300 000
I1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
J1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
K1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
L1	>7 500 000	>7 500 000	>2 300 000	>2 300 000
M1	> 750 000	> 750 000	1 162 900	1 162 900
N1	> 750 000	> 750 000	>2 300 000	>2 300 000

3.2.3. Results selected for study and conclusion of ILS1

The C1 laboratory did not report any results.
B1 and G1 achieved positive results for Level 0.
For all laboratories, for Levels 2 and 3, actual spiking levels were above those targeted and were not usable. The two-dimensional UFC/L log graph for level 1 is presented in the following figure (except for Laboratories B1, C1 and D1). The y=x curve is given for better visualization of the data.



Given the two non-usable levels, it was decided to do a second ILS with the same laboratories and the same matrix/strain torque.

3.3. Interlaboratory study 2 (ILS 2)

3.3.1. Control of experimental parameters

- **Stability of the strain and ancillary flora**

To verify the absence of initial contamination of *Legionella pneumophila* from the matrix, a search was conducted on 10 samples before inoculation. All results were negative.

The stability of the strain was assessed for 3 days at 5 +/- 3 °C. The strain used was *Legionella pneumophila* serogroup 4 (code AdGene Lp4).

The samples were analyzed at D0, D+1 and D+2 using the reference method. The results are summarized in the following table.

	Level 0	Level 1	Level 2	Level 3
D0	0	3.5×10^2	3.8×10^4	3.9×10^6
D+1	0	3.5×10^2	3.8×10^4	3.8×10^6
D+2	0	3.4×10^2	4×10^4	4.2×10^6

The results show that the strain of *Legionella* used is stable at 5 +/- 3°C, in the matrix used.

- **Contamination level and sample coding**

Samples were distributed at a volume of 300ml per sterile vial. Each vial was individually contaminated and then homogenized. Eight samples per laboratory were prepared (2 samples per level of contamination).

The results of the ancillary flora as well as the targeted and actual levels of contamination levels are presented in the table below.

Level of contamination	Sample reference	Ancillary flora (UFC/ml)		Target level	Actual level
		22°C	36°C		
0	2/5	0	0	0	0
1	4/7			2.10^2	$3,4.10^2$
2	6/8			2.10^4	$3,2.10^3$
3	1/3			2.10^6	$3,9.10^5$

- **Temperature control**

The table below includes the main information on the receipt of the package and samples from the collaborating laboratories.

Laboratory	Temperature raised	Temperature recorder	Day and hour reception	Package status	Analysis day
A2	5,4°C	5,4°C	02/04/2019 11H40	Compliant	03/04/2019
B2	9,8°C	7,0°C	02/04/2019 11h45	Compliant	03/04/2019
C2	4,5°C	5,2°C	02/04/2019 13H	Compliant	03/04/2019
D2	4,6°C	6,1°C	02/04/2019 13H46	Compliant	03/04/2019
E2	8,5°C	6,7°C	02/04/2019 14H10	Compliant	03/04/2019
F2	2,1°C	5,9°C	02/04/2019 9H00	Compliant	03/04/2019
G2	8,2°C	7,2°C	02/04/2019 11H30	Compliant	03/04/2019
H2	9,1°C	5,9°C	02/04/2019 11H50	Compliant	03/04/2019
J2	6,8°C	5,6°C	02/04/2019 14H00	Compliant	03/04/2019
K2	7,7°C	6,1°C	02/04/2019 11h15	Compliant	03/04/2019
L2	6,7°C	5,4°C	02/04/2019 9H20	Compliant	03/04/2019
M2	6,2°C	4,7°C	02/04/2019 11H30	Compliant	03/04/2019
N2	7,9°C	5,6°C	02/04/2019 12H30	Compliant	03/04/2019
02	6,7°C	5,9°C	02/04/2019 13H50	Compliant	03/04/2019

Laboratory B measured the temperature 10 minutes after receipt, resulting in a temperature reading above 8°C. Laboratories E, G and H also measured temperatures above 8°C, but the temperatures recorded by the probe during delivery were each time below 8°C.

All of the temperature record curves during transport are shown in appendix 8.

3.3.2. Test results

The results presented in the report are those obtained with the calculation mode 1, which takes into account all the available information of all the count results obtained.

- **Results from the expert laboratory**

Bacterial counts at 36°C and 22°C are 0 UFC/ml.

The results obtained for both methods are as follows:

Level of contamination	Replicates	Reference method	Alternative method
L0	1	<10	<10
	2	<10	<10
L1	1	30	39
	2	10	11
L2	1	45454	3598
	2	850	7628
L3	1	322727	384900
	2	286363	350100

- **Results obtained by collaborating laboratories**

Bacterial counts at 22° and 36°C were, for all collaborating laboratories, between <1 and <300 UFC/mL (appendix 9).

The raw results of the laboratories are presented in appendix 10.

Laboratory	Level 0 ILS 2			
	Reference		Alternative	
	1	2	1	2
A2	<10	<10	<10	<10
B2	<10	<10	<10	<10
C2	<10	<10	<10	<10
D2	<10	<10	<10	<10
E2	<10	<10	<10	<10
F2	<10	<10	<10	<10
G2	<10	<10	<10	<10
H2	<10	<10	<10	<10
J2	<10	<10	<10	<10
K2	<10	<10	<10	<10
L2	<10	<10	<10	<10
M2	<10	<10	<10	<10
N2	<10	<10	<10	<10
O2	<10	<10	<10	<10

Laboratory	Level 1 ILS 2			
	Reference		Alternative	
	1	2	1	2
A2	10	10	11	58
B2	<10	10	39	69
C2	<10	<10	23	35
D2	<10	<10	39	74
E2	<10	30	16	20
F2	65	10	<10	<10
G2	20	<10	<10	75
H2	30	<10	10	11
J2	10	10	11	22
K2	10	10	60	61
L2	300	100	52	61
M2	10	10	10	11
N2	<10	<10	<10	<10
O2	10	30	20	23

Laboratory	Level 2 EIL 2			
	Reference		Alternative	
	1	2	1	2
A2	610	15000	1885	1637
B2	1100	1400	1766	1659
C2	575	421	1660	936
D2	90	0	1033	1132
E2	934	694	2642	2410
F2	5000	5000	<10	<10
G2	1523	5000	1799	2007
H2	743	567	1164	675
J2	10000	725	2527	1591
K2	819	873	2691	2728
L2	243	766	1848	968
M2	836	600	1314	4877
N2	639	459	2133	766
O2	5000	5000	1798	1918

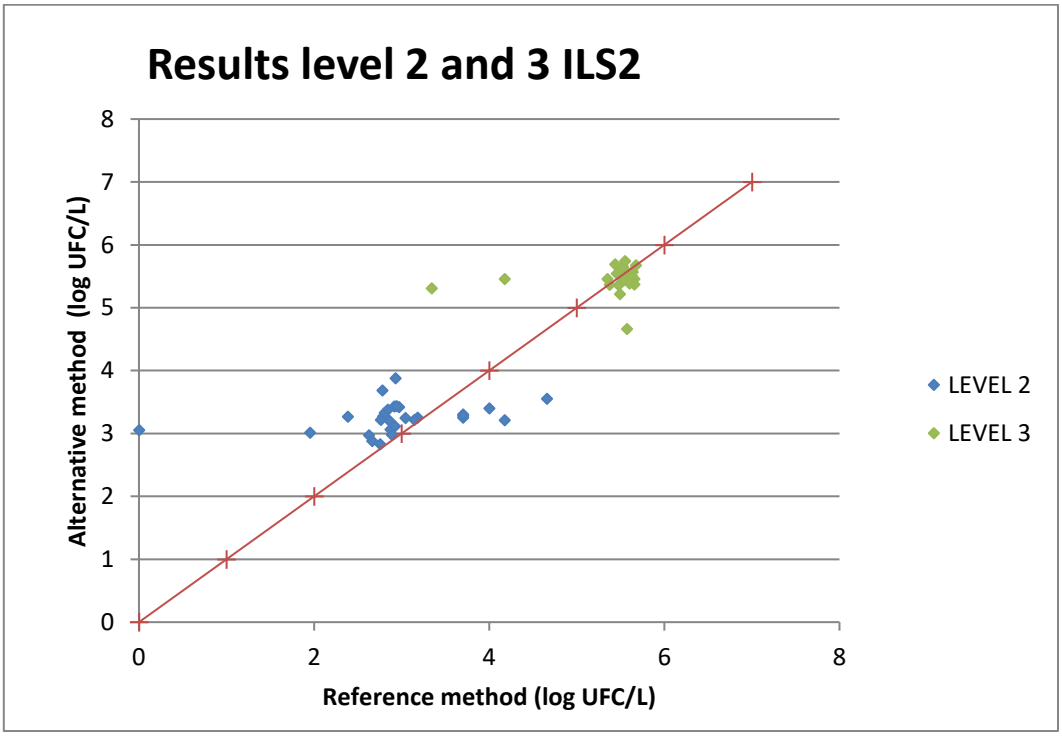
Laboratory	Level 3 EIL 2			
	Reference		Alternative	
	1	2	1	2
A2	336363	354545	448900	554900
B2	340909	318181	397100	422300
C2	272727	236363	491600	232681
D2	2200	15000	204800	286900
E2	372727	422727	46280	373000
F2	259090	240909	<10	<10
G2	390909	422727	339000	317500
H2	318181	454545	328100	236427
J2	454545	413636	286900	350100
K2	309090	400000	164736	242700
L2	386363	422727	307100	317500
M2	340000	345000	277100	277100
N2	300000	222727	230700	286900
O2	436364	472727	373000	477000

3.3.3. Results selected for the study and conclusion of ILS2

The F2 laboratory data was not selected for interpretation because all the results of the alternative method were negative regardless of the level tested. At ILS, this laboratory had tested positive for the alternative method.

Laboratories B2, C2, D2, E2, G2, H2 and N2 performed less than 10 for at least one replica of one of the two Level 1 methods. For this level, the results are not usable because we do not have a minimum of eight data sets. Indeed, for the reference method only 7 laboratories have obtained countable results (compared to 12 for the alternative method).

In contrast, levels 2 and 3 are perfectly usable. The two-dimensional UFC/L log graph for levels 2 and 3 is presented in the following figure (except for the F2 lab). The y=x curve is given for better visualization of the data.



3.4. Combination of data from the two ILS trials

3.4.1. Combined contamination rate

The actual combined contamination rates obtained are as follows:

- Level 0 : 0 UFC/ L,
- Low Level : 10^3 UFC/ L (initially level 2 ILS 2)
- Average level : 10^4 UFC/ L (initially level 1 ILS 1)
- High Level : 10^5 UFC/ L (initialy level 3 ILS 2)

3.4.2. Code matching between the two ILS trials

The table below shows the code matches between the two inter-laboratory tests.

Code ILS1	Code ILS2
A1	N2
B1	L2
C1	A2
D1	B2
E1	G2
F1	E2
G1	M2
H1	C2
I1	F2
J1	K2
K1	O2
L1	H2
M1	D2
N1	J2
O1 (expert lab)	I2 (expert lab)

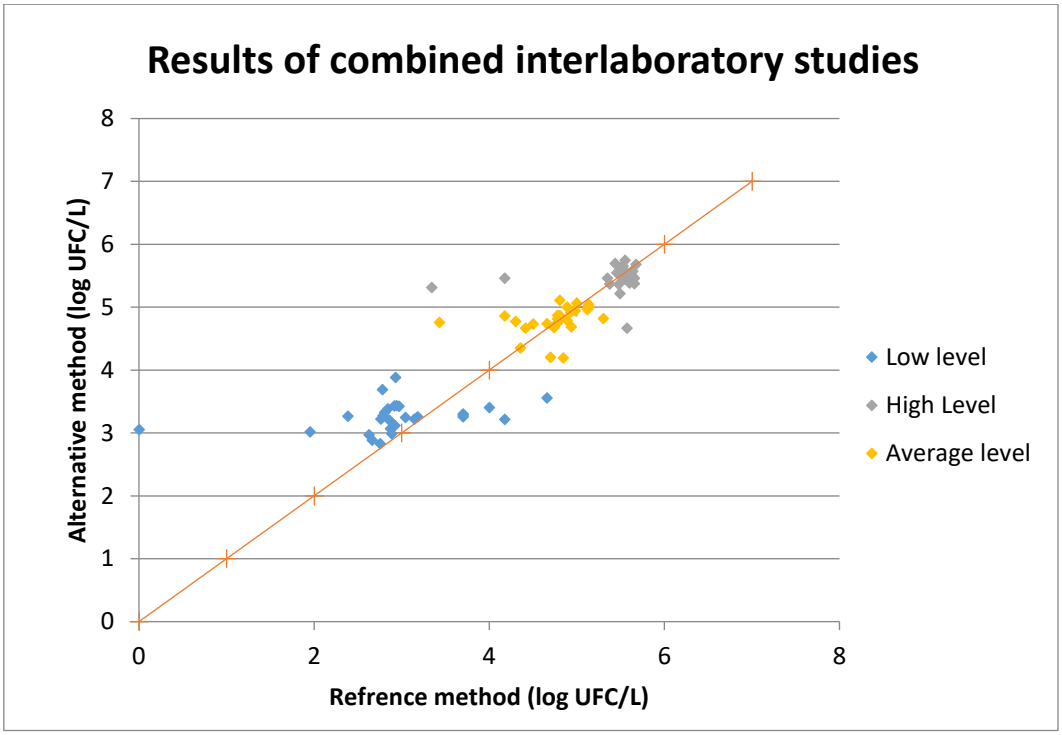
3.4.3. Results of the 2 combined studies

Data from a total of 4 laboratories were removed to interpret the results. The B1 and G1 laboratories (positive results at the L0 level of ILS1), the C1 laboratory (no results at ILS1) and the F2 laboratory (no positive Legiolert results at any level at ILS2).
Note: The M1-D2 laboratory was retained for interpretation of the results despite the fact that it did not test the samples diluted to 1/10th by the reference method. The calculations carried out with or without this laboratory have been carried out and lead to the same results and conclusions.

The table below show the combined results obtained by level.

Laboratory (Code ILS1)	Laboratory (Code ILS2)	Level 0 (ILS 1 & 2)				Low level (level 2 ILS2)				Low level (level 1 ILS 1)				High level (level 3 ILS 2)			
		Reference		Alternative		Reference		Alternative		Reference		Alternative		Reference		Alternative	
		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
A1	N2	<10	<10	<10	<10	639	459	2133	766	77273	60000	100273	73564	300000	222727	230700	286900
D1	B2	<10	<10	<10	<10	1100	1400	1766	1659	200000	85000	65 975	83 140	340909	318181	397100	422300
E1	G2	<10	<10	<10	<10	1523	5000	1799	2007	136363	63636	102382	128145	390909	422727	339000	317500
F1	E2	<10	<10	<10	<10	934	694	2642	2410	131818	136364	91873	97636	372727	422727	46280	373000
H1	C2	<10	<10	<10	<10	575	421	1660	936	2700	86362	57418	48473	272727	236363	491600	232681
J1	K2	<10	<10	<10	<10	819	873	2691	2728	22727	60000	22327	65000	309090	400000	164736	242700
K1	O2	<10	<10	<10	<10	5000	5000	1798	1918	65000	45454	70100	54755	436364	472727	373000	477000
L1	H2	<10	<10	<10	<10	743	567	1164	675	77273	63636	61373	61982	318181	454545	328100	236427
M1	D2	<10	<10	<10	<10	90	1	1033	1132	50000	70000	15917	15578	2200	15000	204800	286900
N1	J2	<10	<10	<10	<10	10000	725	2527	1591	31818	59090	53791	55282	454545	413636	286900	350100
Expert (O1 et I2)		<10	<10	<10	<10	45454	850	3598	7628	20000	15000	59000	72000	322727	286363	384900	350100

The two-dimensional GRAPH in THE UFC/L log is presented in the following figure. The y=x curve is given for better visualization of the data.



3.4.4. Calcul and interpretation

- **Calculation of reference target values and standard deviations**

The table below shows the target values, the average, the standard fidelity deviation, the relative bias and the level bias obtained for the alternative method. Results are expressed in UFC/L and UFC/L log.

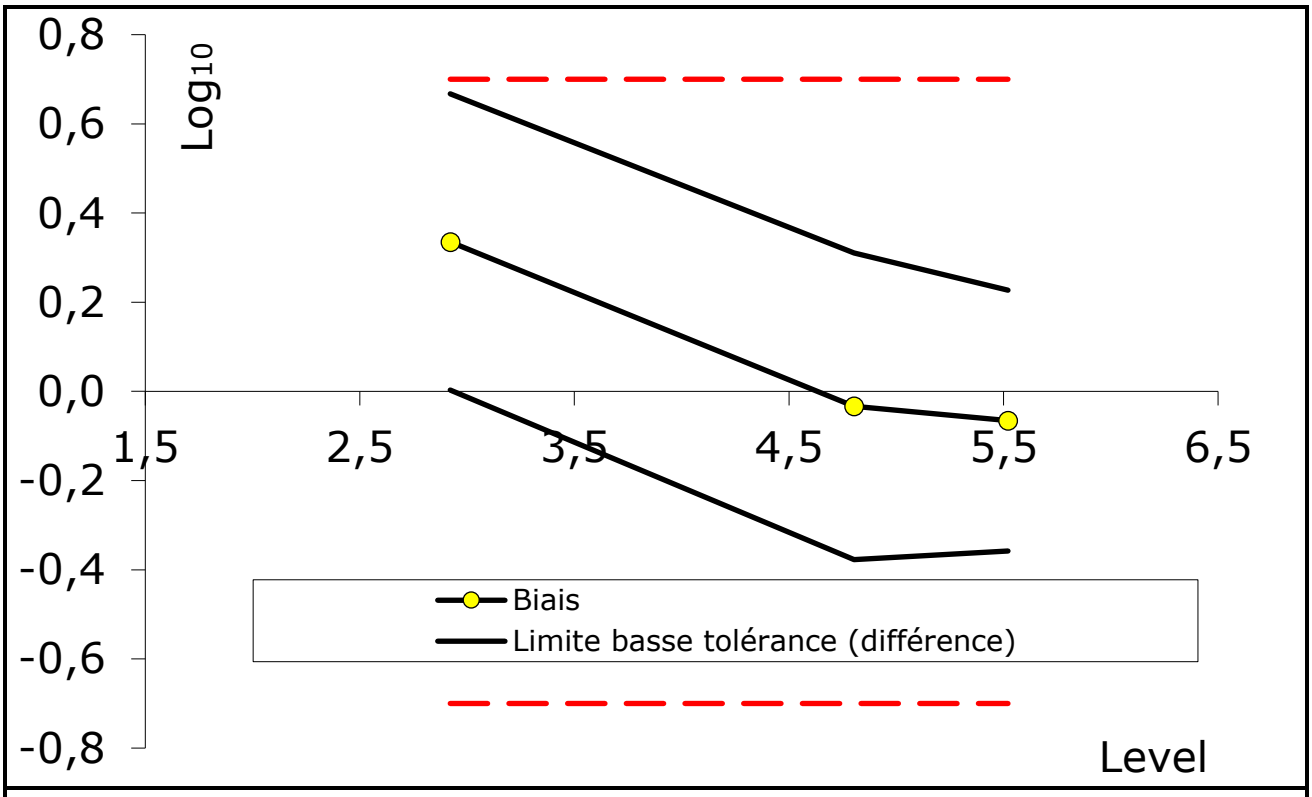
	UFC/L			Log UFC/L		
Level	**Low**	**Average**	**High**	**Low**	**Average**	**High**
Target value	834,5	63636	331818	2,921	4,804	5,521
Average	2102,773	66181,091	310123,818	3,256	4,770	5,455
Standard deviation	1454,558	28344,318	103632,735	0,239	0,244	0,216
Relative bias	251,98%	104,00%	93,46%	11,47%	-0,70%	-1,19%
Bias	1268,273	2545,091	-21694,182	0,335	-0,034	-0,066

- **Building the accuracy profile**

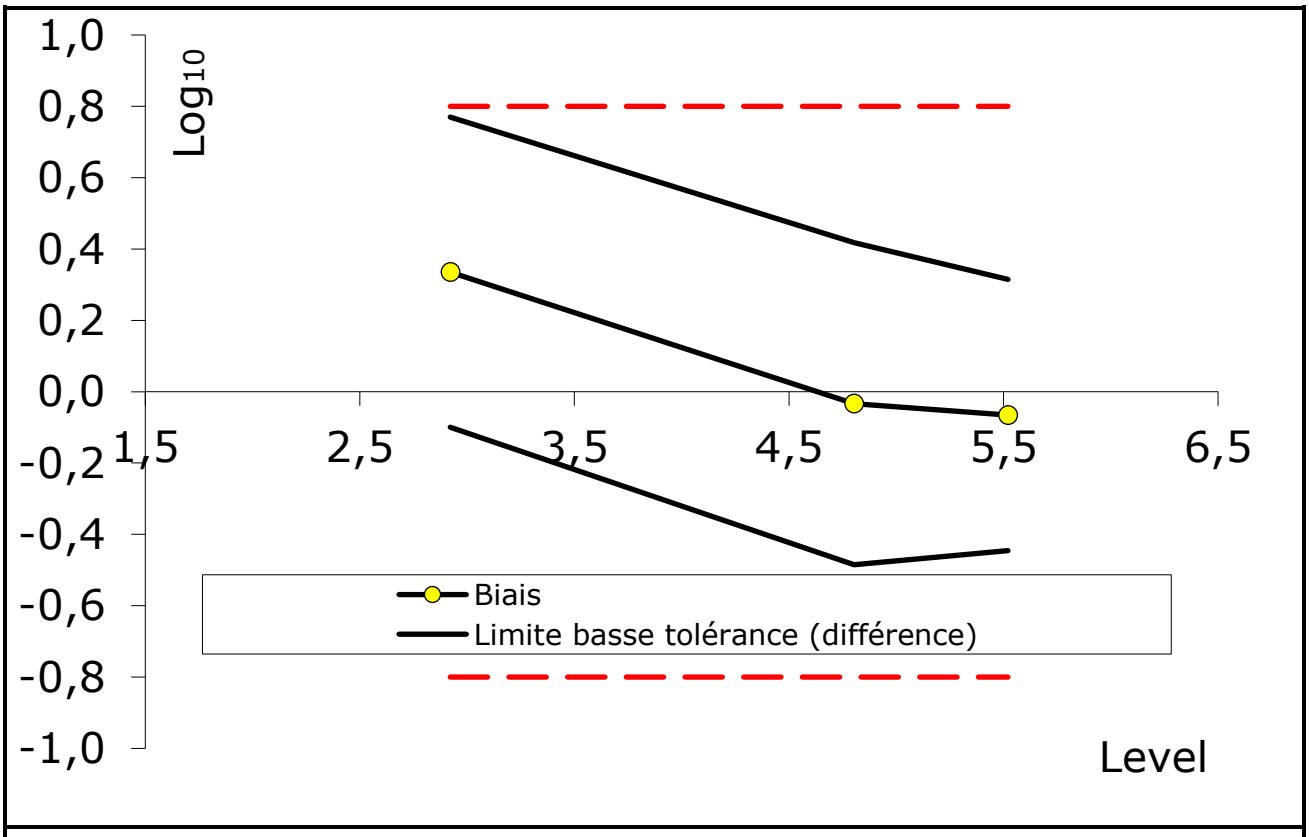
The table below shows the values and tolerance limits obtained with a tolerance probability of 80% and 90%. Results are expressed in UFC/L and UFC/L log.

		CFU/L			Log CFU/L		
		Low	Average	High	Low	Average	High
Probability of tolerance : 80% Acceptability limit: 80% (UFC/L) or 0.7 log UFC/L	Low tolerance value	**10,21%**	**41,36%**	**51,10%**	0.003	-0.377	-0.358
	High-tolerance value	**493,75%**	**166,64%**	**135,82%**	0.667	0.310	0.227
	Low tolerance value	**20,00%**	**20,00%**	**20,00%**	-0.700	-0.700	-0.700
	High-tolerance value	**180,00%**	**180,00%**	**180,00%**	0.700	0.700	0.700
Probability of tolerance : 90% Acceptability limit: 95% (UFC/L) or 0.8 log UFC/L	Low tolerance value	**-64,12%**	**21,76%**	**38,36%**	-0.100	-0.485	-0.446
	High-tolerance value	**568,08%**	**186,24%**	**148,57%**	0.770	0.418	0.315
	Low tolerance value	**5,00%**	**5,00%**	**5,00%**	-0.800	-0.800	-0.800
	High-tolerance value	**195,00%**	**195,00%**	**195,00%**	0.800	0.800	0.800

The following figures show the results of the accuracy profiles.



UFC/L log accuracy profile for 80% tolerance and a tolerance limit of 0.7



UFC/L log accuracy profile for a 90% tolerance probability and a tolerance limit of 0.8

3.5. Conclusion of the interlaboratory study

The accuracy profiles obtained show that the biases obtained for the Legiolert method are acceptable. The tolerance limits for the alternative method for a probability of 80% are between the acceptability limits of 0.7 log.

4- Bibliographical studies

Already 11 studies have been published in peer-reviewed scientific journals and have shown that the Legiolert method is equivalent or superior to the reference methods to which it has been compared. See list below:

Barrette I. Comparison of Legiolert and a conventional culture method for detection of *Legionella pneumophila* from cooling towers in Québec. *J AOAC Int.* 2019;102(4):1235–1240. doi:10.5740/jaoacint.18-0245

Boczek LA, Tang M, Formal C, Lytle D, Ryu H. Comparison of two culture methods for the enumeration of *Legionella pneumophila* from potable water samples. *J Water Health.* 2021;19(3):468–477. doi:10.2166/wh.2021.051

Checa J, Carbonell I, Manero N, Martí I. Comparative study of Legiolert with ISO 11731-1998 standard method-conclusions from a Public Health Laboratory. *J Microbiol Methods.* 2021;186:106242. doi:10.1016/j.mimet.2021.106242

Inoue H, Baba M, Tayama S. Evaluation of Legiolert for quantification of *Legionella pneumophila* from bath water samples. *Biocontrol Sci.* 2020;25(3):179–182. doi:10.4265/bio.25.179

McCuin RM, Bartrand TA, Clancy JL. *Legionella pneumophila* recovery using Legiolert and a traditional culture method. *AWWA Water Sci.* 2021;3(3):e1228. doi:10.1002/aws2.1228

Monteiro SN, Robalo AM, Santos RJ. Evaluation of Legiolert for the detection of *Legionella pneumophila* and comparison with spread-plate culture and qPCR methods. *Curr Microbiol.* 2021;78(5):1792–1797. doi:10.1007/s00284-021-02436-6

Petrisek R, Hall J. Evaluation of a most probable number method for the enumeration of *Legionella pneumophila* from North American potable and nonpotable water samples. *J Water Health.* 2018;16(1):25–33. doi:10.2166/wh.2017.118

Rech MM, Swalla BM, Dobranic JK. Evaluation of Legiolert for quantification of *Legionella pneumophila* from non-potable water. *Curr Microbiol.* 2018;75(10):1282–1289. doi:10.1007/s00284-018-1522-0

Sartory DP, Spies K, Lange B, Schneider S, Langer B. Evaluation of a most probable number method for the enumeration of *Legionella pneumophila* from potable and related water samples. *Lett Appl Microbiol.* 2017;64(4):271–275. doi:10.1111/lam.12719

Scaturro M, Buffoni M, Girolamo A, et al. Performance of Legiolert Test vs. ISO 11731 to confirm *Legionella pneumophila* contamination in potable water samples. *Pathogens.* 2020;9(9):690. doi:10.3390/pathogens9090690

Spies K, Pleischl S, Lange B, et al. Comparison of the Legiolert/Quanti-Tray MPN test for the enumeration of *Legionella pneumophila* from potable water samples with the German regulatory requirements methods ISO 11731-2 and ISO 11731. *Int J Hyg Environ Health.* 2018;221(7):1047–1053. doi:10.1016/j.ijheh.2018.07.006

In 2021, the Legiolert method became the international standard ASTM D8429-2:

- American Society for Testing Materials (ASTM) International: D8429-21 Standard Test Method for *Legionella pneumophila* in Water Samples Using Legiolert. December 22, 2021. Link to ASTM standard https://www.astm.org/d8429-21.html

5- Assessment of the complaints

No complaint from the users of the alternative method was received by AFNOR Certification.

6- State of the intervened modifications and the envisaged modifications

6.1.Technical repository of validation

No modification since the last renewal on the technical repository: “the protocol of validation of an alternative commercial method as compared to a reference method - May 2013”.

6.2.Reference method

No modification was brought to the reference methods NF T90-431 (2017) : “Detection and enumeration of *Legionella* spp and *Legionella pneumophila*” and NF EN ISO 11731 (2017): “Enumeration of *Legionella*”.

6.3.Alternative method

No modification was brought to the alternative method since the initial validation.

7- Presentation of the possible modifications envisaged in the alternative method

No modification is planned.

8- Conclusion

The validation study for the AFNOR Certification validation of the method Legiolert for the enumeration of *Legionella pneumophila* in human consumption and industrial waters was realized in 2015 with a first renewal in 2019.

Concerning the comparative study, the linearity and the relative accuracy of the Legiolert method for the enumeration of *Legionella pneumophila* in water for human consumption and industrial waters are acceptable.

Bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.

The method Legiolert for the enumeration of *Legionella pneumophila* is specific and selective. Except cross reactions were observed with four strains of Proteus tested using the potable protocol.

Results are obtained in 7 days with the alternative method against 12 to 15 days with the two reference methods.

The comparison of the Legiolert test with the French standard NF T90-431 (2017) and the international standard ISO 11731 (2017) allows to conclude that the alternative method give similar results compared to the reference methods for this scope of validation.

The interlaboratory study showed that the bias of the alternative method were more significative for the low values and slightly negative for the medium and high levels of contamination.

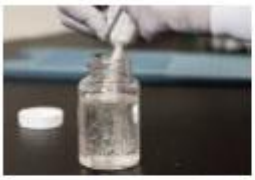
The accuracy profiles obtained show that the biases obtained for the Legiolert method are acceptable. The tolerance limits for the alternative method for a probability of 80% are between the acceptability limits of 0.7 log.

Done at Thury-Harcourt, March 16, 2023

Mickaël MORVAN

Research & Development Engineer

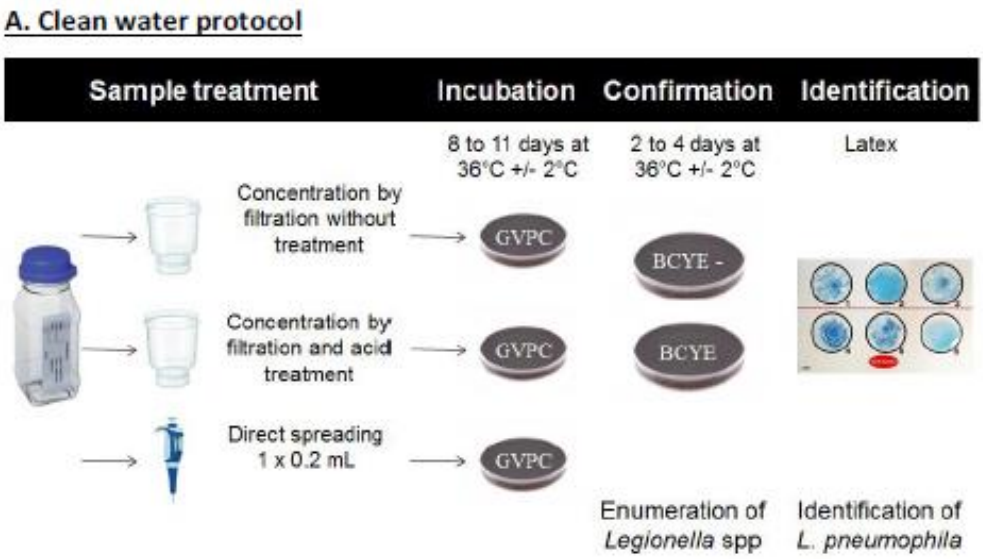
Appendix 1: Alternative method protocol

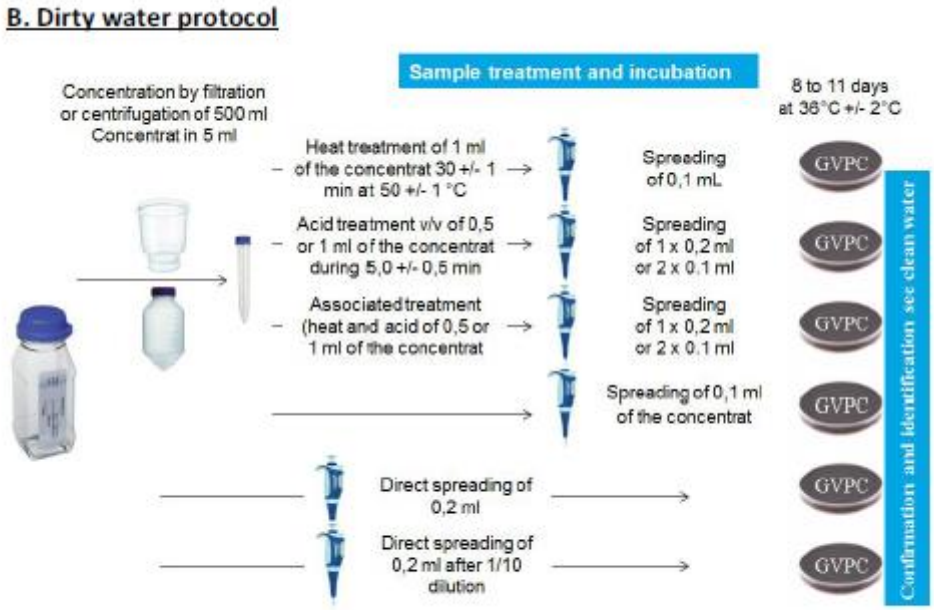
Potable water – 100 ml	Non potable water – 1 ml
Measure hardness and add appropriate volume of reconstituted Legiolert supplement	 Add reagent to sterile diluent
Add reagent to water sample and shake to dissolve	Treat 2 ml nonpotable sample with 2 ml Legiolert pretreatment
	Add 2 ml pretreated sample to 100 ml reagent mixture

Potable water	Non potable water
Pour sample into Quanti-Tray/Legiolert.	
Seal using the Quanti-Tray Sealer PLUS and incubate for 7 days.	
Incubate 7 days at 39°C +/- 0,5°C	Incubate 7 days at 37°C +/- 0,5°C
Read results : any brown and/or turbid wells are positive for *Legionella pneumophila*.	

Appendix 2: Reference method protocol

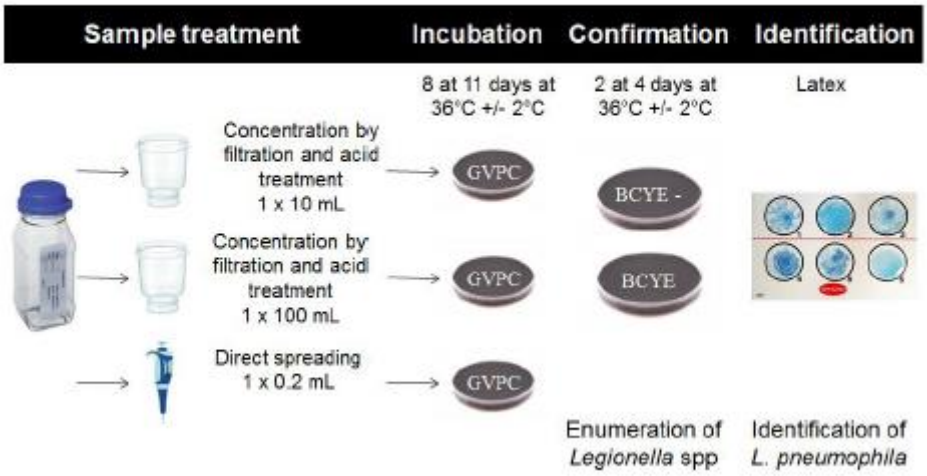
Protocol of the method NF T90-431



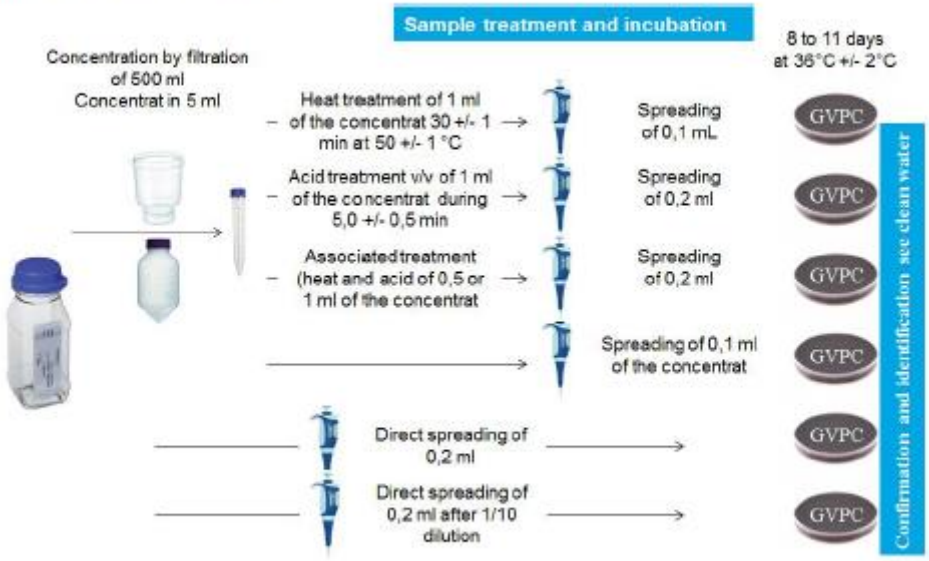


Protocol of the method NF T90-431: protocol applied during the validation study

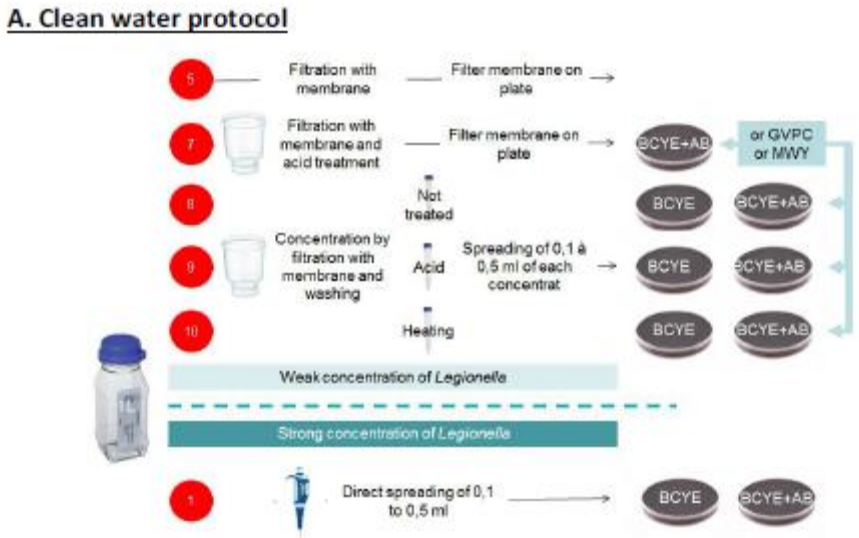
A. Clean water protocol

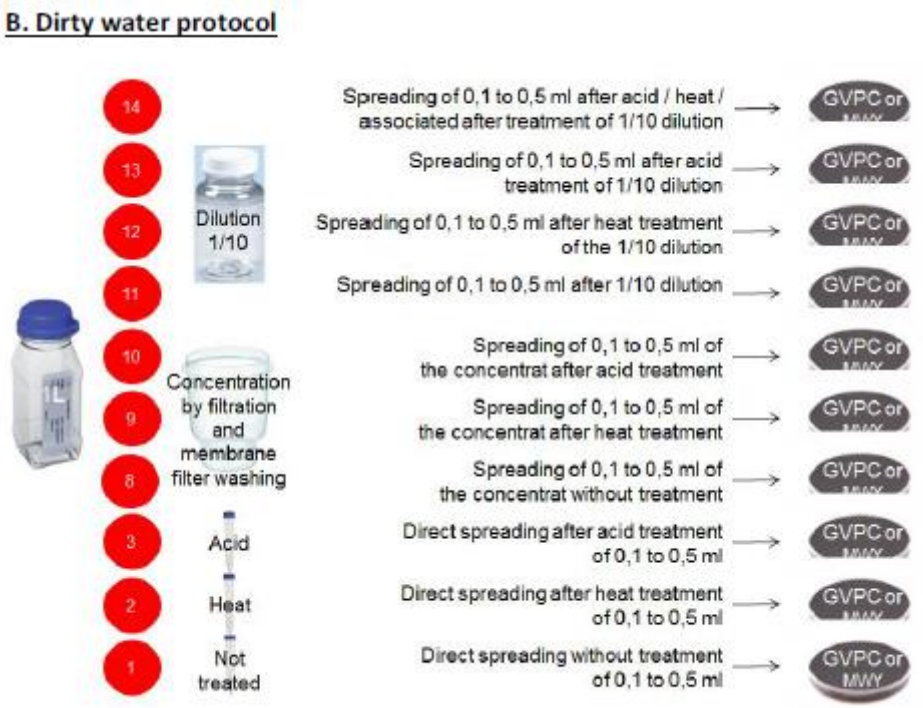


B. Dirty water protocol



Protocol of the method ISO 11731

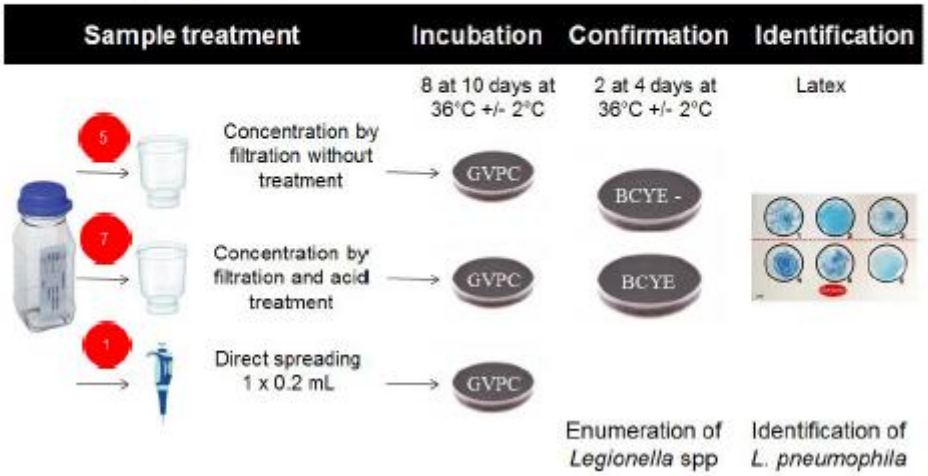




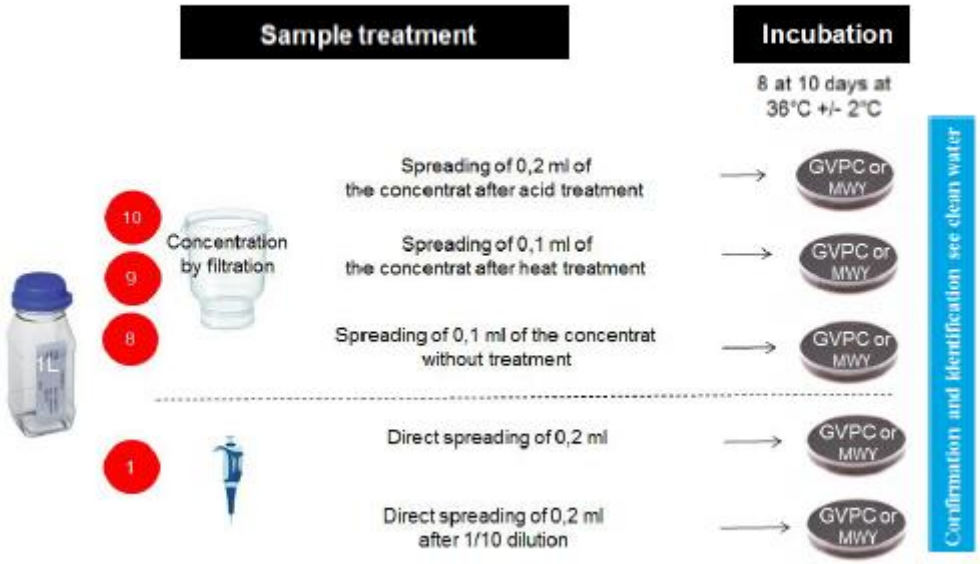
Protocol of the method ISO 11731: protocol applied during the validation study

Procedure of the decisional matrix (appendix J of the NF 11731) chose during the AFNOR technical board

A. Clean water protocol



B. Dirty water protocol



Appendix 3: Artificial contamination

Bacterial stress

Human consumption waters

Code	Strain	Origin	Stress applied	Stress intensity (log)	Sample number	Water
LEG 1.1	*Legionella pneumophila* serogroup 1	Clinical	5 minutes 56°C	0.5	7	Hot water for sanitary use 4
LEG 1.15	*Legionella pneumophila* serogroup13	Clinical	7 minutes 56°C	0.6	22	Hot water for sanitary use 1
LEG 1.9	*Legionella pneumophila* serogroup 7	Clinical	3 minutes 56°C	0.5	23	Tap water 5
LEG 1.11	*Legionella pneumophila* serogroup 9	Clinical	7 minutes 56°C	0.7	26	Hot water for sanitary use 1
LEG 1.31	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.5	32	Hot water for sanitary use 1
LEG 1.31	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.5	33	Fontain water 1
LEG 1.30	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.6	37	Tap water 5
LEG 1.30	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.6	38	Hot water for sanitary use 7
LEG 1.30	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.6	41	Hot water for sanitary use 5
LEG 1.33	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.5	42	Hot water for sanitary use 5
LEG 1.33	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.5	43	Hot water for sanitary use 7
LEG 1.33	*Legionella pneumophila* serogroup 1	Potable water sample	5 minutes 56°C	0.5	44	Fontain water 2
LEG 1.2	*Legionella pneumophila* serogroup 2-14	Clinical	Chlorination	0.8	47	Tap water 7
LEG 1.2	*Legionella pneumophila* serogroup 2-14	Clinical	Chlorination	0.8	48	Fontain water 3
LEG 1.14	*Legionella pneumophila* serogroup 12	Clinical	Chlorination	0.7	49	Tap water 7
LEG 1.14	*Legionella pneumophila* serogroup 12	Clinical	Chlorination	0.7	50	Fontain water 3
LEG 1.25	*Legionella pneumophila* serogroup 1	Potable water sample	Chlorination	0.7	51	Tap water 7
LEG 1.16	*Legionella pneumophila* serogroup 14	Clinical	Chlorination	0.9	54	Spring water 1
LEG 1.16	*Legionella pneumophila* serogroup 14	Clinical	Chlorination	0.9	55	Spring water 1
LEG 1.16	*Legionella pneumophila* serogroup 14	Clinical	Chlorination	0.9	56	Spring water 2
LEG 1.4	*Legionella pneumophila* serogroup 2	Clinical	7 minutes 56°C	0.6	58	Spring water 3
LEG 1.17	*Legionella pneumophila* serogroup 15	Clinical	Chlorination	0.7	59	Spring water 1
LEG 1.17	*Legionella pneumophila* serogroup 15	Clinical	Chlorination	0.7	60	Spring water 2

Industrial waters

Code	Strain	Origin	Stress applied	Stress intensity (log)	Sample number	Water
LEG 1.3	*Legionella pneumophila* serogroupoup 1	Clinical	3 minutes 56°C	0.5	1	Cooling water 1
LEG 1.3	*Legionella pneumophila* serogroupoup 1	Clinical	3 minutes 56°C	0.5	2	Cooling water 2
LEG 1.14	*Legionella pneumophila* serogroup 12	Clinical	5 minutes 56°C	0.6	3	Cooling water 1
LEG 1.14	*Legionella pneumophila* serogroup 12	Clinical	5 minutes 56°C	0.6	4	Cooling water 2
LEG 1.17	*Legionella pneumophila* serogroup 15	Clinical	Chlorination	0.7	5	Cooling water 1
LEG 1.17	*Legionella pneumophila* serogroup 15	Clinical	Chlorination	0.7	6	Cooling water 2
LEG 1.18	*Legionella pneumophila* serogroup 2-14	Cooling tower	Chlorination	0.9	17	Cooling water 4
LEG 1.9	*Legionella pneumophila* serogroup 7	Clinical	Chlorination	0.8	19	Cooling water 5
LEG 1.9	*Legionella pneumophila* serogroup 7	Clinical	Chlorination	0.8	20	Cooling water 6
LEG 1.9	*Legionella pneumophila* serogroup 7	Clinical	Chlorination	0.8	21	Cooling water 7
LEG 1.2	*Legionella pneumophila* serogroup 2-14	Clinical	Chlorination	0.6	22	Purification plant water (inlet)1
LEG 1.2	*Legionella pneumophila* serogroup 2-14	Clinical	Chlorination	0.6	23	Purification plant water (outflow) 1
LEG 1.20	*Legionella pneumophila* serogroupoup 1	Cooling tower	5 minutes 56°C	0.5	24	Cooling water 7
LEG 1.17	*Legionella pneumophila* serogroup 15	Clinical	10 minutes 56°C	0.5	25	Cooling water 7
LEG 1.17	*Legionella pneumophila* serogroup 15	Clinical	10 minutes 56°C	0.5	26	Water spray 1
LEG 1.18	*Legionella pneumophila* serogroup 2-14	Cooling tower	Chlorination	0.7	27	Purification plant water (inlet)1
LEG 1.18	*Legionella pneumophila* serogroup 2-14	Cooling tower	Chlorination	0.7	28	Purification plant water (outflow) 1
LEG 1.19	*Legionella pneumophila* serogroup 2-14	Cooling tower	7 minutes 56°C	0.6	29	Water spay 2
LEG 1.19	*Legionella pneumophila* serogroup 2-14	Cooling tower	7 minutes 56°C	0.6	30	Water spray 1
LEG 1.19	*Legionella pneumophila* serogroup 2-14	Cooling tower	Chlorination	0.7	32	Water spray 2
LEG 1.2	*Legionella pneumophila* serogroup 2-14	Clinical	3 minutes 56°C	0.5	35	Water spray 3
LEG 1.2	*Legionella pneumophila* serogroup 2-14	Clinical	3 minutes 56°C	0.5	36	Water spray 4
LEG 1.19	*Legionella pneumophila* serogroup 2-14	Cooling tower	Chlorination	0.8	39	Water spray 5
LEG 1.20	*Legionella pneumophila* serogroupoup 1	Cooling tower	Chlorination	0.6	40	Water spray 6

RESISTANCE PROTOCOL

The Legiolert non potable protocol employs a pretreatment step to increase selectivity and reduce interference from non-target organisms. Natural isolates of *L. pneumophila*, as found in environmental samples, are resistant to the pretreatment step and detected by Legiolert. In contrast. *L. pneumophila* cultures obtained by cultivation in the laboratory using typical agar plate methods are sensitive to the pretreatment and will not be detected by the Legiolert non potable protocol. This "resistance" protocol enables strains cultivated in the laboratory to be detected with the Legiolert non potable protocol. To allow working with spiked samples during the validation of the Legiolert non potable protocol, a Legiolert non potable resistance protocol should be applied to the culture used for spiking. This treatment has no effect on the reference methods.

Protocol:

Resistance Buffer Preparation

1- Add the chemicals in Table 1 to deionized water, mix on stir plate with stir bar for 30 minutes
2- Check pH to be 6.5±0.05, adjust with HCl or KOH as needed
3- Sterile filter and store at 4°C for up to 1 month

Table 1 : Buffer A composition

Chemical	Concentration (mM)	Concentration g/L	Sigma Product
Magnesium sulfate, anhydrous	4	0.481	M7506
Calcium chloride, dihydrate	0.4	0.059	C3881
Ferrous ammonium sulfate	0.051	0.020	F3754
Sodium monohydrogen phosphate	2.5	0.355	S7907
Potassium dihydrogen phosphate	2.5	0.340	P0662
Sodium citrate	0.42	0.090	71497

Bacterial Strain and culture

1- Revive strains onto designed agar medium and allow for adequate growth
   - *Legionella*: incubate on BCYE for 2 days at 36°C with humidity
   - Non-*Legionella*: incubate on blood-agar media for 1-2 days (as needed) at 36°C
2- Subculture 1 isolated colony from primary culture onto agar media and incubate as stated in 1
3- For each bacterial strain, add 7 mL of Buffer A to a 10 mL glass tube and prepare a cell suspension from the subculture in 2 having an optical density of 0.1±0.01 at 600 nm.
4- Loosely cap the tube and incubate without aeration in a rack at 36°C overnight (20-24 hours)
5- Gently vortex and dilute I buffer A to desired concentration for use

Appendix 4: Relative accuracy results

Relative accuracy: category “water for human consumption”

				MR : T90-431						MR : ISO 11731										MA : Legiolert				
Sample number	Sample	Strain	Stress	Directs	Filtration + acid treatment pH2 (5±0,5min)		Results			Directs		Filtration (without treatment)		Filtration + acid treatment: 30mL pH2.2±0,2 (5±0,5min)		Results			Reading					
				/	C100	C10	*Legionella* spp (CFU/L)	*Legionella pneumophila*		/	/ 1/10	C100ST	C10ST	C100	C10	*Legionella* spp (CFU/L)	*Legionella pneumophila*		*Legionella pneumophila*					
				0,2mL GVPC	filtration 100mL	filtration 10mL		Results (CFU/L)	sero-group	0,2mL GVPC	0,2 mL after dilution 1/10	filtration 100mL	filtration 10mL	filtration 100mL	filtration 10mL		Results (CFU/L)	serogroup	Big wells	Small wells	Dilution	Results (CFU/L)	sero-group
ILL 1 R1	Fontain water 1	LEG 1.1	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	2	2	/	4.70E+01	1
ILL 1 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	4	1	/	5.80E+01	1
ILL 2 R1	Hot water for sanitary use 1	LEG 1.11	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 2 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 3 R1	Hot water for sanitary use 2	LEG 1.11	Chlorination	>150	/	/	~~7.40E+06~~ >750000	~~7.40E+06~~ >750000	2.14	>150	138	/	/	/	/	~~7.40E+06~~ 6.90E+06	~~7.40E+06~~ 6.90E+06	2.14	6	90	/	>22726	2.14
ILL 3 R2				>150	/	/	~~7.10E+06~~ >750000	~~7.10E+06~~ >750000	2.14	>150	132	/	/	/	/	~~7.10E+06~~ 6.60E+06	~~7.10E+06~~ 6.60E+06	2.14	6	90	/	>22726	2.14
ILL 4 R1	Fontain water 3	LEG 1.1	Chlorination	>150	/	/	~~8.70E+06~~ >750000	~~8.70E+06~~ >750000	1	>150	128	/	/	/	/	~~8.70E+06~~ 6.40E+06	~~8.70E+06~~ 6.40E+06	1	6	90	/	>22726	1
ILL 4 R2				>150	/	/	~~8.00E+06~~ >750000	~~8.00E+06~~ >750000	1	>150	149	/	/	/	/	~~8.00E+06~~ 7.50E+06	~~8.00E+06~~ 7.50E+06	1	6	90	/	>22726	1
ILL 5 R1	Fontain water 1	LEG 1.11	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 5 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 6 R1	Tap water 8	LEG 1.11	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 6 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 7 R1	Hot water for sanitary use 4	LEG 1.1	Thermal stress	0	1	0	1.00E+01	**1.00E+01**	1	0	0	83	8	1	0	8.30E+02	**8.30E+02**	1	5	7	/	**2.46E+02**	1
ILL 7 R2				0	0	0	<10	<10	/	0	0	68	5	0	0	6.60E+02	**6.60E+02**	1	5	8	/	**2.75E+02**	1
ILL 8 R1	Hot water for sanitary use 5	LEG 1.1	Thermal stress	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 8 R2				1	0	0	5.00E+03	**5.00E+03**	1	1	0	0	0	0	0	5.00E+03	**5.00E+03**	1	0	0	/	<10	/
ILL 9 R1	Hot water for sanitary use 6	LEG.1.9	Chlorination	0	1	0	1.00E+01	**1.00E+01**	2.14	0	0	1	0	1	0	1.00E+01	**1.00E+01**	2.14	0	0	/	<10	/
ILL 9 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 10 R1	Hot water for sanitary use 7	LEG.1.9	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 10 R2				0	2	0	2.00E+01	**2.00E+01**	2.14	0	0	0	0	2	0	2.00E+01	**2.00E+01**	2.14	0	0	/	<10	/
ILL 11 R1	Tap water 9	LEG1.9	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 11 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 12 R1	Tap water 1	LEG1.15	Chlorination	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 12 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 13 R1	Fontain water 2	LEG 1.1	Thermal stress	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	2	5	/	8.40E+01	1
ILL 13 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	2	2	/	4.70E+01	1
ILL 14 R1	Tap water 2	LEG 1.1	Thermal stress	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	0	4	/	4.00E+01	1
ILL 14 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	2	4	/	7.20E+01	1
ILL 15 R1	Tap water 3	LEG 1.1	Thermal stress	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	2	4	/	7.20E+01	1
ILL 15 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	2	5	/	8.40E+01	1
ILL 16 R1	Tap water 4	LEG.1.9	Thermal stress	0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	4	5	/	1.41E+02	2.14
ILL 16 R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	1	5	/	6.50E+01	2.14
ILL 17 R1	Fontain water 2	LEG.1.9	Thermal stress	0	1	0	1.00E+01	**1.00E+01**	2.14	0	0	0	0	0	0	<10	<10	/	5	3	/	1.46E+02	2.14
ILL 17R2				0	0	0	<10	<10	/	0	0	0	0	0	0	<10	<10	/	5	2	/	1.24E+02	2.14
ILL 18 R1	Tap water 5	LEG.1.9	Thermal stress	0	2	0	2.00E+01	**1.00E+01**	2.14	0	0	0	0	0	0	<10	<10	/	3	3	/	7.90E+01	2.14
ILL 18 R2				0	1	0	1.00E+01	**2.00E+01**	2.14	0	0	0	0	0	0	<10	<10	/	5	1	/	1.04E+02	2.14
ILL 19 R1	Hot water for sanitary use 5	LEG 1.1	Thermal stress	/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 19 R2				/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 20 R1	Tap water 7	LEG 1.1	Thermal stress	/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 20 R2				/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	0	/	<10	/

Non interpretable results

Relative accuracy: category “water for human consumption” (continuation 1)

				MR : T90-431						MR : ISO 11731										MA : Legiolert				
Sample number	Sample	Strain	Stress	Directs	Filtration + acid treatment pH2 (5±0,5min)		Results			Directs		Filtration (without treatment)		Filtration + acid treatment: 30mL pH2.2±0,2 (5±0,5min)		Results				Reading				
				/	C100	C10	*Legionella* spp (CFU/L)	*Legionella pneumophila*		/	/ 1/10	C100ST	C10ST	C100	C10	*Legionella* spp (CFU/L)	*Legionella pneumophila*			*Legionella pneumophila*				
				0,2mL GVPC	filtration 100mL	filtration 10mL		Results (CFU/L)	sero-group	0,2mL GVPC	0.2 mL after dilution 1/10	filtration 100mL	filtration 10mL	filtration 100mL	filtration 10mL		Results (CFU/L)	serogroup	Big wells	Small wells	Dilution	Results (CFU/L)	sero-group
ILL 21 R1	Hot water for sanitary use	1	LEG 1.15	Thermal stress	/	0	0	<10	<10	/	/	/	1	0	0	0	1.00E+01	1.00E+01	2.14	1	0	/	1.10E+01	2.14
ILL 21 R2					/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	1	/	1.00E+01	2.14
ILL 22 R1	Hot water for sanitary use	1	LEG 1.15	Thermal stress	/	1	0	1.00E+01	1.00E+01	2.14	/	/	4	0	2	0	4.00E+01	1.00E+02	2.14	3	5	/	1.08E+02	2.14
ILL 22 R2					/	2	0	2.00E+01	2.00E+01	2.14	/	/	3	0	2	0	3.00E+01	3.00E+01	2.14	5	1	/	1.06E+02	2.14
ILL 23 R1	Tap water	5	LEG 1.9	Thermal stress	/	2	1	~~1.00E+03~~ 2.70E+01	~~1.00E+03~~ 2.70E+01	2.14	/	/	4	1	2	1	~~4.00E+01~~ 4.50E+01	~~4.00E+01~~ 4.50E+01	2.14	4	2	/	9.00E+01	2.14
ILL 23 R2					/	2	1	~~1.00E+03~~ 2.70E+01	~~1.00E+03~~ 2.70E+01	2.14	/	/	3	1	2	0	~~3.00E+01~~ 3.60E+01	~~3.00E+01~~ 3.60E+01	2.14	3	3	/	7.90E+01	2.14
ILL 24 R1	Hot water for sanitary use	2	LEG 1.9	Chlorination	/	4	1	~~1.00E+03~~ 4.50E+01	~~1.00E+03~~ 4.50E+01	2.14	/	/	9	1	4	0	~~1.00E+03~~ 9.10E+01	~~1.00E+03~~ 9.10E+01	2.14	0	0	/	<10	/
ILL 24 R2					/	0	0	<10	<10	/	/	/	5	0	0	0	5.00E+01	5.00E+01	2.14	0	0	/	<10	/
ILL 25 R1	Fontain water	3	LEG 1.11	Chlorination	/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	3	3	/	7.90E+01	2.14
ILL 25 R2					/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	1	3	/	4.30E+01	2.14
ILL 26 R1	Hot water for sanitary use	1	LEG 1.11	Thermal stress	/	11	1	1.10E+02	1.10E+02	2.14	/	/	5	0	11	1	1.10E+02	1.10E+02	2.14	2	9	/	1.35E+02	2.14
ILL 26 R2					/	8	0	8.00E+01	8.00E+01	2.14	/	/	4	0	8	0	8.00E+01	8.00E+01	2.14	5	8	/	2.75E+02	2.14
ILL 27 R1	Tap water	8	LEG 1.11	Thermal stress	/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	3	3	/	7.90E+01	2.14
ILL 27 R2					/	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	1	3	/	4.30E+01	2.14
ILL 28 R1	Tap water	8	LEG 1.32	Thermal stress	0	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 28 R2					0	0	0	<10	<10	/	/	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 29 R1	Hot water for sanitary use	4	LEG 1.32	Thermal stress	0	0	0	<10	<10	1	1	/	0	0	0	0	5.00E+03	5.00E+03	1	1	0	/	1.10E+01	1
ILL 29 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	2	0	/	2.30E+01	1
ILL 30 R1	Spring water	1	LEG 1.32	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	3	1	/	5.20E+02	1
ILL 30 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	3	2	/	6.60E+02	1
ILL 31 R1	Spring water	1	LEG 1.31	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 31 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	1	/	1.00E+01	1
ILL 32 R1	Hot water for sanitary use	1	LEG 1.31	Thermal stress	0	1	0	1.00E+01	1.00E+01	1	0	/	5	1	1	0	~~1.00E+02~~ 5.50E+01	~~1.00E+02~~ 5.50E+01	1	3	4	/	9.40E+01	1
ILL 32 R2					0	3	2	~~3.00E+02~~ 4.50E+01	~~3.00E+02~~ 4.50E+01	1	0	/	6	1	3	2	~~3.00E+03~~ 6.40E+01	~~3.00E+03~~ 6.40E+01	1	4	3		1.06E+02	1
ILL 33 R1	Fontain water	1	LEG 1.31	Thermal stress	0	48	6	~~6.00E+03~~ 4.90E+02	~~6.00E+03~~ 4.90E+02	1	0	/	37	5	53	6	~~6.00E+03~~ 5.40E+02	~~6.00E+03~~ 5.40E+02	1	4	3	/	1.06E+03	1
ILL 33 R2					0	51	7	~~7.00E+02~~ 5.30E+02	~~7.00E+03~~ 5.30E+02	1	0	/	32	6	56	8	~~8.00E+03~~ 5.80E+01	~~8.00E+03~~ 5.80E+01	1	6	2		1.87E+03	1
ILL 34 R1	Spring water	1	LEG 1.30	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	1	0	/	1.10E+01	1
ILL 34 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 35 R1	Spring water	1	LEG 1.30	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	4	3	/	1.06E+02	1
ILL 35 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	5	6	/	2.19E+02	1
ILL 36 R1	Spring water	1	LEG 1.30	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	6	5	/	3.10E+02	1
ILL 36 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	5	11	/	3.67E+02	1
ILL 37 R1	Tap water	5	LEG 1.30	Thermal stress	3	>100	51	1.50E+04	1.50E+04	1	3	/	>100	73	>100	48	1.50E+04	1.50E+04	1	6	83	/	1.29E+04	1
ILL 37 R2					4	>100	37	2.00E+04	2.00E+04	1	4	/	>100	56	>100	29	2.00E+04	2.00E+04	1	6	84		1.37E+04	1
ILL 38 R1	Hot water for sanitary use	7	LEG 1.30	Thermal stress	0	>100	17	1.70E+03	1.70E+03	1	0	/	>100	29	>100	18	2.90E+03	2.90E+03	1	6	57	/	5.07E+03	1
ILL 38 R2					0	>100	21	2.10E+03	2.10E+03	1	0	/	>100	33	>100	19	3.30E+03	3.30E+03	1	5	90		4.21E+03	1
ILL 39 R1	Spring water	2	LEG 1.30	Thermal stress	~~66~~	>100	>100	~~3.65E+05~~ 3.30E+05	~~3.65E+05~~ 3.30E+05	1	~~66~~	/	>100	>100	>100	>100	~~3.65E+05~~ 3.30E+05	~~3.65E+05~~ 3.30E+05	1	6	90	/	>227260	1
ILL 39 R2					53	>100	>100	~~3.35E+05~~ 2.70E+05	~~3.35E+05~~ 2.70E+05	1	53	/	>100	>100	>100	>100	~~3.35E+05~~ 2.70E+05	~~3.35E+05~~ 2.70E+05	1	6	90	/	>227260	1

Non interpretable results

Relative accuracy : category “water for human consumption” (continuation 2)

Sample number	Sample		Strain	Stress	MR : T90-431						MR : ISO 11731									MA : Legiolert				
					Directs	Filtration + acid treatment pH2 (5±0,5min)		Results			Directs		Filtration (without treatment)		Filtration + acid treatment: 30mL pH2.2±0,2 (5±0,5min)		Results			Reading				
					/	C100	C10	*Legionella* spp (CFU/L)	*Legionella pneumophila*		/	/ 1/10	C100ST	C10ST	C100	C10	*Legionella* spp (CFU/L)	*Legionella pneumophila*		*Legionella pneumophila*				
					0,2mL GVPC	filtration 100mL	filtration 10mL		Results (CFU/L)	sero-group	0,2mL GVPC	0.2 mL after dilution 1/10	filtration 100mL	filtration 10mL	filtration 100mL	filtration 10mL		Results (CFU/L)	serogroup	Big wells	Small wells	Dilution	Results (CFU/L)	sero-group
ILL 40 R1	Spring water	2	LEG 1.30	Chlorination	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 40 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 41 R1	Hot water for sanitary use	5	LEG 1.30	Thermal stress	0	2	0	2.00E+01	2.00E+01	1	0	/	3	0	0	0	3.00E+01	3.00E+01	1	3	5	/	1.08E+02	1
ILL 41 R2					0	1	0	1.00E+01	1.00E+01	1	0	/	2	0	0	0	2.00E+01	2.00E+01	1	3	5		1.08E+02	1
ILL 42 R1	Hot water for sanitary use	5	LEG 1.33	Thermal stress	19	>100	>100	9.50E+04	9.50E+04	1	19	/	>100	>100	>100	>100	9.50E+04	9.50E+04	1	6	70	1/10	7.60E+04	1
ILL 42 R2					21	>100	>100	~~1.05E+05~~ 1.10E+05	~~1.05E+05~~ 1.10E+05	1	21	/	>100	>100	>100	>100	~~1.05E+05~~ 1.10E+05	~~1.05E+05~~ 1.10E+05	1	6	69		7.35E+04	1
ILL 43 R1	Hot water for sanitary use	7	LEG 1.33	Thermal stress	11	>100	29	5.50E+04	5.50E+04	1	11	/	>100	>100	>100	43	5.50E+04	5.50E+04	1	6	45	1/10	3.50E+04	1
ILL 43 R2					9	>100	37	4.50E+04	4.50E+04	1	9	/	>100	>100	>100	39	4.50E+04	4.50E+04	1	5	73		3.10E+04	1
ILL 44 R1	Fontain water	2	LEG 1.33	Thermal stress	42	>100	>100	2.10E+05	2.10E+05	1	42	/	>100	>100	>100	>100	2.10E+05	2.10E+05	1	6	87	1/10	1.72E+05	1
ILL 44 R2					49	>100	>100	~~2.45E+05~~ 2.50E+05	~~2.45E+05~~ 2.50E+05	1	49	/	>100	>100	>100	>100	~~2.45E+05~~ 2.50E+05	~~2.45E+05~~ 2.50E+05	1	6	88		1.92E+05	1
ILL 45 R1	Spring water	2	LEG 1.33	Chlorination	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 45 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 46 R1	Spring water	2	LEG 1.33	Chlorination	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 46 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0	/	<10	/
ILL 47 R1	Tap water	7	LEG 1.2	Chlorination	24	>100	>100	1.20E+05	1.20E+05	2.14	24	/	>100	>100	>100	>100	1.20E+05	1.20E+05	2.14	6	78	1/10	1.02E+05	2.14
ILL 47 R2					27	>100	>100	~~1.35E+05~~ 1.40E+05	~~1.35E+05~~ 1.40E+05	2.14	27	/	>100	>100	>100	>100	~~1.35E+05~~ 1.40E+05	~~1.35E+05~~ 1.40E+05	2.14	6	84		1.37E+05	2.14
ILL 48 R1	Fontain water	3	LEG 1.2	Chlorination	0	>100	15	1.50E+03	1.50E+03	2.14	0	/	>100	18	>100	15	1.50E+03	1.50E+03	2.14	6	60	/	5.55E+03	2.14
ILL 48 R2					0	>100	17	1.70E+03	1.70E+03	2.14	0	/	>100	21	>100	17	1.70E+03	1.70E+03	2.14	5	88		4.06E+03	2.14
ILL 49 R1	Tap water	7	LEG 1.14	Chlorination	29	>100	>100	~~1.45E+05~~ 1.50E+05	~~1.45E+05~~ 1.50E+05	2.14	29	/	>100	>100	>100	>100	~~1.45E+05~~ 1.50E+05	~~1.45E+05~~ 1.50E+05	2.14	6	81	1/10	1.16E+05	2.14
ILL 49 R2					31	>100	>100	~~1.55E+05~~ 1.60E+05	~~1.55E+05~~ 1.60E+05	2.14	31	/	>100	>100	>100	>100	~~1.55E+05~~ 1.60E+05	~~1.55E+05~~ 1.60E+05	2.14	6	83		1.29E+05	2.14
ILL 50 R1	Fontain water	3	LEG 1.14	Chlorination	0	>100	19	1.90E+03	1.90E+03	2.14	0	/	>100	29	>100	18	2.90E+03	2.90E+03	2.14	6	64	/	6.27E+03	2.14
ILL 50 R2					0	>100	23	2.30E+03	2.30E+03	2.14	0	/	>100	33	>100	21	3.30E+03	3.30E+03	2.14	6	66		6.68E+03	2.14
ILL 51 R1	Tap water	7	LEG 1.25	Chlorination	142	>100	>100	7.10E+05	7.10E+05	1	142	/	>100	>100	>100	>100	7.10E+05	7.10E+05	1	6	68	1/100	7.12E+05	1
ILL 51 R2					118	>100	>100	5.90E+05	5.90E+05	1	118	/	>100	>100	>100	>100	5.90E+05	5.90E+05	1	6	56		4.92E+05	1
ILL 52 R1	Spring water	1	LEG 1.25	Chlorination	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0		<10	/
ILL 52 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0		<10	/
ILL 53 R1	Spring water	2	LEG 1.16	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0		<10	/
ILL 53 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0		<10	/
ILL 54 R1	Spring water	1	LEG.1.16	Chlorination	2	>100	15	1.00E+04	1.00E+04	2.14	2	/	>100	21	114	12	1.00E+04	1.00E+04	2.14	6	75	/	9.05E+03	2.14
ILL 54 R2					2	98	13	1.00E+04	1.00E+04	2.14	2	/	>100	17	104	14	1.00E+04	1.00E+04	2.14	6	2		8.13E+03	2.14
ILL 55 R1	Spring water	1	LEG.1.16	Chlorination	1	>100	11	5.00E+03	5.00E+03	2.14	1	/	>100	37/6	>100	10	5.00E+03	5.00E+03	2.14	6	56	/	4.92E+03	2.14
ILL 55 R2					1	>100	12	5.00E+03	5.00E+03	2.14	1	/	>100	30/4	>100	11	5.00E+03	5.00E+03	2.14	6	56		4.92E+03	2.14
ILL 56 R1	Spring water	2	LEG.1.16	Chlorination	11	>100	>100	5.50E+04	5.50E+04	2.14	11	/	>100	>100	>100	>100	5.50E+04	5.50E+04	2.14	6	55	1/10	4.77E+04	2.14
ILL 56 R2					9	>100	>100	4.50E+04	4.50E+04	2.14	9	/	>100	>100	>100	>100	4.50E+04	4.50E+04	2.14	6	51		4.22E+04	2.14
ILL 57 R1	Spring water	2	LEG 1.4	Thermal stress	0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0		<10	/
ILL 57 R2					0	0	0	<10	<10	/	0	/	0	0	0	0	<10	<10	/	0	0		<10	/
ILL 58 R1	Spring water	3	LEG.1.4	Thermal stress	2	87	11	1.00E+04	1.00E+04	2.14	2	/	>100	13	>100	9	1.00E+04	1.00E+04	2.14	6	74	/	8.72E+03	2.14
ILL 58 R2					2	96	11	1.00E+04	1.00E+04	2.14	3	/	>100	13	>100	10	1.00E+04	1.00E+04	2.14	6	83		1.29E+04	2.14
ILL 59 R1	Spring water	1	LEG.1.17	Chlorination	6	>100	9	3.00E+04	3.00E+04	2.14	6	/	>100	17	>100	10	3.00E+04	3.00E+04	2.14	6	89	/	2.27E+04	/
ILL 59 R2					5	>100	9	2.50E+04	2.50E+04	2.14	5	/	>100	24	>100	12	2.50E+04	2.50E+04	2.14	6	88		1.92E+04	/
ILL 60 R1	Spring water	2	LEG.1.17	Chlorination	0	27	3	~~2.00E+03~~ 2.70E+02	~~2.00E+03~~ 2.70E+02	2.14	/	/	34	4	23	3	~~4.00E+03~~ 3.50E+02	~~4.00E+03~~ 3.50E+02	2.14	6	11	/	7.23E+02	/
ILL 60 R2					0	24	2	2.40E+02	2.40E+02	2.14	/	/	38	3	31	3	~~3.00E+03~~ 3.70E+02	~~3.00E+03~~ 3.70E+02	2.14	5	18	/	6.06E+02	/

Relative accuracy human consumption water: Idexx Legiolert versus NF T90-431

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
22	10.00	20.00	15.00	7.07	1	108.00	104.00	106.00	2.83	91.000
23	27.00	27.00	27.00	0.00	2	90.00	79.00	84.50	7.78	57.500
26	110.00	80.00	95.00	21.21	3	135.00	275.00	205.00	98.99	110.000
32	10.00	45.00	27.50	24.75	4	94.00	106.00	100.00	8.49	72.500
33	490.00	530.00	510.00	28.28	5	1060.00	1870.00	1465.00	572.76	955.000
37	15000.00	20000.00	17500.00	3535.53	6	12900.00	13700.00	13300.00	565.69	-4200.000
38	1700.00	2100.00	1900.00	282.84	7	5070.00	4210.00	4640.00	608.11	2740.000
41	20.00	10.00	15.00	7.07	8	108.00	108.00	108.00	0.00	93.000
42	95000.00	110000.00	102500.00	10606.60	9	76000.00	73500.00	74750.00	1767.77	-27750.000
43	55000.00	45000.00	50000.00	7071.07	10	35000.00	31000.00	33000.00	2828.43	-17000.000
44	210000.00	250000.00	230000.00	28284.27	11	172000.00	192000.00	182000.00	14142.14	-48000.000
47	120000.00	140000.00	130000.00	14142.14	12	102000.00	137000.00	119500.00	24748.74	-10500.000
48	1500.00	1700.00	1600.00	141.42	13	5550.00	4060.00	4805.00	1053.59	3205.000
49	150000.00	160000.00	150000.00	7071.07	14	116000.00	129000.00	122500.00	9192.39	-27500.000
50	1900.00	2300.00	2100.00	282.84	15	6270.00	6680.00	6475.00	289.91	4375.000
51	710000.00	590000.00	650000.00	84852.81	16	712000.00	492000.00	602000.00	155563.49	-48000.000
54	10000.00	10000.00	10000.00	0.00	17	9050.00	8130.00	8590.00	650.54	-1410.000
55	5000.00	5000.00	5000.00	0.00	18	4920.00	4920.00	4920.00	0.00	-80.000
56	55000.00	45000.00	50000.00	7071.07	19	47700.00	42200.00	44950.00	3889.09	-5050.000
58	10000.00	10000.00	10000.00	0.00	20	8720.00	12900.00	10810.00	2955.71	810.000
59	30000.00	25000.00	27500.00	3535.53	21	22700.00	19200.00	20950.00	2474.87	-6550.000
60	270.00	240.00	255.00	21.21	22	723.00	606.00	664.50	82.73	409.500

q=	22	Mx=	65411.114		My=	57087.409	M=	-8323.705	
n=	2	MEDx=	7500.000		MEDy=	7532.500	MED=	-11.250	
N=qn=	44	SDbx=	144333.470		SDby=	131426.973		Biais	
		MEDwx =		212.132	MEDwy =	629.325			
		SDwx=		19641.888	SDwy=	33804.762			
		rob. SDwx=		314.507	rob. SDwy=	933.037			

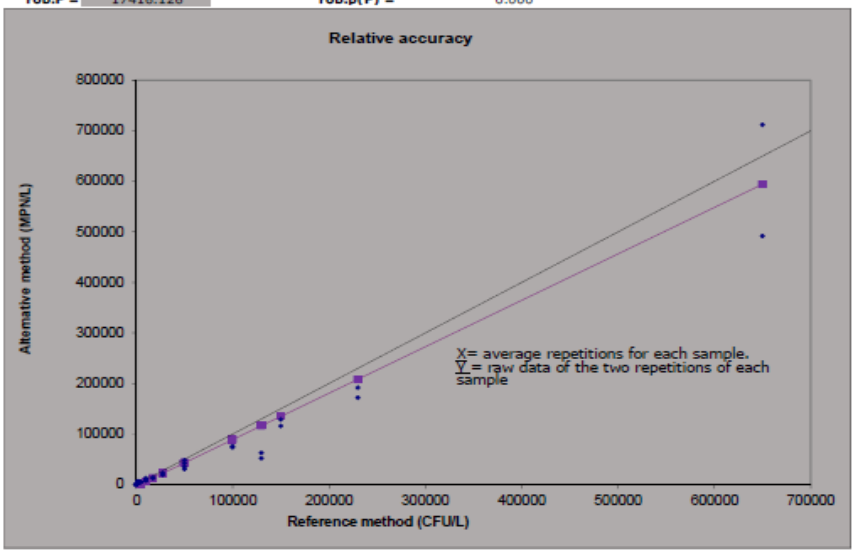
Regression used
OLS1

R=	1.721	Sx=	143476.561
rob. R=	2.967	Sy=	132121.229
r=	0.998		
b=	0.919		
a=	-3022.965	Res. SD=	28642.446
S(b)=	0.030	p(t;b=1)=	0.0110
S(a)=	4755.068	p(t;a=0)=	0.5284
		t(b)=	2.662
		t(a)=	0.636

Repeatability	Reference method	Alternative method
r	54997.285	9465.335
rob. r	880.619	2612.504

Linearity

F =	3.366	p(F) =	0.003
rob.F =	17416.126	rob.p(F) =	0.000



Mx	Alt	Est Y	Deviation
15.000	108.000	-3009.180	3117.180
27.000	90.000	-2998.153	3088.153
95.000	135.000	-2935.663	3070.663
27.500	94.000	-2997.693	3091.693
510.000	1060.000	-2554.294	3614.294
17500.000	12900.000	13058.883	-158.883
1900.000	5070.000	-1276.936	6346.936
15.000	108.000	-3009.180	3117.180
100000.000	76000.000	88873.306	-12873.306
50000.000	35000.000	42925.170	-7925.170
230000.000	172000.000	208338.457	-36338.457
130000.000	52200.000	116442.187	-64242.187
1600.000	5550.000	-1552.624	7102.624
150000.000	116000.000	134821.441	-18821.441
2100.000	6270.000	-1093.143	7363.143
650000.000	712000.000	594302.792	117697.208
10000.000	9050.000	6166.662	2883.338
5000.000	4920.000	1571.849	3348.151
50000.000	47700.000	42925.170	4774.830
10000.000	8720.000	6166.662	2553.338
27500.000	22700.000	22248.510	451.490
255.000	723.000	-2788.629	3511.629
15.000	104.000	-3009.180	3113.180
27.000	79.000	-2998.153	3077.153
95.000	275.000	-2935.663	3210.663
27.500	106.000	-2997.693	3103.693
510.000	1870.000	-2554.294	4424.294
17500.000	13700.000	13058.883	641.117
1900.000	4210.000	-1276.936	5486.936
15.000	108.000	-3009.180	3117.180
100000.000	73500.000	88873.306	-15373.306
50000.000	31000.000	42925.170	-11925.170
230000.000	192000.000	208338.457	-16338.457
130000.000	62700.000	116442.187	-53742.187
1600.000	4060.000	-1552.624	5612.624
150000.000	129000.000	134821.441	-5821.441
2100.000	6680.000	-1093.143	7773.143
650000.000	492000.000	594302.792	-102302.792
10000.000	8130.000	6166.662	1963.338
5000.000	4920.000	1571.849	3348.151
50000.000	42200.000	42925.170	-725.170
10000.000	12900.000	6166.662	6733.338
27500.000	19200.000	22248.510	-3048.510
255.000	606.000	-2788.629	3394.629

Relative accuracy human consumption water: Idexx Legiolert versus NF T90-431 (LOG)

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
22	1.00	1.30	1.15	0.21	1	2.03	2.02	2.03	0.01	0.875
23	1.43	1.43	1.43	0.00	2	1.95	1.90	1.93	0.04	0.495
26	2.04	1.90	1.97	0.10	3	2.13	2.44	2.28	0.22	0.313
32	1.00	1.65	1.33	0.46	4	1.97	2.03	2.00	0.04	0.673
33	2.69	2.72	2.71	0.02	5	3.03	3.27	3.15	0.17	0.441
37	4.18	4.30	4.24	0.09	6	4.11	4.14	4.12	0.02	-0.115
38	3.23	3.32	3.28	0.06	7	3.71	3.62	3.66	0.06	0.388
41	1.30	1.00	1.15	0.21	8	2.03	2.03	2.03	0.00	0.883
42	4.98	5.04	5.01	0.05	9	4.88	4.87	4.87	0.01	-0.136
43	4.74	4.65	4.70	0.06	10	4.54	4.49	4.52	0.04	-0.179
44	5.32	5.40	5.36	0.05	11	5.24	5.28	5.26	0.03	-0.101
47	5.08	5.15	5.11	0.05	12	5.01	5.14	5.07	0.09	-0.040
48	3.18	3.23	3.20	0.04	13	3.74	3.61	3.68	0.10	0.473
49	5.18	5.20	5.19	0.02	14	5.06	5.11	5.09	0.03	-0.103
50	3.28	3.36	3.32	0.06	15	3.80	3.82	3.81	0.02	0.491
51	5.85	5.77	5.81	0.06	16	5.85	5.69	5.77	0.11	-0.039
54	4.00	4.00	4.00	0.00	17	3.96	3.91	3.93	0.03	-0.067
55	3.70	3.70	3.70	0.00	18	3.69	3.69	3.69	0.00	-0.007
56	4.74	4.65	4.70	0.06	19	4.68	4.63	4.65	0.04	-0.045
58	4.00	4.00	4.00	0.00	20	3.94	4.11	4.03	0.12	0.026
59	4.48	4.40	4.44	0.06	21	4.36	4.28	4.32	0.05	-0.118
60	2.43	2.38	2.41	0.04	22	2.86	2.78	2.82	0.05	0.415

q= 22
n= 2
N=qn= 44

Mx= 3.554
MEDx= 3.849
SDbx= 1.475
MEDwx = 0.055
SDwx= 0.127
rob. SDwx= 0.081

My= 3.760
MEDy= 3.872
SDby= 1.172
MEDwy = 0.037
SDwy= 0.080
rob. SDwy= 0.056

M= 0.206
MED= 0.009
Bias

Regression used
GMFR

R= 0.633
rob. R= 0.684

Sx= 1.460
Sy= 1.160

r= 0.992
b= 0.794
a= 0.937

Res. SEM= 0.151
Res. SD= 0.214

S(b)= 0.023
S(a)= 0.100

p(t;b=1)= 0.0000
p(t;a=0)= 0.0000

t(b)= 9.104
t(a)= 9.371

1.71074E-09

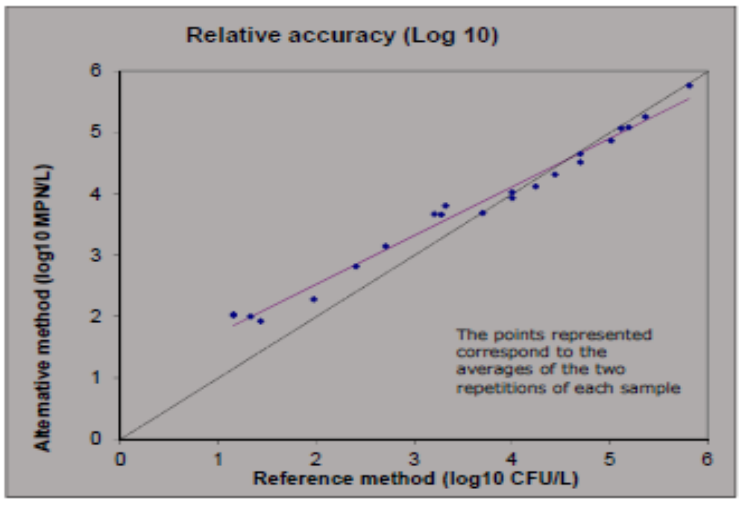
Est. y	Dev.
1.851	0.174
2.074	-0.148
2.503	-0.219
1.991	0.009
3.087	0.061
4.303	-0.180
3.539	0.125
1.851	0.183
4.916	-0.042
4.667	-0.150
5.194	0.065
4.998	0.075
3.481	0.195
5.059	0.028
3.574	0.237
5.552	0.220
4.114	-0.181
3.875	-0.183
4.667	-0.015
4.114	-0.088
4.461	-0.142
2.848	-0.027

Repeatability	Reference method	Alternative method
r	0.355	0.225
rob. r	0.227	0.155

Linearity

F = 4.877
rob.F = 13.480

p(F) = 0.000
rob.p(F) = 0.000



Relative accuracy – water for human consumption: IDEXX Legiolert versus NF T90-431 after concentration by filtration

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
22	10.00	20.00	15.00	7.07	1	108.00	104.00	106.00	2.83	91.000
23	27.00	27.00	27.00	0.00	2	90.00	79.00	84.50	7.78	57.500
26	110.00	80.00	95.00	21.21	3	135.00	275.00	205.00	98.99	110.000
32	10.00	45.00	27.50	24.75	4	94.00	106.00	100.00	8.49	72.500
33	490.00	530.00	510.00	28.28	5	1060.00	1870.00	1465.00	572.76	955.000
38	1700.00	2100.00	1900.00	282.84	7	5070.00	4210.00	4640.00	608.11	2740.000
41	20.00	10.00	15.00	7.07	8	108.00	108.00	108.00	0.00	93.000
48	1500.00	1700.00	1600.00	141.42	13	5550.00	4060.00	4805.00	1053.59	3205.000
50	1900.00	2300.00	2100.00	282.84	15	6270.00	6680.00	6475.00	289.91	4375.000
60	270.00	240.00	255.00	21.21	22	723.00	606.00	664.50	82.73	409.500

q=	10	Mx=	654.450		My=	1865.300		M=	1210.850
n=	2	MEDx=	175.000		MEDy=	434.750		MED=	259.750
N=qn=	20	SDbx=	858.489		SDby=	2458.989			Biais
			MEDwx =	22.981		MEDwy =	90.863		
			SDwx=	135.060		SDwy=	436.891		
			rob. SDwx=	34.072		rob. SDwy=	134.714		

Regression used
OLS1

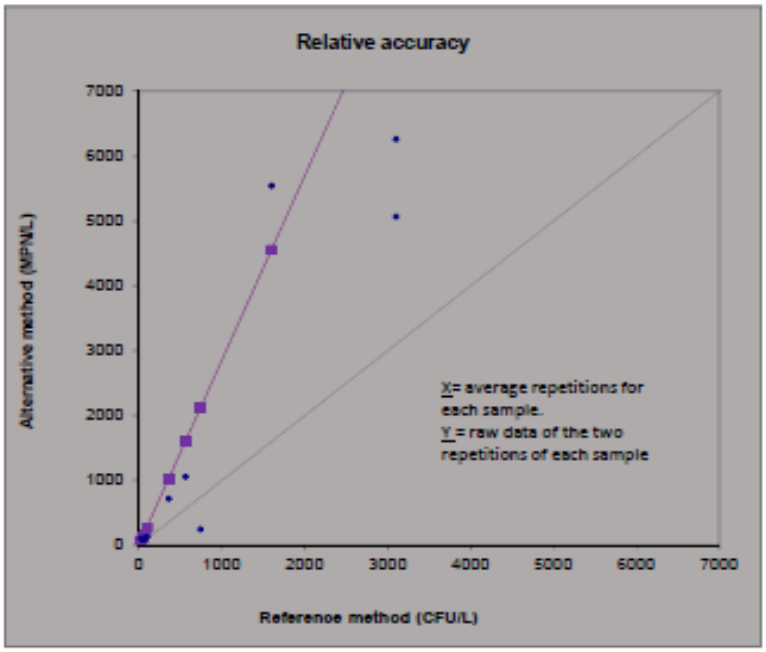
R=	3.235		
rob. R=	3.954		
		Sx=	541.317
		Sy=	2414.300
r=	0.992		
b=	2.845		
a=	3.141	Res. SD=	1624.896
S(b)=	0.443	p(t;b=1)=	0.0006
S(a)=	464.867	p(t;a=0)=	0.9947
		t(b)=	4.165
		t(a)=	0.007

Repeatability	Reference method	Alternative method
r	278.168	1222.294
rob. r	98.400	377.199

Mx	Alt	Est Y	Deviation
745.000	246.00	2122.949	-1876.949
35.000	108.00	102.729	5.271
40.500	90.00	118.379	-28.379
95.000	138.00	273.452	-138.452
89.500	94.00	172.441	-78.441
560.000	1060.00	1596.554	-536.554
3100.000	5070.00	8823.819	-3753.819
25.000	108.00	74.276	33.724
1600.000	5550.00	4555.749	994.251
3100.000	6270.00	8823.819	-2553.819
365.000	723.00	1013.251	-290.251
745.000	246.00	2122.949	-1876.949
35.000	108.00	102.729	5.271
40.500	90.00	118.379	-28.379
95.000	138.00	273.452	-138.452
89.500	94.00	172.441	-78.441
560.000	1060.00	1596.554	-536.554
3100.000	5070.00	8823.819	-3753.819
25.000	108.00	74.276	33.724
1600.000	5550.00	4555.749	994.251
3100.000	6270.00	8823.819	-2553.819
365.000	723.00	1013.251	-290.251

Linearity

F =	324.421	p(F) =	0.000
rob.F =	5116.144	rob.p(F) =	0.000



Relative accuracy – water for human consumption: IDEXX Legiolert versus NF T90-431 (LOG) after concentration by filtration

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
22	1.00	1.30	1.15	0.21	1	2.03	2.02	2.03	0.01	0.875
23	1.43	1.43	1.43	0.00	2	1.95	1.90	1.93	0.04	0.495
26	2.04	1.90	1.97	0.10	3	2.13	2.44	2.28	0.22	0.313
32	1.00	1.65	1.33	0.46	4	1.97	2.03	2.00	0.04	0.67
33	2.69	2.72	2.71	0.02	5	3.03	3.27	3.15	0.17	0.441
38	3.23	3.32	3.28	0.06	7	3.71	3.62	3.66	0.06	0.388
41	1.30	1.00	1.15	0.21	8	2.03	2.03	2.03	0.00	0.883
48	3.18	3.23	3.20	0.04	13	3.74	3.61	3.68	0.10	0.473
50	3.28	3.36	3.32	0.06	15	3.80	3.82	3.81	0.02	0.491
60	2.43	2.38	2.41	0.04	22	2.86	2.78	2.82	0.05	0.415

q= 10
n= 2
Nmqn= 20

Mx= 2.194
MEDx= 2.189
SDbx= 0.902

MEDwx = 0.062
SDwx= 0.180
rob. SDwx= 0.092

My= 2.739
MEDy= 2.553
SDby= 0.780

MEDwy = 0.047
SDwy= 0.098
rob. SDwy= 0.070

M= 0.545
MED= 0.482
Bias

Regression used
GMFR

R= 0.547
rob. R= 0.763

Sx= 0.888
Sy= 0.763

r= 0.982
b= 0.859
a= 0.853

Res. SEM= 0.155
Res. SD= 0.219

S(b)= 0.058
S(a)= 0.150

p(t;b=1)= 0.0260
p(t;a=0)= 0.0000

t(b)= 2.426
t(a)= 5.703

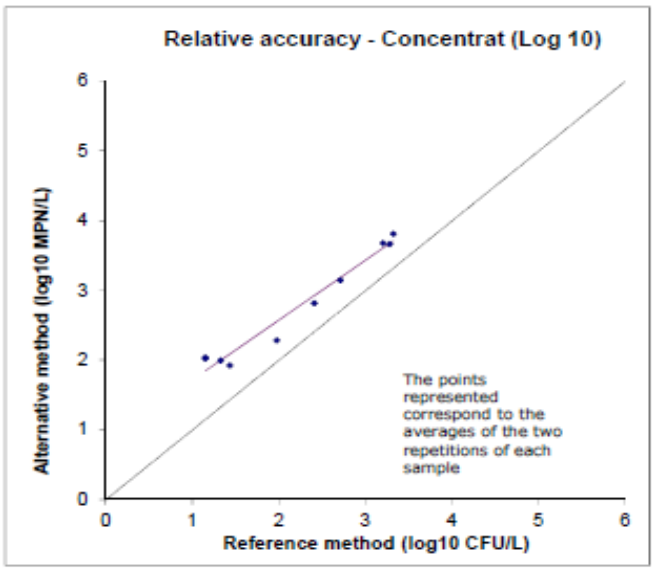
Est. y	Dev.
1.842	0.183
2.083	-0.157
2.548	-0.263
1.993	0.006
3.180	-0.031
3.669	-0.004
1.842	0.191
3.606	0.071
3.706	0.105
2.921	-0.100

Repeatability	Reference method	Alternative method
r	0.504	0.276
rob. r	0.256	0.196

Linearity

F = 2.064
rob.F = 11.568

p(F) = 0.140
rob.p(F) = 0.000



Relative accuracy – water for human consumption water: IDEXX Legiolert versus NF T90-431 direct plating

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
37	15000.00	20000.00	17500.00	3535.53	6	12900.00	13700.00	13300.00	565.69	-4200.000
42	95000.00	105000.00	100000.00	7071.07	9	76000.00	73500.00	74750.00	1767.77	-25250.000
43	55000.00	45000.00	50000.00	7071.07	10	35000.00	31000.00	33000.00	2828.43	-17000.000
44	210000.00	250000.00	230000.00	28284.27	11	172000.00	192000.00	182000.00	14142.14	-48000.000
47	120000.00	135000.00	127500.00	10606.60	12	102000.00	137000.00	119500.00	24748.74	-8000.000
49	150000.00	160000.00	155000.00	7071.07	14	116000.00	129000.00	122500.00	9192.39	-32500.000
51	710000.00	590000.00	650000.00	84852.81	16	712000.00	492000.00	602000.00	155563.49	-48000.000
54	10000.00	10000.00	10000.00	0.00	17	9050.00	8130.00	8590.00	650.54	-1410.000
55	5000.00	5000.00	5000.00	0.00	18	4920.00	4920.00	4920.00	0.00	-80.000
56	55000.00	45000.00	50000.00	7071.07	19	47700.00	42200.00	44950.00	3889.09	-5050.000
58	10000.00	10000.00	10000.00	0.00	20	8720.00	12900.00	10810.00	2955.71	810.000
59	30000.00	25000.00	27500.00	3535.53	21	22700.00	19200.00	20950.00	2474.87	-6550.000

q= 12
n= 2
N=qn= 24

Mx= 119375.000
MEDx= 50000.000
SDbx= 181148.875
MEDwx = 7071.068
SDwx= 26358.901
rob. SDwx= 10483.565

My= 103105.833
MEDy= 38975.000
SDby= 166998.218
MEDwy = 2892.067
SDwy= 45770.127
rob. SDwy= 4287.778

M= -16269.167
MED= -7275.000
Bias

Regression used
OLS2 y=REF

R= 1.736
rob. R= 0.409

Sx= 178187.203
Sy= 166639.909

r= 0.998
b= 1.067
a= 9350.520

Res. SD= 28133.201

S(b)= 0.033
S(a)= 6958.669

p(t;b=1)= 0.0537
p(t;a=0)= 0.1927

t(b)= 2.038
t(a)= 1.344

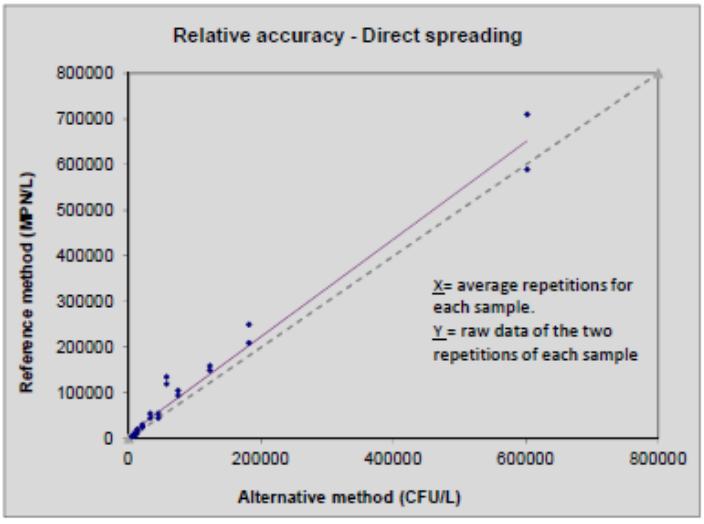
Repeatability	Reference method	Alternative method
r	73804.923	128156.356
rob. r	0.000	12005.779

Mx	Alt	Est Y	Deviation
13300.000	15000.00	23542.981	-8542.981
74750.000	95000.00	89116.423	5883.577
33000.000	55000.00	44564.898	10435.102
182000.000	210000.00	203563.153	6436.847
57450.000	120000.00	70655.551	49344.449
122500.000	150000.00	140070.561	9929.439
602000.000	710000.00	651746.154	58253.846
8590.000	10000.00	18516.929	-8516.929
4920.000	5000.00	14600.663	-9600.663
44950.000	55000.00	57316.772	-2316.772
10810.000	10000.00	20885.896	-10885.896
20950.000	30000.00	31706.315	-1706.315
13300.00	20000.00	23542.981	-3542.981
74750.00	105000.00	89116.423	15883.577
33000.00	45000.00	44564.898	435.102
182000.00	250000.00	203563.153	46436.847
57450.00	135000.00	70655.551	64344.449
122500.00	160000.00	140070.561	19929.439
602000.00	590000.00	651746.154	-61746.154
8590.00	10000.00	18516.929	-8516.929
4920.00	5000.00	14600.663	-9600.663
44950.00	45000.00	57316.772	-12316.772
10810.00	10000.00	20885.896	-10885.896
20950.00	25000.00	31706.315	-6706.315

Linearity

F = 0.369
rob.F = 93.510

p(F) = 0.938
rob.p(F) = 0.000



Relative accuracy – water for human consumption: IDEXX Legiolert versus NF T90-431 (LOG) direct spreading

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
37	4.18	4.30	4.24	0.09	6	4.11	4.14	4.12	0.02	-0.115
42	4.98	5.02	5.00	0.03	9	4.88	4.87	4.87	0.01	-0.126
43	4.74	4.65	4.70	0.06	10	4.54	4.49	4.52	0.04	-0.179
44	5.32	5.40	5.36	0.05	11	5.24	5.28	5.26	0.03	-0.101
47	5.08	5.13	5.10	0.04	12	5.01	5.14	5.07	0.09	-0.032
49	5.18	5.20	5.19	0.02	14	5.06	5.11	5.09	0.03	-0.103
51	5.85	5.77	5.81	0.06	16	5.85	5.69	5.77	0.11	-0.039
54	4.00	4.00	4.00	0.00	17	3.96	3.91	3.93	0.03	-0.067
55	3.70	3.70	3.70	0.00	18	3.69	3.69	3.69	0.00	-0.007
56	4.74	4.65	4.70	0.06	19	4.68	4.63	4.65	0.04	-0.045
58	4.00	4.00	4.00	0.00	20	3.94	4.11	4.03	0.12	0.026
59	4.48	4.40	4.44	0.06	21	4.36	4.28	4.32	0.05	-0.118

q=	12	Mx=	4.686		My=	4.611		M=	-0.075
n=	2	MEDx=	4.697		MEDy=	4.585		MED=	-0.084
N=qn=	24	SDbx=	0.634		SDby=	0.622		Biais	
			MEDwx =	0.045		MEDwy =	0.036		
			SDwx=	0.048		SDwy=	0.061		
			rob. SDwx=	0.067		rob. SDwy=	0.053		

Regression used
GMFR

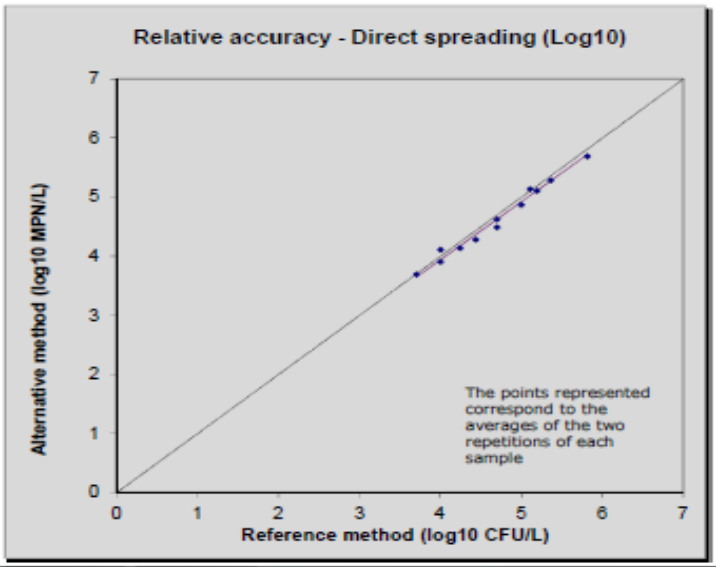
R=	1.281
rob. R=	0.792
Sx=	0.621
Sy=	0.610
r=	0.99591
b=	0.98188
a=	0.00950
Res. SEM=	0.059
Res. SD=	0.084
S(b)=	0.029
S(a)=	0.035
p(t;b=1)=	0.5342
p(t;a=0)=	0.7898
t(b)=	0.631
t(a)=	0.270

Est. y	Dev.
4.171	-0.048
4.918	-0.045
4.621	-0.103
5.272	-0.013
5.022	0.051
5.106	-0.018
5.715	0.057
3.937	-0.004
3.641	0.051
4.621	0.031
3.937	0.089
4.367	-0.047
0.009	-0.009
4.611	0.000

Repeatability	Reference method	Alternative method
r	0.134	0.171
rob. r	0.186	0.147

Linearity

F =	5.565
rob.F =	2.277
p(F) =	0.003
rob.p(F) =	0.089



Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
7	830.00	660.00	745.00	120.21	7	246.00	275.00	260.50	20.51	-484.500
22	40.00	30.00	35.00	7.07	1	108.00	104.00	106.00	2.83	71.000
23	45.00	36.00	40.50	6.36	2	90.00	79.00	84.50	7.78	44.000
26	110.00	80.00	95.00	21.21	3	135.00	275.00	205.00	98.99	110.000
32	55.00	64.00	59.50	6.36	4	94.00	106.00	100.00	8.49	40.500
33	540.00	580.00	560.00	28.28	5	1060.00	1870.00	1465.00	572.76	905.000
37	15000.00	20000.00	17500.00	3535.53	6	12900.00	13700.00	13300.00	565.69	-4200.000
38	2900.00	3300.00	3100.00	282.84	7	5070.00	4210.00	4640.00	608.11	1540.000
41	30.00	20.00	25.00	7.07	8	108.00	108.00	108.00	0.00	83.000
42	95000.00	110000.00	102500.00	10606.60	9	76000.00	73500.00	74750.00	1767.77	-27750.000
43	55000.00	45000.00	50000.00	7071.07	10	35000.00	31000.00	33000.00	2828.43	-17000.000
44	210000.00	250000.00	230000.00	28284.27	11	172000.00	192000.00	182000.00	14142.14	-48000.000
47	120000.00	140000.00	130000.00	14142.14	12	102000.00	137000.00	119500.00	24748.74	-10500.000
48	1500.00	1700.00	1600.00	141.42	13	5550.00	4060.00	4805.00	1053.59	3205.000
49	150000.00	160000.00	155000.00	7071.07	14	116000.00	129000.00	122500.00	9192.39	-32500.000
50	2900.00	3300.00	3100.00	282.84	15	6270.00	6680.00	6475.00	289.91	3375.000
51	710000.00	590000.00	650000.00	84852.81	16	712000.00	492000.00	602000.00	155563.49	-48000.000
54	10000.00	10000.00	10000.00	0.00	17	9050.00	8130.00	8590.00	650.54	-1410.000
55	5000.00	5000.00	5000.00	0.00	18	4920.00	4920.00	4920.00	0.00	-80.000
56	55000.00	45000.00	50000.00	7071.07	19	47700.00	42200.00	44950.00	3889.09	-5050.000
58	10000.00	10000.00	10000.00	0.00	20	8720.00	12900.00	10810.00	2955.71	810.000
59	30000.00	25000.00	27500.00	3535.53	21	22700.00	19200.00	20950.00	2474.87	-6550.000
60	340.00	370.00	355.00	21.21	22	723.00	606.00	664.50	82.73	309.500

q= 23
n= 2
N=qn= 46

Mx= 62922.391
MEDx= 5000.000
SDbx= 141755.121
MEDwx = 141.421
SDwx= 19210.161
rob. SDwx= 209.671

My= 54616.674
MEDy= 6475.000
SDby= 128950.823
MEDwy = 608.112
SDwy= 33061.710
rob. SDwy= 901.587

M= -8305.717
MED= -80.000
Bias

Regression used
OLS1

R= 1.721
rob. R= 4.300

Sx= 140842.412
Sy= 129662.228

r= 0.998
b= 0.919
a= -3193.481

Res. SD= 27633.259

S(b)= 0.029
S(a)= 4470.658

p(t;b=1)= 0.0000
p(t;a=0)= 0.4788

t(b)= 2.778
t(a)= 0.714

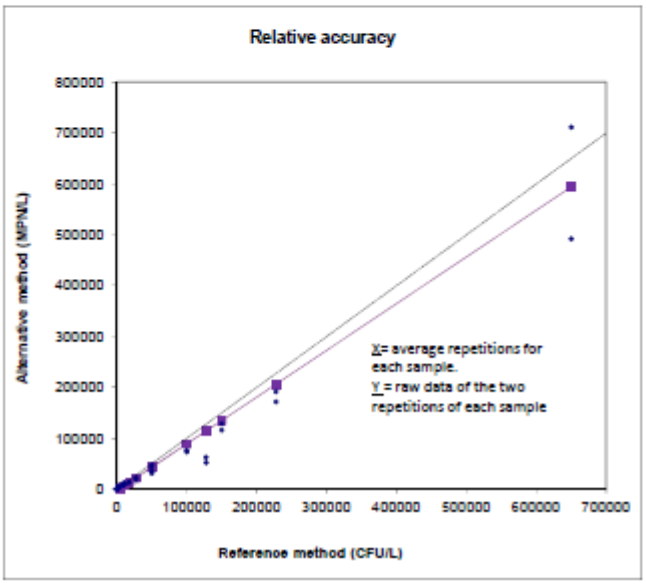
Repeatability	Reference method	Alternative method
r	53758.455	92572.758
rob. r	587.080	2524.442

Linearity

F = 3.240
rob.F = 36392.051

p(F) = 0.004
rob.p(F) = 0.000

Mx	Alt	Est Y	Deviation
665.000	246.000	-2398.760	3644.760
35.000	108.000	-3161.325	3269.325
100.000	90.000	-3101.606	3191.606
95.000	135.000	-3106.200	3241.200
105.000	94.000	-3097.012	3191.012
650.000	1060.000	-2596.292	3656.292
17500.000	12900.000	12884.702	15.298
3100.000	5070.000	-345.346	5415.346
25.000	108.000	-3170.513	3278.513
100000.000	76000.000	88681.850	-12681.850
50000.000	35000.000	42744.184	-7744.184
227500.000	172000.000	205822.897	-33822.897
127500.000	62700.000	113947.566	-51247.566
1600.000	5550.000	-1723.476	7273.476
150000.000	116000.000	134619.515	-18619.515
3100.000	6270.000	-345.346	6615.346
650000.000	712000.000	593996.171	118003.829
10000.000	9050.000	5994.052	3055.948
5000.000	4920.000	1400.285	3519.715
50000.000	47700.000	42744.184	4955.816
10000.000	8720.000	5994.052	2725.948
27500.000	22700.000	22072.235	627.765
350.000	723.000	-2871.918	3594.918
665.000	275.000	-2398.760	2673.760
35.000	104.000	-3161.325	3265.325
100.000	79.000	-3101.606	3180.606
95.000	275.000	-3106.200	3381.200
105.000	106.000	-3097.012	3203.012
650.000	1870.000	-2596.292	4466.292
17500.000	13700.000	12884.702	815.298
3100.000	4210.000	-345.346	4555.346
25.000	108.000	-3170.513	3278.513
100000.000	73500.000	88681.850	-15181.850
50000.000	31000.000	42744.184	-11744.184
227500.000	192000.000	205822.897	-13822.897
127500.000	62700.000	113947.566	-51247.566
1600.000	4060.000	-1723.476	5783.476
150000.000	129000.000	134619.515	-5619.515
3100.000	6680.000	-345.346	7025.346
650000.000	492000.000	593996.171	-101996.171
10000.000	8130.000	5994.052	2135.948
5000.000	4920.000	1400.285	3519.715
50000.000	42200.000	42744.184	-544.184
10000.000	12900.000	5994.052	6905.948
27500.000	19200.000	22072.235	-2872.235
350.000	606.000	-2871.918	3477.918



Relative accuracy – water for human consumption: IDEXX Legiolert versus ISO 11731 (LOG)

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
7	2.92	2.82	2.87	0.07	7.00	2.39	2.39	2.39	0.00	-0.478
22	1.60	1.48	1.54	0.09	1	2.03	2.02	2.03	0.01	0.486
23	1.65	1.56	1.60	0.07	2	1.95	1.90	1.93	0.04	0.331
26	2.04	1.90	1.97	0.10	3	2.13	2.44	2.28	0.22	0.313
32	1.74	1.81	1.77	0.05	4	1.97	2.03	2.00	0.04	0.226
33	2.72	2.76	2.75	0.02	5	3.02	3.27	3.15	0.17	0.401
37	4.18	4.30	4.24	0.09	6	4.11	4.14	4.12	0.02	-0.115
38	3.46	3.32	3.39	0.04	7	3.71	3.62	3.66	0.06	0.274
41	1.48	1.30	1.39	0.12	8	2.03	2.03	2.03	0.00	0.644
42	4.98	5.04	5.01	0.05	9	4.88	4.87	4.87	0.01	-0.136
43	4.74	4.65	4.70	0.06	10	4.54	4.49	4.52	0.04	-0.179
44	5.32	5.40	5.36	0.05	11	5.24	5.28	5.26	0.03	-0.101
47	5.08	5.15	5.11	0.05	12	5.01	5.14	5.07	0.09	-0.040
48	3.18	3.23	3.20	0.04	13	3.74	3.61	3.68	0.10	0.473
49	5.18	5.20	5.19	0.02	14	5.06	5.11	5.09	0.03	-0.103
50	3.46	3.32	3.39	0.04	15	3.80	3.82	3.81	0.02	0.321
51	5.85	5.77	5.81	0.06	16	5.85	5.89	5.87	0.11	-0.059
54	4.00	4.00	4.00	0.00	17	3.96	3.91	3.93	0.03	-0.067
55	3.70	3.70	3.70	0.00	18	3.69	3.69	3.69	0.00	-0.007
56	4.74	4.65	4.70	0.06	19	4.68	4.63	4.66	0.04	-0.045
58	4.00	4.00	4.00	0.00	20	3.94	4.11	4.03	0.12	0.026
59	4.48	4.40	4.44	0.06	21	4.36	4.28	4.32	0.06	-0.118
60	2.83	2.87	2.85	0.03	22	2.86	2.78	2.82	0.06	0.271

q= 23
n= 2
Nmqn= 46

Mx= 3.604
MEDx= 3.699
SDbx= 1.333
MEDwx = 0.047
SDwx= 0.059
rob. SDwx= 0.070

My= 3.700
MEDy= 3.811
SDby= 1.180
MEDwy = 0.037
SDwy= 0.079
rob. SDwy= 0.055

M= 0.097
MED= -0.007
Bias

Regression used
GMFR

Rx= 1.333
rob. Rx= 0.787
Sx= 1.339
Sy= 1.168

r= 0.986
b= 0.873
a= 0.555
Res. SEM= 0.136
Res. SD= 0.192

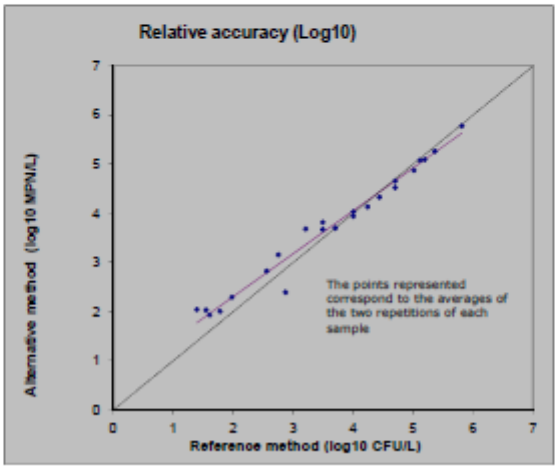
S(b)= 0.022
S(a)= 0.089
p(t;b=1)= 0.000
p(t;a=0)= 0.000
t(b)= 5.874
t(a)= 6.245

Est. y	Dev.
3.060	-0.669
1.899	0.136
1.956	-0.030
2.272	0.008
2.103	-0.104
2.954	0.195
4.399	-0.131
3.602	0.063
1.768	0.266
4.928	-0.054
4.656	-0.137
5.233	0.036
5.018	0.055
2.581	0.255
5.085	0.002
5.003	0.209
5.627	0.145
4.046	-0.113
3.784	-0.042
4.655	-0.002
4.046	-0.021
4.428	-0.109
2.781	0.040

Repeatability	Reference method	Alternative method
r	0.188	0.220
rob. r	0.197	0.155

Linearity

F = 21.258
rob.F = 14.643
p(F) = 0.000
rob.p(F) = 0.000



Relative accuracy – water for human consumption: IDEXX Legiolert versus ISO 11731 after concentration by filtration

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
7	850.00	660.00	745.00	120.21	7	246.00	275.00	260.50	20.51	-484.500
22	40.00	30.00	35.00	7.07	1	108.00	104.00	106.00	2.83	71.000
23	45.00	36.00	40.50	6.36	2	90.00	79.00	84.50	7.78	44.000
28	110.00	80.00	95.00	21.21	3	138.00	275.00	206.50	98.99	110.000
32	55.00	64.00	59.50	6.36	4	94.00	106.00	100.00	8.49	40.500
33	540.00	580.00	560.00	28.28	5	1060.00	1870.00	1465.00	572.76	905.000
38	2900.00	3300.00	3100.00	282.84	7	5070.00	4210.00	4640.00	608.11	1540.000
41	30.00	20.00	25.00	7.07	8	108.00	108.00	108.00	0.00	83.000
48	1500.00	1700.00	1600.00	141.42	13	5550.00	4060.00	4805.00	1053.59	3205.000
50	2900.00	3300.00	3100.00	282.84	15	6270.00	6680.00	6475.00	289.91	3375.000
60	340.00	370.00	355.00	21.21	22	723.00	606.00	664.50	82.73	309.500

qn=	11	Mx=	883.182			My=	1719.409			Mn=	836.227
nn=	2	MEDx=	355.000			MEDy=	260.500			MED=	110.000
Nnqnn=	22	SDbx=	1192.082			SDby=	2382.455			Bias	
			MEDwx =	21.213			MEDwy =	82.731			
			SDwx=	133.597			SDwy=	416.606			
			rob. SDwx=	31.451			rob. SDwy=	122.658			

Regression used
OLS1

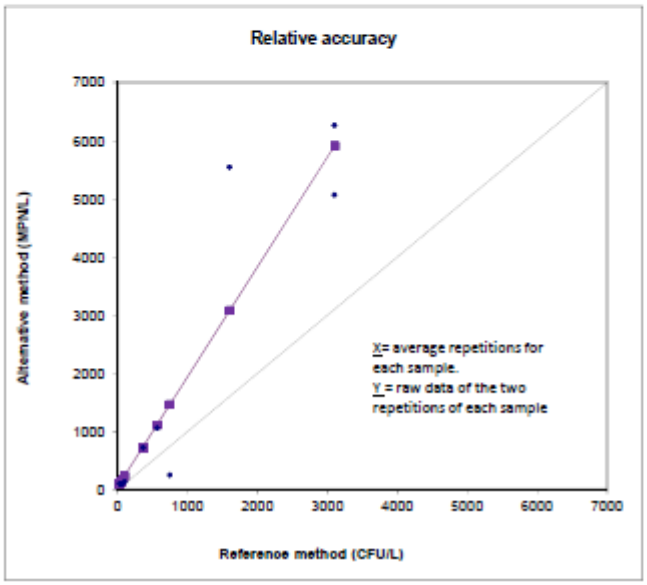
R=	3.118	Sx=	1167.364
rob. R=	3.900	Sy=	2344.507
r=	0.941		
b=	1.890		
a=	50.243	Res. SD=	878.436
S(b)=	0.164	p(t;b=1)=	0.0000
S(a)=	236.870	p(t;a=0)=	0.8342
		t(b)=	5.420
		t(a)=	0.212

Repeatability	Reference method	Alternative method
r	374.073	1166.494
rob. r	88.982	343.442

Mx	Alt	Est Y	Deviation
745.000	246.00	1458.253	-1212.253
35.000	108.00	116.391	-8.391
40.500	90.00	126.786	-36.786
95.000	138.00	229.788	-94.788
59.500	94.00	162.695	-68.695
560.000	1060.00	1108.613	-48.613
3100.000	5070.00	5909.076	-839.076
25.000	108.00	97.492	10.508
1600.000	5550.00	3074.157	2475.843
3100.000	6270.00	5909.076	360.924
355.000	723.00	721.174	1.826
745.000	246.00	1458.253	-1212.253
35.000	108.00	116.391	-8.391
40.500	90.00	126.786	-36.786
95.000	138.00	229.788	-94.788
59.500	94.00	162.695	-68.695
560.000	1060.00	1108.613	-48.613
3100.000	5070.00	5909.076	-839.076
25.000	108.00	97.492	10.508
1600.000	5550.00	3074.157	2475.843
3100.000	6270.00	5909.076	360.924
355.000	723.00	721.174	1.826

Linearity

F =	94.853	p(F) =	0.000
rob.F =	1732.371	rob.p(F) =	0.000



Relative accuracy – water for human consumption: IDEXX Legiolert versus ISO 11731 (LOG) after concentration by filtration

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
7	2.92	2.82	2.87	0.07	7.00	2.39	2.44	2.42	0.03	-0.454
22	1.60	1.48	1.54	0.09	22	2.03	2.02	2.03	0.01	0.486
23	1.65	1.56	1.60	0.07	23	1.95	1.90	1.93	0.04	0.321
26	2.04	1.90	1.97	0.10	26	2.13	2.44	2.28	0.22	0.313
32	1.74	1.81	1.77	0.05	32	1.97	2.03	2.00	0.04	0.226
33	2.73	2.76	2.75	0.02	33	3.03	3.27	3.15	0.17	0.401
38	3.48	3.52	3.49	0.04	38	3.71	3.62	3.66	0.06	0.174
41	1.48	1.30	1.39	0.12	41	2.03	2.03	2.03	0.00	0.644
48	3.18	3.23	3.20	0.04	48	3.74	3.61	3.68	0.10	0.473
50	3.46	3.52	3.49	0.04	50	3.80	3.82	3.81	0.02	0.321
60	2.93	2.87	2.85	0.03	60	2.86	2.78	2.82	0.05	0.271

q=	11	Mx=	2.421		My=	2.710		M=	0.289	
n=	2	MEDx=	2.550		MEDy=	2.415		MED=	0.321	
Nmqn=	22	SDbx=	0.797		SDby=	0.747		Bias		
		MEDwx =		0.047		MEDwy =	0.040			
		SDwx=		0.068		SDwy=	0.094			
		rob. SDwx=		0.069		rob. SDwy=	0.059			

Regression used
GMFR

Rr=	1.395		
rob. Rr=	0.860		
		Sx=	0.779
		Sy=	0.732
r=	0.93629		
b=	0.93916	Res. SEM=	0.281
a=	0.43592	Res. SD=	0.398

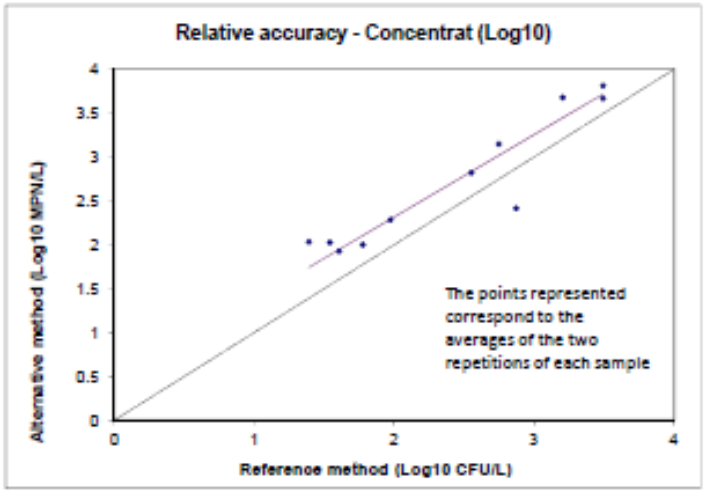
S(b)=	0.114	p(t;b=1)=	0.5999	t(b)=	0.533
S(a)=	0.251	p(t;a=0)=	0.0975	t(a)=	1.736

Est. y	Dev.
3.131	-0.766
1.802	0.143
1.942	-0.017
2.208	-0.003
2.101	-0.102
3.017	0.172
3.714	-0.049
1.740	0.293
2.444	0.232
3.714	0.097
2.851	-0.016

Repeatability	Reference method	Alternative method
r	0.190	0.265
rob. r	0.193	0.166

Linearity

F =	75.443	p(F) =	0.000
rob.F =	72.660	rob.p(F) =	0.000



Relative accuracy : category “industrial waters”

				MR: NF T90-431											MR: ISO 11731											MA: LEGIOLERT				
					Directs		Concentration by filtration				Confirmation	Results				Directs		Concentration by filtration				Confirmation	Results				Reading 1			
					/	/ 1/10	C	CTT	CTA	CTTA			Legionella pneumophila			/	/ 1/10	C	CTT	CTA	CTTA			Legionella pneumophila		Legionella pneumophila			
SN	Sample	Strain	Stress	Type of colonie (showed in the table below)	0,2mL GVPC	0.2 mL after dilution 1/10	Non treated (0.1 mL)	Heat-treatment (50±1°C, 30±1min)	Acide treatment ph2 (V/V: 5±0,5min)	Associated treatment (50±1°C, 30±1min puis V/V ph2: 5±0,5min)	Type of colonie confirmed	Legionella spp (CFU/L)	Results (CFU/L)	zero-group	Type of colonie (showed in the table below)	0,2mL GVPC	0.2 mL after dilution 1/10	non treated (0.1mL)	Heat-treatment (50±1°C, 30±2min)	Acid treatment ph2,2±0,2 (1V / 9V 5±0,5min)	Associated treatment (50±1°C, 30±1min puis V/V ph2: 5±0,5min)	Type of colonie confirmed	Legionella spp (CFU/L)	Results (CFU/L)	zero-group	Big wells	Small wells	Results (CFU/L)	zero-group
ILL 1 R1	Cooling water 1	LEG 1.3	Thermal stress	type a/b	6	2	/	/	/	/	a	3.00E+04	3.00E+04	1	type a/b	6	2	/	/	/	/	a	3.00E+04	3.00E+04	1	6	5	3.10E+04	1
ILL 1 R2				type a/b	8	0	/	/	/	/	a	4.00E+04	4.00E+04	1	type a/b	8	0	/	/	/	/	a	4.00E+04	4.00E+04	1	6	6	3.61E+04	1
ILL 2 R1	Cooling water 2	LEG 1.3	Thermal stress	type a/b	22/1	2	/	/	/	/	a	1.10E+05	1.10E+05	1	type a/b	22/1	2	/	/	/	/	a	1.10E+05	1.10E+05	1	6	14	8.54E+04	1
ILL 2 R2				type a/b	30/2	4	/	/	/	/	a	1.50E+05	1.50E+05	1	type a/b	30/2	4	/	/	/	/	a	1.50E+05	1.50E+05	1	6	22	1.42E+05	1
ILL 3 R1	Cooling water 1	LEG 1.14	Thermal stress	type a/b	35/2	3	/	/	/	/	a	1.75E+05	1.75E+05	2.14	type a/b	35	3	/	/	/	/	a	1.75E+05	1.75E+05	2.14	6	25	1.64E+05	2.14
ILL 3 R2				type a/b	35	2	/	/	/	/	a	1.75E+05	1.75E+05	2.14	type a/b	35	2	/	/	/	/	a	1.75E+05	1.75E+05	2.14	6	24	1.72E+05	2.14
ILL 4 R1	Cooling water 2	LEG 1.14	Thermal stress	type a/b	>150	20	/	/	/	/	a	1.00E+06	1.00E+06	2.14	type a/b	>150	20	/	/	/	/	a	1.00E+06	1.00E+06	2.14	6	75	9.05E+05	2.14
ILL 4 R2				type a/b	>150	21	/	/	/	/	a	1.05E+06	1.05E+06	2.14	type a/b	>150	21	/	/	/	/	a	1.05E+06	1.05E+06	2.14	6	73	8.42E+05	2.14
ILL 5 R1	Cooling water 1	LEG 1.17	Thermal stress	type a/b	5	2	/	/	/	/	a	2.50E+04	2.50E+04	/	type a/b	5	2	/	/	/	/	a	2.50E+04	2.50E+04	/	6	4	2.23E+04	/
ILL 5 R2				type a/b	3	0	/	/	/	/	a	1.50E+04	1.50E+04	/	type a/b	3	0	/	/	/	/	a	1.50E+04	1.50E+04	/	5	2	1.24E+04	/
ILL 6 R1	Cooling water 2	LEG 1.17	Thermal stress	type a/b	19	2	/	/	/	/	a	9.50E+04	9.50E+04	/	type a/b	19	2	/	/	/	/	a	9.50E+04	9.50E+04	/	6	13	7.88E+04	/
ILL 6 R2				type a/b	22	1	/	/	/	/	a	1.10E+05	1.10E+05	/	type a/b	22	1	/	/	/	/	a	1.10E+05	1.10E+05	/	6	15	9.21E+04	/
ILL 7 R1	Cooling water 3	LEG 1.19	Thermal stress	type a/b	/	/	0/1	0/1	1/1	0/1	a	5.00E+01	5.00E+01	2.14	type a	/	/	0/1	0/1	0	/	a	<100	<100	2.14	0	0	<1000	2.14
ILL 7 R2				type a/b	/	/	0	0/2	0	0	/	<100	<100	/	type a	/	/	0	0/2	0	/	/	<100	<100	/	0	0	<1000	/
ILL 8 R1	Cooling water 3	LEG 1.19	Thermal stress	type a/b	/	/	0/1	1/1	1	0/3	a	1.00E+02	1.00E+02	2.14	type a	/	/	0/1	1/1	0	/	a	<100	<100	2.14	0	0	<1000	2.14
ILL 8 R2				type a/b	/	/	0/4	0/5	0	0	/	<100	<100	/	type a	/	/	0/4	0/5	0	/	a	<100	<100	/	0	0	<1000	/
ILL 9 R1	Cooling water 3	LEG 1.19	Thermal stress	type a/b	0	/	1/2	1/1	0/4	0	a	1.00E+03	1.00E+03	2.14	type a	0	/	1/2	1/1	0	/	a	1.00E+02	1.00E+02	2.14	0	0	<1000	2.14
ILL 9 R2				type a/b	0	/	0/4	0	0	0	/	<1000	<1000	/	type a	0	/	0/4	0	0	/	/	<100	<100	/	0	0	<1000	/
ILL 10 R1	Cooling water 3	LEG 1.19	Thermal stress	type a/b	0	/	0/15	0/1	2/3	0	a	1.00E+02	1.00E+02	2.14	type a	0	/	0/15	0/1	0/1	/	a	<100	<100	2.14	0	0	<1000	2.14
ILL 10 R2				type a/b	0	/	0/13	0/4	0/2	0	/	<100	<100	/	type a	0	/	0/13	0/4	0/2	/	/	<100	<100	/	0	0	<1000	/
ILL 11 R1	Cooling water 3	LEG 1.20	Thermal stress	type a/b	/	/	0/>150	0	0	0/1	/	<100	<100	/	type a	/	/	0/>150	0	0	/	/	<100	<100	/	0	0	<1000	/
ILL 11 R2				type a/b	/	/	0/>150	0/2	0	0	/	<100	<100	/	type a	/	/	0/>150	0/2	0	/	/	<100	<100	/	0	0	<1000	/
ILL 12 R1	Cooling water 3	LEG 1.20	Thermal stress	type a/b	/	/	0/>150	0	0/1	0/1	/	<100	<100	/	type a	/	/	0/>150	0	0	/	/	<100	<100	/	0	0	<1000	/
ILL 12 R2				type a/b	/	/	0/>150	0	0	0	/	<100	<100	/	type a	/	/	0/>150	0	0	/	/	<100	<100	/	0	0	<1000	/
ILL 13 R1	Cooling water 3	LEG 1.20	Thermal stress	type a/b	0/59	/	0/>150	0/3	0/4	0/2	a	<100	<100	/	type a	0/59	/	0/>150	0/3	0/1	/	a	5.00E+03	5.00E+03	/	0	0	<1000	/
ILL 13 R2				type a/b	0/76	/	0/>150	0/5	0/3	0/1	/	<100	<100	/	type a	0/76	/	0/>150	0/5	0	/	/	<100	<100	/	0	0	<1000	/
ILL 14 R1	Cooling water 3	LEG 1.20	Thermal stress	type a/b	0/126	/	0/>150	0/3	1/2	0/1	a	1.00E+02	1.00E+02	1	type a	0/126	/	0/>150	0/3	0	/	/	<100	<100	1	0	0	<1000	1
ILL 14 R2				type a/b	0/165	/	0/>150	0/1	0/2	0/4	/	<100	<100	/	type a	0/165	/	0/>150	0/1	0	/	/	<100	<100	/	0	0	<1000	/
ILL 15 R1	Cooling water 4	LEG 1.18	Chlorination	type a/b	/	/	6/16	0/2	1/3	0/1	a	6.00E+02	6.00E+02	2.14	type a	/	/	6/16	0/2	0	/	a	6.00E+02	6.00E+02	2.14	0	0	<1000	2.14
ILL 15 R2				type a/b	/	/	4/22	0/1	0	0	a	4.00E+02	4.00E+02	2.14	type a	/	/	4/22	0/1	0	/	a	4.00E+02	4.00E+02	2.14	0	0	<1000	2.14
ILL 16 R1	Cooling water 4	LEG 1.18	Chlorination	type a/b	/	/	1/6	0/1	2/3	0/2	a	1.00E+03	1.00E+03	2.14	type a	/	/	1/6	0/1	0/1	/	a	1.00E+02	1.00E+02	2.14	0	0	<1000	2.14
ILL 16 R2				type a/b	/	/	2/3	0/3	0	0	a	2.00E+02	2.00E+02	2.14	type a	/	/	2/3	0/3	0	/	a	2.00E+02	2.00E+02	2.14	0	0	<1000	2.14
ILL 17 R1	Cooling water 4	LEG 1.18	Chlorination	type a/b	1	/	7/9	0/3	4/7	0/6	a	5.00E+03	5.00E+03	2.14	type a/b	1	/	7/9	0/3	0/1	/	a	5.00E+03	5.00E+03	2.14	2	1	3.50E+03	2.14
ILL 17 R2				type a/b	1	/	9/17	0/1	4/5	1/4	a	5.00E+03	5.00E+03	2.14	type a/b	1	/	9/17	0/1	0/2	/	a	5.00E+03	5.00E+03	2.14	2	1	3.50E+03	2.14
ILL 18 R1	Cooling water 4	LEG 1.18	Chlorination	type a	0/1	/	4/8	1/2	7/3	0/4	a	4.00E+02	4.00E+02	2.14	type a	0/1	/	4/8	1/2	7/3	/	a	1.00E+03	1.00E+03	2.14	0	0	<1000	2.14
ILL 18 R2				type a	0	/	8/14	2/1	6/3	0/3	a	8.00E+02	8.00E+02	2.14	type a	0	/	8/14	2/1	6/3	/	a	8.00E+02	8.00E+02	2.14	0	0	<1000	2.14
ILL 19 R1	Cooling water 5	LEG 1.9	Chlorination	type a/b	2	/	63/3	17	33	8	a	1.00E+04	1.00E+04	2.14	type a/b	2	/	63/3	17	6	/	a	1.00E+04	1.00E+04	2.14	6	2	9.00E+03	2.14
ILL 19 R2				type a/b	2	/	79/4	13	30	10	a	1.00E+04	1.00E+04	2.14	type a/b	2	/	79/4	13	5	/	a	1.00E+04	1.00E+04	2.14	5	1	1.04E+04	2.14
ILL 20 R1	Cooling water 6	LEG 1.9	Chlorination	type a/b	14	1	/	/	/	/	a	7.00E+04	7.00E+04	2.14	type a/b	14	1	/	/	/	/	a	7.00E+04	7.00E+04	2.14	6	9	5.34E+04	2.14
ILL 20 R2				type a/b	19	1	/	/	/	/	a	9.50E+04	9.50E+04	2.14	type a/b	19	1	/	/	/	/	a	9.50E+04	9.50E+04	2.14	6	13	7.88E+04	2.14

Non interpretable results

V/V: Volume/Volume

1/10 dilution 1/10 of the sample before spreading

Relative accuracy: category “industrial waters” (continuation)

				MR: NF T90-431											MR: ISO 11731											MA: LEGIOLERT				
					Directs		Concentration by filtration				Confirmation	Results				Directs		Concentration by filtration				Confirmation	Results			Reading 1			
					/	/ 1/10	C	CTT	CTA	CTTA			Legionella pneumophila			/	/ 1/10	C	CTT	CTA	CTTA			Legionella pneumophila		Legionella pneumophila			
SN	Sample	Strain	Stress	Type of colonie (showed in the table below)	0,2mL GVPC	0.2 mL after dilution 1/10	Non treated (0.1 mL)	Heat-treatment (50±1°C, 30±1min)	Acide treatment ph2 (V/V: 5±0,5min)	Associated treatment (50±1°C, 30±1min puis V/V ph2: 5±0,5min)	Type of colonie confirmed	Legionella spp (CFU/L)	Results (CFU/L)	sero-group	Type of colonie (showed in the table below)	0,2mL GVPC	0.2 mL after dilution 1/10	non treated (0.1mL)	Heat-treatment (50±1°C, 30±2min)	Acid treatment ph2,2±0,2 (1V / 9V 5±0,5min)	Associated treatment (50±1°C, 30±1min puis V/V ph2: 5±0,5min)	Type of colonie confirmed	Legionella spp (CFU/L)	Results (CFU/L)	sero-group	Big wells	Small wells	Results (CFU/L)	sero-group
ILL21 R1	Cooling water 7	LEG 1.9	Chlorination	type a/b	139	14	/	/	/	/	a	7.00E+05	7.00E+05	2.14	type a/b	139	14	/	/	/	/	a	7.00E+05	7.00E+05	2.14	6	71	7.86E+05	2.14
ILL21 R2				type a/b	118	11	/	/	/	/	a	5.90E+05	5.90E+05	2.14	type a/b	118	11	/	/	/	/	a	5.90E+05	5.90E+05	2.14	6	51	4.23E+05	2.14
ILL22 R1	Purification plant water (inlet)1	LEG 1.2	Chlorination	type a/b	0	/	29/48	57	54	33/2	a	2.90E+03	2.90E+03	2.14	type a/b	0	/	29/48	57	7	/	a	2.90E+03	2.90E+03	2.14	3	2	6.60E+03	2.14
ILL22 R2				type a/b	0	/	16/22	29	26	18	a	1.60E+03	1.60E+03	2.14	type a/b	0	/	16/22	29	4	/	a	1.60E+03	1.60E+03	2.14	3	0	3.90E+03	2.14
ILL23 R1	Purification plant water (outflow) 1	LEG 1.2	Chlorination	type a/b	8	2	/	/	/	/	a	4.00E+04	4.00E+04	2.14	type a/b	8	2	/	/	/	/	a	4.00E+04	4.00E+04	2.14	6	8	4.74E+04	2.14
ILL23 R2				type a/b	5	2	/	/	/	/	a	2.50E+04	2.50E+04	2.14	type a/b	5	2	/	/	/	/	a	2.50E+04	2.50E+04	2.14	6	7	4.16E+04	2.14
ILL24 R1	Cooling water 7	LEG 1.20	Thermal stress	type a/b	86	6	/	/	/	/	a	4.30E+05	4.30E+05	1	type a/b	86	6	/	/	/	/	a	4.30E+05	4.30E+05	1	6	54	4.63E+05	1
ILL24 R2				type a/b	90	9	/	/	/	/	a	4.45E+05	4.45E+05	1	type a/b	90	9	/	/	/	/	a	4.45E+05	4.45E+05	1	6	52	4.36E+05	1
ILL25 R1	Cooling water 7	LEG 1.17	Thermal stress	type a/b	1/5	/	23/41	37/31	37/9	25/3	a	5.00E+03	5.00E+03	2.14	type a/b	1/5	/	23/41	37/31	37/9	/	a	5.00E+03	5.00E+03	2.14	3	1	5.20E+03	2.14
ILL25 R2				type a/b	1/4	/	18/25	30/25	28/2	17/3	a	5.00E+03	5.00E+03	2.14	type a/b	1/4	/	18/25	30/25	28/2	/	a	5.00E+03	5.00E+03	2.14	3	3	7.90E+03	2.14
ILL26 R1	Water spray 1	LEG 1.17	Thermal stress	type a/b	5	2	/	/	/	/	a	2.50E+04	2.50E+04	2.14	type a/b	5	2	/	/	/	/	a	2.50E+04	2.50E+04	2.14	6	2	1.87E+04	2.14
ILL26 R2				type a/b	5/1	0	/	/	/	/	a	2.50E+04	2.50E+04	2.14	type a/b	5/1	0	/	/	/	/	a	2.50E+04	2.50E+04	2.14	6	3	2.23E+04	2.14
ILL27 R1	Purification plant water (inlet)1	LEG 1.18	Chlorination	type a/b	59/1	6	/	/	/	/	a	2.95E+05	2.95E+05	2.14	type a/b	59/1	6	/	/	/	/	a	2.95E+05	2.95E+05	2.14	6	31	2.13E+05	2.14
ILL27 R2				type a/b	63	3	/	/	/	/	a	3.15E+05	3.15E+05	2.14	type a/b	63	3	/	/	/	/	a	3.15E+05	3.15E+05	2.14	6	35	2.49E+05	2.14
ILL28 R1	Purification plant water (outflow) 1	LEG 1.19	Chlorination	type a/b	5/1	/	95/8	84/2	80/1	71	a	2.50E+04	2.50E+04	2.14	type a/b	5/1	/	95/8	84/2	21	/	a	2.50E+04	2.50E+04	2.14	6	3	2.23E+04	2.14
ILL28 R2				type a/b	3/2	/	65/3	59	18	/	a	1.50E+04	1.50E+04	2.14	type a/b	3/2	/	65/3	59	18	/	a	1.50E+04	1.50E+04	2.14	5	3	1.46E+04	2.14
ILL29 R1	Water spray 2	LEG 1.19	Chlorination	type a/b	23/1	5	/	/	/	/	a	1.15E+05	1.15E+05	2.14	type a/b	23/1	5	/	/	/	/	a	1.15E+05	1.15E+05	2.14	6	17	1.06E+05	2.14
ILL29 R2				type a/b	22	1	/	/	/	/	a	1.10E+05	1.10E+05	2.14	type a/b	22	1	/	/	/	/	a	1.10E+05	1.10E+05	2.14	6	16	9.89E+04	2.14
ILL30 R1	Water spray 1	LEG 1.18	Chlorination	type a/b	>150	20/1	/	/	/	/	a	1.00E+06	1.00E+06	2.14	type a/b	>150	20/1	/	/	/	/	a	1.00E+06	1.00E+06	2.14	6	78	1.02E+06	2.14
ILL30 R2				type a/b	>150	24/1	/	/	/	/	a	1.20E+06	1.20E+06	2.14	type a/b	>150	24/1	/	/	/	/	a	1.20E+06	1.20E+06	2.14	6	72	8.12E+05	2.14
ILL31 R1	Water spray 1	LEG 1.19	Chlorination	type a	0/1	/	0/23	0	0	0	/	<100	<100	/	type a	0/1	/	0/23	0	0	/	/	<100	<100	/	0	0	<1000	/
ILL31 R2				type a	0	/	0/11	0	0	0	/	<100	<100	/	type a	0	/	0/11	0	0	/	/	<100	<100	/	0	0	<1000	/
ILL32 R1	Water spray 2	LEG 1.19	Thermal stress	type a/b	/	/	6/5	2	9	1	a	6.00E+02	6.00E+02	2.14	type a/b	/	/	6/5	2	1	/	a	6.00E+02	6.00E+02	2.14	1	0	1.10E+03	2.14
ILL32 R2				type a/b	/	/	5/9	3	8	2	a	5.00E+02	5.00E+02	2.14	type a/b	/	/	5/9	3	2	/	a	5.00E+02	5.00E+02	2.14	0	1	1.00E+03	2.14
ILL33 R1	Water spray 3	LEG 1.19	Thermal stress	type a	0/1	0	/	/	/	/	/	<100	<100	/	type a	0/1	0	/	/	/	/	/	<100	<100	/	0	0	<1000	/
ILL33 R2				type a	0	0	/	/	/	/	/	<100	<100	/	type a	0	0	/	/	/	/	/	<100	<100	/	0	0	<1000	/
ILL34 R1	Water spray 4	LEG 1.20	Thermal stress	type a	0/3	/	0/17	0	0/1	0	/	<100	<100	/	type a	0/3	/	0/17	0	0/1	/	/	<100	<100	/	0	0	<1000	/
ILL34 R2				type a	0/1	/	0/12	0/1	0	0	/	<100	<100	/	type a	0/1	/	0/12	0/1	0	/	/	<100	<100	/	0	0	<1000	/
ILL35 R1	Water spray 3	LEG 1.2	Thermal stress	type a/b	/	/	5/3/1	5	8	5	a	5.00E+02	5.00E+02	2.14	type a/b	/	/	5/3/1	5	1	/	a	5.00E+02	5.00E+02	2.14	1	0	1.10E+03	2.14
ILL35 R2				type a/b	/	/	6/2	4	9	5	a	6.00E+02	6.00E+02	2.14	type a/b	/	/	6/2	4	3	/	a	5.00E+02	5.00E+02	2.14	0	1	1.00E+03	2.14
ILL36 R1	Water spray 4	LEG 1.2	Thermal stress	type a/b	/	/	28	30	22	33	a	3.00E+03	3.00E+03	2.14	type a/b	/	/	28	30	9	/	a	3.00E+03	3.00E+03	2.14	4	0	5.80E+03	2.14
ILL36 R2				type a/b	/	/	35	28/1	26	31/1	a	3.50E+03	3.50E+03	2.14	type a/b	/	/	35	28/1	5	/	a	3.50E+03	3.50E+03	2.14	3	1	5.20E+03	2.14
ILL37 R1	Water spray 5	LEG 1.18	Thermal stress	type a	5	/	125	102	94	107	a	2.50E+04	2.50E+04	2.14	type a	5	/	125	102	16/1	/	a	2.50E+04	2.50E+04	2.14	3	2	6.60E+03	2.14
ILL37 R2				type a	0	/	0	0	0	0	/	<100	<100	/	type a	0	/	0	0	0/1	/	/			/	4	0	5.80E+03	/
ILL38 R1	Water spray 6	LEG 1.18	Thermal stress	type a	0/1	0	/	/	/	/	/	<100	<100	/	type a	0/1	0	/	/	/	/	/	<100	<100	/	0	0	<1000	/
ILL38 R2				type a	0	0	/	/	/	/	/	<100	<100	/	type a	0	0	/	/	/	/	/	<100	<100	/	0	0	<1000	/
ILL39 R1	Water spray 5	LEG 1.19	Chlorination	type a/b	/	/	11	11	7	6	a	1.10E+03	1.10E+03	2.14	type a/b	/	/	11	11	4	/	a	1.10E+03	1.10E+03	2.14	2	0	2.30E+03	2.14
ILL39 R2				type a/b	/	/	18	7	8	6/1	a	1.80E+03	1.80E+03	2.14	type a/b	/	/	18	7	5	/	a	1.80E+03	1.80E+03	2.14	2	1	3.50E+03	2.14
ILL40 R1	Water spray 6	LEG 1.20	Chlorination	type a/b	/	/	23	19	15	17	a	2.30E+03	2.30E+03	1	type a/b	/	/	23	19	2	/	a	2.30E+03	2.30E+03	1	2	2	4.70E+03	1
ILL40 R2				type a/b	/	/	27	31	17	21	a	2.70E+03	2.70E+03	1	type a/b	/	/	27	31	7/1	/	a	2.70E+03	2.70E+03	1	2	2	4.70E+03	1

Non interpretable results

V/V: Volume/Volume

1/10 dilution 1/10 of the sample before spreading

Relative accuracy industrial waters: IDEXX Legiolert vs T90 - 431 and ISO 11731

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
1	30000.00	40000.00	35000.00	7071.07	1	31000.00	36100.00	33550.00	3606.24	-1450.000
2	110000.00	150000.00	130000.00	28284.27	2	85400.00	142000.00	113700.00	40022.24	-16300.000
3	175000.00	175000.00	175000.00	0.00	3	164000.00	172000.00	168000.00	5656.85	-7000.000
4	1000000.00	1050000.00	1025000.00	35355.34	4	905000.00	842000.00	873500.00	44547.73	-151500.000
5	25000.00	15000.00	20000.00	7071.07	5	22300.00	12400.00	17350.00	7000.36	-2650.000
6	95000.00	110000.00	102500.00	10606.60	6	78800.00	92100.00	85450.00	9404.52	-17050.000
17	5000.00	5000.00	5000.00	0.00	7	3500.00	3500.00	3500.00	0.00	-1500.000
19	10000.00	10000.00	10000.00	0.00	8	9000.00	10400.00	9700.00	989.95	-300.000
20	70000.00	95000.00	82500.00	17677.67	9	53400.00	78800.00	66100.00	17960.51	-16400.000
21	700000.00	590000.00	645000.00	77781.75	10	786000.00	422000.00	604000.00	257386.87	-41000.000
22	2900.00	1600.00	2250.00	919.24	11	6600.00	3900.00	5250.00	1909.19	3000.000
23	40000.00	25000.00	32500.00	10606.60	12	47400.00	41600.00	44500.00	4101.22	12000.000
24	430000.00	445000.00	437500.00	10606.60	13	463000.00	436000.00	449500.00	19091.88	12000.000
25	5000.00	5000.00	5000.00	0.00	14	5200.00	7900.00	6550.00	1909.19	1550.000
26	25000.00	25000.00	25000.00	0.00	15	18700.00	22300.00	20500.00	2545.58	-4500.000
27	295000.00	315000.00	305000.00	14142.14	16	213000.00	249000.00	231000.00	25455.84	-74000.000
28	25000.00	15000.00	20000.00	7071.07	17	22300.00	14600.00	18450.00	5444.72	-1550.000
29	115000.00	110000.00	112500.00	3535.53	18	106000.00	98900.00	102450.00	5020.46	-10050.000
30	1000000.00	1200000.00	1100000.00	141421.36	19	1020000.00	812000.00	916000.00	147078.21	-184000.000
32	450.00	500.00	475.00	35.36	20	1100.00	1000.00	1050.00	70.71	575.000
35	500.00	600.00	550.00	70.71	21	1100.00	1000.00	1050.00	70.71	500.000
36	3000.00	3500.00	3250.00	353.55	22	5800.00	5200.00	5500.00	424.26	2250.000
39	1100.00	1800.00	1450.00	494.97	23	2300.00	3500.00	2900.00	848.53	1450.000
40	2300.00	2700.00	2500.00	282.84	24	4700.00	4700.00	4700.00	0.00	2200.000

q= 24
n= 2
N=qn= 48

Mx= 178248.958
MEDx= 28750.000
SDbx= 314843.727
MEDwx = 5303.301
SDwx= 34829.227
rob. SDwx= 7862.674

My= 157677.083
MEDy= 27025.000
SDby= 271335.016
MEDwy = 4560.839
SDwy= 62273.304
rob. SDwy= 6761.900

M= -20571.875
MED= -1525.000
Bias

Regression used
GMFR

R= 1.788
rob. R= 0.860

Sx= 312469.101
Sy= 272096.468

r= 0.997
b= 0.871
a= 2458.818

Res. SEM= 21612.846
Res. SD= 30565.180

S(b)= 0.014
S(a)= 4412.001
p(t;b=1)= 0.0000000000
p(t;a=0)= 0.5800228664
t(b)= 8.959
t(a)= 0.557

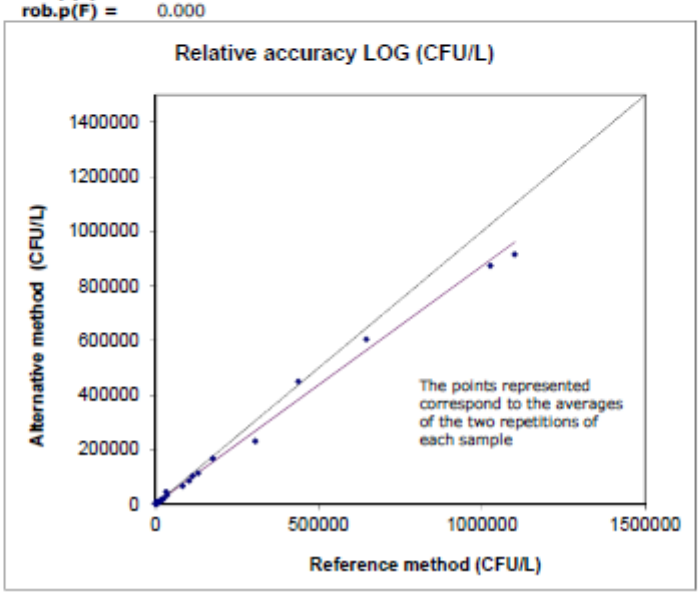
Est. y	Dev.
32936.636	613.364
115662.141	-1962.141
154847.907	13152.093
895023.486	-21523.486
19874.714	-2524.714
91715.284	-6265.284
6812.792	-3312.792
11166.766	-1466.766
74299.388	-8199.388
564121.462	39878.538
4418.106	831.894
30759.649	13740.351
383431.542	66068.458
6812.792	-262.792
24228.688	-3728.688
268051.231	-37051.231
19874.714	-1424.714
100423.232	2026.768
960333.096	-44333.096
2872.445	-1822.445
2937.755	-1887.755
5288.901	211.099
3721.470	-821.470
4635.805	64.195

Repeatability	Reference method	Alternative method
r	97521.836	174365.251
rob. r	22015.487	18933.319

Linearity

F = 0.519
rob.F = 30.506

p(F) = 0.936
rob.p(F) = 0.000



Relative accuracy industrial waters: IDEXX Legiolert vs NF T90 - 431 and ISO 11731 (LOG)

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
1	4.48	4.60	4.54	0.09	1	4.49	4.56	4.52	0.05	-0.015
2	5.04	5.18	5.11	0.10	2	4.93	5.15	5.04	0.16	-0.067
3	5.24	5.24	5.24	0.00	3	5.21	5.24	5.23	0.01	-0.018
4	6.00	6.02	6.01	0.01	4	5.96	5.93	5.94	0.02	-0.070
5	4.40	4.18	4.29	0.16	5	4.35	4.09	4.22	0.18	-0.066
6	4.98	5.04	5.01	0.05	6	4.90	4.96	4.93	0.05	-0.079
17	3.70	3.70	3.70	0.00	7	3.54	3.54	3.54	0.00	-0.155
19	4.00	4.00	4.00	0.00	8	3.95	4.02	3.99	0.04	-0.014
20	4.85	4.98	4.91	0.09	9	4.73	4.90	4.81	0.12	-0.099
21	5.85	5.77	5.81	0.05	10	5.90	5.63	5.76	0.19	-0.048
22	3.46	3.20	3.33	0.18	11	3.82	3.59	3.71	0.16	0.372
23	4.60	4.40	4.50	0.14	12	4.68	4.62	4.65	0.04	0.147
24	5.63	5.65	5.64	0.01	13	5.67	5.64	5.65	0.02	0.012
25	3.70	3.70	3.70	0.00	14	3.72	3.90	3.81	0.13	0.108
26	4.40	4.40	4.40	0.00	15	4.27	4.35	4.31	0.05	-0.088
27	5.47	5.50	5.48	0.02	16	5.33	5.40	5.36	0.05	-0.122
28	4.40	4.18	4.29	0.16	17	4.35	4.16	4.26	0.13	-0.031
29	5.06	5.04	5.05	0.01	18	5.03	5.00	5.01	0.02	-0.041
30	6.00	6.08	6.04	0.06	19	6.01	5.91	5.96	0.07	-0.081
32	2.65	2.70	2.68	0.03	20	3.04	3.00	3.02	0.03	0.345
35	2.70	2.78	2.74	0.06	21	3.04	3.00	3.02	0.03	0.282
36	3.48	3.54	3.51	0.05	22	3.76	3.72	3.74	0.03	0.229
39	3.04	3.26	3.15	0.15	23	3.36	3.54	3.45	0.13	0.305
40	3.36	3.43	3.40	0.05	24	3.67	3.67	3.67	0.00	0.276

q=	24	Mx=	4.438		My=	4.483		M=	0.045
n=	2	MEDx=	4.449		MEDy=	4.417		MED=	-0.024
N=qn=	48	SDbx=	1.014		SDby=	0.896		Bias	
			MEDwx =	0.048		MEDwy =	0.047		
			SDwx=	0.084		SDwy=	0.093		
			rob. SDwx=	0.072		rob. SDwy=	0.070		

Regression used
GMFR

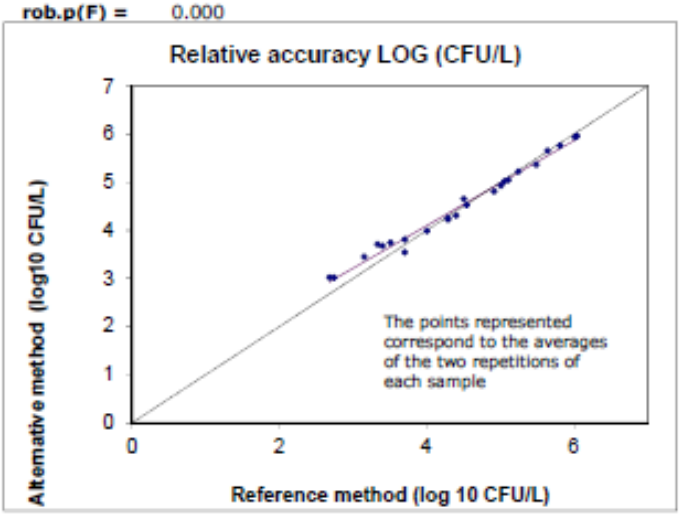
	R=	1.103			
	rob. R=	0.980			
			Sx=	1.005	
			Sy=	0.888	
r=	0.993				
b=	0.884		Res. SEM=	0.106	
a=	0.560		Res. SD=	0.150	
S(b)=	0.022	p(t;b=1)=	0.0000036801	t(b)=	5.258
S(a)=	0.061	p(t;a=0)=	0.0000000000	t(a)=	9.133

Repeatability	Reference method	Alternative method
r	0.235	0.260
rob. r	0.200	0.196

Est. y	Dev.
4.573	-0.049
5.076	-0.034
5.195	0.030
5.873	0.068
4.350	-0.129
4.988	-0.058
3.830	-0.286
4.096	-0.110
4.902	-0.090
5.694	0.066
3.506	0.199
4.538	0.109
5.547	0.106
3.830	-0.023
4.448	-0.138
5.408	-0.046
4.350	-0.093
5.025	-0.015
5.899	0.060
2.926	0.095
2.981	0.040
3.663	0.076
3.343	0.110
3.562	0.110

Linearity

F =	5.592	p(F) =	0.000
rob.F =	8.125	rob.p(F) =	0.000



Relative accuracy industrial waters: IDEXX Legiolert vs NF T90 - 431 and ISO 11731

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
22	2900.00	1600.00	2250.00	919.24	11	6600.00	3900.00	5250.00	1909.19	3000.000
32	450.00	500.00	475.00	35.36	20	1100.00	1000.00	1050.00	70.71	575.000
35	500.00	600.00	550.00	70.71	21	1100.00	1000.00	1050.00	70.71	500.000
36	3000.00	3500.00	3250.00	353.55	22	5800.00	5200.00	5500.00	424.26	2250.000
39	1100.00	1800.00	1450.00	494.97	23	2300.00	3500.00	2900.00	848.53	1450.000
40	2300.00	2700.00	2500.00	282.84	24	4700.00	4700.00	4700.00	0.00	2200.000

q= 6		Mx=	1745.833			My=	3408.333			M= 1662.500
n= 2		MEDx=	1850.000			MEDy=	3800.000			MED= 1825.000
N=qn= 12		SDbx=	1115.170			SDby=	2040.445			Bias
			MEDwx =	318.198			MEDwy =	247.487		
			SDwx=	465.698			SDwy=	871.302		
			rob. SDwx=	471.760			rob. SDwy=	366.925		

Regression used
GMFR

R=	1.871		
rob. R=	0.778		
		Sx=	1117.517
		Sy=	2049.150

Est. y	Dev.
4332.805	917.195
1078.053	-28.053
1215.578	-165.578
6166.468	-666.468
2865.875	34.125
4791.221	-91.221

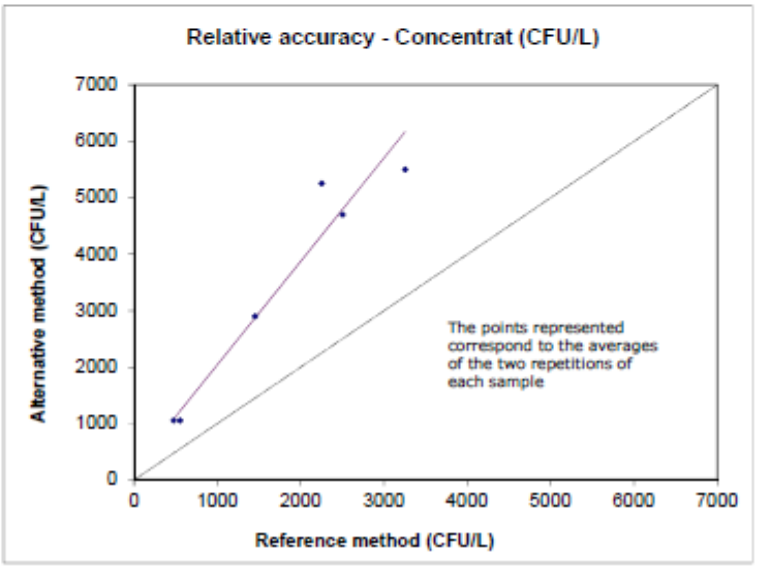
r=	0.96829				
b=	1.83366			**Res. SEM=**	575.134
a=	207.06348			**Res. SD=**	813.362
S(b)=	0.230	**p(t;b=1)=**	0.0047	**t(b)=**	3.622
S(a)=	235.201	**p(t;a=0)=**	0.3993	**t(a)=**	0.880

0.00096

Repeatability	Reference method	Alternative method
r	1303.956	2439.645
rob. r	1320.929	1027.389

Linearity

F =	6.126	**p(F) =**	0.026
rob.F =	5.931	**rob.p(F) =**	0.028



Relative accuracy industrial waters: IDEXX Legiolert vs NF T90 - 431 and ISO 11731 (LOG)

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
22	3.46	3.20	3.33	0.18	11	3.82	3.59	3.71	0.16	0.372
32	2.65	2.70	2.68	0.03	20	3.04	3.00	3.02	0.03	0.345
35	2.70	2.78	2.74	0.06	21	3.04	3.00	3.02	0.03	0.282
36	3.48	3.54	3.51	0.05	22	3.76	3.72	3.74	0.03	0.229
39	3.04	3.26	3.15	0.15	23	3.36	3.54	3.45	0.13	0.305
40	3.36	3.43	3.40	0.05	24	3.67	3.67	3.67	0.00	0.276

q= 6 n= 2 N=qn= 12

Mx= 3.134
MEDx= 3.241
SDbx= 0.351
MEDwx = 0.053
SDwx= 0.104
rob. SDwx= 0.078

My= 3.435
MEDy= 3.562
SDby= 0.336
MEDwy = 0.031
SDwy= 0.087
rob. SDwy= 0.047

M= 0.301
MED= 0.293
Bias

Regression used
GMFR

Est. y	Dev.
3.625	0.080
2.999	0.021
3.059	-0.038
3.794	-0.054
3.449	0.004
3.685	-0.013

R= 0.837
rob. R= 0.597
Sx= 0.344
Sy= 0.327

r= 0.98982
b= 0.95209
a= 0.45149
Res. SEM= 0.054
Res. SD= 0.076

0.44

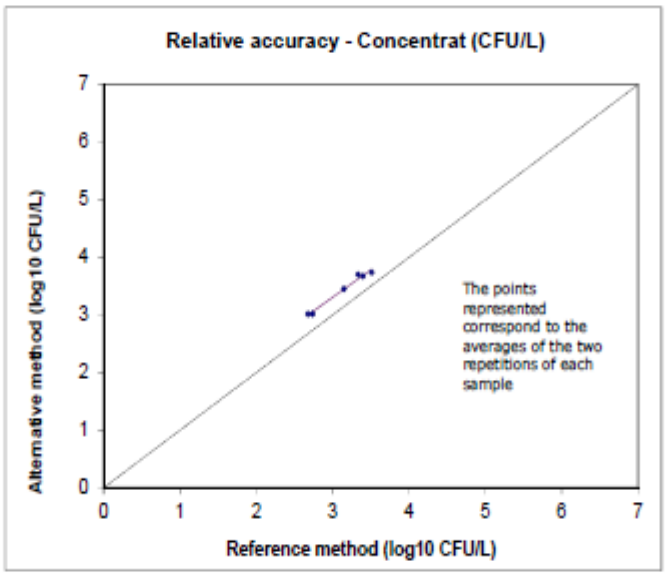
S(b)= 0.070
S(a)= 0.043
p(t;b=1)= 0.5076
p(t;a=0)= 0.0000
t(b)= 0.687
t(a)= 10.576

0.00096

Repeatability	Reference method	Alternative method
r	0.292	0.244
rob. r	0.218	0.130

Linearity

F = 0.177
rob.F = 0.857
p(F) = 0.942
rob.p(F) = 0.539



Relative accuracy industrial waters: IDEXX Legiolert vs direct T90 - 431

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
1	30000.00	40000.00	35000.00	7071.07	1	31000.00	36100.00	33550.00	3606.24	-1450.000
2	110000.00	150000.00	130000.00	28284.27	2	85400.00	142000.00	113700.00	40022.24	-16300.000
3	175000.00	175000.00	175000.00	0.00	3	164000.00	172000.00	168000.00	5656.85	-7000.000
4	1000000.00	1050000.00	1025000.00	35355.34	4	905000.00	842000.00	873500.00	44547.73	-151500.000
5	25000.00	15000.00	20000.00	7071.07	5	22300.00	12400.00	17350.00	7000.36	-2650.000
6	95000.00	110000.00	102500.00	10606.60	6	78800.00	92100.00	85450.00	9404.52	-17050.000
17	5000.00	5000.00	5000.00	0.00	7	3500.00	3500.00	3500.00	0.00	-1500.000
19	10000.00	10000.00	10000.00	0.00	8	9000.00	10400.00	9700.00	989.95	-300.000
20	70000.00	95000.00	82500.00	17677.67	9	53400.00	78800.00	66100.00	17960.51	-16400.000
21	700000.00	590000.00	645000.00	77781.75	10	786000.00	422000.00	604000.00	257386.87	-41000.000
23	40000.00	25000.00	32500.00	10606.60	12	47400.00	41600.00	44500.00	4101.22	12000.000
24	430000.00	445000.00	437500.00	10606.60	13	463000.00	436000.00	449500.00	19091.88	12000.000
25	5000.00	5000.00	5000.00	0.00	14	5200.00	7900.00	6550.00	1909.19	1550.000
26	25000.00	25000.00	25000.00	0.00	15	18700.00	22300.00	20500.00	2545.58	-4500.000
27	295000.00	315000.00	305000.00	14142.14	16	213000.00	249000.00	231000.00	25455.84	-74000.000
28	25000.00	15000.00	20000.00	7071.07	17	22300.00	14600.00	18450.00	5444.72	-1550.000
29	115000.00	110000.00	112500.00	3535.53	18	106000.00	98900.00	102450.00	5020.46	-10050.000
30	1000000.00	1200000.00	1100000.00	141421.36	19	1020000.00	812000.00	916000.00	147078.21	-184000.000

q= 18
n= 2
N=qn= 36

Mx= 237083.333
MEDx= 92500.000
SDbx= 345617.787
MEDwx = 8838.835
SDwx= 40216.428
rob. SDwx= 13104.456

My= 209100.000
MEDy= 75775.000
SDby= 297332.285
MEDwy = 6328.606
SDwy= 71905.258
rob. SDwy= 9382.791

M= -27983.333
MED= -5750.000
Bias

Regression used
GMFR

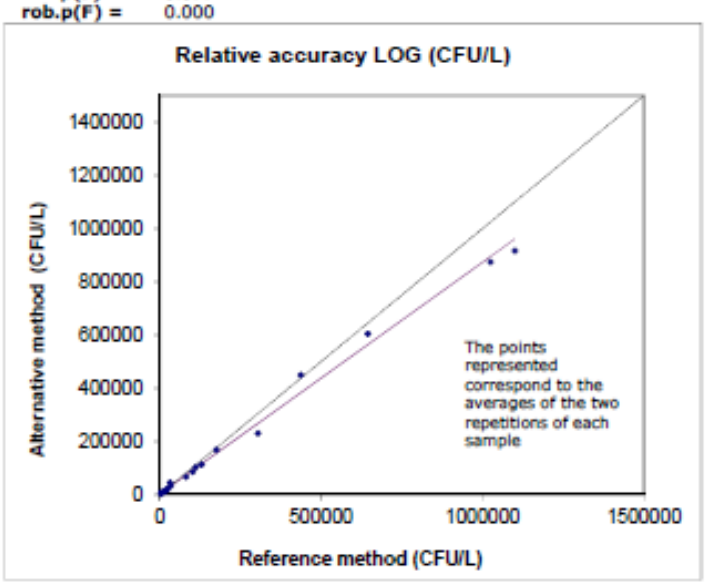
R=	1.788		
rob. R=	0.716		
		Sx=	341863.329
		Sy=	297556.102
r=	0.997		
b=	0.870	Res. SEM=	25306.761
a=	2743.873	Res. SD=	35789.165
S(b)=	0.018	p(t;b=1)=	0.0000000236
S(a)=	5965.087	p(t;a=0)=	0.6484524509
		t(b)=	7.219
		t(a)=	0.460

Est. y	Dev.
33207.695	342.305
115895.212	-2195.212
155062.983	12937.017
894898.657	-21398.657
20151.771	-2801.771
91959.352	-6509.352
7095.848	-3595.848
11447.822	-1747.822
74551.453	-8451.453
564148.591	39851.409
31031.708	13468.292
383541.647	65958.353
7095.848	-545.848
24503.746	-4003.746
268214.321	-37214.321
20151.771	-1201.771
100663.301	1786.699
960178.275	-44178.275

Repeatability	Reference method	Alternative method
r	112605.999	203334.722
rob. r	36692.478	26271.814

Linearity

F =	0.558	p(F) =	0.877
rob.F =	14.725	rob.p(F) =	0.000



Relative accuracy industrial waters: IDEXX Legiolert vs direct T90 – 431 (LOG)

Reference method					Alternative method					Difference
Sample	Repetition 1	Repetition 2	M	SD	Sample	Repetition 1	Repetition 2	M	SD	
1	4.48	4.60	4.54	0.09	1	4.49	4.56	4.52	0.05	-0.015
2	5.04	5.18	5.11	0.10	2	4.93	5.15	5.04	0.16	-0.067
3	5.24	5.24	5.24	0.00	3	5.21	5.24	5.23	0.01	-0.018
4	6.00	6.02	6.01	0.01	4	5.96	5.93	5.94	0.02	-0.070
5	4.40	4.18	4.29	0.16	5	4.35	4.09	4.22	0.18	-0.066
6	4.98	5.04	5.01	0.05	6	4.90	4.96	4.93	0.05	-0.079
17	3.70	3.70	3.70	0.00	7	3.54	3.54	3.54	0.00	-0.155
19	4.00	4.00	4.00	0.00	8	3.95	4.02	3.99	0.04	-0.014
20	4.85	4.98	4.91	0.09	9	4.73	4.90	4.81	0.12	-0.099
21	5.85	5.77	5.81	0.05	10	5.90	5.63	5.76	0.19	-0.048
23	4.60	4.40	4.50	0.14	12	4.68	4.62	4.65	0.04	0.147
24	5.63	5.65	5.64	0.01	13	5.67	5.64	5.65	0.02	0.012
25	3.70	3.70	3.70	0.00	14	3.72	3.90	3.81	0.13	0.108
26	4.40	4.40	4.40	0.00	15	4.27	4.35	4.31	0.05	-0.088
27	5.47	5.50	5.48	0.02	16	5.33	5.40	5.36	0.05	-0.122
28	4.40	4.18	4.29	0.16	17	4.35	4.16	4.26	0.13	-0.031
29	5.06	5.04	5.05	0.01	18	5.03	5.00	5.01	0.02	-0.041
30	6.00	6.08	6.04	0.06	19	6.01	5.91	5.96	0.07	-0.081

q=	18	Mx=	4.873		My=	4.833		M=	-0.040
n=	2	MEDx=	4.960		MEDy=	4.871		MED=	-0.057
N=qn=	36	SDbx=	0.744		SDby=	0.731			Bias
			MEDwx =	0.033		MEDwy =	0.048		
			SDwx=	0.076		SDwy=	0.094		
			rob. SDwx=	0.048		rob. SDwy=	0.071		

Regression used
GMFR

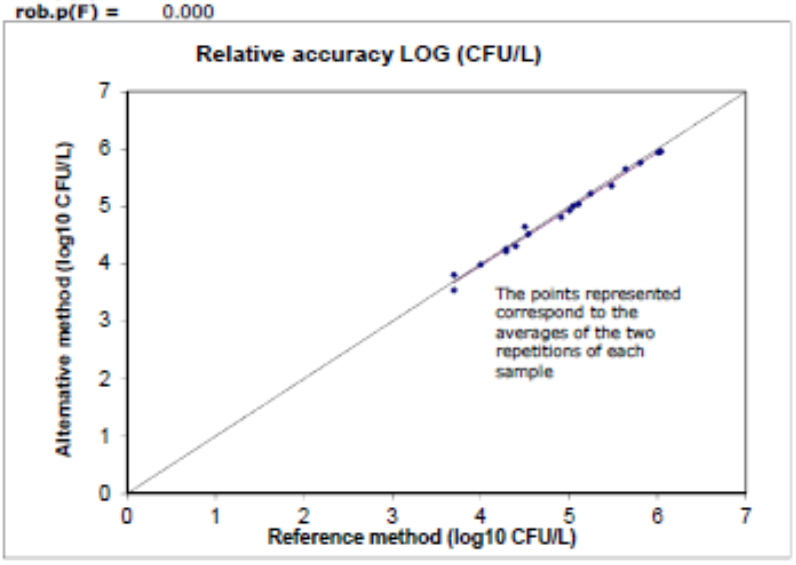
	R=	1.240			
	rob. R=	1.471			
			Sx=	0.736	
			Sy=	0.724	
r=	0.995				
b=	0.984		Res. SEM=	0.074	
a=	0.038		Res. SD=	0.105	
S(b)=	0.025	p(t;b=1)=	0.5175310626	t(b)=	0.654
S(a)=	0.042	p(t;a=0)=	0.3706607985	t(a)=	0.907

Est. y	Dev.
4.505	0.020
5.065	-0.023
5.197	0.028
5.952	-0.011
4.256	-0.035
4.967	-0.037
3.677	-0.133
3.974	0.012
4.870	-0.058
5.753	0.008
4.466	0.182
5.588	0.064
3.677	0.129
4.365	-0.055
5.434	-0.072
4.256	0.000
5.008	0.002
5.981	-0.021

Repeatability	Reference method	Alternative method
r	0.213	0.265
rob. r	0.135	0.199

Linearity

F =	2.916	p(F) =	0.016
rob.F =	8.936	rob.p(F) =	0.000



Appendix 5: Linearity results

Linearity: category "water for human consumption"

				MR: T90-431							MR: ISO 11731									MA : Legiolert				
Sample	Strain	Sample Number	Concentration (CFU/L)	Directs		Filtration + treatment pH2 (5±0,5min)		Results			Directs		Filtration (without treatment)		Filtration + acid treatment: 30mL pH2.2±0,2 (5±0,5min)		Results			Reading				
				/	/ 1/10	C100	C10		*Legionella pneumophilla*		/	/ 1/10	C100ST	C10ST	C100	C10		*Legionella pneumophilla*		*Legionella pneumophilla*				
				0,2mL GVPC	0.2 mL after dilution 1/10	filtration 100mL	filtration 10mL	*Legionella* spp (CFU/L)	Results (CFU/L)	sero-group	0,2mL GVPC	0.2 mL after dilution 1/10	filtration 100mL	filtration 10mL	filtration 100mL	filtration 10mL	*Legionella* spp (CFU/L)	Results (CFU/L)	sero-group	Big well	Small well	Dilution	Results (CFU/L)	sero-group
Hot water for sanitary use	LEG 1.21	ILL 100 R1	100	/	/	4	/	4.00E+01	4.00E+01	2.14	/	/	5	/	4	/	5.00E+01	5.00E+01	2.14	5	1	/	1.04E+02	2.14
		ILL 100 R2		/	/	5	/	5.00E+01	5.00E+01	2.14	/	/	5	/	3	/	5.00E+01	5.00E+01	2.14	5	1	/	1.04E+02	2.14
		ILL 500 R1	500	/	/	11	/	1.10E+02	1.10E+02	2.14	/	/	10	/	8	/	1.00E+02	1.00E+02	2.14	6	12	/	7.23E+02	2.14
		ILL 500 R2		/	/	13	/	1.30E+02	1.30E+02	2.14	/	/	14	/	9	/	1.40E+02	1.00E+02	2.14	6	12	/	7.23E+02	2.14
		ILL 1000 R1	1000	/	/	17	/	1.70E+02	1.70E+02	2.14	/	/	19	/	15	/	1.90E+02	1.90E+02	2.14	6	23	/	1.49E+03	2.14
		ILL 1000 R2		/	/	21	/	2.10E+02	2.10E+02	2.14	/	/	21	/	14	/	2.10E+02	1.90E+02	2.14	6	17	/	1.06E+03	2.14
		ILL 5000 R1	5000	0	/	97	9	9.70E+02	9.70E+02	2.14	0	/	97	14	93	8	1.40E+03	9.70E+02	2.14	6	72	/	8.13E+03	2.14
		ILL 5000 R2		0	/	>100	13	1.30E+03	1.30E+03	2.14	0	/	>100	13	93	8	1.30E+03	9.70E+02	2.14	6	71	/	7.86E+03	2.14
		ILL 10000 R1	10000	1	/	>100	21	5.00E+03	5.00E+03	2.14	1	/	>100	25	>100	22	5.00E+03	5.00E+03	2.14	6	80	/	1.11E+04	2.14
		ILL 10000 R2		2	/	>100	21	1.00E+04	1.00E+04	2.14	2	/	>100	31	>100	23	1.00E+04	5.00E+03	2.14	6	82	/	1.22E+04	2.14
		ILL 50000 R1	50000	18	/	/	/	9.00E+04	9.00E+04	2.14	18	/	/	/	/	/	9.00E+04	9.00E+04	2.14	6	70	1/10	7.60E+04	2.14
		ILL 50000 R2		13	/	/	/	6.50E+04	6.50E+04	2.14	13	/	/	/	/	/	6.50E+04	9.00E+04	2.14	6	68	1/10	7.12E+04	2.14
		ILL 100000 R1	100000	28	/	/	/	1.40E+05	1.40E+05	2.14	28	/	/	/	/	/	1.40E+05	1.40E+05	2.14	6	81	1/10	1.16E+05	2.14
		ILL 100000 R2		22	/	/	/	1.40E+05	1.40E+05	2.14	22	/	/	/	/	/	1.10E+05	1.40E+05	2.14	6	83	1/10	1.29E+05	2.14
		ILL 500000 R1	500000	138	/	/	/	6.90E+05	6.90E+05	2.14	138	/	/	/	/	/	6.90E+05	6.90E+05	2.14	6	63	1/100	6.08E+05	2.14
		ILL 500000 R2		131	/	/	/	6.90E+05	6.90E+05	2.14	131	/	/	/	/	/	6.55E+05	6.90E+05	2.14	6	61	1/100	5.72E+05	2.14

Linearity: “water for human consumption”: IDEXX Legiolert vs T90-431

Level
1
2
3
4
5
6
7
8

Reference method (x)			
Rep.1	Rep.2	M	SD
40,000	50,000	45,000	7,071
110,000	130,000	120,000	14,142
170,000	210,000	190,000	28,284
970,000	1300,000	1135,000	233,345
5000,000	10000,000	7500,000	3535,534
90000,000	65000,000	77500,000	17677,670
140000,000	110000,000	125000,000	21213,203
690000,000	655000,000	672500,000	24748,737

Alternative method (y)			
Rep.1	Rep.2	M	SD
104,000	104,000	104,000	0,000
723,000	723,000	723,000	0,000
1490,000	1057,000	1273,500	306,177
8128,000	7855,000	7991,500	193,040
11097,000	12224,000	11660,500	796,909
75960,000	71150,000	73555,000	3401,184
116290,000	128980,000	122635,000	8973,185
608100,000	572000,000	590050,000	25526,555

q = 8
n = 2
N = qn = 16

Mx = 110498,750
MEDx = 4317,500
SDbx = 231845,176

MEDwx = 1884,440
SDwx = 9312,477
rob. SDwx = 2793,870

My = 100999,063
MEDy = 9826,000
SDby = 202556,583

MEDwy = 551,543
SDwy = 6821,186
rob. SDwy = 817,718

Regression used
OLS; y=ref

Sx = 224190,121
Sy = 195815,020

R = 0,732
rob.R = 0,293

r = 1,000
b = 1,145
a = -25481,342

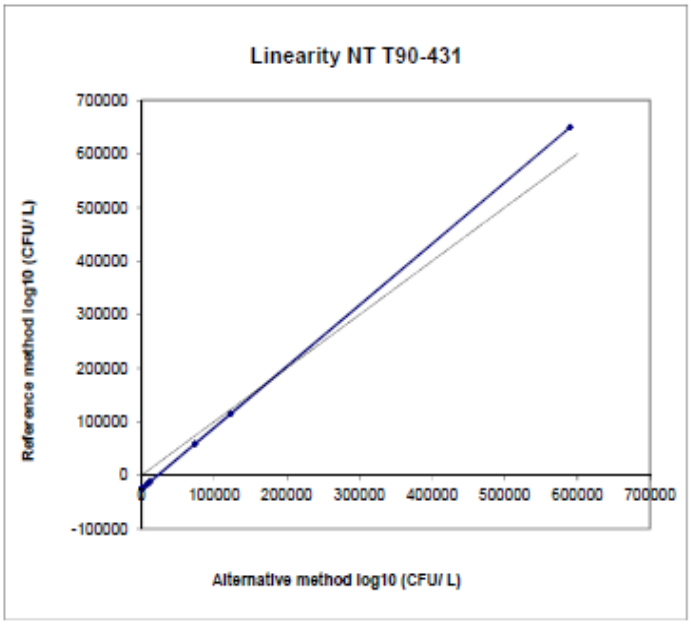
M(Alt.)	Ref	Est. y	Deviation
104,000	40,000	-25362,300	25402,300
723,000	110,000	-24653,773	24763,773
1273,500	170,000	-24023,653	24193,653
7991,500	970,000	-16334,016	17304,016
11660,500	5000,000	-12134,361	17134,361
73555,000	90000,000	58712,064	31287,936
122635,000	140000,000	114890,603	25109,397
590050,000	690000,000	649908,767	40091,233
104,000	50,000	-25362,300	25412,300
723,000	130,000	-24653,773	24783,773
1273,500	210,000	-24023,653	24233,653
7991,500	1300,000	-16334,016	17634,016
11660,500	10000,000	-12134,361	22134,361
73555,000	65000,000	58712,064	6287,936
122635,000	110000,000	114890,603	-4890,603
590050,000	655000,000	649908,767	5091,233

Res.SD = 24481,989

Sb = 0,028
Sa = 6867,857

p(t;b=1) = 0,000
p(t;a=0) = 0,002

t (b) = 5,130
t (a) = 3,710



Linearity

F = 28,724
rob.F = 2090,191

p(F) = 0,000
rob.p(F) = 0,000

Linearity: “water for human consumption”: IDEXX Legiolert vs T90-431 (LOG)

Level	Reference method (x) Rep.1	Rep.2	M	SD	Alternative method (y) Rep.1	Rep.2	M	SD
1	1,602	1,699	1,651	0,069	2,017	2,017	2,017	0,000
2	2,041	2,114	2,078	0,051	2,859	2,859	2,859	0,000
3	2,230	2,322	2,276	0,065	3,173	3,024	3,099	0,105
4	2,987	3,114	3,050	0,090	3,910	3,895	3,903	0,010
5	3,699	4,000	3,849	0,213	4,045	4,087	4,066	0,030
6	4,954	4,813	4,884	0,100	4,881	4,852	4,866	0,020
7	5,146	5,041	5,094	0,074	5,066	5,111	5,088	0,032
8	5,839	5,816	5,828	0,016	5,784	5,757	5,771	0,019

q = 8
n = 2
N = qn = 16

Mx = 3,589
MEDx = 3,450
SDbx = 1,562

MEDwx = 0,071
SDwx = 0,071
rob. SDwx = 0,106

My = 3,959
MEDy = 3,984
SDby = 1,260

MEDwy = 0,019
SDwy = 0,029
rob. SDwy = 0,029

Regression used
OLS; y=ref

Sx = 1,511
Sy = 1,218

R = 0,415
rob.R = 0,273

r = 0,983
b = 1,219
a = -0,418

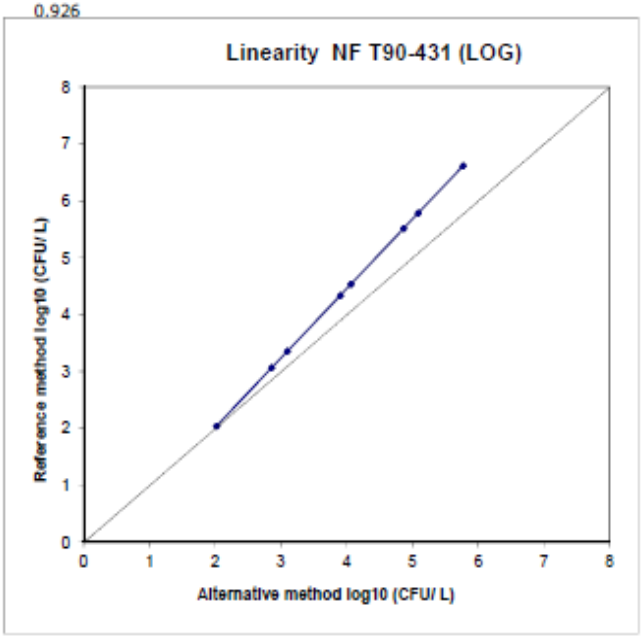
M(alt.)	Ref.	Est. y	Deviation
2,017	1,602	2,042	-0,440
2,859	2,041	3,069	-1,028
3,099	2,230	3,361	-1,131
3,903	2,987	4,341	-1,355
4,066	3,699	4,541	-0,842
4,866	4,954	5,517	-0,562
5,088	5,146	5,787	-0,641
5,771	5,839	6,619	-0,781
2,017	1,699	2,042	-0,343
2,859	2,114	3,069	-0,955
3,099	2,322	3,361	-1,039
3,903	3,114	4,341	-1,227
4,066	4,000	4,541	-0,541
4,866	4,813	5,517	-0,704
5,088	5,041	5,787	-0,746
5,771	5,816	6,619	-0,803

Sb = 0,158
Sa = 0,614

p(t;b=1) = 0,187
p(t;a=0) = 0,507

t (b) = 1,386
t (a) = 0,681

Res.SD = 0,926



Linearity

F = 2306,881
rob.F = 2408,400

p(F) = 0,000
rob.p(F) = 0,000

Linearity: “water for human consumption”: IDEXX Legiolert vs ISO 11731

Level
1
2
3
4
5
6
7
8

Reference method (x)			
Rep.1	Rep.2	M	SD
50,000	50,000	50,000	0,000
100,000	140,000	120,000	28,284
190,000	210,000	200,000	14,142
1400,000	1300,000	1350,000	70,711
5000,000	10000,000	7500,000	3535,534
90000,000	65000,000	77500,000	17677,670
140000,000	110000,000	125000,000	21213,203
690000,000	655000,000	672500,000	24748,737

Alternative method (y)			
Rep.1	Rep.2	M	SD
104,000	104,000	104,000	0,000
723,000	723,000	723,000	0,000
1490,000	1057,000	1273,500	306,177
8128,000	7855,000	7991,500	193,040
11097,000	12224,000	11660,500	796,909
75960,000	71150,000	73555,000	3401,184
116290,000	128980,000	122635,000	8973,185
608100,000	572000,000	590050,000	25526,555

q = 8
n = 2
N = qn = 16

Mx = 110527,500
MEDx = 4425,000
SDbx = 231829,680

MEDwx = 1803,122
SDwx = 9312,310
rob. SDwx = 2673,309

My = 100999,063
MEDy = 9826,000
SDby = 202556,583

MEDwy = 551,543
SDwy = 6821,186
rob. SDwy = 817,718

Regression used
OLS; y=ref

Sx = 224175,157
Sy = 195815,020

R = 0,732
rob.R = 0,306

r = 1,000
b = 1,145
a = -25505,986

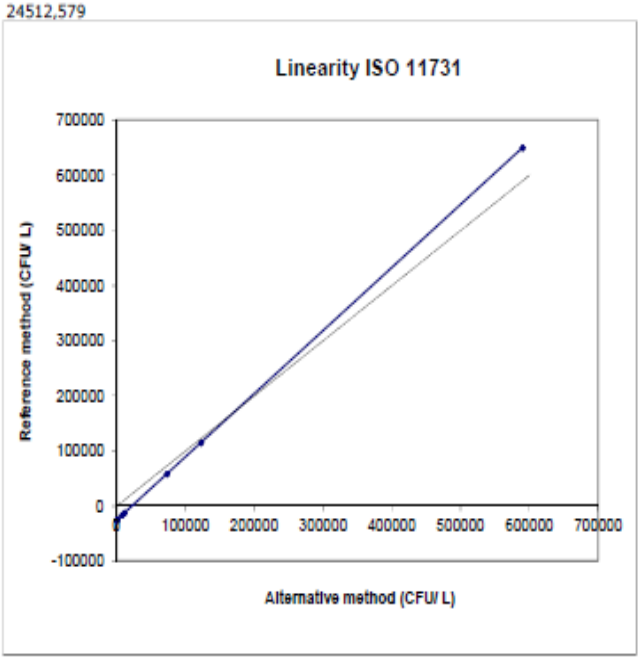
M(Alt.)	Ref	Est. y	Deviation
104,000	40,000	-25386,952	25426,952
723,000	110,000	-24678,471	24788,471
1273,500	170,000	-24048,392	24218,392
7991,500	970,000	-16359,257	17329,257
11660,500	5000,000	-12159,877	17159,877
73555,000	90000,000	58681,921	31318,079
122635,000	140000,000	114856,789	25143,211
590050,000	690000,000	649840,004	40159,996
104,000	50,000	-25386,952	25436,952
723,000	130,000	-24678,471	24808,471
1273,500	210,000	-24048,392	24258,392
7991,500	1300,000	-16359,257	17659,257
11660,500	10000,000	-12159,877	22159,877
73555,000	65000,000	58681,921	6318,079
122635,000	110000,000	114856,789	-4856,789
590050,000	655000,000	649840,004	5159,996

Sb = 0,028
Sa = 6876,901

p(t;b=1) = 0,000
p(t;a=0) = 0,002

t (b) = 5,120
t (a) = 3,709

Res.SD = 24512,579



Linearity

F = 28,799
rob.F = 2095,421

p(F) = 0,000
rob.p(F) = 0,000

Linearity: “water for human consumption”: IDEXX Legiolert vs ISO 11731 (LOG)

Level	Reference method (x) Rep.1	Rep.2	M	SD	Alternative method (y) Rep.1	Rep.2	M	SD
1	1,699	1,699	1,699	0,000	2,017	2,017	2,017	0,000
2	2,000	2,146	2,073	0,103	2,859	2,859	2,859	0,000
3	2,279	2,322	2,300	0,031	3,173	3,024	3,099	0,105
4	3,146	3,114	3,130	0,023	3,910	3,895	3,903	0,010
5	3,699	4,000	3,849	0,213	4,045	4,087	4,066	0,030
6	4,954	4,813	4,884	0,100	4,881	4,852	4,866	0,020
7	5,146	5,041	5,094	0,074	5,066	5,111	5,088	0,032
8	5,839	5,816	5,828	0,016	5,784	5,757	5,771	0,019

Mx = 3,607 **My =** 3,959

q = 8 **MEDx =** 3,490 **MEDy =** 3,984

n = 2 **SDbx =** 1,547 **SDby =** 1,260

N = qn = 16

MEDwx = 0,052 **MEDwy =** 0,019

SDwx = 0,068 **SDwy =** 0,029

rob. SDwx = 0,078 **rob. SDwy =** 0,029

Regression used

OLS; y=ref

Sx = 1,496

Sy = 1,218

R = 0,435 **r =** 0,984

rob.R = 0,371 **b =** 1,209

a = -0,403

M(alt.)	Ref.	Est. y	Deviation
2,017	1,602	2,036	-0,434
2,859	2,041	3,054	-1,013
3,099	2,230	3,344	-1,113
3,903	2,987	4,316	-1,329
4,066	3,699	4,514	-0,815
4,866	4,954	5,481	-0,527
5,088	5,146	5,749	-0,603
5,771	5,839	6,574	-0,736
2,017	1,699	2,036	-0,337
2,859	2,114	3,054	-0,940
3,099	2,322	3,344	-1,022
3,903	3,114	4,316	-1,202
4,066	4,000	4,514	-0,514
4,866	4,813	5,481	-0,668
5,088	5,041	5,749	-0,708
5,771	5,816	6,574	-0,758

Res.SD = 0,900

Sb = 0,155

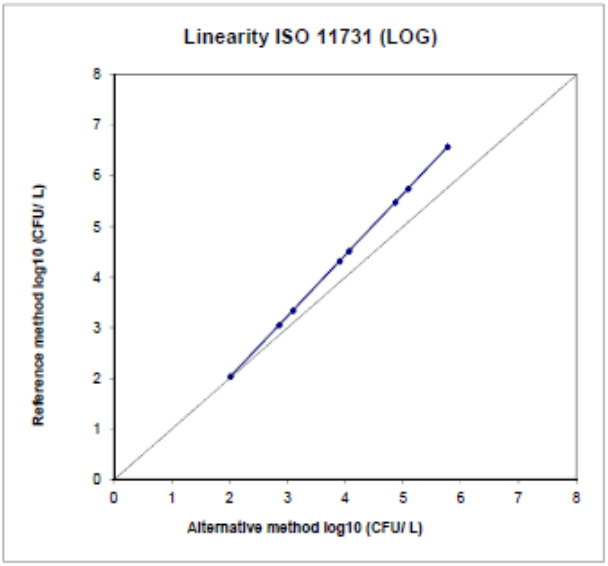
Sa = 0,604

p(t;b=1) = 0,200

p(t;a=0) = 0,516

t (b) = 1,346

t (a) = 0,667



Linearity

F = 2177,264 **p(F) =** 0,000

rob.F = 2273,083 **rob.p(F) =** 0,000

Linearity: category “industrial waters”

				MR: T90-431								MR: ISO 11731								MA: LEGIOLERT			
				Directs	Concentration by filtration				Results			Directs	Concentration by filtration				Results			Reading			
				/	C	CTT	CTA	CTTA		*Legionella pneumophila*		/	C	CTT	CTA	CTTA		*Legionella pneumophila*		*Legionella pneumophila*			
Sample	Strain	Sample number	Concentration (CFU/L)	0,2mL GVPC	Not treated (0.1 mL)	Heat-treatment (50±1°C, 30±1min)	Acid treatment ph2 (V/V: 5±0,5min)	Associated treatment (heat and acid) (50±1°C, 30±1min puis V/V ph2: 5±0,5min)	Legionella spp (CFU/L)	Results (CFU/L)	sero-group	0,2mL GVPC	non treated (0.1mL)	Heat-treatment (50±1°C, 30±1min)	Acid treatment ph2.2±0,2 (9V/1V 5±0,5min)	Associated treatment (50±1°C, 30±1min then 9V/1V ph2.2: 5±0,5min)	Legionella spp (CFU/L)	Results (CFU/L)	serogroup	Big well	Small well	Results (CFU/L)	sero-group
Cooling water	LEG.1.18	ILL 1000 R1	1000	0	2	0	0	1	2.00E+02	2.00E+02	1	0	2	0	0	1	2.00E+02	2.00E+02	/	1	0	1.10E+03	1
		ILL 1000 R2		0	2	0	2	1	2.00E+02	2.00E+02	1	0	2	0	2	1	2.00E+02	1.00E+02	1	1	0	1.00E+00	1
		ILL 5000 R1	5000	1	15	5	9	4	5.00E+03	5.00E+03	1	1	15	5	9	4	5.00E+03	5.00E+03	1	1	1	2.20E+03	1
		ILL 5000 R2		1	15	6	11	7	5.00E+03	5.00E+03	1	1	15	6	11	7	5.00E+03	5.00E+03	1	2	0	2.30E+03	1
		ILL 10000 R1	10000	2	23	7	44	9	1.00E+04	1.00E+04	1	2	23	7	44	9	1.00E+04	1.00E+04	1	4	2	9.00E+03	1
		ILL 10000 R2		1	23	13	50	6	5.00E+03	5.00E+03	1	1	23	13	50	6	5.00E+03	5.00E+03	1	4	1	7.40E+03	1
		ILL 50000 R1	50000	9	/	/	/	/	4.50E+04	4.50E+04	1	9	/	/	/	/	4.50E+04	4.50E+04	1	6	6	3.61E+04	1
		ILL 50000 R2		4	/	/	/	/	2.00E+04	2.00E+04	1	4	/	/	/	/	2.00E+04	2.00E+04	1	5	2	1.24E+04	1
		ILL 100000 R1	100000	22	/	/	/	/	1.10E+05	1.10E+05	1	22	/	/	/	/	1.10E+05	1.10E+05	1	6	14	8.54E+04	1
		ILL 100000 R2		22	/	/	/	/	1.10E+05	1.10E+05	1	22	/	/	/	/	1.10E+05	1.10E+05	1	6	13	7.88E+04	1
		ILL 500000 R1	500000	84	/	/	/	/	4.20E+05	4.20E+05	1	84	/	/	/	/	4.20E+05	4.20E+05	1	6	35	2.49E+05	1
		ILL 500000 R2		91	/	/	/	/	4.55E+05	4.55E+05	1	91	/	/	/	/	4.55E+05	4.55E+05	1	6	38	2.77E+05	1
		ILL 1000000 R1	1000000	137	/	/	/	/	6.85E+05	6.85E+05	1	137	/	/	/	/	6.85E+05	6.85E+05	1	6	51	4.22E+05	1
		ILL 1000000 R2		123	/	/	/	/	6.15E+05	6.15E+05	1	123	/	/	/	/	6.15E+05	6.15E+05	1	6	49	3.97E+05	1

V/V: Volume/volume

Linearity: “industrial waters”: IDEXX Legiolert vs NF T90-431 and ISO 11731

Level	Reference method Rep.1	Rep.2	M	SD	Alternative method Rep.1	Rep.2	M	SD
1	200.00	200.00	200.0	0.000	1100.00	1100.00	1100.0	0.000
2	5000.00	5000.00	5000.0	0.000	2200.00	2300.00	2250.0	70.711
3	10000.00	5000.00	7500.0	3535.534	9000.00	7400.00	8200.0	1131.371
4	45000.00	20000.00	32500.0	17677.670	36100.00	12400.00	24250.0	16758.431
5	110000.00	110000.00	110000.0	0.000	85400.00	78800.00	82100.0	4666.905
6	420000.00	455000.00	437500.0	24748.737	249000.00	277000.00	263000.0	19798.990
7	685000.00	615000.00	650000.0	49497.475	422000.00	397000.00	409500.0	17677.670

q = 7
n = 2
N = qn = 14

Mx = 177528.57
MEDx = 32500.00
SDbx = 260297.60

MEDwx = 3535.53
SDwx = 15555.20
rob. SDwx = 5241.78

My = 112914.29
MEDy = 24250.00
SDby = 160734.66

MEDwy = 4666.90
SDwy = 8487.01
rob. SDwy = 6919.15

Regression used
GMFR

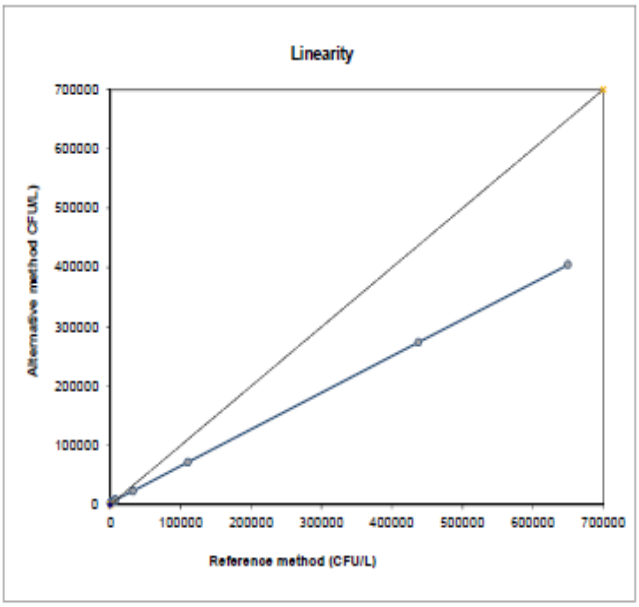
R = 0.546
rob.R = 1.320
Res.SEM = 7404.782
Res.SD = 10471.943

Sx = 250606.275
Sy = 154679.813

Est y	Deviation
3463.115	-2363.115
6425.783	-4175.783
7968.839	231.161
23399.399	850.601
71234.137	10865.863
273374.481	-10374.481
404534.246	4965.754

r = 0.999
b = 0.617
a = 3339.671

Sb = 0.012
Sa = 3524.044
p(t;b=1) = 0.000
p(t;a=0) = 0.362
t (b) = 31.732
t (a) = 0.947



Linearity

F = 2.254
rob.F = 4.097
p(F) = 0.159
rob.p(F) = 0.047

Linearity: “industrial waters”: IDEXX Legiolert vs NF T90-431 and ISO 11731 (LOG)

Level	Reference method				Alternative method			
	Rep.1	Rep.2	M	SD	Rep.1	Rep.2	M	SD
1	2.30	2.30	2.3	0.000	3.04	3.04	3.0	0.000
2	3.70	3.70	3.7	0.000	3.34	3.36	3.4	0.014
3	4.00	3.70	3.8	0.213	3.95	3.87	3.9	0.060
4	4.65	4.30	4.5	0.249	4.56	4.09	4.3	0.328
5	5.04	5.04	5.0	0.000	4.93	4.90	4.9	0.025
6	5.62	5.66	5.6	0.025	5.40	5.44	5.4	0.033
7	5.84	5.79	5.8	0.033	5.63	5.60	5.6	0.019

		Mx =	4.40	My =	4.37	
q =	7	MEDx =	4.48	MEDy =	4.33	
n =	2	SDbx =	1.23	SDby =	1.00	
N = qn =	14					
		MEDwx =	0.02	MEDwy =	0.02	
		SDwx =	0.09	SDwy =	0.09	
		rob. SDwx =	0.04	rob. SDwy =	0.04	

Regression used

GMFR

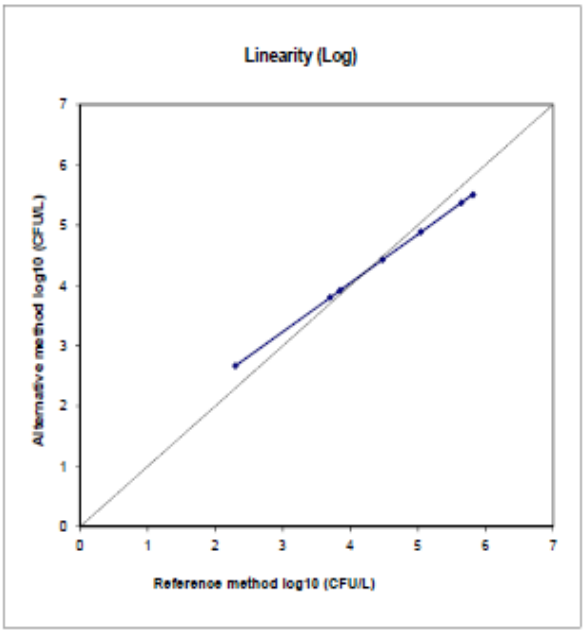
R =	1.020
rob.R =	1.005
Res.SEM =	0.270
Res.SD =	0.381

		Est y	Deviation
Sx =	1.190	2.670	0.372
Sy =	0.962	3.799	-0.447
		3.921	-0.009
		4.428	-0.102
		4.884	0.030
		5.368	0.051
		5.507	0.105
r =	0.970		
b =	0.808		
a =	0.810		

Sb =	0.092	p(t;b=1) =	0.060	t (b) =	2.077
Sa =	0.420	p(t;a=0) =	0.077	t (a) =	0.452

Linearity

F =	41.613	p(F) =	0.000
rob.F =	258.666	rob.p(F) =	0.000



Appendix 6: LOD-LOQ results

Limit of detection (LOD) and limit of Quantification (LOQ) category "water for human consumption"

Raw results Assay 1: category "water for human consumption"

Strain: *Legionella pneumophila (LEG 1.21)*

Target Level (CFU/L)	Real level (CFU/L)*	Replicate					
		1	2	3	4	5	6
		Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)
0	0	0	0	0	0	0	0
10	6	0	0	0	23	0	52
30	20	39	58	104	0	52	23
50	36	74	47	52	58	79	23

Raw results Assay 2: category "water for human consumption"

Strain: *Legionella pneumophila (LEG 1.21)*

Target Level (CFU/L)	Real level (CFU/L)*	Replicate					
		1	2	3	4	5	6
		Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)
0	0	0	0	0	0	0	0
10	8	11	0	23	0	0	0
20	16	0	20	23	10	20	0
40	34	58	65	39	74	35	77

Raw results Assay 3: category "water for human consumption"

Strain: *Legionella pneumophila (LEG 1.21)*

Target Level (CFU/L)	Real level (CFU/L)*	Replicate					
		1	2	3	4	5	6
		Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)
0	0	0	0	0	0	0	0
10	6	0	0	0	11	0	20
20	18	20	0	10	0	11	23
40	32	47	58	52	30	52	43

(*): Level calculated by 30 enumerations

Limit of detection (LOD) and limit of Quantification (LOQ) category “industrial waters”

Raw results: category “industrial waters”

Strain: ***Legionella pneumophila (LEG 1.18)***

Target Level (CFU/L)	Real level (CFU/L)*	Replicate					
		1	2	3	4	5	6
		Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)	Detection (MPN/1L)
0	0	0	0	0	0	0	0
750	780	0	0	1000	0	0	0
1000	1150	1100	0	1100	2300	0	2200
3000	3110	1000	3200	2200	3900	2300	3500

(*): Level calculated by 30 enumerations

Appendix 7: Selectivity results

Inclusivity: Legiolert potable protocol

Strain	Origin	Code	MA : Legiolert			
			Reading			
			Legionella pneumophilla			
			Big well	Small well	Results (CFU/L)	serogroup
Legionella pneumophila serogroup 3	Clinical	LEG 1.5	4	4	123	2.14
Legionella pneumophila serogroup 1	Clinical	LEG 1.3	5	3	146	1
Legionella pneumophila serogroup 7	Clinical	LEG 1.9	4	24	561	2.14
Legionella pneumophila serogroup 13	Clinical	LEG 1.15	3	28	402	2.14
Legionella pneumophila serogroup 1	Clinical	LEG 1.1	3	48	708	1
Legionella pneumophila serogroup 9	Clinical	LEG 1.11	4	2	90	2.14
Legionella pneumophila serogroup 4	Clinical	LEG 1.6	36	3	651	2.14
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.22	27	3	481	2.14
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.26	22	4	512	2.14
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.35	2	35	507	2.14
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.21	2	37	537	2.14
Legionella pneumophila serogroup 1	Potable water sample	LEG 1.32	1	45	550	1
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.37	17	4	393	2.14
Legionella pneumophila serogroup 1	Potable water sample	LEG 1.31	44	3	808	1
Legionella pneumophila serogroup 15	Clinical	LEG 1.17	4	52	970	2.14
Legionella pneumophila serogroup 14	Clinical	LEG 1.16	2	47	693	2.14
Legionella pneumophila serogroup 12	Clinical	LEG 1.14	2	41	599	2.14
Legionella pneumophila serogroup 8	Clinical	LEG 1.10	3	31	556	2.14
Legionella pneumophila serogroup 2-14	Clinical	LEG 1.2	2	67	1019	2.14
Legionella pneumophila serogroup 2	Clinical	LEG 1.4	2	45	661	2.14

Exclusivity: Legiolert potable protocol

Strain	Origin	Code	MA : Legiolert				
			Reading				Observation
			Legionella pneumophila				
			Big well	Small well	Results (CFU/L)	serogroup	
Escherichia coli	Fontain water	ESC 1.111	0	0	<10	/	/
Pseudomonas aeruginosa	Fontain water	PSE 1.4	0	0	<10	/	/
Pseudomonas aeruginosa	Fontain water	PSE 1.8	0	0	<10	/	/
Pseudomonas putida	Water	PSE.3.1	0	0	<10	/	/
Aeromonas hydrophila	Well water	AER 1.1	0	0	<10	/	/
Proteus mirabilis	Water	PRO 1.2	6	72	8128	-	Orange color and precipitate (not characteristic aspect, latex test negatif)
Proteus mirabilis	Food matrix	PRO 1.4	6	78	10176	-	Orange color and precipitate (not characteristic aspect, latex test negatif)
Proteus vulgaris	CIP 103989	PRO.2.1	6	66	6676	-	Orange color and precipitate (not characteristic aspect, latex test negatif)
Proteus penneri	Primary production	PRO.3.1	6	82	12224	-	Orange color and precipitate (not characteristic aspect, latex test negatif)
Staphylococcus aureus	Superficial water	STA 1.5	0	0	<10	/	/
Staphylococcus aureus	Water	STA 1.6	0	0	<10	/	/
Staphylococcus warneri	Nebulizer fruit and vegetable	STA.5.1	0	0	<10	/	/
Staphylococcus warneri	Nebulizer fruit and vegetable	STA.5.2	0	0	<10	/	/
Enterococcus faecalis	River water (Tarnise)	ENTC 1.4	0	0	<10	/	/
Stenotrophomonas maltophilia	Fontain water	STE.1.2	0	0	<10	/	/
Legionella parisiensis	Clinical	LEG 19.1	0	0	<10	/	/
Legionella erythra	Clinical	LEG 9.1	0	0	<10	/	/
Legionella spp	Cooling tower	LEG 2.1	0	0	<10	/	/
Legionella jordanis	Clinical	LEG 13.1	0	0	<10	/	/
Legionella anisa	CIP 103870	LEG 3.1	0	0	<10	/	/
Legionella feeleii	Clinical	LEG 10.1	0	0	<10	/	/
Legionella micdadei	CIP 103882	LEG 17.1	0	0	<10	/	/
Legionella cherrii	Clinical	LEG 6.1	0	0	<10	/	/
Legionella bozemanii	CIP 103872	LEG 5.1	0	0	<10	/	/
Legionella hackeliae	CIP 103844	LEG 12.1	0	0	<10	/	/
Legionella longbeachae	Clinical	LEG 15.1	0	0	<10	/	/
Legionella macrachernii	Clinical	LEG 16.1	0	0	<10	/	/
Legionella oakridgensis	Clinical	LEH 18.1	0	0	<10	/	/
Legionella tucsonensis	Clinical	LEG 21.1	0	0	<10	/	/

Additionnal strains tested as requested by AFNOR technical board

Additional strains tested as requested by AFNOR technical board

Inclusivity: Legiolert non-potable protocol

Strain	Origin	Code	MA : Legiolert			
			Reading			
			Legionella pneumophila			
			Big well	Small well	Results (CFU/L)	serogroup
Legionella pneumophila serogroup 3	Clinical	LEG 1.5	6	15	92100	2.14
Legionella pneumophila serogroup 1	Clinical	LEG 1.3	5	3	14600	1
Legionella pneumophila serogroup 7	Clinical	LEG 1.9	36	13	78800	2.14
Legionella pneumophila serogroup 13	Clinical	LEG 1.15	6	14	85400	2.14
Legionella pneumophila serogroup 1	Clinical	LEG 1.1	6	19	120000	1
Legionella pneumophila serogroup 9	Clinical	LEG 1.11	6	11	65900	2.14
Legionella pneumophila serogroup 4	Clinical	LEG 1.6	6	17	106000	2.14
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.22	6	15	92100	2.14
Legionella pneumophila serogroup 2-14	Potable water sample	LEG 1.26	6	5	31000	2.14
Legionella pneumophila serogroup 2-14	Cooling water	LEG 1.18	6	19	120000	2.14
Legionella pneumophila serogroup 2-14	Cooling water	LEG 1.19	6	9	53400	2.14
Legionella pneumophila serogroup 1	Cooling water	LEG 1.20	6	8	47400	1
Legionella pneumophila serogroup 1	Potable water sample	LEG 1.25	6	25	164000	1
Legionella pneumophila serogroup 1	Potable water sample	LEG 1.23	6	12	72300	1
Legionella pneumophila serogroup 15	Clinical	LEG 1.17	6	6	36100	/
Legionella pneumophila serogroup 14	Clinical	LEG 1.16	6	5	31000	2.14
Legionella pneumophila serogroup 12	Clinical	LEG 1.14	4	3	9000	2.14
Legionella pneumophila serogroup 8	Clinical	LEG 1.10	6	11	65900	2.14
Legionella pneumophila serogroup 2-14	Clinical	LEG 1.2	2	8	47400	2.14
Legionella pneumophila serogroup 2	Clinical	LEG 1.4	6	24	157000	2.14

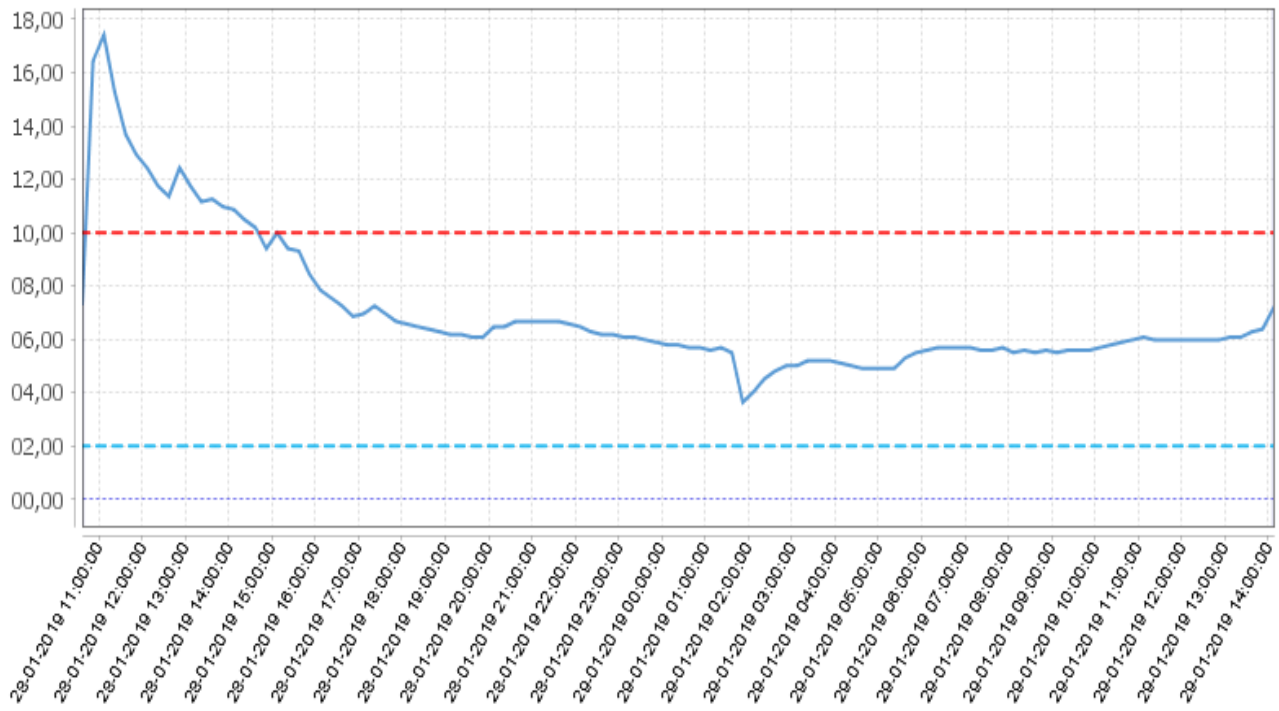
Exclusivity: Legiolert non- potable protocol

			MA : Legiolert				
Strain	Origin	Code	Reading				Observation
			Legionella pneumophilia				
			Big well	Small well	Results (CFU/L)	serogroup	
Escherichia coli	Fontain water	ESC 1.111	0	0	<10	/	/
Pseudomonas aeruginosa	Fontain water	PSE 1.4	0	0	<10	/	/
Aeromonas hydrophila	Well water	AER 1.1	0	0	<10	/	/
Proteus mirabilis	Water	PRO 1.2	0	0	<10	/	/
Staphylococcus aureus	superficial water	STA 1.5	0	0	<10	/	/
Enterococcus faecalis	River water (Tamise)	ENTC 1.4	0	0	<10	/	/
Legionella parisiensis	Clinical	LEG 19.1	0	0	<10	/	/
Legionella erythra	Clinical	LEG 9.1	0	0	<10	/	/
Legionella spp	Cooling tower	LEG 2.1	0	0	<10	/	/
Legionella jordanis	Clinical	LEG 13.1	0	0	<10	/	/
Legionella anisa	CIP 103870	LEG 3.1	0	0	<10	/	/
Legionella feeleii	Clinical	LEG 10.1	0	0	<10	/	/
Legionella micdadei	CIP 103882	LEG 17.1	0	0	<10	/	/
Legionella cherrii	Clinical	LEG 6.1	0	0	<10	/	/
Legionella bozemanii	CIP 103872	LEG 5.1	0	0	<10	/	/
Legionella hackeliae	CIP 103844	LEG 12.1	0	0	<10	/	/
Legionella longbeachae	Clinical	LEG 15.1	0	0	<10	/	/
Legionella maceachernii	Clinical	LEG 16.1	0	0	<10	/	/
Legionella oakridgensis	Clinical	LEG 18.1	0	0	<10	/	/
Legionella tucsonensis	Clinical	LEG 21.1	0	0	<10	/	/

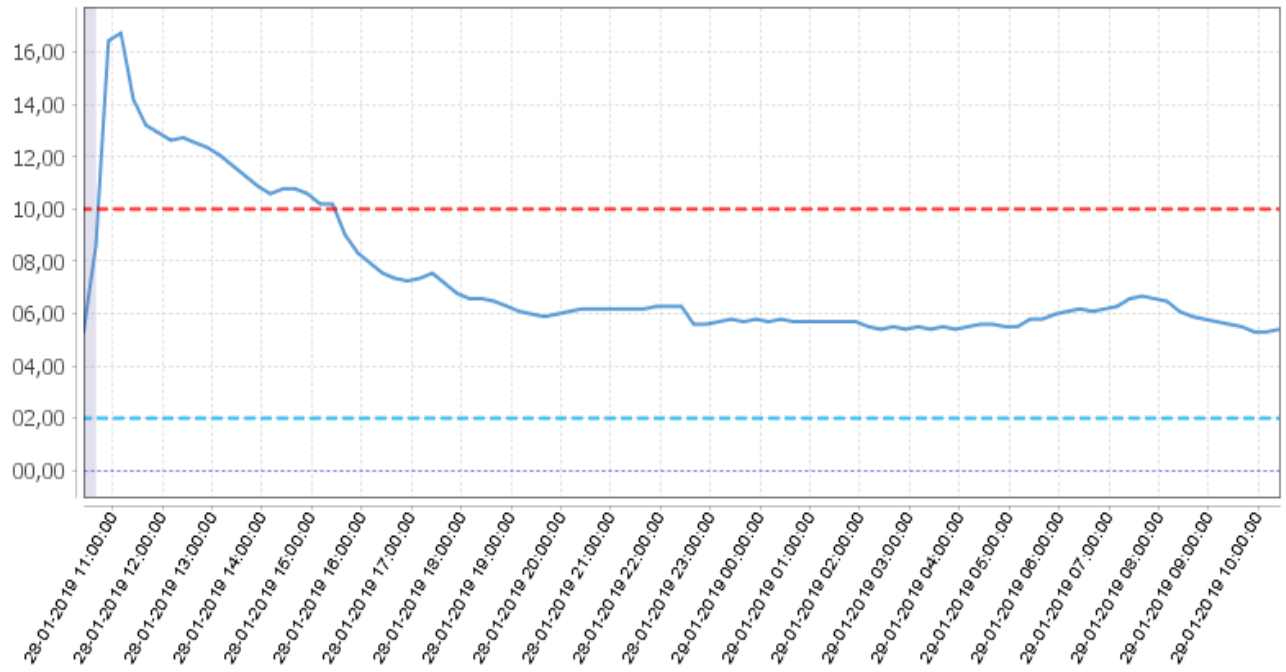
Appendix 8 : Temperature curves ILS1 then ILS2

Temperature curves ILS1

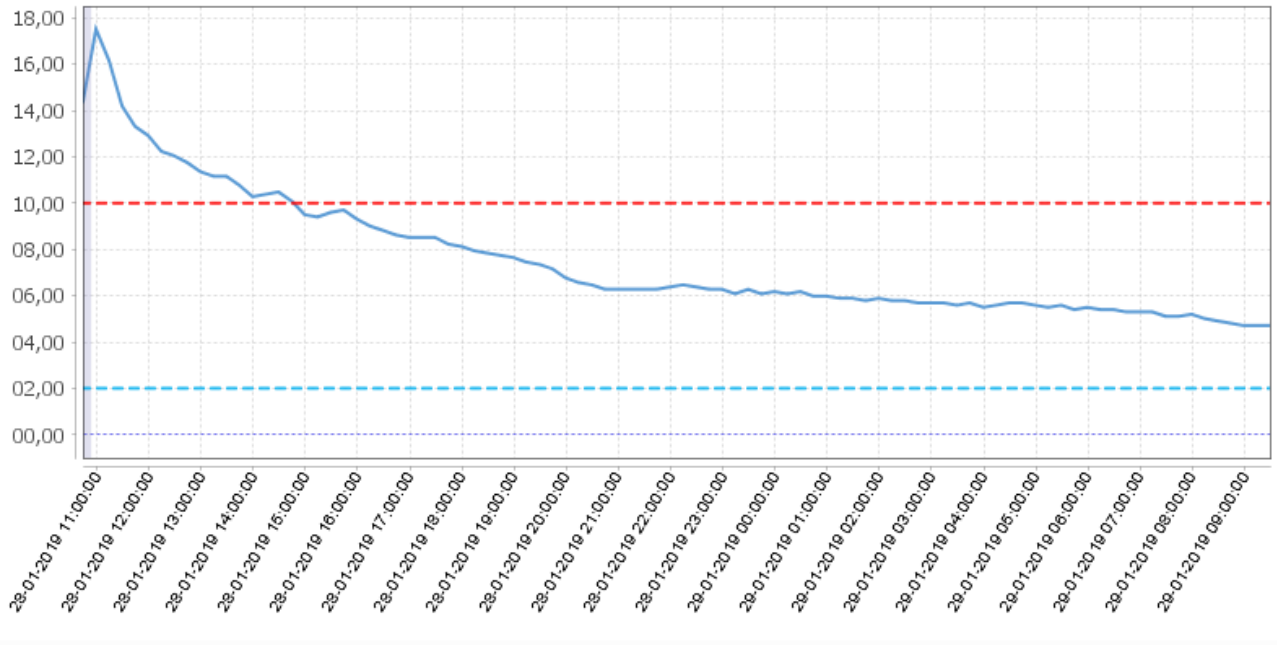
Laboratoire A1



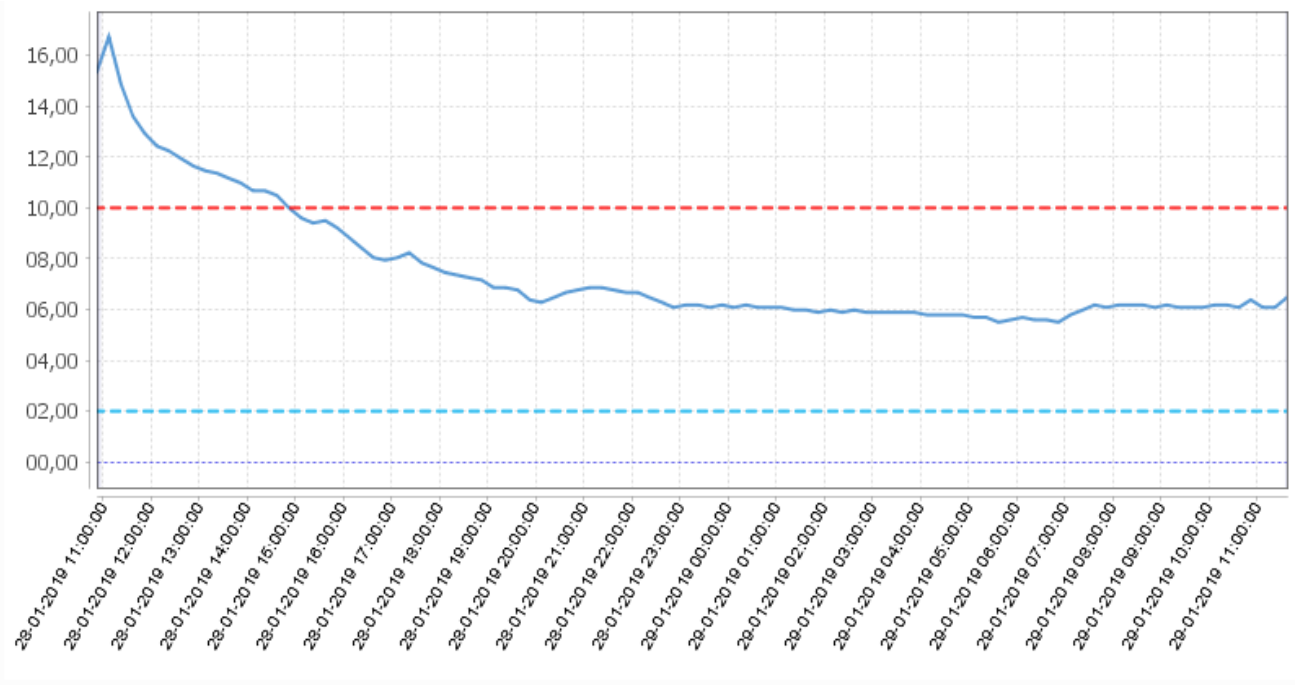
Laboratoire B1



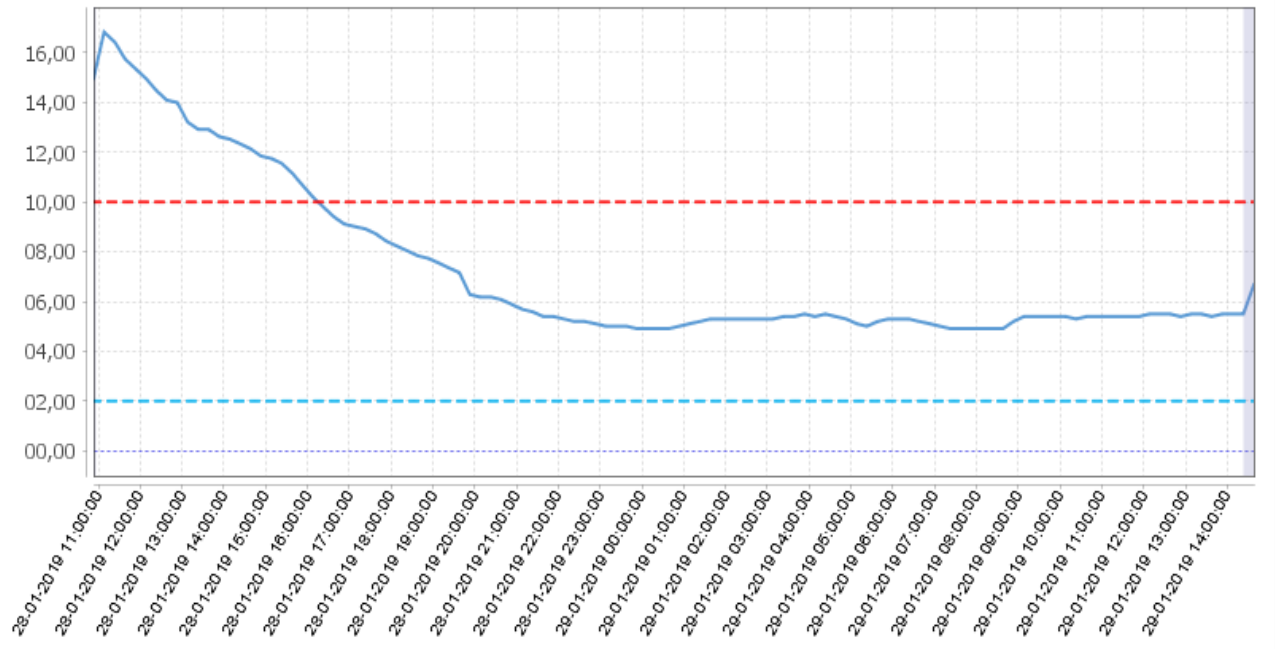
Laboratoire C1



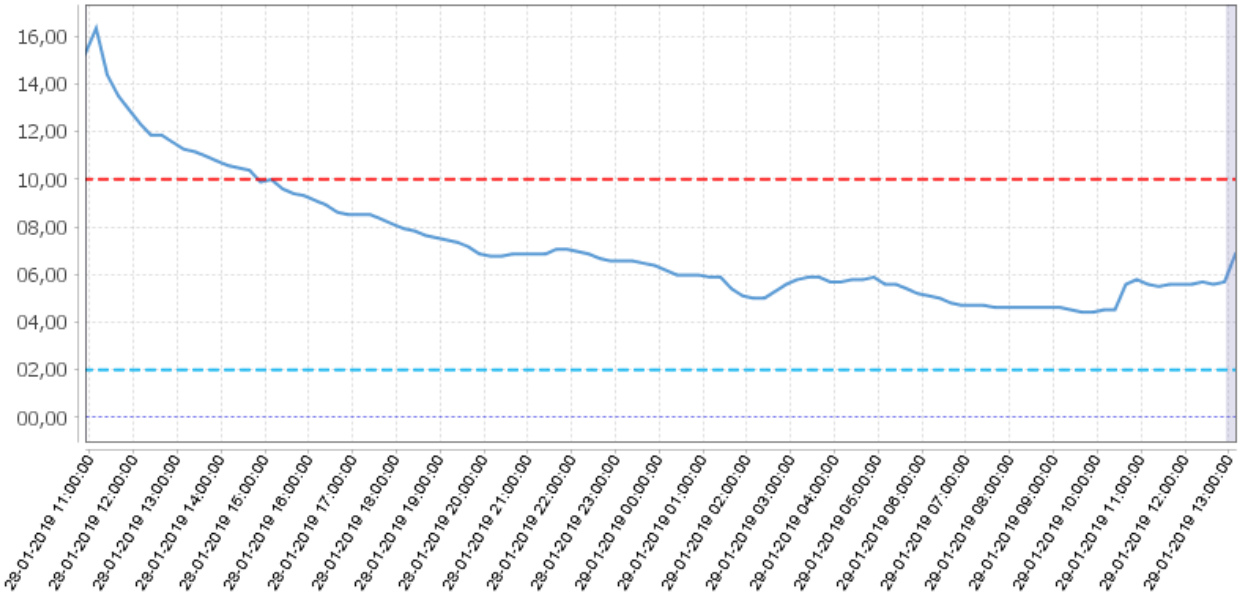
Laboratoire D1



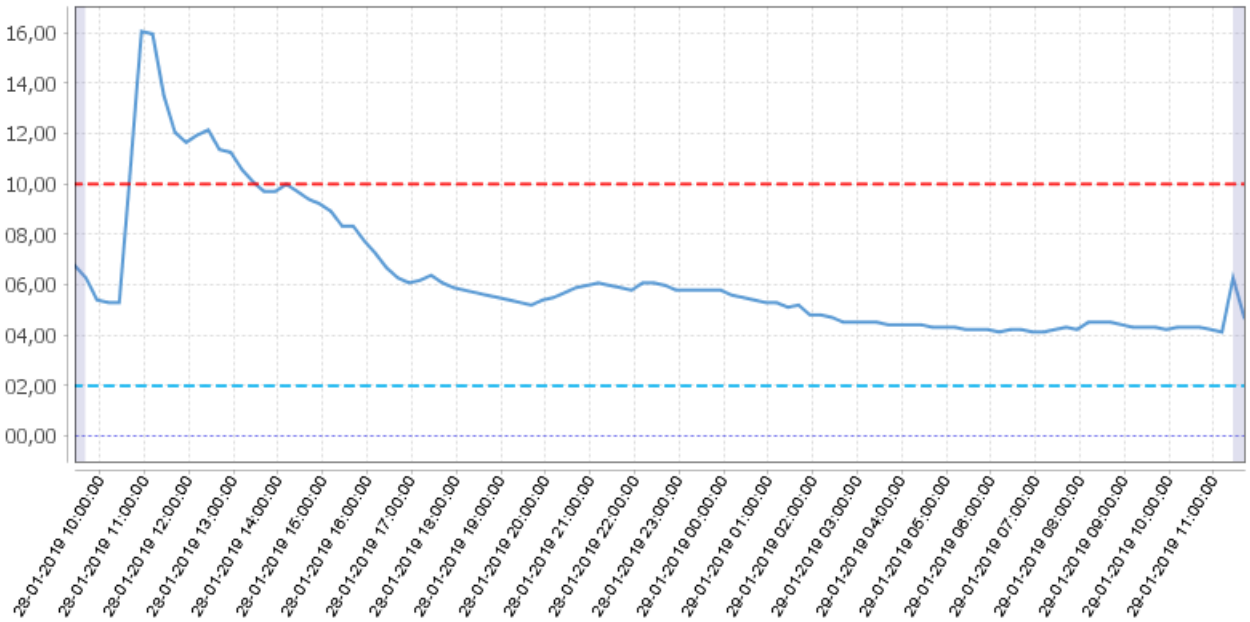
Laboratoire E1



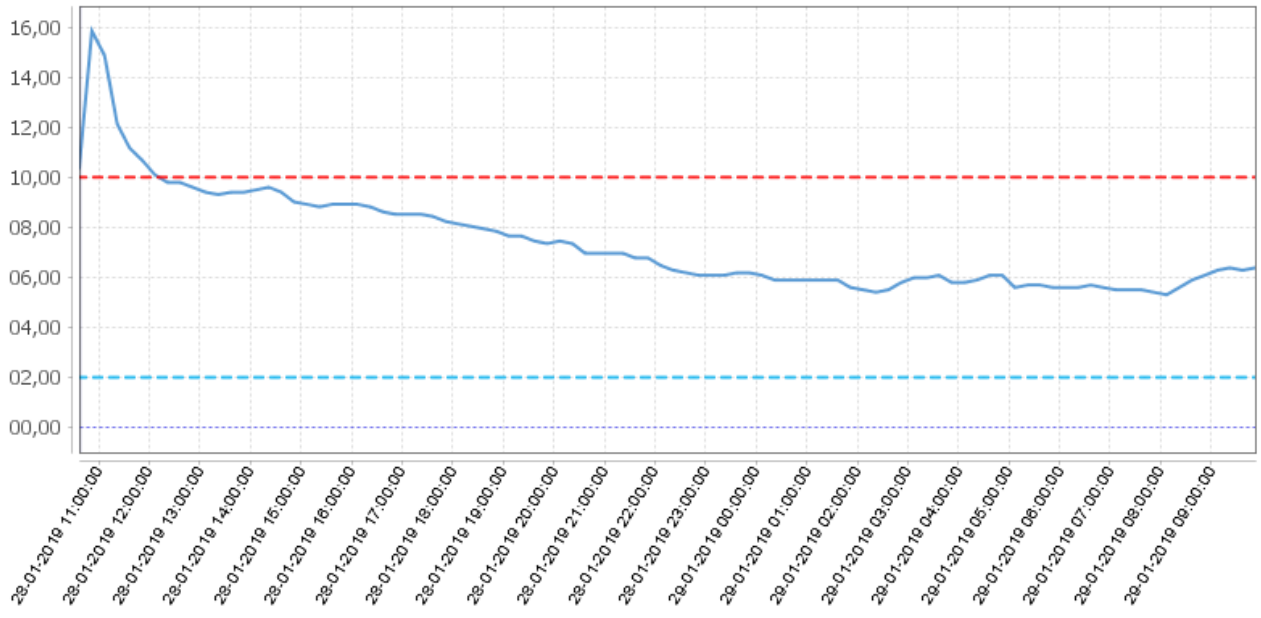
Laboratoire F1

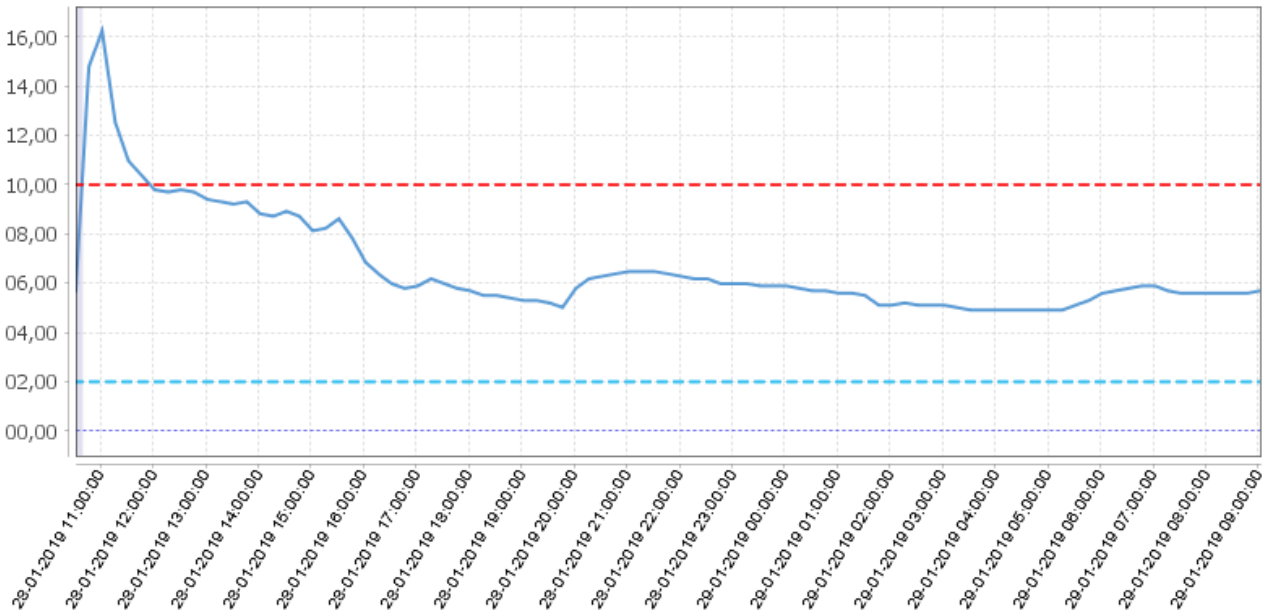


Laboratoire G1



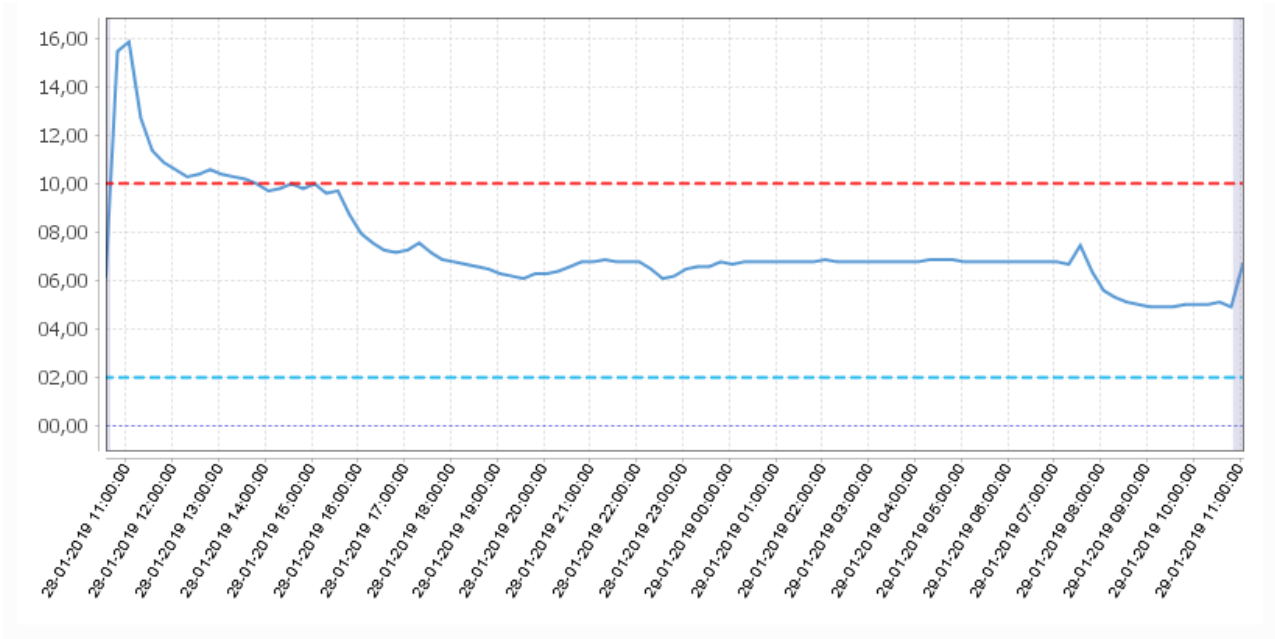
Laboratoire H1



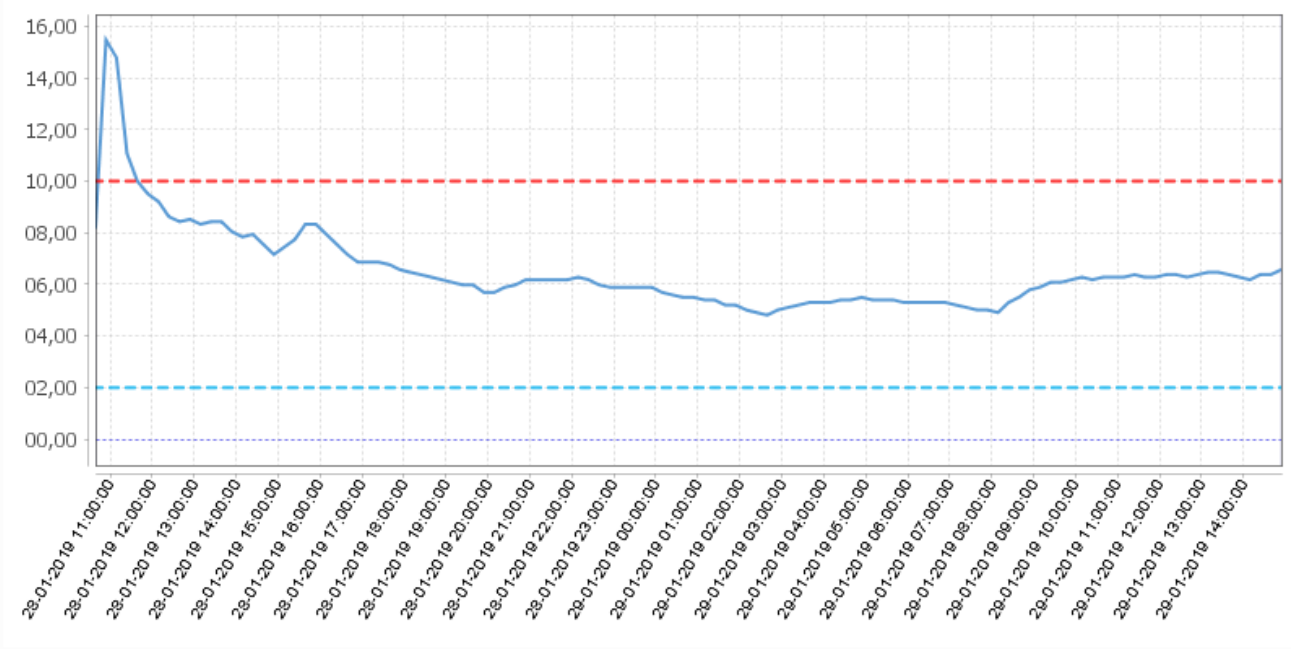


Laboratoire I1

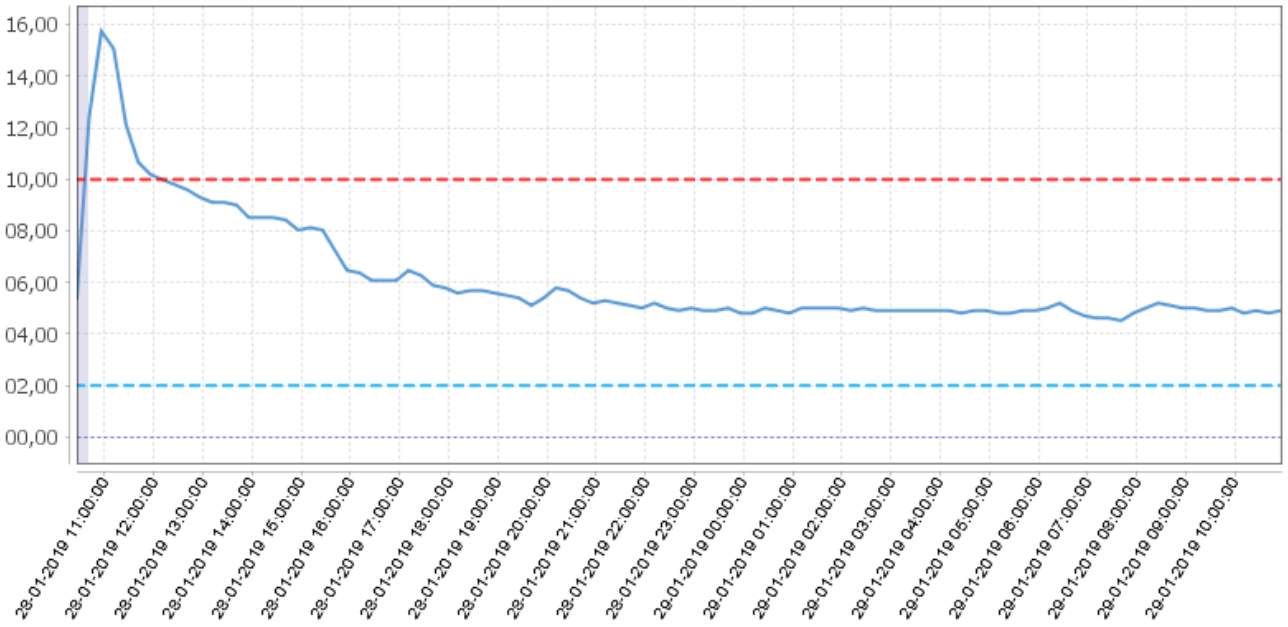
Laboratoire J1



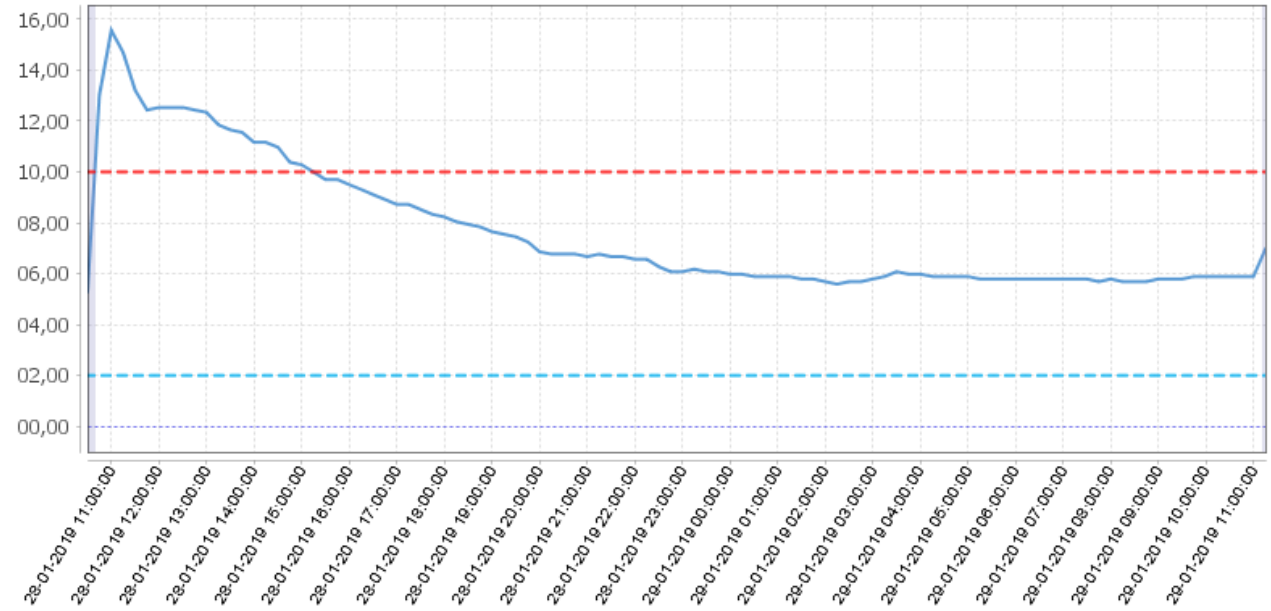
Laboratoire K1



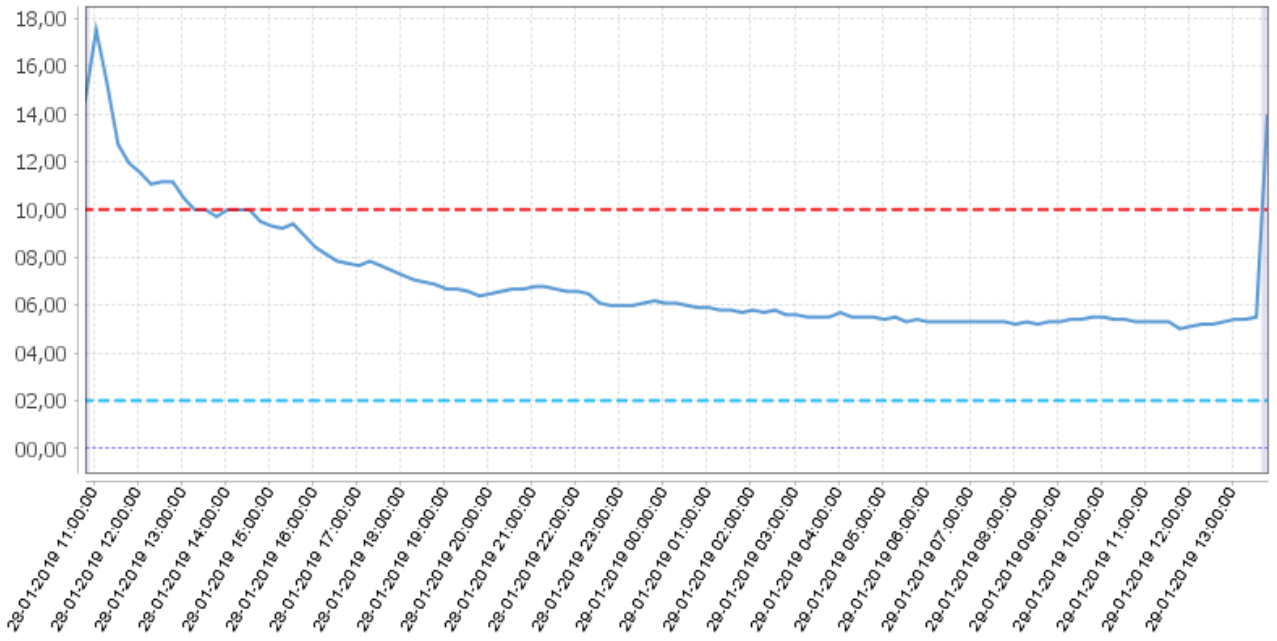
Laboratoire L1



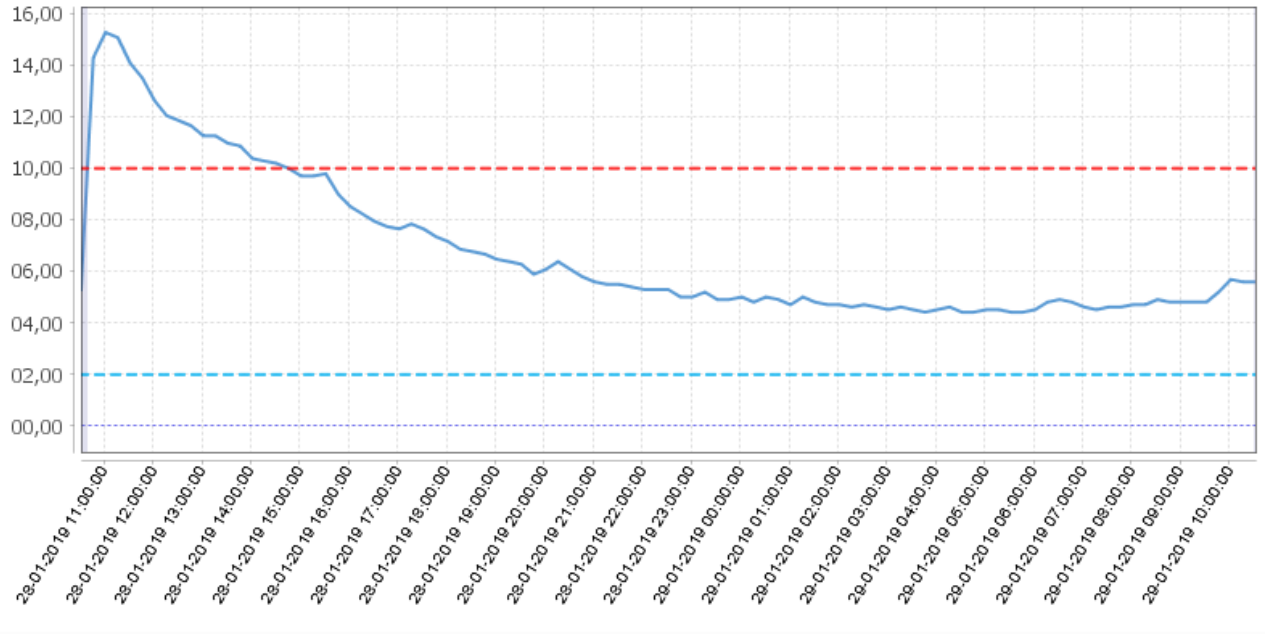
Laboratoire M1



Laboratoire N1

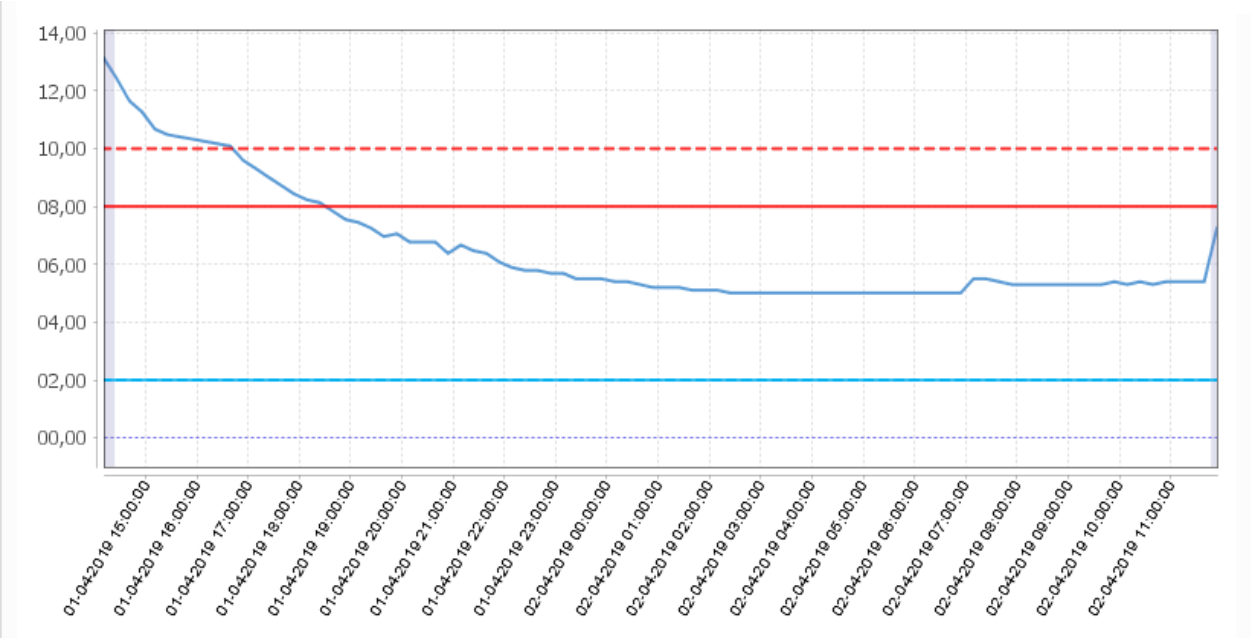


Laboratoire O1

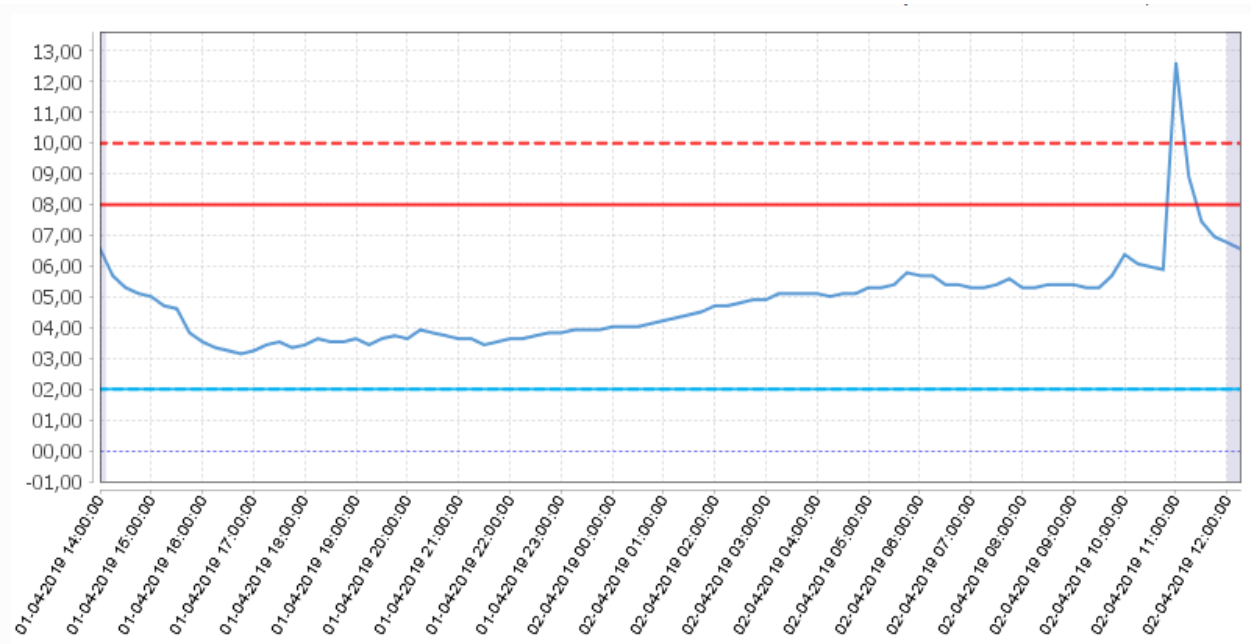


Temperature curves ILS2

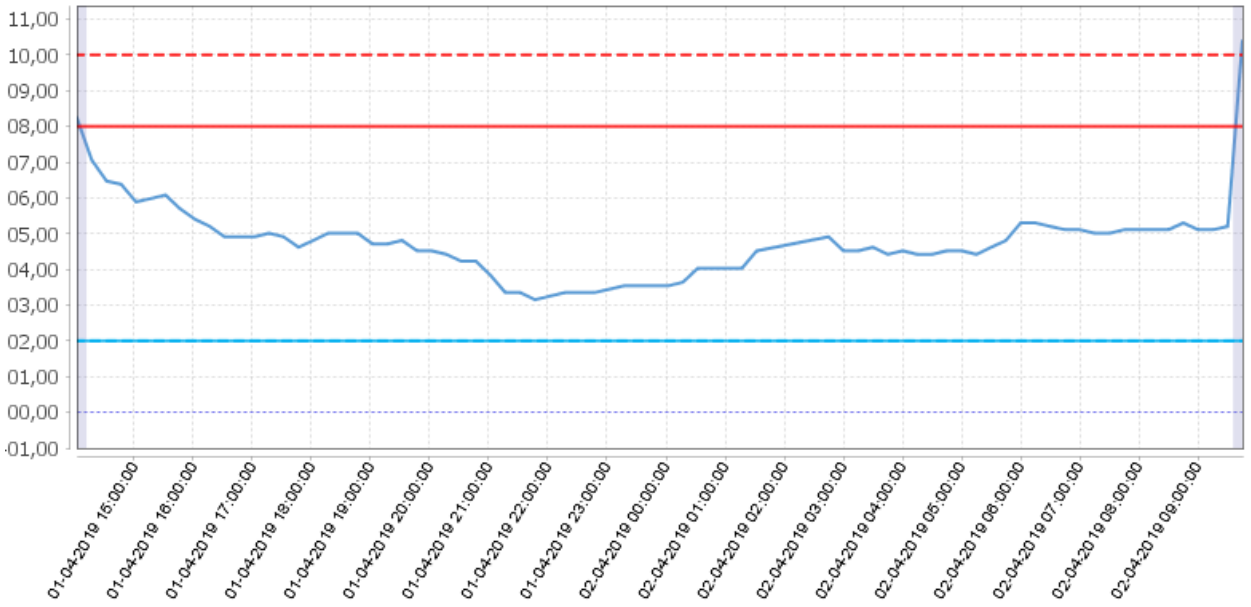
Laboratoire A2



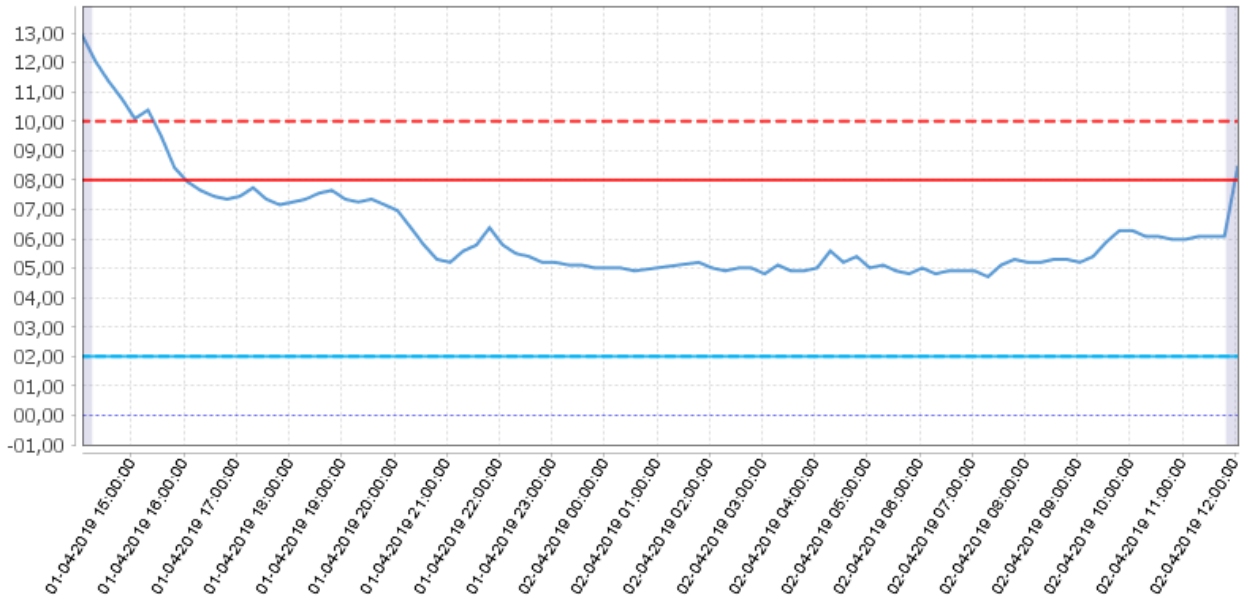
Laboratoire B2



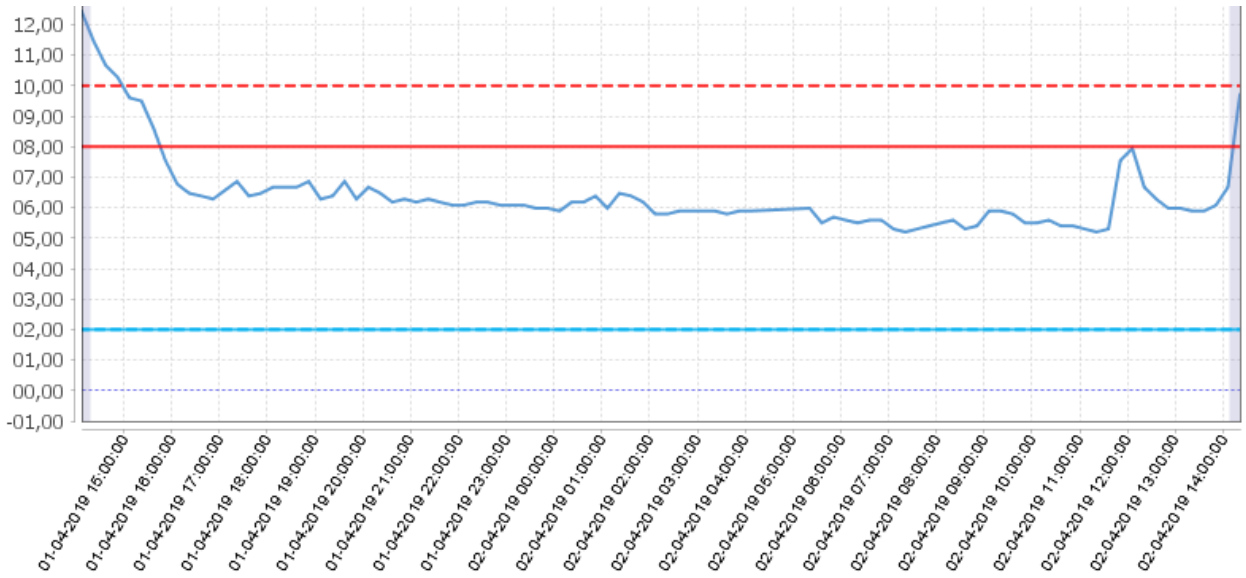
Laboratoire C2



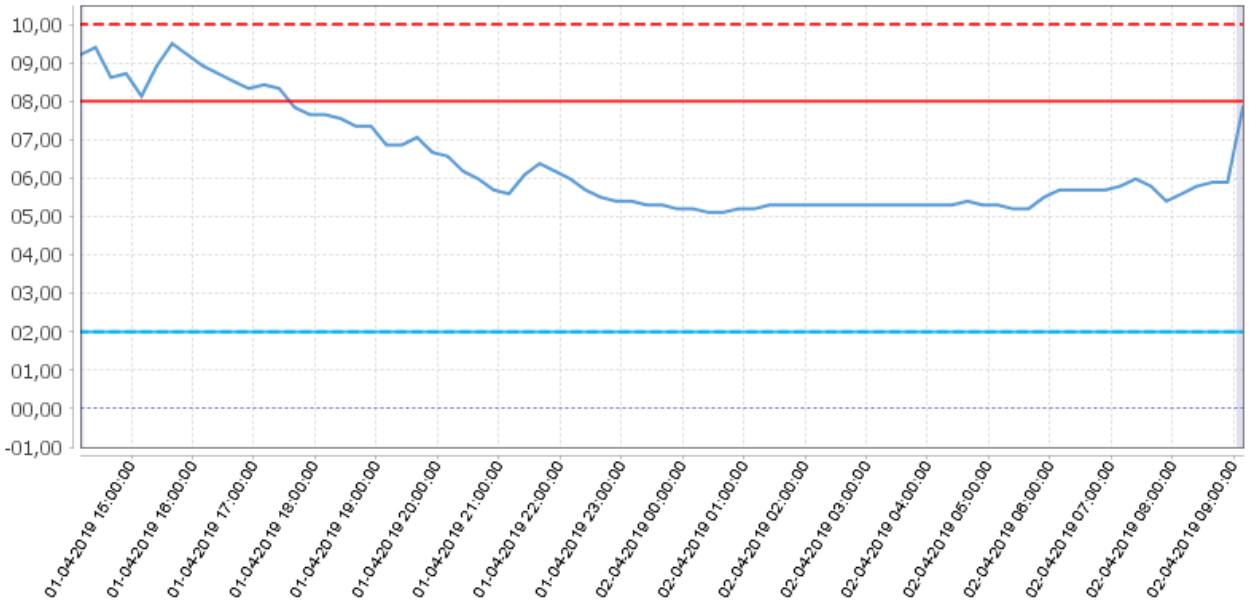
Laboratoire D2



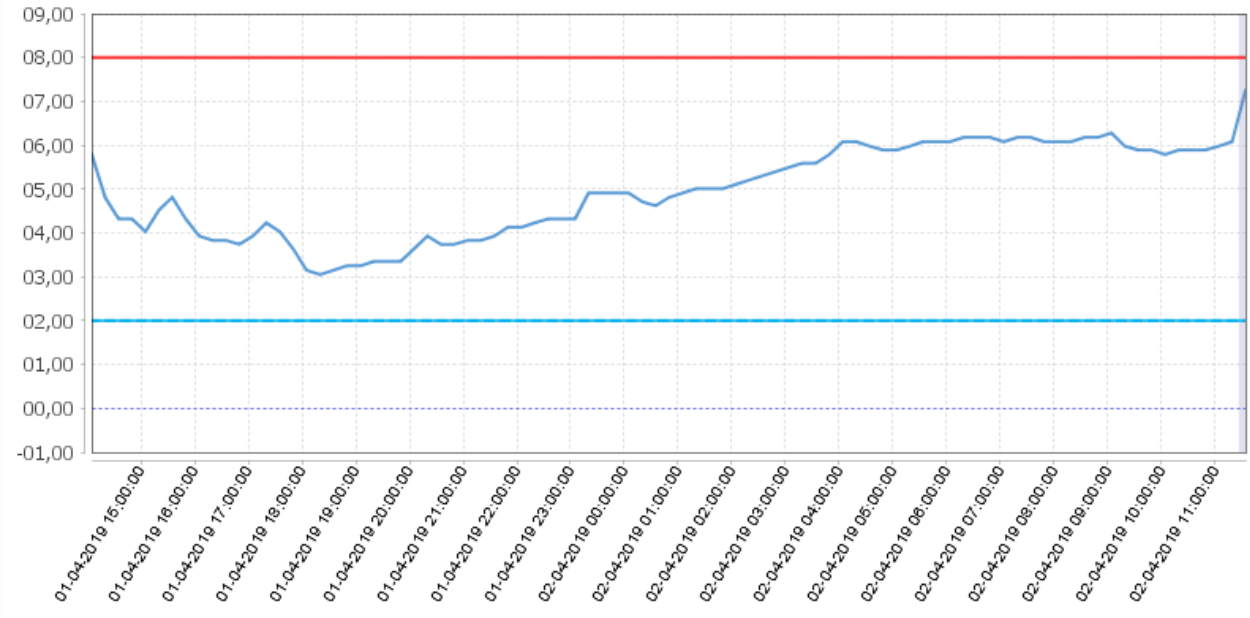
Laboratoire E2



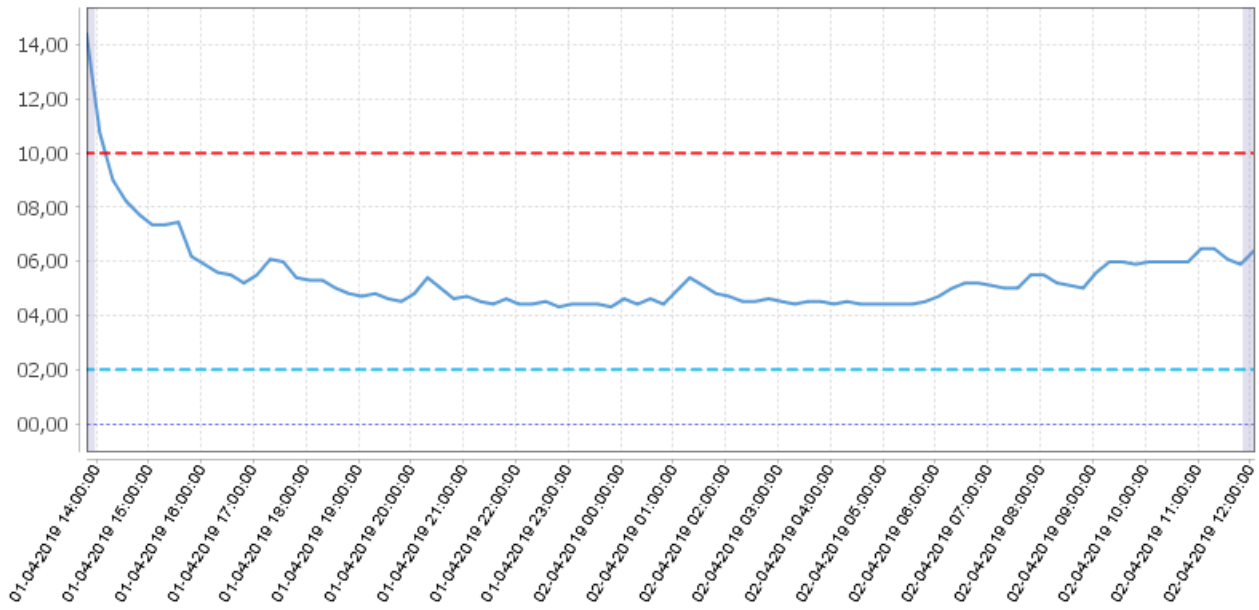
Laboratoire F2



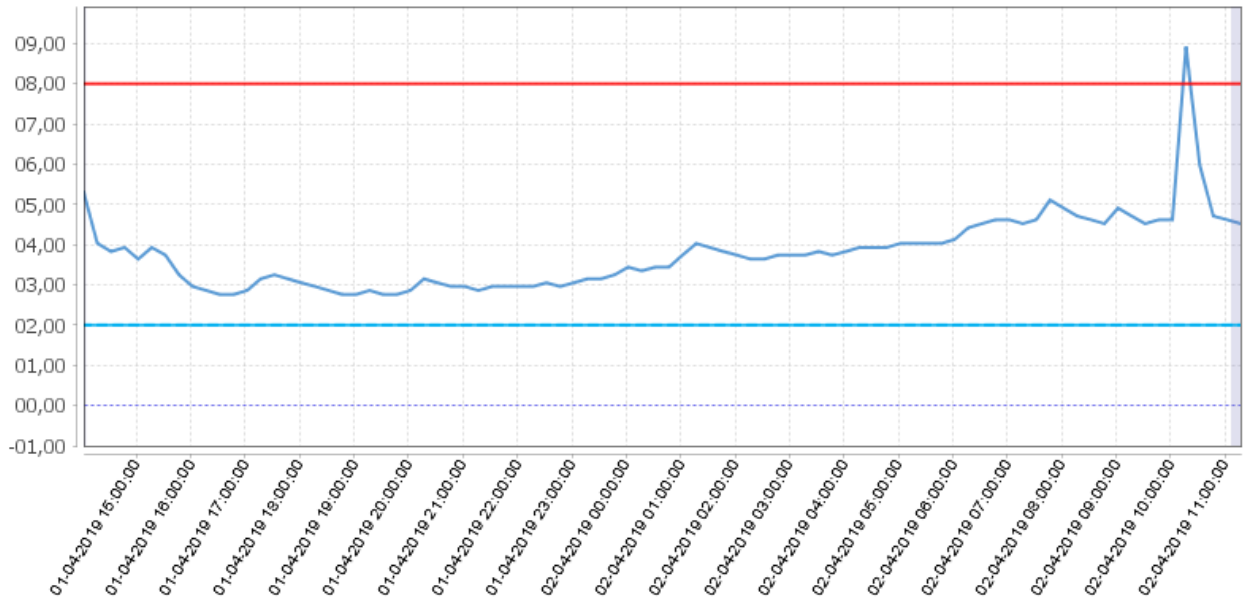
Laboratoire G2



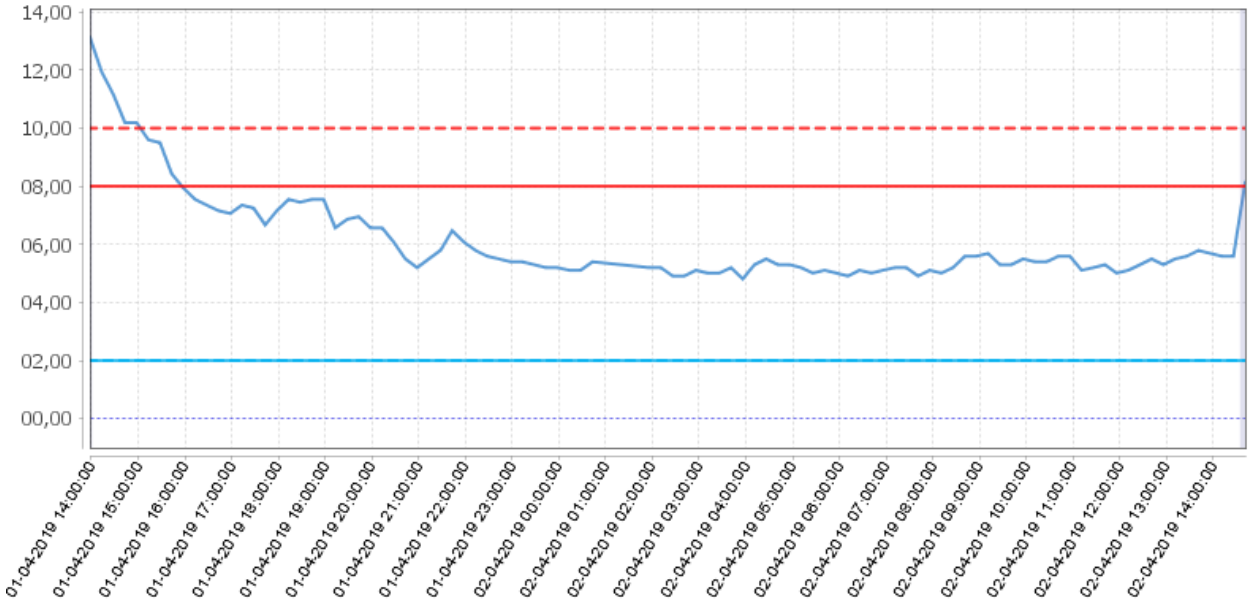
Laboratoire H2



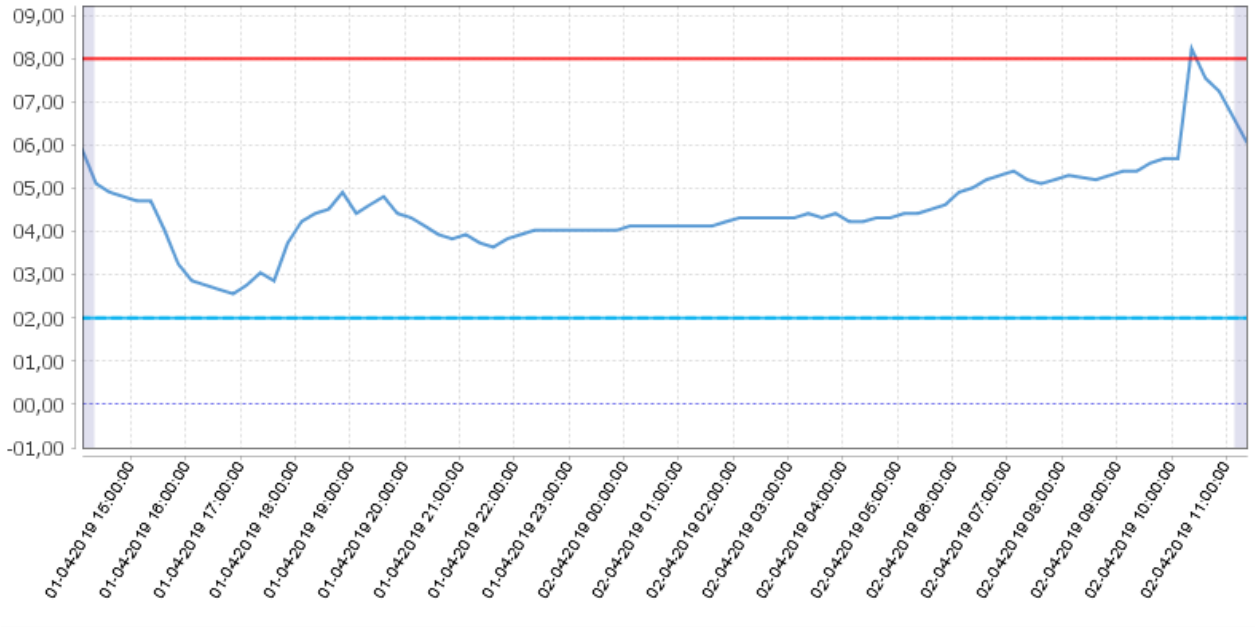
Laboratoire I2



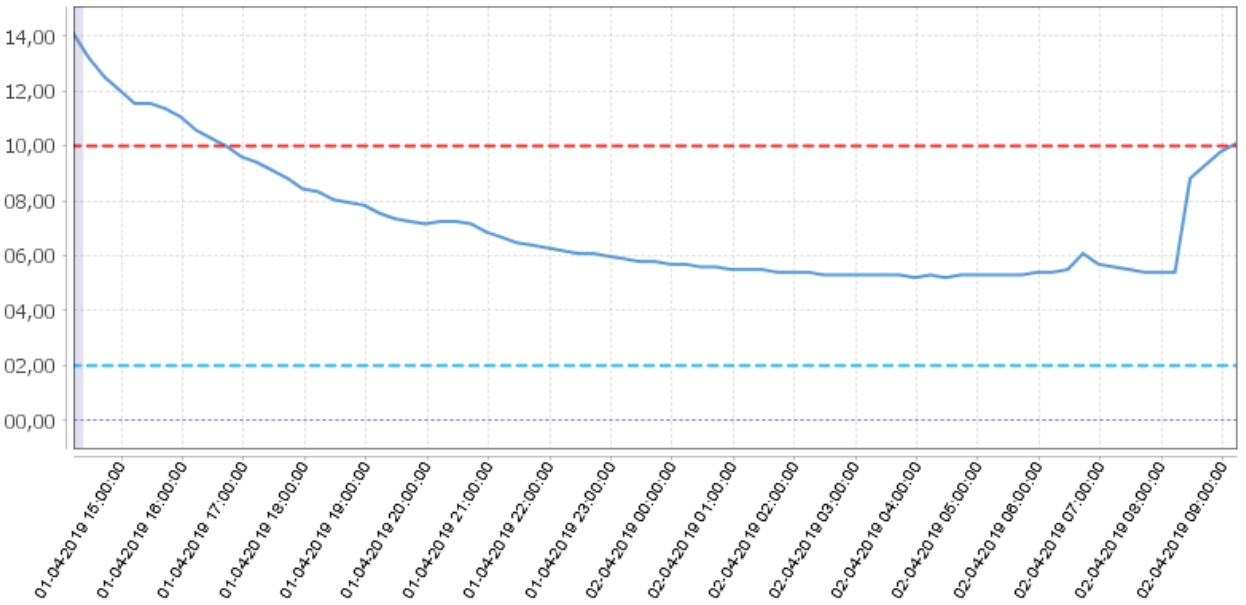
Laboratoire J2



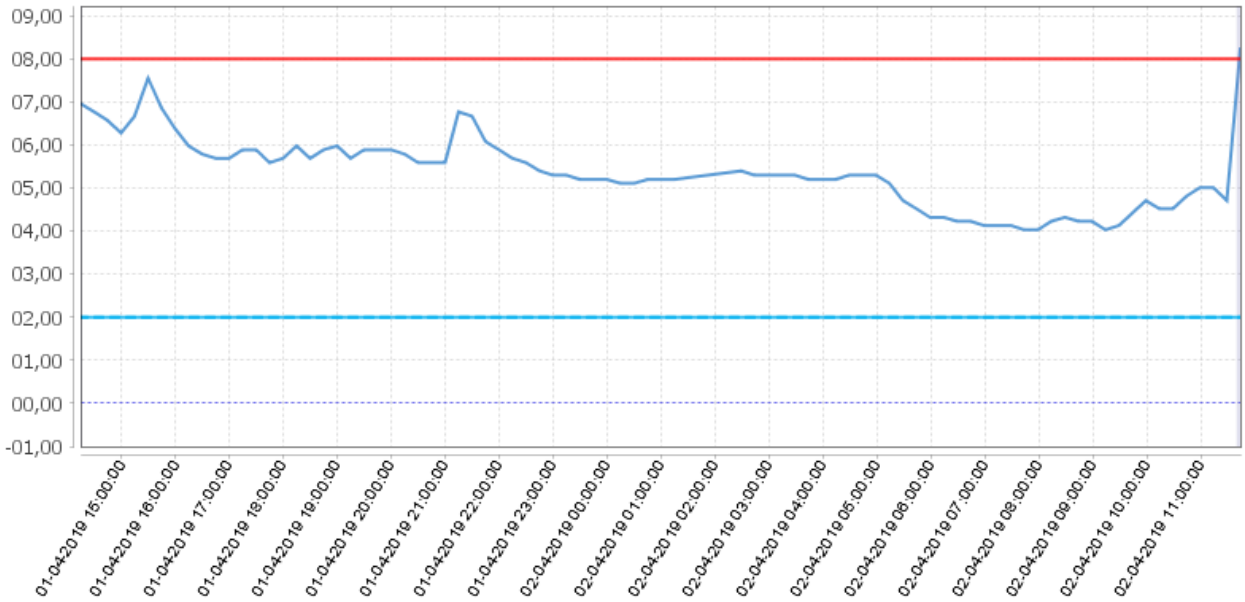
Laboratoire K2



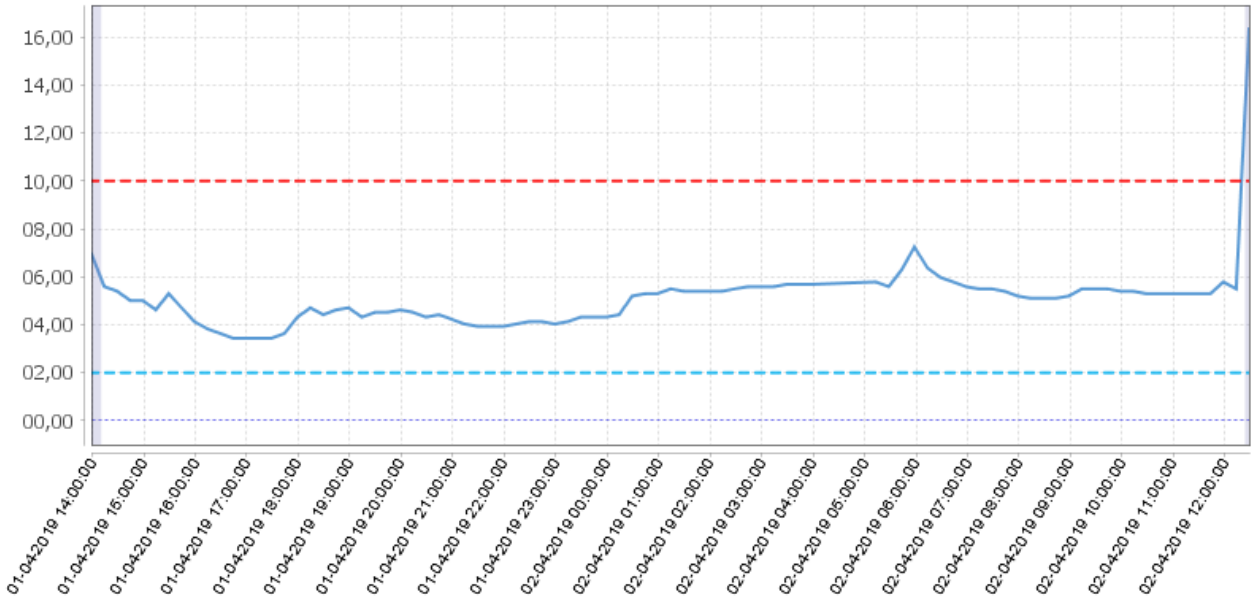
Laboratoire L2



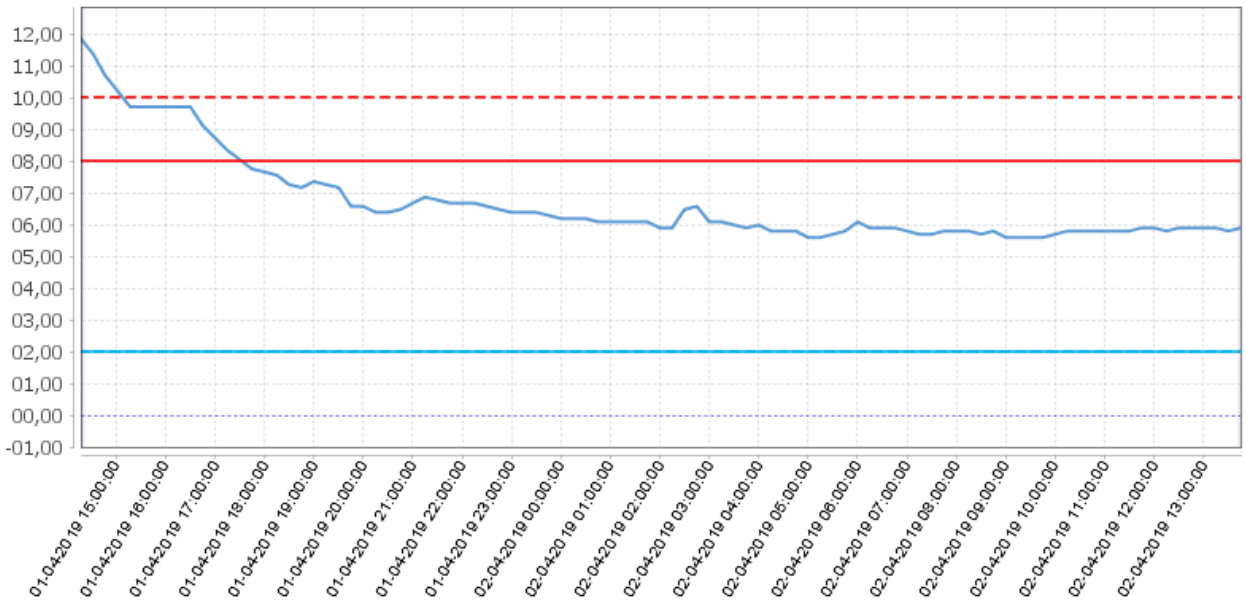
Laboratoire M2



Laboratoire N2



Laboratoire O2



Appendix 9: results - culturable microorganisms

Results of counts of revivable microorganisms at 22°C and 36°C (EIL1)

Laboratory	Result at 22°C (CFU/mL)	Result at 36°C (CFU/mL)
A1	<1	<1
B1	<1	<1
D1	<1	<1
E1	<1	<1
F1	<1	<1
G1	<1	<1
H1	<1	<1
I1	<1	<1
J1	<1	<1
K1	<1	<1
L1	<1	<1
M1	<1	<1
N1	<1	<1
O1 (Expert)	<1	<1

Results of counts of revivable microorganisms at 22°C and 36°C (EIL2)

Laboratory	Result at 22°C (CFU/mL)	Result à 36°C (CFU/mL)
A2	10	15
B2	15-18	140-160
C2	>300	>300
D2	<1	<1
E2	7	7
F2	<1	<1
G2	5	4
H2	1	2
I2 (expert)	<1	<1
J2	8	2
K2	<1	<1
L2	<1	<1
M2	1	2
N2	3	<1
O2	2	0

9- Appendix 10: interlaboratory study raw data ILS1 & ILS2

Glossary : Français English

Lettre + N° : Letter + Number	**nb colonies + / nb de colonies testées : number of positive plates / number of tested plates**
Dilution : Dilution	**Latex ou sérologie : latex or serology**
Type de colonies : types of plates	**Résultats confirmés Legionella pneumophila (CFU/L) : Confirmed results Legionella pneumophila (CFU/L)**
Direct : Direct	**Nb puits positifs : Number of positive wells**
non dilué : not diluted	**Grand puits : High well**
Filtration + traitement acide : Filtration + acid treatement	**Petits puits : Small well**
Confirmation : Confirmation	**Résultat de la table MPN : Results of the MPN table**
Lecture : Enumeration	**Résultats finals MPN/L (Table MPN*dilution*10) : Final results MPN/L (Table MPN x dilution x 10)**

Laboratoire A1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation – Lecture		Latex ou sérologie	Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE – nb colonies + / nb de colonies testées	BCYE + cystéine – nb colonies + / nb de colonies testées				Grand puits	Petit puits		
A1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
A2	Non dilué	a	16	>100	>100	0/5	5/5	2-14	80 000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	78	1017.6	101760
	1/10	a	1	>100	29	0/3	3/3	2-14	50 000					
		b				/	/			1/100	6	14	85.4	85400
		c				/	/							
A3	Non dilué	a	>150	>100	>100	0/5	5/5	2-14	>750 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	136	>100	>100	0/5	5/5	2-14	6 800 000					
		b				/	/			1/100	6	90	>2272.6	>2 272 600
		c				/	/							
A4	Non dilué	a	>150	>100	>100	0/5	5/5	2-14	>750 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	>150	>100	>100	0/5	5/5	2-14	>7 500 000					
		b				/	/			1/100	6	90	>2272.6	>2 272 600
		c				/	/							

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
A5	Non dilué	a	>150	>100	>100	0/5	5/5	2-14	>750 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	>150	>100	>100	0/5	5/5	2-14	>7 500 000					
		b				/	/			1/100	6	90	>2272.6	>2 272 600
		c				/	/							
A6	Non dilué	a	>150	>100	>100	0/5	5/5	2-14	>750 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	110	>100	>100	0/5	5/5	2-14	5 500 000					
		b				/	/			1/100	6	90	>2272.6	>2 272 600
		c				/	/							
A7	Non dilué	a	12	>100	>100	0/5	5/5	2-14	60 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	66	667.6	66 760
	1/10	a	0	>100	24	0/5	5/5	2-14	24 000					
		b				/	/			1/100	6	22	141.6	141 600
		c				/	/							
A8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<1000

Laboratoire B1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation Lecture		Latex ou sérologie	Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées				Grand puits	Petit puits		
B1	Non dilué	a	/	/	/	/	/	/	< 10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<1
	1/10	a	/	/	/	/	/	/						
		b				/	/			1/100	0	0	<1	<1
		c				/	/							
B2	Non dilué	a	25	>100	>100	/	/	Latex	130 000	Non dilué	6	90	> 2 272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	80	1 109,7	110 970
	1/10	a	2	>100	39	/	/	Latex						
		b				/	/			1/100	6	16	98,9	98 900
		c				/	/							
B3	Non dilué	a	>150	Nappe	Nappe	/	/	Latex	> 7 500 000	Non dilué	6	90	> 2 272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	> 2 272,6	>227 260
	1/10	a	>150	>100	>100	/	/	Latex						
		b				/	/			1/100	6	90	> 2 272,6	>2 272 600
		c				/	/							
B4	Non dilué	a	Nappe	Nappe	Nappe	/	/	Latex	> 7 500 000	Non dilué	6	90	> 2 272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	> 2 272,6	>227 260
	1/10	a	Nappe	Nappe	Nappe	/	/	Latex						
		b				/	/			1/100	6	90	> 2 272,6	>2 272 600
		c				/	/							

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation Lecture		Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie		Grand puits	Petit puits		
B5	Non dilué	a	Nappe	Nappe	Nappe	/	/	Latex	> 7 500 000	Non dilué	6	90	> 2 272,6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	>150	>100	>100	/	/	Latex		1/10	6	90	> 2 272,6	>227 260
		b				/	/			1/100	6	90	> 2 272,6	>2 272 600
		c				/	/							
B6	Non dilué	a	>150	>100	>100	/	/	Latex	> 7 500 000	Non dilué	6	90	> 2 272,6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	150	>100	>100	/	/	Latex		1/10	6	90	> 2 272,6	>227 260
		b				/	/			1/100	6	90	> 2 272,6	>2 272 600
		c				/	/							
B7	Non dilué	a	16	>100	>100	/	/	Latex	250 000	Non dilué	6	90	> 2 272,6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	5	>100	20	/	/	Latex		1/10	6	74	872,3	87 230
		b				/	/			1/100	6	15	92,1	92 100
		c				/	/							
B8	Non dilué	a	/	31	7	/	/	Latex	350	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/							
	1/10	a	/	/	/	/	/	/		1/10	0	0	<1	<1
		b				/	/			1/100	0	0	<1	<1
		c				/	/							

Laboratoire D1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie					
D1	Non dilué	a	0	0	0	/	/		<10 ufc/L	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/							
	1/10	a	0	0	0	/	/			1/10	0	0	<1	<100
		b				/	/							
		c				/	/			1/100	0	0	<1	<1000
D2	Non dilué	a	12	>100	>100	0/5	5/5	2-14	200 000	Non dilué	6	90	>2272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	71	785,5	78 550
	1/10	a	4	19	>100	0/6	6/6	2-14						
		b				/	/			1/100	6	9	53,4	53 400
		c				/	/							
D3	Non dilué	a	>150	>100	>100	/	/		5 100 000	Non dilué	6	90	>2272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272,6	>227 260
	1/10	a	101	>100	>100	0/5	5/5	2-14						
		b				/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							
D4	Non dilué	a	>150	>100	>100	/	/		>7 500 000	Non dilué	6	90	>2272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272,6	>227 260
	1/10	a	>150	>100	>100	0/5	5/5	2-14						
		b				/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
D5	Non dilué	a	>150	>100	>100	/	/		>750 0000	Non dilué	6	90	>2272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272,6	>227 260
	1/10	a	>150	>100	>100	0/5	5/5	2-14						
		b				/	/			1/100	6	90	>2272,6	>2 272600
		c				/	/							
D6	Non dilué	a	>150	>100	>100	/	/		6 100 000	Non dilué	6	90	>2272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>2272,6	>227 260
	1/10	a	122	>100	>100	0/5	5/5	2-14						
		b				/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							
D7	Non dilué	a	17	>100	>100	0/5	5/5	2-14	85 000	Non dilué	6	90	>2272,6	>22 726
		b				/	/							
		c				/	/			1/10	6	76	939,8	93 980
	1/10	a	1	13	>100	0/3	3/3	2-14						
		b				/	/			1/100	6	12	72,3	72 300
		c				/	/							
D8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

Laboratoire E1

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
E1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/							
	1/10	a	0	0	0	/	/			1/10	0	0	<1	<10
		b				/	/							
		c				/	/			1/100	0	0	<1	<100
E2	Non dilué	a	28	>100	>100	5/5	5/5	Lp 2-14	140 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	2	>100	31	5/5	5/5	Lp 2-14		1/10	6	77	977.2	97 720
		b				/	/							
		c				/	/			1/100	6	23	149.0	149 000
E3	Non dilué	a	>150	>100	>100	5/5	5/5	Lp 2-14	7 200 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	144	>100	>100	5/5	5/5	Lp 2-14		1/10	6	90	>2272.6	>227 260
		b				/	/							
		c				/	/			1/100	6	90	>2272.6	>2 272 600
E4	Non dilué	a	>150	>100	>100	5/5	5/5	Lp 2-14	>7 500 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	>150	>100	>100	5/5	5/5	Lp 2-14		1/10	6	90	>2272.6	>227 260
		b				/	/							
		c				/	/			1/100	6	90	>2272.6	>2 272 600

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation Lecture		Latex ou sérologie	Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées				Grand puits	Petit puits		
E5	Non dilué	a	>150	>100	>100	5/5	5/5	Lp 2-14	>7 500 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	>150	>100	>100	5/5	5/5	Lp 2-14		1/10	6	90	>2272.6	>227 260
		b				/	/							
		c				/	/			1/100	6	90	>2272.6	>2 272 600
E6	Non dilué	a	>150	>100	>100	5/5	5/5	Lp 2-14	>7 500 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	>150	>100	>100	5/5	5/5	Lp 2-14		1/10	6	90	>2272.6	>227 260
		b				/	/							
		c				/	/			1/100	6	90	>2272.6	>2 272 600
E7	Non dilué	a	14	>100	>100	5/5	5/5	Lp 2-14	70 000	Non dilué	9	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	0	>100	30	5/5	5/5	Lp 2-14		1/10	9	83	1289.8	128 980
		b				/	/							
		c				/	/			1/100	6	19	119.8	119 800
E8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/							
	1/10	a	0	0	0	/	/			1/10	0	0	<1	<10
		b				/	/							
		c				/	/			1/100	0	0	<1	<100

Laboratoire F1

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Latex ou sérologie	Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées							
F1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b	0	0	0	/	/							
		c	0	0	0	/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/							
		b	0	0	0	/	/			1/100	0	0	<1	<100
		c	0	0	0	/	/							
F2	Non dilué	a	0	0	0	/	/		130 000 200 000	Non dilué	6	90	>2272.6	>2272.6
		b	25	>100	>100	0 +/ 5	5 + / 5	2.14						
		c	25	>100	>100	/	/			1/10	6	75	904.9	9049
	1/10	a	0	0	0	/	/							
		b	4	43	>100	0 +/ 9	9 +/ 9	2.14		1/100	6	17	105,7	10570
		c	4	43	>100	/	/							
F3	Non dilué	a	0	0	0	/	/		>750 000 >7 500 000	Non dilué	6	90	>2272.6	>2272.6
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>22726
	1/10	a	0	0	0	/	/							
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14		1/100	6	90	>2272.6	>227260
		c	>150	>100	>100	/	/							
F4	Non dilué	a	0	0	0	/	/		>750 000 >7 500 000	Non dilué	6	90	>2272.6	>2272.6
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>22726
	1/10	a	0	0	0	/	/							
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14		1/100	6	90	>2272.6	>227260
		c	>150	>100	>100	/	/							

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture								
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie			Grand puits	Petit puits		
F5	Non dilué	a	0	0	0	/	/		>750 000	Non dilué	6	90	>2272.6	>2272.6
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>22726
	1/10	a	0	0	0	/	/							
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14	>7 500 000	1/100	6	90	>2272.6	>227260
		c	>150	>100	>100	/	/							
F6	Non dilué	a	0	0	0	/	/		>750 000	Non dilué	6	90	>2272.6	>2272.6
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>22726
	1/10	a	0	0	0	/	/							
		b	>150	>100	>100	0 +/ 5	5 + / 5	2.14	>7 500 000	1/100	6	90	>2272.6	>227260
		c	>150	>100	>100	/	/							
F7	Non dilué	a	0	0	0	/	/		150 000	Non dilué	6	90	>2272.6	>2272.6
		b	29	>100	>100	0 +/ 5	5 + / 5	2.14						
		c	29	>100	>100	/	/			1/10	6	76	939.8	9398
	1/10	a	0	0	0	/	/							
		b	1	33	>100	0 +/ 6	6 + / 6	2.14	50000	1/100	6	21	134.2	13420
		c	1	33	>100	/	/							
F8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b	0	0	0	/	/							
		c	0	0	0	/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/							
		b	0	0	0	/	/			1/100	0	0	<1	<100
		c	0	0	0	/	/							

Laboratoire G1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation – Lecture			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE – nb colonies + / nb de colonies testées	BCYE + cystéine – nb colonies + / nb de colonies testées	Latex ou sérologie			Grand puits	Petit puits		
G1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/							
	1/10	a	0	0	0	/	/			1/10	0	0	<1	<10
		b				/	/							
		c				/	/			1/100	0	0	<1	<10
G2	Non dilué	a	20	>100	>100	20 / 5	20 / 5	2-15	100000	Non dilué	6	90	>2272,6	>23000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/							
	1/10	a	2	>100	6	2 / 2	2 / 2	2-15		1/10	6	80	1109,7	110000
		b			6	5 / 5	5 / 5	2-15						
		c				/	/			1/100	6	25	164,4	160000
G3	Non dilué	a	>150	>100	>100	5 / 5	5 / 5	2-15	>750000	Non dilué	6	90	>2272,6	>23000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/							
	1/10	a	>150	>100	>100	/	/			1/10	6	90	>2272,6	>230000
		b				/	/							
		c				/	/			1/100	6	90	>2272,6	>2300000
G4	Non dilué	a	>150	>100	>100	5 / 5	5 / 5	2-15	>750000	Non dilué	6	90	>2272,6	>23000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/							
	1/10	a	>150	>100	>100	/	/			1/10	6	90	>2272,6	>230000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/			1/100	6	90	>2272,6	>2300000

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation				Nb puits positifs			
						Lecture							
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie	Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Grand puits	Petit puits	Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
G5	Non dilué	a	>150	>100	>100	5 / 5	5 / 5	2-15	>750000	Non dilué	6	90	>2272,6	>23000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/			1/10	6	90	>2272,6	>230000
	1/10	a	>150	>100	>100	/	/							
		b				/	/			1/100	6	90	>2272,6	>2300000
		c				/	/							
G6	Non dilué	a	>150	>100	>100	5 / 5	5 / 5	2-15	>750000	Non dilué	6	90	>2272,6	>23000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/			1/10	6	90	>2272,6	>230000
	1/10	a	>150	>100	>100	/	/							
		b				/	/			1/100	6	90	>2272,6	>2300000
		c				/	/							
G7	Non dilué	a	12	>100	>100	5 / 5	5 / 5	2-15	60000	Non dilué	6	90	>2272,6	>23000
		b		>100		5 / 5	5 / 5	2-15						
		c				/	/			1/10	6	65	646,9	65000
	1/10	a	2	41	>100	2 / 2	2 / 2	2-15						
		b		41		5 / 5	5 / 5	2-15		1/100	6	24	156,6	160000
		c				/	/							
G8	Non dilué	a	0	0	0	/	/		100	Non dilué	0	6	6,0	60
		b				/	/							
		c				/	/			1/10	0	2	2,0	200
	1/10	a	0	1	0	1 / 1	1 / 1	2-15						
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

Laboratoire H1

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie						
H1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1.0	<10
		b				/	/							
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1.0	<1000
		c				/	/							
H2	Non dilué	a	0	>100	27	0/5	5/5	Lp2-15	2700	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	61	572	57200
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	6	10	59.6	59600
		c				/	/							
H3	Non dilué	a	>150	>100	>100	/	/		4800000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>22726
	1/10	a	95	>100	>100	0/5	5/5	Lp2-15						
		b				/	/			1/100	6	90	>2272.6	>22726
		c				/	/							
H4	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1.0	<10
		b				/	/							
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1.0	<1000
		c				/	/							

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
H5	Non dilué	a	>150	>100	>100	/	/		>750000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>22726
	1/10	a	>150	>100	>100	0/2	2/2	Lp2-15						
		b				/	/			1/100	6	90	>2272.6	>22726
		c				/	/							
H6	Non dilué	a	>150	>100	>100	0/5	5/5	Lp2-15	>750000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>22726
	1/10	a	>150	>100	>100	/	/							
		b				/	/			1/100	6	90	>2272.6	>22726
		c				/	/							
H7	Non dilué	a	17	>100	>100	0/5	5/5	Lp2-15	85000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	56	491.6	49160
	1/10	a	2	42	>100	0/2	2/2	Lp2-15						
		b				/	/			1/100	6	7	41.6	41600
		c				/	/							
H8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1.0	<10
		b				/	/							
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1.0	<1000
		c				/	/							

Laboratoire I1

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie						
I1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
I2	Non dilué	a	4	>100	>100	0/4	4/4	2-14	20 000	Non dilué	6	90	>2272,6	>2272,6
		b				/	/							
		c				/	/			1/10	6	53	448,9	4 489
	1/10	a	0	>100	26	0/5	5/5	2-14	26 000					
		b				/	/			1/100	6	10	59,6	5 960
		c				/	/							
I3	Non dilué	a	>150			0/2	2/2	2-14	>750 000	Non dilué	6	90	>2272,6	>2272,6
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 726
	1/10	a	145			0/5	5/5	2-14	7 250 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>227 260
		c				/	/							
I4	Non dilué	a	>150			0/2	2/2	2-14	>750 000	Non dilué	6	90	>2272,6	>2272,6
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 726
	1/10	a	>150			0/2	2/2	2-14	>7 500 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>227 260
		c				/	/							

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
I5	Non dilué	a	>150			0/2	2/2		>750 000	Non dilué	6	90	>2272,6	>2272,6
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 726
	1/10	a	>50			0/2	2/2	2-14	>7 500 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>227 260
		c				/	/							
I6	Non dilué	a	>150			0/2	2/2	2-14	>750 000	Non dilué	6	90	>2272,6	>2272,6
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 726
	1/10	a	50			0/5	5/5	2-14	2 500 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>227 260
		c				/	/							
I7	Non dilué	a	11			0/5	5/5	2-14	55 000	Non dilué	6	90	>2272,6	>2272,6
		b				/	/							
		c		>100	>100	/	/			1/10	6	56	491,6	4 916
	1/10	a	0			/	/		37 000					
		b		>100	37	0/5	5/5	2-14		1/100	6	5	31	3 100
		c				/	/							
I8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<100
		c				/	/							

Laboratoire J1

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
J1	Non dilué	a				/	/		<10 <100	Non dilué	0	0	<1	<10
		b	0	0	0	/	/							
		c	0	0	0	/	/			1/10	0	0	<1	<100
	1/10	a				/	/							
		b	0	0	0	/	/			1/100	0	0	<1	<1000
		c	0	0	0	/	/							
J2	Non dilué	a				/	/		15 000 150 000	Non dilué	6	88	1 922.6	19 226
		b	3	>100	>100	0 / 5	5 / 5	2-14						
		c	3	>100	>100	/	/			1/10	6	55	477	47 700
	1/10	a				/	/							
		b	2	9	>100	0 / 5	5 / 5	2-14		1/100	6	13	78.8	78 800
		c	2	11	>100	/	/							
J3	Non dilué	a				/	/		>750 000 1 700 000	Non dilué	6	87	1717.8	17 178
		b	>150	>100	>100	0 / 5	5 / 5	2-14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>227 260
	1/10	a				/	/							
		b	34	>100	>100	0 / 5	5 / 5	2-14		1/100	6	90	>2272.6	>2 272 600
		c	34	>100	>100	/	/							
J4	Non dilué	a				/	/		>750 000 >7 500 000	Non dilué	6	90	>2272.6	>22 726
		b	>150	>100	>100	0 / 5	5 / 5	2-14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>227 260
	1/10	a				/	/							
		b	>150	>100	>100	0 / 5	5 / 5	2-14		1/100	6	90	>2272.6	>2 272 600
		c	>150	>100	>100	/	/							

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
J5	Non dilué	a				/	/		>750 000 >7 500 000	Non dilué	6	90	>2272.6	>22 726
		b	>150	>100	>100	0 / 5	5 / 5	2-14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>227 260
	1/10	a				/	/							
		b	>150	>100	>100	0 / 5	5 / 5	2-14		1/100	6	90	>2272.6	>2 272 600
		c	>150	>100	>100	/	/							
J6	Non dilué	a				/	/		>750 000 1 850 000	Non dilué	6	90	>2272.6	>22 726
		b	>150	>100	>100	0 / 5	5 / 5	2-14						
		c	>150	>100	>100	/	/			1/10	6	90	>2272.6	>227 260
	1/10	a				/	/							
		b	36	>100	>100	0 / 5	5 / 5	2-14		1/100	6	90	>2272.6	>2 272 600
		c	37	>100	>100	/	/							
J7	Non dilué	a				/	/		60 000 21 000	Non dilué	9	90	>2272.6	>22 726
		b	12	>100	>100	0 / 5	5 / 5	2-14						
		c	12	>100	>100	/	/			1/10	6	66	667.6	66 760
	1/10	a				/	/							
		b	0	19	>100	0 / 5	5 / 5	2-14		1/100	6	8	47.4	47 400
		c	0	21	>100	/	/							
J8	Non dilué	a				/	/		<10 <100	Non dilué	0	0	<1	<10
		b	0	0	0	/	/							
		c	0	0	0	/	/			1/10	0	0	<1	<100
	1/10	a				/	/							
		b	0	0	0	/	/			1/100	0	0	<1	<1000
		c	0	0	0	/	/							

Laboratoire K1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie						
K1	Non dilué	a	<1	<1	<1	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	<1	<1	<1	/	/							
		b				/	/			1/100	0	0	<1	<10
		c				/	/							
K2	Non dilué	a	13			0/5	5/5	Latex	65 000	Non dilué	6	90	>2272,6	>22 726
		b		>100	46	0/2	2/2	Latex						
		c				/	/			1/10	6	68	711,5	71 150
	1/10	a	<1	>100	11	0/5	5/5	Latex	11 000					
		b				/	/			1/100	6	10	59,6	59 600
		c				/	/							
K3	Non dilué	a	>150			0/5	5/5	Latex	>750 000	Non dilué	6	90	>2272,6	>22 726
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 7260
	1/10	a	113			0/5	5/5	Latex	5 700 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							
K4	Non dilué	a	>150			0/5	5/5	Latex	>750 000	Non dilué	6	90	>2272,6	>22 726
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 7260
	1/10	a	>150			0/5	5/5	Latex	>7 500 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture		Latex ou sérologie			Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées							
K5	Non dilué	a	>150			0/5	5/5	Latex	>750 000	Non dilué	6	90	>2272,6	>22 726
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 7260
	1/10	a	>150			0/5	5/5	Latex	>7 500 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							
K6	Non dilué	a	>150			0/5	5/5	Latex	>750 000	Non dilué	6	90	>2272,6	>22 726
		b		>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>22 7260
	1/10	a	91			0/5	5/5	Latex	4 600 000					
		b		>100	>100	/	/			1/100	6	90	>2272,6	>2 272 600
		c				/	/							
K7	Non dilué	a	8			0/5	5/5	Latex	40 000	Non dilué	6	90	>2272,6	>22 726
		b		>100	94	0/2	2/2	Latex						
		c				/	/			1/10	6	60	554,9	55 4900
	1/10	a	2			0/2	2/2	Latex	100 000					
		b		>100	13	0/3	3/3	Latex		1/100	6	8	47,4	47 400
		c				/	/							
K8	Non dilué	a	<1	<1	<1	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	<1	<1	<1	/	/							
		b				/	/			1/100	0	0	<1	<10
		c				/	/							

Laboratoire L1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation Lecture		Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie		Grand puits	Petit puits		
L1	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
L2	Non dilué	a	14			0/4	4/4	Latex	80 000	Non dilué	6	90	>2272.6	>22 726
		b	2			0/1	1/1	latex						
		c		>100	>100	0/2	2/2	latex		1/10	6	62	589.7	58 970
	1/10	a	1			0/1	1/1	latex	50 000					
		b		>100	11	0/4	4/4	latex		1/100	6	14	85.4	85 400
		c				/	/							
L3	Non dilué	a	>150			0/5	5/5	latex	>750 000	Non dilué	6	90	>2272.6	>22 726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	133			0/5	5/5	latex						
		b		>100	>100	0/2	2/2	latex	6 650 000	1/100	6	90	>2272.6	>2 272 600
		c				/	/							
L4	Non dilué	a	>150			0/5	5/5	latex	>750 000	Non dilué	6	90	>2272.6	>22 726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	>150			0/5	5/5	latex						
		b		>100	>100	0/2	2/2	latex	>7 500 000	1/100	6	90	>2272.6	>2 272 600
		c				/	/							

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
L5	Non dilué	a	>150			0/5	5/5	latex	>750 000	Non dilué	6	90	>2272.6	>22 726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	>150			0/5	5/5	latex						
		b		>100	>100	0/2	2/2	latex	>7 500 000	1/100	6	90	>2272.6	>2 272 600
		c				/	/							
L6	Non dilué	a	>150			0/5	5/5	latex	>750 000	Non dilué	6	90	>2272.6	>22 726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	90	>2272.6	>227 260
	1/10	a	116			0/5	5/5	latex						
		b		>100	>100	0/2	2/2	latex	5 800 000	1/100	6	90	>2272.6	>2 272 600
		c				/	/							
L7	Non dilué	a	13			0/5	5/5	latex	65 000	Non dilué	6	90	>2272.6	>22 726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	62	589.7	58 970
	1/10	a	1			0/1	1/1	latex						
		b		>100	17	0/4	4/4	latex	50 000	1/100	6	15	92.1	92 100
		c				/	/							
L8	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/		<100	1/100	0	0	<1	<1000
		c				/	/							

Laboratoire M1

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation Lecture		Résultats confirmés ***Legionella pneumophila*** (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie		Grand puits	Petit puits		
M1	Non dilué	a	0	0	0	/	/		Non dilué	0	0	<1	<10
		b				/	/						
		c				/	/		1/10	0	0	<1	<100
	1/10	a				/	/						
		b				/	/		1/100	0	0	<1	<1000
		c				/	/						
M2	Non dilué	a	10	>100	>100	0/5	5/5	+	Non dilué	6	81	1162.9	11 629
		b				/	/						
		c				/	/		1/10	6	55	477	47 700
	1/10	a				/	/						
		b				/	/		1/100	6	20	126.9	126 900
		c				/	/						
M3	Non dilué	a	>150	>150	>150	0/5	5/5	+	Non dilué	6	81	1162.9	11 629
		b				/	/						
		c				/	/		1/10	6	81	1162.9	116 290
	1/10	a				/	/						
		b				/	/		1/100	6	81	1162.9	1 162 900
		c				/	/						
M4	Non dilué	a	Env.	Env.	Env.	/	/		Non dilué	6	81	1162.9	11 629
		b				/	/						
		c				/	/		1/10	6	81	1162.9	116 290
	1/10	a				/	/						
		b				/	/		1/100	6	81	1162.9	1 162 900
		c				/	/						

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie					
M5	Non dilué	a	Env.	Env.	Env.	/	/		Flore interférente	Non dilué	6	81	1162.9	11 629
		b				/	/							
		c				/	/			1/10	6	81	1162.9	116 290
	1/10	a				/	/							
		b				/	/			1/100	6	81	1162.9	1 162 900
		c				/	/							
M6	Non dilué	a	>150	Env.	Env.	0/5	5/5	+	>750 000 ufc/L	Non dilué	6	81	1162.9	11 629
		b				/	/							
		c				/	/			1/10	6	81	1162.9	116 290
	1/10	a				/	/							
		b				/	/			1/100	6	81	1162.9	1 162 900
		c				/	/							
M7	Non dilué	a	14	>100	>100	0/5	5/5	+	70 000 ufc/L	Non dilué	6	81	1162.9	11 629
		b				/	/							
		c				/	/			1/10	6	57	506.7	50 670
	1/10	a				/	/							
		b				/	/			1/100	6	10	59.6	59 600
		c				/	/							
M8	Non dilué	a	0	0	0	/	/			Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a				/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

Laboratoire N1

Lettre +N°	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE	BCYE + cystéine	Latex ou sérologie						
						nb colonies + / nb de colonies testées	nb colonies + / nb de colonies testées							
1	Non dilué	a	0			/	/		<10	Non dilué	0	0	<1	<10
		b		0		/	/							
		c			0	/	/			1/10	0	0	<1	<100
	1/10	a	0			/	/							
		b		0		/	/			1/100	0	0	<1	<1000
		c			0	/	/							
2	Non dilué	a	6			0/5	5/5	2-14	30 000	Non dilué	6	90	>2272.6	>22726
		b		>100		/	/							
		c			55	0/5	5/5	2-14		1/10	6	59	538.3	53830
	1/10	a	1			0/1	1/1	2-14						
		b		>100		/	/			1/100	6	9	53.4	53400
		c			6	0/5	5/5	2-14						
3	Non dilué	a	ENV			0/5	5/5	2-14	5 400 000	Non dilué	6	90	>2272.6	>22726
		b		ENV		/	/							
		c			ENV	/	/			1/10	6	90	>2272.6	>227260
	1/10	a	107			0/5	5/5	2-14						
		b		ENV		/	/			1/100	6	90	>2272.6	>2272600
		c			>100	0/5	5/5	2-14						
4	Non dilué	a	ENV			/	/		>750 000	Non dilué	6	90	>2272.6	>22726
		b		ENV		/	/							
		c			ENV	/	/			1/10	6	90	>2272.6	>227260
	1/10	a	ENV			0/5	5/5	2-14						
		b		ENV		/	/			1/100	6	90	>2272.6	>2272600
		c			ENV	/	/							

Lettre +N°	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *Legionella pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	BCYE nb colonies + / nb de colonies testées	BCYE + cystéine nb colonies + / nb de colonies testées	Latex ou sérologie					
5	Non dilué	a	ENV			/	/		>750 000	Non dilué	6	90	>2272.6	>22726
		b		ENV		/	/							
		c			ENV	/	/			1/10	6	90	>2272.6	>227260
	1/10	a	ENV			0/5	5/5	2-14						
		b		ENV		/	/			1/100	6	90	>2272.6	>2272600
		c			ENV	/	/							
6	Non dilué	a	ENV			/	/		5 200 000	Non dilué	6	90	>2272.6	>22726
		b		ENV		/	/							
		c			ENV	/	/			1/10	6	90	>2272.6	>227260
	1/10	a	103			0/5	5/5	2-14						
		b		ENV		/	/			1/100	6	90	>2272.6	>2272600
		c			ENV	/	/							
7	Non dilué	a	11			0/5	5/5	2-14	55 000	Non dilué	6	90	>2272.6	>22726
		b		>100		/	/							
		c			38	0/5	5/5	2-14		1/10	6	61	572	57200
	1/10	a	2			/	/							
		b		>100		0/4	4/4	2-14		1/100	6	10	36.1	36100
		c			1	0/1	1/1	2-14						
8	Non dilué	a	0			/	/		<10	Non dilué	0	0	<1	<10
		b		0		/	/							
		c			0	/	/			1/10	0	0	<1	<100
	1/10	a	0			/	/							
		b		0		/	/			1/100	0	0	<1	<1000
		c			0	/	/							

Laboratoire expert O1

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées							
						BCYE	BCYE + cystéine	Latex					
O1	Non dilué	a	0	0	0	/	/		<10 <100	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<10	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<100	<1000
		c				/	/							
O2	Non dilué	a	23	>100	>100	0 / 5	5 / 5	+	120 000 20 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	70	7596	75 960
	1/10	a	4	>100	16	0 / 5	5 / 5	+						
		b				/	/			1/100	4	18	4160	41 600
		c				/	/							
O3	Non dilué	a	>150	>100	>100	0 / 5	5 / 5	+	>750 000 710 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>22726	>227 260
	1/10	a	14	>100	>100	0 / 5	5 / 5	+						
		b				/	/			1/100	6	90	>227260	>2 272 600
		c				/	/							
O4	Non dilué	a	>150	>100	>100	0 / 5	5 / 5	+	>750 000 >7 500 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	6	90	>22726	>227 260
	1/10	a	>150	>100	>100	0 / 5	5 / 5	+						
		b				/	/			1/100	6	90	>227260	>2 272 600

Laboratoire A2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
A1	Non dilué	a	58			0/5	5/5	5/5	290 000	Non dilué	6	90	>2272.6	>22726
		b		>100	>100	0/2	2/2	2/2						
		c				/	/			1/10	6	90	>2272.6	>227260
	1/10	a	16			0/5	5/5	5/5						
		b		>100	89	0/2	2/2	2/2	800 000	1/100	6	53	448.9	448900
		c				/	/							
A2	Non dilué	a	<1	<1	<1	/	/		<10	Non dilué	0	0	<1.0	<10
		b				/	/							
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	<1	<1	<1	/	/							
		b				/	/		<100	1/100	0	0	<1.0	<1000
		c				/	/							
A3	Non dilué	a	72			0/5	5/5	5/5	360 000	Non dilué	6	90	>2272.6	>22726
		b		>100	>100	0/2	2/2	2/2						
		c				/	/			1/10	6	90	>2272.6	>227260
	1/10	a	6			/	/							
		b		>100	47	0/5	5/5	5/5	300 000	1/100	6	60	554.9	554900
		c				/	/							
A4	Non dilué	a	<1	1	<1	0/1	1/1	1/1	10	Non dilué	1	0	1.1	11
		b												
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	<1	<1	<1	/	/							
		b				/	/		<100	1/100	0	0	<1.0	<1000
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture								
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex			Grand puits	Petit puits		
						BCYE	BCYE + cystéine							
A5	Non dilué	a	<1	<1	<1	/	/		<10	Non dilué	0	0	<1.0	<10
		b				/	/							
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	<1	<1	<1	/	/							
		b				/	/		<100	1/100	0	0	<1.0	<1000
		c				/	/							
A6	Non dilué	a	<1	61	4	0/5	5/5	5/5	590	Non dilué	6	24	156.6	1566
		b												
		c				/	/			1/10	6	8	47.4	4740
	1/10	a	<1	6	<1	0/5	5/5	5/5						
		b							600	1/100	3	1	5.2	5200
		c				/	/							
A7	Non dilué	a	<1	1	<1	0/1	1/1	1/1	10	Non dilué	4	0	5.8	58
		b												
		c				/	/			1/10	0	0	<1.0	<100
	1/10	a	<1	<1	<1	/	/							
		b	<1	<1	<1	/	/		<100	1/100	0	0	<1.0	<1000
		c	<1	<1	<1	/	/							
A8	Non dilué	a	3			0/3	3/3	3/3	15 000	Non dilué	6	23	149.1	1491
		b		34	3	0/2	2/2	2/2						
		c				/	/			1/10	6	5	31.0	3100
	1/10	a	<1	4	<1	0/4	4/4	4/4						
		b							400	1/100	0	0	<1.0	<1000
		c				/	/							

Laboratoire B2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE Sans cyst.	BCYE + cystéine							
B1	Non dilué	a	69	-	-	0/5	5/5	2-14	350000	Non dilué	6	90	>2272,6	>22726
		b	-	>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>227260
	1/10	a	6	-	-	0/5	5/5	2-14						
		b	-	>100	82	0/5	5/5	2-14		1/100	6	49	397,1	397100
		c				/	/							
B2	Non dilué	a	0	-	-	/	/		<10	Non dilué	0	0	<1	<10
		b	-	0	0	/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	-	-	/	/							
		b	-	0	0	/	/			1/100	0	0	<1	<1000
		c				/	/							
B3	Non dilué	a	64	-	-	0/5	5/5	2-14	320000	Non dilué	6	90	>2272,6	>22726
		b	-	>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>227260
	1/10	a	6	-	-	0/5	5/5	2-14						
		b	-	>100	56	0/5	5/5	2-14		1/100	6	51	422,3	422300
		c				/	/							
B4	Non dilué	a	0	-	-	/	/		<10	Non dilué	3	0	3,9	39
		b	-	0	0	/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	-	-	/	/							
		b	-	0	0	/	/			1/100	0	0	<1	<1000
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture							
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées				Grand puits	Petit puits		
						BCYE Sans cyst.	BCYE + cystéine	Latex					
B5	Non dilué	a	0	-	-	/	/		<10	Non dilué	0	0	<1	<10
		b	-	0	0	/	/							
		c				/	/							
	1/10	a	0	-	-	/	/			1/10	0	0	<1	<100
		b	-	0	0	/	/							
		c				/	/			1/100	0	0	<1	<1000
B6	Non dilué	a	0	-	-	/	/		1400	Non dilué	6	25	164,4	1644
		b	-	>100	14	0/5	5/5	2-14						
		c				/	/			1/10	5	9	30,5	3050
	1/10	a	0	-	-	/	/							
		b	-	8	0	0/5	5/5	2-14		1/100	1	0	1,1	1100
		c				/	/							
B7	Non dilué	a	0	-	-	/	/		10	Non dilué	3	0	3,9	39
		b	-	1	0	0/1	1/1	2-14						
		c				/	/			1/10	0	1	1,0	100
	1/10	a	0	-	-	/	/							
		b	-	0	0	/	/			1/100	0	0	<1	<1000
		c				/	/							
B8	Non dilué	a	0	-	-	/	/		1600	Non dilué	6	25	164,4	1644
		b	-	>100	16	0/5	5/5	2-14						
		c				/	/			1/10	6	2	18,7	1870
	1/10	a	0	-	-	/	/							
		b	-	12	0	0/5	5/5	2-14		1/100	1	0	1,1	1100
		c				/	/							

Laboratoire C2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture							
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées				Grand puits	Petit puits		
						BCYE	BCYE + cystéine	Latex					
C1	Non dilué	a	55	>100	>100	/	/	Lp 2.-15	280000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>227260
	1/10	a	5	>100	58	/	/							
		b				/	/			1/100	6	56	491.6	491600
		c				/	/							
C2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
C3	Non dilué	a	47	>100	>100	/	/	Lp 2.-15	240000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	89	2272.6	227260
	1/10	a	5	>100	65	/	/							
		b				/	/			1/100	6	39	286.9	286900
		c				/	/							
C4	Non dilué	a	0	0	0	/	/		<10	Non dilué	2	0	2.3	23
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
C5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
C6	Non dilué	a	0	56	10	/	/	Lp 2.-15	600	Non dilué	6	22	141.6	1416
		b				/	/							
		c				/	/			1/10	6	7	41.6	4160
	1/10	a	0	3	0	/	/							
		b				/	/			1/100	1	0	1.1	1110
		c				/	/							
C7	Non dilué	a	0	0	0	/	/		<10	Non dilué	2	1	3.5	35
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
C8	Non dilué	a	0	39	8	/	/	Lp 2.-15	430	Non dilué	6	12	72.3	723
		b				/	/							
		c				/	/			1/10	6	4	26.4	2640
	1/10	a	0	3	1	/	/							
		b				/	/			1/100	3	1	5.2	5200

Laboratoire D2

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
D1	Non dilué	a	0	>100	22	0/5	5/5	+	2 200 ufc/L	Non dilué	6	90	>2272	22 720
		b				/	/							
		c				/	/			1/10	6	89	>2272	227 200
	1/10	a				/	/							
		b				/	/			1/100	6	24	204.8	204 800
		c				/	/							
D2	Non dilué	a	0	0	0	/	/		0 ufc/L	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a				/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
D3	Non dilué	a	3	>100	37	0/3	3/3	+	15 000 ufc/L	Non dilué	6	90	>2272	22 720
		b				/	/							
		c				/	/			1/10	6	88	>2272	227 200
	1/10	a				/	/							
		b				/	/			1/100	6	33	286.9	286 900
		c				/	/							
D4	Non dilué	a	0	0	0	/	/		0 ufc/L	Non dilué	3	0	3.9	39
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a				/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
D5	Non dilué	a	0	0	0	/	/		0 ufc/L	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a				/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
D6	Non dilué	a	0	9	0	0/5	5/5	+	90 ufc/L	Non dilué	6	8	85.4	854
		b				/	/							
		c				/	/			1/10	5	2	24.6	2 460
	1/10	a				/	/							
		b				/	/			1/100	2	0	4.7	4 700
		c				/	/							
D7	Non dilué	a	0	0	0	/	/		0 ufc/L	Non dilué	2	0	4.7	47
		b				/	/							
		c				/	/			1/10	0	1	1	100
	1/10	a				/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
D8	Non dilué	a	0	0	0	/	/		0 ufc/L	Non dilué	6	10	98.9	989
		b				/	/							
		c				/	/			1/10	5	2	24.6	2 460
	1/10	a				/	/							
		b				/	/			1/100	1	0	2.2	2 200
		c				/	/							

Laboratoire E2

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture								
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex			Grand puits	Petit puits		
						BCYE	BCYE + cystéine							
E1	Non dilué	a	74	>100	>100	0/5	5/5	2.15	370 000	Non dilué	6	90	>2272.6	>2272.6
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>22726
	1/10	a	8	>100	65	0/5	5/5	2.15	400 000					
		b				/	/			1/100	6	54	462.8	46280
		c				/	/							
E2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
E3	Non dilué	a	89	>100	>100	0/5	5/5	2.15	450 000	Non dilué	6	90	>2272.6	>2272.6
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>22726
	1/10	a	4	>100	73	0/5	5/5	2.15	200 000					
		b				/	/			1/100	6	47	373.0	37300
		c				/	/							
E4	Non dilué	a	0	0	0	/	/		<10	Non dilué	1	1	2.2	2.2
		b				/	/							
		c				/	/			1/10	1	0	1.0	10
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<100
		c				/	/							

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
E5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
E6	Non dilué	a				/	/		950	Non dilué	6	33	230.7	230.7
		b	0	95	9	0/5	5/5	2.15						
		c				/	/			1/10	6	10	59.6	596
	1/10	a				/	/		820					
		b	0	8	1	0/6	6/6	2.15		1/100	3	0	3.0	300
		c				/	/							
E7	Non dilué	a	0	3	0	0/3	3/3	2.15	30	Non dilué	2	0	2.0	2
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
E8	Non dilué	a				/	/		660	Non dilué	6	32	221.9	221.9
		b	0	67	6	0/5	5/5	2.15						
		c				/	/			1/10	6	7	41.6	416
	1/10	a				/	/		1000					
		b	0	9	2	0/5	5/5	2.15		1/100	4	0	4.0	400
		c				/	/							

Laboratoire F2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE Sans cyst.	BCYE + cystéine							
F1	Non dilué	a	53			0/5	5/5	2-14	265000	Non dilué	0	0	<1	<1
		b		>100	>100	0/4	4/4	2-14						
		c				/	/			1/10	0	0	<1	<10
	1/10	a	4			0/4	4/4	2-14	200000					
		b		>100	>100	0/2	2/2	2-14		1/100	0	0	<1	<100
		c				/	/							
F2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
F3	Non dilué	a	48			0/5	5/5	2-14	240000	Non dilué	0	0	<1	<1
		b		>100	>100	0/2	2/2	2-14						
		c				/	/			1/10	0	0	<1	<10
	1/10	a	5			0/5	5/5	2-14	250000					
		b		>100	>100	0/2	2/2	2-14		1/100	0	0	<1	<100
		c				/	/							
F4	Non dilué	a	0			/	/		65	Non dilué	0	0	<1	<1
		b		1	6	0/5	5/5	2-14						
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<100
		c				/	/							

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
F5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
F6	Non dilué	a	1			0/1	1/1	2-14	5000	Non dilué	0	0	<1	<1
		b				/	/							
		c		>100	20	0/5	5/5	2-14		1/10	0	0	<1	<10
	1/10	a	0			/	/		1900					
		b		18	33	/	/			1/100	0	0	<1	<100
		c				/	/							
F7	Non dilué	a	0	3	0	0/3	3/3	2-15	10	Non dilué	0	0	<1	<1
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<100
		c				/	/							
F8	Non dilué	a	1			0/1	1/1	2-14	5000	Non dilué	0	0	<1	<1
		b		>100	18	0/4	4/4	2-14						
		c				/	/			1/10	0	0	<1	<10
	1/10	a	0			/	/		1500					
		b		15	1	0/5	5/5			1/100	0	0	<1	<100
		C				/	/							

Laboratoire G2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées							
						BCYE	BCYE + cystéine	Latex					
5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/							
	1/10	a	0	0	0	/	/			1/10	0	0	<1	<100
		b				/	/							
		c				/	/			1/100	0	0	<1	<1000
6	Non dilué	a	0	>100	16	5/5	5/5	2-14	1 600	Non dilué	6	26	172.2	1722
		b				/	/							
		c				/	/			1/10	6	4	26.4	2640
	1/10	a	0	13	3	5/5	5/5	2-14						
		b				/	/			1/100	1	0	1.1	1100
		c				/	/							
7	Non dilué	a	0	0	0	/	/		<10	Non dilué	3	0	3.9	39
		b				/	/							
		c				/	/			1/10	1	0	1.1	110
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
8	Non dilué	a	1	>100	8	5/5	5/5	2-14	5 000	Non dilué	6	27	180.1	1801
		b				/	/							
		c				/	/			1/10	5	7	41.6	4160
	1/10	a	0	15	1	5/5	5/5	2-14						
		b				/	/			1/100	1	0	1.1	1100
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture							
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex		Grand puits	Petit puits		
						BCYE	BCYE + cystéine						
1	Non dilué	a	77	>100	>100	5/5	5/5	2-14	450 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/							
	1/10	a	9	>100	>100	5/5	5/5	2-14		1/10	9	90	>2272.6	>227 260
		b				/	/			1/100	6	44	339.0	339 000
		c				/	/							
2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
3	Non dilué	a	87	>100	>100	5/5	5/5	2-14	435 000	Non dilué	6	90	>2272.6	>22 726
		b				/	/							
		c				/	/			1/10	9	90	>2272.6	>227 260
	1/10	a	6	>100	>100	5/5	5/5	2-14						
		b				/	/			1/100	6	42	317.5	317500
		c				/	/							
4	Non dilué	a	0	2	0	2/2	2/2	2-14	20	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture							
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées				Grand puits	Petit puits		
						BCYE	BCYE + cystéine	Latex					
H1	Non dilué	a	67			0/5	5/5	latex	340 000	Non dilué	6	90	>2272,6	>22726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	90	>2272,6	>227260
	1/10	a	2			0/2	2/2	latex	150 000					
		b	1			0/1	1/1	latex		1/100	6	43	328.1	328100
		c		>100	61	0/2	2/2	latex						
H2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
H3	Non dilué	a	87			0/5	5/5	latex	440 000	Non dilué	6	90	>2272,6	>22726
		b		>100	>100	0/2	2/2	latex						
		c				/	/			1/10	6	89	2272,6	227260
	1/10	a	13			0/5	5/5	latex	650 000					
		b		>100	84	0/2	2/2	latex		1/100	6	43	328.1	328100
		c				/	/							
H4	Non dilué	a		3		0/3	3/3	latex	30	Non dilué	0	1	1.0	10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

Laboratoire H2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées							
						BCYE	BCYE + cystéine	Latex					
H5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/							
	1/10	a	0	0	0	/	/		<100	1/10	0	0	<1	<100
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
H6	Non dilué	a		66	14	0/5	5/5	latex	730	Non dilué	6	14	85.4	854
		b				/	/							
		c				/	/			1/10	6	7	41.6	4160
	1/10	a		9	1	0/5	5/5	latex	910					
		b				/	/			1/100	1	1	2.2	2200
		c				/	/							
H7	Non dilué	a	0	0	0	/	/		<10	Non dilué	1	0	1.1	11
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
H8	Non dilué	a		56	10	0/5	5/5	latex	600	Non dilué	6	8	47.4	474
		b				/	/							
		c				/	/			1/10	6	4	26.4	2640
	1/10	a		2	0	0/2	2/2	latex	200					
		b				/	/			1/100	1	0	1.1	1100
		c				/	/							

Laboratoire expert I2

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
I1	Non dilué	a	65	>100	>100	/	/		330000	Non dilué				
		b				/	/							
		c				/	/			1/10				
	1/10	a	6			/	/		300000					
		b		>100	10	/	/			1/100	6	48	38490	380000
		c				/	/							
I2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<10	<100
	1/10	a				/	/		<100					
		b				/	/			1/100	0	0	<100	<1000
		c				/	/							
I3	Non dilué	a	58	>100	>100	/	/		290000	Non dilué				
		b				/	/							
		c				/	/			1/10				
	1/10	a	5	>100	42	/	/		250000					
		b				/	/			1/100	6	45	35010	350000
		c				/	/							
I4	Non dilué	a	0	3	0	/	/		30	Non dilué	3	0	3,9	39
		b				/	/							
		c				/	/			1/10	0	0	<10	<100
	1/10	a	0	0	0	/	/		<100					
		b				/	/			1/100	0	0	<100	<1000

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées							
						BCYE	BCYE + cystéine						
I5	Non dilué	a	0	0	0	/	/	<10	Non dilué	0	0	<1	<10
		b				/	/						
		c				/	/		1/10	0	0	<10	<100
	1/10	a	0	0	0	/	/	<100					
		b				/	/		1/100	0	0	<100	<1000
		c				/	/						
I6	Non dilué	a	1			/	/	5000	Non dilué	6	30	204,8	2000
		b		>100	14	/	/						
		c				/	/		1/10	6	2	187	1900
	1/10	a	9	0	0	/	/	900					
		b				/	/		1/100	0	0	<100	<1000
		c				/	/						
I7	Non dilué	a	NS	1	-	/	/	<10	Non dilué	1	0	1,1	11
		b				/	/						
		c				/	/		1/10	0	0	<10	<100
	1/10	a	0	0	0	/	/	<100					
		b				/	/		1/100	0	0	<100	<1000
		c				/	/						
I8	Non dilué	a	-	>100	6	/	/	6000	Non dilué	6	27	180,1	1800
		b				/	/						
		c				/	/		1/10	6	11	659	6600
	1/10	a	-	11	-	/	/	1100					
		b				/	/		1/100	0	0	<100	<1000

Laboratoire J2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture							
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées				Grand puits	Petit puits		
						BCYE	BCYE + cystéine	Latex					
5	Non dilué	a	0			/	/		<10	Non dilué	0	0	<1	<10
		b		0	0	/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0			/	/							
		b		0	0	/	/			1/100	0	0	<1	<1 000
		c				/	/							
6	Non dilué	a	2			0/2	2/2	Sg2-14	M=10 00 (*) : incohérence	Non dilué	6	35	248.7	2 487
		b		88	5	0/2	2/2	Sg2-14						
		c				/	/			1/10	6	4	26.4	2 640
	1/10	a	1(*)			0/1	1/1	Sg2-14						
		b		11(*)	0(*)	0/2	2/2	Sg2-14		1/100	1	4	5.4	5 400
		c				/	/							
7	Non dilué	a	0			/	/		10	Non dilué	1	1	2.2	22
		b		1	0	0/1	1/1	Sg2-14						
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0			/	/							
		b		0	0	/	/			1/100	0	0	<1	<1 000
		c				/	/							
8	Non dilué	a	0			/	/		M=900	Non dilué	6	20	126.9	1 269
		b		77	1	0/5	5/5	Sg2-14						
		c				/	/			1/10	6	8	47.4	4 740
	1/10	a	0			/	/							
		b		9	0	0/5	5/5	Sg2-14		1/100	2	0	2.3	2 300

Laboratoire K2

N° échantillon	Méthode de référence									Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
K5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/		<100	1/100	0	0	<1	<1000
		c				/	/							
K6	Non dilué	a	0	8	77	0 / 5	5 / 5	2-14	773	Non dilué	6	33	230.7	2 307
		b				/	/							
		c				/	/			1/10	6	9	53.4	5 340
	1/10	a	0	0	6	0 / 5	5 / 5	2-14						
		b				/	/		600	1/100	5	3	14.6	14 600
		c				/	/							
K7	Non dilué	a	0	0	1	0 / 1	1 / 1	2-14	10	Non dilué	1	0	1.1	11
		b				/	/							
		c				/	/			1/10	1	0	1.1	110
	1/10	a	0	0	0	/	/							
		b				/	/		<100	1/100	0	0	<1	<1000
		c				/	/							
K8	Non dilué	a	0	9	82	0 / 5	5 / 5	2-14	827	Non dilué	6	38	277.1	2 771
		b				/	/							
		c				/	/			1/10	6	3	22.3	2 230
	1/10	a	0	0	6	0 / 5	5 / 5	2-14						
		b				/	/		600	1/100	2	1	3.5	3500
		c				/	/							

Laboratoire L2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex					
						BCYE	BCYE + cystéine						
L1	Non dilué	a	78	>100	>100	/	/		390 000	Non dilué	6	90	> 2272,6	> 22 726
		b				/	/							
		c				/	/							
	1/10	a	7	>100	>100	/	/			1/10	6	90	> 2272,6	> 227 260
		b				/	/							
		c				/	/			1/100	6	41	307,1	307 100
L2	Non dilué	a	0	0	0	/	/		< 10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
L3	Non dilué	a	84	>100	>100	/	/		450 000	Non dilué	6	90	> 2272,6	> 22 726
		b				/	/							
		c				/	/			1/10	6	90	> 2272,6	> 227 260
	1/10	a	9	>100	>100	/	/							
		b				/	/			1/100	6	42	317,5	317 500
		c				/	/							
L4	Non dilué	a	0	0	3	/	/		300	Non dilué	3	1	5,2	52
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture				Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex					
						BCYE	BCYE + cystéine						
L5	Non dilué	a	0	0	0	/	/		< 10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<100
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
L6	Non dilué	a	0	12	>100	/	/		1 400	Non dilué	6	24	156,6	1 566
		b				/	/							
		c				/	/			1/10	6	8	47,4	4 740
	1/10	a	0	2	13	/	/							
		b				/	/			1/100	1	0	1,1	1 100
		c				/	/							
L7	Non dilué	a	0	0	1	/	/		100	Non dilué	1	0	1,1	11
		b				/	/							
		c				/	/			1/10	1	0	1,1	110
	1/10	a	0	0	0	/	/							
		b				/	/			1/100	0	0	<1	<1000
		c				/	/							
L8	Non dilué	a	0	9	76	/	/		770	Non dilué	6	13	78,8	788
		b				/	/							
		c				/	/			1/10	6	4	26,4	2 640
	1/10	a	0	7	0	/	/							
		b				/	/			1/100	2	0	2,3	2 300
		c				/	/							

Laboratoire M2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
M1	Non dilué	a	68	>100	>100	0/5	5/5	2-15	340000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272.6	>227260
	1/10	a				/	/							
		b				/	/			1/100	6	38	277.1	277100
		c				/	/							
M2	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0		<10
		b				/	/							
		c				/	/			1/10	0	0		
	1/10	a				/	/							
		b				/	/			1/100	0	0		
		c				/	/							
M3	Non dilué	a	69	>100	>100	0/5	5/5	2-15	345000	Non dilué	6	90	>2272.6	>22726
		b				/	/							
		c				/	/			1/10	6	90	2272.6	227260
	1/10	a				/	/							
		b				/	/			1/100	6	38	277.1	277100
		c				/	/							
M4	Non dilué	a	0	1	0	0/1	1/1	2-15	10	Non dilué	1	0	1.0	10
		b				/	/							
		c				/	/			1/10				
	1/10	a				/	/							
		b				/	/			1/100				
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture					Grand puits	Petit puits		
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex						
						BCYE	BCYE + cystéine							
M5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0		
	1/10	a				/	/							
		b				/	/			1/100	0	0		
		c				/	/							
M6	Non dilué	a	0	82	10	0/5	5/5	2-15	600	Non dilué	6	20	126.9	1269
		b				/	/							
		c				/	/			1/10	4	7	17.8	1780
	1/10	a				/	/							
		b				/	/			1/100	1	0	1.1	1100
		c				/	/							
M7	Non dilué	a	0	1	0	0/1	1/1	2-15	<10	Non dilué	1	0	1.1	11
		b				/	/							
		c				/	/			1/10	0	0		
	1/10	a				/	/							
		b				/	/			1/100	0	0		
		c				/	/							
M8	Non dilué	a	0	61	5	0/5	5/5	2-15	5000	Non dilué	6	55	477.0	4770
		b				/	/							
		c				/	/			1/10	6	10	59.6	5960
	1/10	a				/	/							
		b				/	/			1/100	2	2	4.7	4700
		c				/	/							

Laboratoire N2

N° échantillon	Méthode de référence								Méthode alternative					
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation Lecture nb colonies + / nb de colonies testées		Latex	Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	BCYE	BCYE + cystéine				Grand puits	Petit puits			
N5	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10	>22 726
		b				/	/								
		c				/	/			1/10	0	0	<1	<100	>227 260
	1/10	a	0	0	0	/	/		<100						
		b				/	/			1/100	0	0	<1	<1000	230 700
		c				/	/								
N6	Non dilué	a	0	8	58	0/5	5/5	2-14	600	Non dilué	6	32	221.9	2219	<10
		b				/	/								
		c	0	0	5	0/5	5/5	2-14		1/10	6	0	12.6	1260	<100
	1/10	a				/	/								
		b				/	/		500	1/100	2	0	2.3	2300	<1000
		c				/	/								
N7	Non dilué	a	0	0	0	/	/		<10	Non dilué	0	0	<1	<10	>22 726
		b				/	/								
		c				/	/			1/10	0	0	<1	<100	>227 260
	1/10	a	0	0	0	/	/								
		b				/	/		<100	1/100	0	0	<1	<1000	286 900
		c				/	/								
N8	Non dilué	a	0	3	43	0/5	5/5	2-14	420	Non dilué	6	11	65.9	659	<10
		b				/	/								
		c				/	/			1/10	5	4	16.9	1690	<100
	1/10	a	0	0	5	0/5	5/5	2-14							
		b				/	/		500	1/100	1	1	2.2	2200	<1000
		c				/	/								

Laboratoire O2

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation		Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
			non dilué	100ml	10ml	Lecture nb colonies + / nb de colonies testées BCYE	Lecture nb colonies + / nb de colonies testées BCYE + cystéine	Latex		Grand puits	Petit puits		
O5	Non dilué	a	<1	<1	<1	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/							
	1/10	a	<1	<1	<1	/	/			1/10	0	0	<1	<10
		b				/	/							
		c				/	/			1/100	0	0	<1	<10
O6	Non dilué	a	1			0/1	1/1	2-14	5000	Non dilué	6	26	172,2	1722
		b		89	8	0/4	4/4	2-14						
		c				/	/			1/10	6	4	26,4	2640
	1/10	a	<1	4	<1	0/4	4/4	2-14	40					
		b				/	/			1/100	0	1	1	1000
		c				/	/							
O7	Non dilué	a	<1	3	<1	0/3	3/3	2-14	30	Non dilué	2	2	2,3	23
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	<1	<1	<1	/	/		<10					
		b				/	/			1/100	0	0	<1	<10
		c				/	/							
O8	Non dilué	a	1			0/1	1/1	2-14	5000	Non dilué	6	25	164,4	1644
		b		48	3	0/4	4/4	2-14						
		c				/	/			1/10	6	8	47,4	4740
	1/10	a	<1	8	<1	0/5	5/5	2-14	80					
		b				/	/			1/100	1	0	1,1	1100
		c				/	/							

N° échantillon	Méthode de référence								Méthode alternative				
	Dilution	Type de colonies	Direct	Filtration + traitement acide		Confirmation			Résultats confirmés *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Résultat de la table MPN	Résultats finals MPN/L (Table MPN * dilution * 10)
						Lecture								
			non dilué	100ml	10ml	nb colonies + / nb de colonies testées		Latex			Grand puits	Petit puits		
						BCYE	BCYE + cystéine							
O1	Non dilué	a	89	>100	>100	0/5	5/5	2-14	450000	Non dilué	6	90	>2272,6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272,6	>227260
	1/10	a	7			0/3	3/3	2-14	35000					
		b		>100	30	0/2	2/2	2-14		1/100	6	47	373	373000
		c				/	/							
O2	Non dilué	a	<1	<1	<1	/	/		<10	Non dilué	0	0	<1	<10
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	<1	<1	<1	/	/							
		b				/	/			1/100	0	0	<1	<10
		c				/	/							
O3	Non dilué	a	97	>100	>100	0/5	5/5	2-14	490000	Non dilué	6	90	>2272,6	>22726
		b				/	/							
		c				/	/			1/10	6	90	>2272,6	>227260
	1/10	a	7			0/3	3/3	2-14	35000					
		b		>100	50	0/2	2/2	2-14		1/100	6	55	477	477000
		c				/	/							
O4	Non dilué	a	<1	1	<1	0/1	1/1	2-14	10	Non dilué	2	0	2	20
		b				/	/							
		c				/	/			1/10	0	0	<1	<10
	1/10	a	<1	<1	<1	/	/		<10					
		b				/	/			1/100	0	0	<1	<10
		c				/	/							

Appendix 11 : Explanations of the two calculation methods for determining counts for the reference method and for the alternative method

Two methods of calculation were utilised for the interpretation of the results. An example of each calculation is detailed below from two samples.

Sample N°	Reference Method									Alternative Method				
	Dilution	Type of colonies	Direct	Filtration + acid traitment		Confirmation			Results confirmed *L. pneumophila* (CFU/L)	Dilution	Nb puits positifs		Result of the table MPN	Finals results MPN/L (Table MPN * dilution * 10)
						Reading					High wells	Small wells		
			Undiluted	100ml	10ml	nb colonies + / nb of tested colonies		Latex						
						BCYE	BCYE + cystéine							
C8	Undiluted	a	0	39	8	/	/	Lp 2.-15	430	Undiluted	6	12	72.3	723
		b				/	/							
		c				/	/			1/10	6	4	26.4	2640
	1/10	a	0	3	1	/	/							
		b				/	/			1/100	3	1	5.2	5200
		c				/	/							
B3	Undiluted	a	64	-	-	0/5	5/5	2-14	320000	Undiluted	6	90	>2272,6	>22726
		b	-	>100	>100	/	/							
		c				/	/			1/10	6	90	>2272,6	>227260
	1/10	a	6	-	-	0/5	5/5	2-14						
		b	-	>100	56	0/5	5/5	2-14		1/100	6	51	422,3	422300

Example of calculation mode 1 :

Sample C8 :

➔ Reference Method :

Direct result : <10 CFU/L

Filtration result : (39+3+8+1) / (100 mL + 10 mL + 10 mL + 1 mL) x1000 = **421 UFC/L**

The highest value is retained between the result of the live and the result of the filtration - acid treatment is **421 UFC/L.**

➔ Alternative method : (72.3+26.4+5.2) / (100 mL + 10 mL + 1 mL) x 1000 = **936 UFC/L**

Sample B3:

➔ Reference Method :

Direct result : (64+6)/0,22 x 1000 = **318 181 UFC/L**

Filtration result : 56 x 1000 = 56 000 UFC/L

The highest value is retained between the result of the live and the result of the filtration - acid treatment is **318 181 UFC/L.**

➔ Alternative method : 422,3 x 1000 = **422 300 UFC/L**

Example of calculation mode 2 :

Sample C8 :

➔ Reference Method :

Pur sample result : (39+8)/(100 mL +10 mL) x 1000 = 427 CFU/L

1/10ème sample result : (3+1)/ (10 + 1 mL) x 1000 = 363 CFU/L

(427 + 363)/2 = **395 CFU/L**

The average results of the two samples were used for the interpretation of either **395 CFU/L**.

➔ Alternative method :

723 + 2640 + 5200 = 8563/3 = **2854 CFU/L**

Sample B3:

➔ Reference Method :

Pur sample result : 64/0,2 x 1000 = 320 000 CFU/L

1/10ème sample result: 6/0,02 x 1000 = 300 000 CFU/L

(320 000 + 300 000)/2 = **310 000 CFU/L**

The average results of the two samples were used for the interpretation of either 310 000 CFU/L.

➔ Alternative method :

422,3 x 1000 = **422 300 UFC/L**