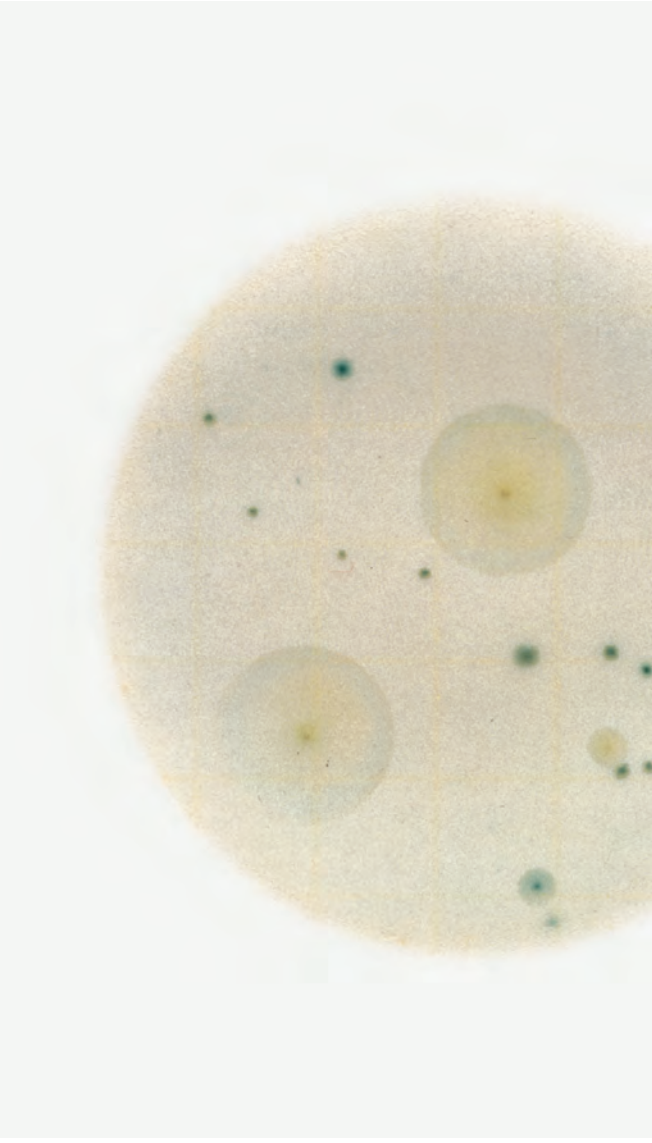
NEOGEN®

Petrifilm®

Interpretation Guide

The Neogen® Petrifilm® Yeast and Mold Count Plate is a sample-ready culture medium system which contains nutrients supplemented with antibiotics, a cold-water-soluble gelling agent, and an indicator that facilitates yeast and mold enumeration. Petrifilm Yeast and Mold Plates are used for the enumeration of yeast and mold in the food and beverage industries.



YM

Yeast and Mold Count Plate

Food and Beverage Applications

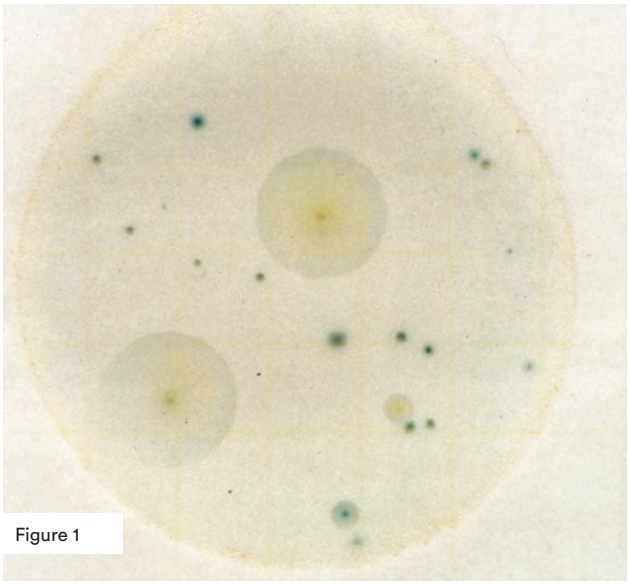


Figure 1

Total count = 20
Yeast count = 16
Mold count = 4

Petrifilm Yeast and Mold Count Plate contains both yeast colonies and mold colonies.

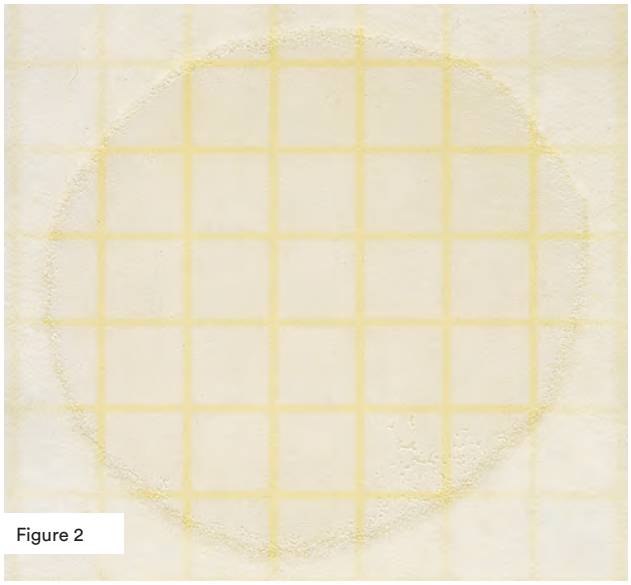


Figure 2

Yeast and mold count = 0

Petrifilm Yeast and Mold Count Plate without yeast or molds. Gridlines are visible with the use of a backlight to assist with estimated enumeration.

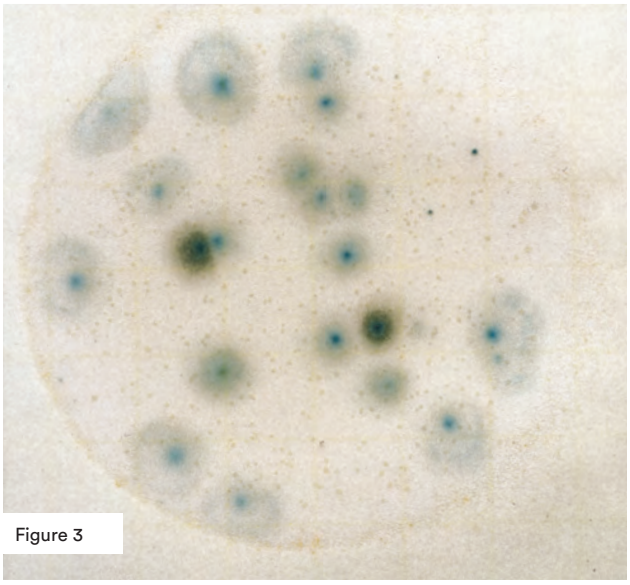


Figure 3

Estimated total count = 500
Estimated yeast count = 480
Estimated mold count = 21

When colonies number more than 150, estimate the count. Gridlines are visible with the use of a backlight to assist with estimated enumeration. Determine the average number of colonies in one square (1 cm^2) and multiply it by 30 to obtain the total count per plate. The inoculated area is approximately 30 cm^2. Yeast colonies may range in color from tan (as in this example) to pink to blue-green.

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

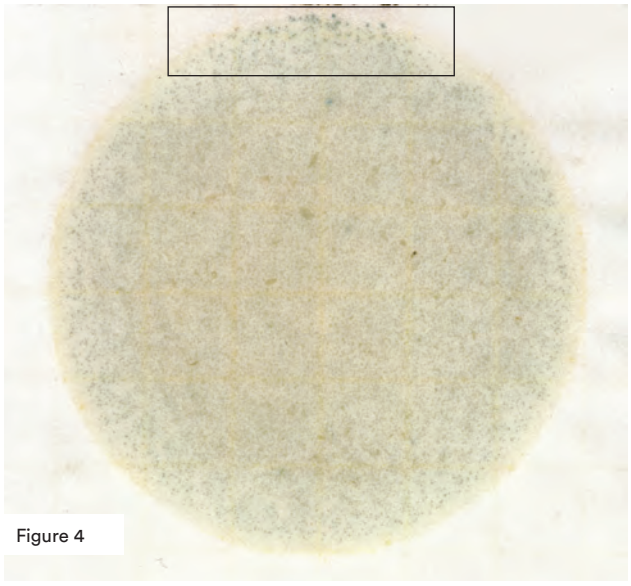


Figure 4

Estimated yeast count = TNTC

Petrifilm Yeast and Mold Count Plate containing yeast colonies too numerous to count (TNTC). The small, blue colonies at the edge of the plate (highlighted in the box) are present throughout the entire plate although less visible.

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

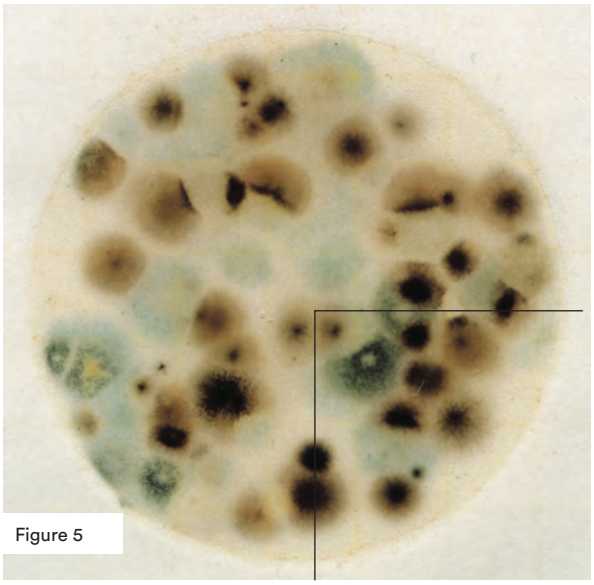


Figure 5

Estimated mold count = 64

Mold colonies are beginning to crowd and overlap each other on the plate. Count each colony margin or focus. The plate can be divided into sections to assist in counting. In this example, approximately 1/4 of the plate was counted, then the number of colonies counted was multiplied by 4 to get the estimated count on the plate. The section shown has 16 molds.

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

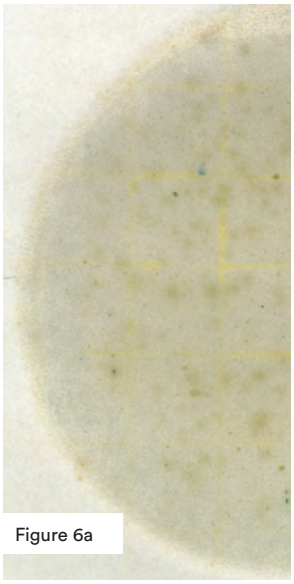


Figure 6a

Mold count = TNTC

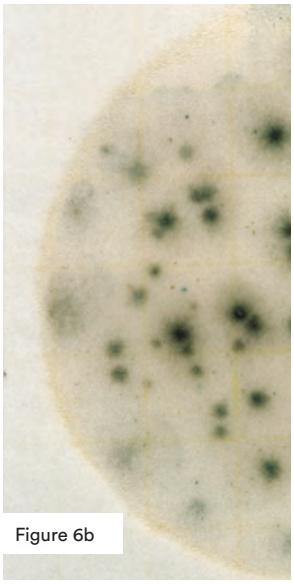


Figure 6b

Mold count = 64

Plates in Figures 6a and 6b are the same sample. Figure 6a is a 1:10 dilution and has colonies that are small, faint and numerous, making it difficult to count. Figure 6b is a 1:100 dilution and shows how diluting a sample to obtain a colony count of less than 150 colonies makes counting easier. As with most growth media, in a highly competitive environment (such as Figure 6a), typical colony growth will be inhibited. For heavily contaminated samples such as these, further dilutions are recommended for a more accurate count and more typical colony growth (as in Figure 6b).

Phosphatase Reaction

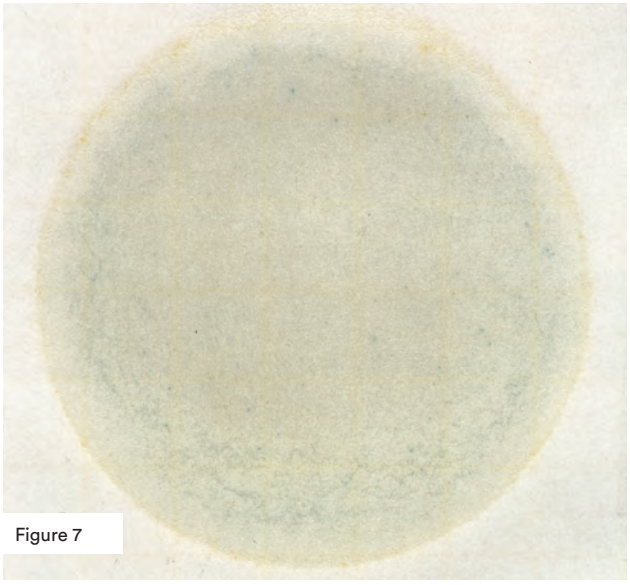


Figure 7

Yeast and mold count = 0

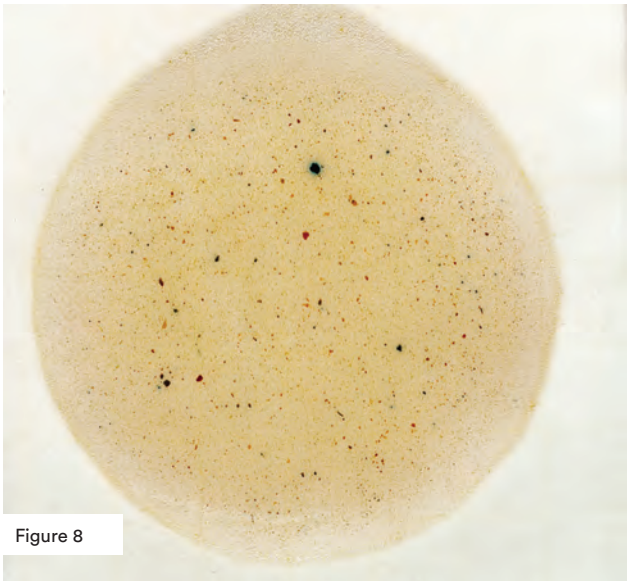


Figure 8

Yeast and mold count = 0

The Petrifilm Yeast and Mold Count Plates utilize a phosphatase indicator dye. All living cells contain phosphatase; therefore, natural phosphatase in samples can cause the indicator to react. Two types of color reactions are sometimes seen: a uniform blue background color or intense, blue spots. Figure 7 shows uniform blue background color and Figure 8 shows intense blue spots which are often seen with spices or granulated products. Figure 8 also shows food particles that yielded phosphatase.

To reduce a phosphatase reaction, follow one or more of these techniques:

1. Dilute Sample: Further sample dilution will minimize blue background color or reduce the number of intense blue spots.
2. Sample Preparation: Mix sample and let settle before plating. Draw sample from center portion of sample container or use filtered homogenizer bag to avoid plating large particles.
3. Check and Note: Observe plates within 24–48 hours of incubation and make note of any color change to aid in final interpretation.

Bottled Water Applications

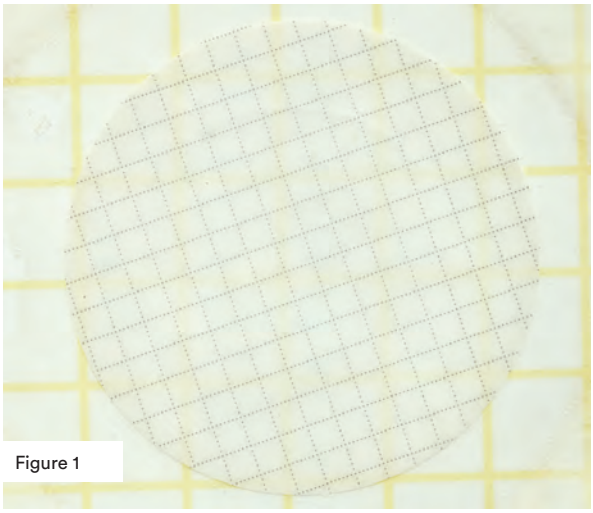


Figure 1

Yeast and mold count: 0

Petrifilm Yeast and Mold Count Plate with no colonies.

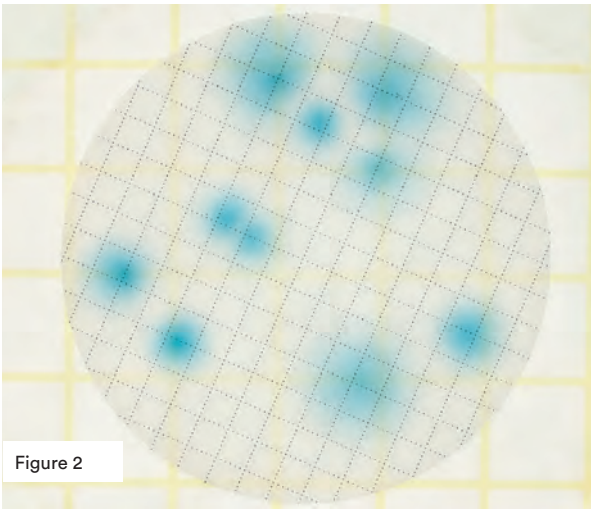


Figure 2

Yeast and mold count: 10

Mold colonies are large with a dark center and diffuse edge.

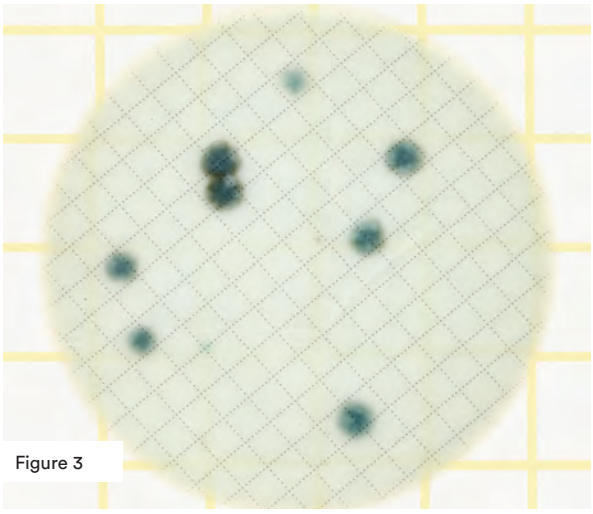


Figure 3

Yeast and mold count: 10

Note two small, faint colonies.

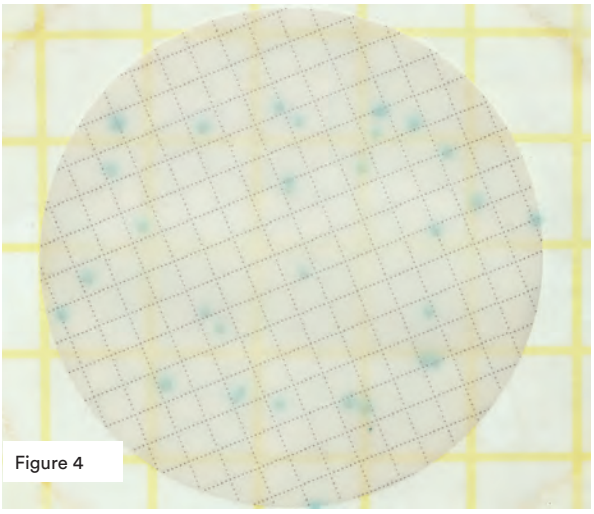


Figure 4

Yeast and mold count: 31

Count colonies partially or totally off of the filter.

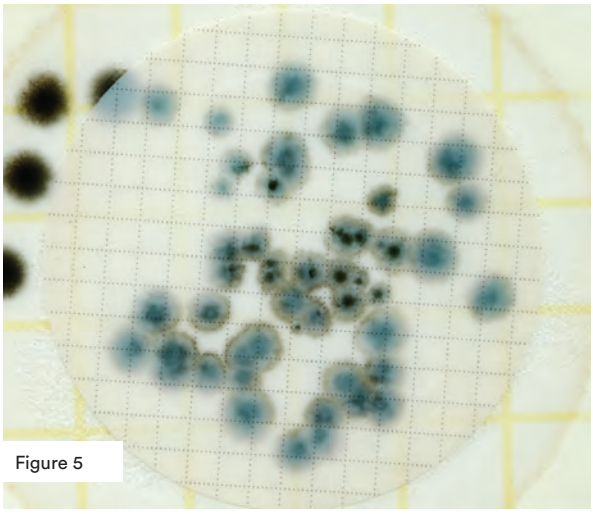


Figure 5

Yeast and mold count: 51

Estimate colony count when molds merge; darker centers can help enumerate colonies. Count colonies partially or totally off of the filter.

For All Applications

Macroscopic Differentiation

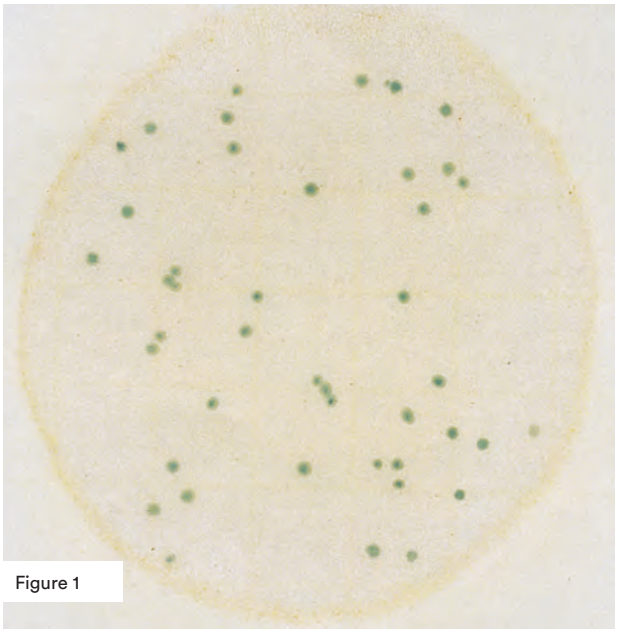


Figure 1

Yeast count = 43

Figure 1 shows typical yeast colonies. Characteristics typical of yeast include:

- Colony is small
- Colony has defined edges
- Colony color can range from pink-tan to blue-green
- Colony may appear raised
- Colony typically is uniform in color, no center focus (dark center)

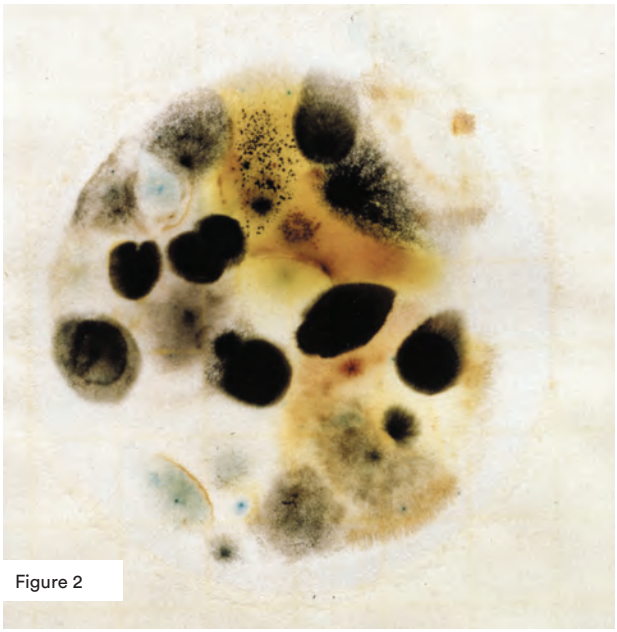


Figure 2

Mold count = 29

Figure 2 shows typical mold colonies. Characteristics typical of mold include:

- Colony grows large
- Colony has diffuse edges
- Colony color may vary as molds produce a variety of pigments (i.e., brown, beige, orange, blue-green)
- Colony appears flat
- Colony usually has a center focus (i.e., usually darker in color, may also be different color)

Microscopic Differentiation

Yeasts and molds are closely related and cannot always be distinguished from each other without microscopic examination.

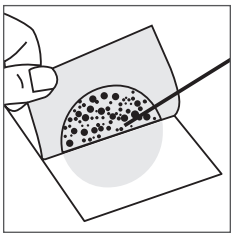


Figure 3

To isolate colonies for further identification, lift the top film and pick from the colony within the gel using a loop or similar device.

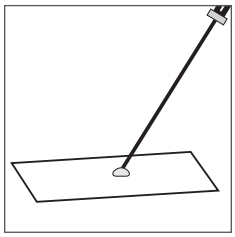


Figure 4

Transfer the colony to a drop of sterile water on a microscope slide, cover with a coverslip, and view under a microscope.

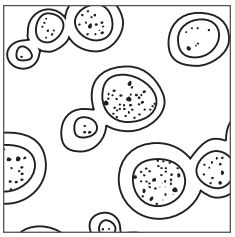


Figure 5

Yeast typically appear oval and may show budding.

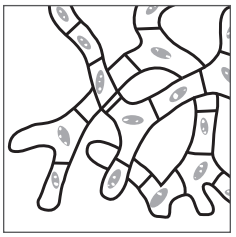


Figure 6

Mold typically appear as branching or thread-like filaments (mycelium).

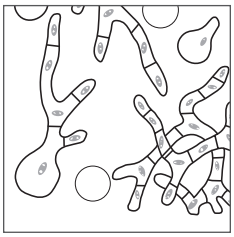


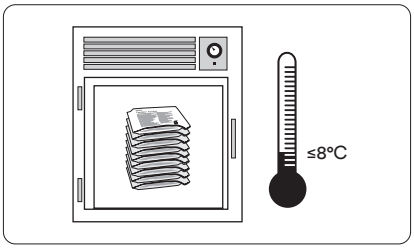
Figure 7

Molds shown above are in various stages of germination.

Reminders For Use

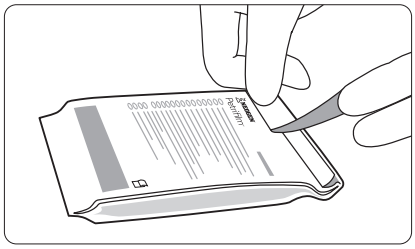
Food and Beverage Applications

Storage



01

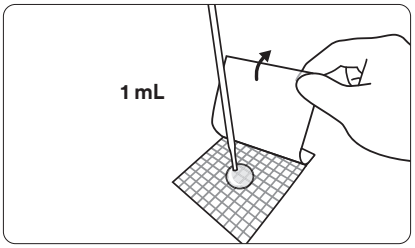
Store the unopened Petrifilm Yeast and Mold Count Plate pouches at refrigerated or frozen at temperatures ≤8°C (≤46°F). Use before expiration date on package. Just prior to use, allow unopened pouches to come to room temperature before opening.



02

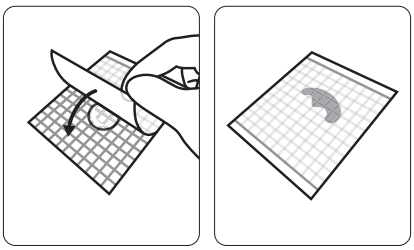
Seal by folding the end of the pouch over and applying adhesive tape. **To prevent exposure to moisture, do not refrigerate opened pouches.** Store resealed pouches in a cool, dry place for no longer than one month.

Inoculation



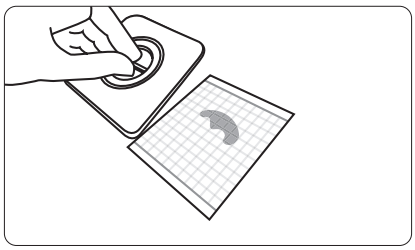
03

Place the Petrifilm Yeast and Mold Count Plate on a flat **level** surface. Lift the top film and with a pipette **perpendicular** to the inoculation area, dispense 1 mL of sample suspension onto the center of the bottom film.



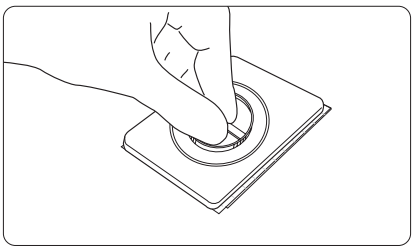
04

Drop the top film down onto the sample.



05

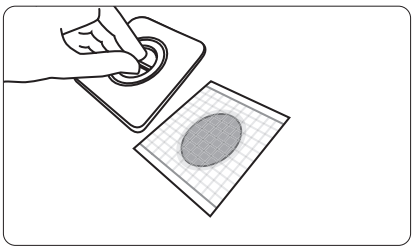
Place the Petrifilm Yeast and Mold Spreader on the center of the plate.



06

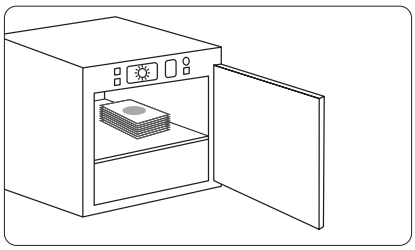
Gently apply pressure on the spreader to distribute the inoculum over circular area. **Do not** twist or slide the spreader.

Incubation



07

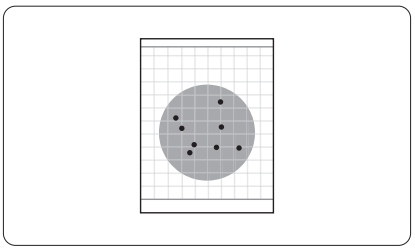
Lift the spreader and leave the plate undisturbed for at least one minute to permit the gel to form.



08

Incubate plates with clear side up in stacks of up to 20. **Please refer to product instructions for third party validated methods.** Because some molds may grow quickly, it may be useful to read and count plates at 3 days as smaller colonies may be obscured by larger, overgrown molds at 5 days. If this happens, the 3 day count may be used; however, it should be reported as an estimated count.

Interpretation



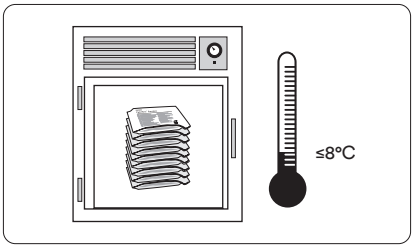
09

Petrifilm Yeast and Mold Count Plates can be counted using a standard colony counter or other illuminated magnifier.

Reminders For Use

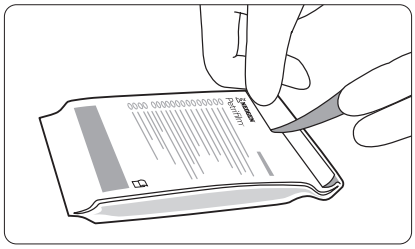
Bottled Water Applications

Storage



01

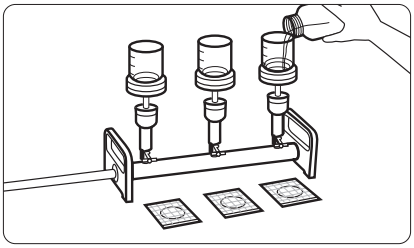
Store unopened pouches at ≤8°C (≤46°F). Use before expiration date on package. Just prior to use, allow unopened pouches to come to room temperature before opening.



02

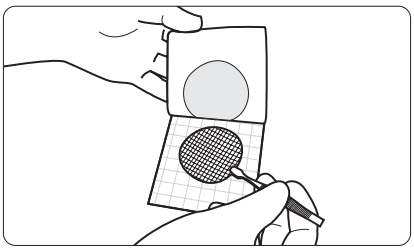
To seal opened pouches, fold end over and apply adhesive tape. **Do not refrigerate opened pouches.** Use Petrifilm Yeast and Mold Count Plates within one month after opening.

Hydration Procedure



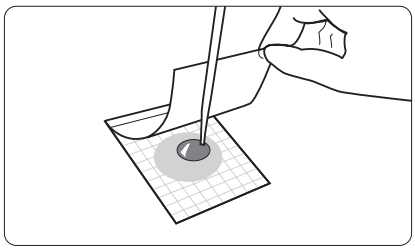
03

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



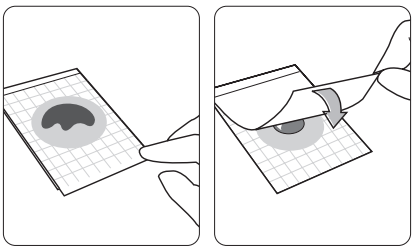
04

Place filter in the center of the plate.



05

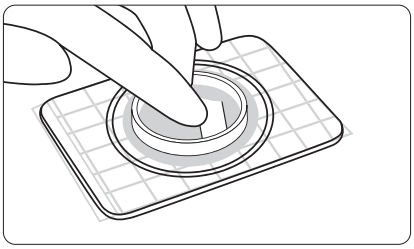
With the pipette **perpendicular** to the Petrifilm Yeast and Mold Count Plate, place 1 mL of hydration diluent onto the center of the filter. Appropriate sterile diluents include distilled water, deionized (DI) water and reverse osmosis (RO) water.



06

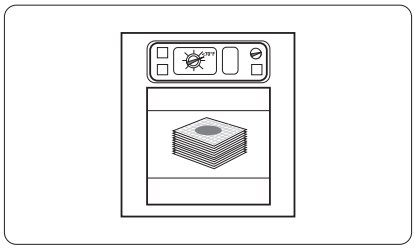
Carefully **roll** top film down onto the filter.

Incubation



07

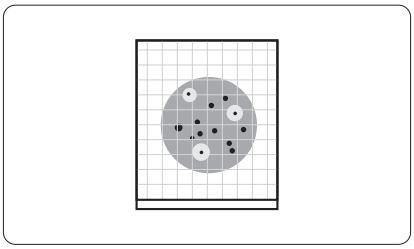
Lightly apply pressure to the Petrifilm Yeast and Mold Spreader to ensure uniform contact of the filter with the gel and to eliminate any air bubbles.



08

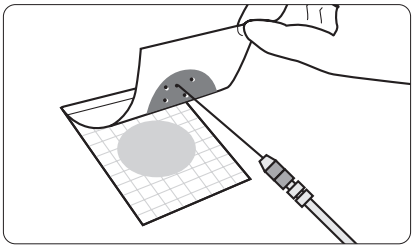
Incubate Petrifilm Yeast and Mold Count Plates in a horizontal position, clear side up, in stacks on no more than 20 plates at 20–25°C for 3–5 days.

Interpretation



09

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



10

Colonies may be isolated for further identification. Lift top film and pick the colony from the gel.

Use Appropriate Sterile Diluents

Butterfield's phosphate buffer, 0.1% peptone water, peptone salt diluent, saline solution (0.85–0.90%), Wide-Spectrum Neutralizer, bisulphite-free letheen broth or distilled water.

Do not use diluents containing citrate, bisulphite or thiosulfate with Petrifilm Yeast and Mold 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 NEOGEN LIABILITY, STORAGE AND DISPOSAL information and INSTRUCTIONS FOR USE, see product instructions.

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