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MOHWM0014.02

Methods of Test for Food Microorganisms- Test of Standard Plate Count (Aerobic Plate Count)

1. Scope: This method is applicable to enumerate microorganisms in food.

2. Method: Samples are serially diluted. Culture each diluted test solution with plate count agar (PCA), then enumerate the colonies.

2.1. Work environment: The working platform needs to be spacious, clean, and well-lit with illumination of cabinet at 100 cd. The air in the closed room is well-ventilated, with as little dust and flowing air as possible. Colonies must not exceed 15 CFU/dish for every 15 min.

2.2. Equipment and materials

2.2.1. Sterilization oven (Hot air sterilizer): capable of operating at 170 ±10 ^{o}C.

2.2.2. Autoclave: capable of operating at 121°C or higher temperature.

2.2.3. Refrigerator: capable of operating at 5 ± 3°C.

2.2.4. Incubator: capable of controlling temperature at ± 1.0 °C.

2.2.5. Water bath: capable of controlling water temperature at ± 1.0 °C.

2.2.6. Blender or stomacher: appropriate for aseptic operation.

2.2.7. Balance: weighing up to 2,000 g with sensitivity of 0.1 g, weighing up to 100 g with sensitivity of 1 mg.

2.2.8. Vortex mixer.

2.2.9. pH meter.

2.2.10. Colony counter: suitable for the calculation of colonies.

2.2.11. Pipette aid.

2.2.12. Pipette: sterile, 1mL pipette with scale of 0.01 mL; 5 and 10 mL with scale of 0.1 mL.

2.2.13. Petri dish: sterile, 90 × 15 mm, surface of the dish should be flat and contain no bubbles or scratches.

2.2.14. Container: screw-capped of flasks, glass bottles or wide-mouth jars made of glass, polyethylene, Teflon or other materials that can be sterilized at 121℃ for more than 20 min, or sterilized bags.

2.2.15. Spatula, scissors, knife and forceps: sterilizable or disposable.

2.2.16. Chemicals

Sodium chloride (NaCl), potassium dihydrogen phosphate (KH_2PO_4), sodium hydroxide (NaOH), glucose ($C_6H_{12}O_6$), polysorbate 80 (Tween 80), reagent grade; peptone, tryptone, yeast extract, agar, microbiological grade.

2.2.17. Preparation of 0.1 N sodium hydroxide solution

Dissolve 4 g of sodium hydroxide in sterile water to 100 mL.

2.2.18. Preparation of diluted solution

2.2.18.1. Saline solution

Dissolve 8.5 g of sodium chloride in distilled water to 1000 mL. Dispense in a dilution container and sterilize at 121°C for 15 min.

2.2.18.2. Butterfield's phosphate-buffered dilution water

Dissolve 34 g of potassium dihydrogen phosphate in 500 mL distilled water. Adjust the pH to 7.2 with 1 N sodium hydroxide solution, and add distilled water to 1000 ml. Sterilize at 121°C for 15 min and store in a refrigerator as a stock solution. When using, take 1.25 mL of the stock solution, dilute in distilled water to 1000 mL, dispense into a dilution container, and sterilize at 121°C for 15 min.

2.2.18.3. 0.1% peptone diluent

Dissolve 1 g of peptone in distilled water to 1000 mL. Dispense into a dilution container and sterilize at 121°C for 15 min. Final pH is 7.0 ± 0.2.

2.2.19. Plate count agar (PCA), or called Standard method agar

Tryptone……..5 g
Yeast extract…… ….. 5 g
Glucose…… ..…..1 g
Agar……..…… .....15 g
Distilled water……..... …..1000 mL
Dissolve the ingredients by heating, dispense into suitable

containers, then sterile at 121°C for 15 min. Final pH is 7.0 ± 0.2.

2.3. Preparation of the test solution(note1~3)

2.3.1. Solid samples

Mince and mingle the sample properly, take 50 g of the sample, and mix well with 450 mL of the diluent as the 10-fold diluted test solution.

2.3.2. Powder, granules or other easily smashed samples

Smash the sample with a sterilized spatulas or other utensils, and mix well. Take 50 g of the sample, and mix well with 450 mL of the diluent as the 10-fold diluted test solution.

2.3.3. Liquid samples

Mix the sample thoroughly, take 50 mL of the sample, and mix well with 450 mL of the diluent as the 10-fold diluted test solution.

2.3.4. Frozen samples

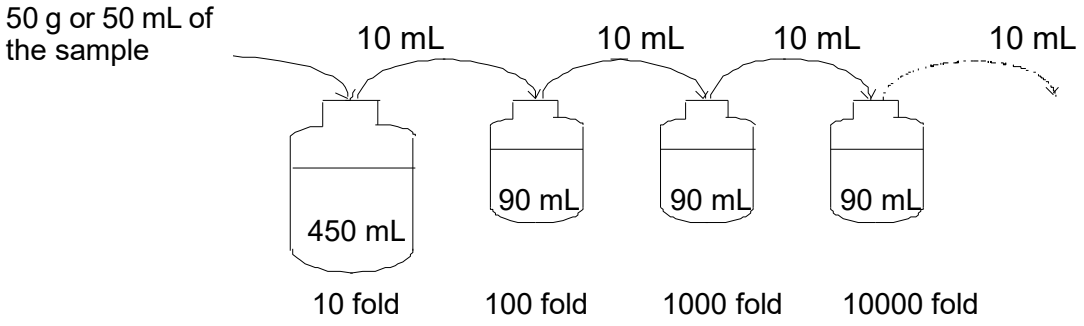
Completely thaw the sample, such as frozen fish meat, poultry meat, fruits, vegetables and dumplings, etc. at refrigerated temperatures (e.g. at 2-5°C within 18 hr), or quickly thaw at higher temperatures (e.g. in a water bath at 45°C for the sample could be thawed within 15 min). Shake the sample frequently to accelerate thawing. After thawing, chop the sample, and mix evenly. Make the sample which do not need to be thawed, such as ice cubes, popsicles and ice cream, etc., into appropriate small pieces, take 50 g of the sample, and mix well with 450 mL of the diluent as the 10-fold diluted test solution.

2.3.5. Condensed and thick liquid samples

Stir and mix thoroughly the sample, such as pudding, condensed milk, etc. Take 50 g of the sample, and mix well with 450 mL of the diluent as the 10-fold diluted test solution.

2.3.6. Serial diluted test solutions

Using a sterilize pipette, pipette 10 mL of the 10-fold diluted test solution into 90 mL of the diluent. Prepare a series of decimal dilutions, such as 100-fold, 1000-fold test solution, and 10000-fold as shown in the following figure.



Note: 1. Dilute only the meat product with 0.1% peptone, and the others with Butterfield's phosphate-buffered dilution, followed by saline solution.

Note 2: In the case of the greasy sample, difficult to disperse and easy to foam, it is necessary to add appropriate amount of sterilized emulsifier (such as Tween 80 at the final concentration in the test solution of 1%), and shake well to emulsify.

Note 3: As the total amount of the sample is less than 50 g (mL), add an appropriate amount of BPW, according to the amount of the sample, to prepare a 10-fold diluted test solution.

2.4. Cultivation of bacteria

2.4.1. Shake the diluted test solution and/or stock solution in section 2.3 thoroughly and mix well.

2.4.2. Pipette 1 mL of each diluted test solution and/or stock solution into the Petri dish, and repeat the process at least twice (two plates).

2.4.3. Pipette 1 mL of the diluent into a Petri dish as a control group (duplicates).

2.4.4. Pour 12 to 15 mL of plate count agar (PCA) which cooled to 45 ± 1 °C into the Petri dishes in section 2.4.2. and 2.4.3. Shake and mix the dishes, and the preparation of the test solution should be completed within 15 min.

2.4.5. Stand the PCA plates of section 2.4.4., until the medium is solidified. Then invert the medium and incubate at 35°C for 48 ± 2 hr.

2.5. Calculation of aerobic plate count

2.5.1. After incubation, select two plates which colonies are between 25-250.

The aerobic plate count unit is CFU/g or CFU/mL. Round off the aerobic plate count to two significant digits, by raising the second digit to the next highest number when the third digit is 6, 7, 8, or 9 and rounding down when the third digit is 1, 2, 3, or 4. (When the third digit is five, round up when the second digit is odd and round down when the second digit is even.)

2.5.2. If only one dilution factor of plates whose colony is between 25-250, the aerobic plate count is the result of the average number of colonies of the two plates multiply the dilution factor (Table: example No.1). If there are two dilution factor of plates whose colonies are between 25-250, calculate aerobic plate count according to the following formula. (Table: example No.2)

Aerobic Plate Count (CFU/g or CFU/mL) =

$$\left[\left(\frac{Aa + Ab}{2}\right) \times A + \left(\frac{Ba + Bb}{2}\right) \times B\right] \times \frac{1}{2}$$

Aa, Ab：The number of colonies of each plate in dilution factor A.
Ba, Bb：The number of colonies of each plate in dilution factor B.
A, B：The dilution factors.

2.5.3. When the number of colonies of each dilution factor plate is less than 25, the aerobic plate count is the result of the average number of colonies of the two lowest dilution factor of plates. It should be indicated the result is an estimate (Table: example No.3).

2.5.4. When the number of colonies in the plate is more than 250 colonies, the number of colonies in a representative portion of the plate is counted first, and the aerobic plate count is calculated, and indicated it as an estimate (Table: example No.4).

2.5.5. When there are diffused colonies on the plate[(note 4)]. Use the following guide:

2.5.5.1. When the area covered by the diffused colonies (Including the growth inhibition range caused by the diffused colonies) more than

1/2 of the plate area or . than 1/4 of the growth inhibition range caused by the diffused colonies, the aerobic plate count should not be calculated and record as diffused colonies. (Table: example No.5).

2.5.5.2. When the diffused colony forms a chain. If only one chain formed, it should be regarded as one colony. If more than two chains formed, it should count separately according to different chain source. If big colonies formed separately each other, it should be counted.

Note 4: Diffused colonies can be divided into 3 types.

(1) Chain-like colonies that cannot be clearly separated, and grew by bacterial division.

(2) The bacteria grow in the layer of steam between the medium and the bottom of the dish.

(3) The bacteria grow on the edge of the medium or on the layer of steam on the surface of the medium.

2.5.6. When each dilution factor has no colony on plate, the number of colonies is less than 1, and multiply by the lowest dilution factor. The value is as aerobic plate count and indicates as an estimate (Table: example No.6).

2.5.7. If one of the numbers of colonies is between 25 and 250, and the other is more than 250 of two repetitions, both should be counted (Table: example No.7).

2.5.8. If one of the numbers of colonies is between 25 and 250, and the other is greater than 250 or less than 25 of two repetitions in two dilution factor of plates, the four plates should be counted and calculated by the formula in the section 2.5.2. (Table: example No.8).

2.5.9. If the numbers of colonies of two repetitions are all between 25 and 250 in one dilution. And one of the numbers of colonies of two repetitions in the other dilution factor is between 25 and 250, and the other is more than 250 or less than 25, the four plates should be

counted and calculated by the formula in the section 2.5.2. (Table: example No.9 and 10).

2.5.10. If the sample is confirmed to be contaminated or deemed inappropriate for other reasons, it shall not be calculated.

Remark: It is allowed to use validated commercial media, biochemical test kits or biochemical identification systems. However, when this test results are disputed, this test method shall prevail.

Reference:

Maturin, L. and Peeler, J. T. 2001. Chapter 3 Aerobic plate count. Bacteriological Analytical Manual. [https://www.fda.gov/food/laboratory-methods-food/bam-chapter-3-aerobicplate-count]

Table. Examples about calculation of aerobic plate count (2 plates/dilution factor).

Example No.	The number of colonies			CFU/g [a]	Calculation basis
	1：100	1：1000	1：10000		
1	TNTC[b] TNTC	175[c] 208	16 17	190000	2.5.2
2	TNTC TNTC	224 245	25 30	250000	2.5.2
3	18 14	2 0	0 0	1600*	2.5.3
4	TNTC TNTC	TNTC TNTC	523 487	5000000*	2.5.4
5	TNTC TNTC	245 230	35 diffusion colonies	290000	2.5.5
6	0 0	0 0	0 0	<100*	2.5.6
7	TNTC TNTC	245 278	23 20	260000	2.5.7
8	TNTC TNTC	225 255	21 40	270000	2.5.8
9	TNTC TNTC	210 240	18 28	230000	2.5.9
10	TNTC TNTC	260 230	30 28	270000	2.5.9

(a) * indicates the estimated value.

(b) TNTC：the number of colonies is too many to counted, and the number of colonies is significantly more than 250.

(c) The underlined number indicates the count.

Test flow chart