CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

032201

The AOAC Research Institute hereby certifies the method known as:

MICA *Legionella* Kit

manufactured by

DIAMIDEX
Grand Luminy Technopole
Zone Luminy Entreprise Biotech, Case 922
163 Avenue de Luminy
13288 Marseille Cedex 09, France

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*[SM] Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*[SM] certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

Bradley A. Stawick, Senior Director
Signature for AOAC Research Institute

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Expiration Date	December 31, 2025

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SUBMITTING COMPANY
DIAMIDEX
Grand Luminy Technopole
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METHOD NAME
MICA *Legionella* Kit

CATALOG NUMBERS
00 917 (Laboratories); 00 916 (non-laboratory customers)

INDEPENDENT LABORATORY
Q Laboratories
1930 Radcliff Dr
Cincinnati, OH 45204 USA

APPLICABILITY OF METHOD
Analytes – *Legionella pneumophila*, all *serogroups*.

Matrixes – Hot domestic/tap water and cooling tower water.

Performance claims - The MICA *Legionella* for *Legionella pneumophila* is a simple and fast kit, which detects and counts only *Legionella pneumophila* bacteria capable of being cultivated (similar to regulatory procedure NF T90-431 or ISO 11731:2017) in samples of select environmental and domestic waters. The sensitivity (inclusivity) of MICA *Legionella* was found to be 94% and specificity (exclusivity) 97%. Performance of the kit is equivalent to the ISO 11731:2017 (2) for the enumeration of *L. pneumophila* in hot domestic waters and can be better than ISO 11731:2017 (2) on cooling tower waters.

ORIGINAL CERTIFICATION DATE
March 11, 2022

CERTIFICATION RENEWAL RECORD
Renewed through December 2025.

METHOD MODIFICATION RECORD
1. **January 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Editorial changes**

Under this AOAC *Performance Tested Methods*^{SM} License Number, 032201 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods*^{SM} License Number, 032201 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
MICA *Legionella* is a detection method allowing detection of culturable microcolonies of *Legionella pneumophila* at 48h of growth instead of 10 days in the standard procedures. The original water sample is concentrated by membrane filtration as in the standard procedure. The membrane is then laid over a drop of culture supplement on a standard *Legionella* agar plate (GVPC). This culture supplement contains Diamidex's patented molecule, a precursor of the legionaminic acid coupled with the bio-orthogonal azido group (pLeg-N3) (23). The molecule will be specifically internalized by growing *L. pneumophila* and integrated in their O-antigen on the surface of the cells. After 48h of bacterial growth, the membrane is transferred onto a drop of tagging solution containing a fluorescent molecule that will bind by click chemistry specifically onto the bio-orthogonal azido group, *i.e.,* the labelled *L. pneumophila*. This specific fluorescent tagging allows the CFU to be detected at the microcolony stage by solid-phase cytometry using the MICA microcolony counter. The MICA *Legionella* AI analyzer then uses multiple parameters to specifically identify *L. pneumophila* microcolonies and gives a result as a concentration of *L. pneumophila* in the original sample. For a better reproducibility, the MICA software provided with the microcolony counter provides a step-by-step protocol guide, including control of the incubation times and reagent traceability. The guidance and analysis by the software allows the MICA *Legionella* method to be used by anyone.

DISCUSSION OF THE VALIDATION STUDY (1)

Since its discovery in 1976, *Legionella pneumophila* has been considered as an important pathogen that should be monitored in domestic hot waters and cooling tower waters. Several detection methods have been developed, but the gold standard remains a culture method, as in ISO 11731:2017. However, the long result delay and high training level for the technicians are important issues that can be addressed by the development of new detection methods. These new detection methods must achieve the same performance level as the standard method.

As shown in this study, MICA *Legionella* can detect all serogroups of *L. pneumophila* and, apart from a very closely related and equally pathogenic *Legionella* species, does not wrongly recognize other species. The protocol proved robust to variations and, additionally, the MICA *legionella* software reduces the risk of deviations from the protocol by providing step-by-step protocol and control of incubation time. Furthermore, the result does not rely on human interpretation, but instead on automatic identification of microcolonies of *L. pneumophila* by the AI analyzer and automatic calculation of contamination density in the original water sample, thus reducing the risk of human mistakes. Another advantage is the use of a single culture plate instead of up to nine for the standard method, which further reduces the human time and skills needed for the analysis.

When compared to ISO 11731:2017, MICA *Legionella* gives similar results in 48h than the standard method in 10 days on simple matrix (hot sanitary water). On complex matrixes (cooling tower water), MICA *Legionella* can perform better than the standard method, thanks to the shorter culture incubation time that makes it less sensitive to background flora interference at reading time.

Importantly for a routine analysis method, the MICA *Legionella* test kit, is reproducible from lot-to-lot and is stable at the recommended storage temperature (4°C) for a long time, up to 18 months according to the accelerated stability study.

Altogether, MICA *Legionella* can be considered as a reliable and fast alternative to the standard methods for enumeration of *L. pneumophila* in hot domestic water and cooling tower waters and should be granted PTM certification.

Supplementary table 1 – Detailed matrix study results (1)

			Log transformed MICA results [log_{10} (CFU/L +1)]					Log transformed ISO results [log_{10} (CFU/L +1)]				
Lab	**Matrix**	**Inoculation density**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
Diamidex	**Hot sanitary water**	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	2.60	2.68	2.86	2.68	2.68	2.72	2.90	2.90	2.88	2.92
		Medium	4.08	4.07	4.08	4.02	3.97	3.76	3.78	3.70	3.76	3.79
		High	4.99	4.99	4.97	4.99	5.00	4.54	4.78	4.78	4.81	4.74
	Cooling tower water	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Low	2.75	3.06	2.51	2.68	3.09	0.00	0.00	3.08	3.15	2.78
		Medium	3.98	3.54	3.45	3.60	3.75	4.18	4.00	4.18	4.70	3.96
		High	4.88	4.98	4.88	4.56	4.80	4.56	4.85	4.70	4.66	4.36
Qlab	**Cooling tower water**	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		Very Low	2.985	3.116	3.055	2.857	3.435	4.602	4.000	4.477	4.477	4.000
		Low	3.022*	4.113	4.201	4.083	4.069	4.301	4.000	4.301	4.301	4.000
		Medium	4.254	4.163	4.160	4.076	4.028	4.699	4.301	4.477	4.477	4.301
		High	4.971	4.955	5.031	4.998	5.080	5.693	5.264	5.743	5.755	5.335
		Very High	5.362	5.000	5.176	5.079	5.114	6.380	6.301	6.415	6.322	6.398

A to J: replicate test portions. See main text for the details.

*Outlying data point determined from Cochran and Grubbs analysis. Removed from final statistical analysis.

Supplementary table 1b – Detailed ISO results for the internal matrix study (1)

Inoculation density		ECS				TAR**			
		100 ml Acid treated	10 ml Acid treated	0.2 ml Untreated	CFU/L*	0.02 ml Untreated	0.2 ml Untreated	Concentrate Heat treated	CFU/L*
0	F	0	0	0	0	0	0	0	0
	G	0	0	0	0	0	0	0	0
	H	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0
	J	0	0	0	0	0	0	0	0
Low	F	53	5	0	527	0	0	0	0
	G	85	2	0	791	0	0	0	0
	H	85	3	0	800	0	0	6	1200
	I	79	4	0	755	0	0	7	1400
	J	84	7	0	827	0	0	3	600
Medium	F	548	57	0	5 700	0	3	35	15000
	G	580	60	0	6 000	0	2	15	10000
	H	590	50	1	5 000	0	3	7	15000
	I	484	57	0	5 700	1	0	2	50000
	J	530	61	1	6 100	1	1	20	9090
High	F	>500	572	7	35 000	3	5	97	36360
	G	>500	558	12	60 000	0	14	149	70000
	H	>500	588	12	60 000	3	8	146	49995
	I	>500	568	13	65 000	1	9	145	45450
	J	>500	560	11	55 000	1	4	120	22725

F to G: replicate test portions. See the main text for the details about the experimental procedure.

*Calculation based on the counts in the grey cells

****The platings of untreated and acid-treated concentrate portions were all overgrown with background flora and gave no result. The platings of the concentrate are equivalent to 5 ml of sample.**

REFERENCES CITED

1. Passot, F., Peslier, S., Benzinger, Jr., M.J., Blackburn, J., Thompson, W., Bastin, B., Dumont, A., and Dukas, S., Validation of MICA Legionella for Enumeration of *Legionella pneumophila* in sanitary waters and cooling tower waters, AOAC *Performance Tested Methods*^{SM} certification 032201.
2. ISO (2017) *ISO 11731-2017 : Water Quality - Enumeration of* Legionella