

INTERNATIONAL
STANDARD

ISO
9363-1

First edition
1994-11-15

Optics and optical instruments — Contact lenses — Determination of cytotoxicity of contact lens material —

Part 1:

Agar overlay test and growth inhibition test

Optique et instruments d'optique — Lentilles de contact — Détermination de la cytotoxicité des matériaux des lentilles de contact —

Partie 1: Essai de recouvrement par de l'agar-agar et essai d'inhibition de croissance



Reference number
ISO 9363-1:1994(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 9363-1 was prepared by Technical Committee ISO/TC 172, *Optics and optical instruments*, Subcommittee SC 7, *Ophthalmic, endoscopic, metrological instruments and test methods*.

Annex A forms an integral part of this part of ISO 9363. Annex B is for information only.

© ISO 1994

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from the publisher.

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

Printed in Switzerland

Optics and optical instruments — Contact lenses — Determination of cytotoxicity of contact lens material —

Part 1:

Agar overlay test and growth inhibition test

1 Scope

This part of ISO 9363 specifies two *in vitro* methods for determining the cytotoxicity of contact lens materials:

- the agar overlay test; and
- the growth inhibition test.

The primary purpose of these tests is to reveal the presence of leachable cytotoxic substances in or on contact lenses.

NOTES

1 Attention is drawn to ISO 10993-5.

2 A minimum of one *in vitro* test is recommended for preclinical evaluation of new types of contact lenses. Either one of the following two *in vitro* tests may be used for the *in vitro* requirement.

2 Principle

The proposed tests are designed to ascertain the absence of extractable cytotoxic substances.

The agar overlay test is designed to assess the presence of leachable toxic substances in solid materials. The test sample is placed in contact with the surface of an agar layer which covers a monolayer of cells treated with a vital stain. After 24 h of incubation, the presence of leachable toxic substances is manifested by the loss of dye from the cells within the diffusion zone of the soluble substance(s) leaching from the sample and by lysis of the cells within the zone if the concentrations and toxicity of the diffusing substance(s) are sufficiently high.

The growth inhibition test is designed to ascertain the presence of extractable cytotoxic substances. The growth rate of mammalian cells is significantly decreased in the presence of toxic substances. Usually, the growth rate is determined by comparing the cell number or the protein content of cells at different time intervals. Since there is a linear relationship between cell number and protein concentration under conditions of the assay to be described, the growth rate is determined by protein measurement. Cell counting can be used as an alternative method of assessment.

In the growth inhibition test, medium extracts of contact lenses are added to the culture medium of cells and the protein content of the cell culture after 72 h in the presence of the extract is compared with the protein content in cell cultures without the extract.

In order to ensure high quality work, the cytotoxicity testing of contact lenses should be carried out in experienced laboratories according to GLP guidelines. The overall assessment of the results should be carried out by an expert in the field of toxicology who is informed about the final product and the conditions of its use and has appropriate chemical and biological data concerning it.

3 Agar overlay test

3.1 Apparatus and solutions

3.1.1 Apparatus

Standard tissue culture facilities, including sterilization equipment (autoclave and membrane filtration), laminar airflow hood, 37 °C carbon dioxide-air