

**MESOPHILIC YEAST AND MOLD
(MEMBRANE FILTER METHOD)****PRINCIPLE**

Mesophilic yeast and mold are quantitated by a membrane filtration technique. Media can be rendered selective for yeast and mold by lowering the pH of the media or by addition of antibiotics. Use of the membrane filtration technique allows accurate quantitation of yeast and mold when low counts are anticipated.

SCOPE

The method is applicable to the sugars and syrups of the corn wet milling industry.

SPECIAL APPARATUS

1. Smooth-tipped, stainless steel forceps
2. 47 mm grid marked, white sterile 0.45 μm membranes and 47 mm absorbent pads (Millipore HAWG 047SO, HAWG S2 or equivalent)
3. Autoclavable 47 mm filtration systems with holder base, funnel assembly and receiver flask (Millipore or equivalent)
4. Vacuum pump capable of 22-27 inches of vacuum
5. Sterile 47 mm plastic petri dishes

MEDIA AND REAGENTS

1. Media:
 - A. Either potato dextrose agar (PDA); plate count agar (PCA); or potato dextrose broth if using alternate procedure in Note 1. Prepare the above media according to the manufacturer's directions. After sterilization, cool the media to 45°C and acidify PCA with 2 mL of sterile 10% tartaric acid per 100 mL of the media immediately before pouring of the plates (pH 3.5-3.7). Adjust the pH of PDA similarly with 1 mL of the sterile tartaric acid solution. Acidify

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potato dextrose broth similarly with 1 mL of the sterile tartaric acid solution.

B. Mycological Agar and YM-11 Agar with chlorotetracycline are acceptable alternatives. No pH adjustment is needed for low pH mycological agar or YM-11 Agar with chlorotetracycline, however pH should be checked.

2. Filter Sterilized Tartaric Acid, 10%
3. Ethanol
4. Sodium Hydroxide Solution, 1*N*
5. 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.

Diluent: Dilute 1.25 mL of stock solution to 1 L volume with purified water. Prepare dilution blanks using this solution.

6. Dilution Blanks: Fill dilution bottles to 50 mL with Butterfield's phosphate diluent. Cap bottles and sterilize at 121°C at 15 pounds of pressure for 15 mins. in a steam autoclave.
7. 47 mm petri dishes plus medium: Prepare 47 petri dishes by pouring approximately 2-3 mL of sterile and properly prepared media into each of the needed number of dishes. Allow the media to solidify (Note 1).

PROCEDURE

1. Aseptically weigh 25 g of the sample into a sterile 50 mL phosphate buffer diluent blank and homogenize.
2. Assemble a sterile 47 mm filtration system with a 47 mm grid marked, white 0.45 μm pore size membrane (Note 2). Connect the receiver flask to the vacuum pump using the vacuum hose.

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3. Aseptically pour all of the homogenous sample solution into the filter funnel and then cover the funnel top opening. Apply vacuum (22-27 psi) and filter the sample solution through the membrane filter. If desired, sterile phosphate buffer can be used for rinsing of the funnel.
4. Using sterile forceps transfer the membrane to a petri dish containing solidified, sterile medium. Roll the filter onto the medium in such a manner to avoid air bubbles forming between the membrane and the medium surface.
5. Incubate the petri dish containing the membrane at 30-32°C for a minimum of 72 hrs. or a maximum of 120 hrs. (3-5 days). Using a lighted magnifier, count separately all the yeast and mold colonies. The membrane grid can be used as an aid when scanning the filter (low power magnification). The total counts of yeast and mold colonies represent the number of each type organism per 25 g of sample (as is).

CALCULATION

The number of yeast and mold can be reported either as is or on a dry solids basis:

$$\text{As is basis (per 10g)} = \frac{\text{Total Count}}{2.5}$$

NOTES AND PRECAUTIONS

1. An alternate method is to prepare potato dextrose broth and disperse 2.2 mL onto an absorbent pad in a 47 mm sterile petri dish. Continue with step 4 of procedure.
2. The membrane can only be handled with sterile forceps. Sterilize the forceps by keeping the forcep blades in ½" of ethanol and then igniting the alcohol to burn itself out just prior to handling the membrane.

REFERENCE

Compendium of Methods for the Microbiological Examination of Foods, Current Edition, American Public Health Association.