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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 concernant les interactions avec le sang

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Contents

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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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 10993-4 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

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

- *Part 1: Guidance on selection of tests*
- *Part 2: Animal welfare requirements*
- *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*
- *Part 4: Selection of tests for interactions with blood*
- *Part 5: Tests for cytotoxicity: in vitro methods*
- *Part 6: Tests for local effects after implantation*
- *Part 7: Ethylene oxide sterilization residuals*
- *Part 8: Clinical investigation*
- *Part 9: Degradation of materials related to biological testing*
- *Part 10: Tests for irritation and sensitization*
- *Part 11: Tests for systemic toxicity*
- *Part 12: Sample preparation and reference materials*

Future parts will deal with other relevant aspects of biological testing.

Annexes A, B and C of this part of ISO 10993 are for information only.

Introduction

The initial source for developing this part of ISO 10993 was the publication, *Guidelines for blood/material interactions: Report of the National Heart, Lung, and Blood Institute working group* [26]; chapters 9 and 10. This publication is being revised [29].

Biological evaluation of medical devices —

Part 4:
Selection of tests for interactions with blood

1 Scope

This part of ISO 10993 gives guidance to agencies, manufacturers, research laboratories and others for evaluating the interactions of medical devices with blood.

It describes:

a) a classification of medical and dental 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, together with the principles and scientific basis of these tests.

Detailed requirements for testing cannot be specified because of the limitations in knowledge and precision of tests for interactions of devices with blood.

2 Normative reference

The following standard contains provisions which, through reference in this text, constitute provisions of this part of ISO 10993. At the time of publication, the edition indicated was valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

3 Definitions

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

3.1 blood/device interaction: Any interaction between blood or any component of blood and a device resulting in effects on the blood, or on any organ or tissue or on the device. Such effects may or may not have clinically significant or undesirable consequences.

3.2 *ex vivo*: Term applied to test systems that shunt blood directly from a human subject or test animal into a test chamber. If using an animal model, the blood may be shunted directly back into the animal (recirculating) or collected into test tubes for evaluation (single pass). In either case, the test chamber is located outside the body.

4 Abbreviations

Table 1 provides a list of abbreviations used in the context of this part of ISO 10993.

Table 1 — Abbreviations

Abbreviation	Meaning
Bb	Product of alternate pathway complement activation
β-TG	Beta-thromboglobulin
C4d	Product of classical pathway complement activation
C3a, C5a	(active) complement split products from C3 and C5
D-Dimer	Specific fibrin degradation products (F XIII cross-linked fibrin)
ECMO	Extracorporeal membrane oxygenator
E.M.	Electron microscopy
FDP	Fibrin/fibrinogen degradation products
FPA	Fibrinopeptide A
F_{1+2}	Prothrombin activation fragment 1 + 2
iC3b	Product of central C complement activation
IL-1	Interleukin-1
IVC	Inferior vena cava
MRI	Magnetic resonance imaging
PAC-1	Monoclonal antibody which recognizes the activated form of platelet surface glycoprotein IIb/IIIa
PET	Positron emission tomography
PF-4	Platelet factor 4
PT	Prothrombin time
PTT	Partial thromboplastin time
RIA	Radioimmunoassay
S-12	Monoclonal antibody which recognizes the alpha granule membrane component GMP140 exposed during the platelet release reaction
SC5b-9	Product of terminal pathway complement activation
TAT	Thrombin-antithrombin complex
TCC	Terminal complement complex
TT	Thrombin time
VWF	von Willebrand factor

5 Devices contacting blood

Devices contacting blood are categorized in ISO 10993-1:1992, clause 5.

5.1 Non-contact devices

(See subclause 5.1.1 of ISO 10993-1:1992.)

An example is *in vitro* diagnostic devices.

5.2 External communicating devices

(See subclause 5.1.3 of ISO 10993-1:1992.)

These are devices that contact the circulating blood and serve as a conduit into the vascular system. Examples include but are not limited to those in 5.2.1 and 5.2.2.

5.2.1 External communicating devices that serve as an indirect blood path [see subclause 5.1.3 a) of ISO 10993-1:1992] include but are not limited to

cannulae,

extension sets,

devices for the collection of blood,

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

5.2.2 External communicating devices in contact with circulating blood [see 5.1.3 c) of ISO 10993-1:1992] include but are not limited to

cardiopulmonary bypass,

extracorporeal membrane oxygenators,

haemodialysis equipment,

donor and therapeutic apheresis equipment,

devices for absorption of specific substances from blood,

interventional cardiology and vascular devices,

percutaneous circulatory support systems,

temporary pacemaker electrodes.

5.3 Implant devices

These are devices (see 5.1.4 of ISO 10993-1:1992) that are placed largely or entirely within the vascular system. Examples include but are not limited to

mechanical or tissue heart valves,

prosthetic or tissue vascular grafts,

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

inferior vena cava filters,

stents,

arteriovenous shunts,

blood monitors,

internal drug delivery catheters,

pacemaker electrodes,

intravascular membrane oxygenators (artificial lungs).

6 Tests

6.1 General recommendations

6.1.1 Where possible, tests should use an appropriate model or system which simulates the geometry and conditions of contact of the device with blood during clinical applications, including duration of contact, temperature, sterile condition and flow conditions. For devices of defined geometry such as vascular grafts of varying lengths, the relation of surface area (length) to test results should be evaluated.

The selected methods and parameters should be in accordance with the current state of the art.

NOTE 1 Only blood-contacting parts should be tested.

6.1.2 Controls shall be used unless their omission can be justified. Where possible, testing should include a device already in clinical use or well-characterized reference materials. Several materials and configurations are available (see ISO 10993-12 [7]).

Reference materials used should include negative and positive controls. All materials tested should meet all quality control and quality assurance procedures of the manufacturer and test laboratory and should be identified as to source, manufacturer, grade and type.

6.1.3 Testing of materials which are candidates to be components of a device should be conducted for screening purposes. However, such tests do not serve as a substitute for the requirement that the complete device be tested under conditions which simulate clinical application.

6.1.4 Tests which do not simulate the conditions of a device during use may not predict accurately the nature of the blood/device interactions which may occur during clinical applications. For example, some short-term *in vitro* or *ex vivo* tests are poor predictors of long-term *in vivo* blood/device interactions [22], [23].

6.1.5 It follows from the above that devices whose intended use is *ex vivo* (external communicating) should be tested *ex vivo* and devices whose intended use is *in vivo* (implants) should be tested *in vivo* in an animal model under conditions simulating where possible clinical use.

6.1.6 *In vitro* tests are regarded as useful in screening external communicating devices or implants but may not be accurate predictors of blood/device interactions occurring upon prolonged or repeated exposure or permanent contact (see 6.3.2). Devices intended for non-contact use only do not require evaluation of blood/device interactions. Devices which come into very brief contact with circulating blood (e.g. lancets, hypodermic needles, capillary tubes) generally do not require blood/device interaction testing.

6.1.7 The two recommendations in 6.1.5 and 6.1.6, together with clause 5, serve as a guide for the selection of tests listed in 6.2.1.

6.1.8 Disposable laboratory equipment used for the collection of blood and performance of *in vitro* tests on blood should be validated to ascertain that there is no significant interference with the test being performed. This can be conducted by performing tests on reference standards and comparing results with those obtained by a clinically approved technique.

6.1.9 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. However, species differences and other factors may limit the predictability of any test.

6.1.10 Because of species differences in blood reactivity, human blood should be used where possible. 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 should be considered. Blood values and reactivity between humans and non-human primates are very similar [23].

NOTE 2 The use of non-human primates for blood compatibility and medical device testing is prohibited by EC law (86/906/EEC) and some national laws.

However, the use of species other than non-human primates such as the pig, calf or sheep may also yield satisfactory results. The canine model has been found useful in the pre-clinical evaluation of prosthetic vascular grafts [43]. Because species differences may be significant (for example platelet adhesion, thrombosis, [17] and haemolysis tends to occur more readily in the canine species than in the human), all results of animal studies should be interpreted with caution.

6.1.11 The use of anticoagulants should be avoided unless the device is designed to perform in their presence. The choice and concentration of anticoagulant used influence blood/device interactions. Devices that are used with anticoagulants shall be assessed using anticoagulants in the range of concentrations used clinically.

6.1.12 Minor modifications in a clinically accepted device may cause significant changes in its clinical functions. Examples of such modifications include changes in design, changes in surface or bulk chemical composition of materials and changes in texture, porosity or other properties of vascular grafts. Therefore the effect of such changes on blood/device interactions shall be considered for clinical significance.

6.1.13 A sufficient number of tests including suitable controls shall 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 interactions. A statistician should be consulted in the early stages of experimental design.

6.2 Test methods

6.2.1 Recommended tests for interactions of devices and materials with blood

Recommended tests are organized on the basis of the type of device:

Table 2: External communicating devices — Level 1 — blood path, indirect

Table 3: External communicating devices — Level 1 — circulating blood

Table 4: External communicating devices — Level 2 — optional

Table 5: Implant devices — Level 1

Table 6: Implant devices — Level 2 — optional

Level 1 and level 2 tests are classified into five categories based on the primary process or system being measured:

a) thrombosis,

b) coagulation,

c) platelets and platelet functions,

d) haematology,

e) immunology.

Two levels of tests are presented. Select one or more test method(s) from each category of level 1 tests (tables 2, 3 and 5), in order to obtain the maximum information about the spectrum of reactions initiated when a device contacts blood.

Additional testing from level 2 (tables 4 and 6) is optional. The principles and scientific basis for these tests are presented in annex B.

6.2.1.1 Non-contact devices

These devices generally do not require blood/device interaction testing. Disposable test kits should be validated to rule out interference of materials with test accuracy.

6.2.1.2 External communicating devices

The external communicating devices and their test methods are listed in tables 2 to 4. These test methods are recommended for devices intended for limited (LI, < 24 h) and prolonged or repeated (PR, 24 h to 30 days) exposure. See also 6.1.6.

6.2.1.3 Implant devices

The implant devices and their test methods are listed in tables 5 and 6. These test methods are recommended for devices intended for prolonged or repeated (PR, 24 h to 30 days) exposure or permanent contact (PC, > 30 days).

6.2.2 Indications and limitations

Table 7 gives a list of commercially available assays validated for use with human blood and tables 2 to 6 present a list of tests. Level 1 tests, which shall be considered, are relatively simple for general use in evaluating the interactions of materials and devices with blood. Level 2 tests are more sophisticated, require special expertise in performance and interpretation, and are regarded as optional. In both categories, strict attention to technical detail is required. RIAs are available for human blood testing but are not generally

available for other species. The human test kits usually do not cross-react with other species except for some non-human primates. Care should be taken when designing test systems to ensure that one is actually measuring activation due to the test material and not an artifact of the system.

Discrepancies in evaluating blood/device interactions may occur because of inadequate materials characterization or inappropriate handling before blood tests are performed. For example the studies may have relied on only one type of test or may have permitted the introduction of foreign material unrelated to the material or device under test. Materials to be used in a low flow (venous) environment may interact with blood quite differently when used in high flow (arterial) situations. Changes in design and/or flow conditions can alter the apparent *in vivo* haemocompatibility of a material.

6.3 Types of tests

6.3.1 *In vitro* tests

Variables that should be considered when using *in vitro* test methods include haematocrit, anticoagulants, sample collection, sample age, aeration and pH, temperature, sequence of test versus control studies, surface-to-volume ratio, and fluid dynamic conditions (especially wall shear rate). Tests should be performed with minimal delay, usually within 4 h, since some properties of blood change rapidly following collection.

6.3.1.1 Platelet tests

Blood collection techniques should be reproducible. Platelets can become hyperreactive under a variety of conditions, including improper blood collection. Tests to verify normal platelet reactivity are usually performed with an aggregometer. Platelet preparations with reduced reactivity are easily detected using this method, but hyperreactive platelets are not normally detected. Platelet aggregation tests can be modified (by appropriately reducing the concentration of platelets or aggregating agents) to determine if platelets become hyperreactive following exposure to a material or device.

6.3.1.2 Coagulation tests

Coagulation methods are based on the use of native (fresh, non-anticoagulated) whole blood, anticoagulated whole blood (usually citrated), platelet-rich plasma or platelet-poor plasma. Since most of the standard coagulation assays are designed to detect clinical coagulation disorders which result in delayed clotting or excessive bleeding, the protocols for evaluating blood/device interactions should be modified appropriately to evaluate accelerated coagulation induced by biomaterials. Reagents for tests based on the activated partial thromboplastin time include an activator such as kaolin, celite, or ellagic acid. Reagents with such activators should be avoided because they tend to mask the acceleration of coagulation which materials and devices cause. The material to be tested serves as the activator; controls (without the material) should be included.

Blood is exposed to test materials either in a static chamber such as a parallel plate cell or in a closed-loop system where the inner surface of the tubing is the test material. After a predetermined contact time with the test surface, tests of the surface and blood can be conducted.

Table 2 — External communicating devices — Level 1 — Blood path, indirect (see 5.2.1)

Test category	Method	Comments
Thrombosis	Light microscopy (adhered platelets, leucocytes, aggregates, erythrocytes, fibrin, etc.)	Light microscopy can be replaced by scanning E.M. if the nature of the material presents technical problems for light microscopy.
Coagulation	PTT (non-activated)	
Platelets	Platelet count	
Haematology	Leucocyte count and differential; haemolysis (plasma haemoglobin)	Haemolysis is regarded as an especially significant screening test to perform in this category because of its measurement of red blood cell membrane fragility in contact with materials and devices. The method used should be one of the normative standard test methods for haemolysis.
Immunology	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9	A panel including the last four tests encompasses the various complement activation pathways.

Table 3 — External communicating devices — Level 1 — Circulating blood (see 5.2.2)

Test category	Method	Comments
Thrombosis	Per cent occlusion; flow reduction; gravimetric analysis (thrombus mass); light microscopy (adhered platelets, leucocytes, aggregates, erythrocytes, fibrin, etc.); pressure drop across device	Light microscopy can be replaced by scanning E.M. if the nature of the material presents technical problems for light microscopy. Pressure drop not recommended for devices intended for PR (see 6.2.1.2).
Coagulation	PTT (non-activated)	
Platelets	Platelet count; platelet aggregation; template bleeding time	
Haematology	Leucocyte count and differential; haemolysis (plasma haemoglobin)	Haemolysis is regarded as an especially significant screening test to perform in this category because of its measurement of red blood cell membrane fragility in contact with materials and devices. The method used should be one of the normative standard test methods for haemolysis.
Immunology	C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9	A panel including the last four tests encompass the various complement activation pathways.

Table 4 — External communicating devices — Level 2 — optional

Test category	Method	Comments
Thrombosis	Scanning E.M. (platelet adhesion and aggregation; platelet and leucocyte morphology; fibrin)	
Coagulation	Specific coagulation factor assays; FPA, D-dimer, F_{1+2}, PAC-1, S-12, TAT	
Platelets	PF-4, β-TG; thromboxane B2; gamma imaging of radiolabelled platelets ^{111}In-labelled platelet survival	^{111}In-labelling is recommended for PR only (see 6.2.1.2).
Haematology	Reticulocyte count; activation specific release products of peripheral blood cells (i.e. granulocytes)	Recommended for PR only (see 6.2.1.2).
Immunology	IL-1 and other cytokines; detection of messenger-RNA specific for cytokines	

Table 5 — Implant devices — Level 1 (see 6.3)

Test category	Method	Comments
Thrombosis	Per cent occlusion; flow reduction; autopsy of device (gross and microscopic); autopsy of distal organs (gross and microscopic)	
Coagulation	PTT (non-activated), PT, TT; plasma fibrinogen, FDP	
Platelets	Platelet count; platelet aggregation	
Haematology	Leucocyte count and differential; haemolysis (plasma haemoglobin)	Haemolysis is regarded as an especially significant screening test to perform in this category because of its measurement of red blood cell membrane fragility in contact with materials and devices. The method used should be one of the normative standard test methods for haemolysis.
Immunology	C3a, C5a, TCC, Bb, iC3b, C4b, SC5b-9	A panel including the last four tests encompassses the various complement activation pathways.

Table 6 — Implant devices — Level 2 — optional

Test category	Method	Comments
Thrombosis	Scannning E.M.; angiography	
Coagulation	Specific coagulation factor assays; FPA, D-dimer, F_{1+2}, PAC-1, S-12, TAT	
Platelets	^{111}In-labelled platelet survival PF-4, β-TG thromboxane B2; gamma imaging of radiolabelled platelets	
Haematology	Reticulocyte count; activation specific release products of peripheral blood cells (i.e. granulocytes)	
Immunology	IL-1 and other cytokines; detection of messenger-RNA specific for cytokines	

6.3.2 *Ex vivo* tests

Ex vivo tests should be performed when the intended use of the device is *ex vivo*, for example an external communicating device. *Ex vivo* testing may also be useful when the intended use is *in vivo*, for example 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 [17], [27], [43]. 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.