INTERNATIONAL STANDARD

ISO 9394

Second edition
1998-08-15

Ophthalmic optics — Contact lenses and contact lens care products — Determination of biocompatibility by ocular study with rabbit eyes

Optique ophtalmique — Lentilles de contact et produits d'entretien pour lentilles de contact — Détermination de la biocompatibilité par évaluation de la tolérance oculaire chez le lapin

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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.

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.

International Standard ISO 9394 was prepared by Technical Committee ISO/TC 172, *Optics and optical instruments*, Subcommittee SC 7, *Ophthalmic optics and instruments*.

This second edition cancels and replaces the first edition (ISO 9394:1994), which has been technically revised.

Annexes A and B form an integral part of this International Standard. Annex C is for information only.

Introduction

The ocular tissue of the rabbit is the system traditionally used to evaluate the irritant properties of materials which come in contact with ocular tissue.

The use of the device under evaluation is governed by the nature, degree, duration, frequency and conditions of exposure of humans to the device in normal intended use.

It is incumbent upon the investigator to conduct such evaluations using good scientific laboratory practices, complying with regulations related to animal welfare and the general principles set forth in the normative references.

ISO 10993-1 is the basic horizontal International Standard for biological evaluation of medical devices, and serves as a framework for planning biological evaluation tests.

ISO 10993-10 assesses possible contact hazards from device-released chemicals that may produce skin and mucosal irritation, eye irritation and delayed contact sensitization.

Usage tests for specific devices are defined in vertical standards. This International Standard describes one of several specific usage tests for contact lenses and contact lens care products.

The existence of this International Standards does not imply that rabbit-eye testing is a requirement in the determination of biocompatibility of contact lenses and contact lens care products, nor that this test is sufficient by itself to determine the biocompatibility of contact lenses and contact lens care products. Taking into consideration animal welfare requirements (ISO 10993-2:1992), it is recommended that this *in vivo* test be carried out after obtaining data of *in vitro* toxicological testing such as described in ISO 9363-1, ISO 10340 and ISO 11986.

Testing by ocular study with rabbit eyes is to be regarded as a "disaster check" before entering human trials; it might be useful in certain situations (e.g. testing of new materials), but will not be required in many cases.

Care should be taken when extrapolating the test results to the human eye.

Ophthalmic optics — Contact lenses and contact lens care products — Determination of biocompatibility by ocular study with rabbit eyes

1 Scope

This International Standard specifies an *in vivo* method of test to assess the ocular safety of contact lenses and contact lens care products. The test assesses the degree of irritation to the ocular tissue produced by the device under test. The test method is described in application to rabbit eyes.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 8321-1:1991, *Optics and optical instruments — Contact lenses — Part 1: Specification for rigid corneal and scleral contact lenses.*

ISO 8321-2:—[1)], *Optics and optical instruments — Contact lenses — Part 2: Specification for single-vision hydrogel contact lenses.*

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

ISO 10993-2:1992, *Biological evaluation of medical devices — Part 2: Animal welfare requirements.*

ISO 10993-10:1995, *Biological evaluation of medical devices — Part 10: Tests for irritation and sensitization.*

ISO/IEC Guide 25:1990, *General requirements for the competence of calibration and testing laboratories.*

3 General requirements

The general principles for biological evaluation and categorization of medical devices given in ISO 10993-1 shall apply. Tests shall be performed in accordance with ISO/IEC Guide 25.

Tests for irritation and sensitization of contact lenses and contact lens care products shall be carried out in accordance with ISO 10993-10.

The assessment of the results shall be carried out by appropriately experienced and competent personnel .

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1) To be published.

4 Animals and husbandry

4.1 New Zealand white strain rabbits (male, female, or mixed sexes) shall be used to test each type of contact lens or contact lens care product. They shall be healthy young adults from a single strain from a single recognized source weighing >2,5 kg. They shall have eyes free from clinically significant ocular irritation or corneal retention of fluorescein stain.

A minimum number of three rabbits shall be used, however a number of six is recommended to ensure an acceptable level of precision of the test results. If less than six rabbits are used, then the quantity shall be justified.

If control articles are included in the evaluation, use the contralateral eye or an additional group of animals with the same number of animals chosen as before for each control article. For contact lens care products, the control group should use the same type of contact lens which has not been treated with the test product.

Positive controls shall not be used.

NOTE In this context, "control article“ should be interpreted as being a device with defined safety and performance characteristics.

4.2 The animal welfare requirements set out in ISO 10993-2 shall be met.

4.3 The animals shall be housed individually and have free access to commercially pelted rabbit feed and tap water. Group housing is not feasible in this test since any lens found expulsed from the eye shall be matched to the specific rabbit which wore the lens and re-inserted into the same eye.

4.4 Each animal shall be identified by one of the following:

a) a numbered ear tag;

b) a tattoo;

c) a microchip; or

d) a permanent ink marking.

The animals shall be acclimatized to the laboratory conditions for at least 5 days prior to testing.

4.5 The nictitating membrane should not be removed from the rabbit’s eye, and the eyelids should not be sutured during lens wear.

NOTE 1 The albino rabbit eye is free of pigment, easily examined and has historically been used for ocular irritation studies.

NOTE 2 If the nictitating membranes are excised from the eyes of the rabbit, this should be done at least two weeks before the experiment. Such treatment shall be mentioned in the final report.

All appropriate regulatory requirements governing the care and use of animals shall be followed.

4.6 During daily treatment, the rabbits shall be minimally restrained.

5 Reagents/Materials

5.1 Sodium fluorescein, as specified by an appropriate pharmacopoeia.

NOTE Attention should be made to the degree of staining and the concentration of fluorescein administered to the eye (e.g. 3 µl of 1 % fluorescein in saline solution).

5.2 Contact lens care products, as recommended by the manufacturers.

5.3 Contact lenses, as recommended by the manufacturer.

6 Apparatus

6.1 Slit lamp microscope, with appropriate filters.

6.2 Magnifying glass, of minimum magnification 6x.

6.3 Balance or weighing machine, capable of weighing up to 5 kg to an accuracy of 100 g.

7 Test specimens

7.1 Contact lens parameters

Contact lenses shall be sufficiently thick to represent either

a) reasonable human-use extremes; or

b) the extreme of the manufacturer's product line.

The contact lens selected shall produce a good fit to a rabbit eye.

NOTE This is necessary to minimize physical irritation and expulsion. In the case where this thickness does not allow a good fit of the contact lens, a contact lens of the greatest thickness which allows a good fit should be used.

Contact lens parameters shall be recorded according to the tolerances specified in either ISO 8321-1 or ISO 8321-2.

7.2 Preparation and storage

If contact lens care products are to be used in the evaluation, contact lenses shall be prepared, cleaned, disinfected, stored and rinsed according to the contact lens manufacturer's instructions using contact lens care products (5.2). If a contact lens falls out during the daily treatment period, it shall be rinsed with rinsing solution (5.2) and re-inserted into the rabbit's eye from which it has fallen out.

NOTE 1 Sufficient additional lenses should be treated using at least one complete daily lens care treatment to replace any lenses that are damaged or lost during the lens-wear day.

NOTE 2 Hydrogel lenses which cannot be immediately reinserted because of drying should be swapped for a similar lens which has been treated in line with the manufacturer's recommendations. Hydrogel lenses which have dried out may be re-used once cleaned and/or rehydrated.

Before insertion, contact lenses should be checked for particulate matter, physical damage and, during hydrogel lens use, for lens inversion. While inserting contact lenses, rabbits shall be observed for reactions different to that during the insertion of a control lens. Such reactions shall be recorded.

Contact lenses shall not be intermixed between rabbits in the same treatment group.

If applicable, lens storage cases shall not be intermixed between treatment groups.

8 Test procedure

8.1 Preliminary examination of animals

8.1.1 The preliminary examination may not be made longer than 24 h before commencement of the test.

8.1.2 Using the balance (6.3), weigh the rabbits and record the mass.

8.1.3 Visually examine both eyes of each rabbit using the slit lamp (6.1) and fluorescein stain (5.1), and record the state of the eyes using the McDonald-Shadduck scoring system (see annex A).

If either eye shows any abnormality, then replace the rabbit.

8.2 Insertion and removal of test lens

8.2.1 Treat the test lens in accordance with 7.2.

8.2.2 Insert the test lens in one eye of the rabbit; the eye should be free of fluorescein at the time of lens insertion. The test lens may be inserted in either eye, although it is recommended that within a test laboratory all testing be carried out on the same side. The contralateral eye serves as either a treated or an untreated control.

NOTE In the case of hydrogel lenses, the lid may be loosely taped near the outer canthus to prevent expulsion of the lens.

8.2.3 On days 1 to 21, after 7 h to 8 h, remove the test lens from the rabbit's eye. After removal, lenses shall be cared for as in step 7.2.

NOTE Designate the first day of lens wear as Day 1.

8.2.4 If, during the course of the day's wearing, a lens requires reinsertion or replacement, this fact shall be recorded. It is recommended that the presence of the lens in the rabbit's eye is checked regularly, e.g. hourly.

8.2.5 Whenever relevant, record any change in the appearance of the contact lens.

8.2.6 Repeat steps 8.2.1 to 8.2.5 on a daily basis.

8.2.7 On day 22, after 4 h to 8 h, remove the test lens from the rabbit's eye.

NOTE The lens may be retained for further examination by the manufacturer.

8.3 Examination of the rabbit's eye

8.3.1 On days 1 to 7, 9 to 14 and 16 to 21, just prior to lens removal visually examine both eyes of each rabbit and record the state of the eyes using the Draize scoring system (see annex B).

Additional visual examinations of the eyes may be conducted, e.g. twice daily, so that if a reaction is found, an early endpoint can be instituted. It is recommended to also record the behaviour of the rabbits. Scratching or pawing at the lens would be an early sign that it is irritant.

8.3.2 On days 8, 15 and 22 after lens removal, visually examine both eyes of each rabbit using the slit lamp (6.1) and fluorescein stain (5.1) and record the state of the eyes using the McDonald-Shadduck scoring system (see annex A).

8.4 Weighing of animals

On day 22, using the balance (6.3), weigh the rabbits and record the mass.

8.5 Histological examination

8.5.1 On day 22, after the lens has been removed and the clinical examination has been completed, the animal should be humanely killed.

8.5.2 Excise the eyes and adnexa and preserve in a suitable fixation solution (e.g. 10 % neutral buffered formalin, Zenker's acetic fixative or Davidson's solution).

8.5.3 Embed the eye and adnexa in paraffin wax.

8.5.4 Section the cornea, conjunctivae, iris and lens of each eye and stain for microscopic evaluation.

Evaluation of the rabbit's eyes and examination of histological sections shall be conducted by appropriately experienced and competent personnel.

8.5.5 Examine the histological sections and record the findings.

8.6 Corneal metabolism

If appropriate, determine effects on corneal metabolism using appropriate chemical or physical methods, taking into account the current state of the art.

NOTE If corneal metabolism is to be evaluated, an additional two rabbits for each test article or control group should be used.

9 Test report

The study report shall be prepared in accordance with ISO/IEC Guide 25 and good laboratory practices (GLP).

10 Assessment of results

10.1 The overall assessment of the test results shall be carried out by appropriately experienced and competent personnel taking into consideration all information in the test report.

10.2 If the appropriately experienced and competent personnel considers the results to be either inconclusive or invalid, consideration shall be given to repeating the test.

10.3 The results of the assessment shall be recorded in the test report.