



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 4789.41-2016

**National food safety standard - Microbiological Examination of
Food Hygiene - Examination of Enterobacteriaceae**

食品安全国家标准 食品微生物学检验 肠杆菌科检验

(English Translation)

Issued on: August 31, 2016

Implemented on: March 1, 2017

**Issued by: National Health and Family Planning Commission of the
People's Republic of China**

Table of Contents

1 Application Scope...	3
2 Terms and Definitions...	3
3 Apparatus and Materials...	3
4 Culture Media and Reagents...	4
5 Test Procedures...	5
6 Operating Procedures...	5
7 Report...	9
8 Test Procedures...	9
9 Operating Procedures...	11
10 Report...	11
Annex A Examples for Calculation of Results...	12
Annex B Culture Media and Reagents...	14

1 Application Scope

This Standard specifies the method for the test of Enterobacteriaceae in foods.

The first method of this Standard applies to the bacterial counts of Enterobacteriaceae in the foods which has a high content of Enterobacteriaceae; and the second method applies to the bacterial counts of Enterobacteriaceae in the foods which has a low content of Enterobacteriaceae.

2.1 Enterobacteriaceae

The oxidase-negative aerobic or facultative anaerobic Gram-negative non-spore- bearing bacillus, which ferments glucose producing acid under given conditions.

2.3 most probable number; MPN

An indirect enumeration method based on Poisson's distribution.

3 Apparatus and Materials

In addition to the conventional sterilization and culture apparatus in the microbiological laboratories, the other apparatus and materials are as follows.

3.1

Thermostatic incubator. $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

3.2

Refrigerator. $2^{\circ}\text{C} \sim 5^{\circ}\text{C}$.

3.3

Water bath. $46^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

3.6

Homogenizer.

3.7

Shaker.

3.8

Sterile pipettes. 1 ml (having graduates of 0.01 ml), 10 ml (having 0.1 ml) or micropipettors and tips.

3.9

Sterile conical flasks or equivalent vessels. capacities 150 ml and 500 ml.

3.12

pH meters or pH colorimetric tubes or precise pH test paper.

4 Culture Media and Reagents

4.1

Buffered peptone water (BPW). see B.1.

4.2

Buffered glucose brilliant green bile broth (EE broth). see B.2.

4.5

Glucose agar. see B.5.

4.6

Gram stain solution. see B.6.

4.7

Oxidase reagent. see B.7.

4.8

Sterile 1 mol/l NaOH. see B.8.

5 Test Procedures

See Figure 1 for the test procedures for the Enterobacteriaceae plate count method.

6.1 Specimen dilution

Test specimen

6.1.1

Solid and semi-solid specimens. take 25 g of specimen to place into a sterile homogeneous cup containing 225 ml of BPW, homogenize for 1 min ~ 2 min at 8 000 r/min ~ 10 000 r/min, or place into a homogeneous bag containing 225 ml of BPW,

6.1.2

Liquid specimens. use a sterile pipette to absorb 25 ml of specimen to pour into a sterile conical flask (in which an appropriate quantity of sterile glass beads are provided in the flask in advance) containing 225 ml of BPW, mix up, and make homogeneous specimen solutions of 1.10.

6.2.1 In accordance with the estimation of the contamination of specimens and

relevant limit requirements, select 2 ~ 3 homogeneous specimen solutions (liquid specimens may be raw solutions) of appropriate continuous dilution and inoculate the solutions of each dilution degree to 2 sterile plates. And Meanwhile, absorb 1 ml of

BPW each to add to two sterile plates as blank control.

6.2.4 Turn over the plate after the upper-layer agar solidifies on the VRBGA plate and culture for 18 h ~ 24 h at $36^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

6.3 Enumeration and confirmation of typical colonies

6.3.1

The typical colonies of Enterobacteriaceae are pink to red or violet colonies with or without precipitation rings. Select the plates with typical colony count between

15 CFU ~ 150 CFU and without spreading growth of colonies and only enumerate the typical colonies. The colony counting is expressed by the colony-forming unit, CFU.

6.3.5

Confirmation of typical colonies.

6.4.1 General principles

If the plate counts of typical colonies of two continuous dilution degrees are within the appropriate counting range, calculate in accordance with Formula (1). See A.1, A.2, A.3 and A.4 for the examples.

6.4.2 Small colony counts

If all the typical colony counts of the plate of minimum dilution degree (including the raw solution of the liquid specimen) are less than 15 CFU and there are confirmed Enterobacteriaceae colonies, then multiply the confirmed colony count by the minimum dilution factor.

6.4.3 Special circumstances

If all the typical colony counts on the plate of the first dilution degree are greater than 150 CFU and there are confirmed Enterobacteriaceae colonies; and if there is no confirmed Enterobacteriaceae colony on the plate of the second dilution degree or the typical colony counts are not between 15 CFU ~ 150 CFU, then multiply the confirmed colony count by the first dilution factor. See A.5 for the example.

7 Report

7.1

When the colony count is less than 100, it shall be reported with integers.

7.2

When the colony count is greater or equal to 100, take the first two digits after rounding off the third digit, and use 0 to replace the places after it;

7.3

The numbers shall be rounded off to the nearest whole numbers.

7.4

If it is impossible to count because of spreading colonies on all the plates, then report the colony spreading.

8 Test Procedures

See Figure 2 for the test procedures for the Enterobacteriaceae most probable number method.