CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

032103

The AOAC Research Institute hereby certifies the method known as:

MicroFast® Aerobic Count Plate (AC)

manufactured by

Beijing Meizheng Bio-Tech Co., Ltd.
No. 2 Building, No. 8 courtyard, Fenggusilu Road
Yanqing District, Beijing, P.R. China

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	December 19, 2022
Expiration Date	December 31, 2023

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 Fax: +1-301-924-7089
Internet e-mail: aoacri@aoac.org * World Wide Web Site: http://www.aoac.org

AUTHORS
ORIGINAL VALIDATION: Lei Lui and Swapna Gone
MODIFICATION NOVEMBER 2022: Lei Lui and Becca Qu

SUBMITTING COMPANY
Shandong Meizheng Bio-Tech Co., Ltd.
No. 69 Zhaoyang North Road
High-tech Zone
Rizhao, Shandong Province, P.R. China

CURRENT COMPANY ADDRESS
Beijing Meizheng Bio-Tech Co., Ltd.
No. 2 Building, No. 8 courtyard
Fenggusilu Road
Yanqing District, Beijing, P.R. China

METHOD NAME
MicroFast® Aerobic Count Plate (AC)

CATALOG NUMBERS
LR1001, LR1301

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

AOAC EXPERTS AND PEER REVIEWERS
Yi Chen[1], Wayne Ziemer[2], Mark Carter[3]
[1] Food and Drug Administration, Center for Food Safety and Applied Nutrition, Maryland, USA
[2] USDA FERN (Retired), Georgia, USA
[3] MC Squared, Tennessee, USA
Modification November 2022 reviewed internally by AOAC Research Institute.

APPLICABILITY OF METHOD
Targe Organism – Aerobic bacteria.

Matrixes
ORIGINAL VALIDATION
(MLG 3.02, 50 g samples) – Frozen ground beef, heat processed chicken drumstick
(BAM Ch. 3) – vegetable chips (50 g), frozen strawberries (50 g), pasteurized liquid milk (2% fat, 11 mL)
(ISO 18593:2018; BAM Ch. 3) – stainless steel environmental surfaces (100 cm^2, sponge)

MODIFICATION NOVEMBER 2022
(MLG 3.02, 50 g samples) – Frozen ground beef, heat processed chicken drumstick
(BAM Ch. 3, 50 g samples) – vegetable chips), frozen strawberries
(SMEDP MCM Ch. 6) – pasteurized liquid milk (2% fat, 11 mL)
(ISO 18593:2018; BAM Ch. 3) – stainless steel environmental surfaces (100 cm^2, sponge)

Performance claims – Performance equivalent to the U.S. Department of Agriculture Food Safety and Inspection Service Microbiology Laboratory Guidebook (USDA/FSIS-MLG) 3.02 *Quantitative Analysis of Bacteria in Foods as Sanitary Indicators* for frozen ground beef and heat processed chicken drumsticks (2), the U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA/BAM) Chapter 3: *Aerobic Plate Count* for vegetable chips, frozen strawberries, and pasteurized liquid milk (2% fat) (3), and the ISO 18593:2018 *Microbiology of the food chain – Horizontal method for surface sampling/*FDA/BAM Chapter 3: *Aerobic Plate Count* for stainless steel environmental surface sponges (4).

REFERENCE METHODS
U.S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG), 3.02, *Quantitative Analysis of Bacteria in Foods as Sanitary Indicators* (2)

Food and Drug Administration Bacteriological Analytical Manual Chapter 3: *Aerobic Plate Count*. (3)

ISO 18593:2018, Microbiology of the food chain – Horizontal methods for surface sampling. (4)

Standard Methods for the Examination of Dairy Products, Microbiological Count Methods Ch. 6, American Public Health Association, Washington, DC 20001-3710, Current Edition (6)

ORIGINAL CERTIFICATION DATE
March 17, 2021

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
1. **December 2021 Level 1**
2. **November 2022 Level 2**
3. **December 2022 Level 1**

SUMMARY OF MODIFICATION
1. **Company address change from Meizheng Group to Beijing Meizheng Bio-Tech Co., Ltd. and editorial updates to insert.**
2. **Change to plate format.**
3. **Editorial/clerical to reformat package insert.**

Under this AOAC *Performance Tested Methods℠* License Number, 032103 this method is distributed by:
1. **PerkinElmer**
2. **Hainan Fosun Trading Co., Ltd.**
3. **Scigiene Corporation**

Under this AOAC *Performance Tested Methods℠* License Number, 032103 this method is distributed as:
1. **MicroFast® Aerobic Count Plate (AC)**
2. **MicroFast® Aerobic Count Plate (AC)**
3. **MicroFast® Aerobic Count Plate (AC)**

PRINCIPLE OF THE METHOD (1)

The MicroFast® AC is a sample-ready culture medium system designed to determine total aerobic bacteria in food matrixes and environmental samples. MicroFast AC plates use rapid diffusion systems and new-generation microbial coloration to achieve rapid proliferation and interpretation of colonies. Aerobic bacteria will appear as red colonies on the plate following 48 ± 1 h of incubation at 36 ± 1°C.

DISCUSSION OF THE VALIDATION STUDY (1)

The MicroFast AC plate evaluated in this study proved reliable and consistent when compared to the USDA/FSIS-MLG 3.02 for frozen ground beef and heat processed chicken, to the FDA/BAM Chapter 3 for vegetable chips, frozen strawberries and pasteurized liquid milk, and to the ISO 18593/BAM Chapter 3 for environmental stainless steel swabs. The results of the statistical analysis using the difference of means with calculated 90% confidence intervals indicate equivalence between the MicroFast AC method and the reference method for all matrices at all contamination levels analyzed. The Cochran and Grubbs test indicated that no outliers were detected in the study. Robustness data show that small variations in the incubation temperature and incubation time of the MicroFast AC films do not affect the method performance. In addition, no statistical differences were seen between production lots for up to one year of shelf life.

The method allows the user to obtain results in 48 hours and get an accurate CFU/g result for the presence of total aerobic bacteria in frozen ground beef, heat processed chicken, vegetable chips, frozen strawberries, pasteurized liquid milk, and stainless steel environmental sponges. The setup is simple, and the procedure is easy to follow, allowing for a technician at any level of training to perform the method and obtain accurate results. The MicroFast AC method requires no additional agar or plates to be used to perform the method, cutting down on supplies and setup time.

Table 1. Matrix study: MicroFast AC results vs reference method results (1)

Matrix	Cont. level[a]	MicroFast AC results			Reference Method results[e]				90% CI[g]		95% CI	
		Mean[b]	s_r[c]	RSD_r[d]	Mean	s_r	RSD_r	Mean diff.[f]	LCL[h]	UCL[i]	LCL	UCL
Frozen raw ground beef (naturally contaminated)	Low	1.963	0.040	2.038	1.933	0.044	2.276	0.030	-0.022	0.081	-0.038	0.097
	Med	2.943	0.010	0.340	2.946	0.014	0.475	-0.003	-0.011	0.006	-0.014	0.008
	High	3.940	0.022	0.558	3.928	0.12	0.305	0.012	-0.002	0.025	-0.006	0.029
Chicken drumstick (*Escherichia coli* ATCC 8739)	Non[k]	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	1.886	0.082	4.348	1.922	0.059	3.070	-0.035	-0.147	0.076	-0.180	0.110
	Med	2.883	0.033	1.145	2.877	0.022	0.765	0.006	-0.036	0.048	-0.048	0.061
	High	3.845	0.034	0.884	3.867	0.013	0.336	-0.021	-0.063	0.020	-0.075	0.032
Vegetable chips (*Staphylococcus epidermidis* ATCC 12228)	Non	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	1.807	0.117	6.475	1.811	0.104	5.743	-0.003	-0.086	0.080	-0.111	0.105
	Med	2.813	0.022	0.782	2.822	0.012	0.425	-0.010	-0.020	0.001	-0.023	0.004
	High	3.855	0.016	0.415	3.856	0.017	1.343	-0.001	-0.021	0.019	-0.027	0.025
Frozen strawberries (naturally contaminated)	Low	1.839	0.084	4.568	1.856	0.052	2.802	-0.017	-0.100	0.067	-0.125	0.092
	Med	2.471	0.022	0.890	2.499	0.028	1.120	-0.028	-0.089	0.033	-0.107	0.051
	High	3.481	0.045	1.293	3.466	0.072	2.077	0.014	-0.036	0.065	-0.052	0.081
Pasteurized liquid milk (2% fat) (*Escherichia coli* ATCC 25922)	Non	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	1.866	0.067	3.590	1.832	0.119	6.496	0.033	-0.018	0.085	-0.034	0.101
	Med	2.485	0.036	1.449	2.523	0.066	2.616	-0.038	-0.081	0.005	-0.094	0.018
	High	3.537	0.048	1.357	3.579	0.056	1.565	-0.042	-0.088	0.003	-0.102	0.017
Stainless steel (*Staphylococcus aureus* ATCC 25923)	Non	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
	Low	1.856	0.052	2.802	1.839	0.084	4.568	0.017	-0.067	0.100	-0.092	0.125
	Med	2.538	0.038	1.500	2.540	0.044	1.732	-0.002	-0.025	0.021	-0.032	0.028
	High	3.526	0.019	0.539	3.538	0.030	0.848	-0.012	-0.036	0.012	-0.044	0.019

[a]All matrices are artificially contaminated when an uncontaminated (Un) level is reported.

[b]Mean of five replicate portions, after logarithmic transformation: $Log_{10}[CFU/g + (0.1)f]$.

[c]Repeatability standard deviation.

[d]Relative standard deviation for repeatability. Reported as a percentage.

[e]Reference methods were USDA/FSIS-MLG 3.02 for frozen ground beef and heat processed chicken drumsticks; FDA/BAM Ch. 3 for vegetable chips, frozen strawberries, liquid milk; ISO 18593:2018/BAM Ch. 3 for stainless steel.

[f]Mean difference between the candidate and reference methods.

[g]Confidence interval.

[h]95% Lower confidence limit for difference of means.

[i]95% Upper confidence limit for difference of means.

[j]American Type Culture Collection (ATCC), Manassas, VA.

[k]Non-inoculated.

DISCUSSION OF MODIFICATION APPROVED NOVEMBER 2022 (5)

The MicroFast AC-New plate evaluated in this study proved reliable and consistent when compared to the USDA/FSIS-MLG 3.02 for frozen ground beef and heat processed chicken, to the FDA/BAM Chapter 3 for vegetable chips, frozen strawberries, to the SMEDP Chapter 6 for pasteurized liquid milk, and to the ISO 18593/BAM Chapter 3 for environmental stainless steel surfaces. The results of the statistical analysis using the difference of means with calculated 90% confidence intervals indicate equivalence between the MicroFast AC-New method and the reference method for all matrices at all contamination levels analyzed. Robustness data show that small variations in the incubation temperature and incubation time of the MicroFast AC-New plates do not affect the method performance.

The method allows the user to obtain accurate results in 48 h for the presence of total aerobic bacteria in frozen ground beef, heat processed chicken, vegetable chips, frozen strawberries, pasteurized liquid milk, and stainless steel environmental sponges. The setup is simple, and the procedure is easy to follow, allowing for a technician at any level of training to perform the method and obtain accurate results. The MicroFast AC-New method requires no additional agar or plates to be used to perform the method, cutting down on supplies and setup time.

Table 1. Matrix study: MicroFast AC-New results vs. MicroFast AC results (5)

		MicroFast AC-New results		MicroFast AC results			90% CI[e]		95% CI	
Matrix	Cont. level[a]	Mean[b]	s_r[c]	Mean	s_r	DOM[d]	LCL[f]	UCL[g]	LCL	UCL
Frozen raw ground beef (naturally contaminated)	Low	1.633	0.001	1.658	0.005	-0.025	-0.032	-0.017	-0.034	-0.016
	Mid	2.372	0.004	2.363	0.006	0.009	0.006	0.012	0.006	0.013
	High	3.633	0.004	3.653	0.023	-0.020	-0.051	0.011	-0.057	0.017
Chicken drumstick (*Escherichia coli* ATCC[h] 8739)	Non	0.000	NA[i]	0.000	NA	NA	NA	NA	NA	NA
	Low	1.629	0.003	1.632	0.001	-0.003	-0.007	0.001	-0.008	0.002
	Mid	2.789	0.017	2.862	0.005	-0.074	-0.092	-0.056	-0.096	-0.052
	High	3.851	0.016	3.859	0.013	-0.008	-0.014	-0.002	-0.015	-0.001
Vegetable chips (*Staphylococcus epidermidis* ATCC 12228)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	1.634	0.001	1.585	0.053	0.049	-0.035	0.134	-0.051	0.150
	Mid	2.750	0.008	2.780	0.004	-0.029	-0.036	-0.022	-0.038	-0.020
	High	3.880	0.004	3.875	0.005	0.005	0.003	0.007	0.003	0.007
Frozen strawberries (naturally contaminated)	Low	1.679	0.006	1.626	0.003	0.053	0.048	0.059	0.047	0.060
	Mid	2.730	0.053	2.676	0.004	0.054	-0.026	0.134	-0.042	0.149
	High	3.660	0.012	3.656	0.015	0.004	-0.001	0.008	-0.001	0.009
Pasteurized liquid milk (2% fat) (*Escherichia coli* ATCC 25922)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	1.633	0.001	1.616	0.005	0.017	0.010	0.025	0.009	0.026
	Mid	2.790	0.050	2.749	0.019	0.041	-0.010	0.092	-0.020	0.101
	High	3.690	0.030	3.683	0.002	0.007	-0.039	0.053	-0.048	0.062
Stainless steel (*Staphylococcus aureus* ATCC 25923)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	1.812	0.061	1.853	0.010	-0.041	-0.125	0.043	-0.141	0.060
	Mid	2.684	0.036	2.683	0.006	0.002	-0.048	0.052	-0.058	0.061
	High	3.747	0.037	3.747	0.012	0.000	-0.041	0.042	-0.049	0.050

[a]Matrixes are artificially contaminated when a non-inoculated (non)level is reported.
[b]Mean of five replicate test portions, after logarithmic transformation: Log10[cfu/g or mL + (0.1)f].
[c]Repeatability standard deviation.
[d]Mean difference between the AC-New plate results and AC plate results.
[e]Confidence interval.
[f]Lower confidence limit for difference of means.
[g]Upper confidence limit for difference of means.
[h]American Type Culture Collection (ATCC), Manassas, VA.
[i]Not Applicable.

Table 2. Matrix study: MicroFast AC-New results vs. reference method results (5)

		MicroFast AC-New results		Reference method[d] results			90% CI[f]		95% CI	
Matrix	Cont. level[a]	Mean[b]	s_r[c]	Mean	s_r	DOM[e]	LCL[g]	UCL[h]	LCL	UCL
Frozen raw ground beef (naturally contaminated)	Low	1.633	0.001	1.616	0.005	0.018	0.011	0.024	0.010	0.026
	Mid	2.372	0.004	2.371	0.002	0.001	-0.002	0.004	-0.002	0.005
	High	3.633	0.004	3.628	0.000	0.005	-0.002	0.011	-0.003	0.012
Chicken drumstick (*Escherichia coli* ATCC[i] 8739)	Non	0.000	NA[j]	0.000	NA	NA	NA	NA	NA	NA
	Low	1.629	0.003	1.629	0.005	0.000	-0.004	-0.003	-0.004	0.004
	Mid	2.789	0.017	2.773	0.003	0.015	-0.007	0.037	-0.011	0.041
	High	3.851	0.016	3.861	0.015	-0.010	-0.013	-0.008	-0.013	-0.007
Vegetable chips (*Staphylococcus epidermidis* ATCC 12228)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	1.634	0.001	1.619	0.006	0.015	0.007	0.022	0.006	0.024
	Mid	2.750	0.008	2.672	0.050	0.078	0.010	0.147	-0.003	0.160
	High	3.880	0.004	3.829	0.048	0.051	-0.023	0.124	-0.037	0.139
Frozen strawberries (naturally contaminated)	Low	1.679	0.006	1.685	0.002	-0.006	-0.013	0.001	-0.014	0.002
	Mid	2.730	0.053	2.732	0.045	-0.002	-0.015	0.010	-0.017	0.013
	High	3.660	0.012	3.662	0.016	-0.003	-0.008	0.003	-0.009	0.004
Pasteurized liquid milk (2% fat) (*Escherichia coli* ATCC 25922)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	1.633	0.001	1.636	0.002	-0.002	-0.005	0.001	-0.006	0.001
	Mid	2.790	0.050	2.816	0.053	-0.026	-0.030	-0.021	-0.030	-0.021
	High	3.690	0.030	3.756	0.024	-0.066	-0.076	-0.056	-0.078	-0.054
Stainless steel (*Staphylococcus aureus* ATCC 25923)	Non	0.000	NA	0.000	NA	NA	NA	NA	NA	NA
	Low	1.812	0.061	1.804	0.072	0.008	-0.010	0.026	-0.013	0.029
	Mid	2.684	0.036	2.631	0.051	0.053	0.028	0.078	0.023	0.083
	High	3.747	0.037	3.715	0.045	0.032	0.018	0.045	0.015	0.048

[a]Matrixes are artificially contaminated when a non-inoculated (non)level is reported.
[b]Mean of five replicate test portions, after logarithmic transformation: Log10[cfu/g or mL + (0.1)f].
[c]Repeatability standard deviation.
[d]Reference methods were USDA/FSIS-MLG 3.02 for frozen ground beef and heat processed chicken drumsticks; FDA/BAM Ch. 3 for vegetable chips, frozen strawberries; SMEDP Ch. 6 liquid milk; ISO 18593:2018/BAM Ch. 3 for stainless steel.
[e]Mean difference between the MicroFast AC-New plate results and reference method results.
[f]Confidence interval.
[g]Lower confidence limit for difference of means.
[h]Upper confidence limit for difference of means.
[i]American Type Culture Collection (ATCC), Manassas, VA.
[j]Not Applicable.

REFERENCES CITED

1. Lui, L., Gone, S., Validation Study for the MicroFast® Aerobic Count Plate (AC), AOAC *Performance Tested Methods℠* certification number 032103.
2. U.S. Department of Agriculture-Food Safety and Inspection Service *Microbiology Laboratory Guidebook* (MLG), 3.02, *Quantitative Analysis of Bacteria in Foods as Sanitary Indicators* MLG 3.02. Quantitative Analysis of Bacteria in Foods as Sanitary Indicators (usda.gov) (Accessed August 2020)
3. Food and Drug Administration Bacteriological Analytical Manual Chapter 3: *Aerobic Plate Count*. January 2001 BAM Chapter 3: Aerobic Plate Count | FDA (Accessed Jan 2021)
4. ISO 18593:2018, Microbiology of the food chain – Horizontal methods for surface sampling. (Accessed August 2020)
5. Lui., L. and Qu, B., Validation Study for the MicroFast® Aerobic Count Plate (AC), AOAC *Performance Tested Methods℠* certification number 032103. Approved November 30, 2022
6. Standard Methods for the Examination of Dairy Products, Microbiological Count Methods Ch. 6, American Public Health Association, Washington, DC 20001-3710, Current Edition.