This guide familiarizes you with results on 3M™ Petrifilm™ Enterobacteriaceae Count Plates. For more information, contact the official 3M Food Safety Products representative nearest you.
An indicator in the 3M™ Petrifilm™ *Enterobacteriaceae* Count Plate colors all colonies red. The top film traps gas produced by some bacteria. Acid-producing bacteria are seen as red colonies surrounded by yellow zones.

Bacteria producing gas and/or acid are considered to be presumptive *Enterobacteriaceae* and will have one of the following characteristics on the 3M Petrifilm *Enterobacteriaceae* Count plate: colonies associated with gas bubbles and no acid zones (see Figure 1, Circle 1), colonies with yellow acid zones but no gas production (see Figure 1, Circle 2), or colonies producing both gas and acid (see Figure 1, Circle 3).

Figure 1 also illustrates how bubble patterns can vary. Sometimes gas disrupts the colony so that the colony “outlines” the gas bubble as in Figure 1, Circle 3.

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**Figure 1**

*Enterobacteriaceae Count = 13*

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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.
Enterobacteriaceae Count = TNTC

In Figure 7, the count is so high that acid zones and gas bubbles are not easily seen. A lightening of the gel color indicates that the result is TNTC. To obtain a more accurate count, dilute the sample further.

Enterobacteriaceae Count = 77

The recommended counting range on 3M Petrifilm Enterobacteriaceae Count Plates is 15–100 colonies. Samples having counts greater than 100 Enterobacteriaceae per plate may be estimated. The circular growth area is approximately 20cm². Estimates can be made by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number of colonies per square by 20 to determine the estimated count per plate.

Enterobacteriaceae Count = 35

Enterobacteriaceae Count = TNTC

3M Petrifilm Enterobacteriaceae Count Plates with more than 100 colonies are considered too numerous to count (TNTC) and have a light background color along with at least one of the following characteristics: many small colonies or many gas bubbles (see Figure 6). To obtain a more accurate count, dilute the sample further.

Enterobacteriaceae Count = 35

Enterobacteriaceae Count = TNTC

In Figure 6, the count is so high that acid zones and gas bubbles are not easily seen. A lightening of the gel color indicates that the result is TNTC. To obtain a more accurate count, dilute the sample further.

Enterobacteriaceae Count = 77

The recommended counting range on 3M Petrifilm Enterobacteriaceae Count Plates is 15–100 colonies. Samples having counts greater than 100 Enterobacteriaceae per plate may be estimated. The circular growth area is approximately 20cm². Estimates can be made by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number of colonies per square by 20 to determine the estimated count per plate.
**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.

**Enterobacteriaceae Count = TN TC**

The 3M Petrifilm *Enterobacteriaceae* Count Plate in Figure 8 has two characteristics indicating TN TC colonies: lightening of the gel color and many small colonies. To obtain a more accurate count, dilute the sample further.

**Enterobacteriaceae Count = 2**

Food particles are often irregularly shaped or filamentous and are not associated with gas bubbles or acid zones (see Figure 10). Do not enumerate.

**Enterobacteriaceae Count = 44**

Artifact bubbles may result from improper inoculation of the 3M Petrifilm *Enterobacteriaceae* Count Plate. They are irregularly shaped and not associated with a red colony (see Figure 9). Do not enumerate.

**Enterobacteriaceae Count = 29**

Food particles also can be seen as dark spots but are not associated with gas bubbles or acid zones (see Figure 11). Do not enumerate.
**Reminders for Use: 3M™ Petrifilm™ Enterobacteriaceae 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 (10F phosphate buffer, KH₂PO₄ @ 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 tryptone 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 6.8–7.2:**
   - For acidic products, adjust the pH with 1N NaOH
   - For alkaline products, adjust the pH with 1N HCl

### Inoculation

7. **Place 3M Petrifilm Enterobacteriaceae 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.**

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 Time and Temperature Vary by Method

Most common approved methods:

- **Compendium of Methods for the Microbiological Examination of Foods**
  Incubate 24±2 hours at 35±1°C

- **AFNOR Validated Method 3M 01/06 09/97**
  Incubate 24±2 hours at 30±1°C, 35±1°C or 37±1°C
  Incubator humidification is required at this elevated temperature

- **AOAC® Official Method 2003.01 Enumeration of Enterobacteriaceae in Selected Foods**
  Incubate 24±2 hours at 37±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.

References
1. ISO7402 1993

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 www.3M.com/foodsafety or call 1-800-328-6553.