# MESOPHILIC YEAST AND MOLD (PETRIFILM YEAST AND MOLD COUNT PLATE METHOD)

## PRINCIPLE

Mesophilic yeast and mold are quantitated by the selective Petrifilm yeast and mold count method based on antibiotic inhibition of bacteria facilitated by the presence of differential indicator dye.

## SCOPE

The method is applicable to starches, sugars, syrups and most coproducts of the corn wet milling industry (Note 1).

## REAGENTS

1. 3M Petrifilm Yeast and Mold Count (YM) Plates. Carefully read manufacturer's instructions prior to use.

2. Butterfield's Phosphate Diluent

   Stock Solution: Dissolve 34 g of potassium dihydrogen phosphate ($KH_2PO_4$) in 500 mL of purified water; adjust to pH 7.2 with about 175 mL of 1 *N* NaOH solution and dilute to 1 L volume. Store under refrigeration at 4°C.

   Diluent: Dilute 1.25 mL of stock solution to 1 L volume with purified water.

3. Sterile Water Dilution Blanks

   Fill water dilution bottles to 90 mL or desired volume with Butterfield's Phosphate Diluent (Note 2).

## APPARATUS

3M Petrifilm for Yeast and Mold or equivalent.

## PROCEDURE

A. Quantitative Procedure for Mesophilic Yeast and Mold

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1. Prepare decimal dilutions by aseptically weighing 10 g of sample into 90 mL sterile diluent (Note 3). The sample is further diluted by factor 10 dilution series, if high counts of yeast and mold are anticipated.

2. Place the YM plates on a flat surface. Lift top film and dispense carefully 1 mL of the sample dilution to the center of bottom film. Carefully roll down the top film onto the sample to prevent the entrapment of air bubbles. Distribute sample evenly within the circular well using the plastic spreader. Let plate stand undisturbed for one minute to permit gel hydration and solidification.

3. Incubate the plates in stacks (20 maximum) at 20-25°C in a horizontal position with the clear side up, checking after 24 and 48 hrs.

4. Count yeast and mold colonies on Petrifilm YM Count plates. An indicator dye stains yeast and mold colonies to provide contrast and facilitate counting. Yeast will be characteristically small, blue-green colonies with defined edges and no foci. Mold will be characteristically large, variable colored colonies with diffuse edges and center foci. Record the results and calculate the number of yeast/mold per gram of sample (Note 4).

### CALCULATION

Number of yeast/mold per g = Average number of yeast/mold × Dilution Factor.

### NOTES AND PRECAUTIONS

1. Although the Petrifilm method is approved by AOAC for all foods, use of the method for highly osmophilic samples should be carefully evaluated against the conventional method for performance verification. A suggested procedure would be to prewet as follows: Place the plate on a flat surface. Carefully lift the top film and wet the center of the bottom film with 1.0 ml of sterile water. Release the top film onto the bottom film with a “rolling down” motion to prevent air bubbles from forming within the plate. Place the plate spreader on the top film and gently press in a downward motion to evenly distribute the sterile water on the bottom film. Allow the plate to gel for 1-2 hours. To

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support reliable results for highly osmophilic samples, a corresponding Mesophilic Yeast and Mold test should be also run for performance verification.

2. When sterilizing dilution blanks a portion of the diluent may be lost. When this occurs, the sterilized blanks are brought to the proper volume with the sterile diluent.

3. When running counts on pregelatinized starches, no more than 5 g of sample per 95 mL of diluent may be used.

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

### REFERENCE

Association of Official Analytical Chemistry, www.aoac.org.