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

Optique ophtalmique — Lentilles de contact et produits d’entretien des lentilles de contact — Essais de cytotoxicité des lentilles de contact en association avec une solution d’entretien des lentilles de contact pour évaluer les interactions solution/lentille
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foreword</td>
<td>iv</td>
</tr>
<tr>
<td>1 Scope</td>
<td>1</td>
</tr>
<tr>
<td>2 Normative references</td>
<td>1</td>
</tr>
<tr>
<td>3 Terms and definitions</td>
<td>1</td>
</tr>
<tr>
<td>4 Principle</td>
<td>1</td>
</tr>
<tr>
<td>5 Direct contact cytotoxicity test for lens/lens care solution combination</td>
<td>1</td>
</tr>
<tr>
<td>5.1 General</td>
<td>1</td>
</tr>
<tr>
<td>5.2 Experimental procedure</td>
<td>2</td>
</tr>
<tr>
<td>5.2.1 Basic procedure</td>
<td>2</td>
</tr>
<tr>
<td>5.2.2 Material</td>
<td>2</td>
</tr>
<tr>
<td>5.2.3 Preparation of test sample</td>
<td>3</td>
</tr>
<tr>
<td>5.2.4 Methods</td>
<td>3</td>
</tr>
<tr>
<td>6 Assessment of results</td>
<td>5</td>
</tr>
<tr>
<td>7 Test report</td>
<td>5</td>
</tr>
<tr>
<td>Annex A (normative) Measurement of zone of cell lysis for the direct contact cytotoxicity test method for testing contact lens in combination with lens care solution</td>
<td>7</td>
</tr>
<tr>
<td>Bibliography</td>
<td>9</td>
</tr>
</tbody>
</table>
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 (see 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 (see 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.

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 World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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 contact lens care products — Cytotoxicity testing of contact lenses 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 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 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 contact 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 Dulbecco's Phosphate Buffered Saline with Ca²⁺ and Mg²⁺ (DBPS)-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 International Standard, 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 (MEM) supplemented with 5 % fetal bovine serum (FBS).

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 MEM supplemented with 5 % FBS.

The tissue culture plates are incubated at 37 °C ± 1 °C in 5 % ± 1 % CO₂ 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 or equivalent, 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 % ± 1 % CO₂/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.