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National Food Safety Standard - Food Microbiological Examination - Counting of fecal coliform

食品安全国家标准 食品微生物学检验 粪大肠菌群计数

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1 Scope

This Standard specifies the method for counting of fecal coliform in food.

This Standard applies to the counting of fecal coliform in various foods.

2 Terms and definitions

2.1 Fecal coliform

A group of aerobic and facultative anaerobic gram-negative bacillus bacteria – cultivated at 44.5 °C for 24 h ~ 48 h – that can ferment lactose, and produce acid and gas. The flora comes from the feces of humans and warm-blooded animals, and is used as an indicator of fecal contamination to evaluate the hygiene status of food and to infer the possibility of contamination by enteric pathogens in food.

3 Equipment and materials for technical requirements

In addition to the routine sterilization and culture equipment in the microbiology laboratory, other equipment and materials are as follows:

a) Constant temperature incubator: 36 °C ± 1 °C;

b) Refrigerator: 2 °C ~ 5 °C;

c) Constant temperature water bath: 44.5 °C ± 0.2 °C;

d) Balance: sensitive 0.1 g;

e) Homogenizer;

f) Oscillator;

g) Sterile pipette: 1 mL (scale of 0.01 mL), 10 mL (scale of 0.1 mL), or micropipette and tip;

h) Sterile conical flask: capacity 500 mL;

i) Sterile petri dish: diameter 90 mm;

6 Operation steps

6.1 Dilution of samples

6.1.1 Solid and semi-solid samples: Weigh 25 g of the sample; put it in a sterile homogenizing cup containing 225 mL of phosphate buffer saline or normal saline; homogenize it at 8 000 r/min ~ 10 000 r/min for 1 min ~ 2 min to make a 1:10 sample homogenate solution; OR put it in a sterile homogenizing bag containing 225 mL of diluent; use a flapping homogenizer to beat it for 1 ~ 2 min to make a 1:10 sample homogenate solution.

6.1.2 Liquid sample: Use a sterile pipette to take 25 mL of sample; put it in a sterile conical flask containing 225 mL of phosphate buffer saline or normal saline (preset an appropriate number of sterile glass beads in the bottle); mix well to make a 1:10 sample homogenate solution.

6.1.3 The pH value of the sample homogenate solution shall be 6.5 ~ 7.5; use 1 mol/L NaOH or 1 mol/L HCl to adjust if necessary.

6.1.4 Use a 1 mL sterile pipette or micropipette to draw 1 mL of the 1:10 sample homogenate solution; slowly inject it along the tube wall into a sterile test tube containing 9 mL of phosphate buffer saline or normal saline (the pipette or tip shall not touch the diluent liquid level); shake the test tube or use a 1 mL sterile pipette for repeated pipetting to make it evenly mixed, to make a 1:100 sample homogenate solution.

6.1.5 According to the estimation of the contamination status of the sample, make a serial dilution of homogenate sample in ten-fold increments. For each incremental dilution, use a new 1 mL sterile pipette or tip. From the preparation of the sample homogenate solution to the completion of the sample inoculation, the whole process shall not exceed 15 minutes.

6.2 Initial fermentation test

For each sample, select 3 appropriate serial dilutions of sample homogenate solutions (a stock solution can be selected for the liquid sample); for each dilution, inoculate 3 tubes of lauryl sulfate tryptone (LST) broth; for each tube, inoculate 1 mL (if the inoculum volume needs to exceed 1 mL, use double LST broth); incubate at 36 °C ± 1 °C for 24 h ± 2 h; observe whether there are bubbles in the inverted tube; perform a re-fermentation test for those which produce gas within 24 h; if no gas is produced, continue to cultivate to 48 h ± 2 h. Record the number of LST broth tubes producing gas within 24 h and 48 h. Those producing no gas are negative for fecal coliform; those producing gas shall be subjected to a re-fermentation test.

If multiple dilutions are used, see Appendix B for the final determination of the optimal three serial dilutions.

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