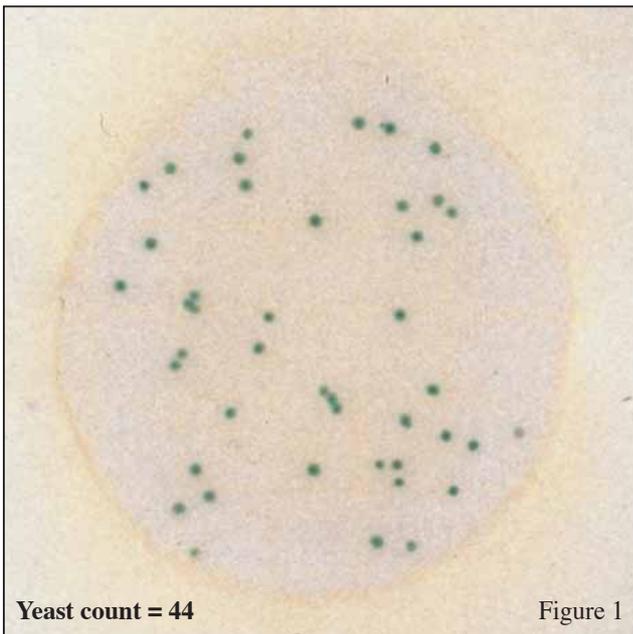
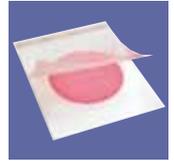




# Petrifilm™

## Yeast and Mould Count Plate



Yeast count = 44

Figure 1

It is easy to count yeast and mould colonies on Petrifilm Yeast and Mould count plates. An indicator dye stains yeast and mould colonies to provide contrast and facilitate counting.

To differentiate yeast and mould colonies on Petrifilm Yeast and Mould count plates, look for one or more of the following typical characteristics:

### YEAST

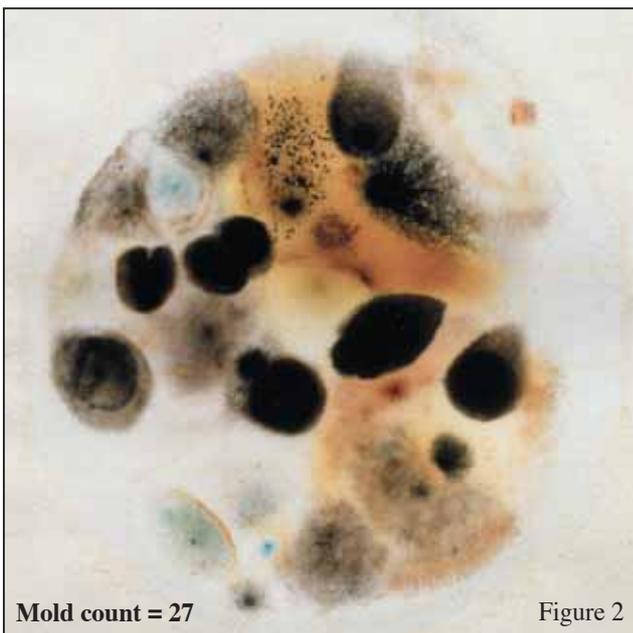
- **Small** colonies
- Colony has **defined edges**
- **Pink-tan to blue-green** in colour
- Colony may appear raised ("3D")
- Usually no focus (dark centre) in middle of colony

### MOULD

- **Large** colonies
- Colony has **diffuse edges**
- Variable colour (moulds may produce their own pigments)
- Colonies appear flat
- Usually a **focus** in centre of colony

The colonies in figure 1 are characteristic examples of yeasts: **small, blue-green** colonies, with defined edges, and no foci. (**Yeast count = 44**)

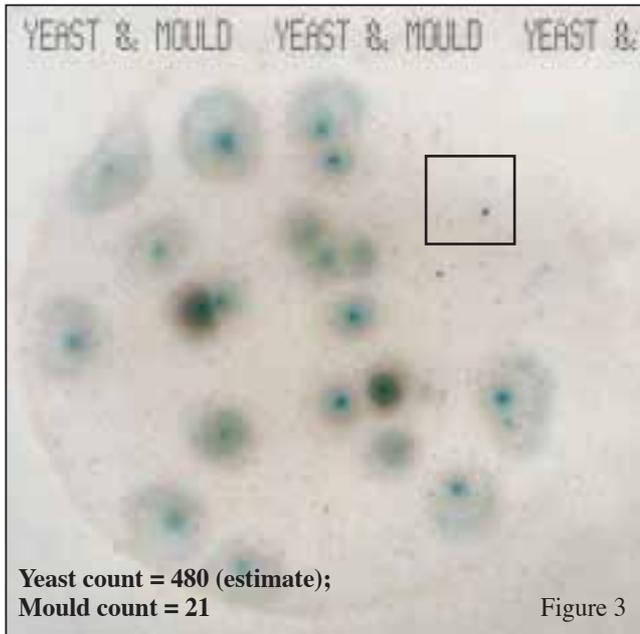
The colonies in figure 2 are characteristic examples of moulds: **large, variable coloured** colonies, with **diffuse edges**, and centre **foci**. (**Mould count = 27**)



Mold count = 27

Figure 2

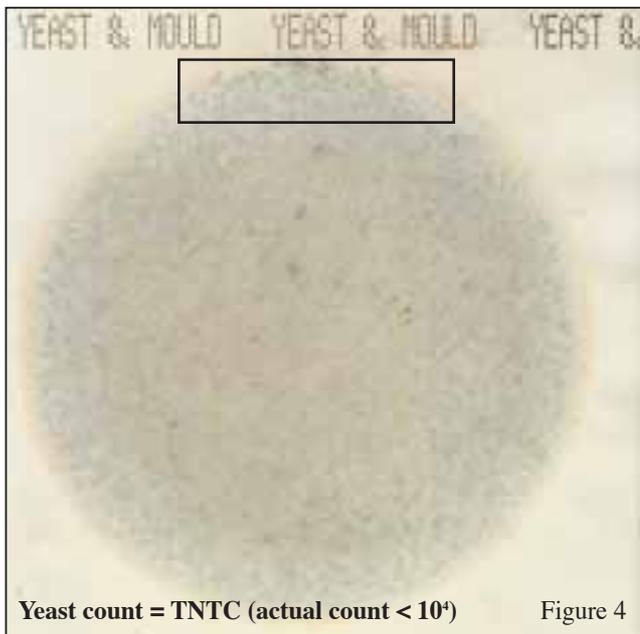
# Yeast



The 3M™ Petrifilm™ Yeast and Mould Count Plate in figure 3 contains an easily countable number of mould colonies, (large, green colonies with diffuse edges, and centre foci) and a high number of yeast colonies. The yeast colonies are **small, tan colonies with defined edges, and no foci**. When colonies number more than 150, estimate the count. (**Yeast count = 480 (estimate); Mould count = 21**)

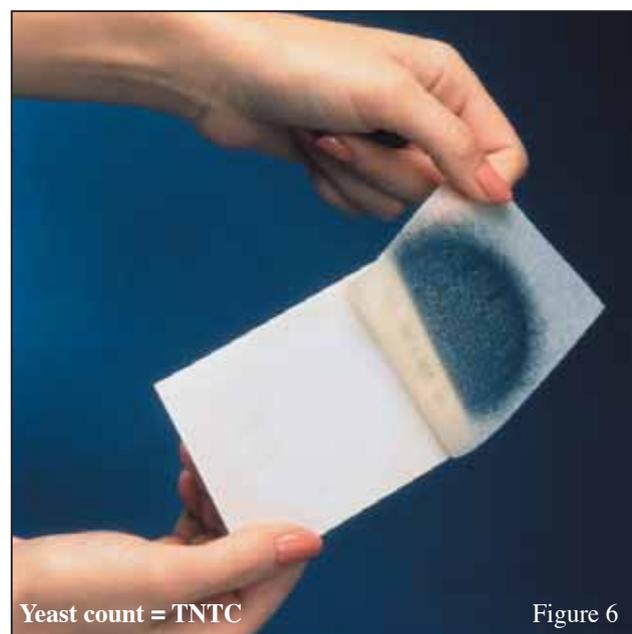
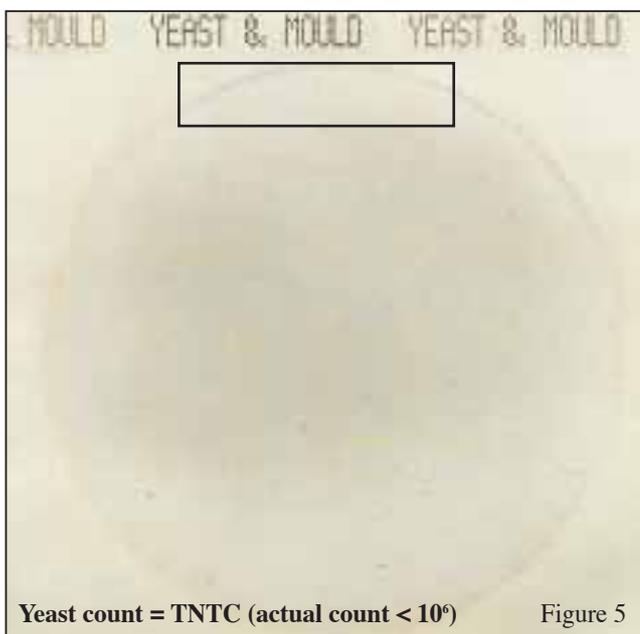
Determine the average number of colonies in one square (1 cm<sup>2</sup>) and multiply it by 30 to obtain the total count per plate. The inoculated area of a Petrifilm Yeast and Mould count plate is approximately 30 cm<sup>2</sup>.

The Petrifilm Yeast and Mould count plate in figure 4 contains a high number of yeast colonies - too numerous to count (TNTC). The **small, blue colonies outlined at the edge of the plate** differentiate the plate from a TNTC mould count. (**Yeast count = TNTC actual count > 10<sup>6</sup>**)

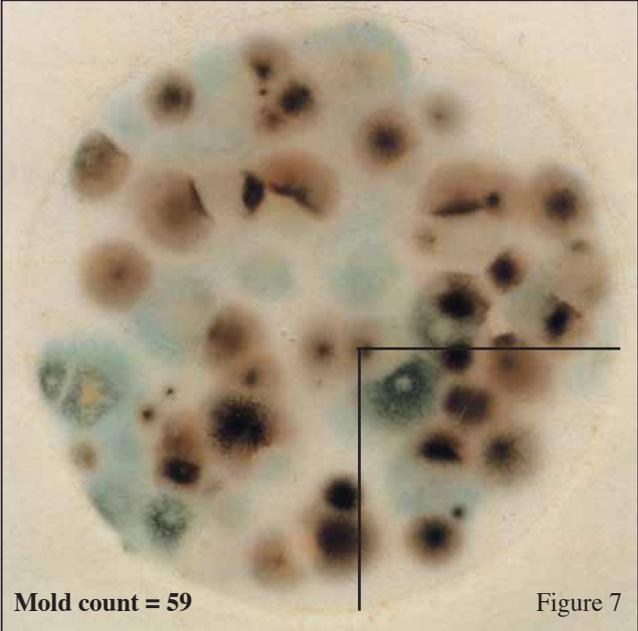


Sometimes Petrifilm Yeast and Mould count plates with high numbers of yeast colonies may appear to have blue growth only around the edges (figure 5). This is also recorded as a yeast count of TNTC. (**Yeast count = TNTC actual count > 10<sup>6</sup>**)

If Petrifilm Yeast and Mould count plates appear to have no growth, lift the top film and examine the gel that adheres to the top film (figure 6). If numerous yeast are present, you will see white colonies in the gel. This is recorded as a yeast count of TNTC. (**Yeast count = TNTC**)



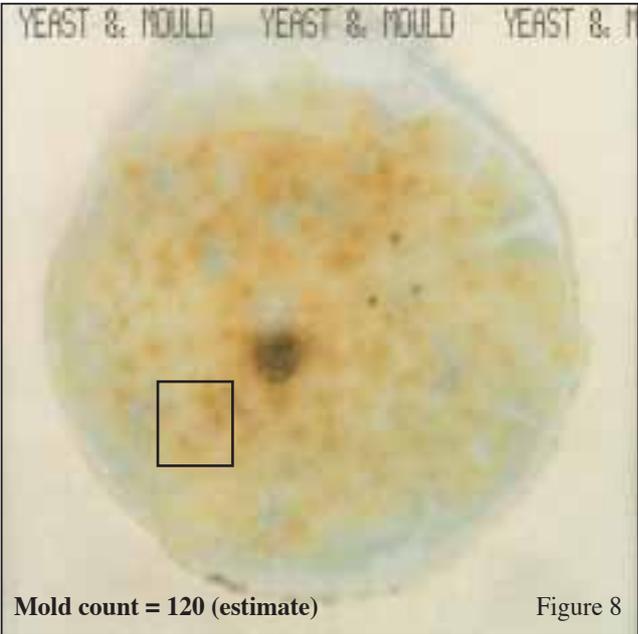
# Moulds



The mould colonies on the 3M™ Petrifilm™ Yeast and Mould Count Plate in figure 7 are **variably pigmented** colonies, with **diffuse edges**, and centre **foci**. They are **large**, and beginning to crowd, sporulate, and overlap each other on the plate. For ease in counting, divide the plate into sections and look for foci to help distinguish individual colonies. **(Mould count = 59)** The section shown has 15 moulds.

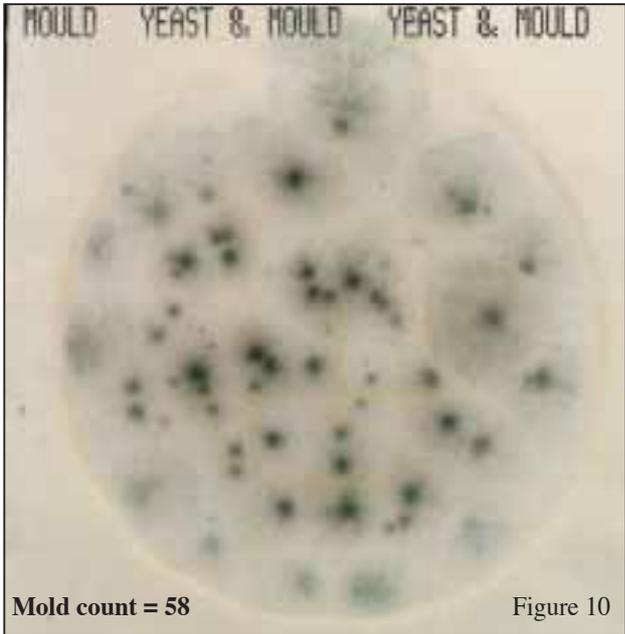
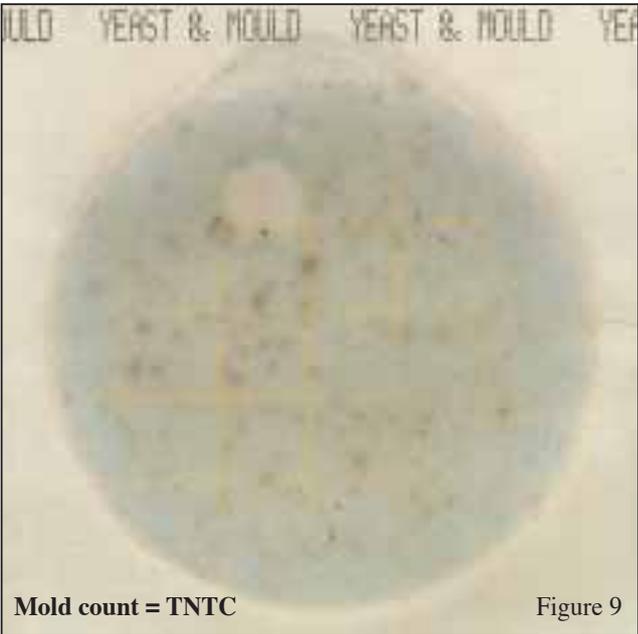
Note the **variable pigmentation**, and **fuzzy edges** of the plate in figure 8, caused by the high numbers of mould colonies and sporulation that has taken place. Estimate the count by counting the foci. There are 4 colonies in the square shown. **(Mould count = 120 estimate)**

As with all plate count methods, crowded plates may show atypical colony characteristics. Proper dilution is important to ensure an accurate count.



The Petrifilm Yeast and Mould count plates in figures 9 and 10 are 1 : 10 and 1 : 100 dilutions respectively, of the same product. The colonies in the figure 9 are small, faint and numerous making the count difficult to estimate. An artifact bubble is present. **(Mould count = TNTC)**

Dilution of the product to obtain a colony count within the desired counting range (15-150 colonies), makes counting easy. The moulds in figure 10 are **large**, with **diffuse edges** and centre **foci**. **(Mould count = 58)**. The over-crowding on the plate in figure 9 prevented their typical growth.



# Phosphatase Reaction

All living cells contain the enzyme phosphatase. In the presence of phosphatase, the indicator in the 3M™ Petrifilm™ Yeast and Mould Count Plates is activated and stains the yeast and mould colonies a blue colour.

Some raw and processed food products that contain living cells (and therefore, phosphatase) may also cause this blue colour reaction to occur. Two types of colour reaction from products are sometimes seen: a uniform blue background colour, or intense, pinpoint blue spots (often seen with spices or granulated products).

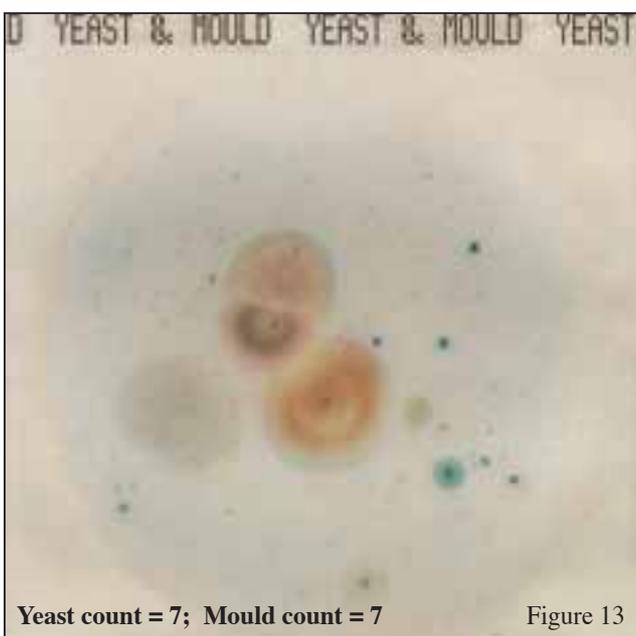
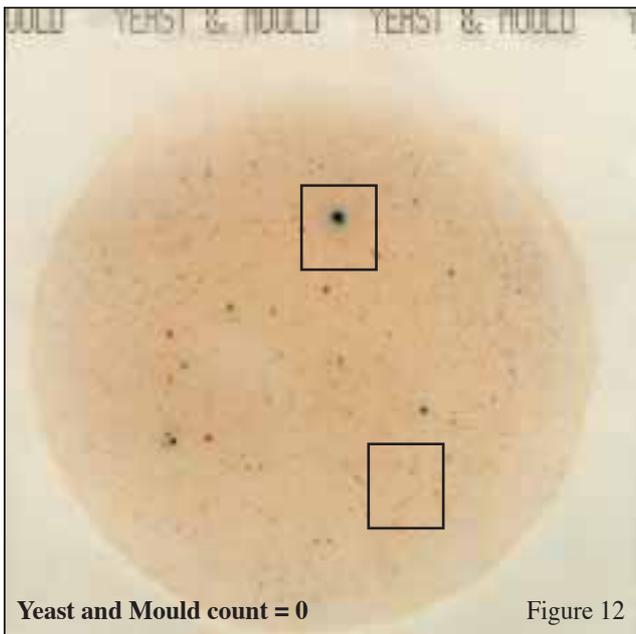
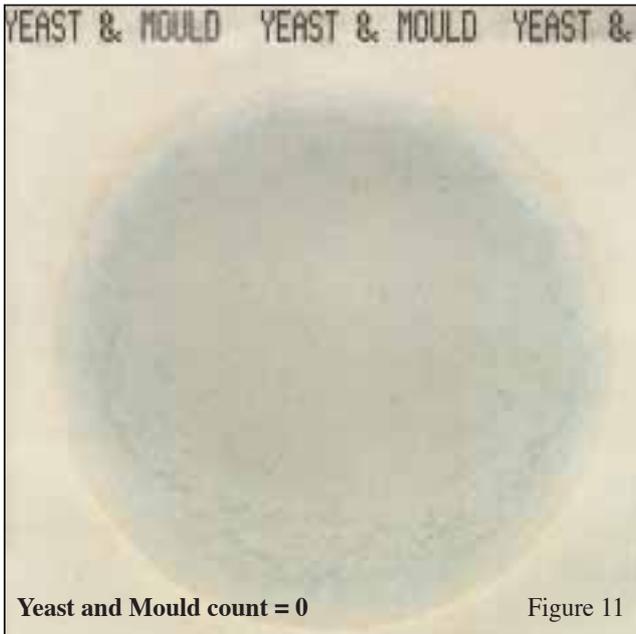
A colour reaction caused by natural phosphatase in a product can be distinguished from yeast and mould colonies by one or more of the following techniques:

- 1. Dilution:** When possible, further dilution will eliminate blue background colour, or reduce the number of pinpoint blue spots.
- 2. Late Supernate:** Mix sample and let settle 3-5 minutes to eliminate large product particles that can often cause the pinpoint colour reactions.
- 3. Incubation Temperature:** Incubate plates at the proper temperature 20-25°C. Enzyme (phosphatase) reactions occur faster as temperatures increase.
- 4. Check & Note:** Check Petrifilm Yeast and Mould count plates after 24-48 hours of incubation. Product colour change can occur within 24-48 hours. Make note of any colour seen, to aid in final interpretation.

The Petrifilm Yeast and Mould count plate in figure 11, is an example of a plate with uniform background colour caused by the "natural phosphatase" present in the sample tested. The "grainy" appearance is due to particles of product in the dilution plated. To help distinguish from the TNTC yeast or mould count, note the edges of the plate. (**Yeast and Mould count = 0**)

Figure 12 is an example of intense, pinpoint blue spot reactions seen occurring from the "natural phosphatase" in some food products. Note their SHAPE - tiny, pinpoints or irregularly shaped, and COLOUR - deep blue, that often look faint, or smeared around the edges of some of the larger particles. (**Yeast and Mould count = 0**)

Another example of intense blue pinpoint colour reactions is shown in figure 13. The pinpoint dots are very bright, tiny, and irregularly shaped. The yeast colonies are **small, blue-green** colonies with **defined edges**. The mould colonies are **large, variably pigmented** colonies with diffuse edges and centre foci. (**Yeast count = 7; Mould count = 7**)



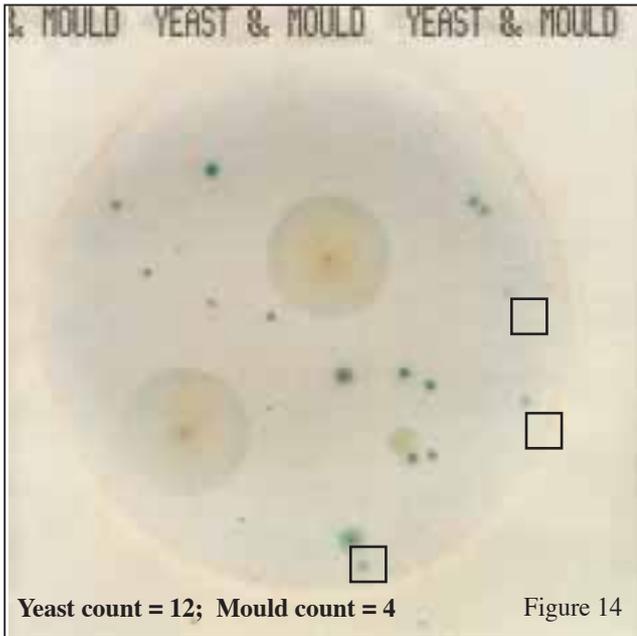


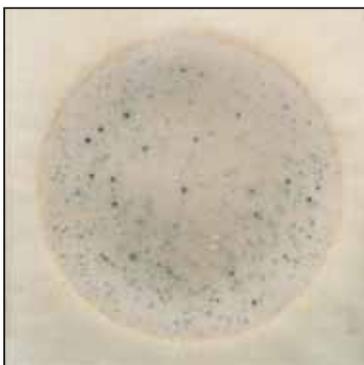
Figure 14 is the same product as shown in figure 13, plated after allowing the product particulates to settle 3-5 minutes before plating. There are still a few pinpoint spots (in the squares above) caused by product particles, but most product interference was eliminated. (**Yeast count = 12**  
**Mould count = 4**)

## Time & Temperature

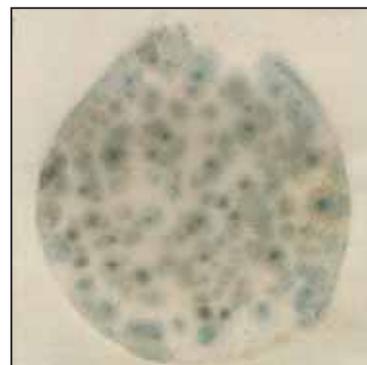
Proper incubation **TIME** and **TEMPERATURE** are important to ensure growth of the types of yeast and mould that can cause spoilage. These yeast and moulds are generally slow growing, and sensitive to high temperatures, regardless of the method used.

To ensure optimum growth, incubate 3M™ Petrifilm™ Yeast and Mould Count Plates at **20°C-25°C** (room temperature), and check plates for growth at both **3 and 5 days**. Since mould colonies grow between the films, inspecting Petrifilm plates will not dislodge spores and cause additional colonies.

Incubating yeast and mould plates at a higher temperature may not mean a faster result - it may mean an **inaccurate** result as illustrated in the Petrifilm Yeast and Mould count plates in figures 15 and 16. They are duplicate plates of the same product and dilution, but were incubated for different times at different temperatures.



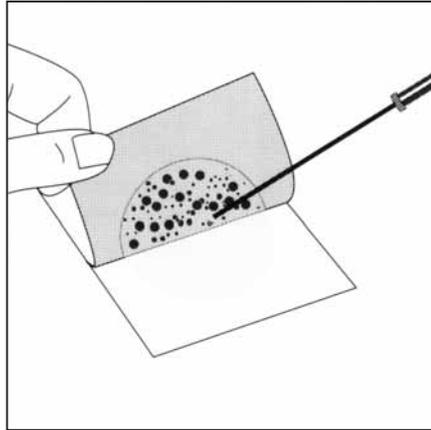
**Yeast count = TNTC**  
Incubated 3 days at 35°C



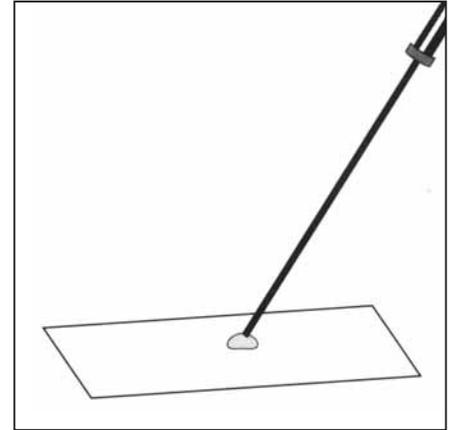
**Yeast count = TNTC (actual count >10<sup>7</sup>)**  
**MOULD count = 120 (estimate)**  
Incubated 5 days at room temperature.

# Microscopic Differentiation

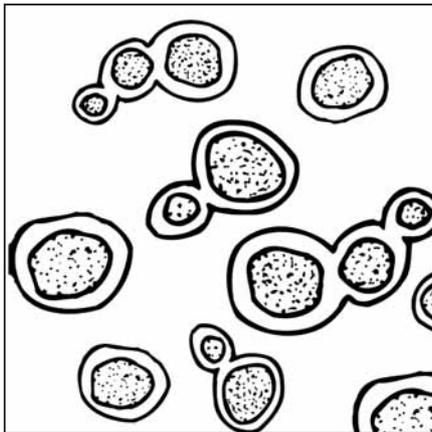
Yeasts and moulds are very diverse organisms, and cannot always be distinguished from each other macroscopically. As with any method, positive differentiation can be made with microscopic examination.



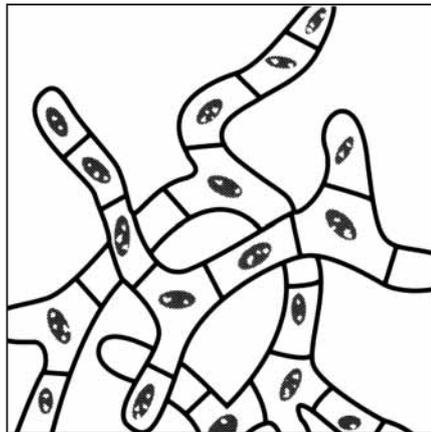
To isolate colonies for further identification, lift the top film and pick the colony from the gel.



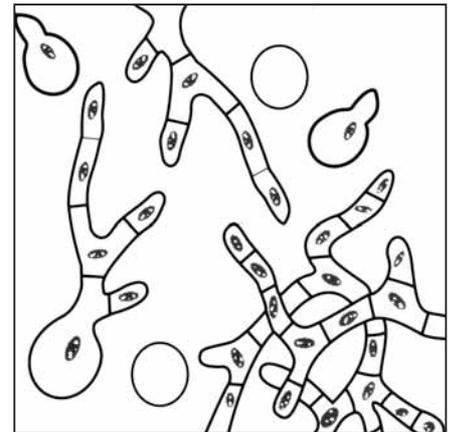
Transfer the colony to a drop of sterile water on a microscope slide, cover with a coverslip, and view under oil immersion power.



Look for oval shaped, budding  
**YEAST**



branching, thread-like filaments  
(mycelium) – **MOULD**

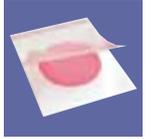


or **MOULD** in various stages of  
germination.

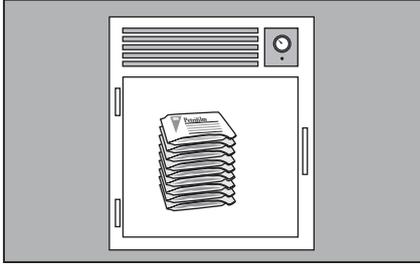
# 3M™ Petrifilm™ Yeast & Mould Count Plates

For detailed warnings, cautions, disclaimer of warranties / limited remedy, limitation of 3M liability, storage and disposal information and instructions for use, see product's package insert.

Reminders  
for use



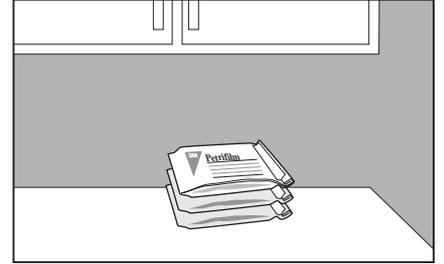
## Storage



1 Refrigerate unopened packages. Use before expiration date on package.



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

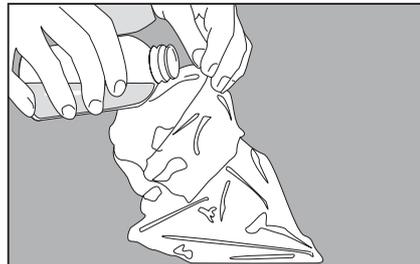


3 Keep resealed package at  $\leq 21^{\circ}\text{C}$  ( $\leq 70^{\circ}\text{F}$ ),  $\leq 50\% \text{RH}$ . Do not refrigerate opened packages. Use Petrifilm count plates within one month after opening.

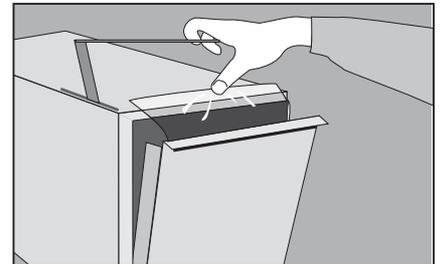
## Sample Preparation



4 Prepare a 1:10 or greater dilution of food product.\* Weigh or pipette food product into Whirl-Pak® bag, stomacher bag, dilution bottle or other appropriate sterile container.



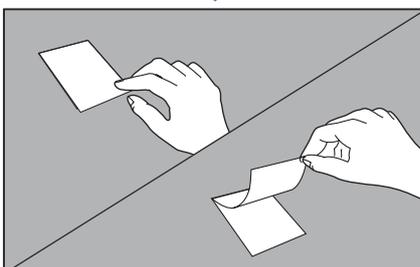
5 Add appropriate quantity of diluent. These include Standard Methods phosphate buffer, 0.1% peptone water, distilled water, phosphate buffered saline, and Butterfield's buffer. Do not use buffers containing sodium citrate or thiosulfate.



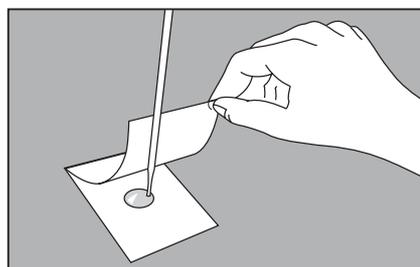
6 Blend or homogenize sample per current procedure.

\* If greater sensitivity is required with dairy or juice products please refer to Petrifilm Dairy & Juice Products sheet.

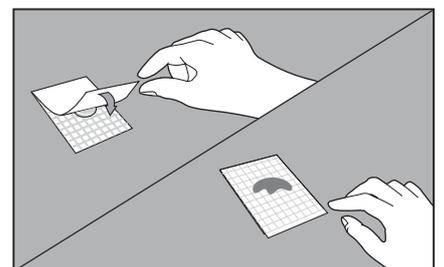
## Inoculation



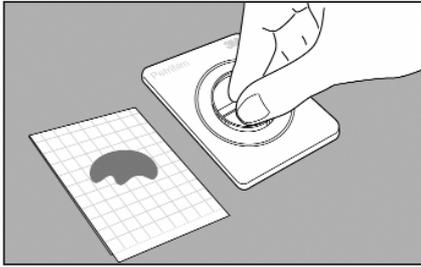
7 Place Petrifilm count plate on flat surface. Lift top film.



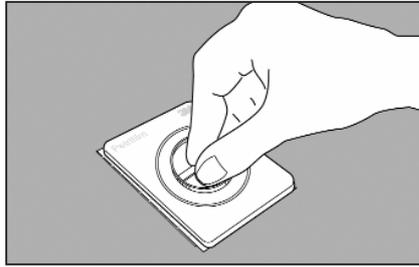
8 With pipette perpendicular to Petrifilm count plate, place 1 ml of sample onto centre of bottom film.



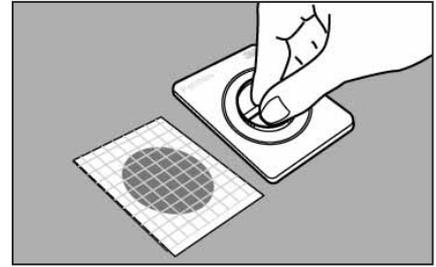
9 Release top film; allow it to DROP. DO NOT roll top film down.



**10** Holding crossbar, position Petrifilm Yeast & Mould spreader over Petrifilm count plate.

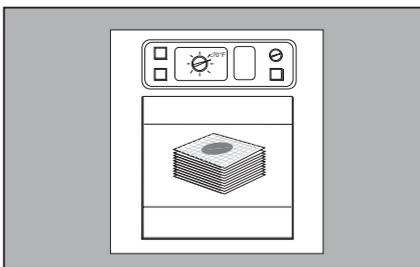


**11** Apply pressure on spreader to distribute inoculum over circular area. Do not twist or slide the spreader.



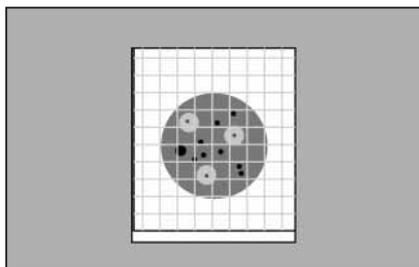
**12** Lift spreader. Wait one minute for gel to solidify.

## Incubation



**13** Incubate Petrifilm count plates with the clear side up in stacks of 20 or less at a temperature of 25°C for 3-5 days.

## Interpretation



**14** Read Petrifilm count plates on a standard Quebec-type colony counter or other magnified light source. Refer to Guide to Interpretation when reading results.

## Additional Comments

- **Note:** Remember to inoculate and spread each Petrifilm plate before going on to the next.
- Steps 9 and 10 are unique to Petrifilm Yeast & Mould count plates.
- Incubate Petrifilm Yeast & Mould count plates in a plastic container or plastic bag to optimize colony development.

Date	Version
January 2006	1.0

**3M**

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