National Standard of the People's Republic of China

GB 4789.2-2022

National Standards For Food Safety
Food Microbiological Analysis Aerobic Plate Count

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Forward

This standard replaces GB4789.2-2016 "National Food Safety Standard for Food Microbiological Analysis Detection Of Aerobic Plate Count".Compared with GB4789.2-2016, the main changes in this standard are as follows:

——Added Appendix B;

——Equipment and materials have been modified;

——Modified the culture medium and reagents;

——Revised inspection procedures;

——Modified the operating steps;

——Revised Appendix A.

National Standards For Food Safety
Food Microbiological Analysis Aerobic Plate Count

1 Scope

This standard specifies the method for determining the total bacterial count (Aerobic plate count) in food.

This standard is applicable to the determination of total bacterial count in food.

2 Terms and definitions

2.1 Aerobic Plate Count

The total number of microbial colonies formed in each g (mL) of the food sample after treatment and cultivation under certain conditions (such as culture medium, culture temperature, and culture time).

3 Equipment and Materials

In addition to conventional sterilization and cultivation equipment in the microbiological laboratory, other equipment and materials are as follows:

a) Thermostatic incubator: 36 ℃± 1 ℃, 30 ℃± 1 ℃.

b) Refrigerator: 2 ℃~5 ℃.

c) Thermostatic device: 48 ℃± 2 ℃.

d) Balance: Sensitivity is 0.1g.

e) Homogenizer.

f) Oscillator.

g) Sterile pipette: 1mL (with a 0.01mL scale), 10mL (with a 0.1mL scale), or micropipette and pipette.

h) Sterile conical flask: capacity 250mL, 500mL.

i) Sterile culture dish: diameter 90mm.

j) PH meter or pH colorimetric tube or precision pH test paper.

k) Magnifier or/and colony counter.

4 Culture medium and reagents

4.1 Plate counting agar medium: see A.1.

4.2 Total bacterial count test piece: It should comply with the quality control requirements of plate counting agar medium in GB4789.28, and the main nutritional components should be consistent with the formula of plate counting agar medium.

4.3 Sterile phosphate buffer solution: see A.2.

4.4 Sterile physiological saline: see A.3.

5 Inspection Procedure

The inspection procedure for the total aerobic plate count is shown in Figure 1.

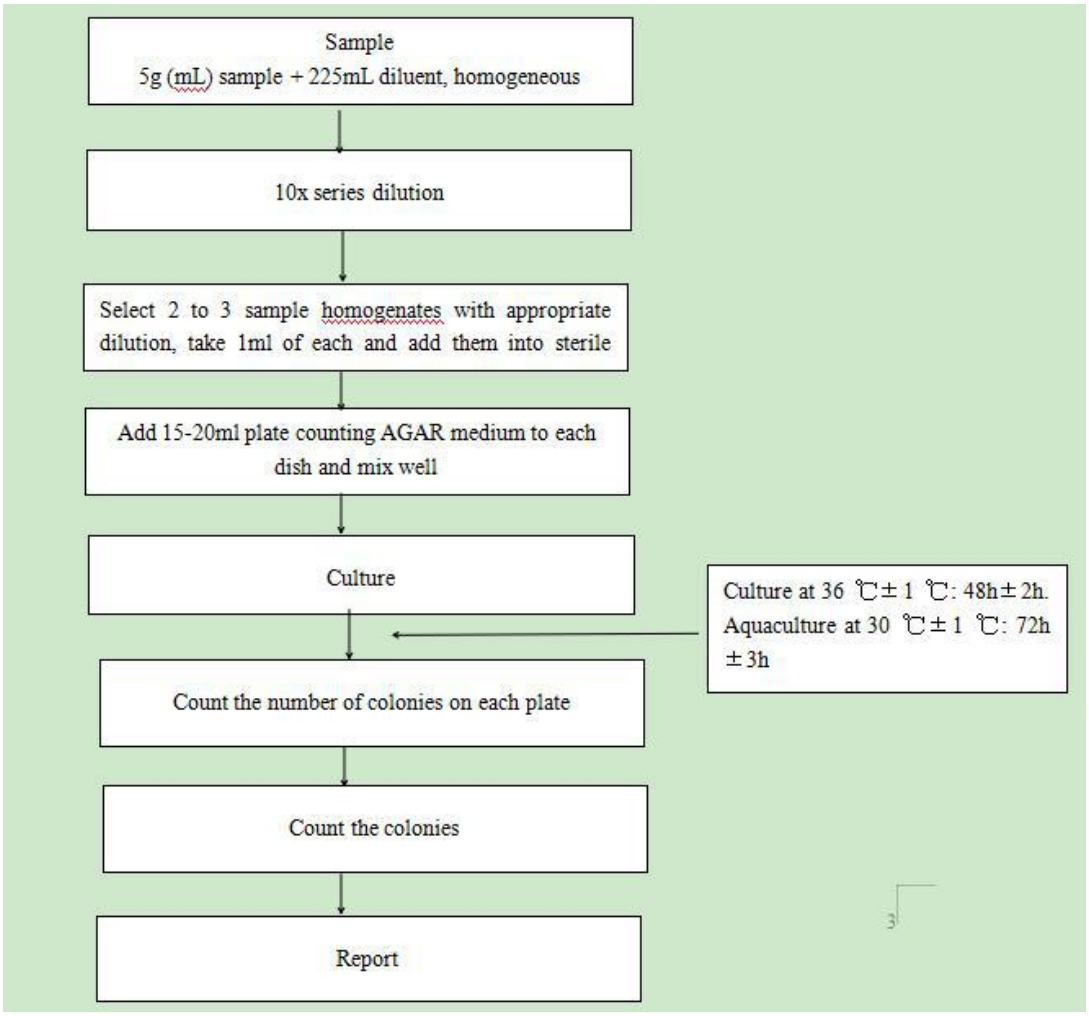

Figure 1 Inspection Procedure for aerobic plate count .

6 Operation steps

6.1 Dilution of samples

6.1.1 Solid and semi solid samples: Weigh 25g of the sample and place it in a sterile homogenizing cup containing 225mL of sterile phosphate buffer or sterile physiological saline. Homogenize at 8000r/min to 10000r/min for 1-2 minutes, or place it in a sterile homogenizing bag containing 225mL of diluent. Use a tapping homogenizer to beat for 1-2 minutes, prepare a 1:10 sample homogenization.

6.1.2 Liquid samples: Take 25mL of the sample with a sterile straw and place it in a sterile conical flask containing 225mL of sterile phosphate buffer or sterile physiological saline (an appropriate number of sterile glass beads can be pre placed in the bottle). Mix well, or place it in a sterile homogenizing bag containing 225mL of diluent. Use a tapping homogenizer to beat for 1-2 minutes to produce a 1:10 sample homogenization. When the result requires the total number of bacterial colonies per g of sample, operate according to 6.1.1.

6.1.3 Use a 1mL sterile pipette or micropipette to draw 1mL of a 1:10 sample homogenization solution. Slowly pour along the tube wall into a sterile test tube containing 9mL of dilution solution (note that the tip of the pipette or suction head should not touch the dilution liquid surface). Shake and mix on an oscillator to produce a 1:100 sample homogenization solution.

6.1.4 According to 6.1.3, prepare a 10 fold series of diluted sample homogenization. Replace with a 1mL sterile pipette or nozzle for each incremental dilution.

6.1.5 Based on the estimation of the contamination status of the sample, select 1 to 3 sample homogenizations with appropriate dilution (liquid samples can include the original solution), draw 1mL of the sample homogenization into a sterile culture dish, and make two culture dishes for each dilution. At the same time, take 1mL of blank diluent and add it to two sterile culture dishes for blank control.

6.1.6 Timely pour 15mL~20mL of plate counting agar culture medium (which can be placed in a 48 ℃± 2 ℃ constant temperature device for insulation) cooled to 46 ℃～

50 ℃ into the culture dish, and rotate the culture dish to mix evenly.

6.2 Cultivation

6.2.1 After the agar solidifies, flip the plate horizontally and incubate at 36 ℃ ± 1 ℃ for 48 hours ± 2 hours. Aquatic products are cultured at 30 ℃ ± 1 ℃ for 72 hours ± 3 hours.If the sample may contain colonies that spread and grow on the surface of the agar medium, a thin layer of plate counting agar medium (about 4mL) can be covered on the surface of the solidified agar medium. After solidification, the plate can be flipped for cultivation.

6.2.2 If using a total bacterial count test piece, the relevant technical procedures provided by the test piece should be followed for operation

6.3 Colony count

6.3.1 It can be observed with the naked eye, and if necessary, a magnifying glass or colony counter can be used to record the dilution factor and the corresponding number of colonies. The colony count is expressed in colony forming unit (CFU).

6.3.2 Select a plate with a colony count between 30CFU and 300CFU and no spreading colony growth to count the total number of colonies. Record the specific number of bacterial colonies on plates below 30CFU, while those above 300CFU can be recorded as numerous and not counted.

6.3.3 When one of the plates has a large area of colony growth, it is not suitable to use it. Instead, the plate without a large area of colony growth should be used as the dilution of the number of colonies; If the number of plate shaped colonies is less than half of the plate, and the distribution of colonies in the other half is very uniform, it can be calculated by multiplying half of the plate by 2 to represent the number of plate shaped colonies.

6.3.4 When there is chain like growth without clear boundaries between colonies on the plate, count each single chain as a colony.

7 Results and Reports

7.1 Calculation method for total bacterial count

7.1.1 If the bacterial count on only one dilution plate is within the appropriate counting range, calculate the average of the bacterial count on the two plates, and then multiply the average by the corresponding dilution factor to obtain the result of the total bacterial count per g (mL) of the sample. For example, see B.1.

7.1.2 If there are two consecutive dilution plates with a suitable counting range, calculate according to formula (1), as shown in B.2 for an example.

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

In the formula:

N---Number of colonies in the sample;

$\sum C$---The total number of colonies in the plate (plate containing the appropriate range of colonies)

n1---Number of the plates with the first dilution (low dilution ratio)

n2---Number of the plates with the second dilution (high dilution ratio)

d---Dilution factor (first diluton)

7.1.3 If the number of colonies on all dilution plates is greater than 300CFU, the plate with the highest dilution will be counted. Other plates can be recorded as many but not counted, and the results will be calculated by multiplying the average number of colonies by the highest dilution factor. For example, see B.3

7.1.4 If the bacterial count of all dilution plates is less than 30CFU, the average bacterial count with the lowest dilution should be multiplied by the dilution factor. For example, see B.4.

7.1.5 If all dilutions (including liquid sample stock solution) are sterile and grow on the plate, calculate by multiplying the minimum dilution factor by less than 1. For example, see B.5

7.1.6 If the bacterial count of all dilution plates is not between 30CFU and 300CFU, and some of them are less than 30CFU or more than 300CFU, then the average bacterial count closest to 30CFU or 300CFU is multiplied by the dilution factor. For

example, see B.6.

7.2 Report on aerobic plate count

7.2.1 When the total number of bacterial colonies is less than 100CFU, it should be rounded off according to the "rounding" principle and reported as an integer.

7.2.2 When the total number of bacterial colonies is greater than or equal to 100CFU, the third digit is rounded off using the "rounding" principle, and two significant digits are used, followed by 0 to replace the digits; It can also be expressed in the form of an index of 10, rounded according to the "rounding" principle and using two significant digits.

7.2.3 If there is bacterial growth on the blank control, the test result is invalid.

7.2.4 Weighing sampling is reported in CFU/g units, and volume sampling is reported in CFU/mL units.

Appendix A
Culture medium and reagents

A.1 Plate Count Agar culture medium

A.1.1 Composition

Tryptone (main nutritional component)	5.0g
Yeast extract (main nutritional component)	2.5g
Glucose (main nutritional component)	1.0g
Agar	15.0g
Distilled water	1000mL

A.1.2 Preparation method

Add the above ingredients to distilled water, boil and dissolve, and adjust the pH to 7.0 ± 0.2. Divide into suitable containers and sterilize under high pressure at 121 ℃ for 15 minutes.

A.2 Sterile phosphate buffer solution

A.2.1 Composition

Potassium dihydrogen phosphate (KH2PO4)	34.0g

Distilled water 500mL

A.2.2 Preparation method

Storage solution: Weigh 34.0g of potassium dihydrogen phosphate and dissolve it in 500mL of distilled water. Adjust the pH to 7.2 with approximately 175mL of 1mol/L sodium hydroxide solution. Dilute to 1000mL with distilled water and store in a refrigerator.

Dilution solution: Take 1.25mL of the storage solution and dilute it to 1000mL with distilled water. Divide it into suitable containers and sterilize it under high pressure at 121 ℃ for 15min.

A.3 Sterile saline

A.3.1 Composition

Sodium chloride 8.5g

Distilled water 1000mL

A.3.2 Preparation method

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

Appendix B
Example

B.1 Example 1

Dilutability	1:10	1:100	1:1000	Calculation results
Aerobic plate count/CFU	Too much to count, too much to count	124,138	11,14	13 100

After rounding off according to 7.2.2, the above data is represented as 13 000 or 1.3 × 10^4.

B.2 Example 2

Dilutability	1:100(First dilution)	1:1000(Secondary dilution)	Calculation results
Aerobic plate count/CFU	232,244	33,35	24 727

After rounding off according to 7.2.2, the above data is represented as 25 000 or 2.5 × 10^4.

B.3 Example 3

Dilutability	1:10	1:100	1:1000	Calculation results
Aerobic plate count/CFU	Too much to count, too much to count	Too much to count, too much to count	442,420	43 100

After rounding off according to 7.2.2, the above data is represented as 4300 00 or 4.3 × 10^5.

B.4 Example 4

Dilutability	1:10	1:100	1:1000	Calculation results
Aerobic plate count/CFU	14,15	1,0	0,0	145

After rounding off according to 7.2.2, the above data is represented as 150 or 1.5 × 10^2.

B.5 Example 5

Dilutability	1:10	1:100	1:1000	Calculation results
Aerobic plate count/CFU	0,0	0,0	0,0	<10

The above data is expressed as < 10.

B.6 Example 6

Dilutability	1:10	1:100	1:1000	Calculation results
Aerobic plate count/CFU	312,306	14,19	2,4	3 090

After rounding off according to 7.2.2, the above data is represented as 31 00or 3.1 × $10^{3.}$