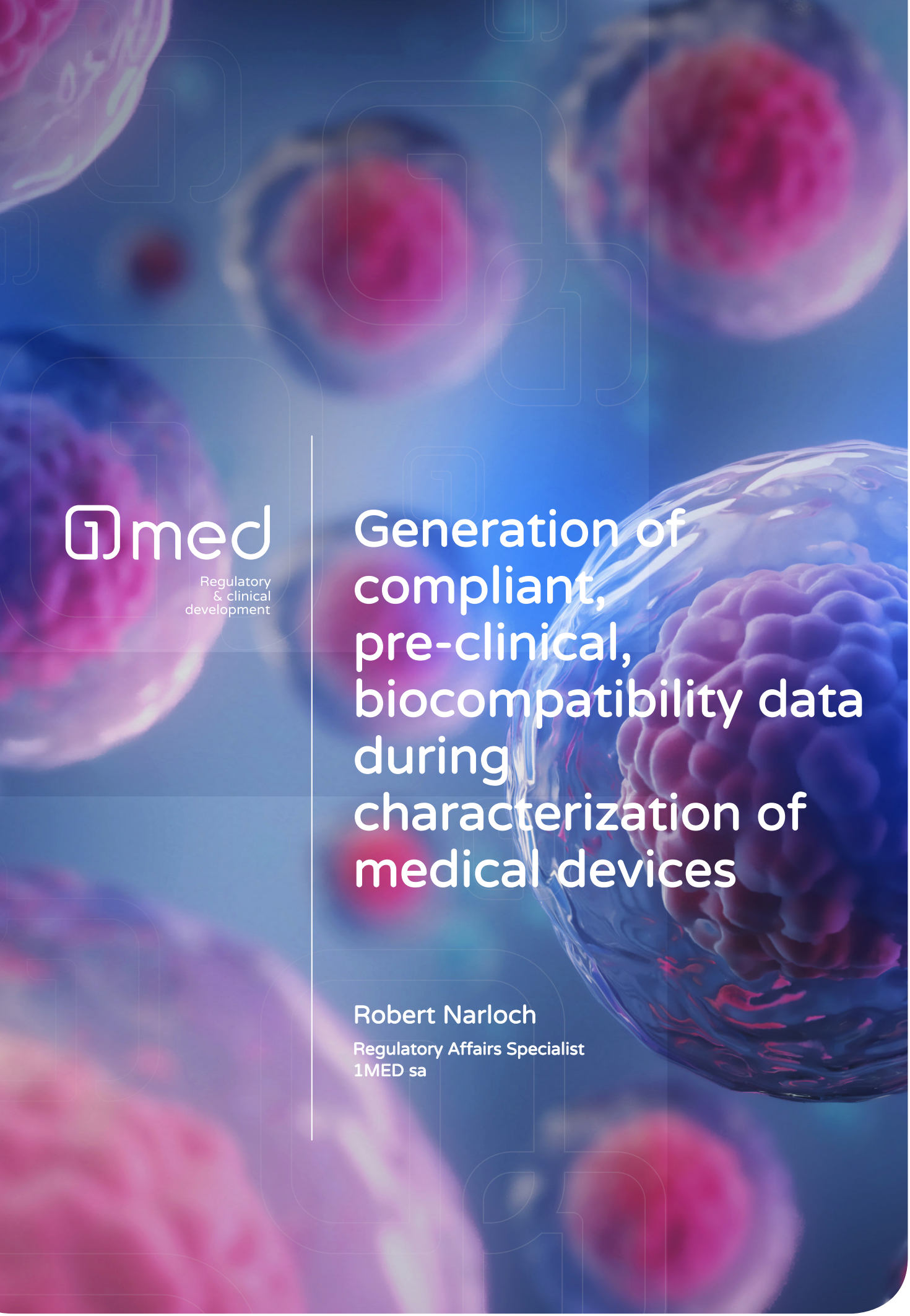

1med
Regulatory
& clinical
development

Generation of compliant, pre-clinical, biocompatibility data during characterization of medical devices

Robert Narloch
Regulatory Affairs Specialist
1MED sa

Contents

page 4 **1 Introduction**

5 1.1 Medical device definition
1.2 Responsibility of the Manufacturer
1.3 Responsibility of the Notified Body
6 1.4 Importance of characterization of the device for equivalence claim
7 1.5 ISO standards

8 **2 STEP 1 - Biological Evaluation Plan**

9 **3 STEP 2 - ISO 10993-18:2020 - Chemical characterization**

10 **4 STEP 3 - ISO 10993-17 - Toxicological risk assessment (based on chemical characterization outcome)**

11 **5 STEP 4 – Laboratory testing**

11 5.1 ISO 10993-12 – Extracts preparation and reference materials
5.2 In vitro testing
5.2.1 ISO 10993-5 - Cytotoxicity
13 5.2.2 ISO 10993-3 - Genotoxicity
5.3 Ex vivo testing
5.3.1 ISO 10993-4 - Hemocompatibility
14 5.4 In vivo testing
5.4.1 ISO 10993-10 - Allergic sensitization
15 5.4.2 ISO 10993-23 – Irritation
5.4.3 EP/USP/JP - Material-mediated pyrogenicity
16 5.4.4 ISO 10993-11 - Systemic toxicity
5.4.5 ISO 10993-6 - Implantation

17 **6 Accurate generation of reliable pre-clinical data**

6.1 Aseptic handling and limited manipulation
6.2 Correct sample labelling, even if temporary
6.3 Selection of the testing facility
18 6.4 Golden rules during lab work
6.5 False results and test artifacts
6.6 Unexpected result
19 6.7 Interference with assay method
6.8 Worst-case scenario
20 6.9 Importance of the predicate device
6.10 Correct use of the device in laboratory conditions

21 **7 Conclusions**

Disclaimer

No warranties

This White Paper is an informative document and does not constitute an official or agreed position. The information contained within this document is the product of professional research and reflects a professional judgment and has been compiled from sources deemed to be both expert and reliable, however the views expressed are entirely those of the authors. No representation or warranty is made or given as to the completeness of its content.

Limitation of liability

While every care has been taken in developing and compiling this paper, in no event 1MED shall be liable in relation to the contents of, or use of, its documents or for any consequential loss or damage, incidental, indirect or direct in connection with reliance on its contents. You agree that the limitation of liability set out in this disclaimer is reasonable; if you do not think it is reasonable, you should not make reference to 1MED's documents.

Copyright

1 Introduction

Successful testing is the one that brings to the successful and conform result through a planned approach, in line with current regulatory requirements and normative references.

Experts from technical committees have been constantly and tirelessly working on assuring the efficient test methods for the safety of medical devices and on the applicability of those methods to various typologies of medical devices. With a number of standards to follow, there is plenty of work to do before safely placing a medical device on the market. Unfortunately, choosing the right testing approach is not enough.

In this White Paper, a schematic and planned approach to biocompatibility is discussed, aimed to minimize the unforeseen events that may occur during the laboratory phase and exclude the risk of unnecessary and expensive repetition of tests, especially those involving laboratory animals. The additional advantages could be savings of both time and economical resources and a generation of reliable data sets to support a medical device's biological safety.

1.1 Medical device definition

Chapter I, Article 2 of the Medical Device Regulation (EU) 2017/745 (MDR) provides the following definition for medical device:
'medical device' means any instrument, apparatus, appliance, software, implant, reagent, material or other article intended by the manufacturer to be used, alone or in combination, for human beings for one or more of the following specific medical purposes:

- diagnosis, prevention, monitoring, prediction, prognosis, treatment or alleviation of disease,
- diagnosis, monitoring, treatment, alleviation of, or compensation for, an injury or disability,
- investigation, replacement or modification of the anatomy or of a physiological or pathological process or state,
- providing information by means of in vitro examination of specimens derived from the human body, including organ, blood and tissue donations, and which does not achieve its principal intended action by pharmacological, immunological or metabolic means, in or on the human body, but which may be assisted in its function by such means.

The following products shall also be deemed to be medical devices:

- devices for the control or support of conception,
- products specifically intended for the cleaning, disinfection or sterilization of devices as referred to in Article 1(4) and of those referred to in the first paragraph of this point.

1.2 Responsibility of the Manufacturer

According to the MDR Annex II, pt. 6.1. "Pre-clinical and clinical data", the manufacturer is required to provide detailed information regarding test design, complete test or study protocols, methods of data analysis, in addition to data summaries and test conclusions regarding in particular:

- the **biocompatibility** of the device, including the identification of all materials in direct or indirect contact with the patient or user,
- physical, **chemical,** and microbiological characterization.

Under the MDR, it is a responsibility of the manufacturer to demonstrate that the finished device, which is the result of materials used and processes, is safe for humans.

1.3 Responsibility of the Notified Body

As specified in the MDR Annex VII, pt. 4.5.4. "Pre-clinical evaluation", the notified body shall have documented procedures in place for the review of the manufacturer's **procedures** and documentation relating to the evaluation of pre-clinical aspects.
The notified body shall examine, validate and verify that the manufacturer's procedures and documentation adequately address:

- the planning, conduct, assessment, reporting, and - where appropriate - updating of the pre-clinical evaluation, in particular of the scientific pre-clinical literature search, and the pre-clinical testing, for example laboratory testing, simulated use testing, computer modelling, the use of animal models,

- the nature and duration of body contact and the specific associated biological risks,
- the interface with the risk management process, and
- the appraisal and analysis of the available pre-clinical data and its relevance with regard to demonstrating conformity with the relevant requirements of Annex I.

The notified body's assessment of pre-clinical evaluation procedures and documentation shall address the results of literature searches and all validation, verification and testing performed and conclusions drawn, and shall typically include considering the use of alternative materials and substances and take account of the packaging, stability, including shelf life, of the finished device. Where no new testing has been undertaken by a manufacturer or where there are deviations from procedures, the notified body in question shall critically examine the justification presented by the manufacturer.

1.4 Importance of characterization of the device for equivalence claim

As laid down in the MDR Annex XIV, "Clinical Evaluation and Post-Market Clinical Follow-Up", Part A, pt. 3, clinical evaluation may be based on clinical data relating to a device for which **equivalence** to the device in question can be demonstrated. The following technical, biological and clinical characteristics shall be taken into consideration for the demonstration of equivalence:

- Technical: the device is of similar design; is used under similar conditions of use; has similar specifications and properties including physicochemical properties such as intensity of energy, tensile strength, viscosity, surface characteristics, wavelength and software algorithms; uses similar deployment methods, where relevant; has similar principles of operation and critical performance requirements,

- Biological: the device uses the **same materials or substances in contact with the same human tissues or body fluids for a similar kind and duration of contact and similar release characteristics of substances, including degradation products and leachables,**

- Clinical: the device is used for the same clinical condition or purpose, including similar severity and stage of disease, at the same site in the body, in a similar population, including as regards age, anatomy and physiology; has the same kind of user; has similar relevant critical performance in view of the expected clinical effect for a specific intended purpose.

The characteristics of equivalent devices shall be similar. There should be **no clinically significant difference** in the safety and clinical performance of the device. Importantly, considerations of equivalence shall be based on proper scientific justification. In this case, both the equivalent device and the proposed device must be appropriately characterized. In the context of biological evaluation, the equivalence is established based on the criteria described in Annex C of ISO 10993-18 and takes into consideration endpoint equivalence (intended clinical use) and material equivalence (e.g., extractables profile). This requirement underlines the importance of a correctly conducted chemical characterization and evaluation of in vitro and in vivo endpoints.

1.5 ISO standards

International Organization for Standardization has put at the disposal of medical device manufacturers and industry professionals a set of consensus-based standards. These standards, such as ISO 14971:2019, allow to analyze and manage the risks associated with medical devices. ISO 10993-1:2018 is aimed to provide a structured approach to plan a biological evaluation within a risk assessment process that minimizes the use of test animals: preference is given to the assessment of chemical/physical properties through testing using in vitro models. These methods are employed when the results provide equally relevant information to that obtained from animal models. A responsible and justified use of animals according to 3Rs (Replacement, Reduction, Refinement) is regulated by ISO 10993-2:2006 (Animal welfare requirements), currently under revision. The need to characterize a medical device is underlined in this standard and described in detail in the entire family of ISO 10993 standards for each of the endpoints, such as chemical characterization, cytotoxicity, irritation, sensitization, systemic toxicity, pyrogenicity, hemocompatibility, genotoxicity, implantation, just to name a few.

2 STEP 1 Biological Evaluation Plan

The first step of a successful biocompatibility testing program is a Biological Evaluation Plan (BEP) - a part of the Risk Management Plan. A simple execution of laboratory tests without taking into account various aspects of biocompatibility listed in Annex A of ISO 10993-1:2018 is not sufficient. Such approach does not meet the requirements of ISO 14971:2019. It is worth reminding that Table A.1 (Endpoints to be addressed in a biological risk assessment) is not a checklist and should not be treated as one: rather, it serves as a tool for the selection of the proper battery of tests and so to build a suitable strategy that can always differ between medical devices, especially based on their nature and materials.

The aim of the BEP is to plan the biological evaluation of a medical device based on its intended use, biocompatibility, and toxicological data available on the constituents, manufacturing process, packaging, sterilization, and current regulatory requirements.

BEP should consider a series of risks, where the biological risk is only one of the aspects that must be taken into consideration. The plan should start with an evaluation of the physical and chemical characteristics of the medical device in the light of its intended use. Any available data - from all reliable sources - such as toxicological and biological safety data should be gathered and evaluated. Additional tests, especially those involving laboratory animals, thus in harmony with 3Rs definition, should only be performed if necessary. The laboratory testing should ideally cover all gaps in the characterization of the medical device.

BEP should depict a suitable strategy for device testing, based on the literature search and already available data on the medical device under evaluation and its constituents.

ISO 10993-1 prioritizes chemical characterization of the medical device as a first-line test. Further investigations can be planned as a next step based on the outcome of the chemical tests. In case chemical data are conflicting with biological data, then the biological tests should be given bigger weight as their results are directly derived from complex biological systems.

The implementation of the strategy defined in the Biological Evaluation Plan gives rise to the Biological Evaluation Report, discussing the outcome of literature searches and all the results obtained from laboratory studies supporting the biological safety of the medical device.

3 STEP 2 - ISO 10993-18:2020 Chemical characterization

Chemical characterization (CC) is performed with the aim to investigate the nature of materials that constitute the medical device in its final form. CC can address the systemic effects rather than local ones; for example, systemic toxicity is more likely to be suitably addressed by chemical characterization than irritation.

Chemical characterization consists in verifying of the identity and the number of chemical substances extracted from the device under exaggerated conditions or leached from the device under conditions of simulated clinical use. It is important that CC considers the manufacturing process, in particular additives and residues. There is a number of compounds belonging to various classes (Volatile, Semi-Volatile and Non-Volatile Organic Compounds, Inorganic) that can be identified by means of different chromatographic techniques (HS-GC/MS, GC/MS, LC/MS, ICP/MS). These compounds represent a “chemical signature” of the medical device under investigation and based on their concentration, may play a key role in the biological response of the human body.

Once the chemical profile of the device is obtained (qualitative and quantitative analysis of substances, where applicable), a toxicological assessment can be performed.

The new release of ISO 10993-18:2020 on chemical characterization is better integrated and harmonized with ISO 10993-1, ISO 10993-12 on sample preparation, and ISO 10993-17 on the allowable limits for leachable substances. Authors from the Technical Committee ISO/TC 194 underline that analytical testing (especially in vivo) is not necessarily required if chemical characterization results do not pose any doubts or concerns to medical device safety.

4 STEP 3 - ISO 10993-17 Toxicological risk assessment (based on chemical characterization outcome)

Pre-clinical chemical data generated from the medical device in chemical characterization studies must be evaluated by an experienced professional (toxicologist) to assess whether an exposure dose of chemical constituents is without appreciable harm to health. Guidelines and criteria for toxicologists are specified in ISO 10993-17 and describe the toxicological risk assessment process used within the biological evaluation process. If any risk is identified, in vivo tests may respond to the concerns on the safety of the device as they provide results deriving directly from a biological system.

The toxicological evaluation is performed by calculating the Permitted Daily Exposure, Tolerable Intake, and values like No Observed Adverse Effect Level (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) from the toxicological literature databases.
A favorable toxicological profile of the device may eliminate some unnecessary testing (e.g., genotoxicity).

5 STEP 4 - Laboratory testing

5.1 ISO 10993-12 – Extracts preparation and reference materials

One of the most critical aspects of medical device testing is extraction. The choice of the extraction procedure should always be justified in every study plan and final report (both GLP and non-GLP) for all types of studies where the extraction is performed. ISO 10993-12 provides guidance on the preparation of samples and selection of reference materials for medical device testing; the standard is applicable to all parts of ISO 10993 series.

Testing should be performed on the final (finished) product and, if possible, the device should be tested according to its clinical use. However, if testing of the device "as it is" becomes impossible, then the device may be cut, and the representative portions (represented proportionally) of the product can be tested. This choice must be justified in the final report. This aspect is one of the most critical issues of medical device extract preparation. Particular attention should also be paid while cutting the device as it would expose parts that are not in contact with the human body. Additional attention must be paid to device surface area, as it changes after cutting - it increases as new parts of the device are exposed.

The extraction should be performed in inert (not impacting the result) and closed (not allowing media evaporation or alteration) containers. The device should be completely covered or filled, based on the selected approach and the clinical use. Positive and negative controls should be processed in the same way as the test item. In the case of devices with coating, both coating and substrate must be tested. Experimental controls (negative, positive, vehicle) are always included to validate the test procedure and must meet the acceptability criteria.

Extraction is a complex process impacted by time, temperature, surface-area-to-volume ratio and extraction vehicle and should not cause test material degradation (although some swelling may be acceptable). The extraction must be performed in dynamic conditions with agitation or circulation to maximize the extraction potential of the solvent. Among extraction media, the following can be listed: culture medium (cytotoxicity), saline solution, cottonseed oil, sesame oil (in vivo tests). The extraction ratio (surface area/volume) must be chosen based on the type of medical device and on the clinical use. Mass to extract volume ratio should only be used if surface area cannot be calculated by the manufacturer, or if use of mass/volume ratio will result in a worst-case approach if compared to surface area/volume.

Extracts should be used straight away after preparation, and it is not allowed to manipulate them by pH adjustment, filtration, or centrifugation. The aspect of the extract and any visible changes (clouding, particulate, sediment, color change) must be described in the final report.

5.2 In vitro testing

5.2.1 ISO 10993-5 - Cytotoxicity

Cell culture methods have shown an excellent sensitivity. They are complementary to animal testing and provide a predictive measure of the toxic potential of substance-based medical devices or medical devices' extracts. In vitro viability test is recommended for assessing the cytotoxic potential when evaluating new materials or formulations for possible use in medical applications or as part of quality control for established medical materials and devices.

Cytotoxicity, described in ISO 10993-5:2009, is a cost-effective test that may help identify – among test products – the best candidate for further testing or test the materials before their employment in medical device production. Cytotoxicity should be performed after chemical characterization and before in vivo testing; it is generally required for all body-contacting medical devices and represents the first test of the "big three" biocompatibility panel, together with sensitization and irritation. Cytotoxicity is performed on immortalized and commercially available cell lines and does not pose any concern regarding animal welfare or ethical issues.

The cytotoxicity test is based on an assessment of cell death by the use of vital dyes (Neutral Red, MTT, XTT), allowing to measure cell proliferation and viability via their metabolic activity after treatment with the test item.

The cellular response to the medical device extract (or direct application) is cell death, which may not always reflect the device's toxic potential or its effects in the long run. That is why cytotoxicity test cannot be interpreted as "pass" or "fail"; though, it gives an important hint to further testing.

In case of a cytotoxic result, the cause of elevated cell death rate should be investigated, and it should be demonstrated (by dilution testing) at what concentration the medical device or its extract is no more cytotoxic. The cytotoxic result can be justified by the successful in vivo and in vitro testing results that can overturn the unfavorable cytotoxicity outcome. This approach makes cytotoxicity a useful tool for other in vitro and ex vivo tests (e.g., in vitro irritation, genotoxicity, hemocompatibility) and contributes to a more detailed characterization of a medical device and to the understanding of the biological response of a test system.

5.2.2 ISO 10993-3 - Genotoxicity

Genotoxicity assays are based on induction of DNA damage, producing a genotoxic response from the in vitro cellular system (eukaryotic or mammalian) or in the animal model. Genotoxicity testing allows detection of gene mutations, changes in chromosomes and gene toxicities caused by the components of a medical device (substance-based medical devices) or by extractable and leachable compounds. However, genotoxic effects may not always derive from mutations. In general, all mutagens are genotoxic, but not all genotoxic substances are necessarily mutagenic. As stated in ISO 10993-3, no single test can detect all relevant genotoxic agents; therefore, the usual approach is to conduct a battery of in vitro tests and - under certain circumstances - additional in vivo tests.

When genotoxicity testing is performed, the test battery shall include (modified for medical devices, ISO/TR 10993-33):

- test for gene mutations in bacteria (AMES test, OECD 471),

and one of the following in vitro tests in mammalian cells:

- test with cytogenetic evaluation of chromosomal damage with mammalian cells (OECD 473), or
- mouse lymphoma tk assay (OECD 476), or
- mammalian cell micronucleus test for chromosomal damage and aneugenicity (OECD 487).

If any result in the mammalian test system is positive, the decision of performing an additional in vitro or in vivo genotoxicity study should be taken into consideration.
For example, an in vivo assay is not necessary if it can be demonstrated that the quantities of extractables from the test article are less than the amount of material that would induce a positive response with a potent, well-characterized in vivo micronucleus genotoxin ("positive toxicological profile", paragraph 4).

5.3 Ex vivo testing

5.3.1 ISO 10993-4 - Hemocompatibility

ISO 10993-4 provides guidelines for the evaluation of hemocompatibility for blood-contacting medical devices. The selection of the right test should be based on the intended use of the medical device.
The test methods and evaluation criteria are available in various standards, such as ASTM F756. ISO 10993-4 gives a broader look at the device's typology regarding ISO 10993-1. It enlists a wide range of blood-contacting devices and suggests various tests to investigate the activation of molecular mechanisms leading to hemolysis, coagulation, blood cells depletion, or complement activation. Although ISO 10993-4 defines the categories of tests, it is up to the manufacturer to prepare a testing strategy and decide which of the hemocompatibility methods to employ and to demonstrate the safety of the medical device.

Hemolysis

The hemolysis test measures the hemolytic potential of the medical device in a qualitative way.
The test item is tested indirectly (physiological saline extract) and directly by contact with the blood substrate. Hemoglobin from hemolyzed red blood cells is released and measured by photometric detection.

Coagulation – Thrombin-antithrombin (TAT) complex formation
Medical devices should not possess any coagulation activity. Coagulation begins thanks to the contact activation pathway, with the formation of active thrombin, which promotes the formation of fibrin and initiating the coagulation cascade. Increased TAT levels indicate a high thrombogenic potential.

Hematology
The complete blood count is used to estimate the concentrations of White Blood Cells (WBC), Red Blood Cells (RBC) and Platelets (PLT) in blood. The analysis is performed before and after direct blood contact with a tested medical device. Both data are compared to help understand the medical device's impact on blood cells and phenomena, such as platelet sequestration (activation, aggregation, adhesion) or lower number of erythrocytes (possible hemolysis). The blood count provides an insight into the thrombogenic potential of the medical device.

Platelet activation - β-Thromboglobulin activation (β-TG)
Activated platelets release β-Thromboglobulin. This test, together with complete blood count, determines - by measurement of levels of β-TG - the activation of platelets after the exposure of the medical device to blood.

Complement system activation (C3, SC5b-9)
The complement system is an integral part of the immune response and the coagulation cascade. It is a major effector within innate immunity and a universally distributed defense mechanism. Once activated, the complement system works via the formation of membrane attack complex (MAC) to clear the body from foreign elements through lysis (directly) or phagocytosis (indirectly, by recruiting cells). Augmented levels of complement activation can be found in a variety of pathologic states, including inflammatory and autoimmune diseases, such as Systemic Lupus Erythematosus and Rheumatoid Arthritis.

5.4 In vivo testing

5.4.1 ISO 10993-10 - Allergic sensitization

Generally, all medical devices need to be evaluated for skin sensitization endpoint according to ISO 10993-1:2018.
Sensitization is the allergic response following repeated exposure to an allergen (antigen). Hypersensitivity reaction can happen after a single or multiple contact with the allergen. The sensitization test evaluates the sensitizing capacity to the exposure of a tested medical device or its extract (both polar and non-polar). Three types of test can be distinguished:

- Magnusson and Kligman Guinea Pig Maximization Test (GPMT; e.g., extractable devices),
- Buehler Sensitization (patch test; e.g., substance-based medical devices), and
- Murine Local Lymph Node Assay (to determine sensitizing potential of chemicals).

All of them involve the use of laboratory animals.

Currently, there is no approved, official, in vitro test for the assessment of hypersensitivity for medical devices. Efforts are made by the scientific community (both private and state-owned) together with The European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), aimed

to provide a reliable in vitro test for an errorless identification of mild, medium and strong sensitizers, with concomitant replacement of tests involving animals.
On a molecular level, the key chemical and biological events underlying skin sensitization have been extensively described in an OECD document on an Adverse Outcome Pathway (AOP). This AOP recapitulates the impact of skin exposure and lists all key events starting from the molecular initiating event, e.g., covalent binding of a chemical to skin protein, via intermediate key events like dendritic cell (DC) and keratinocyte, to the final event which is the initiation and activation of T cells that, after being challenged, will be the effector cells in the clinical manifestation in the living organism: Allergic Contact Dermatitis (ACD).
Nowadays, the need to replace animal use in skin sensitization testing must be of utmost importance, and it would allow to set up the "big three" biocompatibility panel (cytotoxicity, sensitization, and irritation) with the exclusive use of only in vitro techniques and by eliminating the animal testing, in the name of 3Rs: 'refine, reduce, replace'.
The pre-validation of SENS-IS® (EpiSkin™) assay is one of the most promising approaches. During the experimental testing, the assay correctly identifies the presence of sensitizers in medical devices extracts. Another promising example of in vitro skin sensitization test that employs human dendritic-like cell line is the in vitro GARD® (Genomic Allergen Rapid Detection, SenzaGen) skin medical devices assay, able to detect leachables as either skin sensitizers or non-sensitizers in polar and non-polar extraction of medical devices. These methods are highly developed and may soon find their use in completely replacing the animals in skin sensitization assays.

5.4.2 ISO 10993-23 – Irritation

Assessment of irritation effects is generally necessary for all patient-contacting medical devices. Irritation tests (skin, ocular, nasal, vaginal, penile, rectal) are proposed to evaluate the local response and the irritation potential of extractables or substance-based medical devices (e.g., vaginal creams, eye drops, skin gels, nasal sprays, etc.).
Intracutaneous reactivity test is performed whether the type of medical device under evaluation is not suitable for irritation tests. This test is preferred for medical devices or composing materials that contact body tissues or body fluids (e.g., external communicating devices, implantable devices, etc.). The test consists in the administration of polar and non-polar extracts from the medical device into the skin on the dorsal part of a test animal (rabbit) and in evaluating the inflammation induction or the tissue response (damage).
ISO 10993-23 has been published in January 2021 and includes alternative methods to animal testing for evaluation of skin irritation and intracutaneous reactivity. The new ISO standard was designed to refine, reduce and replace the use of animals for irritation by the employment of validated in vitro tests with reconstructed human epidermis (RhE).
A stepwise testing approach is desired by starting with the in vitro RhE model.
For confirmation or further categorization of the irritant activity, in vivo animal tests or human irritant tests should be taken into consideration.

5.4.3 EP/USP/JP - Material-mediated pyrogenicity

Medical devices must be free from pyrogens (pyrogenic – 'producing fever'). Pyrogens can derive from materials used in medical device design (leachables) and from bacterial endotoxins accumulated during the production process (lipopolysaccharide, LAL test), which can potentially cause a febrile reaction in patients using the device.
The most used methods for the rabbit pyrogen test are described in European (EP), United States

(USP) and Japanese (JP) Pharmacopoeia. The test involves the measurement of the temperature rise in rabbits following the intravenous injection of the test solution. Based on the result obtained from all tested animals, the device tested can be stated as “pyrogen free” (not giving rise to the body temperature) or “pyrogen” (causing the temperature rise, febrile state or fever).

5.4.4 ISO 10993-11 - Systemic toxicity

Systemic toxicity testing is generally necessary for all devices in contact with internal organs and their tissues. Medical devices must not show any systemic adverse effect.
Systemic toxicity test evaluates the hypothetical adverse effects of a medical device on the organs and relative tissues of the body. These effects are measured after different exposure regimens: a single, multiple or continuous. Various test methods can be distinguished based on the intended use of the device and duration of contact: acute, sub-acute, sub-chronic, and chronic. Acute systemic toxicity is evaluated after the administration of polar/non-polar extracts via topical, oral, intravenous or intraperitoneal route or implantation of the device. Sub-chronic and sub-acute toxicity studies can last up to 26 weeks and consist in constant or repeated exposure. If chronic toxicity data with a duration up to 12 months are required, the acute, sub-acute and sub-chronic data should be obtained first.

5.4.5 ISO 10993-6 - Implantation

The implantation test evaluates local effects on tissues and organs at a macroscopic and microscopic level, including an eventual absorption or degradation.
Implantation studies with an evaluation of local biological effects might provide information otherwise available only in systemic toxicity studies or carcinogenicity studies. Moreover, implantation tests can address some endpoints also covered by systemic toxicity, which can reduce the number of animals requested by eliminating unnecessary, double testing.

6 Accurate generation of reliable pre-clinical data

6.1 Aseptic handling and limited manipulation

The result of the tests is impacted not only by the materials used and by the medical device's production process, but also by the way medical devices are manipulated before testing. Before sending the medical device to the laboratory that will perform the test, it shall be ensured that the sampling will be performed minimizing the contamination. If the device is supposed to be sterile, it must be sterile when it reaches the testing site. Also, while handling samples that are not supposed to be sterile, gloves shall be worn, and adequate packaging shall be used. This is of utmost importance, especially for in vitro tests. Culture media used for the extraction usually contain antibiotics that prevent bacterial growth, but the percentage is low and highly influenced by the initial bioburden of the analyzed sample. Nonsterile samples must never be pre-treated with disinfectants if not foreseen by the production process, as it will negatively impact the result. Entrusting the qualified personnel with this initial part is crucial for delivering an intact, eligible test material.

6.2 Correct sample labelling, even if temporary

All GLP-accredited facilities store samples in rooms with controlled temperature with temperature monitoring systems. The storage is essential for all medical devices, as exposing them to extreme or uncontrolled temperatures may cause irreversible damage, such as degradation. Data generated from such devices are not reliable. Medical devices shall be correctly labelled, and the temperature of storage, expiry date and batch number shall be reported on the labelling and on the accompanying documentation. Also, the manufacturer should not test the device beyond the expiry date for obvious reasons, so the testing facility shall be provided with samples that have an expiry date covering the whole testing period (up to 6 months, depending on laboratory scheduling).

6.3 Selection of the testing facility

If GLP testing is needed, then the testing partner should be checked for a valid GLP certificate from national Authorities. It is also advisable to organize an inspection visit on site. A visit to the testing facility is not only a way of knowing better the team responsible for the delivery of results, but also a good way for verification of the working standards (SOPs for workflow, sample handling, validation and maintenance of instruments, training provided to the personnel, etc.). One of the features of a successful project is the collection and sharing of data and information. When the information is not shared, the risk of not keeping earlier arrangements is high. This is why another crucial element of testing is an efficient Project Management. A Project Manager (PM) assures that the specific goals (testing) are achieved within the given constraints (time). An efficient PM is a 'meeting point' of information from all stakeholders (manufacturer, sponsor, monitor, technical staff, accounting department, etc.).

6.4 Golden rules during lab work

Data generated is only considered reliable when the internal controls included in the experimental design meet the acceptability criteria. It is important to conduct a periodic check of positive and negative controls that verify if the desired characteristics are maintained, regardless of the expiry date or correct storage conditions. For this reason, as a part of a good practice, the suitability tests should be scheduled, conducted and kept in the archives to demonstrate the validity of controls at the disposal of the laboratory. Another point is a generation of a sufficient amount of data. A good example can be the in vitro test: cytotoxicity. The test is performed with cell lines in a multi-well plate. The absolute minimum for test sample is a triplicate, but the more replicates there are the more robust the data. It is a good practice to include also blanks to subtract the possible background noise.

6.5 False results and test artifacts

Even in validated testing procedures, the risk of generating false positive or false negative results cannot be excluded. Regardless of internal controls used and acceptability criteria, some events may occur that could put hardly obtained result in the discussion. One example of an artifact from in vivo testing is represented by histologic lesions associated with intravenous infusions of large volumes of isotonic saline solution in rats. Histologic lesions related to treatment with negative control included pulmonary infiltrates of eosinophils, pulmonary inflammation and granulomas, endothelial hypertrophy and hyperplasia within pulmonary arterial vessels, and pulmonary arterial medial thickening. These findings show that histologic lesions were already observed in the negative control treatment group and were ascribed to the infusion of large volumes in test animals. A different example could be testing of non-sterile samples for sterility or non-intentionally sterile samples for bioburden test. In both cases the result obtained is false, even if it is reliable.

6.6 Unexpected result

If the chemical characterization and subsequent toxicological evaluation resulted in a favorable profile of the medical device, then a bad result in in vitro or in vivo tests can be a real surprise. Nevertheless, in the case of a positive result (cytotoxic, pyrogenic, hemolytic, coagulant) it would be better to take a step back and analyze the possible causes.
A positive result in the cytotoxicity testing can be outweighed by in vivo assays. The cytotoxicity test is not a “pass” or “fail” kind of test, but it gives the idea of the cytotoxic potential of the device that usually, when positive, must be confirmed or overruled by further testing (irritation, sensitization). Medical devices, such as some vaginal creams, often give a cytotoxic result, but this does not necessarily mean they are not eligible to be marketed once they pass the irritation and sensitization tests. Some substance-based devices containing high percentages of alcohol are known to be cytotoxic, too.
When it comes to pyrogens (pyrogen test, LAL test), these two tests do not necessarily have to fail when the pyrogenicity is suspected. If a device passes the LAL test but fails the pyrogen test, the explanation could be that not all pyrogens are endotoxins. These tests distinguish between pyrogenic bacterial endotoxins and material-mediated pyrogenicity. The medical device must pass both tests: the pyrogen test in rabbit and bacterial endotoxin-detecting LAL test as these tests

detect pyrogenicity from different sources: from device design (induced by substances released from materials) or production process (inefficient sterilization process resulting in endotoxin presence). Hemocompatibility is another crucial element for the biocompatibility of blood-contacting devices. In the case of ex vivo test with blood, some unexpected results could be caused by the geometry of the device (protruding or sharp ends, also as the result of cutting before extraction procedure), impacting the integrity of blood cells used in the assay. Moreover, some laboratory techniques during sample processing (which would not occur during the clinical use) could cause unexpected lysis of red blood cells resulting in a false positive, hemolytic result. In general, the analytical approach should be revised, and the result should be discussed. In many circumstances, the benefit for the patient outweighs the risk carried by the device; in this case, a clinical expert statement should be obtained. However, the manufacturer should verify and prove by all means the safety of the device in a certified, external laboratory. Special attention should be paid to hemostatic medical devices during hemocompatibility testing.

6.7 Interference with assay method

Not all tests are feasible for all medical devices. It may happen that a substance-based device contains ingredients interfering with vital dyes, so the cytotoxicity assay may not give a reliable result. This is something to be aware of when selecting the proper cytotoxicity assay. Sometimes the dyes (or other ingredients) used to manufacture the medical device may migrate to or simply dissolve in the solution, making it impossible to perform qualitative and/or quantitative evaluation. In this case, the substance released from the device can interfere with vital dyes such as MTT, XTT or NRU or even make the visual evaluation with a naked eye impossible.
Another issue may be the incompatibility of non-sterile medical devices and in vitro assays. Depending on their initial bioburden of spores, they may interfere with assays such as cytotoxicity or AMES test, creating a bacterial lawn and interfering with cell line (cytotoxicity) or bacterial strains used in the assay for mutagenicity (AMES). Not always sterilization of the device is a good idea, as sterilization of natural products may result in their degradation (high temperatures) and should not be performed, if not foreseen by the production process. Some other attempts, such as sterilization by irradiation, may not be suitable and causes discussion. In addition, sterilization by filtration is not always feasible for substance-based medical devices and depends highly on their ingredients and parameters, such as density.

6.8 Worst-case scenario

In general, exaggerated extraction conditions are preferred by reviewers. This approach can guarantee that the extract contains a combination of all extractable substances and the absence of undesired biological effect, in this case, is comforting.
However, “worst-case scenario” is not always the winning approach. The testing should be as close to the clinical use as possible, so the biological response is closest to the true state. Sometimes the complexity of the device does not allow the application of the same extraction conditions to all of its parts. For example, in the case of enteral administration sets, the extraction should be separate for the implanted part, the part in contact with the enteral solution and the part in contact with tissues but not with the enteral solution.

6.9 Importance of the predicate device

Comparator device inclusion for parallel testing is crucial for hemocompatibility testing but is also applicable to other tests from the biocompatibility panel, especially for (but not limited to) the US market. Predicate device (or comparator device) is a registered, legally marketed medical device to which equivalence is drawn. A claim of substantial equivalence does not mean the device must be identical. Equivalence is established with respect to intended use, design, energy used or delivered, materials, performance, safety, effectiveness, labeling, biocompatibility, standards, and other applicable characteristics. Let's suppose that the predicate device (already on the market) performs fairly and the test item (not yet on the market) performs in the same manner. Still, the result is out of specification for both devices. In this case, a formal opinion can be asked to an expert in a subject matter (e.g., clinical hematologist for blood-contacting devices), aimed to justify the "out of specification" result of the new test item seen a similar performance of already marketed comparator. This could avoid repeated, unnecessary and expensive re-testing.

6.10 Correct use of the device in laboratory conditions

To ensure the correct handling of the device in the testing facility, the manufacturer should provide as much information as possible to guarantee the testing as by clinical use, providing the technical staff reliable information on the sample assembly and handling.
This is of great importance, especially in the case of complex administration sets, but also for substance-based medical devices where some initial steps of preparation are required before the application. For example, some medical devices in dentistry need to be mixed before use. During the mixing, a temporary appearance of fumes or vapors can be observed: these substances may negatively impact the biological reaction of cell lines (cytotoxicity). Some mixing reactions are exothermic and may cause the extraction medium degradation (cell culture medium) or evaporation. The provision of device Instructions For Use (IFU) and other supporting documentation, including videos, can be of great help for the laboratory staff.

7 Conclusions

The approach to biological evaluation for medical devices has significantly changed, and the release of ISO 10993-1 in 2018 put a stronger emphasis on biological safety planning, including physical and chemical characterization of medical devices. Moreover, the EU Regulation 2017/745 (MDR) stresses the importance of chemical, physical and biological properties of medical devices.

1MED team includes skilled and experienced personnel dedicated to the definition of the proper pre-clinical and regulatory pathway for your medical devices.
We are faithful to the motto: *"Right strategy is cost-effective and eliminates unnecessary testing"*.
Our tests are performed in 1LAB and in external testing laboratories in Europe.

For more information on our pre-clinical and regulatory services, please visit: https://1med.ch

References

- Regulation (EU) 2017/745 of the European Parliament and of the Council of 5 April 2017 on medical devices amended by Regulation (EU) 2020/561 of the European Parliament and of the Council of 23 April 2020;
- ISO 14971:2019 – Medical devices - Application of risk management to medical devices;
- EN ISO 13485:2016 – Medical Devices - Quality management systems - Requirements for regulatory purposes;
- ISO 10993-1:2018 – Biological evaluation of medical devices – Part 1: Evaluation and testing within a risk management process;
- ISO 10993-2:2006 — Biological evaluation of medical devices – Part 2: Animal welfare requirements;
- EN ISO 10993-3:2014 – Biological evaluation of medical devices – Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity;
- EN ISO 10993-4:2017 - Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood;
- EN ISO 10993-5:2009 – Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity;
- EN ISO 10993-6:2016 – Biological evaluation of medical devices - Part 6: Tests for local effects after implantation;
- ISO 10993-10:2010 – Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization;
- EN ISO 10993-11:2018 – Biological evaluation of medical devices - Part 11: Tests for systemic toxicity;
- EN ISO 10993-12:2012 – Biological evaluation of medical devices - Part 12: Sample preparation and reference materials;
- ISO 10993-17:2009 – Biological evaluation of medical devices - Part 17: Establishment of allowable limits for leachable substances;
- ISO 10993-18:2020 – Biological evaluation of medical devices - Part 18: Chemical characterization of medical device materials within a risk management process;
- ISO 10993-23:2021 – Biological evaluation of medical devices - Part 23: Tests for irritation;
- ISO/TR 10993-33:2015 – Biological evaluation of medical devices - Part 33: Guidance on tests to evaluate genotoxicity - Supplement to ISO 10993-3;

- U.S. Food and Drug Administration (FDA) 2020. Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process";

- Morton D, Safron JA, Glosson J, Rice DW, Wilson DM, White RD. Histologic lesions associated with intravenous infusions of large volumes of isotonic saline solution in rats for 30 days. Toxicol Pathol. 1997 Jul-Aug;25(4):390-4;

- Cedzyński M, Thielens NM, Mollnes TE, Vorup-Jensen T. Editorial: The Role of Complement in Health and Disease. Front Immunol. 2019; 10: 1869;

- https://www.fda.gov/medical-devices/premarket-notification-510k/how-find-and-effectively-use-predicate-devices;

- https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/ validated-test-methods/skin-sensitisation;

- Pellevoisin C, Cottrez F, Johansson J, Pedersen E, Coleman K, Groux H. Pre-validation of SENS-IS assay for in vitro skin sensitization of medical devices, Toxicology in Vitro, Volume 71, 2021;

- In vitro skin sensitization testing of Medical Devices using GARD®. Rose-Marie Jenvert, Angelica Johansson, Olivia Larne, Emelie Danefur, Emil Altonen, Anders Jerre, Robin Gradin, Gunilla Grundström. SenzaGen, Lund, Sweden. Poster presented at Eurotox, Helsinki, Sep 9, 2019;

- OECD (2020), Test No. 471: Bacterial Reverse Mutation Test ,OECD Guidelines for the Testing of Chemicals, Section 4. OECD Publishing, Paris;

- OECD (2016), Test No. 473: In Vitro Mammalian Chromosomal Aberration Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris;

- OECD (2016), Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris;

- OECD (2016), Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris;

- ASTM F756 - 17 Standard Practice for Assessment of Hemolytic Properties of Materials;

- United States Pharmacopoeia (USP 43–NF 38);

- European Pharmacopoeia (Eur. Ph.) 10th Edition;

- Japanese Pharmacopoeia (17th Edition with Supplements);
Organisation for Economic Co-operation and Development. The Adverse Outcome Pathway for Skin Sensitisation Initiated by Covalent Binding to Proteins. ENV/JM/MONO(2012)10/PART1&PART2. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2012)10/PART1&docLanguage=En

Contributors

1MED is grateful to all the professionals involved in the development of this document.

Author

Robert Narloch, Regulatory Affairs Specialist at 1MED
Mr. Narloch has eight years of experience in medical device pre-clinical testing with background in molecular biology. He is currently in charge of regulatory activities as a Regulatory Affairs Specialist at 1MED; he has experience in the preparation of biological evaluations and clinical evaluations for various types and classes of medical devices.
Mr. Narloch holds a M.Sc. in Medical Biotechnology from the University of Gdansk, Poland and a PhD in Molecular and Translational Medicine from the University of Milano-Bicocca, Italy.

Peer reviewers

Michela Mori, Regulatory Affairs Manager at 1MED
Mrs. Mori has more than ten years of experience in testing and regulatory aspects pertaining to substance-based products. In her current role as Regulatory Affairs Manager at 1MED, she constantly supports clients in the preparation of technical documents and in providing regulatory consultancy with particular focus on pre-clinical and clinical evaluation processes. Thanks to her regulatory and pre-clinical expertise, Mrs. Mori also supports the design and development of in vitro tests conducted at 1LAB laboratory.
Mrs. Mori holds a M.Sc. in Pharmacy and a II Master Degree in Pharmaceutical Technology and Regulatory Affairs from the University of Pavia, Italy. She also took a PhD in Biopharmaceutic and Pharmacokinetics from the University of Parma, Italy.

Sara Fosforani, Regulatory Intelligence and Strategy Manager at 1MED
Mrs. Fosforani has more than seven years of professional experience in regulatory affairs. She currently manages regulatory intelligence activities at 1MED. She has experience in the evaluation of products' feasibility and classification and, as a Regulatory Affairs Manager, she supported clients in the definition of a proper regulatory strategy and in the preparation of technical documentation, including biological and clinical evaluations.
Mrs. Fosforani holds a B.A in Biotechnology and a M.Sc. in Medical and Pharmaceutical Biotechnology from the University of Pavia, Italy.

1med
Regulatory
& clinical
development

About 1MED

1MED is a consultancy Company and a full service CRO based in Tessin (Switzerland), providing regulatory strategy, clinical trial management and quality assurance services to the medical device and pharmaceutical industries.

For more information please visit:
https://1med.ch

1MED SA
Via Campagna, 13
6982 Agno
Switzerland

+41 (0)91 605 40 50