INTERNATIONAL STANDARD

ISO 14730

First edition
2000-09-15

Ophthalmic optics — Contact lens care products — Antimicrobial preservative efficacy testing and guidance on determining discard date

Optique ophtalmique — Produits d'entretien des lentilles de contact — Essais de l'efficacité de conservation antimicrobienne et lignes directrices pour la détermination de la durée d'utilisation après première ouverture

Reference number
ISO 14730:2000(E)

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Contents

Page

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

International Standard ISO 14730 was prepared by Technical Committee ISO/TC 172, *Optics and optical instruments*, Subcommittee SC 7, *Ophthalmic optics and instruments*.

Annexes A to F of this International Standard are for information only.

Introduction

Contact lens care products (CLCP) are used with contact lenses. These products rinse, clean, disinfect, store, wet, aid the comfort of, and condition contact lenses. Some products have one function, whilst others are multifunctional.

Usually products manufactured for use with hydrogel lenses may be used with rigid gas-permeable (RGP) or poly(methyl methacrylate) (PMMA) lenses, but products specifically used for RGP or PMMA contact lenses are not usually suitable for hydrogel lenses.

Most CLCPs are manufactured as solutions and are commonly packaged and sold in multidose containers. Dry products are sold as tablets or granules and must be dissolved in a suitable solvent immediately prior to use.

If the contact lens care product solution does not have any antimicrobial activity itself, an antimicrobial preservative may be added to the product to inhibit the growth of microorganisms that may be introduced from repeated dispensing during use and subsequent storage. All antimicrobial agents have the potential for toxicity to the user. For maximum protection to the user, the concentration of the preservative should be such that it provides adequate preservative activity with minimum toxicity.

There are differences between ophthalmic preparations and contact lens care products and some of these differences are significant in relation to preservative efficacy testing. Typically, ophthalmic preparations are packaged in small-volume containers and are for use for short periods on compromised eyes. Contact lens care products are distributed in larger volume containers and are used with contact lenses on a long term basis on healthy eyes. The potential risks for contact lens care products are the solution/lens interaction causing ocular irritation and the risks of the solution contamination by the repeated (daily) use of the product.

Thus when contact lens care products are formulated, the risk of adverse patient reaction due to the lens and/or solution interaction has to be weighed against the benefits of safety derived from the maintenance of the antimicrobial activity of the solution.

This International Standard gives the test procedure and performance criteria for preservative efficacy. It has been adapted from Pharmacopoeias which give a time limitation in their test procedure of 28 days. The informative annexes give four examples of preservative efficacy test procedures developed by contact lens care product manufacturers to show preservative efficacy for products whose discard dates are over 28 days.

Ophthalmic optics — Contact lens care products — Antimicrobial preservative efficacy testing and guidance on determining discard date

1 Scope

This International Standard specifies a procedure to be used in evaluating the antimicrobial preservative activity of all preserved multidose contact lens care products, and provides guidance on methods to be used for determination of discard date as informative annexes.

This test is applicable to products for up to a 28-day discard date.

The test is not applicable to sterile products packaged in unit doses for single use or multidose containers designed with physical barriers to microbial contamination (e.g. aerosol containers).

NOTE 1 Principles of the test may be used to extend discard dating beyond 28 days. See annexes B, C, D and E.

NOTE 2 Use of multiple or mixed microbial challenges and/or inclusion of contact lenses or other organic load can influence the apparent antimicrobial activity of a particular product. The evaluation of these variables together with testing against a larger panel of microorganisms and testing of samples from partially used containers may be of value in developing a contact lens care product, but are excluded from the scope of this International Standard.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 8320-2:—[1)], *Contact lenses and contact lens care products — Vocabulary — Part 2: Contact lens care products.*

ISO 14534:1997, *Ophthalmic optics — Contact lenses and contact lens care products — Fundamental requirements*.

3 Terms and definitions

For the purposes of this International Standard, the terms and definitions given in ISO 8320-2 apply.

4 Principle

4.1 The test consists of challenging the preparation with a specified inoculum of suitable microorganisms at the commencement of the test and then rechallenging at day 14. The inoculated preparations are stored at a specified

1) To be published.

temperature. Samples are withdrawn from the inoculated preparations at specified time intervals and are cultured for determination of viable organisms. The capability of the product to prevent re-growth is confirmed by counting of viable organisms over longer time periods.

4.2 The size of the microbial challenge chosen in this test is not intended to be representative of the likely challenge in practice, but to provide countable numbers from which estimation of the rate and extent of viability loss can be determined.

4.3 The antimicrobial preservative properties of the product are adequate if, in the conditions of the test, there is significant reduction of bacteria and no increase in yeasts and moulds in the inoculated preparation after the times and at the temperatures specified. The performance criteria are given in 5.6.

4.4 Appropriate measures shall be taken to inactivate or remove residual antimicrobial agents during culturing and counting of survivors. The effectiveness of these measures shall be validated.

5 Test methods

5.1 Materials and reagents

5.1.1 Test organisms.

The strains listed in Table 1 shall be used.

NOTE Test organisms from other culture collections that may be used are listed in Annex F.

Table 1 — Test organisms

Pseudomonas aeruginosa	ATCC 9027
Staphylococcus aureus	ATCC 6538
Escherichia coli	ATCC 8739
Candida albicans	ATCC 10231
Aspergillus niger	ATCC 16404

5.1.2 Culture media and reagents.

5.1.2.1 Tryptone Soya Agar (TSA).

5.1.2.2 Sabouraud Dextrose Agar (SDA).

5.1.2.3 **Dulbecco's Phosphate-Buffered Saline** without calcium chloride and magnesium chloride (DPBS).

Combine 200 mg/l KCl, 200 mg/l KH_2PO_4, 8 000 mg/l NaCl, and 2 160 mg/l $Na_2HPO_4 \cdot 7H_2O$ or suitable diluent.

5.1.2.4 **Dulbecco's Phosphate Buffered Saline** plus 0,05 % volumic mass polysorbate 80 (DPBST) or suitable diluent.

5.1.2.5 Validated neutralizing agents/media as required, for example, Dey-Engley Neutralizing Broth (DEB) and Letheen Broth.

5.1.3 Laboratory equipment.

The following common laboratory equipment is required*:* Sterile pipettes, swabs, tubes, Petri dishes (90 mm to 100 mm × 20 mm), etc. and suitable instruments for spectrophotometric determination of cell density, for colony counting, and for centrifugation.

5.2 Test sampling and culture maintenance

The product to be tested shall be representative of the product to be marketed. Aliquots should be taken directly from the final product container immediately prior to testing.

Three lots of product shall be tested. Each lot of product shall be tested with a separate inoculum preparation for each challenge organism.

Maintain the test cultures as recommended by the curator of the appropriate culture collection.

Cultures should be no greater than five passes removed from the depository stock (ATCC, NCIB, NCTC, NCPF or other recognized culture depository; see annex F). Each pass is a subculture of the previous pass.

5.3 Preparation of microbial challenge (Inoculum)

Culture each test organism on agar slopes under the conditions given in Table 2.

Table 2 — Media and incubation conditions for growth of challenge organisms

Organism	Medium	Temperature °C	Incubation time
P. aeruginosa	TSA	30 to 35	18 h to 24 h
S. aureus	TSA	30 to 35	18 h to 24 h
E. coli	TSA	30 to 35	18 h to 24 h
C. albicans	SDA	either 20 to 25	42 h to 48 h
		or 30 to 35	18 h to 24 h
A. niger	SDA	20 to 25	7 d to 10 d

Use sterile DPBST or suitable diluent to harvest each culture; wash the surface growth, transfer it to a suitable vessel and vortex. Filter the spore suspensions through sterile glass wool, cheesecloth or gauze to remove hyphal fragments.

After harvesting, the cultured organisms may be washed using centrifugation. The bacterial suspensions may be filtered (e.g. 3 µm to 5 µm pore size) to produce a single cell dispersion. Then adjust all challenge cell suspensions with DPBST or other suitable diluent to a concentration of between 1×10^7 cfu/ml and 1×10^8 cfu/ml. Estimate the approximate cell concentration of each suspension by measuring the turbidity of the suspension or a dilution of the suspension using a spectrophotometer. The actual concentration of colony-forming units per millilitre shall be determined for each suspension, e.g. by the plate-count method, at the time of the test.

If centrifugation is used, each centrifugation should be conducted at 20 °C to 25 °C for no longer than the equivalent of 10 min at 4 000 *g* or less.

Use bacterial and yeast cell suspensions on the day of preparation.

NOTE 1 Longer centrifugation times may be required at lower speeds.

NOTE 2 Spore suspensions may be used up to seven days following preparation by storage under refrigeration (2 °C to 8 °C).

5.4 Inoculum challenge test procedure

5.4.1 Prepare one or more tubes (for each lot tested) containing a minimum of 10 ml of test solution per challenge organism.

NOTE Sample tubes are used rather than lens cases to allow effective technical execution of the test. Since incompatibilities can exist between solution ingredients and tube materials, tubes of an appropriate material which is compatible with the ingredients should be considered.

Inoculate the sample tube of the product to be tested with a suspension of test organisms sufficient to provide a final count of between $1{,}0 \times 10^5$ cfu/ml and $1{,}0 \times 10^6$ cfu/ml. Ensure that the volume of inoculum does not exceed 1 % of the sample volume. Ensure complete dispersion of the inoculum by adequate mixing.

5.4.2 Store the inoculated product at 20 °C to 25 °C. The temperature must be monitored using a calibrated device and the temperature documented.

If the product is sensitive to light, it should be protected during the period of the test.

5.4.3 Take 1,0 ml aliquots of the inoculated product for determination of viable count at 7 d and 14 d.

5.4.4 After taking the 14 d sample, each sample is rechallenged as in 5.4.1 by using an inoculum level of $1{,}0 \times 10^4$ cfu/ml to $1{,}0 \times 10^5$ cfu/ml.

5.4.5 Take 1,0 ml aliquots of the inoculated product for determination of the viable count at 21 d and 28 d.

5.4.6 Subject each of the 1,0 ml aliquots, removed at the specified time intervals, to a suitable series of decimal dilutions in validated neutralizing media. Mix the suspension well by vortexing vigorously and let stand to allow neutralization to be completed. Neutralization conditions shall be based on recovery-medium control testing (see 5.5.2).

If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure (see annex A).

5.4.7 Determine the viable count of organisms in appropriate dilutions by preparation of triplicate plates (unless otherwise justified) of a suitable recovery medium (e.g. TSA for bacteria and SDA for mould and yeast).

If membrane filtration has been employed to remove or neutralize antimicrobial agents, culture the membranes on these media as appropriate.

If the pour-plate method is utilized, keep the agar for pour plates below 50 °C prior to pouring.

NOTE The agar media used for determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

5.4.8 Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Incubation times for optimal recovery of bacteria,

yeast and moulds shall be determined. Minimum incubation times shall be based on recovery medium control testing (see 5.5.2). Record the number of cfu observed on countable plates.

Plates should be observed periodically during incubation to prevent the occurrence of uncountable plates due to overgrowth.

5.4.9 Determine the average number of colony-forming units on countable plates. Calculate the microbial reduction at the specified time points.

NOTE Countable plates refer to 30 cfu to 300 cfu per plate for bacteria and yeast, and 8 cfu to 80 cfu per plate for moulds, except when colonies are observed only for the 10^0 or 10^{-1} dilution plates.

5.4.10 The absence of microorganisms shall be documented, e.g., by recording a “0” or “NR” (no recovery), when plates for all dilutions of a sample at a single time point have zero colonies.

5.4.11 The concentration of survivors is calculated at each point of time. The concentration of viable organisms following the 14 d rechallenge is the sum of the rechallenge inoculum concentration and the 14 d survivor concentration.

5.5 Controls

5.5.1 Inoculum controls

The initial and rechallenge inoculum concentrations are calculated by dispersing an identical aliquot of the inoculum into the same volume as used in 5.4.1 of a suitable diluent to achieve a final concentration not less than $1{,}0 \times 10^5$ cfu/ml to $1{,}0 \times 10^6$ cfu/ml for the initial inoculum or $1{,}0 \times 10^4$ to $1{,}0 \times 10^5$ cfu/ml for the rechallenge. The volume of inoculum does not exceed 1 % of the sample volume. Ensure dispersion of the inoculum by adequate mixing. Evaluate this control sample for cfu/ml at the beginning of the test in order to demonstrate the suitability of the medium used for growth of the test organism and provide an estimate of the initial inoculum concentration. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

5.5.2 Recovery medium control

Vortex a 1/10 dilution of the preserved product in the validated neutralizing broth (1 ml into 9 ml). Let it stand to allow neutralization to be completed. Prepare a second control tube with 10 ml of a suitable diluent (e.g. DPBST). Inoculate the tubes with sufficient inoculum to result in 10 cfu to 100 cfu of challenge organism per plate. Incubate for an appropriate period of time at ambient temperature. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Determine minimum incubation times for optimal recovery of bacteria, yeast and moulds.

Check that the recovery from the neutralizer broth is at least 50 % of the recovery in the second control tube. Perform this control for each challenge organism.

If a dilution of greater than 1/10 is required for neutralization, then membrane filtration should be used.

Validate the neutralization of the product with each challenge organism initially and as appropriate.

5.6 Performance criteria

5.6.1 General

Products shall be capable of meeting these criteria throughout their labelled shelf life and at the discard date.

Meeting the criteria of 5.6.2 and 5.6.3 shall justify a 28 d period of use after opening (discard date).

NOTE Refer to annexes B, C, D and E for suggested methods, if a discard date longer than 28 d is desired.

5.6.2 Bacteria

The number of each challenge organism recovered per millilitre shall be reduced by a mean value of not less than 3,0 logs at 14 d. After the rechallenge at 14 d, the concentration of each challenge organism shall be reduced again by at least a mean value of 3,0 logs by 28 d.

5.6.3 Moulds and yeasts

The number of each challenge organism recovered per millilitre shall remain at, or below, the initial concentrations (within an experimental error of $\pm$ 0,5 logs) within 14 d. At 28 d, the concentration of each challenge organism shall remain at, or below, the concentrations (within an experimental error of $\pm$ 0,5 logs) of each challenge organism after the rechallenge.

5.7 Test report

The test report shall include:

a) the title of this International Standard;

b) the identification of the product, including name of the product, batch number, expiry date, manufacturer, storage conditions and active substances(s) and its/their concentration(s) (as available);

c) the name(s) of the operator(s);

d) deviations from the protocol;

e) date and time of incubation;

f) storage time for inoculated product;

g) results obtained, including numbers of organisms recovered at each time point.

Annex A
(informative)

Example of a membrane filtration procedure

A.1 Materials and reagents

A.1.1 Culture media and reagents

A.1.1.1 **Diluting fluid,** with or without neutralizers.

A.1.1.2 **Tryptone Soya Agar (TSA).**

A.1.1.3 **Dulbecco's Phosphate-Buffered Saline** without calcium chloride and magnesium chloride (DPBS): 200 mg/l KCl, 200 mg/l KH_2PO_4, 8 000 mg/l NaCl, and 2 160 mg/l $Na_2HPO_4 \cdot 7H_2O$ or suitable diluent.

A.1.1.4 **Dulbecco's Phosphate-Buffered Saline** plus 0,05 % polysorbate 80 (DPBST) or suitable diluent.

A.1.1.5 **Validated neutralizing agents/media** as required, for example, Dey-Engley Neutralizing Broth (DEB) and Letheen Broth.

A.1.2 Test equipment

Usual laboratory equipment (such as sterile pipettes, Petri dishes, containers) together with the following.

A.1.2.1 **Sterile membrane filter.**

A.1.2.2 **Suitable sterile apparatus** for holding the sterile membrane filter and filtrate.

A.1.2.3 **Suitable equipment for creating a vacuum or pressure** to cause the liquid phase of the inoculated test solution to pass through the membrane filter aseptically.

The membrane filter should have a nominal pore size of not greater than 0,45 µm, a diameter of at least 47 mm and should be free of chemicals which could be toxic to microbial cells.

A.2 Test method and results

A.2.1 Moisten the sterile membrane filter (A.1.2.1) in a sterile filter assembly (A.1.2.2) with sterile DPBST (A.1.1.4), or suitable diluent.

A.2.2 Aseptically transfer a measured volume of the inoculated test solution into sterile DPBST (A.1.1.4) or diluting fluid.

A.2.3 Transfer the diluted solution to the membrane and filter immediately with the aid of vacuum or pressure. Dilute the sample applied to the filter with 50 ml to 100 ml of dilution fluid and thoroughly mix to ensure uniform distribution of the sample over the entire area of the filter.

NOTE This will decrease the probability of multiple colony-forming units being placed on the filter at the same location.

A.2.4 Wash the membrane filter with several volumes of diluting fluid which may contain additional neutralizing agents as needed. The actual volume should be determined empirically for each formulation for each challenge organism.

NOTE Three volumes of diluting fluid (100 ml each) are usually sufficient to remove and/or dilute the antimicrobial agent.

A.2.5 Incubate the membrane filter with appropriate media to allow growth of colony-forming units on the surface of the filter.

NOTE This may be accomplished by aseptic removal of the membrane filter from the filter assembly unit and placement of the membrane on the surface of a sterile agar plate which does not have obvious liquid on the surface; or the membrane may be enclosed in an agar sandwich. Alternatively, a sterile membrane filter unit may be used which requires addition of sterile media to the sealed filter and incubation of the membrane *in situ*. Media should be used which are appropriate for the type of challenge organism and the specific formulation under test. Time of the incubation should be established.

A.2.6 Determine the average number of colony-forming units on the countable membrane filters (3 cfu to 100 cfu/47 mm filter for bacteria and yeast and 3 cfu to 10 cfu/47 mm filter for moulds). Calculate and document the number of colony-forming units per millilitre of inoculated solution.

A.3 Controls

Confirm neutralizer efficacy by transferring an aliquot of the uninoculated test solution into 50 ml to 100 ml of sterile diluting fluid using the same ratio of volume of test solution to volume of diluting fluid. Apply the entire volume to the membrane and filter using vacuum or pressure. Wash the filter with several volumes of the diluting fluid using the same volume as used for the test procedure. Transfer 5 cfu to 100 cfu challenge organisms (one species per filter) into 100 ml of diluting fluid and apply to the membrane. Incubate the membrane filter in contact with media as described in the test procedure (see A.2.5).

Repeat the procedure using diluting fluid not exposed to the test solution. Compare counts with those derived by the same method but using a suitable diluent (e.g. DPBST), instead of the test solution. Confirm the inoculum on a suitable medium in triplicate (unless otherwise justified). Ensure that the recovery in the neutralizer broth is at least 50 % of the inoculum.

Annex B
(informative)

Discard date procedure I

B.1 Principle

B.1.1 The test consists of inoculating the test samples with a high level of organism (approximately 10^6 cfu/ml) on 0 d. The test samples are rechallenged with a low organism inoculum level (approximately 10^3 cfu/ml) in the test formulation.

B.1.2 Inoculation times are at initial, 2 weeks, 25 %, 50 %, 75 % and 100 % of the proposed discard date.

B.1.3 Sampling times should include 1, 2, 3, 4 weeks, and 25 %, 50 %, 75 % and 100 % of the proposed discard date, and 14 d after the proposed discard date.

B.1.4 Test samples should meet the test criteria for ISO Preservative Efficacy of Multidose Preserved Contact Lens Care Products at 28 d.

B.1.5 Stasis should be shown after rechallenges.

B.2 Test methods

B.2.1 Materials and reagents

B.2.1.1 Test organisms

Test organisms should be as specified in 5.1.1.

B.2.1.2 Test media

Test media should be as specified in 5.1.2.

B.2.1.3 Laboratory equipment

Laboratory equipment should be as specified in 5.1.3.

B.2.1.4 Test samples

Challenge tests are conducted to support discard after opening date, using three lots of contact lens care solutions representative of the product to be marketed.

Culture maintenance should be as specified in 5.2.

B.2.2 Preparation of microbial challenge (Inoculum)

Test organisms should be cultured and harvested as specified in 5.3.

B.2.3 Inoculum challenge test procedure

B.2.3.1 Prepare a composite sample of greater than 250 ml from individual test samples.

B.2.3.2 Prepare a separate 50 ml quantity of test formulation for each test organism. Place the sample into a 250 ml flask or specimen container.

B.2.3.3 Prepare 50 ml containers of a suitable diluent for bacteria, yeast and mould controls.

B.2.3.4 Inoculate formulation samples and controls with 0,5 ml aliquot of the 10^8 cfu/ml organism suspension to achieve a final concentration of approximately 10^6 cfu/ml. Ensure complete dispersion of the inoculum by adequate mixing.

B.2.3.5 Store the inoculated product at 20 °C to 25 °C. The temperature shall be monitored using a calibrated device and the temperature documented.

If the contact lens care product is sensitive to light, it should be protected during the period of the test.

B.2.3.6 At 1 week, 2 weeks, 3 weeks, 4 weeks, and at 25 %, 50 %, 75 % and 100 % of the proposed discard date, and 14 d after the proposed discard date, take 1,0 ml aliquots of the inoculated product for determination of viable counts.

B.2.3.7 Rechallenge the formulation samples at 2 weeks, 25 %, 50 %, 75 % and 100 % of the proposed discard date, using a 0,05 ml/50 ml ratio of a 10^6 cfu/ml organism suspension to provide a final concentration of approximately 10^3 cfu/ml. Repeat the viable count determinations at each time point prior to rechallenging.

B.2.3.8 Subject each of the 1,0 ml aliquots, removed at the specified time intervals, to a suitable series of decimal dilutions in validated neutralizing media. Mix the suspension well by vortexing vigorously and let stand to allow neutralization to be completed.

If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure (see annex A).

B.2.3.9 Determine the viable count of organisms in appropriate dilutions by preparation of plates in triplicate (unless otherwise justified) of a suitable recovery medium (e.g. TSA for bacteria and SDA for mould and yeast).

If membrane filtration has been employed to remove or neutralize antimicrobial agents, culture the membranes on these media as appropriate.

If the pour-plate method is utilized, keep the agar for pour plates below 50 °C prior to pouring.

NOTE The agar media used for determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

B.2.3.10 Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Incubation times for optimal recovery of bacteria, yeast and moulds should be determined. Minimum incubation times should be based on recovery medium control testing (see B.2.4.2).

B.2.3.11 Determine the average number of colony-forming units on countable plates. Calculate the microbial reduction at the specified time points.

NOTE Countable plates refer to 30 cfu to 300 cfu/plate for bacteria and yeast, and 8 cfu to 80 cfu/plate for moulds, except when colonies are observed only for the 10^0 or 10^{-1} dilution plates.

B.2.3.12 When plates for all dilutions of a sample at a single time point indicate no recovery of microorganisms, it should be documented.

B.2.3.13 The concentration of survivors is calculated at each time point.

B.2.4 Controls

B.2.4.1 Inoculum controls

Fresh control samples are run at each inoculation date by inoculating the saline (for bacteria and yeast) or saline/Tween®[2] 80 for mould in the same manner as for the sample inoculation. The theoretical numbers can be found by calculating the cumulative sum of the last inoculum, plus the last survivor count in the formulation sample.

B.2.4.2 Recovery medium control

Vortex a 1/10 dilution of the preserved product in the validated neutralizing broth (1 ml into 9 ml). Let it stand to allow neutralization to be completed. Prepare a second control tube with 10 ml of a suitable diluent (e.g. DPBST). Inoculate the tubes with sufficient inoculum to result in 10 cfu to 100 cfu of challenge organism per plate. Incubate for an appropriate period of time at ambient temperature. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Determine minimum incubation times for optimal recovery of bacteria, yeast and moulds.

Check that the recovery in the neutralizer broth is at least 50 % of the recovery in the second control tube. Perform this control for each challenge organism.

If a dilution of greater than 1/10 is required for neutralization, then membrane filtration shall be used.

Qualify the neutralization of the product initially and periodically.

B.2.5 Performance criteria

The discard date is supported if the number of surviving bacteria are reduced by 3 logs by day 14 and the number of bacteria and fungi do not increase in numbers thereafter.[3]

B.2.6 Test report

The test report should be as specified in 5.7.

2) Tween is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) The number of organisms recovered from a test sample does not exceed the sum of the microbial counts from the inoculation plus the last count of survivors within a ± 0,5 log variance (i.e. multiplication of the organisms did not occur).

Annex C
(informative)

Discard date procedure II

C.1 Principle

C.1.1 The test consists of challenging the preparation with a specified inoculum of suitable microorganisms, storing the inoculated preparation at a specified temperature, withdrawing samples from the container at specified time intervals and counting the organisms in the samples so removed. The capability of the product to prevent re-growth is confirmed by counting of viable organisms over longer time periods.

C.1.2 The size of the microbial challenge chosen in this test is not intended to be representative of the likely challenge in practice, but to provide countable numbers from which estimation of the rate and extent of viability loss can be determined.

C.1.3 The preparation shall meet the requirements for an adequately preserved contact lens care product initially, and following a simulated use for the intended discard date interval (performance criteria are included in C.2.7).

C.1.4 Appropriate measures should be taken to inactivate or remove residual antimicrobial agents during culturing and counting of survivors, and the effectiveness of these measures should be validated. The action of this process during the test should be demonstrated by the construction of suitable controls.

C.2 Test methods

C.2.1 Materials and reagents

C.2.1.1 Test organisms

Test organisms should be as specified in 5.1.1.

C.2.1.2 Test media

Test media should be as specified in 5.1.2.

C.2.1.3 Laboratory equipment

Laboratory equipment should be as specified in 5.1.3.

C.2.1.4 Test samples

The contact lens care product to be tested should be representative of the product to be marketed. Three lots of product should be tested. Aliquots should be taken directly from the final product container immediately prior to testing. A suitable number of containers should be utilized to ensure that enough product will be available for challenge testing after simulated use (i.e. containers may be pooled in order to provide sufficient product for testing).

Culture maintenance should be as specified in 5.2.

C.2.2 Preparation of microbial challenge (Inoculum)

Test organisms should be cultured and harvested as specified in 5.3.

C.2.3 Inoculum challenge test procedure

C.2.3.1 Prepare one tube containing a minimum of 10 ml of test solution per challenge organism. Inoculate the sample tube of the product to be tested with a suspension of test organisms sufficient to provide a final count of between $1{,}0 \times 10^5$ cfu/ml and $1{,}0 \times 10^6$ cfu/ml. Ensure that the volume of inoculum does not exceed 1 % of the sample volume. Ensure complete dispersion of the inoculum by adequate mixing.

C.2.3.2 Store the inoculated product at 20 °C to 25 °C. The temperature shall be monitored using a calibrated device and the temperature documented.

If the contact lens care product is sensitive to light, it should be protected during the period of the test.

C.2.3.3 Take 1,0 ml aliquots of the inoculated product for determination of viable count at 7 d and 14 d.

C.2.3.4 After taking the 14 d sample, each sample is rechallenged as in C.2.3.1 by using an inoculum level of $1{,}0 \times 10^4$ cfu/ml to $1{,}0 \times 10^5$ cfu/ml.

C.2.3.5 Take 1,0 ml aliquots of the inoculated product for determination of the viable count at 21 d and 28 d.

C.2.3.6 Subject each of the 1,0 ml aliquots, removed at the specified time intervals, to a suitable series of decimal dilutions in validated neutralizing media. Mix the suspension well by vortexing vigorously and let stand to allow neutralization to be completed.

If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure (see annex A).

C.2.3.7 Determine the viable count of organisms in appropriate dilutions by preparation of plates in triplicate (unless otherwise justified) of a suitable recovery medium (e.g. TSA for bacteria and SDA for mould and yeast).

If membrane filtration has been employed to remove or neutralize antimicrobial agents, culture the membranes on these media as appropriate.

If the pour-plate method is utilized, keep the agar for pour plates below 50 °C prior to pouring.

NOTE The agar media used for determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

C.2.3.8 Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Incubation times for optimal recovery of bacteria, yeast and moulds should be determined. Minimum incubation times should be based on recovery medium control testing (see C.2.6.2).

C.2.3.9 Determine the average number of colony-forming units on countable plates. Calculate the microbial reduction at the specified time points.

NOTE Countable plates refer to 30 cfu to 300 cfu/plate for bacteria and yeast, and 8 cfu to 80 cfu/plate for moulds, except when colonies are observed only for the 10^0 or 10^{-1} dilution plates.

C.2.3.10 When plates for all dilutions of a sample at a single time point indicate no recovery of microorganisms, it should be documented.

C.2.3.11 The concentration of survivors is calculated at each time point. The concentration of viable organisms following the 14 d rechallenge is the sum of the rechallenge inoculum concentration and the 14 d survivor concentration.

C.2.4 Simulated use

Using unchallenged product in original containers, subject the product to a simulated use by dispensing an appropriate aliquot from the containers for a period of not less than the target discard date (i.e. dispense 1 ml every 3 d for three months).

A suitable number of containers shall be utilised to ensure that enough product will be available for challenge testing after simulated use (i.e. containers may be pooled in order to provide sufficient product for testing).

C.2.5 Inoculum challenge following simulated use

At the end of the simulated use period, repeat the inoculum challenge test procedure as described in C.2.3.

C.2.6 Controls

C.2.6.1 Inoculum controls

The initial and rechallenge inoculum concentrations are calculated by dispersing an identical aliquot of the inoculum into the same volume of a suitable diluent used in C.2.3.1 to achieve a final concentration of not less $1{,}0 \times 10^5$ cfu/ml to $5{,}0 \times 10^6$ cfu/ml for the initial inoculum or $1{,}0 \times 10^4$ cfu/ml to $1{,}0 \times 10^5$ cfu/ml for the rechallenge. The volume of inoculum shall not exceed 1 % of the sample volume. Ensure dispersion of the inoculum by adequate mixing. Evaluate this control sample for colony-forming units per millilitre at the beginning of the test, in order to demonstrate the suitability of the medium used for growth of the test organism and provide an estimate of the initial inoculum concentration. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

C.2.6.2 Recovery medium control

Vortex a 1/10 dilution of the preserved product in the validated neutralizing broth (1 ml into 9 ml). Let it stand to allow neutralization to be completed. Prepare a second control tube with 10 ml of a suitable diluent (e.g. DPBST). Inoculate the tubes with sufficient inoculum to result in 10 cfu to 100 cfu of challenge organism per plate. Incubate for an appropriate period of time at ambient temperature. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Determine minimum incubation times for optimal recovery of bacteria, yeast and moulds.

Check that the recovery in the neutralizer broth is at least 50 % of the recovery in the second control tube. Perform this control for each challenge organism.

If a dilution of greater than 1/10 is required for neutralization, then membrane filtration shall be used.

Qualify the neutralization of the product initially and periodically.

C.2.7 Performance criteria

C.2.7.1 General

Products shall meet the performance criteria for both the initial challenge and the final challenge following simulated use.

C.2.7.2 Bacteria

The number of organisms recovered per millilitre should be reduced by a value of not less than 3,0 logs at 14 d. After the rechallenge at 14 d, the concentration of bacteria should be reduced again by at least a mean value of 3,0 logs by 28 d.

C.2.7.3 Moulds and yeasts

The number of organisms recovered per millilitre should remain at, or below, the initial concentrations (within an experimental error of ± 0,5 logs) within 14 d. At 28 d, the concentration of moulds and yeast should remain at, or below, the concentrations (within an experimental error of ± 0,5 logs) of moulds and yeasts after the rechallenge.

C.2.8 Test report

The test report shall be as specified in 5.7.

Annex D
(informative)

Discard date procedure III

D.1 Principle

D.1.1 The test consists of challenging the preparation with a specified inoculum of suitable microorganisms, storing the inoculated preparation at a specified temperature, withdrawing samples from the container at specified time intervals and counting the organisms in the samples so removed. The capability of the product to prevent re-growth is confirmed by counting of viable organisms over longer time periods.

D.1.2 The size of the microbial challenge chosen in this test is not intended to be representative of the likely challenge in practice, but to provide countable numbers from which estimation of the rate and extent of viability loss can be determined.

D.1.3 The preparation shall meet the requirements for an adequately preserved contact lens care product throughout its intended discard date period (for performance criteria see D.2.5).

D.1.4 Appropriate measures should be taken to inactivate or remove residual antimicrobial agents during culturing and counting of survivors, and the effectiveness of these measures should be validated. The action of this process during the test should be demonstrated by the construction of suitable controls.

D.2 Test methods

D.2.1 Materials and reagents

D.2.1.1 Test organisms

Test organisms should be as specified in 5.1.1.

D.2.1.2 Test media

Test media should be as specified in 5.1.2.

D.2.1.3 Laboratory equipment

Laboratory equipment should be as specified in 5.1.3.

D.2.1.4 Test samples

The contact lens care product to be tested should be representative of the product to be marketed. Three lots of product should be tested. Testing should be conducted in the actual product container. The largest container size proposed for the product should be utilized.

Culture maintenance should be as specified in 5.2.

D.2.2 Preparation of microbial challenge (Inoculum)

Test organisms should be cultured and harvested as specified in 5.3.

D.2.3 Inoculum challenge test procedure

D.2.3.1 Inoculate the sample product to be tested with a suspension of test organisms sufficient to provide a final count of between $1,0 \times 10^5$ cfu/ml and $1,0 \times 10^6$ cfu/ml. Ensure that the volume of inoculum does not exceed 1 % of the sample volume. Ensure complete dispersion of the inoculum by adequate mixing.

D.2.3.2 Store the inoculated product at 20 °C to 25 °C. The temperature shall be monitored using a calibrated device and the temperature documented.

If the contact lens care product is sensitive to light, it should be protected during the period of the test.

D.2.3.3 Take 1,0 ml aliquots of the inoculated product for determination of viable count at 7 d, 14 d, 21 d and 28 d, and continue sampling at 7-d intervals until the product contents are depleted.

D.2.3.4 Subject each of the 1,0 ml aliquots, removed at the specified time intervals, to a suitable series of decimal dilutions in validated neutralizing media. Mix the suspension well by vortexing vigorously and let stand to allow neutralization to be completed.

If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure (see annex A).

D.2.3.5 Determine the viable count of organisms in appropriate dilutions by preparation of plates in triplicate (unless otherwise justified) of a suitable recovery medium (e.g. TSA for bacteria and SDA for mould and yeast).

If membrane filtration has been employed to remove or neutralize antimicrobial agents, culture the membranes on these media as appropriate.

If the pour-plate method is utilized, keep the agar for pour plates below 50 °C prior to pouring.

NOTE The agar media used for determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

D.2.3.6 Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Incubation times for optimal recovery of bacteria, yeast and moulds should be determined. Minimum incubation times should be based on recovery medium control testing (see D.2.4.2).

D.2.3.7 Determine the average number of colony-forming units on countable plates. Calculate the microbial reduction at the specified time points.

NOTE Countable plates refer to 30 cfu to 300 cfu/plate for bacteria and yeast, and 8 cfu to 80 cfu/plate for moulds, except when colonies are observed only for the 10^0 or 10^{-1} dilution plates.

D.2.3.8 When plates for all dilutions of a sample at a single time point indicate no recovery of microorganisms, it should be documented.

D.2.3.9 The concentration of survivors is calculated at each time point.

D.2.4 Controls

D.2.4.1 Inoculum controls

The initial inoculum concentration is calculated by dispersing an identical aliquot of the inoculum into the same volume of a suitable diluent used in D.2.3.1 to achieve a final concentration not less than $1,0 \times 10^4$ to $5,0 \times 10^5$ cfu/ml. The volume of inoculum does not exceed 1 % of the sample volume. Ensure dispersion of the inoculum by adequate mixing. Evaluate this control sample for cfu/ml at the beginning of the test in order to demonstrate the suitability of the medium used for growth of the test organism and provide an estimate of the initial inoculum concentration. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

D.2.4.2 Recovery medium control

Vortex a 1/10 dilution of the preserved product in the validated neutralizing broth (1 ml into 9 ml). Let it stand to allow neutralization to be completed. Prepare a second control tube with 10 ml of a suitable diluent (e.g. DPBST). Inoculate the tubes with sufficient inoculum to result in 10 cfu to 100 cfu of challenge organism per plate. Incubate for an appropriate period of time at ambient temperature. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Determine minimum incubation times for optimal recovery of bacteria, yeast and moulds.

Check that the recovery in the neutralizer broth is at least 50 % of the recovery in the second control tube. Perform this control for each challenge organism.

If a dilution of greater than 1/10 is required for neutralization, then membrane filtration shall be used.

Qualify the neutralization of the product initially and periodically.

D.2.5 Performance criteria

D.2.5.1 Bacteria, moulds and yeasts

The number of organisms recovered per millilitre should remain at, or below, the initial concentrations.

D.2.5.2 Determination of discard date

The product discard date should correspond to the interval prior to a time point which reveals an increase in number for any organism.

EXAMPLE

Organism	Count at Day					
	0	7	14	21	28	35
E.coli	10^5	10^1	10^2	10^2	10^3	10^3
P. aeruginosa	10^5	10^3	10^3	10^2	10^3	10^4
S. aureus	10^5	<10	<10	<10	<10	<10
C. albicans	10^5	10^5	10^4	10^2	<10	<10
A. niger	10^5	10^4	10^3	10^4	10^4	10^5

Interval prior to time point which reveals an increase in growth:

E. coli = 7 d

P. aeruginosa = 21 d

A. niger = 14 d

The discard date in the above hypothetical product is 7 d after opening.

D.2.6 Test report

The test report should be as specified in 5.7.

Annex E
(informative)

Discard date procedure IV

E.1 Principle

E.1.1 The test consists of inoculating product bottles with a low level of organisms. The test samples are repeatedly rechallenged with a low level of organisms inoculated into the product bottle according to schedule.

E.1.2 Inoculation times are at initial, 24 h, 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 12 weeks, 16 weeks, 18 weeks, 24 weeks and further at six-week intervals and/or until twice the desired or labelled discard date is reached.

E.1.3 Sampling times begin at 24 h and immediately before each rechallenge and at the endpoint of the test.

E.1.4 To pass the test, all sample point counts shall be less than the total of the most recent challenge plus the previous survivor count.

E.1.5 Discard-date testing is done as a supplement to Preservative Efficacy of Multidose Preserved Contact Lens Care Products testing performed to verify the labelled shelf life. Discard testing requirements shall be met throughout the labelled shelf life.

E.2 Test methods

E.2.1 Materials and reagents

E.2.1.1 Test organisms

Test organisms should be as specified in 5.1.1.

E.2.1.2 Test media

Test media should be as specified in 5.1.2.

E.2.1.3 Laboratory equipment

Laboratory equipment should be as specified in 5.1.3.

E.2.1.4 Test samples

Challenge tests are conducted directly into final product containers. A minimum of two sample containers for each test organism, from each of three individual product lots representative of the product to be marketed, are selected for testing at each interval in the stability-testing schedule selected for discard dating.

Culture maintenance should be as specified in 5.2.

E.2.2 Preparation of microbial challenge (Inoculum)

Test organisms should be cultured and harvested as specified in 5.3.

E.2.3 Inoculum challenge test procedure

E.2.3.1 On day 0, inoculate a series of the final product containers being tested with a single strain of test organisms suspended to a level to produce a 1×10^3 cfu/ml to 2×10^3 cfu/ml count in the volume of product being tested. The volume of inoculum should not exceed 0,5 % of the sample volume at each challenge period, to reduce the risk of diluting the test product sample over repeated rechallenges. Ensure dispersion of inoculum by adequate mixing.

E.2.3.2 Store the inoculated product at 20 °C to 25 °C. The temperature shall be monitored using a calibrated device and the temperature documented.

If the contact lens care product is sensitive to light, it should be protected during the period of the test.

E.2.3.3 At 24 h, take 1,0 ml aliquots of the inoculated product for determination of viable counts.

E.2.3.4 Rechallenge with fresh cultures of the same inoculum organism, level and quantity as in E.2.3.1, store at 20 °C to 25 °C until day 7 and repeat the viable count determination, rechallenge, and storage steps. Duplicate this process at 2 weeks, 4 weeks, 6 weeks, 8 weeks, 12 weeks, 18 weeks, 24 weeks and as needed at six-week intervals, and/or until two times the desired or labelled discard date has been reached.

E.2.3.5 Subject each of the 1,0 ml aliquots, removed at the specified time intervals, to a suitable series of decimal dilutions in validated neutralizing media. Mix the suspension well by vortexing vigorously and let stand to allow neutralization to be completed.

If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure (see annex A).

E.2.3.6 Determine the viable count of organisms in appropriate dilutions by preparation of plates in triplicate (unless otherwise justified) of a suitable recovery medium (e.g. TSA for bacteria and SDA for mould and yeast).

If membrane filtration has been employed to remove or neutralize antimicrobial agents, culture the membranes on these media as appropriate.

If the pour-plate method is utilized, keep the agar for pour plates below 50 °C prior to pouring.

NOTE The agar media used for determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

E.2.3.7 Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20° C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Incubation times for optimal recovery of bacteria, yeast and moulds should be determined. Minimum incubation times should be based on recovery medium control testing (see E.2.4.2).

E.2.3.8 Determine the average number of colony-forming units on countable plates. Calculate the microbial reduction at the specified time points.

NOTE Countable plates refer to 30 cfu to 300 cfu/plate for bacteria and yeast, and 8 cfu to 80 cfu/plate for moulds, except when colonies are observed only for the 10^0 or 10^{-1} dilution plates.

E.2.3.9 When plates for all dilutions of a sample at a single time point indicate no recovery of microorganisms, it should be documented.

E.2.3.10 The concentration of survivors is calculated at each time point.

E.2.4 Controls

E.2.4.1 Inoculum controls

At each time interval duplicate matched bottles containing a suitable diluent (e.g. DPBST) are inoculated at the same time as the test samples for the determination of inoculum levels. The volume in the control container should approximate the remaining volume in the test container. Ensure dispersion of the inoculum by adequate mixing. Evaluate this control sample for cfu/ml at the beginning of the test in order to demonstrate the suitability of the medium used for growth of the test organism and provide an estimate of the initial inoculum concentration. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

E.2.4.2 Recovery medium control

Vortex a 1/10 dilution of the preserved product in the validated neutralizing broth (1 ml into 9 ml). Let it stand to allow neutralization to be completed. Prepare a second control tube with 10 ml of a suitable diluent (e.g. DPBST). Inoculate the tubes with sufficient inoculum to result in 10 cfu to 100 cfu of challenge organism per plate. Incubate for an appropriate period of time at ambient temperature. Plate the appropriate aliquot from each tube onto the recovery agar plates in triplicate (unless otherwise justified).

Incubate bacterial recovery plates at 30 °C to 35 °C. Incubate yeast recovery plates at 20 °C to 25 °C or 30 °C to 35 °C. Incubate mould recovery plates at 20 °C to 25 °C. Determine minimum incubation times for optimal recovery of bacteria, yeast and moulds.

Check that the recovery in the neutralizer broth is at least 50 % of the recovery in the second control tube. Perform this control for each challenge organism.

If a dilution of greater than 1/10 is required for neutralization, then membrane filtration shall be used.

Qualify the neutralization of the product initially and periodically.

E.2.5 Performance criteria

E.2.5.1 The test is carried out to two times the desired or labelled discard time.

E.2.5.2 The numbers of organisms recovered should not be more than the sum of the most recent inoculum plus the previous survivor count within a $\pm$ 0,5 log variance, (i.e. multiplication of organisms has not occurred).

E.2.5.3 The products should be capable of meeting these criteria throughout the labelled shelf life and for the additional time of the discard-after-opening date at the end of the shelf life.

E.2.6 Test report

The test report should be as specified in 5.7.

Annex F
(informative)

Test organisms from other culture collections

Table F.1 — Test organisms from other culture collections

Pseudomonas aeruginosa	MUAVCR 278	CCM 1961	CIP 82.118	DSM 1128	IAM 10374
	IFO 13275	NCIMB 8626	NRRL B-800		
Staphylococcus aureus	CIP 4.83	DSM 799	IFO 13276	NCIB 9518	NCTC 10788
Escherichia coli	CIP 53.126	DSM 1576	NCDO 904	NCIB 8545	
Candida albicans	CBS 6431	CCY 29-3-106	CIP 48.72	DSM 1386	IFO 1594
	NCPF 3179	NCYC 1363	VTT C-85161		
Aspergillus niger	CBS 733.88	DSM 1988	IMI 149007	IFO 9455	NCPF 2275
NOTE Cultures from various collections should be equivalent to ATCC strains.					

Table F.2 — Culture collections and institutions

ATCC	American Type Culture Collection, Rockville, MD, USA
MUAVCR	Microbiologický ústav Akademie ved. Ceské republiky, Prague, Czech Republic
CBS	Centraalbureau voor Schimmelcultures, Baarn, The Netherlands
CCM	Czech Collection of Microorganisms, Ceská sbirka mikroorganizmu, Prírodovedecká fakulta Masarykovy univerzity, Brno, Czech Republic
CCY	Culture Collection of Yeasts, Chemický ústav SAV, Bratislava, Slovakia
CIP	Collection de bactéries de l'Institut Pasteur, Paris, France
DSM	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany
IAM	Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan
IFO	Institute for Fermentation, Osaka, Japan
IMI	International Mycological Institute, Kew, Surrey, UK
NCDO	National Collection of Dairy Organisms, Shinfield, Reading, Berkshire, UK
NCIB	National Collection of Industrial Bacteria, Aberdeen, Scotland, UK
NCIMB	National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland, UK
NCPF	National Collection of Pathogenic Fungi, Mycological Reference Laboratory, Central Public Health Laboratory, London, UK
NCTC	National Collection of Type Cultures, Central Public Health Laboratory, London, UK
NCYC	National Collection of Yeast Cultures, Nutfield, Surrey, UK
NRRL	Northern Regional Research Center, U.S. Department of Agriculture, Peoria, IL, USA
VTT	Technical Research Centre of Finland, VTT Collection of Industrial Microorganisms, Espoo, Finland

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ICS 11.040.70

Price based on 23 pages