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Biological evaluation of medical devices —

Part 4: Selection of tests for interactions with blood

Évaluation biologique des dispositifs médicaux —
Partie 4: Choix des essais pour les interactions avec le sang

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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).

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For an explanation on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices.*

This third edition cancels and replaces the second edition (ISO 10993-4:2002), which has been technically revised.

It also incorporates the Amendment ISO 10993-4:2002/Amd 1:2006.

The following changes were made:

a) some definitions have been revised and new definitions have been added;

b) Tables 1 and 2 have been consolidated into a single new Table 1 with test categories and headers reorganized to emphasize and include material and mechanical-induced haemolysis testing and *in vitro* and *in vivo* testing for assessment of risk for thrombosis;

c) Tables 3 and 4 have been consolidated into a single new Table 2 with a simplified list of suggested and most common tests;

d) Annex B has been updated to cover only the most common practiced tests for assessing blood interactions;

e) Annex C has been added to cover the topic of *in vivo* thrombosis and methods for testing;

f) Annex D, which was Annex C in the previous edition, has been updated and now includes added information on mechanically-induced haemolysis;

g) Annex E has been added to cover the topic of complement testing and best test method practices;

h) Annexes F and G have been added to present the less common tests used to assess interactions with blood and the tests that are not recommended for preclinical assessment of medical device blood interaction, respectively. Many of these methods were previously included in Annex B;

i) subtle language refinements can be found throughout the revised document;

j) the Bibliography has been reorganized by common subjects of interest and updated with additional and more current references.

Introduction

The selection and design of test methods for the interactions of medical devices with blood should take into consideration device design, materials, clinical utility, usage environment and risk benefit. This level of specificity can only be covered in vertical standards.

The initial source for developing this document was the publication, *Guidelines for blood/material interactions*, Report of the National Heart, Lung, and Blood Institute[14] chapters 9 and 10. This publication was subsequently revised[15].

Biological evaluation of medical devices —

Part 4:
Selection of tests for interactions with blood

1 Scope

This document specifies general requirements for evaluating the interactions of medical devices with blood.

It describes

a) a classification of medical devices that are intended for use in contact with blood, based on the intended use and duration of contact as defined in ISO 10993-1,

b) the fundamental principles governing the evaluation of the interaction of devices with blood,

c) the rationale for structured selection of tests according to specific categories, together with the principles and scientific basis of these tests.

Detailed requirements for testing cannot be specified because of limitations in the knowledge and precision of tests for evaluating interactions of devices with blood. This document describes biological evaluation in general terms and may not necessarily provide sufficient guidance for test methods for a specific device.

The changes in this document do not indicate that testing conducted according to prior versions of this document is invalid. For marketed devices with a history of safe clinical use, additional testing according to this revision is not recommended.

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-1, ISO 10993-12 and the following apply.

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

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

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

3.1
anticoagulant
agent which prevents or delays blood coagulation

EXAMPLE Heparin, ethylenediaminetetraacetic acid (EDTA), sodium citrate.

3.2
blood/device interaction
interaction between blood or a blood component and a device

3.3
coagulation
phenomenon that results from activation of the clotting (coagulation) factor cascade

Note 1 to entry: Factors of the coagulation cascade and fibrinolytic systems can be measured following exposure to devices either *in vitro* or *in vivo*.

3.4
complement system
part of the innate immune system consisting of over 30 distinct plasma proteins, including enzymes, cofactors, and cellular receptors which may be involved in the promotion of thrombosis

Note 1 to entry: Effector molecules produced from complement components are possible components in the phenomena of inflammation, phagocytosis and cell lysis. Complement activation related to immunotoxicity, hypersensitivity and generation of anaphylatoxins is not covered in this document. (See ISO/TR 10993-20.)

Note 2 to entry: The focus in this document is complement activation as it can promote and accelerate haemolysis, platelet and leukocyte activation and thrombosis on device material surfaces. (See also Annex E on complement activation.)

3.5
direct blood contact
term used when the device or device material comes into physical contact with blood or blood constituents

3.6
embolization
process whereby a blood thrombus, or foreign object, is carried in the bloodstream and which may become lodged and cause obstructed blood flow downstream

3.7
***ex vivo* test system**
term applied to a test system that shunts blood directly from a human subject or test animal into a test chamber located outside the body

Note 1 to entry: If using an animal model, the blood may be shunted directly back into the animal (recirculating) or collected in test tubes for evaluation (single pass). In either case, the test chamber is located outside the body.

3.8
haematology
study of blood that includes quantification of cellular and plasma components of the blood

3.9
haematocrit
ratio of the volume of erythrocytes to that of whole blood in a given sample

3.10
haemolysis
liberation of haemoglobin from erythrocytes, either by destruction or through a partially damaged but intact cell membrane

3.11
haemocompatible
<device or device material> able to come into contact with blood without any appreciable clinically-significant adverse reactions such as thrombosis, *haemolysis* (3.10), platelet, leukocyte, and complement activation, and/or other blood-associated adverse event occurring

3.12
indirect blood contact
nature of devices that contact the patient's blood path at one point and serve as a conduit for entry into the vascular system

EXAMPLE Drug and parenteral nutrition solution delivery devices.

3.13
legally-marketed comparator device
LMCD
approved, or cleared long-established, and recognized-to-be-safe medical device used as a reference control in an *in vitro* or *in vivo* safety evaluation of a test device of similar design, material(s), and clinical use

Note 1 to entry: It may be necessary that the LMCD be legally marketed in the same region as the regulatory submission for the test device.

3.14
non-blood-contact
nature of the device or material contact with the patient's body where the device or potentially extracted material does not have direct or indirect contact with blood

3.15
colloidal osmotic pressure
total influence of the proteins or other large molecular mass substances on the osmotic activity of plasma

3.16
platelets
anuclear, cellular bodies that are present in blood and contribute to the process of thrombosis by adhering to surfaces, releasing factors, and/or aggregating to form a haemostatic plug

3.17
platelet adherent
<material or device> having the tendency to allow or promote *platelets* (3.16) to attach to its surface

Note 1 to entry: This is often characterized relative to a negative control, positive control, and/or LMCD upon blood contact due to its surface properties.

Note 2 to entry: Platelet adherent does not necessarily mean platelet activating, i.e. platelets on a surface may or may not be activated.

3.18
thrombin generating
<material or device> due to its surface properties, having the tendency to promote or show increased thrombin formation

Note 1 to entry: This is often characterized relative to a negative control, positive control, and/or LMCD upon blood contact.

3.19
thrombogenic
<material or device> due to its surface properties, having the tendency to form or promote thrombus formation

Note 1 to entry: This is often characterized relative to a negative control, positive control, and/or LMCD upon blood contact.

3.20
thromboembolization
process where a dislodged *thrombus* (3.21) is carried downstream, where it may cause subsequent vascular blockage or occlusion

3.21
thrombus
coagulated mixture of red blood cells, aggregated *platelets* (3.16), fibrin and other cellular elements

3.22
thrombosis
formation of a *thrombus* (3.21) under *in vivo*, *ex vivo*, or *in vitro* simulated conditions, caused by activation of the coagulation system and *platelets* (3.16) in flowing whole blood

Note 1 to entry: Thrombosis can also occur in regions of a blood vessel or device where there is stasis.

3.23
whole blood
unfractionated blood drawn from a human donor or test animal

Note 1 to entry: The blood may be non-anticoagulated or anticoagulated, e.g. contain sodium citrate or heparin as an anticoagulant.

4 Abbreviated terms

Bb	enzymatically active fragment of Factor B produced by cleavage (by Factor D) in the activation of the alternative pathway
β-TG	beta-thromboglobulin
C4d	degradation product of C4 by classical pathway complement activation
C3a, C5a	complement split products from C3 and C5
CH-50	amount of complement required to lyse 50 % of a RBC suspension
D-Dimer	specific fibrin degradation products (F XIII cross-linked fibrin) consisting of D-fragment dimer
ELISA	enzyme-linked immunosorbent assay
FDP	fibrin/fibrinogen degradation products
FPA	fibrinopeptide A
F1.2	the non-catalytic fragment split off from prothrombin in its conversion to thrombin (also referred to as F1+2)
iC3b	inactive form of C3b, a sub-fragment of C3
IFU	instruction for use
IVC	inferior vena cava
MRI	magnetic resonance imaging
PET	positron emission tomography
PF-4	platelet factor 4

PRP	platelet-rich plasma
PT	prothrombin time
PTT	partial thromboplastin time
SC5b-9	product of terminal pathway complement activation
SEM	scanning electron microscopy
TAT	thrombin-antithrombin complexes
TCC	terminal complement complex; also called membrane attack complex (MAC); estimated by measuring SC5b-9
TT	thrombin time
TxB2	thromboxane B2

5 Types of devices in contact with blood (as categorized in ISO 10993-1)

5.1 Non-blood-contact devices

Non-blood-contact devices are devices that do not have direct or indirect contact with either blood or blood constituents that reside in the body or that are returned to the body. An *in vitro* diagnostic device and a blood-collection tube are examples of non-blood-contact devices. Some devices, such as introducer systems for implants, may contain both blood-contacting and non-blood-contacting components.

5.2 External communicating devices

5.2.1 General

These are devices that contact the circulating blood and serve as a conduit into the vascular system. Some devices may have components or portions with different types of contact (direct and indirect). Examples include but are not limited to the following.

5.2.2 External communicating devices that serve as an indirect blood path

— blood collection devices;

— cannulae;

— cell savers;

— devices for the storage and administration of blood and blood products (e.g. tubing and bags);

— extension sets;

— intravascular catheters.

5.2.3 External communicating devices directly contacting circulating blood

— atherectomy devices;

— blood monitoring devices with direct or indirect blood contact;

— cardiopulmonary bypass circuitry;

— devices for adsorption of specific substances from blood;

— donor and therapeutic apheresis equipment;

— extracorporeal membrane oxygenators;

— haemodialysis/haemofiltration devices;

— interventional cardiology and vascular devices;

— intravascular catheters (balloon, imaging, laser, ultrasound);

— leukocyte removal filters;

— percutaneous circulatory support devices;

— retrograde coronary perfusion catheters;

— vascular guide wires.

5.3 Implant devices

Implant devices are placed largely or entirely within the vascular system. Examples include but are not limited to the following:

— annuloplasty rings;

— arteriovenous shunts;

— blood monitors (implantable);

— circulatory support devices (ventricular-assist devices, artificial hearts, intra-aortic balloon pumps);

— embolization devices;

— endovascular synthetic vascular grafts;

— implantable defibrillator and cardioverter leads;

— inferior vena cava filters;

— internal drug delivery catheters;

— intravascular oxygenators (artificial lungs);

— mechanical or tissue heart valves;

— pacemaker leads;

— surgical synthetic or tissue vascular grafts;

— vascular stents.

6 Characterization of blood interactions

6.1 General requirements

IMPORTANT — Since this is a horizontal International Standard, sound rationales can be supplied to justify the choice of test category(ies) based on the device being characterized. For example, *in vivo* testing for evidence of thrombosis is frequently the preferred method for device characterization in the thrombosis category. However, in some cases, written rationales that include a combination of tests from the categories of coagulation, platelets, haematology and complement can be used as a substitute for thrombosis testing.

6.1.1 Figure 1 illustrates a decision tree that can be used to determine whether testing for interaction with blood is necessary. Blood interactions can be divided into several categories based on the primary process or system being measured. Table 1 lists examples of devices which contact circulating blood and the categories of testing appropriate to each device. The list is not all inclusive and sound judgement shall be applied to devices not listed in the tables.

For medical devices where a specific International Standard (vertical standard) exists, the biological evaluation requirements and test methods set forth in that vertical standard shall take precedence over the general requirements suggested in this document.

6.1.2 Where possible, tests shall use an appropriate model or system which simulates the geometry and conditions of contact of the device with blood during clinical applications. The simulation should include an appropriate duration of contact, temperature, sterile condition, anticoagulant (and level; see 6.1.12) and flow conditions. For example, for devices of defined geometry such as a vascular stent, the surface area used in the test, in cm^2, shall be given consideration relative to the fluid volume of the *in vitro* test system. For devices with undefined or complicated geometry (such as a dispersion of PVA particles used as an embolization agent), mass should be used instead of surface area to determine the amount of sample used in test system.

Only direct or indirect blood-contacting parts should be tested. The selected test methods and parameters should be in accordance with the current state of the art.

Appropriate type and level of anticoagulant may be case specific depending on both the device use indication and the type of test conducted. Include information on the specific type and level of anticoagulation used and provide a discussion on the ability to discern positive and negative responses. For further information, see 6.1.6 and C.2 for animal studies, 6.1.12 for *in vivo* and *ex vivo* tests, 6.3.1 for *in vitro* tests and A.3 for catheters and guide wires.

As many tests for haemocompatibility are recognized to be strictly surface-contact dependent, such tests (e.g. complement activation) will not apply to indirect contact applications.

6.1.3 Controls (positive and negative) shall be used unless their omission can be justified. Where possible, testing should include a relevant predicate device already in clinical use (i.e. a LMCD) or a well-characterized material[6].

Controls should include negative and positive reference materials. All materials and LMCDs tested shall meet all quality control and quality assurance specifications of the manufacturer and test laboratory. All materials and devices tested shall be identified as to source, manufacturer, grade and type.

6.1.4 Testing of materials which are candidates for components of a device may be conducted for screening purposes. However, such preliminary tests do not serve as a substitute for the requirement that the complete sterilized device or device component should be tested under conditions which simulate or exaggerate clinical application.

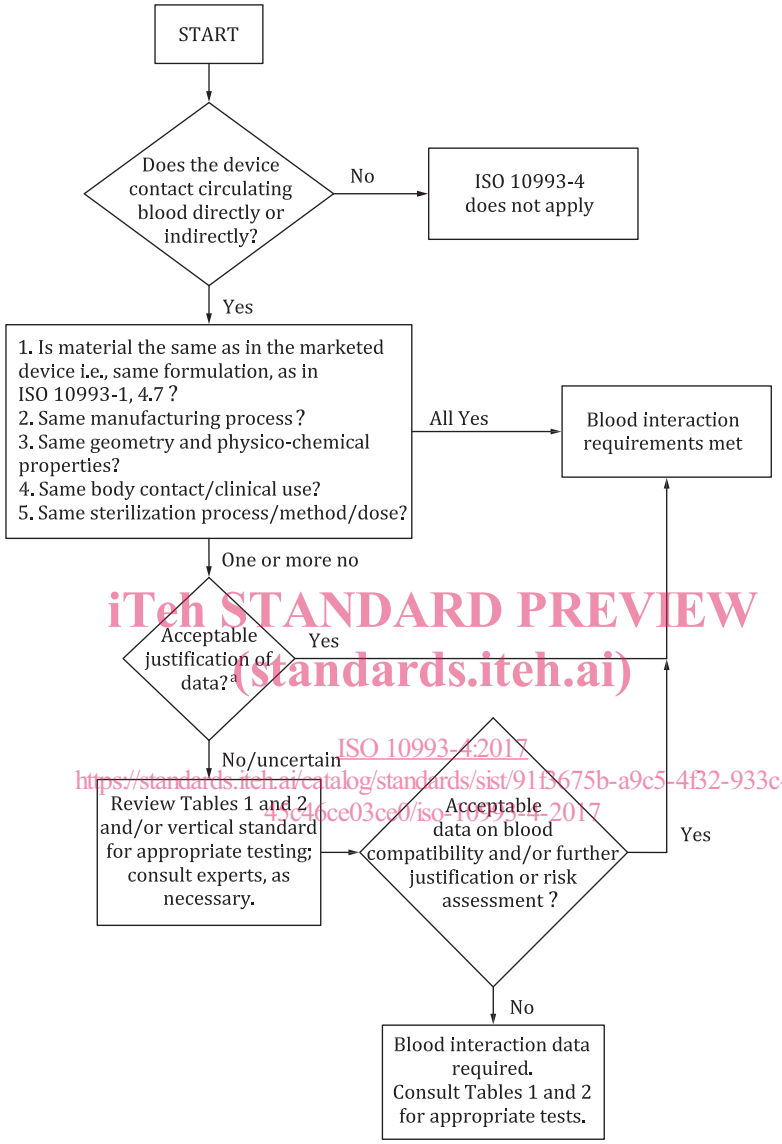
NOTE 1 Changes in manufacturing process (including use of manufacturing aids) that could affect the surface properties, or chemistry of the complete sterilized device, could also impact haemocompatibility.

NOTE 2 Where aging could impact the final device properties, use of aged samples can also be necessary. (For example, the properties of biologically active coatings such as heparin could change over time.)

6.1.5 Tests which do not simulate the conditions of a device during use may not predict accurately the nature of the blood/device interactions which can occur during clinical applications. In addition, the capacity of short-term *in vitro* or *ex vivo* tests to predict performance in actual clinical applications is thought to be higher when the clinical application involves limited exposure rather than prolonged or permanent exposure.

NOTE Simplified testing of candidate device materials (e.g. surface geometric and functional chemical modifications) can serve as a crucial step in device material identification, optimization and selection.

6.1.6 If an animal study is to be conducted, devices whose intended use is *ex vivo* (external communication) should be tested *ex vivo* and devices whose intended use is *in vivo* (implants) should be tested *in vivo* in an animal model simulating as closely as possible conditions of clinical use. Protocols in such investigations should specifically call out each test category (see 6.2.1) being evaluated and describe the specific method(s) of assessment.



[a] For direct and indirect contact devices, the necessity for haemocompatibility testing should be considered based upon appropriate risk analysis, including prior haemocompatibility testing, clinical data, extractable/leachable data, and/or information on surface characteristics. For example, for devices with direct contact, extractable/leachable testing may not be sufficient if the surface morphology is changed, even if the extractable/leachable chemistry is the same (see ISO 10993-1).

Figure 1 — Decision tree to help determine whether testing for interaction with blood is necessary

Table 1 — Circulating blood-contacting devices or device components and the categories of appropriate testing for consideration — External communicating devices and implant devices

Device examples	Test category						
	Haemolysis		Thrombosis				
			in vitro				*In vivo*/ *Ex vivo*[a]
	Material-induced	Mechanically-induced	Coagulation	Platelet activation	Complement [d]	Haematology	
External communicating devices							
Blood monitors (temporary/*ex vivo*)[b]	X		X	X		X	
Blood storage and administration equipment (e.g. infusion/transfusion sets), blood collection devices, extension sets	X		X	X		X	
Catheters in place for less than 24 h (e.g. atherectomy devices, intravascular ultrasound catheters, antegrade/retrograde coronary perfusion catheters, guide wires); cannulae	X		X[c]	X[c]		X[c]	X[c]
Catheters in place for more than 24 h (e.g. parenteral nutrition catheters, central venous catheters); cannulae	X		X[c]	X[c]		X[c]	X[c]
Cell savers[b]	X		X	X			
Devices for adsorption of specific substances from blood[b]	X	X	X	X	X		
Donor and therapeutic aphaeresis equipment and cell separation systems[b]	X	X	X	X	X		
Cardiopulmonary bypass system[b]	X	X	X[c]	X[c]	X	X[c]	X[c]
Haemodialysis/haemofiltration equipment[b]	X	X	X[c]	X[c]	X	X[c]	X[c]
Leukocyte removal filter[b]	X		X[c]	X[c]	X	X[c]	X[c]
Percutaneous circulatory support devices[b]	X	X	X[c]	X[c]	X	X[c]	X[c]
Implant devices							
Annuloplasty rings, mechanical heart valves	X	X					X
Embolization devices	X						X
Endovascular grafts	X						X
Implantable defibrillator and cardioverter leads	X						X
Intra-aortic balloon pumps[b]	X	X					X
Pacemaker leads	X						X
Prosthetic (synthetic) vascular grafts and patches, including arteriovenous shunts	X						X

[a] Thrombosis is an *in vivo* or *ex vivo* phenomenon, but can be simulated with *in vitro* conditions. *In vivo* or *ex vivo* testing might not be necessary if clinically relevant *in vitro* thrombosis testing is performed.

[b] Direct or indirect blood-contacting components only. For components that have only indirect blood contact, *in vivo* thrombogenesis and mechanical haemolysis or complement activation might not be necessary.

[c] It is recognized that coagulation, platelet and leucocyte responses are primarily involved in the process of thrombosis. Therefore, it is up to the manufacturer to decide if specific testing in the coagulation, platelet and haematology test categories is appropriate as an alternate to *in vivo* testing.

[d] See also ISO/TS 10993-20 for information on when complement activation should be considered for other end points such as anaphylaxis.