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Antimicrobial property testing and evaluation methods for nano-inorganic materials

纳米无机材料抗菌性能检测方法及评价

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Table of Contents

Antimicrobial property testing and evaluation methods for nano-inorganic materials

1 Scope

This document specifies the test methods, antimicrobial property evaluation, test report and safety operation requirements for the antibacterial property of nano-inorganic materials.

This document applies to nano-inorganic materials with antibacterial functions, as well as products with nano-inorganic materials as antibacterial components (structural units), such as fibers, fabrics, plastics, coatings and ceramics. The antibacterial property testing and evaluation of other materials can also be carried out in accordance with this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the version corresponding to that date is applicable to this document; for undated references, the latest version (including all amendments) is applicable to this document.

GB 4789.2, National food safety standard -Microbiological examination of food: Aerobic plate count

GB/T 30544 (all parts), Nanotechnologies - Vocabulary

3 Terms and definitions

For the purposes of this document, the following terms and definitions, as well as those given in GB/T 30544 (all parts), apply.

3.1

antibacterial

The process of killing microorganisms such as bacteria and fungi and/or hindering their growth, reproduction and activity by chemical or physical methods.

3.2

nano-inorganic materials

Inorganic materials whose three-dimensional spatial scale has at least one dimension at the nanoscale (1 nm ~ 100 nm).

4 Test method

4.1 Procedure

4.1.1 The test of the antibacterial property of powdered nano-inorganic materials shall be carried out according to the method specified in Appendix A.

4.1.2 The test of the surface antibacterial property of non-porous products containing nano-inorganic antibacterial components shall be carried out according to the method specified in Appendix B or Appendix C.

4.1.3 The test of the antibacterial property of porous products containing nano-inorganic antibacterial components shall be carried out according to the method specified in Appendix B or Appendix D.

4.2 Test data processing

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

4.3 Calculation of test results

4.3.1 Calculation of colony counts

Multiply the colony counts on each plate by the dilution factor to get the actual number of bacteria recovered from the sample.

4.3.2 Calculation of antibacterial rate

Calculate the antibacterial rate R according to Formula (1).

$$R = \frac{A - B}{A} \times 100\% \qquad \cdots\cdots (1)$$

Where:

R – antibacterial rate;

A – the average number of bacteria recovered after the control sample has been in contact with the test bacteria for a certain period of time, in colony forming units per milliliter (cfu/mL);

B – the average number of bacteria recovered after the test sample has been in contact with the test bacteria for a certain period of time, in colony forming units per milliliter (cfu/mL).

microbial culture collection centers or national corresponding culture collection management centers.

A.2.5 Control samples

Silicon dioxide powder, the powder size is required to be no more than 100 nm and the purity to be 98% ~ 99%. It has no antibacterial effect and has no influence on the determination of the test results.

A.3 Test procedure

A.3.1 Preparation of bacterial slants

A.3.1.1 Bacterial activation

Take a tube of dry culture; open it under sterile operation; add an appropriate amount of nutrient broth with a capillary pipette; gently pipette several times to melt and disperse the culture. Take a test tube containing 5.0 mL ~ 10.0 mL of nutrient broth; drop a small amount of bacterial suspension into it; culture it at 37 ℃ ± 1 ℃ for 18 h ~ 24 h.

A.3.1.2 Separation

Use an inoculating loop to take the bacterial suspension of the first-generation culture; streak it onto a nutrient agar plate; culture it at 37 ℃ ± 1 ℃ for 18 h ~ 24 h.

A.3.1.3 Purification

Pick a typical colony from the second-generation culture mentioned above; inoculate it on a nutrient agar slant; culture it at 37 ℃ ± 1 ℃ for 18 h ~ 24 h to obtain the third-generation culture.

A.3.1.4 Preservation of bacterial species

Inoculate the species on a slant of nutrient agar medium; culture at 37 ℃ ± 1 ℃ for 24 h; then store at 0 ℃ ~ 5 ℃. Generally, transfer the species once no more than one month. When contamination is suspected, identification shall be carried out using methods such as colony morphology, Gram staining and biochemical tests.

A.3.2 Test steps

A.3.2.1 Preparation of bacterial suspension

Take 18 h ~ 24 h fresh culture of the nutrient agar medium slant of the third to eighth generations of bacterial species; use a 5.0 mL pipette to draw 3.0 mL ~ 5.0 mL of 0.03 mol/L phosphate buffer into the slant test tube; repeatedly aspirate and blow to wash off the bacterial lawn. Transfer the washed bacterial solution to another test tube; use an oscillator to mix it; use 0.03 mol/L phosphate buffer to dilute it to an appropriate

concentration (about 105 cfu/mL). The bacterial vegetative suspension shall be stored in a 4 °C refrigerator for use and shall not be stored for more than 4 h.

A.3.2.2 Preparation of control group sample solution

Weigh 0.50 g ± 0.05 g of the control sample powder and put it into a conical flask; add 95 mL of phosphate buffered saline containing 0.1% (mass fraction) Tween-80; mix well; then, add 5.0 mL of the pre-made bacterial suspension.

A.3.2.3 Preparation of test sample solution

Weigh 0.50 g ± 0.05 g of the test sample powder and put it into a conical flask; add 95 mL of phosphate buffered saline containing 0.1% (mass fraction) Tween-80; mix well; then, add 5.0 mL of the pre-made bacterial suspension.

A.3.2.4 Viable count of control sample at "0" contact time

Before shaking, dilute the control sample solution appropriately; aspirate 1.0 mL and inoculate it into a sterile plate; inoculate 3 plates in parallel with each sample solution; pour in nutrient agar medium dissolved at 45 ℃ ~ 55 ℃; turn the plate over after the agar medium solidifies; place the plate in a constant temperature incubator at 37 ℃ ± 1 ℃ to count the colonies.

A.3.2.5 Oscillating contact culture

Fix the conical flasks containing the control sample and the test sample on the shaker of a constant temperature shaking incubator. Under the condition of an action temperature of 37 ℃ ± 1 ℃, shake at a speed of 150 r/min. The materials that need to be diluted before use should be shaken for 1 h ~ 4 h, and the materials that do not need to be diluted and used directly should be shaken for 4 h ~ 24 h.

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

After appropriate dilution of the shaken control sample and test sample, take 1.0 mL of each sample and inoculate it in a sterile plate. Inoculate 2 plates in parallel with each sample. Pour the dissolved nutrient agar medium at 45 ℃ ~ 55 ℃. After the agar medium solidifies, turn the plate over and place the plate in a constant temperature incubator at 37 ℃ ± 1 ℃ to count the viable bacteria.

A.3.2.7 Negative control group

For the negative control group, respectively take dilution solution and culture medium from the same batch of test samples and place in a constant temperature incubator at 37 ℃ ± 1 ℃ for culture. Observe for contamination.

A.3.2.8 Observations

Appendix B

(Normative)

Test method for antibacterial properties of porous and non-porous materials containing nano-inorganic antibacterial components - Oscillation method

B.1 Applicability

This test method is applicable to the determination of the antibacterial property of porous and non-porous materials (including dissolvable and non-dissolvable fibers, fabrics, plastic powders, microporous filter materials, etc.) containing antibacterial components of nano-inorganic materials.

B.2 Test equipment and materials

B.2.1 Test equipment, test apparatuses and standard species for testing

The requirements for test equipment, test apparatuses and standard species for testing shall comply with the provisions of A.2.1 ~ A.2.4.

B.2.2 Control samples

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

B.3 Test procedure

B.3.1 Preparation of bacterial slants

The preparation of bacterial slant shall comply with the requirements of A.3.1.

B.3.2 Test steps

B.3.2.1 Select the sterilization method according to the sample. Generally, use high-pressure steam sterilization; place the sample in an appropriate container and place it in a high-pressure sterilizer for sterilization (121°C, 103 kPa, 20 min). If the sample is not suitable for sterilization by high-pressure steam, it can be sterilized by other methods such as high-temperature moist heat, dry heat or ethylene oxide. However, the sterilization method used shall not affect the antibacterial performance and test results, and shall be stated in the report.

Appendix C

(Normative)

Test method for antibacterial property of non-porous materials containing nano-inorganic antibacterial components - Film method

C.1 Applicability

This test method is applicable to the determination of the antibacterial property of non-porous materials (such as plastics, ceramics, paint films, plates, metals and other hard surface materials) containing antibacterial components of nano-inorganic materials.

C.2 Test equipment and materials

C.2.1 Test equipment

Type A_2 secondary biological safety cabinet, constant temperature incubator (37 ℃ ± 2 ℃), pressure steam sterilizer (pressure 103 kPa, temperature 121 ℃), electric constant temperature dry oven (0 ℃ ~ 250 ℃), refrigerator (2 ℃ ~ 8 ℃), microwave oven (output power ≥ 700 W), ultraviolet lamp (30 W, 253.7 nm).

C.2.2 Test apparatuses

Erlenmeyer flask (capacity 500 mL, 250 mL), petri dish (diameter 90 mm), test tube (18 mm × 180 mm), measuring cylinder (100 mL), pipette (10 mL, 5 mL, 1 mL), pipette (accuracy 0.01 mL), alcohol lamp, test tube rack, 70% ethanol and polyethylene film, etc.

C.2.3 Culture medium, reagents and standard bacterial species for testing

Culture media, reagents and standard bacterial species for testing shall comply with the requirements of A.2.3 and A.2.4.

C.3 Test procedure

C.3.1 Preparation of bacterial slants

The preparation of bacterial slant shall comply with the requirements of A.3.1.

C.3.2 Test steps

C.3.2.1 Preparation of covering film

The covering film is made of polyethylene film with a size of (40±2) mm × (40±2) mm and a thickness of 0.05 mm ~ 0.10 mm. If the test sample is small, the size of the covering film can be reduced according to its surface area to prevent the bacterial suspension from overflowing.

C.3.2.2 Control samples

Use sanitary high-density polyethylene (HDPE) to perform injection molding on the control sample to a standard size of (50±2) mm × (50±2) mm and a thickness of no more than 5 mm. It is required that it itself has no antibacterial effect and has no influence on the determination of the test results.

C.3.2.3 Preparation of test group samples

Make the test samples into standard size of (50±2) mm × (50±2) mm. If the test samples are smaller, they shall not be less than 20 mm × 20 mm.

C.3.2.4 Sample pretreatment

Take the control sample and the test sample; use 70% ethanol solution to wipe their surfaces; rinse with sterile distilled water after 5 minutes; dry naturally. If the sample is not suitable for treatment with disinfectants, it can be directly rinsed with sterile distilled water or disinfected by other methods according to the characteristics of the sample, but it shall not affect its antibacterial properties and interfere with the test results.

C.3.2.5 Preparation of bacterial suspension

Take 18 h ~ 24 h fresh culture of the nutrient agar medium slant of the third to eighth generations of bacterial species; use a 5.0 mL pipette to draw 3.0 mL ~ 5.0 mL of 0.03 mol/L phosphate buffer into the slant test tube; repeatedly aspirate and blow to wash off the bacterial lawn. Transfer the washed bacterial solution to another test tube; use an oscillator to mix it; use 0.03 mol/L phosphate buffer to dilute it to an appropriate concentration (about 105 cfu/mL). The bacterial vegetative suspension shall be stored in a 4 °C refrigerator for use and shall not be stored for more than 4 h.

C.3.2.6 Inoculation of bacterial solution

Place the control sample and the test sample in sterile dishes respectively; draw 0.2 mL ~ 0.5 mL of the test bacterial solution and drop it on the surface of the control sample and the test sample respectively; make 3 parallel samples for each sample. Use sterile tweezers to pick up the covering film and cover the surface of the sample; make sure it is flat without any bubbles. Make sure the bacterial solution is in uniform-contact with the sample. Cover the plate and culture at 37 ℃ ± 1 ℃ and relative humidity of 90% for 16 h ~ 24 h. If the sample to be tested uses a photocatalyst antimicrobial agent, a light source shall be installed in the constant temperature incubator according to the sample test requirements.

C.3.2.7 Colony count

For samples that have been cultured in contact for 16 h ~ 24 h, add 20 mL of elution solution respectively, and elute the samples and covering film repeatedly for 3 times.

Appendix D

(Normative)

Test method for antibacterial property of porous materials containing nano-inorganic antibacterial components - Absorption method

D.1 Applicability

This test method is applicable to the determination of the antibacterial property of porous products (such as fabrics, cotton wool, down, yarn, carpets or similar structural products) containing antibacterial components of nano-inorganic materials.

D.2 Test equipment and materials

D.2.1 Test equipment

Secondary biological safety cabinet, constant temperature incubator (37 ℃ ± 2 ℃), water bath (46 ℃ ± 2 ℃ and 70 ℃ ~ 90 ℃), pressure steam sterilizer (pressure 103 kPa, temperature 121 ℃), electric constant temperature dry oven (0 ℃ ~ 250 ℃), refrigerator (2 ℃ ~ 8 ℃), microwave oven (output power ≥700 W), and vortex oscillator.

D.2.2 Test apparatuses

Erlenmeyer flask (capacity 500 mL, 250 mL), petri dish (diameter 90 mm), glass bottle (flat-bottom cylindrical, 30 mL), test tube (18 mm × 180 mm), measuring cylinder (100 mL), pipette (10 mL, 5 mL, 1 mL), pipette (accuracy 0.01 mL), alcohol lamp, test tube rack, 70% ethanol, aluminum foil, etc.

D.2.3 Culture medium, reagents and standard bacterial species for testing

Culture media, reagents and standard bacterial species for testing shall comply with the requirements of A.2.3 and A.2.4.

D.3 Test procedure

D.3.1 Preparation of bacterial slants

The preparation of bacterial slant shall comply with the requirements of A.3.1.

D.3.2 Preparation of bacterial suspension

The preparation of bacterial suspension shall comply with the requirements of A.3.1.2.

D.3.3 Test steps

D.3.3.1 Preparation of samples

D.3.3.1.1 Mass and shape of sample

Weigh 0.40 g ± 0.05 g as a sample and cut it into appropriate size. Take 6 samples to be tested for antibacterial property and 6 control samples, respectively.

Note: Three control samples and three test samples are used to determine the bacterial count at "0" time, and the remaining three control samples and three test samples are used to determine the bacterial count after 18 h ~ 24 h of incubation.

D.3.3.1.2 Placement of sample

Place the test samples individually in glass vials and choose the appropriate placement method based on the properties of the samples.

a) If the samples are prone to curling or contain lint or down, place a glass rod on the samples or secure the sides with string.

b) If the samples are yarn, tie the samples together and place a glass rod on top.

c) For samples of carpet or similar structures, cut the pile part as the sample and place a glass rod on it.

If necessary, the test samples may be washed in accordance with ISO 6330 or other suitable method. After washing, use water to rinse the sample to remove the detergent.

D.3.3.2 Sterilization of test samples

If contamination of the sample is found or suspected, sterilize the sample in an autoclave. Use aluminum foil to wrap the opened glass bottle and bottle cap and place them in a high-pressure sterilizer for 15 ~ 20 minutes. Remove the glass bottle and bottle cap from the autoclave; remove the aluminum foil; dry on a clean workbench or other place without contamination risk for 60 min; then, cover the bottle with the cap.

D.3.3.3 Inoculation of bacterial solution

Use a pipette to accurately take 0.2 mL of the test bacterial solution (D.3.2) and inoculate it on the sample in each vial (D.3.3.1.2), making sure that the bacterial solution does not stick to the bottle wall; tightly close the bottle cap.

D.3.3.4 Elution

Quickly add 20 mL of nutrient broth medium (A.2.3.1) or physiological saline to the 3 control sample and 3 test sample vials (D.3.3.3) inoculated with the test bacterial solution; cover the bottles; shake by hand or with a mixer to wash out the bacteria.

D.3.3.5 Cultivation

Incubate 6 vials (3 control samples and 3 test samples) at 37 ℃ ± 2 ℃ for 18 h ~ 24 h.

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