NATIONAL STANDARD OF THE

PEOPLE'S REPUBLIC OF CHINA

GB 4789.3-2025

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National food safety standard - Food microbiological examination: Enumeration of coliforms

食品安全国家标准 食品微生物学检验 大肠菌群计数

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2 Terms and definitions

2.1 Coliforms: aerobic and facultatively anaerobic, Gram-negative, non-spore-forming bacilli that ferment lactose with the production of acid and gas under given culture conditions.

2.2 Most probable number (MPN) technique: a quantitative detection method combining statistics and microbiology. After the sample under test has been serially diluted and incubated, the most probable number of coliforms in it is worked out by statistical probability theory from the lowest dilution at which no growth occurs and the highest dilution at which growth occurs.

3 Equipment and materials

Besides the sterilizing and incubating equipment usual in a microbiological laboratory, the following are used.

3.1 Incubators at 36 °C +/- 1 °C and 30 °C +/- 1 °C. 3.2 Refrigerator, 2 °C to 8 °C. 3.3 Thermostatic device, 48 °C +/- 2 °C. 3.4 Balance, readability 0.1 g.

3.5 Homogenizer with sterile homogenizing bags and cups, and a vortex mixer.

3.6 Test tubes of 15 mm by 150 mm, 18 mm by 180 mm or another suitable size, with small inverted tubes (Durham tubes) or another suitable gas collecting device.

3.7 Sterile pipettes of 1 mL graduated in 0.01 mL, 2 mL graduated in 0.02 mL, 5 mL graduated in 0.05 mL and 10 mL graduated in 0.1 mL, or micropipettes with sterile tips.

3.8 Sterile conical flasks of 500 mL. 3.9 Sterile Petri dishes of 90 mm diameter. 3.10 pH meter or precision pH paper. 3.11 Sterile inoculating loop of 10 µL, about 3 mm in diameter. 3.12 Magnifier or colony counter.

4 Culture media and reagents

4.1 Phosphate buffer solution, see A.1 of Annex A. 4.2 Physiological saline, see A.2. 4.3 Lauryl sulfate tryptose (LST) broth, see A.3. 4.4 Brilliant green lactose bile (BGLB) broth, see A.4. 4.5 Violet red bile agar (VRBA), see A.5. 4.6 Sodium hydroxide solution, 1 mol/L, see A.6. 4.7 Hydrochloric acid solution, 1 mol/L, see A.7.

4.8 Coliform count test plate, for which the criterion of a positive result shall be the fermentation of lactose with production of acid and gas, and whose performance shall meet the quality requirements for the corresponding culture media in GB 4789.28.

6 First method, operating steps

6.1.1 Solid and semi-solid samples. Under aseptic conditions 25 g of sample is weighed out and placed in a sterile homogenizing cup holding 225 mL of phosphate buffer solution or physiological saline and homogenized for 1 min to 2 min at 8 000 r/min to 10 000 r/min; or it is placed in a sterile homogenizing bag holding 225 mL of the same diluent and beaten for 1 min to 2 min in a paddle homogenizer, giving a 1 to 10 sample homogenate.

6.1.2 Liquid samples. With a sterile pipette, 25 mL of sample is taken into a sterile conical flask holding 225 mL of phosphate buffer solution or physiological saline, in which a suitable number of sterile glass beads may be placed beforehand, and mixed thoroughly; or it is placed in a sterile homogenizing bag holding 225 mL of the same diluent and beaten for 1 min to 2 min in a paddle homogenizer, giving a 1 to 10 sample homogenate. Where the sample does not lend itself to sampling by volume, 6.1.1 is followed.

6.1.3 Where necessary, the pH of the sample homogenate or of the undiluted liquid sample is adjusted to between 6.5 and 7.5 with 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid.

6.1.4 With a sterile pipette or a micropipette, 1 mL of the 1 to 10 sample homogenate is drawn and run slowly down the wall of a sterile tube holding 9 mL of phosphate buffer solution or physiological saline, care being taken that the tip of the pipette does not touch the surface of the diluent; the tube is mixed on the vortex mixer to give a 1 to 100 sample homogenate.

6.1.5 Depending on the estimated level of contamination of the sample, tenfold serial dilutions are made in turn following 6.1.4, a fresh sterile pipette or tip being used at each dilution step.

6.2 Presumptive fermentation test. For each sample, three suitable consecutive dilutions of the homogenate are chosen, a liquid sample being usable undiluted, and three tubes of LST broth are inoculated at each dilution, each tube receiving 1 mL of homogenate; if the inoculum exceeds 1 mL it is added to an equal volume of double-strength LST broth. The whole operation from preparing the homogenate to finishing the inoculation of the LST broth shall not exceed 15 min. The inoculated LST broth tubes are incubated at 36 °C +/- 1 °C for 24 h +/- 2 h and examined for gas production. Gas production is judged where a bubble has formed in the small inverted tube or the gas collecting device, or where fine bubbles rise continuously in the tube when the LST broth tube is shaken gently; tubes producing gas go on to the confirmed fermentation test. Tubes that have not produced gas are incubated on to 48 h +/- 2 h and examined again; those producing gas go on to the confirmed test and those still not producing gas at 48 h +/- 2 h are judged negative for coliforms. Where at 48 h +/- 2 h none of the LST broth tubes has produced gas, the MPN of coliforms per gram or millilitre of sample is reported from the MPN table of Annex B, expressed as MPN/g or MPN/mL.

6.3 Confirmed fermentation test. Each gas-producing LST broth tube is shaken gently, one loopful of culture is taken with an inoculating loop and transferred to a BGLB broth tube, which is incubated at 36 °C +/- 1 °C for 24 h +/- 2 h and examined for gas production; tubes producing gas are judged positive for coliforms. Tubes that have not produced gas are incubated on to 48 h +/- 2 h and examined again; those producing gas are judged positive and those still not producing gas are judged negative.

7 Results and report. From the number of tubes positive for coliforms at the three suitable consecutive dilutions in the confirmed fermentation test, and where more than three consecutive dilutions have been used the most suitable three consecutive dilutions being settled as laid down in Annex C, the MPN of coliforms per gram or millilitre of sample is reported from the MPN table of Annex B, expressed as MPN/g or MPN/mL.

9 Second method, operating steps

9.1 The sample is diluted as laid down in 6.1.

9.2.1 Depending on the estimated level of contamination of the sample, two or three suitable consecutive dilutions of the homogenate are chosen, a liquid sample being usable undiluted, and two sterile Petri dishes are inoculated at each dilution with 1 mL each. At the same time, phosphate buffer solution or physiological saline is added to two sterile Petri dishes, 1 mL each, as a blank control.

9.2.2 VRBA cooled to 48 °C +/- 2 °C is poured into the dishes as quickly as possible, 15 mL to 20 mL per dish. The dish is swirled carefully so that the medium and the inoculated homogenate mix thoroughly and is then left level to set. The whole operation from preparing the homogenate to finishing the pouring of the VRBA shall not exceed 15 min. Once the agar has set, a further 3 mL to 4 mL of VRBA is spread evenly over the whole plate surface; once the overlay has set, the plate is inverted and incubated at 36 °C +/- 1 °C for 18 h to 24 h. For milk and milk products the plates are incubated at 30 °C +/- 1 °C for 18 h to 24 h.

9.2.3 Where a coliform count test plate is used, it is operated as its instructions lay down.

9.3.1 The colonies are observed and counted, with a magnifier or a colony counter where necessary. Plates with a total colony count between 15 CFU and 150 CFU are chosen and the typical and suspect coliform colonies on them counted separately. A typical colony is red to purplish red, may be surrounded by a ring of red precipitate, and is generally more than 0.5 mm in diameter. A suspect colony is red to purplish red and is generally less than 0.5 mm in diameter.

9.3.2 Where two dilutions give plate counts between 15 CFU and 150 CFU, and in the other cases of choosing the colony count, Annex D applies.

9.4 Confirmation test. From the VRBA plates of the same dilution, five typical and five suspect colonies are picked; where there are fewer than five typical or suspect colonies, all of them are picked. Each colony is inoculated into one BGLB broth tube, which is incubated at 36 °C +/- 1 °C for 24 h +/- 2 h and examined for gas production; tubes producing gas are positive for coliforms. Tubes that have not produced gas are incubated on to 48 h +/- 2 h and examined again; those producing gas are positive and those still not producing gas are negative.

10.1 The colony count of coliforms is the mean of the sum of the products of the number of typical colonies and of suspect colonies at the chosen dilution with their respective positive ratios, multiplied by the dilution factor. Where the colony count of coliforms is below 100 CFU it is rounded by the round-half-up rule and reported as a whole number. Where it is 100 CFU or more, the third digit is rounded by the round-half-up rule, the first two digits are kept and the remaining places filled with zeros; the result may also be expressed as a power of ten, rounded by the round-half-up rule and kept to two significant figures. Annex D