NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.38-2025

Replacing GB 4789.38-2012

National food safety standard - Food microbiological examination - Enumeration 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

Issued on: March 16, 2025 **Implemented on: September 16, 2025**

Issued by: State Administration for Market Regulation;
Standardization Administration of the PRC

Table of Contents

1 Scope

The standard lays down methods for counting Escherichia coli in food.

The first method applies to the counting of Escherichia coli in foods with a comparatively low content of the organism. The second method applies to the counting of Escherichia coli in foods with a comparatively high content, and does not apply to the counting of Escherichia coli in shellfish and shellfish products.

2 Principle of the examination

Escherichia coli ferments lactose at 44.5 °C with the production of acid and gas, has beta-glucuronidase activity, can break down 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide, and forms blue-green colonies on tryptone bile X-glucuronide (TBX) agar. On the strength of these properties the organism is counted by MPN and by plate count.

A note records that the great majority of Escherichia coli strains have beta-glucuronidase activity but that strains such as Escherichia coli O157 do not, and that some Shigella and Salmonella strains within the Enterobacteriaceae also have beta-glucuronidase activity.

3 Equipment and materials

Besides the sterilisation and incubation equipment routine to a microbiology laboratory, the following are listed: a constant temperature incubator at 36 °C +/- 1 °C; a refrigerator at 2 °C to 8 °C; a constant temperature water bath at 44.5 °C +/- 0.2 °C; a constant temperature device at 48 °C +/- 2 °C; a balance reading to 0.1 g; a homogeniser with sterile homogenising bags and cups, and a shaker; test tubes of 15 mm x 150 mm, 18 mm x 180 mm or other suitable sizes, together with Durham tubes or other suitable devices for collecting gas; sterile pipettes of 1 mL graduated in 0.01 mL, 2 mL in 0.02 mL, 5 mL in 0.05 mL and 10 mL in 0.1 mL, or micropipettes with sterile tips; sterile conical flasks of 500 mL; sterile Petri dishes of 90 mm diameter; a pH meter or precision pH paper; and a sterile inoculating loop of 10 µL, about 3 mm in diameter.

4 Culture media and reagents

Phosphate buffered solution (Annex A, A.1); physiological saline (A.2); lauryl sulfate tryptose (LST) broth (A.3); EC broth (A.4); tryptone bile X-glucuronide (TBX) agar (A.5); 1 mol/L NaOH (A.6); and 1 mol/L HCl (A.7).

5 Examination procedure (first method)

The examination procedure for the MPN count of Escherichia coli is given in Figure 1. The figure is a flow chart and its content is not reproduced in the extracted text.

6 Operating steps (first method)

6.1 Dilution of the sample. Solid and semi-solid samples: 25 g is weighed aseptically into a sterile homogenising cup holding 225 mL of phosphate buffered solution or physiological saline and homogenised at 8 000 r/min to 10 000 r/min for 1 min to 2 min, or placed in a sterile homogenising bag holding 225 mL of the same and paddle-blended for 1 min to 2 min, giving a 1 in 10 sample homogenate. Liquid samples: 25 mL is pipetted aseptically into a sterile conical flask holding 225 mL of phosphate buffered solution or physiological saline, in which sterile glass beads may be placed beforehand, and mixed thoroughly, or placed in a sterile homogenising bag and paddle-blended for 1 min to 2 min, giving a 1 in 10 homogenate; where the sample is not suited to volumetric sampling, 6.1.1 is followed instead. Where necessary the pH of the homogenate or of the undiluted liquid sample is adjusted to 6.5 to 7.5 with 1 mol/L NaOH or 1 mol/L HCl. 1 mL of the 1 in 10 homogenate is run slowly down the wall of a sterile tube holding 9 mL of phosphate buffered solution or physiological saline, taking care that the tip of the pipette or the tip does not touch the surface of the diluent, and the tube mixed on a shaker to give a 1 in 100 homogenate. Following 6.1.4, a ten-fold dilution series is made up as the estimated level of contamination requires, changing the sterile pipette or tip at each step.

6.2 Presumptive test. For each sample three suitable consecutive dilutions of the homogenate are chosen, or the undiluted liquid sample may be chosen, and three tubes of LST broth inoculated per dilution, five tubes per dilution for shellfish and shellfish products, with 1 mL of homogenate per tube; where the volume inoculated exceeds 1 mL it is added to an equal volume of double strength LST broth. The whole operation from the preparation of the homogenate to the completion of inoculation into LST broth shall not exceed 15 min. The inoculated LST broth tubes are held at 36 °C +/- 1 °C for 24 h +/- 2 h and examined for gas. Bubbles in the Durham tube or gas collecting device, or a stream of fine bubbles rising when the LST broth tube is shaken gently, is judged as gas production, and gas-producing tubes go forward to the confirmatory test. Tubes with no gas are incubated on to 48 h +/- 2 h; gas producers then go forward to the confirmatory test, and tubes still without gas at 48 h +/- 2 h are judged Escherichia coli negative. Where no LST broth tube has produced gas by 48 h +/- 2 h, the MPN of Escherichia coli per gram or millilitre of sample is reported from the MPN table of Annex B or Annex C, expressed as MPN/g or MPN/mL.

6.3 Confirmatory test. Each gas-producing LST broth tube is shaken gently, one loopful of culture transferred to a tube of EC broth, and the tube placed in a covered water bath whose water level stands at least 1 cm to 2 cm above the level of the EC broth, at 44.5 °C +/- 0.2 °C for 24 h +/- 2 h, and examined for gas; tubes without gas are incubated on to 48 h +/- 2 h. The number of EC broth tubes producing gas within 48 h +/- 2 h is recorded and those tubes go forward to the confirmation step. Where no EC broth tube has produced gas by 48 h +/- 2 h the result is judged Escherichia coli negative and the MPN reported from the table of Annex B or Annex C as MPN/g or MPN/mL.

6.4 Confirmation. Each gas-producing EC broth tube is shaken gently and a loopful streaked onto a TBX agar plate, which is incubated at 36 °C +/- 1 °C for 18 h to 24 h and the colonies examined; Escherichia coli colonies on TBX agar are blue-green. An EC broth gas tube whose TBX plate carries blue-green colonies is Escherichia coli positive, otherwise negative.

7 Results and report (first method)

From the number of positive tubes at the three suitable consecutive dilutions, the MPN of Escherichia coli per gram or millilitre of sample is reported from the MPN table of Annex B or Annex C, expressed as MPN/g or MPN/mL.

8 Examination procedure (second method)

The examination procedure for the plate count of Escherichia coli is given in Figure 2. The figure is a flow chart and its content is not reproduced in the extracted text.

9 Operating steps (second method)

9.1 Dilution of the sample follows 6.1.

9.2 Inoculation and incubation. Two or three suitable consecutive dilutions of the homogenate are chosen as the estimated level of contamination requires, or the undiluted liquid sample may be chosen, and two sterile Petri dishes inoculated per dilution with 1 mL per dish; at the same time phosphate buffered solution or physiological saline is added to two sterile dishes as a blank control, 1 mL per dish. TBX agar cooled to 48 °C +/- 2 °C is poured into the dishes as quickly as possible, 15 mL to 20 mL per dish, the dishes rotated gently so that the medium and the inoculated homogenate mix thoroughly, and left level to set. The whole operation from the preparation of the homogenate to the completion of pouring shall not exceed 15 min. Once the agar has set the plates are inverted and incubated at 36 °C +/- 1 °C for 18 h to 24 h.

9.3 Selection of plate counts. Typical colonies of Escherichia coli on TBX agar are blue-green; plates carrying between 15 CFU and 150 CFU of typical colonies are selected and the typical colonies counted.

9.4 Calculation of the Escherichia coli count. Where the typical colony count of only one dilution falls within the counting range, the mean typical colony count at that dilution is multiplied by the corresponding dilution factor. Where the typical colony counts of two consecutive dilutions fall within the range, the result is calculated by formula (1), whose legend gives N as the count of Escherichia coli in the sample, C as the typical colony count on the plates selected, n1 as the number of plates selected at the first dilution, that is the lower dilution factor, n2 as the number of plates selected at the second dilution, that is the higher dilution factor, and d as the dilution factor of the first dilution. Where every dilution gives typical colony counts above 150 CFU, the plates of the highest dilution are counted, the counts on the other plates may be recorded as too numerous to count, and the result calculated from the mean typical colony count at the highest dilution multiplied by the corresponding dilution factor. Where every dilution gives typical colony counts below 15 CFU, the plates of the lowest dilution are counted and the result calculated from the mean typical colony count at the lowest dilution multiplied by the corresponding dilution factor. Where no plate at any dilution, the undiluted liquid sample included, carries typical colonies, the result is calculated as less than 1 multiplied by the lowest dilution factor. Where no dilution gives typical colony counts between 15 CFU and 150 CFU, some falling below 15 CFU and some above 150 CFU, the result is calculated from the mean typical colony count nearest to 15 CFU or to 150 CFU multiplied by the corresponding dilution factor.

10 Results and report (second method)

Where the colony count of Escherichia coli is below 100 CFU it is rounded by the rule of rounding half up and reported as a whole number. Where it is 100 CFU or more, the third digit is rounded by the same rule, the first two digits kept and the remaining places filled