



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
082101

The AOAC Research Institute hereby certifies the method known as:

Petrifilm® Coliform Count (CC) Plate

manufactured by

Neogen Corporation
620 Leshar Place
Lansing, Michigan 48912
USA

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.

A handwritten signature in black ink, appearing to read "Bradley A. Stawick".

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

Issue Date
Expiration Date

December 06, 2024
December 31, 2026

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METHOD NAME Neogen® Petrifilm® Coliform Count (CC) Plate Formerly 3M™ Petrifilm™ Coliform Count (CC) Plate		CATALOG NUMBERS 6410, 6411, 6416, 6443	
INDEPENDENT LABORATORY SGS Vanguard Sciences 224 North Derby Lane, North Sioux City, SD 57049 USA		APPLICABILITY OF METHOD Analytes – Coliforms. Matrixes – (BAM Ch. 4, 100 mL) - purified water, treated spring water, non-carbonated natural spring water, carbonated natural spring water. OMA Approved Matrixes (OMA 986.33) Milk (OMA 989.10) Dairy Products (OMA 991.14) Foods Performance claims – No statistical difference detected compared to the U.S. Food and Drug Administration (FDA) <i>Bacteriological Analytical Manual</i> (BAM) Chapter 4 (2).	
ORIGINAL CERTIFICATION DATE August 03, 2021		CERTIFICATION RENEWAL RECORD Renewed through December 2026.	
METHOD MODIFICATION RECORD 1. October 2021 Level 1 2. November 2021 Level 1 3. December 2022 Level 1 4. January 2024 Level 1		SUMMARY OF MODIFICATION 1. Editorial changes. 2. Editorial changes. 3. Editorial changes. 4. Editorial changes to rebrand method from 3M to Neogen Corporation.	
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PRINCIPLE OF THE METHOD (1)

The Neogen® Petrifilm® Coliform Count (CC) Plate is a sample-ready-culture medium system which contains modified Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. Petrifilm CC Plates are used for the enumeration of coliforms in the food and beverage and bottled water industries.

DISCUSSION OF THE VALIDATION STUDY (1)

In the matrix studies conducted in this evaluation, the Petrifilm Coliform Count Plate method was compared to the FDA/BAM Chapter 4, Section III.D for the enumeration of coliform count in bottled water reference method at 24 hours. Four bottled water matrixes – purified water, treated spring water, non-carbonated natural spring water, and carbonated natural spring water were inoculated with coliforms at low, medium, and high levels. The log counts from the Petrifilm Coliform Count Plate method were compared with log counts from the reference method.

The mean differences of log transformed results between the Petrifilm CC Plate and the reference method was less than 0.5 log₁₀ and had a 90% confidence interval containing values between (-0.5, 0.5) for all contamination levels for purified water, treated spring water, and carbonated natural spring water. The confidence intervals were calculated on the mean differences for each comparison and were between (-0.5, 0.5). In fact, the differences were 0.37 log₁₀ and lower and had a 95% confidence interval between (-0.5, 0.5) indicating that the Petrifilm CC Plate is statistically equivalent to the reference method for purified water, treated spring water, and carbonated natural spring water. The mean differences of log₁₀ transformed results between the Petrifilm CC Plate and the reference method did not meet the equivalency requirements of less than 0.5 log₁₀ and a 90% confidence interval containing values between (-0.5, 0.5) for the non-carbonated natural spring water; however, this was due to the fact that *K. pneumonia* (ATCC 4352) was not recovered on the reference media at any of the inoculation levels and the difference in recovery is in favor of the Petrifilm CC Plate method. The root cause of the poor recovery of *K. pneumonia* (ATCC 4352) on the reference method was not determined.

In strain studies, of the 50 coliform inclusivity isolates tested, 47 out of 50 strains had typical coliform growth (red colonies associated with gas) on Petrifilm CC Plates. Three strains, *C. braakii* ATCC 51113, *C. braakii* ATCC 43162 and *C. freundii* ATCC 8090 had atypical coliform growth (red colonies without associated gas) that were confirmed as coliforms per the product instructions. All 30 exclusivity strains had no growth or atypical growth (red colonies without associated gas) that did not confirm as coliforms per the product instructions.

Table 1. Inclusivity Results for Petrifilm CC Plates – Bottled Water Method (1)

Number	Organism	Source	Origin	Results ^a	Confirmation ^b
1	<i>Citrobacter braakii</i>	ATCC ^c 51113	snake	G, G-	+
2	<i>Citrobacter braakii</i>	ATCC 43162	Clinical	G, G-	+
3	<i>Citrobacter freundii</i>	ATCC 8090	unknown	G, G-	+
4	<i>Citrobacter freundii</i>	ATCC 43864	unknown	G, G+	NA
5	<i>Cronobacter muytjensii</i>	ATCC 51329	Unknown	G, G+	NA
6	<i>Cronobacter sakazakii</i>	ATCC 29544	Clinical, child's throat	G, G+	NA
7	<i>Enterobacter aerogenes</i> ^d	U37 ^e	unknown	G, G+	NA
8	<i>Enterobacter aerogenes</i>	ATCC 13048	Clinical, sputum	G, G+	NA
9	<i>Enterobacter aerogenes</i>	ATCC51697	unknown	G, G+	NA
10	<i>Enterobacter amnigenus</i> ^d	ATCC 51816	Milk	G, G+	NA
11	<i>Enterobacter amnigenus</i>	ATCC 51818	Milk	G, G+	NA
12	<i>Enterobacter cancerogenus</i>	ATCC 49817	Poplar	G, G+	NA
13	<i>Enterobacter cloacae</i>	ATCC 23355	Unknown	G, G+	NA
14	<i>Enterobacter cloacae</i>	ATCC 35030	Unknown	G, G+	NA
15	<i>Enterobacter cloacae</i>	ATCC 13047	Clinical, spinal fluid	G, G+	NA
16	<i>Enterobacter cloacae</i>	NCTC 13464	Unknown	G, G+	NA
17	<i>Enterobacter hormaechei</i>	ATCC 700323	Unknown	G, G+	NA
18	<i>Enterobacter gergoviae</i>	ATCC 33028	Clinical, urine	G, G+	NA
19	<i>Escherichia coli</i>	ATCC 51813	Food, MN	G, G+	NA
20	<i>Escherichia coli</i>	ATCC 11229	unknown	G, G+	NA
21	<i>Escherichia coli</i>	NCTC ^f 13216	unknown	G, G+	NA
22	<i>Escherichia coli</i>	Neogen PWV4 ^e	Wastewater	G, G+	NA
23	<i>Escherichia coli</i>	Neogen FR1 ^e	food	G, G+	NA
24	<i>Escherichia coli</i>	AFLCC ^g	food	G, G+	NA
25	<i>Escherichia coli</i>	ATCC 35218	Clinical, canine	G, G+	NA
26	<i>Escherichia coli</i>	ATCC 11229	Unknown	G, G+	NA
27	<i>Escherichia coli</i>	ATCC 25922	Clinical	G, G+	NA
28	<i>Escherichia coli</i>	ATCC 8739	Clinical, feces	G, G+	NA
29	<i>Escherichia coli</i> ^d	ATCC 10536	unknown	G, G+	NA
30	<i>Escherichia coli</i> ^d	ATCC 14948	unknown	G, G+	NA
31	<i>Escherichia coli</i> ^d	FSD723 ^e	unknown	G, G+	NA
32	<i>Escherichia coli</i> ^d	FR2 ^e	unknown	G, G+	NA
33	<i>Escherichia coli</i> ^d	ATCC 35218	Canine	G, G+	NA
34	<i>Escherichia vulneris</i>	ATCC 33821	Clinical, wound	G, G+	NA
35	<i>Hafnia alvei</i>	ATCC 51815	Milk	G, G+	NA
36	<i>Klebsiella aerogenes</i>	ATCC 35029	Unknown	G, G+	NA
38	<i>Klebsiella aerogenes</i>	NCIMB 10102	Clinical, sputum	G, G+	NA
39	<i>Klebsiella oxytoca</i>	ATCC 43165	Clinical	G, G+	NA
40	<i>Klebsiella oxytoca</i> ^d	ATCC 43086	Unknown	G, G+	NA
41	<i>Klebsiella oxytoca</i>	ATCC 13182	Clinical, tonsil	G, G+	NA
42	<i>Klebsiella oxytoca</i> ^d	ATCC 700324	Unknown	G, G+	NA
43	<i>Klebsiella oxytoca</i> ^d	U33 ^e	Unknown	G, G+	NA
44	<i>Klebsiella oxytoca</i> ^d	ATCC 8724	Unknown	G, G+	NA
45	<i>Klebsiella oxytoca</i> ^d	ATCC 51817	Milk	G, G+	NA
46	<i>Klebsiella pneumoniae</i>	ATCC 4352	Cow's milk	G, G+	NA
47	<i>Klebsiella pneumoniae</i> ^d	ATCC 23357	Cow's milk	G, G+	NA
48	<i>Klebsiella pneumoniae</i> ^d	U29 ^e	unknown	G, G+	NA
49	<i>Klebsiella pneumoniae</i> ^d	U28 ^e	unknown	G, G+	NA
50	<i>Kluyvera georgiana</i>	ATCC 51603	Clinical, human sputum	G, G+	NA

^aResults on Petrifilm CC Plates for growth with gas is indicated by G, G+ and growth with no gas is indicated by G, G-.^bThe confirmation method was completed and the strain was confirmed as a coliform (+) or was not confirmed as a coliform (-). No confirmation steps were completed (NA) because the coliform result was confirmed by the presence of gas on the Petrifilm CC Plate.^cATCC = American Type Culture Collection, Manassas, VA.^dStrain tested by Neogen Company Laboratory.^eNeogen Company Laboratory Culture Collection.^fNCTC = National Collection of Type Cultures.^gSGS Vanguard Sciences, Inc Culture Collection.

Table 2. Exclusivity Results for Petrifilm CC Plates – Bottled Water Method (1)

Number	Organism	Source	Origin	Results ^a	Confirmation ^b
1	<i>Acinetobacter baumannii</i>	ATCC ^c 19606	Urine	-	NA
2	<i>Aeromonas hydrophila</i>	ATCC 35654	Unknown	G, G-	-
3	<i>Alcaligenes faecalis</i>	ATCC 8750	Unknown	G, G-	-
4	<i>Bacillus cereus</i>	ATCC 11778	Unknown	-	NA
5	<i>Bacillus subtilis</i>	ATCC 27370	Unknown	G, G-	-
6	<i>Bordetella parapertussis</i>	ATCC 15311	Clinical	NG	NA
7	<i>Carnobacterium maltaromaticum</i>	ATCC 27865	Raw milk	NG	NA
8	<i>Edwardsiella tarda</i>	ATCC 15947	Clinical, feces	G, G-	-
9	<i>Enterococcus faecalis</i>	ATCC 14506	Unknown	G, G-	-
10	<i>Enterococcus faecium</i>	NRRL ^d B2354	Dairy utensils	G, G-	-
11	<i>Haemophilus influenzae</i>	ATCC 35056	Clinical	G, G-	-
12	<i>Lactococcus garvieae</i>	ATCC 43921	Mastitis	G, G-	-
13	<i>Leuconostoc mesenteroides</i>	ATCC 8293	Olives	G, G-	-
14	<i>Listeria innocua</i>	ATCC 33090	Cow brain	G, G-	-
15	<i>Listeria ivanovii</i>	ATCC 19119	Sheep	G, G-	-
16	<i>Listeria monocytogenes</i>	ATCC 19115	Clinical	G, G-	-
17	<i>Micrococcus luteus</i>	NCIMB ^e 8166	air	G, G-	-
18	<i>Microbacterium testaceum</i>	ATCC 15829	Paddy	G, G-	-
19	<i>Proteus mirabilis</i>	ATCC 25933	Clinical	-	NA
20	<i>Proteus vulgaris</i>	ATCC 6380	Unknown	-	NA
21	<i>Pseudomonas aeruginosa</i>	ATCC 15442	Animal room water bottle	G, G-	-
22	<i>Salmonella</i> Anatum	ATCC 9270	Pork liver	-	NA
23	<i>Salmonella</i> Typhimurium	ATCC 13311	Clinical, feces	G, G-	-
24	<i>Shigella flexneri</i>	ATCC 9199	Unknown	-	NA
25	<i>Shigella sonnei</i>	ATCC 25931	Clinical, feces	-	NA
26	<i>Shigella boydii</i>	ATCC 9207	Unknown	-	NA
27	<i>Staphylococcus aureus</i>	ATCC 6538	Clinical	G, G-	-
28	<i>Streptococcus pyogenes</i>	ATCC 12384	Unknown	-	NA
29	<i>Vibrio parahaemolyticus</i>	NCTC ^f 10885	oyster	G, G-	-
30	<i>Yersinia enterocolitica</i>	ATCC 23715	Clinical, eye	-	NA

^aResults on Petrifilm CC Plates with no growth (-) and growth with no gas (G, G-).^bThe confirmation method was completed and the strain was not confirmed as a coliform (-). No confirmation steps were completed (NA) because there was no growth on the Petrifilm CC Plate.^cATCC = American Type Culture Collection, Manassas, VA.^dNRRL = Agricultural Research Service Culture Collection.^eNCIMB = National Collection of Industrial Food and Marine Bacteria^fNCTC = National Collection of Type Cultures

Table 4. Matrix study: Petrifilm Coliform Count Plate Bottled Water Method vs Reference Method Results (1)

Matrix	Cont. level ^a	Petrifilm CC Plate			Reference Method ^e				90% CI ^g		95% CI ^j	
		Mean ^b	s _r ^c	RSD _r ^d	Mean	s _r	RSD _r	DOM ^f	LCL ^h	UCL ⁱ	LCL	UCL
Carbonated	Un	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
Natural Spring	Low	1.48	0.08	5.41	1.72	0.03	1.74	-0.24	-0.31	-0.17	-0.33	-0.15
Water (<i>C. freundii</i>	Med	1.82	0.04	2.25	2.09	0.01	0.72	-0.27	-0.30	-0.23	-0.32	-0.22
ATCC ^k 8090)	High	1.91	0.12	6.28	2.28	0.05	2.02	-0.37	-0.49	-0.26	-0.53	-0.22
Non-carbonated	Un	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
Natural Spring	Low	1.17	0.13	11.47	-1.00	0.00	0.00	2.17	2.04	2.30	2.00	2.34
Water (<i>K. pneumonia</i>	Med	1.79	0.04	2.29	-1.00	0.00	0.00	2.79	2.75	2.89	2.74	2.84
ATCC 4352)	High	1.99	0.04	1.86	-1.00	0.00	0.00	2.99	2.95	3.02	2.94	3.03
Treated Spring	Un	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
Water (<i>E. coli</i>	Low	1.50	0.14	9.1	1.49	0.06	3.95	0.01	-0.14	0.15	-0.18	0.20
ATCC 8739)	Med	1.78	0.03	1.6	1.99	0.04	1.91	-0.21	-0.26	-0.17	-0.27	-0.15
	High	1.98	0.05	2.3	2.20	0.03	1.32	-0.23	-0.29	-0.16	-0.32	-0.14
Purified Water ^l (<i>E. cloacae</i>	Un	0.000	NA	NA	0.000	NA	NA	NA	NA	NA	NA	NA
ATCC 13047)	Low	1.11	0.22	0.90	0.99	0.42	42.10	0.13	-0.13	0.39	-0.21	0.47
	Med	1.72	0.49	28.36	1.70	0.33	19.51	0.02	-0.13	0.17	-0.18	0.22
	High	2.23	0.05	2.38	2.06	0.04	1.75	0.17	0.12	0.23	0.10	0.25

^aAll matrices are artificially contaminated when an uncontaminated (Un) level is reported.^bMean of five replicate portions after logarithmic transformation: Log₁₀[CFU/100 mL + (0.1)f].^cRepeatability standard deviation.^dRelative standard deviation for repeatability. Reported as a percentage.^eReference methods were BAM Ch. 4, Section III.D.^fMean difference between the candidate and reference methods.^g90% Confidence interval based on paired statistical analysis. If the confidence interval of the methods does not fall between -0.5 and 0.5, then the methods would not be considered equivalent.^hLower confidence limit for difference of means.ⁱUpper confidence limit for difference of means.^j95% confidence interval based on paired statistical analysis.^kAmerican Type Culture Collection, Manassas, VA.^lPurified water samples were tested by Neogen company**Table A1. INDEPENDENT LABORATORY Matrix Study: Petrifilm Coliform Count Plate Bottled Water Method vs Reference Method Results. (1)**

Matrix	Cont. level ^a	Petrifilm CC Plate			Reference Method ^e				90% CI ^g		95% CI ^j	
		Mean ^b	s _r ^c	RSD _r ^d	Mean	s _r	RSD _r	DOM ^f	LCL ^h	UCL ⁱ	LCL	UCL
Purified	Un	0.00	NA	NA	0.00	NA	NA	NA	NA	NA	NA	NA
Water ^k (<i>E. cloacae</i>	Low	1.04	0.23	2.24	1.59	0.11	7.13	-0.54	-0.83	-0.25	-0.92	-0.16
ATCC ^l 23355)	Med	1.50	0.30	19.97	2.06	0.02	0.97	-0.56	-0.86	-0.26	-0.95	-0.17
	High	1.83	0.08	4.54	2.23	0.03	1.35	-0.40	-0.489	-0.301	-0.518	-0.272

^aAll matrices are artificially contaminated when an uncontaminated (Un) level is reported.^bMean of five replicate portions after logarithmic transformation: Log₁₀[CFU/100 mL + (0.1)f].^cRepeatability standard deviation.^dRelative standard deviation for repeatability. Reported as a percentage.^eReference methods were BAM Chpt 4, Section III.D.^fMean difference between the candidate and reference methods.^g90% Confidence interval based on paired statistical analysis. If the confidence interval of the methods does not fall between -0.5 and 0.5, then the methods would not be considered equivalent.^hLower confidence limit for difference of means.ⁱUpper confidence limit for difference of means.^j95% confidence interval based on paired statistical analysis.^kPurified water samples were tested by SGS Vanguard Sciences^lAmerican Type Culture Collection, Manassas, VA.**REFERENCES CITED**

- Schumacher, A. and Lingle, C., Validation of the 3M™ Petrifilm™ Coliform Count Plate for Enumeration of Coliforms in Bottled Water, AOAC Performance Tested MethodsSM certification number 082101.
- US Food and Drug Administration Bacteriological Analytical Manual (BAM). Chapter 4. October 2020.