

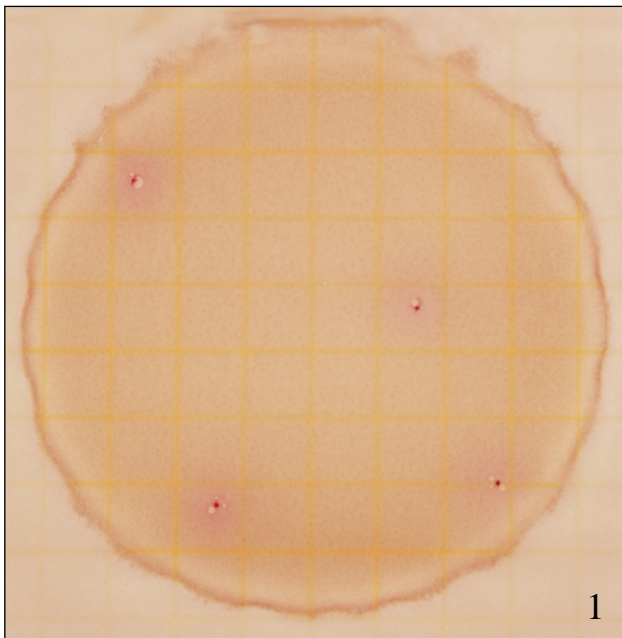


# Petrifilm™

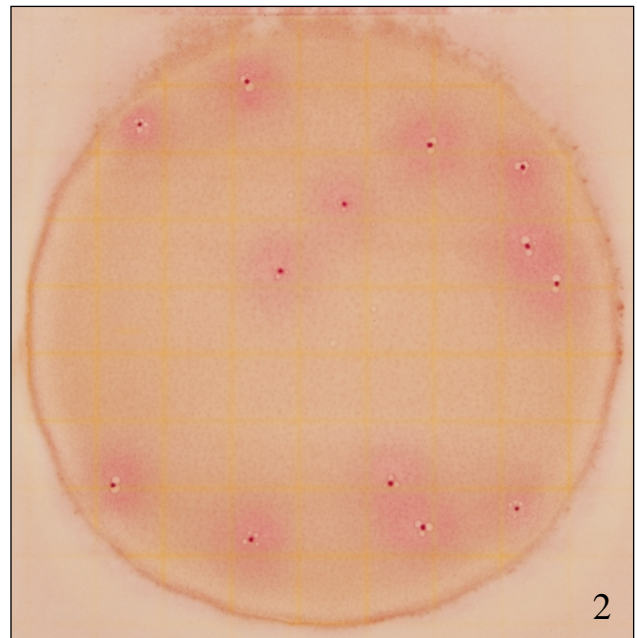
## High-Sensitivity Coliform Count Plate

This guide familiarizes you with results on 3M™ Petrifilm™ High-Sensitivity Coliform Count plates. For more information, contact the 3M Microbiology representative nearest you.

AOAC® INTERNATIONAL and U.S. FDA Bacteriological Analytical Manual (BAM) define coliforms as gram-negative rods which produce acid and gas from lactose during fermentation. Gas production is used to differentiate coliform from non-coliform colonies. Gas trapped around red colonies indicates coliforms on the Petrifilm High-Sensitivity Coliform Count plate. Acid production causes the pH indicator to deepen the gel color to a more pink-red background color.



**Coliform count = 4**



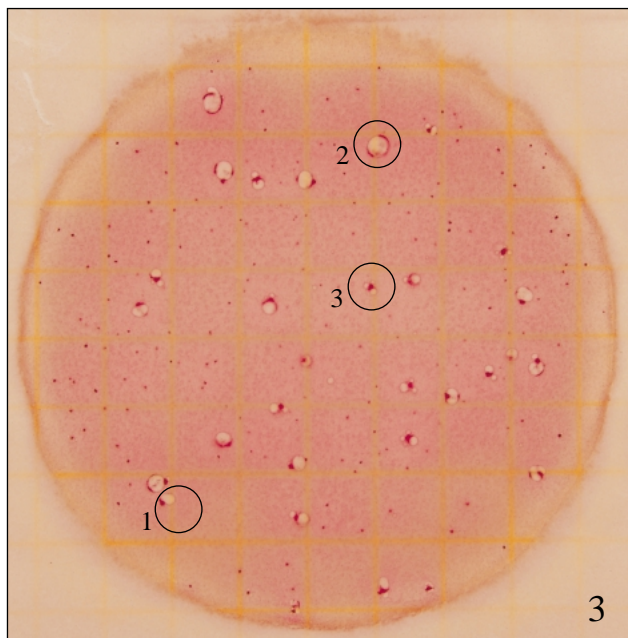
**Coliform count = 13**

It is easy to count coliform colonies on Petrifilm High-Sensitivity Coliform Count plates. A red indicator dye in the plate colors gram-negative colonies and the top film traps gas produced by the coliforms.

When coliforms produce acid, the gel surrounding the colony becomes more pink, as shown in figure 2.

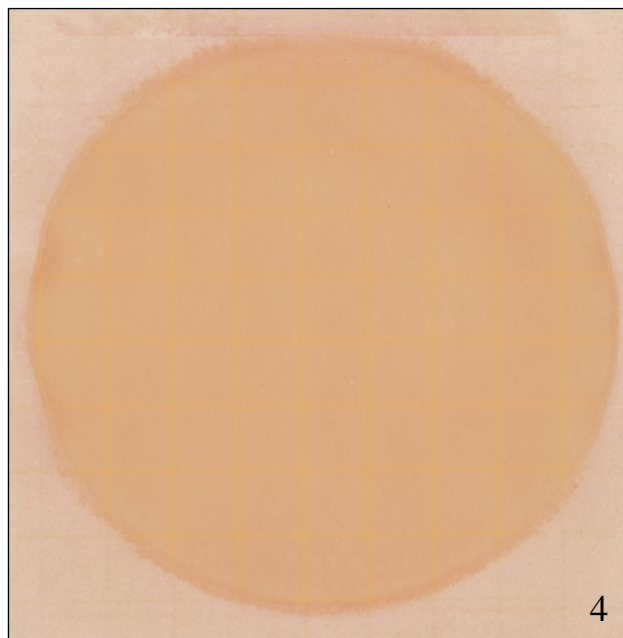
Look for pink-red zones around the colony to aid in counting. Count red colonies that are associated with gas bubbles as coliforms.

# 3M™ Petrifilm™ High-Sensitivity Coliform Count Plate



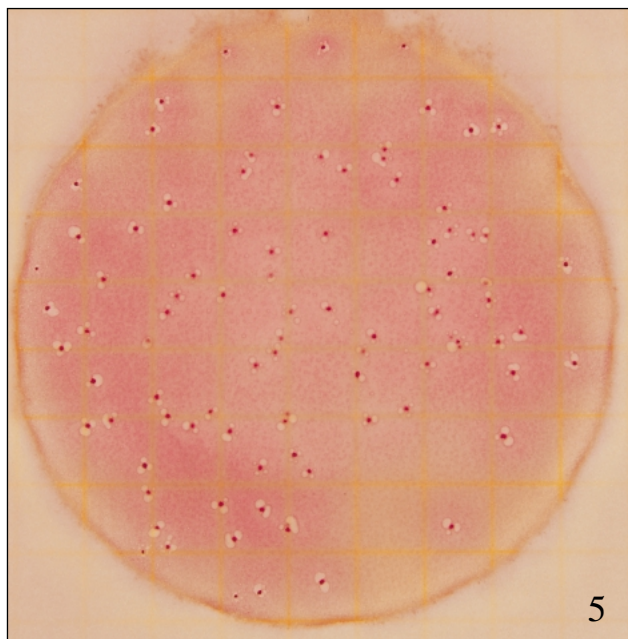
## Coliform count = 30

Gas production is used to differentiate coliform from non-coliform colonies. Circles 1, 2 and 3 show how bubble patterns may vary. The gas bubble in circle 1 is adjacent to the colony. In circle 2, the gas disrupts the coliform colony so that the colony “outlines” the bubble. In circle 3, three small gas bubbles circle the colony. All of these examples are coliforms. Red colonies which are not associated with gas bubbles should not be counted as coliforms.

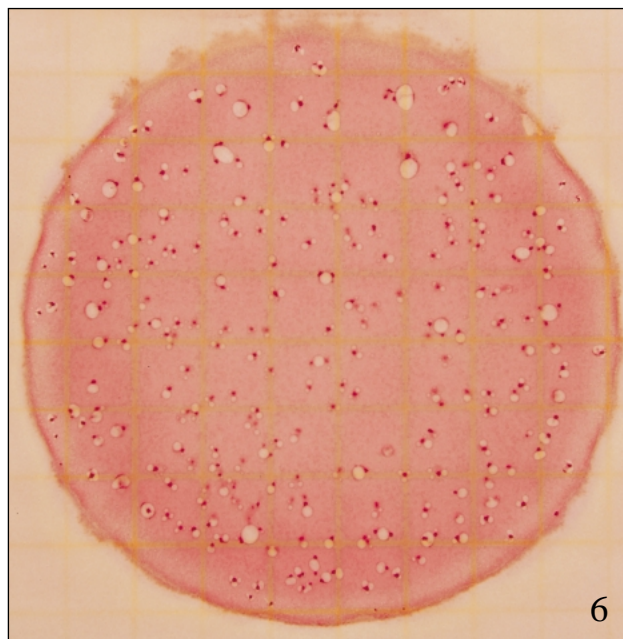


## Coliform count = 0

Notice the change in gel color in figures 4 through 9. As the coliform count and acid production increases, the color of the gel deepens from a light orange in figure 4 to a bright pink-red in figure 9. Plating and incubating a negative control will aid in differentiating changes in gel color.



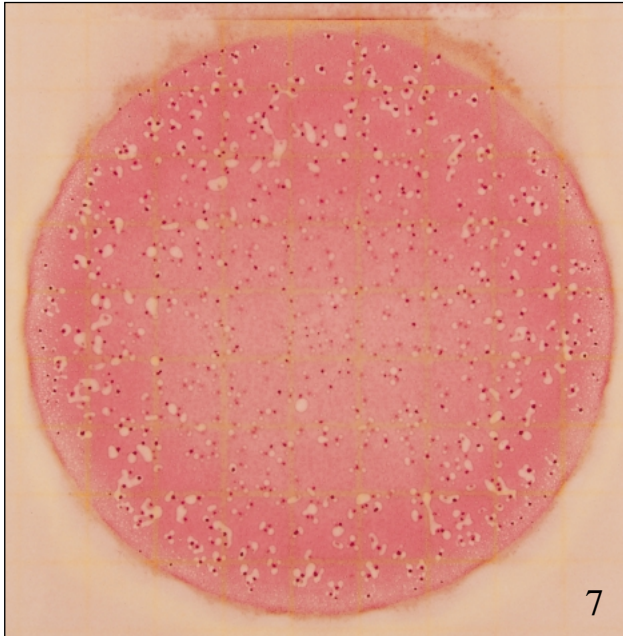
## Coliform count = 90



## Estimated coliform count = 320

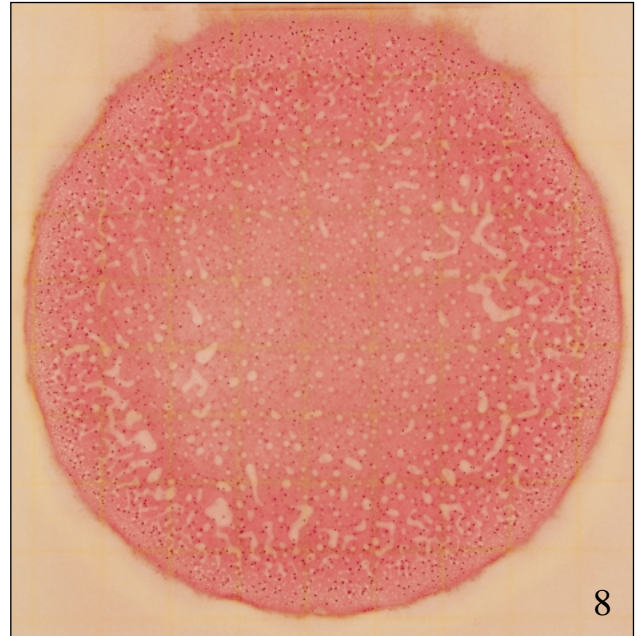
The circular growth area is approximately 60 cm<sup>2</sup>. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 60 to determine the estimated count per plate.





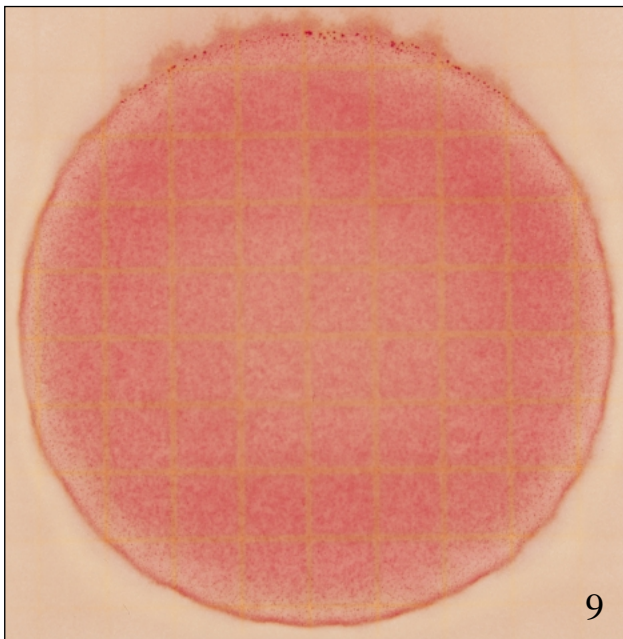
**Estimated coliform count = 840**

Colonies and gas bubbles may be smaller around the edge of the inoculated area, as noted in figure 7. The different gel appearance around the edge of the inoculum does not affect colony counts.



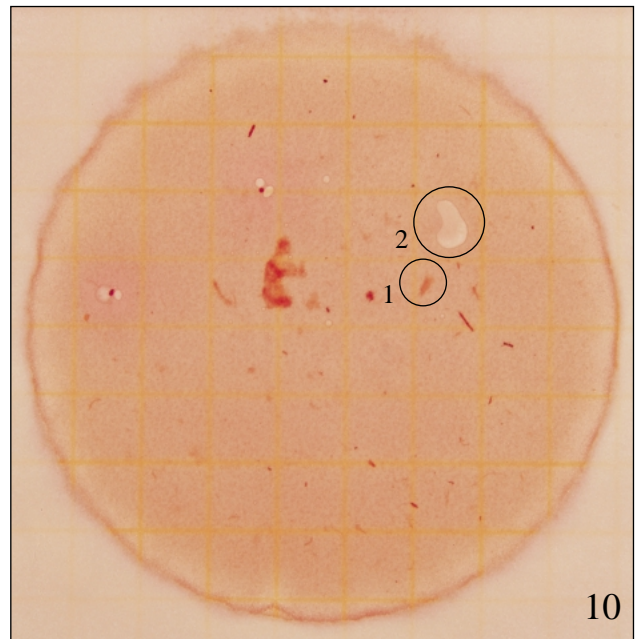
**Coliform count = TNTC (Estimated Count > 10<sup>5</sup>)**

A TNTC Coliform count will cause the gel to turn a darker pink-red color. Additionally, one may observe many small colonies and/or many gas bubbles. The higher the count the less prominent the gas and colonies may be. All three characteristics are shown in figure 8.



**Coliform count = TNTC (Estimated Count > 10<sup>4</sup>)**

Figure 9 shows many small colonies and a deepening of the gel color.



**Coliform count = 2**

Food particles often are irregularly shaped and are not associated with gas bubbles. See circle 1.

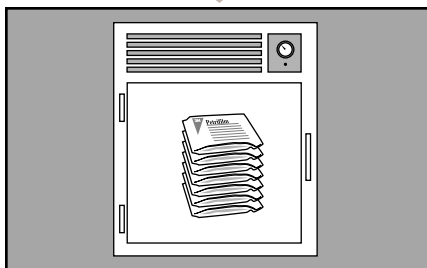
Artifact bubbles may result from improper inoculation of the Petrifilm High-Sensitivity Coliform Count plate. They are irregularly shaped and not associated with a red colony. See circle 2.

# 3M Petrifilm™ High-Sensitivity Coliform Count Plates

## Reminders for Use

For detailed WARNING, CAUTIONS, DISCLAIMER OF WARRANTIES / LIMITED REMEDY, LIMITATION OF 3M LIABILITY, STORAGE AND DISPOSAL information, and INSTRUCTIONS FOR USE see PRODUCT PACKAGE INSERT.

### Storage



- 1 **Store** unopened pouches 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.

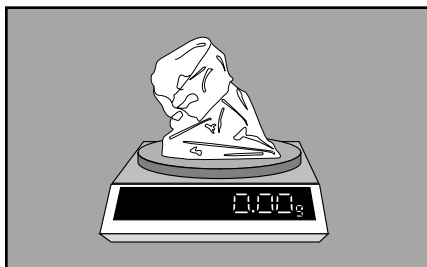


- 2 To seal opened package, fold end over and tape shut.

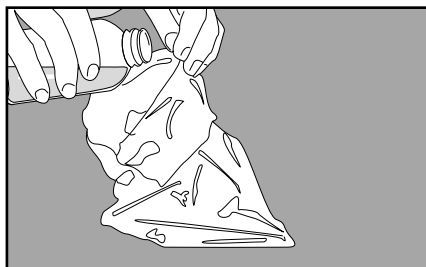


- 3 To prevent exposure to moisture, do not refrigerate opened pouches. Store resealed pouches in a cool, dry place for no longer than one month. Avoid exposing plates to temperature  $> 25^{\circ}\text{C}$  ( $> 77^{\circ}\text{F}$ ) and/or relative humidity  $> 50\%$ .

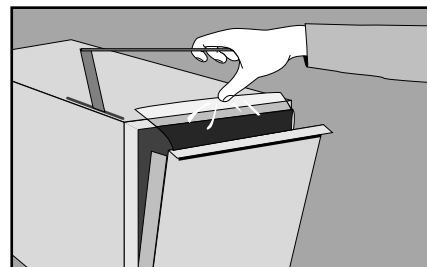
### Sample Preparation



- 4 Prepare dilution of food product. Weigh or pipette food product into a sterile container such as a homogenizer bag or dilution bottle.



- 5 Add appropriate quantity of one of the following **sterile** diluents: Butterfield's phosphate buffer, (IDF phosphate buffer,  $\text{KH}_2\text{PO}_4$  @ 0.0425 g/L, adjust to pH 7.2), 0.1% peptone water, peptone salt diluent (ISO method 6887), saline solution (0.85 - 0.90%), or distilled water.



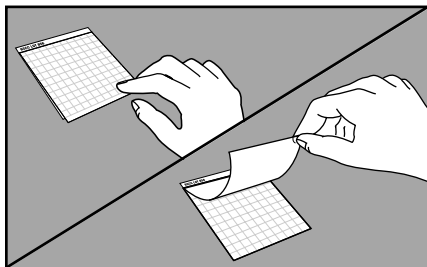
- 6 Blend or homogenize sample per current procedure.

For acidic products, adjust pH of the diluted sample to 6.5 - 7.5 with 1N NaOH. For alkaline products adjust pH with 1N HCl.

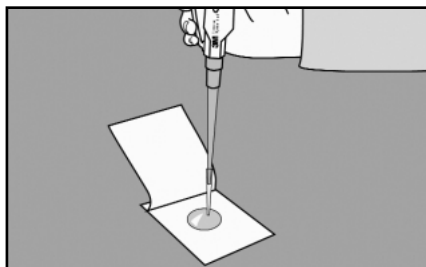
Do not use buffered peptone water or buffers containing citrate, bisulfite, or thiosulfate; they can inhibit growth.

### Inoculation

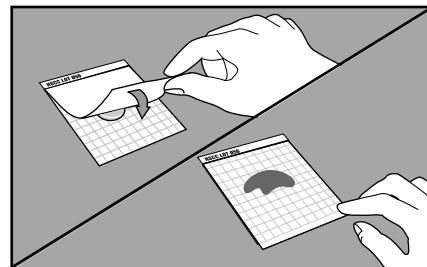
Remember to inoculate and spread each Petrifilm plate before going on to the next plate.



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

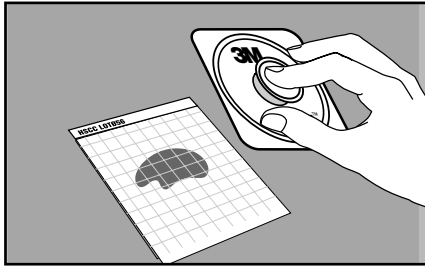


- 8 With 3M™ Electronic Pipettor or equivalent **perpendicular** to Petrifilm plate, place **5 mL** of sample or diluted sample onto center of bottom film.

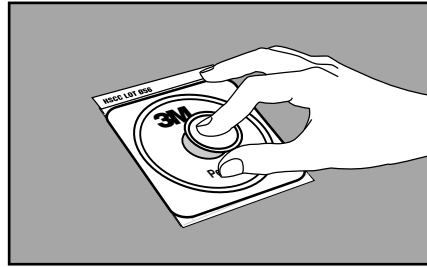


- 9 **ROLL** top film down onto sample **GENTLY** to prevent pushing sample off film and to avoid entrapping air bubbles. Do **NOT** let top film drop.

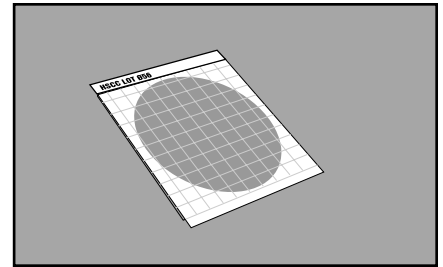
Continued - over



**10** Place the High-Sensitivity plate spreader on top film over inoculum.

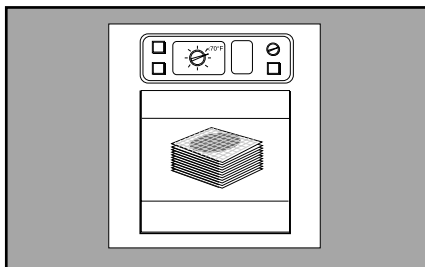


**11** Distribute sample with a **gentle** downward pressure on the handle of the spreader. Do not twist or slide the spreader.



**12** Lift spreader. Wait 2-5 minutes for gel to solidify.

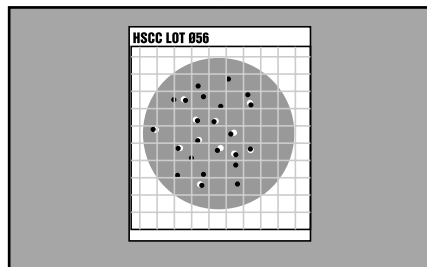
## Incubation



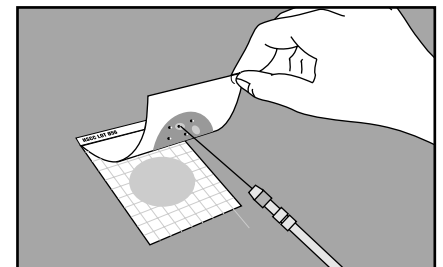
**13** Incubate plates with clear side up in stacks of up to 10 at temperature and time indicated below. For complete listing refer to package insert.

Humidify incubator to minimize loss of moisture.

## Interpretation



**14** Petrifilm plates can be counted on a standard colony counter or other illuminated magnifier.



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

## Dilutions

Recommended dilutions for dairy products:

**1:10 minimum dilution required for: cultured dairy products and dry dairy products.**

1:10 dilution yields a 2 coliform per gram sensitivity.

**1:5 minimum dilution required for: chocolate milk, evaporated milk, heavy and light cream, ice cream, and sweetened condensed milk.**

1:5 dilution yields a 1 coliform per gram sensitivity.

**Pasteurized milk and raw milk may be plated directly. For further information, refer to 3M™ Petrifilm™ Plate Guide to Dilution Preparation or 3M™ Petrifilm™ Plates Use with Dairy and Juice Products.**

Incubation time and temperature vary by method.

### Most common methods used in the United States:

- AOAC Official Method 996.02  
Coliform Count in Dairy Products:  
Incubate 24h ± 2h at 32°C ± 1°C.
- All foods except dairy products:  
Incubate 24h ± 2h at 35°C ± 1°C.

### Most common methods used in Europe:

- AFNOR validated method  
3M 01/7 - 03/99:  
Incubate 24h ± 2h at 30° C ± 1° C  
or 35° C ± 1° C, or 37° C ± 1° C  
for total coliforms.
- Incubate 24h ± 2h at 44° C ± 1° C  
for thermotolerant coliforms.  
Incubator humidification is  
required at this elevated  
temperature.

## Additional Information

- Questions? U.S., call **1-800-328-6553**.
- To order Petrifilm plates in the U.S., call **1-800-328-1671**.
- Canada, call **1-800-563-2821** for technical service.
- Latin America / Africa and Asia Pacific regions, call **1-651-733-7562**.
- 3M Microbiology offers a full line of products to accomplish a variety of your microbial testing needs. For more product information, visit us at **[www.3M.com/microbiology](http://www.3M.com/microbiology)**.



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