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National Food Safety Standard - Food Microbiological Examination - Counting of Escherichia Coli

食品安全国家标准 食品微生物学检验 大肠埃希氏菌计数

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1 Scope

This Standard specifies the method for counting Escherichia coli in foods.

The Method I of this Standard is applicable to the counting of Escherichia coli in foods with low Escherichia coli content; the Method II is applicable to the counting of Escherichia coli in foods with high Escherichia coli content, and is not applicable to the counting of Escherichia coli in shellfish and products.

2 Inspection Principle

Escherichia coli ferments lactose at 44.5℃ to produce acid and gas; has *β*-glucuronidase activity, can decompose 5-bromo-4-chloro-3-indole-*β*-D-glucuronide; and forms blue-green colonies on Tryptone Bile X-glucuronide (TBX) agar. Based on the above characteristics of Escherichia coli, MPN count and plate count are performed on it.

NOTE: Most Escherichia coli have *β*-glucuronidase activity, but strains such as Escherichia coli O157 do not have *β*-glucuronidase activity. In addition, some strains of Shigella and Salmonella in the Enterobacteriaceae family also have *β*-glucuronidase activity.

3 Equipment and Materials

In addition to routine sterilization and culture equipment in microbiological laboratories, other equipment and materials are as follows.

3.1 Constant temperature incubator: 36℃ ± 1℃.

3.2 Refrigerator: 2℃ ~ 8℃.

3.3 Constant temperature water bath: 44.5℃ ± 0.2℃.

3.4 Constant temperature device: 48℃ ± 2℃.

3.5 Balance: With sensitivity of 0.1 g.

3.6 Homogenizer and sterile homogenizing bag, homogenizing cup, oscillator.

3.7 Test tube: 15 mm × 150 mm, 18 mm × 180 mm or other suitable specifications, as well as small inverted tube (Durham's tube) or other suitable gas collection device.

3.8 Sterile pipette: 1 mL (with 0.01 mL scale), 2 mL (with 0.02 mL scale), 5 mL (with 0.05 mL scale), 10 mL (with 0.1 mL scale) or micro pipettor and sterile sucker.

3.9 Sterile conical flask: With capacity of 500 mL.

3.10 Sterile culture dish: With diameter of 90 mm.

3.11 pH meter or precision pH test paper.

3.12 Sterile inoculation loop: 10 μL (about 3 mm in diameter).

4 Culture Medium and Reagents

4.1 Phosphate buffer: See A.1 in Appendix A.

4.2 Physiological saline: See A.2.

4.3 Lauryl sulfate tryptone (LST) broth: see A.3.

4.4 EC broth: see A.4.

4.5 Tryptone bile X-glucuronide (TBX) agar: see A.5.

4.6 1 mol/L NaOH: see A.6.

4.7 1 mol/L HCl: see A.7.

Method I MPN Counting Method of Escherichia Coli

5 Inspection Procedures

The inspection procedures for the MPN counting method of Escherichia coli are shown in Figure 1.

6.1.2 Liquid samples: Use a sterile pipette to draw 25 mL of sample and place it in a sterile conical flask containing 225 mL of phosphate buffer or physiological saline (an appropriate number of sterile glass beads can be pre-placed in the flask) and mix thoroughly. Or place it in a sterile homogenizing bag containing 225 mL of phosphate buffer or physiological saline and beat it with a slapping homogenizer for 1 min ~ 2 min to make a 1:10 sample homogenate. When the sample is not suitable for volume sampling, follow 6.1.1.

6.1.3 If necessary, use 1 mol/L NaOH or 1 mol/L HCl to adjust the pH of the sample homogenate or liquid sample stock solution to 6.5~7.5.

6.1.4 Use a sterile pipette or micro pipettor to draw 1 mL of 1:10 sample homogenate; and slowly inject it into a sterile test tube containing 9 mL of phosphate buffer or physiological saline along the tube wall (be careful not to let the tip of the pipette or sucker touch the diluent surface); and oscillate the test tube on an oscillator to mix well and make a 1:100 sample homogenate.

6.1.5 Based on the estimation of the sample contamination status, refer to the operation of 6.1.4 and make a 10-fold incremental serial dilution of the sample homogenate. After each incremental dilution, replace a sterile pipette or sucker.

6.2 Primary fermentation test

For each sample, select 3 appropriate sample homogenates with continuous dilutions (the stock solution can be selected for liquid samples); inoculate 3 tubes of LST broth for each dilution (5 tubes of LST broth for each dilution of shellfish and products); and inoculate 1 mL of sample homogenate into each tube of LST broth (if the inoculation volume exceeds 1 mL, add it to an equal volume of double-material LST broth). The whole process from the preparation of sample homogenate to the completion of inoculation into LST broth shall not exceed 15 min. Place the LST broth tube with the inoculated sample at 36℃ ± 1℃ for 24 h ± 2 h, and check the gas production. If bubbles are generated in the small inverted tube or the gas collection device, or if fine bubbles are continuously rising in the test tube when the LST broth tube is gently shaken, it is judged to be gas production; then the re-fermentation test is carried out. If no gas is produced, continue to culture for 48 h ± 2 h. If gas is produced, perform a re-fermentation test. If no gas is produced after 48 h ± 2 h, it is judged as negative for Escherichia coli. If no gas is produced in all LST broth tubes after 48 h ± 2 h, report the MPN value of Escherichia coli per gram (mL) of sample according to the MPN retrieval table as per Appendix B or C, expressed as MPN/g (mL).

6.3 Re-fermentation test

Gently shake each gas-producing LST broth tube; take 1 loop of culture with an inoculation loop, respectively; transfer it to the EC broth tube; and place it in a covered water bath. The water level of the water bath shall be at least 1 cm ~ 2 cm higher than the liquid level of the EC broth medium. Cultivate at 44.5℃ ± 0.2℃ for 24 h ± 2 h; check the gas production; and continue to culture for 48 h ± 2 h if no gas is produced. Record the number of EC broth tubes

9 Operation Steps

9.1 Dilution of sample

Perform according to 6.1.

9.2 Inoculation and culture

9.2.1 Based on the estimation of sample contamination, select 2 ~ 3 appropriate sample homogenates with continuous dilutions (the stock solution can be selected for liquid samples); and inoculate 2 sterile culture dishes for each dilution; 1 mL in each dish. At the same time, take phosphate buffer or physiological saline and add it to 2 sterile culture dishes as blank controls; 1 mL in each dish.

9.2.2 Pour TBX agar cooled to 48℃ ± 2℃ into the culture dishes as soon as possible; 15 mL ~ 20 mL in each dish. Carefully rotate the culture dishes to mix the culture medium and the inoculated sample homogenate thoroughly; and let it stand horizontally until it solidifies. From the preparation of the sample homogenate to the pouring of TBX agar, the whole process shall not exceed 15 min. After the agar solidifies, turn the plate over and place it at 36℃ ± 1℃ to culture for 18 h ~ 24 h.

9.3 Selection of plate colony counts

If the typical colonies of Escherichia coli on TBX agar plates are blue-green, plates with typical colony counts between 15 CFU ~ 150 CFU shall be selected; and count the typical colonies.

9.4 Calculation of Escherichia coli colony counts

9.4.1 If the typical colony count on only one dilution plate is within the counting range, the result shall be calculated by the average of the typical colony counts of that dilution multiplied by the corresponding dilution multiple.

9.4.2 If the typical colony counts on two continuous dilution plates are within the counting range, the result shall be calculated according to Formula (1):

$$N = \frac{\sum C}{(n_1 + 0.1n_2)d} \qquad \cdots\cdots(1)$$

Where:

N - the Escherichia coli colony count in the sample;

C - the typical colony count on the selected plate;

n_1 - the number of selected plates for the first dilution (low dilution multiple);

n_2 - the number of selected plates for the second dilution (high dilution multiple);

d - the dilution factor (the first dilution).

9.4.3 If the typical colony count on the plates of all dilutions is greater than 150 CFU, the plate with the highest dilution shall be counted; and the number of colonies on the plates of other dilutions may be recorded mostly uncountable. The result shall be calculated by multiplying the average typical colony count of the highest dilution by the corresponding dilution multiple.

9.4.4 If the typical colony count on the plates of all dilutions is less than 15 CFU, the plate with the lowest dilution shall be counted; and the result shall be calculated by multiplying the average typical colony count of the lowest dilution by the corresponding dilution multiple.

9.4.5 If no typical colony grows on the plates of all dilutions (including the stock solution of liquid sample), the result shall be calculated as less than 1 multiplied by the lowest dilution multiple.

9.4.6 If the typical colony count on the plates of all dilutions is not between 15 CFU ~ 150 CFU, and some of them are less than 15 CFU and some are greater than 150 CFU, the result shall be calculated by multiplying the average of typical colony count closest to 15 CFU or 150 CFU by the corresponding dilution multiple.

10 Results and Report

10.1 When the Escherichia coli colony count is less than 100 CFU, it shall be rounded off according to the principle of "rounding off" and reported as an integer.

10.2 When the Escherichia coli colony count is greater than or equal to 100 CFU, the third digit shall be rounded off according to the principle of "rounding off", and the first two digits shall be taken, and the digits behind shall be replaced by 0; it shall also be expressed in the form of 10 exponentials, and two significant digits shall be retained after rounding off according to the principle of "rounding off".

10.3 If colonies grow on the blank control, the test result is invalid.

10.4 The results of weight sampling shall be reported in CFU/g, and the results of volume sampling shall be reported in CFU/mL.

Sodium lauryl sulfate: 0.1 g

Distilled water: 1,000 mL

A.3.2 Preparation method

Add all compositions (except distilled water for double-composition LST broth, double the amount of each composition) to distilled water and heat to dissolve. Adjust pH if necessary. Dispense into test tubes with small inverted tubes, 10 mL per tube. Autoclave at 121℃ for 15 min. The pH of the culture medium after sterilization is 6.8 ± 0.2 at 25℃.

A.4 EC broth

A.4.1 Compositions

Tryptone or trypticase: 20.0 g

No.3 bile or mixed bile: 1.5 g

Lactose: 5.0 g

Dipotassium hydrogen phosphate: 4.0 g

Potassium dihydrogen phosphate: 1.5 g

Sodium chloride: 5.0 g

Distilled water: 1,000 mL

A.4.2 Preparation method

Dissolve each component in distilled water by heating; and adjust pH, if necessary. Dispense into test tubes with small inverted tubes; 10 mL per tube. Autoclave at 121 ℃ for 15 min. The pH of the culture medium after sterilization is 6.9 ± 0.2 at 25 ℃.

A.5 Tryptone bile X-glucuronide (TBX) agar

A.5.1 Compositions

Tryptone: 20.0 g

No.3 Bile: 1.5 g

5-Bromo-4-chloro-3-indole-β-D-glucuronide (BCIG) Cyclohexylamine Salt or Sodium Salt: 144 μmol

Agar: 9.0 g~18.0 g

Distilled Water: 1,000 mL

A.5.2 Preparation method

Add 0.5 mL of 1 mol/L sodium hydroxide solution to 2.5 mL of 95% ethanol; then add 144 μmol (0.075 g) of BCIG cyclohexylamine salt and mix well; and add it to distilled water together with other compositions. Alternatively, add 144 μmol of BCIG sodium salt to distilled water together with other compositions. Heat until completely dissolved and adjust pH, if necessary. Autoclave at 121℃ for 15 min. The pH of the culture medium after sterilization is 7.2 ± 0.2 at 25℃. The culture medium after sterilization can be stored in a sealed refrigerator away from light for no more than 1 month.

A.6 1 mol/L NaOH

A.6.1 Compositions

Sodium hydroxide: 40.0 g

Distilled water: 1,000 mL

A.6.2 Preparation method

Weigh 40 g of sodium hydroxide and dissolve it in 1,000 mL of distilled water.

A.7 1 mol/L HCl

A.7.1 Compositions

Concentrated hydrochloric acid: 89 mL

Distilled water: 1,000 mL

A.7.2 Preparation method

Take 89 mL of concentrated hydrochloric acid and dilute it to 1,000 mL with distilled water.

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