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Biological evaluation of medical devices —

Part 23: Tests for irritation

Évaluation biologique des dispositifs médicaux —

Partie 23: Essais d'irritation

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Foreword

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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 of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 206, *Biological and clinical evaluation of medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

A list of all parts in the ISO 10993 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document assesses possible contact hazards from medical devices, which can produce irritation.

Some materials that are included in medical devices have been tested, and their skin or mucosal irritation potential has been demonstrated. Other materials and their chemical components have not been tested and can induce adverse effects when in contact with human tissue. The manufacturer is thus obliged to evaluate each device for potential adverse effects prior to marketing.

The irritation potential of a medical device or its components can be predicted either by an *in vivo* animal irritation test or by an *in vitro* irritation test if qualified for use with medical devices.

ISO 10993-2 describes animal welfare aspects for performing animal studies for the biological evaluation of medical devices thereby also emphasizing the 3R's for replacement, reduction, and refinement of animal studies. This document describes tests to determine the irritancy of medical devices, materials or their extracts either by *in vitro* tests or *in vivo* tests. *In vitro* tests have preference over *in vivo* tests when appropriately validated and providing equally relevant information to that obtained from *in vivo* tests (see ISO 10993-1 and ISO 10993-2).

Traditionally, tests in small animals have been performed prior to testing on humans to help predict human responses. More recently, *in vitro* tests as well as human tests have been added as adjuncts or alternatives. For skin irritation testing of neat chemicals *in vitro* tests were developed using reconstructed human epidermis (RhE) models[31]. The method was adapted for detection of irritant chemicals in medical device extracts. The results of a large round robin study that tested two types of RhE models showed that these models can also be used to detect the presence of irritant chemicals extracted from polymeric materials [polyvinylchloride (PVC) and silicone] commonly used in the manufacture of medical devices[6]. This method was found to be equally sensitive in the detection of low concentrations of some strong irritant compounds when compared to the human patch testing and intracutaneous rabbit test[14]. Therefore, a stepwise approach for irritant testing can start with the *in vitro* RhE model.

The developed and validated RhE models are appropriate to predict skin tissue irritation response. It is recommended to explore the use of other alternative *in vitro* models to assess the irritation potential for mucosal or eye epithelial applications.

It is intended that, for regulatory submission, these studies be conducted using GLP or ISO/IEC 17025 as applicable to the respective country and comply with regulations related to animal welfare. Statistical analysis of data is recommended and can be used whenever appropriate.

This document is intended for use by professionals, appropriately qualified by training and experience, who are able to interpret its requirements and judge the outcomes of the evaluation for each medical device, taking into consideration all the factors relevant to the device, its intended use and the current knowledge of the medical device provided by review of the scientific literature and previous clinical experience.

The tests included in this document are important tools for the development of safe products, provided that they are executed and interpreted by trained personnel.

This document is based on numerous standards and guidelines, including OECD Test Guidelines (TG), U.S. Pharmacopoeia[40] and the European Pharmacopoeia[39]. It is intended to be the basic document for the selection and conduct of tests enabling evaluation of irritation responses relevant to the safety of medical materials and devices.

Instructions are given in normative Annex A for the preparation of materials specifically in relation to the above tests. In normative Annex D several special *in vivo* irritation tests are described for application of medical devices in areas other than skin. In addition, normative Annex E provides information for conducting human skin irritation testing.

Biological evaluation of medical devices —

Part 23: **Tests for irritation**

1 Scope

This document specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation. The tests are designed to predict and classify the irritation potential of medical devices, materials or their extracts according to ISO 10993-1 and ISO 10993-2.

This document includes:

— pre-test considerations for irritation, including *in silico* and *in vitro* methods for dermal exposure;

— details of *in vitro* and *in vivo* irritation test procedures;

— key factors for the interpretation of the results.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

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

ISO 10993-9, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products*

ISO 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-13, *Biological evaluation of medical devices — Part 13: Identification and quantification of degradation products from polymeric medical devices*

ISO 10993-14, *Biological evaluation of medical devices — Part 14: Identification and quantification of degradation products from ceramics*

ISO 10993-15, *Biological evaluation of medical devices — Part 15: Identification and quantification of degradation products from metals and alloys*

ISO 10993-18, *Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process*

ISO 14155, *Clinical investigation of medical devices for human subjects — Good clinical practice*

OECD 404, *Acute Dermal Irritation/Corrosion*

OECD 439, *In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
blank
solution prepared in the same way as the sample measuring solution but so that it does not contain the analyte to be determined

[SOURCE: ISO 10136-1:1993, 3.8, modified — the term has been changed from "blank test solution" to "blank".]

3.2
dose
dosage
amount of *test sample* (3.14) administered (e.g. mass, volume) expressed per unit of body weight or surface area

Note 1 to entry: The terms are often used interchangeably (more commonly dosage).

3.3
erythema
reddening of the skin or mucous membrane

3.4
eschar
scab or discoloured slough of skin

3.5
extract
liquid or suspension that results from exposing a test or control material to an extraction *vehicle* (3.16) under controlled conditions

3.6
irritant
agent that produces *irritation* (3.7)

3.7
irritation
localized non-specific inflammatory response to single, repeated or continuous application of a substance/material

Note 1 to entry: Skin irritation is a reversible reaction and is mainly characterized by local *erythema* (3.3) (redness) and swelling [*oedema* (3.9)] of the skin.

3.8
necrosis
cell death as a direct result of irreversible changes caused by injury or disease

Note 1 to entry: Tissue repair will occur either resulting in complete functional restoration or resulting in scar formation.

3.9
negative control
well-characterized material or substance that, when evaluated by a specific test method, demonstrates the suitability of the procedure to yield a reproducible, appropriately negative, non-reactive or minimal response in the test system

Note 1 to entry: In practice, negative controls (NC) include *blanks* (3.1), *vehicles* (3.16)/solvents and reference materials.

3.10
oedema
swelling due to abnormal infiltration of fluid into the tissue

3.11
positive control
well-characterized material or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system

3.12
skin corrosion
production of irreversible damage to the skin, manifested as visible *necrosis* (3.8) through the epidermis and into the dermis, following application of a *test sample* (3.14)

EXAMPLE The action of a compound, chemical or a test sample resulting in ulceration of skin (see 3.15).

3.13
test material
material, device, device portion or component thereof that is sampled for biological or chemical testing

3.14
test sample
material, device, device portion, component, *extract* (3.5) or portion thereof that is subjected to biological or chemical testing or evaluation

3.15
ulceration
open sore representing loss of superficial tissue

3.16
vehicle
liquid used to moisten, dilute, suspend, *extract* (3.5) or dissolve the test substance/material

3.17
vehicle control
extraction *vehicle* (3.16) not containing the *test material* (3.13), retained in a vessel identical to that which holds the test material and subjected to identical conditions to which the test material is subjected during its extraction

Note 1 to entry: The purpose of the vehicle control (VC) is to evaluate possible confounding effects due to the extraction vessel, the vehicle and the extraction process.

4 General principles — Step-wise approach

The available methods for testing irritation were developed specifically to detect skin and mucous membrane irritation potential. Other types of adverse effects, such as sensitization, are generally not predicted by these tests. Historically irritation testing was done on rabbits. For medical devices that are used as implants or external communicating devices, intradermal testing is more relevant in approaching the application and so for detection of irritation activity, intracutaneous testing is indicated as described in 7.2.

Preference for *in vitro* tests instead of *in vivo* tests in accordance with ISO 10993-2, shall be considered, with replacement of the latter as new *in vitro* tests are scientifically validated and qualified for use with medical devices and become reasonably and practicably available. The results of a large round robin study that tested two types of RhE models showed that these models can also be used to detect the presence of irritant chemicals extracted from polymeric materials [polyvinylchloride (PVC) and silicone] commonly used in the manufacture of medical devices[6]. This method was found equally sensitive to detect low concentrations of some strong irritant compounds when compared to the human patch testing and intracutaneous rabbit test[14]. Therefore, the *in vitro* irritation test shall be performed before animal testing or human patch test is considered.

NOTE It can be relevant to provide detailed information of the applicability of the RhE model for the specific medical device being tested.

This document describes a stepwise approach, which shall include one or more of the following:

a) chemical characterization, supplemented where needed with chemical testing of samples in accordance with the general principles specified in ISO 10993-9, ISO 10993-13, ISO 10993-14, ISO 10993-15 and ISO 10993-18;

b) literature review, as indicated in ISO 10993-1, including an evaluation of chemical and physical properties, and information on the irritation potential of any product constituent as well as structurally-related chemicals and materials;

NOTE *In silico* methods (structure activity relationship, QSAR, read across) can indicate potential irritant activity.

c) *in vitro* alternative test using validated RhE per the methods in 6.2 to 6.12;

NOTE For special irritation tests relevant for medical devices intended to be applied to a specific area (Annex D), i.e. mucosal or eye epithelia, the RhE models are not adapted and it is recommended to explore the use of other *in vitro* models with relevant cells or tissues if qualified for use with medical devices.

d) *in vivo* animal tests;

NOTE *In vivo* animal tests are appropriate when test materials cannot be characterized and risk assessments cannot be undertaken using information obtained by the means set out in a), b) and c).

e) clinical studies according to ISO 14155 and ethics principles governing human clinical research, shall not be performed before the irritancy potential of a device has been established through one or more of the evaluations described in a) to d).

5 Pre-test considerations

5.1 General

It is important to emphasize that pre-test considerations can result in the conclusion that testing for irritation is not necessary. For example, if the pH of the test sample is ≤2,0 or ≥11,5 the material shall be considered an irritant and no further irritant testing is required according to OECD 404.

The requirements specified in ISO 10993-1:2018, Clause 5 on the categorization of medical devices and the following apply.

Non-sterile samples shall be investigated *in vivo* by topical investigation only, as the possibility of microbial contamination of the test sample could confound the final assay interpretation. In cases where the sterility of a test sample cannot be guaranteed, but the sample is still considered to be non-contaminated, intradermal administration should be justified.

5.2 Types of material

5.2.1 Initial considerations

It shall be taken into consideration that during manufacture and assembly of medical devices, additional chemical components can be used as processing aids, for example, lubricants or mould-release agents. In addition to the chemical components of the starting material and manufacturing process aids, adhesive/solvent residues from assembly, sterilant residues or reaction products resulting from the sterilization process can be present in a finished product. Whether these components pose a health hazard/risk depends on the leaching or degradation characteristics of the finished products. These components shall be taken into account for their potential irritation activity. The following types of materials are often used in medical devices and could introduce risks for irritation.

5.2.2 Ceramics, metals and alloys

These materials are normally less complex than polymers and biologically derived materials in terms of the number of chemical constituents.

5.2.3 Polymers

These materials are normally chemically more complex than ceramics, metals and alloys in terms of composition. A number of reaction products, impurities, and additives can be present and the completeness of polymerization can vary.

5.2.4 Biologically derived materials

These materials are inherently complex in their composition. They often also contain process residues, for example, cross-linkers and anti-microbial agents. Biological materials can be inconsistent from sample to sample.

5.3 Information on chemical composition

5.3.1 General

A description of the medical device chemical constituents shall be established according to ISO 10993-18. As described in ISO 10993-1, the extent of physical and/or chemical characterization required depends on what is known about the material formulation and on the nature and duration of body contact with the medical device. At a minimum, the characterization shall address the constituent chemicals of the medical device and possible residual process aids or additives used in its manufacture. The rigour necessary in the characterization of the chemical constituents is principally determined by the nature, degree, frequency and duration of the exposure and the hazards identified for the medical device or material. Where relevant to biological safety, quantitative data shall also be obtained. If quantitative data are not obtained, the rationale shall be documented and justified.

5.3.2 Existing data sources

Qualitative and quantitative information on the composition shall be obtained where possible from the supplier of the starting material. For polymers, this often requires access to proprietary information; provision should be made for the transfer and use of such confidential information.

Qualitative information about any additional processing additives (e.g. mould-release agents) shall also be obtained from appropriate members of the manufacturing chain, including converters and component manufacturers.

In the absence of any data on composition, a literature search is recommended to establish the likely nature of the starting material(s) and any additives, so as to assist in the selection of the most appropriate methods of analysis for the material concerned.

The chemical characterization of a medical device shall be conducted in accordance with ISO 10993-18.

NOTE The composition of ceramics, metals and alloys can be specified in accordance with ISO or American Society of Testing Materials (ASTM) standards or it can be specified by the user, or both. However, in order to obtain full qualitative and quantitative details on composition, it can be necessary to request these from the supplier or manufacturer of the starting material and also from component manufacturers to ensure that processing aids are also identified. Material master files held by regulatory authorities are another source of data, where they are accessible.

6 *In vitro* irritation tests

6.1 General

The *in vitro* method with RhE models for testing irritation was developed specifically to detect skin irritation potential for neat chemicals[3][12](see OECD 439). The method was adapted and validated with two RhE models for detection of irritant chemicals in medical device extracts[5][6][12][13][17][18][19]. This method was found equally sensitive to detect low concentrations of some strong irritants in extracts from polymeric medical materials (PVC and silicone) when compared to the human patch testing and intracutaneous rabbit test[20]. Hence, the RhE test as described in this document can replace the *in vivo* rabbit test for irritation by skin exposure and by intracutaneous (intradermal) administration.

NOTE It can be relevant to provide detailed information of the applicability of the RhE model for the specific medical device being tested.

6.2 *In vitro* reconstructed human epidermis model

6.2.1 Test system — Reconstructed human epidermis model

The RhE model shall consist of normal human-derived epidermal keratinocytes, which have been cultured to form a multi-layered highly differentiated model of the human epidermis. It shall consist of organized basal, spinous and granular layers, and a multi-layered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*. Normal human keratinocytes obtained from healthy volunteer donors shall be cultured for a number of days on a membrane or filter at an air-liquid interface to form the three-dimensional epidermal model comprising the main basal, supra basal, spinous and granular layers and a functional stratum corneum. The model system shall allow for both polar (e.g. saline) and non-polar (e.g. sesame oil) extracts to be directly added to the apical surface of RhE constructs.

Materials not suitable for extraction (e.g. liquids, gels, pastes, and particulates) might be suitable for the test system. If used, validation data should be provided to demonstrate the ability of the assay to detect irritant activity of these forms of materials prior to testing.

6.2.2 Principle of the method

Endpoints: cell viability determination is based on cellular reduction of MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) and subsequent conversion to a purple formazan salt that is quantitatively measured after extraction from the tissues[9][16]. The cell viability in treated tissues is expressed as a percentage of the negative control. The percent reduction in viability is used to predict the irritation potential.

NOTE 1 Reduced tissue survival can be accompanied by IL-1α release[13][17][19]. Tissue culture media from the exposure can be collected and kept frozen at ≤−20 °C for possible analysis of cytokines.

Brief procedure: studies performed with polymeric biomaterials specifically manufactured to contain irritant chemicals at low concentrations indicated that a prolonged exposure is needed compared to the OECD 439 protocol for neat chemicals. An incubation period of no less than 18 h up to 24 h exposure at 37 °C for exposure to potentially low concentrations of irritants in extracts from biomaterials is sufficient for predicting irritation *in vitro* by reduction of tissue viability below 50 %[4][6][13][17][19]. Both

18 h and 24 h exposure showed similar results in both RhE models evaluated in the round robin study using medical device extracts[13][17][19].

Tissues are incubated at 37 °C, 5 % CO_2 in a humidified incubator following the addition of the test and control extracts.

Exposure to the test sample extract is terminated by rinsing with Dulbecco's phosphate buffered saline (DPBS), or PBS without Ca^{2+} and Mg^{2+}. After washing, the tissues are manually dried. The viability is assessed by incubating the tissues for 3 h with MTT solution in a 24-well plate (1 mg/ml; 300 µl per well). The formazan crystals are extracted using an appropriate amount (depending on the RhE model used) of isopropanol for at least 2 h at room temperature. Two or three aliquots (depending on the instructions of the supplier) per tissue of extracted formazan is then added to 96-well plates (200 µl/well) and quantified spectrophotometrically at 570 nm.

For direct inoculation assays, a solution with a 1 % volume fraction of sodium dodecyl sulfate (SDS, see 6.4.4) in saline solution of NaCl 0,9 % can be used as positive controls (PCs) and DPBS or PBS without Ca^{2+} and Mg^{2+} treated epidermis are used as the negative control, respectively. For extracted assays, a verified irritant infused control extracted in sesame oil and in saline solutions of NaCl 0,9 % can be used as positive controls.

NOTE 2 Aliquots of culture media collected after 18 h or 24 h exposure can be stored frozen (at a minimum of −20 °C) for potential cytokine (IL-1α) measurements as a complementary endpoint to cell viability. IL-1α measurement determines the inflammation component to the assessment of skin irritation in addition to the cell damage component determined indirectly by the MTT test for cell viability.

Vehicle controls shall include saline (NaCl 0,9 %) solution and sesame oil that have undergone the ISO 10993-12 medical device extraction procedure. For each treated tissue the viability is expressed as a percent relative to negative DPBS or PBS treated control tissues (mean).

Known limitations of the method: The method is not applicable to gases and aerosols. It is also not considered applicable to evaluate irritation by direct contact of solid materials as close contact over the whole test surface cannot be guaranteed.

Known cases of test-compounds requiring specific controls: some chemicals can directly reduce the MTT reagent (e.g. electrophiles, test articles with high pH), while other chemicals can directly colour the tissue or the cells. Such test sample properties can only interfere if sufficient amounts of the chemical are still present on the tissue at the end of the exposure period. In these cases, a special procedure allowing the quantification of the "true" MTT reduction should be applied. A protocol for the determination of possible interactions with MTT is provided in References [20] and [21]. The use of specific and adapted controls enables the calculation of true tissue viability after subtracting the unspecific optical densities (OD) due to direct chemical MTT reduction or chemical residual colour extracted from the tissues, or both.

6.2.3 Prediction model

This prediction model is based on the prediction model of the OECD 439 and data further generated during the optimization of the medical device protocol[4][6][14][17][19].

If cell viability after the exposure is ≤50 %: the test sample is classified as an irritant (I).

If cell viability after the exposure is >50 %: the test sample is classified as a non-irritant (NI).

The cell viability test shall be conducted with both polar (e.g. saline) and non-polar (e.g. sesame oil) test extracts. If at least one of the extracts shows a positive effect (viability ≤50 %) the test sample of the medical device is considered to have irritant potential. The device or the device component tested shall then be considered to induce irritant activity. *In vivo* testing might be considered to further evaluate the categorization of the irritant activity when necessary. If the result is non-irritant (viability >50 %) with the two solvents, the device or the device component shall be considered as non-irritant.

6.3 Materials

6.3.1 Reconstructed human epidermis models — Product description

The epidermal cells are taken from healthy volunteer donors negative to anti-HIV 1 and 2, and to hepatitis C antibodies, and to hepatitis B antigens. Nevertheless, normal handling procedures for biological materials should be followed.

For test samples based on medical device extracts the application of two RhE models described and recognized in the OECD 439 was evaluated in a large international round robin study[6]. In this study the EpiDerm™ tissues EPI-200 model[1)] and the SkinEthic™ RHE model[2)] were used. Specific protocols of these models are available as supplementary material[6][20][21].

Both models have been validated by EURL ECVAM for determining skin irritation of chemicals and are included in OECD 439 and EU Guideline B.46[41]. These models were validated with neat industrial chemicals for the purpose of classification and labelling. The other described and recognized models included in OECD 439, can be used for medical device skin irritation if validated for testing medical device extracts for skin and tissue irritation. The validation of a new RhE based method similar to a reference method is called catch-up validation[3][37].

The validation for a new RhE model listed in the OECD 439 for *in vitro* skin irritation must demonstrate equal performances in terms of predictive capabilities and within- and between-laboratory variability to those of the initial round robin study[6]. The inter-laboratory study (minimum three laboratories) must be carried out blind with three runs (three production batches of the RhE model)[37] on a set of irritant and non-irritant materials equivalent to that of the original round robin study.

6.3.2 Preparation of medical device extracts

The preparation of medical device and/or biomaterial extracts shall be performed in accordance with ISO 10993-12.

— Polar extracts shall be prepared in 0,9 % saline solution (900 mg in 100 ml ultrapure or deionized water).

— Non-polar extracts shall be prepared in sesame oil from *sesamum indicum* (examples of quality that are acceptable: super refined and pharmaceutical grade).

NOTE 1 In the round robin study[6] 0,9 % saline solution and sesame oil were demonstrated to be suitable extractants for irritants present in PVC or silicone polymers, or both, to be applied in the *in vitro* RhE irritation assay. Therefore, these vehicles are recommended as extract solutions.

If other vehicles are used for extraction, validation data shall be provided to confirm that a change in the extraction vehicle will not impact the ability of the test system to differentiate between negative, weak, moderate, and strong irritants.

The extraction time and temperature should be justified based on ISO 10993-12.

NOTE 2 In the round robin study to evaluate the RhE for irritation testing of the medical device, extracts spiked polymers (PVC and silicone) were used and extraction was performed at (37 ± 1) °C for (72 ± 2) h with continuous agitation/shaking.

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1) EpiDerm™ is a trademark of a product supplied by MatTek *In Vitro* Life Science Laboratories (Bratislava, Slowakia) and MatTek Corporation, (Ashland, MD, USA). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

2) SkinEthic™ RHE is a trademark of a product supplied by EpiSkin SA (Lyon, France), EpiSkin Brazil (Rio, Brazil) and Shanghai EPISKIN Biotechnology (Shanghai, China). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Materials not suitable for extraction (e.g. liquids, gels, pastes, and particulates) can be suitable for the test system. If used, validation data should be provided to demonstrate the ability of the assay to detect irritant activity of these forms of materials prior to testing.

6.4 Methods

6.4.1 General

CAUTION — The procedure described in this document requires the use of hazardous reagents. This document does not claim to cover all related safety issues. It is the responsibility of the user of this document to take appropriate precautionary and occupational health and safety measures and to ensure compliance with regulatory and regulatory requirements.

MTT poses the following hazards:

— H302: harmful if swallowed;

— H315: causes skin irritation;

— H319: causes serious eye irritation;

— H330: fatal if inhaled;

— H335: may cause respiratory irritation;

— H341: suspected of causing genetic defects <state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard>.

Isopropanol poses the following hazards:

— H225: highly flammable liquid and vapour;

— H319: causes serious eye irritation;

— H336: may cause drowsiness or dizziness.

6.4.2 Test procedure

Annex B provides a check list for *in vitro* irritation testing using RhE models.

All procedures performed during the RhE irritation test shall be documented. An example of a method documentation sheet (MDS) is presented in Annex C. A description of the various steps involved in performing the *in vitro* skin irritation test is presented below. The following steps shall be followed, with any deviations justified and accompanied with validation data.

— Prepare device/biomaterial test and control samples as extracts in a polar (saline) and non-polar (sesame oil) solvent starting prior to tissue arrival. The timing of initiation of extraction should be based on the time chosen for extraction in accordance with ISO 10993-12 and the time the tissue is ready for treatment (depending on the time of arrival and necessary pre-incubations of the RhE tissues). The test material/medical device extract should be used within the timeframe allowed in ISO 10993-12 for use of the extracts for biocompatibility testing. Apply during the extraction period continuous agitation/shaking.

— Upon receipt transfer RhE tissues from transport plates medium to medium according to tissue manufacturer directions for use. If required by tissue manufacturer's instructions, pre-incubate tissues by placing the tissues in a suitable sized culture plates with assay medium (see 6.5.1).

— If extracts of positive control materials are not included, spike on the day of the experiment the positive control (SDS) into polar (saline) solvent at the concentrations specified. Lower concentrations of SDS can be used (e.g. 0,25 % to 0,5 %) as positive control after demonstrating suitability and positive outcomes[17][19].