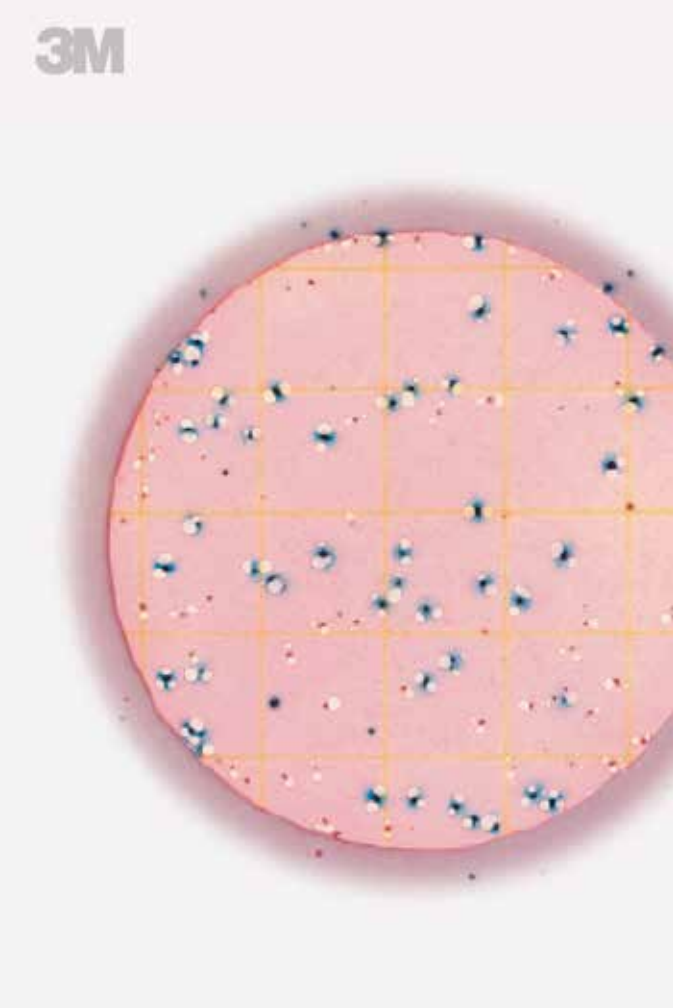
3M

Petrifilm™

Interpretation Guide

The 3M™ Petrifilm™ *E. coli*/Coliform Count Plate is a sample-ready-culture medium system which contains modified Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG), and a tetrazolium indicator that facilitates colony enumeration. 3M Petrifilm *E. coli*/Coliform Count Plates are used for the enumeration of *Escherichia coli (E. coli)* and coliforms in the food and beverage industries.



EC

***E. coli*/Coliform Count Plate**

The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) define coliforms as Gram negative rods, which produce acid and gas from lactose fermentation.

Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony indicated by the blue to red-blue colonies. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, as indicated by colonies associated with entrapped gas (within approximately one colony diameter). Blue colonies without gas are not counted as *E. coli*.* Other coliform colonies are red and closely associated with entrapped gas. The total coliform count consists of both the red and blue colonies associated with gas.*

Most *E. coli* O157 strains are atypical, for example they are glucuronidase negative; they will not produce a blue color, and will not be detected on 3M Petrifilm *E. coli*/Coliform Count Plates.

*Validated through AOAC® *Official Methods of Analysis*℠ program. The definition of *E. coli* and coliform varies by regional method. In particular, the confirmation of *E. coli* may vary by country.

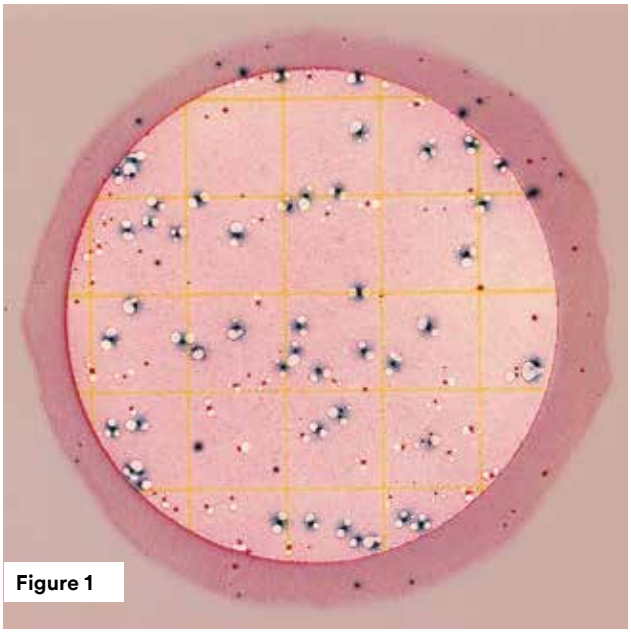
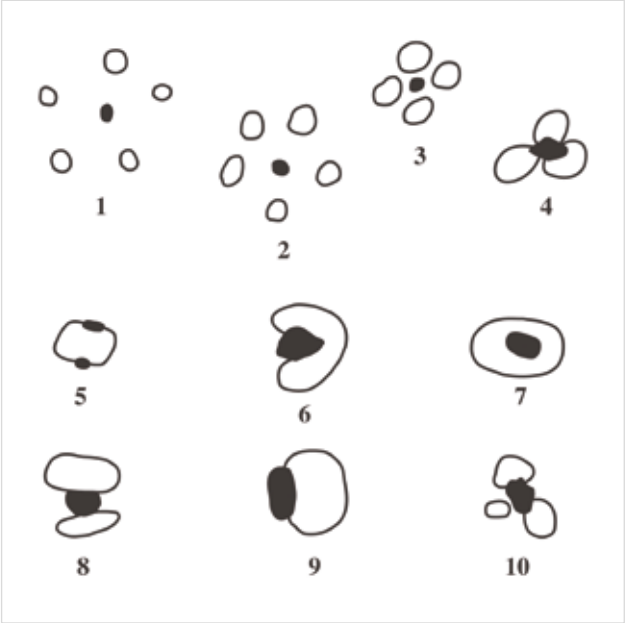


Figure 1

***E. coli* count = 49 (blue colonies with gas)**

Total coliform count = 87 (red and blue colonies with gas)

All figures have been counted according to AOAC® *Official Methods of Analysis*℠ #998.08 and #991.14. The confirmation of *E. coli* may vary by country.



Bubbles

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

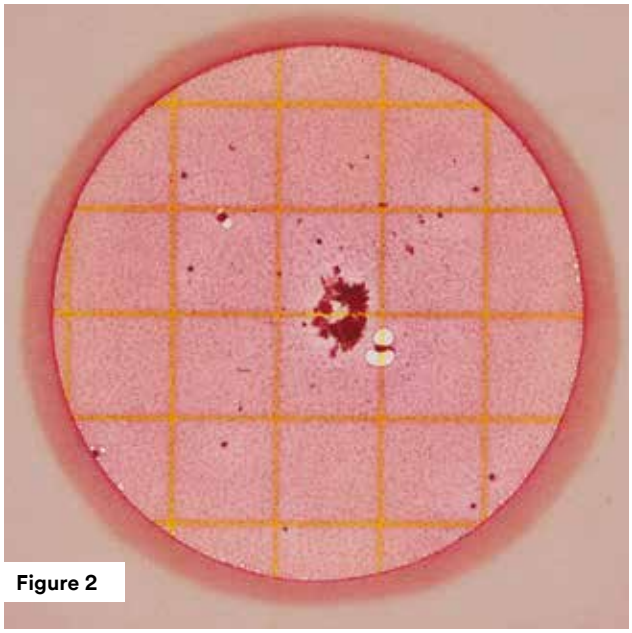


Figure 2

Total coliform count = 3 (red colonies with gas)

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

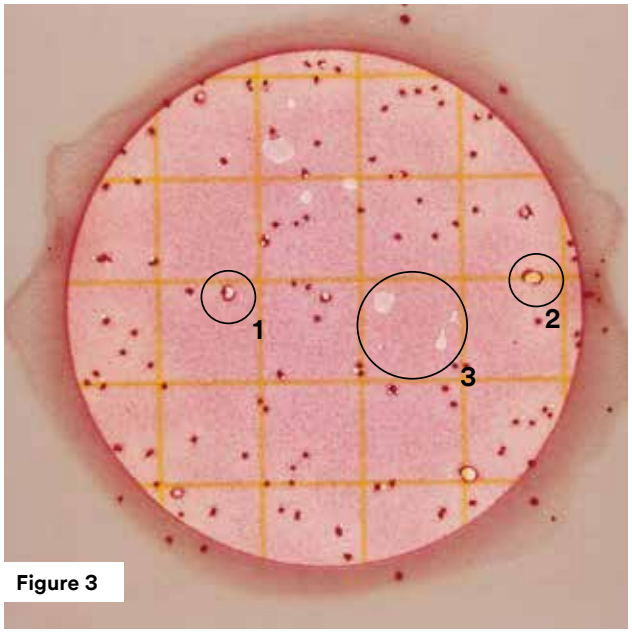


Figure 3

Estimated total coliform count = 150

The recommended counting limit on a 3M Petrifilm *E. coli*/ Coliform Count Plate is 150 colonies.

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.

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

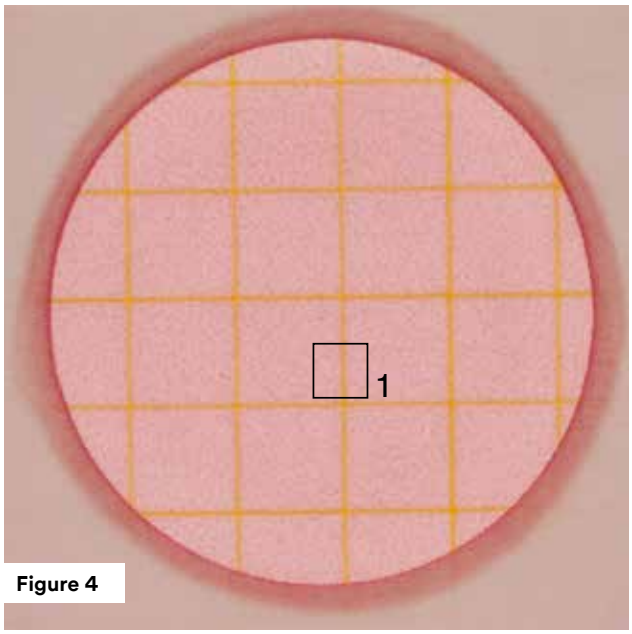


Figure 4

No growth = 0

Notice the changes in gel color in Figures 4 through 10. As the *E. coli* or coliform count increases, the color of the gel turns to dark red or purple-blue. Background bubbles are a characteristic of the gel and are not a result of *E. coli* or coliform growth. See Square 1.

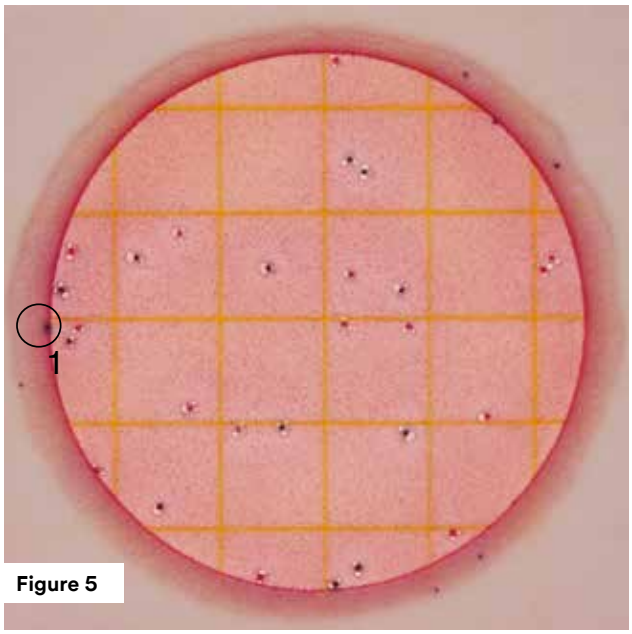


Figure 5

E. coli count = 13 (blue colonies with gas)

Total coliform count = 28 (red and blue colonies with gas)

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 6a

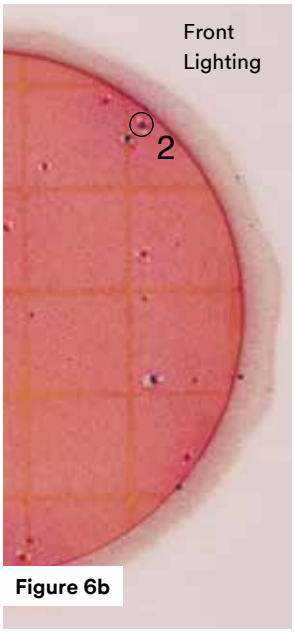


Figure 6b

E. coli count = 3 (blue colonies with gas)

Any blue in a colony (blue to red-blue) with gas indicates the presence of *E. coli*. Front lighting may enhance the detection of blue precipitate formed by a colony. Circle 1 shows a red-blue colony counted using back lighting. Circle 2 shows the same colony with front lighting. The blue precipitate is more evident in Circle 2.

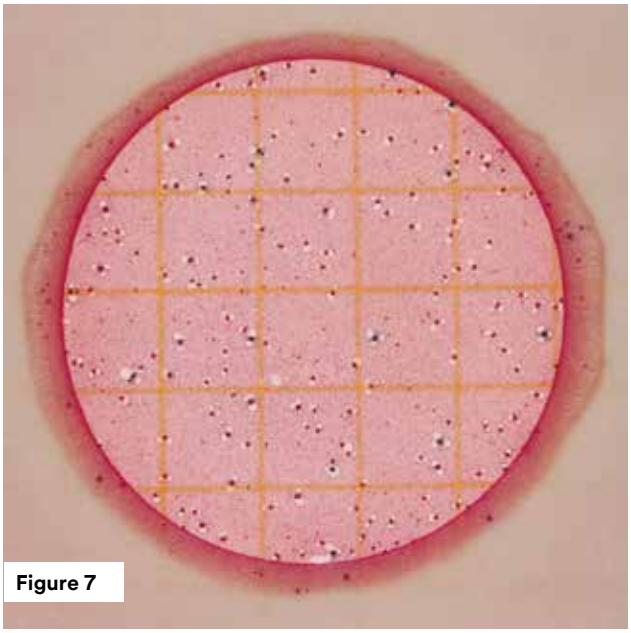


Figure 7

Estimated *E. coli* = 17 (blue colonies with gas)

Estimated total coliform count = 150

The circular growth area is approximately 20cm^2. 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 the sample may be necessary.

Too Numerous to Count (TNTC)

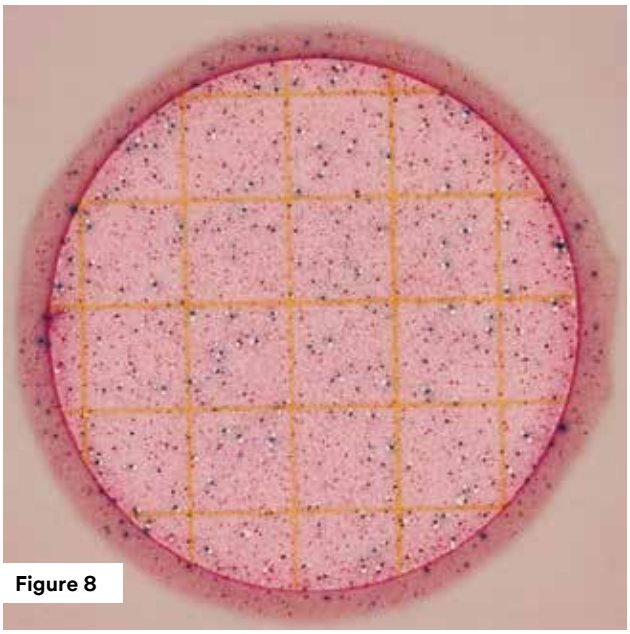


Figure 8

Total coliform count = TNTC

3M Petrifilm *E. coli*/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 from red to purple-blue.

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

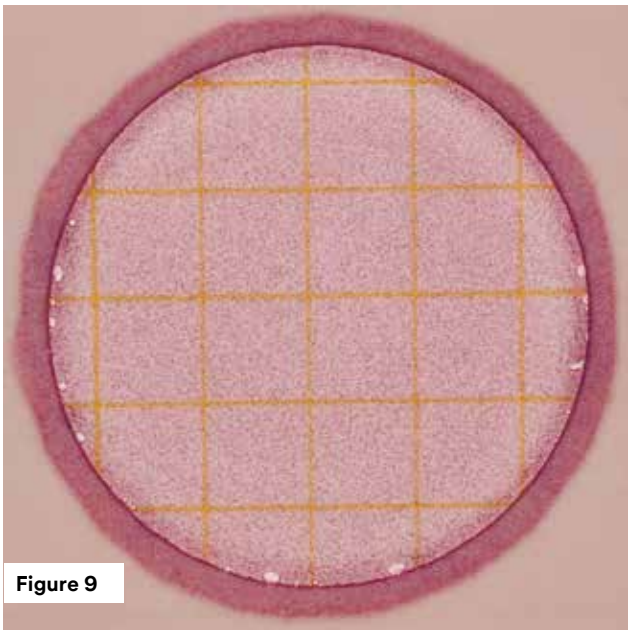


Figure 9

Total coliform count = TNTC

High concentrations of *E. coli* may cause the growth area to turn purple-blue.

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

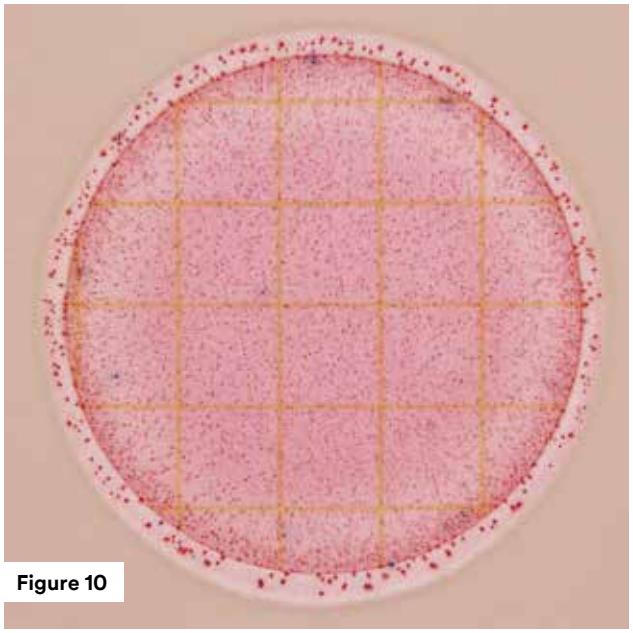


Figure 10

Total coliform count = TNTC

High concentration of coliforms may cause a deepening of the gel color and many small, indistinct colonies.

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

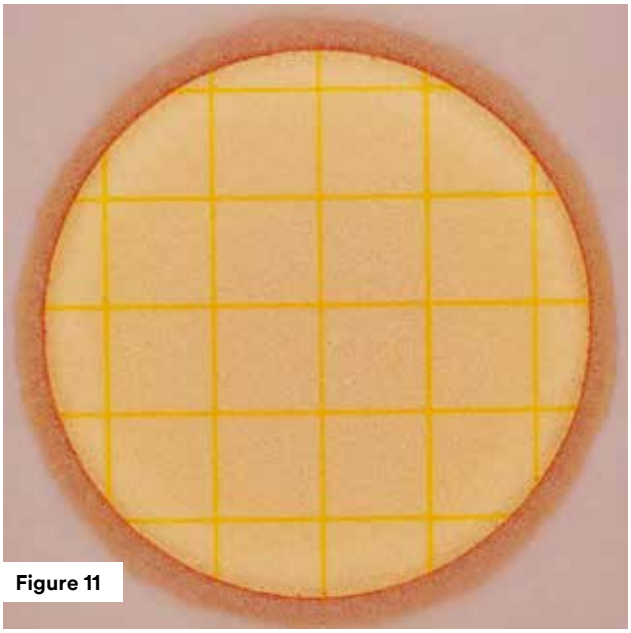


Figure 11

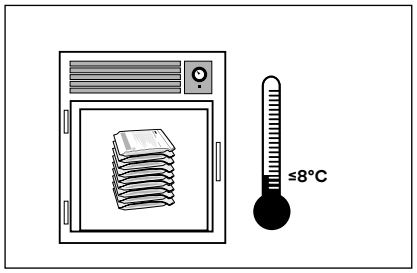
Total coliform count = TNTC

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

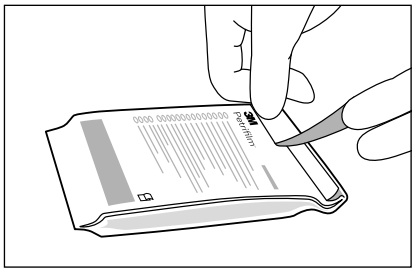
For a more accurate count, further dilution of the sample may be necessary.

Reminders for Use

Storage

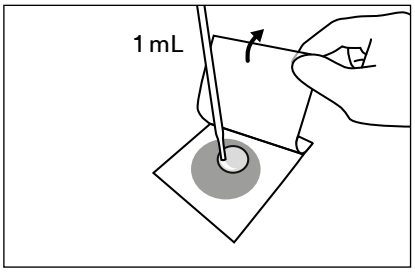


1. Store the unopened pouches of plates at frozen or refrigerated temperatures ≤8°C (≤46°F). Use before expiration date on package. It is best to allow pouches to reach room temperature before opening.

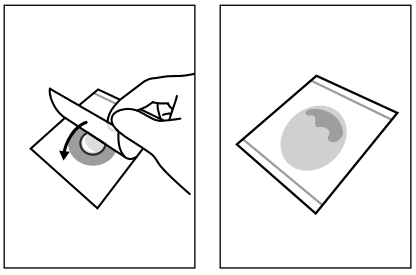


2. Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store sealed pouches in a cool dry place for no longer than four weeks.

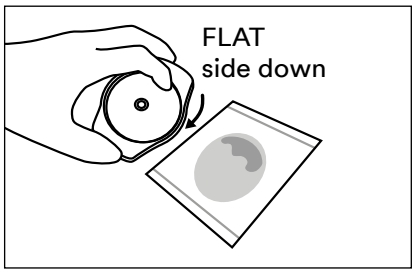
Inoculation



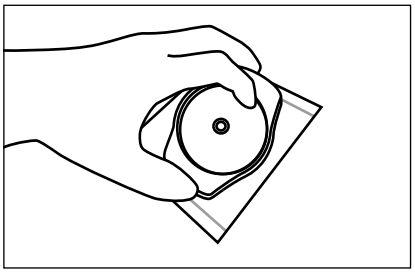
3. Place 3M Petrifilm *E. coli*/Coliform Count Plate on flat, level surface. Lift the top film and with a 3M™ Electronic Pipettor or equivalent held perpendicular to plate, dispense 1 mL of sample suspension onto center of bottom film.



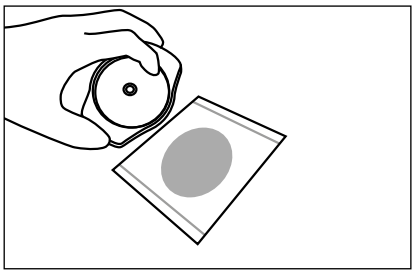
4. Roll the top film down onto sample gently to prevent pushing sample off film and to avoid entrapping air bubbles. Do not let top film drop.



5. With flat side down, place 3M™ Petrifilm™ Spreader on the center of the 3M Petrifilm *E. coli*/Coliform Count Plate.

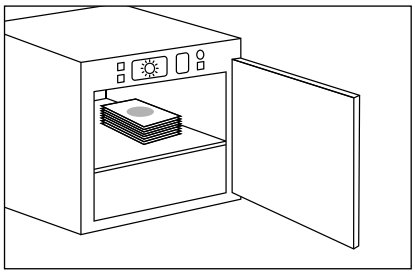


6. Press firmly on the center of the spreader to distribute the sample evenly. Spread the inoculum over the growth area before the gel is formed. Do not slide the spreader across the top film.



7 Remove the spreader and leave the plate undisturbed for one minute to permit the gel to form.

Incubation



8 Incubate 3M Petrifilm *E. coli*/Coliform Count Plates with clear side up in stacks of up to 20. It may be necessary to humidify incubator to minimize moisture loss. **Please refer to the product instructions for third party validated methods.**

Use Appropiate Sterile Diluents

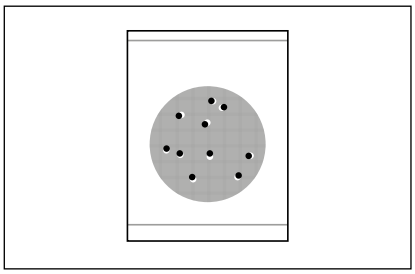
Butterfield's phosphate buffered dilution water, 0.1% peptone water, peptone salt diluent, quarter-strength Ringer's solution, saline solution (0.85-0.90%), bisulfite-free letheen broth or distilled water.

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 *E. coli*/Coliform 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.

Interpretation



9 3M Petrifilm *E. coli*/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.

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.

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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.

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.

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