*E. coli/*Coliform Count

Interpretation Guide

This guide familiarizes you with results on 3M™ Petrifilm™ *E. coli*/Coliform Count Plates. For more information, contact the official 3M Food Safety representative nearest you.

Do not use this plate alone for the detection of *E. coli* O157. Like most other *E. coli*/Coliform media, this plate will not specifically indicate whether any O157 strain is present.

3M
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3M™ Petrifilm™ EC Plate Overview

3M™ Petrifilm™ *E. coli*/Coliform Count (EC) Plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E. coli.* About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the 3M Petrifilm EC Plate (within approximately one colony diameter).

U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) define coliforms as Gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the 3M Petrifilm EC Plate produce acid which causes the pH indicator to make the gel color darker red. Gas trapped around red coliform colonies indicates confirmed coliforms.
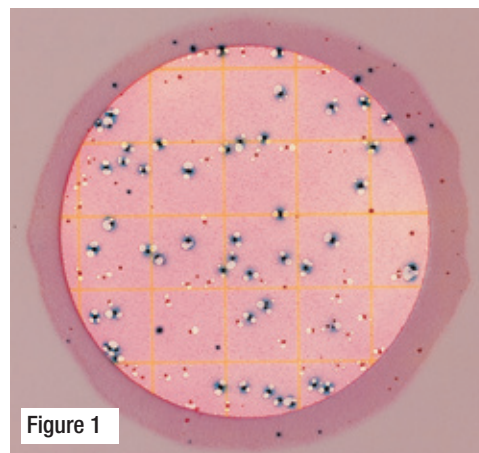

Figure 1

E. coli count = 49 (blue colonies with gas)
Total coliform count = 87 (red and blue colonies with gas)
Validated through AOAC® *Official Method of Analysis*℠ program. The confirmation of *E. coli* may vary by country.

Bubbles

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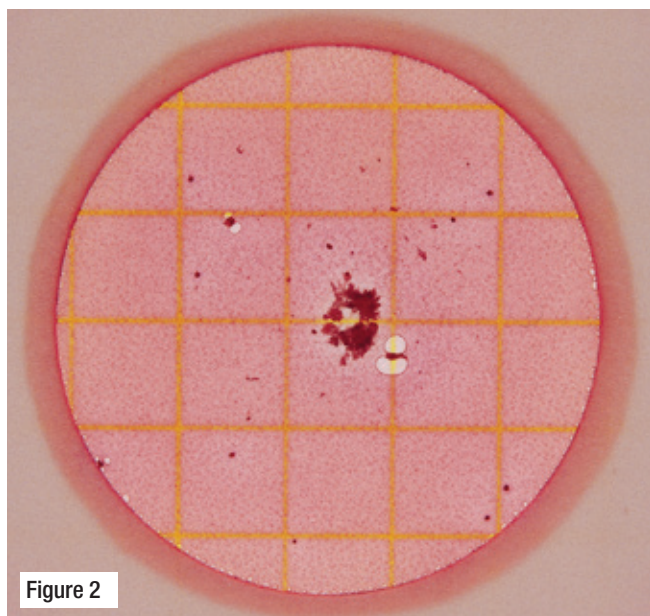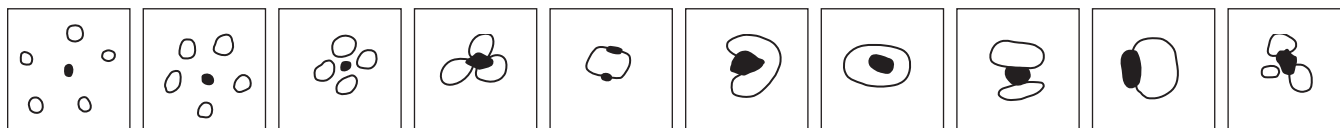

Figure 2

Total coliform count = 3
Food particles are irregularly shaped and are not associated with gas bubbles.
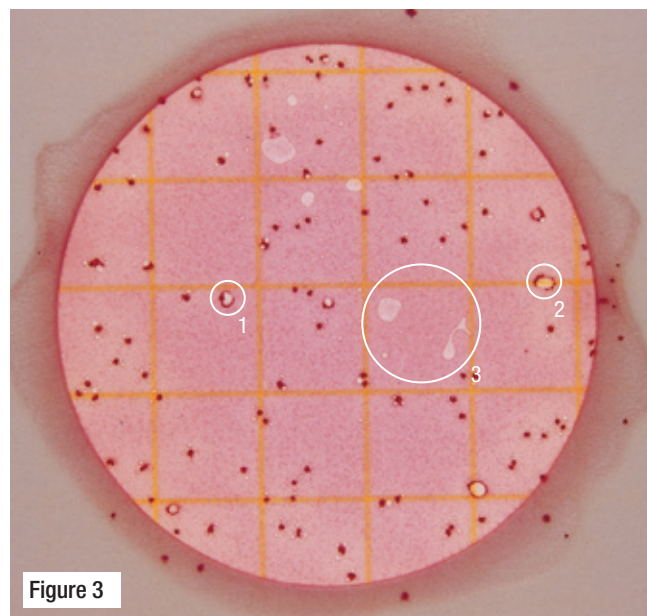

Figure 3

Total coliform count = ~150
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.

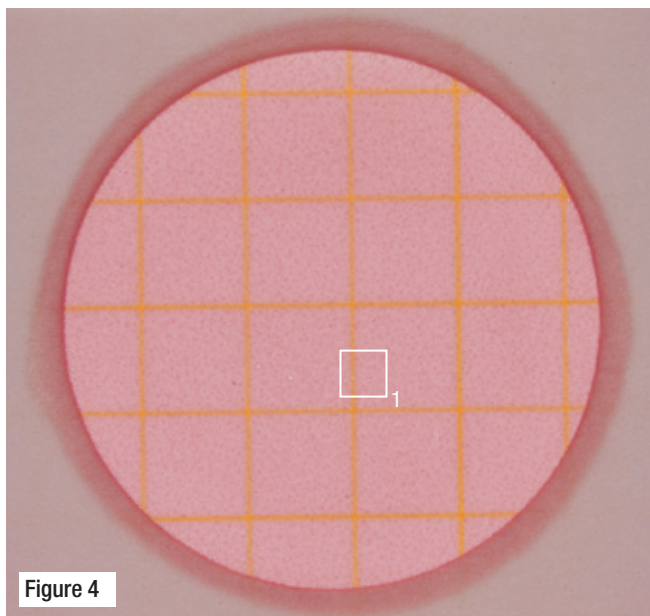# 3M™ Petrifilm™ *E. coli*/Coliform Count Plate Interpretation

Figure 4

Figure 5

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.
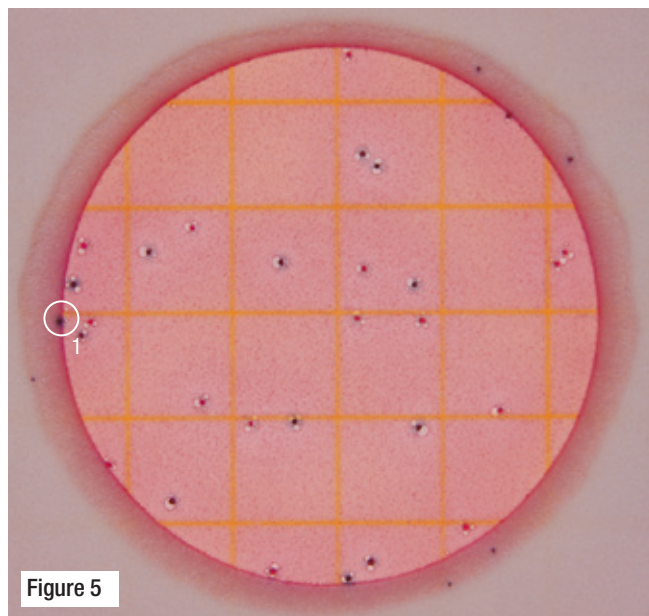
***E. coli* count = 13**
Total coliform count = 28
The recommended counting limit on a 3M Petrifilm EC Plate is 150 colonies. Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the medium. See Circle 1.
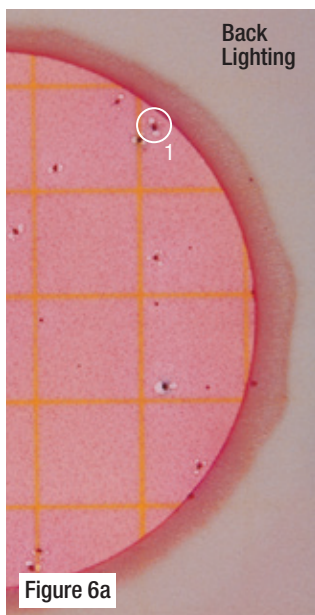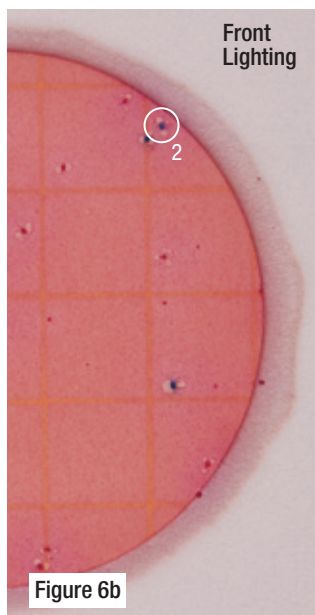

Back Lighting
Figure 6a

Front Lighting
Figure 6b

Figure 7

***E. coli* count = 3**
Any blue in a colony (blue to red-blue) 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.
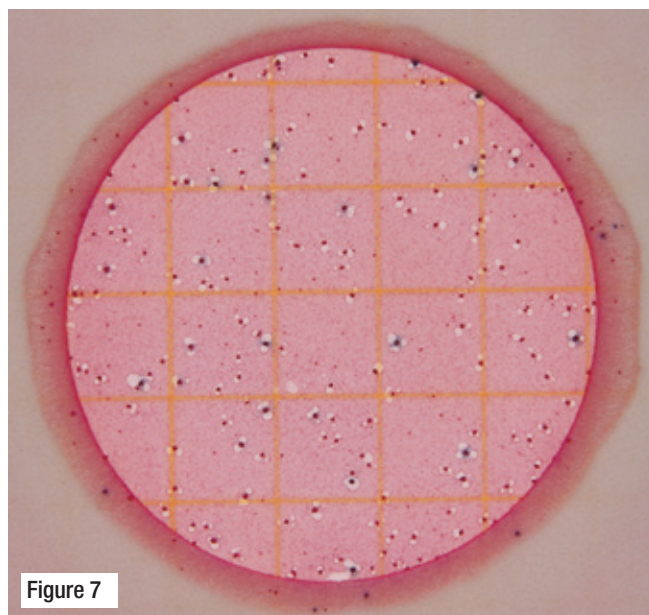
***E. coli* count = 17**
Estimated total coliform count = ~150
The circular growth area is approximately $20cm^2$. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.

TNTC (Too Numerous to Count) To obtain a more accurate count, dilute the sample further.
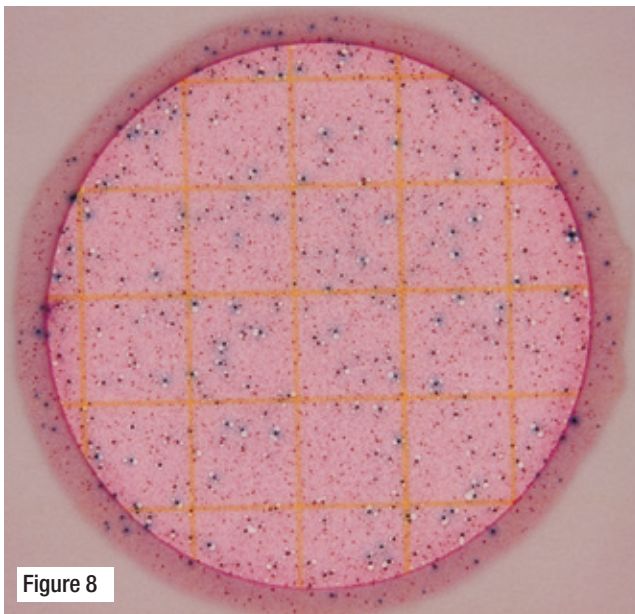

Figure 8

Actual count = ~10^6

3M Petrifilm EC Plates with colonies that are 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.
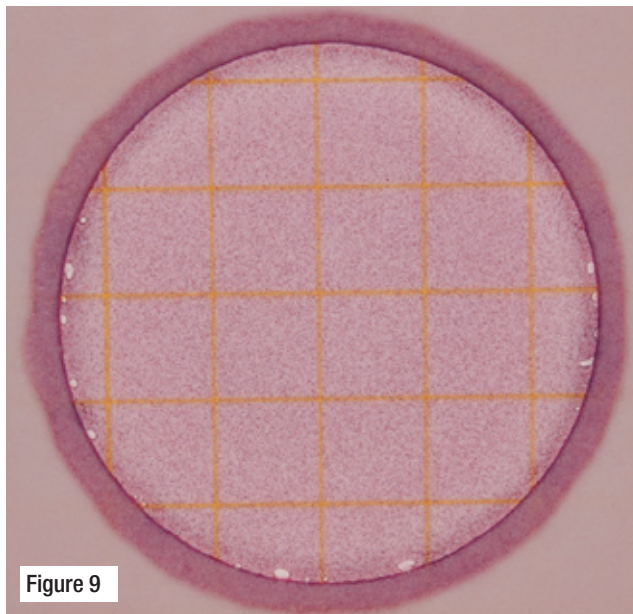

Figure 9

Actual count = ~10^8

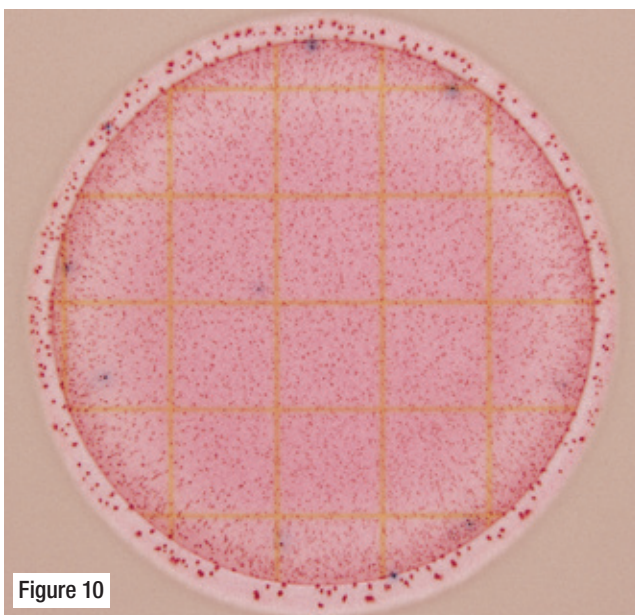High concentrations of *E. coli* may cause the growth area to turn purple-blue.

Figure 10

Presumptive *E. coli* count = ~8
Estimated total coliform count = ~10^8

When high levels of coliforms are present (>10^8), some strains of *E. coli* may produce less gas and blue colonies may be less definitive. Count all blue colonies without gas and/or blue zones as presumptive *E. coli.* Pick blue colonies without gas and confirm if necessary.
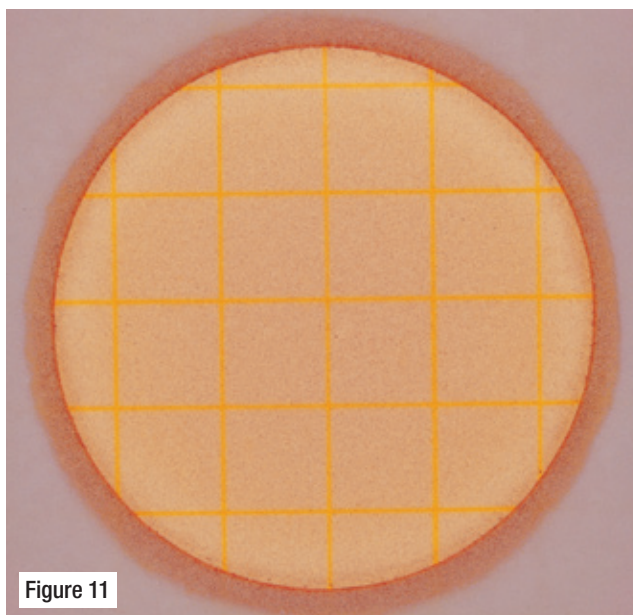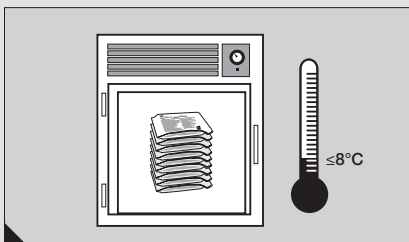

Figure 11

Actual count = ~10^8

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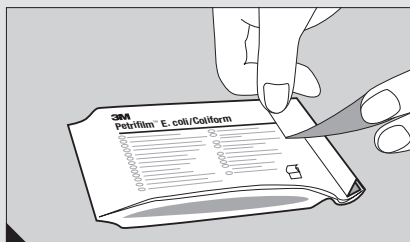
Reminders for Use: 3M™ Petrifilm™ *E. coli*/Coliform Count Plate

Storage

1 **Store** unopened pouches of plates at ≤8°C (≤46°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.
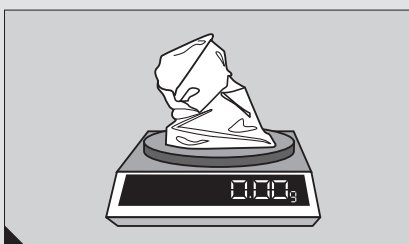
2 To seal opened pouch, fold end over and tape shut.
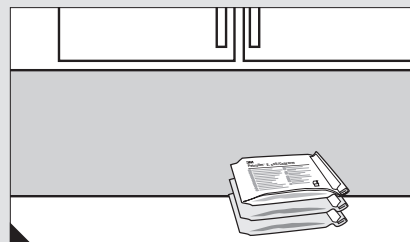
3 **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place. Use plates within one month after opening. Avoid exposure of plates to temperatures >25°C (>77°F) and/or relative humidity >50%.

Sample Preparation

4 Prepare a 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.

*See Petrifilm Use with Dairy and Juice Products sheet for recommended dilutions.

5 Add appropriate quantity of one of the following **sterile** diluents: Butterfield's phosphate buffer (IDF phosphate buffer, KH_2PO_4 @ 0.0425 g/L, adjust to pH 7.2), 0.1% peptone water, peptone salt diluent (ISO method 6887-1), buffered peptone water (ISO 6887-1), saline solution (0.85–0.90%), bisulfite-free letheen broth or distilled water. **Do not use buffers containing citrate, bisulfite or thiosulfate; they can inhibit growth.**
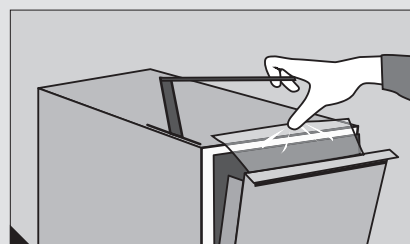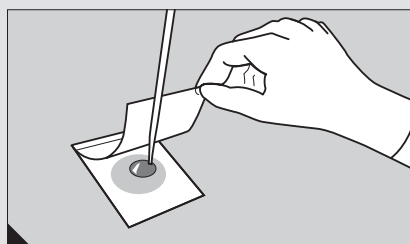
6 Blend or homogenize sample per current procedure. **For optimal growth and recovery of microorganisms, adjust the pH of the sample suspension to 6.6–7.2:**
• For acidic products, adjust the pH with *1N* NaOH
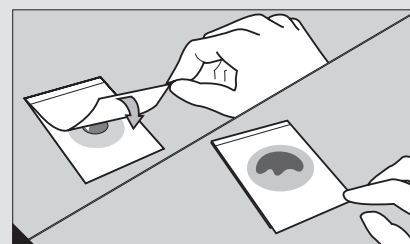• For alkaline products, adjust the pH with *1N* HCl

Inoculation
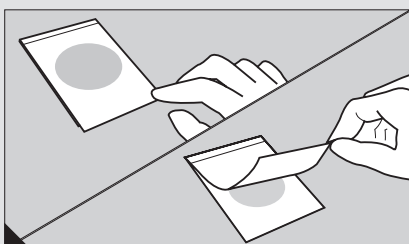
**7** Place 3M Petrifilm *E. coli*/Coliform Count Plate on **level** surface. Lift top film.

8 With 3M™ Electronic Pipettor or equivalent held **perpendicular** to plate, place 1mL of sample or diluted sample onto center of bottom film.

9 **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.

RIDGE side
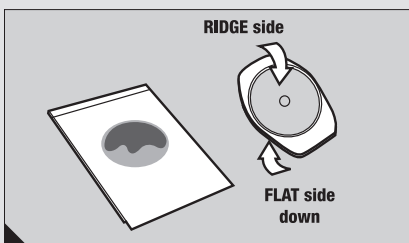
FLAT side down

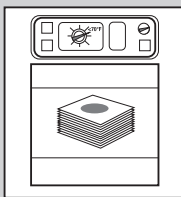**10** With **flat** side down, place 3M™ Petrifilm™ Spreader on top film over inoculum.
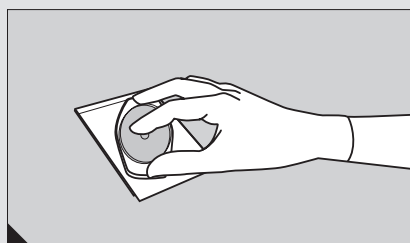
11 **Gently** apply pressure on 3M Petrifilm Spreader to distribute inoculum over circular area before gel is formed. **Do not** twist or slide the spreader.
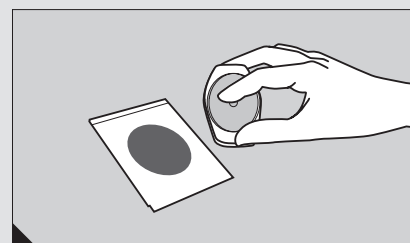
12 Lift 3M Petrifilm Spreader. Wait a minimum of 1 minute for gel to solidify.
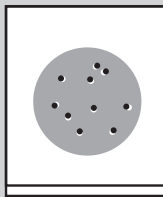
Incubation

**13** Incubate plates with clear side up in stacks of up to 20 at time and temperature listed below. It may be necessary to humidify incubator to minimize moisture loss.

Interpretation

14 3M Petrifilm *E. coli*/Coliform Count Plates can be counted on a standard colony counter or other illuminated magnifier. Refer to the **Interpretation Guide** section when reading results.
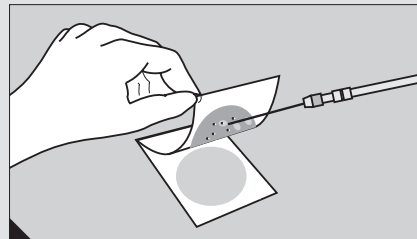
15 Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Incubation Time and Temperature Vary by Method

Most common approved methods:

AOAC® Official Method 991.14
- Coliforms: Incubate 24±2 hours at 35°C±1°C
- *E. coli*: Incubate 48±2 hours at 35°C±1°C

AOAC® Official Method 998.08
- Coliforms and *E. coli* (for meat, poultry and seafood): Incubate 24±2 hours at 35°C±1°C

NMKL Method 147.1993
- *E. coli*: Incubate 48±2 hours at 37°C±1°C

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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