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

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**Evaluating Method for Efficacy of Antibacterial and
Bacteriostasis
(English Translation)**

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Foreword

This standard is written in accordance with the provisions of GB/T 1.1-2009.

This standard is provided by Jiangsu Provincial Center for Disease Control and Prevention, China Center for Disease Control and Prevention, Environmental and Health-related Product Safety Institute, Shandong Province the Center for Disease Control and Prevention and the Heilongjiang Provincial Center for Disease Control and Prevention are responsible for drafting.

1 Scope

This standard specifies the selection principles and uses of antibacterial and bacteriostatic evaluation methods.

This standard is applicable to the identification of antibacterial and bacteriostatic effects of products with antibacterial and/or bacteriostatic functions.

2 Normative references

The following documents are indispensable for the application of this document. For dated references, only the dated version applies to this article.

Pieces. For undated references, the latest edition (including all amendments) applies to this document.

GB 15979 Hygienic standard for single-use hygiene products FZ/T 73023 antibacterial knitwear Disinfection Technical Specifications (2002 Edition) Ministry of Health (Wei Fa Jian

Fa [2002] No. 282)

3 Terms and definitions

The following terms and definitions apply to this document.

Antibacterial the use of chemical or physical methods to kill or hinder the growth and reproduction of bacteria can reduce the number and activity of the process.

Bacteriostatic bacteriostasis the process of inhibiting or hindering the growth and reproduction of bacteria and its activity by chemical or physical means.

Sustained antibacterial action the antibacterial product with antibacterial effect is applied to the surface of the object, and it still kills or hinders the growth and reproduction of bacteria for more than 7 days.

Neutralizer

In the microbicide test, the disinfectant used to eliminate the test microorganisms and disinfectants, and the disinfectant remaining on the surface of the microorganisms, it removes the agent that inhibits and kills microorganisms.

4.1 Difference between antibacterial test and antibacterial test

Antibacterial test. The antibacterial effect of the antibacterial product on bacteria and fungi is determined. The neutralizing agent is required to terminate the bactericidal action during the test.

Bacteriostatic test. The antibacterial effect of the antibacterial product on bacteria and fungi was determined, and the neutralizing agent was not needed to terminate the antibacterial action during the test.

4.2.1 Principles for selection of methods for evaluating bacteriostasis

Antibacterial products are selected for antibacterial effect evaluation methods. Liquid antibacterial products, selective suspension bacteriostatic test, paste or semi-solid gel, Viscous antibacterial products select carrier soaking quantitative antibacterial test, wet wipes or other products containing their own antibacterial ingredients Test, antibacterial ring test for soap solid antibacterial products, textile selective dipping bacteriostatic test containing soluble antibacterial ingredients, containing continuous Antibacterial washing solution for bacteriostatic action was selected for retention and bacteriostatic test.

4.2.2 Selection principle of antibacterial effect evaluation method

Antibacterial products are selected for evaluation of antibacterial effects. Liquid antibacterial product selection suspension quantitative sterilization test, gelatinous or paste antibacterial product Select carrier soak quantitative bactericidal test, wet wipes or other products

containing antibacterial ingredients to select carrier sterilization test, containing soluble anti-drug the textiles of the bacterial components were selected for the impregnation test.

Textiles, non-woven fabrics, fibers, etc. containing insoluble antibacterial ingredients, after being identified as containing no soluble antibacterial ingredients, are shaken by flasks test.

Tiles, plastics, metals, paints, etc. containing insoluble antibacterial ingredients, using a film test; for application to the surface, after drying Continue antibacterial tests for products that continue to be antibacterial.

5.1.1.1 Scope of application

It is suitable for the determination of microbial bacteriostatic effect of liquid antibacterial preparations such as sanitary lotion and antibacterial spray.

5.1.1.2.1 Test strain

Staphylococcus aureus (ATCC 6538), *Escherichia coli* (8099), *Candida albicans* (ATCC 10231) and according to bacteriostatic agents Other strains used for specific purposes.

5.1.1.2.2 Reagents

Diluent. 0.03 mol/L phosphate buffered saline (PBS) (pH 7.2-7.4), medium. *Staphylococcus aureus* and large intestine rod the culture was carried out using a nutrient agar medium, and the culture of *Candida albicans* was carried out using Sabouraud agar medium.

5.1.1.2.3 Equipment

Constant temperature water bath, timer, Class II biological safety cabinet, etc.

5.1.1.3 Test procedure

The fresh slant culture of the test bacteria for 24 hours was washed with PBS and diluted with PBS to about 5.0×10^5 CFU/mL to 4.5×10^6 CFU/mL bacterial suspension.

spare. Take a sterile test tube, first add 5.0 mL of sample (use the original solution or diluent according to the instructions for use), set $20^\circ \text{C} \pm 1^\circ \text{C}$ water bath After 5 minutes, add 0.1 mL of the test bacterial suspension, mix quickly and time immediately. The bacteria to be tested interact with the sample to the specification at a fixed time, inoculate 1.0 mL of the test bacteria and the sample mixture to inoculate 2 plates, and pour the medium. When the amount of bacteria cannot be counted, do 10 with PBS.

Double dilution, select appropriate dilution to absorb 1.0 mL to inoculate 2 plates, and count the viable culture. Simultaneously replace the sample with PBS Parallel test as a positive control. The number of colonies recovered from the positive control was between 1.0×10^4 CFU/mL and 9.0×10^4 CFU/mL. Take the same batch PBS and medium were used as

negative controls. All test samples and control samples were cultured at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$, and the bacterial propagules were cultured for 48 hours.

Results. *Candida albicans* culture for 72 hours to observe the final results. The test was repeated 3 times and the inhibition rate was calculated.

5.1.1.4 Calculation of inhibition rate

10 x

In the formula.

X-bacteriostatic rate, %;

The amount of bacteria recovered in the A0-positive control group, the unit is CFU/mL;

A1 - The amount of bacteria recovered in the test group, the unit is CFU/mL.

5.1.1.5 Result determination

The inhibition rate is $\geq 50\%$ ~90%, and the bacteriostasis effect is determined; the inhibition rate is $\geq 90\%$, and it has strong antibacterial effect.

5.1.2.1 Scope of application

It is suitable for the determination of microbial bacteriostasis effect of paste or semi-solid gel and viscous antibacterial products.

5.1.2.2 Reagents, media and equipment

The carrier is a 10 mm x 10 mm skim white plain cloth, and the degreasing method is carried out according to the "Disinfection Technical Specification" (2002 edition).

Force steam sterilization standby, electronic balance ($d = 0.01\text{g}$), the same as 5.1.1.2.

5.1.2.3 Operation steps

The fresh slant culture of the test bacteria for 24 hours was washed with PBS and diluted with PBS to about 5.0×10^6 CFU/mL to 5.0×10^7 CFU/mL.

The suspension is ready for use. Dilute 10 μL of the bacterial suspension on a sterile carrier with a micropipette, dry at $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ or air dry at room temperature for use.

The sample was weighed in a sterile dish at a rate of 5 g/tablet, placed in a water bath at $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 5 min, and the carrier was inoculated with sterile forceps to complete the carrier.

Fully immersed in the sample and timed immediately. The carrier to be infected and the sample interact with the sample for the specified time, respectively, and the carrier is added