

In Vitro Biocompatibility Testing of Biomaterials and Medical Devices

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Biomaterials used for medical devices must be thoroughly tested according to ISO 10993¹ before their introduction so that any negative effects on the body are known about and prevented. By using in vitro laboratory tests, dangers for patients and unnecessary animal experiments can be avoided. Here, in vitro tests for cell compatibility (cytotoxicity) and blood compatibility (haemocompatibility) are described.

Image: iStockphoto

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Practical application

For many manufacturers and developers of medical devices, ISO 10993, Biological Evaluation of Medical Device,¹⁻⁵ is a necessary evil. However, those who are fully conversant with the different parts of the standard and able to apply it will gain a significant advantage in their new product development and production processes.

This article outlines methods of biological evaluation, describing haemocompatibility and cytotoxicity testing. The article also makes recommendations from the perspective of an accredited test laboratory that focuses on in vitro testing of the biocompatibility of biomaterials and medical devices, and these serve to highlight important aspects of applying the standard.

The basic requirements of the clinical evaluation of a medical device are set out in the Medical Device Directive⁶ and the Active Implantable Medical Device Directive.⁷ Checking biocompatibility of

a product and/or its components based on ISO 10993 is one way of meeting these requirements.

ISO 10993 is a “horizontal” standard and therefore it is applicable to approximately 70 000 different medical devices. For practical use, it offers different starting points. The biological evaluation of medical devices is one important way to gain the CE mark. Particularly with in vitro tests according to ISO 10993-4, Selection of Tests for Interactions With Blood,³ and ISO 10993-5, Tests for In Vitro Cytotoxicity,⁴ the standard offers the opportunity to test early in product development the materials that are being used for the medical device. Potential toxicity can be tested and therefore expensive and undesirable development work can be avoided. In addition, products that are already on the market can be tested regularly with regard to cytotoxicity. In this way, the production process is monitored and, for example, toxic residues from the production and

cleaning process can be detected.

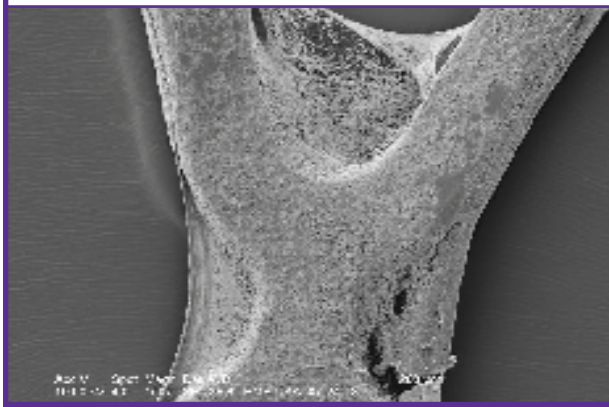
The following discussion focuses on the practical aspects of employing Parts 4 and 5^{3,4} of ISO 10993 in the development and manufacture of medical devices.

Haemocompatibility

Part 4 of ISO 10993 deals with the requirements of evaluating interactions of medical devices with blood.³ The so-called “blood-device interaction” is defined as “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. These effects may or may not have clinically significant or undesirable consequences. Generally, it is valid that: Testing shall be performed on the sterile final product, or representative samples from the final product or materials processed in the same manner as the final product (including sterilisation).”³

However, single components of the products can be tested by →

Figure 1: SEM shot of investigated stent sample after direct contact with human blood (deposition of blood components).



→ a quicker screening procedure to exclude haemoincompatibility and this is best undertaken during the development process. Testing of controls used for validation is required together with proof of the validity of the different methods. The properties of the reference materials must be well known and comply with the regimes of the manufacturers and the test laboratory in terms of quality control and quality assurance.

Because there are no limiting values for the evaluation of haemocompatibility, evaluating the test results is even more difficult. Therefore, it is useful to test a reference device that is well known and/or already available on the market at the same time as the device under development. The qualification and experience of the laboratory staff also play an important role.

ISO 10993-4 requires that human blood is used for in vitro tests where possible because of species differences in blood reactivity. Pig and baboon are suitable animal models, but species differences may be significant; for example, platelet adhesion, thrombosis and haemolysis tend to occur more readily in the canine species than in the human. Thus, all results of animal studies should be interpreted with caution because they run the risk of misjudgement. Animal blood should only be preferred if special methods or test parameters

require it.

Therefore, many test laboratories only test with human blood. Taking into account the different reactions of the different donors of human blood, several test runs with different donors are performed. In addition to the

consideration of different variables such as the circumstances of the sampling, the addition of anti-coagulants or the flow conditions in a dynamic testing system, particularly the shear forces at the blood vessel epithelium, mean fast processing after blood collection is important. ISO 10993-4³ requires that tests are performed with minimal delay, usually within 4 hours, because some properties of blood change rapidly following collection. However, from experience, it is recommended that tests are started within 30 minutes to help ensure valid results.

Caution with the haemolysis test

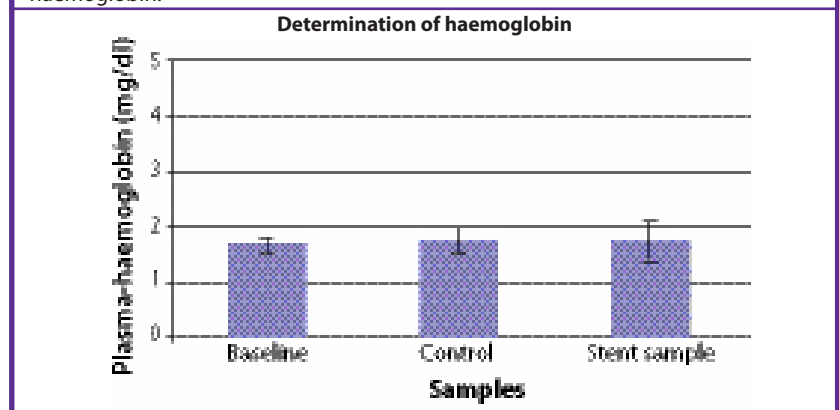
The test for haemolysis should be regarded with care. It is the only recommended test for some medical devices such as atherectomy or embolisation devices, as stated

in Part 4 of ISO 10993.³ Experience has shown that evaluation by haemolysis is insufficient for many devices with direct blood contact, because important reactions of blood, for example, coagulation activation, cannot be detected by this single test. In addition to haemolysis, determination of the blood count is highly recommended. With a blood count, the interaction between the blood and the reference material can be determined and the change in the quantity of erythrocytes, leukocytes and thrombocytes can indicate important foreign body reaction.

For evaluation of thrombocyte adhesion, the investigation of the surface of the medical device by scanning electron microscopy (SEM) is ideal to determine the activation of thrombocytes, the deposition of blood components or the formation of fibrin. This information, which directly influences the haemocompatibility of the medical device, cannot be gained by a single haemolysis test. Thus, evaluation for prolonged exposure and permanent blood contact that is limited to only this test is definitely not advisable.

Testing strategies should always be orientated towards the intended use (nature of body contact, duration of contact) of the device and include the necessary blood properties. The choice of technological investigation parameters is important: if the

Figure 2: Influence of the stent sample on haemolysis measured as free plasma haemoglobin.



medical device is to be employed in a dynamic system, the tests should also be done in a dynamic system and not in a static one.

Figure 1 shows stent material after contact with human blood. There is a massive deposition of blood components such as fibrin meshes with caught erythrocytes and leukocytes, whereas the haemolysis test showed no significant influence (Figure 2). This example shows that only investigation of several parameters delivers a safe result with respect to the haemocompatibility of biomaterials and medical devices.

Cytotoxicity

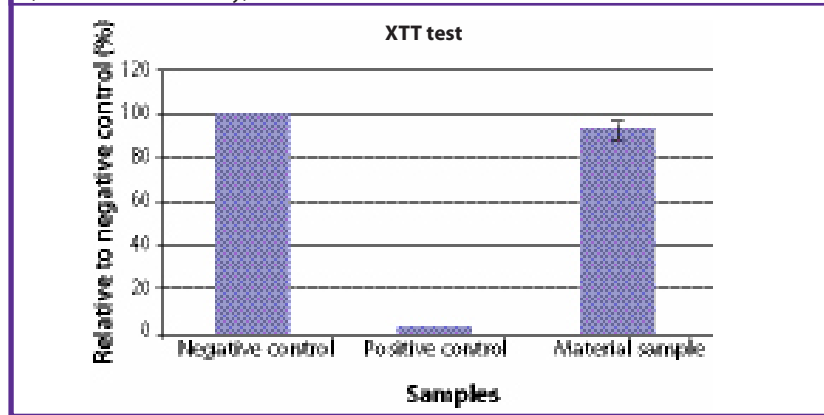
Part 5 of ISO 10993⁴ describes tests for in vitro cytotoxicity. Methods for direct and/or indirect cell contact can be used depending on the nature and duration of the device contact with the body. Quantitative and qualitative analysis methods are available.

As in testing haemocompatibility, sample preparation and a product orientated choice of test system is important. If the investigation sample is, for example, an implant, a combination of direct and indirect cell contact should be chosen. It is important to bear in mind, for example, that unleachable toxic substances that do not pass into the extraction medium can be proved by the direct cell contact, but not by testing indirectly via extraction method.

As an example, Figures 3 and 4 show a combination of direct and indirect test methods with different results of cytotoxicity for the investigated implant material. Comprehensive evaluation of a possible cell rejection response is only possible by considering the results of direct contact.

Positive and negative controls should be employed with the tests wherever possible to evaluate the effect of the investigated material compared with the controls. Before the tests, the morphology and the subconfluency of used cells should be controlled with the microscope.

Figure 3: Nontoxic result after indirect contact of implant material (mitochondrial activity).



The samples must be sterile to avoid contamination of the cells and a resulting misjudgement of cytotoxicity.

If the sample is delivered in an unsterilised state, it must be sterilised using the manufacturer's recommended method. The choice of the sterilisation method plays an important role, because it has a direct influence on the materials used to fabricate the medical device. For example, plasma sterilisation can influence the polymer structure and lead to negative effects. If extracts of nonsterile test items are sterilised by sterile filtration, there is a risk that toxic components remain in the syringe filters and the test results will be falsified.

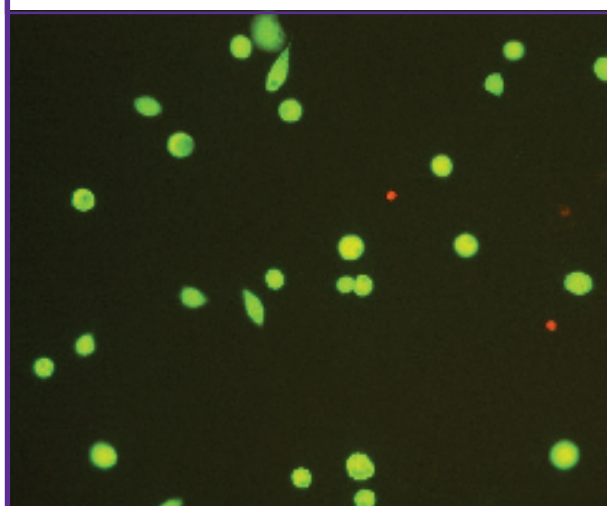
New medical devices show a marked increase in complexity. In many cases, they consist of several materials from different material groups. Here, the choice of the right testing strategy is important. The component with the toxic influence needs to be found and this can only be achieved by testing each single component.

Reasons for repeat testing

A general requirement of ISO 10993-12⁵ is that there are several repetitions of in vitro tests. This not only refers to the validation of the analytical methods, but also to cells (ISO 10993-5⁴) and blood (ISO 10993-4³), which are biological media and can fluctuate within their normal fluctuation margin and are restricted to only generalising validity.

For some manufacturers, who of course have to meet budgets as well as biocompatibility, repetition of several test runs involving different samples of one product, the different cell passage and using a testing strategy that employs more than one property of the cells seems to

Figure 4: Slight toxic result after direct contact of implant material (vitality with some rounded cells and some red-coloured damaged cells).



be exaggerated and too expensive. There follows the counter arguments to this view.

■ As already mentioned, the blood of different donors can react differently on the tested material. A secure result can only be achieved by repetitions with blood of different donors combined with different samples of one product.

■ When the cell passage shows a hidden source of errors and the tests are performed with one test run, the cytotoxicity results do not refer to the product, but to the cell passage itself. Therefore, for every replication, an additional, separate cell passage has to be used.

■ When there is only one sample that is tested, and if that one sample shows a cytotoxic effect, the sample will be declared cytotoxic in the investigation report. But what happens if this effect is only on that one sample and the samples of the other test runs show no toxicity? This would suggest an unstable batch and the manufacturer is then able to optimise the production process. In this way, the simultaneous testing of several products from one batch can be a validation of the properties of the production process. Experience in recent years has shown that this is a considerable advantage for manufacturers, because the production process can be changed at an early stage.

■ In recent years, testing systems have been established. These include evaluation of morphology and membrane integrity, and metabolism efficiency of the cells by measuring proliferation activity of the cells (BrdU-test) and mitochondrial activity of the cells by measuring dysfunction of mitochondrial activity as a sensor for a disturbed cell function (XTT-test). These complex systems allow, for example, a clear and comprehensive evaluation of biological efficiency of materials with nonadhesive properties such as some coronary stents. Unsuitable materials can be eliminated by in vitro screenings, and the subsequent

animal experiments can be reduced because unsuitable materials can be excluded from animal experiments.

Batch testing of cytotoxicity

Employing in vitro tests for cytotoxicity after the CE-marking of products offers manufacturers and patients increased confidence in the safety of the products.

One element of batch testing is the microbiological (bioburden) test, but this not the only aspect of the production monitoring. Microbiological tests, for example, do not show residues of the production or cleaning processes. Small changes within the cleaning and/or production processes can lead to devastating effects such as increased endoprosthesis loosening. In most cases, technical defects or human errors within the production process cannot be detected by the microbiological test. That is why manufacturers are increasingly expanding their quality assurance with one efficient and simultaneous inexpensive method: batch testing by cytotoxicity tests.

Today's advantages

This article describes the main aspects of in vitro biocompatibility testing according to the ISO 10993 series for obtaining the CE mark and for use after the introduction of the medical device onto the market. The ISO 10993 series is developing continuously. The development of in vitro tests for cytotoxicity and haemocompatibility within the last years has increased their validity and adaptation to specific product applications. The substitution of animal experiments by specially developed in vitro test methods and the use of tests that minimise any pain and distress for animals is reflected in this ISO 10993 series.² In addition to the development of the methods, the requirements for the test laboratories have increased and are reflected in the last revision of ISO 10993-1 (ISO/DIS 10993-1:2006).¹ All tests must be conducted in compliance with current best laboratory/

quality practices, for example, Good Laboratory Practices, or ISO 17025,⁸ where applicable.

The specific application of in vitro biocompatibility tests for cytotoxicity and haemocompatibility ultimately saves money, but as a first priority, leads to a significant increase in the safety of the product and the patient.

References

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