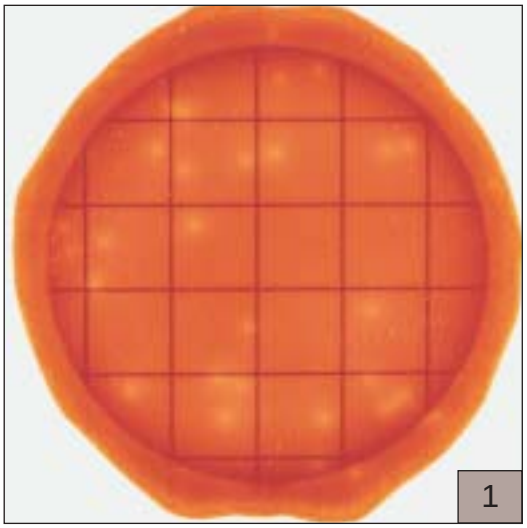
Interpretation Guide

Petrifilm™ Rapid Coliform Count Plate

This guide familiarizes you with results on 3M™ Petrifilm™ Rapid Coliform Count (RCC) plates, as defined by three of the most globally accepted coliform enumeration methods. For more information, contact the official 3M Microbiology Products representative nearest you.

AOAC INTERNATIONAL and **U.S. Food and Drug Administration, Bacteriological Analytical Manual (BAM)** define coliforms as gram-negative rods which produce acid and gas from lactose during metabolic fermentation. As colonies grow on the Petrifilm RCC plate and produce acid, the pH indicator in the plate changes from red-orange to yellow, providing a presumptive indication of coliforms. Gas trapped around coliform colonies indicates confirmed coliforms.

ISO defines coliforms by their ability to grow in method-specific, selective media. **ISO method 4832**, enumerating coliforms by the colony count technique, defines coliforms by colony size and acid production on VRB with lactose (VRBL) agar. On Petrifilm RCC plates, these acid-producing coliforms are indicated by yellow acid zones, or red colonies with or without gas. **ISO method 4831**, enumerating coliforms by the Most Probable Number (MPN) method, defines coliforms by their ability to grow and produce gas from lactose in a selective broth. On Petrifilm RCC plates these coliforms are indicated by red colonies associated with gas. **AFNOR** has validated Petrifilm RCC plates as a method in comparison to ISO method 4831 and ISO method 4832.



At 6 hours of incubation.

Coliform enumeration by acid zones (6-14 hours)

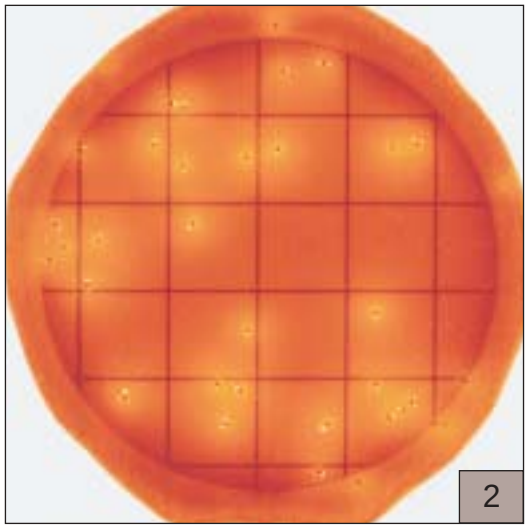
Yellow acid zones may begin to appear as early as 6 hours. If coliforms are present, yellow zones will appear and diffuse throughout incubation.

Interpretation when comparing to AOAC/BAM methods

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

Interpretation when comparing to ISO 4832 (VRBL)

- Count yellow acid zones with or without red centers as coliforms.
- Final results at 14 hours (AFNOR validation)



At 14 hours of incubation.

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.

Interpretation when comparing to AOAC/BAM methods

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

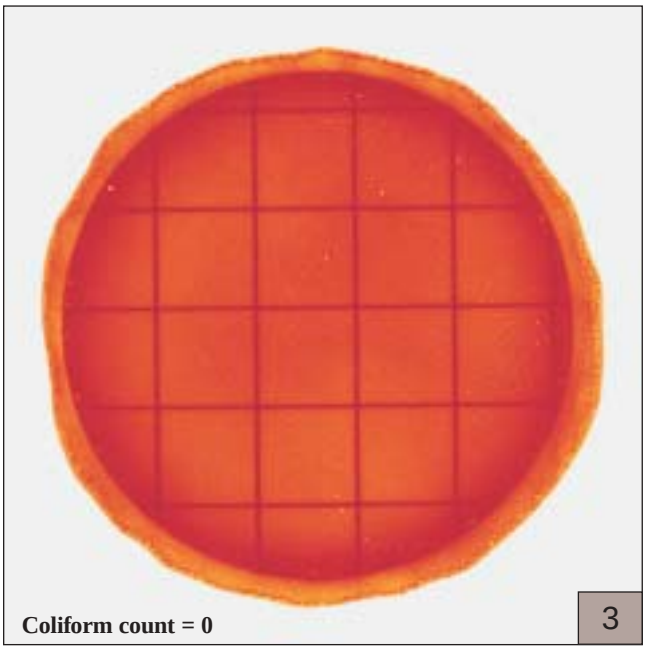
Interpretation when comparing to ISO 4831 (MPN)

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

Interpretation when comparing to ISO 4832 (VRBL)

- Count red colonies with or without gas as coliforms.
- Final results at 24 ± 2 hours (AFNOR validation)

Early reading of bacterial growth on Petrifilm Rapid Coliform Count plates (measured by acid and gas production)



Coliform count = 0 3

Enumeration of acid zones (6-14 hours)

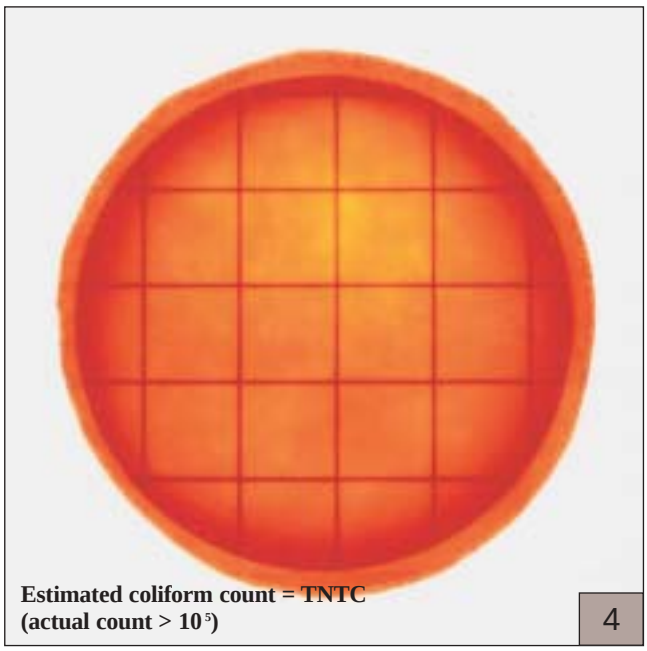
Notice the gel changes in figures 3 through 10. As the coliforms produce acid, the color of the gel changes from red-orange to orange-yellow.

High concentrations of coliforms (>1000 colonies/plate) may cause the entire growth area to turn yellow after 4 hours of incubation. See figure 4. When this occurs, further dilution of the sample may be required to obtain an accurate count.

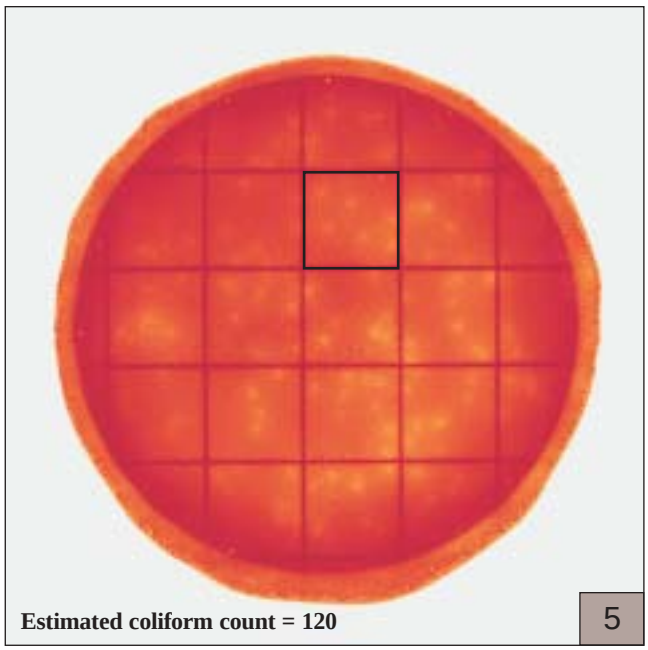
Some coliforms produce large amounts of acid. For these organisms, fusion of the acid zones could occur with as few as 20 colonies per plate. Estimates can be made on plates containing greater than 50 discreet acid zones.

The circular growth area on a Petrifilm RCC plate is approximately 20cm^2. 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.

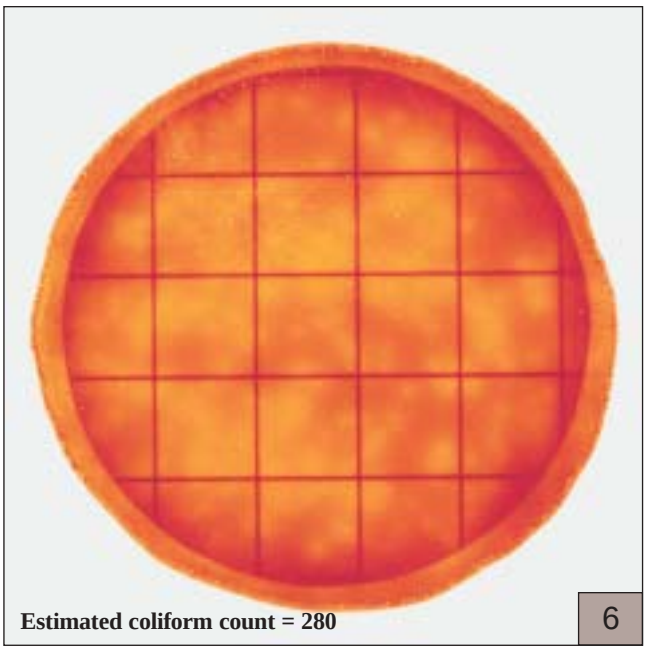
Red colonies will begin to appear within the acid zones as the coliforms continue to grow. See figure 6.



Estimated coliform count = TNTC (actual count > 10^5) 4



Estimated coliform count = 120 5



Estimated coliform count = 280 6

depends on the type of bacteria, their metabolic state and their concentration.

Enumeration of colonies and gas (8-24 hours)

Figures 7 and 8 show the results from the same concentration of different organisms incubated the same amount 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.

Count colonies with or without gas depending on the method you are following. A colony is associated with a gas bubble(s) if it is within one colony diameter away or in a ring pattern around the colony. See circles 1 and 2 respectively in figure 7.

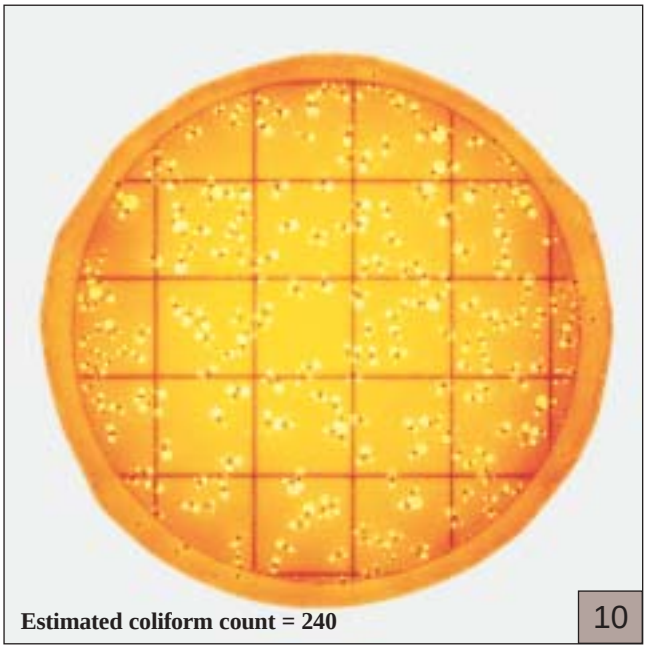
Figure 9* is another example of counting colonies with or without gas bubbles. The count depends on the method you follow.

As compared to AOAC/BAM methods, confirmed coliform colonies with gas = 72

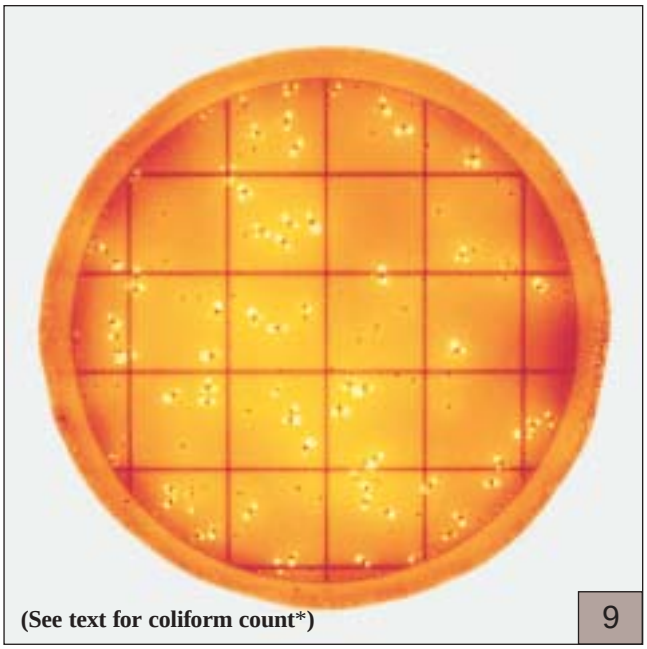
As compared to ISO 4831, coliforms are colonies with gas = 72

As compared to ISO 4832, coliforms are colonies with and without gas = 128

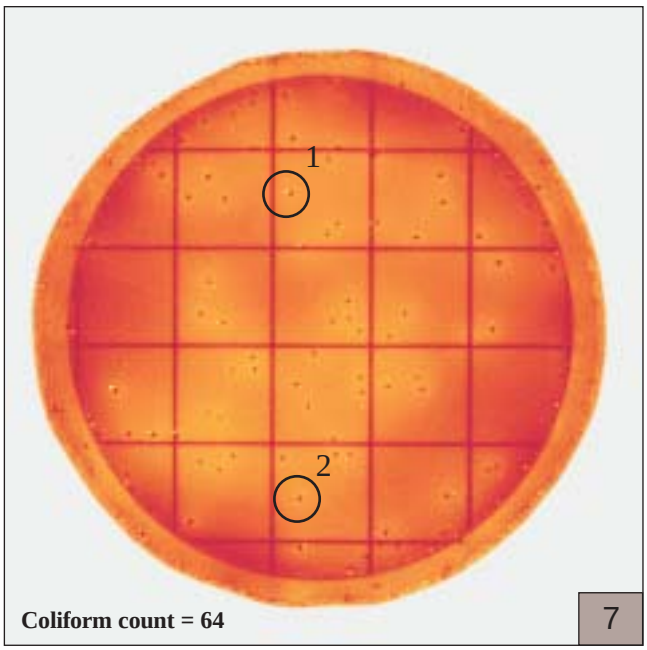
When colonies number more than 150 per plate, estimate the count. Do not count colonies which appear on the foam barrier because they are removed from the selective influence of the medium. See figures 7-10.



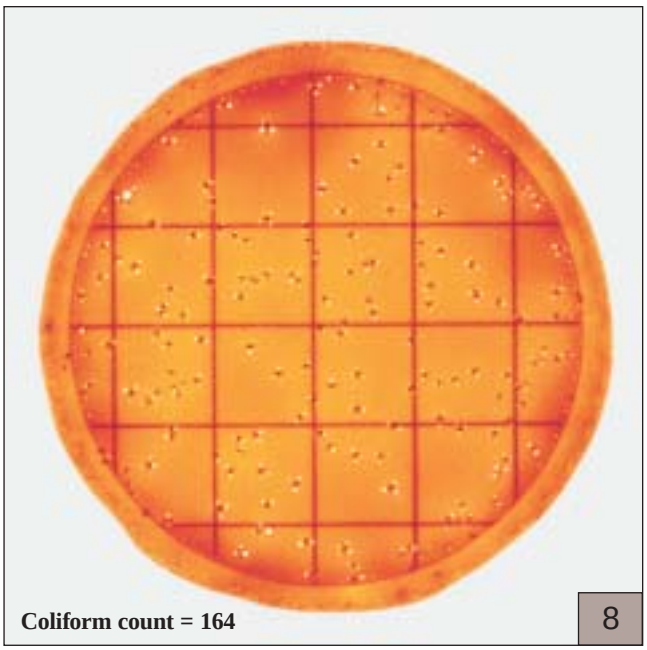
Estimated coliform count = 240 10



(See text for coliform count*) 9

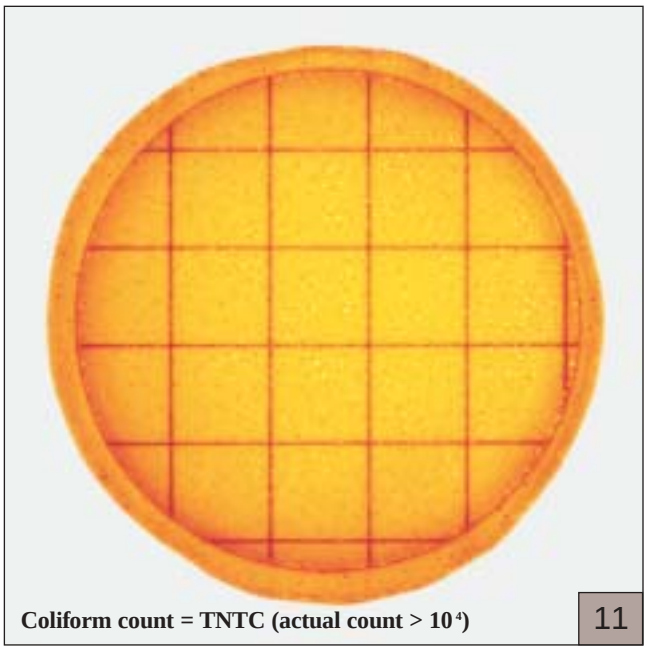


Coliform count = 64 7



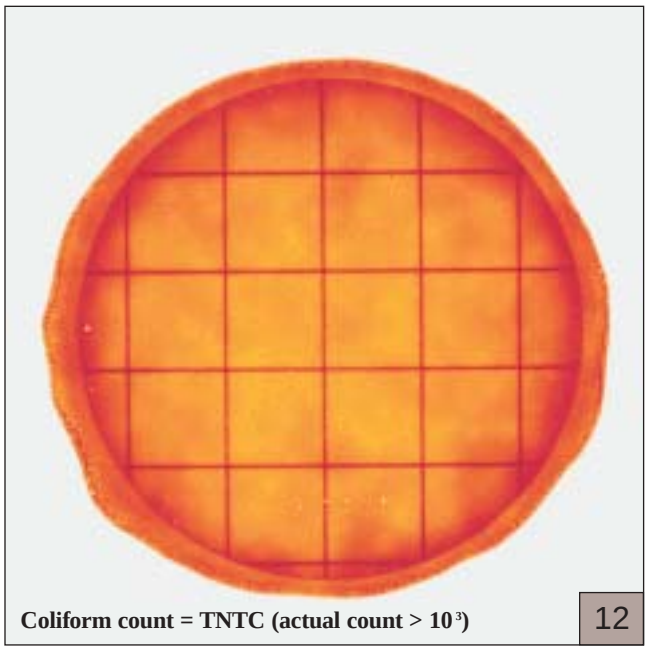
Coliform count = 164 8

TNTC (Too Numerous To Count) 1000 colonies/plate



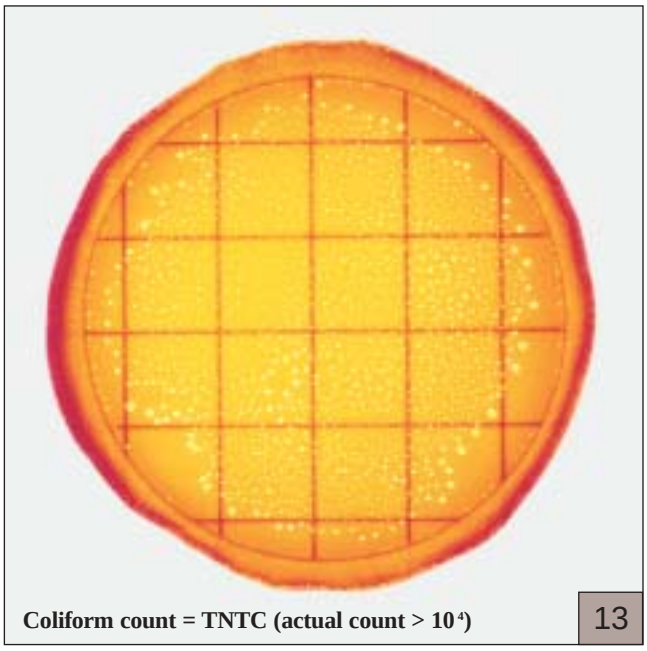
Coliform count = TNTC (actual count > 10^4) 11

Petrifilm RCC plates with colonies that are too numerous to count (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. See figure 11.



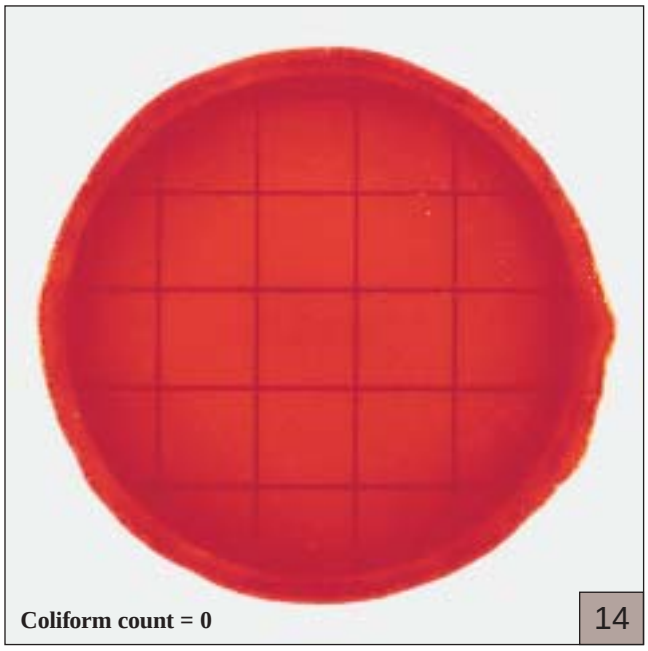
Coliform count = TNTC (actual count > 10^3) 12

The Petrifilm RCC plate in figure 12 has two characteristics indicating TNTC colonies: change in gel color and many small colonies.



Coliform count = TNTC (actual count > 10^4) 13

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 TNTC colonies.



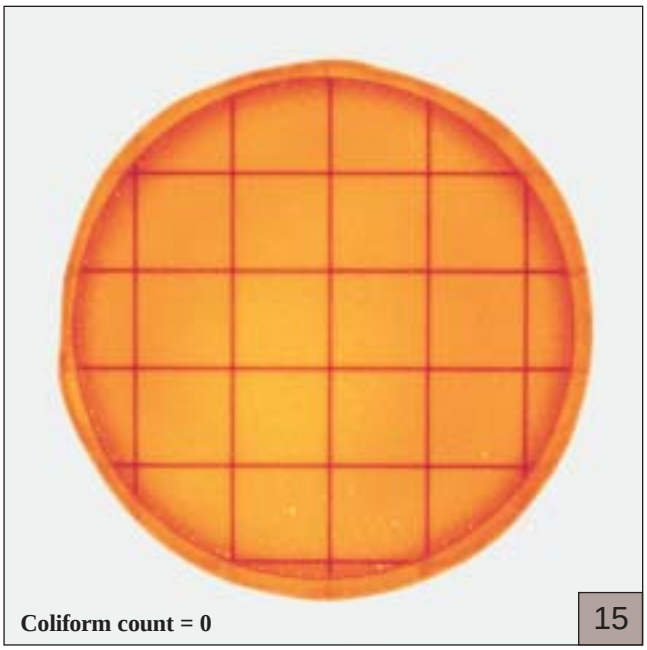
Coliform count = 0 14

Figure 14 shows a Petrifilm RCC 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.

pH

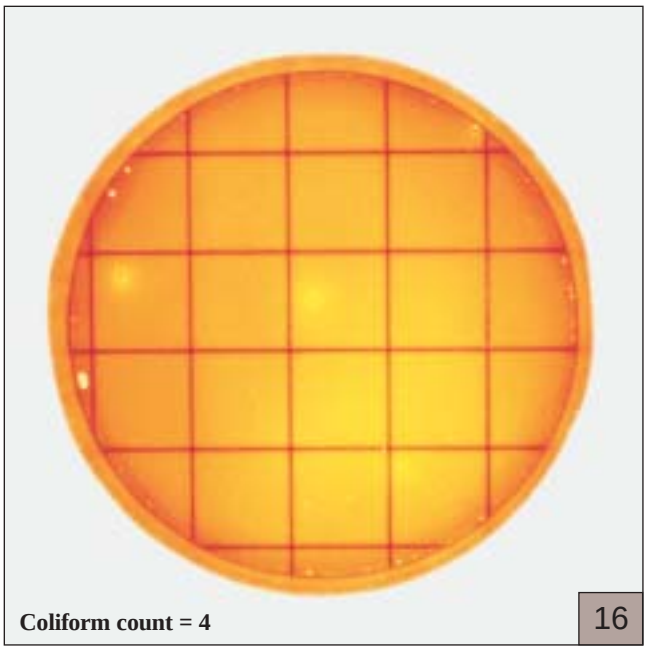
Most bacteria show optimum growth at a pH near 7.0. Dilutions of low pH products require pH adjustments to pH 6.5 - 7.5 before plating on Petrifilm plates.

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 indications of TNTC coliform growth.



Coliform count = 0 15

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.

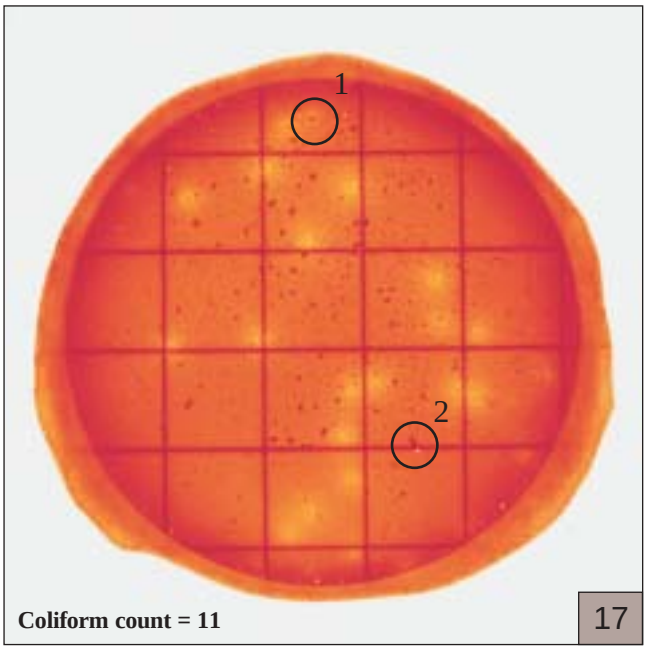


Coliform count = 4 16

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

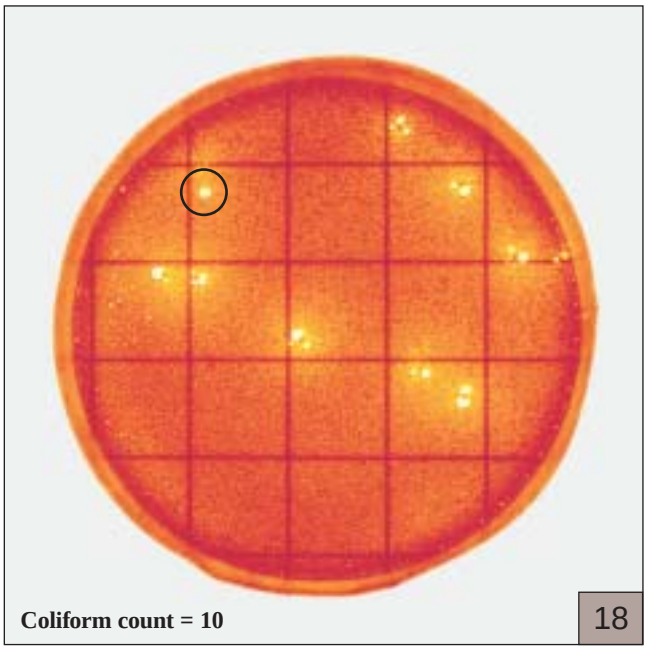
Product

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



Coliform count = 11 17

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.



Coliform count = 10 18

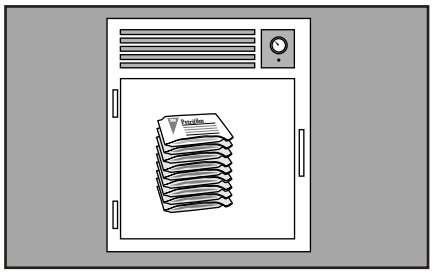
A dilution of chocolate is shown in figure 18. Zones of acid associated with colonies will continue to expand during incubation. Gas bubbles associated with colonies are another criteria that will aid in the identification of coliforms. Gas bubbles may outline the colony as shown in the circle. Enumeration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

3M Petrifilm™ Rapid Coliform Count Plates

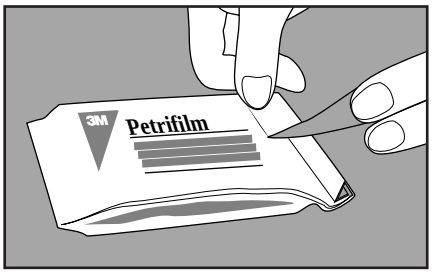
Reminders for Use

For detailed CAUTIONS, LIMITED WARRANTY and LIMITED REMEDIES, STORAGE AND DISPOSAL information, and INSTRUCTIONS FOR USE see Product's package insert.

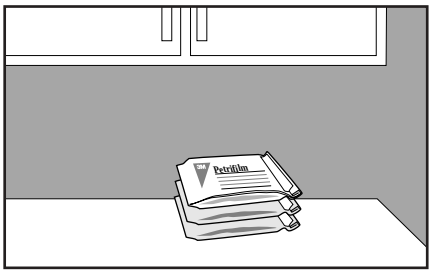
Storage



1 **Store** unopened packages at ≤8°C (≤46°F) . Use before expiration date on package.

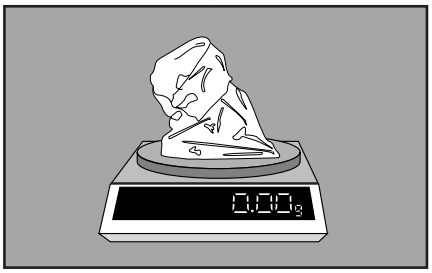


2 To seal opened package, fold end over and tape shut.

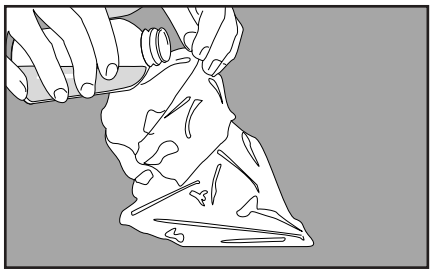


3 Keep resealed package at ≤25°C (≤77°F) and ≤50%RH. **Do not refrigerate opened packages.** Use Petrifilm plates within one month after opening.

Sample Preparation

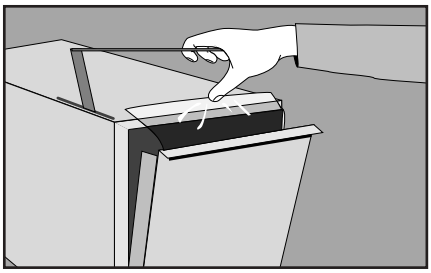


4 High and lowfat milk may be plated directly. For other food and dairy products prepare at least a 1:10 dilution of sample. Weigh or pipette food product into an appropriate container such as a stomacher bag, dilution bottle, Whirl-Pak® bag, or other sterile container.



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), saline solution (0.85 - 0.90%), or distilled water.

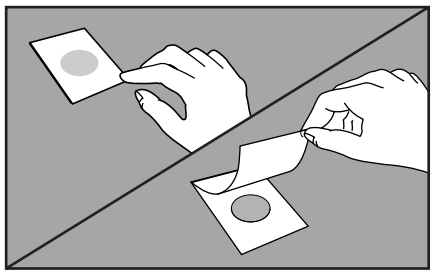
Do not use buffers containing citrate, bisulfite, or thiosulfate; they can inhibit growth.



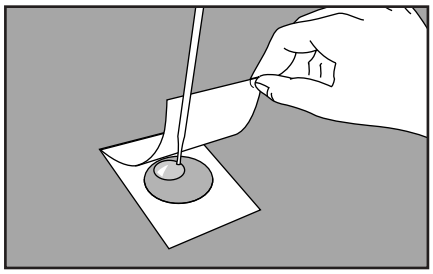
6 Blend or homogenize sample per current procedure.

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.

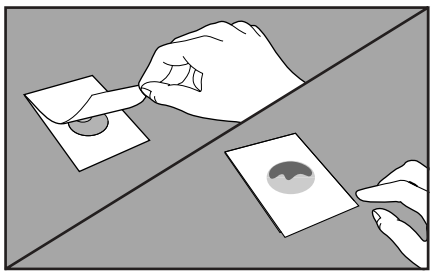
Inoculation



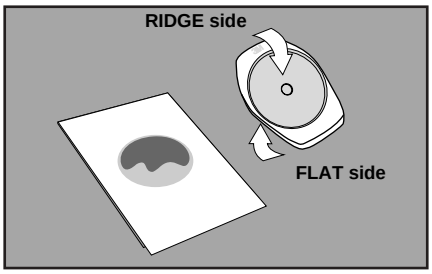
7 Place Petrifilm plate on **level** surface. Lift top film.



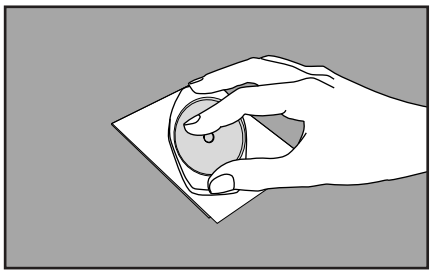
8 With pipette **perpendicular** to Petrifilm plate, place 1 mL of sample onto center of bottom film.



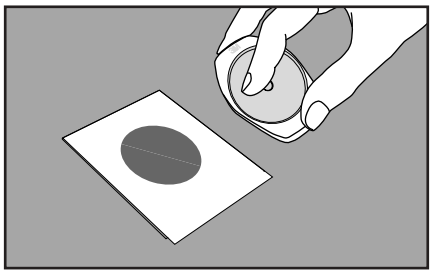
9 Carefully **ROLL** top film down to avoid entrapping air bubbles. Do **NOT** let top film drop.



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

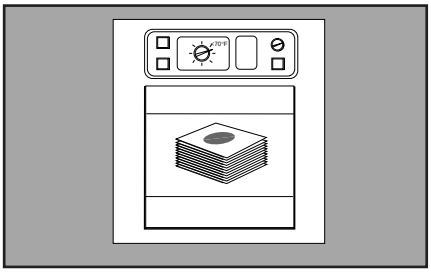


11 **GENTLY** apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.



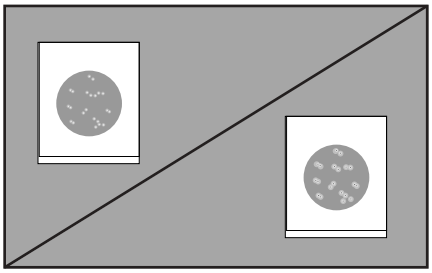
12 Lift spreader. Wait at least one minute for gel to solidify.

Incubation

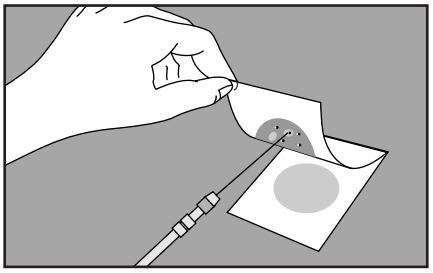


13 Incubate plates with clear side up in stacks of up to 20 at a temperature of 35°±1°C* for up to 24 ± 2 hours, examining plates at timed intervals depending on desired information (refer to package insert).

Interpretation



14 Read Petrifilm plates using indirect light for early detection. Petrifilm plates can be counted on a standard colony counter or other magnified light source. 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.

Incubate plates up to 24 h at 35±1°C.
AOAC Official Method 2000.15

AFNOR Validated Method 3M 01/5-03/97A
14 h result (as compared to VRBL 30°C method)
(Incubate at 30°C for processed pork products.)
AFNOR Validated Method 3M 01/5-03/97B
24 h result (as compared to VRBL 30°C method)
(Incubate at 30°C for processed pork products.)
AFNOR Validated Method 3M 01/5-03/97C
24 h result (as compared to MPN method)

Additional Information

- Questions? U.S., call **1-800-328-6553**.
- To order, call **1-800-328-1671.**
- Canada, call **1-800-563-2821** for technical service.
- Latin America / Africa and Asia Pacific regions, call **1-651-733-7562.**
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