A Practical Guide to ISO 10993-4: Hemocompatibility

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ISO 10993-4

Manufacturers can use the structured selection system in the standard on blood/device interactions to tailor hemocompatibility testing to their products. Note: this article is part of an ongoing series on ISO 10993. The previous installment covered genotoxicity. [5]

Blood represents one of the most complex biochemical systems in living organisms, and its various components play integral roles in several life functions, including the transport of oxygen, destruction of invading pathogens, and repair of damaged tissue. Because these functions are critical, medical devices that contact blood during routine use must be hemocompatible. That is, they must not adversely interact with any blood components so as to cause their inappropriate activation or even destruction.

ISO 10993-1, the international guidance on the selection of biocompatibility tests for medical devices that has been developed by the International Organization for Standardization (ISO), requires an evaluation of
hemocompatibility for any medical device that has contact with circulating blood, directly or indirectly, during routine use. Guidelines for such evaluations are subsequently presented in ISO 10993-4, "Selection of Tests for Interactions with Blood." This standard provides a structured test-selection system based on the intended use of the device. Although it does not provide detailed test methods or evaluation criteria, it cites various applicable references.

THE COMPLEXITY OF BLOOD

Blood is composed of a multitude of cell types, ranging from simple oxygen-carrying erythrocytes to sophisticated antigen-specific lymphocytes. The various cells participate in a vast array of functions, including tissue repair and immune responses as well as oxygen transport. Because of the range and criticality of these functions, any source of cytotoxicity to blood cells can cause significant harm. For example, hemolysis—a breakdown of red blood cells, which can be material mediated or result from mechanical damage—directly impairs the ability of the circulatory system to carry oxygen to body tissues. Likewise, adverse interactions with white blood cells can impair the body's ability to eliminate invading pathogens efficiently.

Blood also contains several soluble multicomponent protein systems that systematically interact in various ways to perform critical functions. For example, the complement system participates in inflammatory reactions and facilitates removal of invading pathogens. The clotting cascade operates to initiate coagulation, thereby preventing excessive loss of fluid and facilitating tissue repair. Inhibition of either of these cascade systems can have significant adverse effects on the body.

HEMOCOMPATIBILITY TESTING

As mentioned above, ISO 10993-4 provides a structured test-selection system that is based on clinical concerns. The types of tests required by the standard depend on the blood contact category of the device or material (external communicating devices—blood path indirect, external communicating devices—circulating blood, and implant devices). For each contact category, primary (Level 1) and optional (Level 2) tests are recommended from the following list of general test categories: thrombosis, coagulation, platelets/platelet function, hematology, and immunology. Each test category represents a specific blood function. Table I is a representative Level 1 test selection from the standard.
<table>
<thead>
<tr>
<th>category</th>
<th>Method</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombosis</td>
<td>Light microscopy (adhered platelets, leukocytes, aggregates, erythrocytes, fibrin, etc.)</td>
<td>Light microscopy can be replaced by scanning electron microscopy if the nature of the material presents technical problems for light microscopy.</td>
</tr>
<tr>
<td>Coagulation</td>
<td>Partial thromboplastin time (nonactivated)</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>Platelet count</td>
<td></td>
</tr>
<tr>
<td>Hematology</td>
<td>Leukocyte count and differential; hemolysis (plasma hemoglobin)</td>
<td>Hemolysis 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 hemolysis.</td>
</tr>
<tr>
<td>Immunology</td>
<td>C3a, C5a, TCC, Bb, iC3b, C4d, SC5b-9</td>
<td>A panel including the last four tests encompasses the various complement activation pathways.</td>
</tr>
</tbody>
</table>

Table I. A sample of primary tests for the contact category referred to as external communicating devices—blood path indirect. Reproduced with permission of the International Organization for Standardization. Some words have been adapted to American spelling. The standard can be obtained from the American National Standards Institute (11 W. 42nd St., 13th Fl., New York, NY 10036 or from the Central Secretariat, ISO, Case Postal 56, 1211 Geneva 20, Switzerland. Copyright remains with ISO.

Once a device's contact category is determined, one proceeds to the appropriate Level 1 table. In order to maximize hemocompatibility information about the device, one or more tests from each category included in the table should be performed. Several laboratory methods are available for each type of test, and rapid advances in technology in this area are creating more test options. Unfortunately, in the absence of standardized and validated test methods, the requirement for testing across all categories is frequently
All Level 1 tables include in vitro complement activation (immunology), hemolysis (hematology), and partial thromboplastin time (coagulation) tests. Such in vitro test methods are usually quicker and less costly than in vivo methods and do not require the use of animals. Complement activation is the most relevant immunology test for devices exposed to circulating blood. An increase in a downstream complement component over baseline levels indicates activation of the complement cascade. Acceptable complement activation limits have not been established, but comparative data are valuable. ASTM F 756, a standardized ASTM hemolysis test method, is available for determining the hemolytic potential of a device or material, and a suggested ISO method may be issued soon. These in vitro tests involve a quantitative measurement of plasma hemoglobin. An increase in plasma hemoglobin correlates with lysis of red blood cells, thereby indicating hemolytic activity of the material exposed to the cells. Such testing is frequently performed using rabbit blood. A device’s effects on blood coagulation may be measured in vitro by determining the rate of clot formation or the partial thromboplastin time of plasma exposed to the biomaterial or device during an incubation period. The reaction of white blood cells to materials can also be used as an effective hematology test (Figures 1 and 2).

**Figure 1. Normal neutrophils and lymphocytes following exposure to a negative control material.**

**Figure 2. Cell nuclei fragmenting into degenerative pieces following exposure to a positive control material.**

Thrombosis may be addressed by performing either an in vivo or ex vivo test. An evaluation of the thrombogenic potential of a device typically involves placing the device in a simulated clinical setting for a period of time, then removing the device and evaluating the extent of thrombus formation on or in it. The use of an appropriate control article is essential to the interpretation of results in these tests. The Lee White clotting-time test is also sometimes used to satisfy the requirement for a test in this category, but it is at best a gross screen because the dynamics of circulating blood are not present.

After completing the appropriate Level 1 tests, the device manufacturer must determine if any Level 2 tests are necessary. ISO 10993-4 does not indicate when such further testing is required. Because these tests are typically more complicated than Level 1 tests, and require specialized knowledge for their performance and interpretation, they are presumably most appropriate for situations when Level 1 test results indicate the device has an effect on a particular blood component. In such cases, Level 2 testing is usually a viable option for investigating the specific details of an observed effect. Level 2 tests also may be considered if an investigation into systemic effects, such as the immunotoxicity potential of a device, is warranted.
CONCLUSION

It is critical that any medical device having contact with blood be hemocompatible. The concept of hemocompatibility can best be viewed as a series of checks and balances. If a medical device upsets a balance or improperly activates a blood component, it can present a danger to the patient. ISO 10993-4 provides a structured test-selection system that manufacturers can use to customize a blood compatibility analysis to their devices, thus ensuring patient safety.

The responsible ISO committee (Working Group 9 of Technical Committee 194) is currently working toward making significant improvements to this standard. Because of the complex nature of blood compatibility, however, deference to vertical standards for specific devices may be more appropriate than attempting to develop a single comprehensive standard.

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Continue to the next installment, Sample Preparation and Reference Materials.

Tags:
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