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AOAC Official Method 2018.13
Enumeration of *Escherichia coli* and Coliform
in a Broad Range of Foods and Select Environmental Surfaces
3M Petrifilm Rapid *E. coli/*Coliform Count Plate
First Action 2018

[Applicable to the enumeration of *E. coli* and coliform from pasteurized whole milk, butter, non-fat dry milk, raw ground pork, raw lamb chop, raw ground chicken, chicken carcass rinsate, shell eggs, liquid egg whites, powdered egg whites, fresh raw bean sprouts, frozen cranberries, infant formula with probiotics, infant formula without probiotics, infant rice cereal without probiotics, dry dog kibble, dry cat food, all-purpose flour, chocolate chip cookie dough, raw ground beef (73% lean), raw frozen chicken wings, raw milk, whole liquid egg, tuna sushi, smoked salmon, bunched raw spinach, pasteurized carrot juice, ready-made sandwiches (bread, deli meat and cheese), raw vegetable salad with mayonnaise-based dressing, chicken feed, soybean meal, stainless steel environmental sponges and sealed concrete environmental sponges.]

See Tables **2018.13A** and **B** for a summary of results of the collaborative study.

A. Principle

The 3M Petrifilm Rapid *E. coli*/Coliform Count Plate is a self-contained, sample-ready-culture-medium system which contains a cold-water-soluble gelling agent and two different indicators; 5-bromo-4-chloro-3-indolyl-D-glucuronide that indicates glucuronidase activity and tetrazolium that facilitates colony enumeration. The 3M Petrifilm Rapid *E. coli*/Coliform Count Plate is intended for the use for the enumeration of both *Escherichia coli* and coliforms in various food and beverage products and from environmental surfaces. The 3M Petrifilm Rapid *E. coli*/Coliform Count Plates can be incubated for 18 - 24 hours at 30 ± 1°C or 32 ± 1°C, for *E.* coli and coliforms, and 42 ± 1°C for *E. coli* for dairy products*;* for all other foods; 35 ± 1°C or 37 ± 1°C for for *E.* coli and coliforms, and 42 ± 1°C for *E. coli*. The typical colony morphology for *E. coli* is blue to blue-green colonies with or without gas production, regardless of size or color intensity. Other coliform isolates will appear as red colonies with entrapped gas (within approximately one colony diameter) for enumeration according to FDA BAM, and red colonies with and without gas production according to ISO 4832:2006. Plates containing more than 100 CFU for the total coliform count or more than 100 CFU for the *E. coli* count can either be estimated or recorded as TNTC.

B. Apparatus and Reagents

- **a.** *3M Petrifilm Rapid E. coli/Coliform Count Plates* –. Available from 3M Food Safety - CAT# 6436/6437.
- **b.** 3M Petrifilm Spreader – CAT# 1223767436
- **c.** *Sterile Diluent*- Peptone Salt Solution & Butterfield's Phosphate Buffered Diluent
- **d.** *Pipettes*- capable of pipetting 1,000 µL or a serological pipette
- **e.** *Sterile pipette tips*- capable of 1,000 µL
- **f.** *Laboratory paddle-blender*- Seward 400 or equivalent
- **g.** *Filter Stomacher bags*- Seward or equivalent
- **h.** *Incubators* – Capable of maintaining 30 ± 1°C, 32 ± 1°C, 35 ± 1°C, 37 ± 1°C or 42 ± 1°C
- **i.** *Refrigerator* - capable of maintaining 2-8°C, for storing plates
- **j.** *Freezer* – capable of maintaining -20-0°C for storing plates
- ***k.*** *Standard Colony Counter or Illuminated Magnifier*
- **l.** *Top-loading balance* – capable of weighing 1-2000 g

C. General Instructions

a. Read the instruction manual carefully before use

b. Storage conditions: Store the plates at -20-8°C. Allow the plates to warm to ambient temperature (20-25°C/<60% relative humidity) prior to use. After opening the pouch, unused plates should be placed back in the pouch, sealed and stored at ambient temperature for no longer than 4 weeks. If the temperature of the site is > 25°C with a relative humidity greater than 50%, it is recommended to place the plates in a sealed container and store in a freezer for no more than 4 weeks.

c. Plates containing more than 100 CFU for the total coliform count or more than 100 CFU for the *E. coli* count can either be estimated or recorded as TNTC Note, the enumeration for *E. coli* or total coliforms may occur on separate dilutions.

Safety Precautions

Do not use this plate for the specific detection of *E. coli* O:157 because most *E. coli* O:157 strains are atypical, glucuronidase negative, and will not be detected as *E. coli* but only as coliform. After use, 3M Petrifilm Rapid *E. coli*/Coliform Count Plates may contain microorganisms that may be a potential biohazard. Follow current industry standards and local regulations for disposal of biohazardous waste.

To reduce the risks associated with release of contaminated product:

- Follow all product storage instructions contained in the instructions for use.
- Do not use beyond the use by date
- Do not use 3M Petrifilm Rapid *E. coli*/Coliform Count Plates that show discoloration
- Do not use diluents containing citrate, bisulfate or thiosulfate; they can inhibit growth

To reduce the risks associated with bacterial infection and workplace contamination:

- Perform testing in a properly equipped laboratory under the control of a skilled microbiologist
- The user must train its personnel in current proper testing techniques

To reduce the risk associated with misinterpretation of results:

- 3M has not documented 3M Petrifilm Rapid *E. coli*/Coliform Count Plates for use in industries other than food and beverage. 3M has not documented 3M Petrifilm Rapid *E. coli*/Coliform Count Plates for testing water, pharmaceuticals or cosmetics
- Do not use 3M Petrifilm Rapid *E. coli*/Coliform Count Plates in the diagnosis of conditions in humans or animals
- 3M Petrifilm Rapid *E. coli*/Coliform Count Plates do not differentiate any one *E. coli* or coliforms strain from another
- A few strains of bacteria can produce β-glucuronidase such as *Shigella, Salmonella, Enterobacter, Citrobacter* and *Klebsiella* and will produce blue to blue-green colonies on the 3M Petrifilm Rapid *E. coli*/Coliform Count Plate.
- Foods with high sugar content may increase the potential for gas production from non-coliform *Enterobacteriaceae*

D. Sample Preparation

1. Use appropriate sterile diluents (Butterfield's Phosphate Buffer Diluent (BPBD) or Peptone Salt Solution (PPS)). **Do not use diluents containing citrate, bisulfate, or thiosulfate with the 3M Petrifilm Rapid *E. coli/*Coliform Count Plates, as they can inhibit growth.**

2. For food samples, prepare test portion (10, 11, or 50g) or equivalent ratio of sample to diluent to create a 1:10 dilution as appropriate to the sample being tested.

3. For environmental surface samples, add 25 mL of the appropriate diluent to each sponge sample.

4. Blend or homogenize sample as appropriate.

5. For optimal growth and recovery of microorganisms in acidic products (< pH 5), adjust the pH of the sample suspension to greater than pH 5. For acidic products, adjust with pH 1N NaOH.

6. Remove all required plates and allow to come to ambient temperature (20-25^{o}C).

7. Retract the top film to fully expose the culture medium and dispense 1.0 mL of sample onto the center of the plate.

8. Reapply the cover by rolling down the film. Place the 3M Petrifilm Flat Spreader on the center of the plate and press gently to allow sample to spread evenly over the medium causing gel to form. Let the plate sit undisturbed for at least 1 minute.

9. Incubate the plates

   a) For dairy products: 30 ± 1^{o}C or 32 ± 1^{o}C for coliforms and *E. Coli* or 42 ± 1^{o}C for *E. coli* for 18-24 hours.

   b) for all other foods: 35 ± 1^{o}C, 37 ± 1^{o}C or for coliforms and *E. Coli* or 42 ± 1^{o}C for *E. coli* for 18-24 hours.

10. Enumerate all blue to blue-green colonies with or without gas regardless of size or intensity of color as *E. coli.*

11. Interpretation of non-*E. coli* coliform colonies varies by reference method.

    a. The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) Chapter 4 defines coliforms as Gram negative rods, which produce acid and gas from lactose during metabolic fermentation. Enumerate red colonies which are closely associated with entrapped gas within one colony diameter of the colony. Colonies not associated with gas (a distance greater than one colony diameter between colony and gas bubble) are not counted as coliforms. The total coliform count consists of both the red colonies with gas and blue colonies with and without gas, estimates can be made on plates containing greater than 100 colonies.
    b. ISO defines coliforms by their ability to grow in method-specific, selective media. ISO 48324 enumerates typical coliform colonies on Violet Red Bile Lactose (VRBL) agar, with confirmation of atypical colonies. Enumerate red colonies with and without gas production. The total coliform count is indicated by red colonies with or without gas

production and blue colonies with and without gas production, estimates can be made on plates containing greater than 100 colonies.

12. Multiply the count by the dilution factor to get final coliform and *E. coli* count.

Reference: *J. AOAC Int*. (future issue)

Table 2018.13A Interlaboratory Study Results of 3M Petrifilm Rapid *E. coli*/Coliform Count Plate vs. FDA BAM Chapter 4												
3M Petrifilm Rapid *E. coli*/Coliform Count Plate - Coliform (35°C)						**FDA BAM Chapter 4 Coliform**					**Difference of Means[b]**	**Difference of Means[c] 95% LCL, UCL**
Matrix	**Lot**	**N[a]**	**Mean Log_{10} CFU/g**	**s_r**	**s_R**	**Lot**	**N[a]**	**Mean Log_{10} CFU/g**	**s_r**	**s_R**		
Dry Pet Kibble	Uninoculated	9	0.000	0.00	0.00	Uninoculated	9	0.000	0.00	0.00	0.00	0.00, 0.00
	Low	9	1.645	0.22	0.25	Low	9	1.617	0.18	0.31	-0.30	-0.20, 0.14
	Medium	9	2.687	0.12	0.16	Medium	9	2.660	0.10	0.12	-0.30	-0.09, 0.03
	High	9	3.458	0.10	0.17	High	9	3.474	0.12	0.17	0.02	-0.06, 0.10
3M Petrifilm Rapid *E. coli*/Coliform Count Plate – *E. coli* (35°C)						**FDA BAM Chapter 4 *E. coli***					**Difference of Means[b]**	**Difference of Means[c] 95% LCL, UCL**
Dry Pet Kibble	Uninoculated	9	0.000	0.00	0.00	Uninoculated	9	0.000	0.00	0.00	0.00	0.00, 0.00
	Low	9	1.422	0.22	0.31	Low	9	1.448	0.20	0.32	0.02	-0.17, 0.22
	Medium	9	2.298	0.12	0.26	Medium	9	2.292	0.11	0.22	-0.01	-0.12, 0.11
	High	9	3.236	0.14	0.22	High	9	3.220	0.15	0.17	-0.02	-0.14, 0.10

[a]Number of collaborators that reported complete results.

[b]A Difference of means <0.5 indicates no statistically significant difference be
methods

[c] 95% Lower and Upper Confidence Limits

s_r – Repeatability

s_R – Reproducibility

Table 2018.13B Interlaboratory Study Results of 3M Petrifilm Rapid *E. coli*/Coliform Count Plate vs. ISO 4832 & 16649-2												
3M Petrifilm Rapid *E. coli*/Coliform Count Plate – Coliform (37°C)						**ISO 4832**					**Difference of Means[b]**	**Difference of Means[c] 95% LCL, UCL**
Matrix	**Lot**	**N[a]**	**Mean Log_{10} CFU/g**	**s_r**	**s_R**	**Lot**	**N[a]**	**Mean Log_{10} CFU/g**	**s_r**	**s_R**		
Dry Pet Kibble	Uninoculated	9	0.000	0.00	0.00	Uninoculated	9	0.000	0.00	0.00	0.00	0.00, 0.00
	Low	9	1.616	0.28	0.34	Low	9	1.692	0.22	0.24	0.08	-0.09, 0.25
	Medium	9	2.583	0.09	0.19	Medium	9	2.557	0.09	0.23	-0.03	-0.12, 0.07
	High	9	3.359	0.09	0.16	High	9	3.352	0.09	0.12	-0.01	-0.10, 0.08
3M Petrifilm Rapid *E. coli*/Coliform Count Plate – *E. coli* (37°C)						**ISO 16649-5**						
Dry Pet Kibble	Uninoculated	9	0.000	0.00	0.00	Uninoculated	9	0.000	0.00	0.00	0.00	0.00, 0.00
	Low	9	1.419	0.28	0.28	Low	9	1.542	0.22	0.22	0.12	-0.04, 0.29
	Medium	9	2.291	0.12	0.19	Medium	9	2.296	0.11	0.17	0.01	-0.11, 0.12
	High	9	3.140	0.11	0.14	High	9	3.202	0.16	0.21	0.04	-0.07, 0.16
3M Petrifilm Rapid *E. coli*/Coliform Count Plate – *E. coli* (42°C)						**ISO 16649-5**					**Difference of Means[b]**	**Difference of Means[c] 95% LCL, UCL**
Dry Pet Kibble	Uninoculated	9	0.000	0.00	0.00	Uninoculated	9	0.000	0.00	0.00	0.00	0.00, 0.00
	Low	9	1.400	0.27	0.27	Low	9	1.542	0.22	0.22	0.14	0.02. 0.27
	Medium	9	2.342	0.13	0.15	Medium	9	2.296	0.11	0.17	-0.05	-0.15. 0.06
	High	9	3.133	0.14	0.14	High	9	3.202	0.16	0.21	0.05	-0.09, 0.19

[a]Number of collaborators that reported complete results.

[b]A Difference of means <0.5 indicates no statistically significant difference between r

[c] 95% Lower and Upper Confidence Limits

s_r – Repeatability

s_R – Reproducibility