



National Standard of the People's Republic of China

GB 4789.3—2010

National Food Safety Standard

Food microbiological examination: Enumeration of coliforms

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issued by the Ministry of Health of People's Republic of China

Foreword

This standard substitutes GB/T 4789.3-2008 《Food hygiene microbiological examination: Enumeration of coliforms》

Compared with GB/T 4789.3-2008, the main modifications of this standard are as follows:

- Modify Chinese-English titles in the standard;
- The scope of plate colony counts in “Method 2 Coliform Plate Counts” is modified to “15 CFU~150 CFU”;
- Delete “Method 3 Coliform Petrifilm™ Method”

The Annex A and Annex B here of the standard are informative annex.

The releases of all editions substituted by this Standard are as follows:

- GB 4789.3-1984、GB 4789.3-1994、GB /T 4789.3-2003、GB /T 4789.3-2008。

National Foods Safety Standard

Food microbiological examination: Enumeration of coliforms

1. Scope

This standard provides the method for enumeration of coliforms in foods

This standard is applicable to enumeration of coliforms in various foods.

2. Terms and Definitions

The following terms and definitions are applicable to this standard.

2.1 Coliforms

A cluster of concurrently aerobic and anaerobic gram negative sporeless bacilli which can ferment lactose and generate acid and gas if cultured in 36□ for 24 hours.

2.2 Most probable number; MPN

A Poisson distribution-based indirect counting method

3. Devices and Materials

Devices and materials except conventional sterilizing and culturing devices for microbiological laboratory are as follows:

3.1 Thermostatic incubator: 36°C±1°C

3.2 Refrigerator: 2□~5°C

3.3 Thermostatic water bath: 46°C±1°C

3.4 Balance: sensitive to 0.1g

3.5 Homogenizer

3.6 Oscillator

3.7 Aseptic suction tube: 1ml (with 0.01ml graduation), 10ml (with 0.1ml graduation) or micro pipettor and sucker

3.8 Aseptic conical flask: 500ml in volume

3.9 Aseptic culture dish: 90mm in diameter

3.10 pH meter or pH colorimetric tube or precision pH test paper

3.11 Colony counter

4. Culture Media and Reagents

4.1 Lauryl sulfate tryptose (LST) broth: See Chapter A.1 of Annex A

4.2 Brilliant green lactose bile (BGLB) broth: See Chapter A.2 of Annex A

4.3 Violet red bile agar (VRBA): See Chapter A.3 of Annex A

4.4 Phosphate buffer solution: See Chapter A.4 of Annex A

4.5 Aseptic physiological saline: See Chapter A.5 of Annex A

4.6 1mol/L Sodium hydroxide (NaOH): See Chapter A.6 of Annex A

4.7 1mol/L Hydrochloric acid (HCL): See Chapter A.7 of Annex A

4.8 Petrifilm™ coliform examination test wafer and plate

The First Method: Coliform MPN Counts

5. Examination Procedures

See Figure 1 for Coliform MPN counts examination procedures.

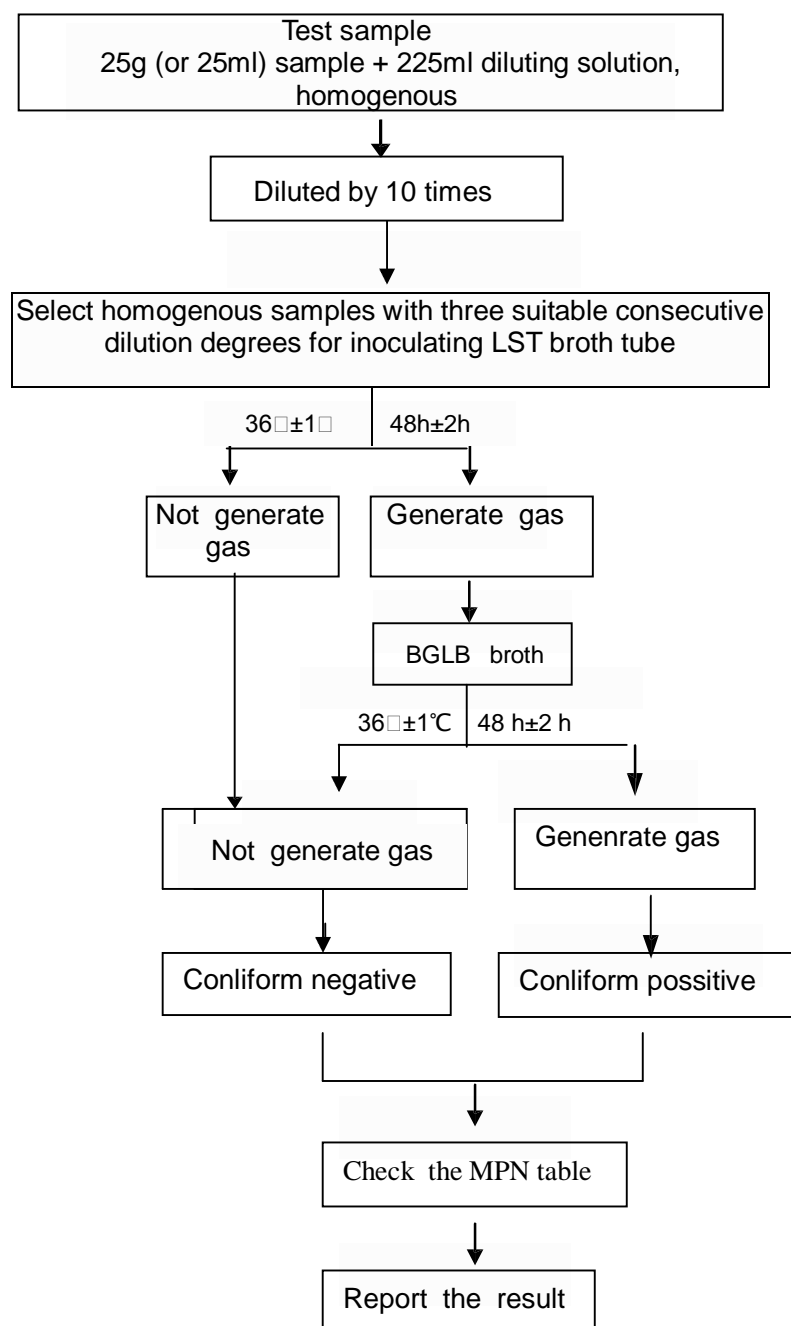


Figure 1: Coliform MPN counts examination procedures

6. Operating Steps

6.1 Diluting the samples

6.1.1 Solid and semi-solid samples: Weigh and take 25g sample, put it in an aseptic homogenizing cup which contains 225ml phosphate buffer solution or physiological

saline, and homogenize it 8000r/min to 10000r/min for 1 to 2 minutes; or put it in an aseptic homogenizing bag which contains 225ml phosphate buffer solution or physiological saline and homogenize it by flapping with a smack type homogenizer for 1 to 2 minutes to get 1:10 homogenous sample liquor.

- 6.1.2 Liquid samples: Suck 25ml sample with an aseptic suction tube, put it in an aseptic conical flask (with a certain number of aseptic glass beads placed inside beforehand) which contains 225ml phosphate buffer solution or physiological saline, and blend the solution properly to get 1:10 homogenous sample liquor.
- 6.1.3 pH value of the homogenous sample liquor should be between 6.5 and 7.5. Regulate its pH value with 1mol/L sodium hydroxide (NaOH) or 1mol/L hydrochloric acid (HCL) respectively, when necessary.
- 6.1.4 Suck 1ml 1:10 homogenous sample liquor with a 1ml aseptic suction tube or micro pipettor, empty it in an aseptic test tube (attention: the pointed end of test tube or sucker should not touch the diluting liquid) which contains 9ml phosphate buffer solution or physiological saline slowly along the tube wall, jolt the test tube or beat upon it with a 1ml aseptic suction tube so that it will be homogenized properly to get 1:100 homogenous sample liquor.
- 6.1.5 According to estimation of sample pollution, make homogenous sample liquor series diluted by 10 times and above as per the above-stated operating steps. For every increased diluting degree, replace one 1ml aseptic suction tube or sucker. From preparation of homogenous sample liquor to completion of inoculation, the whole process should be within 15 minutes.

6.2 Primary fermentation test

For every sample, select homogenous sample liquors with three suitable consecutive dilution degrees (stock solution may be chosen in case of liquid sample), and for every dilution degree, inoculate 3 tubes of lauryl sulfate tryptone (LST) broth, 1ml each tube (if more than 1ml is inoculated, double LST broth should be adopted). Make them cultured in $36^{\circ}\pm 1^{\circ}$ for $24\text{h} \pm 2\text{h}$ and observe whether bubbles are generated in the tubes; if there is no any bubble, make them cultured for $48\text{h} \pm 2\text{h}$ in total. Tubes without bubbles are coliform negative and tubes with bubbles go through secondary fermentation test.

6.3 Secondary fermentation

Take 1 circle of cultures from each of all LST broth tubes which ferment and generate gas within $48\text{h} \pm 2\text{h}$ respectively with an inoculation ring, transfer-inoculate them to brilliant green lactose bile (BGLB) broth, culture them in $36^{\circ}\pm 1^{\circ}$ for $48\text{h} \pm 2\text{h}$, observe bubble-generation. Tubes which generate bubbles are recorded as coliform positive.

6.4 Reporting most probable number (MPN) of coliforms

According to the number of tubes which are coliform positive verified through 6.3, search the MPN Table (see Annex B) to report coliform MPN counts in every gram (or ml) of sample.

The Second Method: Coliform Plate Counts

7. Examination Procedures

See Figure 2 for coliform plate counts examination procedures.

Test sample
25g (or 25ml) sample + 225ml diluting
solution, homogenous

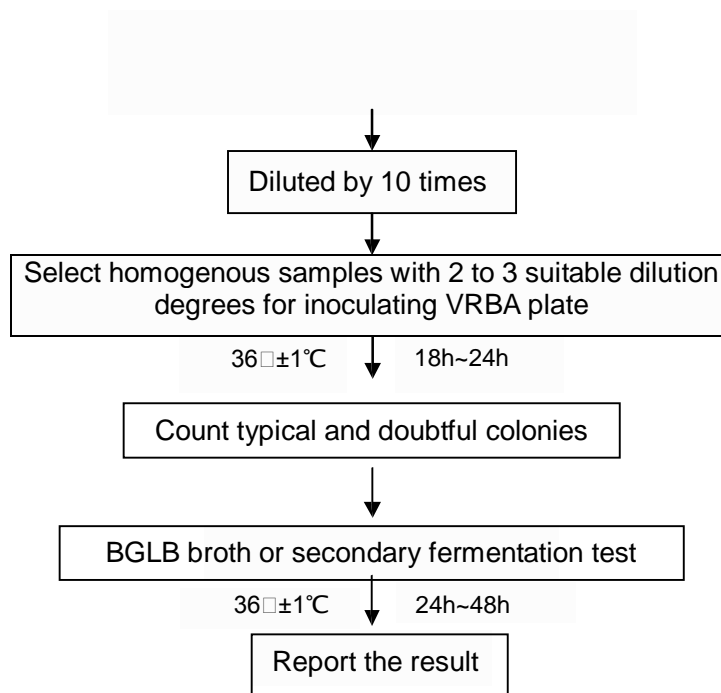


Figure 2: Coliform plate counts examination procedures

8. Operating Steps

8.1 Diluting the samples

Dilute the samples as per clause 6.1.

8.2 Plate count

8.2.1 Select 2 to 3 suitable consecutive dilution degrees, for each of which, inoculate two aseptic flat dishes, 1ml per dish, and at the same time, add 1ml physiological saline in the two aseptic flat dishes for blank control.

8.2.2 Pour 15ml to 20ml violet red bile agar (VRBA) which is cooled to 46°C in each of the flat dishes in time, turn the flat dishes carefully to blend the culture medium with the sample liquor properly. After agar is coagulated, add 3ml to 4ml VRBA to cover the plate surface. Flip the plate and put it in 36±1°C for 18h to 24h.

8.3 Selecting colony plate counts

Select plates with colony counts between 15 to 150 and count typical and doubtful coliforms appearing on plates. Typical coliforms are in purple, 0.5mm in diameter or bigger, surrounded by red bile salt deposit circle.

8.4 Verification test

Select 10 typical and doubtful coliforms of different types on VRBA plates, transfer-inoculate them in BGLB broth tubes respectively, and culture them in 36±1°C for 24h to 48h and observe bubble generation. All BGLB broth tubes which generate gas are reported as coliform positive.

8.5 Reporting coliform plate counts

The percentage of coliform positive test tubes finally confirmed are multiplied by the coliform plate counts in clause 8.3 and dilution multiples to get the number of coliforms per gram (or ml) sample. E.g.: For 10⁻⁴ diluted sample liquor, 100 typical and doubtful coliforms exist on VRBA plates, and 10 of them are selected and inoculated to BGLB

broth tubes, and 6 positive tubes are confirmed. According to the foregoing, it's determined that the number of coliforms in this sample is: $100 \times \frac{6}{10} \times 10^4/\text{g (ml)} = 6.0 \times 10^5 \text{ CFU/g (CFU/ml)}$.

Appendix A
(Regulatory annex)
Culture Media and Reagents

A.1 Lauryl sulfate tryptose (LST) broth

A.1.1 Ingredients

Typtone or Trypticase	20.0 g
Sodium chloride	5.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate	2.75 g
Monopotassium phosphate	2.75 g
Lauryl sodium sulfonate	0.1 g
Distilled water	1000 ml
pH 6.8±0.2	

A.1.2 Preparation method

Dissolve the above-stated ingredients in distilled water and regulate the pH. Separately fill the solution in test tubes having small glass backward tubes, 10ml each, and sterilize them under high pressure in 121°C for 15 minutes.

A.2 Brilliant green lactose bile (BGLB) broth

A.2.1 Ingredients

Peptone	10.0 g
Lactose	10.0 g
Oxgall or oxbile solution	200.0 ml
0.1% brilliant green water solution	13.3 ml
Distilled water	1000 ml
Ph 7.2±0.1	

A.2.2 Preparation method

Dissolve peptone and lactose in about 500ml distilled water, add in 200ml oxgall solution (dissolve 20.0g dehydrated oxgall powder in 200ml distilled water, regulate pH 7.0 to 7.5), dilute it with distilled water to 975ml, regulate its pH to 7.4, and then add in 13.3ml 0.1% brilliant green water solution, dilute it with distilled water to 1000ml, filter the solution, and separately fill the filtrate in test tubes having small glass backward tubes, 10ml each. Sterilize them under high pressure in 121°C for 15 minutes.

A.3 Violet red bile agar (VRBA)

A.3.1 Ingredients

Peptone	7.0 g
Yeast cream	3.0 g
Lactose	10.0 g
Sodium chloride	5.0 g
Bile salt or No.3 bile salt	1.5 g
Neutral red	0.03 g
Crystal violet	0.002 g
Agar	15g- 18g

Distilled water	1000 ml
pH 7.4±0.1	

A.3.2 Preparation method

Dissolve the foregoing ingredients in distilled water, put the solution in stillness for several minutes, blend it fully, and regulate the pH. Boil it for 2 minutes, cool the culture medium to 45℃~50℃ pour plate. Prepare it for immediate use no more than 3 hours later.

A.4. Buffer phosphate**A.4.1 Ingredients**

Monopotassium phosphate (KH ₂ PO ₄)	34.0 g
Distilled water	500 ml
pH 7.2	

A.4.2 Preparation method

Stock solution: Weigh and take 34.0g monopotassium phosphate and dissolve it in 500ml distilled water. Regulate its pH to 7.2 with about 175ml 1mol/L sodium hydrochloride solution, dilute with distilled water to 1000ml, and store it in refrigerator.

Diluted solution: Take 1.25ml stock solution, dilute it with distilled water to 1000ml, separately fill the solution in a suitable container, and sterilize it under high pressure in 121℃ for 15 minutes.

A.5 Aseptic physiological saline**A.5.1 Ingredients**

Sodium chloride	8.5 g
Distilled water	1000 mL

A.5.2 Preparation method

Weigh 8.5g sodium chloride, dissolve it in 1000ml distilled water, and sterilize it under high pressure in 121℃ for 15 minutes.

A.6 1 mol/L Sodium hydroxide**A.6.1 Ingredients**

Sodium hydroxide	40.0 g
Distilled water	1000 mL

A.6.2 Preparation method

Weigh 40g sodium hydroxide, dissolve it in 1000ml distilled water, and sterilize it under high pressure in 121℃ for 15 minutes.

A.7 1 mol/L hydrogen chloride**A.7.1 Ingredients**

HclHydrogen chloride	90 mL
Distilled water	1 000 mL

A.7.2 Prepaation method

Take 90ml concentrated hydrochloric acid, dilute it with distilled water to 1000ml, and sterilize it under high pressure in 121℃ for 15 minutes.

Appendix B
(Regulatory annex)

Most Probable Number (MPN) of Coliform Retrieval Table

B.1 Most Probable Number (MPN) of Coliform Retrieval Table

See Table 1 for the most probable number (MPN) of coliforms per gram (or millimeter) test sample.

Table 1: Most probable number (MPN) of coliforms per gram (or millimeter) test sample

Number of positive tubes			MPN	95% confidence		Number of positive tubes			MPN	95% confidence	
0.10	0.01	0.001		Lower limit	Upper limit	0.10	0.01	0.001		Lower limit	Upper limit
0	0	1	<	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	3.0	1.2	18	2	3	0	29	8.7	94
0	2	0	6.1	1.2	18	2	3	1	36	8.7	94
0	3	0	6.2	3.6	38	3	0	0	23	4.6	94
1	0	0	9.4	0.17	18	3	0	1	38	8.7	110
1	0	1	3.6	1.3	18	3	0	2	64	17	180
1	0	2	7.2	3.6	38	3	1	0	43	9	180
1	1	0	11	1.3	20	3	1	1	75	17	200
1	1	1	7.4	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	11	4.5	42	3	2	0	93	18	420
1	3	0	15	4.5	42	3	2	1	150	37	420
2	0	0	16	1.4	38	3	2	2	210	40	430
2	0	1	9.2	3.6	42	3	2	3	290	90	1000
2	0	2	14	4.5	42	3	3	0	240	42	1000
2	1	0	20	3.7	42	3	3	1	460	90	2000
2	1	1	15	4.5	42	3	3	2	1100	180	4100
2	1	2	20	8.7	94	3	3	3	> 1100	420	
			27								

Note 1: This table adopts three dilution degrees [0.1g (or 0.1ml), 0.01g (or 0.01ml) and 0.001g (or 0.001ml)], for which of which, three tubes are inoculated.

Note 2: If the tested amounts as shown in this table are changed to 1g (or 1ml), 0.1g (or 0.1ml) and 0.01g (or 0.01ml), figures in this table should be decreased by 10 times accordingly; if the tested amounts are changed to 0.01g (or 0.01ml), 0.001g (or 0.001ml) and 0.0001g (or 0.0001ml), figures in this table should be increased by 10 times accordingly, so on and so forth.