CLOSTRIDIUM PERFRINGENS
PRESUMPTIVE ANAEROBIC PLATE COUNT METHOD

PRINCIPLE

Presumptive *Clostridium perfringens* are quantitated by a selective medium under anaerobic conditions. Caution must be exercised when applying this method since *Clostridium perfringens* are pathogenic and other potential isolates may also be pathogenic.

SCOPE

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

SPECIAL APPARATUS

Anaerobic jars, BBL GasPak or equivalent, equipped with GasPak hydrogen + CO_2 generator envelopes and anaerobic indicator.

MEDIA AND REAGENTS

1. Use one of the following:

   a. Tryptose-Sulfite-Cycloserine (TSC) Agar Base

Tryptose	15 g
Yeast extract	5 g
Soytone	5 g
Ferric ammonium citrate (NF Brown Pearls)	1 g
Sodium metabisulfite	1 g
Agar	20 g
Distilled water	900 mL

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Mix ingredients and heat to dissolve. Adjust pH to 7.4-7.8 with 1*N* NaOH or 1*N* HCl. Autoclave at 121°C under 15 pounds of pressure for 15 mins. Cool medium to 48-50°C.

b. Shahidi-Ferguson-Perfringens (SFP) Agar Base

2. D-cycloserine solution, 0.5%

Dissolve 1 g D-cycloserine (white crystalline powder) in 200 mL distilled water. Sterilize by filtration through a .45 micron filter and store at 4°C until use. Add 20 mL of this solution to 250 mL base medium of choice.

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 appropriate volume of Butterfield's phosphate diluent. Cap bottles and sterilize at 121°C at 15 pounds of pressure for 15 mins. in a steam autoclave (Note 1).

PROCEDURE

Aseptically weigh 20 g of 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 80 mL water blank, and the sample is diluted by a factor of 25. The number of dilutions depends on the individual sample and may be determined by past experience (Note 2).

Pour 6-7 mL media of choice into each petri dish, swirl the dish and spread the agar evenly on bottom. When agar has solidified, pipet 1 mL of each sample dilution to the center of duplicate agar surface. Pour additional 15 mL media of choice into each dish, swirl the plates and allow to solidify. Invert the plates and place them in an anaerobic jar.

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Incubate the anaerobic jar at 35-37°C for 22-26 hrs. (Note 3). Count the number of black colonies on those plates showing 25-250 colonies (Note 4). Record and calculate the number of presumptive *Clostridium perfringens* per gram of sample (Note 5).

CALCULATION

$$\text{Number of C. perfringens per gram} = \text{Average number of colonies} \times \text{Dilution factor}$$

NOTES AND PRECAUTIONS

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

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

3. Observe the anaerobic jar without opening after 22-26 hrs. If no growth is evident, incubate an additional 22-26 hrs.

4. If the low dilution shows less than 25 colonies, then any black colonies must be counted and reported.

5. If characteristic black colonies are found, the appropriate biochemical and morphological tests must be performed by an experienced microbiological laboratory to confirm the isolates as positive *Clostridium perfringens*.

REFERENCE

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