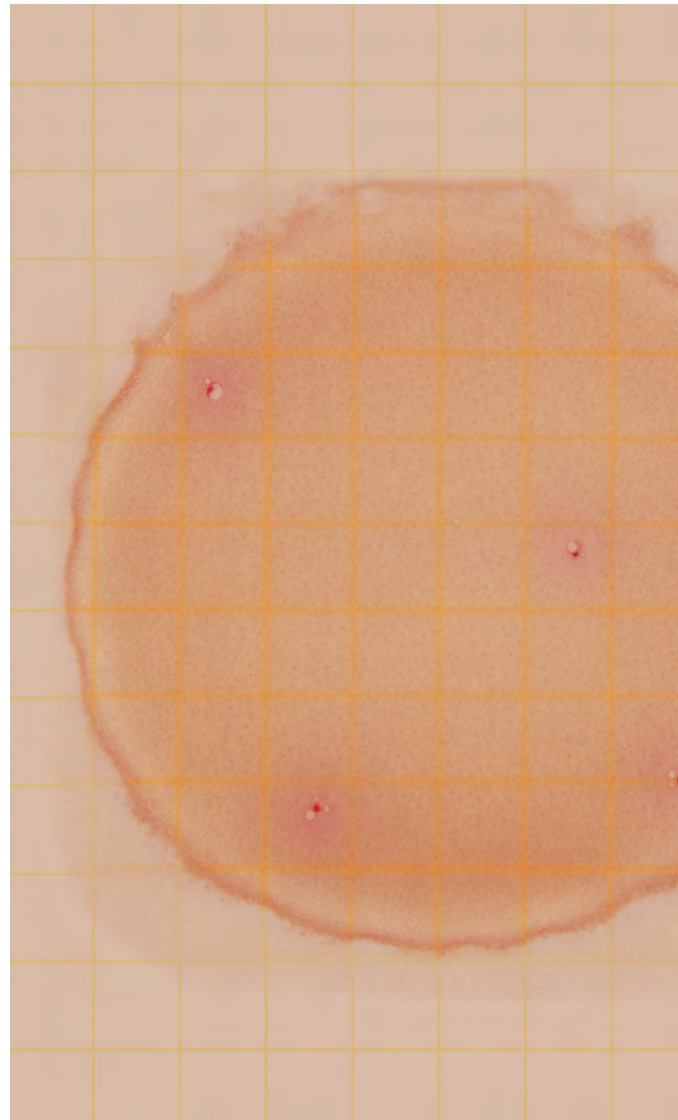




Petrifilm®

Interpretation Guide

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



HSCC

High-Sensitivity Coliform Count Plate

The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) defines coliforms as Gram-negative rods which produce acid and gas from lactose during fermentation. Gas production is used to differentiate coliform from non-coliform colonies. Gas trapped around red colonies indicates coliforms on the Petrifilm High-Sensitivity Coliform Count Plate. Acid production causes the pH indicator to deepen the gel color to a more pink-red background color.

ISO defines coliforms by their ability to grow in method-specific, selective media. 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 High-Sensitivity Coliform Count Plate, these coliforms are indicated by red colonies with gas.

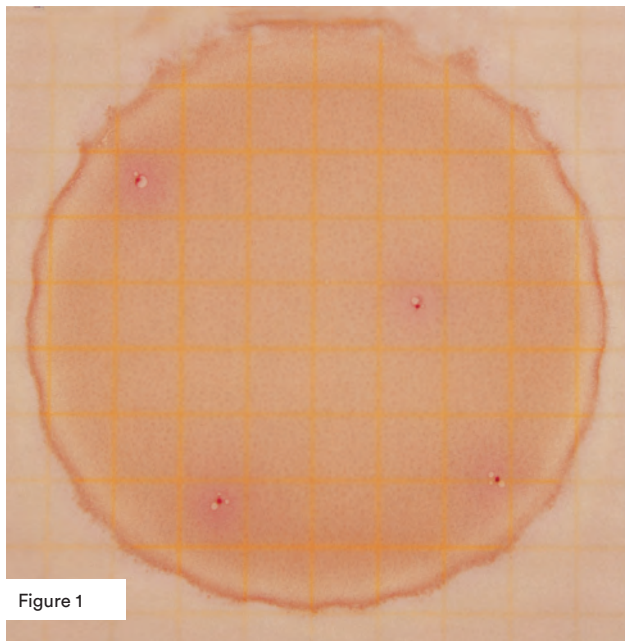


Figure 1

Coliform count = 4

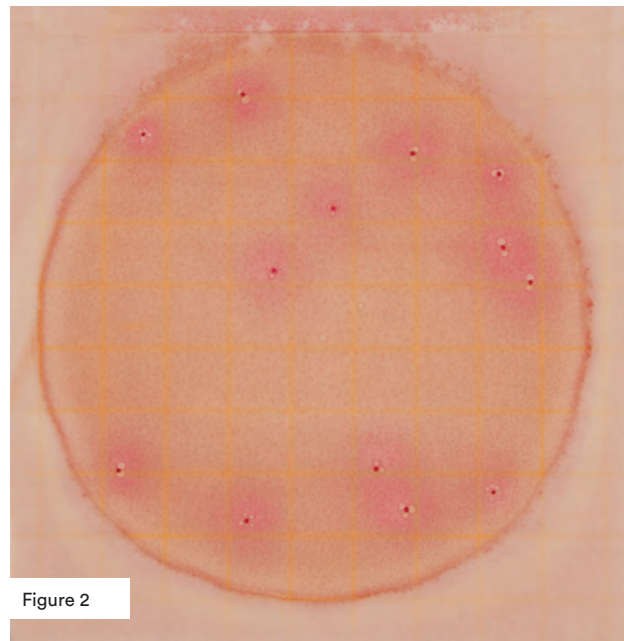


Figure 2

Coliform count = 13

It is easy to count coliform colonies on Petrifilm High-Sensitivity Coliform Count Plates. A red indicator dye in the plate colors Gram-negative colonies and the top film traps gas produced by the coliforms.

When coliforms produce acid, the gel surrounding the colony becomes pinker, as shown in Figure 2.

Look for pink-red zones around the colony to aid in counting. Count red colonies that are associated with gas bubbles as coliforms.

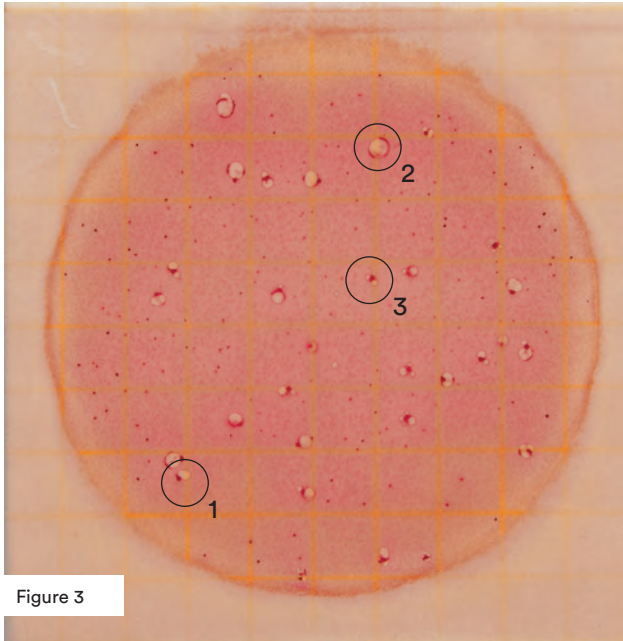


Figure 3

Coliform count = 30

Gas production is used to differentiate coliform from non-coliform colonies. Circles 1, 2 and 3 show how bubble patterns may vary. The gas bubble in Circle 1 is adjacent to the colony. In Circle 2, the gas disrupts the coliform colony so that the colony “outlines” the bubble. In Circle 3, three small gas bubbles circle the colony. All of these examples are coliforms. Red colonies which are not associated with gas bubbles should not be counted as coliforms.

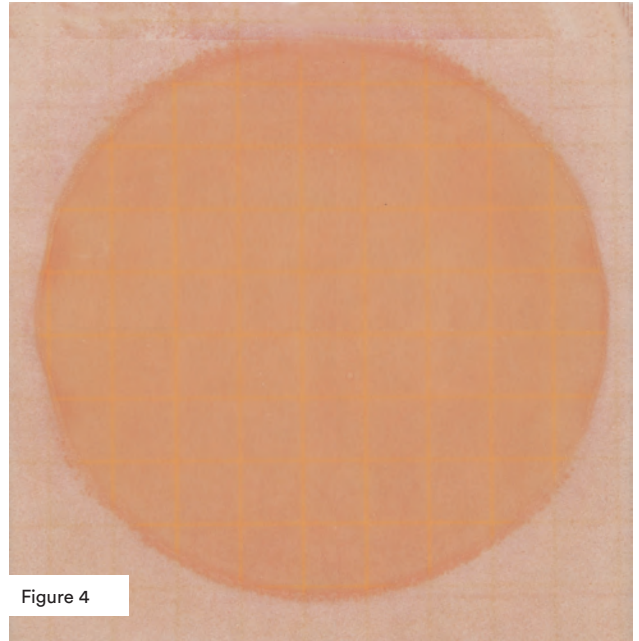


Figure 4

Coliform count = 0

Notice the change in gel color in Figures 4 through 9. As the coliform count and acid production increases, the color of the gel deepens from a light orange in Figure 4 to a bright pink-red in Figure 9. Plating and incubating a negative control will aid in differentiating changes in gel color.

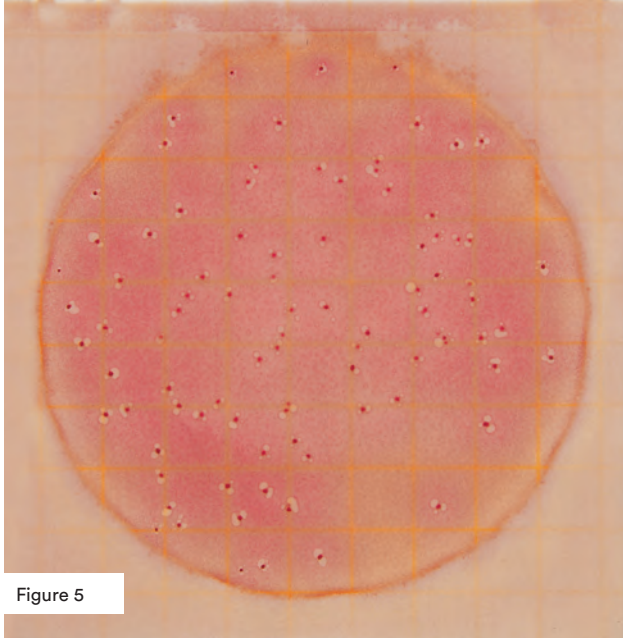


Figure 5

Coliform count = 90

The countable range on Petrifilm High-Sensitivity Coliform Count Plate is less than or equal to 150 colonies.

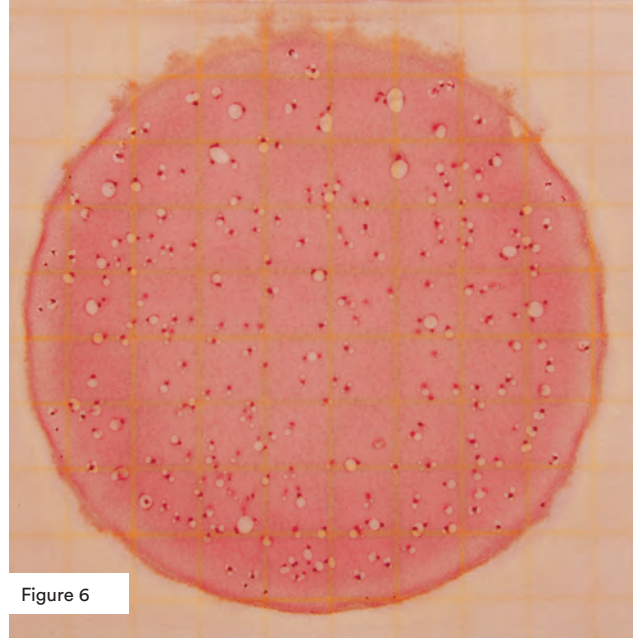


Figure 6

Estimated coliform count = 320

The circular growth area is approximately 60 cm². 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 60 to determine the estimated count per plate.

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

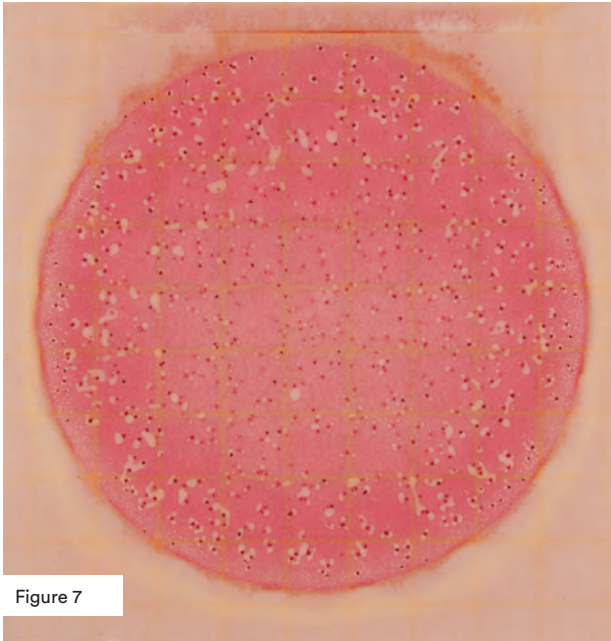


Figure 7

Estimated coliform count = 840

Colonies and gas bubbles may be smaller around the edge of the inoculated area, as noted in Figure 7. The different gel appearance around the edge of the inoculum does not affect colony counts.

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

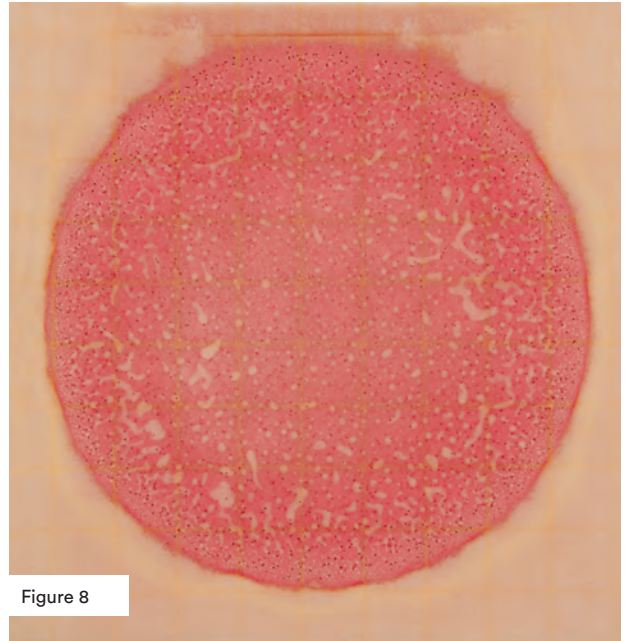


Figure 8

Coliform count = too numerous to count (TNTC)

A TNTC coliform count will cause the gel to turn a darker pink-red color. Additionally, one may observe many small colonies and/or many gas bubbles. The higher the count the less prominent the gas and colonies may be. All three characteristics are shown in Figure 8.

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

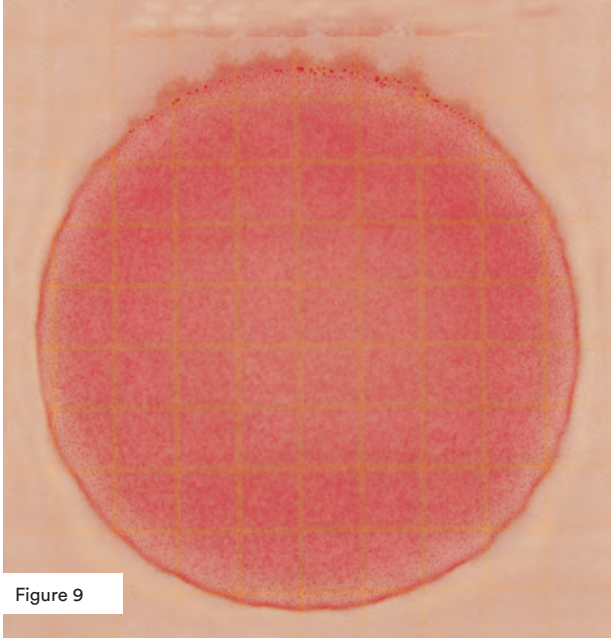


Figure 9

Coliform count = TNTC

Figure 9 shows many small colonies and a deepening of the gel color.

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

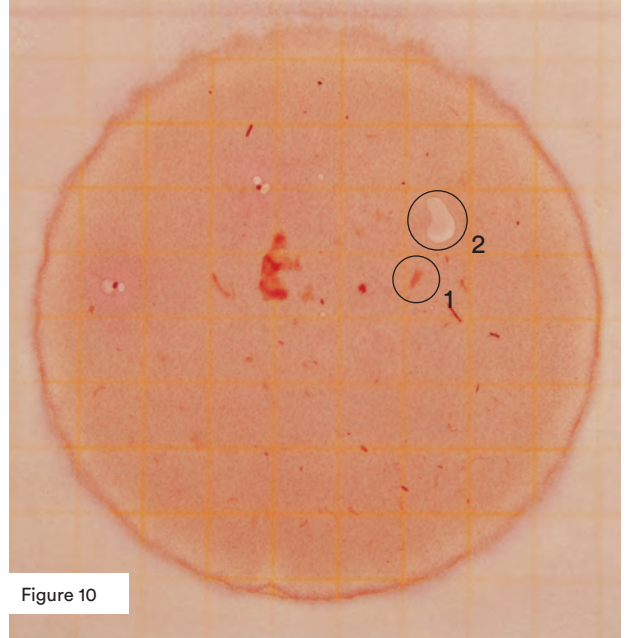


Figure 10

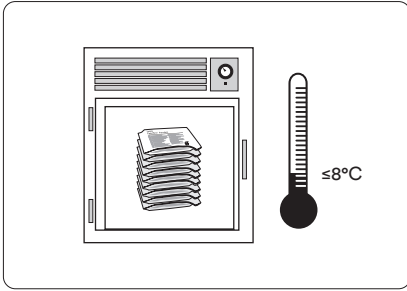
Coliform count = 2

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

Artifact bubbles may result from improper inoculation of the Petrifilm High-Sensitivity Coliform Count Plate. They are irregularly shaped and not associated with a red colony. See Circle 2.

Reminders For Use

Storage



01

Store unopened Petrifilm High-Sensitivity Coliform Count Plate pouches frozen or refrigerated at temperatures $\leq 8^{\circ}\text{C}$ ($\leq 46^{\circ}\text{F}$). Use before expiration date on package. Just prior to use, allow unopened pouches to come to room temperatures before opening. Return unused plates to pouch.

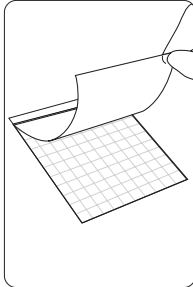
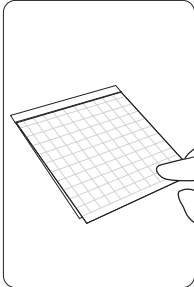


02

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.

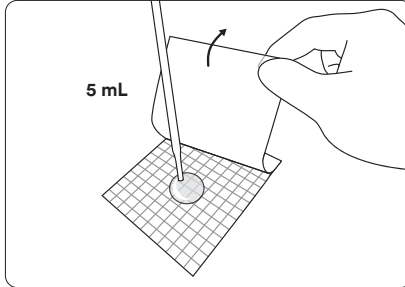
Inoculation

Remember to inoculate and spread each Petrifilm Plate before going on to the next plate.



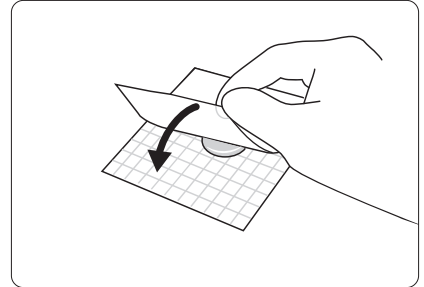
03

Place the Petrifilm High-Sensitivity Coliform Count Plate on flat, level surface. Lift top film.



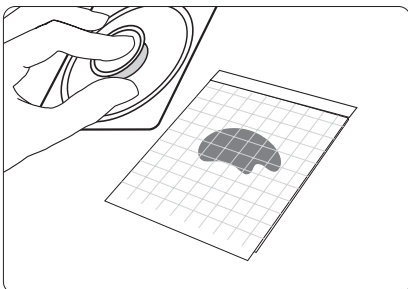
04

Lift the top film and, with the pipette perpendicular to the inoculation area, dispense 5 mL of sample suspension onto the center of the bottom film.



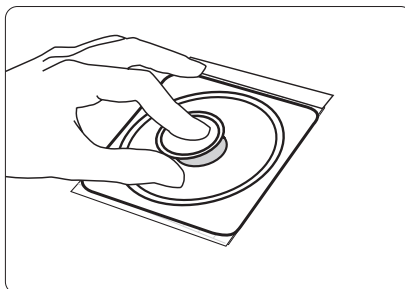
05

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



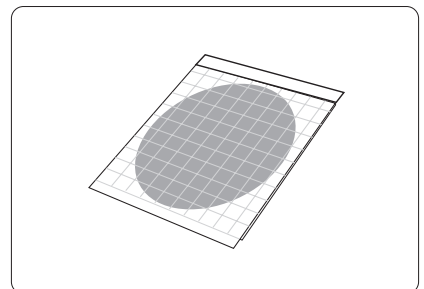
06

Place the Petrifilm High-Sensitivity Plate Spreader on top film over inoculum.



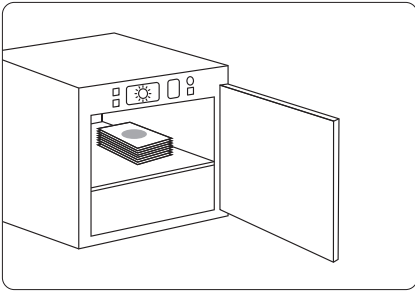
07

Distribute sample with a gentle downward pressure on the handle of the spreader. **Do not** twist or slide the spreader.



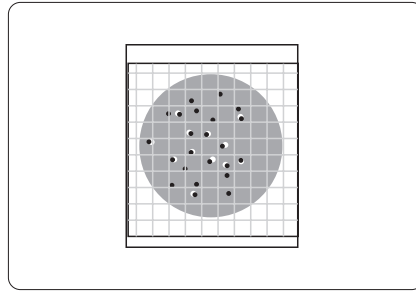
08

Lift spreader. Wait **2–5 minutes** for gel to form.



09

Incubate plates with clear side up in stacks of up to 10. It may be necessary to humidify incubator to minimize moisture loss. **See product instructions for third party validated methods.**



10

Petrifilm High-Sensitivity Coliform Count Plates can be counted 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 diluent, quarter-strength Ringer's solution, dipotassium hydrogen phosphate, 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 6.5–7.5.

Do not use buffers containing citrate, bisulfite or thiosulfate; 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.

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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