Official Methods of Analysis of AOAC INTERNATIONAL (22nd Edition)

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CHAPTER

17.2.12 AOAC Official Method℠ 2015.13 Enumeration of Aerobic Bacteria in Food and on Selected Surfaces: Neogen® Petrifilm® Rapid Aerobic Count (RAC) Plate

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First Action 2015

Final Action 2018

Revised First Action 2022 (for Selected Surfaces Only)

[Applicable to the enumeration of aerobic bacteria from raw ground beef, raw ground pork, raw ground turkey, chicken carcass rinsate, fresh swai, fresh tuna, fresh tiger shrimp, raw easy-peel shrimp, cherry tomato wash, frozen blueberries, Mediterranean apricots, creamy salad dressing, fresh pasta, vanilla ice cream, instant nonfat dry milk (NFDM), pasteurized skim milk, stainless steel, sealed concrete, and rubber.]

Caution: After use, the diluents and Petrifilm RAC Plates may contain microorganisms that may be a potential biohazard. When testing is complete, follow current industry standards for disposal of contaminated waste. Consult Material Safety Data Sheet for additional information and local regulations for disposal.

To reduce risks associated with bacterial infection and workplace contamination, perform Petrifilm RAC Plate testing in properly equipped laboratory under control of skilled microbiologist. The user must train personnel in current proper testing techniques—for example, good laboratory practices, ISO 17025, or ISO 7218.

See Tables 2015.13A and B for results of the interlaboratory study supporting acceptance of the method.

See Table 2015.13C for results of the matrix study supporting extension of the method.

A Principle

The Petrifilm RAC Plate is a sample-ready culture medium system that contains nutrients, cold-water-soluble gelling agent, and indicator system that facilitates aerobic bacterial enumeration. Petrifilm RAC Plates are used for the enumeration of aerobic bacteria in as little as 24 h for most food matrixes. Neogen is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

B Apparatus and Reagents

(a) *Petrifilm RAC Plate.*—Cat. No. 6478/6479; Product SKUs 700006478/700006479 (Neogen Corp., Lansing, MI, USA; www.neogen.com).

(b) *Sterile diluent.*—Butterfield's Phosphate-Buffered Diluent (BPBD).

(c) *Pipets.*—Capable of pipetting 1000 μL or serological pipet.

(d) *Sterile pipet tips.*—Capable of 1000 μL.

(e) *Stomacher.*—Seward or equivalent.

(f) *Filter stomacher bags.*—Seward or equivalent.

(g) *Petrifilm Flat Spreader.*—Cat. No. 6425.

(h) *Swab Sampler with 10 mL Letheen broth.*—Cat. No. RS96010LET; Product SKU 700002039 (Neogen Corp.) or equivalent.

(i) *Incubators.*—Capable of maintaining 32 ± 1°C and 35 ± 1°C and having solid front to maintain dark interior.

(j) *Refrigerator or freezer.*—Capable of maintaining temperature between −20 and 8°C for storing unopened Petrifilm RAC Plates.

(k) *Freezer.*—Capable of maintaining temperature at less than −15°C for storing Petrifilm RAC pouches after incubation.

(l) *Standard colony counter or illuminated magnifier.*

C General Instructions

(a) *Storage conditions.*—Store Petrifilm RAC Plates at −20 to 8°C. After opening Petrifilm RAC Plate pouches, seal pouch, and store at ambient temperature, <60% relative humidity. Post-incubation Petrifilm RAC Plates can be stored at less than −15°C for up to 1 week.

(b) *Spreader.*—Place Petrifilm Flat Spreader on center of plate when preparing sample aliquot to prevent trapping air bubbles.

(c) Follow all instructions carefully. Failure to do so may lead to inaccurate results.

D Sample Preparation

(a) Aseptically prepare 1:10 dilution of each test portion.

(1) *Dairy products.*—Pipet 11 mL or weigh 11 g sample into 99 mL sterile BPBD.

(2) *All other foods.*—Weigh 50 g test portion into sterile stomacher bag and dilute with 450 mL BPBD; blend or homogenize per standard.

(3) *Environmental surfaces.*—Mix or shake swab in Letheen vigorously.

(b) Prepare 10-fold serial dilutions in BPBD. Environmental surface samples may be plated directly as needed.

(c) Place Petrifilm RAC Plates on flat, level surface for each dilution to be tested.

(d) Lift film. With the pipet perpendicular, dispense 1 mL of each dilution onto center of bottom film of plate.

(e) Roll film down onto sample.

(f) Place Petrifilm Flat Spreader on center of plate. Press gently on center of spreader to distribute sample evenly. Spread inoculum over entire Petrifilm RAC Plate growth area before gel is formed. Do not slide spreader across film.

(g) Remove spreader and leave plate undisturbed for at least 1 min to permit gel to form.

(h) Incubate Petrifilm RAC Plate at either 32 ± 1°C (seafood and dairy products) or 35 ± 1°C (all other foods and environmental surfaces) in horizontal position with clear side up in stacks of no more than 20 (for dairy products) or 40 (for all other foods). Enumerate plates after 24 ± 2 h of incubation (or 48 ± 3 h in the case of dairy powders, including whey powder). Petrifilm RAC Plates can be counted using standard colony counter with use of backlight or illuminated magnifier to assist with estimated enumeration.

(i) Enumerate all colonies regardless of size, color, or intensity.

(j) Circular growth area is approximately 30 cm^2. Plates containing >300 colonies can be either estimated or recorded as "too numerous to count" (TNTC). Estimation can be done only by counting the number of colonies in one or more representative squares and determining the average number per square. The average number can be multiplied by 30 to determine the estimated count per plate. If a more accurate count is required, the sample may need to be retested at higher dilutions.

(k) Report final results as colony-forming units per gram or milliliter (CFU/g or CFU/mL).

Note: If there are two dilutions within the countable range, use the following calculation to determine the final count: $N = \Sigma C / (1.1 \times d)$

where N = number of colonies per milliliter or per gram of product; ΣC = sum of all colonies on both plates; and d = dilution from which first counts were obtained.

(l) Food samples may occasionally show interference on Petrifilm RAC Plates—for example:

(1) Uniform blue background color (often seen from organisms used in cultured products). These should not be counted as TNTC.

(2) Intense pinpoint blue specs (often seen with spices or granulated products).

(m) When necessary, colonies may be isolated for further identification test using standard procedures. Lift top film and pick colony from gel.

Revised: October 2018; October 2019 [***D*** *(11): Deleted "Average the counts between the replicate plates" for clarity]; May 2022 (Matrix extension to selected environmental surfaces)*

Table 2015.13A. Interlaboratory study results of Neogen Petrifilm RAC Plate vs FDA BAM Chapter 3 method for raw easy-peel shrimp

Matrix	Petrifilm RAC Plate					FDA BAM Chapter 3					Difference of means	Difference of means 95% LCL, UCL[d, e]	Reverse-transformed difference of mean, CFU/g	Reverse-transformed difference of means LCL, UCL
	Lot	N[a]	Mean log_{10} CFU/g	s_r[b]	s_R[c]	Lot	N	Mean log_{10} CFU/g	s_r	s_R				
Raw easy-peel shrimp 32°C	Low	16	2.96	0.132	0.280	Low	16	3.02	0.218	0.356	0.06	−0.11, 0.24	139.47	0.77, 1.72
	Medium	16	4.29	0.202	0.215	Medium	16	4.23	0.095	0.298	−0.06	−0.18, 0.06	−2424.10	0.67, 1.15
	High	16	5.56	0.110	0.248	High	16	5.76	0.097	0.214	0.20	−0.01, 0.42	214352.79	0.97, 2.61
Raw easy-peel shrimp 35°C	Low	16	2.80	0.121	0.335	Low	16	3.02	0.218	0.356	0.22	−0.03, 0.48	422.68	0.92, 3.03
	Medium	16	4.22	0.172	0.273	Medium	16	4.23	0.095	0.298	0.01	−0.08, 0.11	539.37	0.83, 1.28
	High	16	5.67	0.141	0.174	High	16	5.76	0.097	0.214	0.09	−0.09, 0.26	105217.30	0.82, 1.83

a *N* = Number of laboratories that reported complete results.

b s_r = Repeatability.

c s_R = Reproducibility.

d LCL, UCL = 95% Lower and upper confidence limits, respectively.

e A 95% confidence interval that contains the point 0 indicates no statistical significant difference between methods.

Table 2015.13B. Interlaboratory study results of Neogen Petrifilm RAC Plate vs SMEDP Chapter 6 method for pasteurized skim milk and instant NFDM

Matrix	Petrifilm RAC Plate					SMEDP Chapter 6					Difference of means	Difference of means 95% LCL, UCL[d, e]	Reverse-transformed difference of mean, CFU/g	Reverse-transforme difference means LCL, UCL
	Lot	N[a]	Mean log_{10} CFU/g	s_r[b]	s_R[c]	Lot	N	Mean log_{10} CFU/g	s_r	s_R				
Pasteurized skim milk	Low	13	2.51	0.131	0.310	Low	13	2.47	0.123	0.301	−0.04	−0.08, 0.01	24.56	0.83, 1.03
	Medium	13	3.53	0.180	0.242	Medium	13	3.48	0.119	0.264	−0.05	−0.13, 0.03	346.20	0.75, 1.08
	High	13	4.63	0.136	0.232	High	13	4.58	0.116	0.196	−0.05	−0.11, 0.01	4936.41	0.78, 1.00
Instant NFDM	Low	15	2.42	0.096	0.126	Low	15	2.34	0.129	0.179	−0.08	−0.16, 0.01	42.05	0.69, 1.02
	Medium	15	3.04	0.059	0.148	Medium	15	2.98	0.104	0.195	−0.06	−0.14, 0.01	153.18	0.73, 1.02
	High	15	4.26	0.174	0.190	High	15	4.19	0.185	0.197	−0.07	−0.14, 0.01	2806.94	0.71, 1.00

a *N* = Number of laboratories that reported complete results.

b s_r = Repeatability.

c s_R = Reproducibility.

d LCL, UCL = 95% Lower and upper confidence limits, respectively.

e A 95% confidence interval that contains the point 0 indicates no statistical significant difference between methods.

Table 2015.13C. Matrix study: Neogen Petrifilm RAC Plate vs BAM Ch. 3

Matrix/organism	Contamination level[a]	Petrifilm RAC Plate		FDA/BAM Ch. 3[d]		DOM[e]	SE[f]	90% CI[g]		95% CI	
		Mean[b]	s_r[c]	Mean	s_r			LCL[h]	UCL[i]	LCL	UCL
Stainless steel/*Listeria innocua* (ATCC[j] 33090)	Low	1.609	0.297	1.531	0.244	0.078	0.078	–0.088	0.244	–0.139	0.294
	Med	2.144	0.156	2.093	0.133	0.051	0.057	–0.072	0.173	–0.109	0.210
	High	3.123	0.107	3.191	0.095	–0.067	0.017	–0.103	–0.032	–0.114	-0.021
Rubber/*Klebsiella aerogenes* (ATCC 35029)	Low	1.823	0.056	1.803	0.101	0.020	0.047	–0.080	0.119	–0.110	0.150
	Med	2.862	0.042	2.819	0.070	0.043	0.041	–0.044	0.130	–0.070	0.156
	High	3.871	0.033	3.801	0.087	0.070	0.029	0.008	0.131	–0.010	0.150
Sealed concrete/*Klebsiella oxytoca* (ATCC 43165)	Low	1.659	0.243	1.788	0.110	–0.129	0.136	–0.419	0.160	–0.506	0.247
	Med	2.136	0.017	2.141	0.042	–0.005	0.016	–0.038	0.028	–0.048	0.038
	High	3.105	0.055	3.124	0.062	–0.019	0.008	–0.036	–0.002	–0.041	0.003

a All surfaces are artificially contaminated, 100 cm^2 test areas.

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

c s_r = Repeatability standard deviation.

d FDA/BAM Ch. 3, Aerobic Plate Count.

e DOM = Difference of means between candidate and reference methods.

f SE = Standard error on mean difference.

g CI = Confidence interval.

h LCL = Lower confidence limit for difference of means.

i UCL = Upper confidence limit for difference of means.

j ATCC = American Type Culture Collection, Manassas, VA, USA.

References:

J. AOAC Int. **99**, 664(2016) (First Action) DOI: 10.5740/jaoacint.15-0260

J. AOAC Int. **106**, 165(2023) (Matrix extension) DOI: https://doi.org/10.1093/jaoacint/qsac122

*Revised: October 2018; October 2019 [**D**(11): Deleted "Average the counts between the replicate plates" for clarity]; May 2022 (Matrix extension to selected environmental surfaces); November 2023 (brand update and minor editorial corrections)*