
Kemična razkužila in antiseptiki - Metode za dezinfekcijo površin v prostorih z delci v zraku z avtomatiziranim postopkom - Določevanje baktericidne, mikobaktericidne, sporicidne, fungicidne, virucidne, tuberkulocidne in fagocidne aktivnosti ter aktivnosti kvasovk

Chemical disinfectants and antiseptics - Methods of airborne room disinfection by automated process - Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities

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Chemische Desinfektionsmittel und Antiseptika - Verfahren zur luftübertragenen Raumdesinfektion durch automatisierte Verfahren - Bestimmung der bakteriziden, mykobakteriziden, sporiziden, fungiziden, levuroziden, viruziden, tuberkuloziden, und Phagen-Wirksamkeit

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Antiseptiques et désinfectants chimiques - Méthodes de désinfection des pièces par voie aérienne par des procédés automatisés - Détermination de l'activité bactéricide, fongicide, levuricide, sporicide, tuberculocide, mycobactéricide, virucide et phagocide

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Chemical disinfectants and antiseptics - Methods of airborne room disinfection by automated process - Determination of bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal and phagocidal activities

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European foreword

This document (EN 17272:2020) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2020, and conflicting national standards shall be withdrawn at the latest by October 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document describes a Phase 2 step 2 method designed:

- to check, under standardized laboratory conditions close to real-world practice, that the proposed airborne surface disinfection processes meet the objective for which they were devised;
- to cross-compare different processes under reproducible conditions;
- to provide an experimental design within specified limits when real-world-practice conditions depart from the conditions given in the text below.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

EN 17272:2020 (E)**Introduction**

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

The proposed test method consists of 2 parts:

- Part 1 - Efficacy test: intended to ensure that minimum efficacy requirements are fulfilled for each type of activity claimed and for the targeted application area(s) (CEN/TC 216 WG 1 and/or WG 2 and/or WG 3).
- Part 2 - Distribution test: intended to ensure efficacy of the process throughout the enclosure. It is performed with a reference test organism at 4 sampling positions.

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

Every automated airborne disinfection cycle/application is unique and the purpose of this document is to provide a defined challenge for the automated airborne disinfection system to successfully meet in order 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.

The method is used to qualify the process, i.e. the device(s) and product(s) needed for implementation. For such chemical processes, the combination of device and product cannot be separated.

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

The manufacturer:

- specifies the limitations and precautions for use of the process;
- ensures that the specified test conditions are representative for the recommended application(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 test method defined below. Additional conditions are also proposed.

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

Generally, the processes are implemented after a cleaning procedure and then tested, according to the application areas, under clean or low-level soiling conditions. For specified applications and/or according to the manufacturer recommendations, test methods with other interfering substance can also be envisaged as additional conditions.

The tests described in this document are based on measuring the reduction (expressed as decimal logarithm lg) in terms of numbers of surviving test organisms of different strains of bacteria, mycobacteria, bacterial spores, fungal spores, yeasts, viruses or bacteriophages and under specified conditions. Test organisms may be supplemented by other test organisms. The experimental design described in this document is expected to be followed, but the conditions can be varied according to the needs of the practical application(s).

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

CEN/TC 216 phase 2, step 1 suspension tests for evaluating the irreversible inactivation by the product cannot be performed as the product is changed by the diffusion through the air (e.g. liquid state vs vapour state).

1 Scope

The test methods described are designed to determine the disinfectant activity of processes used in the 1) medical area, 2) veterinary area, 3) food, industrial, domestic and institutional area using automated processes for distributing chemicals by air diffusion with no operator manually applying the disinfectant. This document covers the disinfection of nonporous surfaces but not that of the air.

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 a defined challenge.

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

- vegetative bacteria,
- mycobacteria,
- bacterial spores,
- yeasts,
- fungal spores,
- viruses,
- bacteriophages.

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This document does not cover processes for which the mode of action is based on immersing and/or circulation, flooding, spraying, wiping or other processes where the product is directly applied to the surfaces and not via air dispersion.

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 10088-2, *Stainless steels — Part 2: Technical delivery conditions for sheet/plate and strip of corrosion resisting steels for general purposes*

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*

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3 Terms and definitions

For the purposes of this document, the terms of EN 14885 and the following definitions apply:

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

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

3.1

chemical process

process in which the active substance is a chemical agent (product) diffused in gas, liquid and/or solid form

Note 1 to entry: The product and the diffusion system (device) cannot be evaluated separately.

3.2

automated airborne disinfection process

process diffusing a product in the form of a gas, vapour and/or an aerosol (excluding aqueous steam) from a device, without the need for human intervention, targeting surfaces and not the air

3.3

airborne disinfection contact time

ADC time

time from the first release of the product (disinfectant) to the point where carriers are recovered or to the point where aeration starts, if an aeration time is considered necessary

Note 1 to entry: The carriers can be recovered at the end of ADC time, or during the aeration time, with suitable personal protective equipment (PPE) where necessary

3.4

aeration time

period of time during which an air exchange of the enclosure achieves an appropriate concentration of the product in the enclosure based on the manufacturer's use instructions and risk assessment, to enable the recovery of the carriers

Note 1 to entry: The duration of this aeration time is dependent of the air treatment system characteristics.

3.5

distribution test

placement of test-carriers loaded with test organisms in such a way that the distribution of a product by the combination of a device (machine) and the product achieves its claimed activity throughout the enclosure

Note 1 to entry: This test is performed after or in parallel with the efficacy test.

3.6

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 excluding the obligatory test conditions

3.7

sensitive test organism

test organism where the drying causes a lg reduction of more than 1,5 by the end of the aeration time

4 Requirements

4.1 Efficacy tests

The automated airborne disinfection process to be tested under the obligatory experimental conditions defined in 5.5 shall lead to the following reductions in terms of numbers, expressed in decimal log (lg):

Bactericidal activity:

- 5 lg or greater reduction on test-carriers compared to control-carriers not exposed to the process, for each of the four specified bacterial test organisms.

For medical area, refer to 5.2.1.2, 5.2.1.3, 5.2.1.4, 5.2.1.5.

For veterinary area, refer to 5.2.1.1, 5.2.1.2, 5.2.1.3, 5.2.1.6.

For food, industrial, domestic and institutional area, refer to 5.2.1.1, 5.2.1.2, 5.2.1.3, 5.2.1.4.

Mycobactericidal activity:

- 4 lg or greater reduction for medical area and food industry and laboratory area on test-carriers comparative to control-carriers not exposed to the process for the two test organisms implemented, refer to 5.2.1.15, 5.2.1.16.
- 4 lg or greater reduction for veterinary area on test-carriers comparative to control-carriers not exposed to the process for the specified test organism, refer to 5.2.1.15.

Sporicidal activity:

- 4 lg or greater reduction for medical area, 3 lg for veterinary area and food, industrial, domestic and institutional area on test-carriers compared to control-carriers not exposed to the process for the specified bacterial spore test organism, refer to 5.2.1.7.

Fungicidal activity:

- 4 lg or greater for medical area, veterinary area and food, industrial, domestic and institutional area on test-carriers compared to control-carriers not exposed to the process for the two specified fungal test organisms (yeast and fungal spore), refer to 5.2.1.8 and 5.2.1.9.

Yeasticidal activity:

- 4 lg or greater reduction for medical area, veterinary area and food industry and laboratory area on test-carriers comparative to control-carriers not exposed to the process for the specified yeast test organism, refer to 5.2.1.8.

Virucidal activity:

- 4 lg or greater reduction for medical area and food industry and laboratory area on test-carriers comparative to control-carriers not exposed to the process, for the two specified test organisms, refer to 5.2.1.10 and 5.2.1.11.
- 4 lg or greater reduction for veterinary area on test-carriers comparative to control-carriers not exposed to the process, for the specified test organism, refer to 5.2.1.12.

Phagocidal activity:

- 4 lg or greater reduction for food industry and laboratory area on test-carriers comparative to control-carriers not exposed to the process for the specified two test organisms, refer to 5.2.1.13 and 5.2.1.14.

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The requirements and test methods are summarized in Annex A.

The reductions are expressed as decimal logarithmic values (lg).

The reductions indicated in the table of Annex A are the minimum reductions to be obtained on test-carriers in comparison to control-carriers not exposed to the process.

These activities may be determined independently, i.e. by separate testing of the different microbiological groups, e.g. mycobacteria, and do not all shall be undertaken simultaneously. Each activity can be claimed independently but passing both bactericidal and yeasticidal activity as described in Annex A is the minimum requirement to claim compliance with this document.

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

Additional specific bactericidal, mycobactericidal, sporicidal, fungicidal, yeasticidal, virucidal, and phagocidal activity can, as appropriate, be determined under other conditions (e.g. other ADC times, type of carriers, test organisms, volume of the enclosure, temperature, humidity), for specific intended uses. These additional conditions shall be described, recorded and reported in the test report.

Even if an automated airborne disinfection system has passed all or part of the test method described here, the system and its delivered cycles shall then be validated in the individual enclosure (e.g. hospital room, or animal house) in practice to be disinfected using appropriate biological or validated chemical indicators.

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 (refer to 5.2.1.2) to achieve 5 lg or greater reduction on each test-carrier. Test-carriers shall be located in accordance with Annex A, part 2. Test conditions shall replicate those used in the efficacy tests.

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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 product transferred via the carriers into the subculture media (agar plates and/or membranes) and to find a method for eliminating this effect. This procedure should ensure that the results of the efficacy test are based only on the irreversible inactivation of the test organisms and not on an inhibitory (static) effect.

Non-contaminated carriers are exposed to the process after deposition and drying of the interfering substance used in the assay.

Recovery into 100 ml (20 ml for virucidal activity) of sterile liquid medium, and testing for microbiostatic (e.g. bacteriostatic) effects due to traces of the product residing on the carriers, which could generate an inhibitory effect in agar medium and/or on the filter membranes or a decrease in the residual viral titre.

5.1.2 Efficacy test

Using test organism suspensions containing interfering substance, deposit 50 µl per carrier prepared as described in 5.2.3.

Spread and air-dry the inoculum as described in 5.5.1.2.2, then expose the prepared test-carriers to the product diffused by the tested automated airborne disinfection process, under defined conditions.

Recover the surviving bacteria, mycobacteria, bacterial spores, yeasts, fungal spores, viruses or bacteriophages by mechanical action, such as scraping (e.g. with a glass pipette or scalpel), or if required sonicate, from the carriers, into the recovery liquid:

For bacteria, mycobacteria, bacterial spores, fungal spores and yeasts, dilute and inoculate the agar with a fraction of the recovery liquid. Incubate and count the colonies. Filtrate the remaining recovery liquid using membrane filtration, then rinse to eliminate as much product as possible. Transfer onto agar medium. Place the carrier into agar medium to capture any remaining surviving test organisms. Incubate and count the number of colonies.

For viruses and bacteriophages, dilute a fraction of the recovery liquid, incubate on a cell line and determine the viral titre.

5.1.3 Distribution test

The distribution test is a replication of the efficacy test except that only *Staphylococcus aureus* is used as the test organism and the test carriers are located in other defined positions and orientations.

5.2 Materials and reagents

5.2.1 Test organisms

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

These test organisms can be obtained from culture collections. The test organisms are:

For bactericidal activity tests:

— 5.2.1.1 *Pseudomonas aeruginosa* ATCC 15442 = CIP 103-467 (DSM 937)

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

— 5.2.1.3 *Enterococcus hirae* ATCC 10541 = CIP 5855 (DSM 3320)

— 5.2.1.4 *Escherichia coli* ATCC 10536 = CIP 54127 (DSM 682)

— 5.2.1.5 *Acinetobacter baumannii* ATCC 19606 = CIP 70.34 (DSM 30007)

— 5.2.1.6 *Proteus hauseri* ATCC 13315 = CIP 58.60 (DSM 30118)

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 (DSM 1988)

For virucidal activity tests:

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- 5.2.1.10 Murine Norovirus souche S99, Friedrich Loeffler Institut, Berlin. MNV cultured on RAW 264.7 (ATCC TIB-71) 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 phagocidal activity:

- 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 bacteriophages 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 test organisms shall be tested, except for the veterinary area where only *Mycobacterium avium* is required.

These strains can be obtained from biological collection resource centres.

The use of other test organisms

If additional test organisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere, media) and noted in the test report. If the additional test organisms selected do not correspond to the specified strains, 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 five years.

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 organisms.

NOTE Commercial information (e.g. product name...) are provided by CEN as a convenience to users of this document and does not represent an endorsement by CEN of this product. Equivalent products can be used if it is demonstrated that they lead to the same results.

5.2.2.2 Culture media for bacteria, mycobacteria, spores, fungal spores and yeasts**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).

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

NOTE If distilled water of adequate quality is not available, water for injection 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 (see 5.2.2.2.1) to	1 000 ml

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

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

Agar for the preservation of test organisms 5.2.1.1 to 5.2.1.6 (refer to 5.2.2.2.2) without neutralizer.

5.2.2.2.3 Reconstituted milk for use with sensitive test organisms

Prepare the reconstituted skimmed milk (15 g/l fat) as follows:

Skimmed powdered milk, guaranteed antibiotic-free and additive-free, reconstituted at 100 g powder per 1 l distilled water.

Sterilize 30 min at (105 ± 3) °C or 5 min at (121 ± 3) °C

5.2.2.2.4 Malt extract agar (MEA)

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

Malt extract (technical grade)	30,0 g
Agar	15,0 g
Water (see 5.2.2.2.1) to	1 000 ml

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

Sterilize in the autoclave. After sterilization, pH of the medium shall be equivalent to $5,6 \pm 0,2$ when measured at (20 ± 1) °C.

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

5.2.2.2.5 Medium for the preservation of test organisms 5.2.1.8 and 5.2.1.9 without neutralizer (5.2.2.2.4)

5.2.2.2.6 Agar for mycobacteria (refer to 5.2.1.15. and 5.2.1.16)

Middlebrook and Cohn 7H10 medium + 10 % oleic acid dextrose-albumin complex (OADC) (hereinafter referred to as 7H10)

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To carry out the counts of viable mycobacteria:

—	Middlebrook 7H10 agar	19 g
—	glycerol	5 ml
—	water (see 5.2.2.2.1) to	895 ml

Heat to boiling point to achieve total dissolution. Sterilize in an autoclave for 10 min at 121 °C and allow to cool to a temperature of 50 °C to 55 °C in a water bath.

Add 100 ml of Middlebrook OADC enrichment medium under aseptic conditions. Final pH = 6,6 at (20 ± 1) °C.

5.2.2.2.7 Medium for spores (refer to 5.2.1.7)

Tryptone Soy Agar (TSA) for counting of viable *Bacillus* spores:

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
Sodium Chloride (NaCl)	5,0 g
Agar	15,0 g
Water (see 5.2.2.2.1) to	1 000 ml

Sterilize in the autoclave (5.3.2.1). After sterilization the pH of the medium shall be equivalent to (7,2 ± 0,2) measured at (20 ± 1) °C.

Other media may be used for spores: see Annex D

5.2.2.2.8 Diluent for microbial suspensions

Bacteria, fungi, yeast and spores standards.iteh.ai/catalog/standards/sist/45a0bb40-3d1c-4fca-aa7e-89760e186c26/sist-en-17272-2020

Tryptone salt:

—	Tryptone, pancreatic digest of casein	1,0 g
—	Sodium chloride	8,5 g
—	Water (see 5.2.2.2.1) 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 ± 1) °C.

Mycobacteria: Tryptone salt (5.2.2.2.9) or distilled water (5.2.2.2.1).

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

5.2.2.2.9 Liquid for test organism recovery and rinsing liquid for membrane filtration**Composition:**

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride	8,5 g
Water (see 5.2.2.2.1) to	1 000 ml

Preparation:

Dissolve the sodium chloride and tryptone in the water. Add any neutralizing agent specifically designed for the product under test.

Prepare the diluent in large-volume batches, then distribute into smaller adapted flasks. Sterilize in the autoclave.

For mycobacteria, water (refer to 5.2.2.2.1) is used instead of diluents.

5.2.2.3 Culture media and reagents for preparation of virus (test organisms 5.2.1.10 to 5.2.1.12.)

5.2.2.3.1 Growth and preservation media

For viruses, Eagle minimum essential medium (MEM) or equivalent should be used, supplemented with a suitable concentration of mycoplastm-free inactivated foetal calf serum, and antibiotics and other growth factors if required (refer to EN 14476 and EN 14675).

5.2.2.4 Culture media and reagents for preparation of bacteriophages (test organisms 5.2.1.13 to 5.2.1.14.)

5.2.2.4.1 M17 culture broth

For the preservation of the host bacterial strain, bacteriophage multiplication and bacteriophage diluent preparation:

Phytone peptone (derived from soybean meal)	5,0 g
Polypeptone peptone (derived from casein and animal tissue)	5,0 g
Beef extract powder	5,0 g
Yeast extracts	2,5 g
D(+)-lactose	5,0 g
Ascorbic acid	0,5 g
Sodium -glycerophosphate	19,0 g
Magnesium sulfate, 7 H ₂ O	0,2 g
Water (see 5.2.2.2.1) to	1 000 ml

Sterilize by autoclaving. After sterilization, pH of the medium shall be equivalent to $(7,0 \pm 0,2)$ when measured at $(20 \pm 1) ^\circ\text{C}$.

When M17 broth is the diluent used to prepare the neutralizer (refer to EN 13610), double-concentrated prepared M17 broth should be used (i.e. the concentration of all the ingredients added to the 1 000 ml of water should be doubled).

5.2.2.4.2 M17 agar (underlay agar)

Underlay agar for quantitative counting of the lysis zones (plaques) each obtained from a single infectious bacteriophage particle, formed in the host bacterial lawn.

Add 15 g of agar to 1 000 ml of M17 culture broth. Dissolve the agar by bringing it to the boil while stirring continuously.

Sterilize by autoclaving. After sterilization, pH of the medium shall be equivalent to $7,0 \pm 0,2$ when measured at $(20 \pm 1) ^\circ\text{C}$. Once the agar has cooled to $(45 \pm 1) ^\circ\text{C}$, add 10 ml of sterile 1 mol/l CaCl₂ stock solution (refer to 5.2.2.4.6). Mix gently and pour 15 ml to 18 ml of agar into Petri dishes.

5.2.2.4.3 Overlay agar (surface agar, soft agar)

For counts of bacteriophages: dissolve 6,5 g of agar in 1 000 ml of M17 culture broth (bring to the boil while stirring continuously). Distribute the molten agar into test tubes (2,5 ml to 3 ml per tube).