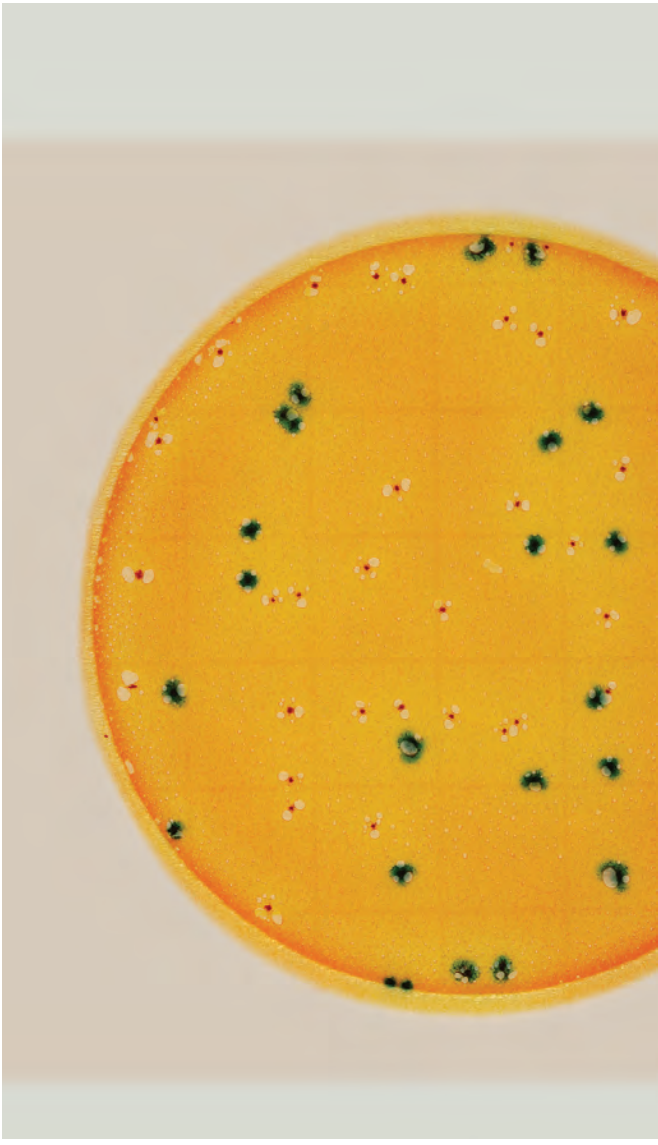
NEOGEN®

Petrifilm®

Interpretation Guide

The Neogen® Petrifilm® Rapid *E. coli*/Coliform Count Plate is a selective and differential sample-ready-culture medium system which contains proprietary nutrients, a cold-water-soluble gelling agent, 5-bromo-4-chloro-3-indolyl-D-glucuronide (BCIG) an indicator of glucuronidase activity, and a tetrazolium indicator that facilitates colony enumeration. Petrifilm Rapid *E. coli*/Coliform Count Plates are used for the enumeration of *Escherichia coli* (*E. coli*) and coliforms in the food and beverage industries.



REC

Rapid *E. coli*/Coliform Count Plate

Rapid

Most *E. coli* (about 97%) produce beta-glucuronidase, which produces a blue precipitate associated with the colony on the Petrifilm Rapid *E. coli*/Coliform Count Plate. The top film on the Petrifilm Rapid *E. coli*/Coliform Count Plate traps carbon dioxide gas produced by the lactose fermenting coliforms and *E. coli*. A small percentage of *E. coli* do not produce carbon dioxide gas as a result of lactose fermentation; these are known as anaerogenic *E. coli*. On the Petrifilm Rapid *E. coli*/Coliform Count Plate, blue to blue-green colonies associated with and without entrapped gas (within approximately one colony diameter) are counted as *E. coli*.

Most *E. coli* O157 strains are atypical, for example they are glucuronidase negative, they will not produce a blue color, and therefore will be detected as coliforms on Petrifilm Rapid *E. coli*/Coliform Count Plates.

The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) define coliforms as Gram-negative rods, which produce acid and gas from lactose during metabolic fermentation. Non-*E. coli* coliform colonies growing on the Petrifilm Rapid *E. coli*/Coliform Count Plate produce acid and gas, which causes the pH indicator to change the gel color to light yellow and gas trapped around red colonies. In this interpretation guide, the number of coliforms per the FDA BAM definition is the number of red colonies with gas production and blue colonies with and without gas production.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832 enumerates typical coliform colonies on Violet Red Bile Lactose (VRBL) agar, with confirmation of atypical colonies. On the Petrifilm Rapid *E. coli*/Coliform Count Plate, coliforms are indicated by red colonies with or without gas production and blue colonies with and without gas production.

Please refer to the product instructions for additional information.

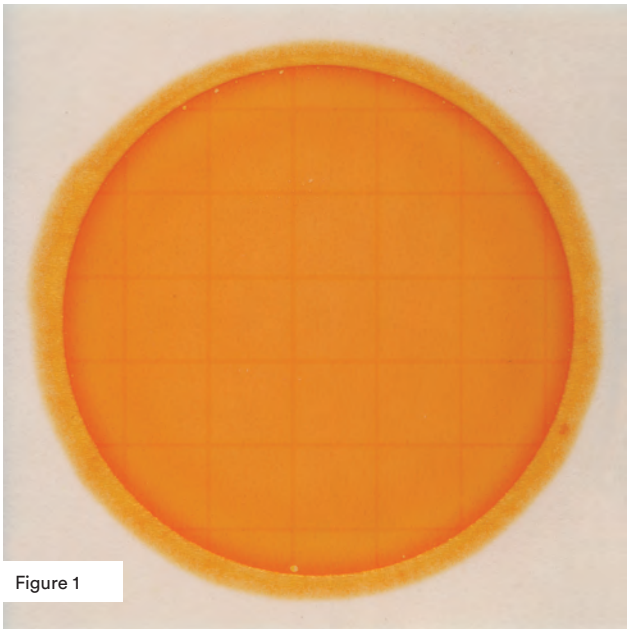


Figure 1

***E. coli* count = 0**
Total coliform count = 0

Petrifilm Rapid *E. coli*/Coliform Count Plate without colonies.

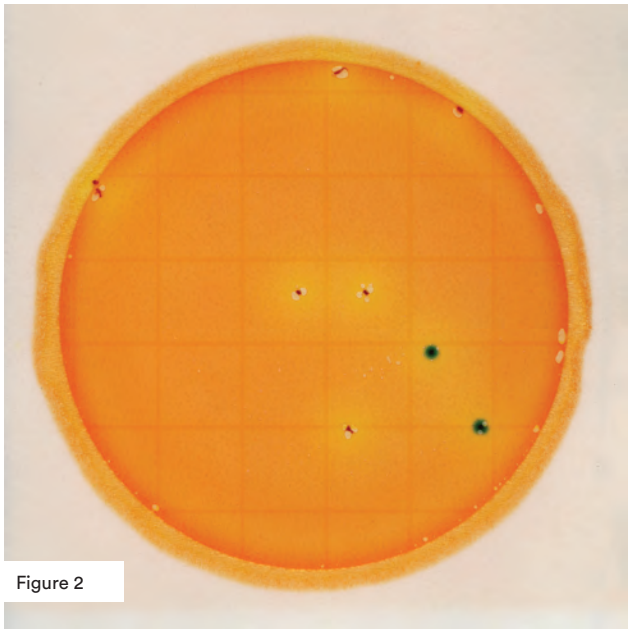


Figure 2

***E. coli* count = 2**
(blue colonies with and without gas)
Total coliform count = 8
(red colonies with gas and blue colonies)
Total coliform count = 8
(red colonies and blue colonies)

E. coli are blue to blue-green colonies with and without associated gas bubbles. The definition of coliforms may vary by country. Please refer to the section above and product instructions for definitions.

Do not count colonies on the foam barrier because they are removed from the selective influence of the medium.

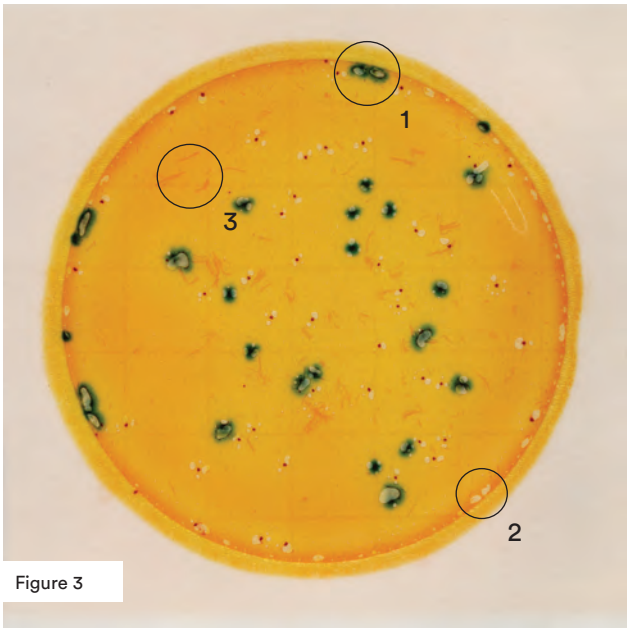


Figure 3

***E. coli* count = 25** (blue colonies with and without gas)
Total coliform count = 71 (red colonies with gas and blue colonies)
Total coliform count = 75 (red and blue colonies)

Two distinct colonies can be seen in Circle 1, although the blue precipitate from both colonies have merged together. The gas bubble formed by each colony has disrupted the colony so that the colony "outlines" the bubble.

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 (Circle 2).

Food particles are irregularly shaped and are not associated with gas bubbles (Circle 3).

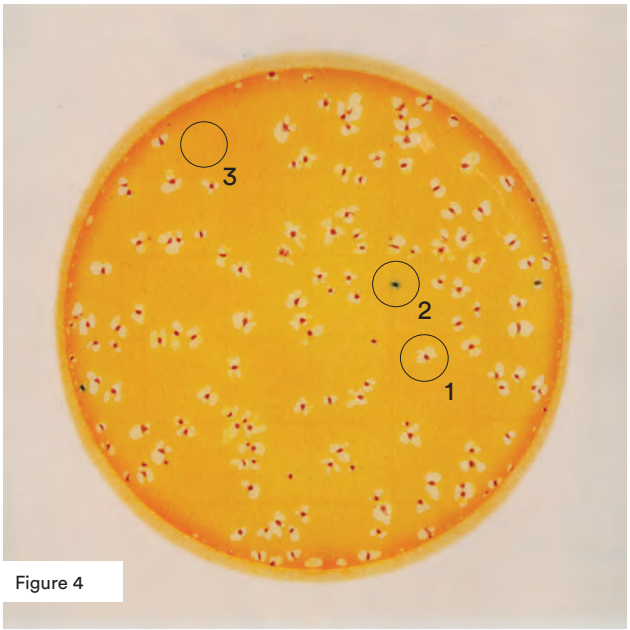


Figure 4

***E. coli* count = 3** (blue colonies with and without gas)
Estimated total coliform count = 145

The circular growth area is approximately 30 cm^2. Estimates can be made on Petrifilm Rapid *E. coli*/Coliform Count Plates containing greater than 100 colonies. Count the number of colonies in one or more representative squares and determine the average number per square. Multiply the average number by 30 to determine the estimated count. In this picture, three types of colonies can be seen: Red colonies with gas (Circle 1), blue colonies with gas (Circle 2), and very tiny, pale pink colonies without gas. The tiny, pale pink colonies without gas are non-coliform and should not be counted (Circle 3).

Further a more accurate count, further dilution of the sample may be necessary.

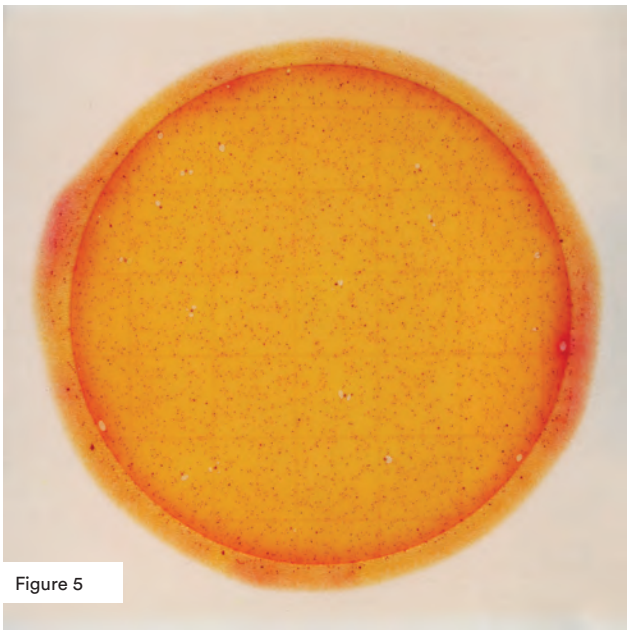


Figure 5

***E. coli* count = 0**
Total coliform count = Too Numerous To Count (TNTC)

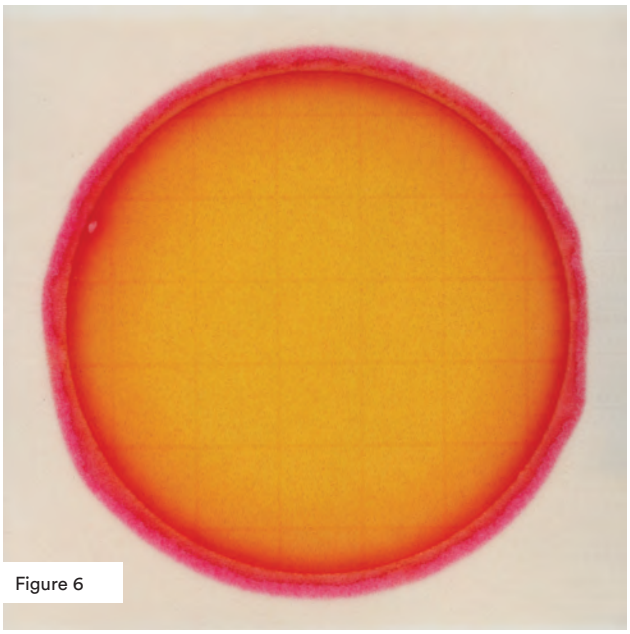


Figure 6

***E. coli* count = cannot be determined**
Total coliform count = TNTC

The counting range for total coliform is less than or equal to 100 total colonies. Plates with colony counts TNTC may have one or more of the follow characteristics: lightening of the gel color to yellow, many small, indistinct red or blue colonies and/or many gas bubbles. High concentrations of *E. coli* or coliforms may cause the outer edge of the growth area to turn pink to pink orange.

For a more accurate count, further dilution of the sample may be necessary.

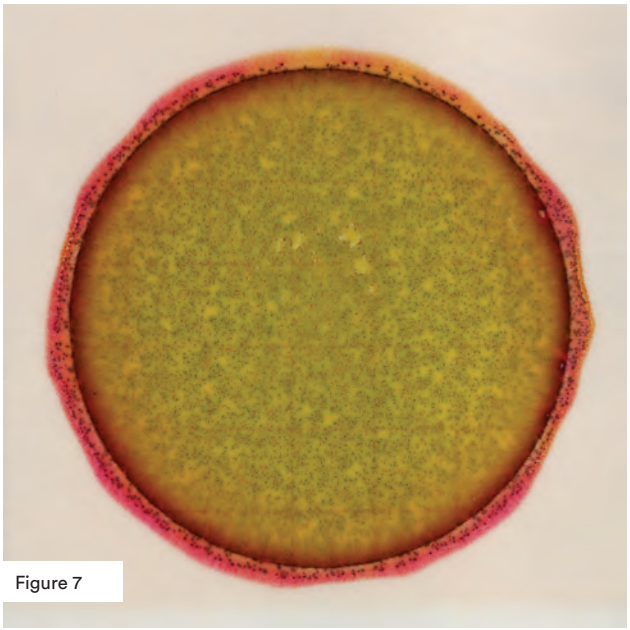


Figure 7

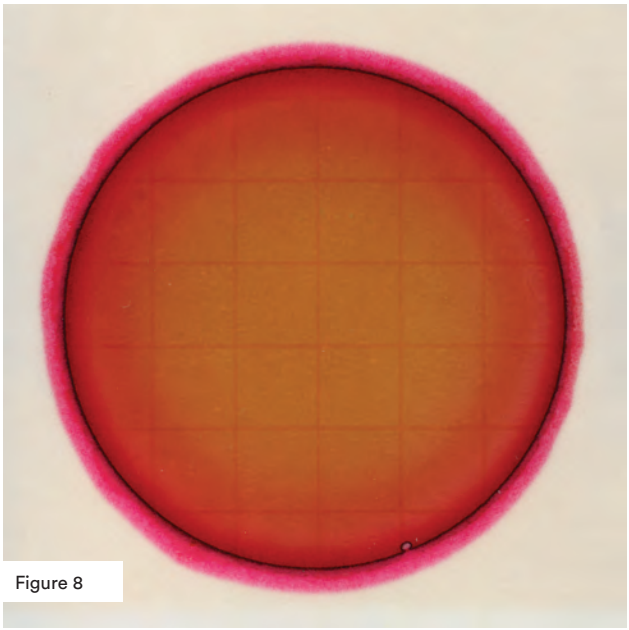


Figure 8

***E. coli* count = TNTC** (blue colonies with and without gas)
Total coliform count = TNTC

The counting range for *E. coli* is less than or equal to 100 blue to blue-green colonies. Plates with colony counts TNTC may have one or more of the follow characteristics: lightening of the gel color to yellow, many small, indistinct red or blue colonies and/or many gas bubbles. High concentrations of *E. coli* or coliforms may cause the outer edge of the growth area to turn pink to pink orange.

For a more accurate count, further dilution of the sample may be necessary.

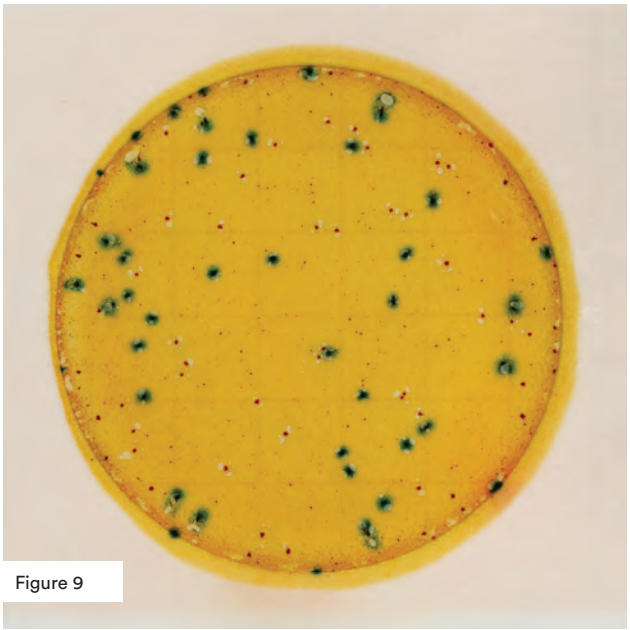


Figure 9

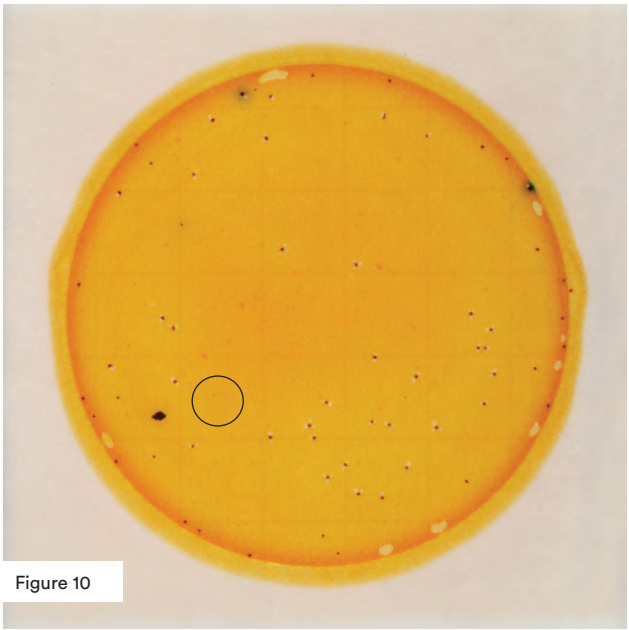


Figure 10

***E. coli* count = 41** (blue colonies with and without gas)
Total coliform count = TNTC (red colonies with gas and blue colonies)
Total coliform count = TNTC (red and blue colonies)

The counting range for *E. coli* on the Petrifilm Rapid *E. coli*/Coliform Count Plate is less than or equal to 100 blue to blue-green colonies regardless of the number of total colonies. The countable range for total coliform may occur on a separate dilution.

For a more accurate count, further dilution of the sample may be necessary.

***E. coli* count = 2** (blue colonies with and without gas)
Total coliform count = 36 (red colonies with gas and blue colonies)
Total coliform count = 58 (red and blue colonies)

This figure shows the next serial dilution for the sample plated in Figure 9. The countable range for total coliforms on Petrifilm Rapid *E. coli*/Coliform Count Plate is lower than or equal to 100 total colonies. Do not count tiny, pale pink colonies without gas (see circle). These colonies are non-coliform colonies and should not be counted.

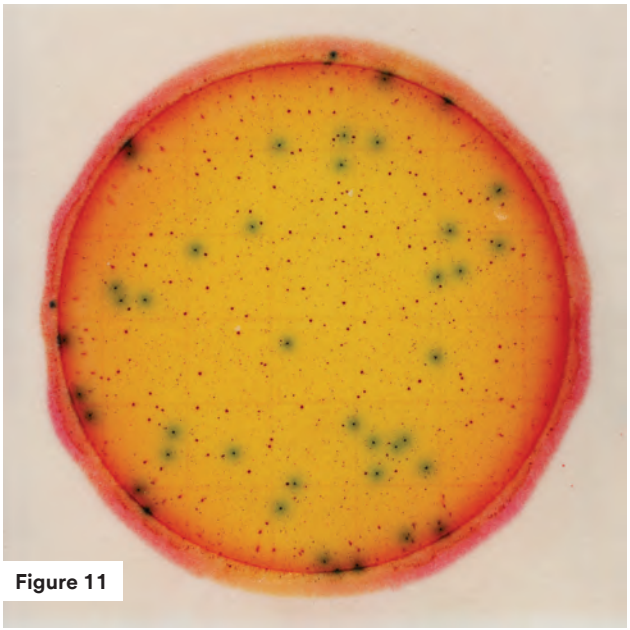


Figure 11

***E. coli* count = 42** (blue colonies with and without gas)
Total coliform count = TNTC (red colonies with gas and blue colonies)
Total coliform count = TNTC (red and blue colonies)

The counting range for *E. coli* on the Petrifilm Rapid *E. coli*/Coliform Count Plate is less than or equal to 100 blue to blue-green colonies regardless of the number of total colonies. The countable range for total coliform may occur on a separate dilution.

For a more accurate count, further dilution of the sample may be necessary.

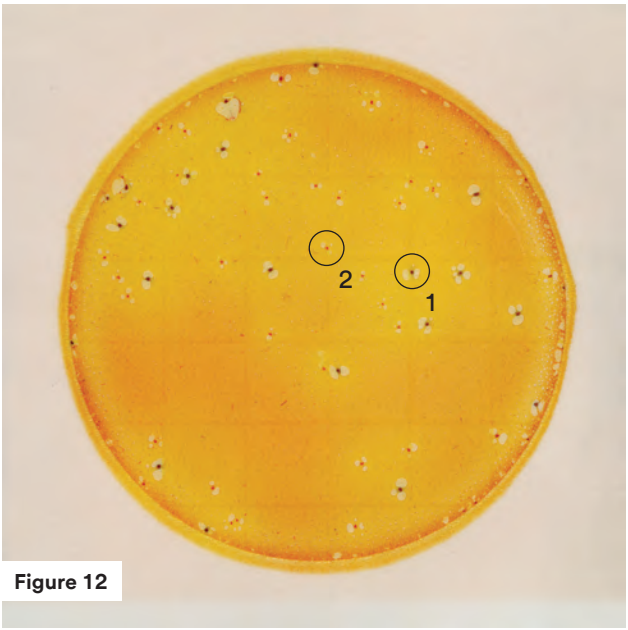


Figure 12

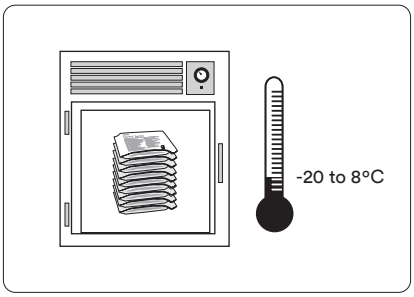
***E. coli* count = 21** (blue colonies with and without gas)
Total coliform count = 60 (red colonies with gas and blue colonies)
Total coliform count = 60 (red and blue colonies)

A 1:10 dilution of some food matrices, for example those containing fruit such as cherries and blueberries, may affect ß-glucuronidase production by *E. coli* rendering them faint blue-green. Additional incubation and/or a 1:20 dilution will restore the appearance of the blue green color.

In this figure, *E. coli* colonies can be identified by their darkened colony color surrounded by a faint blue-green color (Circle 1). Compare this colony to a non-*E. coli* coliform colony which is bright red (Circle 2).

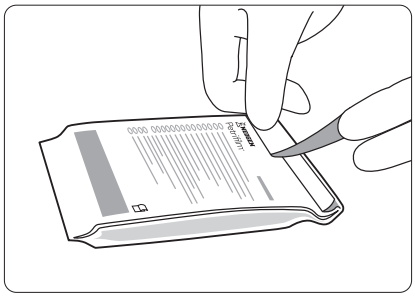
Reminders For Use

Storage



01

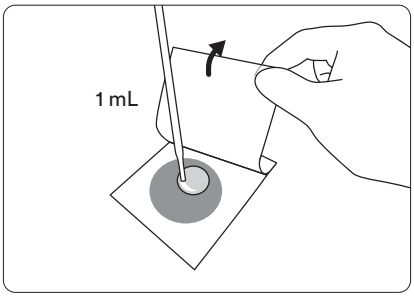
Store the unopened Petrifilm Rapid *E. coli*/Coliform Count Plate pouches at frozen or refrigerated temperature equal to -20 to 8°C (-4 to 46°F). Use before expiration date on package. Just prior to use, all unopened pouches to come to room temperature before opening.



02

Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than four weeks.

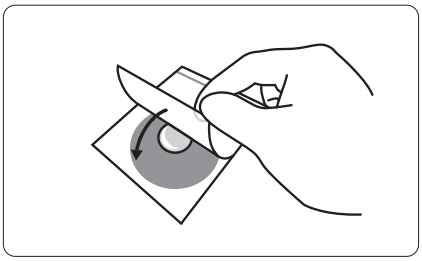
Inoculation



03

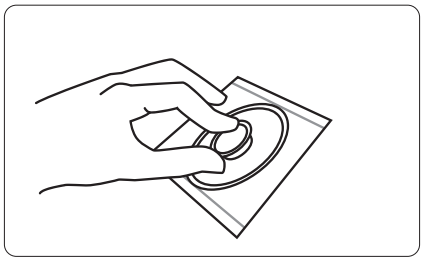
Place the Petrifilm Rapid *E. coli*/Coliform Count Plate on a level surface. Lift the top film and with the pipette perpendicular to the inoculation area, dispense 1 mL of sample suspension onto the center of the bottom film.

Incubation



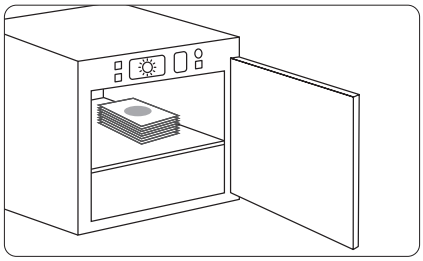
04

Roll the top film down onto the sample gently to prevent trapping air bubbles.



05

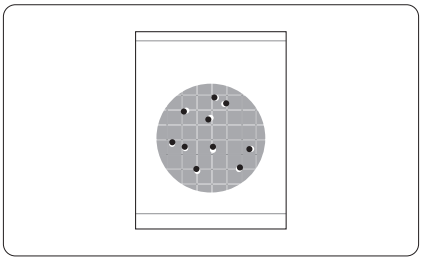
Place the Petrifilm Flat Spreader on the center of the Petrifilm Rapid *E. coli*/Coliform Count Plate. Press firmly on the center of the spreader to distribute the sample evenly. Do not twist or slide the spreader.



06

Incubate plates with clear side up in stacks of no more than 20 plates. It may be necessary to humidify the incubator to minimize moisture loss. **Please refer to the product instructions for third party validated methods.**

Interpretation



07

Petrifilm Rapid *E. coli*/Coliform Count Plates can be counted with the Petrifilm Plate Reader Advanced or with a standard colony counter or other illuminated magnifier. Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Use Appropriate Sterile Diluents

Butterfield's phosphate-buffered dilution water, 0.1% peptone water, peptone salt diluent (Maximum Recovery Diluent), buffered peptone water, 0.85%–0.9% saline, phosphate buffered saline (PBS), Wide-Spectrum Neutralizer, distilled water or bisulfite-free letheen broth.

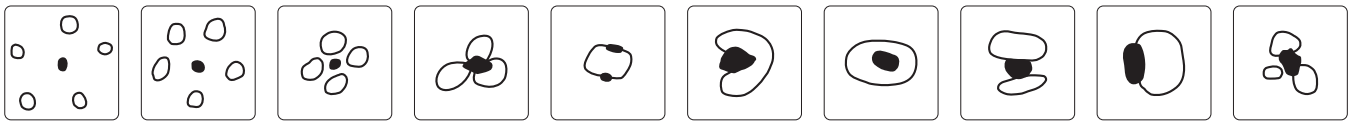
For optimal growth and recovery of microorganisms in acidic products (< pH 5), adjust the pH of the sample suspension to greater than pH 5.

Do not use diluents containing citrate, bisulfate or thiosulfate with the Petrifilm Rapid *E. coli*/ Coliform Count Plates; they can inhibit growth.

If citrate buffer is indicated in the standard procedure, substitute with Butterfield's phosphate-buffered dilution water, warmed to 40–45°C.

Bubbles

The illustrations below show examples of various bubble patterns associated with gas producing colonies. All should be enumerated.



Neogen offers a full line of products to accomplish a variety of your microbial testing needs.

For more product information, visit **info.neogen.com/petrifilm**

User's Responsibilities: Neogen 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.

For detailed CAUTIONS, DISCLAIMER OF WARRANTIES/LIMITED REMEDY and LIMITATION OF NEOGEN LIABILITY, STORAGE AND DISPOSAL information and INSTRUCTIONS FOR USE, see product instructions.

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