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Antimicrobial property detection methods for nano-inorganic materials

纳米无机材料抗菌性能检测方法

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Antimicrobial property detection methods for nano-inorganic materials

1 Scope

This Standard specifies the terms and definitions, test methods, test data processing, calculation of detection results, detection report and precautions for antimicrobial property of nano-inorganic materials.

This Standard is applicable to nano antimicrobial powder and materials that use nano antimicrobial powder as antimicrobial functional components (structural units), such as fibers, fabrics, plastics, coatings and ceramics. The detection for antimicrobial property of other materials can also refer to this Standard.

2 Normative references

The provisions in following documents become the provisions of this Standard through reference in this Standard. For dated references, the subsequent amendments (excluding corrigendum) or revisions do not apply to this Standard, however, parties who reach an agreement based on this Standard are encouraged to study if the latest versions of these documents are applicable. For undated references, the latest edition of the referenced document applies.

GB/T 19619, *Terminology for nanomaterials*

GB/T 13221, *Nanometer powder - Determination of particle size distribution - Small angle X-ray*

"Cosmetics Hygiene Standards" of the Ministry of Health of the People's Republic of China (Edition 2002)

3 Terms and definitions

For the purposes of this document, the terms and definitions established in GB/T 19619 as well as the followings apply.

3.1 antibacterial

the effect that can inhibit or hinder the growth and reproduction of bacteria or fungi and their activity.

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3.2 bactericidal

the effect that kills the growth and reproduction of bacteria or fungi.

3.3 antimicrobial

the effect that kills or hinders the growth and reproduction of bacteria or fungi and their activity.

3.4 nano antimicrobial powder

an assembly of discrete nanoparticles that meets the requirements of GB/T 13221 and has antibacterial effects.

3.5 nano antimicrobial material

nano antibacterial powder and materials that use nano antibacterial powder as antibacterial functional components (structural units).

4 Test methods

4.1 The test method for the antimicrobial property of nano powder shall be carried out according to the method specified in Annex A.

4.2 The test method for the antimicrobial property of materials such as fibers, fabrics, plastic powders and microporous filter materials shall be carried out according to the method specified in Annex B.

4.3 The test method for the antimicrobial property of hard surface materials such as plastics, ceramics, paint films, plates and metals are carried out in accordance with the methods specified in Annex C.

5 Test data processing

Multiply the number of colonies on each plate by the dilution factor to obtain the actual number of colonies recovered from the sample.

6 Calculation of detection results

6.1 Calculation of the number of colonies

Multiply the number of colonies on each plate by the dilution factor to obtain the actual number of bacteria recovered from the sample.

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Annex A

(normative)

Test method for powder antimicrobial property - Oscillation method

A.1 Scope of application

This test method is applicable to determination of the antimicrobial property of nano powder.

A.2 Test equipment and materials

A.2.1 Test equipment

Type A_2 secondary biological safety cabinet, constant temperature shaking incubator (300r/min), constant temperature incubator, pressure steam sterilizer, electric heating constant temperature dry oven [(0~250)°C], refrigerator, microwave oven (output power ≥700W), balance (resolution of 0.001g).

A.2.2 Test equipment

Erlenmeyer flask (capacity of 500ml, 250ml, 150ml), petri dish (diameter of 90mm), test tube (18mm×180mm), graduated cylinder (100mL), straw (10mL, 5mL, 1mL), alcohol lamp, test tube rack and so on.

A.2.3 Medium and reagents

A.2.3.1 Ordinary nutrient broth medium

Peptone	10g
Beef extract	5g
Sodium chloride	5g
Distilled water	1000mL

Adjust the pH value to 7.2~7.4. Perform high-pressure steam sterilization at 121°C for 20min.

Usage: for the cultivation of staphylococcus aureus and Escherichia coli enrichment.

A.2.3.2 Ordinary nutrient agar medium

Peptone	10g

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Wash off the lawn. Transfer the washed bacteria liquid to another test tube. After mixing with a shaker, use 0.03mol/L phosphate buffer to dilute to an appropriate concentration (about 10^5cfu/mL). The suspension of bacterial propagules shall be stored in a refrigerator at 4°C for later use and shall not exceed 4h.

A.3.2.2 Preparation of control sample solution

Weigh 0.5g±0.05g of control sample powder into an Erlenmeyer flask. Add 95ml of phosphate buffer containing 0.1% Tween-80. After mixing, add 5.0mL of pre-prepared bacteria suspension.

A.3.2.3 Preparation of test group sample solution

Weigh 0.5g±0.05g of test sample powder into an Erlenmeyer flask. Add 95mL of PBS containing 0.1% Tween-80. After mixing, add 5.0mL of pre-prepared bacteria suspension.

A.3.2.4 Count of live bacteria in the control sample "0"

Before oscillation, properly dilute the control sample solution. Pipette 1.0mL and inoculate it in a sterile dish. Inoculate 2 plates in parallel for each sample solution. Pour the nutrient agar medium that has been melted at 45°C~55°C. Turn the plate over after the agar medium has solidified. Place the above plate in a 37°C±1°C constant temperature incubator for colony count.

A.3.2.5 Oscillation contact cultivation

Fix the Erlenmeyer flask containing the control sample and the test sample on the shaking bed of the constant temperature shaking incubator. Under the condition of action temperature 37°C±1°C, at the speed of 150r/min, oscillate and contact the material that test sample needs to be diluted before use 1h~4h; oscillate and contact the material that does not need to be diluted and is directly used 4h~24h.

A.3.2.6 Viable bacteria count after oscillation for a certain period of time

After appropriate dilution of oscillated control sample solution and test solution, respectively take 1.0 mL of the sample solution and inoculate it in a sterile dish. Inoculate 2 plates in parallel for each sample solution. Pour the nutrient agar medium that has been melted at 45°C~55°C. Turn the plate over after the agar medium has solidified. Place the above plate in a 37°C±1°C constant temperature incubator and count the live bacteria culture.

A.3.2.7 Negative control group

The negative control group draws the same batch of dilutions, culture medium

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Annex B

(normative)

Test method for material antimicrobial property - Oscillation method

B.1 Scope of application

This test is applicable to the determination the antimicrobial property of dissolvable and non-dissolvable fibers, fabrics, plastic powders, and microporous filter materials.

B.2 Test equipment and materials

B.2.1 Test equipment, test device and standard strains for test

The requirements for test equipment, test device and standard strains for test shall meet the provisions of A.2.1~A. 2.4.

B.2.2 Control sample

The control sample is a piece of pure cotton plain white cloth (32 yarns). The sample itself has no antibacterial effect and has no influence on the determination of test results. Degreasing treatment shall be carried out before the test: Boil the pure cotton plain weave cloth in water with detergent for 30min; Rinse 3 times with tap water; Then boil it with distilled water for 5min, rinse, dry and iron; Before cutting, remove the warp and weft according to the size of the prepared sample, and then cut it according to the draw marks.

B.3 Test procedures

B.3.1 Preparation of strain slope

The preparation of the strain slope shall comply with the provisions of A.3.1.

B.3.2 Test steps

B.3.2.1 Cut the antimicrobial fabric sample into 10mm×10mm. Antimicrobial plastics, microporous filter materials, filaments, staple fibers, and yarns are used as they are. Weigh 1.0g±0.05g of antimicrobial sample into an Erlenmeyer flask. Add 95 mL of PBS containing 0.1% Tween-80. After mixing, add 5.0mL of pre-prepared bacteria suspension.

B.3.2.2 Preparation of bacterial suspension, preparation of control sample solution, count of viable bacteria of control sample "0" contact time, oscillation contact culture, viable bacteria count after oscillation contact for a certain period

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the bacterial suspension from overflowing.

C.3.2.2 Control sample

The control sample is injection-molded with sanitary high-density polyethylene (HDPE). The standard size is 50mm×50mm (±2 mm). The thickness is not more than 5mm. It is required that it has no antibacterial effect and has no influence on the judgment of test results.

C.3.2.3 Preparation of test group samples

Make the test sample into a standard size of 50mm×50mm (±2mm). If the test sample size is small, it shall not be less than 20mm×20mm.

C.3.2.4 Sample pretreatment

Take control samples and tested samples. Use 70% ethanol solution to wipe the surfaces. After 5min, use sterile distilled water to rinse. Dry naturally. If the sample is not suitable for disinfectant treatment, it can be directly rinsed with sterile distilled water or disinfected by other methods according to the characteristics of the sample. But it must not affect its antimicrobial property and interfere with the test results.

C.3.2.5 Preparation of bacterial suspension

Take the nutrient agar medium slope from the third to the eighth generation of strains for 18h-24h fresh culture. Use a 5.0mL pipette to suck 3.0mL~5.0mL of 0.03mol/L phosphate buffer into the inclined tube. Repeatedly suck and blow. Wash off the lawn. Transfer the washed bacteria liquid to another test tube. After mixing with an oscillator, use 0.03mol/L phosphate buffer to dilute to an appropriate concentration (about 10^5cfu/mL). The suspension of bacterial propagules shall be stored in a refrigerator at 4°C for later use and shall not exceed 4h.

C.3.2.6 Inoculation bacteria solution

Put the control sample and the tested sample into a sterile dish. Pipette 0.2mL~0.5mL test bacteria solution and drip it on the surface of control sample and tested sample respectively. Make 3 parallel samples for each sample. Use a sterile tweezer to pick up the cover film and cover the sample surface separately and flatten it. There must be no bubbles. Make the bacteria solution evenly contact the sample. Cover the plate. Conduct contact cultivation at 37°C±1°C and relative humidity of 90% for 16h~24h. If the tested sample is a photocatalyst antibacterial agent, a light source shall be installed in a constant temperature incubator according to the sample test requirements.

C.3.2.7 Colony count

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