National Food Safety Standard of the People's Republic of China

GB4789.2-2010

National Food Safety Standard
Food microbiological examination：
Aerobic plate count

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Preface

The standard substitutes the GB/T4789.2-2008 'Microbiological Examination in Foods: Aerobic Plate Count'

Compared with the GB /T4789.2-2008 main changes are following:

- The Chinese and English names of the standard are revised.
- The explanation of the calculation formula of Aerobic Plate Count is revised.
- The culture mediums and reagents are revised.
- The 2nd method: Petrifilm™ for aerobic plate count is deleted.

The appendix A of the standard is the normative appendix.

The replaced former editions are:

- GB 4789.2-1984, GB/T 4789.2-1994, GB/T 4789.2-2003, GB/T 4789.2-2008.

National Food Safety Standard

Food microbiological examination: Aerobic Plate Count

1. Scope

This Standard defines the determination method of aerobic plate count in foods.

This Standard is applicable to the determination method of aerobic plate count in all kinds of foods.

2. Terms and Definitions

2.1 Aerobic plate count

The aerobic plate count obtained from 1ml(or 1g) of sample under certain cultivation conditions (such as the ingredients of culture medium, cultivation temperature and time, pH, and aerobic, etc) after proper treatment.

3. Equipment and Materials

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

3.1 Thermostatic cultivator: 36±1℃,30±1℃

3.2 Refrigerator: 2℃~5℃.

3.3 Thermostatic water bath: 46±1℃

3.4 Balance: accuracy of 0.1g.

3.5 Homogenizer.

3.6 Oscillator.

3.7 Sterile pipette: 1ml (with a scale of 0.01ml), 10ml(with a scale of 0.1ml) or micropipettor and tips.

3.8 Sterile conical beaker: 250ml, 500ml.

3.9 Sterile culture plate: with a diameter of 90mm.

3.10 pH meter or pH colorimetric tube or precise pH indicator paper.

3.11 Magnifying glass or/ and bacterial colony counter.

4. Culture Medium and Reagents

4.1 Agar Culture Medium for plate count: please refer to Appendix A.1.

4.2 Phosphate buffer solution: please refer to Appendix A.2.

4.3 Sterile normal saline solution: please refer to Appendix A.3.3

5. Examination Procedures

For the examination procedures of aerobic plate count, please refer to Fig.1.

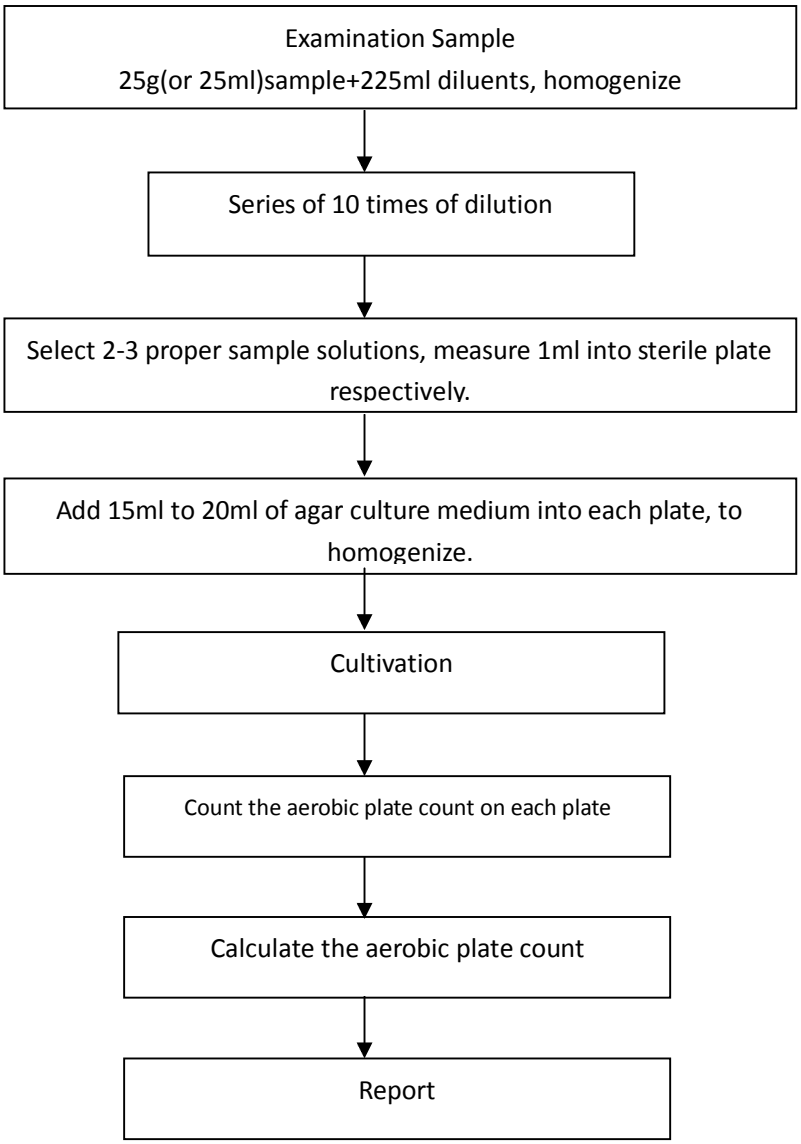

Figure 1. The Examination Procedures of plate count

6. Operation Procedures

6.1 Dilution of samples

6.1.1 Solid and semi solid samples: measure 25g sample into a sterile homogenizing cup containing 225ml phosphate buffer solution or normal saline solution, homogenize with 8000-10000r/min for 1-2min, or place into sterile homogenizing bag containing 225ml diluent, beating with slaping type homogenizer for 1min to 2min, and then formulate into 1:10 sample solution.

6.1.2 Liquid Sample: Measure 25ml sample with sterile pipette into a sterile conical beaker containing 225ml phosphate buffer solution or normal saline solution (proper amount of sterile beads are placed in the beaker in advance), and then homogenize and formulate into 1:10 sample solution.

6.1.3 Absorb 1ml of 1:10 sample solution with 1ml sterile pipette or micropipettor, drip the solution into the sterile tube containing 9ml diluent along the wall of the tube (it is noted that the tip of the pipette shall not touch the diluent solution surface), shake up the test tube or place a piece of sterile pipette, blow repeatedly to homogenize, and then formulate into 1:100 sample

solution.

6.1.4 Follow the operation procedures in 6.1.3, formulate the sample solution with series of dilution of 10 times. For each dilution, one piece of 1ml sterile pipette or tip is replaced.

6.1.5 As per the estimation of contamination status of samples, select 2 to 3 sample solutions with proper dilution (for liquid sample, original liquid shall be applied), when carrying out the escalating 10 times series of dilution, for each dilution, 1ml of sample solution is placed into two sterile plates. At the same time, measure 1ml of diluents into two sterile plate respectively to serve as blank controls.

6.1.6. Timely cool down the agar culture medium plates with 15-20ml content in each plate to 46℃(which are placed into 46±1℃ water bath), decant the plates, and then rotate the plates to homogenize.

6.2 Cultivation

6.2.1 After the solidification of agar, turn the plates up-side-down, cultivate at 36±1℃ for 48h±2h. For aquatic products, cultivate at 30±1℃ for 72h±3h.

6.2.2 If the samples possibly contain bacteria that could spread growing on the surface of agar culture medium, a thin layer of agar culture medium is covered on the agar surface after solidification (about 4ml), and then turn the plate up-side-down after solidification, and cultivate it as per 6.2.1.

6.3 Plate Count

It could be observed with naked eyes, apply magnifying glass or bacteria colony counter when necessary, and record the dilution times and corresponding plate count. Plate count number is represented by colony-forming units (CFU).

6.3.1 Select the plates for total plate count with colony number between 30-300CFU, and without spreading growth on the plate. For plate with plate count under 30CFU, the number of colony is recorded, while for plate count over 300, it shall be recorded as uncountable. For each dilution degree, the average number of two plates shall be applied.

6.3.2 For those plates with large piece of colony growing, they shall not be applied. However, the plates without large piece of colony growth shall be applied for plate count; If the piece of colony covers less than one half of the plate area, and the colonies on the remaining half of the plate area scatter evenly, it shall be counted of this half of the plate and then multiply by 2, to represent the entire plate count.

6.3.3 When there occurs chain like growth on the plate without evident border line between colonies on the plate, each chain shall be calculated as one colony.

7. Results and reports

7.1 Calculation method for aerobic plate count

7.1.1 If there is only one dilution degree whose plate count fall in the proper counting scope, the average plate count of both plate shall be calculated, and then multiply the average value by corresponding dilution times, to serve as the total plate count in one gram (or ml) of sample.

7.1.2 If there are two continuous dilution degrees whose plate count falls in the proper counting scope, they shall be calculated as in Formula (1):

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

Where,

N - Plate Count in sample;

$\sum C$ - The total number of colonies on the plates (including the plates within the range of proper plate count;

N_1 - The number of colonies on the plates of the first proper dilution degree;

N_2 - The number of colonies on the plates of the second proper dilution degree;

d - Dilution Factor (the first dilution degree).

Example:

Dilution degree	1:100 (the first dilution degree)	1:1000 (the second dilution degree)
Number of colonies	232,244	33,35

$$N = \sum C / (n_1 + 0.1 n_2) d$$

$$= \frac{232 + 244 + 33 + 35}{[2 + (0.1 \times 2)] \times 10^{-2}} = \frac{544}{0.022} = 24\ 727$$

The values mentioned above are round-up, and then represented as 25000 or 2.5×10^4.

7.1.3 If the colony numbers on the plates of all dilution degrees are all over 300CFU, count the plates with the maximum dilution degree. For other plates, they shall be recorded as uncountable, and the results shall be obtained by multiplying the average colony number by the maximum dilution times.

7.1.4 If the colony numbers on the plates of all dilution degree are all less than 30CFU, it shall be calculated by multiplication of average colony number on the minimum dilution degree plates by the dilution times.

7.1.5 If, for plates of all dilution degrees (including the original liquid samples), there is no colony growth, then it shall be calculated as multiplying the minimum dilution degree by a factor smaller than 1.

7.1.6 If, for plates of all dilution degrees, the colony number falls outside the range between 30CFU and 300CFU, part of which are less than 30CFU or more than 300CFU, then it shall be calculated for the plates whose colony number is closest to 30CFU or 300CFU, as the average colony number multiply by dilution times.

7.2 Reports of plate count

7.2.1 When the plate count falls within 100CFU, it shall be rounded up and reported as interger.

7.2.2 When the plate count is larger than or equal to 100CFU, the third digit shall be rounded up, and take the first two digits, while the following digits are replaced by 0; it could also be indicated as exponential of 10CFU, round-up and then take the two significant digits.

7.2.3 When all the plates are covered by spreading colonies, making it unable to calculate, it shall be reported as colony spreading.

7.2.4 When there are colonies growing on the blank control, the examination result is invalid.

7.2.5 For sampling by weight, CFU/g is applied as the report unit, while for sampling by volume, CFU/ml is applied as the report unit.

Appendix A
(Normative Appendix)
Culture Mediums and Reagents

A.1 Plate count agar (PCA) culture medium

A.1.1 Ingredients

Tryptone	5.0g
Yeast Extract	2.5g
Glucose	1.0g
Agar	15.0g
Distilled Water	1000ml
pH 7.0±0.2	

A.1.2 Formulation method

Add the above mentioned ingredients into distilled water, boil for dissolving, and then adjust the pH value. Distribute into tubes or conical beakers, autoclave at 121℃ for 15 min.

A.2 Phosphate Buffer Solution

A.2.1 Ingredients:

KH_2PO_4	34.0g
Distilled Water	500ml
pH 7.2	

A.2.2 Formulation Method

Stock Solution: Measure 34.0 g KH_2PO_4 to dissolve in 500ml distilled water, adjust the pH value to 7.2 with about 175ml of 1mol/L NaOH solution, then dilute with distilled water to a volume of 1000ml, and then store in the refrigerator.

Diluent Solution: Measure 1.25ml of the Stock Solution, dilute with distilled water to 1000ml, distribute into proper containers, and then autoclave at 121℃ for 15min.

A.3 Sterile normal saline solution

A.3.1 Ingedients

NaCl	8.5g
Distilled Water	1000ml

A.3.2 Formulation Method

measure 8.5g NaCl to dissolve in 1000ml distilled water, and autoclave at 121□ for 15 minutes.