AOAC Official Method 997.02
Yeast and Mold Counts in Foods
Dry Rehydratable Film Method (Petrifilm™ Method)
First Action 1997
Final Action 2000

(Applicable to enumeration of total yeasts and molds in foods.)

See Tables **997.02A** and **B** for the results of the interlaboratory study supporting the acceptance of the method.

A. Principle

Method uses culture plates of dry medium supplemented with antibiotics, dye to enhance visualization of growth, and cold H_2O-soluble gelling agent. Undiluted or diluted suspensions are added to plates at a rate of 1 mL/plate. Suspension is spread over ca 30 cm^2 growth area. Gelling agent is allowed to solidify, plates are incubated, and yeasts and molds are counted.

B. Apparatus and Reagent

(**a**) *Yeast and mold count plates*.—Contain nutrients supplemented with chlortetracycline, chloramphenicol, cold H_2O-soluble gelling agent, and dye sensitive to presence of phosphatase (5-bromo-4-chloro-3-indolyl phosphate) that enhances visualization of yeast and mold growth. Circular growth area of single plate contains thirty 1×1 cm squares outlined on film base. (Available as 3M™ Petrifilm™ Yeast and Mold Count plates from 3M Microbiology Products, 3M Center, Bldg. 275-5W-05, St. Paul, MN 55144-1000, USA.)

(**b**) *Plastic spreader*.—Provided with Petrifilm plates, designed to spread suspension evenly over plate growth area.

(**c**) *Pipets*.—Serological pipet or pipetting syringe accurately delivering 1.0 mL.

(**d**) *Colony counter*.—Standard apparatus, Quebec model preferred, or one providing equivalent magnification (1.5×) and visibility.

(**e**) *Blender*.—High speed mechanical blender rotating at 10 000–12 000 rpm, or stomacher.

(**f**) *Dilution water*.—Butterfield's phosphate-buffered dilution water. Place 34 g KH_2PO_4 into 1 L volumetric flask and dissolve in 500 mL H_2O. Adjust pH to 7.2 with 1M NaOH (40 g/L) and dilute to volume with H_2O. Autoclave 15 min at 121°C. Store stock solution in refrigerator. Prepare dilution blanks by pipetting 1.25 mL stock solution into 1 L volumetric flask and dilute to volume with H_2O. Dispense 90 or 99 ± 1 mL into bottles. Autoclave 15 min at 121°C.

C. General Instructions

Store unopened yeast and mold count plate foil pouches at ≤8°C. After opening, return unused plates to foil pouch. Seal pouch by folding and taping the open end. Store resealed foil pouch at ≤8°C in a dry place. Use plates within 1 month after opening. Exposure of yeast and mold count plates to temperatures >25°C and/or humidities >50% RH can affect performance of plates.

After use, plates contain viable yeast and/or mold cultures. Autoclave used plates 15 min at 121°C prior to discarding.

D. Preparation of Test Suspension

Aseptically prepare 1:10 or greater dilution of food product with dilution H_2O. Blend or stomach 2 min and plate. Prepare additional dilutions as required.

E. Analysis

Place yeast and mold count plate on flat surface. Lift top film, hold pipet perpendicular to plate, and carefully inoculate 1 mL test suspension onto center of film base. Place top film down onto inoculum.

Lift plastic spreader using circular handle. Align center of spreader with approximate center of plate. Distribute suspension evenly using gentle downward pressure on center of spreader. *Do not slide spreader across film.* Remove spreader and leave plate undisturbed 1 min to let gel solidify.

Place plates in incubator in horizontal position, clear side up, in stacks not exceeding 20 units. Incubate plates 5 days at 20–25°C.

Count plates promptly after incubation period. Yeasts appear as blue-green or off-white in color and form small defined colonies. Mold colonies are usually blue but may also assume their natural pigmentation (e.g., black, yellow, green). They tend to be larger and more diffuse than yeast colonies.

To calculate yeast and mold count, multiply total number of yeast and mold colonies/plate (or average number of colonies/plate, if counting duplicate plates of same dilution) by appropriate dilution factor. When counting colonies on duplicate plates of consecutive dilutions, calculate mean number of colonies for each dilution before determining average yeast and mold count.

Estimated counts can be made on plates with >150 colonies and should be reported as estimated counts. In making such counts, determine average count/1 cm^2 and multiply by 30 (circular growth area is ca 30 cm^2).

High numbers of yeast colonies may cause the entire growth area to turn blue. High numbers of mold colonies may cause growth area to turn blue, black, yellow, etc. When this occurs, do not make estimated counts, but further dilute and plate test suspension to obtain more accurate count.

Reference: *J. AOAC Int.* **80,** 806(1997).

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