MICA Advance

Legionella

SCIENTIFIC REPORT

DIAMIDEX

CONTENTS

I/ Study for AOAC certification

I.1 Scope

The aim of this study is to demonstrate the reliability of the MICA *Legionella* solution, as well as its performance compared with the standard method. The design of this study was validated by the AOAC international and the results are being submitted for certification.
This study is made of four parts:

- Inclusivity / Exclusivity
- Ruggedness
- Product consistency and stability
- Comparison with the ISO 11731-2017 method

The validation study is now published: Fanny Passot, Sabine Peslier, M Joseph Benzinger, Jonathan Blackburn, Wesley Thompson, Benjamin Bastin, Audrey Dumont, Sam Dukan, Validation of MICA *Legionella* for Enumeration of *Legionella pneumophila* in Sanitary Waters and Cooling Tower Waters: AOAC *Performance Tested Method*[SM] 032201, *Journal of AOAC INTERNATIONAL*, Volume 106, Issue 3, May-June 2023, Pages 725–736, https://doi.org/10.1093/jaoacint/qsac150

The accuracy of the method is evaluated based on the 0.5 log10 rule:
The 0.5 log10 rule is adapted from a publication by Basil Jarvis (Sampling for Microbiological Analysis in 'The Microbiological Safety and Quality of Food' Volume II, 2000, edited by Lund, Baird-Parker and Gould). Although the 0.5 log10 rule is based on microbiological criteria, it is also statistically valid: for example, if the expected count on a plate is 10 colonies, and the organisms are randomly distributed, then 95% of results would be between 4 and 16 colonies. When the range is converted to a log10 scale it would be between 0.60 and 1.20, with a median of 1.00, i.e. the lower and upper limits are within 0.5 log10 units. For Legionella, 0.5 log10 rule is expanded to 0.7 log10 to reflect the greater variation inherent in the standard method.

I.2 Inclusivity / Exclusivity

I.2.1 Method

The purpose of inclusivity testing is to ensure that MICA *Legionella* can identify many different strains of the *Legionella pneumophila* species. 35 different isolates are tested.
The purpose of exclusivity testing is to determine the ability of MICA *Legionella* to discriminate target organisms from non-target organisms. 29 non- *Legionella pneumophila* species are used to demonstrate that MICA *Legionella* specifically detects only *Legionella pneumophila*.
Inclusivity and exclusivity evaluations are performed together as one study. Inclusivity and exclusivity test samples are blind coded and intermingled so the analysts cannot know the identity of the test samples.

I.2.2 Results

I.2.2.1 Inclusivity strains

All strains are *Legionella pneumophila* (Lp) strains.
Inclusivity strains are prepared as vegetative cells. The strains are grown in a non-selective media broth as pure cultures. Serial dilutions are prepared of an overnight culture and the number of cells determined by counting colony forming units (CFU's). Approximately 1,000 to 5,000 cells (100 times LOD of the method) per test portion (20ml) are spiked into filter-sterilized phosphate buffer.

Strain identity			Detection by MICA *Legionella*
Serogroup	**Strain number**	**Blind code**	
1	ATCC 33152	C15	++
1	CIP 107629	A13	++
1	CIP 108286	A1	++
1	CIP103854T	C14	++
1	CIP105349	A5	++
1	Environmental	B19	++
1	Environmental	B20	++
1	Environmental	B21	++
1	LG 0901 3003	A4	++
1	LG 0905 2014	A3	++
2	CIP103856	C13	++
3	CIP103857	A15	++
4	LG 0905 1005	A19	++
5	LG 0905 3003	A20	++
6	LG 08463022	A21	++
6	LG12105012	C8	++
6	LG12312024	A27	++
6	LG13173014	C9	++
7	CIP 103861	B1	-
7	LG06054003	C3	++
7	LG06111002	C2	+
7	LG06154008	C1	++
7	LG08244007	C5	-
7	LG09455040	C4	++
8	LG 14023007	B2	++
9	CIP103863	B3	++
10	16174522401	C7	++

Strain identity			Detection by MICA *Legionella*
Serogroup	**Strain number**	**Blind code**	
11	LG 1221 5012	B8	++
12	LG 14133007	B9	++
13	DMS 25225	B13	++
14	CIP 103869	B14	++
15	LG 08393017	B15	++
2-14	Environmental	B25	++
2-14	Environmental	B26	++
2-14	Environmental	B27	++

I.2.2.2 Exclusivity strains

Exclusivity strains are prepared as vegetative cells. The strains are grown in a non-selective media broth as pure cultures to their growth limit. Serial dilutions are prepared of an overnight culture and the number of cells determined by counting colony forming units (CFU's). Approximately 10,000 to 50,000 cells (10x inclusivity testing levels) per test portion (20ml) are spiked into filter-sterilized phosphate buffer.

Strain identity			Detection by MICA *Legionella*
Species	**Strain**	**Blind code**	
Legionella anisa		A7	-
Legionella bozemanii	HL 0538 2034	A8	-
Legionella cincinnatiensis	HL 0524 4030	A9	-
Legionella feeleii	HL 0418 4001	A10	-
Legionella geestiana	DSM-21217	A24	-
Legionella gormanii	HL 0540 3034	A11	-
Legionella jordanis	HL 0450 5001	C12	-
Legionella longbeachae	LG 0902 4055	A16	-
Legionella maceachernii	HL 0540 2029	A17	-
Legionella micdadei	HL 0522 5034	A18	-
Legionella moravica	DSM-105104	B5	-
Legionella nagasakiensis	DSM-24727	A28	-
Legionella norrlandica	DSM-23087	B4	++
Legionella spiritensis	DSM-19324	A29	-
Legionella tucsonensis	HL 0438 3117	A22	-
Legionella waltersii	DSM-21908	A23	-
Acinetobacter baumanii	ATCC 19606	B30	-
Bacillus cereus	ATCC 14579	C6	-

Strain identity			Detection by MICA *Legionella*
Species	**Strain**	**Blind code**	
Candida albicans	ATCC 10231	C10	-
Citrobacter freudii	ATCC 8090	B6	-
Escherichia coli Migula	ATCC 8739	B11	-
Enterococcus faecelis	ATCC 19433	B10	-
Listeria monocytogenes	ATCC 35152	B12	-
Pseudomonas aeruginosa	ATCC 10145	B16	-
Pseudomonas fluorescens	ATCC 13525	C11	-
Staphylococcus aureus	ATCC 6538	B22	-
Staphylococcus epidermidis	ATCC 12228	B23	-
Klebsiella pneumoniae	ATCC 13883	B28	-
Salmonella typhimurium	ATCC 13311	B18	-

On 32 *Legionella pneumophila* strains, 2 of them are not recognized. Both are serogroup 7. But 4 other *Legionella pneumophila* Sg 7 are well recognized by MICA. Note that the serogroup 7 represents less than 1% in the environmental isolates (V. L. Yu et al., J. Infect. Dis. 2002, 186, 127 – 128; A. Doleans, H. Aurell, M. Reyrolle, G. Lina, J. Freney, F. Vandenesch, J. Etienne, S. Jarraud, J. Clin. Microbiol. 2004, 42, 458 – 460; T. G. Harrison, B. Afshar, N. Doshi, N. K. Fry, J. V. Lee, Eur. J. Clin. Microbiol. Infect. Dis. 2009, 28, 781 – 791.)

Of all 29 exclusivity strains tested, the only one that is detected by MICA *Legionella*, is *Legionella norrlandica*. This species was described in 2015 from 14 isolates coming from the biopurification system of three different wood processing plants in Sweden (Rizzardi, K. *et al. Legionella norrlandica* sp. nov., isolated from the biopurification systems of wood processing plants. *International Journal of Systematic and Evolutionary Microbiology* **65**, 598–603 *2015*). The species is more closely related to *L. pneumophila* than the other *Legionella* species (such as *L. anisa*, *L. longbeachae* or *L. moravica*) and harbours the majority of the known virulence genes of *L. pneumophila* as well as resistance genes to several antibiotics (macrolides, sulfonamide, chloramphenicol). It is less virulent than *L. pneumophila* on amoebae. These characteristics led the DSMZ database to classify *L. norrlandica* as a class-2 pathogen (Leibniz Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH ; Curators of the DSMZ; DSM 105104). Thus, *L. norrlandica* has a low occurrence and should be treated with the same caution as *L. pneumophila*.

I.3 Ruggedness

This study evaluates the ability of the method to remain unaffected by small variations in method parameters that might be expected to occur when the MICA *Legionella* kit is performed by an end user.
Statistically significant findings in this experiment are not indicative of a faulty method, and the discovery of significance is not a roadblock to successful method validation. In fact, significant findings should be celebrated as indications of a well-designed experiment. Any

findings at this stage will be used to modify method kit instructions, emphasize areas of caution, or even possibly to widen specific parameter options. Success of this experiment is not conditional on a conclusion of "no significant differences were observed."

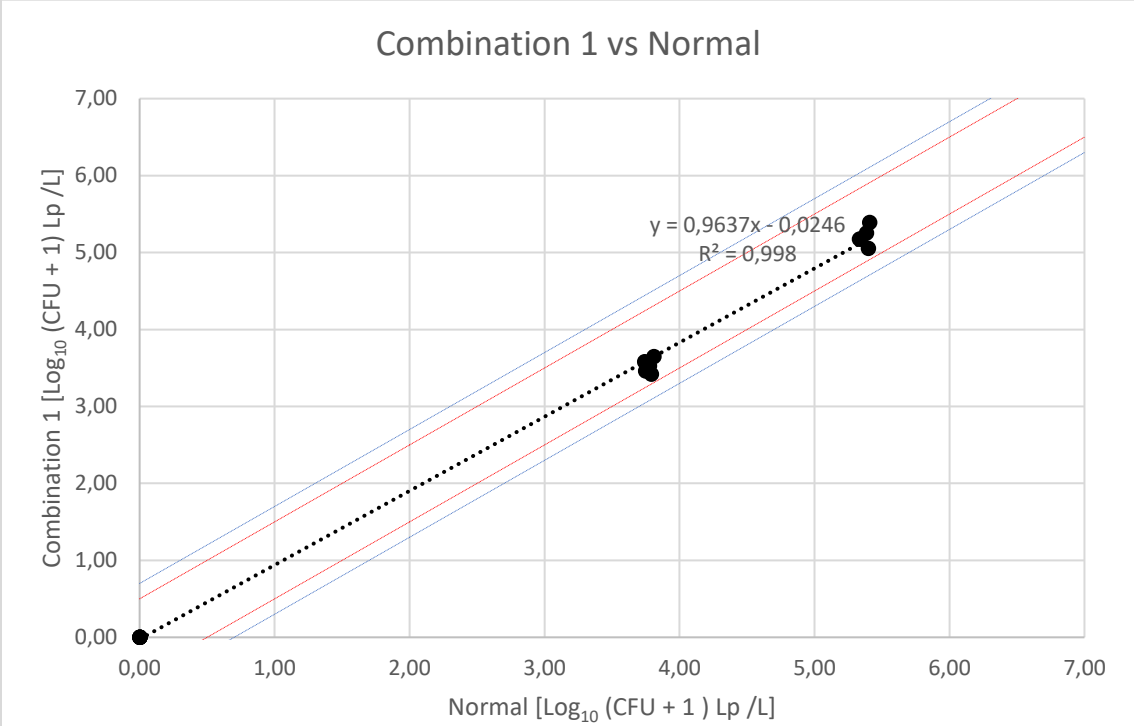
Robustness Parameter to be varied

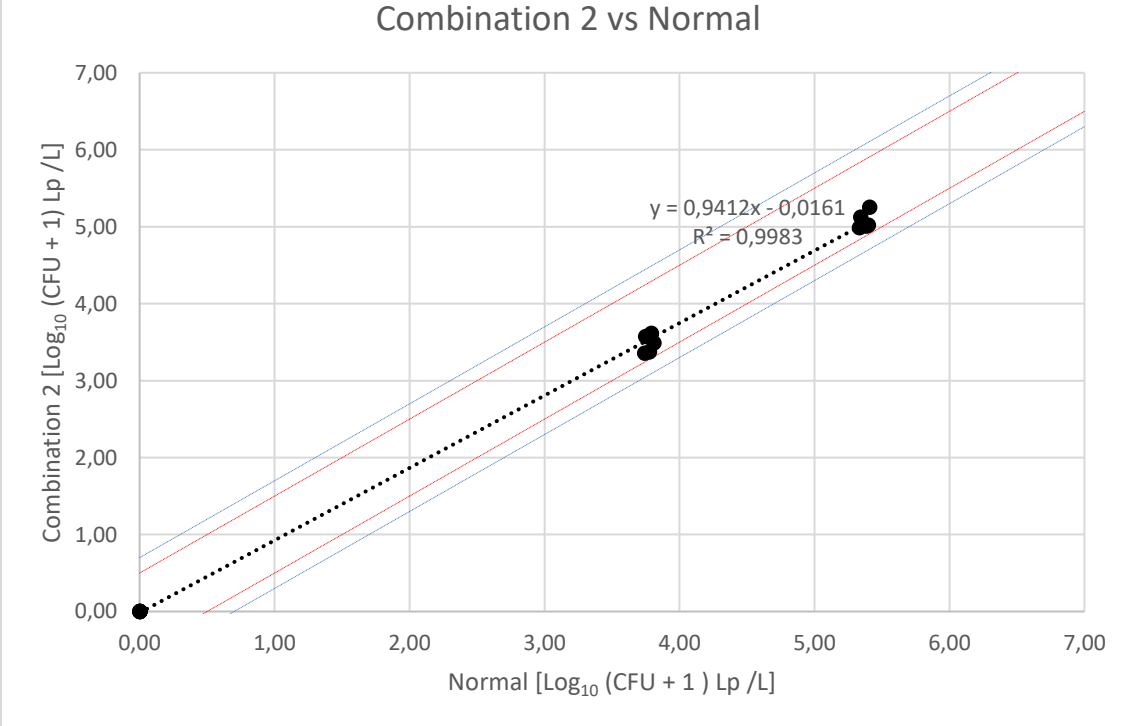
Robustness Test Parameter	Low Value	Normal Value (Package Insert Directions)	High Value
Growth Incubation Time	46 h	48 h	50 h
Growth Incubation Temperature	35±1°C	37±1°C	39±1°C
Labeling Time	10 min	15 min	30 min

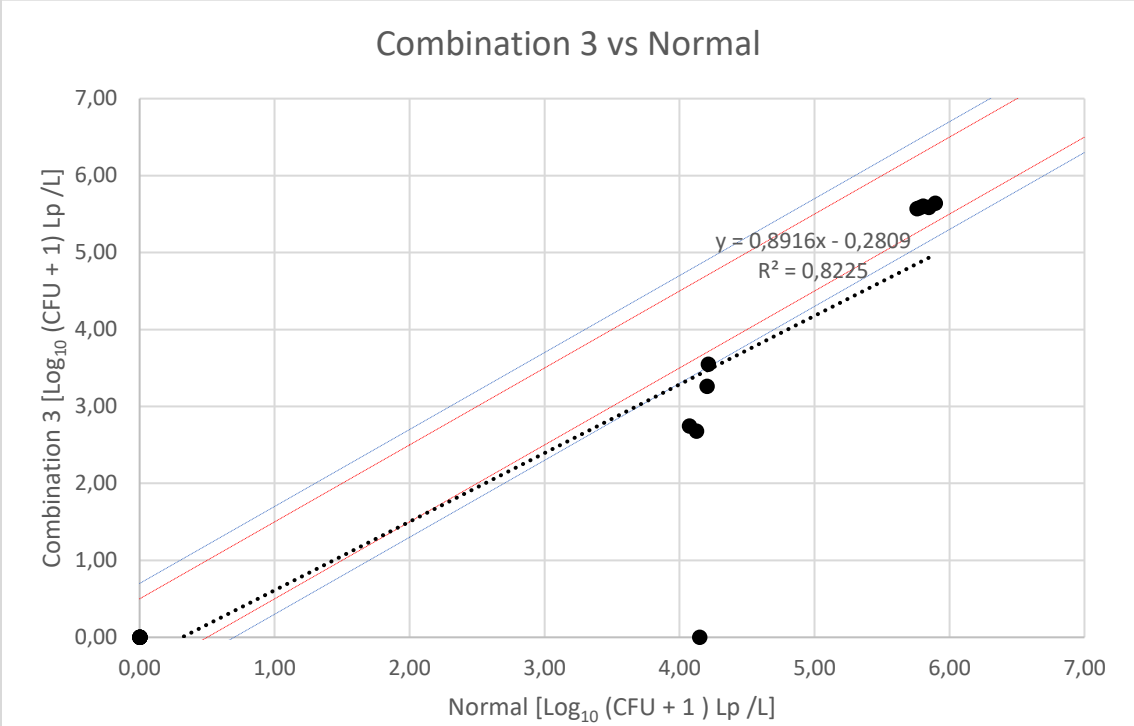
Factorial design for robustness experiment

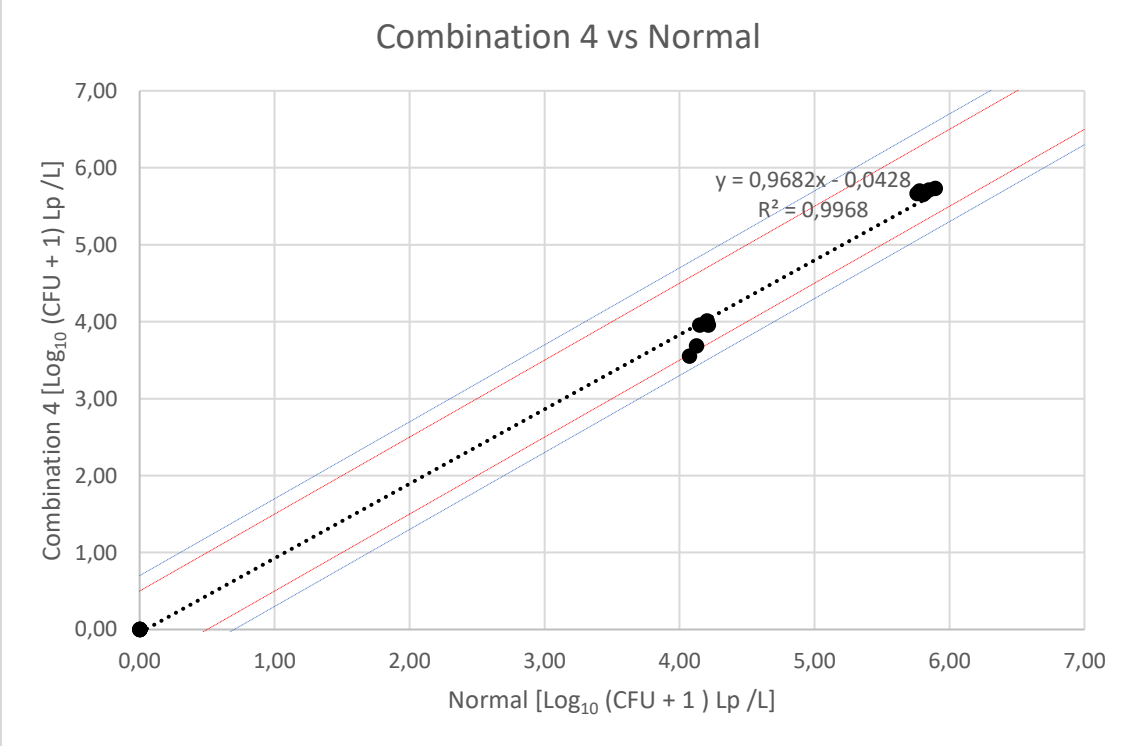
Treatment Combination	Incubation Time	GVPC Petri Dish Incubation Temperature	Labeling Time
1	46 h	39±1°C	10 min
2	46 h	39±1°C	30 min
3	46 h	35±1°C	10 min
4	46 h	35±1°C	30 min
5	50 h	39±1°C	10 min
6	50 h	39±1°C	30 min
7	50 h	35±1°C	10 min
8	50 h	35±1°C	30 min
9 (Normal)	48 h	37±1°C	15 min

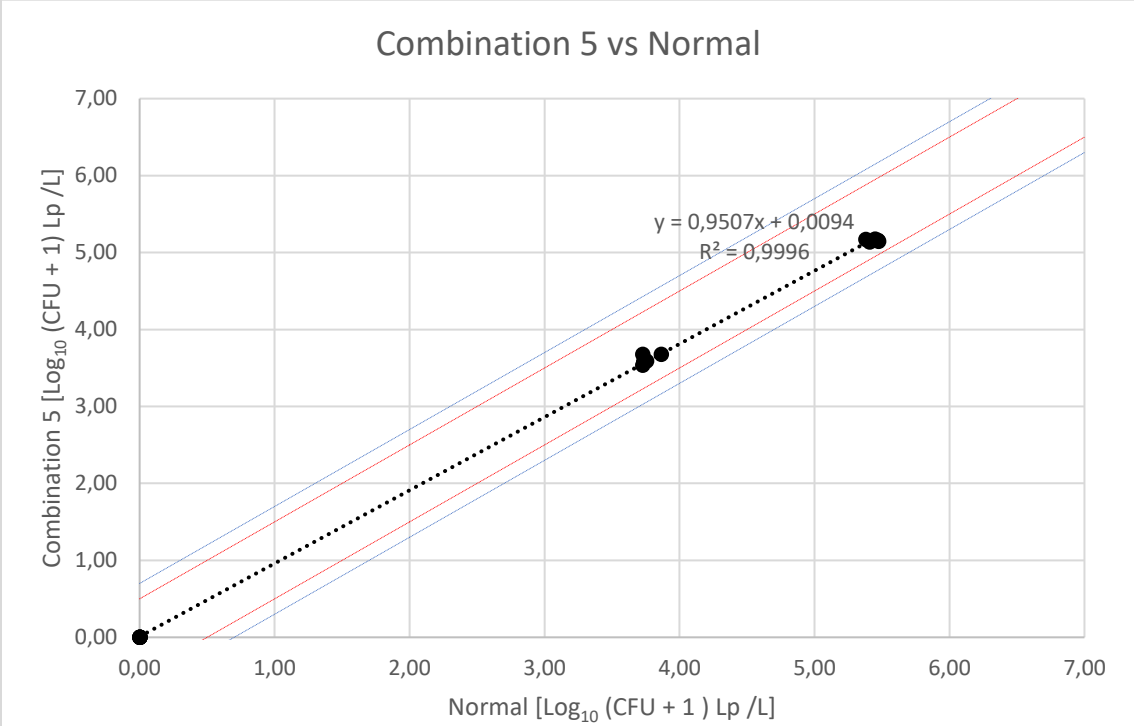
Bottled mineral water is spiked with approximately 0, 5000 or 250000 Lp/L from a pure culture (Lp6 LG08463022). Five test portions are processed for each cell density and each treatment combination. The normal procedure is included in each of the experiment rounds and the modified treatment combination are compared with the normal one from the same round.

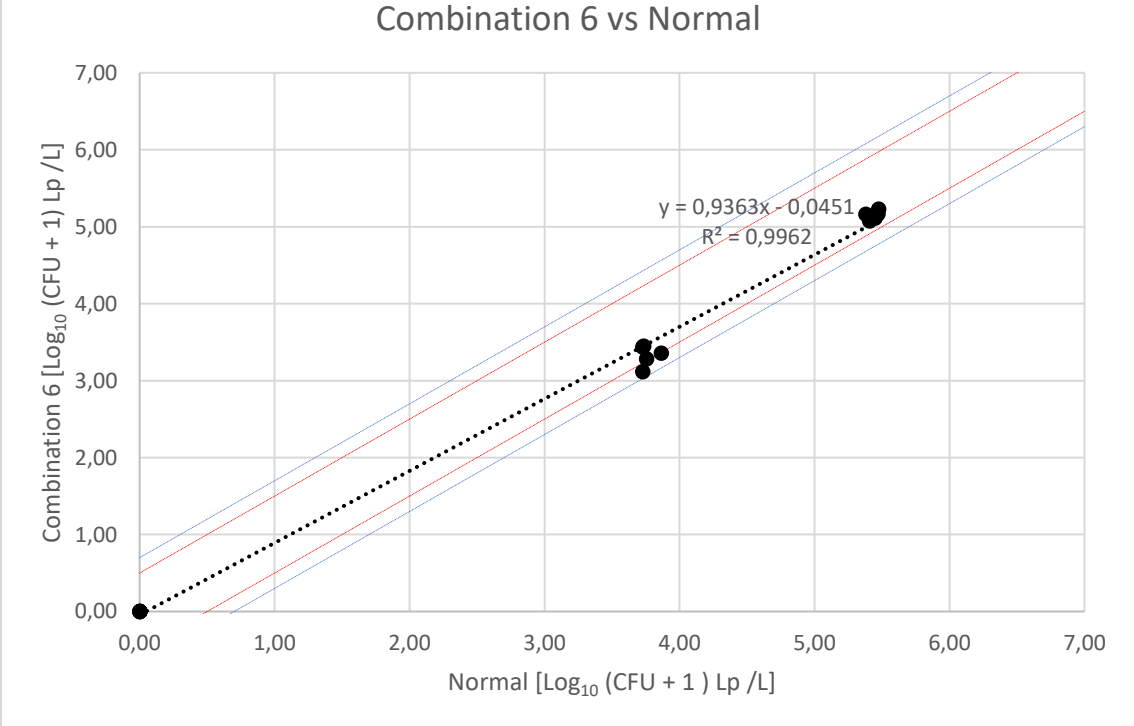

Combination 1 vs Normal
y = 0,9637x - 0,0246
R² = 0,998
Combination 1 [Log10 (CFU + 1) Lp /L]
Normal [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00

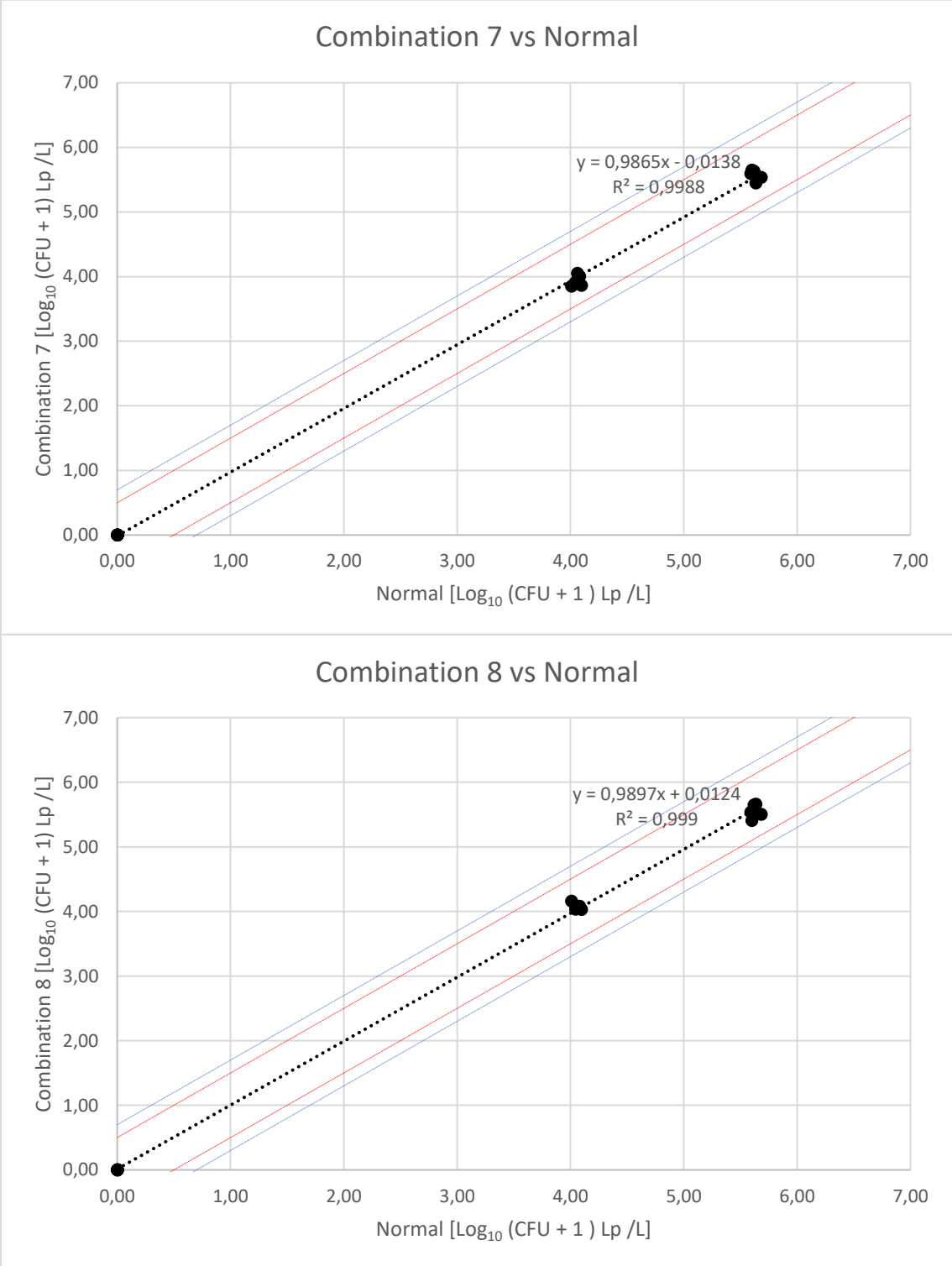

Combination 2 vs Normal
y = 0,9412x - 0,0161
R² = 0,9983
Combination 2 [Log10 (CFU + 1) Lp /L]
Normal [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00


Combination 3 vs Normal
y = 0,8916x - 0,2809
R² = 0,8225
Combination 3 [Log10 (CFU + 1) Lp /L]
Normal [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00


Combination 4 vs Normal
y = 0,9682x - 0,0428
R² = 0,9968
Combination 4 [Log10 (CFU + 1) Lp /L]
Normal [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00


Combination 5 vs Normal
y = 0,9507x + 0,0094
R² = 0,9996
Combination 5 [Log10 (CFU + 1) Lp /L]
Normal [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00


Combination 6 vs Normal
y = 0,9363x - 0,0451
R² = 0,9962
Combination 6 [Log10 (CFU + 1) Lp /L]
Normal [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00



Combination of a shorter growth incubation time at a lower temperature and a shorter labelling time is the worst possible combination tested, with 4 out of 5 replicates at 10^4 Lp/L that fall out of the 0.7log ratio. Apart from that, the MICA protocol is robust, nearly all data points falling within the 0.5log ratio.
A more detailed statistical analysis is presented in Appendix.

I.4 Product consistency and stability

This study will examine 3 lots of MICA *Legionella* test kit for lot-to-lot variability.

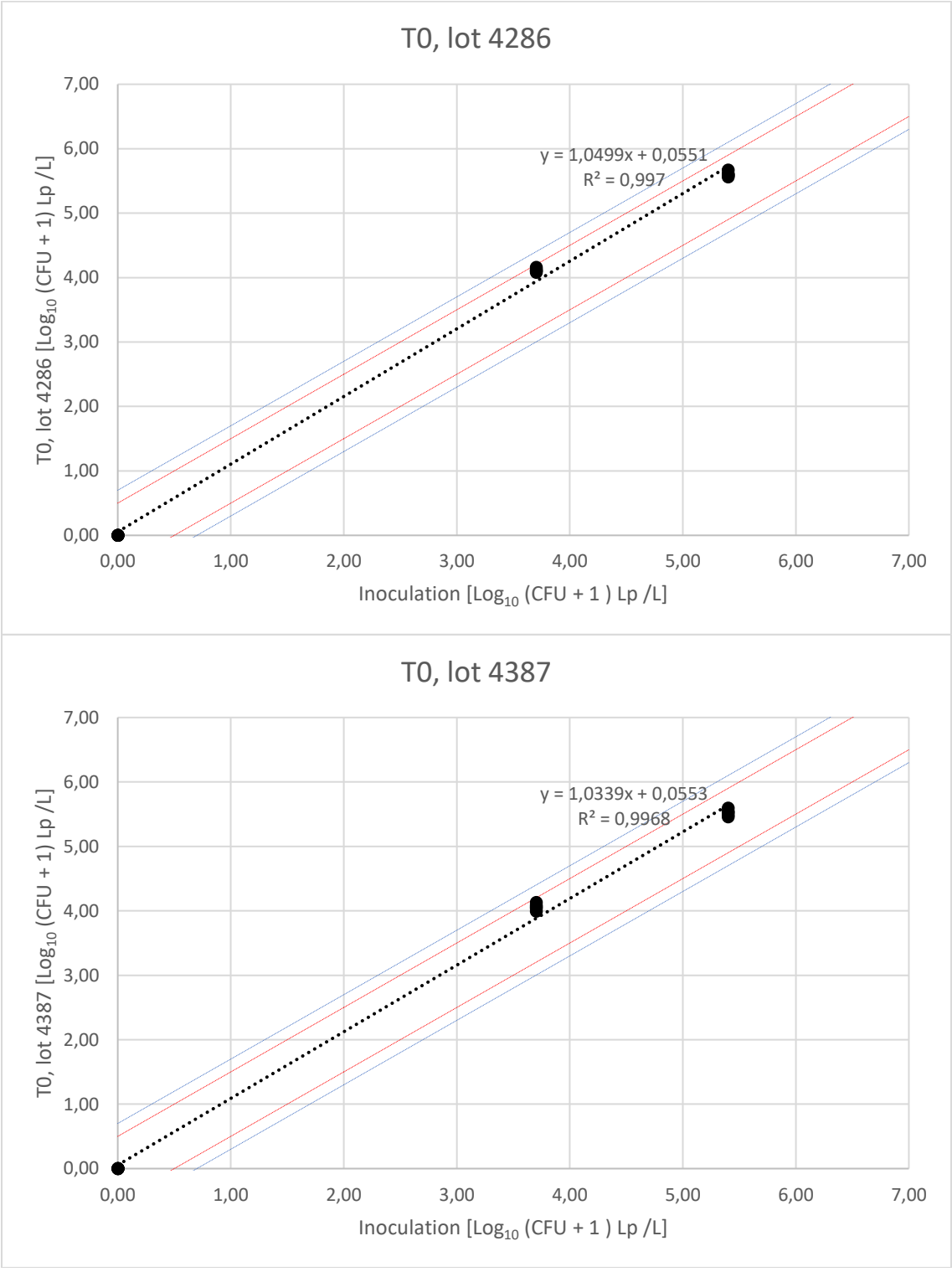
For product stability, data will be submitted for three time points. Accelerated stability data for the time points will be submitted for certification and real-time data will be submitted no later than the first annual renewal. Data demonstrating no statistical difference in detection between lots and no significant time slope is required. This study is carried out with pure cultures of *Legionella pneumophila* strains spiked into bottled mineral water at approximately 5000 and 250 000 Lp/L (Lp1 CIP105349) and with negative control samples, which are bottled mineral water. The bottled mineral water contains approximately 10^7 cells/100 mL and natural background flora. Five test portions are processed for each Lp density.

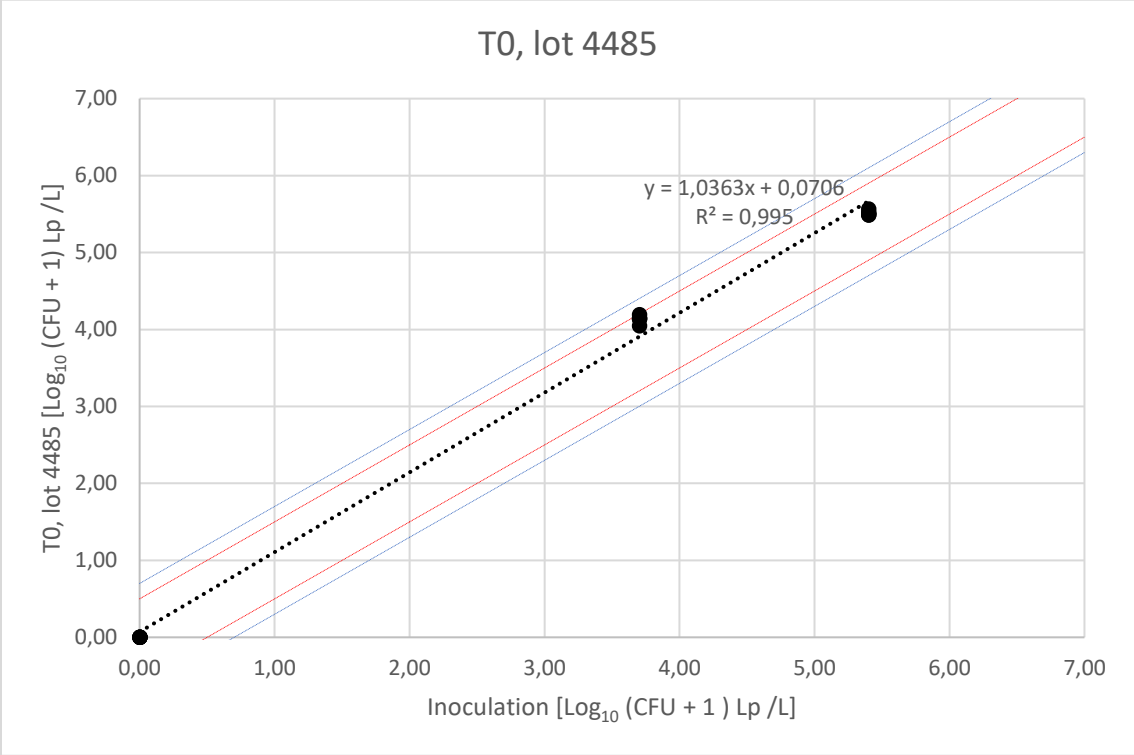
Stability data will be initially generated through accelerated studies based on the Arrhenius model and must support the shelf life of 12 months for kits. Assuming E_a = 20 kcal, 1 year at 5°C ≈ 32 days at 25°C.

For combined product consistency and stability testing, three lots of kits are stored at the following temperatures and test one lot at the specified time points:

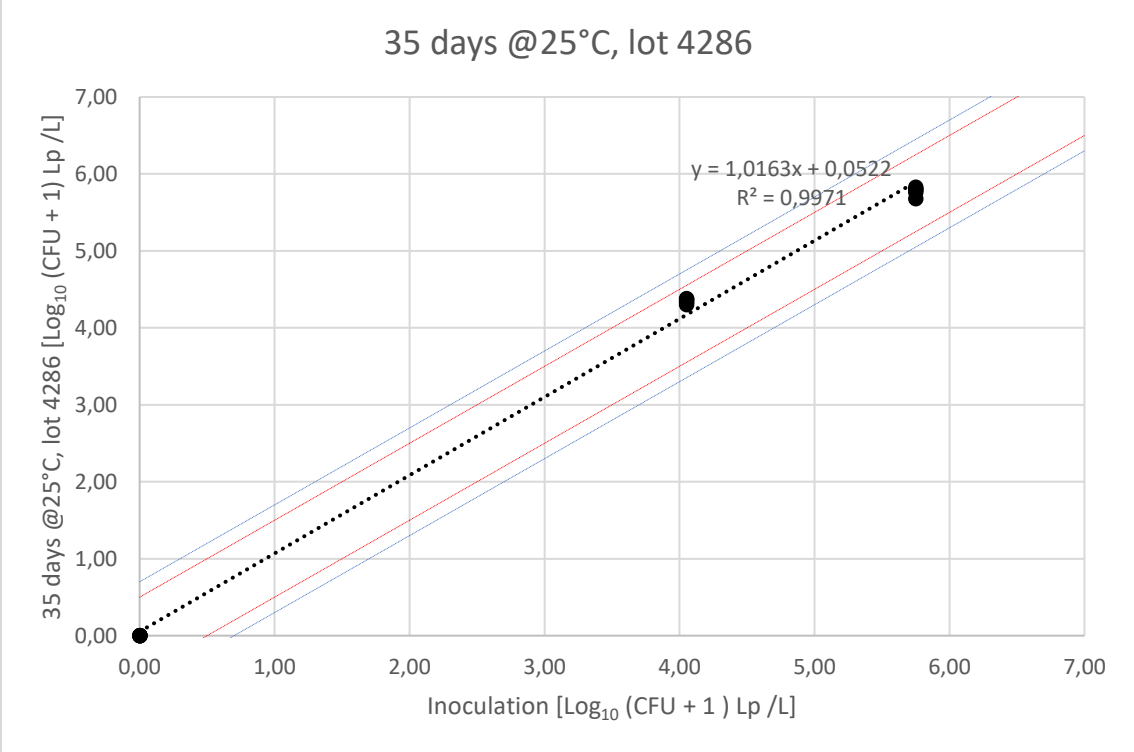
Microcolony Counter Kit	Storage Temperature	Time Points (from the date of production)
Real time	2-8 ± 1°C	0
		12 months
		18 months
Accelerated	25 ± 1°C	0
		32 days
		48 days

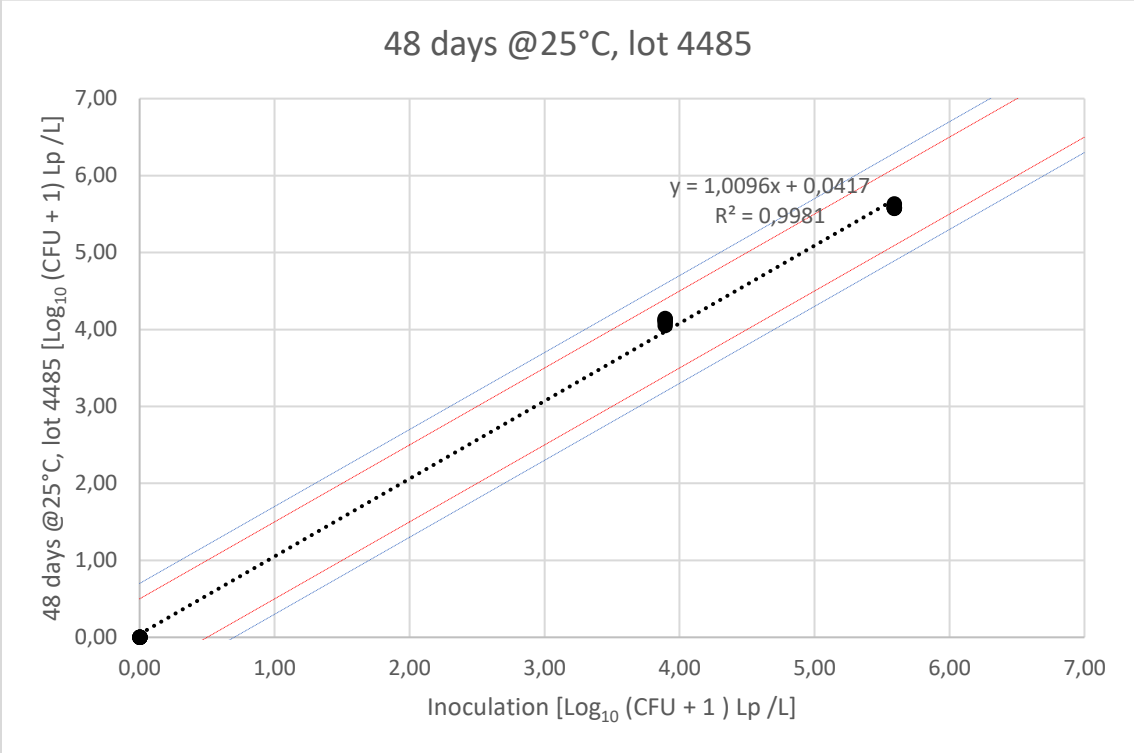
If unexpected results are observed when conducting the product consistency and stability study as one study, three separate lots of similar age will need to be tested for consistency and a separate stability study will need to be conducted.


T0, lot 4286
y = 1,0499x + 0,0551
R² = 0,997
T0, lot 4286 [Log10 (CFU + 1) Lp /L]
Inoculation [Log10 (CFU + 1) Lp /L]
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
T0, lot 4387
y = 1,0339x + 0,0553
R² = 0,9968
T0, lot 4387 [Log10 (CFU + 1) Lp /L]
Inoculation [Log10 (CFU + 1) Lp /L]



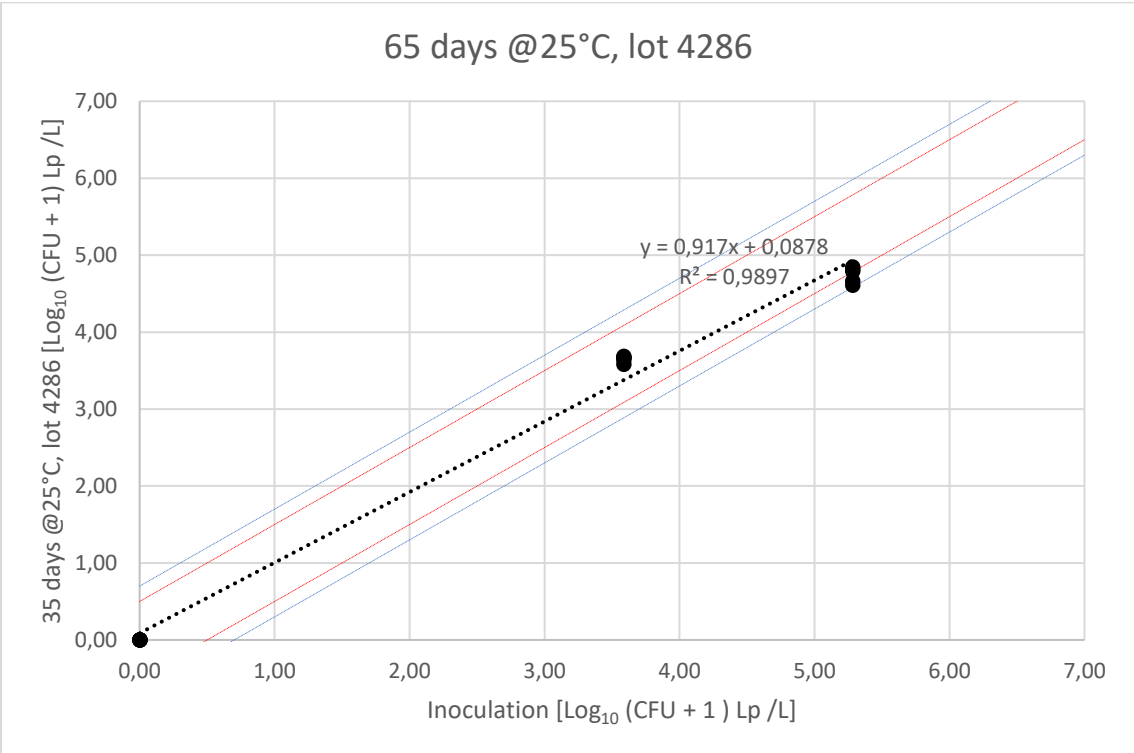
All three tested lots give similar results, proving the MICA *Legionella* test kit consistency.

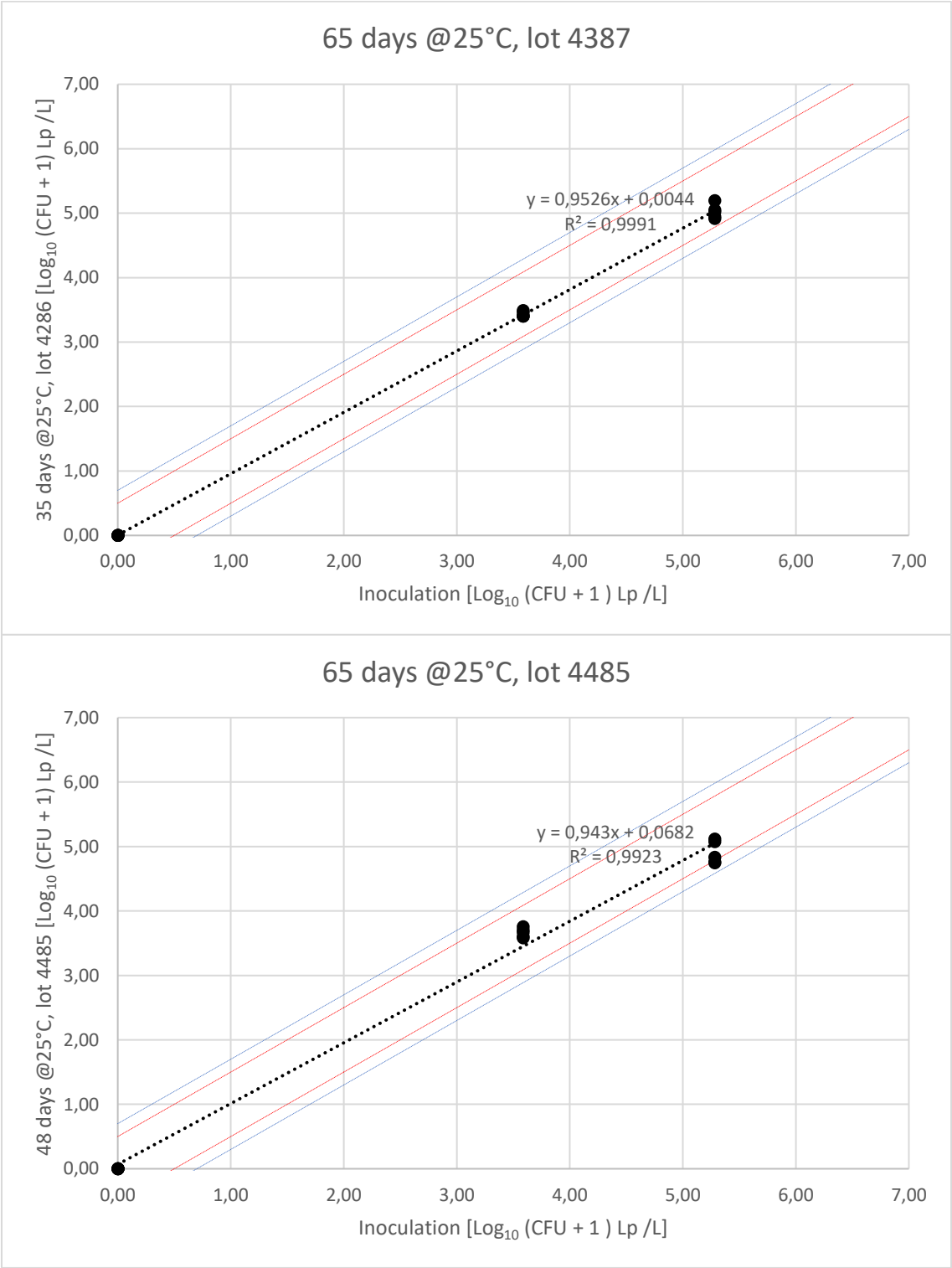




The MICA *Legionella* test kit is stable at least 48 days at 25°C, *i.e.* approximately 18 months at 4°C.

Considering the very good results obtained after 48 days at 25°C, an extra test point was added at 65 days (corresponding to approximately 24 months at 4°C), with all three tested lots.



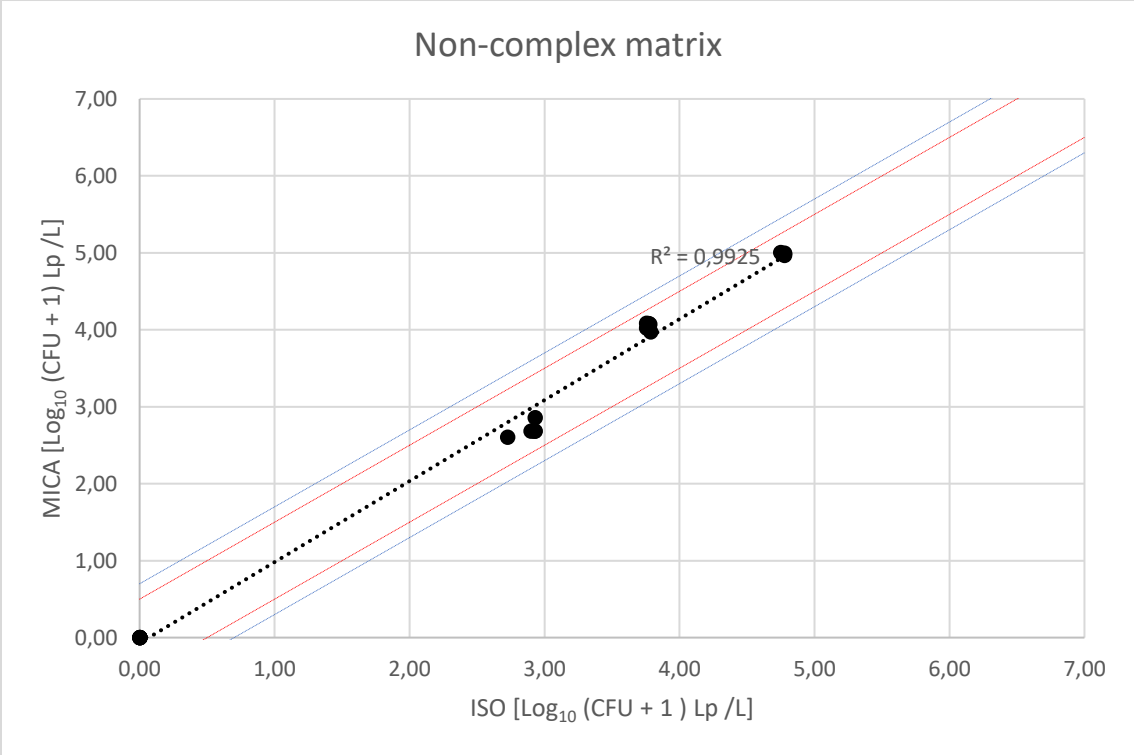


After 65 days at 25°C, the detection efficiency decreases, particularly for high density samples.

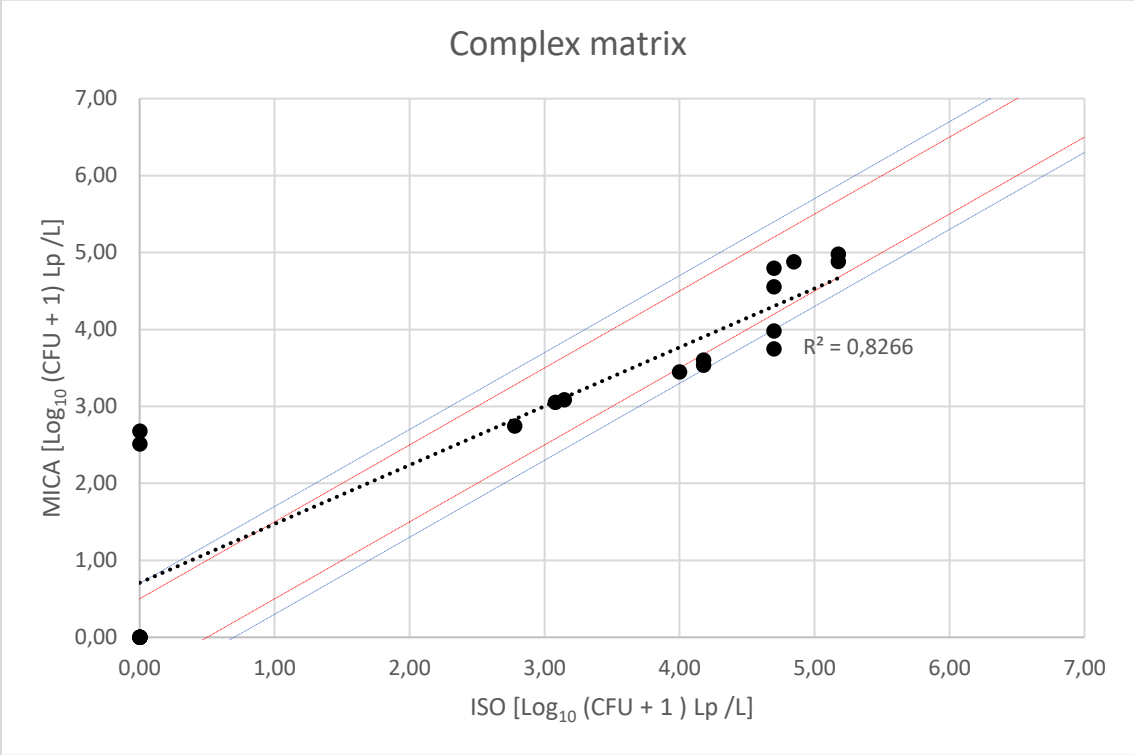
I.5 Comparison with the ISO 11731-2017 method

The matrix study is the most important part of the validation process for the *Performance Tested Methods* certification. It is necessary that the appropriate reference method be used as the basis for comparison. For the purposes of this study the MICA *Legionella* kit will be compared to the reference method procedures found in the ISO 11731-2017 *"Water quality – Enumeration of Legionella"*.

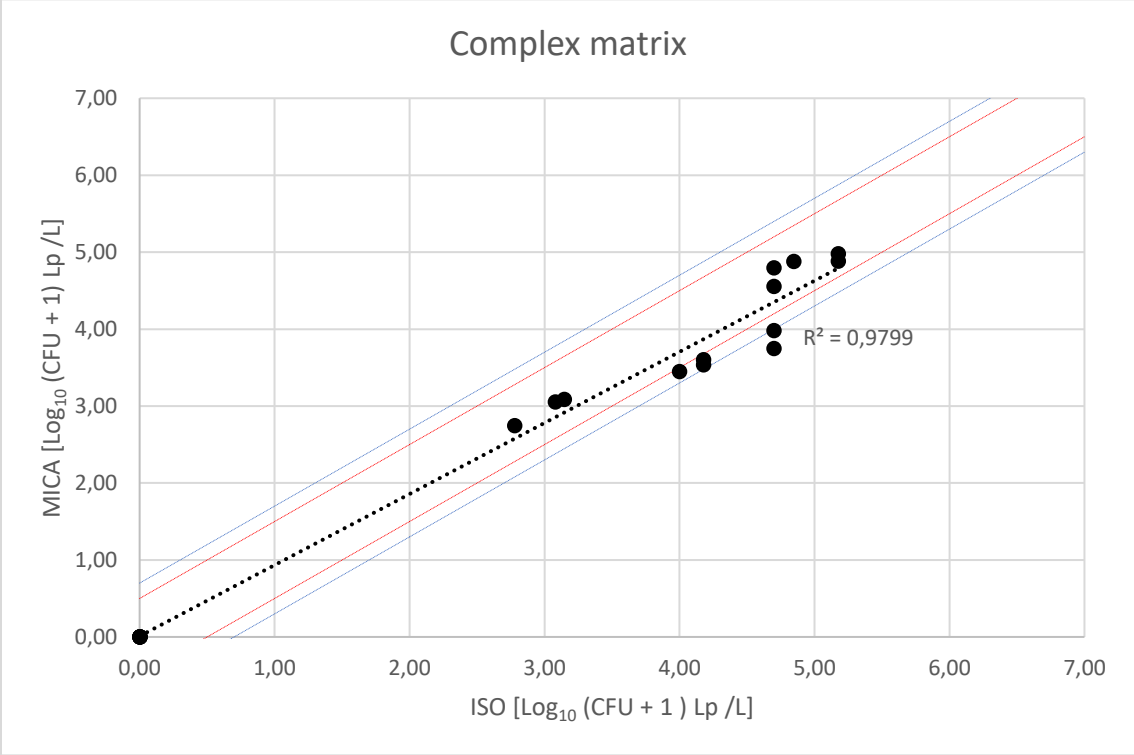
Two types of matrix are tested: a non-complex matrix (domestic hot water) and a complex matrix (cooling tower water). For each matrix, the study includes 5 replicate test portions at each contamination level. There is an uncontaminated level, a low level (10^3 Lp/L), a medium level (10^4 Lp/L) and a high level (10^5 Lp/L), so that the range of levels covers the range of the method. The non-complex matrix is spiked with a lab culture of Lp6 LG08463022, the complex matrix is spiked with a lab culture of Lp1 CIP105349.



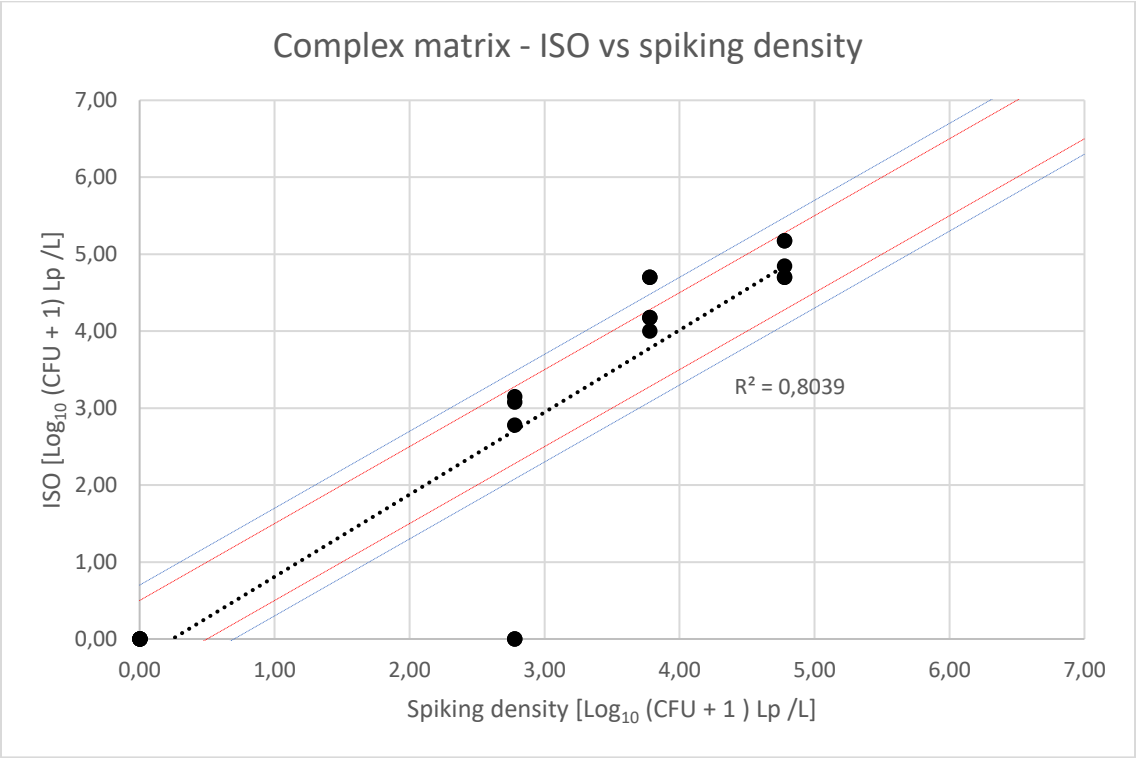
With a non-complex matrix, all data points fall within a 0.5log ratio and the correlation between MICA *Legionella* and the ISO 11731-2017 is very good (R^2=0.55).

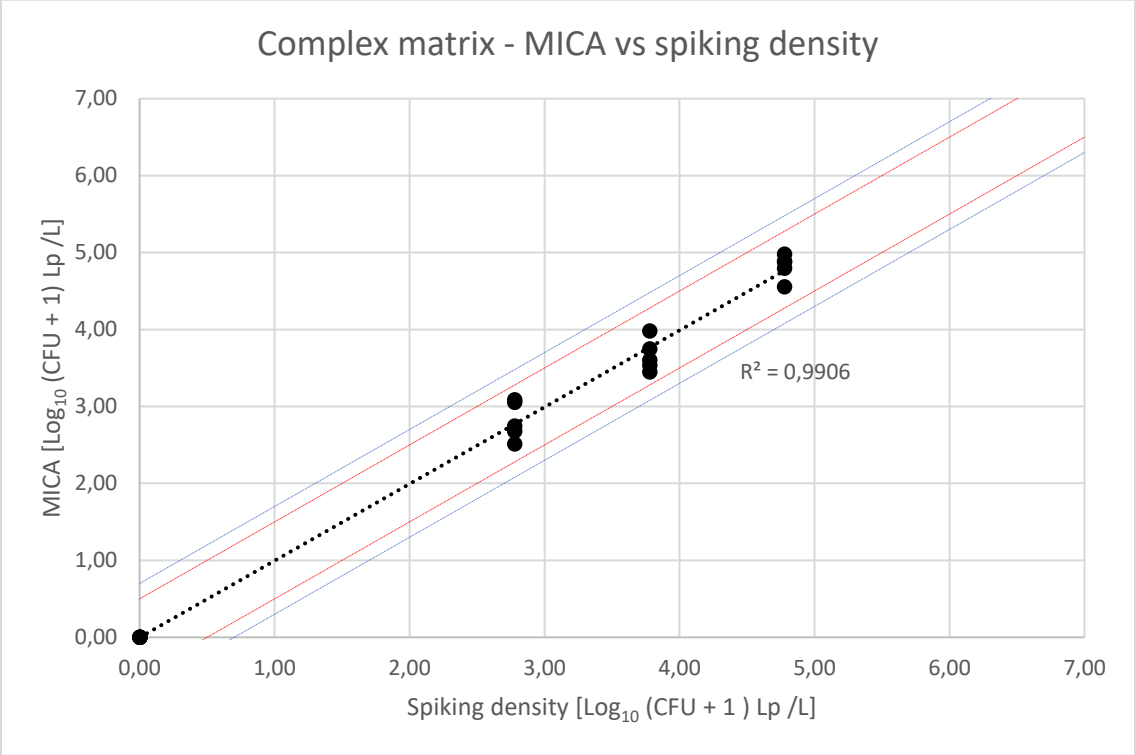


With a complex matrix, the high amount of background flora gives a high variability of results. 16 out of 20 data points (80%) fall within a 0.7log ratio. Two of the problem points are false negative of the ISO method on the low density sample: out of the five replicates, two gave a negative result, while three gave a positive result. MICA, however, gave consistent results for all five replicates. This shows that the ISO method is not as reliable as MICA on this sample. Without the two ISO false negatives, the R^2 is much better:



The dispersion of the datapoints can be due to a low performance of the MICA method on this type of matrix, or to a low performance of the ISO method. To discriminate between the two possibilities, each method is compared with the spiking density, calculated from the density of the initial lab culture. The two graphs below show that the correlation of the MICA *Legionella* with the spiking density is much better (R^2=0.99) than that of the ISO method (R^2=0.80). Thus, on a complex matrix, MICA *Legionella* performs better than the ISO 11731-2017.



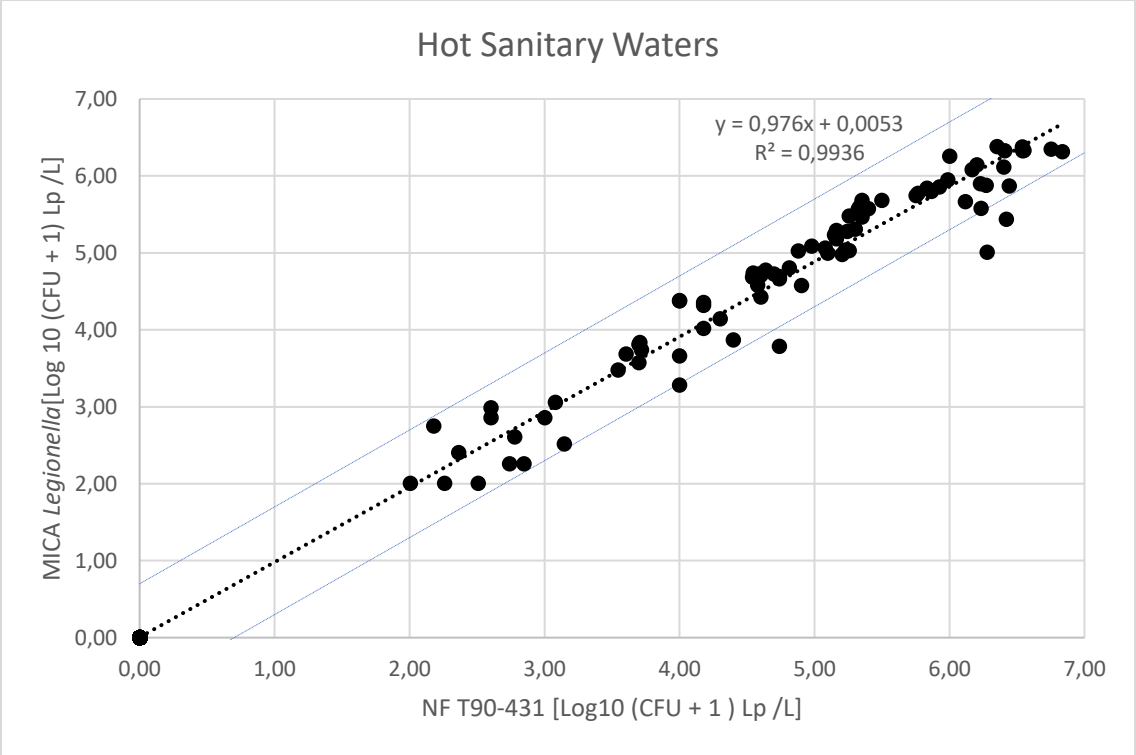


A more detailed statistical analysis is presented in Appendix.

II/ Comparison of MICA *Legionella* and ISO 11731-2017 on real samples

II.1 Hot sanitary water samples

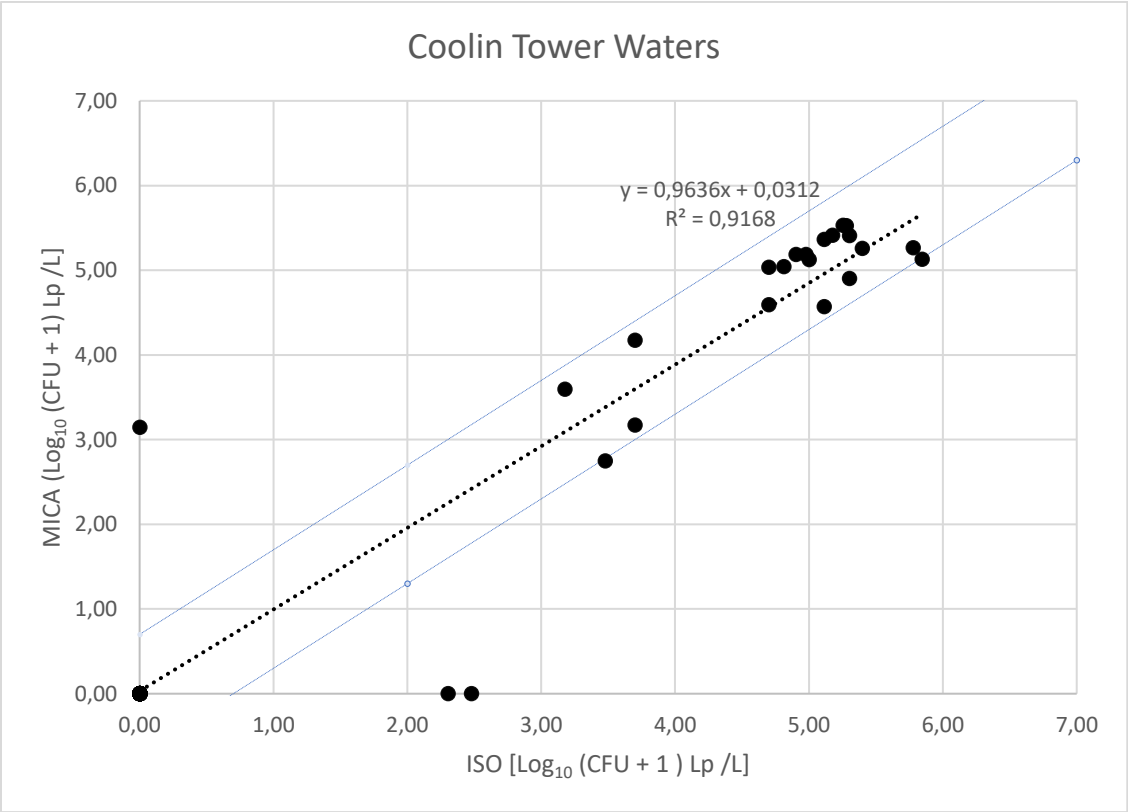
237 hot sanitary water samples are tested with both the MICA *Legionella* and the ISO 11731-2017 method. Out of the tested samples, 153 are negative samples (without *L. pneumophila*) and 84 samples are naturally contaminated with *L. pneumophila* (between 100 and 10 000 000 CFU/L). All filtrations are of 20ml of sample, which leads to a detection threshold of 100 CFU/L for MICA *Legionella*.



For 231 samples (97.4%) the MICA results are within a 0.7 log ratio of ISO 11731-2017.
Specificity: 100%
Sensibility: 97.2%
VPN: 98.7%
VPP: 100%

II.2 Cooling tower water samples

124 cooling tower water samples are tested with both the MICA *Legionella* and the ISO 11731-2017 method. 103 are negative samples (without *L. pneumophila*) and 21 samples are contaminated with *L. pneumophila*. The contamination can be natural or from a second filtration on the same filter membrane of a naturally contaminated sanitary water sample. The double filtration method allows to get the background microorganisms of the cooling water, while avoiding any weird chemical reaction or physiological shock to the Lp that could occur when mixing 2 water samples with different chemical properties.
All filtrations are of 20ml of sample, which leads to a detection threshold of 100 CFU/L for MICA *Legionella*.



95,7 % of cooling tower samples are within 0,7 log ratio of ISO 11731-2017 method.
Specificity: 99%
Sensibility: 90%
VPN: 98%
VPP: 95 %

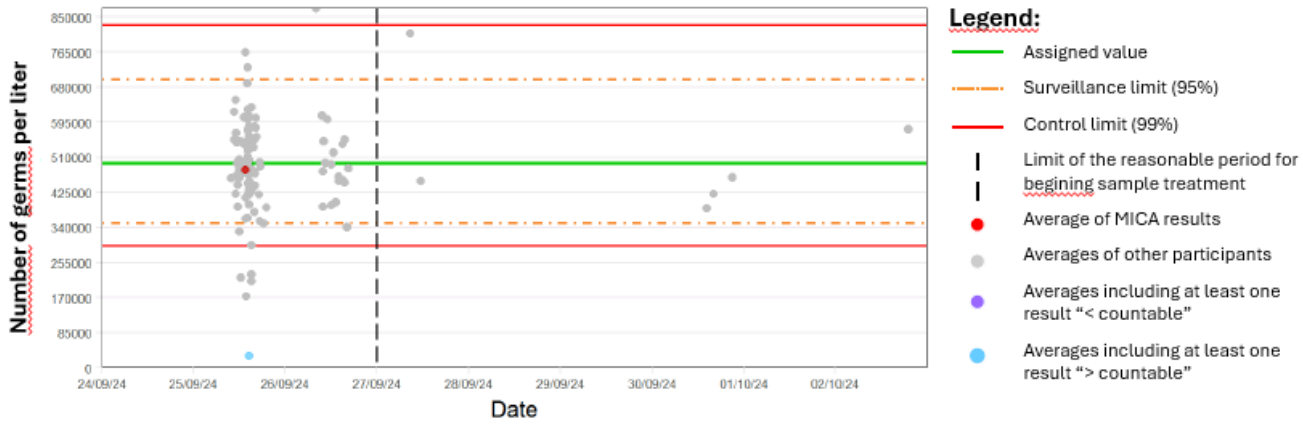
II.3 Proficiency tests

In 2024, we started participating in the Proficiency Test scheme organized by AGLAE, accredited by COFRAC (French Accreditation Committee) according to the ISO 17043 standard to conduct proficiency testing schemes. The other participants mainly used the French standard method NF-T90 431 or the international standard ISO 11731 to enumerate *Legionella pneumophila*.
Here is a summary of the results we obtained with MICA Advance *Legionella* in this Proficiency Test scheme:

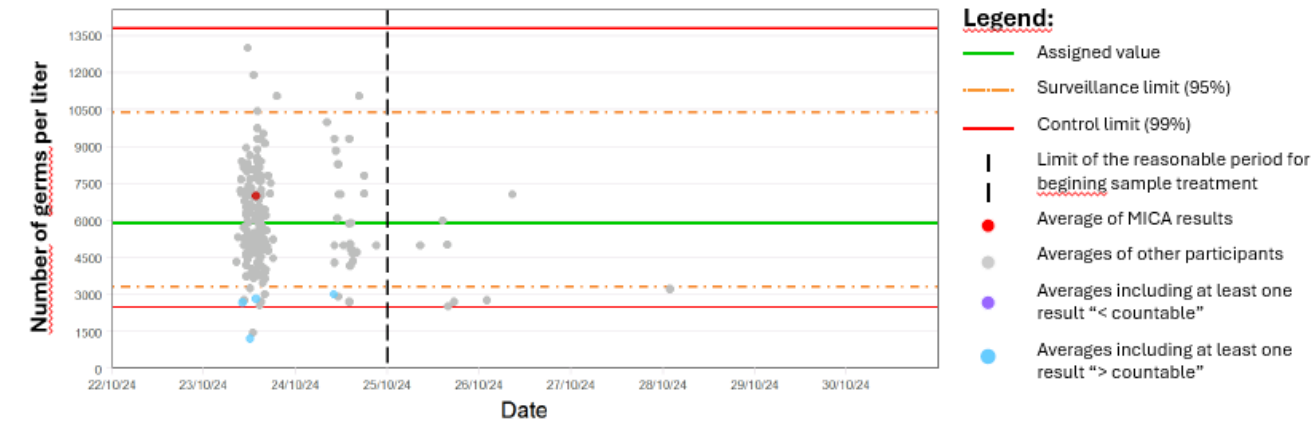
Round	24M33.3	24M32.3	25M32.1	25M32.2
Type of water	Dirty	Clean	Clean	Clean
Contaminating strains in the PT samples	Lp6 (environment) + *E. coli* (WDCM 00090)	Lp6 (ATCC 33215)	Lp6 (environment) + *L. dumoffii*	Lp10 (ATCC 43283)
MICA Replica 1	658 100 Lp CFU/L	7 354 Lp CFU/L	14 034 Lp CFU/L	19 892 Lp CFU/L
MICA Replica 2	295 500 Lp CFU/L	6 942 Lp CFU/L	17 116 Lp CFU/L	16 381 Lp CFU/L
MICA Replica 3	501 850 Lp CFU/L	6 804 Lp CFU/L	17 251 Lp CFU/L	20 427 Lp CFU/L
MICA Replica 4	543 650 Lp CFU/L	6 922 Lp CFU/L	15 800 Lp CFU/L	22 023 Lp CFU/L
Mean MICA Result	479 939 Lp CFU/L	7 002 Lp CFU/L	15 996 Lp CFU/L	19 567 Lp CFU/L
Number of participating labs	121	220	217	230
Consensus Value	495 198 Lp CFU/L	5 883 Lp CFU/L	19 129 Lp CFU/L	26 938 Lp CFU/L
MICA z-score	-0.18	+0.61	-0.59	-1.23
Qualitative ranking	A	A	A	A

II.3.1 Graphical representation of the results

Round 24M33.3

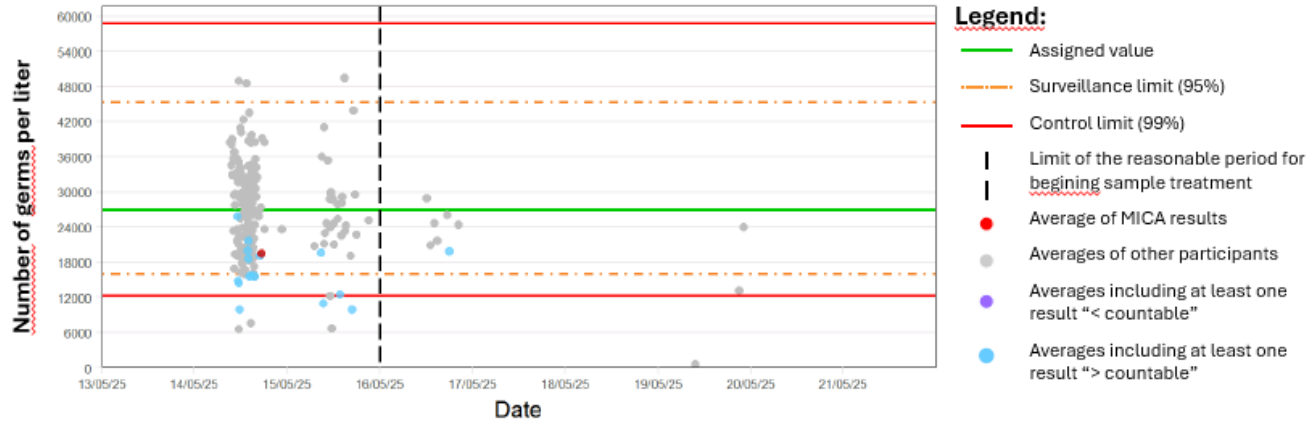


Round 24M32.3



Round 25M32.1

Round 25M32.2



III/ Appendix: Statistical analyses of the experiments for the AOAC certification

III.1 method comparison data

Matrix	Inoculation Density (CFU/L)	MICA *Legionella* Number of replicates	MICA *Legionella* Mean Log(CFU/L)	MICA *Legionella* Std Dev	ISO 11731-2017 Number of replicates	ISO 11731-2017 Mean Log(CFU/L)	ISO 11731-2017 Std Dev	Difference of Mean (ISO - MICA)	95% Confidence Interval Lower Limit	95% Confidence Interval Upper Limit
Non-complex	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	low (~1000)	5	2.709	0.092	5	2.887	0.087	0.178	0.020	0.335
	Medium (~10000)	5	4.046	0.048	5	3.770	0.013	-0.276	-0.338	-0.215
	High (~100000)	5	4.988	0.014	5	4.768	0.014	-0.220	-0.244	-0.195
Complex	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	low (~1000)	5	2.824	0.244	5	2.203	1.107	-0.620	-2.028	0.788
	*low (~1000)**	*5*	*2.824*	*0.244*	*3*	*3.006*	*0.194*	*0.182*	*-0.315*	*0.680*
	Medium (~10000)	5	3.665	0.208	5	4.350	0.326	0.685	0.205	1.166
	High (~100000)	5	4.820	0.160	5	4.919	0.242	0.099	-0.261	0.460

*without the two false negative from the ISO method.

With the non-complex matrix, the 95% confidence interval of the difference of mean falls in between -0.5 and +0.5 log, showing that both methods are similar.
On the complex matrix, the confidence interval expands outside of -0.5 and +0.5, but this is largely due to the high variability of results on such a matrix. Note that the variability (as shown by the Standard Deviation) is higher for the ISO method than for MICA *Legionella*.

III.2 Ruggedness data

All data from the ruggedness combinations are compared with the control (standard) condition performed on the same day.

Condition	Inoculation Density (CFU/L)	Ruggedness			Control (same day)			Difference of Mean (Ctrl - Rgd)	95% Confidence Interval	
		Number of replicates	Mean Log(CFU/L)	Std Dev	Number of replicates	Mean Log(CFU/L)	Std Dev		Lower Limit	Upper Limit
1 (46h, 39°C / 10min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	3.530	0.092	5	3.773	0.029	0.244	0.124	0.363
	~250000	5	5.211	0.124	5	5.373	0.034	0.162	0.001	0.322
2 (46h, 39°C / 30min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	3.482	0.114	5	3.773	0.029	0.291	0.145	0.438
	~250000	5	5.078	0.111	5	5.373	0.034	0.295	0.150	0.439
3 (46h, 35°C / 10min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	2.652	0.990	5	4.153	0.058	1.501	0.269	2.733
	~250000	5	5.597	0.028	5	5.816	0.055	0.218	0.142	0.295
4 (46h, 35°C / 30min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	3.831	0.201	5	4.153	0.058	0.321	0.062	0.581
	~250000	5	5.692	0.032	5	5.816	0.055	0.124	0.045	0.203
5 (50h, 39°C / 10min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	3.614	0.061	5	3.761	0.059	0.147	0.041	0.252
	~250000	5	5.158	0.016	5	5.436	0.042	0.278	0.223	0.333
6 (50h, 39°C / 30min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	3.330	0.135	5	3.761	0.059	0.431	0.248	0.614
	~250000	5	5.147	0.058	5	5.436	0.042	0.289	0.200	0.378
7 (50h, 35°C / 10min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	3.939	0.088	5	4.058	0.034	0.120	0.002	0.237
	~250000	5	5.573	0.082	5	5.625	0.036	0.052	-0.059	0.164
8 (50h, 35°C / 30min)	0	5	1.000	0.000	5	1.000	0.000	0.000	0.000	0.000
	~5000	5	4.072	0.052	5	4.058	0.034	-0.014	-0.091	0.063
	~250000	5	5.550	0.106	5	5.625	0.036	0.076	-0.063	0.215

The combinations for which the Confidence interval of the difference of means expands outside of -0.5 log - +0.5log are the conditions that combine a shorter growth and a lower growth temperature (Combinations 3 and 4), leading to smaller colonies, and the condition combining a longer growth at a higher temperature with a longer tagging step, leading to bigger brighter objects that are not properly recognized.

MICA Advance

Legionella

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