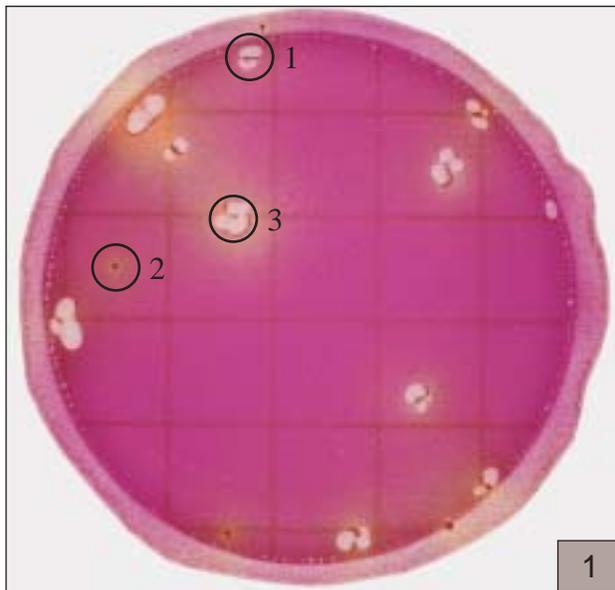


Petrifilm™

Enterobacteriaceae Count Plate

This guide familiarizes you with results on 3M™ Petrifilm™ Enterobacteriaceae Count plates. For more information contact the official 3M Microbiology Products representative nearest you.

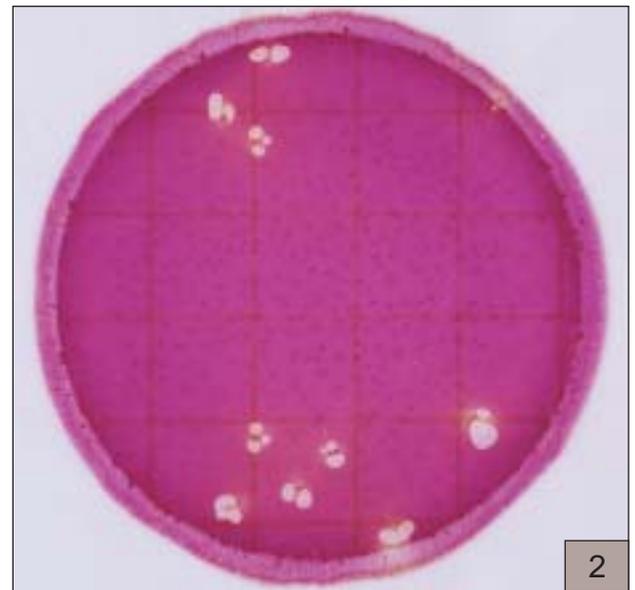


***Enterobacteriaceae* count = 13**

An indicator in the Petrifilm Enterobacteriaceae Count plate colors all colonies red. The top film traps gas produced by some bacteria. Acid-producing bacteria are seen as red colonies surrounded by yellow zones.

Bacteria producing gas and/or acid are considered to be presumptive *Enterobacteriaceae* and will have one of the following characteristics on the Petrifilm Enterobacteriaceae Count plate: colonies associated with gas bubbles and no acid zones (see figure 1, circle 1), colonies with yellow acid zones but no gas production (see figure 1, circle 2), or colonies producing both gas and acid (see figure 1, circle 3).

Figure 1 also illustrates how bubble patterns can vary. Sometimes gas disrupts the colony so that the colony “outlines” the gas bubble as in figure 1, circle 3.

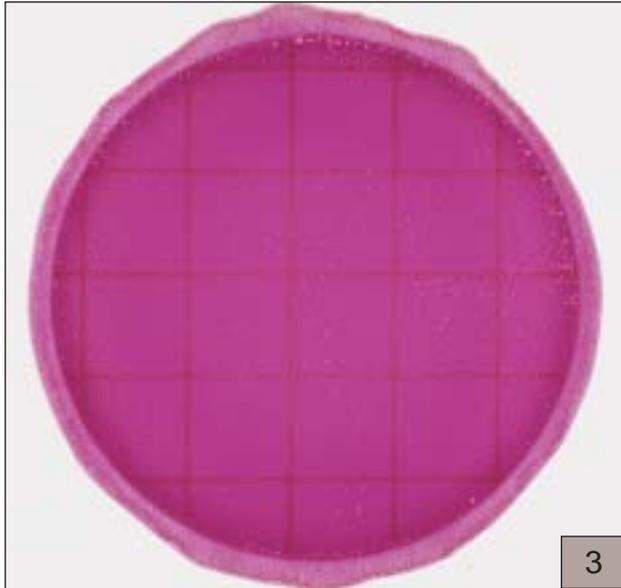


***Enterobacteriaceae* count = 9**

Figure 2 shows a Petrifilm Enterobacteriaceae Count plate with a few *Enterobacteriaceae* colonies and a high number of non-*Enterobacteriaceae*, gram-negative colonies.

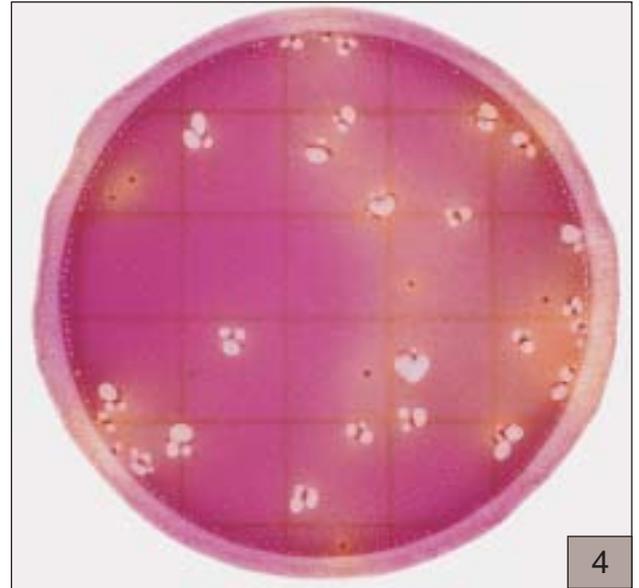
Do not count colonies on the foam dam since they are removed from the selective influence of the medium.

3M™ Petrifilm™ Enterobacteriaceae Count Plate

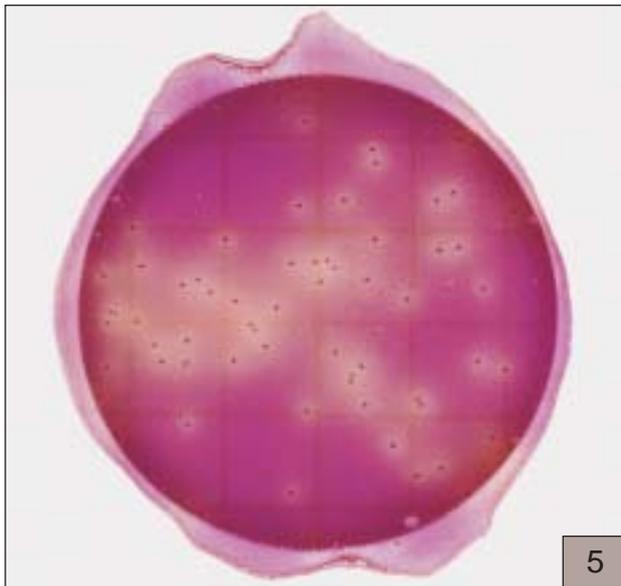


***Enterobacteriaceae* count = 0**

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

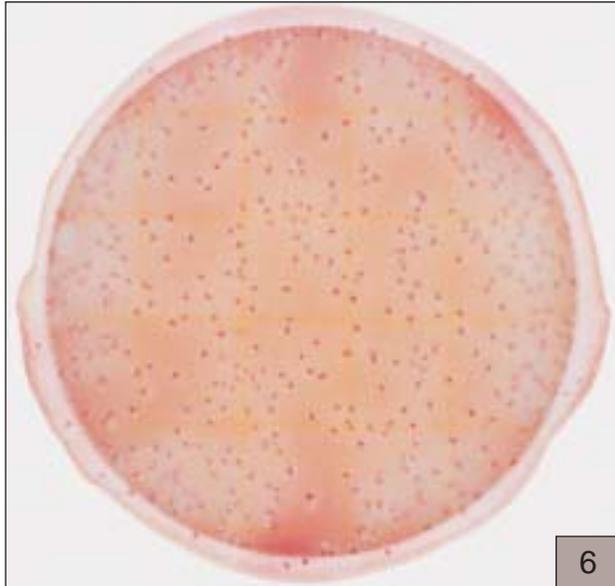


***Enterobacteriaceae* count = 35**



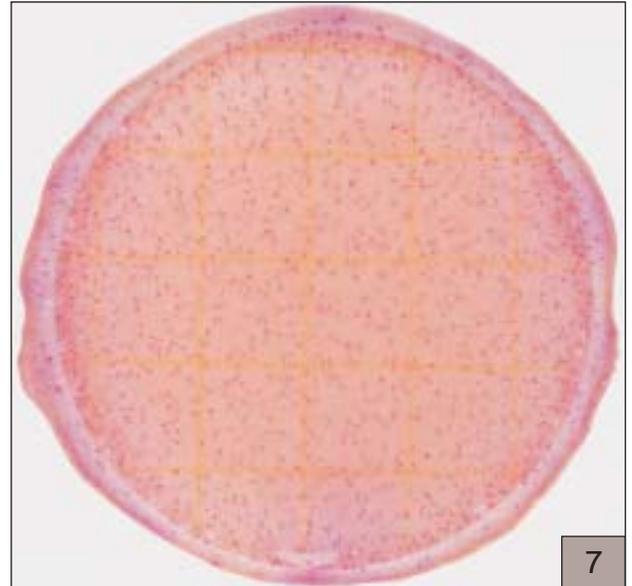
***Enterobacteriaceae* count = 77**

The recommended counting range on Petrifilm Enterobacteriaceae Count plates is 15–100 colonies. Samples having counts greater than 100 *Enterobacteriaceae* per plate may be estimated. The circular growth area is approximately 20 cm². 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.



***Enterobacteriaceae* count = TNTC**

Petrifilm Enterobacteriaceae Count plates with more than 100 colonies are considered too numerous to count (TNTC) and have a light background color along with at least one of the following characteristics: many small colonies or many gas bubbles. See figure 6.



***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.



***Enterobacteriaceae* count = TNTC**

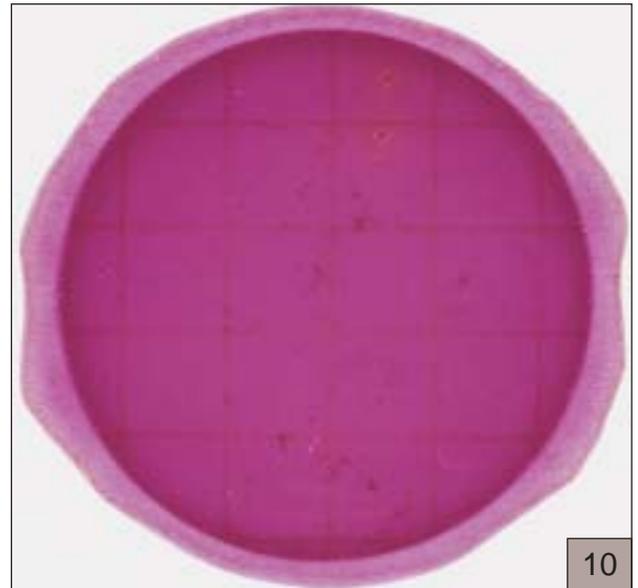
The Petrifilm Enterobacteriaceae Count plate in figure 8 has two characteristics indicating TNTC colonies: lightening of the gel color and many small colonies.

Artifact Bubbles and Food Particles Do Not Enumerate



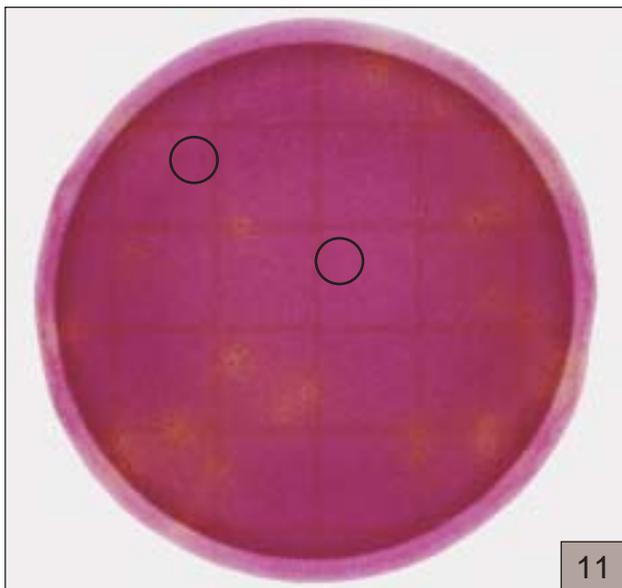
***Enterobacteriaceae* count = 44**

Artifact bubbles may result from improper inoculation of the Petrifilm Enterobacteriaceae Count plate. They are irregularly shaped and not associated with a red colony. See figure 9.



***Enterobacteriaceae* count = 2**

Food particles are often irregularly shaped or filamentous and are not associated with gas bubbles or acid zones. See figure 10.



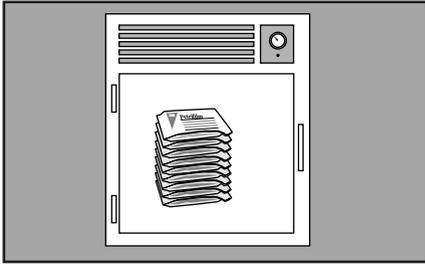
***Enterobacteriaceae* count = 29**

Food particles also can be seen as dark spots but are not associated with gas bubbles or acid zones. See figure 11.

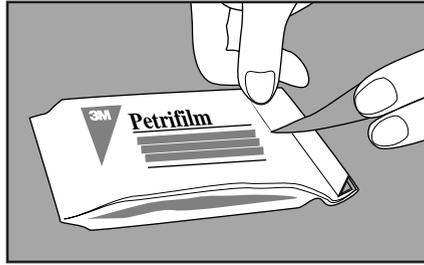
3M Petrifilm™ Enterobacteriaceae Count Plates Reminders for Use

For detailed CAUTIONS, DISCLAIMER OF WARRANTIES / LIMITED REMEDY, and LIMITATION OF 3M LIABILITY, STORAGE AND DISPOSAL information, and INSTRUCTIONS FOR USE see Product's package insert.

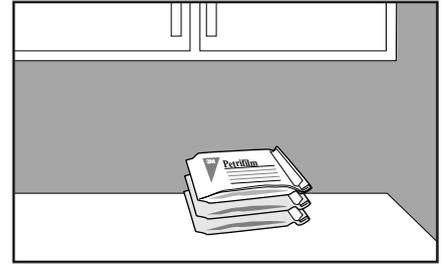
Storage



1 Store unopened packages 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 packages to reach room temperature before opening.

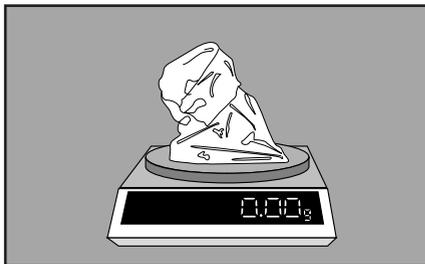


2 To seal opened package, fold end over and tape shut.

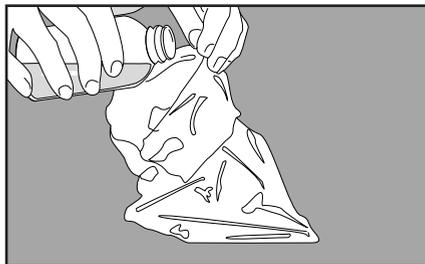


3 Keep resealed package at $\leq 25^{\circ}\text{C}$ ($\leq 77^{\circ}\text{F}$) and $\leq 50\% \text{RH}$. **Do not refrigerate opened packages.** Use Petrifilm plates within one month after opening.

Sample Preparation

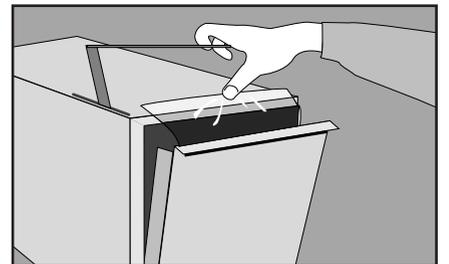


4 Prepare a 1:10 or greater dilution of food product. Weigh or pipette food product into an appropriate container such as a stomacher bag, dilution bottle, Whirl-Pak® bag, or other sterile container.



5 Add appropriate quantity of one of the following sterile diluents: Butterfield's phosphate buffer (IDF phosphate buffer, 0.0425 g/L of KH_2PO_4 , adjust to pH 7.2), 0.1% peptone water, peptone salt diluent (ISO method 6887), buffered peptone water (ISO method 6887-1), saline solution (0.85-0.90%), sodium bisulfate-free letheen broth or distilled water.

Do not use buffers containing citrate, bisulfite, or thiosulfate; they can inhibit growth.

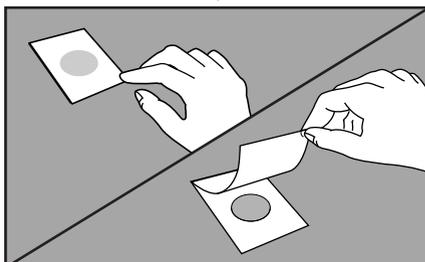


6 Blend or homogenize sample per current procedure.

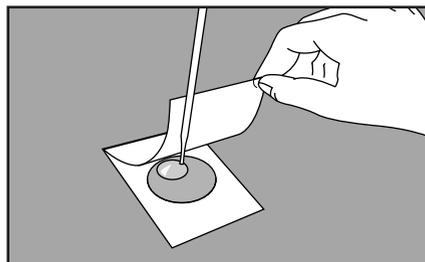
Adjust pH of the diluted sample between 6.5 and 7.5 :

- for acid products, use 1N NaOH,
- for alkaline products, use 1N HCl.

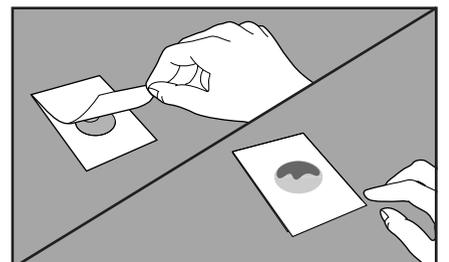
Inoculation



7 Place Petrifilm plate on **level** surface. Lift top film.

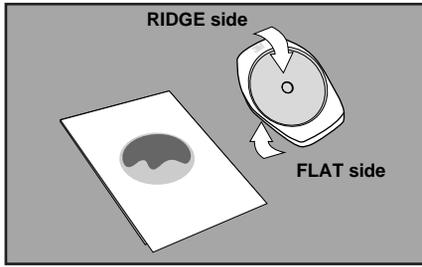


8 With pipette **perpendicular** to Petrifilm plate, place 1 mL of sample onto center of bottom film.

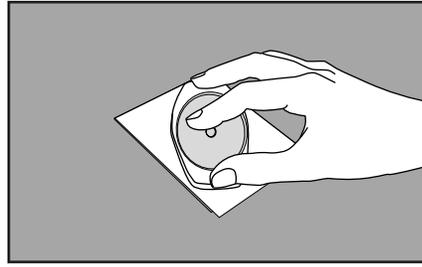


9 Carefully **ROLL** top film down to avoid entrapping air bubbles. Do **NOT** let top film drop.

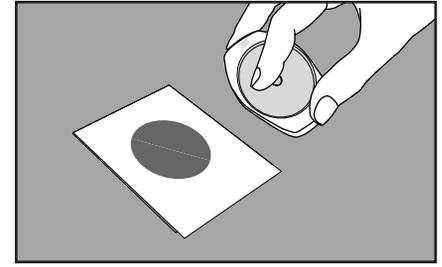
Continued - over



10 With **FLAT** side down, place spreader on top film over inoculum.

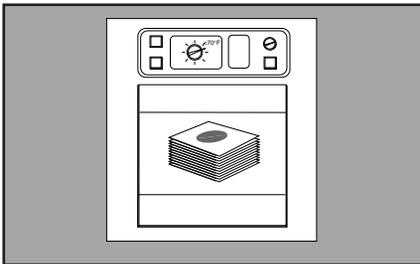


11 **GENTLY** apply pressure on spreader to distribute inoculum over circular area before gel is formed. Do not twist or slide the spreader.



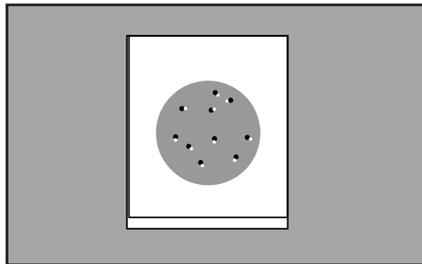
12 Lift spreader. Wait a minimum of one minute for gel to solidify.

Incubation

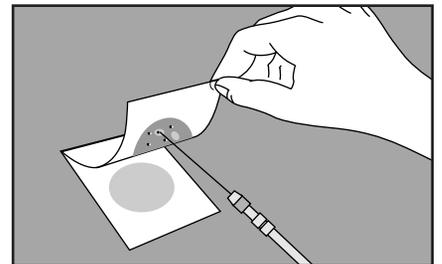


13 Incubate plates with clear side up in stacks of up to 20. It may be necessary to humidify incubator to minimize moisture loss during incubation.

Interpretation



14 Petrifilm plates can be counted on a standard colony counter or other illuminated magnifier. Refer to the *Interpretation Guide* when reading results.



15 Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Incubation temperature varies by method. Most common approved methods:

- **Compendium of Methods for the Microbiological Examination of Foods**
Incubate 24h ± 2h at 35°C ± 1°C

- **AFNOR Validated Method**
3M 01/06 09/97
Incubate 24h ± 2h at 30°C ± 1°C
35°C ± 1°C or 37°C ± 1°C

Additional Comments

- Questions? U.S., call **1-800-328-6553**, Canada, call **1-800-265-1840 x6574** for technical service.
- To order Petrifilm plates in the U.S., call **1-800-328-1671**.
- Latin America / Africa and Asia Pacific regions, call **1-651-733-7562**.

References
1. ISO7402 1993

3M

3M Microbiology

3M Center
Bldg. 275-5W-05
St. Paul, MN 55144-1000
USA
1 800 228-3957
www.3M.com/microbiology
Email: microbiology@3M.com

3M Canada

Post Office Box 5757
London, Ontario N6A 4T1
Canada
1 800 563-2921

3M Europe

Laboratoires 3M Santé
Boulevard de l'Oise
F-95029 Cergy-Pontoise Cedex
France
33 1 30 31 8571

Petrifilm is a trademark of 3M.
Whirl-Pak is a registered trademark of Nasco.

 40% Pre-consumer waste paper
10% Post-consumer waste paper

Printed in U.S.A.
©2003 70-2008-8668-0 (23.5)ii