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Evaluation of bactericidal activity of a non-porous antimicrobial surface used in a dry environment

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 330, *Surfaces with biocidal and antimicrobial properties*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The surfaces in our environment can constitute significant reservoirs of bacteria and a risk to health that is essential to control.

In healthcare establishments, healthcare-associated infections (HAI) are a major cause of mortality and disability the world over. HAI affects 5 % to 15 % of hospital patients in developed countries and can affect 9 % to 37 % of patients admitted to intensive care units. Each year, about 1 in 25 U.S. hospital patients is diagnosed with at least one infection related to hospital care alone. At least five million HAI are estimated to occur in acute care hospitals in Europe, causing 135 000 deaths per year and approximately 25 million additional days spent in hospital, representing a financial cost of €13 billion to €24 billion.

In public transport, the risks of contamination between travellers are extremely high due to the frequency and number of contacts points. In establishments receiving the public, all common areas and collective spaces constitute a risk of the spread of pathogens microorganisms.

In the agri-food sector, microbial hazards are the highest risk amongst those that can impact consumer health, above malnutrition and chemical contaminants. In addition to the impact on health, the consequences of a case of contamination can be disastrous for the image and sustainability of a company. For instance:

— an estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420 000 die every year, resulting in the loss of 33 million healthy life years [disability-adjusted life years];

— US$110 billion is lost each year in productivity and medical expenses resulting from unsafe food in low- and middle-income countries;

— children under 5 years of age carry 40 % of the foodborne disease burden, with 125 000 deaths every year;

— diarrhoeal diseases are the most common illnesses resulting from the consumption of contaminated food, causing 550 million people to fall ill and 230 000 deaths every year.

The drive to control microbial risks extends to many other sectors (transport, pharmaceuticals, aeronautics, cosmetics, the phytosanitary sector, services, etc.), and even to the entire industrial manufacturing sector.

Pathogenic agents can be transmitted in a variety of ways, such as via food and water, via air (aerosols), via body contact with infected person and via surfaces contaminated by body secretions and fomites. Healthcare-associated infections, or infections acquired in healthcare settings are the most frequent adverse event in healthcare delivery worldwide. Hundreds of millions of patients are affected by healthcare-associated infections worldwide each year, leading to significant mortality and financial losses for health systems. Of every 100 hospitalized patients at any given time, 7 in developed and 10 in developing countries will acquire at least one healthcare-associated infection. Such infections annually account for 37 000 attributable deaths in Europe and potentially many more that can be related, and they account for 99 000 deaths in the USA. Amongst the available tools for reducing microbial risks, surfaces with biocidal properties can help to control cross-contaminations when used in combination with standard cleaning and disinfecting practice.

This document was written to address the need to demonstrate the biocidal efficacy of a surface under ambient conditions close to the conditions found in the field. The method described in this document simulates the contamination of a surface by a microdroplet. For example, this pathway of contamination is representative of a surface contamination following a sneeze (microdroplet post sneeze might have a mobility of several meters). The temperatures and humidity required for the test were defined in relation to conditions that are representative of the atmosphere in the healthcare facilities.

Numerous non-porous surfaces claim to perform a bactericidal function:

— surfaces of materials that have been treated with or include a biocidal product in order to give the material bactericidal properties, either temporarily or permanently.

— surfaces of materials, such as certain metals, that claim to have intrinsic bactericidal properties.

The method prescribes the representative basic strains to be tested. Additional strains may be tested, depending on the intended use. For a given use, further experiments including in use condition (soiling substances, ageing, adaption of environmental condition) are needed for demonstrating bactericidal activity of the tested surface as claimed.

This method will be revised in the near future to include interfering substances and to adapt strains/efficacy/contact time specifications to the needs of different sectors, in addition to the medical area. Ageing simulation testing will be produced soon by ISO/TC 330.

Surfaces with bactericidal properties supplement, but do not replace, the regular use of surface-treatment products, such as detergents, detergent-disinfectants and surface disinfectants which must be demonstrated to be compatible with maintaining the surface bactericidal activity.

Evaluation of bactericidal activity of a non-porous antimicrobial surface used in a dry environment

WARNING — Persons using this document shall have technical microbiological knowledge.

1 Scope

This document specifies the test conditions and the levels of activity to determine the bactericidal activity of non-porous surfaces used in a dry environment. It defines a protocol to validate the bactericidal character of a surface and to measure its performance. It is not intended to be used to substantiate cleaning or disinfecting properties.

This document is applicable to surfaces claiming to have an activity against vegetative bacteria. The obligatory test conditions are defined in this document. It does not apply to porous surfaces.

It does not refer to methods for testing the toxicological and ecotoxicological properties of the surfaces. This document is used to measure bactericidal action, not bacteriostatic activity of a surface.

2 Normative reference

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 edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
bactericidal activity
capability of a surface to produce a reduction in the number of viable bacterial cells of representative *test organisms* (3.6) under defined conditions without soil load, nor ageing test nor test conditions adaptation to a specific sector.

Note 1 to entry: Note to entry: At the moment this document does not include soiling or ageing condition.

3.2
bacteriostatic activity
capability of a surface to inhibit the growth of viable bacterial cells of representative *test organisms* (3.6) under defined conditions

Note 1 to entry: The term "bacteriostatic activity" cannot be used for claims according to this document.

3.3
additional condition
test conditions that are optional and not obligatory, that can be used for additional claims of *bactericidal activity* (3.1) regarding a surface

3.4
cleaning
all operations that achieve a level of cleanliness, appearance, comfort and hygiene

Note 1 to entry: Such operations use, to varying degrees, the following combined factors: chemical action, mechanical action, temperature, duration of action

3.5
neutralizer
chemical agent or formulation that suppresses the residual microbicidal activity of a product or active substance from the surface to be tested for a specific test but does not inactivate or inhibit the *test organism* (3.6)

3.6
test organism
strain of a microorganism selected to evaluate the antimicrobial activity of a surface for a standardized test

3.7
bactericidal surface
surface that irreversibly kill vegetative bacteria under defined conditions

Note 1 to entry: The adjective “bactericidal” corresponds to the noun “bactericide”.

3.8
reference surface
surface without any *bactericidal activity* (3.1) or properties, that is used to evaluate the quantity of culturable bacteria present at the moment when the bactericidal activity of the *test surface* (3.9) is evaluated

3.9
test surface
surface claiming *bactericidal activity* (3.1)

Note 1 to entry: This method is suitable for testing any type of non-porous surfaces (such as metals, plastic, glass, coated surfaces, etc.) as long as recovery of bacteria from the test surface shall not lose more than 1 log at T=0.

Note 2 to entry: Very hydrophobic surfaces, for which a drying time greater than 10 min (see 6.2.2) is necessary, cannot be tested according this method.

3.10
ambient light
light corresponding to a maximum value of 2 000 lux

Note 1 to entry: Any specific light or light spectrum (e.g. strong light, UV, etc.) is not considered as ambient.

3.11
porous surface
surface permeable to water, air, or other fluids

4 Apparatus, reagents and materials

4.1 Test organisms

Bactericidal activity shall be evaluated using the following four strains:

— *Pseudomonas aeruginosa* ATCC 15442 = CIP 103-467;

— *Staphylococcus aureus* ATCC 6538 = CIP 4.83;

— *Enterococcus hirae* ATCC 10541 = CIP 5855;

— *Escherichia coli* ATCC 10536 = CIP 54127.

The reference strain numbers in other culture collections shall be in accordance with the strains specified in Annex A.

The activity data can be completed with other strains using the experimental design described in this document, varying the conditions to meet the needs of the intended -practice application(s). If additional strains are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere) to be recorded in the test report.

Their suitability for supplying the inoculum and controls with a sufficient concentration shall be verified. If these additional test strains are not classified at a culture collection centre, their identification characteristics shall be stated. In addition, they shall be held by the testing laboratory or national culture collection under a reference for a five-year period.

NOTE ISO/TC 330 is currently adapting the method in terms of strains/efficacy levels/contact times/ interfering substances according to different sectors' needs and to include not only the medical area.

4.2 Culture media and reagents

The reagents shall be of analytical grade and/or appropriate for microbiological purposes. They shall be free from substances that are toxic or inhibitory to the test organisms.

4.2.1 Water, which shall be free from any substances that are toxic or that inhibit the bacteria. It shall be freshly glass-distilled water or water for injection or possibly deionized or demineralized water.

Sterilize in the autoclave (4.4.1).

NOTE If the water is sterilized during the sterilization of the reagents, this is not necessary.

4.2.2 Microbial suspension diluents

Tryptone-salt solution:

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride	8,5 g
Water (see 4.2.1)	1,000 ml

Sterilize in the autoclave (see 4.4.1). After sterilization, the pH of the medium shall be equivalent to 7,0 ± 0,2 when measured at 20 °C.

4.2.3 Liquid for bacteria recovery and for membrane rinsing

4.2.3.1 Composition

Tryptone, pancreatic digest of casein	1,0 g

Sodium chloride	8,5 g
Polysorbate 80	5,0 g
Water (see 4.2.1)	1 000 ml

4.2.3.2 Preparation

a) Dissolve the sodium chloride and tryptone in the water (see 4.2.2). Add the Polysorbate 80, mix and complete up to 1 000 ml with water

b) distribute into smaller adapted flasks. Sterilize in the autoclave.

c) If necessary, add a neutralizer to the recovery liquid

The use of a validated neutralizer of the respective antimicrobial is compulsory to warrant a correct test time for bacteria recovery and for membrane rinsing (see 4.2.3): The functionality of the respective neutralizer shall be validated beforehand. The neutralizer:

— shall be validated for the tested surface, as per 6.2.4 and it shall be sterile;

— shall be mentioned in the test report.

Examples of neutralizers are given in Annex D.

4.2.4 Agar for bacteria maintenance and counting [tryptone soya agar (TSA)]

Use agar to preserve the bacterial strains and count the viable bacteria.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of soybean meal	5,0 g
Sodium chloride	5,0 g
Agar	15,0 g
Water (see 4.2.1)	1 000 ml

Sterilize in the autoclave. After sterilization, the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at 20 °C. Pre pored plate TSA are acceptable if they meet the composition and pH value described in 4.2.4.

4.3 Reference and test surfaces

4.3.1 General

The surfaces shall be used only once.

4.3.2 Reference surfaces

4.3.2.1 Description

Inox 304 stainless steel, disk 2 cm in diameter, of which both sides have a grade 2B finish. The surfaces shall be flat.

If the coated surface without antimicrobial agent (the test surface) exist, it can be used as a reference surface similar to inox and therefore can be used for log reduction calculation.

The reference surface used during the test shall be specified in the report.

4.3.2.2 Cleaning — Disinfection/sterilization

The reference surfaces shall be cleaned and disinfected before use.

If a specific preparation protocol is applied to the test surface (see examples in Annex C), the same protocol should be applied to the reference surface (see 4.3.3.2). Otherwise, the protocol in example 1 of Annex C should be used.

No residual action or changes of the antimicrobial properties shall result from disinfection/cleaning procedure on the reference surface.

The reference surface treatment used during the test shall be specified in the report.

4.3.3 Test surfaces

4.3.3.1 Identification and production

The characteristics (e.g. dimensions, thickness, etc.) and references of the finished product shall be defined and specified in the final test report.

If the test surface is treated with a bactericide, the nature of the active substance(s) shall be specified to the lab for safety and security reasons, and indicated in the final test report. For coated surfaces with unknown porosity (e.g. paint), recovery of the microorganisms shall be checked by the laboratory, comparing the coated surface with the antimicrobial agent and the reference surface (C0 vs T0) or the coated surface with and without the antimicrobial agent, to ensure the accuracy of bacteria reduction evaluation. Recovery of bacteria from the test surface shall not lose more than 1 log at T=0 compared to the reference one.

The test surface shall be produced from the materials intended for the finished product claiming bactericidal activity, and according to the same steps of the final production process. If it is impossible to perform the steps on the same equipment, it is permitted to use a representative simulation of these steps.

The test surface shall have flat surfaces and measure between 12 mm and 25 ± 2 mm on the sides or in diameter. If it is impossible to cut the test surface into squares or disks of this size, other sizes and shapes can be used. In this case, the actual dimensions used will be stated in the test report. Any changes made to the protocol (recovery volume, etc.) due to the dimensions of the tested test surface shall be specified in the test report.

The uniformity of each test surface shall be visually inspected. Test surfaces with anomalies on the surface or edges shall be discarded (e.g. corrosion/rust, slivers, deep grooves or ridges, etc.).

4.3.3.2 Cleaning – disinfection/sterilization

All the test surfaces shall be at least rinsed with sterile distilled water before performing the tests.

If necessary, disinfection or sterilization can be performed according to the nature of the surface, indicated by the manufacturer and detailed in the test report. The manufacturer shall propose/validate the complete treatment. If necessary, preliminary tests shall be performed to define the appropriate treatment to avoid impacting the efficacy of the tested surfaces. These tests can result in the methodology described in 4.3.2.2 being adapted with identical treatment for the reference surface and the test surface.

No residual action or changes of the antimicrobial properties shall result from disinfection/cleaning procedure on the reference and tested surface.

Annex C contains examples of surface preparation protocols.

The test surface treatment used during the test shall be specified in the report.

4.4 Apparatus and materials

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods:

— in the autoclave (see 4.4.1), by keeping them at between 121 °C and 124 °C for at least 15 min;

— in the hot air oven (see 4.4.1), by keeping them at 180 °C for at least 30 min, at 170 °C for at least 1 h or at 160 °C for at least 2 h.

Usual microbiological laboratory equipment, and in particular:

4.4.1 Apparatus for sterilization

— For moist heat sterilization, an autoclave capable of being maintained at between 121 °C and 124 °C for at least 15 min.

— For dry heat sterilization, a hot air oven capable of being maintained at between 180 °C and 185 °C for at least 30 min, at between 170 °C and 175 °C for at least 1 h, or at between 160 °C and 165 °C for at least 2 h.

4.4.2 Water baths. Thermostat-controlled bath at 45 °C ± 1 °C.

4.4.3 Incubators

— Incubators capable of being maintained in the range of 35 °C to 38 ± 1 °C

— Thermostat-controlled incubators at the defined test temperatures (excluding ambient temperature).

4.4.4 pH-meter, accurate to ±0,1 pH units at 20 °C ± 1 °C

For measuring the pH of the agar media, a puncture electrode or a flat membrane electrode shall be used.

4.4.5 Calibrated thermometer or temperature probe accurate to ±1 °C.

4.4.6 Calibrated relative humidity meter accurate to ±2 % RH .

4.4.7 Light measuring apparatus.

4.4.8 Stopwatch.

4.4.9 Mechanical or electromechanical shaker.

4.4.10 Refrigerator, temperature-controllable to between 2 °C and 8 °C.

4.4.11 Graduated pipettes of nominal capacities of 10 ml, 2 ml, 1 ml and 0,1 ml. Calibrated automatic pipettes may be used, in particular for small volumes (inoculum deposit).

4.4.12 Petri dishes, 90 mm to 100 mm in diameter. 55 mm Petri dishes can be used for the membranes.

4.4.13 Glass beads (diameter: 3 mm to 5 mm).

4.4.14 Analytical balance of suitable operating range.

4.4.15 Spectrophotometer fitted with a monochromator.