

**THERMOPHILIC SPORE-FORMING BACTERIA
ANAEROBIC THERMOPHILIC SPORES
NON H₂S PRODUCING**

PRINCIPLE

Vegetative cells present in the sample are thermally destroyed, which concurrently activates the thermophilic spores. The thermophilic anaerobic spores are quantified by the presence of gas production in a selective and differential medium.

SCOPE

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

MEDIA AND REAGENTS

1. P.E.-II Medium (PE2), Prepare as follows:

Yeast extract	3.0 g
Peptone	20.0 g
Bromocresol purple 1% ethanol solution	4.0 mL
Purified water	1.0 L

Mix the ingredients together until dissolved. Pipet 15 mL of PE2 into 20 × 150 mm culture tubes containing 8-10 Alaska seed peas each (6 tubes per sample). (Peas are available through all major seed companies) Allow to stand for at least 1 hour for hydration of the peas. When the peas have hydrated, sterilize the tubes at 121°C for 15 minutes. Cool to 53-57°C in a water bath.

2. Butterfield's Phosphate Diluent

Stock Solution: Dissolve 34 g of potassium dihydrogen phosphate (KH₂PO₄) 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.

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

3. Dilution Blanks. Fill dilution bottles to 80 mL or desired volume with Butterfield's Phosphate Diluent (Note 1).
4. 2% Plain Agar. Prepare agar 2% by weight. Pour mixture in 100 mL volumes in bottles and autoclave for 15 minutes at 121°C.

PROCEDURE

Aseptically weigh 20 g of sample into a sterile 80 mL water blank and homogenize (Note 2).

Evenly distribute 20 mL of the suspension among the 6 PE2 tubes (Note 3). The tubes should be swirled gently several times.

Place in a boiling water bath and boil for 15 minutes (Note 4). Gently rotate the tubes frequently for the first 5 minutes to ensure even distribution.

Cool the tubes rapidly in cold water. Pour a thin layer (about 3 mL) of 2% agar in each PE2 tube and solidify (Note 5). Incubate at 53-57°C for 70-74 hours. Any evidence of gas production (often reflected in the peas rising to the surface) is considered positive for the presence of non H₂S producing thermophilic anaerobes.

CALCULATION

Count the number of tubes showing gas production. Calculate the percent of positive as follows:

$$\% \text{ positive} = \frac{\text{number positive}}{6} \times 100$$

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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 grams of sample per 95 mL of diluent may be used.
3. If the medium is not prepared the day of the test, the PE2 tubes must be exhausted. To exhaust the PE2 tubes, place them in an autoclave with flowing steam for 20 minutes.
4. For sugars and syrups, boil for 5 minutes.
5. Caution should be used when pouring plain agar in tubes to prevent mixing of media and agar. To avoid this, tilt the tube while gently pouring the agar down the inside wall of the tube.

REFERENCE

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