

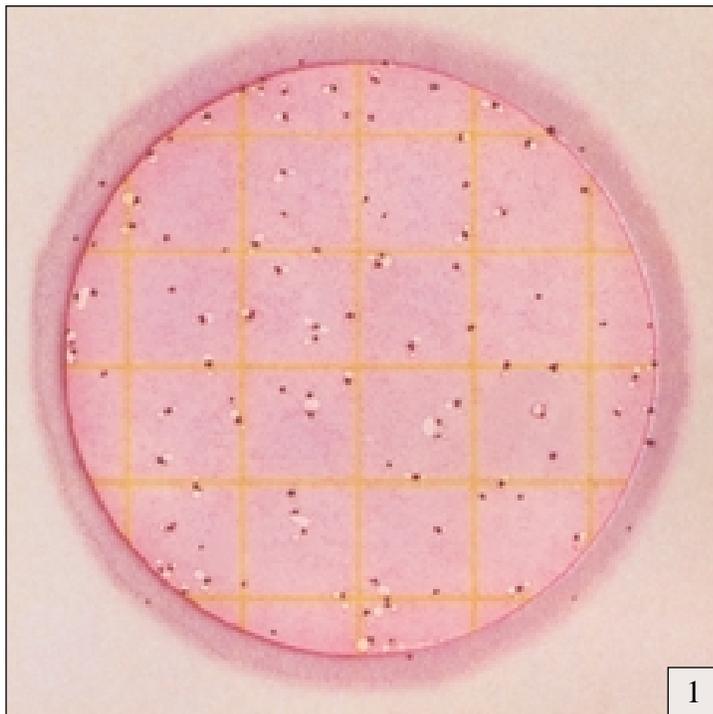


Petrifilm™ Coliform Count Plate

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

Petrifilm Coliform Count (CC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, and a tetrazolium indicator that facilitates colony enumeration. The top film traps gas produced by the lactose fermenting coliforms.

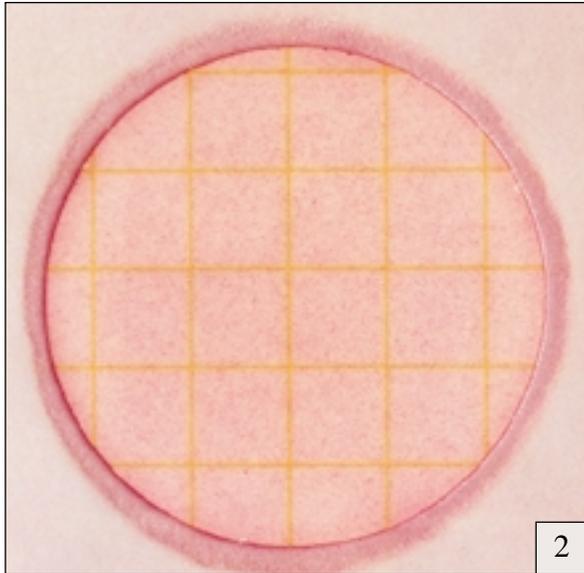
AOAC INTERNATIONAL and U.S. FDA Bacteriological Analytical Manual (BAM) define coliforms as gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm CC plate produce acid which causes the pH indicator to deepen the gel color. Gas trapped around red coliform colonies indicates confirmed coliforms.



The identification of coliforms may vary by country (see Reminders for Use section for incubation times and temperatures):

AOAC INTERNATIONAL validated method
Total coliform = 69 (colonies with gas)

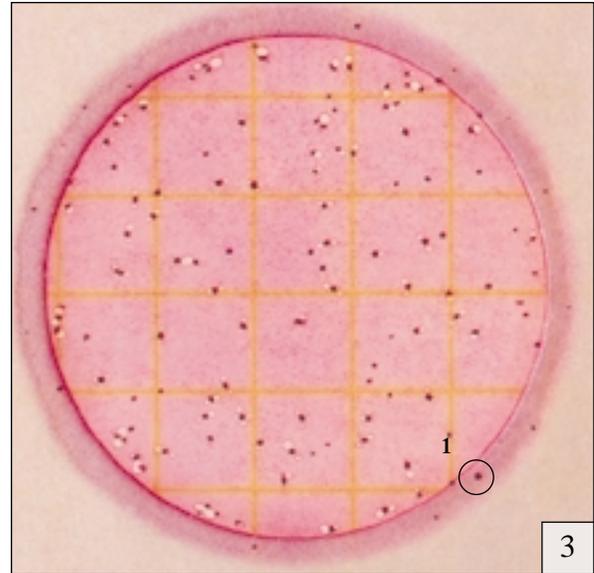
3M™ Petrifilm™ Coliform Count Plate



No growth = 0

Notice the changes in gel color in figures 2 through 5. As the coliform count increases, the gel color deepens.

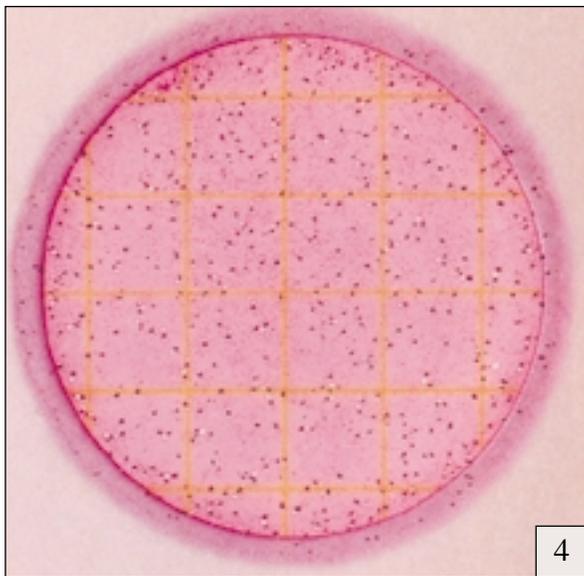
Background bubbles are a characteristic of the gel and are not a result of coliform growth.



Total coliform count = 79

The counting range for the total population on Petrifilm CC plates is 15–150.

Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the medium. See circle 1.



Estimated total coliform count = 220

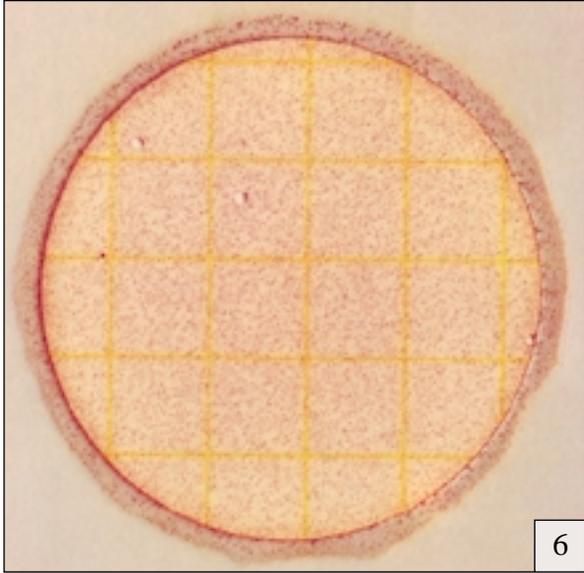
The circular growth area is approximately 20 cm². Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per plate.

Further dilution of the sample is recommended for an accurate count.



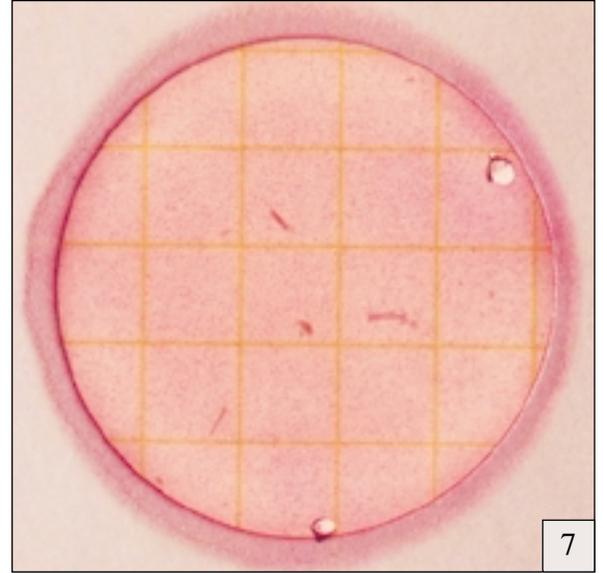
TNTC

Petrifilm CC 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.



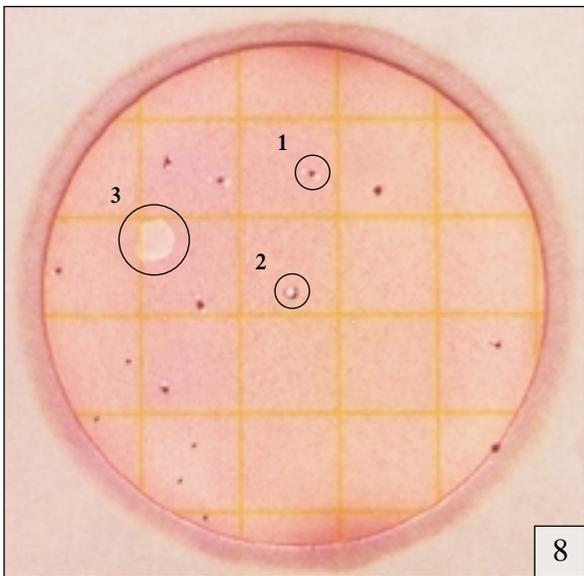
Actual count = 4

When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm CC plates, the gel may turn yellow.



Total coliform count = 2

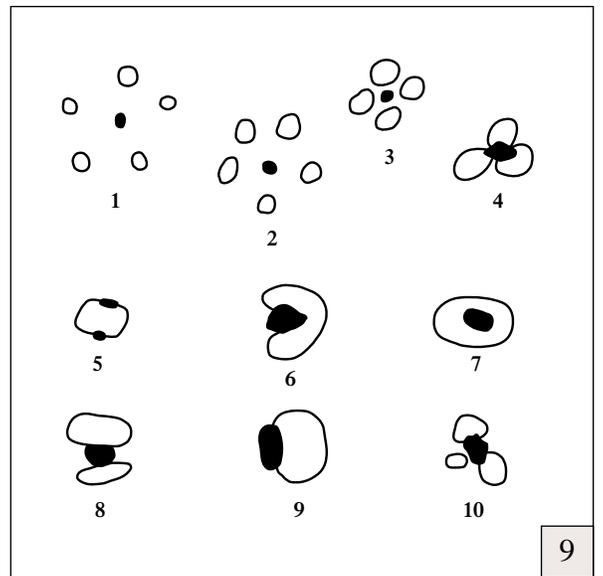
Food particles are irregularly shaped and are not associated with gas bubbles.



Total coliform count = 8

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.



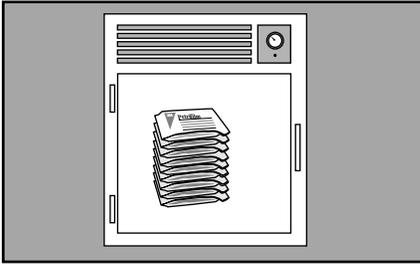
Examples 1–10 show various bubble patterns associated with gas producing colonies. All should be enumerated.

3M Petrifilm™ Coliform Count Plates

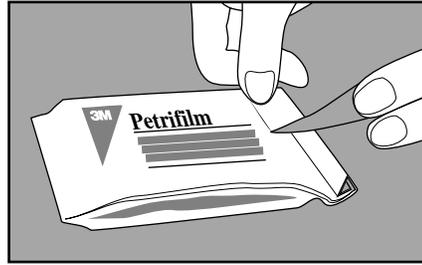
Reminders for Use

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

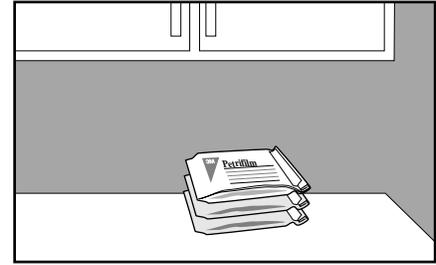
Storage



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.

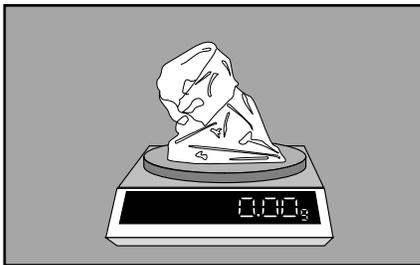


2 To seal opened package, fold end over and tape shut.



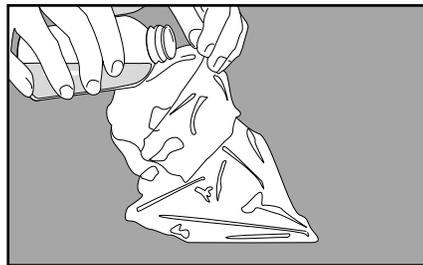
3 Do not refrigerate opened packages. Use Petrifilm plates within one month after opening.

Sample Preparation



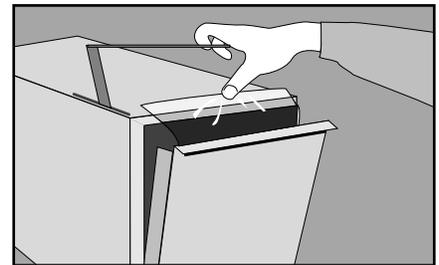
4 Prepare a dilution of food product.* Weigh or pipette food product into an appropriate container such as a stomacher bag, dilution bottle, Whirl-Pak® bag, or other sterile container.

*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, 0.0425 g/L of KH_2PO_4 adjusted to pH 7.2, 0.1% peptone water, peptone salt diluent (ISO method 6887), buffered peptone water (ISO method 6579), 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.

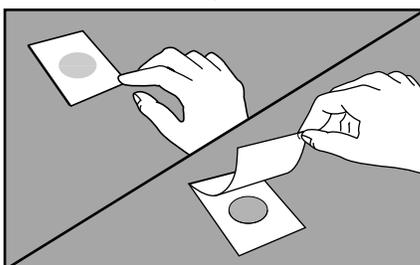


6 Blend or homogenize sample per current procedure.

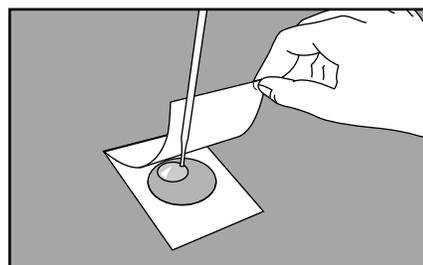
Adjust pH of the diluted sample between 6.6 and 7.2 :

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

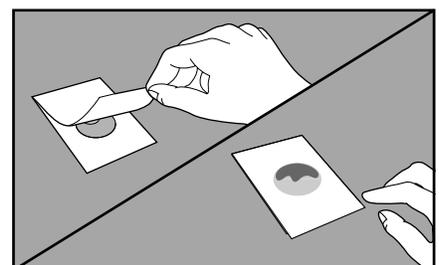
Inoculation



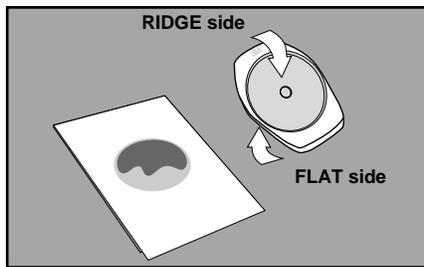
7 Place Petrifilm plate on level surface. Lift top film.



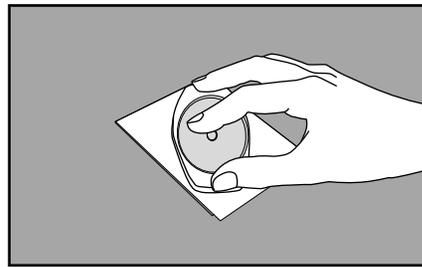
8 With pipette **perpendicular** to Petrifilm plate, place 1 mL of sample onto center of bottom film.



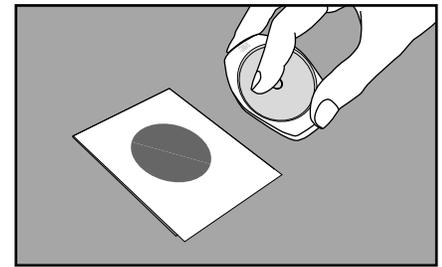
9 Carefully **roll** top film down to avoid entrapping air bubbles. Do **not** let top film drop.



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

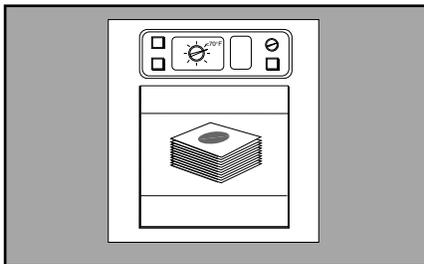


11 **Gently** apply pressure on spreader to distribute inoculum over circular area before gel is formed. Do not twist or slide the spreader.



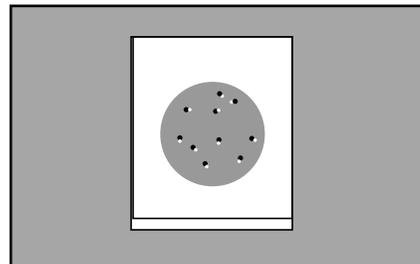
12 Lift spreader. Wait a minimum of one minute for gel to solidify.

Incubation

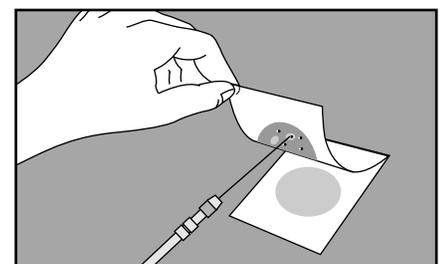


13 Incubate plates in stacks of up to 20.

Interpretation



14 Petrifilm 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 varies by method. Most common approved methods:

Total coliforms

- AOAC Official Methods 986.33 and 989.10 (milk, raw milk, other dairy products):
Incubate 24h ± 2h at 32°C ± 1°C.
- AOAC Official Method 991.14 (foods):
Incubate 24h ± 2h at 35°C ± 1°C.
- NMKL Method 147.1993:
Incubate 24h ± 2h at 37°C ± 1°C.
- AFNOR validated methods 3M 01/2 - 09/89A and B (all food types except shellfish):
Incubate 24h ± 2h at 30°C ± 1°C.

Thermotolerant (fecal) coliforms

- AFNOR validated method 3M 01/2 - 09/89C (all food types):
Incubate 24h ± 2h at 44°C ± 1°C.
Incubator humidification is required at this elevated temperature.

Additional Comments

- Questions? U.S., call **1-800-328-6553**, Canada, call **1-800-563-2921** 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**.

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