# SLOVENSKI STANDARD
# oSIST prEN 17272:2018

**01-september-2018**

---

**[Not translated]**

Chemical Disinfectants and Antiseptics - Quantitative Carrier test for Airborne Room Disinfection by Automated Processes - Determination of Bactericidal, Fungicidal, Yeasticidal, Sporicidal, Tuberculocidal, Mycobactericidal, Virucidal and Phagocidal Activities in the Medical Area, Veterinary Area and Food, Industrial, Domestic and Institutional Areas - Test Methods and Requirements (phase 2, step 2)



Chemische Desinfektionsmittel und Antiseptika - Quantitative Keimträgerprüfung zur luftübertragenen Raumdesinfektion durch automatisierte Verfahren - Bestimmung der bakteriziden, fungiziden, levuroziden, sporiziden, tuberkuloziden, mykobakteriziden, viruziden und Phagen-Wirksamkeit im humanmedizinischen Bereich, Veterinärbereich, in den Bereichen Lebensmittel, Industrie, Haushalt und öffentliche Einrichtungen - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Essai quantitatif de porteur pour la désinfection de l□air des pièces par des processus automatisés - Détermination de l□activité bactéricide, fongicide, levuricide, sporicide, tuberculocide, mycobactéricide, virucide et phagocide

**Ta slovenski standard je istoveten z:** **prEN 17272**

---

**ICS:**

| | | |
|---|---|---|
| 11.080.20 | Dezinfektanti in antiseptiki | Disinfectants and antiseptics |
| 71.100.35 | Kemikalije za dezinfekcijo v industriji in doma | Chemicals for industrial and domestic disinfection purposes |

**oSIST prEN 17272:2018** **en,fr,de**

**CEN/TC 216**

Date: 2018 –02

**prEN XXXXX: XXXX**

Secretariat: AFNOR

# Methods of airborne room disinfection by automated process - determination of bactericidal, yeasticidal, fungicidal, sporicidal, mycobactericidal and virucidal activities

**Einführendes Element — Haupt-Element — Ergänzendes Element**

**Élément introductif — Élément central — Élément complémentaire**

**ICS:**

# Contents

Page

## European foreword

This document (prEN 17272:2018) has been prepared by Technical Committee CEN/TC 216 “Chemical antiseptics and disinfectants”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

# Introduction

The purpose of this document is to describe a method for assessing the disinfectant activity of airborne surface disinfection processes under a specific set of experimental conditions.

The processes concerned include those involving chemical disinfectants in dispersed gaseous, vapour and/or aerosolised form.

This document does not cover processes for which the mode of action is based on submerging and/or contact optionally associated with a mechanical wiping action. The above effects result in the displacement of all or part of the microorganisms away from the carrier surface as defined in the document, without inactivating these microorganisms.

It must be understood that every automated airborne disinfection cycle / application is unique and the purpose of this standard is to provide a defined challenge that must be met for the automated airborne disinfection system to be considered an efficacious process. This standard method should therefore be regarded as a useful starting point and not as a validation for all intended treatments with a particular automated airborne disinfection system.

# 1 Scope

The method described is designed to determine the disinfectant activity of processes used in the 1) human health, 2) veterinary, 3) food, industrial and institutional sectors using chemical processes.

The objective of the described processes is to disinfect the surfaces of the overall area including the external surfaces of the equipment contained in such rooms. Air handling and products or processes specifically designed for the disinfection of medical devices are excluded from the scope of this document.

The test methods and volumes described provide the defined challenge. Even if an automated airborne disinfection system has passed all or part of the test described here, the system and its delivered cycles should then be validated in the individual enclosure to be disinfected using appropriate biological or chemical indicators.

This document describes a Phase 2 step 2 methods designed:

— to check, under standardized laboratory conditions approaching real-world practice, that the proposed airborne surface disinfection processes meet the objective for which they were devised;

— to cross-compare the different processes under reproducible conditions;

— to provide an experimental design within specified boundaries when real-world-practice conditions depart from the conditions given in the text below.

The method is applicable to processes to be used in the area of human medicine, the veterinary area and in food, industrial, domestic and institutional areas (WG1, WG2 and WG3).

It applies to the process of whole enclosure / room disinfection achieved by automated processes (i.e. with no operator manually applying the disinfectant). This document covers the disinfection of nonporous surfaces but not that of air;

This method can be used as a basis for biosecurity applications in laboratories.

The method is used to qualify the process, i.e. the set of components and consumables needed for implementation. By way of example, for chemical processes, the device product combination cannot be separated.

This document is applicable to processes for which activity is claimed against the following categories of microorganisms:

— vegetative bacteria including mycobacteria,

— bacterial spores,

— yeasts,

— fungal spores and,

— viruses.

Each activity can be claimed independently but passing both bactericidal and yeasticidal activity as per text in Annex A tests is the minimum requirement to claim compliance.

An activity tested for each domain of application may only be claimed if the criteria are achieved against all of the specified organisms associated with that activity.

The proposed method includes 2 parts:

— Part 1 - Efficacy test: intended to ensure that minimal efficacy requirements are obtained for each type of activity claimed and for the targeted application(s) (WG1 and/or WG2 and/or WG3).

— Part 2 - Distribution test: intended to ensure efficacy of the process throughout the enclosure. It is performed with a reference microorganism at 4 sampling positions.

The tests described in this document are based on measuring the logarithmic reduction of different species of bacteria, yeasts, moulds, bacterial spores, bacteriophages or viruses and mycobacteria under a specified set of conditions. As the panel of microorganisms selected may prove to be too narrow for certain applications, it may be supplemented by other strains based on the experimental design described in this document, by varying the conditions according to the needs of the practical application(s) envisaged.

For the defined test conditions the number of test position of the carriers can be increased according to specific needs of a given application or local requirements.

The manufacturer:

— specifies the limitations for use and precautions for use of the process;

— ensures that the documented test conditions are representative of the recommended practice(s).

The aim of this document is to simulate practical conditions of airborne disinfection in a laboratory situation; obligatory conditions are defined according to the method defined below. Additional conditions are also proposed.

The test report specifies and summarizes the conditions under which the tests are carried out.

The processes are generally implemented after a cleaning procedure and are then tested, according to the application sectors, under clean or low-level soiling conditions. For some specific applications, and according to the manufacturer's recommendations, evaluation methods in the presence of other interfering substances can also be envisaged under additional conditions.



## 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 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*

EN 14885, *Chemical disinfectants and antiseptics - Application of European Standards for chemical disinfectants and antiseptics*

# 3 Terms and definitions

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

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

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

**3.1**
**chemical process**
process in which the active substance is a chemical agent diffused in gas, liquid or solid form, the process includes the chemical product and the diffusion system which cannot be evaluated separately

**3.2**
**automatic airborne disinfection process**
process diffusing a gas, vapour and/or an aerosol from a release source (i.e. an airborne disinfection process) without the need for human intervention, with the airborne disinfection process employed on surfaces and not for disinfection of the air itself

**3.3**
**disinfectant activity of an airborne disinfection process**
biocidal process with the ability to reduce the number of viable microorganisms that can be recovered and counted under conditions described in this document, with the data obtained from specified strains belonging to the following seven groups of microorganisms: bacteria, bacteria spores, yeasts, moulds, viruses, bacteriophages and mycobacteria, under defined conditions



Note 1 to entry: Activities should be specified from the following list: bactericidal activity, sporicidal activity, fungicidal activity, yeasticidal activity, virucidal activity, virucidal activity against bacteriophages, tuberculocidal activity and mycobactericidal activity



**3.4**
**distribution test**
placement of microbiological challenge carriers in such a way as to ensure the device product combination achieves the claimed disinfectant activity throughout the enclosure and performed after or in parallel to the efficacy test

**3.5**
**supplementary obligatory conditions**
test conditions used instead of the obligatory conditions where the practical use of the process and the manufacturers claims are clearly and unambiguously outside of the obligatory test conditions

**3.6**
**sensitive strain**
loss at drying greater than 1,5 lg after the drying phase, outside the exposure period

# 4 Requirements

## 4.1 Efficacy tests

The process being submitted for testing under the obligatory experimental conditions defined in 5.5.4.2 shall lead to the following reduction rates, expressed in log base-10:

**Bactericidal activity:**

— 5-lg or greater reduction for human health area on test -carriers comparative to control-carriers non-exposed to the process, for the 4 bacterial strains implemented (see 5.2.1.2 - 5.2.1.3 - 5.2.1.4 - 5.2.1.5)

— 5-lg or greater reduction for veterinary area on test -carriers comparative to control-carriers non-exposed to the process, for the 5 bacterial strains implemented (see 5.2.1.1 - 5.2.1.2 - 5.2.1.3 - 5.2.1.4 - 5.2.1.6.) As above

— 5-lg or greater reduction for food industry laboratory area on test -carriers comparative to control-carriers non-exposed to the process for the 4 bacterial strains implemented (see 5.2.1.1 - 5.2.1.2 - 5.2.1.3 - 5.2.1.4.) As above

**Sporicidal activity:**

— 4-lg or greater reduction for human health area, veterinary area and food industry laboratory area on test -carriers comparative to control-carriers non-exposed to the process for the spore-forming bacterial strain implemented (see 5.2.1.7)



**Fungicidal activity:**

— 4-lg or greater reduction for human health area, veterinary area and food industry laboratory on test -carriers comparative to control-carriers non-exposed to the process for the two fungal strains (yeast and mould) implemented (see 5.2.1.8 - 5.2.1.9)



**Yeasticidal activity:**

— 4-lg or greater reduction for human health area, veterinary area and food industry laboratory on test -carriers comparative to control-carriers non-exposed to the process for the yeast strain implemented (see 5.2.1.8).

**Virucidal activity:**

— 4-log or greater reduction for human health area and food industry laboratory on test -carriers comparative to control-carriers non-exposed to the process, for the 2 strains implemented (see 5.2.1.10 – 5.2.1.11)

— 4-lg or greater reduction for veterinary area on test -carriers comparative to control-carriers non-exposed to the process, for the strain implemented (see 5.2.1.12)

**virucidal activity against bacteriophages:**

— 4-lg or greater reduction for human health area, veterinary area and food industry laboratory on test -carriers comparative to control-carriers non-exposed to the process for the 2 strains implemented (see 5.2.1.13 - 5.2.1.14).

**Mycobactericidal activity:**

— 4-lg or greater reduction for human health area and food industry laboratory on test -carriers comparative to control-carriers non-exposed to the process for the 2 strains implemented (see 5.2.1.15 - 5.2.1.16).

The requirements and test method are summarized in Annex A.

The reduction rates sought are expressed as log base-10 values and are dependent on the application.

The reduction rates indicated in the table in Annex A are the minimal reduction rates to be obtained on test-carriers in comparison to control-carriers non-exposed to the process

These activities may be determined independently, i.e. by separate testing of the different microbiological groups, and do not all have to be undertaken simultaneously.

These seven activities shall be determined under the standard-reference experimental conditions defined in 5.5.

Additional specific bactericidal, sporicidal, fungicidal, yeasticidal, virucidal (human viruses), bacteriophagicidal and mycobactericidal activity can, as appropriate, be determined under other condition variables, from contact time to type of carriers, microorganisms, volume of the test room or temperature and humidity, in order to account for conditions governing the specific usages the processes are intended to provide.

These additional conditions shall be described, recorded and reported in the test report.

### 4.2 Distribution tests

The reduction in the number of viable bacterial cells throughout the enclosure shall be determined with-*Staphylococcus aureus* ATCC 6538 = CIP 4.83 (5.2.1.2) to achieve 5-log or greater reduction on each test –carrier. Carriers shall be located in accordance with Annex A, part 2. Test conditions shall replicate those used in the efficacy tests.



## 5 Test method

### 5.1 Principle



#### 5.1.1 Preliminary test to validate absence of residual effect

The aim of the preliminary test is to identify whether there is any residual activity due to residual disinfectant transferred via the carriers into the subculture media (in agar plates and on membranes) and to find a method for eliminating this effect. As such, it is the test itself that determines whether there is any real bactericidal, fungicidal, yeasticidal, sporicidal, virucidal, including against bacteriophages, and mycobactericidal activity rather than a simple localized growth-inhibitory effect.

Non-contaminated carriers are exposed to a gas, vapour and/or aerosol.

Recovery into 100 ml (20 ml for virucidal activity) of sterile liquid medium, and testing for bacteriostatic (or fungistatic or spore germination inhibition or cell susceptibility reduction) effects due to disinfectant adhering to the carriers, which could generate an inhibitory effect in agar medium and on the filter membranes or a decrease in the residual viral titration.

### 5.1.2 The test itself

Using microorganism suspensions, deposition of 50 µl on an inert carrier prepared as described in 5.2.3.

Spread and air-dry the inoculum as described in 5.5.1.2.1, then hold the carrier set in contact exposure with the product diffused by the tested process, under defined conditions.

Recover bacteria, mycobacteria, yeasts, moulds, spores or viruses or bacteriophages by scraping, shaking and sonicate if required, using the carriers, in the recovery liquid:

— for bacteria, mycobacteria, yeasts, moulds and spores, dilution and agar-plating of a fraction of the recovery liquid. Incubation followed by colony counting. Membrane-filtration of the remaining recovery liquid, then rinsing to eliminate as much disinfectant as possible. Membrane transferred on agar medium. Carrier placed in agar medium. Incubation followed by final colony counting.

— for viruses, dilution of fraction of the recovery liquid and testing for viable virus by incubation and determination of viral titre.

## 5.2 Materials and reagents

### 5.2.1 Test microorganisms

Depending on the type of activity targeted, tests shall use all or some of the following strains.

These strains can be obtained from biological collection resource centres. The test strains are:



**For bactericidal activity tests:**

— 5.2.1.1 *Pseudomonas aeruginosa* ATCC 15442 = CIP 103-467 (DSM937)

— 5.2.1.2 *Staphylococcus aureus* ATCC 6538 = CIP 4.83 (DSM 799)

— 5.2.1.3 *Enterococcus hirae* ATCC 10541 = CIP 5855 (DSM3320)



— 5.2.1.4 *Escherichia coli* ATCC 10536 = CIP 54127 (DSM682)

— 5.2.1.5 Acinetobacter baumanii ATCC 19606 = CIP 70.34 (*DSM30007*)

— 5.2.1.6 Proteus vulgaris…..ATCC 13315 = CIP 58.60(*DSM30118*)

**For sporicidal activity tests:**

— 5.2.1.7 *Bacillus subtilis* spores ATCC 6633 = CIP 52 62;

**For yeasticidal activity tests:**

— 5.2.1.8 *Candida albicans* ATCC 10231 = IP 4872 (DSM 1386)

**For fungicidal activity tests:**

— 5.2.1.8 *Candida albicans* ATCC 10231 = IP 4872 (DSM 1386)

— 5.2.1.9 *Aspergillus brasiliensis* ATCC 16404 = IP 1431-83 (DSM1988)

**For virucidal activity tests:**

— 5.2.1.10 Murine Parvovirus Crawford strain ATCC VR-1346 cultured on A9 cells line

— 5.2.1.11 Adenovirus type 5, adenoid strain, ATCC VR-5. *Adenovirus* cultured on HeLa cells or other lines of suitable susceptibility.

— 5.2.1.12 Porcine Parvovirus NADL2 strain cultured on ST cells or other appropriate cells line

**For virucidal activity against bacteriophage tests:**

— 5.2.1.13 Bacteriophage for *Lactococcus lactis* subspecies *lactis* P001 (DSM 4262);

— 5.2.1.14. Bacteriophage for *Lactococcus lactis* subspecies *lactis* P008 (DSM 10567);

The multiplication of these two phages shall be obtained from the following host strain: *Lactococcus lactis* subspecies *lactis* F7/2 (DSM 4366).

**For mycobactericidal activity:**

— 5.2.1.15. Mycobacterium avium ATCC 15769 = CIP 105415

— 5.2.1.16 *Mycobacterium terrae* ATCC 15755 (tuberculocidal activity)

Both organisms shall be tested, except for veterinary area where only *Mycobacterium avium* is required

These strains can be obtained from biological collection resource centres.

**The use of other microorganisms**

If additional test microorganisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere, media) which shall be recorded in the test report. If the additional test microorganisms chosen do not correspond to the specified species, their suitability for supplying the required inocula shall be verified. If these additional test organisms are not classified at a reference 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.

### 5.2.2 Culture media and reagents

#### 5.2.2.1 General



All weights of chemical substances given in this document refer to the anhydrous salts.

Hydrated forms may be used as an alternative, but the weights required shall be adjusted to allow for consequent molecular weight differences.

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 microorganisms.

#### 5.2.2.2 Culture media for bacteria, yeasts, moulds, spores and mycobacteria

##### 5.2.2.2.1 Water

The water shall be freshly glass-distilled water and not demineralized water.

Sterilize in the autoclave (5.3.2.1a).

NOTE 1 Sterile distilled water is necessary for preparation of suspensions tests. Sterilization is not necessary if the water is used for e.g. preparation of culture media and subsequently sterilized

NOTE 2 If distilled water of adequate quality is not available, water for injections can be used.

##### 5.2.2.2.2 Agar for bacterial counts (Tryptone Soya Agar: TSA)

For counts of viable bacterial cells (5.2.1.1 to 5.2.1 6)

| | |
|---|---|
| Tryptone, pancreatic digest of casein | 15,0 g |
| Soya peptone, papaic digest of soybean meal | 5,0 g |

| | |
|---|---|
| NaCl | 5,0 g |
| Agar | 15,0 g |
| Water to | 1 000 ml |

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

If necessary, add a neutralizer to the medium (and record the addition in the test report).

#### 5.2.2.3 Agar for the preservation of strains 5.2.1.1 to 5.2.1.6 (see 5.2.2.1.2) without neutralizer

##### 5.2.2.3.1 Diluent for microbial suspensions

Bacteria, fungi, yeast and spores

Tryptone sel:

| | | |
|---|---|---|
| — | Tryptone, pancreatic digestif of casein | 1,0 g |
| — | Sodium chloride | 8,5 g |
| — | Water (see 5.2.2.1.2) to | 1 000 ml |

Sterlize in the autoclave. After sterilization, pH of the medium shall be equivalent to (7,2 ± 0,2) when measured at (20 ± 1)°C

Mycobacteria**: Tryptone sel (see 5.2.2.1.4.1) or distilled water (see 5.2.2.1.1)**

The diluent shall not induce interference between the test microorganisms and the disinfectant product under test.



##### 5.2.2.3.2 Malt extract agar (MEA)

For counts on the number of viable yeast and mould cells (5.2.1.8. and 5.2.1.9).



| | |
|---|---|
| Malt extract (technical grade) | 30,0 g |
| Agar | 15,0 g |
| Water (see 5.2.2.1.2) to | 1 000 ml |

The malt extract shall be of food grade (e.g. Cristomalt powder, available from Difal) or of equivalent grade, not highly purified and not containing only maltose (e.g. malt extract available from OXOID).

NOTE This information is provided by Afnor as a convenience to users of this document and does not represent an commitment by Afnor of this product equivalent products can be used if it is demonstrated that they lead to the same results

Sterilize in the autoclave. After sterilization, pH of the medium shall be equivalent to 5.6 ± 0.2 when measured at (20 ± 1)°C

If necessary, add a disinfectant neutralizer to the medium (and record the addition in the test report).

##### 5.2.2.3.3 Medium for the preservation of strains 5.2.1.8 and 5.2.1.9 (see 5.2.2.1.6) without neutralizer (5.2.2.1.5)

##### 5.2.2.3.4 Liquid for microorganism recovery and for membrane rinsing

**Composition:**

| | |
|---|---|
| Tryptone, pancreatic digest of casein | 1,0 g |
| Sodium chloride | 8,5 g |