TC 172

INTERNATIONAL STANDARD

ISO 9394

First edition
1994-11-15

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.

International Organization for Standardization
Case Postale 56 • CH-1211 Genève 20 • Switzerland

Printed in Switzerland

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.

Annex B
(normative)

McDonald-Shadduck score system — Slit lamp

Conjunctival congestion

0 = Normal. May appear blanched to reddish pink without perilimbal injection (except at 12:00 and 6:00 o'clock positions) with vessels of the palpebral and bulbar conjunctiva easily observed.

+ 1 = A flushed, reddish colour predominantly confined to the palpebral conjunctival with some perilimbal injection but primarily confined to the lower and upper parts of the eye from the 4:00 and 7:00 and 11:00 to 1:00 o'clock positions.

+ 2 = Bright red colour of the palpebral conjunctiva with accompanying perilimbal injection covering at least 75 % of the circumference of the perilimbal region.

+ 3 = Dark, beefy red colour with congestion of both the bulbar and the palpebral conjunctiva along with pronounced perilimbal injection and the presence of petechia on the conjunctiva. The petechia generally predominate along the nictitating membrane.

Conjunctival swelling

0 = Normal or no swelling of the conjunctival tissue.

+ 1 = Swelling above normal without eversion of the lids (can be easily ascertained by noting that the upper and lower eyelids are positioned as in the normal eye), swelling generally starts in the lower cul-de-sac near the inner canthus, which needs slit-lamp examination.

+ 2 = Swelling with misalignment of the normal approximation of the lower and upper eyelids; primarily confined to the upper eyelid so that in the initial stages the misapproximation of the eyelids begins by partial eversion of the upper eyelid. In this stage, swelling is confined generally to the upper eyelid, although it exists in the lower cul-de-sac (observed best with the slit-lamp).

+ 3 = Swelling definite with partial eversion of the upper and lower eyelids essentially equivalent. This can be easily ascertained by looking at the animal head-on and noticing the positioning of the eyelids; if the eye margins do not meet, eversion has occurred.

+ 4 = Eversion of the upper eyelid is pronounced with less pronounced eversion of the lower eyelid. It is difficult to retract the lids and observe the perilimbal region.

Conjunctival discharge

Discharge is defined as a whitish, gray precipitate, which should not be confused with the small amount of clear, inspissated, mucoid material that can be formed in the medial canthus of a substantial number of rabbit eyes.

0 = Normal. No discharge.

+ 1 = Discharge above normal and present on the inner portion of the eye but not on the lids or hairs of the eyelids. One can ignore the small amount that is in the inner and outer canthus.

+ 2 = Discharge is abundant, easily observed, and has collected on the lids and around the hairs of the eyelids.

+ 3 = Discharge has been flowing over the eyelids so as to wet the hairs substantially on the skin around the eye.

Aqueous flare

The intensity of the Tyndall phenomenon is scored by comparing the normal Tyndall effect observed when the slit-lamp beam passes through the lens with that seen in the anterior chamber. The presence of aqueous flare is presumptive evidence of breakdown of the blood-aqueous barrier.

0 = The absence of visible light beam light in the anterior chamber (no Tyndall effect).

+ 1 = The Tyndall effect is barely discernible. The intensity of the light beam in the anterior chamber is less than the intensity of the slit beam as it passes through the lens.

+ 2 = The Tyndall beam in the anterior chamber is easily discernible and is equal in intensity to the slit beam as it passes through the lens.

+ 3 = The Tyndall beam in the anterior chamber is easily discernible; its intensity is greater than the intensity of the slit beam as it passes through the lens.

Iris involvement

In the following definitions the primary, secondary, and tertiary vessels are utilized as an aid to determining a subjective ocular score for iris involvement. The assumption is made that the greater the hyperemia of the vessels and the more the secondary and tertiary vessels are involved, the greater the intensity of iris involvement. The scores range from 0 to + 4.

0 = Normal iris without any hyperemia of the iris vessels. Occasionally around the 12:00 to 1:00 o'clock position near the pupillary border and the 6:00 and 7:00 o'clock position near the pupillary border there is a small area around 1 mm to 3 mm in diameter in which both the secondary and tertiary vessels are slightly hyperamic.

+ 1 = Minimal injection of secondary vessels but not tertiary. Generally, it is uniform, but may be of greater intensity at the 1:00 or 6:00 o'clock position. If it is confined to the 1:00 or 6:00 o'clock position, the tertiary vessels must be substantially hyperamic.

+ 2 = Minimal injection of tertiary vessels and minimal to moderate injection of the secondary vessels.

+ 3 = Moderate injection of the secondary and tertiary vessels with slight swelling of the iris stroma (this gives the iris surface a slightly rugose appearance, which is usually most prominent near the 3:00 and 9:00 o'clock positions).

+ 4 = Marked injection of the secondary and tertiary vessels with marked swelling of the iris stroma. The iris appears rugose; may be accompanied by hemorrhage (hyphema) in the anterior chamber.

Cornea

The scoring scheme measures the severity of corneal cloudiness and the area of the cornea involved. Severity of corneal cloudiness is graded as follows:

0 = Normal cornea. Appears with the slit-lamp as having a bright gray line on the epithelial surface and a bright gray line on the endothelial surface with a marblelike gray appearance of the stroma.

+ 1 = Some loss of transparency. Only the anterior half of the stroma is involved as observed with an optical section of the slit-lamp. The underlying structures are clearly visible with diffuse illumination, although some cloudiness can be readily apparent with diffuse illumination.

+ 2 = Moderate loss of transparency. In addition to involving the anterior stroma, the cloudiness extends all the way to the endothelium. The stroma has lost its marblelike appearance and is homogeneously white. With diffuse illumination, underlying structures are clearly visible.

+ 3 = Involvement of the entire thickness of the stroma. With optical section, the endothelial surface is still visible. However, with diffuse illumination the underlying structures are just barely visible (to the extent that the observer is still able to grade flare, iritis, observe for pupillary response, and note lenticular changes).

+ 4 = Involvement of the entire thickness of the stroma. With the optical section, cannot clearly visualize the endothelium. With diffuse illumination, the underlying structures cannot be seen. Cloudiness removes the capability for judging and grading aqueous flare, iritis, lenticular changes, and pupillary response.

The surface area of the cornea relative to the area of cloudiness is divided into five grades from 0 to + 4.

0 = Normal cornea with no area of cloudiness.
+ 1 = 1 % to 25 % area of stromal cloudiness.
+ 2 = 26 % to 50 % area of stromal cloudiness.
+ 3 = 51 % to 75 % area of stromal cloudiness.
+ 4 = 76 % to 100 % area of stromal cloudiness.

Pannus is vascularization or the penetration of new blood vessels into the corneal stroma. The vessels are derived from the limbal vascular loops. Pannus is divided into three grades.

0 = No pannus.
+ 1 = Vascularization is present but vessels have not invaded the entire corneal circumference. Where localized vessel invasion has occurred, they have not penetrated beyond 2 mm.
+ 2 = Vessels have invaded 2 mm or more around the entire corneal circumference.

The use of fluorescein is a valuable aid in defining epithelial damage. The area can be judged on a 0 to + 4 scale using the same terminology as for corneal cloudiness.

0 = Absence of fluorescein staining.
+ 1 = Slight fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are easily visible. (The outline of the pupillary margin is as if there were no fluorescein staining.)
+ 2 = Moderate fluorescein staining confined to a small focus. With diffuse illumination the underlying structures are clearly visible, although there is some loss of detail.
+ 3 = Marked fluorescein staining. Staining may involve a larger portion of the cornea. With diffuse illumination underlying structures are barely visible but are not completely obliterated.
+ 4 = Extreme fluorescein staining. With diffuse illumination the underlying structures cannot be observed.