
**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*

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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.

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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.

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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.

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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.

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Table 1 — Test organisms

<i>Pseudomonas aeruginosa</i>	ATCC 9027
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Escherichia coli</i>	ATCC 8739
<i>Candida albicans</i>	ATCC 10231
<i>Aspergillus niger</i>	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 KH₂PO₄, 8 000 mg/l NaCl, and 2 160 mg/l Na₂HPO₄ · 7H₂O 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 Lethen 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
<i>P. aeruginosa</i>	TSA	30 to 35	18 h to 24 h
<i>S. aureus</i>	TSA	30 to 35	18 h to 24 h
<i>E. coli</i>	TSA	30 to 35	18 h to 24 h
<i>C. albicans</i>	SDA	either 20 to 25	42 h to 48 h
		or 30 to 35	18 h to 24 h
<i>A. niger</i>	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

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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.

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