National Standard of the People's Republic of China

GB 4789.2-2016

National Food Safety Standard

Food MicrobiologicalExamination: Aerobic Plate Count

食品安全国家标准

食品微生物学检验：菌落总数测定

- Date of Publication: 2016-12-23
- Date of Implementation: 2017-06-23
- Issued by: NHFPC & CFDA

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Foreword

This standard supersedes GB 4789.2-2010 “Food Safety National Standards Food Hygiene Microbiological Examination- Aerobic Plate Count”.

National Food Safety Standard

Food Microbiological Examination: Aerobic Plate Count

1. Scope

This standard specifies the method for the determination of aerobic plate count in foods.
This standard applies to the determination of aerobic plate count in foods.

2. Terms and definitions

Aerobic plate count
The aerobic plate count produced in 1g (or 1mL) of test sample after the sample of food test was treated and incubated under certain conditions (such as culture medium, cultivation temperature and time, etc.).

3. Equipment and materials

Except for conventional sterilization and cultivation apparatus in microbiological laboratory, other equipment and materials are as follows:

3.1 Constant temperature incubator: 36℃±1℃, 30℃±1℃

3.2 Refrigerator: 2℃~5℃

3.3 Constant temperature water bath: 46℃±1℃

3.4 Balance: with sensitivity of 0.1g

3.5 Homogenizer

3.6 Vibrator

3.7 Sterile pipette: with nominal capacities of 1mL (graduated in 0.01mL division) and 10mL (graduated in 0.1mL division), or micropipette and pipette tips

3.8 Sterile conical flask: with nominal capacity of 250mL and 500mL

3.9 Sterile culture dish: with diameter of 90mm

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

3.11 Magnifying glass or/and bacterial colony counter.

4. Culture mediums and reagents

4.1 Plate count agar medium: please refer to A.1.

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

4.3 Sterile normal saline solution: please refer to A.3.

5. Examination procedures

Refer to Figure 1 for the examination procedures of aerobic plate count.

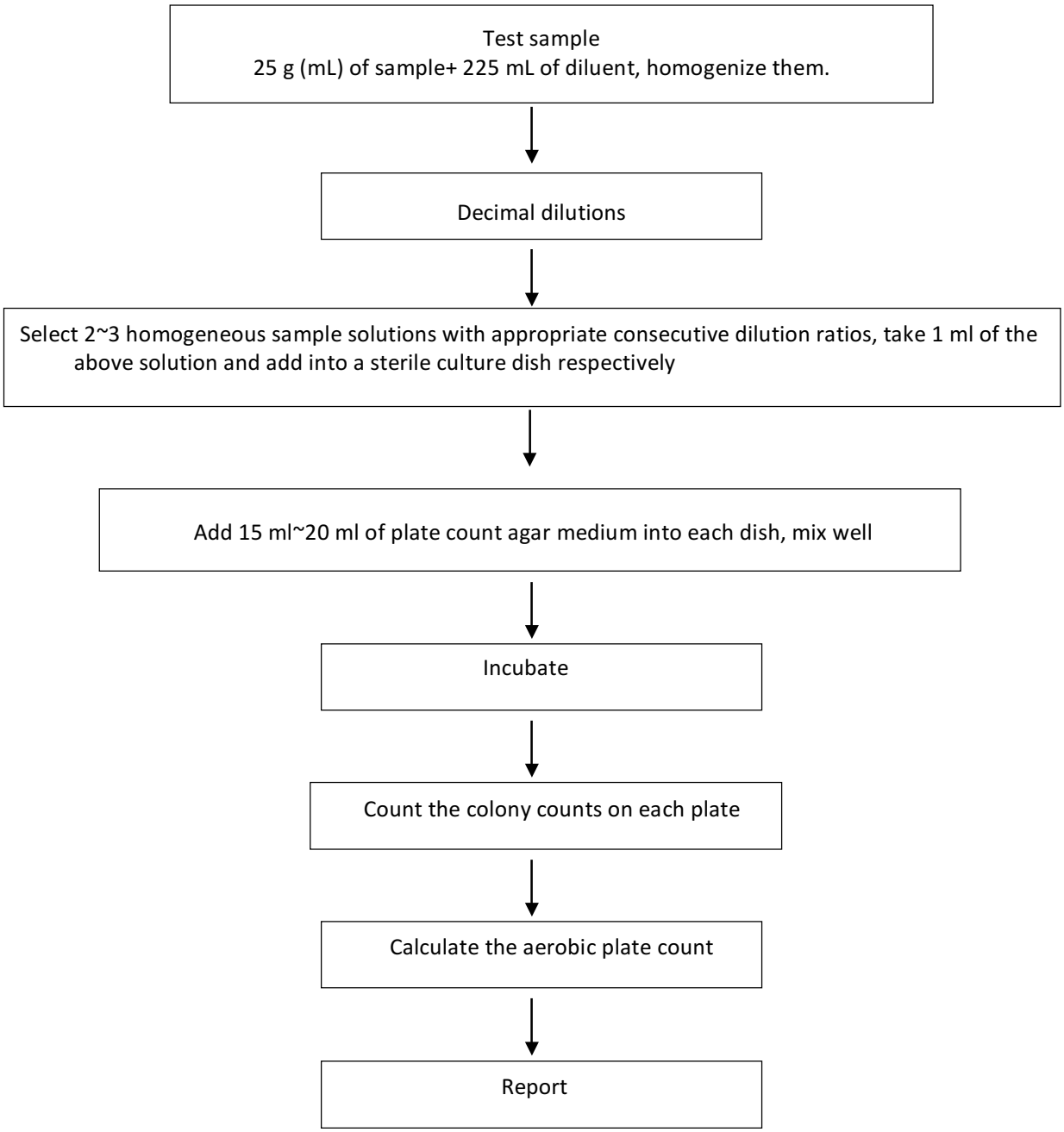

Figure 1 Examination procedures of aerobic plate count

6. Operation procedures

6.1 Dilution of sample

6.1.1 Solid and semisolid sample: Weigh 25g of sample into a sterile homogenization cup filled with 225mL of phosphate buffer solution or normal saline solution, homogenize at the speed of 8000r/min~10000r/min for 1

min~2min, or place into a sterile homogenization bag filled with 225mL of diluent, homogenize it with a slapping homogenizer for 1 min~2min to get a 1:10 homogeneous sample solution.

6.1.2 Liquid sample: Using a sterile pipette to pipette 25mL of sample into a sterile conical flask filled with 225mL of phosphate buffer solution or normal saline solution (a suitable quantity of sterile glass beads should be put in the beaker in advance), and then oscillate and mix uniformly to get a 1:10 homogeneous sample solution.

6.1.3 Pipette 1mL of the above 1:10 homogenous sample solution with a 1mL sterile pipette or micropipette, pour the solution slowly into a sterile tube filled with 9mL of diluent along the tube wall (Make sure that the pipette or tip of it never touch the diluent), oscillate or blow and hit with another 1mL sterile pipette repeatedly to mix uniformly and get a 1:100 homogeneous sample solution.

6.1.4 Following the operation procedures in Section 6.1.3, prepare homogeneous sample solution of 10 times serial dilution. Use a new 1mL sterile pipette or pipette tip before the next dilution.

6.1.5 Select 2~3 homogenous sample solutions with appropriate consecutive dilution ratios after referring to the estimation of sample contamination status, (including liquid sample itself).When carrying out 10 times increasing dilution, pipette 1mL of homogenous sample solution into two sterile plates for every dilution ratio. Meanwhile, pipette 1mL of blank diluents into two sterile plates respectively, as control group.

6.1.6 Opportunely put plate count agar medium of 15 mL~20mL which has been cooled down to 46℃ (or placed into 46℃±1℃ water bath to keep warm), and then revolve the plate to mix uniformly.

6.2 Incubation

6.2.1 After the agar solidified, turn over the plate, incubate at 36℃±1℃ for 48h±2h. For aquatic products, incubate at 30℃±1℃ for 72h±3h.

6.2.2 When the samples likely contain bacteria that could spread and grow on the surface of agar culture medium, cover a thin layer of agar culture medium (about 4mL) on the agar surface when it is solidified, inverse the plate up-side-down after solidification, and cultivate under the conditions in Section 6.2.1.

6.3 Colony count

6.3.1 It could be observed with naked eyes, magnifier or bacteria colony counter can be used if necessary. Record the dilution ratios and the corresponding colony count. Colony count is expressed as colony-forming units (CFU).

6.3.2 Select the plates with colony count in the range of 30CFU~300CFU and without spreading growth of colony for total plate count. The count of the plate lower than 30CFU would be recorded, instead those which are higher than 300CFU should be recorded as “too numerous to count (TNTC)”. For each dilution ratio, the average colony count of two plates shall be applied.

6.3.3 The plate should not be applied while one plate appearing large piece of colony growing. And the other plate without large piece of colony growing can be applied to plate count for the corresponding dilute ratio; if the colony pieces covers less than one half of the plate area, and the left half is distributed evenly by the colonies, it can be counted and multiply the obtained number by 2, to represent the count of the plate.

6.3.4 When chain-like growth of colonies appears on the plate without obvious boundary between colonies, take each single chain as one colony for counting.

7. Results and reports

7.1 Calculation method for aerobic plate count

7.1.1 If there is only one dilution ratio of which the plate count falls in the proper counting range, calculate the average colony count of the two plates, and then multiply the average value by the corresponding dilution ratio, and take the obtained value as the total plate count in each gram (or ml) of sample.

7.1.2 If there are two continuous dilution ratios of which the plate count falls in the proper counting range, it can be calculated according to Formula(1):

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

Where,
N——colony count in the sample;
∑C——the total colony counts on the plates (the plates containing colony count within the proper range);
n1——the number of the plates for the first dilution ratio (low dilution ratio); *n2*——the number of the plates for the second dilution ratio (high dilution ratio); *d*——the dilution factor (for the first dilution ratio).

Example:

Dilution ratio	1:100 (the first dilution ratio)	1:1000 (the second dilution ratio)
Colony count (CFU)	232,244	33,35

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

$$= \frac{232 + 244 + 33 + 35}{[2 + (0.1 \times 2)] \times 10^{-2}} = \frac{544}{0.022} = 24727$$

The data mentioned above are rounded-up according to Section 7.2.2, and expressed as 25000 or 2.5×10^4.

7.1.3 If the colony counts on the plates of all dilution ratios are all over 300CFU, count the plates with the highest dilution ratio. Other plates can be recorded as TNTC, and the result should be calculated by multiplying the average colony count by the highest dilution ratio.

7.1.4 If the colony counts on the plates of all dilution ratios are all less than 30CFU, the result should be calculated by multiplying the average colony count on the lowest dilution ratio plates by the dilution ratio.

7.1.5 If there is no colony growing on the plates of all dilution ratios (including the liquid sample itself), then it should be calculated as “less than 1 multiplying by the lowest dilution ratio”.

7.1.6 If the colony counts on plates of all dilution ratios fall outside the range of 30CFU~300CFU, part of which are less than 30CFU or more than 300CFU, then it should be calculated by multiplying the average colony count on the plate with colony count closest to 30CFU or 300CFU by the corresponding dilution ratio.

7.2 Aerobic plate count reports

7.2.1 When the colony count is less than 100CFU; it should be rounded up according to the rounding principle and reported as an integer.

7.2.2 When the colony count is larger than or equal to 100CFU, the third figure should be rounded up according

to the rounding principle, and the first two figures should be taken, and the following digits should be substituted by 0; or could be expressed as the exponential of 10 as well, and rounded up in accordance with the rounding principle, with two significant digits.

7.2.3 When all the plates are covered by spreading colonies and make it unable to count, report it as colony spreading.

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

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

Annex A

Culture mediums and reagents

A.1 Plate count agar (PCA) culture medium

A.1.1 Composition

Tryptone	5.0g
Yeast Extract	2.5g
Glucose	1.0g
Agar	15.0g
Distilled Water	1,000mL

A.1.2 Preparation method

Put all the above components into distilled water, boil to dissolve, and then adjust the pH to 7.0±0.2. Distribute into test tubes or conical flasks, sterilize in an autoclave set at 121℃ for 15 min.

A.2 Phosphate buffer solution

A.2.1 Composition

Potassium dihydrogen phosphate (KH_2PO_4)	34.0g
Distilled water	500mL

A.2.2 Preparation method

Stock solution: Weigh 34.0 g of potassium dihydrogen phosphate and dissolve in 500mL of distilled water, adjust the pH to 7.2 with about 175mL of 1mol/L sodium hydroxide solution, dilute to 1,000mL with distilled water, and then store in therefrigerator.

Diluent: Take 1.25mL of the stock solution, dilute to 1,000mL with distilled water, distribute into proper containers, and then sterilize in an autoclave set at 121℃ for 15min.

A.3 Sterile normal saline solution

A.3.1 Composition

Sodium chloride	8.5g
Distilled water	1,000mL

A.3.2 Preparation method

Weigh 8.5g of sodium chloride and dissolve in 1,000mL of distilled water, and sterilize in an autoclave set at 121℃ for 15 minutes.