

**TOTAL AND FECAL COLIFORM GROUP OF BACTERIA  
(PETRIFILM COLIFORM COUNT METHOD)**

**PRINCIPLE**

Coliform bacteria are quantitated by the selective Petrifilm Coliform count method based on gas formation.

**SCOPE**

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

**REAGENTS**

1. 3M Petrifilm Coliform Count (PCC) Plates. Carefully read manufacturers instructions prior to use.

2. Butterfield's Phosphate Diluent

Stock Solution: Dissolve 34 g of potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ ) 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 at 4°C.

Diluent: Dilute 1.25 mL of stock solution to 1 L volume with purified water.

3. Sterile Water Dilution Blanks

Fill water dilution bottles to 90 mL or desired volume with Butterfield's Phosphate Diluent (Note 1).

**PROCEDURE**

- A. Quantitative Procedure for Total Coliforms

1. Prepare decimal dilutions by aseptically weighing 10 g of sample into 90 mL sterile diluent (Note 2). The sample is further diluted by factor 10 dilution series, if high counts of coliform are anticipated. Adjust pH of the diluted sample between 6.5 and 7.5. For acid products use 1N

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NaOH, for alkaline products use 1N HCl, per manufacturer's instructions.

2. Place the PCC plates on a flat surface. Lift top film and dispense carefully 1 mL of the sample dilution to the center of bottom film. Carefully roll down the top film onto the sample to prevent the entrapment of air bubbles. Distribute sample evenly within the circular well using the plastic spreader. Let plate stand undisturbed for one minute to permit gel hydration and solidification.
3. Incubate the plates in stacks (20 maximum) at 35-37°C in a horizontal position with the clear side up for 22-26 hrs.
4. Total coliforms include red colonies with gas and blue colonies. Red colonies associated with entrapped gas are treated as presumptive coliforms. Colonies with associated gas bubbles and blue coloring should be counted as fecal coliforms. Record the results and calculate the number of coliforms per gram of sample. Confirm the colonies if it is deemed necessary by the qualitative tests.

**CALCULATION**

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

**REFERENCES**

Association of Official Analytical Chemistry, [www.aoac.org](http://www.aoac.org).