National Standard of the People's Republic of China

GB 4789.3-2016

National Standards For Food Safety

Food Microbiological Analysis Enumeration Of Coliforms

2016-12-23 Issue 2017-06-23 Implementation

Forward

This standard replaces GB4789.3-2010 “National Food Safety Standard for Microbiological Examination of Food - Coliform Count”,GB/T4789.32-2002 "Microbiological examination of food hygiene - Rapid detection of coliform bacteria" and SN/T0169-2010 "Detection methods for coliform bacteria, fecal coliform bacteria, and coliform bacteria in imported and exported foods" - Coliform count section.

Compared with GB4789.3-2010, the main changes in this standard are as follows:

——Added inspection principles;

—— Modified the scope of application;

—— Modified the morphological description of typical colonies;

——Modified the selection of plate colony count for the second method;

——Modified the second method validation test;

——Revised the report for the second method of plate counting.

National Standards For Food Safety

Food Microbiological Analysis Enumeration Of Coliforms

1 Scope

This standard specifies the method for counting Coliforms in foods.

The first method of this standard is applicable to the count of coliforms in foods with low coliforms content; The second method is applicable to the count of coliform in foods with high coliform content.

2 Terms and definitions

2.1 Coliforms

Under certain culture conditions, aerobic and facultative anaerobic gram-negative Bacillus spp. can ferment lactose, produce acid and produce gas.

2.2 The most probable number :MPN

An indirect counting method based on Poisson distribution.

3 Testing principle

3.1 MPN method

MPN is a quantitative method combining statistics and microbiology. After a series of dilutions and cultures, the maximum probable number of coliforms in the tested samples was deduced by using statistical probability theory according to the minimum dilution degree and the maximum dilution degree of growth.

3.2 Plate Count Method

Coliforms ferment lactose to produce acid in solid medium, and form countable red or purple colony with or without sedimentation ring under the action of indicator.

4 Equipment and materials

In addition to routine sterilization and culture equipment in microbiology laboratory, other equipment and materials are as follows:

4.1 Constant temperature incubator: 36℃ ±1℃.

4.2 Refrigerator: 2℃~5℃.

4.3 Constant Temperature Water Bath Box: 6℃±1℃.

4.4 Balance: 0.1g of sensibility.

4.5 Homogenizer.

4.6 Oscillator.

4.7 Sterile pipette: 1mL (with 0.0 mL scale), 10 mL (with 0.1mL scale) or micro pipette and suction head.

4.8 Aseptic conical bottle: capacity 500mL.

4.9 Sterile Petri dish: 90mm in diameter.

4.10 PH meter or pH colorimeter or precise pH test paper.

4.11 Colony counter.

5 Medium and Reagents

5.1 January Lauryl sulfate tryptone (LST) broth: see A.1.

5.2 Briliant Green Lactose Bile (BGLB) broth: see A.2.

5.3 Violet violet neutral biliary agar (VRBA): see A.3.

5.4 Aseptic phosphate buffer: see A.4.

5.5 Sterile saline: see A.5.

5.6 1mol/L NaOH solution: see A.6.

5.7 1mol/LHCl solution: see A.1

First Method: Coliforms MPN Counting Method

6 Inspection procedure

The inspection procedure for the MPN count of coliform bacteria is shown in Figure 1.

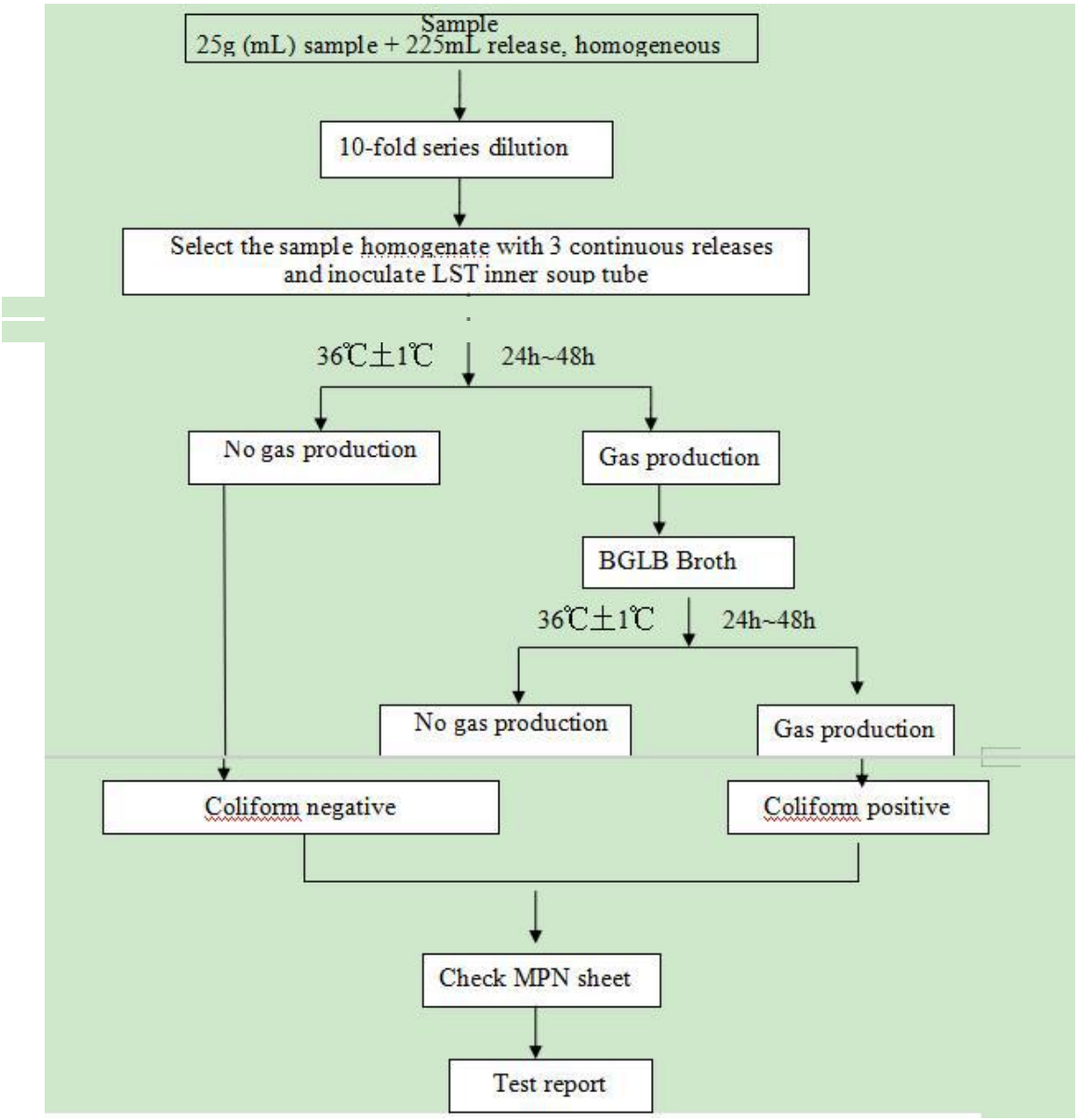

Figure 1. MPN Counting Procedure for Coliform Bacteria

7 Operation steps

7.1. Sample dilution

7.1.1 Solid and semi-solid samples: 25g samples were weighed and put into sterile homogeneous cups containing 225mL phosphate buffer or saline, 8000r/min~10000r/min for 1min~2min, or in sterile homogeneous bags containing 225mL phosphate buffer or saline, beating for 1min~2min with a beating homogenizer to make sample homogeneous solution of 1:10.

7.1.2 Liquid Samples: 25mL Samples were taken by sterile straw and placed in sterile

conical flasks containing 225mL phosphate buffer or saline solution.
(A proper number of sterile glass beads are pre-arranged in the bottle) or other sterile containers are fully shaken or placed in a mechanical oscillator to shake, fully mix, and make a sample homogenate of 1:10.

7.1.3 The pH of sample homogenate should be between 6.5 and 7.5, adjusted by 1mol/L NaOH or 1mol/LHCl respectively when necessary.

7.1.4 Take 1:10 sample homogenate 1mL with 1mL sterile suction tube or micro-pipette, slowly inject 9mL phosphate buffer or saline along the tube wall (pay attention to the suction tube or the tip of the suction head do not touch the diluted liquid surface), shake the test tube or replace it with 1mL sterile suction tube and blow repeatedly to make it mix evenly, and make 1:100 sample uniform. Liquid.

7.1.5 Based on the estimation of sample contamination, a series of diluted sample homogenates with ten-fold increment were prepared according to the above operation. For each incremental dilution, 1mL sterile straw or suction head was used. The whole process should not exceed 15minutes from homogenate preparation to inoculation.

7.2 Initial Fermentation Test

Each sample was inoculated with 3 tubes of lauryl sulfate tryptone broth (LST) broth at each dilution, and 1mL of LST broth was inoculated in each tube (if the inoculation volume exceeded 1mL, then double-feed LST broth was used). The samples were cultured at 36℃± 1℃ for 24h±2h. Whether bubbles were produced in the inverted tube or not was observed for24h±2h. Recurrent fermentation test (confirmatory test) was performed in Gas patients, if not, gas production continued to be cultured for 48h±2h. Gas producers were subjected to a relapse fermentation test. Those who did not produce gas were coliform negative.

7.3 Recurrent Fermentation Test (Confirmation Test)

One ring of culture was taken from the gas-producing LST broth tube by inoculation ring and transplanted into the BGLB tube. The gas production was observed by incubation at 36℃±1℃ for 48h±2h. Gas producers are counted as coliform positive tubes.

7.4 Report on the Most Possible Number of Coliforms (MPN)

According to the number of BGLB positive tubes confirmed by 7.3, the MPN table was retrieved (see Appendix B), and the MPN values of coliforms in each g (mL) sample were reported.

Second Method: Plate Counting of Coliform Bacteria

8 Inspection Procedure

The procedure of coliform plate counting is shown in Figure 2.

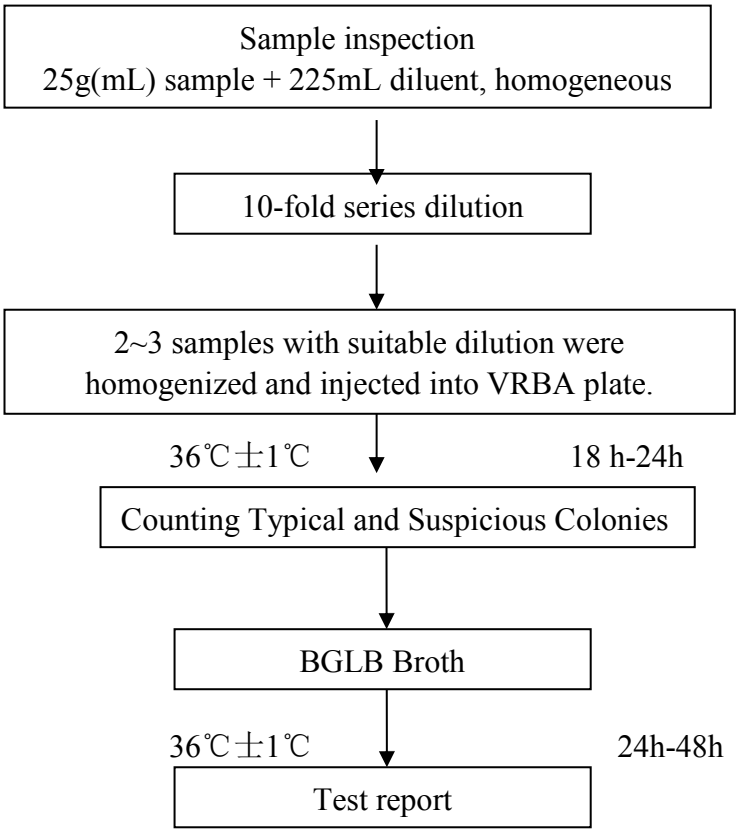

Fig. 2 Inspection procedure of plate counting method for coliform bacteria

9 Operation steps

9.1 Sample dilution: Proceed according to 7.1.

9.2 Plate Counting

9.2.1 Two to three suitable continuous dilutions were selected, and two sterile dishes were inoculated with each dilution, 1mL per dish. At the same time, 1mL saline was added to sterile dishes as blank control.

9.2.2 The crystal violet neutral red biliary salt agar (VRBA) was melted at 15mL ~ 20mL in time and then poured into each dish at constant temperature to 46 C. Carefully rotate the dish and mix the medium with the sample solution. After agar solidification, add 3mL~4mLVRBA to cover the surface of the plate. The plate was reversed and incubated at 36℃±1℃ for 18 h to 24 h.

9.3 Selection of Plate Colony Number

The typical and suspicious coliform colonies (e.g. smaller colony diameter) were counted on the plate with the number of colonies between 15CFU and 150CFU. Typical colonies are purple red with red bile salt precipitation rings around the colonies. The diameter of the colonies is 0.5mm or larger. The minimum dilution plate is lower than the specific number of colonies recorded by 15CFU.

9.4 Confirmation Test

Ten different types of typical and suspicious colonies were selected from VRBA plate, and all the typical and suspicious colonies were selected from less than 10 colonies. BGLB broth tube was transplanted and cultured at 36℃±1℃ for 24h-48h to observe the gas production. Where BGLB broth tube produces gas, it can be reported to be positive for coliform bacteria.

9.5 Report on Plate Counting of Coliforms

The proportion of coliform positive test tubes multiplied by the number of planar colonies counted in 9.3, and then multiplied by the dilution multiple, that is, the number of coliform colonies per g (mL) sample. Example: 10^{-4} sample diluent 1mL, 100 typical and suspicious colonies on VRBA plate, 10 of which were inoculated with BGLB broth tube. 6 positive tubes were confirmed. The coliform group number of the sample was 100 *6/10 */10^4g(mL) = 6.0 *10^5CFU/g(mL). If all dilutions (including the original liquid sample) of the plate are aseptic, the minimum dilution factor is calculated by multiplying the dilution factor by less than 1.

Appendix A: Culture Medium and Reagents

A.1 Lauryl sulfate tryptone (LST) broth

A.1.1 Composition

Tryptone or casein	20.0g
Sodium chloride	5.0g
Lactose	5.0g
Dipotassium hydrogen phosphate (K2HPO4)	2.75g
Potassium dihydrogen phosphate (KH2PO4)	2.75g
Sodium lauryl sulfate	0.1g
Distilled water	1000mL

A.1.2 Preparation Method

The above-mentioned components were dissolved in distilled water and the pH was adjusted to 6.8+0.2. Separated into small glass inverted tubes, each 10mL. High-pressure sterilization at 121℃for 15minutes.

A.2 Huanghuang Green Lactose Bile Salt (BGLB) Broth

A.2.1 Composition

Peptone	10.0g
Lactose	10.0g
Oxgall or Oxbill solution	200mL
0.1% brilliant green aqueous solution	13.3mL
Distilled water	800mL

A.2.2 Preparation Method

The peptone and lactose were dissolved in about 500 mL distilled water, and the solution of bovine bile powder was added to 200 mL (20.0 g dehydrated bovine bile powder was dissolved in 200 mL distilled water, the pH was adjusted to 7.0-7.5), the distilled water was diluted to 975 mL, the pH was adjusted to 7.2+0.1, the 0.1% brilliant green water solution was added to 13.3 mL, the distilled water was

supplemented to 1000 mL, and filtered with cotton, and then the glass was separated into small pours. In the test tube, each tube is 10 mL. High-pressure sterilization at 121℃ for 15 minutes.

A.3 Crystal Violet Neutral Red Bile Salt Agar (VRBA)

A.3.1 Composition

Peptone	7.0g
Yeast extract	3.0g
Lactose	10.0g
Sodium chloride	5.0g
Bile salt or No.3 bile salt	1.5g
Neutral Red	0.03g
Crystal Purple	0.002g
Agar	15g~18g
Distilled water	1000mL

A.3.2 Preparation Method

Dissolve the above ingredients in distilled water for a few minutes, stir well, and adjust the pH to 7.4 + 0.1. After boiling for 2 minutes, the medium was melted and poured into the plate at 45℃~50℃ at constant temperature. Temporary preparation before use shall not exceed 3h.

A.4 Phosphate buffer

A.4.1 Composition

Potassium dihydrogen phosphate (KH2PO4)	34.0g
Distilled water	500mL

A.4.2 Preparation Method

Storage solution: 34.0g potassium dihydrogen phosphate was dissolved in 500 mL distilled water, the pH was adjusted to 7.2±0.2 with 1 mol/L sodium hydroxide solution of about 175mL, and diluted to 1000mL with distilled water and stored in refrigerator. Diluting solution: Take 1.25mL of storage liquid, dilute it to 1000mL with distilled water, and separate it into suitable containers. Sterilize it at 121℃ for

15 minutes under high pressure.

A.5 Aseptic Saline

A.5.1 Composition

Sodium chloride	8.5g
Distilled water	1000mL

A.5.2 Preparation Method

8.5g sodium chloride was dissolved in 1000mL distilled water and sterilized at 121℃for 15 min under high pressure.

A.6 1mol NaOH solution

A.6.1 Composition

NaOH	40.0g
Distilled water	1000mL

A.6.2 Preparation Method

Sodium hydroxide 40 g was weighed and dissolved in 1000 mL sterile distilled water.

A.7 1mol HCl solution

A.7.1 Composition

HCl	90mL
Distilled water	1000mL

A.7.2 Preparation Method

Remove 90mL of concentrated hydrochloric acid and dilute it to 1000mL with sterile distilled water.

Appendix B:
Search Table for Most Probable Number of Coliforms (MPN)

B. 1 Search Table for Most Probable Number of Coliforms (MPN)

The most probable number of coliforms (MPN) in each g (mL) sample is shown in Table B.1.

Table B.1 Search Table for Most Possible Number of Coliforms (MPN)

Negative tube count			MPN	95% Confidence limit		Positive tube count			MPN	95% Confidence limit	
0.10	0.01	0.001		Lower limit	Upper limit	0.10	0.01	0.001		Lower limit	Upper limit
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	1000
2	0	2	20	4.5	42	3	3	0	240	42	1000
2	1	0	15	3.7	42	3	3	1	460	90	2000
2	1	1	20	4.5	42	3	3	2	1100	180	4100
2	1	2	27	8.7	94	3	3	3	>1100	420	—

Note 1: Three dilutions [0.1g(mL), 0.01g(mL), 0.001g(mL)] are used in this table, and no dilutions are inoculated into three tubes.

Note 2: If the sample size listed in the table is changed to 1g(mL), 0.1g(mL.) and 0.01g(mL), the number in the table should be reduced by 10 times accordingly; if use 0,01 (mL.), 0.001g(ml.) and 0,0001g (mL) are changed, the number in the table should be increased by 10 times accordingly, and the rest of the analogies are made.