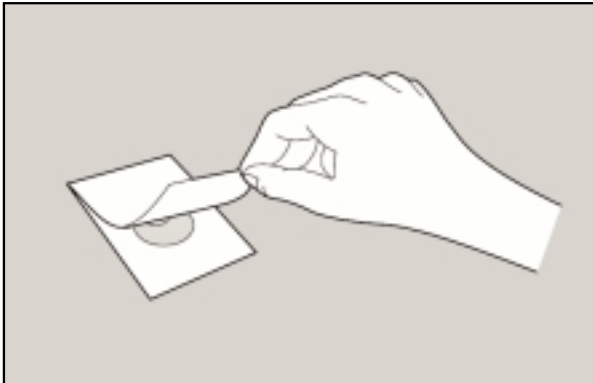


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# 3M<sup>™</sup> Petrifilm<sup>™</sup> E. coli/Coliform Count Plates

Instruction Manual



## Description

The 3M™ Petrifilm™ E. coli/Coliform Count (EC) Plate is a ready-made culture medium system designed to facilitate counting coliforms and *E. coli*. Petrifilm EC Plates contain nutrients, a cold-water-soluble gelling agent, and indicator dyes. Petrifilm plates are prepared on a grid background to facilitate counting colonies.

Petrifilm EC plates are designed to allow organisms to grow in an oxygen-limited environment. Their system of nutrients and dyes enables the user to identify colonies formed by coliforms and by *E. coli*. For more information, please see **Interpretation**. Petrifilm plates can be used in a variety of ways:

- Petrifilm plates can be hydrated with a bacterial culture, or a dilution of a culture, for counting the viable organisms present. See Method A.
- Petrifilm plates can be hydrated first with water or buffer, then inoculated by swabbing, streaking, or touching to surfaces. See Method B.
- Antibiotics can be added to the hydration fluid to select for resistant organisms in Method A or B.
- Cells can be removed from colonies growing on Petrifilm plates and used to inoculate additional cultures or for staining.
- Experimental results on Petrifilm plates can be saved for future reference by scanning the plates on a standard computer-linked scanner. Do not open the plates for scanning.

Petrifilm plates were developed for use in the food and beverage industry. They have been certified for official analyses in many countries. For more information about these applications, see the 3M page on the World Wide Web at [www.3m.com](http://www.3m.com).

## Storage

**Refrigerate unopened packages at  $\leq 8^{\circ}\text{C}$  ( $\leq 46^{\circ}\text{F}$ ).** Use before expiration date on package.

To seal an opened package, fold the end over and tape it shut (fig. 1).



**Figure 1**

Keep resealed packages at room temperature and less than 50% relative humidity. **Do not refrigerate opened packages.** Use plates from the opened package within one month after opening.

## Directions for Use

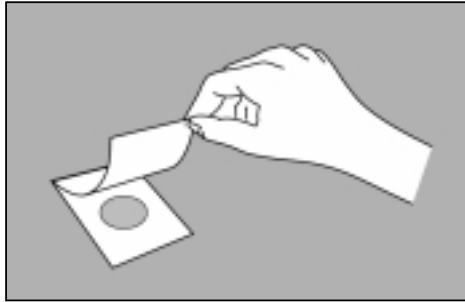
Use sterile technique when handling Petrifilm plates. Disinfect the work area by wiping thoroughly with alcohol or other disinfectant before and after use. Used Petrifilm plates may contain viable organisms. Do not open the plates unnecessarily. The procedures in this instruction booklet should be used for educational purposes only.

### **Method A. Inoculation with liquid sample**

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

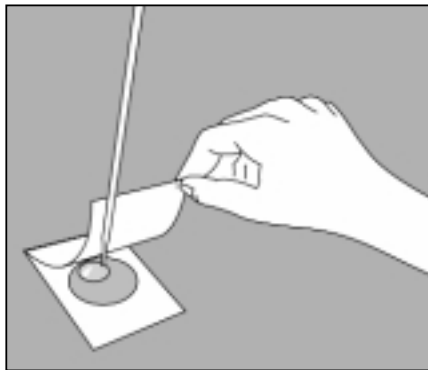
1. If a Petrifilm plate pack has been stored in the refrigerator, let the package come to room temperature before opening it. This step prevents condensation from forming inside the package.

2. Place the Petrifilm plate on a level surface, with the gridded side down. Lift the top film (fig. 2).



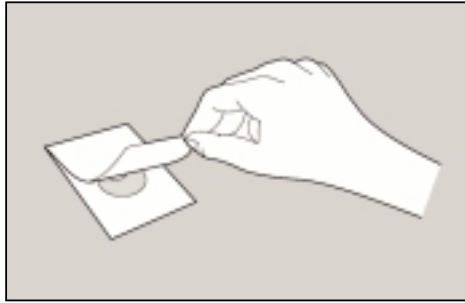
**Figure 2**

3. With pipet perpendicular to the Petrifilm plate, place 1 mL of sample onto the center of the bottom film (fig. 3). If necessary, samples can be diluted with distilled water, liquid culture medium, or buffers with pH between 6.6 and 7.2.\* If antibiotics are to be added to the medium, add them to the inoculating liquid at the working concentration.



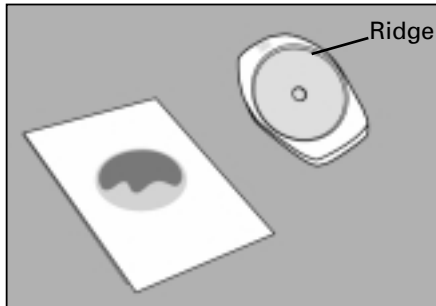
**Figure 3**

4. **Roll** the top film onto the bottom film. Do not drop the top film down (fig. 4).



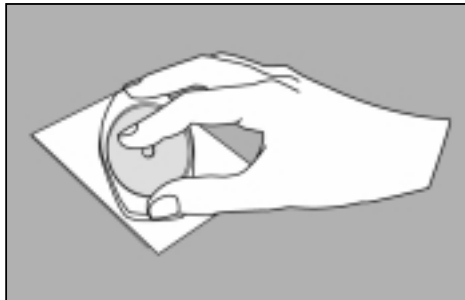
**Figure 4**

5. With the flat side down (not the side with the circular ridge), place the spreader on the top film over the inoculum (fig. 5).



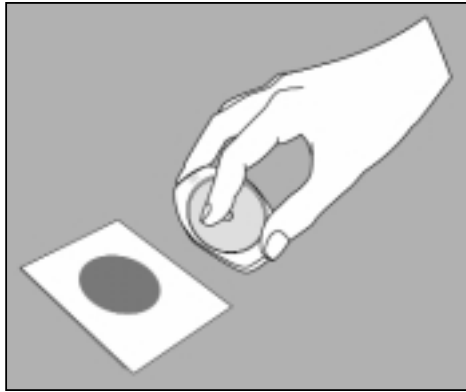
**Figure 5**

6. **Gently** apply pressure on the spreader to distribute the inoculum over a circular area. Do not twist or slide the spreader (fig. 6).



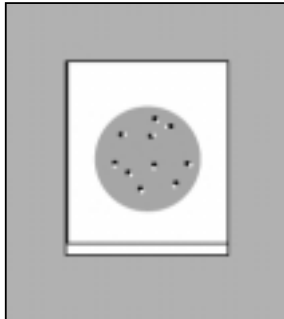
**Figure 6**

7. Lift the spreader. Wait at least 1 minute for the gel to solidify (fig. 7).



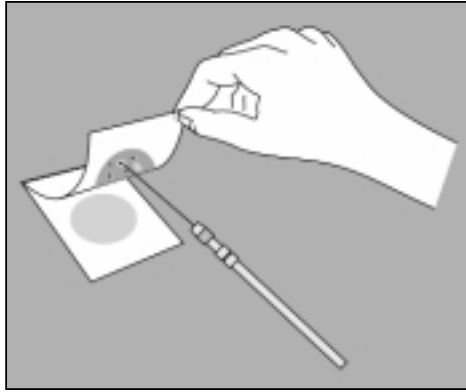
**Figure 7**

8. Incubate plates with the gridded side down in stacks of up to 20 plates. Incubation time and temperature will vary according to the application and equipment available.
9. Colonies on Petrifilm plates can be counted on a standard colony counter or other light source (fig. 8). Bacterial colonies on Petrifilm EC plates are red or blue because of the indicator dyes in the medium. Refer to the Interpretation section on pg. 8 and 9 for more details.



**Figure 8**

10. Colonies may be isolated for further study or to inoculate additional cultures. Lift the top film and pick the colony from the gel. The medium will adhere to the top film (fig. 9).



**Figure 9**

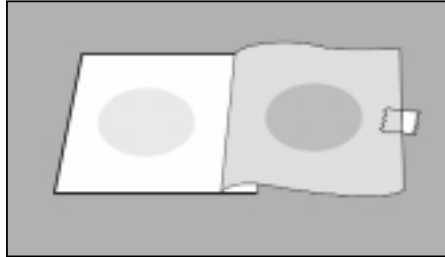
11. Disinfect before disposal. Petrifilm plates can be disinfected by autoclaving or by soaking in 20% bleach for 1 hour. Then, they can be placed in the trash. Alternatively, they can be taken to a facility such as a hospital or given to a school nurse for disposal with other biohazardous material.

\*Do not use diluents that contain citrate or sodium thiosulfate because they can inhibit growth on the Petrifilm plates. These substances are not found in common microbiological media such as Luria broth or nutrient broth.

### **Method B. Hydrating and using as solid medium**

1. Follow steps 1–6 from Method A, using 1 mL of distilled water, liquid culture medium, or buffers with pH between 6.6 and 7.2 to hydrate the Petrifilm plate.\* If antibiotics are to be added to the medium, add them to the hydration liquid at the working concentration.
2. Lift the spreader. Wait at least 2 hours for the gel to solidify.
3. Hydrated Petrifilm EC plates can be stored in a sealed bag in the refrigerator for up to 7 days before use.

- To inoculate the medium, lift the top film. The circular gel area will adhere to the top film (fig. 10). Tape the Petrifilm plate to a flat surface in the open position for streaking. Streak with a sterile inoculating loop more gently and with less pressure than you would use on a standard agar plate.



**Figure 10**

- Incubate the plates with the gridded side down, in stacks of up to 20 plates.

Colonies may be isolated for further study or to inoculate additional cultures. Lift the top film and pick the colony from the gel (See Method A, step 10).

\*Do not use diluents that contain citrate or sodium thiosulfate. These substances are not found in common microbiological media such as Luria broth or nutrient broth.

## Interpretation

The U.S. Food and Drug Administration Bacteriological Analytical Manual defines coliforms as gram-negative rods that produce acid and gas from lactose fermentation. *E. coli* is a coliform, but unlike other coliforms, most strains of *E. coli* produce the enzyme beta-glucuronidase. Petrifilm EC Plates are formulated to differentiate non-coliform, coliform, and *E. coli* colonies.

Petrifilm EC Plates contain an indicator dye in the medium that stains bacterial colonies red. The red color helps to distinguish them from dust particles or other environmental contaminants. The EC Plate growth medium contains lactose, and the top film of the plates traps gas produced by coliforms. Finally, EC Plates contain an indicator of beta-glucuronidase activity, 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide (BCIG). The enzyme beta-glucuronidase cleaves BCIG to produce a blue product that precipitates, causing a beta-glucuronidase-producing bacterial colony (such as *E. coli*) to appear blue. With this formulation, non-coliform colonies growing on

Petrifilm EC plates appear red with no gas bubbles, coliform colonies appear red with gas bubbles, and *E. coli* colonies appear blue with gas bubbles.

Sometimes gas bubbles are trapped when the inoculate is spread on Petrifilm plates. These bubbles are irregularly shaped and not associated with a bacterial colony, while gas bubbles produced by lactose-fermenting bacteria are associated with a colony (see fig. 11.)

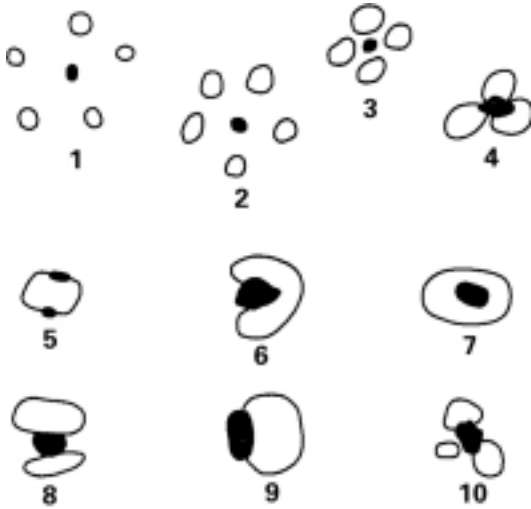


Figure 11. Gas bubbles produced by colonies

### Sample Plate

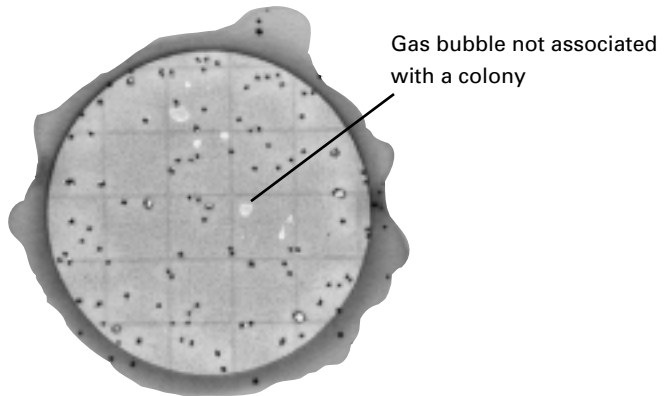


Figure 12.





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