

Determination of Coliforms, Faecal coliforms and Escherichia coli in Foods and Beverages ,Total Coliform Count(TCC).

Fecal Coliform bacteria: A gram-negative, non-spore-forming rod-shaped bacterium, able to ferment lactose with gas production within 48 hours. at 44.5°C. The fecal coliforms consist primarily of *E. coli*, but a few *Enterobacter* and *Klebsiella* strains can produce gas in lactose broth at 44.5°C . The fecal coliforms are relatively specific for fecal material of warm-blooded animals. Fecal coliforms can be found in animals, plants, and foods derived from them, as well as in contaminated soil and water . Also the fecal coliform group is indicative of organisms originating in the intestinal tract of humans and some animals . Since most microorganisms that are shed in the feces of humans and animals share these characteristics, fecal coliforms are used as indicators of the presence of fecal contamination in foods.

Procedure

1- Test for Coliforms

Coliforms in foods may be enumerated by the solid medium method or by the Most Probable Number (MPN) method.

Solid medium method

Preparation of food homogenate

Make a 1:10 dilution of the well mixed sample, by aseptically transferring sample to the desired volume of diluent.

Measure **non-viscous liquid samples** (i.e., viscosity not greater than milk) volumetrically and mix thoroughly with the appropriate

volume of diluent (11 ml into 99 ml, or 10 ml into 90 ml or 50ml into 450 ml).

Weigh **viscous liquid sample** and mix thoroughly with the appropriate volume of diluent (11 \pm 0.1g into 99ml; 10 \pm 0.1g into 90ml or 50 \pm 0.1g into 450ml .

Weigh 50 \pm 0.1g of **solid or semi-solid sample** into a sterile blender jar or into a stomacher bag. Add 450 ml of diluent. Blend for 2 minutes at low speed (approximately 8000 rpm) or mix in the stomacher for 30-60 seconds.

Powdered samples may be weighed and directly mixed with the diluent. Shake vigorously (50 times through 30 cm arc).

In most of the food samples particulate matter floats in the dilution water. In such cases allow the particles to settle for two to three minutes and then draw the diluent from that portion of dilution where food particles are minimum and proceed.

Dilution:

If the count is expected to be more than 2.5×10^6 per ml or g, prepare decimal dilutions as follows. Shake each dilution 25 times in 30 cm arc. For each dilution use fresh sterile pipette. Alternately use auto pipette. Pipette 1 ml of food homogenate into a tube containing 9 ml of the diluent. From the first dilution transfer 1ml to second dilution tube containing 9ml of the diluent.

Repeat using a third, fourth or more tubes until the desired dilution is obtained.

Pour Plating

Pipette 1ml of the food homogenate (prepared sample) and of

each dilution into each of the appropriately marked duplicate petri dishes.

Pour into each petri-dish 10-12 ml of **VRBA(Violet red bile salt agar)**

OR MaConkey Agar (tempered to 48 C) and swirl plates to mix.

Allow to solidify. Overlay with 3 to 5 ml VRBA and allow to solidify.

Incubate the dishes, inverted at 35 C for 18 to 24 hours.

Counting the colonies

Following incubation, count all colonies that are purple red in colour, (Purplish red colonies), 0.5 mm in diameter or larger and are surrounded by a reddish zone of precipitated bile acids were counted as *coliforms*. Optimally the plates should have 30 to 100 colonies.

Calculation

Multiply the total number of colonies per plate with the reciprocal of the dilution used and report as coliforms per g or ml.