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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

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.

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](http://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](http://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 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](http://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<sup>[14]</sup> chapters 9 and 10. This publication was subsequently revised<sup>[15]</sup>.

# 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<sup>2</sup>, 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.

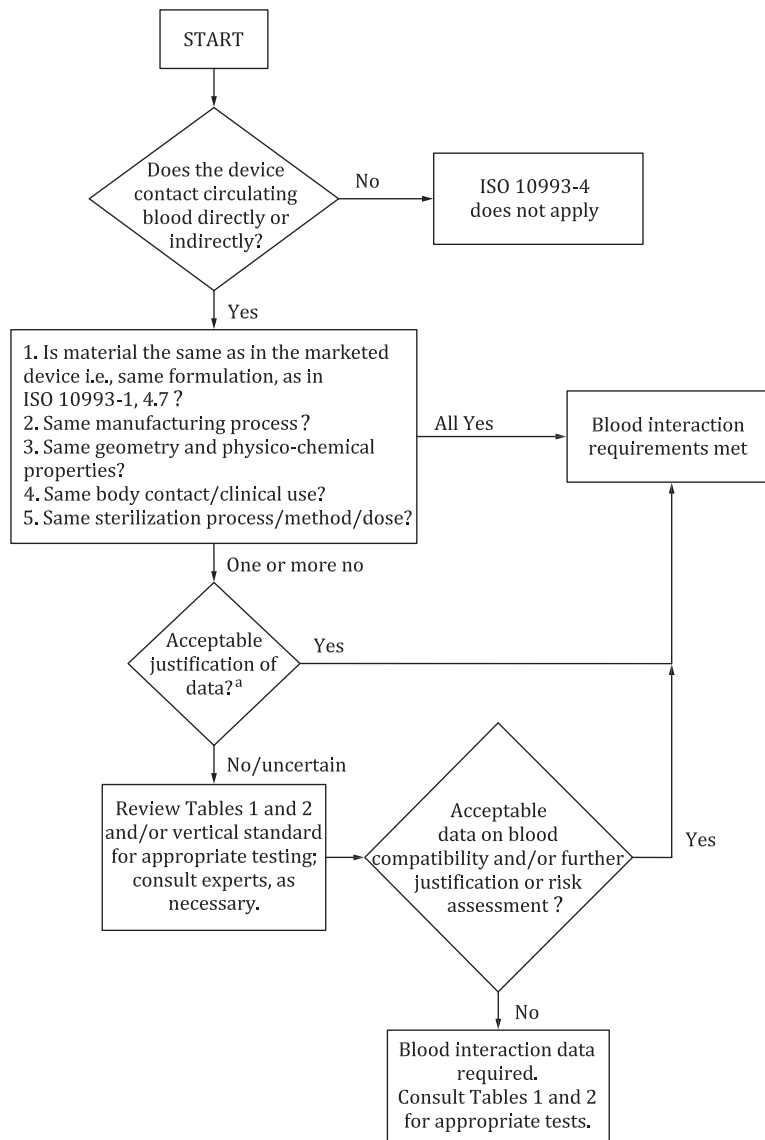
**NOTE 1** Changes in manufacturing process (including use of manufacturing aids) that could affect the surface properties, or chemistry of the complete sterilized device, could also impact haemocompatibility.

**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.



<sup>a</sup> 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      |                        |                         |             |                                                |
|                                                                                                                                                                                                                                                                                                                       | Material-<br>induced | Mechanically-<br>induced | <i>in vitro</i> |                        |                         |             | <i>In vivo/</i><br><i>Ex vivo</i> <sup>a</sup> |
|                                                                                                                                                                                                                                                                                                                       |                      |                          | Coagulation     | Platelet<br>activation | Complement <sup>d</sup> | Haematology |                                                |
| External communicating devices                                                                                                                                                                                                                                                                                        |                      |                          |                 |                        |                         |             |                                                |
| Blood monitors (temporary/ <i>ex vivo</i> ) <sup>b</sup>                                                                                                                                                                                                                                                              | 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 <sup>c</sup>  | X <sup>c</sup>         |                         |             | X <sup>c</sup>                                 |
| Catheters in place for more than 24 h (e.g. parenteral nutrition catheters, central venous catheters); cannulae                                                                                                                                                                                                       | X                    |                          | X <sup>c</sup>  | X <sup>c</sup>         |                         |             | X <sup>c</sup>                                 |
| Cell savers <sup>b</sup>                                                                                                                                                                                                                                                                                              | X                    |                          | X               | X                      |                         |             |                                                |
| Devices for adsorption of specific substances from blood <sup>b</sup>                                                                                                                                                                                                                                                 | X                    | X                        | X               | X                      | X                       |             |                                                |
| Donor and therapeutic apheresis equipment and cell separation systems <sup>b</sup>                                                                                                                                                                                                                                    | X                    | X                        | X               | X                      | X                       |             |                                                |
| Cardiopulmonary bypass system <sup>b</sup>                                                                                                                                                                                                                                                                            | X                    | X                        | X <sup>c</sup>  | X <sup>c</sup>         | X                       |             | X <sup>c</sup>                                 |
| Haemodialysis/haemofiltration equipment <sup>b</sup>                                                                                                                                                                                                                                                                  | X                    | X                        | X <sup>c</sup>  | X <sup>c</sup>         | X                       |             | X <sup>c</sup>                                 |
| Leukocyte removal filter <sup>b</sup>                                                                                                                                                                                                                                                                                 | X                    |                          | X <sup>c</sup>  | X <sup>c</sup>         | X                       |             | X <sup>c</sup>                                 |
| Percutaneous circulatory support devices <sup>b</sup>                                                                                                                                                                                                                                                                 | X                    | X                        | X <sup>c</sup>  | X <sup>c</sup>         | X                       |             | X <sup>c</sup>                                 |
| 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 <sup>b</sup>                                                                                                                                                                                                                                                                               | X                    | X                        |                 |                        |                         |             | X                                              |
| Pacemaker leads                                                                                                                                                                                                                                                                                                       | X                    |                          |                 |                        |                         |             | X                                              |
| Prosthetic (synthetic) vascular grafts and patches, including arteriovenous shunts                                                                                                                                                                                                                                    | X                    |                          |                 |                        |                         |             | X                                              |
| Thrombosis is an <i>in vivo</i> or <i>ex vivo</i> phenomenon, but can be simulated with <i>in vitro</i> conditions. <i>In vivo</i> or <i>ex vivo</i> testing might not be necessary if clinically relevant <i>in vitro</i> thrombosis testing is performed.                                                           |                      |                          |                 |                        |                         |             |                                                |
| Direct or indirect blood-contacting components only. For components that have only indirect blood contact, <i>in vivo</i> thrombogenesis and mechanical haemolysis or complement activation might not be necessary.                                                                                                   |                      |                          |                 |                        |                         |             |                                                |
| 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 <i>in vivo</i> testing. |                      |                          |                 |                        |                         |             |                                                |
| See also ISO/TS 10993-20 for information on when complement activation should be considered for other end points such as anaphylaxis.                                                                                                                                                                                 |                      |                          |                 |                        |                         |             |                                                |

Table 1 (continued)

| Device examples                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Test category    |                      |             |                     |                         |                                               |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|----------------------|-------------|---------------------|-------------------------|-----------------------------------------------|
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Haemolysis       |                      | Thrombosis  |                     |                         |                                               |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Material-induced | Mechanically-induced | Coagulation | Platelet activation | Complement <sup>d</sup> | <i>In vivo</i><br><i>Ex vivo</i> <sup>a</sup> |
| Stents (vascular)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | X                |                      |             |                     |                         | X                                             |
| Tissue heart valves, vascular grafts and patches and AV shunts                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | X                |                      |             |                     |                         | X                                             |
| Total artificial hearts                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | X                | X                    |             |                     |                         | X                                             |
| Vena cava filters                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | X                |                      |             |                     |                         | X                                             |
| Ventricular-assist devices <sup>b</sup>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | X                | X                    |             |                     |                         | X                                             |
| <sup>a</sup> Thrombosis is an <i>in vivo</i> or <i>ex vivo</i> phenomenon, but can be simulated with <i>in vitro</i> conditions. <i>In vivo</i> or <i>ex vivo</i> testing might not be necessary if clinically relevant <i>in vitro</i> thrombosis testing is performed.<br><sup>b</sup> Direct or indirect blood-contacting components only. For components that have only indirect blood contact, <i>in vivo</i> thrombogenesis and mechanical haemolysis or complement activation might not be necessary.<br><sup>c</sup> 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 <i>in vivo</i> testing.<br><sup>d</sup> See also ISO/TS 10993-20 for information on when complement activation should be considered for other end points such as anaphylaxis. |                  |                      |             |                     |                         |                                               |

**6.1.7** *In vitro* tests are regarded as useful in screening external communicating devices or implants and potential early interactions between devices/materials with blood, but may not be accurate predictors of blood/device interactions occurring upon prolonged or repeated exposure or permanent contact (see [6.3.1](#)).

NOTE For new devices or devices where there is a change in geometry, testing under physiologic flow can be needed. For long-term catheters or permanent implants, *in vitro* test systems might not be sufficient due to blood stability issues.

**6.1.8** Devices or device components which come into very brief/transient contact with circulating blood (e.g. lancets, hypodermic needles, capillary tubes that are used for less than 1 min) generally do not require blood/device interaction testing.

NOTE 1 For products made with materials such as coatings that could be left in contact with blood after the device is removed, blood/device interaction testing might be necessary.

NOTE 2 If some device components (e.g. syringe bodies) are in contact with fluids that will ultimately be injected into the patient, and the storage time is unspecified or greater than 1 min, haemolysis testing of the fluid-contacting component would be needed, even though the device itself would be in contact with circulating blood for less than 1 min.

**6.1.9** Disposable laboratory equipment used for the collection of blood and performance of *in vitro* tests on blood shall be evaluated to ascertain that there is no significant interference with the test being performed.

**6.1.10** If tests are selected in the manner described and testing is conducted under conditions which simulate clinical applications, the results of such testing have the greatest probability of predicting clinical performance of devices. For devices that operate over a range of conditions, the extreme and the average conditions should be considered. However, species differences and other factors may limit the predictability of any test.

**6.1.11** Because of species differences in blood reactivity, human blood should be used where possible (with the exception of established test methods with animal blood, such as some haemolysis tests). When animal models are necessary, for example for evaluation of devices used for prolonged or repeated exposure or permanent contact, species differences in blood reactivity shall be considered.

Blood values and reactivity in humans and non-human primates are very similar<sup>[204]</sup>. The use of animals, such as the rabbit, pig, calf, sheep or dog, can also be acceptable for a particular type of test. However, since species differences may be significant (for example, platelet adhesion<sup>[148][150]</sup>, thrombosis<sup>[44]</sup> and haemolysis<sup>[47]</sup> tend to occur more readily in the canine than in the human), all results of animal studies shall be interpreted with caution. The species selected and the number of animals used shall be justified (see also ISO 10993-2).

NOTE The use of non-human primates for *in vivo* blood compatibility and medical device testing is prohibited by EU law (86/609/EEC) and some national laws.

**6.1.12** The use of anticoagulants in *in vivo* and *ex vivo* tests should be avoided unless the device is designed to perform in their presence. The type and concentration of anticoagulant used influence blood/device interactions and their selection shall be justified. Devices that are used with anticoagulants should be assessed using anticoagulants in the range of concentrations used clinically and/or described in the product IFU or other appropriate literature. Species differences should also be considered when determining the appropriate level of anticoagulation.

**6.1.13** Modifications in a clinically accepted device shall be considered for their effect on blood/device interactions and clinical functions. Examples of such modifications include changes in design, geometry, changes in surface or bulk chemical composition of materials and changes in texture, porosity or other properties. An *in vitro* flow model with application-consistent exposure conditions and relevant measurements can be used to evaluate the effect of modifications to a clinically accepted device.

**6.1.14** A sufficient number of replications of a test including suitable controls should be performed to permit statistical evaluation of the data. The variability in some test methods requires that those tests be repeated a sufficient number of times to determine significance. In addition, repeated studies over an extended period of blood/device contact provide information about the time-dependence of the blood-device interactions<sup>[213]–[216]</sup>. Balance should be considered between statistical evaluation and animal welfare when applying *in vivo* testing; see ISO 10993-2.

**6.1.15** The recommendations within 6.1, together with [Figure 1](#) and [Table 1](#), serve as a guide for the selection of tests listed in [Table 2](#). Further guidance on pre-clinical evaluations is given in [Annexes A](#) to [G](#). In summary, the following procedure shall be performed:

- a) determine which potential blood interaction categories (see [6.2](#)) are appropriate for consideration to establish safety of the particular device (see examples in [Table 1](#));
- b) evaluate the *existing information* in each test category for the device;
- c) where *sufficient safety information exists*, prepare an appropriate rationale to support this conclusion and that further testing is not necessary;

NOTE Any difference in formulation, geometry, surface properties, fabrication methods, sterilization technique and/or clinical use could limit the use of safety information on a similar product.

- d) where *insufficient information exists* under a test category(ies), select appropriate tests, based upon examples in [Tables 1](#) and [2](#), to supply the additional safety information.

## 6.2 Categories of tests and blood interactions

### 6.2.1 Recommended tests for interactions of devices with blood

Recommended tests are organized on the basis of the type of device (see examples in [Table 1](#)). The tests are divided into the following categories based on the primary process or system being measured:

- haemolysis
  - material-induced
  - mechanically-induced
- thrombosis
  - *in vitro*
    - coagulation
    - platelet activation
    - complement
    - haematology
  - *in vivo/ex vivo*

The principles and scientific bases for these tests are given in [Annexes A](#) to [E](#).

**Table 2 — Common tests used to assess interaction with blood**

| Tests by categories                                                                                                                                                          |                                                                                                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Haemolysis</b>                                                                                                                                                            | Material-induced (e.g. ASTM[17], NIH[28], MHLW[22])                                                                                                                      |
|                                                                                                                                                                              | Mechanical-induced                                                                                                                                                       |
| <b>Thrombosis</b><br>( <i>in vivo</i> , <i>ex vivo</i> )                                                                                                                     | Gross analysis <sup>a</sup> , percentage occlusion, light microscopy, SEM                                                                                                |
| <b><i>In vitro</i> thrombosis</b>                                                                                                                                            |                                                                                                                                                                          |
| <b>Coagulation</b>                                                                                                                                                           | Thrombin (e.g. TAT, F1.2), fibrin (e.g. FPA) assays, PTT assay                                                                                                           |
| <b>Platelet activation</b>                                                                                                                                                   | Platelet count (% loss) and some indicator of activation (e.g. release products or platelets surface markers such as $\beta$ TG, PF4, TxB2) or SEM (platelet morphology) |
| <b>Haematology</b>                                                                                                                                                           | Complete blood count (CBC), leucocyte activation                                                                                                                         |
| <b>Complement system</b>                                                                                                                                                     | SC5b-9 (C3a optional)                                                                                                                                                    |
| <sup>a</sup> Included in all animal studies (see B.2.1 and ISO 10993-6).<br>Not all tests are needed for each category and testing in each category might not be equivalent. |                                                                                                                                                                          |

### 6.2.2 Non-contact devices

These devices do not require blood/device interaction testing.

### 6.2.3 External communicating devices and implant devices

After using Table 1 to align a new device under investigation with similar existing devices and noting the test categories for consideration, use Table 2, Annexes A and E to guide the selection of appropriate tests for assessing blood interactions.

### 6.2.4 Limitations

Testing and study design parameters may present certain practical limitations/considerations based upon science, technology and the particular application. For example:

- materials/devices in a high blood flow (arterial) environment may interact with blood differently in a low blood flow (venous) environment;
- blood interactions may occur with all materials, i.e. the test materials/test devices *and* the non-test materials (e.g. test system). Caution shall be taken to not confound blood interactions associated with the test materials to those contributed by other factors;
- studies that rely on just one type of test for blood interactions may be less predictive of the true response than studies that include several different tests for blood interactions;
- immunoassays for detecting protein indicators of haemocompatibility, e.g. TAT, C3a, etc., are often available for human blood testing but are not generally available for use or functional with blood from other species.

## 6.3 Types of tests

### 6.3.1 *In vitro* tests

*In vitro* testing (models) should consider designs to simulate the anticipated worst-case clinical use conditions of each device application. Variables that shall be considered when using *in vitro* test methods

include haematocrit, anticoagulant (type and amount), test sample preparation, test sample age, blood/blood component age, test sample storage, aeration and pH, temperature, proper randomization, test sample surface area to blood volume ratio and for dynamic studies, fluid flow conditions, especially flow rate, wall shear rate and pressure(s). Tests shall be started with minimal delay, usually within 4 h of blood draw, since some properties of blood change rapidly following collection. Alternatives to the latter may be feasible if validated. In some cases, the resulting samples can also be frozen using appropriate techniques for future analysis if the freeze/thaw process does not affect the analyte being assessed.

**NOTE** Clinically relevant types and amounts of anticoagulant may or may not be appropriate, depending on the test system and the ability to discern positive and negative responses.

When used to evaluate the haemocompatibility of device modifications, *in vitro* testing for haemolysis, thrombus formation, platelet and coagulation responses may be assessed and compared between the modified device and the clinically accepted device (see [A.1.4](#)).

### 6.3.2 *Ex vivo* tests

*Ex vivo* tests shall be performed when the intended use of the device is *ex vivo*, e.g. an external communicating device. *Ex vivo* testing can also be useful when the intended use is *in vivo*, e.g. to assess the acute response to an implant such as a vascular graft. Such use should not however substitute for an implant test.

*Ex vivo* test systems are available for monitoring platelet adhesion, emboli generation, fibrinogen deposition, thrombus mass, white-cell adhesion, platelet consumption and platelet activation[44][46][47][50][54][70][78][80]. Blood flow rates can be measured with either Doppler or electromagnetic flow probes. Alterations in flow rates may indicate the extent and course of thrombus deposition and embolization. Simple thrombus build-up can be assessed by gross and or microscopic visualization. Other more advanced and technically demanding tools have also been used[53][69][73][74][79].

### 6.3.3 *In vivo* tests

*In vivo* testing involves implanting the material or device in animals. Vascular patches, vascular catheters, vascular grafts, vascular stents, annuloplasty rings, heart valves and circulatory assist devices are examples of devices tested *in vivo*. Given the diversity of blood-contacting medical device applications, *in vivo* test models are expected to be equally diverse, in order to appropriately mimic each clinical application.

“Patency of a conduit or device (i.e. the unimpeded flow of blood through the device)” is a common measure of success or failure for some *in vivo* experiments. The percent occlusion and thrombus mass are determined after the device is removed. The tendency of thrombi formed on a device to embolize to distal organs should be assessed by careful gross as well as microscopic examination of organs downstream from the device. In addition, histopathological evaluation of the surrounding tissue and organs is useful. The kidneys are especially prone to trap thrombi which have embolized from devices implanted upstream from the renal arteries (e.g. ventricular-assist devices, artificial hearts, aortic prosthetic grafts)[184][187][236][237].

Methods to evaluate *in vivo* interactions without terminating the experiment are available. Arteriograms or imaging from intravascular ultrasound (IVUS) catheters are used to determine patency or thrombus deposition on devices. Radioimaging can be used to monitor platelet deposition at various time periods *in vivo*; platelet survival and consumption can be used as indicators of blood/device interactions and passivation due to neointima formation or protein adsorption[46][72][79].

In some *in vivo* test systems, the material's properties may not be major determinants of the blood/device interactions. Rather, flow parameters, compliance, porosity and implant design may be more important than blood compatibility with the material itself. As an example, low flow rate systems may give substantially different results when compared with the same material evaluated in a high flow rate system. In such cases, test system performance *in vivo* should carry more importance than *in vitro* test results.

*In vivo* test protocols should contain precise and stand-alone sections stating how each test category identified for testing, i.e. haemolysis, thrombosis, coagulation, platelets, haematology and complement system, will be evaluated.

## Annex A (informative)

### Preclinical evaluation of cardiovascular devices and prostheses

#### A.1 General considerations

##### A.1.1 Background

This annex provides background for selecting tests to evaluate the interactions of cardiovascular devices with blood. [Clause 6](#) contains guides to determine when testing is necessary, which blood interaction categories might be appropriate for specific devices, and a list of tests for evaluating blood/device interactions of non-contact, external communicating- and implant devices. The classification of blood/device interactions in [A.1.2](#) is provided as background.

##### A.1.2 Classification

**A.1.2.1** Interactions which mainly affect the device and which may or may not have an undesirable effect on the animal or human are as follows:

- a) adsorption of plasma proteins, lipids, calcium or other substances from the blood onto the surface of the device; or absorption of such substances into the device;
- b) adhesion of platelets, leukocytes or erythrocytes onto the surface of the device, or absorption of their components into the device;
- c) formation of pseudointima or neointima on the blood contacting surface and tissue capsule on the surface of the device;
- d) alterations in mechanical and other properties of the device.

**A.1.2.2** Interactions which have a potentially undesirable effect on the animal or human are as follows:

- a) activation of platelets, leukocytes or other cells, or activation of the coagulation, fibrinolytic, or complement pathways;
- b) formation of thrombus on the device surface;
- c) embolization of thrombotic or other material from the device's surface to another site within the circulation;
- d) injury to circulating blood cells resulting in anaemia, haemolysis, leucopenia, thrombocytopenia or altered function of blood cells;
- e) injury to cells and tissues adjacent to the device;
- f) intimal hyperplasia or accumulation of other tissue on or adjacent to the device, resulting in reduced flow or affecting other functions of the device;
- g) adhesion and growth of bacteria or other infectious agents on or near the device.

NOTE For items b), c) and d) above, some devices such as embolization coils require thrombus formation to be functional.

### A.1.3 Advantages and limitations of animal models

Animal models permit the closest end use simulation of clinical devices prior to actual testing in humans. They permit continuous device monitoring and a systematic controlled investigation of important variables. However, the choice of an animal model may be restricted by size requirements, the availability of certain species and cost. For example, a device may not be operated under the full range of clinical use conditions in an animal model due to anatomical limitations. It is critical that the investigators be mindful of the physiological differences and similarities of the species chosen with those of the human, particularly those relating to coagulation, platelet functions and fibrinolysis, and the response to pharmacological agents such as anaesthetics, anticoagulants, thrombolytic and antiplatelet agents and antibiotics. Because of species differences in reactivity, subject differences in reactivity and variable responses to different devices, data obtained from a single species should be interpreted with caution. Non-human primates such as baboons exhibit a close similarity to the human in haematological values, blood coagulation mechanism and cardiovascular system<sup>[50]</sup>. An additional advantage of a non-human primate is that many of the immunological probes for thrombosis assays developed for humans are suitable for use in primates. These probes include PF-4,  $\beta$ -TG, FPA, TAT and F1.2. The dog is a commonly used species and has provided useful information; however, device-related thrombosis in the dog tends to occur more readily than in the human, a difference which can be viewed as an advantage (as a challenging or accelerated model) when evaluating this complication. Pigs and sheep are generally regarded as suitable animal models because of their haematological and cardiovascular similarities to the human<sup>[71][148][149][150]</sup>. The effect of the surgical implant procedure on results should be kept in mind and appropriate controls included. The final decision on the use of an animal or *in vitro* model ultimately involves consideration of the availability and ethical use of animals (see ISO 10993-2), the availability and limits of *in vitro* blood models and the applicability of proper statistics for sound conclusions<sup>[213][214][215][216]</sup>.

### A.1.4 Advantages and limitations of *in vitro* models

*In vitro* blood-exposure models are attractive approaches to testing the haemocompatibility of medical materials and cardiovascular devices because they allow

- a) avoidance of costly animal models,
- b) high replication testing of test objects alongside controls and reference materials using the same batch of blood and at the same time,
- c) use of human or animal blood where flow, temperature and anticoagulation is standardized,
- d) worst case scenario testing, where activation products accumulate without clearance by kidneys or liver or other organs and activation-inhibiting functions of endothelial cells are absent, and
- e) isolation from confounding factors associated with device implantation/tissue injury associated with *in vivo* usage.

Such testing of medical materials and devices should simulate as best as possible the range of clinical conditions of blood exposure to the device, since testing on blood under clinically inapplicable conditions, e.g. non-clinical anticoagulation (types or levels) and flow conditions, can make interpretation of results difficult. Whenever possible, consult product IFU brochures or common medical practice literature for applicable anticoagulant type(s) and amount(s). When appropriate, testing over the full range of labelled use conditions for the device should be considered. For example, to evaluate mechanically-induced haemolysis and platelet activation, testing is often performed at the highest blood flow rate. For thrombosis testing, the minimum labelled blood flow rate may be important to characterize the safety of the device. Since it has been shown that responses in blood may differ considerably between various species<sup>[47][148][149][150]</sup>, the use of human blood is more relevant to the interpretation of results. Another advantage in using human blood is that it offers a more detailed array of test methods, since most contemporary bioanalytical methods are based on human blood components/epitopes. Conversely, there are certain limitations in the volume of blood that can be obtained from a single human donor. Thus, the use of blood from a single large animal may be more practical in cases where the model designed to simulate clinical-relevant conditions presents a large volume capacity.