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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB/T 18204.4-2013

**Examination methods for public places - Part 4: Microorganism
on a surface of public articles**

公共场所卫生检验方法 第4部分：公共用品用具微生物

(English Translation)

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1 Scope

This Part of GB/T 18204 specifies the sampling and test methods for the total bacterial count, total fungi count, coliforms, staphylococcus aureus and streptococcus hemolyticus of public articles in public places.

This Part is applicable to the test of the total number of bacteria, total bacterial number, total fungi count, coliforms, staphylococcus aureus and streptococcus hemolyticus of public articles in public places, and other places can be implemented by reference.

2 Terms and Definitions

For the purposes of this Document, the following terms and definitions apply.

2.1 Total bacterial count

The total number of mesophilic aerobic and facultative anaerobic colonies grown and developed on the nutrient agar medium at 35°C ~ 37°C for 48h after the sampling and processing of the public articles.

2.2 Coliforms

Cultivate gram-negative non-spore bearing bacilli that can ferment lactose, produce acid, produce gas, produce aerobic and facultative anaerobic at 37°C for 24h.

2.3 Staphylococcus aureus

Plasma coagulase-positive Gram-positive Staphylococcus that grows well on Baird Parker medium or blood plate medium, and decomposes mannitol to produce acid.

2.4 Total fungi count

The total number of colonies formed on Rose Bengal or Sabouraud agar medium at 25°C ~28°C for 3d~7d.

2.5 Streptococcus hemolyticus

It belongs to the genus streptococcus and is the gram-positive bacteria that

Pork bile salt (or beef and sheep bile salt): 5g

Lactose: 10g

Bromocresol purple aqueous solution (mass concentration=0.04%): 25mL

Distilled water: 1000mL

Preparation method: Dissolve peptone, bile salt and lactose in distilled water; adjust the pH to 7.4; add bromocresol purple solution; and mix well. Then dispense into test tubes with inverted tubes, 10 mL per tube. After autoclaving at 68.96kPa (115°C, 10 lb) for 20min.

NOTE: The other compositions in the double-material lactose bile salt fermentation tube has doubled except distilled water.

4.12 Eosin-methylene blue agar

Compositions:

Peptone: 10g

Lactose: 10g

Dipotassium hydrogen phosphate: 2g

Agar: 17g

Eosin aqueous solution (mass concentration = 2%): 20mL

Methylene blue aqueous solution (mass concentration=0.5%): 10mL

Distilled water: 1000mL

Preparation method: Dissolve peptone, phosphate and agar in distilled water; adjust the pH to 7.2; and dispense into flasks. After autoclaving at 68.96kPa (115°C, 10 lb) for 20min, add lactose and heat to melt the agar before use; cool to 50°C ~55°C; add eosin and methylene blue solution; shake well; and pour into the plate.

4.1.3 Lactose fermentation tube

Compositions:

Peptone: 20g

Lactose: 10g

Bromocresol purple aqueous solution (mass concentration=0.04%): 25mL

5.3.4 Pick typical colonies for smear staining microscopy, which are gram-positive and arranged in grapes.

5.3.5 Plasma coagulase test: Pipette 0.5mL of 1:4 fresh plasma into a sterile cuvette; and then add 0.5mL of 24h broth culture of the bacteria to be tested. Mix well, put it in a $36^{\circ}\text{C}\pm 1^{\circ}\text{C}$ incubator or water bath; and observe once every 30min. If there is a clot within 24h, it is positive. At the same time, 0.5 mL of broth culture and broth medium of known plasma coagulase positive and negative strains are respectively added to a sterile cuvette and mixed with 0.5 mL of 1:4 plasma as a control.

5.4 Result report

Where there is suspicious colony growth on the above-mentioned selected plate, the staining microscopic examination proves that it is Gram-positive staphylococcus, and the plasma coagulase test is positive, the the detection of staphylococcus aureus can be reported.

6.1.1 Normal saline

See 3.1.1.

6.1.2 Rose Bengal medium

Compositions:

Peptone: 5g

Glucose: 10g

Potassium dihydrogen phosphate: 1g

Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$): 0.5g

Agar: 20g

Chloramphenicol: 0.1g

Distilled water: 1000mL

Rose Bengal aqueous solution (mass concentration=1:3000): 100mL

Preparation method: Dissolve peptone, glucose, potassium dihydrogen phosphate, magnesium sulfate and agar in distilled water; then add Rose Bengal solution;

Minced beef: 500g

Sodium chloride: 5g

Peptone: 10g

Dipotassium hydrogen phosphate: 2g

Distilled water: 1000mL

Preparation method: Add 500g of minced fascia-free fat-free beef to 1000mL of distilled water; mix them and put in the refrigerator overnight. Remove the slick oil on the liquid surface; and boil it in water for 30min to completely condense the meat residue into a block; filter it with a flannel; and squeeze it and collect all the filtrate under pressure;

and add water to make up the original amount. Add peptone, sodium chloride and phosphate; adjust the pH to 7.4~7.6 after dissolving; boil and filter; add 1% glucose;

autoclave at 121°C for 15min.

7.1.2 Pick's Broth

Compositions:

Tryptone's beef heart infusion (mass concentration = 1%): 200mL

Crystal violet saline solution (mass concentration=0.04%): 10mL

Sodium trinitride solution (mass concentration = 0.125%): 10mL

Defibrinated rabbit blood (or sheep blood): 10mL

Preparation method: The above-mentioned sterilized ingredients are mixed in sequence by aseptic operation and stored in the refrigerator for later-use.

7.1.3 Blood agar: see 5.1.4.

7.1.4 Potassium oxalate human plasma: Put 0.01g of potassium oxalate into a sterile

cuvette; then add 5 mL of human blood; mix well. Centrifuge for precipitation, and pipette the supernatant to obtain potassium oxalate human plasma.

7.1.5 Calcium chloride (mass concentration = 0.25%).

7.1.6 Sterilized normal saline (mass concentration = 0.85%).

7.1.7 Gram staining solution: see 4.1.4.