INTERNATIONAL STANDARD

**ISO 14729**

First edition
2001-04-15

# Ophthalmic optics — Contact lens care products — Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses

*Optique ophtalmique — Produits d'entretien des lentilles de contact — Exigences microbiologiques et méthodes d'essai des produits et protocoles d'entretien des lentilles de contact*

Reference number
ISO 14729:2001(E)

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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 14729 was prepared by Technical Committee ISO/TC 172, *Optics and optical instruments*, Subcommittee SC 7, *Ophthalmic optics and instruments*.

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

# Introduction

Products for contact lens disinfection by chemical means are intended to reduce microbial contamination introduced during lens wear and removal, cleaning and storage and are required to contain antimicrobial agents capable of achieving this.

It is essential that all liquid contact lens care products are sterile until opened. Dry products (tablets, granules, etc.) should be subject to control of microbial contamination and should be dissolved in a suitable diluent immediately prior to use. Multidose contact lens care products must be adequately preserved or be packaged in a container designed and labelled to minimize the risk of injury resulting from in-use contamination.

Contact lenses are normally subject to a regimen of cleaning and contact lens disinfection between periods of wear. Aqueous solutions containing cleaning and/or disinfecting agents are commonly used for this purpose. These products may be marketed as solutions or as tablets for dissolution immediately prior to use in a suitable diluent such as saline.

The past 20 years of experience in the use and regulation of contact lens disinfecting products has shown distinct disinfecting antimicrobial criteria for this class of medical devices. Ocular toxicology concerns, process convenience and product comfort on the eye, have meant an evolution of products which maintain a low incidence of contact lens associated ocular infection when used as instructed by the manufacturer. This International Standard gives these distinct contact lens disinfecting antimicrobial criteria along with annexes to explain why viruses (annex C) and Acanthamoeba (annex D) are not included as challenges. Organic soil is not required for evaluation of contact lens care disinfecting products but may be used; an informative annex (annex E) is included to discuss organic soil in the context of contact lenses and contact lens care products.

# Ophthalmic optics — Contact lens care products — Microbiological requirements and test methods for products and regimens for hygienic management of contact lenses

## 1 Scope

This International Standard specifies two test methods for evaluating the antimicrobial activity of products to be marketed for contact lens disinfection by chemical means and for products that are part of a contact lens care regimen.

This International Standard is not applicable to the hygienic management of trial lenses.

NOTE General disinfection product standards are not applicable to contact lens care products, e.g. EN 1040:1997 and EN 1275:1997.

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

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

## 3 Terms and definitions

For the purposes of this International Standard, the definitions given in ISO 8320 apply together with the following.

**3.1**
**contact lens disinfecting product**
product that possesses cidal activity (kills, destroys and/or inactivates) meeting the primary criteria of the stand-alone test specified in this International Standard

**3.2**
**contact lens disinfecting regimen**
contact lens care regimen designed to meet both the secondary criteria of the stand-alone test and the regimen test as specified in this International Standard

1) To be published. (Revision of ISO 8320:1986)

**3.3**
**contact lens disinfection**
chemical or physical process to reduce the number of viable microorganisms as specified in the performance requirement sections of this International Standard

# 4 Principle

## 4.1 General

The stand-alone test is designed to qualify individual solutions with a suitable level of antimicrobial activity as contact lens disinfection products. The regimen test is designed to qualify individual solutions as part of a contact lens disinfecting regimen. Products meeting the regimen test criteria shall also meet the minimum performance requirements of the stand-alone test. It is fundamental that such products (unopened containers) are capable of meeting the requirements of the test throughout their labelled shelf life.

As described in Figure 1, contact lens care solutions which are designed to possess disinfecting properties shall be tested in the stand-alone test first. If the respective primary criteria are met (see 5.1), the product may be labelled as a contact lens disinfecting product. If the product fails the primary criteria of the stand-alone test, the product must exhibit sufficient antimicrobial activity to meet the secondary criteria of the stand-alone test as listed in 5.2. If these secondary criteria are met, the regimen test shall be performed in order to qualify the product as part of a contact lens disinfecting regimen by meeting the regimen criteria (see 5.3). If the product meets both the secondary criteria of the stand-alone test and the regimen test but fails the primary criteria of the stand-alone test, it shall be labelled as part of a contact lens disinfecting regimen.

The design of contact lens care products for cleaning and contact lens disinfection shall take into consideration the needs of patient compliance and the probability of non-compliance. For example, disinfecting time must be appropriate for contact lens wear.

NOTE Use of multiple or mixed microbial challenges can influence the apparent disinfecting 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. (See annexes C and D).

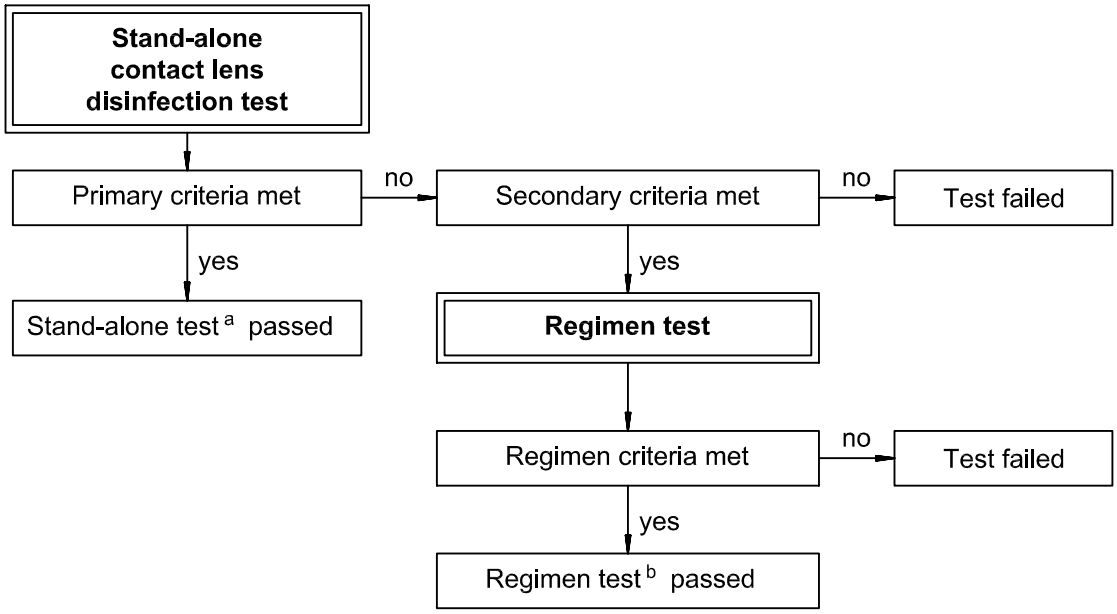
## 4.2 Stand-alone test (Inoculum challenge test)

The stand-alone test challenges a disinfecting product with a standard inoculum of a representative range of microorganisms and establishes the extent of their viability loss at pre-determined time intervals comparable with those during which the product may be used. 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.

In carrying out the test for antimicrobial activity the qualitative and quantitative compositions of the product have to be known at the time of testing by either analytical testing or extrapolation.

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

NOTE For information about virus testing, see annex C, and for *Acanthamoeba* testing, see annex D.




a Product may be labelled as a contact lens disinfecting product.

b Product shall be labelled as part of a contact lens disinfecting regimen.

**Figure 1 — Flow chart for stand-alone test and regimen test**

### 4.3 Regimen test

This is a test which challenges a multifunctional disinfecting regimen with a standard inoculum of a representative range of micro-organisms and which measures the viability loss after a predetermined time interval; the inoculum is carried through the regimen by being applied to a contact lens.

This procedure is applicable to multifunctional disinfecting regimens which may include the steps of cleaning, rinsing and soaking. In carrying out the regimen test procedure, the products are used in the manner and quantity recommended in product labelling and/or patient instructions.

The disinfecting stage of any proposed contact lens disinfecting regimen evaluated by this test shall satisfy the minimum requirements of the stand-alone test as described in Figure 1. Only those products that satisfy the minimum performance requirements for the stand-alone test may be incorporated into a disinfecting regimen.

In carrying out the test, qualitative and quantitative composition of all products used in the test regimen have to be known at the time of testing, either by analytical testing or extrapolation.

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

NOTE For problems associated with the use of human-worn lenses, see annex E.

## 5 Performance requirements

### 5.1 Stand-alone test: Primary criteria (see also Table 1)

#### 5.1.1 Bacteria

The number of each challenge organism recovered per millilitre shall be reduced by an average value of not less than 99,9 % (3,0 logs) within the minimum recommended soaking period.

NOTE The value is determined by taking the average of the log reductions for each challenge organism for the individual lots tested.

#### 5.1.2 Moulds and yeasts

The number of each challenge organism recovered per millilitre shall be reduced by an average value of not less than 90 % (1,0 log) within the minimum recommended soaking period with no increase at not less than four times the minimum recommended soaking period within an experimental error of ± 0,5 logs.

NOTE The value is determined by taking the average of the log reductions for each challenge organism for the individual lots tested.

### 5.2 Stand-alone test: Secondary criteria (see also Table 1)

Products failing to meet the criteria in 5.1.1 or 5.1.2 shall be evaluated by the regimen test procedure described in 6.4, provided the sum of the averages is a minimum of 5,0 log units reduction for the three species of bacteria within the recommended soaking period with a minimum average of 1,0 log unit reduction for any single bacteria. Stasis for the yeast and mould shall be observed for the recommended soaking period within an experimental error of ± 0,5 logs.

### 5.3 Regimen test: Regimen criteria (see also Table 1)

For each microbial species, the average regimen recovery count (for all lots tested) shall be no more than 10 cfu for each lens type/storage solution combination.

Data from more than one lens type should not be combined to calculate the average.

NOTE When qualifying a lens care product regimen for use with one lens type, the average count for each species requires averaging the data from the 24 inoculated and treated lenses of the one lens type. When qualifying a lens care product regimen for use with more than one lens type, the average count for each species by lens type would require averaging the data from the 12 inoculated and treated lenses for each lens type. See Table 4 for numbers of lenses to be used.

**Table 1 — Summary of performance requirements criteria for contact lens disinfection procedures**

| Test | Average log reduction at soaking time | | | | |
|---|---|---|---|---|---|
| | Fungi | | Bacteria | | |
| | FS[a] | CA[a] | SM[a] | PA[a] | SA[a] |
| Stand-alone test: Primary Criteria | 1 | 1 | 3 | 3 | 3 |
| Stand-alone test: Secondary Criteria | b | b | c | c | c |
| Regimen test: Regimen criteria[d] | ≈ 4 to 5 | ≈ 4 to 5 | ≈ 4 to 5 | ≈ 4 to 5 | ≈ 4 to 5 |

[a] PA = *P. aeruginosa* ATCC 9027;

SA = *S. aureus* ATCC 6538;

SM = *S. marcescens* ATCC 13880;

CA = *C. albicans* ATCC 10231;

FS = *F. solani* ATCC 36031.

[b] Stasis at the soaking time.

[c] The minimum acceptable log reduction for all three bacteria combined is 5. The minimum acceptable log reduction for any single bacterial type is 1.

[d] Equivalent to an average of not more than 10 cfu per lens type/storage solution combination.

# 6 Test methods

## 6.1 Materials and reagents

The materials and reagents (i.e. the test organisms, media and reagents, equipment and samples) are common to both the stand-alone procedure for disinfecting products and the regimen procedure for contact lens disinfection.

### 6.1.1 Test organisms

The strains listed in Table 2 shall be used.

NOTE Test organisms from other culture collections that may be used are listed in annex A.

**Table 2 — Test organisms**

| | |
|---|---|
| *Pseudomonas aeruginosa* | ATCC 9027 |
| *Staphylococcus aureus* | ATCC 6538 |
| *Serratia marcescens* | ATCC 13880 |
| *Candida albicans* | ATCC 10231 |
| *Fusarium solani* | ATCC 36031 |

### 6.1.2 Culture media and reagents

#### 6.1.2.1 **Potato dextrose agar** (PDA).

**6.1.2.2 Tryptone soya agar** (TSA).

**6.1.2.3 Sabouraud Dextrose Agar** (SDA).

**6.1.2.4 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.

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

**6.1.2.6 Validated neutralizing agents/media** as required, for example, Dey-Engley Neutralising Broth (DEB) and Letheen Broth[2)].

### 6.1.3 Test equipment

The following common laboratory equipment is required.

**6.1.3.1 Sterile pipettes.**

**6.1.3.2 Swabs.**

**6.1.3.3 Tubes.**

**6.1.3.4 Petri dishes** (90 mm to 100 mm × 20 mm).

**6.1.3.5 Incubator**.

**6.1.3.6 Spectrometer,** for determination of cell density.

**6.1.3.7 Instrument for colony counting.**

**6.1.3.8 Centrifuge.**

### 6.1.4 Test samples

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.

### 6.1.5 Culture maintenance

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

Cultures should be no greater than 5 passes removed from the depository stock (ATCC, NCIB, NCTC, NCPF or other recognized culture depository; see annex A). Each pass is a subculture of the previous pass.

## 6.2 Preparation of microbial challenge (Inoculum)

The preparation of the microbial challenge organisms (inoculum) is common to both the stand-alone procedure for disinfecting products and the regimen procedure for contact lens disinfection.

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2) Dey-Engley Neutralising Broth (DEB) and Letheen Broth are examples of suitable products 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.

For the regimen procedure for contact lens disinfection, organic soil may be included as part of the inoculum. See annex E for an example.

Culture each test organism on agar slopes under the conditions given in Table 3.

**Table 3 — 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 |
| *S. marcescens* | TSA | 30 to 35 | 18 h to 24 h |
| *C. albicanx* | SDA | 20 to 25 | 42 h to 48 h |
| or *C. albicans* | SDA | 30 to 35 | 18 h to 24 h |
| *F. solani* | PDA | 20 to 25 | 10 d to 14 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 *F. solani* suspensions through sterile glass wool, cheese cloth 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 $1 \times 10^7$ cfu/ml and $1 \times 10^8$ 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 a centrifuge is used, each centrifugation should be conducted at 20 °C to 25 °C for no longer than the equivalent of 10 min at $4\,000 \times g$ or less. Longer centrifugation times may be required at lower speeds.

Use bacterial and yeast cell suspensions on the day of preparation. Spore suspensions may be used up to seven days following preparation if stored under refrigeration (2 °C to 8 °C).

## 6.3 Stand-alone procedure

### 6.3.1 Inoculum challenge test procedure

**6.3.1.1** Prepare one or more tubes (for each lot tested) containing a minimum of 10 ml of test product 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 are compatible with the ingredients, should be used.

Inoculate the sample tube of the product to be tested with a suspension of test organisms sufficient to provide a final count of between $1{,}0 \times 10^5$ 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.

**6.3.1.2** 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 product is sensitive to light it should be protected during the period of the test.

**6.3.1.3** Take 1,0 ml aliquots of the inoculated product for determination of viable count at 25 %, 50 %, 75 % and 100 % of the minimum recommended disinfecting time for all organisms, and, in addition, not less than 400 % of the minimum recommended disinfecting time for yeast and mould. If overnight contact lens disinfection is recommended, use a soaking time of 8 h.

**6.3.1.4** 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. Neutralization conditions shall be based on recovery medium control testing (see 6.3.2.2).

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

**6.3.1.5** 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 determination of viable counts may also contain antimicrobial inactivators or neutralizers, if required.

**6.3.1.6** 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 shall be determined. Minimum incubation times shall be based on recovery medium control testing (see 6.3.2). Record the number of cfu observed on countable plates.

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

**6.3.1.7** 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/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.

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

### 6.3.2 Controls

#### 6.3.2.1 Inoculum Control

Prepare an inoculum count by dispersing an identical aliquot of the inoculum into the same volume as used in 6.3.1.1 of a suitable diluent (e.g. DPBST) to achieve a final concentration of $1{,}0 \times 10^5$ cfu/ml to $1{,}0 \times 10^6$ cfu/ml. Ensure that 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).

#### 6.3.2.2 Recovery medium control

Vortex a 1/10 dilution of the disinfecting 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/ml to 100 cfu of challenge organism per plate. Incubate for an appropriate period of time at ambient temperature but not long enough to allow multiplication of the

inoculated organisms. 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 shall be used.

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

#### 6.3.2.3 Control specification

If any control value is outside that specified, repeat the procedure as the associated test is invalid.

### 6.3.3 Test report

The test report shall specify:

a) reference to this International Standard;

b) the identification of the product:

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

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

d) deviations from the protocol;

e) period of incubation;

f) storage time for inoculated product;

g) results obtained.

If a product passes the primary criteria of the stand-alone test, it may be labelled as a contact lens disinfecting product. If the product only passed the secondary criteria of the stand-alone test, and the regimen test, it shall be labelled as part of a contact lens disinfecting regimen.

## 6.4 Regimen procedure

### 6.4.1 Lens inoculation

Carry out the test using lens types representative of those with which the regimen is intended to be used, e.g. low water non-ionic, high water ionic, silicone acrylate, etc. New and unused lenses should be used for this test. When qualifying a lens care product regimen with a single lens type, inoculate each of eight lenses for each microbial species per lot of test product; this results in testing a total of 24 lenses per formulation per species. When qualifying a lens care product regimen for use with all hydrophilic lens types, inoculate each of four lenses from Group 1 (low water content non-ionic) and four lenses from Group 4 (mid- and high water content ionic) for each microbial species per lot of test product; this results in testing a total of 12 lenses per lens type per formulation per species. Additional hydrophilic lens types may be tested; however, a minimum of four lenses per lens type per species per lot of formulation shall be used. In qualifying a lens care product regimen for use with all non-hydrophilic lens types, inoculate four silicone acrylate lenses and four fluorosilicone acrylate lenses per microbial species per lot of test product for a total of 12 lenses per lens type per formulation per species. Qualification of a lens care product regimen with all hydrophilic and all non-hydrophilic lenses requires testing with Group 1 and 4 hydrophilic lens types and silicone acrylate and fluorosilicone acrylate non-hydrophilic lenses.

The number of lenses required for the test is given in Table 4.

Place test and control lenses, with concave surface uppermost, in a sterile petri dish. Inoculate each lens by placing 0,01 ml of inoculum on the underside of the lens at the point of contact between the petri dish and the lens. Also inoculate the upper surface by application of 0,01 ml of the same inoculum directly on to the concave surface of the lens.

Allow the inoculum to adsorb onto each lens for between 5 min and 10 min at 20 °C to 25 °C.

**Table 4 — Number of lenses required**

| Number of lenses per microbial species | | | | | |
|---|---|---|---|---|---|
| Test sample[a] | Qualification for a single lens type[c] | Qualification for all hydrophilic lenses[b, d] | | Qualification for all non-hydrophilic lenses[b, d] | |
| | (e.g. Group 1) | Group 1 | Group 4 | Silicone acrylate | Fluoro-silicone acrylate |
| Solution LOT 1 | 8 | 4 | 4 | 4 | 4 |
| Solution LOT 2 | 8 | 4 | 4 | 4 | 4 |
| Solution LOT 3 | 8 | 4 | 4 | 4 | 4 |
| Total[d] | 24 | 12 | 12 | 12 | 12 |

[a] Minimum of three lots of lens care product to be tested.

[b] If testing more than one lens type, a minimum of four lenses per lens type per lot of lens care product per microbial species shall be used.

[c] If testing only one lens type, a minimum of eight lenses per lens type per lot of lens care product per microbial species shall be used.

[d] Qualification of a lens care product regimen with all hydrophilic lenses and all non-hydrophilic lenses would require, at a minimum, testing the product with four lenses from each of the following lens types: Group 1 and Group 4 hydrophilic lenses and silicone acrylate and fluorosilicone acrylate non-hydrophilic lenses.

### 6.4.2 Lens treatment

After adsorption of the inoculum, treat the lenses as described in the manufacturer's instructions to the consumer for contact lens disinfection, including all steps of cleaning, rinsing and soaking as specified by the manufacturer. Test protocols should specify the parameters of the cleaning and rinsing procedures (e.g. rubbing and rinsing times and rinse volumes).

**6.4.3 Recovery of surviving challenge organisms** (see annex B for an example of a membrane filtration procedure).

**6.4.3.1** Dispense a suitable volume of a validated neutralizing medium into the filtration apparatus. (B.1.2.1). Neutralization conditions shall be based on recovery medium control testing (see 6.4.4.2).

**6.4.3.2** Transfer the entire contents of each test lens case (lens and solution) into the neutralizing medium in the filtration apparatus (B.1.2.2) having determined the neutralization exposure time prior to filtration (see annex B).

**6.4.3.3** Apply reduced pressure and filter the solution. Rinse the filter with a suitable volume of neutralizing medium.

**6.4.3.4** Aseptically transfer the contact lens onto a bed of agar medium appropriate for recovery of the test organism. Pour some of the same agar medium (kept below 50 °C) over the lens and allow to cool.

**6.4.3.5** Apply the test filter to the surface of a plate of appropriate solid media (could be the same as used in 6.3.1.5).

**6.4.3.6** 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 shall be determined. Minimum incubation times shall be based on recovery medium control testing (see 6.4.4). 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.

### 6.4.4 Controls

#### 6.4.4.1 Lens inoculation control

For each microbial species tested, transfer three inoculated lenses to tubes of a suitable diluent (e.g. DPBST). Vortex for 30 s. Serially dilute and plate out appropriate dilutions in triplicate (unless otherwise justified) to permit a count of viable cells present. This count confirms that the number of organisms on the lens at the time of the regimen challenge is adequate. The mean of the counts should be not less than $2 \times 10^5$ cfu/lens and not greater than $2 \times 10^6$ cfu/lens.

#### 6.4.4.2 Recovery medium control

Prepare filtration apparatus in triplicate (unless otherwise justified) as described in 6.4.3 with suitable volumes of the neutralizing medium and disinfecting product (see annex B). Let it stand to allow neutralization to be completed. Add 5 cfu to 100 cfu challenge organisms (one organism per filter*),* filter and cultivate as described in 6.4.3.

Confirm the inoculum on a suitable medium in triplicate (unless otherwise justified).

Ensure that the recovery on the filter from the neutralizer broth is at least 50 % of the inoculum.

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

### 6.4.5 Test report

The test report shall include:

a) reference to this International Standard;

b) the identification of the product:

— name of the product;

— batch number;

— expiry date;

- manufacturer;
- storage conditions;
- active substances(s) and its/their concentration(s) (as available);

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

d) deviations from the protocol;

e) period of incubation;

f) storage time for inoculated product;

g) results obtained.

If a product passes the primary criteria of the stand-alone test, it may be labelled as a contact lens disinfecting product. If the product only passed the secondary criteria of the stand-alone test, and the regimen test, it shall be labelled as part of a contact lens disinfecting regimen.

# Annex A
(informative)

# Test organisms from other culture collections

## A.1 General

Details of test organisms and culture collections and institutions are given in Tables A.1 and A.2 respectively.

Cultures from various collections should be equivalent to ATCC strains.

**Table A.1 — Test organisms from other culture collections**

| | | | | | |
|---|---|---|---|---|---|
| *Pseudomonas aeruginosa* | MUAVCR 278<br>IAM 10374 | CCM 1961<br>IFO 13275 | CIP 82.118<br>NCIMB 8626 | DSM 1128<br>NRRL B-800 | DSM 1385 |
| *Staphylococcus aureus* | CIP 4.83 | DSM 799 | IFO 13276 | NCIB 9518 | NCTC 10788 |
| *Serratia marcescens* | CCM 303<br>NCTC 10211 | DSM 47 | DSM 30121 | CDC 813-60 | NCIB 9155 |
| *Candida albicans* | CBS 6431<br>NCPF 3179 | CCY 29-3-106<br>NCYC 1363 | CIP 48.72<br>VTT C-85161 | DSM 1386 | IFO 1594 |

**Table A.2 — Culture collections and institutions**

| | |
|---|---|
| ATCC | American Type Culture Collection, Rockville, Md., USA |
| MUAVCR | Mikrobiologicky ustav Akademie ved Ceske republiky, Prague, Czech Republic |
| CBS | Centraalbureau voor Schimmelcultures, Baarn, The Netherlands |
| CCM | Ceska sbirka mikroorganizmu, Pnrodovedecka fakulta Masarykovy univerzity, Brno, Czech Republic |
| CCY | Culture Collection of Yeasts, Chemicky ustav SAV, Bratislava, Slovakia |
| CDC | Centers for Disease Control, Atlanta, Georgia, USA |
| 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 |
| NCIB | National Collection of Industrial Bacteria, Aberdeen, Scotland, U.K. |
| NCIMB | National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland, U.K. |
| NCPF | National Collection of Pathogenic Fungi, Mycological Reference Laboratory, Central Public Health Laboratory, London, U.K. |
| NCTC | National Collection of Type Cultures, Central Public Health Laboratory, London, U.K. |
| NCYC | National Collection of Yeast Cultures, Nutfield, Surrey, U.K. |
| NRRL | Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois, USA |
| VTT | Technical Research Centre of Finland, VTT Collection of Industrial Microorganisms, Espoo, Finland |

# Annex B
(informative)

# Example of a membrane filtration procedure

## B.1 Materials and reagents

### B.1.1 Culture media and reagents

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

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

**B.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.

**B.1.1.4 Dulbecco's Phosphate Buffered Saline plus 0,05% w/v polysorbate 80 (DPBST)** or suitable diluent.

**B.1.1.5 Validated neutralizing agents/media,** as required, for example, Dey-Engley Neutralizing Broth (DEB) and Letheen Broth[3)].

### B.1.2 Test equipment

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

**B.1.2.1 Sterile apparatus for holding the sterile membrane filter and filtrate.**

**B.1.2.2 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.

## B.2 Test method and results

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

**B.2.2** Aseptically transfer a measured volume of the inoculated test solution into 50 ml to 100 ml of sterile DPBST (B.1.1.4) or diluting fluid and thoroughly mix.

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

**B.2.3** Transfer the diluted solution to the membrane and filter immediately with the aid of vacuum or pressure.

**B.2.4** Wash the membrane filter with several volumes of diluting fluid which may contain additional neutralizing agents as needed.

---

3) Dey-Engley Neutralising Broth (DEB) and Letheen Broth are examples of suitable products 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.

NOTE Three volumes of diluting fluid (100 ml each) are usually sufficient to remove and/or dilute the antimicrobial agent. The actual volume should be determined empirically for each formulation for each challenge organism.

**B.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.

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

## B.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 B.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 on the filter from the neutralizer broth is at least 50 % of the inoculum.

# Annex C
(informative)

# Technical report: Virus testing

Due to fundamental differences in life forms, viruses do not replicate and proliferate on contact lenses, in lens cases, or in lens care solutions the way gram-negative bacteria such as *Pseudomonas and Serratia* or *Acanthamoeba* can. This is because viruses are obligate intracellular parasites and require living cells in which to multiply (see reference [1] in the bibliography). The mode of transmission of viral keratitis caused by *Herpes simplex* usually occurs in childhood and 80 % of the population have already been infected with *Herpes simplex* by the age of 15. Recurrent infection in adults is triggered by stress, fever or UV light because of the reactivation of the latent virus already present in nerves and other tissues (see references [2] and [3]). Transferring the infection from one body site to another is possible.

There is a risk of measurable transmission of viruses, such as HIV, hepatitis or adenovirus from trial lenses in the practitioner's office (see references [4], [5] and [6]), because the virus can be associated with the surface of the lens. A literature search showed that no reports were found which implicated transmission of viruses by contact lenses for individual use or a direct association between contact lens wear and viral infections of the outer eye.

This International Standard is intended to provide a means for the evaluation of contact lens disinfecting systems for individual use only. Since incidents of viral transmission via contact lens wear have not been documented and viruses cannot proliferate on contact lenses or in lens cases, this International Standard does not recommend virucidal testing.

In the event of a viral ocular infection during a period of contact lens wear, it is recommended that the contact lenses and the contact lens case be discarded to avoid the possibility of reinfection.

# Annex D
(informative)

# Technical report: *Acanthamoeba* testing

*Acanthamoeba* may cause a rare but serious keratitis. This form of keratitis occurs mainly in contact lens wearers and is associated with the use of contaminated contact lens care systems. Use of tap water or homemade saline prepared from nonsterile distilled water with contact lenses are important risk factors associated with the disease. (see references [7], [8], [9] and [10]). Persons wearing contact lenses should always follow the manufacturers' lens care instructions carefully (see references [11] and [12]).

It is thought that the amoeba grows on bacteria adhering to the contact lens, the contact lens case and/or in the care solution. When a contaminated contact lens is removed from the case and placed in the eye, it provides the route for the *Acanthamoeba* to penetrate the corneal epithelium (see references [13], [14], [15], [16] and [17]).

A literature search reveals that the amoebae are very resistant to freezing, desiccation and antimicrobial agents including antibacterial, antifungal, antiprotozoal, antiviral and anticancer agents (see reference [14]). In addition, there are no standard methods for testing products, recovery and quantitation of survivors, species type or stage of the *Acanthamoeba* organism to test (see references [18] and [19]). Heat or strong antimicrobials with long soaking times are needed to kill *Acanthamoeba,* and these antimicrobials may be toxic to the eye. Therefore, most contact lens disinfecting products do not kill *Acanthamoeba,* especially cysts, at their recommended soaking time (see references [14], [20], [21], [22], [23], [24], [25], [26], [27] and [28]). Since bacteria are the food source for *Acanthamoeba* (see reference [23]), cleaning and rinsing lenses with a sterile contact lens care solution, storing the lenses in a sterile preserved solution, keeping the lens case clean and dry and replacing the lens case frequently can greatly help in the prevention of *Acanthamoeba* contamination (see references [9] and [30]). A lens care system that eliminates contamination with bacteria can reduce the incidence of *Acanthamoeba* contamination (see references [30] and [31]).

Based on this information, i.e. the rare incidence of infection, preventable source of contamination and lack of standard methodology, this International Standard does not recommend Acanthamoebicidal testing.

# Annex E
(informative)

# Technical report: Artificial tears (organic soil) in laboratory testing

Organic soil is not required for evaluation of contact lens disinfecting products but may be used; this informative annex is included to discuss organic soil in the context of contact lenses and contact lens care products.

It has been established that the presence of organic material may affect the microbicidal activity of some contact lens disinfecting products (see reference [51]). In the regimen test, organic soil may be added to the lenses to mimic deposits that may be present in actual patient use situations. Inclusion of organic load allows for an evaluation of the cleaning step to remove debris and associated microorganisms, as well as the interaction of any remaining organic material with the soaking solution.

There have been numerous attempts to develop and standardize an artificial tear model, or organic soil, for use in evaluating contact lens care products. The intent was to imitate or mimic the natural tear fluid; however, no model completely represents the very complex characteristics of human tears or a natural tear film on a contact lens.

The tear film is composed of a superficial lipid layer, an aqueous phase and a mucous layer. The aqueous phase contains at least 60 protein components, including lysozyme, lactoferrin, tear lipacalin, transferrin, albumin, caeruloplasmin, complement, glycoproteins, antiproteinases and a variety of immunoglobulins; specifically, secretory IgA. (see references [32], [33], [34] and [35]). Although many laboratory artificial tear or organic soil models are available (see references [36], [37], [38], [39] and [40]) none of these contain all the components found in natural tears (see reference [13]). Furthermore, tear component concentrations, activity and sources (e.g. egg-white compared with human lysozyme) in artificial models do not fully correlate with human tears (see references [32], [33], [42] and [43]).

In addition to tear component composition, several additional factors need to be considered when attempting to simulate a natural tear film. The composition of human tears is variable in time and from individual to individual (see reference [53]). More importantly, once tears are absorbed onto a lens, the activity of the tear film may be dissimilar to its original activity.

Additional inconsistencies may be found when depositing artificial tears onto the lens surface:

a) the nature and composition of a macromolecular film on a lens will depend on the chemical nature of the lens polymer matrix (see references [46], [47], [48], [49] and [50]); and

b) lens deposits will be dependent on the deposition procedure used (see references [41] and [45]). Artificial tears on a lens may not accurately represent human deposited tears, especially when an eye-air interface and mechanical blinking are not duplicated *in vitro*.

Because the addition of organic soil has not been standardized for use with this method at this time, an artificial tear or organic soil is not required during the evaluation of contact lens care products. It is recognized that some governmental health authorities (e.g. the United States Food and Drug Administration) recommend the use of organic soil for product registrations. Therefore, this International Standard encompasses regimen testing conducted either with or without organic soil. An example for preparation and use of organic soil follows (see reference [52]).

Prepare organic soil as follows: Culture *S. cerevisiae* on SDA at 20 °C to 25° C for 48 h. Harvest as in 6.2. Heat kill the suspension at 100 °C $\pm$ 2 °C for 10 min. Centrifuge at no more than 5 000 $\times g$ for a maximum of 30 min. Resuspend in bovine serum which has been heat treated at 56 °C for 30 min to inactivate complement. The concentration of *S. cerevisiae* in serum should be $1 \times 10^7$ to $10^8$ cfu/ml.

After harvesting the challenge organism, centrifuge test organism suspension. Resuspend in organic soil to a concentration of $1 \times 10^7$ to $10^8$ cfu/ml. This is the inoculum to be used in 6.4.

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