COLIFORM GROUP OF BACTERIA (STANDARD PLATE COUNT METHOD)

PRINCIPLE

Coliform bacteria are quantitated by the fractional gram pour plate technique (Note 1).

SCOPE

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

SPECIAL APPARATUS

Test tubes containing gas collector tubes (Durham Tubes)

MEDIA AND REAGENTS

1. Brilliant green lactose bile broth (BGLB), 2% (Note 2)

2. Violet Red Bile Agar (VRB) or equivalent

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 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.

4. Dilution Blanks

   Fill dilution bottles to 90 mL or desired volume with Butterfield's Phosphate Diluent (Note 3).

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PROCEDURE

A. Quantitative Procedure for Total Coliforms

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.

1. 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.

2. Factor Ten Dilution Series (FTS): The same as the 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.

Pipet 1 mL of each dilution to appropriately marked duplicate Petri dishes. Pour 15-20 mL of VRB into each dish, which has been cooled to 45ºC. Swirl the plates, allow to solidify and overlay the plates with 3-4 mL of VRB. Invert the plates and incubate at 35-37ºC for 18-24 hours. Incubation in excess of 24 hours must be avoided.

Count the dark-red colonies having an estimated diameter of 0.5 mm or more (colony size may be affected by the number of colonies per plate) which have a reddish zone of precipitated bile. Record the results and calculate the number of coliforms per gram of sample. Confirm these colonies, if it is deemed necessary, by the qualitative test described below.

B. Coliform Confirmation

Select two or more typical colonies from the VRB plate. Inoculate each colony into a separate tube containing 10 mL of BGLB broth plus a gas collector tube. Incubate the tubes at 35-37ºC and examine the tubes for gas production at 24 and 48 hours. All gassing tubes are considered to be positive for the presence of coliforms.

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CALCULATION

Number of coliforms per gram = Average number of coliforms x Dilution factor

NOTES AND PRECAUTIONS

1. To further support results for individual samples, a corresponding MPN or plate count test may be run. A flow chart of the different methods is attached.

2. The test should be run with a positive control. A 10 g sample is required for USP testing and 1 g for food standards.

3. 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.

REFERENCE

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

QUANTITATIVE METHODS FOR TOTAL COLIFORMS