TC 172

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Optics and optical instruments — Determination of biological compatibility of contact lens material — Testing of the contact lens system by ocular study with rabbit eyes

Optique et instruments d'optique — Détermination de la compatibilité biologique des matériaux des lentilles de contact — Essai du système des lentilles de contact par évaluation de la tolérance oculaire chez le lapin

Reference number
ISO 9394:1994(E)

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, endoscopic, metrological instruments and test methods.*

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

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Introduction

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

Optics and optical instruments — Determination of biological compatibility of contact lens material — Testing of the contact lens system 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. The method may be adapted to assess contact lens care products.

The assessment of the results should be carried out by an appropriately qualified toxicologist.

NOTE 1 Attention is drawn to ISO 10993-1, regarding biological testing and ISO 10993-2 regarding animal welfare.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the edition indicated was 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 edition of the standard 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.*

3 Animals and husbandry

3.1 A minimum of six New Zealand white strain rabbits shall be used to test each type of contact lens. They shall be healthy adults of either sex weighing between 2,5 kg and 3,5 kg. They shall have eyes free of clinically significant ocular irritation. They shall have eyes free from corneal retention of fluorescein stain. If corneal metabolism (see 7.6) is to be evaluated, an additional two rabbits shall be used.

3.2 The animals shall be housed individually and have free access to commercially pelleted rabbit feed and tap water.

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

a) a numbered ear tag;

b) a tattoo; or

c) a microchip.

The animals shall be acclimatized in the individual cages in the animal laboratory for at least seven days prior to testing.

3.4 The nictitating membrane should not be removed from the rabbits' eye.

NOTES

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

3 If the nictitating membranes are excised from the eyes of the rabbits this should be done at least two weeks before the experiment.

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

4 Reagents

4.1 Fluorescein stain.

4.2 Contact lens care solutions, as recommended by the manufacturers.

5 Apparatus

5.1 Slit lamp.

5.2 Magnifying glass, of minimum magnification 6 x.

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

6 Test specimens

6.1 Lens parameters

Contact lens shall be sufficiently thick to represent either

a) reasonable human use extremes; or

b) the extreme of the manufacturers product line.

Lens parameters shall be recorded according to the tolerances specified in ISO 8321-1.

NOTE 4 The lens selected should produce a good fit to the rabbit eye, in order to minimize physical irritation and expulsion.

6.2 Preparation and storage regime

Lenses shall be prepared, cleaned, disinfected, stored and rinsed according to the lens manufacturer's instructions using contact lens care solutions (4.2). If a lens falls out during the daily treatment period it shall be rinsed with rinsing solution (4.2) and reinserted into the rabbit's eye.

NOTES

5 Additional lenses should be treated to the appropriate lens care treatment on a daily basis to replace any lenses that are damaged or lost during the lens wear day.

6 Soft 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.

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

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

7 Test procedure

7.1 Preliminary examination of animals

7.1.1 Using the balance (5.3) weigh the rabbits and record the mass.

7.1.2 Visually examine the rabbit's eyes using the slit lamp (5.1) and fluorescein stain (4.1) and record the state of the eyes using the McDonald-Shadduck scoring system (see annex B).

7.1.3 Visually examine the rabbit's eyes macroscopically and record the state of the eyes using the Draize scoring system (see annex A).

7.2 Insertion and removal of test lens

7.2.1 Treat the test lens in accordance with 6.2.

7.2.2 Visually examine the rabbit's eyes macroscopically using the Draize scoring system (annex A) and record the findings.

7.2.3 Insert the test lens in one eye of the rabbit.

NOTES

7 The 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.

8 In the case of soft lenses, the lid may be loosely taped near the temporal canthus to prevent expulsion of the lens.

9 If control lenses are used, additional animals should be used.

7.2.4 On days 1 to 21, after 7 $^{+1}_{0}$ h, remove the test lens from the rabbit's eye. Before removal of the lens examine the rabbit's eyes macroscopically as described in 7.2.2.

7.2.5 If, during the course of the days wearing, a lens requires reinsertion or replacement this fact shall be recorded.

7.2.6 Record any change in the appearance of the contact lens.

7.2.7 Repeat steps 7.2.1 to 7.2.6 on a daily basis.

7.2.8 On day 22, after 4 $^{+4}_{0}$ h, remove the test lens from the rabbit's eye.

NOTE 10 The lens may be retained for further examination by the manufacturer.

7.3 Examination of the rabbit's eye

7.3.1 Each day just prior to lens removal visually examine the rabbit's eye and record the state of the eyes using the Draize scoring system (see annex A).

NOTE 11 Lenses may be removed prior to examination if debris on the lenses obscures the observations. Removal of the lenses prior to examination may result in increased scores due to irritation of the eyes during lens removal.

7.3.2 On days 8, 15 and 22 after lens removal visually examine the rabbit's eyes using the slip lamp (5.1) and fluorescein stain (4.1) and record the state of the eyes using the McDonald-Shadduck scoring system (see annex B).

7.4 Weighing of animals

On days 8, 15, 22 using the balance (5.3) weigh the rabbits and record the mass.

7.5 Histological examination

7.5.1 After the lens has been removed and the clinical examination has been completed, the animal should be humanely killed.

7.5.2 Excise the eyes and preserve in fixation solution.

NOTE 12 Suitable fixative solutions are 10 % neutral buffered formalin, Zenker's acetic fixative or Davidson's solution.

7.5.3 Embed the eye in paraffin wax.

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

7.5.5 Examine the histological sections and record the findings.

7.6 Corneal metabolism

If appropriate, determine effects on corneal metabolism (see 3.1) using appropriate chemical or physical methods.

8 Test report

8.1 The results shall be recorded in a test report which includes a complete record of all procedures followed and any other relevant data necessary for the assessment of results as described in clause 9.

8.2 If more than the minimum number of animals complete the test, all shall be considered as part of the test.

9 Assessment of results

9.1 The overall assessment of the test results shall be carried out by a toxicologist, taking into consideration all information in the test report.

9.2 If the toxicologist considers the results to be either inconclusive or invalid, consideration shall be given to repeating the test.

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

Annex A
(normative)

Draize scale for scoring ocular lesions

(1) Cornea

(A) Opacity-degree of density (area most dense taken for reading):

No opacity: 0

Scattered or diffuse area, details of iris clearly visible: 1

Easily discernible translucent areas, details of iris slightly obscured: 2

Opalescent areas, no details of iris visible, size of pupil barely discernible: 3

Opaque, iris invisible: 4

(B) Area of cornea involved:

One quarter (or less) but not zero: 1

Greater than one quarter, but less than half: 2

Greater than half, but less than three quarters: 3

Greater than three quarters, up to whole area: 4

Score equals A × B × 5 Maximum = 80

(2) Iris

(A) Values:

Normal: 0

Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive): 1

No reaction to light, hemorrhage, gross destruction (any or all of these): 2

Score equals A × 5 Maximum = 10

(3) Conjunctivae

(A) Redness (refer to palpebral and bulbar conjunctivae excluding cornea and iris):

Vessels normal: 0

Vessels definitely injected above normal: 1

More diffuse, deeper crimson red, individual vessels not easily discernible: 2

Diffuse beefy red: 3

(B) Chemosis:

No swelling: 0

Any swelling above normal (includes nictitating membrane): 1

Obvious swelling with partial eversion of lids: 2

Swelling with lids about half closed: 3

Swelling with lids half closed to completely closed: 4

(C) Discharge:

No discharge: 0

Any amount different from normal (does not include small amounts observed in inner canthus of normal animals): 1

Discharge with moistening of the lids and hairs adjacent to lids: 2

Discharge with moistening of the lids and hairs, and considerable area around the eye: 3

Score equals (A + B + C) × 2 Maximum = 20

The maximum total score is the sum of all scores obtained for the cornea, iris, and conjunctivae. Total maximum score possible = 110 per eye.