DRAFT INTERNATIONAL STANDARD

ISO/DIS 18189

ISO/TC **172**/SC **7**

Secretariat: **DIN**

Voting begins on:
2015-08-17

Voting terminates on:
2015-11-17

Ophthalmic optics — Contact lenses and contact lens care products — Cytotoxicity testing of contact lenses and contact lens care solutions

Titre manque

ICS: 11.040.70

Reference number
ISO/DIS 18189:2015(E)

COPYRIGHT PROTECTED DOCUMENT

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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.

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. www.iso.org/directives

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received. www.iso.org/patents

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

Ophthalmic optics — Contact lenses and lens care products — Cytotoxicity testing of contact lens in combination with lens care solution to evaluate lens - solution interactions

1 Scope

This International Standard describes an in vitro test method to assess the potential cytotoxic effects that may arise due to interaction of contact lenses with contact lens care solutions.

NOTE The potential of a contact lens or a contact lens care solution to cause cytotoxicity by itself can be evaluated in accordance with general guidance in ISO 10993-5.

2 Normative references

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

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

3 Terms and definitions

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

3.1
room temperature
temperature defined as 18 °C to 25 °C

4 Principle

The chemicals in a lens care solution can cause cytotoxic effects by direct contact with ocular tissues or by indirect contact through contact lenses. Uptake of the care product preservative or other solution ingredients by the lens and subsequent release of these chemicals in the ocular environment can compromise ocular biocompatibility. The potential interactions between a lens care product and various contact lens materials should be taken into account in designing the tests to fully evaluate the cytotoxicity potential of a new contact lens or a lens care product.

5 Direct contact cytotoxicity test for lens-lens care solution combination

5.1 General

The following protocol describes the test method for evaluating potential cytotoxic effects of contact lenses exposed to contact lens care solution. The cytotoxicity can result from contact lens – lens care solution interactions.

With the exception of daily disposable contact lenses, the potential interaction of a new contact lens with marketed representative multipurpose solutions to produce cytotoxicity shall be evaluated.

For evaluating a new contact lens care solution, the potential interaction of new contact lens care solution with representative conventional and silicone hydrogel lenses to produce cytotoxicity shall be evaluated.

5.2 Experimental procedure

5.2.1 Basic procedure

The test contact lens is incubated in ~10 ml of contact lens care solution in a sterile compatible container for 24 h ± 2 h at room temperature. Similarly, a DPBS (Dulbecco's Phosphate Buffered Saline with Ca^{2+} and Mg^{2+})-treated control lens ("Lens Control") is prepared by incubating the contact lens in ~10 ml of DPBS in the same type of container for 24 h ± 2 h at room temperature.

For the purpose of this document, a compatible container refers to a container in which there is little to no uptake of the disinfecting agent and/or preservative. Rinsing of the container with the contact lens care product may be used to reduce uptake by the container.

Following the 24 h ± 2 h soak period, the lenses may be cut in a pinwheel fashion (3 to 4 cuts approximately 1/3 to 1/2 into the lens) and immediately used for cytotoxicity testing. If the lens is not cut, it shall be placed on the cells in a concave manner. Each lens is placed in the centre on the cell surface in a 60 mm diameter tissue culture plate containing subconfluent monolayer of L-929 cells in 1,6 ml Minimal Essential Medium supplemented with 5 % serum (MEM).

Similarly, negative and positive controls are placed in the designated 60 mm diameter tissue culture plates containing subconfluent monolayer of L-929 cells in 1,6 ml Minimal Essential Medium supplemented with 5 % serum (MEM).

The tissue culture plates are incubated at 37 °C ± 1 °C in 5 % ± 1 % CO_2 for 24 h ± 2 h.

Following incubation, the lenses and the controls are removed from each plate and the cells are stained with Trypan Blue to facilitate observation of dead or damaged cells. The cytotoxicity is assessed by evaluating the cells macroscopically and microscopically (100×) for any abnormal cell morphology and lysis around the test article and controls to determine the zone of lysis (if any).

5.2.2 Material

5.2.2.1 Cell line

L-929 cells (NCTC clone 929: CCL 1, American Type Culture Collection [ATCC], Manassas, VA, USA; ECACC No. 88102702, European Collection of Cell Cultures, Salisbury, Wiltshire SP4 0JG, UK). Cell cultures shall be free of mycoplasma.

The passage number of the cells for testing should be 10 – 30.

5.2.2.2 Technical equipment

5.2.2.2.1 Incubator, 37 °C ± 1 °C, humidified, 5 % CO_2/air.

5.2.2.2.2 Laminar flow cabinet, standard: "biological hazard".

5.2.2.2.3 Water bath, 37 °C.

5.2.2.2.4 Inverse phase contrast microscope.

5.2.2.2.5 Laboratory burner.

5.2.2.2.6 Centrifuge.

5.2.2.2.7 Laboratory balance.

5.2.2.2.8 Cell counter or hemocytometer.

5.2.2.2.9 Tissue culture flasks and 60 mm diameter tissue culture petri dishes.

5.2.2.2.10 Pipetting aid.

5.2.2.2.11 Pipettes.

5.2.2.3 Chemicals, media, and sera

5.2.2.3.1 Eagle minimal essential medium.

5.2.2.3.2 Fetal bovine serum (FBS).

5.2.2.3.3 Trypsin/EDTA solution.

5.2.2.3.4 Dulbecco’s Phosphate Buffered Saline with Ca^{2+} and Mg^{2+} (DPBS).

5.2.2.3.5 Penicillin/streptomycin solution.

5.2.2.3.6 Trypan Blue

5.2.3 Preparation of test sample

The contact lenses should be handled aseptically with forceps. Each lens is individually soaked in ~10 ml of appropriate contact lens solution in a sterile compatible container for 24 h ± 2 h with gentle stirring (continual agitation on a shaker at ~50 rpm) at room temperature. Aseptic procedure should be followed.

Each lens may be cut in a pinwheel fashion (3 to 4 cuts approximately 1/3 to 1/2 into the lens) immediately following the 24 h soak period. Both sides of the lens are then tapped gently on a sterile gauze to remove excess fluid and used for cytotoxicity testing immediately. If the lens is not cut, it shall be placed on the cells in a concave manner. The cytotoxicity test method is described in 5.2.4.3.

5.2.4 Methods

5.2.4.1 General

The cells should be maintained and cultured using the routine cell culture methods.

5.2.4.2 Quality check of the assay: Positive, negative, and lens controls

5.2.4.2.1 General

Positive control, negative control, and lens control shall be included in each test.

5.2.4.2.2 Positive control

Latex glove is recommended as a positive control. A 1 cm × 1 cm portion shall be placed on the cells in each designated positive control tissue culture plate for testing. Other validated positive control may be used.

5.2.4.2.3 Negative control

High density polyethylene (HDPE) (0,5 mm thickness) is recommended as a negative control. A 1 cm × 1 cm portion shall be placed on the cells in each designated negative control tissue culture plate for testing. Other validated negative control may be used.

5.2.4.2.4 Lens control

The test contact lens soaked in Dulbecco's Phosphate Buffered Saline with Ca^{2+} and Mg^{2+} (DPBS) shall be used as a lens control. The contact lenses should be handled aseptically with forceps. Each lens is individually soaked in ~10 ml of DPBS solution in a sterile compatible container for 24 h ± 2 h with gentle stirring (continual agitation on a shaker at ~50 rpm) at room temperature. Aseptic procedure should be followed.

Each lens may be cut in a pinwheel fashion (3 to 4 cuts approximately 1/3 to 1/2 into the lens) immediately following the 24 h ± 2 h soak period. Both sides of the lens are then tapped gently on a sterile gauze to remove excess fluid and used for cytotoxicity testing immediately. If the lens is not cut, it shall be placed on the cells in a concave manner. The cytotoxicity test method is described in 5.2.4.3.

5.2.4.2.5 Test acceptance criteria

For a test to be considered valid, the following test acceptance criteria shall be met:

a) the negative control shall have grades of ≤ 1 in all four wells

b) the lens control shall have grades of ≤ 1 in all four wells

c) the positive control shall have grades of ≥ 3 in all four wells.

5.2.4.3 Test procedure

5.2.4.3.1 Seed the L-929 cells at a density of ~6 × 105 cells per plate in the 60 mm diameter tissue culture plates in 6 ml of Minimal Essential Medium supplemented with 5 % fetal bovine serum (MEM) and incubate at 37 °C ± 1 °C in 5 % ± 1 % CO_2 for approximately 24 h to obtain subconfluent monolayers of cells prior to use. If antibiotics are used in the MEM medium, it should be documented in the worksheet.

5.2.4.3.2 Verify the subconfluency (~80 %) and morphology of the cultures microscopically (100×) before starting the test. Four cultures (i.e. four 60 mm plates with the cells) shall be used for each test and control article. Only a single test/control article section shall be placed in each plate.

5.2.4.3.3 Discard the medium in each plate and replace with 1,6 ml of MEM.

5.2.4.3.4 Place the test/control article in the centre on the cell surface in the designated plates.

Place the lens which has been soaked in ~10 ml of contact lens solution for 24 h ± 2 h and may have been cut in a pinwheel fashion as described in 5.2.3 in the centre on the cell surface in each of four 60 mm "Test article" tissue culture plates.

Place a 1 cm × 1 cm portion of latex (positive control) in the centre on the cell surface in each of four 60 mm "positive control" tissue culture plates.

Place a 1 cm × 1 cm HDPE (negative control) in the centre on the cell surface in each of four 60 mm "negative control" tissue culture plates.

Place the lens which has been soaked in ~10 ml of DPBS solution for 24 h ± 2 h and may have been cut in a pinwheel fashion as described in 5.2.4.2.4 in the centre on the cell surface in each of four 60 mm "Lens control" tissue culture plates.

To aid in assessing the movement of the test/control article, the location of the test/control article should be marked on the bottom of each plate with a dot in the approximate centre of the test/control article location.

5.2.4.3.5 Incubate the plates at 37 °C ± 1 °C in 5 % ± 1 % CO_2 for 24 h ± 2 h.

Extreme care should be taken to minimize the movement of the lens during handling because it can cause physical trauma to the cells. Also, if the lens does not stay in place, it would be difficult to accurately measure the reactivity zone around the lens.

5.2.4.3.6 Following incubation, remove the lenses and the controls from each plate and replace the medium in each plate with 1,6 ml of 0,4 % Trypan Blue in DPBS to stain dead cells.

To facilitate the measurement of the zone of lysis beyond the specimen, the location of the edges of the test and control articles should be marked on the bottom of the plates prior to removal of the test and control articles from the plates.

5.2.4.3.7 Expose the cells to Trypan Blue solution for approximately 2 min at room temperature.

5.2.4.3.8 Remove the Trypan Blue solution and rinse the cells with 1,6 ml DPBS.

5.2.4.3.9 Examine the cells macroscopically and microscopically (100×) for any abnormal cell morphology and lysis around the test article and controls to determine the zone of lysis (if any). Trypan blue facilitates observation of dead or damaged cells; membrane damage and dead cells allow uptake of Trypan blue since it is an exclusion dye. Cells which are dead or have damaged membranes will appear blue compared to the cells on the negative control and lens control plates.

5.2.4.3.10 Assess the cytotoxicity using the criteria described in Table 1. It is considered a cytotoxic effect if a grade greater than 2 is observed. For guidance on how to measure zone of cell lysis, see Annex A.

Table 1 — Reactivity grades

Grade	Reactivity	Conditions of all cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen
3	Moderate	Zone extending beyond the specimen, which may extend up to 10 mm beyond the specimen
4	Severe	Zone extending greater than 10 mm beyond specimen

6 Assessment of results

The overall assessment of the results shall be carried out by a person capable of making informed decisions based on the test data. Any cytotoxic effect can be of concern. However, it is primarily an indication of potential for in vivo toxicity and the device cannot necessarily be determined to be unsuitable for a given clinical application based solely on cytotoxicity data. Cytotoxicity data shall be assessed in relation to other biocompatibility data and the intended use of the product.