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Report Name: National Food Safety Standard Food Microbiological Examination Enumeration of Escherichia coli

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Report Highlights:

On March 27, 2025, China's NHC and the SAMR jointly released the National Food Safety Standard Food Microbiological Examination Enumeration of Escherichia coli (GB 4789.38-2025). This updated standard applies to the testing of E. coli counts in foods. The final standard will enter into force on September 16, 2025. At the time of the report, China hadn't notified WTO of this revised standard. This report provides unofficial translation of the final standard. Stakeholders should conduct their own review of the regulations to assess any market or regulatory effect on their business.

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Report Summary:

On March 27, 2025, The People's Republic of China (China's) National Health Commission (NHC) and the State Administration for Market Regulation (SAMR) jointly released the National Food Safety Standard Food Microbiological Examination Enumeration of Escherichia coli (GB 4789.38-2025) (link in Chinese).

The updated standard applies to the testing of *Escherichia coli* (*E. coli*) counts in foods. The final standard will enter into force on September 16, 2025, to replace the current implementing standard GB 4789.38-2012, which was implemented since July 2012. At the time of the report, China hasn't notified WTO of this revised standard.

This report provides an unofficial translation of the final standard. Stakeholders should conduct their own review of the regulations to assess any market or regulatory effect on their business.

BEGIN TRANSLATION

National Food Safety Standard

Food Microbiological Examination

Enumeration of Escherichia coli

Foreword

This standard supersedes GB 4789.38-2012 National Food Safety Standard Food Microbiological Examination Enumeration of Escherichia coli. Compared with GB 4789.38-2012, the main changes in this standard are as follows:

– Modified the scope;

– Deleted terms and definitions;

– Added testing principles;

– Modified equipment, materials, culture medium, and reagents;

– Modified testing procedures, operational steps, results, reports, and appendixes.

National Food Safety Standard

Food Microbiological Examination

Enumeration of Escherichia coli

1. Scope

This standard specifies the method for counting *Escherichia coli* in foods.

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

2. Testing Principles

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

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

3. Equipment and Materials

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

3.1 Constant temperature incubator: 36°C±1°C.

3.2 Refrigerator: 2℃~8°C.

3.3 Thermostatic water bath box: 44.5°C±0.2°C.

3.4 Thermostatic device: 48°C±2°C.

3.5 Balance: sensitivity 0.1g.

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

3.7 Test tube: 15 mm × 150 mm, 18 mm ×180 mm or other suitable specifications, and small inverted tube (Duchenne tube) or other suitable gas production 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 micropipette and sterile tip

3.9 Sterile conical flask: with a capacity of 500 mL.

3.10 Sterile culture dish: 90 mm in diameter.

3.11 pH meter or precision pH test paper

3.12 Sterile inoculation ring: 10 μL with 3mm in diameter.

4. Medium and Reagent

4.1 Phosphate buffer: see A.1 in Appendix A

4.2 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. Testing Procedures

The testing procedure for *Escherichia coli* MPN counting method is shown in Figure 1.

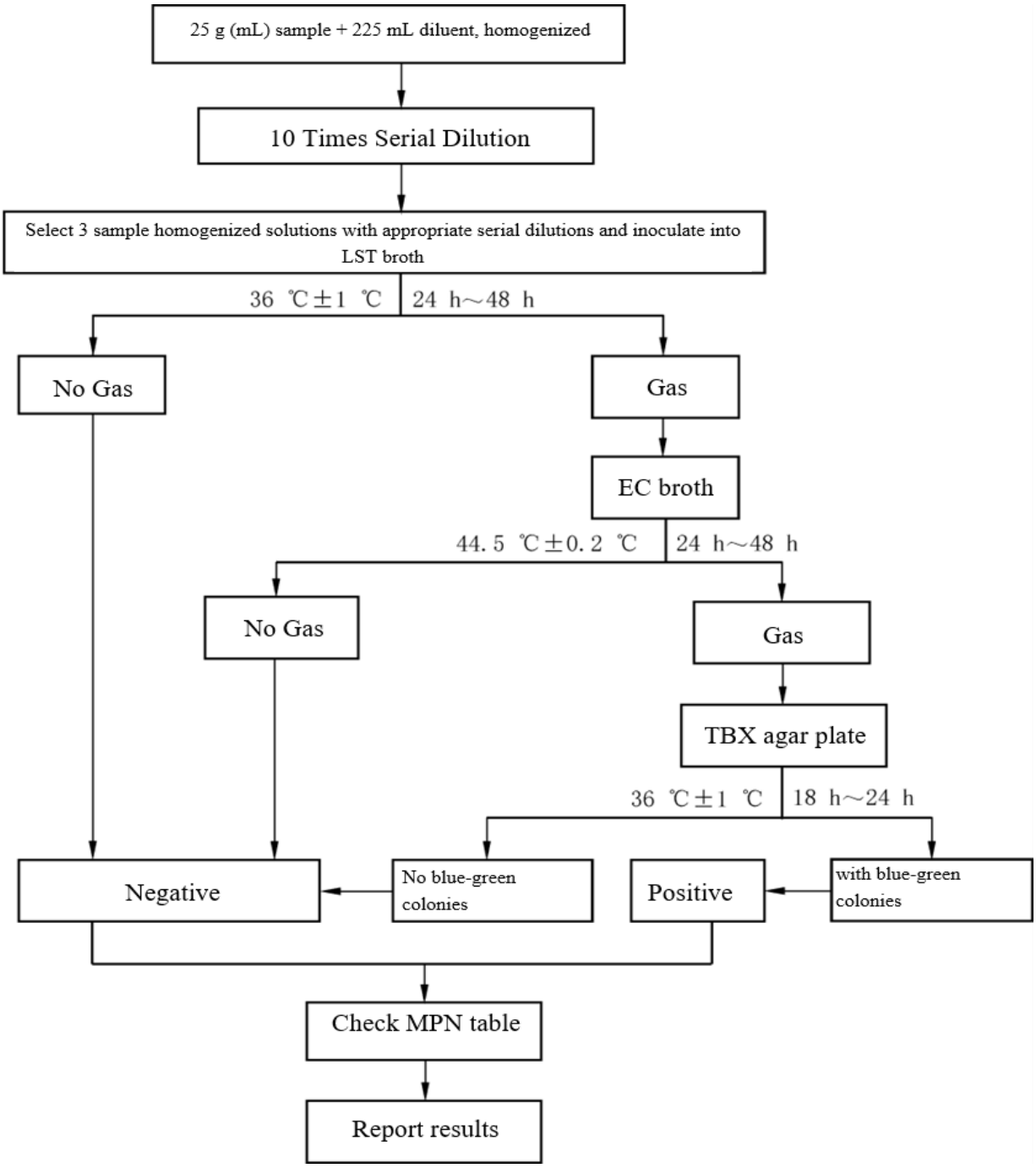

Figure 1: Testing Procedure of Escherichia coli MPN Counting Method

6. Operational Steps

6.1 Dilution of samples

6.1.1 Solid and semi-solid samples: in sterile operation, weigh 25 g of samples, put it into a sterile homogenization cup containing 225 mL of phosphate buffer or saline, and homogenize it at 8,000 r/min~10,000 r/min for 1 minute to 2 minutes; Or put it into a sterile homogenization bag containing 225 mL phosphate buffer or saline, and beat it with a tapping homogenizer for 1 to 2 minutes to make a 1:10 sample homogenization solution.

6.1.2 Liquid sample: use a sterile straw to take 25 mL of sample, put it into a sterile conical bottle containing 225 mL phosphate buffer or saline (appropriate number of sterile glass beads can be preset in the bottle), and mix it well, or put it into a sterile homogenization bag containing 225 mL phosphate buffer or saline, and beat it for 1 to 2 minutes with a tapping homogenizer to make a 1:10 sample homogenization solution. When the sample is not suitable for volume sampling, follow 6.1.1.

6.1.3 When necessary, adjust the pH value of sample homogenization solution or liquid sample stock solution to 6.5~7.5 with 1 mol/L NaOH or 1 mol/L HCl.

6.1.4 Take 1 mL of 1:10 sample homogenization solution with a sterile pipette or micropipette, slowly inject it along the wall of the tube into a sterile test tube containing 9 mL of phosphate buffer or saline (be careful not to let the tip of the pipette or pipette touch the surface of the diluent). Shake the test tube on an oscillator to mix and make a 1:100 sample homogenization solution.

6.1.5 According to the estimation of the sample contamination status, and with reference to the operation of 6.1.4, prepare 10 times of increasing serial diluted sample homogenization solution. For each incremental dilution, use a new sterile pipette or tip.

6.2 Preliminary fermentation test

For each sample, select 3 sample homogenization solution with appropriate serial dilutions (liquid samples can be stock solution), inoculate 3 tubes of LST broth with each dilution (each dilution of shellfish and products are inoculated with 5 tubes of LST broth), inoculate 1 mL of sample homogenization solution in each tube of LST broth (if the inoculation volume exceeds 1 mL, add it to an equal volume of double LST broth). The whole process from preparation of sample homogenization solution to end of inoculation of LST broth shall not exceed 15 minutes.

Place the LST broth tube with inoculated samples at temperature of 36℃ ± 1℃ for 24 ± 2 hours and check the gas production. If there are bubbles in the small-inverted tube or the gas

production collection device, or there are fine bubbles rising in the test tube by gently shaking the LST broth tube, it is determined as gas production, and the re-fermentation test shall be conducted for the gas production samples. If no gas is produced, continue to culture it until 48 ± 2 hours, and the re-fermentation test shall be conducted for the gas production samples; after incubation for 48 ± 2 hours, those who still did not produce gas are determined to be *Escherichia coli* negative samples. If no gas is produced in all LST broth tubes after incubation for 48 ± 2 hours, report the MPN value of *Escherichia coli* per gram (mL) of sample in MPN/g (mL) according to the MPN table in Appendix B or Appendix C.

6.3 Re-fermentation test

Gently shake each gas producing LST broth tube and take 1 ring of culture with the inoculation ring; transfer to EC broth tube and put it into the water bath box with a cover. The water level of the water bath box shall be at least 1 to 2 centimeters higher than the level of EC broth medium, incubate it at temperature of 44.5℃ ± 0.2℃ for 24 ± 2 hours, check the gas production, and continue to incubate to 48 ± 2 hours if no gas production is found. Record the number of EC broth tubes which produced gas within 48 ± 2 hours and conduct the confirmation tests. If the culture time is 48 ± 2 hours, all EC broth tubes do not produce gas, then it is determined to be negative for *Escherichia coli*. According to the MPN table in Appendix B or Appendix C, report the MPN values of *Escherichia coli* in each gram (mL) of sample, in MPN/g (mL).

6.4 Confirmation test

Gently shake the EC broth tubes that produce gas and respectively use the inoculation ring to take the culture to be inoculated on the TBX agar plate, incubate at temperature of 36 ℃ ± 1 ℃ for 18 to 24 hours, and observe the colonies on the TBX agar plate. The colonies of *Escherichia coli* on the TBX agar plate are blue-green. If there are blue-green colonies on the TBX agar plate, the corresponding gas producing tube of EC broth is positive for *Escherichia coli*, otherwise it is negative.

7. Results and Reports

According to the number of positive tubes of *Escherichia coli* in three appropriate serial dilutions and the MPN table in Appendix B or Appendix C, report the MPN value of *Escherichia coli* in each gram (mL) of the samples, expressed in MPN/g (mL).

Method II: Escherichia coli Plate Counting Method

8. Testing Procedures

See Figure 2 for the testing procedures of *Escherichia coli* plate counting method.

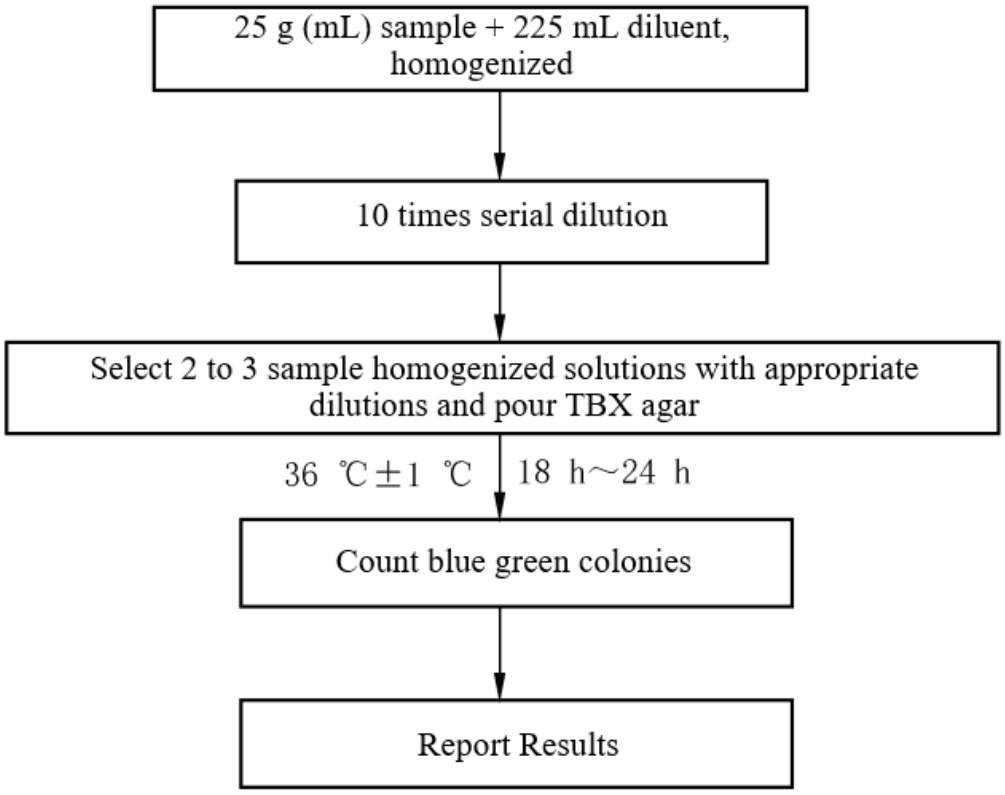

Figure 2: Testing procedure of Escherichia coli plate counting method

9. Operation Steps

9.1 Dilution of samples

According to 6.1.

9.2 Inoculation and culture

9.2.1 According to the estimation of sample contamination, select 2 to 3 sample homogenization solution with appropriate serial dilutions (liquid samples can be the stock solution), inoculate 2 sterile Petri dishes for each dilution with 1 mL of each dish. At the same time, add phosphate buffer or saline to 2 sterile Petri dishes as blank control, 1 mL for each dish.

9.2.2 Inject the TBX agar cooled to 48 ± 2℃ into Petri dishes as soon as possible, 15 mL to 20 mL each dish. Carefully rotate the Petri dish, mix the culture medium with the inoculated sample homogenization solution thoroughly and set it aside horizontally until it solidifies. The whole process from the preparation of sample homogenization solution to the end of TBX agar

injection shall not exceed 15 minutes. After agar solidified, turn the plate over and incubate it at temperature of 36℃± 1℃ for 18 to 24 hours.

9.3 Selection of plate colony number

The color of typical colonies of *Escherichia coli* on TBX agar plate is blue green. Select the plate with typical colony number between 15 CFU and 150 CFU and count the typical colonies.

9.4 Calculation of Escherichia coli colony number

9.4.1 If only one dilution plate has a typical colony count within the counting range, the result is calculated by taking the average of the typical colony counts of that dilution and multiplying it by the corresponding dilution multiple.

9.4.2 If the number of typical colonies on the plates of two serial dilutions is within the counting range, the result shall be calculated according to formula (1):
In which:

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

In which:
N: colony number of *Escherichia coli* in the sample;
C: the number of typical colonies on the selected plate;
n_1: number of plates selected for the first dilution (low dilution ratio);
n_2: number of plates selected for the second dilution (high dilution ratio);
d: dilution factor (first dilution).

9.4.3 If the number of typical colonies on plates of all dilution is greater than 150 CFU, count the plate with the highest dilution. The colony counts on plates of other dilutions can be recorded as too many to be counted. The result is calculated by multiplying the average of typical colony counts of the highest dilution by the corresponding dilution multiple.

9.4.4 If the number of typical colonies on plates of all dilutions are less than 15 CFU, count the plate with the lowest dilution, and the result can be calculated by multiplying the average of typical colony counts at the lowest dilution by the corresponding dilution multiple.

9.4.5 If there is no typical colony growth in plates of all dilutions (including liquid sample stock solution), the result shall be calculated by multiplying the value less than 1 by the lowest dilution multiple.

9.4.6 If the typical colony counts on plate of all dilutions are not between 15 CFU and 150 CFU, some of which are less than 15 CFU and some are more than 150 CFU, then multiply the average number of typical colonies closest to 15 CFU or 150 CFU by the corresponding dilution factor to calculate the result.

10. Results and Reports

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

10.2 When the colony counts of *Escherichia coli* is greater than or equal to 100 CFU, the first two digits shall be retained, the third digit shall be rounded off and represented by 0; it can also be expressed in the form of an index of 10, after rounding off, two significant figures shall be retained.

10.3 If there is colony grown on the blank control, the test result is invalid.

10.4 Report the results in CFU/g for weighing sampling and in CFU/mL for volume sampling.

Appendix A

Culture Medium and Reagents

A. 1 Phosphate Buffer

A. 1.1 Composition

Potassium dihydrogen phosphate	34.0 g
Distilled water	500 mL

A. 1.2 Preparation

Storage solution: weigh 34.0 g of potassium dihydrogen phosphate and dissolve it in 500 mL of distilled water (or other qualified experimental water, the same below), adjust the pH value to 7.2 ± 0.2 with about 175 mL of 1 mol/L sodium hydroxide solution, dilute it to 1,000 mL with distilled water, and seal and store it in a refrigerator. Diluent: take 1.25 mL of storage solution, dilute it to 1,000 mL with distilled water, dispense it in a suitable container, autoclave at temperature of 121℃ for 15 minutes, and set aside as sample dilution.

A. 2 Saline

A.2.1 Composition

Sodium Chloride	8.5g
Distilled Water	1,000 mL

A.2.2 Preparation

Dissolve sodium chloride in distilled water and sterilized by high pressure at 121 ℃ for 15minutes.

A.3 LST Broth

A.3.1 Composition

Tryptone	20.0 g
Sodium chloride	5.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g
Sodium lauryl sulfate	0.1 g

Distilled water	1, 000 mL

A.3.2 Preparation

Add each component (except distilled water, the amount of each component in double LST broth is doubled) to distilled water for heating and dissolution and adjust the pH values if necessary. Dispense it into a test tube with small-inverted tubes, 10 mL per tube. Autoclave at temperature of 121 ℃ for 15 minutes, and the pH value of the sterilized medium at temperature of 25 ℃ is 6.8 ± 0.2.

A. 4 EC Broth

A. 4.1 Composition

Tryptone	20.0 g
No. 3 bile salt or mixed bile salt 1	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

Heat and dissolve the components in distilled water and adjust the pH value if necessary. Dispense them into test tubes with small-inverted tubes, 10 mL each. Autoclave at temperature of 121 ℃ for 15 minutes, and the pH value of the sterilized medium at temperature 25 ℃ is 6.9 ± 0.2.

A. 5 Tryptone Bile Salts X-Glucuronide (TBX) Agar

A.5.1 Composition

Tryptone	20.0 g
Bile salt No.3	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

Add 0.5 mL of 1 mol/L sodium hydroxide solution to 2.5 mL of 95% ethanol, then add 144 μ mol (0.075 g) of BCIG cyclohexylamine salt, mix well and add it to distilled water together with other components. Or add 144 μ mol of BCIG sodium salt to distilled water together with other components. Heat to dissolve completely, adjust the pH value when necessary. Autoclave at temperature of 121℃ for 15 minutes, and pH value of medium at temperature of 25℃ after sterilization is 7.2 ± 0.2. The sterilized medium can be stored in a refrigerator without light for no more than 1 month.

A.6 1 mol/L NaOH

A.6.1 Composition

Sodium hydroxide	40.0 g
Distilled water	1,000 mL

A. 6.2 Preparation

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

A.7 1 mol/L HCl

A. 7.1 Composition

Concentrated hydrochloric acid	89 mL
Distilled water	1,000 mL

A. 7.2 Preparation

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

Appendix B

Table for the Most Probable Number (MPN) of Escherichia coli in Foods

The most probable number (MPN) of *Escherichia coli* in food samples per gram (mL) is as shown in table B.1.

Table B.1: Table for the Most Probable Number (MPN) of Escherichia Coli in Foods

Number of positive tubes			MPN	95% confidence limit		Number of positive tubes			MPN	95% confidence limit	
0.1	0.01	0.001		Lower limits	Upper limits	0.1	0.01	0.001		Lower limits	Upper limits
0	0	0	<3.0	-	9.5	2	2	0	21	4.5	42
0	0	1	3.0	0.15	9.6	2	2	1	28	8.7	94
0	1	0	3.0	0.15	11	2	2	2	35	8.7	94
0	1	1	6.1	1.2	18	2	3	0	29	8.7	94
0	2	0	6.2	1.2	18	2	3	1	36	8.7	94
0	3	0	9.4	3.6	38	3	0	0	23	4.6	94
1	0	0	3.6	0.17	18	3	0	1	38	8.7	110
1	0	1	7.2	1.3	18	3	0	2	64	17	180
1	0	2	11	3.6	38	3	1	0	43	9	180
1	1	0	7.4	1.3	20	3	1	1	75	17	200
1	1	1	11	3.6	38	3	1	2	120	37	420
1	2	0	11	3.6	42	3	1	3	160	40	420
1	2	1	15	4.5	42	3	2	0	93	18	420
1	3	0	16	4.5	42	3	2	1	150	37	420
2	0	0	9.2	1.4	38	3	2	2	210	40	430
2	0	1	14	3.6	42	3	2	3	290	90	1 000
2	0	2	20	4.5	42	3	3	0	240	42	1 000
2	1	0	15	3.7	42	3	3	1	460	90	2 000
2	1	1	20	4.5	42	3	3	2	1 100	180	4 100
2	1	2	27	8.7	94	3	3	3	>1 100	420	-

Note 1: Three dilutions are used in this table, inoculates three tubes for each dilution. The sample volume of each tube in the three dilutions is 0.1g (mL), 0.01g (mL) and 0.001 g (mL), respectively.
Note 2: When the sample volume inoculated in three dilutions is changed to 1g (mL), 0.1g (mL) and 0.01g (mL), the value in the table should be reduced by 10 times accordingly; If 0.01 g (mL), 0.001 g (mL) and 0.000 1 g (mL) are used, the values in the table should be expanded by 10 times accordingly, and so on.
Note 3: If necessary, the value in this table can be multiplied by 100 to report the most probable number of *Escherichia coli* in every 100 g (mL) sample, expressed in MPN/100 g (mL).

Appendix C

Table for the Most Probable Number (MPN) of Escherichia coli in Shellfish and Products

The most probable number (MPN) of *Escherichia coli* in shellfish and product samples per gram (mL) is as shown in table C.1.

Table C.1: the Most Probable Number (MPN) of Escherichia coli in Shellfish and Products

Number of positive tubes			MPN	95% confidence limit		Number of positive tubes			MPN	95% confidence limit	
0.1	0.01	0.001		Lower limits	Upper limits	0.1	0.01	0.001		Lower limits	Upper limits
0	0	0	<1.8	-	6.8	2	2	2	14	5.9	36
0	0	1	1.8	0.09	6.8	2	3	0	12	4.1	26
0	1	0	1.8	0.09	6.9	2	3	1	14	5.9	36
0	1	1	3.6	0.7	10	2	4	0	15	5.9	36
0	2	0	3.7	0.7	10	3	0	0	7.8	2.1	22
0	2	1	5.5	1.8	15	3	0	1	11	3.5	23
0	3	0	5.6	1.8	15	3	0	2	13	5.6	35
1	0	0	2.0	0.1	10	3	1	0	11	3.5	26
1	0	1	4.0	0.7	10	3	1	1	14	5.6	36
1	0	2	6.0	1.8	15	3	1	2	17	6.0	36
1	1	0	4.0	0.7	12	3	2	0	14	5.7	36
1	1	1	6.1	1.8	15	3	2	1	17	6.8	40
1	1	2	8.1	3.4	22	3	2	2	20	6.8	40
1	2	0	6.1	1.8	15	3	3	0	17	6.8	40
1	2	1	8.2	3.4	22	3	3	1	21	6.8	40
1	3	0	8.3	3.4	22	3	3	2	24	9.8	70
1	3	1	10	3.5	22	3	4	0	21	6.8	40
1	4	0	11	3.5	22	3	4	1	24	9.8	70
2	0	0	4.5	0.79	15	3	5	0	25	9.8	70
2	0	1	6.8	1.8	15	4	0	0	13	4.1	35
2	0	2	9.1	3.4	22	4	0	1	17	5.9	36
2	1	0	6.8	1.8	17	4	0	2	21	6.8	40

2	1	1	9.2	3.4	22	4	0	3	25	9.8	70
2	1	2	12	4.1	26	4	1	0	17	6.0	40
2	2	0	9.3	3.4	22	4	1	1	21	6.8	42
2	2	1	12	4.1	26	4	1	2	26	9.8	70

Table C.1: the Most Probable Number (MPN) of Escherichia Coli in Shellfish and Products (Continued)

Number of positive tubes			MPN	95% confidence limit		Number of positive tubes			MPN	95% confidence limit	
0.1	0.01	0.001		Lower limits	Upper limits	0.1	0.01	0.001		Lower limits	Upper limits
4	1	3	31	10	70	5	2	1	70	22	170
4	2	0	22	6.8	50	5	2	2	94	34	230
4	2	1	26	9.8	70	5	2	3	120	36	250
4	2	2	32	10	70	5	2	4	150	58	400
4	2	3	38	14	100	5	3	0	79	22	220
4	3	0	27	9.9	70	5	3	1	110	34	250
4	3	1	33	10	70	5	3	2	140	52	400
4	3	2	39	14	100	5	3	3	180	70	400
4	4	0	34	14	100	5	3	4	210	70	400
4	4	1	40	14	100	5	4	0	130	36	400
4	4	2	47	15	120	5	4	1	170	58	400
4	5	0	41	14	100	5	4	2	220	70	440
4	5	1	48	15	120	5	4	3	280	100	710
5	0	0	23	6.8	70	5	4	4	350	100	710
5	0	1	31	10	70	5	4	5	430	150	1 100
5	0	2	43	14	100	5	5	0	240	70	710
5	0	3	58	22	150	5	5	1	350	100	1 100
5	1	0	33	10	100	5	5	2	540	150	1 700
5	1	1	46	14	120	5	5	3	920	220	2 600
5	1	2	63	22	150	5	5	4	1 600	400	4 600
5	1	3	84	34	220	5	5	5	>1 600	700	--
5	2	0	49	15	150						

Note 1: This table uses three dilutions, and for each of which, it inoculates five tubes. The sample volume of each tube in the three dilutions is 0.1g (mL), 0.01g (mL), and 0.001 g (mL), respectively.
Note 2: When the sample volume inoculated in three dilutions is changed to 1g (mL), 0.1g (mL), and 0.01g (mL), the value in the table should be reduced by 10 times accordingly; if 0.01 g (mL), 0.001 g (mL) and 0.000 1 g (mL) are used, the values in the table should be expanded by 10 times accordingly, and so on.
Note 3: If necessary, the value in this table can be multiplied by 100 to report the most probable number of *Escherichia coli* in every 100 g (mL) sample, expressed in MPN/100 g (mL).

END TRANSLATION

Attachments:

GB 4789.38-2025 E Coli Count.pdf