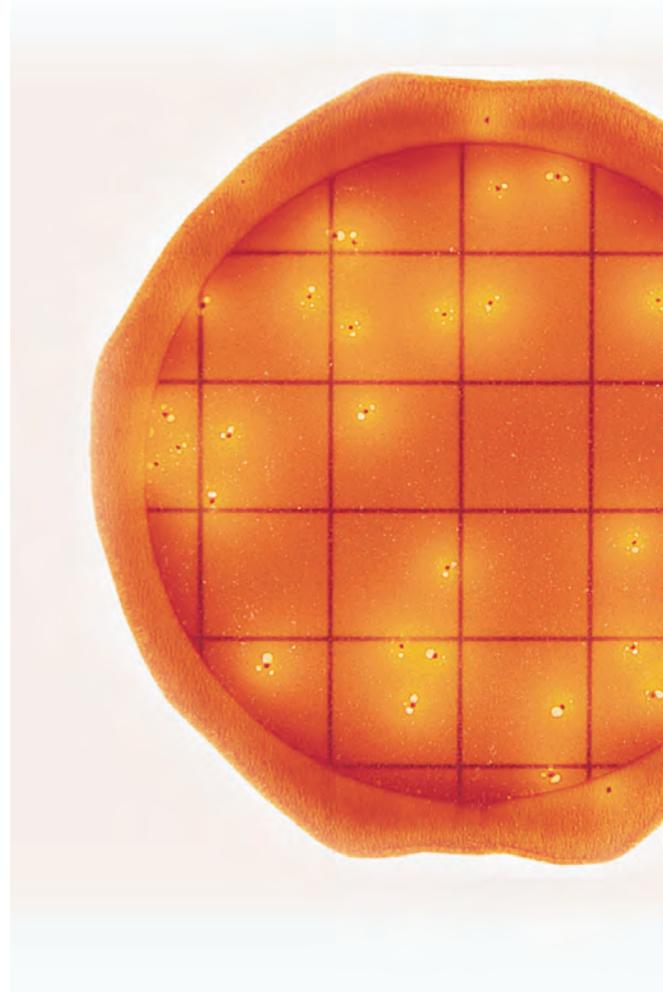




Petrifilm®

Interpretation Guide

The Neogen® Petrifilm® Rapid Coliform Count Plate is a sample-ready culture medium system that contains Violet Red Bile (VRB) nutrients, a cold-water-soluble gelling agent, a pH indicator to detect acid and a tetrazolium indicator that facilitates colony enumeration. Petrifilm Rapid Coliform Count Plates are useful for the enumeration of coliform bacteria in the food and dairy industries.



RCC

Rapid Coliform Count Plate



The United States Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM)¹ defines coliforms as Gram-negative rods, which produce acid and gas from lactose fermentation, and is one of the reference methods used to validate alternative methods to achieve AOAC® *Official Methods of Analysis*SM status. Per the Petrifilm Rapid Coliform Count Plate AOAC® OMA 2000.15, as colonies grow they produce acid and the pH indicator in the plate changes from red-orange to yellow, providing a presumptive indication of coliforms. Gas trapped around colonies indicates confirmed coliforms.

ISO coliform reference methods define coliforms by their ability to grow in method-specific, selective media. ISO 4832:2006² enumerates typical coliform colonies on Violet Red Bile Lactose (VRBL) agar, with confirmation of atypical colonies. The Petrifilm Rapid Coliform Count Plate has been validated in comparison to ISO 4832:2006 to achieve NF Validations by AFNOR Certification 3M 01/05-03/97 A and 3M 01/05-03/97 B. When following these validations, coliforms are indicated by yellow acid zones or red colonies with or without gas production. ISO 4831:2006³ enumerates coliforms by the most probable number (MPN) method and defines coliforms by their ability to grow and to produce gas. The Petrifilm Rapid Coliform Count Plate has been validated in comparison to ISO 4831:2006 to achieve NF Validation by AFNOR Certification 3M 01/05-03/97 C. When following this validation, coliforms are indicated by red colonies with gas.

Please refer to the product instructions for additional information.

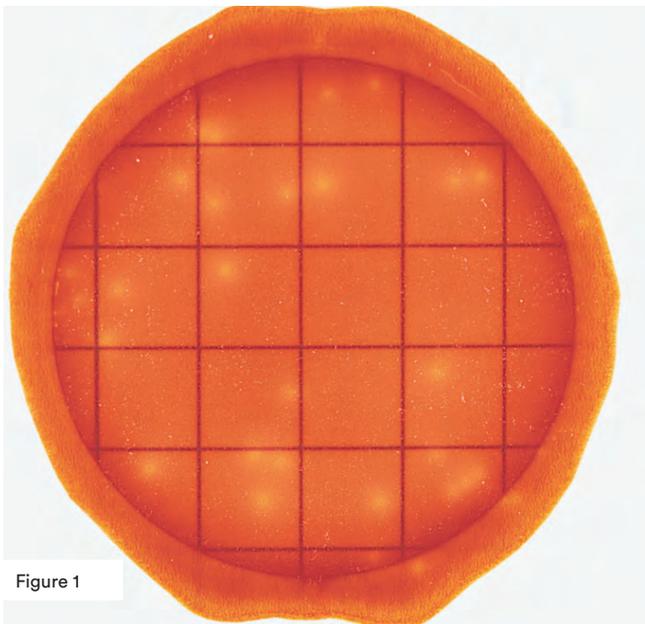


Figure 1

Coliform enumeration by acid zones (6–14 hours)

Yellow acid zones may begin to appear as early as 6 hours. If coliforms are present, yellow zones will appear and diffuse throughout incubation. The coliform count will depend on which validated method you follow.

AOAC OMA 2000.15:

Count yellow acid zones with or without red centers as presumptive coliforms.

NF Validation 3M 01/05-03/97 A and B:

Count yellow acid zones with or without red centers as coliforms. Final results at 14 hours.

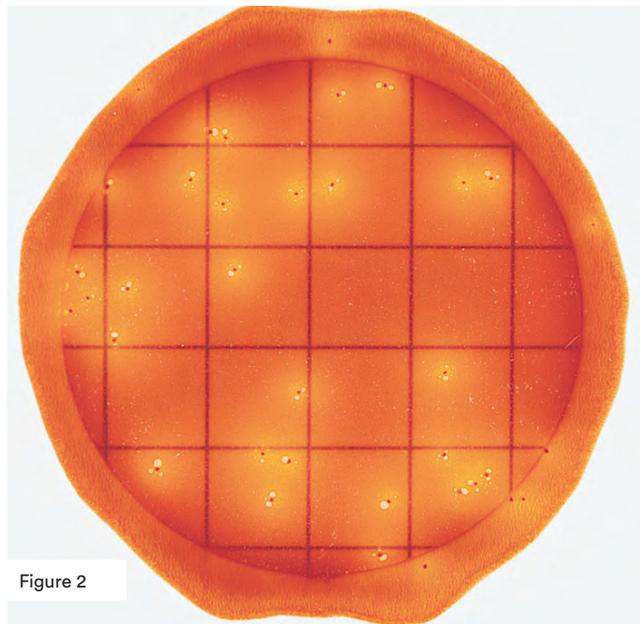


Figure 2

Coliform colony enumeration (8–24 hours)

Red colonies with or without gas may begin to appear as soon as 8 hours and continue to grow throughout incubation. The coliform count will depend on which validated method you follow.

AOAC OMA 2000.15:

Count red colonies associated with gas as confirmed coliforms whenever they appear.

NF Validation 3M 01/05-03/97 C:

Count red colonies associated with gas as coliforms. Final results at 24 ± 2 hours, except for processed pork.

NF Validation 3M 01/05-03/97 A and B:

Count red colonies with or without gas as coliforms. Final results at 24 ± 2 hours.

Early reading of bacterial growth on Petrifilm Rapid Coliform Count Plates (measured by acid and gas production) will depend on the type of bacteria present in the sample, their metabolic state and their concentration.

Enumeration of Acid Zones (6–14 Hours)

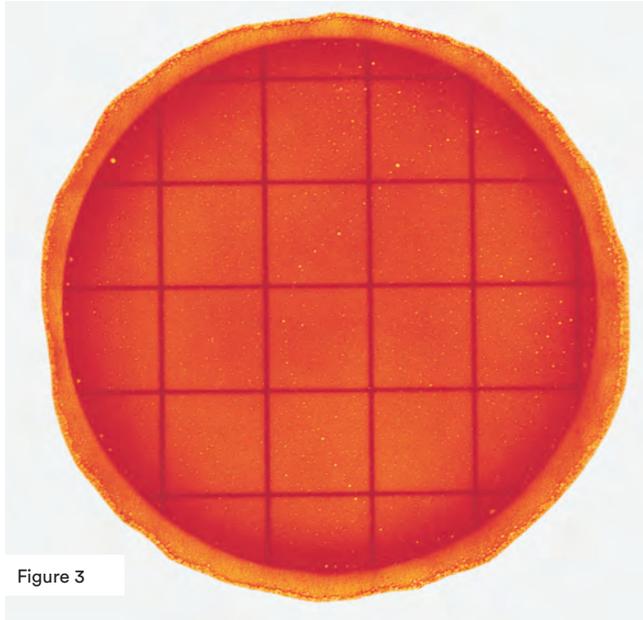


Figure 3

Coliform count = 0

Notice the gel changes in figures 3 through 10. As the coliforms produce acid, the color of the gel changes from red-orange to orange-yellow.

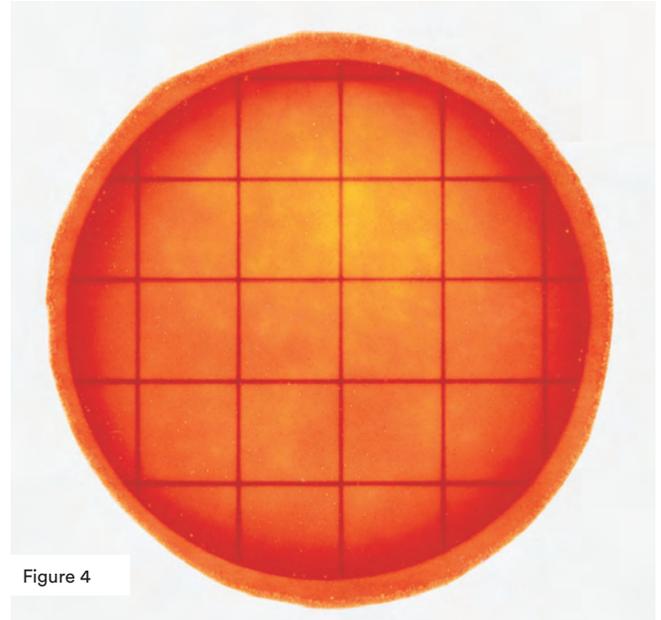


Figure 4

Estimated coliform count = TNTC

High concentrations of coliforms may cause the entire growth area to turn yellow after 6 hours of incubation.

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

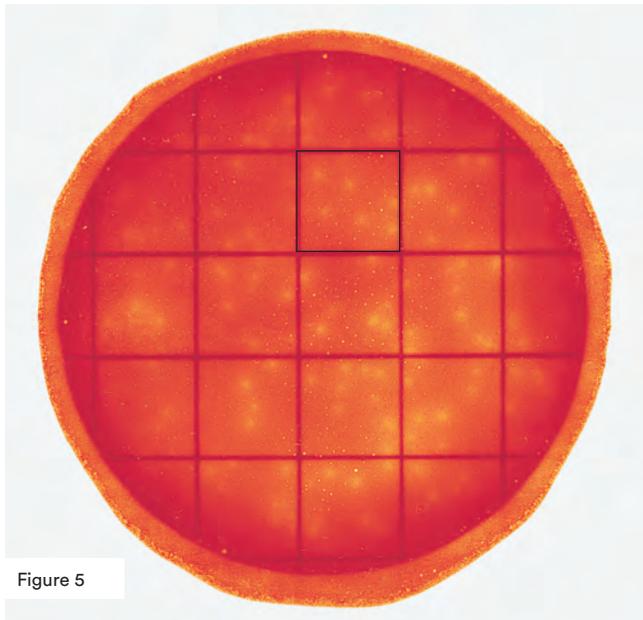


Figure 5

Estimated coliform count = 120

Some coliforms produce large amounts of acid. For these organisms, fusion of the acid zones could occur with as few as 20 colonies per plate. Estimates can be made on plates containing greater than 50 discreet acid zones.

The circular growth area on a Petrifilm Rapid Coliform Count Plate is approximately 20cm². Estimates can be made on plates by counting the number of acid zones in one or more representative squares, determining the average number per square and multiplying by 20. There are 6 acid zones in the square outlined in Figure 5.

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

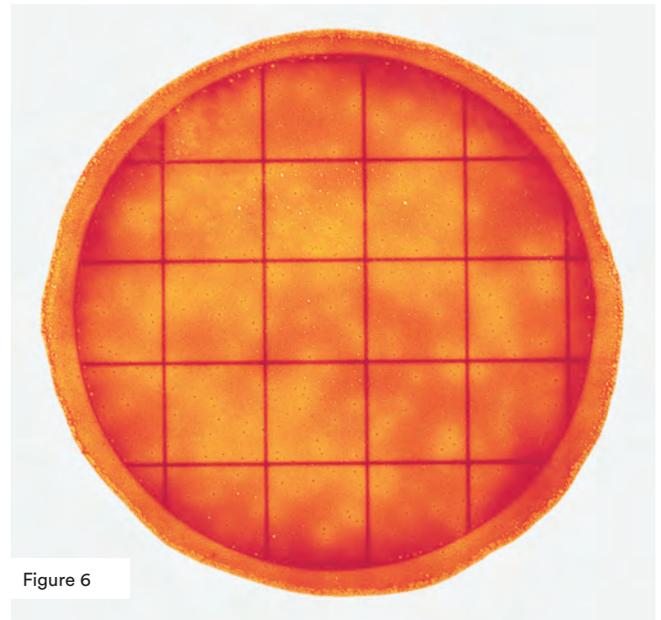


Figure 6

Estimated coliform count = 280

Red colonies will begin to appear within the acid zones as the coliforms continue to grow.

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

Enumeration of Colonies with Gas (8–24 Hours)

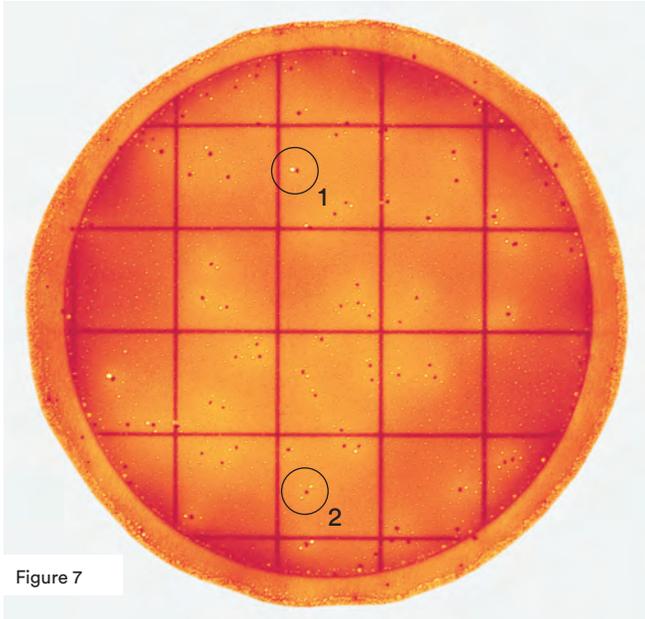


Figure 7

Total coliform count = 64

Colonies and gas bubbles may be smaller around the edge of the growth area. Count colonies with, or without gas depending on which validated method to which you are comparing (FDA BAM or ISO method). A colony is associated with a gas bubble(s) if it is within one colony diameter away from the colony or in a ring pattern around the colony. See Circles 1 and 2.

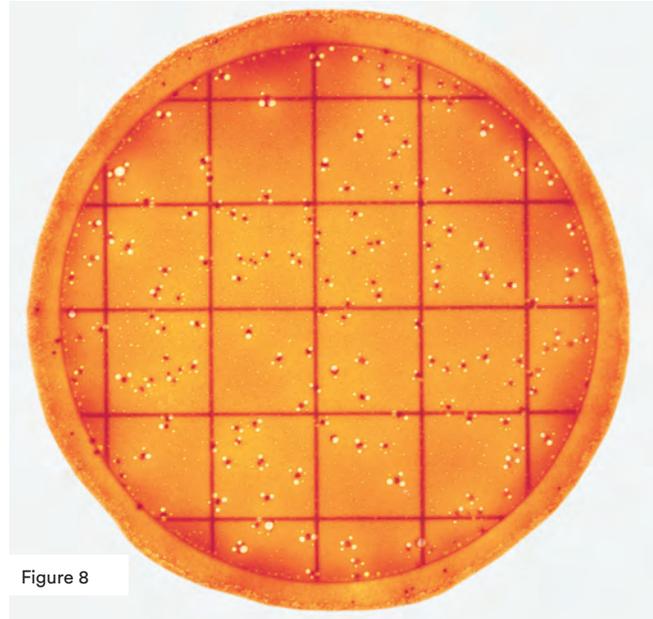


Figure 8

Estimated coliform count = 164

Figures 7 and 8 show the results from the same concentration of different organisms incubated the same length of time. Distinct red colonies with acid zones appear on both plates. The organisms in Figure 8 appear to ferment lactose to produce gas more readily than those in Figure 7.

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

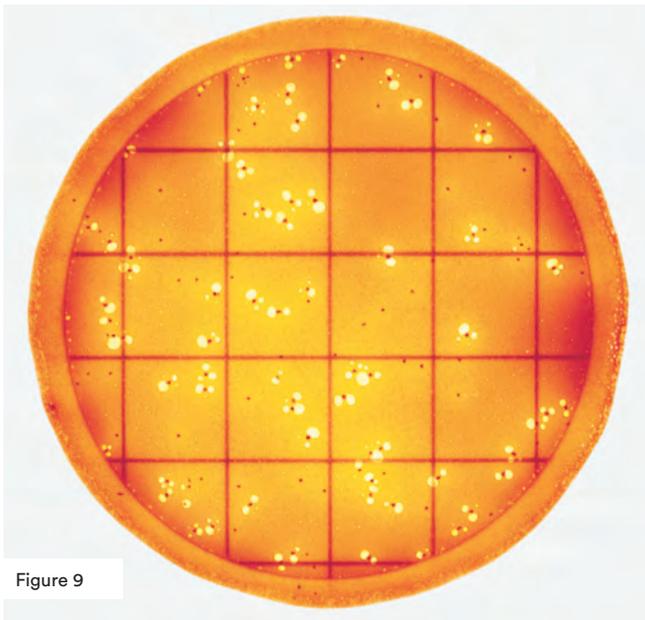


Figure 9

Method Specific Counts

Figure 9 is another example of counting colonies with or without gas bubbles. The count depends on which validated method you follow.

AOAC OMA 2000.15: Coliforms are colonies with gas = 72.

NF Validation 3M 01/05-03/97 C: Coliforms are colonies with gas = 72.

NF Validation 3M 01/05-03/97 A and B: Coliforms are colonies with and without gas = 128.

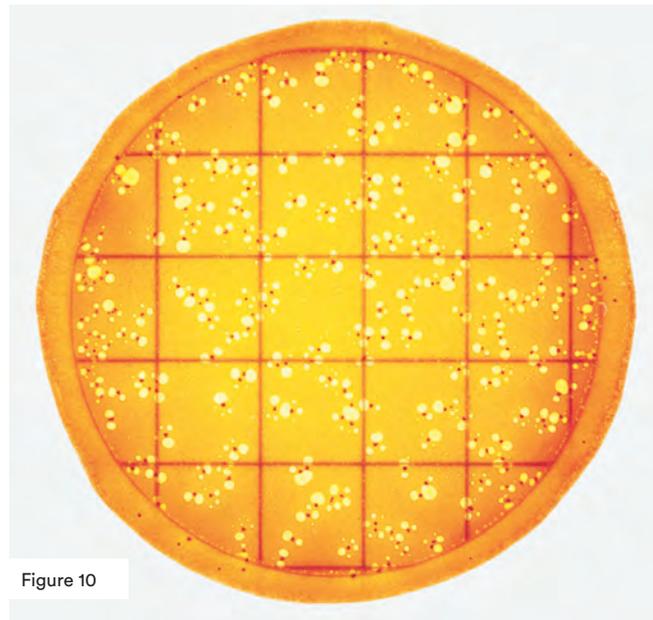


Figure 10

Estimated coliform count: 240

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

Too Numerous To Count (TNTC)

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

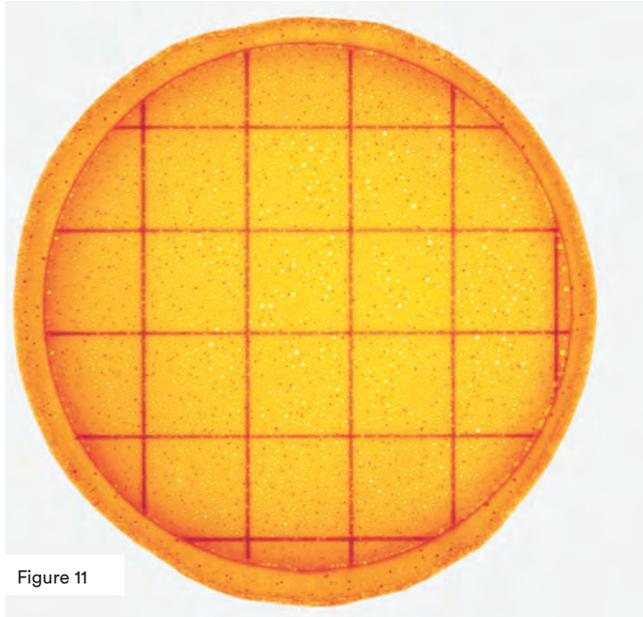


Figure 11

Coliform count = TNTC

Petrifilm Rapid Coliform Count Plates with colonies that are TNTC have one or more of the following characteristics: change in gel color from red-orange to orange-yellow, many small colonies, many gas bubbles.

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

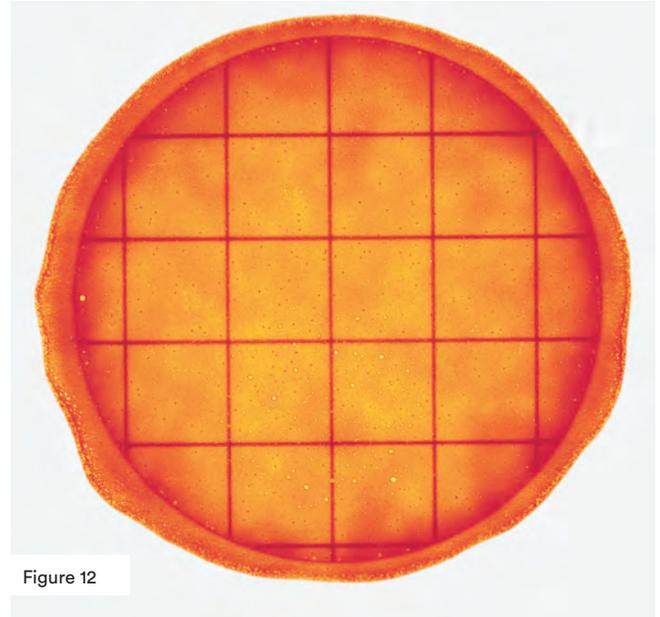


Figure 12

Coliform count = TNTC

The Petrifilm Rapid Coliform Count Plate in Figure 12 has two characteristics indicating a TNTC result: change in gel color and many small colonies.

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

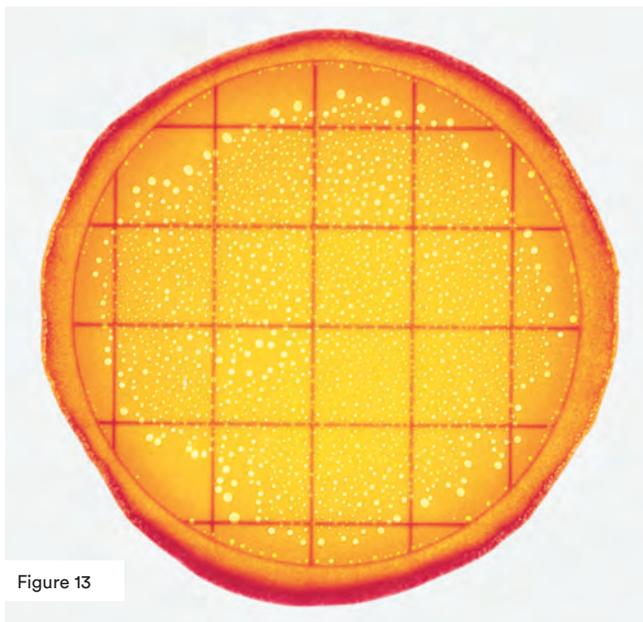


Figure 13

Coliform count = TNTC

In Figure 13, the count is so high that individual colonies do not show. A change in the gel color to yellow and the many gas bubbles indicate a TNTC result.

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

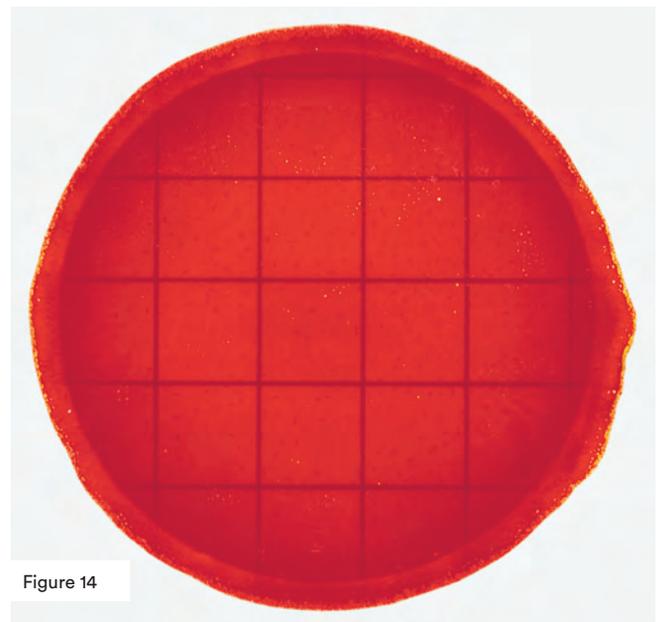


Figure 14

Coliform count: 0

Figure 14 shows a Petrifilm Rapid Coliform Count Plate with a high number of Gram-negative non-coliform colonies. When high numbers of organisms that do not ferment lactose are present, the gel may appear dark red.

The optimal growth for most bacteria occurs near pH 7.0. Dilutions of low pH products require pH adjustments to pH 6.5 – 7.5.

Figures 15 and 16 show examples of fresh yogurt plated after pH adjustment. Inhibitors in the media prevent the Gram-positive starter culture from growing, but acid produced by the starter culture may still change the background color of the gel from red-orange to orange-yellow, mimicking an early TNTC result. Monitor plates containing fresh yogurt culture during incubation for further indication of TNTC coliform growth.

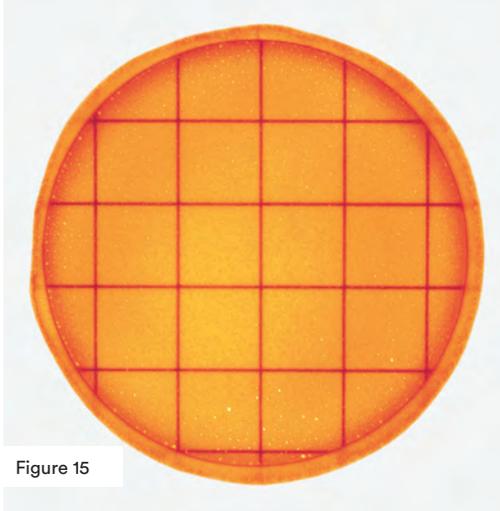


Figure 15

Coliform count: 0

Compare the negative plate above to the TNTC plates on the previous page. Note that no colonies or gas bubbles are present in Figure 15 to indicate a TNTC result.

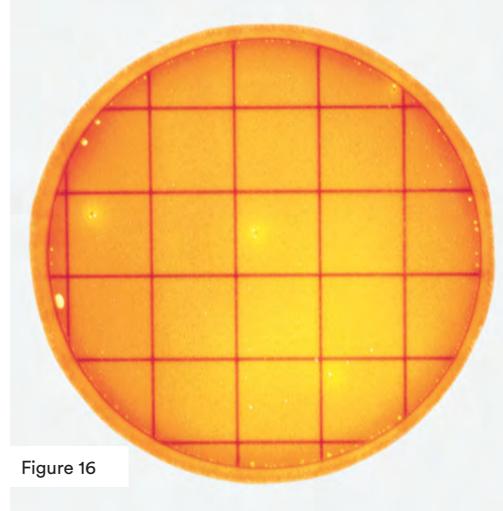


Figure 16

Coliform count: 4

Despite the change in the gel color, acid produced by the coliforms is still easily seen, as shown in Figure 16.

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

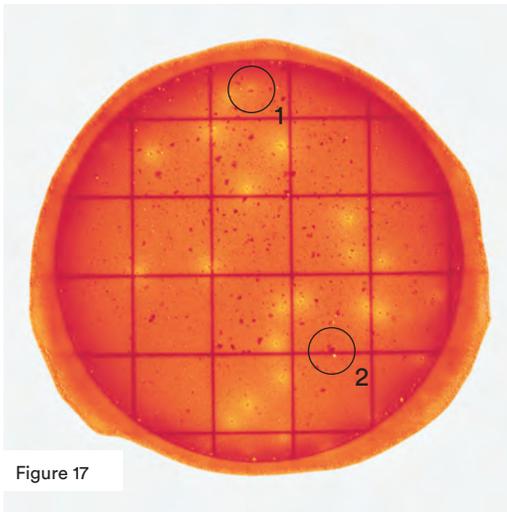


Figure 17

Coliform count: 11

Figure 17 is an early reading of a dilution of paprika. Circle 1 shows an acid zone around a red, irregularly-shaped food particle. Some foods may contain acidic particles that react with the pH indicator. Circle 2 shows a bubble near a red, irregularly-shaped food particle — but no acid zone. Neither should be counted as a colony.

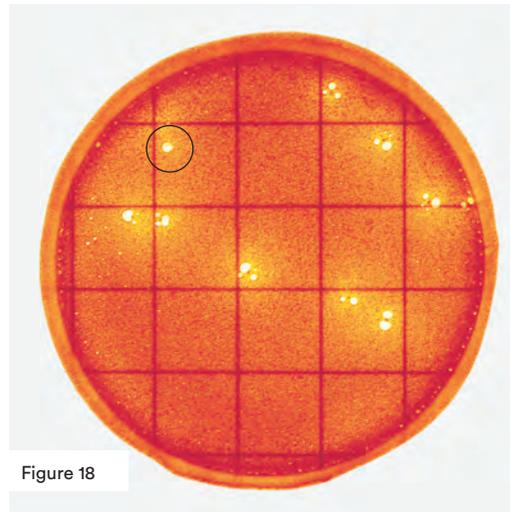


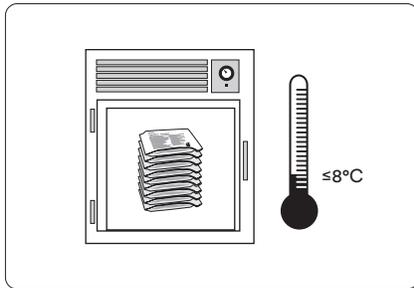
Figure 18

Coliform count: 10

A dilution of chocolate is shown in Figure 18. Zones of acid associated with colonies will continue to expand during incubation. Gas bubbles associated with colonies are another criteria that will aid in the identification of coliforms. Gas bubbles may outline the colony as shown in the circle. Enumeration with or without gas is method dependent.

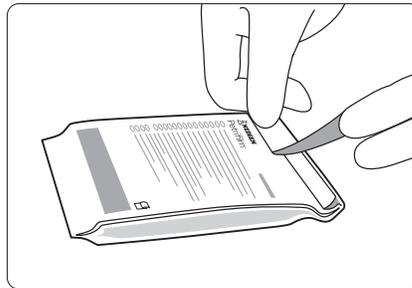
Reminders For Use

Storage



01

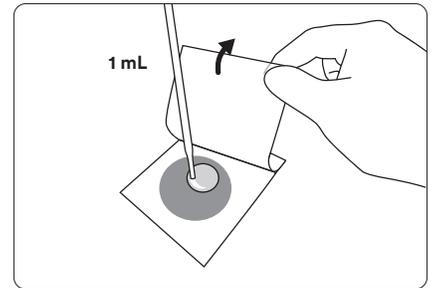
Store unopened pouches of plates at $\leq 8^{\circ}\text{C}$ ($\leq 46^{\circ}\text{F}$). Use before expiration date on package.



02

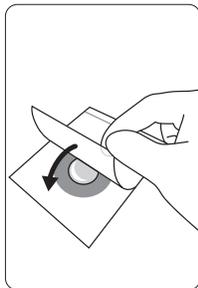
To seal opened package, fold the end of the pouch over and apply adhesive tape. Keep resealed package at $\leq 25^{\circ}\text{C}$ ($\leq 77^{\circ}\text{F}$) and $\leq 50\%$ RH. **Do not refrigerate opened packages.** Use plates within four weeks after opening.

Inoculation



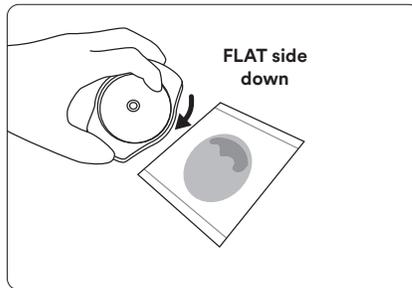
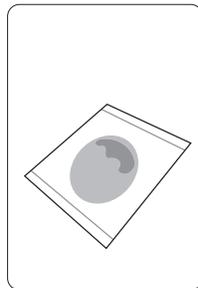
03

Place Petrifilm Rapid Coliform Count Plate on **level** surface. Lift top film. With pipetter **perpendicular** to the plate, place 1 mL of sample onto center of bottom film.



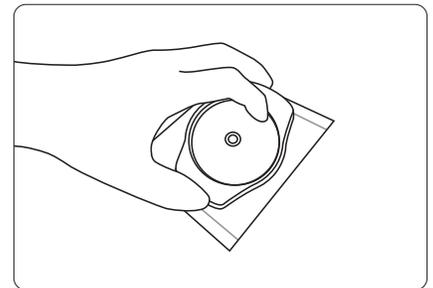
04

Carefully **roll** top film down to avoid trapping air bubbles.



05

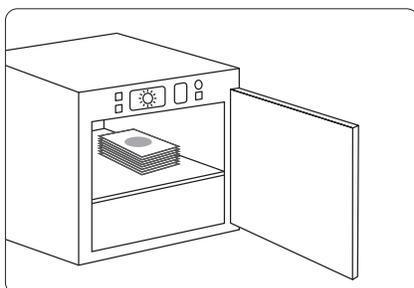
With **flat** side down, place Petrifilm Spreader on top film over inoculum.



06

Gently apply pressure on Petrifilm Spreader to distribute over circular area before gel is formed. **Do not** twist or slide the spreader. Lift the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.

Incubation



07

Incubate plates with clear side up in stacks of up to 20. **See product instructions for third party validated methods.**

Interpretation

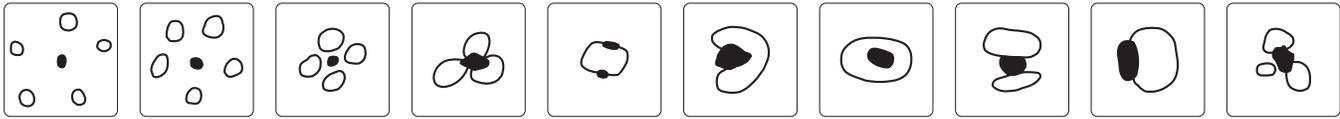


08

Petrifilm Rapid 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.

Bubbles

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



Use Appropriate Sterile Diluents

Butterfield's phosphate buffered dilution water, 0.1% peptone water, peptone salt diluent, saline solution (0.85-0.90%), 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 diluents containing citrate, bisulfite or thiosulfate with the Petrifilm Rapid 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.

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 3M LIABILITY, STORAGE AND DISPOSAL information and INSTRUCTIONS FOR USE, see product instructions.



¹ U.S. Food and Drug Administration Bacteriological Analytical Manual. Chapter 4: Enumeration of *Escherichia coli* and the *Coliform* Bacteria.

² ISO 4832:2006. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *coliforms* - Colony count technique.

³ ISO 4831:2006. Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *coliforms* - Most probable number technique.

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