MICA Highlight

Total Viable Count

SCIENTIFIC REPORT

DIAMIDEX

CONTENTS

I. Introduction

Simple and rapid solution that detects culturable bacteria, yeasts and molds in different types of non-sterile water.

MICA Highlight TVC water allows the analysis of drinking water (tap water, well water, bottled water), process water, rinsing water and personal care products in between 24 to 72 hours.

MICA Highlight TVC Water enumerates all microorganisms (bacteria, yeasts and molds) in colony-forming units (CFU).

II. Principle and procedure

a. Principle

The principle of the MICA Highlight TVC Water solution procedure is the same as that of the standard methods APHA 9215D for drinking water, ISO:11737-1 for bioburden initial assessment of medical devices before sterilization and US pharmacopeia chapters 61 and 1231 for process water and personal care products. All procedures are by membrane filtration with the main difference that the incubation times are much shorter, and the enumeration done automatically.

b. Procedure

Fig 1: MICA Highlight TVC water process based on membrane filtration

c. Comparison of cultural conditions and incubation times with standard methods

APHA 9215 is a standard method used for the enumeration of heterotrophic bacteria in water. It is part of the Standard Methods for the Examination of Water and Wastewater manual. Part D of APHA 9215 specifically describes the membrane filtration method. This method is particularly accurate and

suitable for quantitative microbiological analyses in drinking water, surface water, and industrial water environments.

The culture media used in this standard and addressed by the MICA solution are PCA and R2A. On PCA, the membrane is incubated at 35 ± 0.5°C for 48 hours. For slower-growing colonies, the standard recommends incubating the membrane at 20 to 28°C for 5 to 7 days.

	APHA 9215 D		MICA Highlight TVC according to APHA 9215D	
	Drinking water and swimming pool			
Culture media	PCA	R2A	PCA	R2A
Temperature	35°C	20-28°C	35°C	22°C
Incubation	2 days	5 to 7 days	24 hours	72 hours

Fig 2: APHA 9215D versus MICA Highlight TVC

ISO 11737 deals with the determination of microbial populations on medical products. It is primarily used in the context of sterilization control of medical devices.

Part 1 of ISO 11737 concerns the quantification of microorganisms present on medical devices prior to sterilization. The objective is to ensure that the microbial load is controlled and will not compromise the effectiveness of the sterilization process.

The culture media used in this standard and addressed by the MICA solution are TSA for aerobic bacteria and SDA for yeasts and molds. Incubation conditions are 30-35°C for aerobic bacteria and 20-25°C for fungi. The analysis time is generally 3 to 7 days, depending on the type of microorganisms being tested.

	ISO 11737-1:2018		MICA Highlight TVC according to ISO 11737-1:2018	
	Water used for Bioburden assessment prior to sterilization of medical devices			
Culture media	TSA	SDA	TSA	SDA
Temperature	30-35°C	20-25°C	35°C	22°C
Incubation	3 to 7 days	5 to 7 days	24 hours	48 hours

Fig 3: ISO 11737-1:2018 versus MICA Highlight TVC

Chapter 61 of the United States pharmacopeia establishes methods for determining the total microbial load in nonsterile products. The matrices covered by this chapter of the US Pharmacopeia are nonsterile pharmaceutical products, raw materials, cosmetics, dietary supplements, and nonsterile medical devices. Enumeration is performed on mesophilic aerobic bacteria (Total Aerobic Microbial Count, TAMC) and on yeasts and molds (Total Yeast and Mold Count, TYMC).

The culture media used in this standard and addressed by the MICA solution are, for mesophilic aerobic bacteria, Tryptic Soy Agar (TSA) and for yeasts and molds, Sabouraud Dextrose Agar (SDA). In TSA, the

incubation temperature is between 30 and 35 °C for an incubation time of 3 to 5 days. In SDA, the incubation temperature is between 20 and 25 °C for an incubation time of 5 to 7 days.

	USP 61		MICA Highlight TVC according to USP 61	
	Personal care products			
Culture media	TSA	SDA	TSA	SDA
Temperature	30-35°C	20-25°C	35°C	22°C
Incubation	3 to 5 days	5 to 7 days	24 hours	48 hours

Fig 4: U.S. pharmacopeia chapter 61 versus MICA Highlight TVC

Chapter 1231 of the United States Pharmacopeia addresses the microbiological management and control of water used in the pharmaceutical industry and more generally high-purify water. It provides detailed recommendations to ensure the microbiological quality of water for pharmaceutical use. MICA Highlight TVC doesn't address water for pharmaceutical use but water used in other sectors requiring high-purity water, such as: cosmetics industry, food industry (particularly for sensitive ingredients or sterile processes) and semiconductor industry (which requires ultrapure water).

Among the common methods, membrane filtration is used for samples with low levels of contamination. Incubation is performed on R2A medium for slow-growing water bacteria at an incubation temperature of 20 to 25°C for an incubation time of 5 to 7 days.

	USP 1231	MICA Highlight TVC according to USP 1231
	Process Water	
Culture media	R2A	R2A
Temperature	20-25°C	22°C
Incubation	5 to 7 days	72 hours

Fig 5: U.S. pharmacopeia chapter 1231 versus MICA Highlight TVC

III. Performances

a. Selected strains

23 different microorganisms were tested, 13 bacteria strains from 12 bacterial genera (8 laboratory strains and 5 environmental strains), 3 yeasts (1 laboratory and 2 environmental strains) and 7 molds (4 laboratory and 3 environmental strains). Laboratory strains come from the collection of microorganisms and cell cultures of the Leibniz Institute in Germany (DSMZ), the Biological Resources Center of the Pasteur Institute in France (CRBIP), and from the American Type Culture Collection in the United States (ATCC). Some strains have also been isolated from real matrices from customers or prospects around the world. These strains were all evaluated according to the method developed by Diamidex and the results compared to those obtained with the standards.

Chosen bacteria (1)(2), yeasts and molds (3)(4)(5)(6)(7) are well documented as part of the most prevalent microorganisms present in contaminated main water samples.

Strains type	Prevalence in Water samples	Strains number	Strains ID	Prevalence of bacterial genera in main water
Bacteria	High	13	*Pseudomonas fluorescens (ATCC® 13525)* and *Pseudomonas aeruginosa (ATCC® 9027)* *Brevundimonas vesicularis (ATCC® 11426)* *Sphingomonas parapaucimobilis (Environmental strain)* *Bacillus subtilis (ATCC® 6633)* *Micrococcus luteus (Environmental strain)* *Ralstonia pickettii (Environmental strain)* *Stenotrophomonas maltopilia (Environmental strain)* *Burkholderia cepacia (ATCC® 25416)* *Acinetobacter calcoaceticus (Environmental strain)* *Escherichia coli* (ATCC® 25922) *Enterobacter cloacae (ATCC® 13047)* *Serratia marcescens (ATCC® 13880 & ATCC® 14756)*	24% 14% 9% 7% 7% 6% 4% 4% <1% <1% <1% <1%
Yeasts	Low	3	*Candida parapsilosis (Environmental strain)* *Aureobasidium pullulans (Environmental strain)* *Cryptococcus neoformans (ATCC® 32045)*	
Molds	Very low	7	*Aspergillus brasiliensis (ATCC® 16404)* *Aspergillus fumigatus (ATCC® 204305)* *Penicillium commune* (ATCC® 10428) *Paecilomyces variotti (Environmental strain)* *Fusarium oxysporum (Environmental strain)* *Alternaria spp. (ATCC® 20084)* *Geotrichum candidum (Environmental strain)*	

Fig 6: Strains tested with MICA Highlight TVC Water solution.

<u>Standard conditions to address considering standards:</u>

The cultural conditions in which the chosen microorganisms were tested correspond to those in which these same microorganisms would be detectable by eye within the time frame of the reference method.

Strains type	Strains species	APHA 9215 D		USP61 & ISO 11737-1:2018		USP1231
		PCA 35°C 48h	R2A 22°C 7 days	TSA 35°C 3 days	SDA 22°C 5 days	R2A 22°C 7 days
Bacteria	*E. coli*	x	x	x	x	x
	P. aeruginosa	x	x	x		x
	P. fluorescens		x	x	x	x
	B. vesicularis		x	x		x
	S. parapaucimobilis	x	x	x		x
	B. subtilis	x	x	x		x
	M. luteus	x	x	x		x
	R. pickettii	x	x			x
	S. maltophila	x	x	x	x	x
	B. cepacia	x				
	A. calcoaceticus	x	x	x	x	x
	E. cloacae	x	x	x	x	x
	S. marcescens	x	x		x	x
Yeasts	*C. parapsilosis*	x	x	x	x	x
	A. pullulans	x	x	x	x	x
	C. neoformans	x	x		x	x
Molds	*A. brasiliensis*	x	x	x	x	x
	A. fumigatus	x	x	x	x	x
	P. commune		x		x	x
	P. variotti	x	x		x	x
	F. oxysporum	x	x	x	x	x
	A. species	x	x		x	x
	G. candidum	x	x		x	x

Fig 7: Representative table of the conditions under which the selected organisms grow within the timeframe of the reference standard.

IV. Accuracy performed on laboratory strains

The results shown in the graphs below are from culture conditions on sterile, white, non-gridded 0.45µm MCE membranes with the following references: Advantec A045G047A, Filtratech MF047ME045S and Sartorius 11306-47-ACN. The culture media used are ready prepared plates: Plate Count Agar (PCA), Tryptone Soya Agar (TSA), Sabouraud Dextrose Agar (SDA) and Reasoner's 2A Agar (R2A) with the references: Oxoid PO5013A for PCA, Oxoid PO5012A for TSA, VWR 100884ZA for SDA and Oxoid PO5149A for R2A.

An acceptability threshold with a criterion of ± 0.5 log deviation was used in the validation of the microbiological method to establish equivalence with the various standards addressed. This criterion is found in ISO 16140-2:2016 – Validation of alternative methods establishing the requirements for comparing an alternative method with a reference method. It indicates that the acceptable difference between the two methods is generally ± 0.5 log (i.e. a factor of 3 in number of microorganisms). This acceptability threshold is also present in the European Pharmacopoeias (Ph. Eur. 5.1.6) & USP <1223> – Validation of alternative and American methods as well as in the standard NF EN ISO 13843:2017 – Validation of microbiological methods for water.

a. Enumération results compared to APHA 9215 D

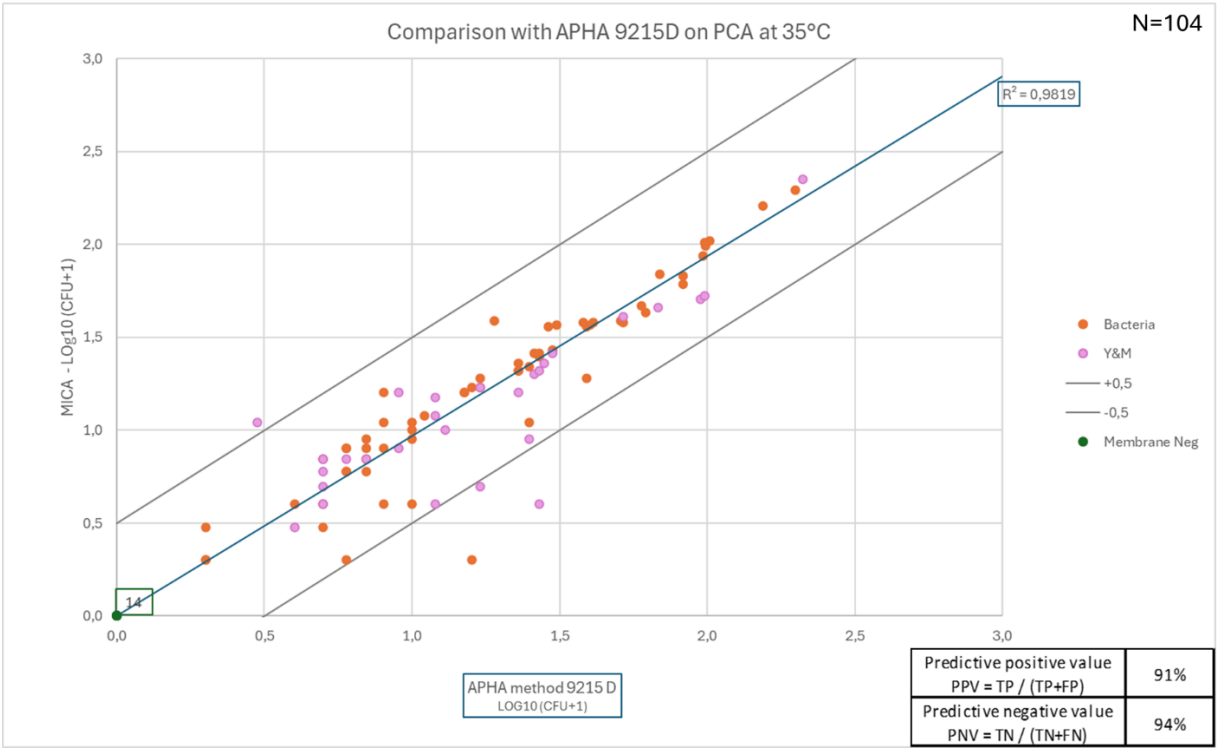


Fig 8: Results obtained on PCA plates at 35°C in 24h for MICA Highlight TVC Water solution and 48h for APHA 9215 procedure D. The numbers in the green squares at the base of the x- and y-axes correspond to the number of negative membranes tested.

b. Enumeration results compared to ISO:11737-1 & USP 61

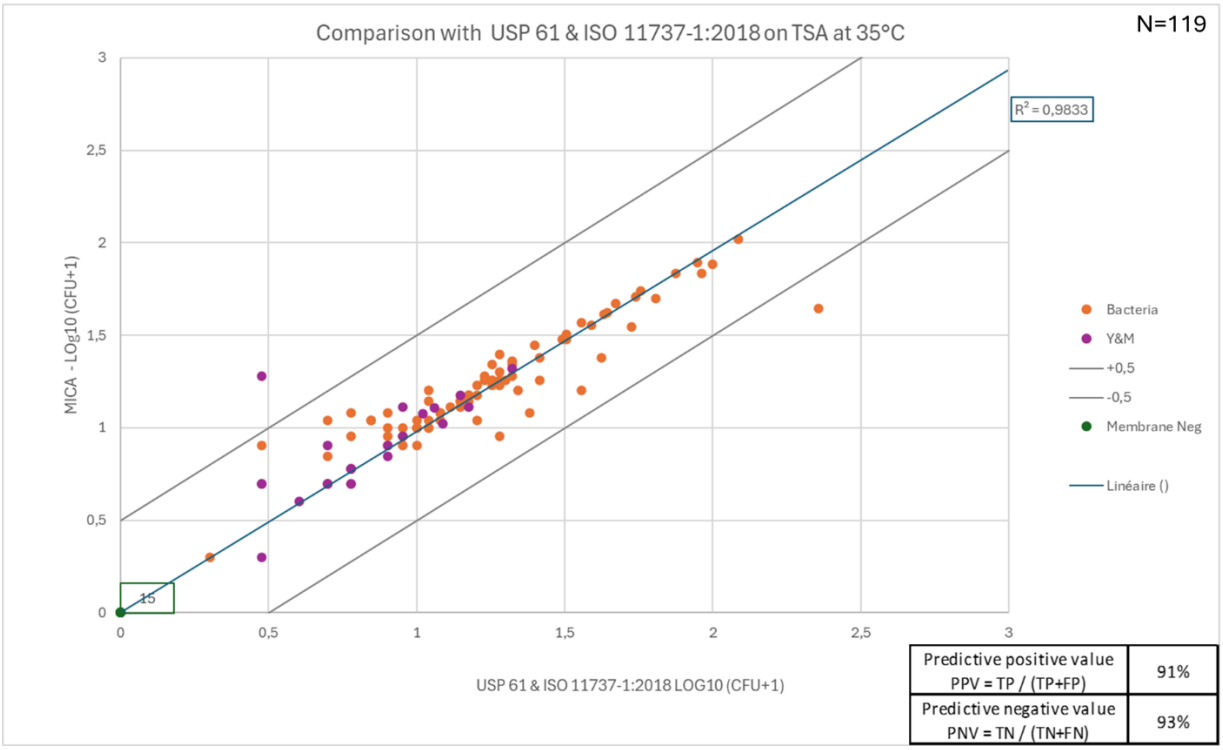


Fig 9: Results obtained on TSA plates at 35°C in 24h for MICA Highlight TVC Water solution and 3 days for USP 61 and ISO 11737-1:2018. The numbers in the green squares at the base of the x- and y-axes correspond to the number of negative membranes tested.

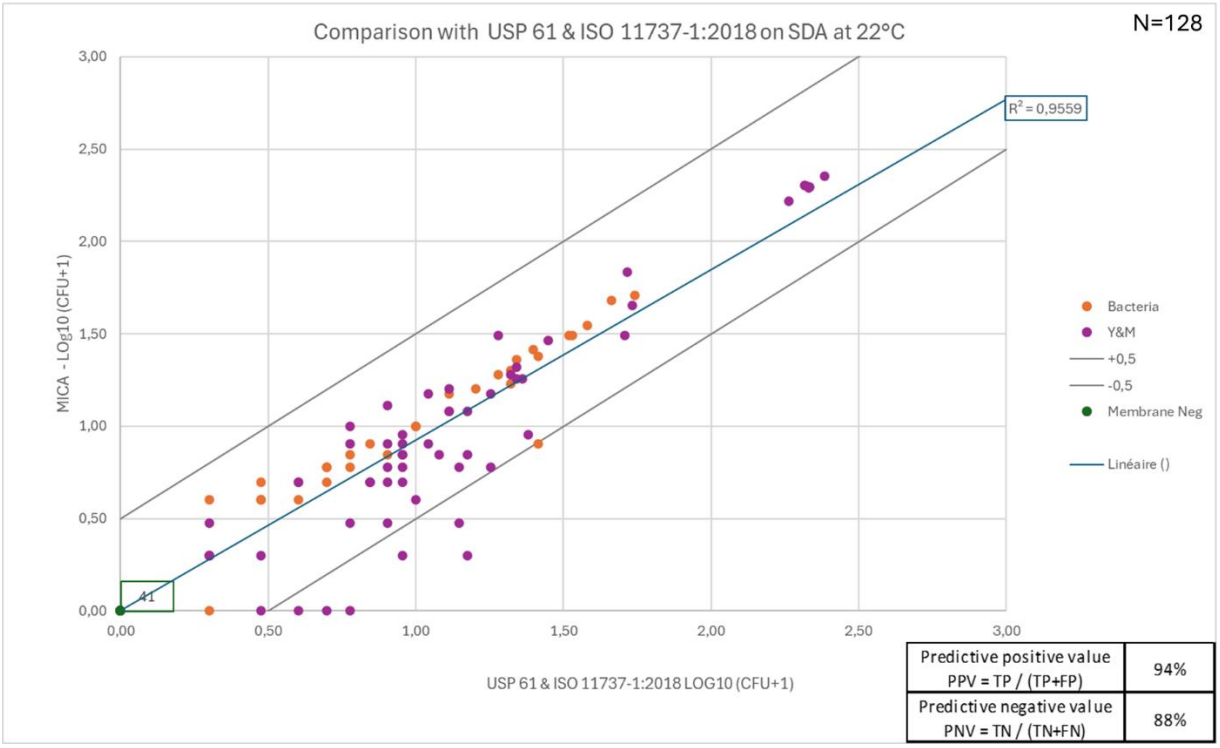


Fig 10: Results obtained on SDA plates at 22°C in 48h for MICA Highlight TVC Water solution and 5 days for USP 61 and ISO 11737-1:2018. The numbers in the green squares at the base of the x- and y-axes correspond to the number of negative membranes tested.

c. Enumeration results compared to USP 1231

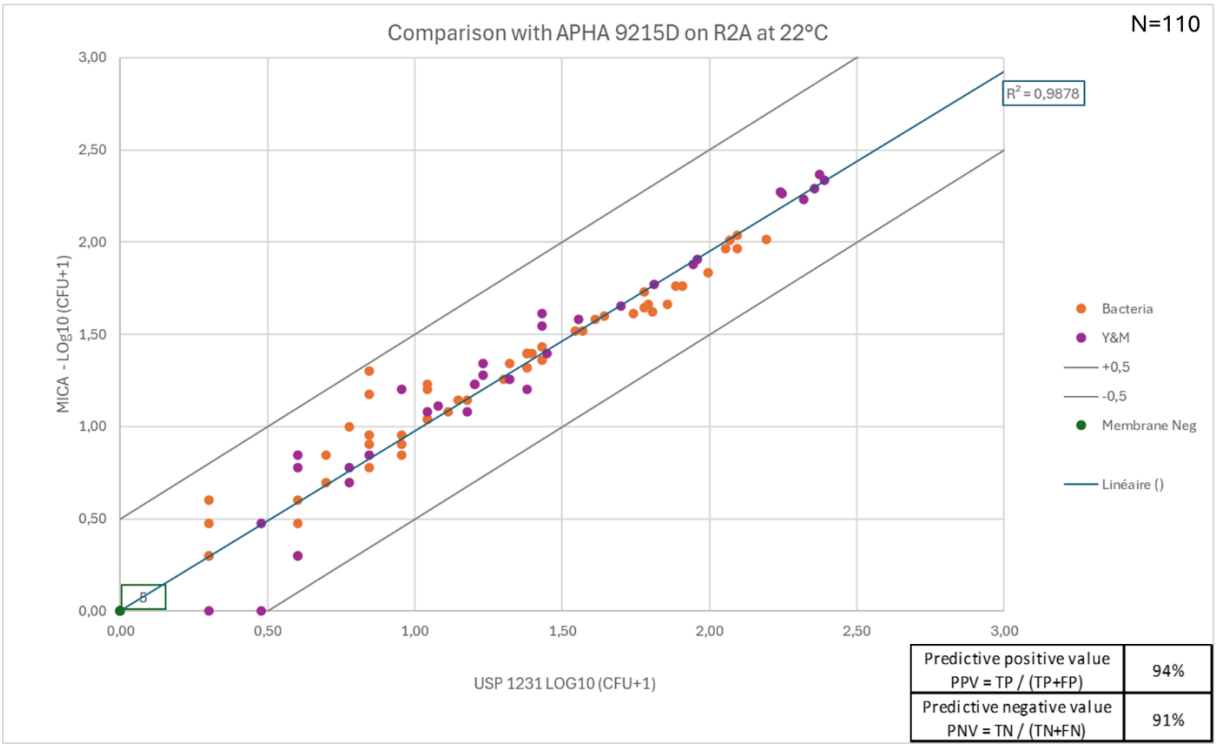


Fig 11: Results obtained on R2A plates at 22°C in 72h for MICA Highlight TVC Water solution and 7 days for USP 1231. The numbers in the green squares at the base of the x- and y-axes correspond to the number of negative membranes tested.

Conclusion: Enumeration results for MICA Highlight TVC water solution are comparable to those obtained with standard method.

V. Accuracy performed on water matrices

The results shown in the graph below are from real water matrices on sterile, white, non-gridded 0.45µm MCE membranes with the following references: Advantec A045G047A, Filtratech MF047ME045S and Sartorius 11306-47-ACN. Tested real matrices are from potable water, bottled water and process water.

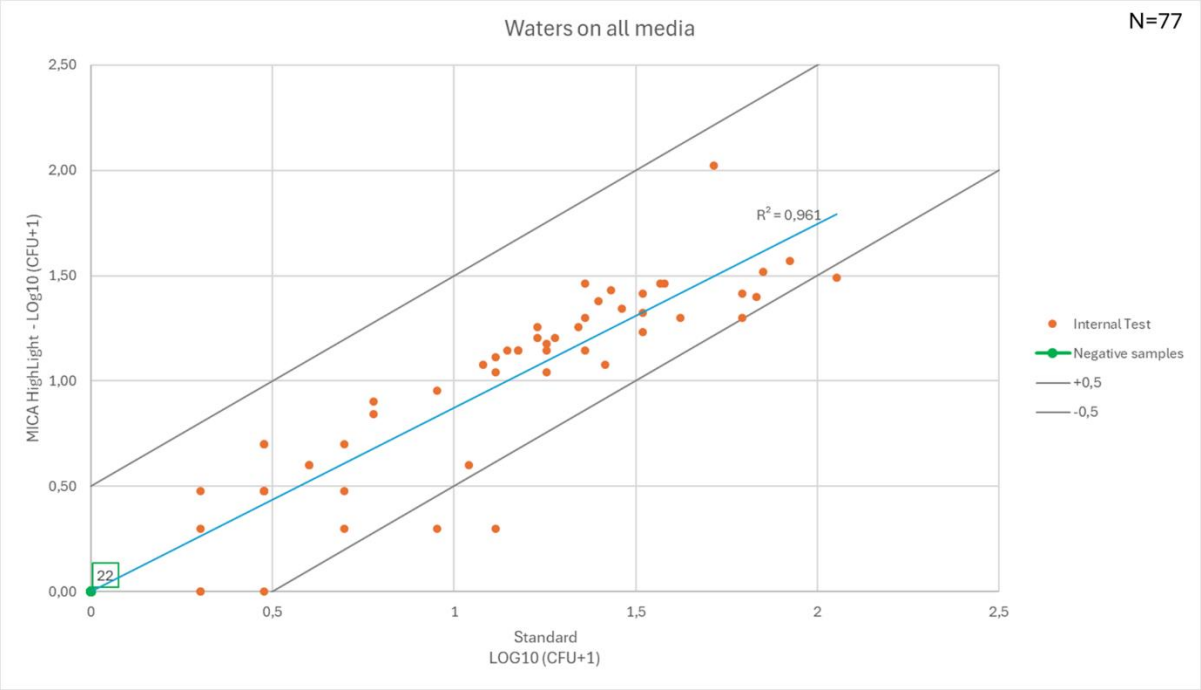


Fig 12: Results obtained on all agar media for MICA Highlight TVC Water solution and compared to standard timing depending on the type of tested matrix.

Conclusion: Enumeration results for MICA Highlight TVC water solution on real water matrices are comparable to those obtained with standard method.

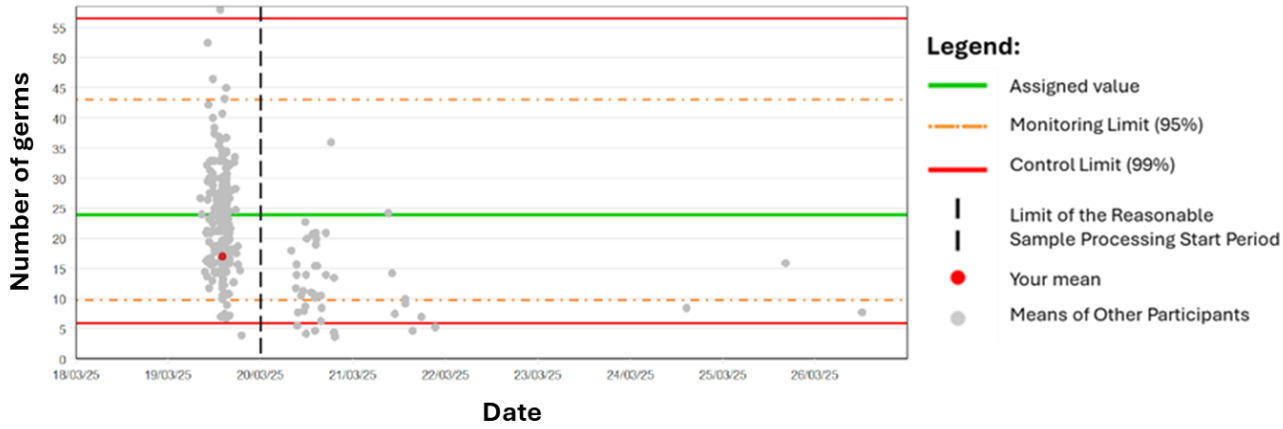
VI. Proficiency tests

In early 2025, we initiated proficiency testing campaigns on water samples, focusing on Total Viable Count (TVC) with MICA Highlight in both clean and bacteriologically controlled waters. These campaigns were conducted in collaboration with AGLAE, a French organization accredited by COFRAC (French Accreditation Committee) is according to the NF EN ISO/CEI 17043 standard to conduct proficiency testing schemes (PTS), which provided spiked water samples containing microorganisms of interest. The proficiency tests aimed to evaluate the accuracy and reliability of our solution in detecting microbial contamination under controlled conditions. The results obtained are presented below with a distinction between clean water and bacteriologically controlled water.

The clean water samples were inoculated with a specific load of *Enterococcus faecalis* (strain reference WDCM 00176) and tested in parallel by 262 participating laboratories. The bacteriologically controlled water samples were inoculated with a specific load of *Enterococcus faecalis* (strain reference WDCM 0087) and *Staphylococcus aureus* (strain isolated from the environment) and tested by 74 participants. The results below present the values obtained with MICA Highlight TVC Water solution compared to the expected target values, as well as the results reported by the other participants, the majority of whom tested the samples according to water standards.

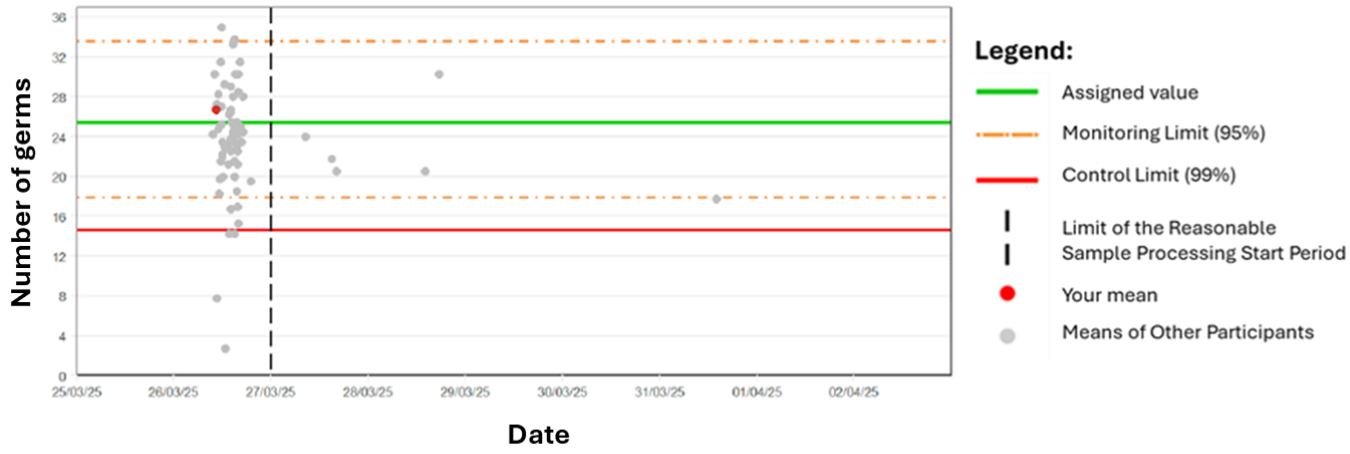
Clean Water	Mean of Diamidex results (4 replicates)	Mean of assigned values (4 replicates)	Z-score	Qualitative ranking
Revivable microorganims at 36°C	21	22	-0,03	A
Revivable microorganims at 22°C	17	24	-0,78	A

Results on clean water for revivable microorganisms at 36°C.

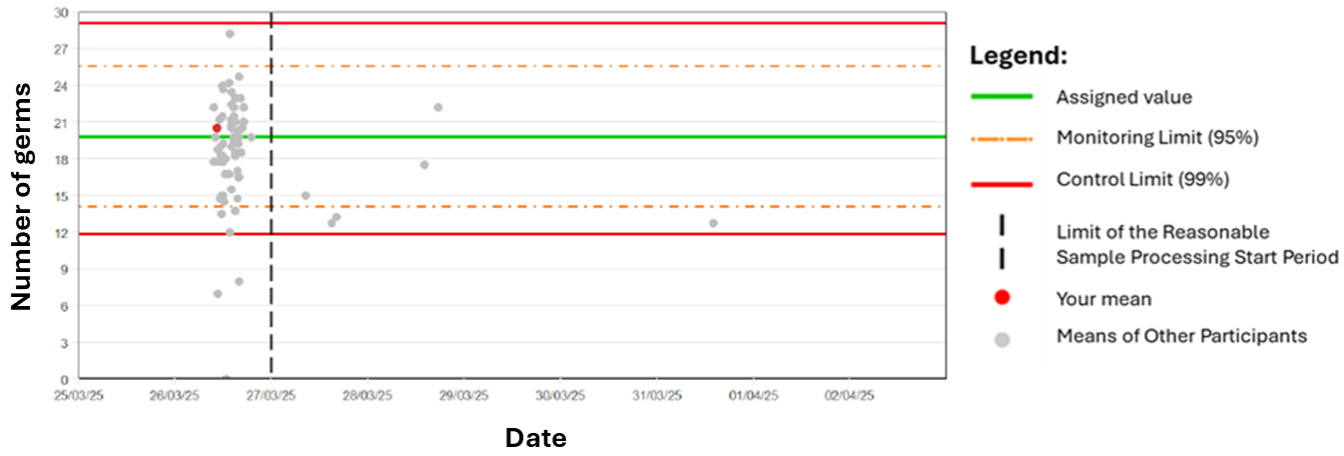


Results on clean water for revivable microorganisms at 22°C.

Bacteriologically controlled water	Mean of Diamidex results (4 replicates)	Mean of assigned values (4 replicates)	Z-score	Qualitative ranking
Revivable microorganims at 36°C	27	25	+0,41	A
Revivable microorganims at 22°C	21	20	+0,32	A



Results on bacteriologically controlled water for revivable microorganisms at 36°C.



Results on bacteriologically controlled water for revivable microorganisms at 22°C.

Conclusion: Enumeration results for MICA Highlight TVC water solution obtained in the framework of proficiency tests on clean water and bacteriologically controlled water are close to the expected target values and within the range of those obtained by the other participating laboratories.

ANNEX 1:

Bibliographic references

(1) Sandle, T. (2015) Characterizing the Microbiota of a Pharmaceutical Water System-A Metadata Study, *SOJ Microbiology & Infectious Diseases*, 3 (2): 1-8

(2) Zanetti F, et al. (2014) Occurrence of non-fermenting gram-negative bacteria in drinking water dispensed from point-of-use microfiltration devices, *Annals of Agricultural and Environmental Medicine*, 2014;21(1):29-34.

(3) (2011) A review of fungi in drinking water and the implications for human health, Department for environment Food and Rural Affairs

(4) Arroyo MG, et al. (2020) Broad Diversity of Fungi in Hospital Water, *Scientific World Journal,* Jul 4; 2020:9358542.

(5) Afonso TB, et al. (2021) Occurrence of filamentous fungi in drinking water: their role on fungal-bacterial biofilm formation, *Research in Microbiology*, Volume 172, Issue 1, January–February 2021, 103791.

(6) Góralska K, et al. (2020) The occurrence of potentially pathogenic filamentous fungi in recreational surface water as a public health risk, *Journal of Water & Health,* 18 (2): 127–144.

(7) Qiqi Wan, et al. (2023) Occurrence and control of fungi in water: New challenges in biological risk and safety assurance, Science of The Total Environment, Volume 860, 20 February 2023, 16053.

MICA Highlight

Total Viable Count

Technical support

Email : **support@diamidex.com**

Tel : +33 7 61 90 45 95

Monday to Friday – 9.00 to 18.00
Central European Time (UTC+1)

DIAMIDEX