INTERNATIONAL STANDARD

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# Ophthalmic optics — Contact lens care products — Method to assess contact lens care products with contact lenses in a lens case, challenged with bacterial and fungal organisms

*Optique ophtalmique — Produits d'entretien de lentilles de contact — Méthode d'évaluation des produits d'entretien des lentilles de contact avec les lentilles de contact dans leur étui, en présence de contamination par des bactéries et champignons*



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# Contents

Page

# Foreword

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

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 172, *Optics and photonics*, Subcommittee SC 7, *Ophthalmic optics and instruments*.



 

# Ophthalmic optics — Contact lens care products — Method to assess contact lens care products with contact lenses in a lens case, challenged with bacterial and fungal organisms

## 1 Scope

This International Standard specifies an antimicrobial efficacy end point methodology to determine compatibility of contact lens solutions, lens cases and hydrogel lenses for disinfection. This provides a process for evaluating compatibility of solutions used for disinfection with contact lenses and lens cases using an antimicrobial efficacy end point. Specifically, the microbiological effect of the antimicrobial agent(s) while in the presence of the lens cases and/or lenses will be evaluated as described in the soak step of the label instructions.

For practical purposes, this does not apply to oxidative systems.

## 2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 14729, *Ophthalmic optics — Contact lens care products — Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses*

ISO 18369-1, *Ophthalmic optics — Contact lenses — Part 1: Vocabulary, classification system and recommendations for labelling specifications*

ISO 18369-3, *Ophthalmic optics — Contact lenses — Part 3: Measurement methods*

## 3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 18369-1 apply.

## 4 Principle

The antimicrobial efficacy of the test solution in combination with a lens and a lens case will be evaluated at various times following inoculation with organisms in the presence of organic soil. New lenses and new lens cases shall be used unless otherwise justified. This test will simulate microbial contamination introduced by patient handling.

Place a lens in a well of a lens case and inoculate each lens with $1{,}0 \times 10^5$ to $1{,}0 \times 10^6$ cfu; leave inoculum in contact with the lens for 3 min to 10 min and dispense the appropriate volume (minimum of 2 ml) of the test solution into each well. The inoculated lenses in solutions will be allowed to soak for various storage times (the labelled regimen soaking period, at 24 h, at 7 days and at the maximum labelled storage in the lens case) in order to evaluate the effects of the lens case and the lens on the antimicrobial activity of the test solution. A separate set of lens case wells shall be prepared for each time point; three wells shall be evaluated for each unique test condition. Additional time points can be evaluated.

A variety of lenses shall be evaluated, e.g. Group I, Group IV, and Group V. The lens case(s) recommended for use with the test solution shall be evaluated at a minimum.

All five challenge organisms specified in ISO 14729 shall be used.



 

Log reductions will be evaluated for all exposure times.

The data generated from this method should be assessed in conjunction with preservative uptake and release data (ISO 11986).

## 5 Rationale

These studies are designed to simulate the recommended soaking and storage periods wherein the contaminating microorganisms are introduced by patient handling.

## 6 Methodology

### 6.1 General

Use ISO 14729 for media, challenge organisms, culture maintenance, test equipment and other details for conducting the Stand Alone Test with the exception of using lens cases for the microbial challenge.

### 6.2 Test procedure

**6.2.1** Conduct the test using lens types representative of those with which the solution is intended to be used, e.g. low-water non-ionic lens (Group I), high-water ionic lens (Group IV), and representative silicone hydrogel lens (Group V). Use −3,00 D lenses. New and unused lenses shall be used unless otherwise justified.

The lens cases recommended for use with the test solution shall be evaluated at a minimum. The lens cases used in this test shall be new and unused with no preconditioning unless otherwise justified. Prepare three lens case wells per lens type per time point to be examined for the test samples; additionally, prepare control lens cases for each evaluation time point without lenses. Therefore, for the evaluation of one lens case with one solution and one lens type, a total of 6 lens case wells (three wells for the test and three wells for the control) will be prepared for each of the five challenge organisms for each time point or 24 lens case wells for a minimum of four time points per challenge organism are to be evaluated. Additional time points can be evaluated.

If more than one lens type is evaluated in a test, only one set of control lens cases is required per inoculum preparation.

The inoculum shall be prepared using organic soil as specified in Annex A.

**6.2.2** Aseptically remove a new, unused lens from its sterile packaging. Aseptically blot the lens on sterile gauze. Each lens will be soaked for 24 h ± 1 h in ≥10 ml ISO saline/lens directly out of the blister pack prior to use in the assay. See ISO 18369-3 for ISO saline formulation.

**6.2.3** Prepare the lens cases (test and control) by removing the caps. Aseptically remove a lens soaked in ISO saline and aseptically blot the lens on sterile gauze and place one lens inside each test well with the concave side up. Prepare three lens case wells without lenses for use as controls.

Care should be taken to keep the shape of the lens concave.

**6.2.4** Inoculate each test and control well with 0,10 ml of the inoculum suspension prepared with organic soil to result in a final count of between $1,0 \times 10^5$ and $1,0 \times 10^6$ cfu per well. Gently dispense the inoculum directly onto the concave surface of the lens for the test wells and into the well for each control well then cover the wells.

**6.2.5** Leave the inoculum in contact with the test lens for 3 min to 10 min and then aseptically dispense a known volume of the test solution gently into each test and control lens case well so that each lens



 

is completely immersed in the solution. Each well shall contain a minimum of 2 ml unless otherwise justified. Do not agitate the contents of the lens case at this time since contamination of the cap can occur.

**6.2.6** Place the caps on the inoculated lens case wells tightly and store the inoculated lens cases at 20 °C to 25 °C. The temperature shall be monitored using a calibrated device and the temperature documented.

Care should be taken in moving the inoculated lens cases since contamination of the caps can occur.

**6.2.7** Prepare an inoculum baseline check for each challenge organism suspension.

It is suggested that 0,10 ml of the inoculum suspension be dispensed into a sterile tube containing a volume of PBST equivalent to the volume of test solution contained in the lens case well. Vortex and serially dilute a 1,0 ml aliquot of the inoculated PBST and plate out the dilutions in triplicate.

**6.2.8** Solutions will be sampled at least at the minimum regimen soaking time(s) (±10 min), 24 h ± 1 h, at 7 ± 0,25 days and at the proposed maximum lens storage period of ±0,25 days. Additional time points can be evaluated. If a regimen time point is less than 30 min, the sample shall be taken within +30 s. Solution from both test and control wells will be sampled at each time point. Each lens case well will be sampled at one time point only. The following procedure will be used at each sampling time:

**6.2.8.1** Ensure each well is tightly capped.

**6.2.8.2** Orient the case well perpendicular to the vortexing surface (hold the case vertically with the inoculated well in contact with the surface of the vortex instrument) and vortex each well separately immediately before sampling on the high speed setting for a minimum of 30 s.

**6.2.8.3** Using aseptic technique, immediately remove the lens taking care to shake the excess liquid from the lens into the lens case well. Discard the lens.

**6.2.8.4** Using aseptic technique, immediately remove a 1 ml aliquot from the vortexed lens case well using a sterile pipet and dilute in 9 ml of a validated neutralizing media.

**6.2.8.5** Perform serial dilutions in validated neutralizing media. Mix each dilution well by vortexing vigorously prior to preparing the subsequent dilutions. Let stand to allow neutralization to be completed. Neutralization conditions shall be based on recovery medium control testing according to ISO 14729.

**6.2.8.6** If an antimicrobial agent in the formulation cannot be adequately inactivated or neutralized, eliminate it using a validated membrane filtration procedure.

NOTE For procedures that can be appropriate, see ISO 14729, Annex B.

**6.2.9** 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.

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

**6.2.10** 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 shall be determined. Minimum incubation times shall be based

on recovery medium control testing (according to ISO 14729). Record the number of all cfu observed on countable plates.

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

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

**6.2.11** Determine the average number of colony forming units on countable plates from 6.2.10.

**6.2.12** Calculate the average cfu/ml of test solution and the log reduction for each replicate well.

**6.2.13** Calculate the average log reduction for the three replicate wells for each unique test sample.

## 7 Performance criteria

This provides data for risk assessment and does not replace the requirements of ISO 14729.

# Annex A
(normative)

# Preparation of challenge organisms in organic soil

## A.1 Materials and reagents

### A.1.1 Organism for organic soil

Saccharomyces cerevisiae (S.c.) of unspecified strain.

### A.1.2 Culture media and reagents

**A.1.2.1** 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 $NA_2PO_4 \bullet 7H_2O$.

**A.1.2.2** Sabouraud Dextrose Agar (SDA) slants.

**A.1.2.3** Heat inactivated bovine serum.

### A.1.3 Test equipment

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

## A.2 Preparation of heat killed yeast

Culture the S.c. on SDA slants for 42 h to 48 h at 20 °C to 25 °C. Harvest S.c. from the SDA slants in DPBS, centrifuge, decant supernatant, add fresh DPBS, centrifuge again, and resuspend in DPBS. Adjust the concentration to 1 × $10^7$ cfu/ml to 1 × $10^8$ cfu/ml and heat kill at 100 °C ± 2 °C for 10 min. No further manipulations of the heat killed yeast shall take place. Store under refrigeration (2 °C to 8 °C) until day of use.

## A.3 Preparation of organic soil

**A.3.1** On the day of use, centrifuge an aliquot of the heat killed yeast preparation (A.2) at no more than 5 000 × *g* for a maximum of 30 min and decant the supernatant; add an equal aliquot of the heat inactivated bovine serum and resuspend the heat killed yeast.

EXAMPLE If centrifuging 1 ml of heat killed yeast above, use 1 ml of heat inactivated bovine serum to resuspend the heat killed yeast pellet.

**A.3.2** Dilute 1 ml of this organic soil preparation into 9 ml DPBS. This constitutes what is called organic soil preparation.

## A.4 Preparation of challenge organisms in organic soil

Prepare individual challenge organisms per ISO 14729, 6.2. After harvesting, the cultured organisms can be washed using centrifugation. The bacterial suspensions can be filtered (e.g. 3 µm to 5 µm pore size)