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Insights into *in-vitro* Hemocompatibility testing according to ISO 10993-4

Hemocompatibility testing of blood-contacting medical devices is one of the most important criteria for successful clinical application. Catheters, guide wires, dialyzers, oxygenators, heart-supporting systems, vascular grafts, stents, and heart valves are examples of widely used medical devices coming in direct contact with a patient's blood.

Blood is a complex "organ" composed of 55% plasma, 44% erythrocytes and 1% leukocytes and platelets. In plasma, numerous proteins are present that take part in the regulation of the coagulation and inflammation pathways. The interaction of medical devices with blood leads to cellular and humoral reactions, which can result in undesired inflammation and/or coagulation. Thus, these interactions should be carefully analyzed to prevent the adverse activation or damage of blood components, in order to minimize the risk of harm to patients [1].

Using fresh human blood and adequate in-vitro models, it is possible to analyze the hemocompatibility of medical devices. In-vitro hemocompatibility analysis offers many advantages in comparison to in-vivo animal models. In-vitro models allow for analysis under well-controlled conditions, such as blood flow and anticoagulation, as the blood contact is more intense and products generated due to reaction of the blood components are not cleared [1].

ISO 10993-4:2017 [2] defines the framework for hemocompatibility testing of medical devices to ensure an adequate biological and safety profile prior to clinical application. This standard recommends the testing endpoints that should be evaluated for different types of medical devices depending on the type and duration of blood contact. The recommended endpoint measurements are classified into five categories, depending on the blood components analyzed:

1. Hemolysis: Analysis of the integrity and damage to red blood cells. Example: ASTM Hemolysis.

2. Coagulation: Analysis of the coagulation pathway. Examples: Analysis of the Partial Thromboplastin Time (PTT), Analysis of the Thrombin-Antithrombin complex (TAT).

3. Platelets: Analysis of platelet loss or activation. Examples: ß-Thromboglobulin Activity (ß-TG), Platelet Count.

4. Hematology: Measurement of cell depletion and/or leukocyte activation. Examples: Complete Blood Count (CBC), Release of PMN-Elastase.

5. Complement System: Analysis of the complement pathway (part of the innate immune system). Example: Quantification of Sc5b-9.

The typical workflow for hemocompatiblity testing is shown in Figure 1. The first step is to set the test design for extraction of the device according to the recommendations of ISO 10993-4, ISO 10993-21, and for clinical use [2],[3]. Determining the adequate experimental conditions for hemocompatibility testing of a medical device is a necessary challenge. In general, test design strategies should represent the worst-case approach and simulate conditions under clinical use [2]. For example, it is important to determine which parts of the device may come in direct or indirect contact with circulating blood during clinical use and for how long. Another important aspect is to determine the blood volume used for extraction. ISO 10993-21 can provide important guidance in this area.

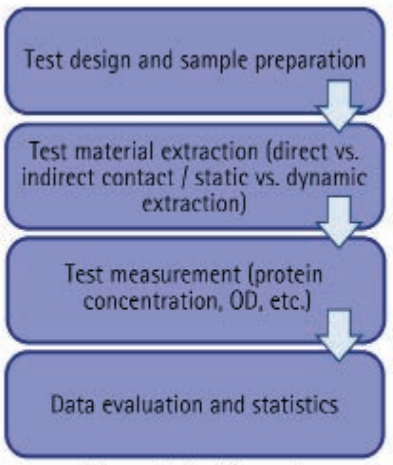

Figure 1. Workflow of hemocompatibility testing

For the analysis of a medical device, the relevant parts of the device are incubated with blood, in either direct or indirect contact. For direct contact, the medical device is incubated directly with blood. For indirect contact, the test device is extracted using an isotonic solution (NaCl or PBS). Afterwards, the resulting extracts are incubated with blood. The extraction can be performed under static conditions or using a dynamic model, such as the Chandler Loop or a pump-based recirculating system. Once the extraction is completed, the blood is collected and prepared for analysis. Testing endpoints are then selected from the tests recommended in ISO 10993-4:2017 [2], as listed in points one through five above.

In summary, the following aspects should be taken into consideration when designing a hemocompatibility testing strategy:

- Type and duration of blood contact.
- Surface, weight, and filling capacity of the device.
- Parts of the device with direct or indirect blood contact.
- Type of material (especially important for porous materials with high surface area and volume ratios)
- Type of blood anticoagulant.
- Number of blood donors
- Static vs. dynamic testing conditions.

Although ISO 10993-4 provides some guidance on how to perform hemocompatibility testing, key steps of the experimental design such as type of blood anticoagulant, blood volume and duration of blood contact remain open. Eurofins Medical Device Testing's experts in hemocompatibility testing can help design adequate testing strategies for a particular type of medical device based on clinical use, regulatory requirements, and testing experience.

One particular challenge for hemocompatibility testing is the high donor-to-donor variability observed for the blood parameters analyzed. This variability makes the establishment of specific threshold values or pass/fail criteria difficult. The lack of defined pass/fail values in hemocompatibility testing poses an important challenge for data evaluation and interpretation.
To solve this challenge, Eurofins Medical Device Testing bases a testing strategy on:

1. Simultaneous testing and comparison to validated controls, reporting data as fold-change values in comparison to control samples.
2. Use of a self-generated database of positive and negative control values including multiple donors, which covers for donor-to-donor variation.
3. Simultaneous testing of a predicate sample, which defines the range of values observed in response to marketed, clinically safe devices.

Specifically, using a predicate sample with comparable material composition, geometry and clinical use as the test item provides a basis for data interpretation and discussion [2] and is highly recommended by national authorities like the FDA.

In conclusion, hemocompatibility of medical devices can be reliably evaluated using the right combination of different in-vitro models and an appropriate test design. The test design should consider regulatory requirements, as well as clinical use and worst case conditions. Thus, individual test designs for each specific device, together with adequate controls, are essential to understanding the interaction and compatibility of a device with human blood and its components.

References:

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2.ISO 10993-4: Biological Evaluation of Medical Devices — Part 4: Selection of tests for interactions with blood. N.p.: International Organization for Standardization, 2017

3. ISO 10993-21: Biological evaluation of Medical Devices - Part 12: Sample preparation and reference materials; International Organization for Standardization; 2021.