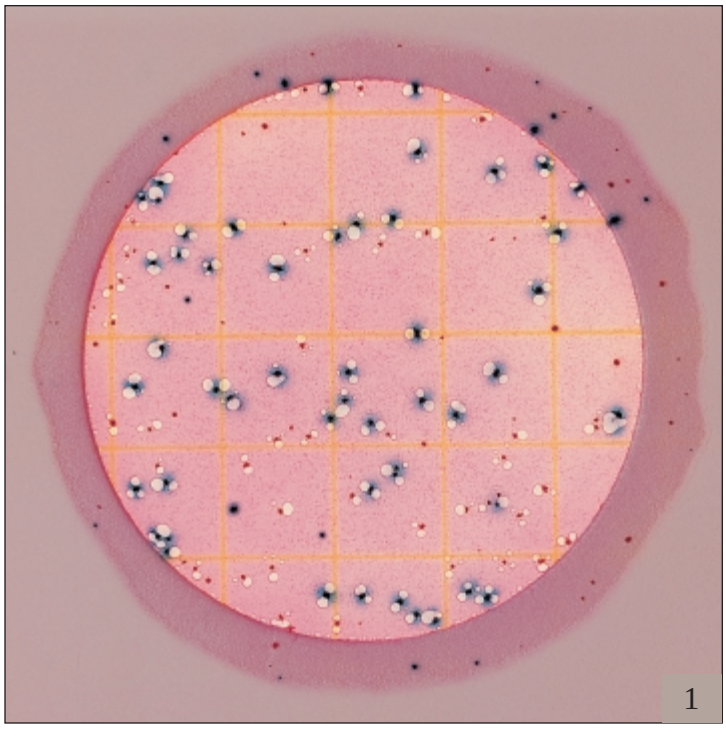
Interpretation Guide

Petrifilm™
E. coli/Coliform Count Plate

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

Petrifilm E. coli/Coliform Count (EC) plates contain Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, an indicator of glucuronidase activity, and an indicator that facilitates colony enumeration. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, indicated by blue to red-blue colonies associated with entrapped gas on the Petrifilm EC plate (within approximately one colony diameter).

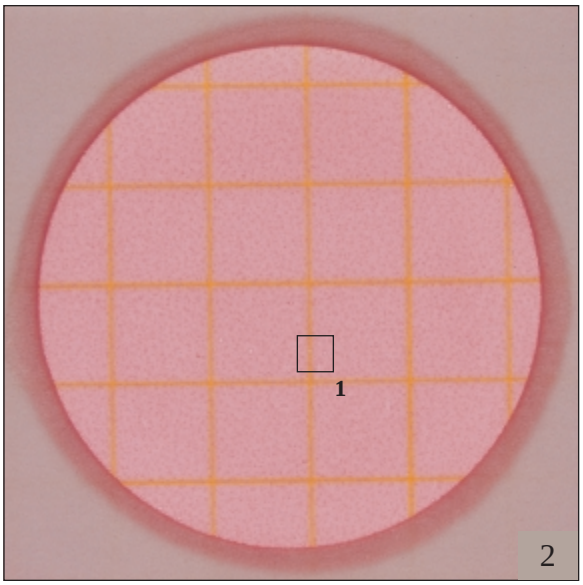
AOAC INTERNATIONAL and U.S. FDA Bacteriological Analytical Manual (BAM) define coliforms as gram-negative rods which produce acid and gas from lactose during metabolic fermentation. Coliform colonies growing on the Petrifilm EC plate produce acid which causes the pH indicator to make the gel color darker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


1

The identification of *E. coli* may vary by country (see Reminders for Use section for incubation times and temperatures):

AOAC INTERNATIONAL validated method
***E. coli* = 49** (blue colonies with gas)
Total coliform = 87 (red and blue colonies with gas)

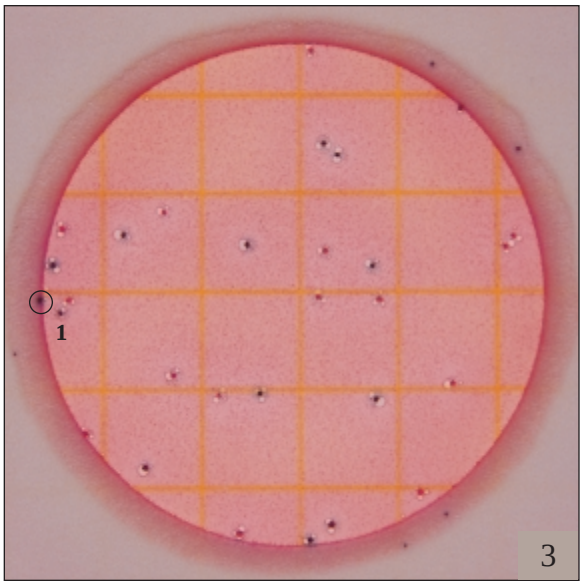
Do not use this plate alone for the detection of *E. coli* O157. Like most other *E. coli*/coliform media, this plate will not specifically indicate whether any O157 strain is present.



No growth = 0

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

Background bubbles are a characteristic of the gel and are not a result of *E. coli* or coliform growth. See square 1.

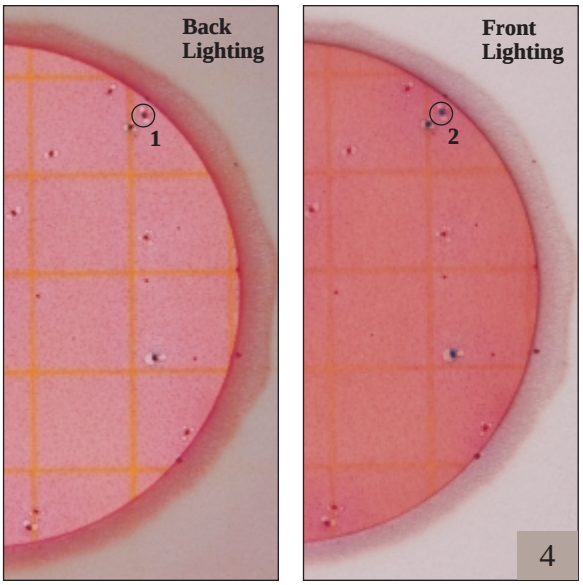


***E. coli* count = 13**

Total coliform count = 28

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

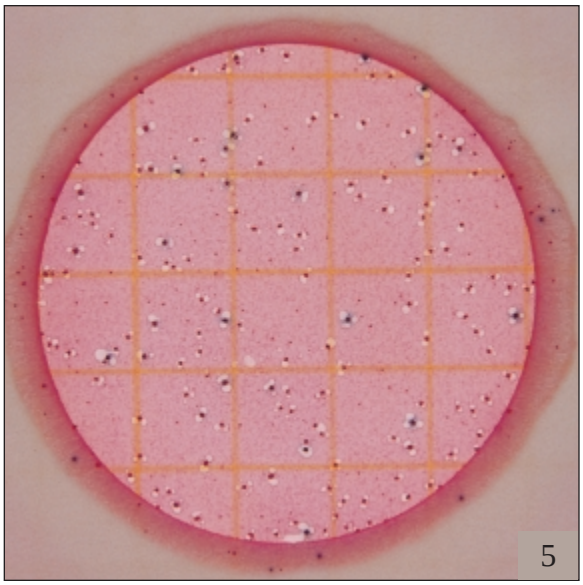
Do not count colonies that appear on the foam barrier because they are removed from the selective influence of the medium. See circle 1.



***E. coli* count = 3**

Any blue in a colony (blue to red-blue) indicates the presence of *E. coli*. Front lighting will enhance the detection of blue precipitate formed by a colony.

Circle 1 shows a red-blue colony counted using back lighting. Circle 2 shows the same colony with front lighting. The blue precipitate is more evident in circle 2.

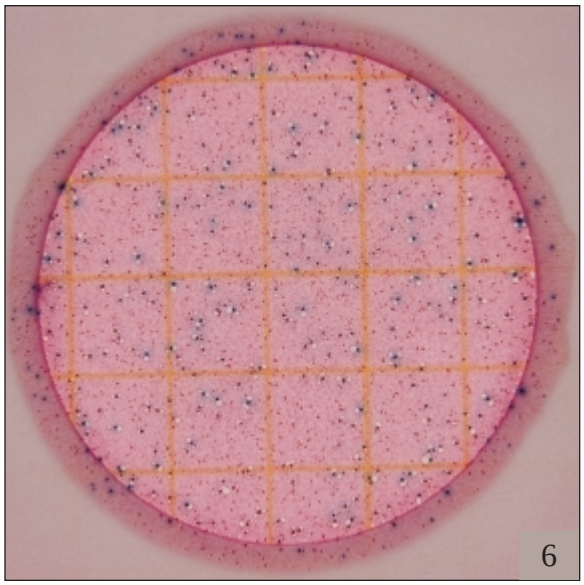


***E. coli* count = 17**

Estimated total coliform count = 150

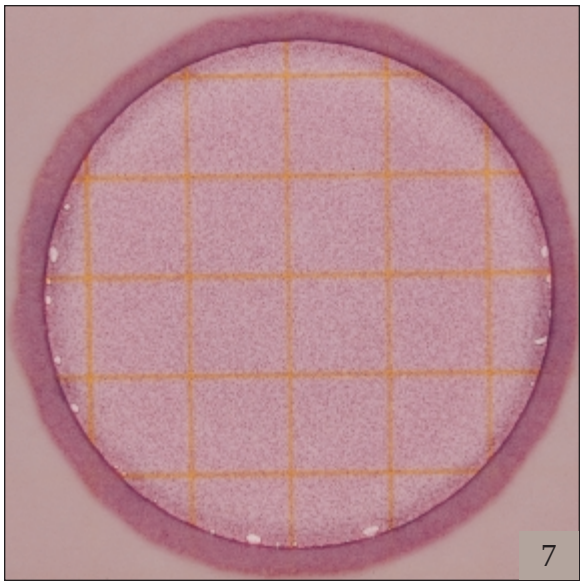
The circular growth area is approximately 20 cm^2. Estimates can be made on plates containing greater than 150 colonies by counting the number of colonies in one or more representative squares and determining the average number per square. Multiply the average number by 20 to determine the estimated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

TNTC (Too Numerous to Count) *To obtain a more accurate count, dilute the sample further.*



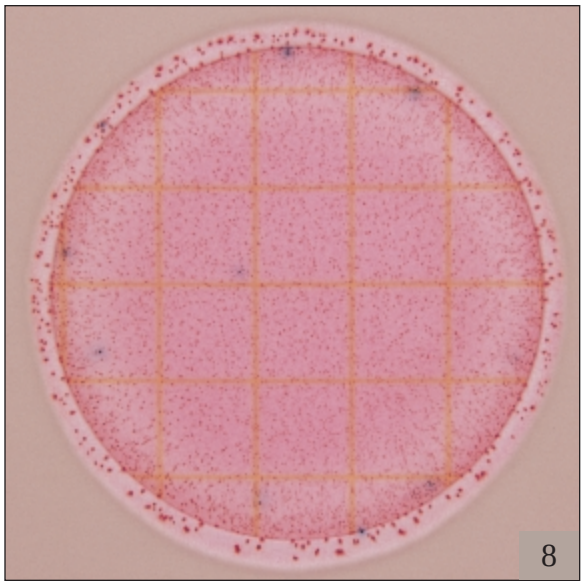
Actual count ~ 10^6

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 color from red to purple-blue.



Actual count ~ 10^8

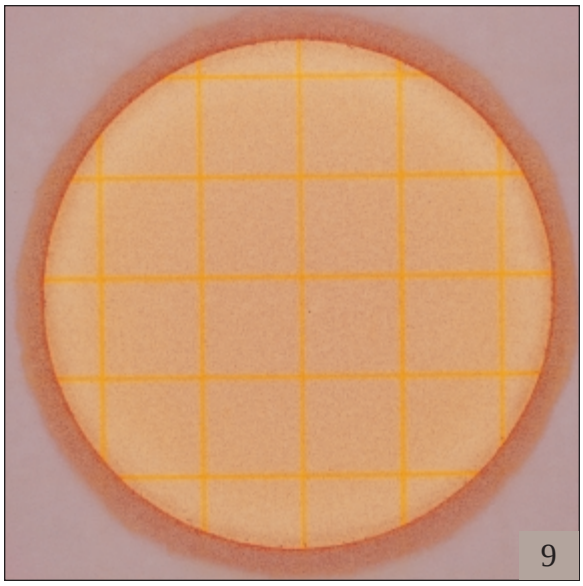
High concentrations of *E. coli* may cause the growth area to turn purple-blue.



Presumptive *E. coli* count ~ 8

Estimated total coliform count ~ 10^8

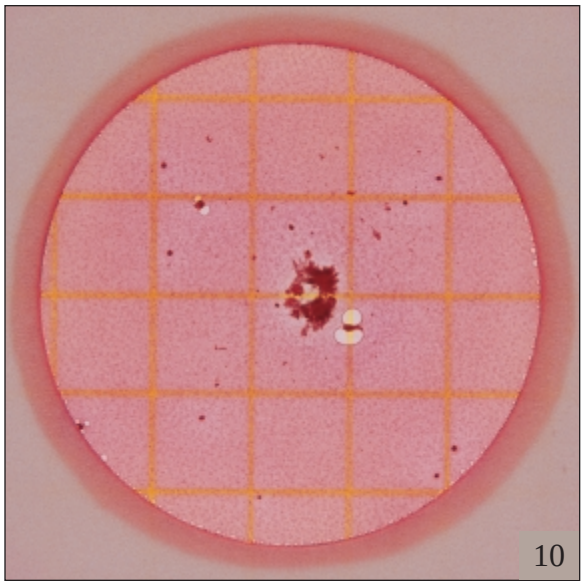
When high levels of coliforms are present (>10^8), some strains of *E. coli* may produce less gas and blue colonies may be less definitive. Count all blue colonies without gas and/or blue zones as presumptive *E. coli.* Pick blue colonies without gas and confirm if necessary.



Actual count ~ 10^8

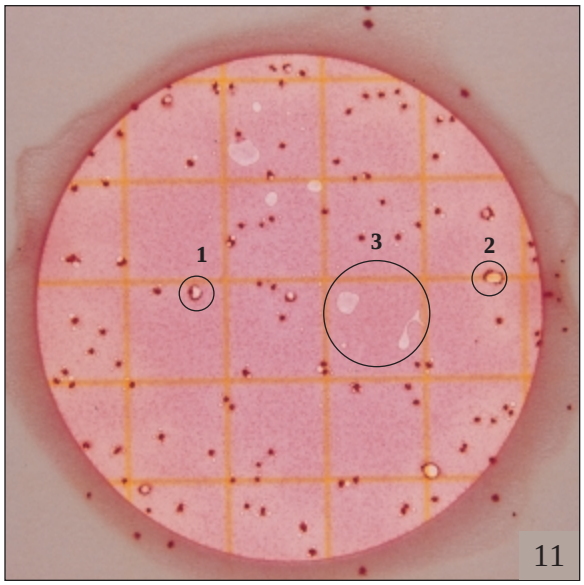
When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm EC plates, the gel may turn yellow.

Bubbles



Total coliform count = 3

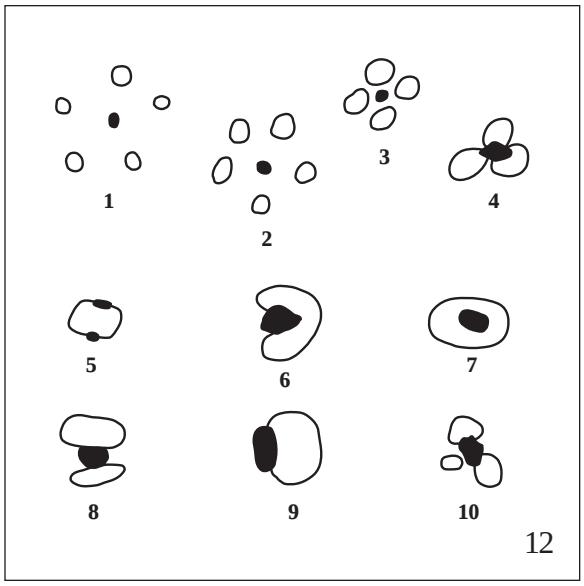
Food particles are irregularly shaped and are not associated with gas bubbles.



Total coliform count = 78

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.

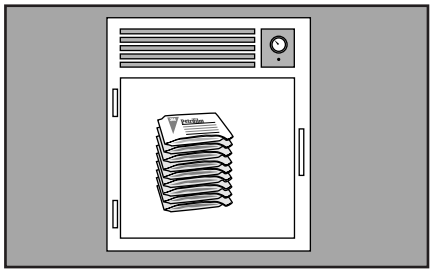


Examples 1–10 show various bubble patterns associated with gas producing colonies. All should be enumerated.

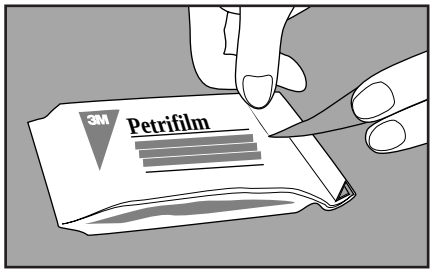
3M Petrifilm™ E. coli / Coliform Count Plates Reminders for Use

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.

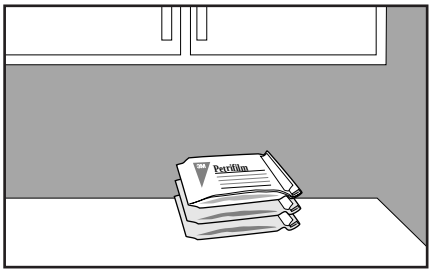
Storage



1 **Store** unopened packages at ≤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 packages to reach room temperature before opening.

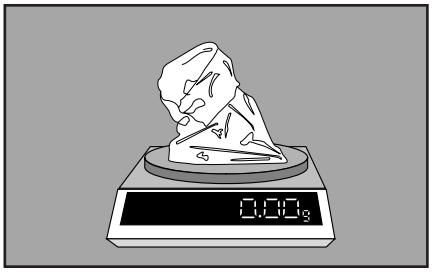


2 To seal opened package, fold end over and tape shut.

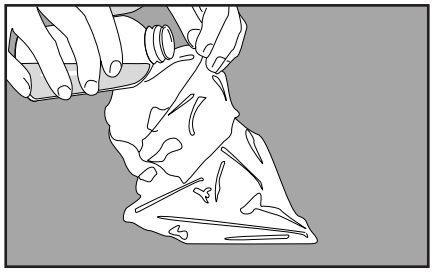


3 Keep resealed package at ≤25°C (≤77°F) and <50%RH. **Do not refrigerate opened packages.** Use Petrifilm plates within one month after opening.

Sample Preparation

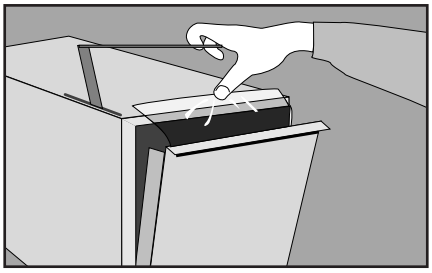


4 Prepare a 1:10 or greater dilution of food product. Weigh or pipette food product into an appropriate container such as a stomacher bag, dilution bottle, Whirl-Pak® bag, or other sterile container.



5 Add appropriate quantity of one of the following sterile diluents: Butterfield's phosphate buffer (IDF 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 6887-1), saline solution (0.85-0.90%), bisulfite-free letheen broth, or distilled water.

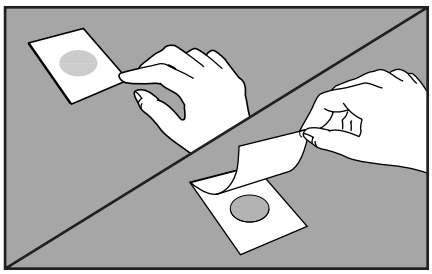
Do not use buffers containing citrate, bisulfite, or thiosulfate; they can inhibit growth.



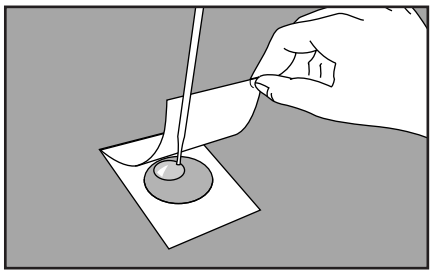
6 Blend or homogenize sample per current procedure.

Adjust pH of the diluted sample between 6.5 and 7.5.
- for acid products, use 1*N* NaOH,
- for alkaline products, use 1*N* HCl.

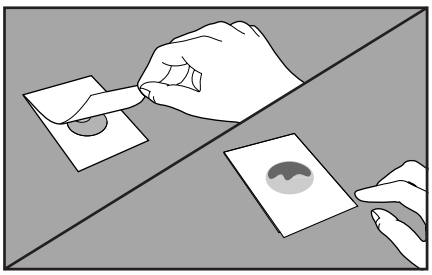
Inoculation



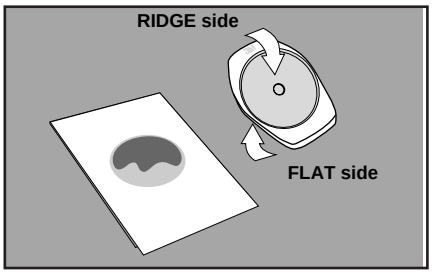
7 Place Petrifilm plate on **level** surface. Lift top film.



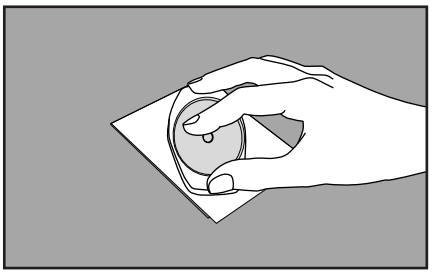
8 With pipette **perpendicular** to Petrifilm plate, place 1 mL of sample onto center of bottom film.



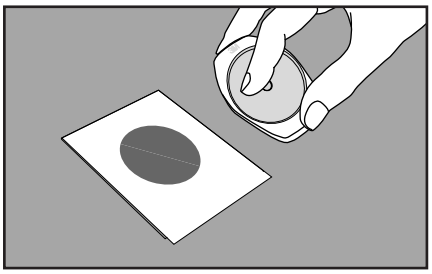
9 Carefully **ROLL** top film down to avoid entrapping air bubbles. Do **NOT** let top film drop.



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

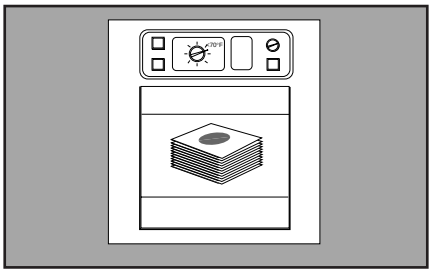


11 **GENTLY** apply pressure on spreader to distribute inoculum over circular area before gel is formed. Do not twist or slide the spreader.



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

Incubation



13 Incubate plates with clear side up in stacks of no more than 20. It may be necessary to humidify incubator to minimize moisture loss.

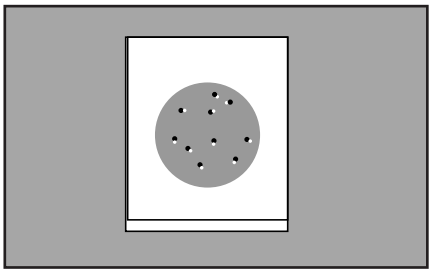
Incubation time and temperature varies by method. Most common approved methods:

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

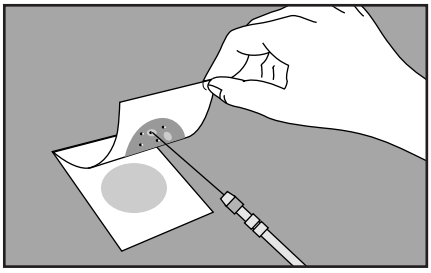
AOAC Official Method 998.08
for *E.coli* (for meat, poultry and seafood)*:*
incubate 24h ± 2h at 35°C ± 1°C

NMKL method (147.1993)
for coliforms: incubate 24h ± 2h at 37°C
for *E.coli:* incubate 48h ± 2h at 37°C

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.

Additional Comments

- Questions? U.S., Call **1-800-328-6553**, Canada, call **1-800-265-1840 x6574** 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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