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Methods of Test for Food Microorganisms-Test of *Pseudomonas aeruginosa* in Bottled and Packaged Drinking Water

1. Scope

This method is applicable to examine *Pseudomonas aeruginosa* in bottled and packaged drinking water.

2. Method

Filter water sample through a membrane, and put the membrane on selective medium, then count the colonies after incubation.

2.1. Work environment: The working platform needs to be spacious, clean and well-lit with illumination of cabinet over 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 every 15 min.

2.2. Equipment and materials

2.2.1. Dry heat sterilizer.

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. Balance: weighting up to 2,000 g with sensitivity of 0.1 g, weighting up to 120 g with sensitivity of 5 mg.

2.2.6. Membrane filter: The funnel and vacuum holder base can be placed for filtering film, and the funnel should be sterile or sterilizable.

2.2.7. Vortex mixer.

2.2.8. Filter membrane: Pore size 0.45 μm nitrocellulose filter film (for water quality inspection, white, latticed, sterilized) or equivalent, suitable for the membrane filter device in section 2.2.6.

2.2.9. pH meter.

2.2.10. Pipette aid.

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

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

2.2.13. Container: sterile bag or 1000 mL, 500 mL, 99 mL and 90 mL sterilizable wide-mouth bottles with labeled caps (plugs).

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

2.2.15. Inoculating needle and inoculating loop (3 mm i.d.): made of nichrome,

platinum-iridium or chromel wire material, or a disposable product.

2.2.16. pH test paper: Range 6-8.

2.2.17. Chemicals: L-lysine HCl, sodium chloride, xylose, sucrose, lactose, phenol red, ferric ammonium citrate, sodium thiosulfate, magnesium sulfate ($MgSO_4 \cdot 7H_2O$), kanamycin and nalidixic acid, reagent grade; yeast extract, instant skim milk, nutrient broth, and agar, microbiological grade.

2.2.18. Media

2.2.18.1. M-PA-C agar

L-Lysine · HCl……5.0 g
Yeast extract……...2.0 g
Sodium chloride……5.0 g
Xylose……1.25 g
Sucrose……1.25 g
Lactose……..1.25 g
Phenol red……0.08 g
Ferric ammonium citrate……...0.8 g
Sodium thiosulfate……5.0 g
$MgSO_4 \cdot 7H_2O$……1.5 g
Kanamycin……..0.008 g
Nalidixic acid……...0.037 g
Agar……12.0 g
Distilled water……1000 mL
Heat and stir until boiling to completely dissolve the medium. Continue heating for 1 min. Final pH is 7.2 ± 0.1. Dispense into Petri dishes.

2.2.18.2. Milk agar

Mixture A:
Skim milk……100 g
Distilled water……500 mL
Mixture B:
Nutrient broth……12.5 g
Sodium chloride……..2.5 g
Agar……15.0 g

Distilled water……1000 mL

Dissolve mixture A completely, sterilize at 115°C for 20 min, dissolve mixture B completely, sterilize at 121°C for 15 min, after cooling A and B to 55°C, mix under sterile condition and dispense into Petri dishes.

2.3. Sampling

Take sample 100 mL from a sterilized container or sterile bag, and filter it, if sample is in severe contamination, then filter sample 10 mL is available, and examine at least two replicates for each sample.

2.4. Filtration

Filter the sample under reduced pressure with a 0.45 μm nitrocellulose membrane.

2.5. Culture

Take out the membrane in section 2.4, and place it on M-PA-C agar, invert and incubate at 42°C for 24 hr.

2.6. Observation

After incubation, observe whether a diameter of 0.8 to 2.2 mm colony is formed. A typical colony is flattened with brown to dark green center, and has bright edges.

2.7. Confirmation test

Select 3 to 5 typical colonies for confirmation test, inoculate the isolated typical colonies into milk agar, draw a line of 2 to 4 cm in length, and incubate at 35 °C for 24 hr. If it can hydrolyze the casein of cow's milk and show yellow to green spread, it is *Pseudomonas aeruginosa.*

2.8. Counting

Calculate the number of *Pseudomonas aeruginosa* according to the ratio of the confirmation test, and the bacteria count is expressed as CFU/100 mL.

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

Test flow chart