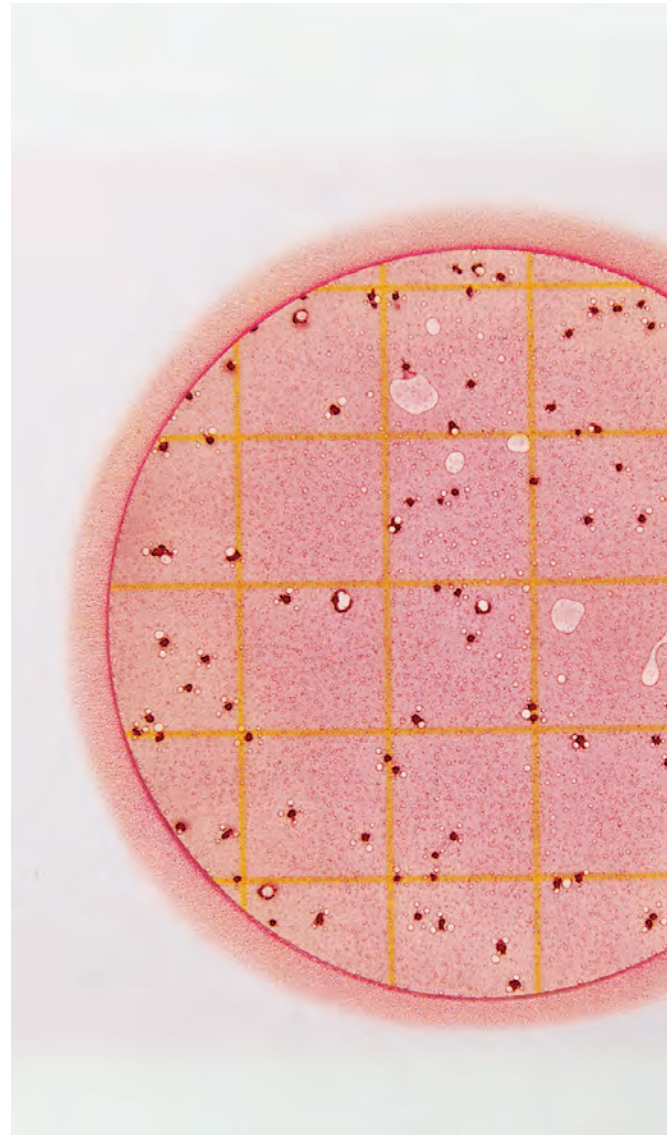




Petrifilm®

Interpretation Guide

The Neogen® Petrifilm® Coliform Count Plate is a sample-ready culture medium system which contains modified Violet Red Bile nutrients, a cold-water-soluble gelling agent and a tetrazolium indicator that facilitates colony enumeration.



CC

Coliform Count Plate

Food and Beverage Applications

Coliform Definitions by Method

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 fermentation. Coliform colonies growing on the Petrifilm Coliform Count Plate produce acid, which causes the pH indicator to deepen the gel color, 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.

ISO defines coliforms by their ability to grow in method-specific, selective media. ISO method 4832:2006 enumerates typical coliform colonies on Violet Red Bile Lactose (VRBL) agar, with confirmation of atypical colonies. On the Petrifilm Coliform Count Plate, these coliforms are indicated by red colonies with or without gas production. ISO method 4831, enumerating coliforms by the most probable number (MPN) method, defines coliforms by their ability to grow and produce gas in the conditions described in the standard. On the Petrifilm Coliform Count Plate, these coliforms are indicated by red colonies with gas.

It is also possible to enumerate thermotolerant coliforms on the Petrifilm Coliform Count Plate. Typically thermotolerant coliforms can be selected with an elevated incubation temperature. One example of a method for enumeration of thermotolerant coliforms is described in NF V80 060. Reading the total of red colonies on a Petrifilm Coliform Count Plate incubated at $44^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for $24\text{h} \pm 2\text{h}$ yields results equivalent to enumeration with NF V08 060.

Please refer to the product instructions for additional information.

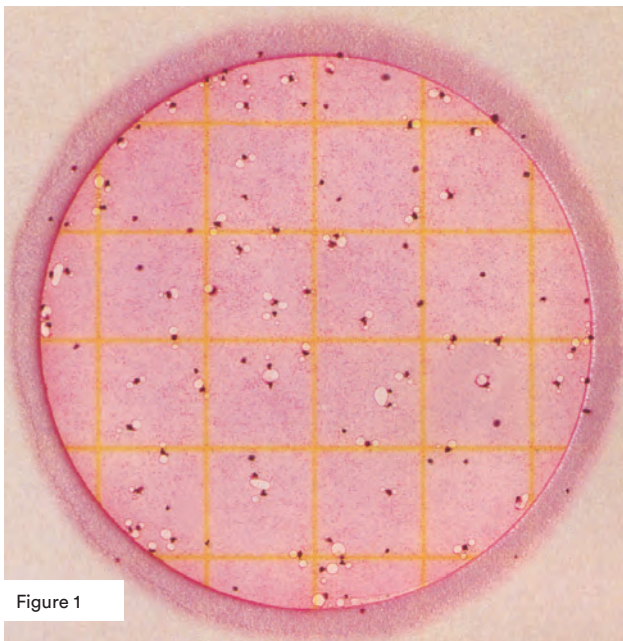


Figure 1

Total colonies with gas = 69
Total colonies = 94

The definition of coliforms may vary by country.

Please refer to section above and product instructions for definitions.

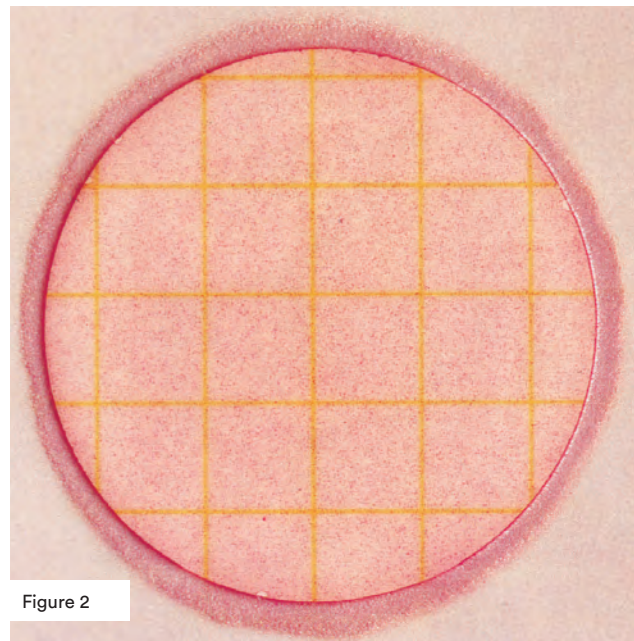


Figure 2

No growth = 0

Notice the changes in gel color in Figures 2–5. As the coliform count increases, the gel color deepens.

Background bubbles are a characteristic of the gel and are not a result of coliform growth.

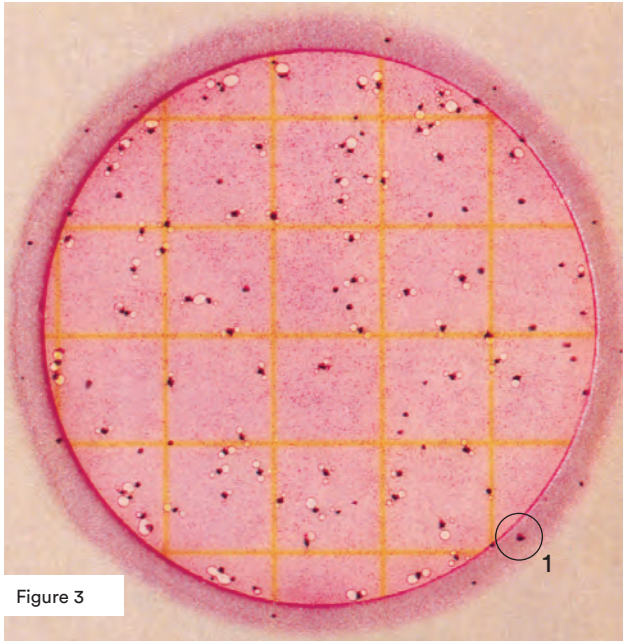


Figure 3

Total colonies with gas = 79
Total colonies = 109

The recommended counting limit on Petrifilm Coliform Count Plates is less than 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).

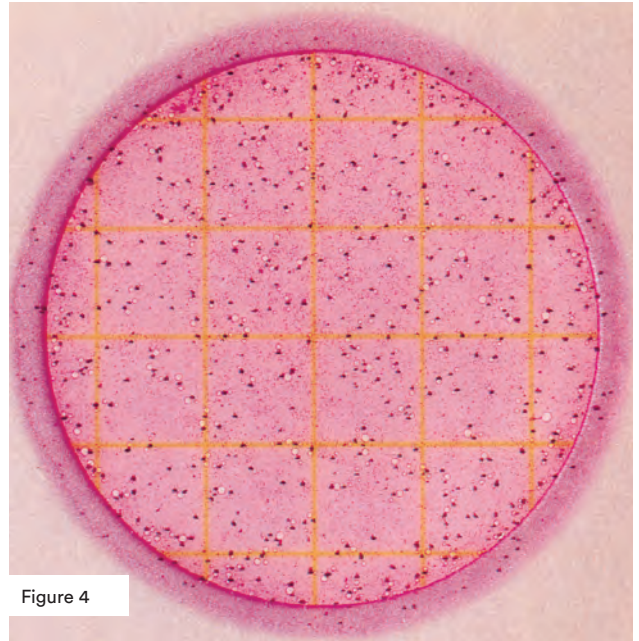


Figure 4

Estimated total coliform count = 220

The circular growth area is approximately 20cm². 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.

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

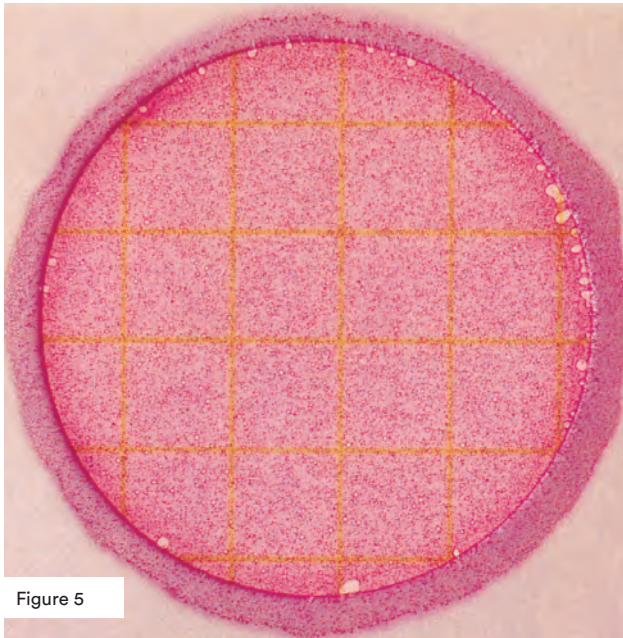


Figure 5

Total coliform count = TNTC

Petrifilm Coliform Count 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.

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

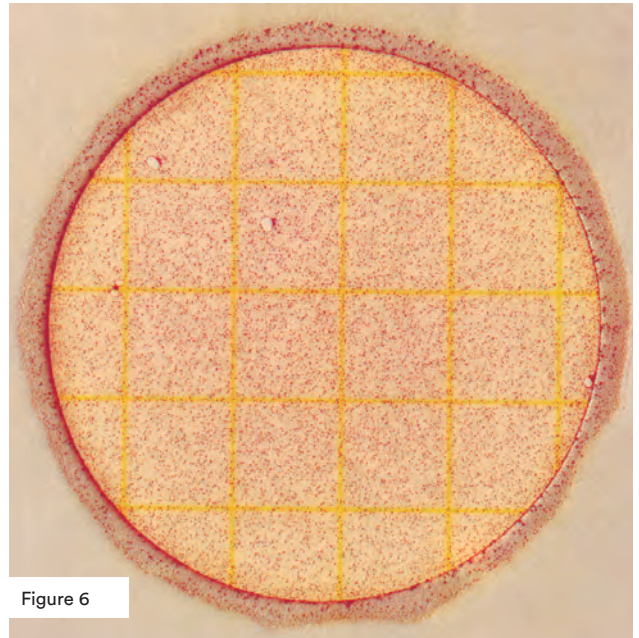


Figure 6

Actual count = TNTC

When high numbers of non-coliform organisms such as *Pseudomonas* are present on Petrifilm Coliform Count Plates, the gel may turn yellow. Further dilution of the sample is recommended.

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

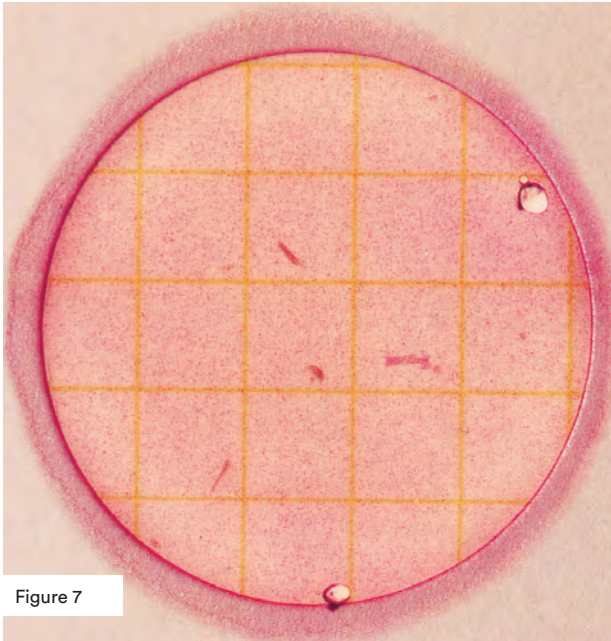


Figure 7

Total colonies with gas = 2
Total colonies = 2

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

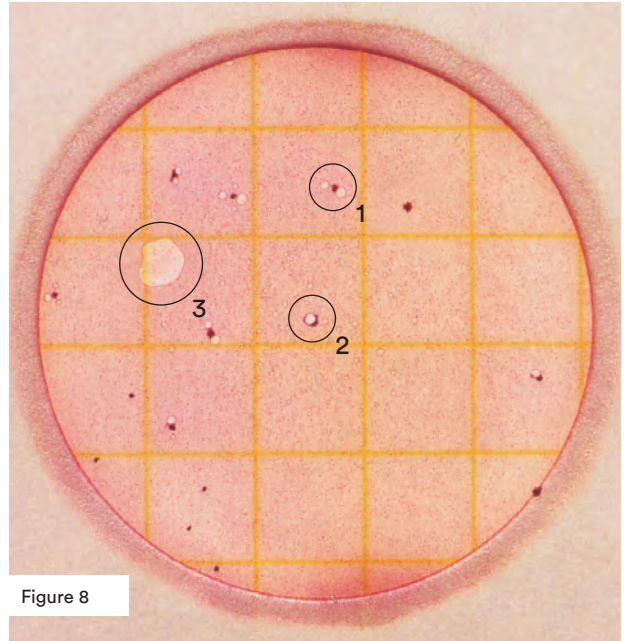


Figure 8

Total colonies with gas = 8
Total colonies = 15

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).

Bottled Water Applications

Coliform colonies are indicated by red colonies associated with gas for bottled water samples plated to Petrifilm Coliform Count Plates.

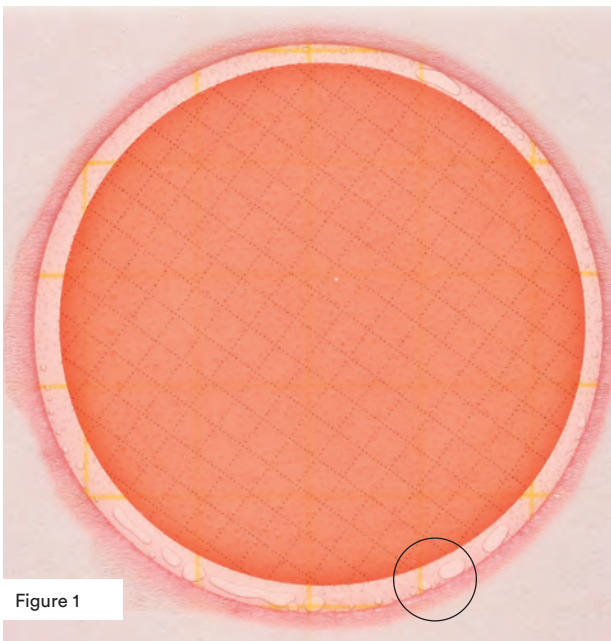


Figure 1

Coliform count: 0

Gas bubbles surrounding filter do not indicate microbial growth. See circle for example.

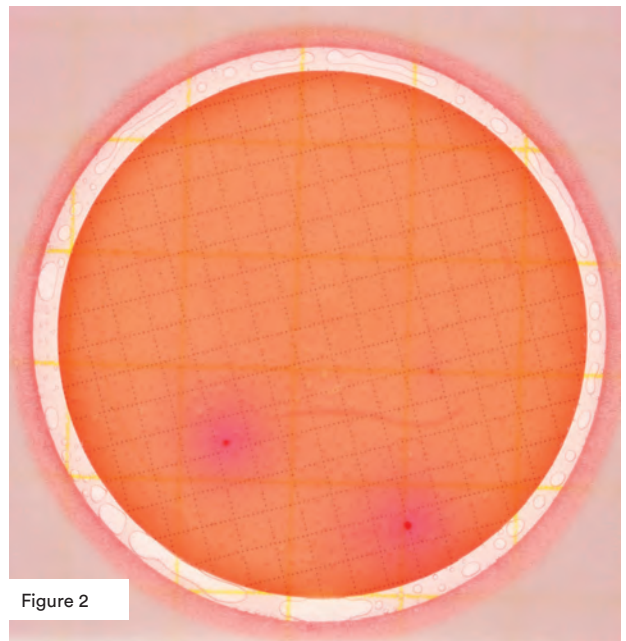


Figure 2

Coliform count: 0

Red colonies without closely associated gas bubbles may be coliforms and should be picked and tested with appropriate confirmation methods.

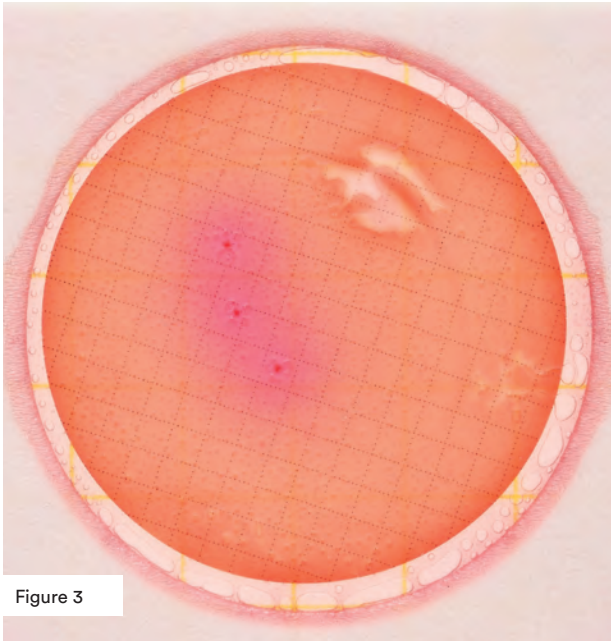


Figure 3

Coliform count: 3

Coliforms produce acid (faint pink halo associated with colonies) and are associated with gas bubbles.

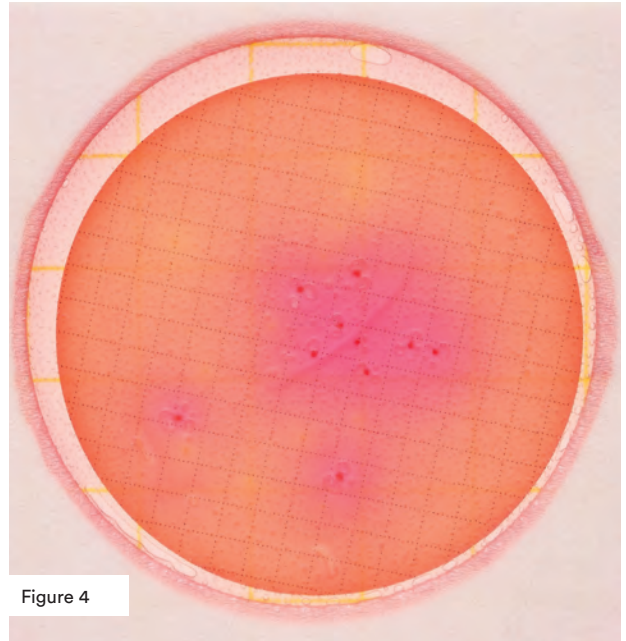


Figure 4

Coliform count: 10

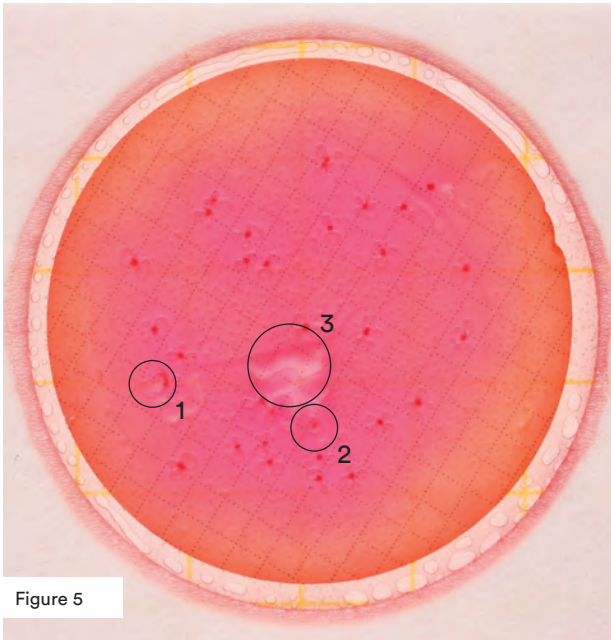


Figure 5

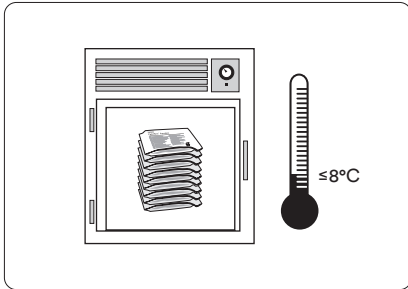
Coliform count: 30

Gas bubbles may influence colony morphologies. The colony in Circle 1 is distorted by the gas bubble. In Circle 2, a faint colony is underneath the gas bubble. Note large artifact gas bubble in the center of the plate (see Circle 3).

Reminders For Use

Food and Beverage Applications

Storage



01

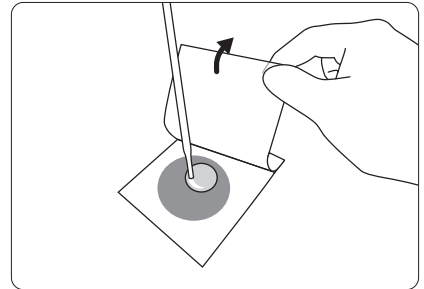
Store unopened pouches of plates at $\leq 8^{\circ}\text{C}$ ($\leq 46^{\circ}\text{F}$). Use before expiration date on package. In areas of high humidity where condensate may be an issue, it is best to allow pouches to reach room temperature before opening.



02

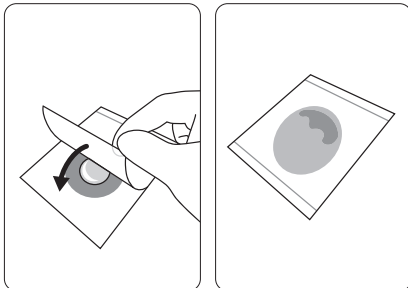
To seal opened pouch, fold end over and apply adhesive tape.

Inoculation



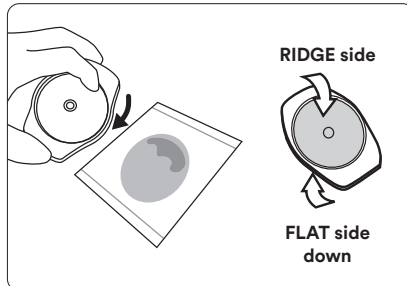
03

Place Petrifilm Coliform Count Plate on **level** surface. Lift top film. With Pipettor held **perpendicular** to plate, place 1mL of sample or diluted sample onto center of bottom film.



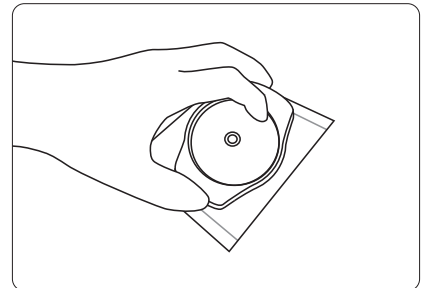
04

Roll the top film down onto sample **gently** to prevent pushing sample off film and to avoid entrapping air bubbles.



05

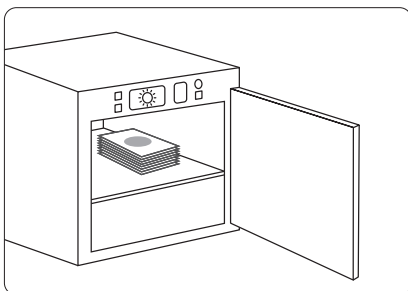
With **flat** side down, place Petrifilm Spreader on top film over inoculum.



06

Gently apply pressure on Petrifilm Spreader to distribute inoculum over circular area before gel is formed. **Do not** twist or slide the spreader. Lift Petrifilm Spreader. Wait a minimum of 1 minute for gel to solidify.

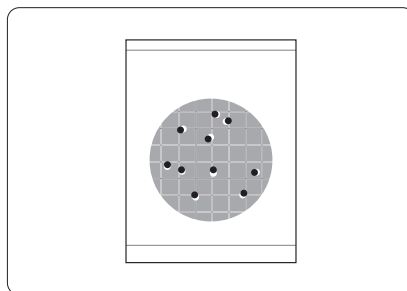
Incubation



07

Incubate plates with clear side up in stacks of up to 20. It may be necessary to humidify incubator to minimize moisture loss. See **product instructions for third party validated methods**.

Interpretation



08

Petrifilm Coliform Count Plates can be counted using the Petrifilm Plate Reader Advanced, on 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 diluents, buffered peptone water, saline solution (0.85–0.90%), Wide-Spectrum Neutralizer, bisulfite-free letheen broth or distilled water.

For optimal growth and recovery of the microorganisms, adjust the pH of the sample suspension to 6.6–7.2.

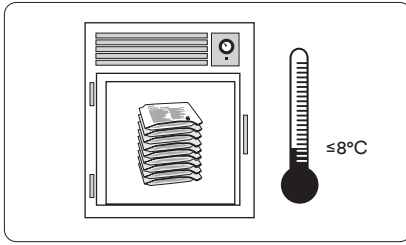
Do not use diluents containing citrate, bisulfite or thiosulfate with the Petrifilm Coliform Count Plates; they can inhibit growth.

If citrate buffer is indicated in the standard procedure, substitute with one of the buffers listed above, warmed to 40–45°C.

Reminders For Use

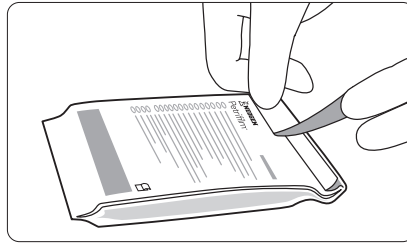
Bottled Water Applications

Storage



01

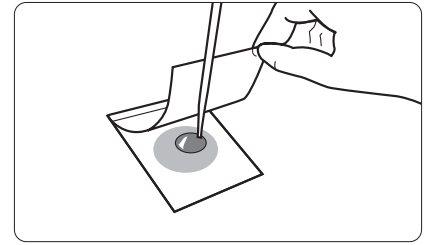
Store unopened packages at $\leq 8^{\circ}\text{C}$ ($\leq 46^{\circ}\text{F}$). Use before expiration date on package. Just prior to use, allow unopened pouches to come to room temperature before opening.



02

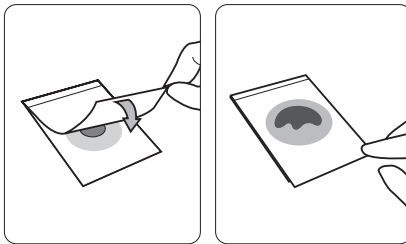
To seal opened package, fold end over and apply adhesive tape. **Do not refrigerate opened packages.** Use Petrifilm Coliform Count Plates within one month after opening.

Hydration Procedure



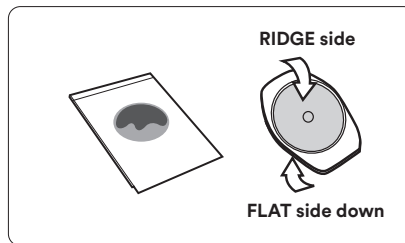
03

Place Petrifilm Plate on a flat, level surface. With the pipette **perpendicular** to the Petrifilm Plate, place hydration diluent onto the center of the bottom film. Hydration diluents include distilled water, deionized (DI) water and reverse osmosis (RO) water.



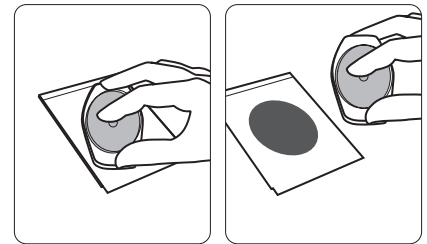
04

Roll the top film down onto sample gently to prevent pushing sample off film and to avoid entrapping air bubbles.



05

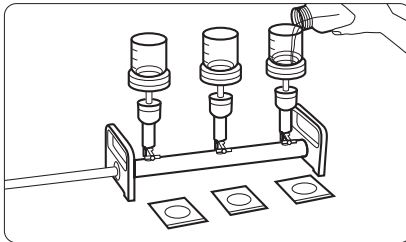
With **flat** side down, place Petrifilm Spreader on top film over hydration diluent.



06

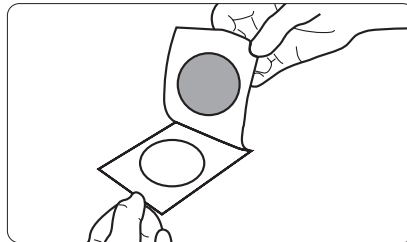
Gently apply pressure on Petrifilm Spreader to distribute inoculum or hydration diluent over circular area before gel is formed. **Do not** twist or slide the spreader. Lift spreader.

- Allow the hydrated plates to remain closed for a minimum of one hour before use.
- Any additional hydrated Petrifilm Coliform Count Plates may be stored in a sealed pouch or plastic bag. Protect plates from light and refrigerate at $2\text{--}8^{\circ}\text{C}$ ($36\text{--}46^{\circ}\text{F}$) for up to 7 days.



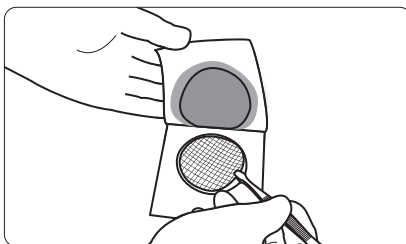
07

Following standard procedures for water analysis, membrane filter water sample using a 47 mm, 0.45 micron pore size Mixed Cellulose Ester (MCE) filter.



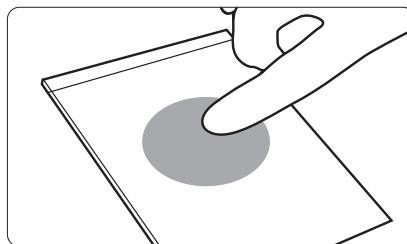
08

Carefully lift top film.



09

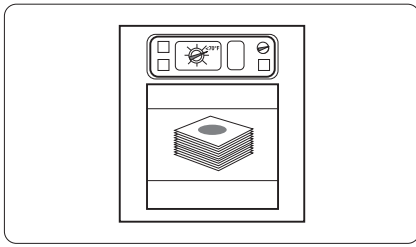
Place filter in the center of the well. Roll top film down to minimize air bubbles or gaps between the filter and the Petrifilm Coliform Count Plate.



10

Lightly apply pressure to ensure uniform contact of the filter with the gel and to eliminate any air bubbles.

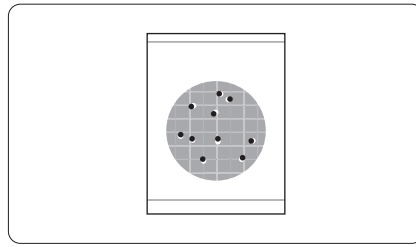
Incubation



11

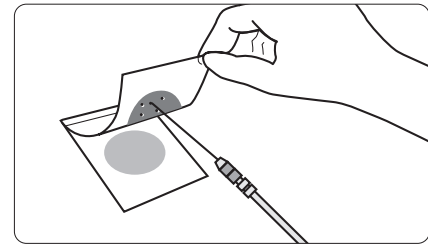
Incubate Petrifilm Coliform Count Plates in a horizontal position, clear side up, in stacks on no more than 20 plates at $35^{\circ}\pm 1^{\circ}\text{C}$ for 24 ± 2 hours or $36^{\circ}\pm 1^{\circ}\text{C}$ for 24 ± 2 hours. **Please refer to the product instructions for third party validated methods.**

Interpretation



12

Petrifilm Coliform Count Plates can be counted on a standard colony counter or other illuminated magnifier.

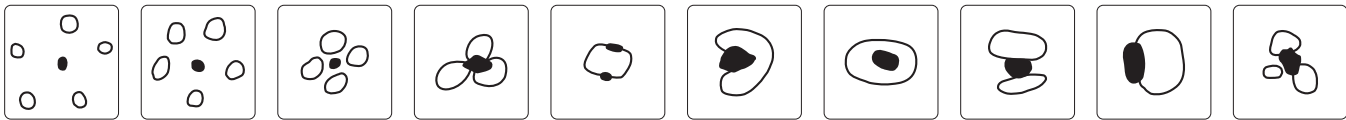


13

Colonies may be isolated for further identification. Lift top film and pick the colony from the gel. Red colonies without closely associated gas bubbles may be coliforms and should be picked and tested with appropriate confirmation methods.

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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