

**MESOPHILIC YEAST AND MOLD
(STANDARD PLATE COUNT OR TOTAL PLATE COUNT)**

PRINCIPLE

Yeast and mold are quantitated by the fractional gram pour plate technique. Media can be rendered selective for yeast and mold by lowering the pH of the media or by addition of antibiotics.

SCOPE

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

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 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 the pH should be checked.
2. Filter Sterilized Tartaric Acid Solution, 10%

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3. 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 1N 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. Water Dilution Blanks. Fill water dilution bottles to appropriate volume of Butterfield's Phosphate Diluent. Cap bottles and sterilize at 120°C, at 15 pounds pressure for 15 mins. in a steam autoclave (Note 1).

7. Sodium Hydroxide Solution, 1N.

PROCEDURE

Two common fractional gram sample dilution techniques may be used for any given sample. The number of dilutions depends on the individual sample and may be determined by past experience.

A. Factor Five Dilution Series (FFS): Aseptically weigh 20 g of the sample into a sterile 80 mL water blank and homogenize. This is the primary 1:5 dilution blank (PDB). Twenty mL of the PDB can be aseptically transferred to another sterile 80 mL water blank, and the sample is diluted by a factor of 25.

B. Factor Ten Dilution Series (FTS): The same as FFS only 10 g of sample and 90 mL sterile water blanks are used. The sample is diluted by a factor of 10, 100, 1000, etc.

Aseptically dilute the sample by either FFS or FTS (Note 3). Pipet aseptically 1 mL of each dilution to appropriately marked duplicate Petri dishes. Pour 15-20 mL of the media of choice which has been cooled to 45°C into each dish. Swirl plates and allow to solidify.

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Invert the plates and incubate at 30-32°C. Count the plates after 72 hrs. (3 days), but if the colonies are too small, extend the incubation time to 96-120 hrs. (4-5 days). Average the count of the duplicate plates, multiply by the dilution factor and record as the number of yeast or mold per gram.

When counting mold or yeast, use the following criteria:

- A. To Count Mold: Use the plates which have the maximum number of colonies and whose surfaces are not completely covered with mold and/or where each individual surface and subsurface mold colony is discernible (Note 4).
- B. To Count Yeast: Any plate containing not more than 200 colonies and preferably not less than 20 colonies where possible (Notes 5 and 6). The yeast count may be subject to interference from molds, etc. If so, count the plates where the yeast colonies are most discernible.

CALCULATION

Number of yeast/mold per gram = Average number of yeast/mold x Dilution Factor

NOTES AND PRECAUTIONS

1. 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. This instruction applies to all methods employing dilution bottles.
2. Do not hold agar of low pH in the molten state for more than eight hrs. to avoid acid hydrolysis.
3. When running counts on pregelatinized starches, no more than 5 g of sample per 95 mL of diluent may be used.
4. Mold is difficult to accurately assay in a given sample by virtue of mycelial structure. A microscopic examination (970X oil immersion) of a sample is at times more appropriate.

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5. The membrane filter technique (II-B) may be used for sugars and syrups if low counts are anticipated.
6. Starches having mycotic densities less than 100 per g may be quantitated by aseptically weighing 1 g of sample into 99 mL sterile dilution blank. Homogenize the starch, raise the temperature to 45°C and aseptically transfer the contents to 100 mL of sterile double strength (doubly acidified) agar, which has been cooled to 45°C. Homogenize the mixture and distribute among seven sterile Petri dishes. Incubate the dishes as specified under FFS and FTS. The total count of all seven plates equals the density per g.

REFERENCE

Bacteriological Analytical Manual, Current Edition, Food and Drug Administration.

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