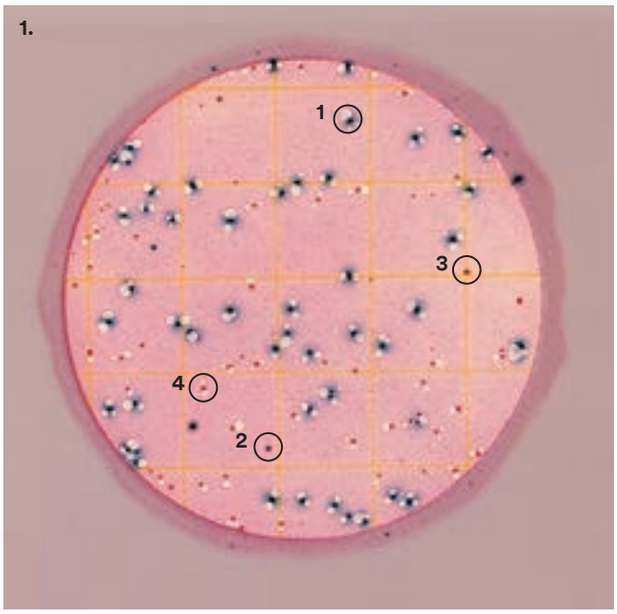
3M Science. Applied to Life.™

Interpretation Guide

3M™ Petrifilm™ E. coli and Coliform Count Plates

This guide should familiarize you with results on 3M™ Petrifilm™ *E. coli* and Coliform Count plates (EC). For more information contact the official 3M Food Safety Products representative in your area.

Petrifilm EC plates contain VRB nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity BCIG, and a tetrazolium indicator that facilitates colony enumeration. The top film traps gas produced by the lactose fermenting Coliforms and *E. coli*.



- ***Time and Temperature of incubation as well as interpretation of the Petrifilm EC plates vary by method. Therefore, this may give slightly different results. Temperatures of incubation used in the most common methods are mentioned in the package insert, and examples are shown in the technical section (pages 2 and 3) of this Guide.***

- ***Do not count colonies on the foam dam since they are removed from the selective influence of the medium.***

- Follow the time and temperature usually used in the laboratory. These are the most common used temperatures (E. coli and coliform): 35, 37, 42 or 44 °C during 24 to 48h.

<u>*E. coli:*</u>
E. coli are able to grow on media containing Violet Red Bile (VRB) nutrients. Most *E. coli* (about 97%) produce beta-glucuronidase which reacts with a BCIG indicator dye in the Petrifilm EC plate that makes the colony turn blue to red-blue. About 95% of *E. coli* produce gas from lactose, this is indicated by colonies associated (within approximately one colony diameter) with entrapped gas. See Circle 1. *E. coli* colonies appear blue to red-blue and produce gas, confirm blue to red-blue colonies without gas. See Circle 2. In some validation processes, this interpretation has been modified: See pages 2 and 3 of this Interpretation Guide.

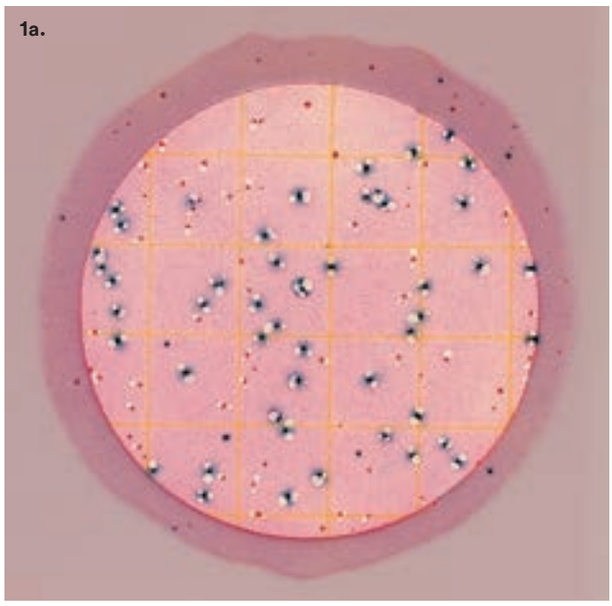
<u>Remark:</u>
Because most *E. coli* O157:H7 strains are atypical: they do not grow at temperatures ≥ 44.5 °C, are glucuronidase negative, and therefore will not produce a blue precipitate. They will appear as non-*E. coli* Coliforms (red with gas).

<u>Coliforms:</u>
The Petrifilm EC plates can also be used to search for Coliforms.

- **ISO** defines Coliforms by their ability to grow in method-specific, selective media. **ISO method 4832**, enumerating Coliforms by the colony count technique, defines Coliforms by colony size and acid production on VRB w ith lactose (VRBL) agar. On Petrifilm EC plates, these acid-producing Coliforms are indicated by red colonies with or without gas (within approximately one colony diameter). See Circle 3. **ISO method 4831**, enumerating Coliforms by the Most Probable Number (MPN) method, defines Coliforms by their ability to grow and produce gas from lactose in a selective broth. On Petrifilm EC plates these Coliforms are indicated by red colonies associated (within approximately one colony diameter) with gas. See Circle 4.

- **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 EC plate produce acid which deepen the gel color. Gas trapped around Coliform colonies (within approximately one colony diameter) indicates confirmed Coliforms. See Circle 4.

Interpretations of 3M™ Petrifilm™ E. coli Coliform Count Plates

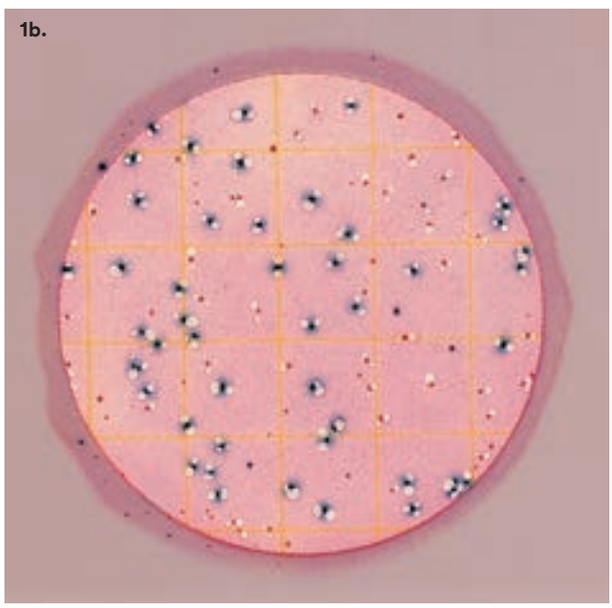


53 *E. coli*.

Recommended method in France

Incubation:
- 24h +/- 2h at 42 °C +/- 1 °C

Interpretation:
- *E. coli*: Count all blue colonies with and without gas.



47 *E. coli*, AOAC official method
87 confirmed Coliforms, AOAC official method

Reading following AOAC.
International all foods (method 991.14)

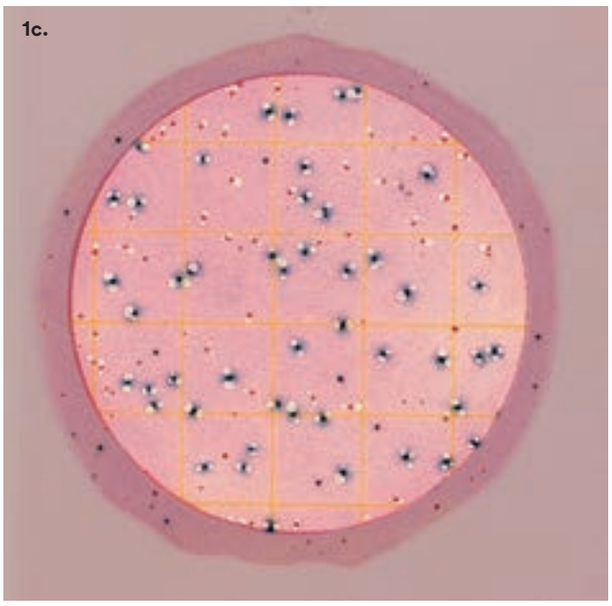
Incubation:
- Coliforms in all foods: incubate 24h +/- 2h at 35 °C +/- 1 °C.
- Enumeration of *E. coli* in all foods, except those here under: incubate 48h +/- 4h at 35 °C +/- 1 °C.

Reading following AOAC International, meat, poultry and seafood (method 998.08)

Incubation:
- Enumeration of *E. coli* in Meat, Poultry and Seafood, and Coliforms in all foods: incubate 24h +/- 2h at 35°C +/- 1°C.

Interpretation (Methods 991.14 and 998.08)
- *E. coli*: blue colonies with gas.
- Confirmed Coliforms: all colonies with gas (blue and red).

3M™ Petrifilm™ E. coli and Coliform Count Plates

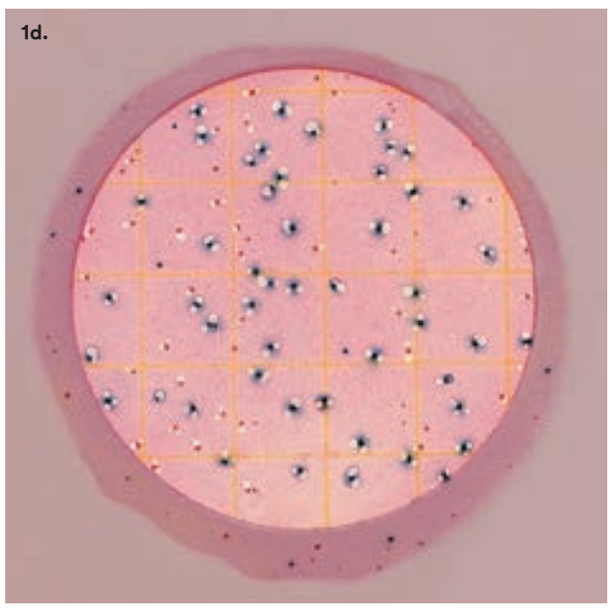


53 *E. coli*, NORDVAL validated method.
95 Total Coliforms, NORDVAL validated method.

Reading following NORDVAL validated method (certificate n° 14)

Incubation:
- 37°C +/- 1°C

Interpretation:
- *E. coli*: Count all blue colonies, with and without gas after 48h +/- 2h of incubation.
- Coliforms : Count red colonies with gas and all blue colonies with or without gas after 24h +/- 2h of incubation.



53 *E. coli*, EMMAS assessed method.

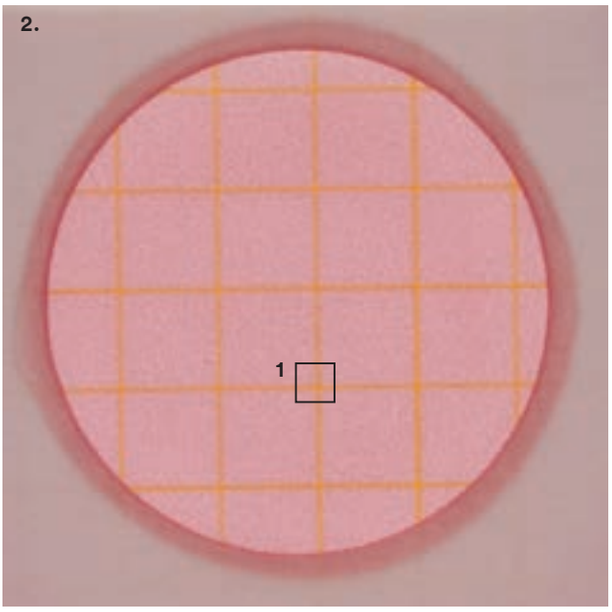
Reading following EMMAS assessed method

Incubation:
- 48h +/- 2h at 37°C +/- 1°C

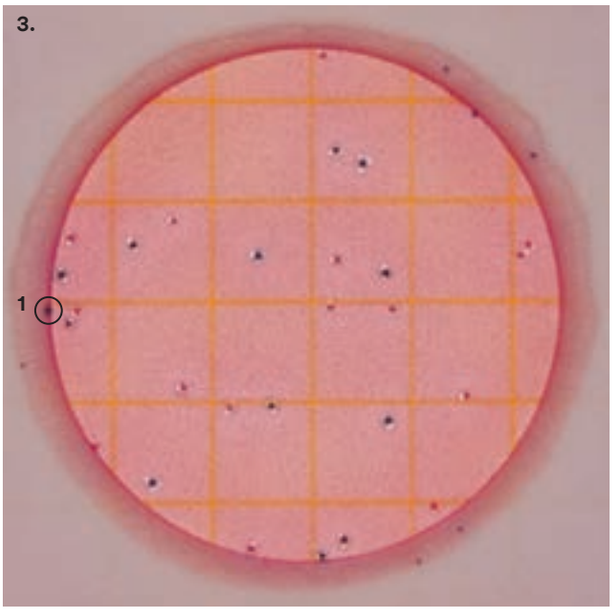
Interpretation:
- *E. coli*: Count all blue colonies with and without gas. *It is advisable to confirm blue colonies without gas, particularly when they are present in high proportion.*

3M™ Petrifilm™ E. coli and Coliform Count Plates

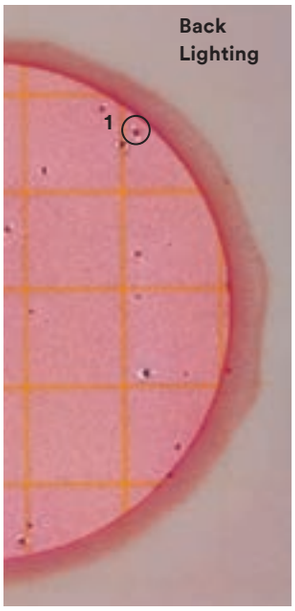
Notice the change in gel colour in figures 2 through 8. As the *E. coli* or Coliform count increases, the colour of the gel turns to dark red or purple-blue.

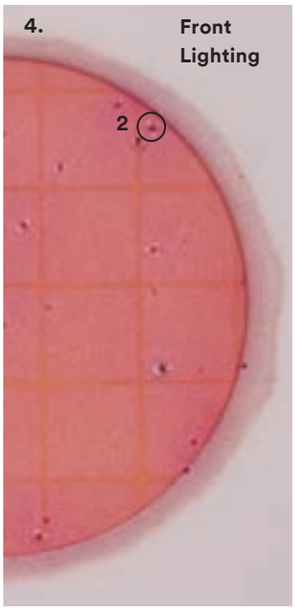


No growth
***E. coli* count = 0**
Background bubbles are a characteristic of the gel and are not a result of *E. coli* or Coliform growth. Background gas bubbles are small to pin-point in size, regular in shape and do not have a colony associated with them. See Square 1.



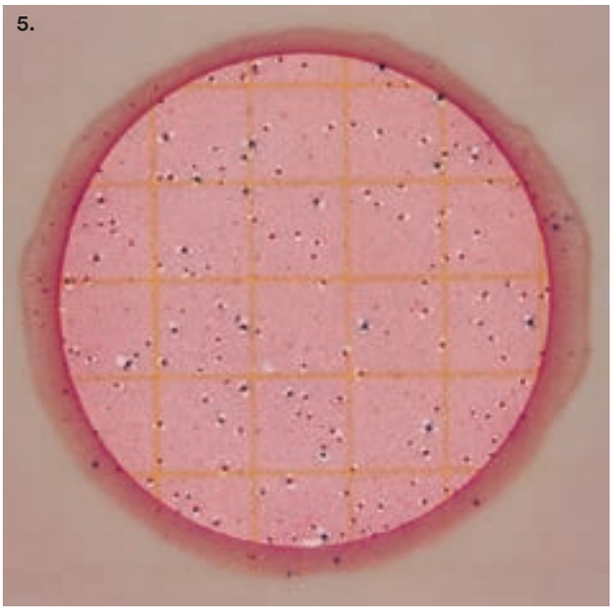
***E. coli* count = 13**
Gas producing Coliforms count = 28
As with VRB agar plates, the preferable counting range (total colony population) on Petrifilm EC plates is 15 - 150.
Do not count colonies that appear on the foam dam since they are removed from the selective influence of the medium.
See Circle 1.





***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 using back lighting.
- Circle 2 shows the same colony with front lighting.

The blue precipitate is more evident in this case.

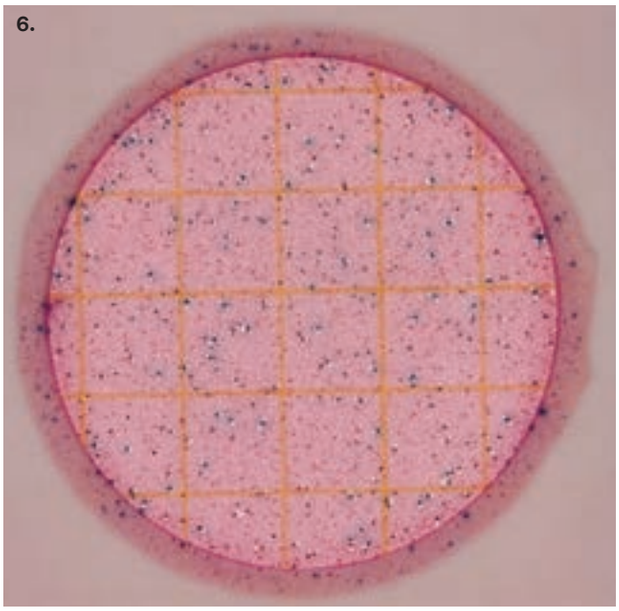


***E. coli* count = 20**
Estimated total count = 150
The Petrifilm EC plate circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated count per Petrifilm EC plate.

TNTC (Too Numerous To Count) plates.

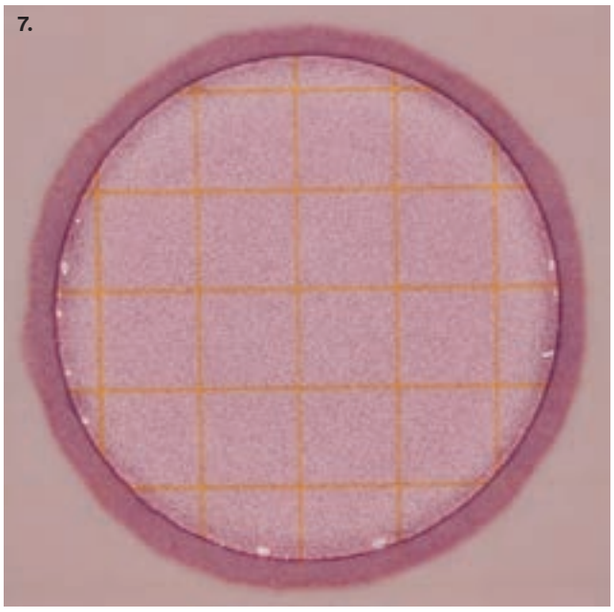
To obtain an accurate count, dilute the sample further.

6.



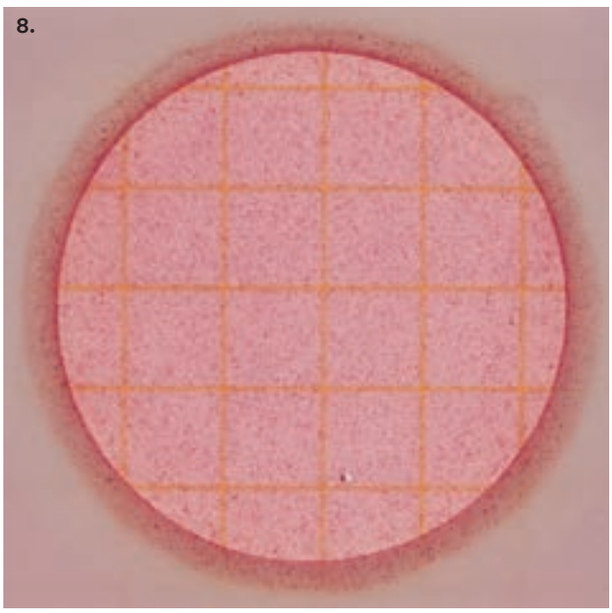
Actual count ~ 10^6
Petrifilm EC plates with colonies that are TNTC have one or more of the following characteristics: many small colonies, many gas bubbles, and a deepening of the gel colour from red to purple-blue.

7.



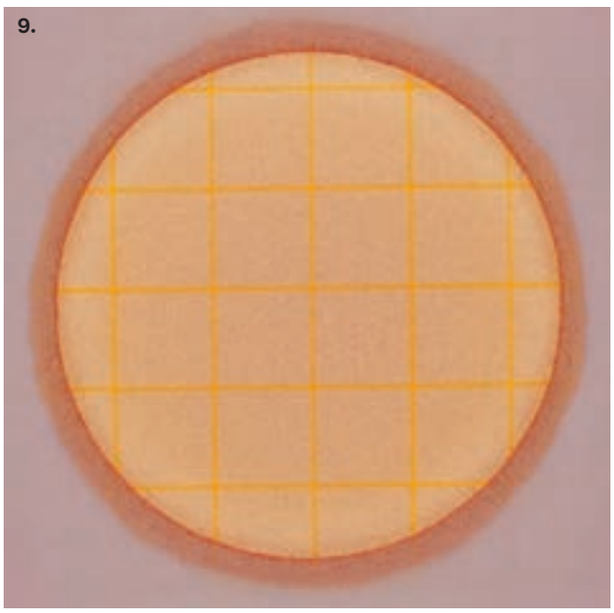
Actual count ~ 10^8
High concentrations of *E. coli* will cause the growth area to turn purple-blue.

8.



Actual count ~ 10^8
High concentrations of Coliforms (non *E. coli*) will cause the growth area to turn dark red. Additional dilutions are required to determine if *E. coli* are present.

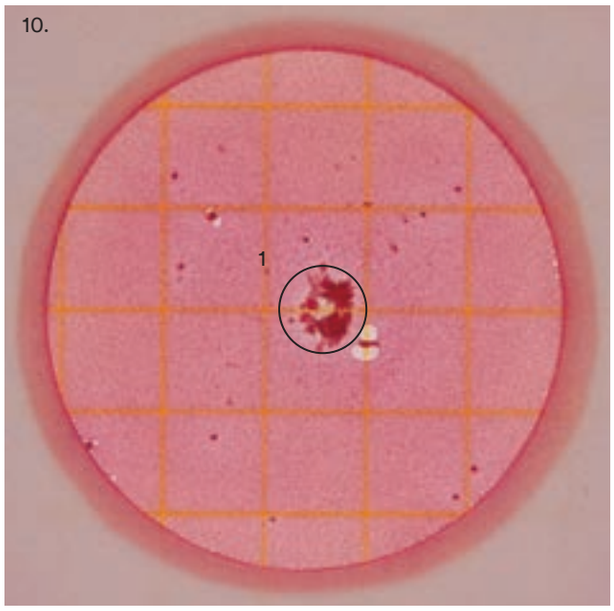
9.



Actual count ~ 10^8
When high numbers of non-Coliforms organisms such as *Pseudomonas* are present on Petrifilm EC plates, the gel may turn yellow.

3M™ Petrifilm™ E. coli and Coliform Count Plates

Bubbles



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

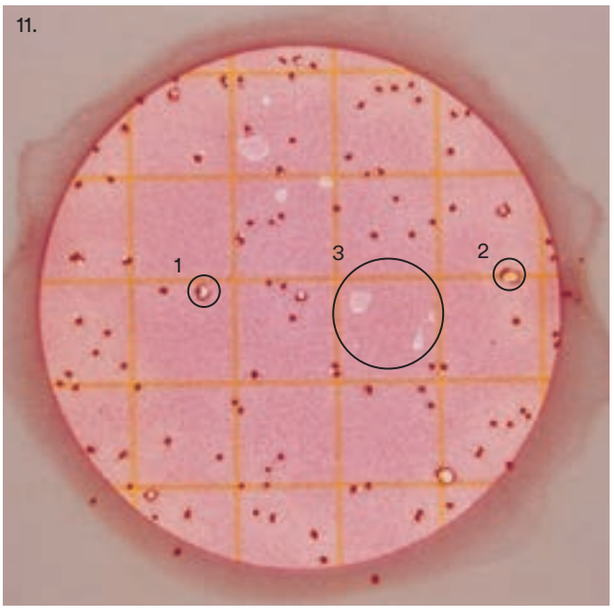
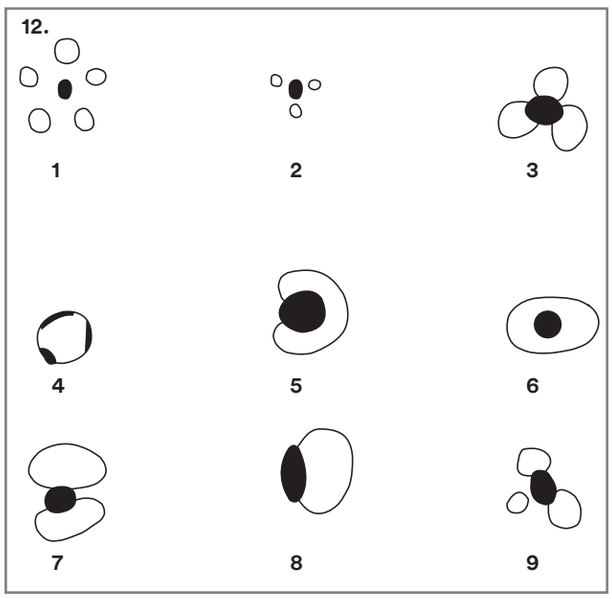


Figure 11 shows how bubble patterns may vary. Sometimes gas disrupts the colony so that the colony "outlines" the bubble. See Circles 1 and 2.
Artifact bubbles may result from improper inoculation of the Petrifilm EC plate or from trapped air within the sample. They are irregularly shaped and are not associated with a colony. See Circle 3.
Do not count colonies on the foam dam since they are removed from the selective influence of the medium.

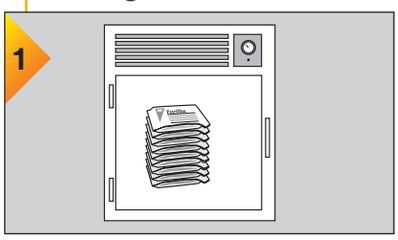


The following are additional examples of various bubble patterns associated with a colony. All of them should be taken into account.

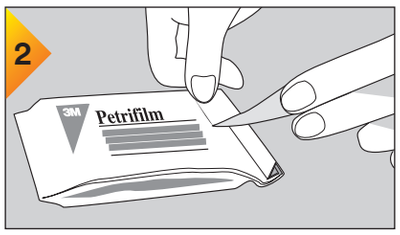
3M™ Petrifilm™ E. coli and Coliform Count Plates

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

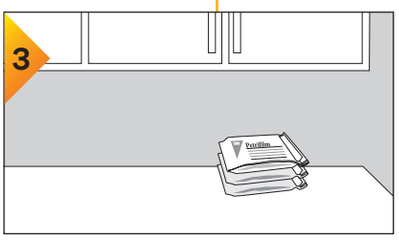
Storage



Store unopened packages at ≤8°C. Use before expiration date on package.



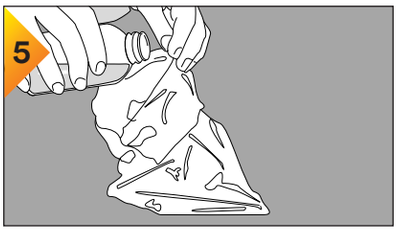
To seal opened package, fold end over and tape shut.



Keep resealed package at ≤25°C and ≤50% RH. **Do not refrigerate opened packages.** Use Petrifilm plates within one month after opening.

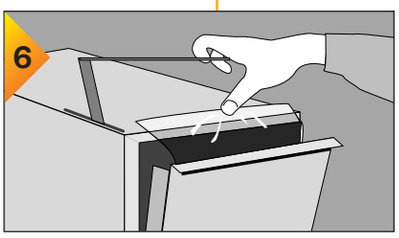
Sample Preparation

Weigh or pipette food product into an appropriate sterile container such as stomacher bag, dilution bottle, or other sterile container.



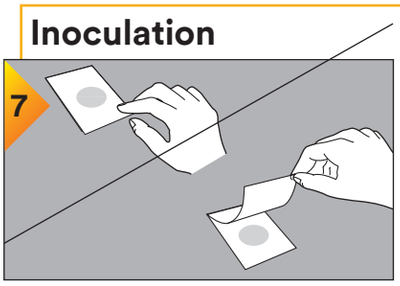
Add appropriate quantity of one of the following sterile diluents : Butterfield's phosphate buffer (IDF phosphate buffer, KH_2PO_4 at 0.0425g/L, adjust pH to 7.2), 0.1% peptone water, Maximal Recovery Diluant, peptone salt diluent (ISO method 6887), saline solution (0.85 - 0.90%), or distilled water.

Do not use buffers containing citrate, bisulphite or thiosulphate; they can inhibit growth.
Adjust pH of the diluted sample between 6.6 and 7.2:
- for acid products, use NaOH 1*N*,
- for alkaline products, use HCl 1*N*.

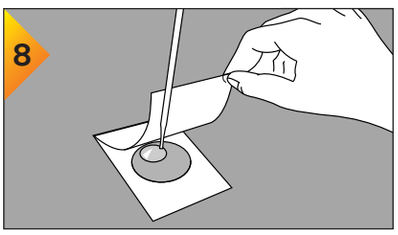


Blend or homogenize sample as per current procedure.

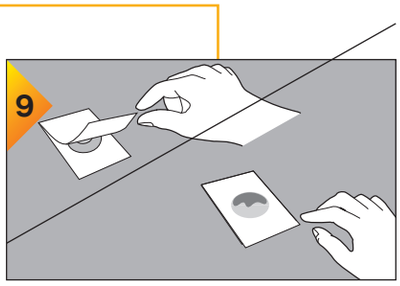
Inoculation



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



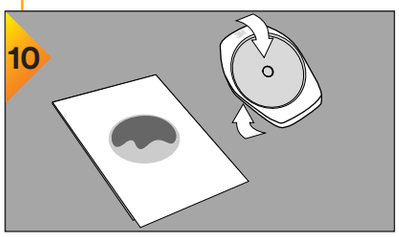
With pipette **perpendicular** to Petrifilm plate, place 1mL of sample onto center of bottom film.



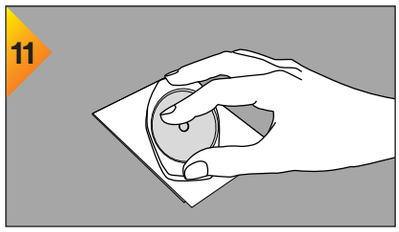
Carefully roll top film down to avoid trapping air bubbles.
Do **not** let top film drop.

3M™ Petrifilm™ E. coli and Coliform Count Plates

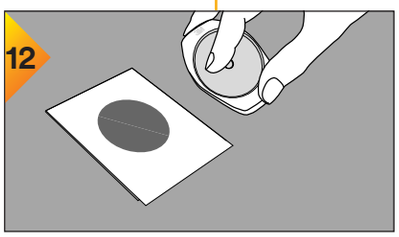
Inoculation



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

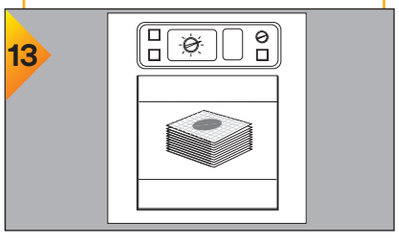


Gently apply pressure on spreader to distribute over circular area. Do not twist or slide the spreader.



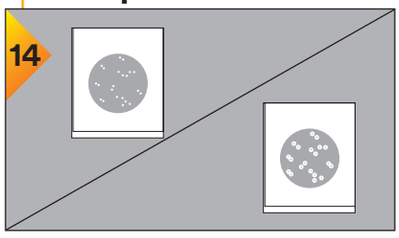
Lift spreader. Wait at least one minute for gel to solidify.

Incubation

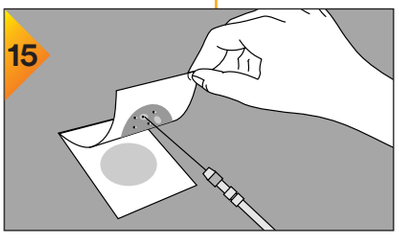


Incubate plates with clear side up in stacks of up to 20. Incubation time and temperature vary by method*.

Interpretation



Petrifilm plates can be counted with a standard colony counter or other magnifier. Refer to the Interpretation Guide section when reading results.



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

Most common approved methods:

- AOAC Official Method 991.14 : for coliforms, incubate 24h ± 2h at 35°C ± 1°C ; for *E. coli*, incubate 48h ± 2h at 35°C ± 1°C.
- AOAC Official Method 998.08 : for *E. coli* in Meat, Poultry and Seafood, and Coliforms in all foods, incubate 24 h +/- 2 h at 35°C +/-1°C
- NORDVAL approved method (certificate n° 14) :
  for Coliforms, incubate 24h ± 2h at 37°C ± 1°C ; for *E. coli*, incubate 48h ± 2h at 37°C ± 1°C.

* See product instruction for use.

Additional Comments

- **Remember to inoculate and spread each Petrifilm plate before going on to the next plate.**
- **Incubation time and temperature vary by method, see product instruction for use.**

User's responsibility :
3M plate has not been evaluated with all combinations and food matrices. It is the user's responsibility to determine that any test methods and results meet the user's requirement

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