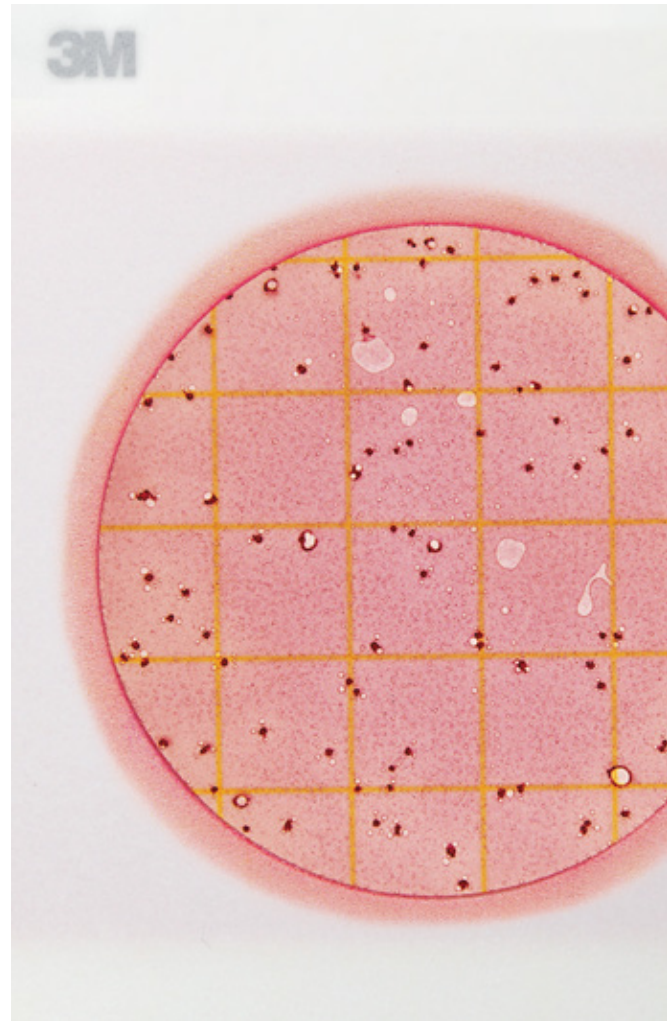




Petrifilm™

Interpretation Guide

The 3M™ Petrifilm™ Coliform Count Plate is a sample-ready-culture medium system which contains modified Violet Red Bile nutrients, a cold-water-soluble gelling agent and a tetrazolium indicator that facilitates colony enumeration.



Coliform Count Plate

Food and Beverage Applications

Coliform Definitions by Method

The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) defines coliforms as Gram negative rods, which produce acid and gas from lactose fermentation. Coliform colonies growing on the 3M™ Petrifilm™ Coliform Count Plate produce acid, which causes the pH indicator to deepen the gel color, and gas trapped around red colonies. In this interpretation guide, the number of coliforms per the FDA BAM definition is the number of red colonies with gas.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832 enumerates typical coliform colonies on Violet Red Bile Lactose (VRBL) agar, with confirmation of atypical colonies. On the 3M Petrifilm Coliform Count Plate, these coliforms are indicated by red colonies with or without gas production. ISO method 4831, enumerating coliforms by the most probable number (MPN) method, defines coliforms by their ability to grow and produce gas in the conditions described in the standard. On the 3M Petrifilm Coliform Count Plate, these coliforms are indicated by red colonies with gas.

It is also possible to enumerate thermotolerant coliforms on the 3M Petrifilm Coliform Count Plate. Typically thermotolerant coliforms can be selected with an elevated incubation temperature. One example of a method for enumeration of thermotolerant coliforms is described in NF V08 060. Reading the total of red colonies on a 3M Petrifilm Coliform Count Plate incubated at $44^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for $24\text{h} \pm 2\text{h}$ yields results equivalent to enumeration with NF V08 060.

Please refer to the product instructions for additional information.

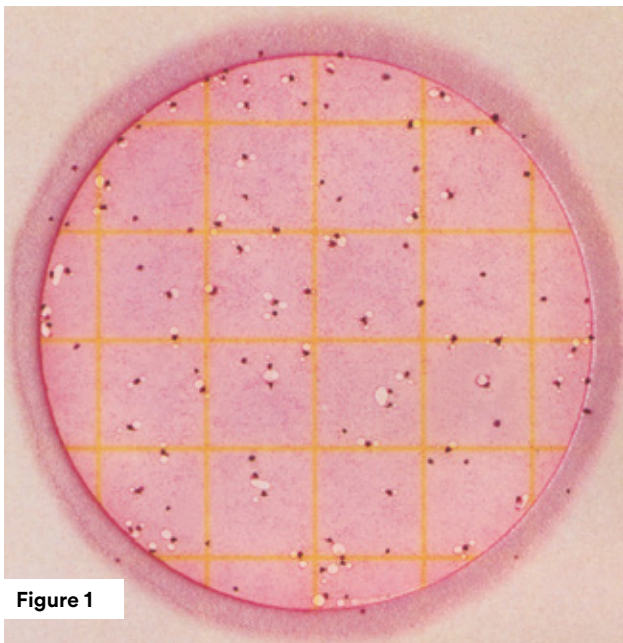


Figure 1

Total colonies with gas = 69
Total colonies = 94

The definition of coliforms may vary by country.

Please refer to section above and product instructions for definitions.

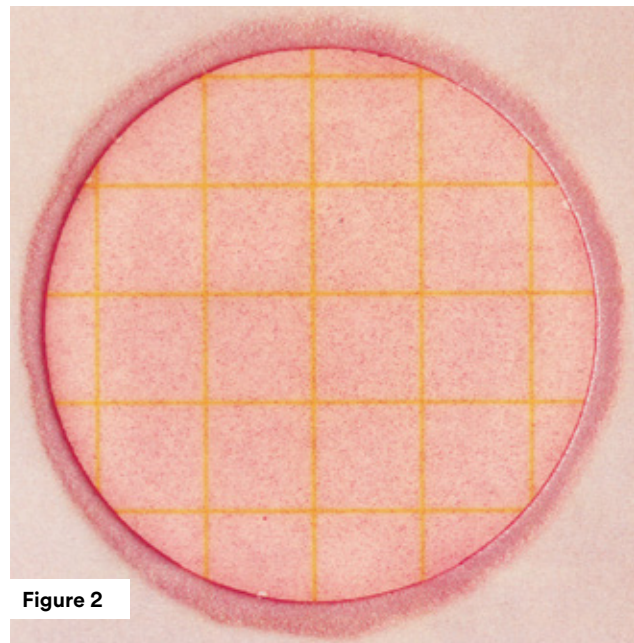


Figure 2

No growth = 0

Notice the changes in gel color in Figures 2–5. As the coliform count increases, the gel color deepens.

Background bubbles are a characteristic of the gel and are not a result of coliform growth.

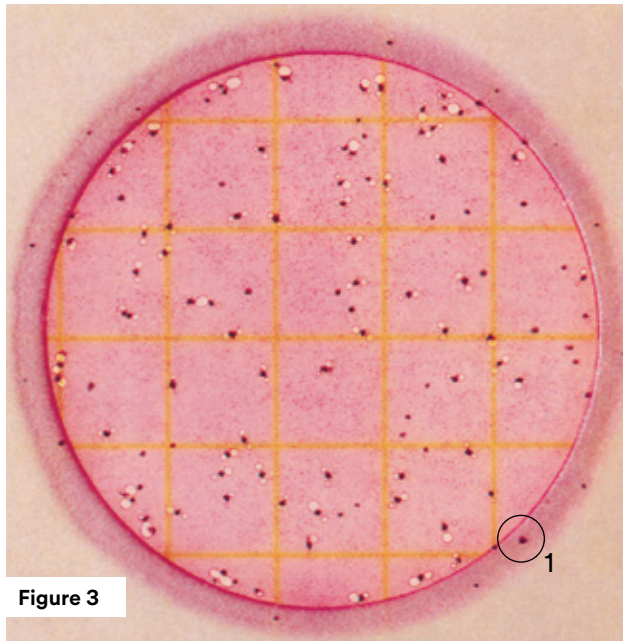


Figure 3

Total colonies with gas = 79
Total colonies = 109

The recommended counting limit on 3M Petrifilm Coliform Count Plates is less than 150.

Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the medium (see Circle 1).

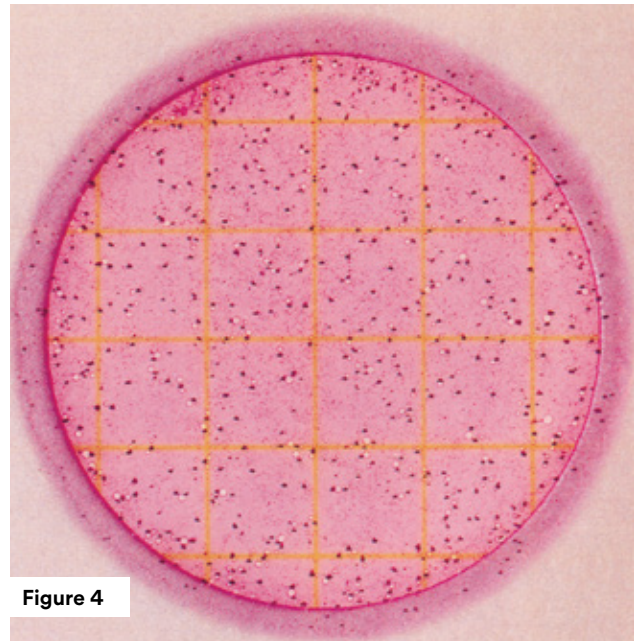


Figure 4

Estimated total coliform count = 220

The circular growth area is approximately 20cm². Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.

For a more accurate count, further dilution of sample may be necessary.

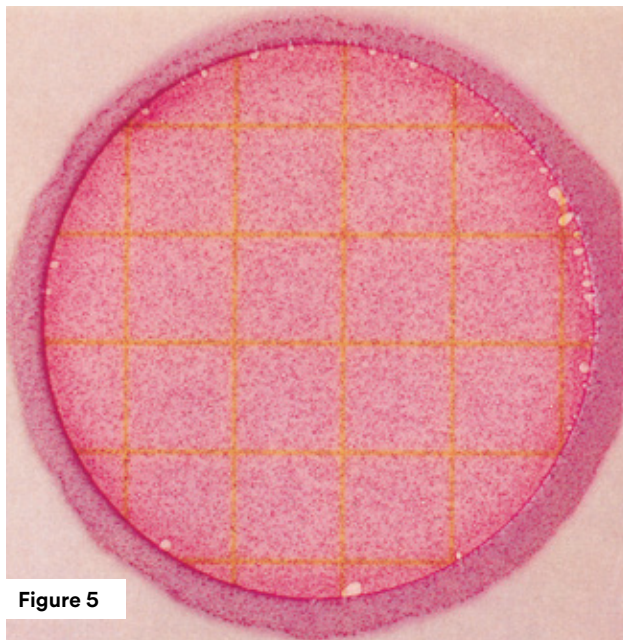


Figure 5

Total coliform count = TNTC

3M Petrifilm Coliform Count Plates with colonies that are too numerous to count (TNTC) have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel color.

For a more accurate count, further dilution of sample may be necessary.

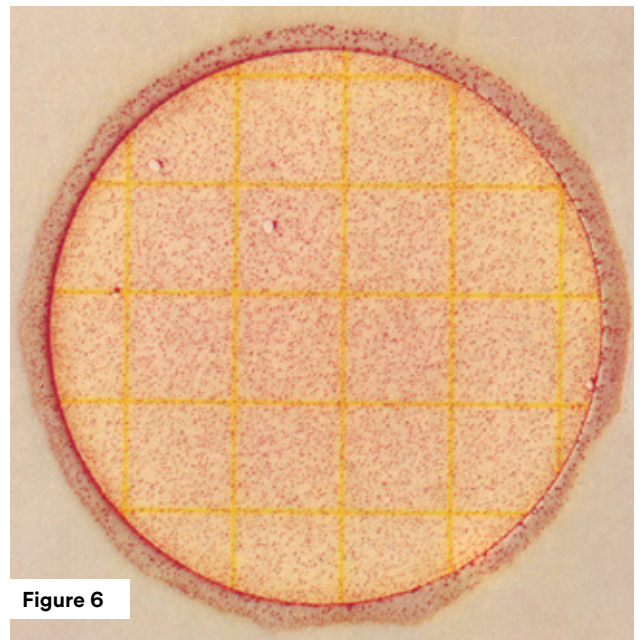


Figure 6

Total count = TNTC

When high numbers of non-coliform organisms such as *Pseudomonas* are present on 3M Petrifilm Coliform Count Plates, the gel may turn yellow.

For a more accurate count, further dilution of sample may be necessary.

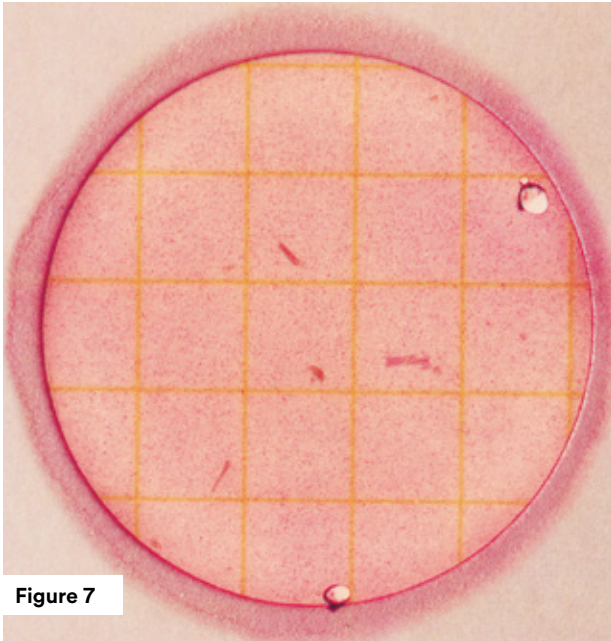


Figure 7

Total colonies with gas = 2
Total colonies = 2

Food particles are irregularly shaped and are not associated with gas bubbles.

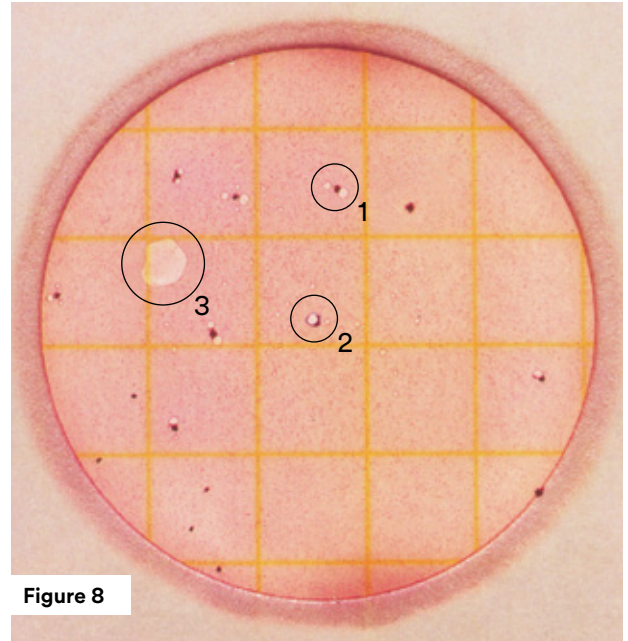


Figure 8

Total colonies with gas = 8
Total colonies = 15

Bubble patterns may vary. Gas may disrupt the colony so that the colony "outlines" the bubble (see Circles 1 and 2). Artifact bubbles may result from improper inoculation or from trapped air within the sample. They are irregularly shaped and are not associated with a colony (see Circle 3).

Bottled Water Applications

Coliform colonies are indicated by red colonies associated with gas for bottled water samples plated to 3M Petrifilm Coliform Count Plates.

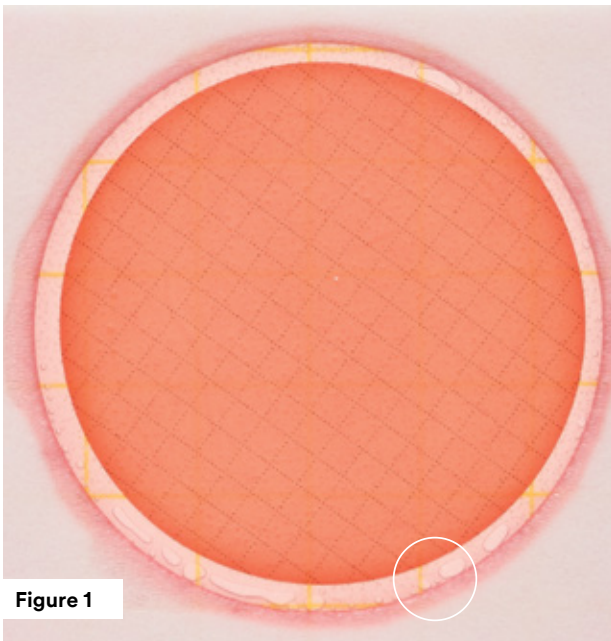


Figure 1

Coliform count: 0

Gas bubbles surrounding filter do not indicate microbial growth. See circle for example.

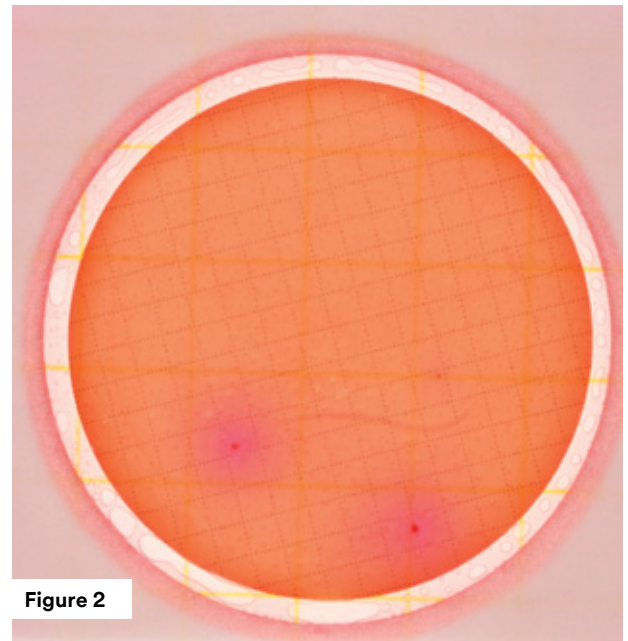


Figure 2

Coliform count: 0

Red colonies without closely associated gas bubbles may be coliforms and should be picked and tested with appropriate confirmation methods.

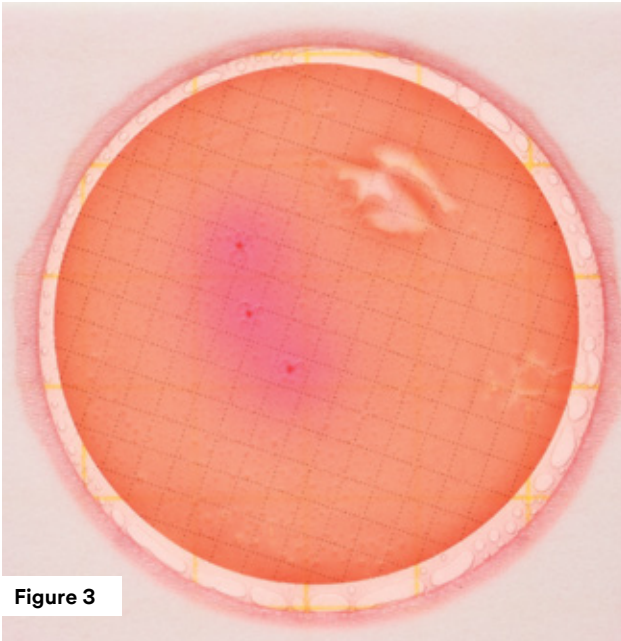


Figure 3

Coliform count: 3
 Coliforms produce acid (faint pink halo associated with colonies) and are associated with gas bubbles.

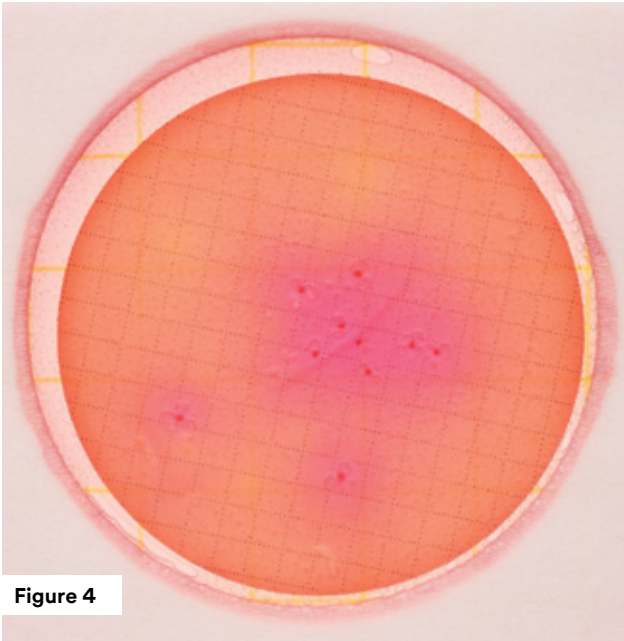


Figure 4

Coliform count: 10

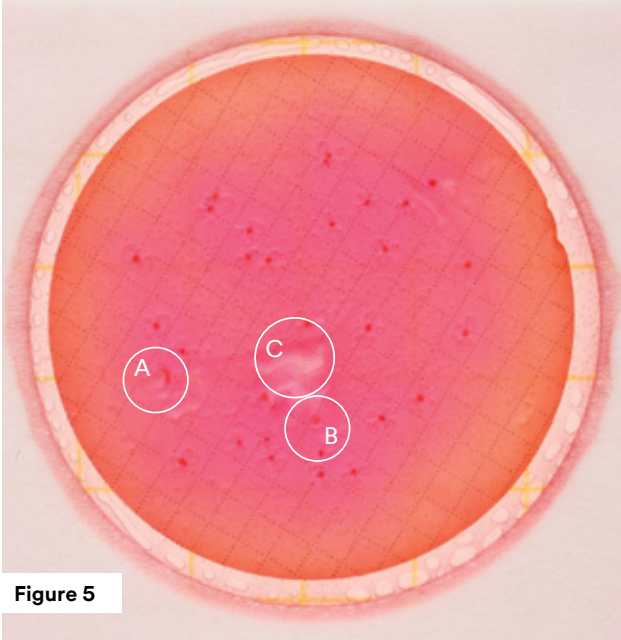
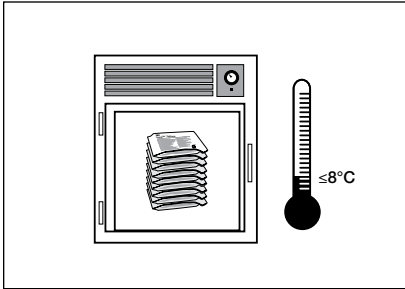


Figure 5

Coliform count: 30
 Gas bubbles may influence colony morphologies. The colony in Circle A is distorted by the gas bubble. In Circle B, a faint colony is underneath the gas bubble. Note large artifact gas bubble in the center of the plate (Circle C).

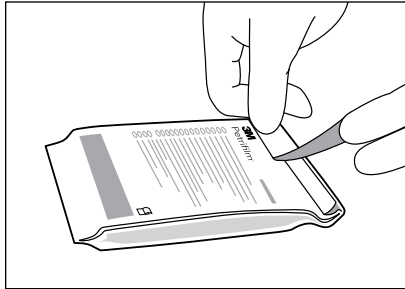
Reminders for Use: Food and Beverage Applications

Storage

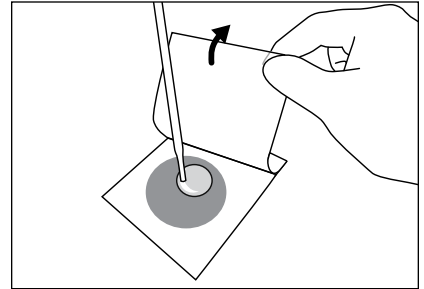


- 1 Store unopened pouches of plates at $\leq 8^{\circ}\text{C}$ ($\leq 46^{\circ}\text{F}$). Use before expiration date on package. In areas of high humidity where condensate may be an issue, it is best to allow pouches to reach room temperature before opening.

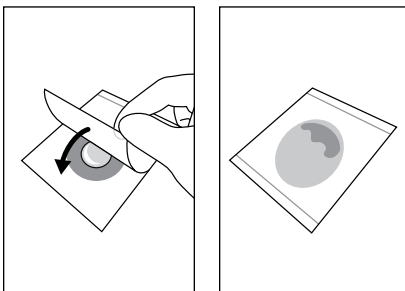
Inoculation



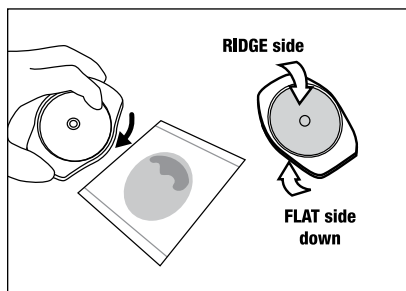
- 2 To seal opened pouch, fold end over and apply adhesive tape.



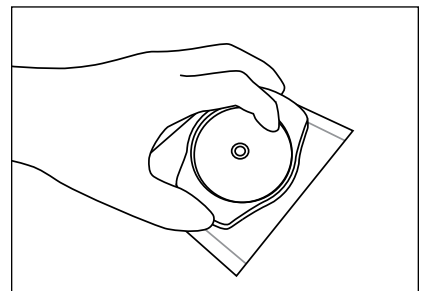
- 3 Place 3M Petrifilm Coliform Count Plate on level surface. Lift top film. With a pipette **perpendicular** to plate, place 1mL of sample or diluted sample onto center of bottom film.



- 4 Roll top film down onto sample **gently** to prevent pushing sample off film and to avoid entrapping air bubbles. **Do not** let top film drop.

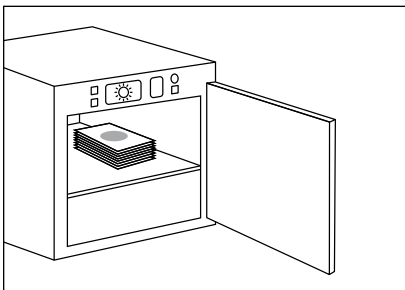


- 5 With **flat** side down, place 3M™ Petrifilm™ Spreader on top film over inoculum.



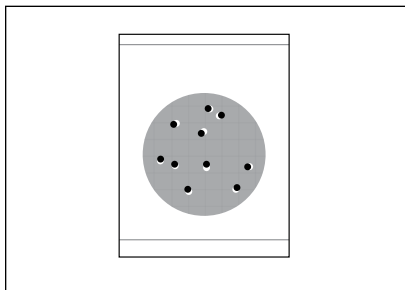
- 6 **Gently** apply pressure on 3M Petrifilm Spreader to distribute inoculum over circular area before gel is formed. **Do not** twist or slide the spreader. Lift 3M Petrifilm Spreader. Wait a minimum of 1 minute for gel to solidify.

Incubation



- 7 Incubate plates with clear side up in stacks of up to 20. It may be necessary to humidify incubator to minimize moisture loss. **See product instructions for third party validated methods.**

Interpretation



- 8 3M Petrifilm Coliform Count Plates can be counted using the 3M™ Petrifilm Plate Reader, on a standard colony counter or other illuminated magnifier. Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Use Appropriate Sterile Diluents

Butterfield's phosphate buffered dilution water, 0.1% peptone water, peptone salt diluents, buffered peptone water, saline solution (0.85–0.90%), bisulfite-free letheen broth or distilled water.

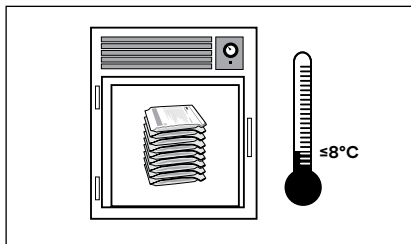
For optimal growth and recovery of the microorganisms, adjust the pH of the sample suspension to 6.6–7.2.

Do not use diluents containing citrate, bisulfite or thiosulfate with the 3M Petrifilm Coliform Count Plates; they can inhibit growth.

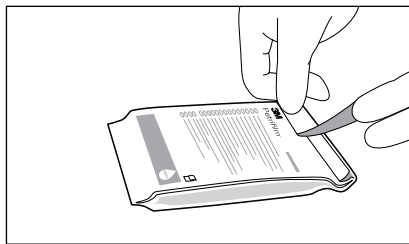
If citrate buffer is indicated in the standard procedure, substitute with one of the buffers listed above, warmed to 40–45°C.

Reminders for Use: Bottled Water Applications

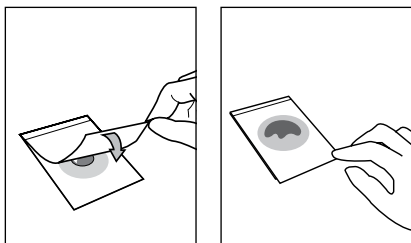
Storage



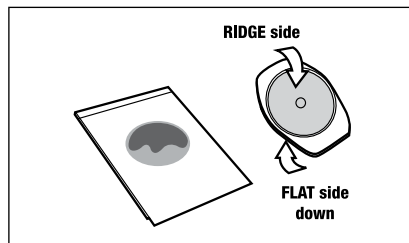
- 1 Store unopened packages at $\leq 8^{\circ}\text{C}$ ($\leq 46^{\circ}\text{F}$). Use before expiration date on package. Just prior to use, allow unopened pouches to come to room temperature before opening.



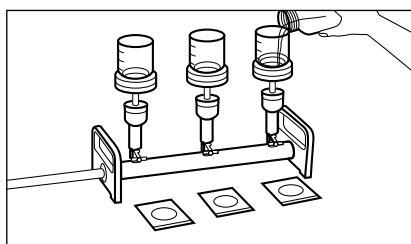
- 2 To seal opened package, fold end over and apply adhesive tape. **Do not refrigerate opened packages.** Use 3M Petrifilm Coliform Count Plates within one month after opening.



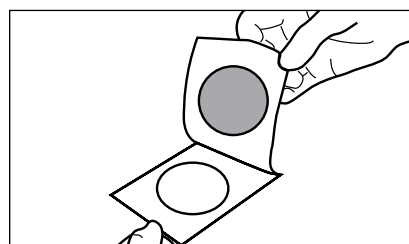
- 4 Carefully roll top film down to avoid entrapping air bubbles. Do not let top film drop.



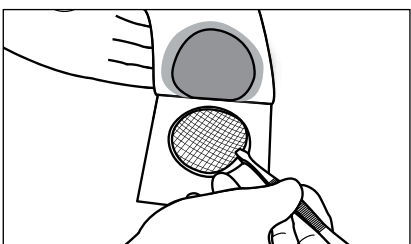
- 5 With flat side down, place 3M™ Petrifilm™ Spreader on top film over hydration diluent.



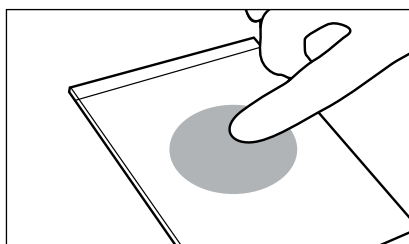
- 7 Following standard procedures for water analysis, membrane filter water sample using a 47 mm, 0.45 micron pore size Mixed Cellulose Ester (MCE) filter.



- 8 Carefully lift top film.

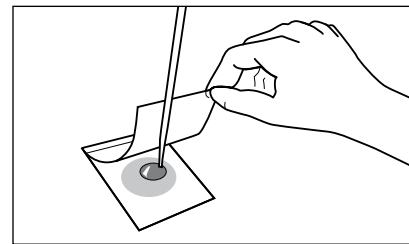


- 9 Place filter in the center of the well. Roll top film down to minimize air bubbles or gaps between the filter and the 3M Petrifilm Coliform Count Plate.

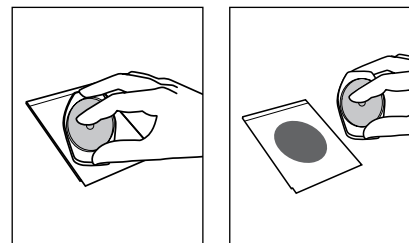


- 10 Lightly apply pressure to ensure uniform contact of the filter with the gel and to eliminate any air bubbles.

Hydration Procedure



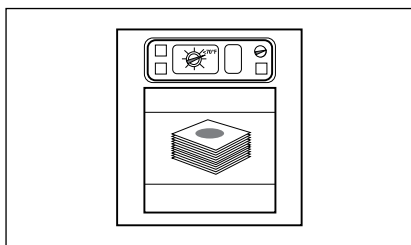
- 3 Place 3M Petrifilm Plate on a flat, level surface. With the pipette perpendicular to the 3M Petrifilm Plate, place hydration diluent onto the center of the bottom film. Hydration diluents include distilled water, deionized (DI) water and reverse osmosis (RO) water.



- 6 Gently apply pressure on spreader to distribute inoculum or hydration diluent over circular area before gel is formed. Do not twist or slide spreader. Lift spreader.

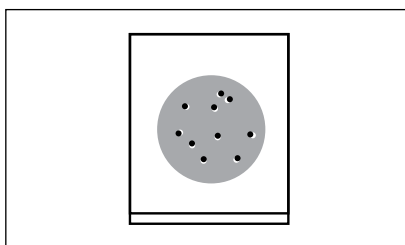
- Allow the hydrated plates to remain closed for a minimum of one hour before use.
- Any additional hydrated 3M Petrifilm Coliform Count Plates may be stored in a sealed pouch or plastic bag. Protect plates from light and refrigerate at $2-8^{\circ}\text{C}$ ($36-46^{\circ}\text{F}$) for up to 7 days.

Incubation

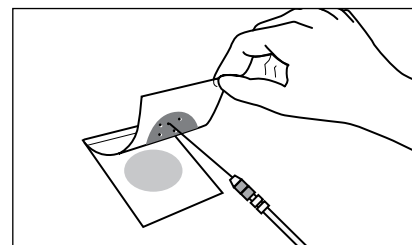


- 11** Incubate 3M Petrifilm Coliform Count Plates in a horizontal position, clear side up, in stacks on no more than 20 plates at $35^{\circ}\pm 1^{\circ}\text{C}$ for 24 ± 2 hours or $36^{\circ}\pm 1^{\circ}\text{C}$ for 24 ± 2 hours. **Please refer to the product instructions for third party validated methods.**

Interpretation



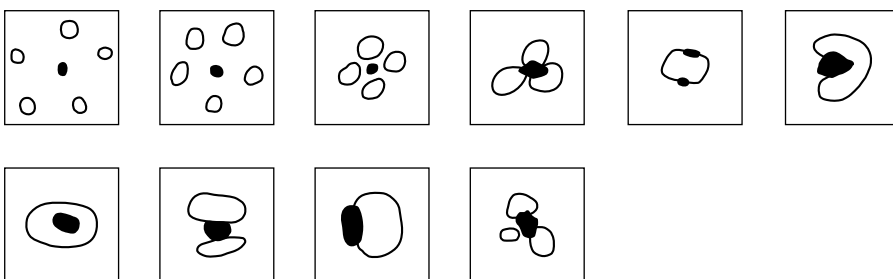
- 12** 3M Petrifilm Coliform Count Plates can be counted on a standard colony counter or other illuminated magnifier.



- 13** Colonies may be isolated for further identification. Lift top film and pick the colony from the gel. Red colonies without closely associated gas bubbles may be coliforms and should be picked and tested with appropriate confirmation methods.

Bubbles

The illustrations below show examples of various bubble patterns associated with gas producing colonies. All should be enumerated.



3M Food Safety offers a full line of products to accomplish a variety of your microbial testing needs. For more product information, visit us at 3M.com/foodsafety/Petrifilm or call 1-800-328-6553.



3M

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