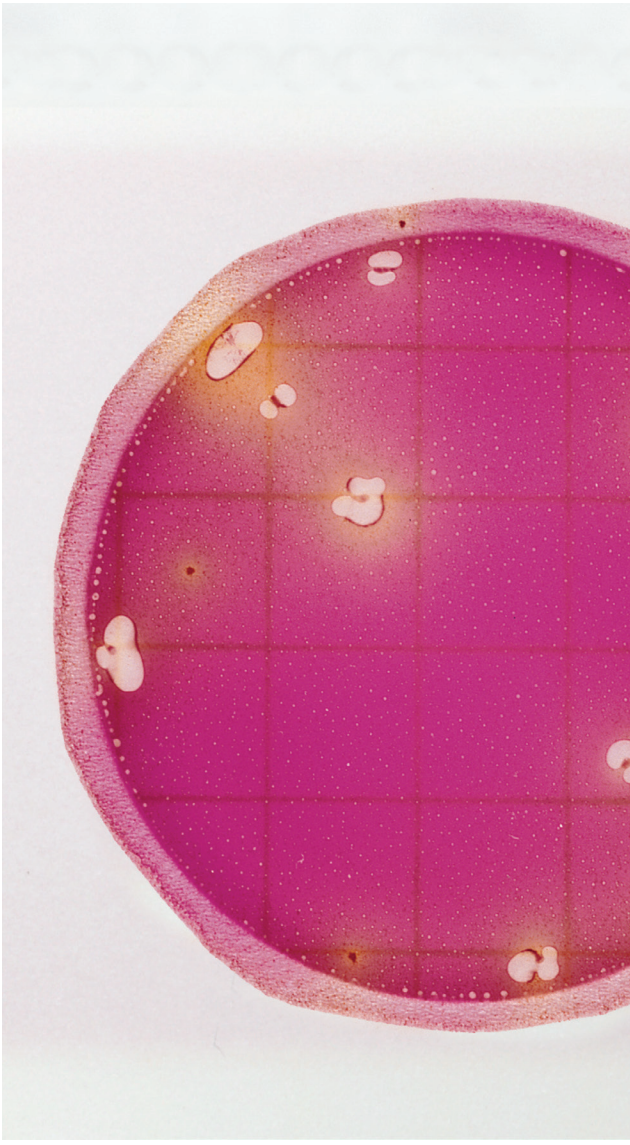
NEOGEN®

Petrifilm®

Interpretation Guide

The Neogen® 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. 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, or red colonies associated with yellow zones and with gas bubbles.



EB

***Enterobacteriaceae* Count Plate**

Food and Beverage Applications

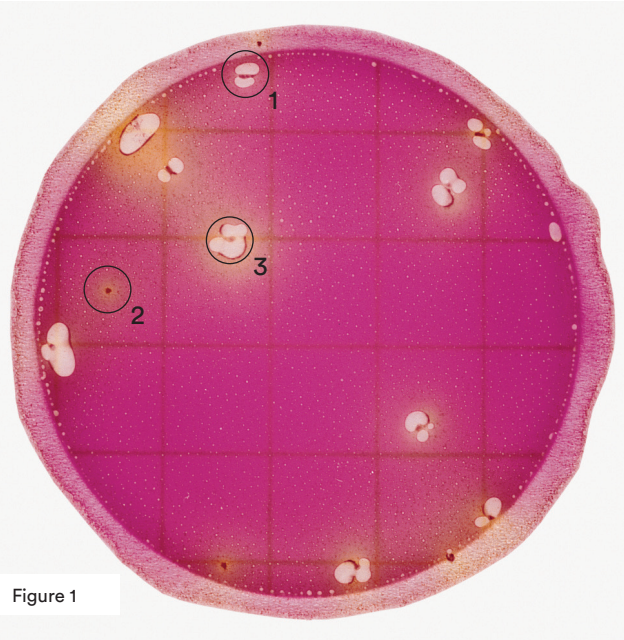


Figure 1

***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.

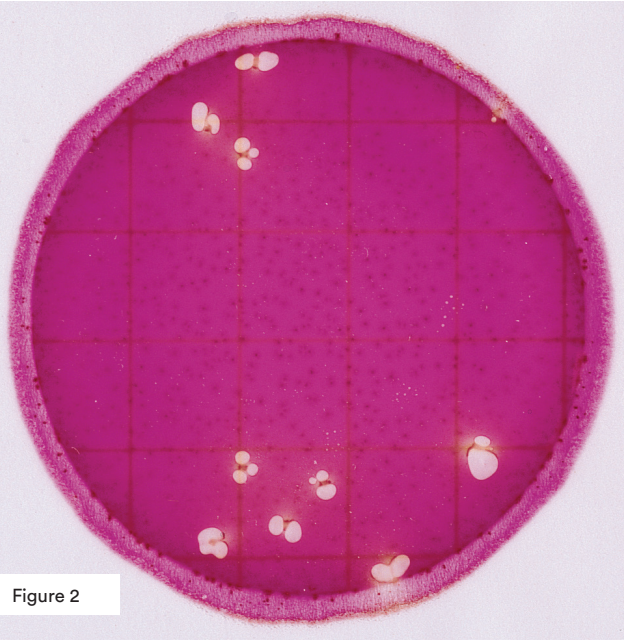


Figure 2

***Enterobacteriaceae* count = 9**

Figure 2 shows a 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.

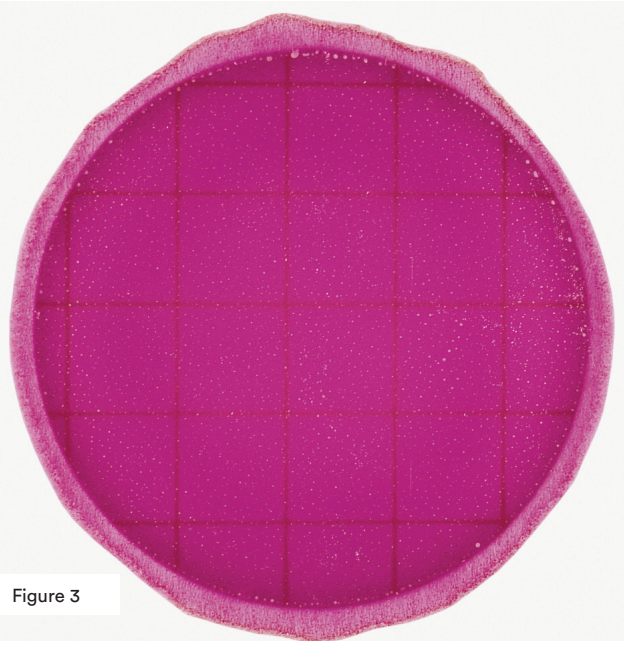


Figure 3

***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.

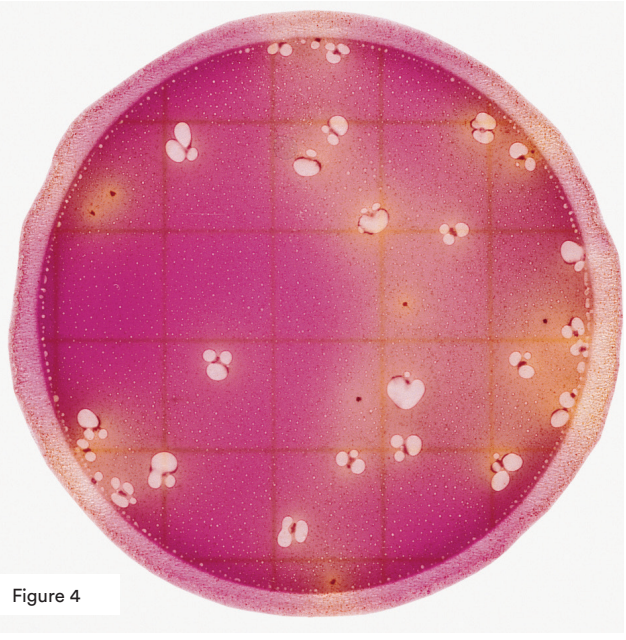


Figure 4

***Enterobacteriaceae* count = 35**

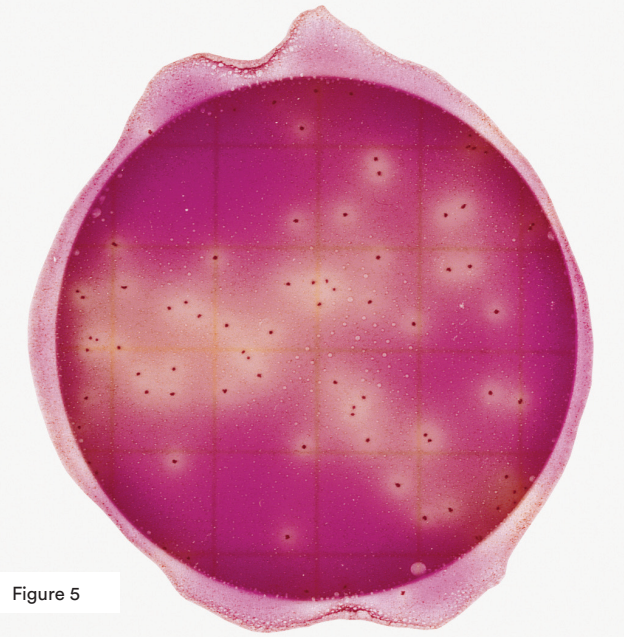


Figure 5

Enterobacteriaceae count = 77

The recommended counting range on Petrifilm *Enterobacteriaceae* Count Plate is less than or equal to 100 colonies.

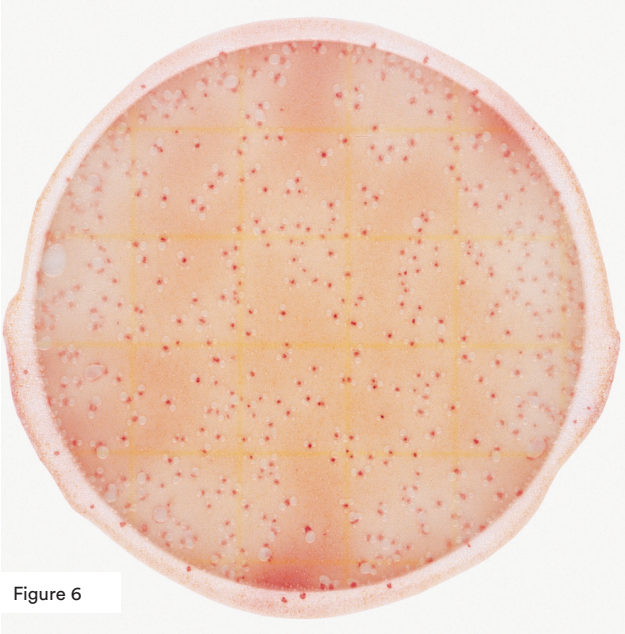


Figure 6

Enterobacteriaceae count = TNTC

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.

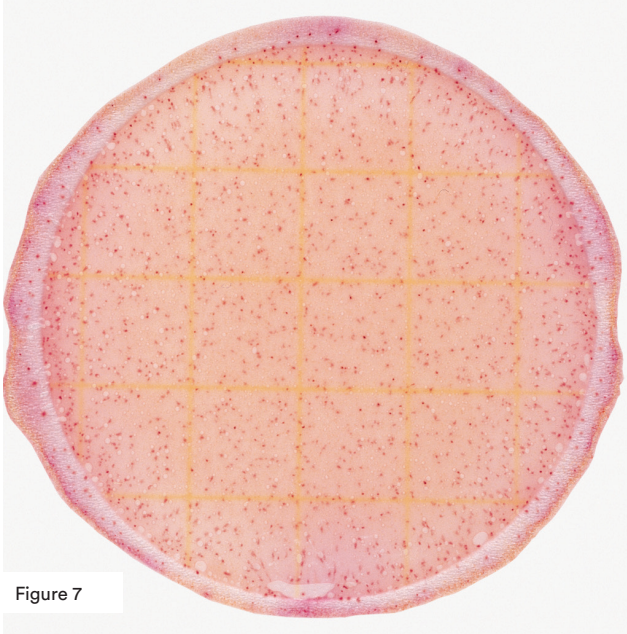


Figure 7

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.

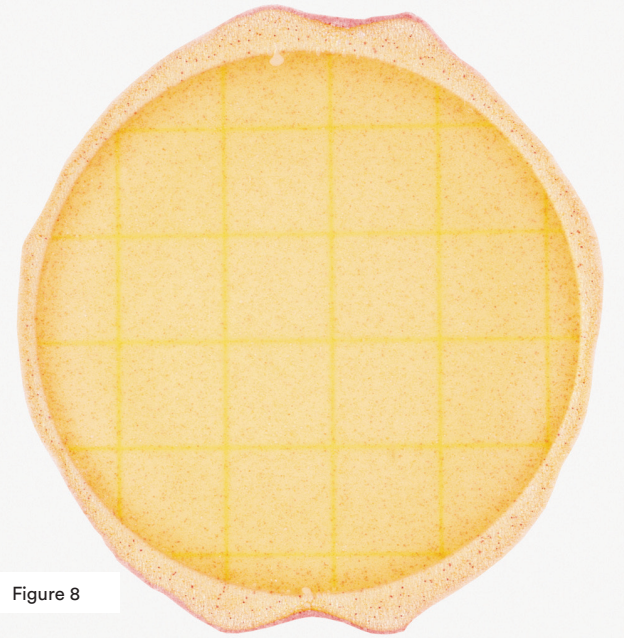


Figure 8

Enterobacteriaceae count = TNTC

The 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.

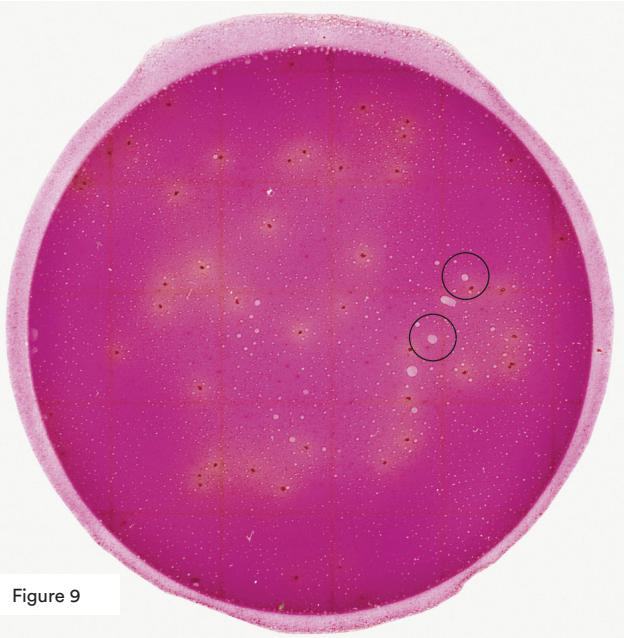


Figure 9

Enterobacteriaceae count = 44

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

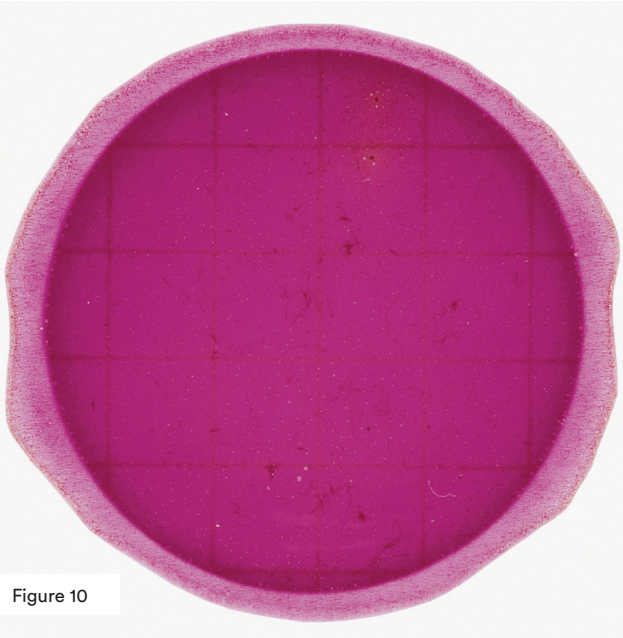


Figure 10

Enterobacteriaceae count = 2

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

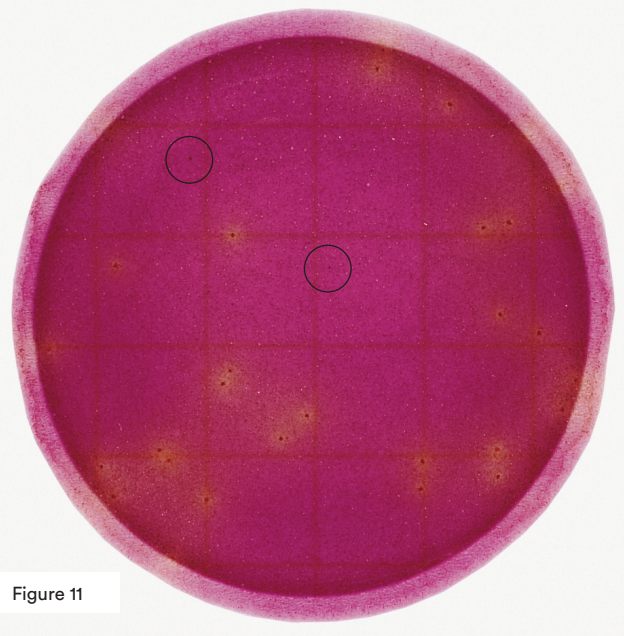


Figure 11

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 Applications

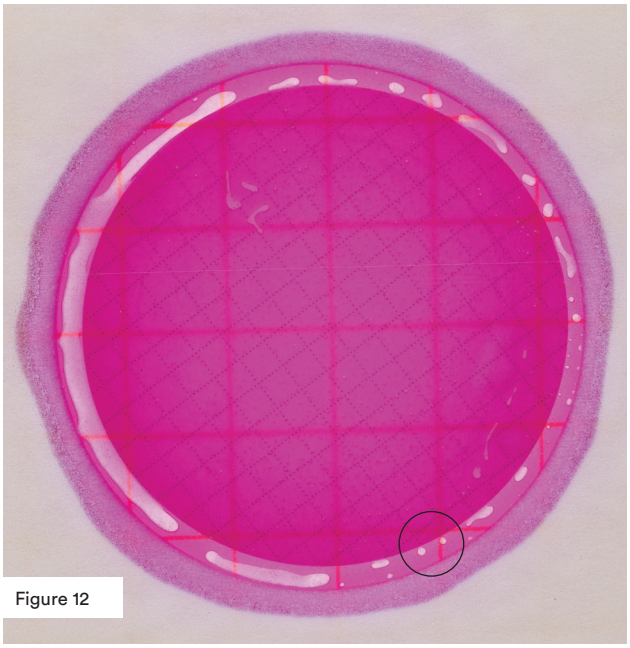


Figure 12

Enterobacteriaceae count = 0

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

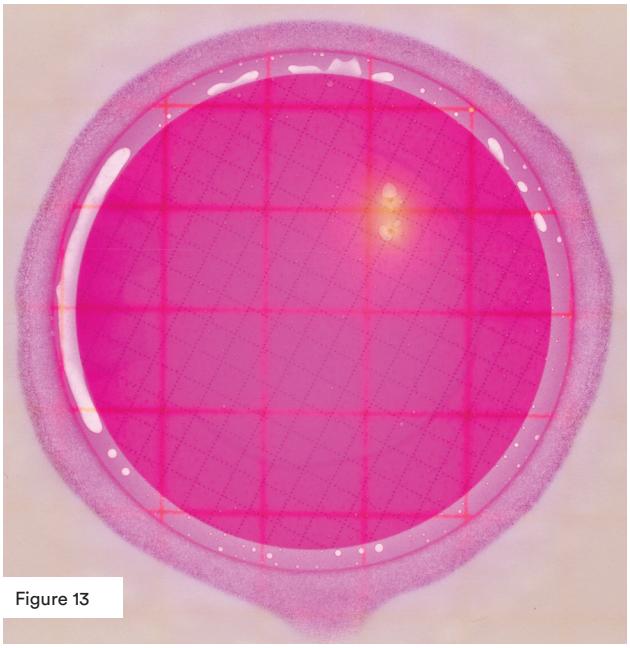


Figure 13

Enterobacteriaceae count = 2

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

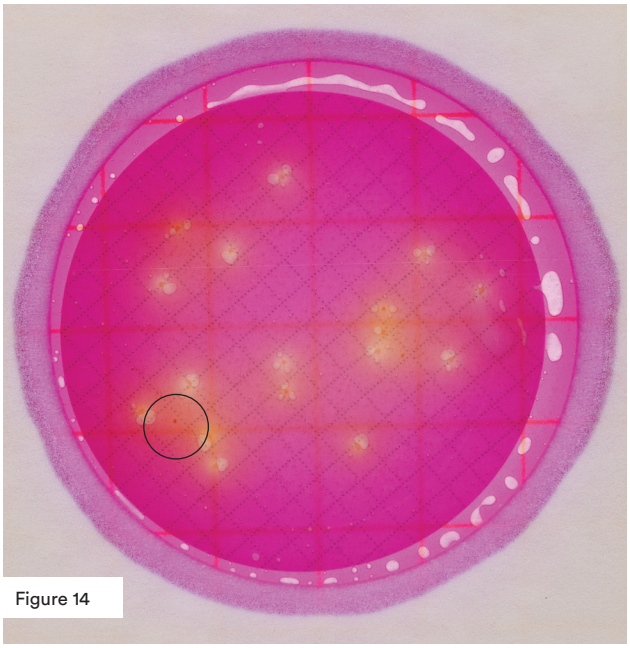


Figure 14

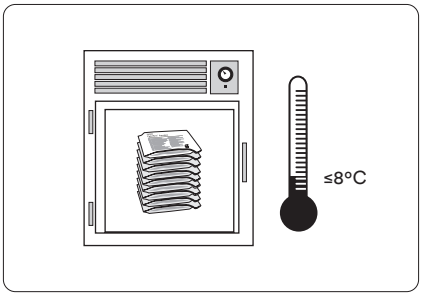
Enterobacteriaceae count = 17

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

Reminders For Use

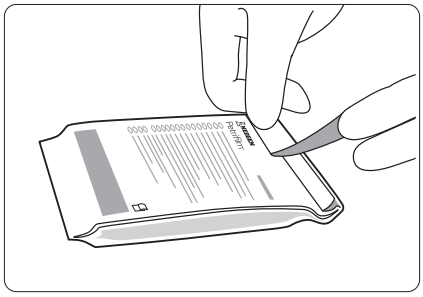
Food and Beverage Applications

Storage



01

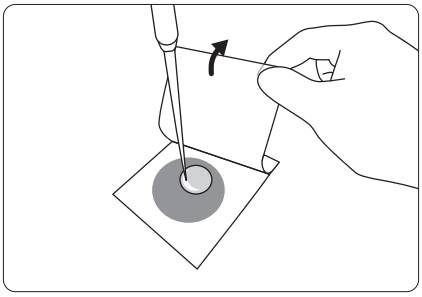
Store the **unopened** Petrifilm *Enterobacteriaceae* Count Plate pouches at frozen or refrigerated temperatures lower than or equal to 8°C (46°F). Use before expiration date on package. It is best to allow pouches to reach room temperature before opening.



02

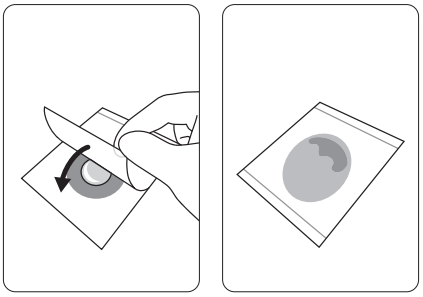
Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than four weeks.

Inoculation



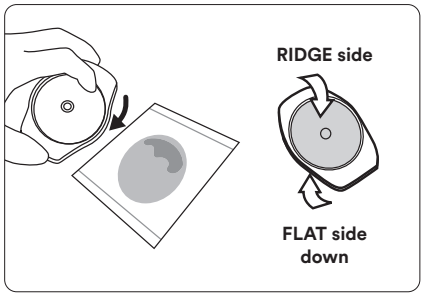
03

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



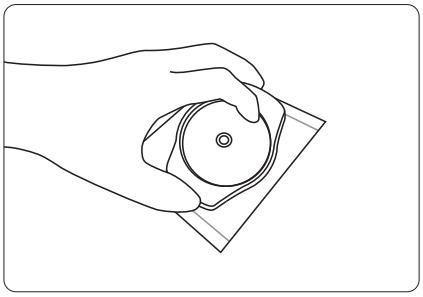
04

Roll top film down onto sample **gently** to prevent trapping air bubbles.



05

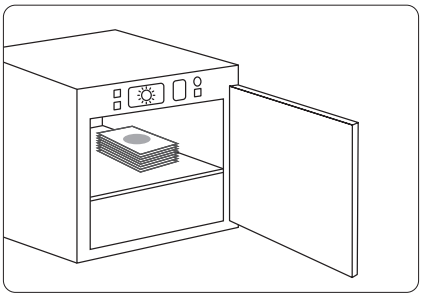
With **flat** side down, place Petrifilm Spreader on top film over inoculum.



06

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

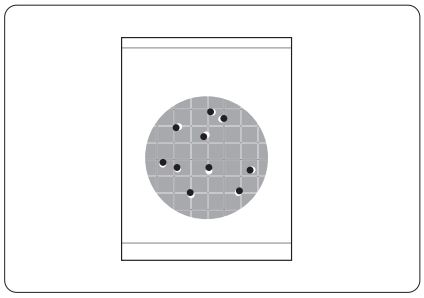
Incubation



07

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

Interpretation



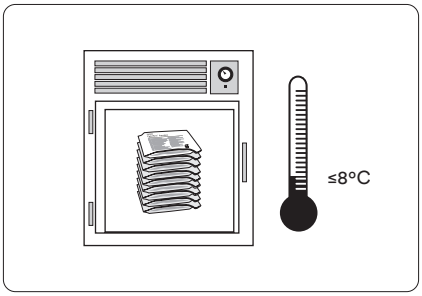
08

Petrifilm *Enterobacteriaceae* Count Plates can be counted using the Petrifilm Plate Reader Advanced, 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.

Reminders For Use

Bottled Water Applications

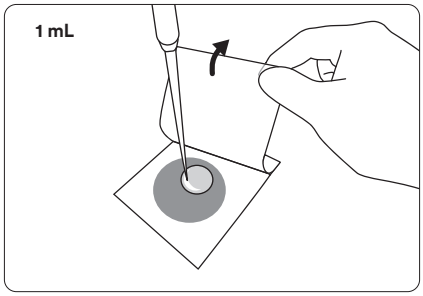
Storage



01

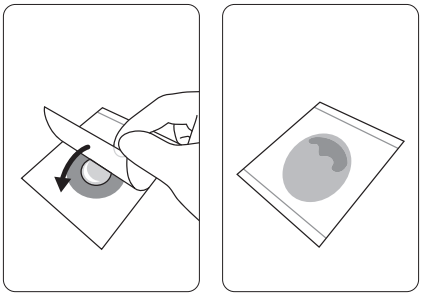
Follow steps 1 and 2 of Food and Beverage Application Reminders for Use.

Hydration



02

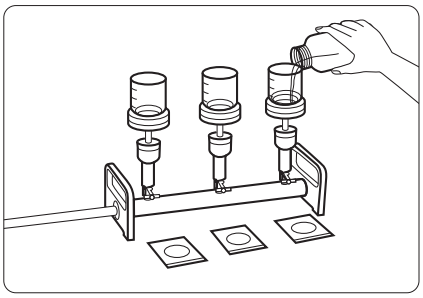
Place Petrifilm *Enterobacteriaceae* Count Plate on a flat, **level** surface. Lift the top film and dispense 1 mL of an appropriate sterile hydration diluent onto the center of the bottom film. Appropriate sterile diluents include sterile water, deionized (DI) water and reverse osmosis (RO) water.



03

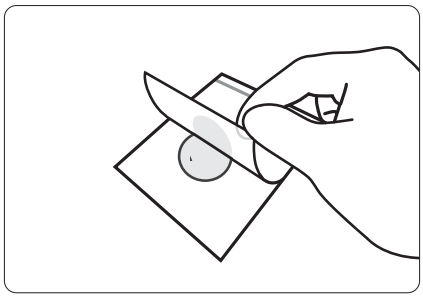
Carefully roll the top film down onto the sample to prevent trapping air bubbles.

Filtration



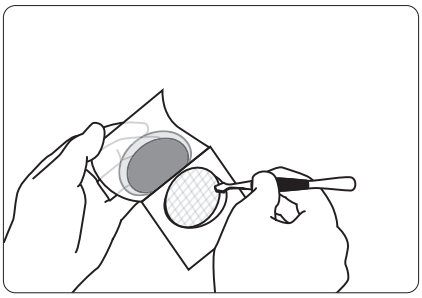
04

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



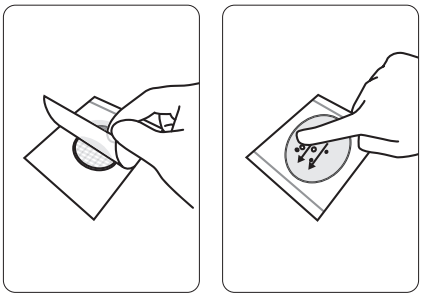
05

Carefully lift the top film.



06

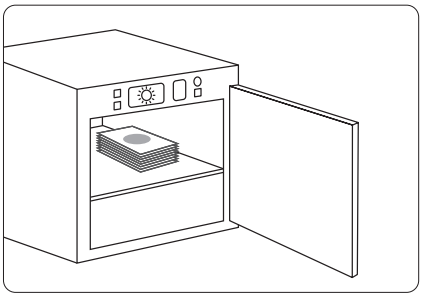
Place filter in the center of the hydrated area. Roll top film down to minimize air bubbles or gaps between the filter and the Petrifilm *Enterobacteriaceae* Count Plate.



07

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

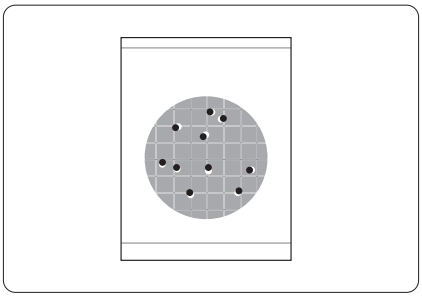
Incubation



08

Incubate 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

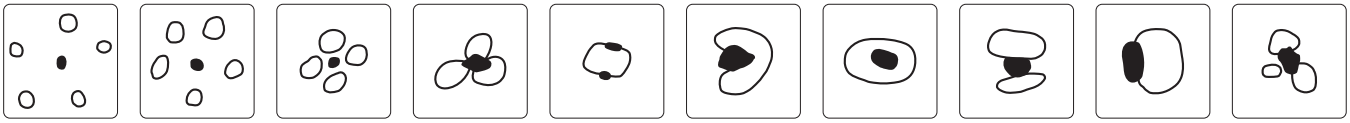


09

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.



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%), Wide-Spectrum Neutralizer, bisulfite-free letheen 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 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.

Neogen offers a full line of products to accomplish a variety of your microbial testing needs.

For more product information, visit **info.neogen.com/petrifilm**

User's Responsibilities: Neogen 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.

For detailed CAUTIONS, DISCLAIMER OF WARRANTIES/LIMITED REMEDY and LIMITATION OF NEOGEN LIABILITY, STORAGE AND DISPOSAL information and INSTRUCTIONS FOR USE, see product instructions.

NEOGEN

Neogen Corporation, 620 Lesher Place, Lansing, MI 48912 USA.

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