CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

012002

The AOAC Research Institute hereby certifies the method known as:

PixeeMo™

manufactured by

AFI Corporation
2rd Fl, SR Bldg Umeshin
6-7-5 Nishitenma
Kita-ku, Osaka 530-0047 JAPAN

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*[SM] Program and found to perform as stated in the applicability of the method. 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date November 30, 2023

Expiration Date December 31, 2024

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SUBMITTING COMPANY
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METHOD NAME
PixeeMo™

CATALOG NUMBERS
ELS-002, ELC121 and ELB100N

INDEPENDENT LABORATORY
Japan Food Research Laboratories
52-1 Motoyoyogi-cho
Shibuya-ku, Tokyo 151-0062 Japan

APPLICABILITY OF METHOD
Analyte – Aerobic Bacteria

Matrixes – Drinking Water

Performance claims - Performance of the PixeeMo™ method is equivalent to that of the Standard Method for the Examination of Water and Wastewater (SMEWW) 9060, *Samples* (2) and SMEWW 9215, *Heterotrophic Plate Count, Part B – Pour Plate Method* (3) for enumeration of aerobic bacteria in drinking water.

ORIGINAL CERTIFICATION DATE
January 14, 2020

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2024.

METHOD MODIFICATION RECORD
1. **September 2022 Level 1**

SUMMARY OF MODIFICATION
1. **Corporate address change from Sakyo-ku, Kyoto Japan to Kita-ku, Osaka Japan.**

Under this AOAC *Performance Tested Methods℠* License Number, 012002 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods℠* License Number, 012002 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
PixeeMo™ method consists of feeding a liquid sample to a microchannel chip, capturing bacteria contained in the sample, observing microscopically, and counting the captured bacteria. "Fluid, electric filtering and sorting technology (FES)" is used for capturing bacteria. FES is a selection technology that integrates electrical measurement and fluid control. A sample is fed into an appropriately designed microchannel, AC electric field is generated by the electrode in the microchannel, and the electric force acts to attract bacteria when passing over the electrode. This method uses the intracellular ion homeostasis of the bacteria, therefore intact cellular membrane is required for capture on the electrodes, implying only viable organisms will be captured. To perform the method, the water sample is first centrifuged, then the water replaced with buffer, and the sample is then loaded into a syringe, which is placed on the instrument and brought into contact with the chip. The software controls the settings and operation of the instrument for drinking water analysis. While the syringe plunger expels liquid at a constant flow rate for a specified time period, the voltage supply is turned on and microbes are captured on the electrodes. At the end of the collection period, digital images are taken and analyzed by software to determine the microbial concentration. By collecting the bacteria on the electrode, it is possible to directly observe and count the bacteria within one or two fields of view. With this method, the bacterial concentration of a sample is determined by counting the captured bacteria from 1 mL of sample fed through the microchip. Feeding time is approximately 17 min, therefore it is possible to determine the bacterial concentration of a sample within 1 hour.

DISCUSSION OF THE VALIDATION STUDY (1)

The method of SMEWW 9215B takes 7 days to get the results. On the other hand, the PixeeMo method can provide the results within 1 hour because incubation is unnecessary. Obtaining the results on the day of testing is a notable advantage of the PixeeMo method. Furthermore, the test procedure using AFI PixeeMo is quite simple and easy.

The maximum repeatability standard deviation of the PixeeMo method was 14.8%. The difference of mean log10 values between the PixeeMo and SMEWW 9215B methods ranged from -0.015 to 0.258. Similar results have been obtained in the independent laboratory study. However, there was a slight difference only from the lower confidence limit for the difference of means between the two methods at the low contamination level by the independent laboratory. On the other hand, the scatter plot of both methods indicated a high correlation between the two methods from the developer and the independent laboratory. Overall, there is no statistically significant difference between the PixeeMo™ and SMEWW 9215B methods.

In the product consistency and stability study, chips and buffers within the expiration date are considered to have no change in performance. In the robustness study, three parameters (centrifugation of sample, sample conductivity and output voltage of Channel 2) were varied, it was confirmed that there was no effect within the expected range. As a result of comparing the measurement results of the 3 PixeeMo instruments by analysis of variance, there is no significant difference among the data of 3 PixeeMo instruments.

Table 1. Method comparison data summary and statistics (1)

Matrix (organism)	Cont. level	N	PixeeMo™			SMEWW 9215B			DOM[a]	95% CI[b]	
			Mean	s_r	RSD_r, %	Mean	s_r	RSD_r, %		LCL[c]	UCL[d]
Drinking water[e] (naturally contaminated)	Low	5	1.761	0.260	14.8	1.704	0.028	1.7	-0.057	-0.362	0.248
	Medium	5	3.006	0.189	6.3	2.992	0.025	0.8	-0.015	-0.221	0.191
	High	5	3.755	0.139	3.7	4.012	0.023	0.6	0.258	0.069	0.446
Drinking water[f] (naturally contaminated)	Low	5	1.806	0.039	2.2	2.205	0.077	3.5	-0.399	-0.506	-0.292
	Medium	5	2.989	0.106	3.5	3.142	0.111	3.5	-0.153	-0.343	0.039
	High	5	3.937	0.091	2.3	4.119	0.172	4.2	-0.182	-0.423	0.059

[a]DOM = Difference of Means
[b]CI = Confidence Interval for DOM
[c]LCL = Lower confidence limit for DOM
[d]UCL = Upper confidence limit for DOM
[e]Method Developer data
[f]Independent Laboratory data

REFERENCES CITED

1. Wakizaka, Y., Itoi, T., Takano, M., Kato, E., Sato, Y., Morita, S, and Enjoji, T., AFI Corp. PixeeMo™ for Enumeration of Aerobic Bacteria in Drinking Water, AOAC *Performance Tested Methods℠* certification number 012002.
2. Standard Method for the Examination of Water and Wastewater (2017) Method 9060, *Samples*, American Public Health Association (Washington, D.C.), American Water Works Association (Denver, CO), and Water Environment Federation (Alexandria, VA), https://www.standardmethods.org/doi/10.2105/SMWW.2882.184
3. Standard Method for the Examination of Water and Wastewater (2017) Method 9215, *Heterotrophic Plate Count, Part B – Pour Plate Method*, American Public Health Association (Washington, D.C.), American Water Works Association (Denver, CO), and Water Environment Federation (Alexandria, VA), https://www.standardmethods.org/doi/abs/10.2105/SMWW.2882.188