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17.2.12

AOAC Official Method 2015.13
Enumeration of Aerobic Bacteria in Food
3M™ Petrifilm™ Rapid Aerobic Count Plate
First Action 2015
Final Action 2018

[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 (NFD), and pasteurized skim milk.]

Caution: After use, the diluents and 3M Petrifilm RAC Plates may contain microorganisms that may be a potential biohazard. When testing is complete, follow current industry standards for the disposal of contaminated waste. Consult the Material Safety Data Sheet for additional information and local regulations for disposal.

To reduce the risks associated with bacterial infection and workplace contamination: Perform 3M Petrifilm RAC Plate testing in a properly equipped laboratory under the control of a 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.

A. Principle

The 3M Petrifilm Rapid Aerobic Count (RAC) Plate is a sample-ready culture medium system that contains nutrients, a cold-water-soluble gelling agent, and an indicator system that facilitates aerobic bacterial enumeration. 3M Petrifilm RAC Plates are used for the enumeration of aerobic bacteria in as little as 24 h for most food matrices. 3M™ Food Safety is certified to ISO (International Organization for Standardization) 9001 for design and manufacturing.

B. Apparatus and Reagents

- (a) *3M Petrifilm RAC Plate*.—Twenty-five plates/pouch, two pouches/box (3M Food Safety, St. Paul, MN, USA; Cat. No. 6478).
- (b) *Sterile diluent*.—Butterfield’s Phosphate-Buffered Diluent.
- (c) *Pipets*.—Capable of pipetting 1000 µL or a serological pipet.
- (d) *Sterile pipet tips*.—Capable of 1000 µL.
- (e) *Stomacher*.—Seward or equivalent.
- (f) *Filter Stomacher bags*.—Seward or equivalent.
- (g) *3M Petrifilm Flat Spreader*.—Cat. No. 6425.
- (h) *Incubators*.—Capable of maintaining 32 ± 1°C and 35 ± 1°C and having a solid front to maintain a dark interior.
- (i) *Refrigerator or freezer*.—Capable of maintaining temperature between –20 to 8°C for storing unopened 3M Petrifilm RAC Plates.
- (j) *Freezer*.—Capable of maintaining temperature at less than –15°C for storing 3M Petrifilm RAC pouches after incubation.
- (k) *Standard colony counter or illuminated magnifier*.

C. General Instructions

- (a) *Storage conditions*.—Store the 3M Petrifilm RAC Plates at –20 to 8°C. After opening the 3M Petrifilm RAC Plate pouches, seal the pouch and store at ambient temperature, <60% relative humidity. Post-incubation 3M Petrifilm RAC Plates can be stored at less than –15°C for up to 1 week.
- (b) *Spreader*.—Place the 3M Petrifilm Flat Spreader on the center of the 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

- (1) Aseptically prepare a 1:10 dilution of each test portion.
 - (a) *Dairy products*.—Pipet 11 mL or weigh 11 g sample into 99 mL sterile Butterfield’s phosphate-buffered Diluent.
 - (b) *All other foods*.—Weigh a 50 g test portion into a sterile stomacher bag and dilute with 450 mL Butterfield’s Phosphate-Buffered Diluent; blend or homogenize per standard.
- (2) Prepare 10-fold serial dilutions in Butterfield’s Phosphate-Buffered Diluent.
- (3) Place the 3M Petrifilm RAC Plates on a flat, level surface for each dilution to be tested.
- (4) Lift the film. With the pipet perpendicular, dispense 1 mL of each dilution onto the center of the bottom film of the plate.

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

Matrix	3M Petrifilm RAC Plate					FDA BAM Chapter 3					Difference of means 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 3M Petrifilm RAC Plate vs SMEDP Chapter 6 method for pasteurized skim milk and instant NFDM

Matrix	3M Petrifilm RAC Plate					SMEDP Chapter 6					Difference of means 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				
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.

(5) Roll the film down onto the sample.

(6) Place the 3M Petrifilm Flat Spreader on the center of the plate. Press gently on the center of the spreader to distribute the sample evenly. Spread the inoculum over the entire 3M Petrifilm RAC Plate growth area before the gel is formed. Do not slide the spreader across the film.

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

(8) Incubate the 3M Petrifilm RAC Plate at either 32 ± 1°C (seafood and dairy products) or 35 ± 1°C (all other foods) in a horizontal position with the clear side up in stacks of no more than 20 (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). 3M Petrifilm RAC Plates can be counted using a standard colony counter with the use of a back-light or an illuminated magnifier to assist with the estimated enumeration.

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

(10) The circular growth area is approximately 30 cm². Plates containing >300 colonies can be either estimated or recorded as Too Numerous To Count (TNTC). Estimation can only be done 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.

(11) Average the counts between the replicate plates. 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.

(12) Food samples may occasionally show interference on the 3M Petrifilm RAC Plates, for example:

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

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

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

Reference: *J. AOAC Int.* **99**, 664(2016)

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