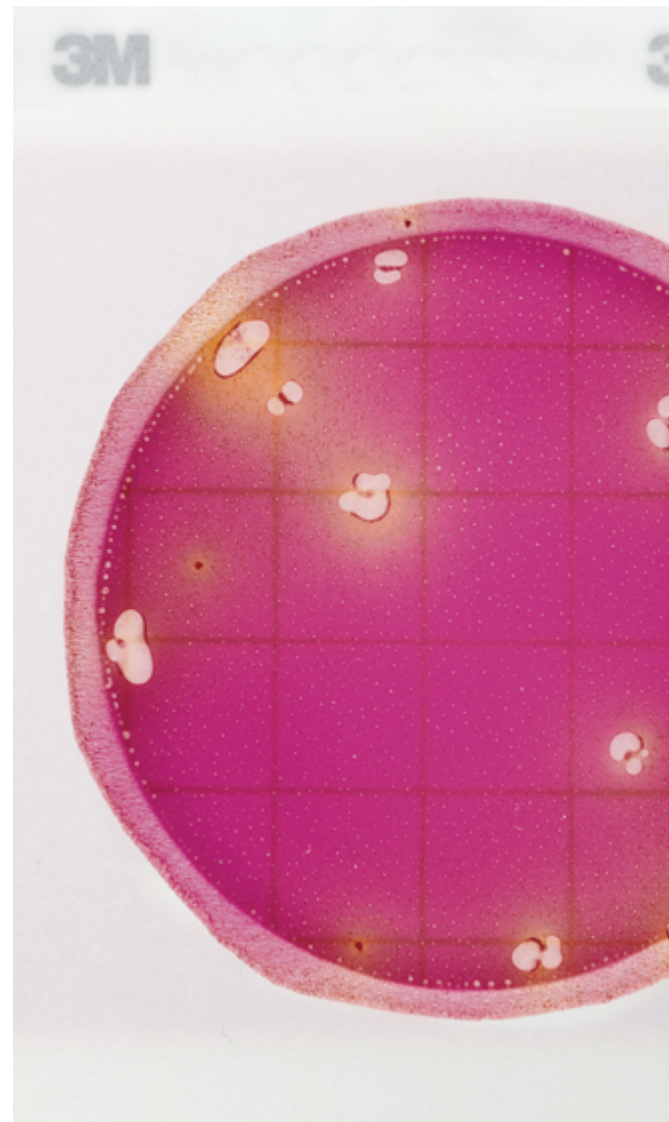




Petrifilm™

# Interpretation Guide

The 3M™ Petrifilm™ *Enterobacteriaceae* Count Plate is a sample-ready-culture medium system that contains modified Violet Red Bile Glucose (VRBG) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm *Enterobacteriaceae* Count Plates are used for the enumeration of *Enterobacteriaceae* in the food, beverage and bottled water industries. *Enterobacteriaceae* are oxidase-negative, Gram-negative rods that ferment glucose to produce acid and/or gas. *Enterobacteriaceae* colonies will appear as red colonies associated with yellow zones, red colonies associated with gas bubbles, red colonies associated with yellow zones and with gas bubbles.



**EB**

*Enterobacteriaceae* Count Plate

# Food and Beverage Application

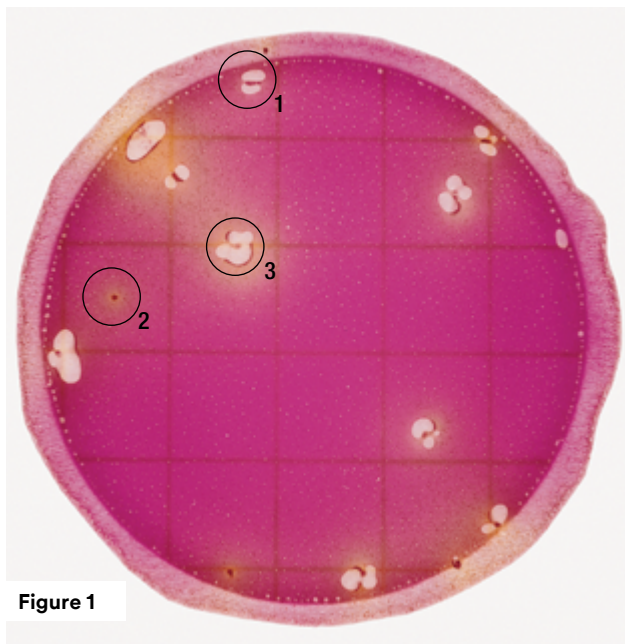


Figure 1

***Enterobacteriaceae* count = 13**

Figure 1 illustrates the three types of typical colonies. Sometimes gas disrupts the colony so that the colony "outlines" the gas bubbles.

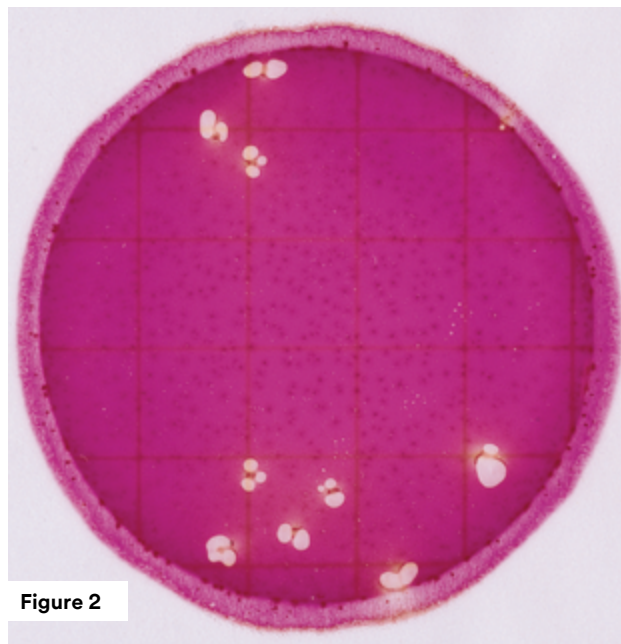


Figure 2

***Enterobacteriaceae* count = 9**

Figure 2 shows a 3M™ Petrifilm™ *Enterobacteriaceae* Count Plate with a few *Enterobacteriaceae* colonies and a high number of non-*Enterobacteriaceae*, Gram-negative colonies. Do not count colonies on the foam dam since they are removed from the selective influence of the medium.

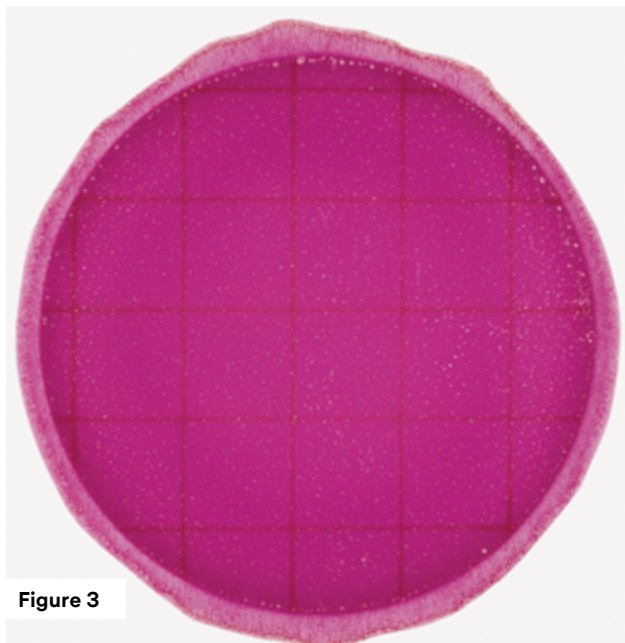


Figure 3

***Enterobacteriaceae* count = 0**

Notice the change in gel color in Figures 3 to 8. As the *Enterobacteriaceae* count increases, the color of the gel lightens from purple to yellow or cream colored.

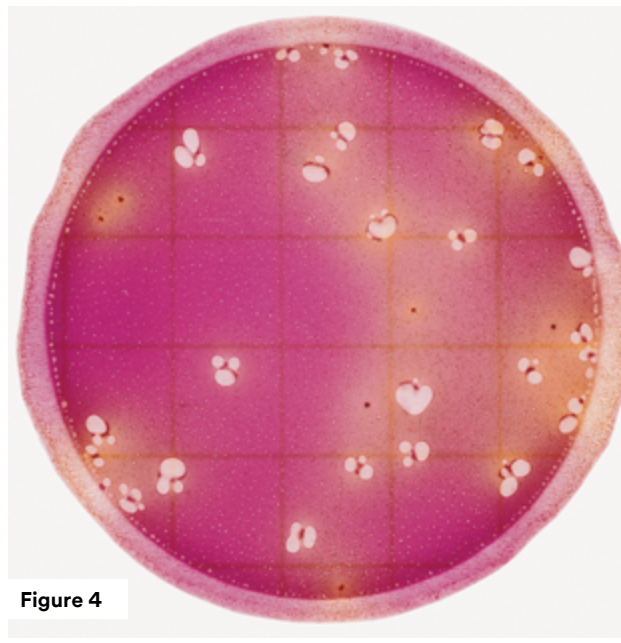
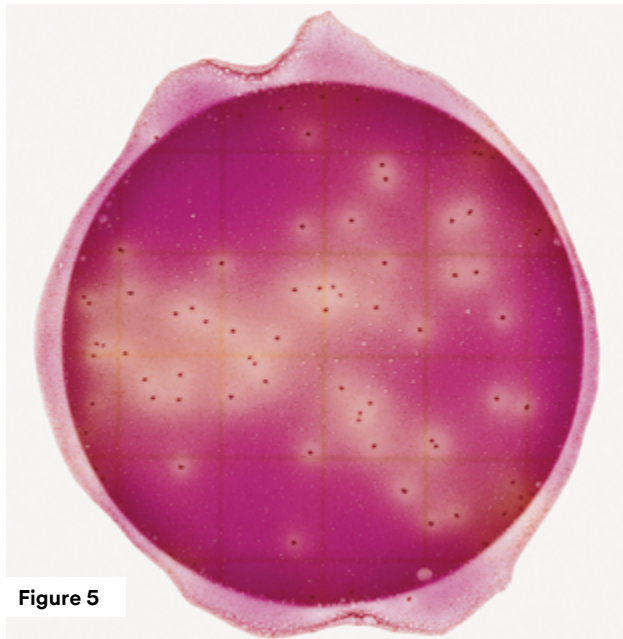


Figure 4

***Enterobacteriaceae* count = 35**

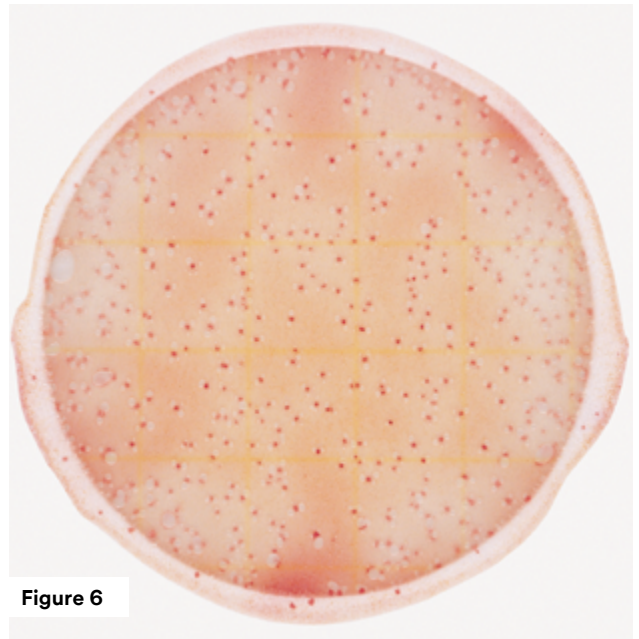


**Figure 5**

**Enterobacteriaceae count = 77**

The recommended counting range on 3M Petrifilm *Enterobacteriaceae* Count Plate is less than or equal to 100 colonies. Samples having counts greater than 100 *Enterobacteriaceae* per plate may be estimated. The circular growth area is approximately 20cm<sup>2</sup>. Estimates can be made by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number of colonies per square by 20 to determine the estimated count per plate.

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

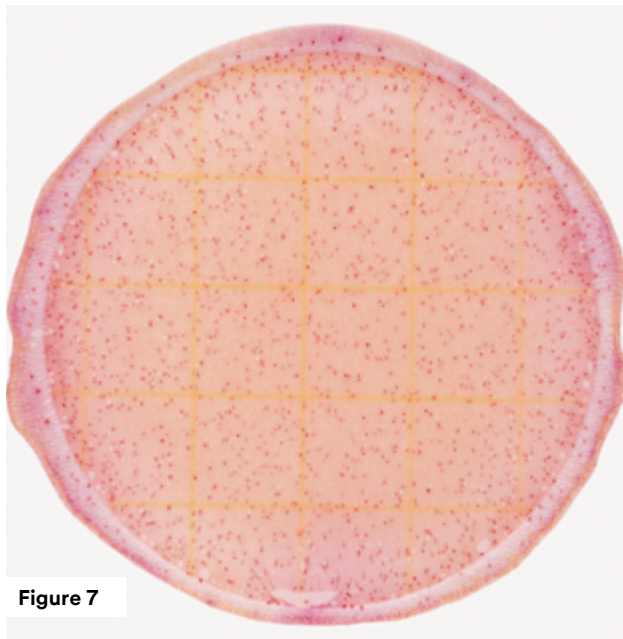


**Figure 6**

**Enterobacteriaceae count = TNTC**

3M Petrifilm *Enterobacteriaceae* Count Plates with more than 100 colonies are considered too numerous to count (TNTC). When colonies are present in large numbers, plates will have a deepening of the gel color or may turn completely yellow, and either or both of the following characteristics: many small, indistinct colonies and/or many gas bubbles. When this occurs, record results as TNTC.

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

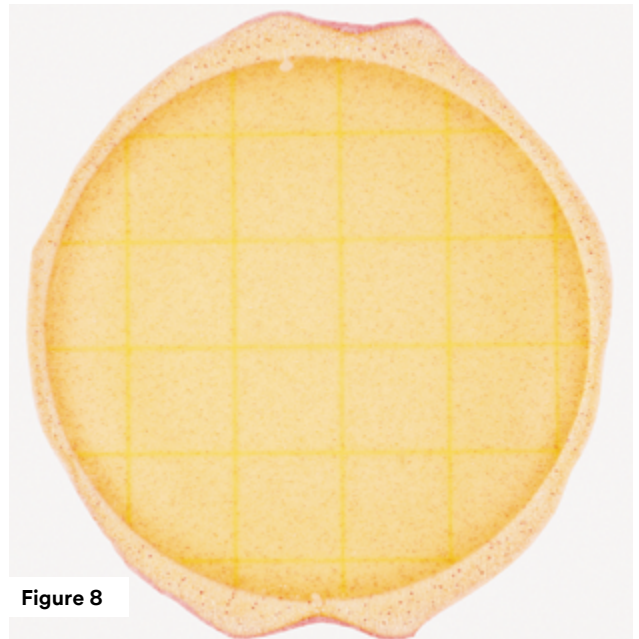


**Figure 7**

**Enterobacteriaceae count = TNTC**

In Figure 7, the count is so high that acid zones and gas bubbles are not easily seen. A lightening of the gel color indicates that the result is TNTC.

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



**Figure 8**

**Enterobacteriaceae count = TNTC**

The 3M Petrifilm *Enterobacteriaceae* Count Plate in Figure 8 has two characteristics indicating TNTC colonies: lightening of the gel color and many small colonies.

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

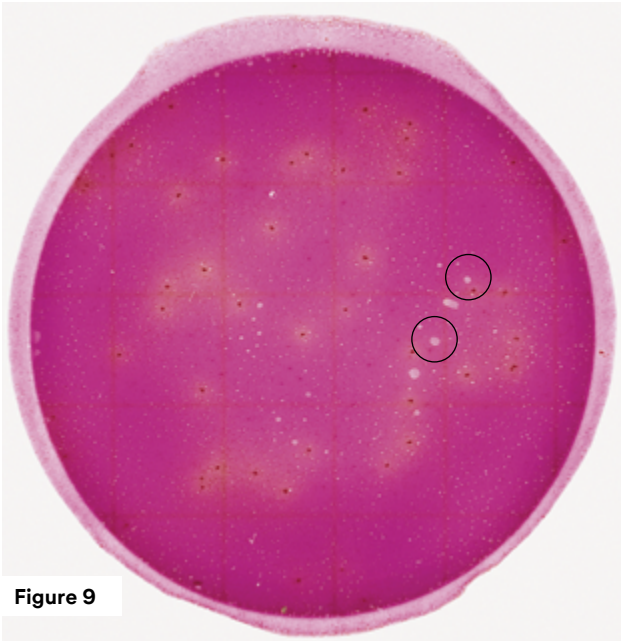


Figure 9

***Enterobacteriaceae* count = 44**

Artifact bubbles may result from improper inoculation of the 3M Petrifilm *Enterobacteriaceae* Count Plate. They are irregularly shaped and not associated with a red colony. Do not enumerate.

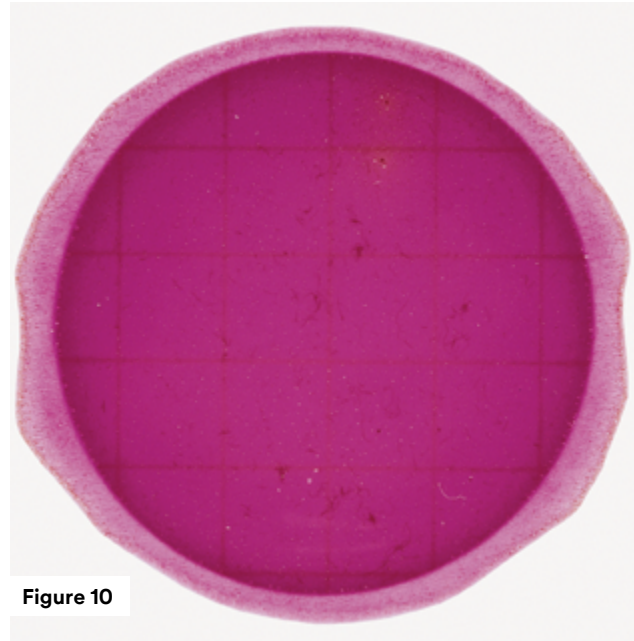


Figure 10

***Enterobacteriaceae* count = 2**

Food particles are often irregularly shaped or filamentous and are not associated with gas bubbles or acid zones. Do not enumerate.

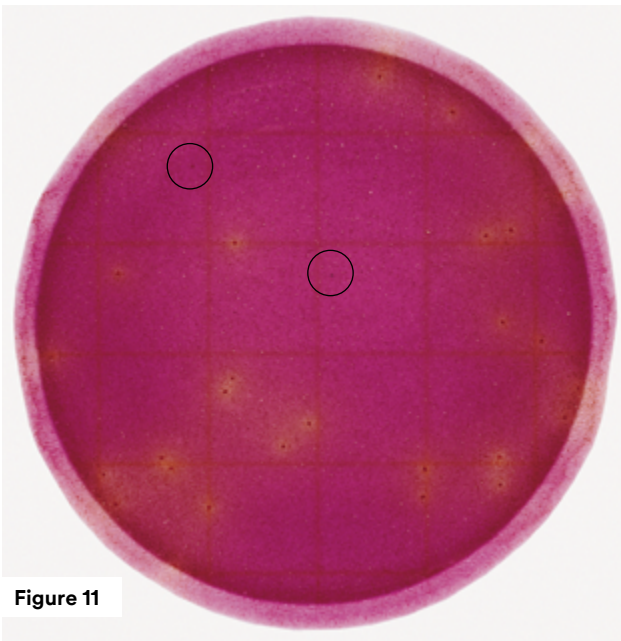


Figure 11

***Enterobacteriaceae* count = 29**

Food particles also can be seen as dark spots but are not associated with gas bubbles or acid zones. Do not enumerate.

# Bottled Water Application

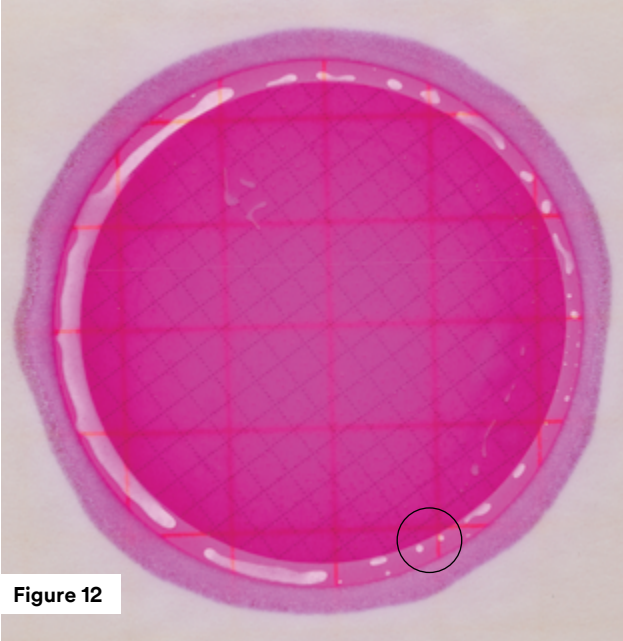


Figure 12

***Enterobacteriaceae* count = 0**

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

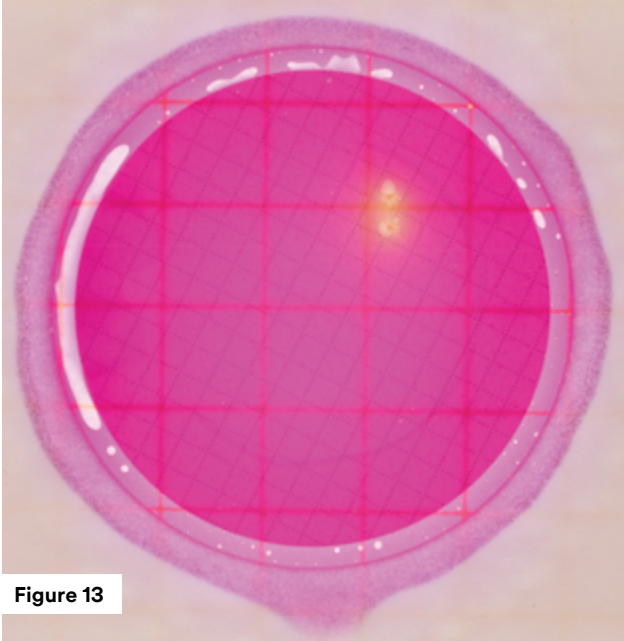


Figure 13

***Enterobacteriaceae* count = 2**

*Enterobacteriaceae* are identified by the presence of acid (yellow halo) and/or colony-associated gas bubbles.

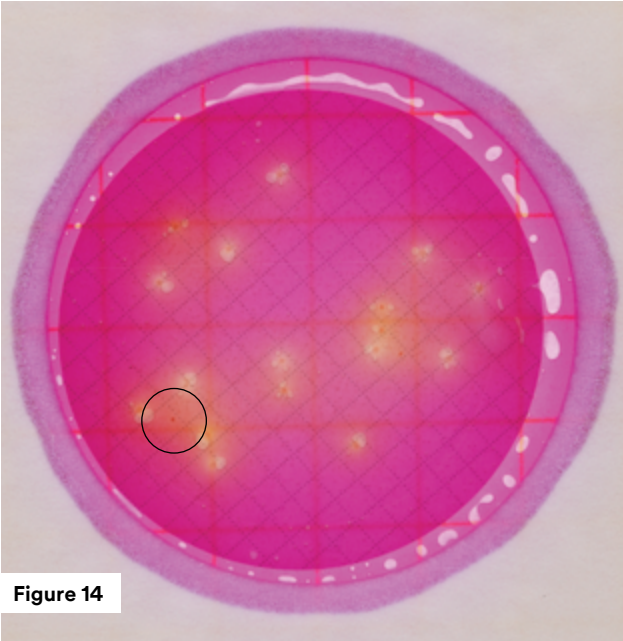


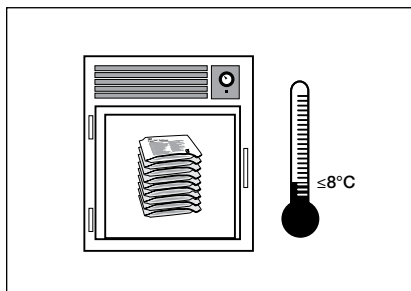
Figure 14

***Enterobacteriaceae* count = 17**

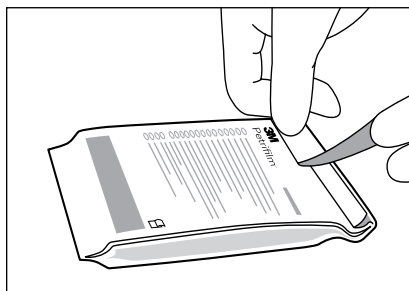
Red colonies without acid or gas production (circle) are not counted as *Enterobacteriaceae*.

# Reminders for Use: Food and Beverage Applications

## Storage

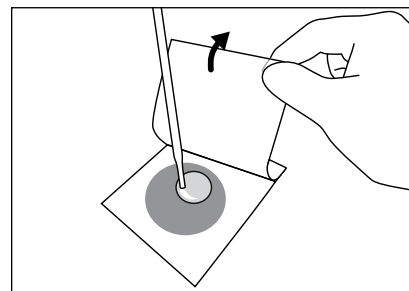


- 1 Store the unopened 3M Petrifilm *Enterobacteriaceae* Count Plate pouches at frozen or refrigerated temperatures lower than or equal to 8°C (46°F). Use before expiration date on package. It is best to allow pouches to reach room temperature before opening.

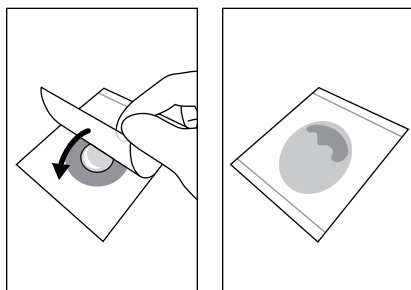


- 2 Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than four weeks.

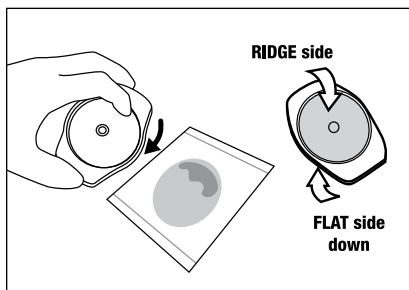
## Inoculation



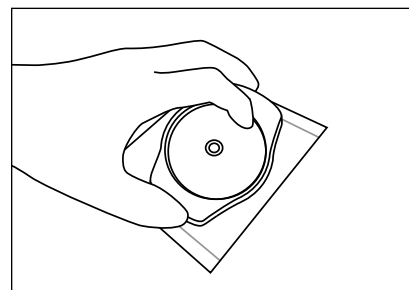
- 3 Place the 3M Petrifilm *Enterobacteriaceae* Count Plate on a level surface. Lift the top film and with the pipette perpendicular to the inoculation area, dispense 1mL of sample suspension onto the center of the bottom film.



- 4 Roll the top film down onto the sample gently to prevent trapping air bubbles. Do not let the top film drop.

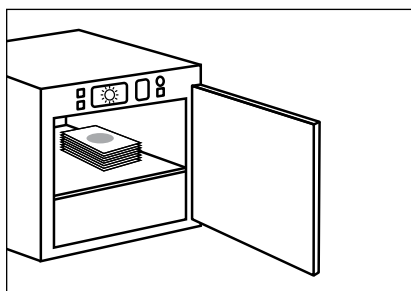


- 5 With flat side down, place 3M™ Petrifilm™ Spreader on top film over inoculum.



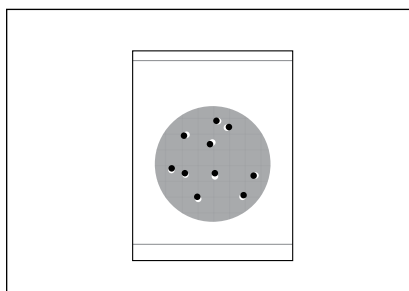
- 6 Gently apply pressure on 3M Petrifilm Spreader to distribute inoculum over circular area before gel is formed. Do not twist or slide the spreader. Lift 3M Petrifilm Spreader. Wait a minimum of 1 minute for gel to solidify.

## Incubation



- 7 Incubate plates with clear side up in stacks of no more than 20 plates. It may be necessary to humidify the incubator to minimize moisture loss. **See product instructions for third party validated methods.**

## Interpretation



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

## Use Appropriate Sterile Diluents

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

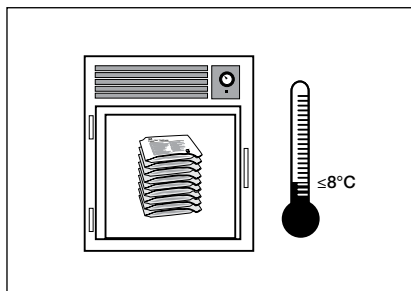
For optimal growth and recovery of the microorganisms, adjust the pH of the sample suspension to 6.5 to 7.5.

**Do not use diluents containing citrate, bisulfite or thiosulfate with the 3M Petrifilm *Enterobacteriaceae* 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.

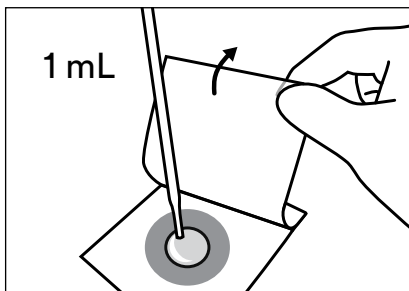
# Reminders for Use: Bottled Water Applications

## Storage

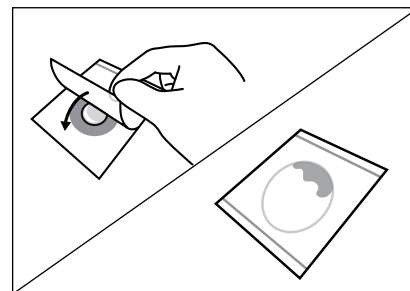


- 1 Follow steps 1 and 2 of Food and Beverage Application Reminders for Use.

## Hydration

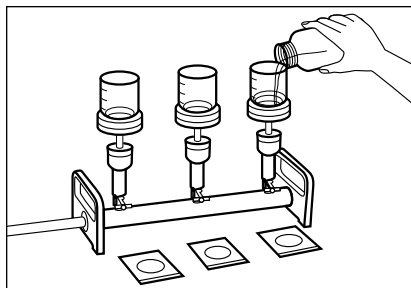


- 2 Place 3M Petrifilm *Enterobacteriaceae* Count Plate on a flat, level surface. Lift the top film and dispense 1 mL of an appropriate sterile hydration diluent onto the center of the bottom film. Appropriate sterile diluents include sterile water, deionized (DI) water and reverse osmosis (RO) water.

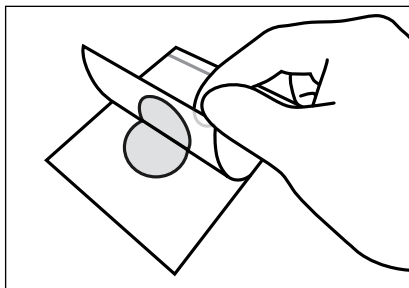


- 3 Roll the top film down onto the sample to prevent trapping air bubbles. Place the 3M™ Petrifilm™ Spreader with the flat side down on the center of the plate. Press gently on the center of spreader to distribute the diluent evenly. Remove the spreader and allow the plates to remain closed for a minimum of 1 hour before use.

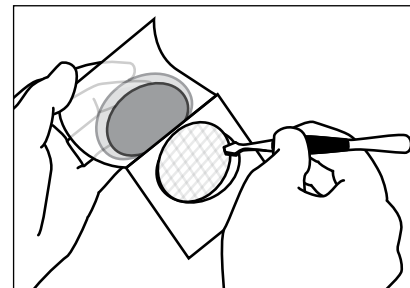
## Filtration



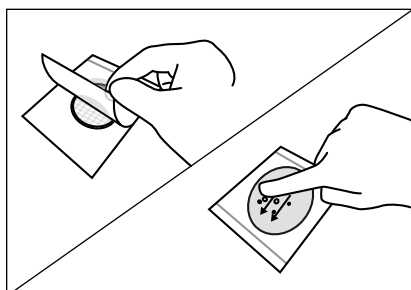
- 4 For membrane filtration of water samples use a 47mm, 0.45 micron pore size Mixed Cellulose Ester (MCE) filter.



- 5 Carefully lift the top film.

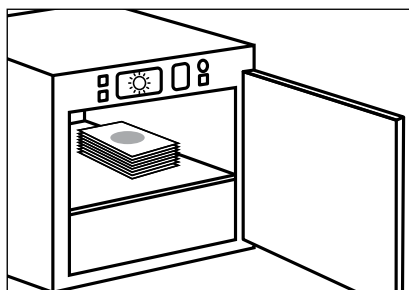


- 6 Place filter in the center of the hydrated area. Roll top film down to minimize air bubbles or gaps between the filter and the 3M Petrifilm *Enterobacteriaceae* Count Plate.



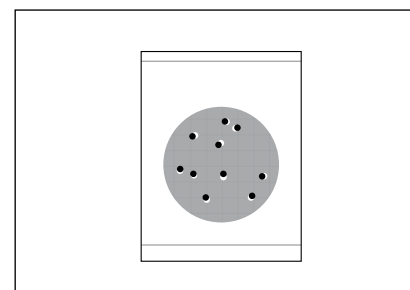
- 7 Lightly apply pressure to ensure uniform contact of the filter with the gel and to eliminate any air bubbles.

## Incubation



- 8 Incubate 3M Petrifilm *Enterobacteriaceae* Count Plates in a horizontal position, clear side up, in stacks of no more than 20 plates at 34-37°C for 24 ± 2 hours.

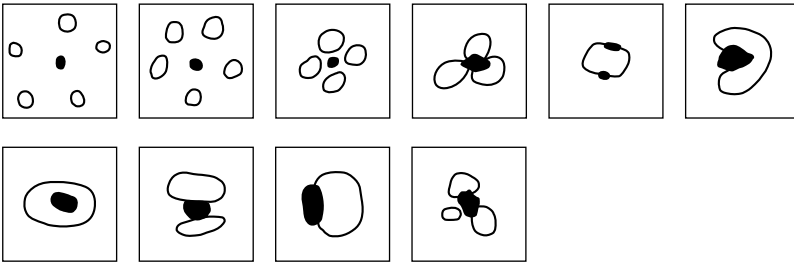
## Interpretation



- 9 3M Petrifilm *Enterobacteriaceae* Count Plates can be counted 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.

# 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](http://3M.com/foodsafety/Petrifilm) or call 1-800-328-6553.



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London, Ontario N6A 4T1  
Canada  
1-800-364-3577

**User's Responsibilities:** 3M Petrifilm Plate performance has not been evaluated with all combinations of microbial flora, incubation conditions and food matrices. It is the user's responsibility to determine that any test methods and results meet the user's requirements. Should re-printing of this Interpretation Guide be necessary, user's print settings may impact picture and color quality.

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