TECHNICAL SPECIFICATION

ISO/TS 10993-20

First edition
2006-08-01

Biological evaluation of medical devices —

Part 20: Principles and methods for immunotoxicology testing of medical devices

Évaluation biologique des dispositifs médicaux —

Partie 20: Principes et méthodes relatifs aux essais d'immunotoxicologie des dispositifs médicaux

Reference number
ISO/TS 10993-20:2006(E)

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Published in Switzerland

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. 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.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of normative document:

— an ISO Publicly Available Specification (ISO/PAS) represents an agreement between technical experts in an ISO working group and is accepted for publication if it is approved by more than 50 % of the members of the parent committee casting a vote;

— an ISO Technical Specification (ISO/TS) represents an agreement between the members of a technical committee and is accepted for publication if it is approved by 2/3 of the members of the committee casting a vote.

An ISO/PAS or ISO/TS is reviewed after three years in order to decide whether it will be confirmed for a further three years, revised to become an International Standard, or withdrawn. If the ISO/PAS or ISO/TS is confirmed, it is reviewed again after a further three years, at which time it must either be transformed into an International Standard or be withdrawn.

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.

ISO/TS 10993-20 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

ISO/TS 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Evaluation and testing*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 5: Tests for* in vitro *cytotoxicity*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Framework for identification and quantification of potential degradation products*

— *Part 10: Tests for irritation and delayed-type hypersensitivity*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymeric medical devices*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from metals and alloys*

— *Part 16: Toxicokinetic study design for degradation products and leachables*

— *Part 17: Establishment of allowable limits for leachable substances*

— *Part 18: Chemical characterization of materials*

— *Part 19: Physico-chemical, morphological and topographical characterization of materials*

— *Part 20: Principles and methods for immunotoxicology testing of medical devices*

Introduction

International and European Standards are the main focus for demonstration of the safety and compliance of medical devices. There has been increasing attention over the past few years on the potential for medical devices to cause changes in the immune system. It was felt necessary to provide guidance on how to address adverse effects of medical devices on the immune system. As there are no standardized tests available, this document provides a framework on how to approach the evaluation of immunotoxicity.

The intention of this document is:

— to summarize the current state of knowledge in the area of immunotoxicology, including information on methods of assessment of immunotoxicity and their predictive value;

— to identify what the problems are and how they have been dealt with in the past.

For clinical indications of immune alterations due to medical devices, an extensive literature review has been performed, primarily through Medline. The key areas which have been researched are:

— immunosuppression;

— immunostimulation;

— hypersensitivity;

— chronic inflammation;

— autoimmunity.

These key words are linked with the following materials:

— plastics and other polymers;

— metals;

— ceramics, glasses and composites;

— biological materials.

NOTE See also Table 1 for possibilities of interaction of materials with the immune system.

Biological evaluation of medical devices —

Part 20: Principles and methods for immunotoxicology testing of medical devices

1 Scope

This part of ISO 10993 presents an overview of immunotoxicology with particular reference to the potential immunotoxicity of medical devices. It gives guidance on methods for testing for immunotoxicity of various types of medical devices.

This part of ISO 10993 is based on several publications written by various groups of immunotoxicologists over the last few decades in which the development of immunotoxicology as a separate entity within toxicology took place.

The current state of knowledge with regard to immunotoxicity is described in Annex A. A summary of clinical experience to date with immunotoxicology associated with medical devices is given in Annex B.

NOTE See also Bibliographic Reference [11].

2 Normative references

The following referenced documents are indispensable for the application 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 14971, *Medical devices — Application of risk management to medical devices*

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing*

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

ISO 10993-6, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation*

ISO 10993-10, *Biological evaluation of medical devices — Part 10: Tests for irritation and delayed-type hypersensitivity*

ISO 10993-11:2006, *Biological evaluation of medical devices — Part 11: Test for systemic toxicity*

3 Terms and definitions

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

3.1
immunotoxicology
study of the adverse health effects that result, directly or indirectly, from the interaction of xenobiotics with the immune system

3.2
medical device
any instrument, apparatus, appliance, material or other article, including software, whether used alone or in combination, intended by the manufacturer to be used on human beings solely or principally for the purpose of:

— diagnosis, prevention, monitoring, treatment or alleviation of disease;

— diagnosis, monitoring, treatment, alleviation of, or compensation for an injury or handicap;

— investigation, replacement or modification of the anatomy or of a physiological process;

— control of conception

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which can be assisted in its function by such means.

NOTE 1 Devices are different from drugs and their biological evaluation requires a different approach.

NOTE 2 Use of the term "medical device" includes dental devices.

3.3
xenobiotic
substance foreign to the human body or living organisms

3.4
immunogenic
able to stimulate cells of the immune system resulting in an antigen specific immune response

4 Risk assessment and risk management

Risk assessment includes hazard identification, dose response assessment and exposure assessment, which together allow characterization of the risk. Based on this risk characterization, risk management shall be applied.

Because of the difficulties in predicting immunotoxicity of new chemicals and materials, effort and interest need to be focused on the assessment and management of risks arising from known immunotoxic chemicals contained in medical devices. Application of risk management to medical devices shall be performed in accordance with ISO 14971. Possible immunotoxic hazards of the chemicals contained in the medical device shall be identified first by an extensive literature search. Examples of such hazards are the production of anaphylactic shock by chlorohexidine in medicines and by proteins in latex rubber. Subsequently the overall risk management/reduction procedures shall be considered, together with the various possible actions that could be taken to further reduce remaining risks such as indicating contra-indications on the label, product recall, design-change and restrictions of use or application.

5 Identification of hazards

Immunological hazards should be identified by assessing exposure to medical device materials to identify the presence of (potentially) immunotoxic agents. There are many sources from which information on immunological hazards can be obtained. These sources include but are not limited to:

— material characterization;

— residue characterization;

— characterization of the leachable materials;

— characterization of drugs and other substances added to the medical device;

— characterization of exposure duration and route;

— observations made during previous exposure to chemicals, drugs or materials;

— toxicity testing.

Most immunological reactions identified to date relate to the additives to materials. Therefore exposure assessment for these chemicals is important in order to identify the immunological hazard. Details of potential outcomes with various materials from different types of medical devices are given in Table 1.

Table 1 — Potential responses of the immune system

Medical device categorization by			Immune system responses					
Nature of body contact		Contact duration						
Category	Contact	A:limited (⩽ 24 h) B:prolonged (> 24 h to 30 d) C:permanent (> 30 d)	Irritation/ acute inflammation	Chronic inflammation	Immunosuppression	Immunostimulation	Hypersensitivity	Autoimmunity
Surface device	Skin	A	×	–	–	×	×	–
		B	×	×	–	×	×	–
		C	×	×	×	×	×	×
	Mucosal membrane	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
	Breached or compromised surface	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
External communicating device	Blood path, indirect	A	×	–	–	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
	Tissue, bone, dentin communicating implant devices	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×
Implant device	Tissue, bone and other body fluids	A	×	–	×	×	×	×
		B	×	×	×	×	×	×
		C	×	×	×	×	×	×

NOTE This table is a framework for consideration of the potential interaction of materials from different types of medical devices with various parts of the immune system, and is not a checklist for testing.

Effects on the immune system (immunotoxicity) occur due to an encounter of immunologically competent cells with foreign substances that are toxic and kill the cells, or result from foreign substances that interact with the early events of the immune response and alter subsequent responses. Prediction of the likelihood of immunotoxicity is difficult but can be based on known events in immunology.

First of all, for a substance to stimulate the immune response, it must be recognized as foreign to the host. The likelihood of being immunogenic is greatest with proteins, then polysaccharides, then nucleic acids and then lipids. Small molecular weight substances are generally not immunogenic. However, these substances may become immunogenic by binding to host proteins and altering the structure of the protein. These substances are usually referred to as haptens.

It is possible that polymeric materials, ceramic materials, and metallic materials may have leachable, wear or degradation products that bind to host proteins. Materials of biological origin, such as collagens, natural latex proteins, albumins and animal tissues are known to stimulate the immune response, and efforts must be taken to make these materials non-immunogenic. In order for large substances (size $>$ 1 000 000 daltons) to be immunogenic, they must be broken down and delivered as smaller substances.

The foregoing are examples of substances and materials which may have immunogenic potential and thus should be considered for their adverse effects on the immune system.

Body contact: every body contact listed in ISO 10993-1 is capable of manifesting an inappropriate immune response (immunotoxicity). Skin and mucous membranes are particularly likely to develop Type I and Type IV reactions. Other routes are likely to give systemic responses including Type I and Type IV reactions.

Duration of contact: in general, the longer the material is in contact with the body, the greater the likelihood of immunogenic substances forming. However, some chemicals will act rapidly and immune responses from materials in contact with the body for less than 24 hours can be immunogenic.

6 Methods of assessment of immunotoxicity

6.1 General

Immunotoxicity testing can be carried out using *in vivo* and *in vitro* assays. In contrast to *in vivo* immunotoxicity testing, possibilities for *in vitro* testing are limited as the models lack the complexity of the intact immune system. The value of *in vitro* methods in assisting extrapolation of animal data to man (by elucidating mechanisms of toxicity) is further limited because they are not yet sufficiently developed and standardized. However, they can be useful as mechanistic studies.

An important focus of immunotoxicology is the detection and evaluation of undesired effects of substances by means of tests on rodents. When animal tests are considered, to satisfy the provisions of ISO 10993-2 all reasonable and practically available replacement, reduction and refinement alternatives should be identified and implemented. Although there are validated laboratory tests, in many cases the biological significance and predictive value of immunotoxicity tests require careful consideration. The potential for effects on the immune system can be indicated by alterations in lymphoid organ weight or histology, changes in total or differential peripheral leukocyte counts, depressed cellularity of lymphoid tissues, increased susceptibility to infections by opportunistic organisms or neoplasia. The prime concern within the area of immunotoxicology is therefore to identify such changes and assess their significance with regard to human health.

In the context of immunotoxicity two kinds of assays can be distinguished: non-functional and functional. Non-functional assays have a descriptive character in that they measure, in morphological and/or quantitative terms, alterations in the extent of lymphoid tissue, the number of lymphoid cells and levels of immunoglobulins or other markers of immune function. In contrast, functional assays determine activities of cells and/or organs, such as proliferative responses of lymphocytes to mitogens or specific antigens, cytotoxic activity and specific antibody formation (e.g. in response to sheep erythrocytes).

A new development in this area is the application of “-omics” for the detection of alterations in the expression of genes involved in immune functions.

The evaluation of immunotoxicological hazards should be planned in accordance with the flow chart given in Annex C. Examples of tests for and indicators of immune responses are given in Table 2.

Although there are specific materials that are known or suspected to be immunotoxic, immunotoxicity testing related to immunosuppression or immunostimulation shall initially be limited to those assays carried out in the phase of general toxicity testing. Only those agents that show evidence of causing immunosuppression or immunostimulation shall be subjected to further investigation. Sub-acute tests are useful for obtaining general indications of potential immunosuppression or immunostimulation. If they are performed, they shall be carried out in accordance with ISO 10993-11.

6.2 Inflammation

Agents can interact with components of the non-specific arm of the immune system, i.e. granulocytes, macrophages and other cell types that are capable of producing and releasing inflammatory mediators. It should be noted that after implantation of a foreign body, a local inflammatory response is quite common. The duration and degree of the response determines whether it indicates an adverse effect. The most direct and adequate method of assessing the degree of induction of inflammation after exposure to agents is histopathology of the injection or implantation site of the agent. Chronic inflammation associated with immunotoxicity is a lesion which is predominant in lymphocytic cells as opposed to the foreign body reaction which is composed of macrophages and foreign body giant cells at the tissue/material interface. Initial tests for local inflammation are described in ISO 10993-6. Other useful tests include serum assays for C-reactive protein and acute phase protein.

6.3 Immunosuppression

For the detection of immunosuppression a tiered approach is warranted in order to reflect the complexity of the immune system with its variety of functions and components. This tiered approach comprises a first tier of immunosuppression testing using non-functional assays, followed by a second tier, that includes functional assays. This tiered approach does not provide the most sensitive approach available as functional assays are more sensitive than non-functional assays. The rationale for including less sensitive indicators as a first tier and more sensitive indicators as a second tier is not because it best assesses the immune system, but rather because it reduces the need for additional test animals.

In the first tier, indications for immunosuppression are induced alterations in, for instance, weight of immune organs, in cell numbers and/or cell populations and in immunoglobulins.

In the second tier, more specific immune function assays can then be utilized, such as determination of the influence of the agent on NK cell activity and/or on immune function during active immunization, for instance, the assay of antigen-specific antibody production after sensitization. In some guidelines some of these assays are already included in the first tier (antibody response to T-cell dependent antigens such as sheep red blood cells).

The real consequence of immunosuppression can probably be best determined by assessing effects on resistance against infection in bacterial, viral and/or parasitic animal models, and/or effects on resistance against tumours. The importance of these types of assays is that they assess the immune system as a complete and functional entity. However, since it is not practical to evaluate all immunologically relevant parameters in a single toxicity or immunosuppression study, the most important predictive parameters need to be identified and a practical approach chosen to assess immunosuppression for a particular agent.

As the general malaise of an individual also affects the immune system, immunosuppression is considered to exist when immune alterations are detected at dose levels inducing no overt general toxicity. Therefore, immunosuppression testing is best performed in the context of general toxicity testing, since general toxicity testing uses a range of doses of an agent and evaluates all major organ systems.

For the detection of general toxicity of chemicals after sub-acute exposure OECD 407[1] was recently adapted to include several immunotoxicological parameters for the determination of an immunotoxic effect of the compound under investigation.