Chapter 11

Medical Device Preclinical Testing

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OBJECTIVES

- Provide an overview of preclinical testing and its role in medical device design and development
- Discuss the objectives of the different types of preclinical testing
- Provide examples of preclinical testing of devices

DIRECTIVES, REGULATIONS AND GUIDELINES COVERED IN THIS CHAPTER

- Manual on borderline and classification in the Community regulatory framework for medical devices (only for the Medical Devices Directive)
- NBOG BPG 2009-4 Guidance on notified body’s tasks of technical documentation assessment on a representative basis
- GHTF SG1/N011 2008: Summary Technical Documentation (STED)

Introduction

The purpose of preclinical testing is to ensure the device meets all its requirements, including user requirements, regulatory requirements, requirements from (harmonised/recognised) standards and risk management requirements. During the preclinical testing process, the product design might have to be modified to meet these requirements. Both the US Food and Drug Administration (FDA) and the latest version of ISO 13485 require that design outputs meet design inputs.

Preclinical testing covers both the in vitro and in vivo tests to ensure a device’s safety and efficacy. This chapter discusses the regulatory aspects of the preclinical studies required before a device proceeds to the clinical trial stage or achieves marketing approval.

Preclinical Test Plan

During design, the manufacturer’s selection of materials to be used to produce the device should fit the device’s purpose, i.e., mechanical, physical, chemical and toxicological.

Current literature and other available information should be reviewed to determine what standard testing, purity data or biocompatibility studies associated with the (raw) materials already exists. The manufacturer’s choice of materials should depend partly on the sterilisation method, as some materials and some intended uses are more suited for certain sterilisation methods.
As stated above, inputs for the preclinical test plan include specific performance requirements in relation to the intended use of the device, requirements from regulatory bodies and requirements identified during the risk management process. These requirements are not fixed but can change as a result of the outcome of preclinical testing.

Table 11-1 shows how the design traceability matrix connects the requirements to the test results by listing the requirements on the y-axis and the design inputs and outputs in the columns in the x-direction.

**User Requirements**

User requirements, or customer requirements, should meet the needs of the user. The user is by default the patient, but depending on the type of the device, the medical professional also can be a user. User requirements are closely related to the device’s intended use, actual use and are often performance requirements.

User requirements are often described in a “soft” way but need to be measurable to become a testable requirement. For example, “widening the trachea” can be translated into a testable minimum percentage of diameter increase. In the preclinical phase, device performance can be tested through patient simulations. A discussion of the clinical trial process for medical devices falls outside the scope of this chapter.

**Regulatory Requirements**

In Europe, the main regulatory requirements are contained in the General Safety and Performance Checklist (EU MDD Annex I), replacing the well-known EU MDD Essential Requirements. The manufacturer must determine which of these general requirements are applicable to its device. The list does not contain any link to standards, so the manufacturer also must determine which standards are applicable. This is different from the system in the US, where FDA provides guidance on the standards that apply to each type of device.

The EU MDD lists mandatory “harmonised standards.” A similar list will be available in the EU MDR. As the list of harmonised standards in the EU MDD has not been updated in the last three years, notified bodies will require manufacturers to begin using the latest version of the applicable standards one year after the standard has been published. In addition, manufacturers will need to comply with a longer list of mandatory standards compared to the EU MDD’s list of harmonised standards.

Further, under the EU MDD, compliance with MEDDEV guidances also were considered mandatory. Currently, the Medical Device Coordination Group is working to publish a new set of guidances. In addition, the EU MDR has introduced “common specifications,” which can be considered similar to FDA’s guidances.

**Requirements from Standards**

One of the first steps in the preclinical testing planning process as well as in the development process is to determine and evaluate the current relevant industry standards. In the EU, many International Organization for Standardization (ISO) and International Electrical Commission (IEC) standards have been adopted, and these have been published as EN-ISO and EN-IEC standards, e.g., the ISO 10993-X (biocompatibility) series and the IEC 60601-1-X³ (electrical safety) series. However, only a limited number of these standards has been incorporated into the EU MDD’s harmonised standards list, which can be confusing for non-EU companies. In practice, there is little difference between the EU MDD and FDA-recognised standards, although FDA is quicker to recognise new standards and provides specific mandatory standards per product type (code), while the EU does not.

Apart from the very limited list of harmonised standards, there are many additional industry standards for conducting both in vivo and in vitro experiments, which could be considered “best practices.”

**Risk Management Requirements**

Risk management should follow the EN ISO 14971⁴ standard (2019 version), starting with a risk management plan, followed by the risk assessment process and finally the report. The risk management file (including the risk assessment) is updated during the development process, and new requirements may become known during the process.

During the risk assessment process, uncertainties are identified, which need to be mitigated by risk-reducing measures. This often involves testing, so these requirements will appear in the preclinical test plan and preferably also in the design traceability matrix.

Increasingly, standards are explicitly requiring risk management assessments to be conducted prior to setting up the test plan, including those contained in the Electrical Safety and EMC standards (EN IEC 60601 series) and the new main biocompatibility standard (EN ISO 10993-1).⁵ The output of the risk management assessment is the input for the preclinical test plan.

**Nonclinical Studies**

A nonclinical laboratory study is an *in vivo* or *in vitro* test in which samples of the medical device are studied prospectively in test systems to determine whether the device meets all requirements as determined during the planning phase.
Depending on the type of device, preclinical testing will contain one or more of the following tests:

- biocompatibility studies according to the EN ISO 10993-X series (and device-specific standards)
- performance testing
- packaging validation and transport studies
- sterilisation validation
- electrical safety and electrical magnetic disturbance testing (EN IEC 60601-X and -X-X series)
- shelf life (aging) studies
- software verification
- usability (human factors) studies

These test types will be discussed in more detail below.

### Biocompatibility Testing

All devices that are expected to have direct contact with a patient and/or user require a biocompatibility evaluation according to EN ISO 10993-1. This standard requires an analysis of the device type, the contact and the duration of the contact, and a risk assessment to determine the possible risks to the patient and/or user. Data can then be sought from toxicological databases and literature. If existing information is not sufficient to address concerns about the biological safety of the device, as is often the case, preclinical biocompatibility testing may be needed. The EN ISO 10993-1 standard addresses the different test types that should be considered.

These tests are described in detail in the EN ISO 10993-X series. Examples are:

- cytotoxicity testing
- sensitisation and irritation studies
- acute, subchronic, chronic studies
- carcinogenicity studies
- genotoxicity testing
- implantation
- hemocompatibility testing
- pyrogenicity testing

Special attention must be given to the possible influence of the production and sterilisation methods. It is not possible to leverage data from competitors because this information is often proprietary. Also, it is often not possible to use datasheets of raw materials as evidence because production methods and sterilisation of the device may alter its biological properties. However, it is possible to leverage data from the company’s similar products, in case the type of contact, the production methods and the sterilisation methods are (nearly) identical.

The EN ISO 10993-X series is not the only source for determining the type of tests to be performed. For some devices, e.g., ventilation tubes and transfusion sets, product standards require specific biocompatibility testing.

If the product is made of a new material that has not been used in healthcare applications before, a full suite of testing may be required. Some of these tests are lengthy and may take considerable numbers of devices to run, so proper planning for this segment is essential to meeting timelines. Even when a device is made of well-known materials, biocompatibility testing may be a long and costly process.

### Performance Testing

Each medical device will have to meet its intended performance, which will have to be proven by testing. For some device types, specifically for devices used on a large scale, there are specific product standards with predefined performance criteria. For example, for intravascular catheters, the EN ISO 10555-X series of standards describes performance requirements, including test setups. Other devices with specific product standards are transfusion sets for single use (EN ISO 1135-4) and x-ray equipment (EN IEC 60601-2-54).
Packaging Validation
The product packaging should be made of robust materials to protect the product under all shipping and temperature conditions because a compromised product may have unintended functionality and safety effects. Packaging material selection should be based on a few key areas: product integrity, sterilisation method and product functionality. The objective is to design a packaging system that will allow the user ease of access while also protecting the product from internal or external breaches in sterility.

Sterile products are a special case, for which there are several standards addressing packaging for these products, starting with the EN ISO 11607-1 and -2 standards. The pouch or blister needs to keep the product sterile during the product’s whole lifetime; therefore, the integrity of the packaging must be tested. These tests consist of seal strength of the packaged product and integrity of the complete pouch or blister, i.e., determining whether there are any leaks in the packaging. EN 868-5 provides requirements regarding the required seal strength. Often, sterile packaging testing is performed along with shelf life testing, as the packaging needs to remain intact until the end of the product’s shelf life.

Transport Studies
In the EU, there are no harmonised standards for transport studies, although EN ISO 11607-1 provides some general requirements. This may be the reason that transport study requirements have not been addressed as thoroughly as other studies. However, recently, notified bodies have become much stricter in their review of transport studies.

Transport studies are conducted to ensure the packaging system (inner and outer packaging) will maintain the product’s integrity until it reaches the end user. For this reason, several harsh tests should be performed, ranging from drop tests to extensive vibration tests with a range of vibration frequencies. The two most commonly applied test standards/methods are ISTA 2 series and ASTM D4169.

In addition, there is more emphasis on studying possible temperature influences on the product. Some companies place their products in temperature test chambers and simulate a range of temperatures, from cold weather in winter to high temperatures and humidity in hot climates. Whether this has any added value should be analysed in the risk assessment phase. For example, when shelf life tests are performed at elevated temperatures, performing temperature tests during transport may not offer added value.

Sterilisation Validation
Manufacturers of sterile devices or of devices that will be cleaned and sterilised between uses, must validate the sterilisation method to be used. Just as the materials chosen to build a product can affect its safety and efficacy, so can sterilisation methods. Whether the manufacturer sterilises the product or provides instructions to the end-user, the chosen method must be demonstrated to be safe and effective. A manufacturer’s choice of materials should depend partly on the sterilisation method used. Some materials and some intended uses are more suited for certain sterilisation methods.

Steam sterilisation is the most common sterilisation method, because hospitals use this method for their routine sterilisation processes; however, it is not popular among medical device manufacturers. Manufacturers commonly use Ethylene Oxide (EO) gas sterilisation because almost all materials are EO product friendly. Exceptions are materials that cannot withstand 100% humidity at 40°C, e.g., cyanoacrylate glues (superglues). Gamma-irradiation also is commonly used, but the radiation can cause material degradation (cracks) and discoloration. Although there are other methods (e.g., E-beam sterilisation), these play a marginal role.

The (harmonised) EN 556-1 standard requires that the chance that a device is not sterile as one device in one million or better. Therefore, sterilisation validation should prove the Sterility Assurance Level (SAL) is 10⁻⁶ or that there is less than one in a million chance the product is not sterile.

For EO sterilisation, the most applied validation method is the “overkill” method (Annex B of EN ISO 11135). The basis of this method is to prove that half the EO sterilisation cycle is sufficient to already achieve a SAL of 10⁻⁶. For this test, test-strips (biological indicators) containing a million EO resistant bacteria are placed inside the product in a difficult-to-reach area. This is considered a worst-case situation. Then, the product with the test strips is exposed to a half cycle, which according to the requirement, is already sufficient to kill a million bacteria. The full cycle will then always result in a 10⁻⁶ SAL or better. The second test during this sterilisation method is performance qualification (PQ). PQ will determine whether the product meets its performance requirements after the sterilisation process.

Often, product performance is tested after two sterilisation cycles so that if something went wrong during the first cycle, the product can be sterilised a second time. In addition, the residuals of the EO gas sterilisation process need to be determined after the sterilisation process because these remnants could be detrimental to the patient.

In contrast to the EO sterilisation validation, the gamma-irradiation sterilisation validation is based on
a theoretical model. The basis is a table in the EN ISO 11137-2 standard showing the relationship between the bioburden of the device and the required SAL. The consequence is the bioburden must be closely monitored, in number and gamma-irradiation resistance.

For gamma-sterilisation, the most applied validation method is the VDmax method (EN ISO 11137-2). In this method, 10 products are exposed to the (low) gamma-irradiation dose corresponding to a 10% chance (SAL 10^-1) that the product is not sterile. This dose is based on the average bioburden of three batches. In case 9 out of the 10 products are sterile, the test is a pass. By applying a table from EN ISO 11137-2, the corresponding SAL 10^-6 can then be found.

Since this is a theoretical model, the resistance of the contamination of the product needs to be tested every quarter (quarterly dose audits) to determine whether the original sterilisation validation is still valid.

**Electrical Safety and Electrical Magnetic Disturbance Testing**

Testing of active (electrical) devices is one of the most complicated subjects in medical device testing. The basic requirements are described in the General Electrical Safety Standard (EN IEC 60601-1) and in the corresponding collateral standards (EN IEC 60601-1-X). In addition, several device-related standards (EN IEC 60601-2-X) contain electrical safety requirements that are device-specific.

The general requirements applicable to medical electrical devices address their safety and impact on other medical electrical devices. If the device (or a part of the device) contacts the patient, the device is considered to have an “applied part,” and the general standard for electrical safety applies (IEC 60601-1). If there is no contact with the patient, this standard is in principle not applicable, and the general low-voltage standards are applicable. However, even if there is no contact with the patient (e.g., a medical power supply), manufacturers often choose to test according to the general standard to provide assurance of safety to their customers.

The influence on other devices is covered in the electrical magnetic disturbance (EMC) standard (EN IEC 60601-1-2). Although there are some exceptions, all electrical devices must meet this standard.

Interestingly, electrical safety tests are usually performed on one device, which is considered to be representative for serial produced devices.

**Shelf Life (Aging) Studies**

Shelf life studies basically involve putting the device on the shelf, waiting until the expiry date of the device and conducting tests to determine the device’s safety and efficacy after aging. As such, shelf life studies are impractical, as they can take several years before they are completed. Fortunately, it is commonly accepted to speed up the process by elevating the temperature, using the Arrhenius equation. The general rule is that every 10-degree increase will double the aging process (see the ASTM F1980 standard). Thus, a 30°C temperature increase would shorten the waiting time by a factor of eight. In this way, a shelf life test of several years can be performed in a few months.

Although the accelerated aging test method is generally accepted when starting with a product, it is only accepted by notified bodies in combination with a plan for real-time aging. Thus, a shelf life test plan should contain both accelerated aging and real-time aging tests.

**Software Verification and Validation**

Software can be a medical device if it is embedded in the device. Many devices today contain software. Software verification is different from the other preclinical tests described, as this is usually part of the development process and is performed by the manufacturer itself.

Software development, including verification, is described in detail in the specific standard for medical software, the EN IEC 62304.

The software validation method used depends on the type of device. Standalone software is usually validated in a simulated user environment, e.g., a hospital, or with possible users. Embedded software is usually validated as part of the complete device validation.

**Usability (Human Factors) Studies**

The usability of devices continues to receive increased attention. The usability standard (EN IEC 62366-1), although an IEC standard, is applicable to all medical devices.

Usability testing should focus on safety, as the EN IEC 62366-1 explicitly states that medical device usability testing relates to safety, only under normal use. The standard is risk based, so in principle, the analysis can focus on the safety risks during all steps described in the instructions for use.

The two main steps in the usability process are the formative evaluation and the summative evaluation. During the formative evaluation, different designs or parts of the design can be tested before a final design has been reached. In the summative evaluation, the final design is tested by a representative user group and set up. In addition to EN IEC 62366-1, Part 2 of the standard provides guidance on the application of usability engineering to medical devices.
Chapter 11

Statistical Methods
For every preclinical test, the sample size and acceptance criteria need to be defined and explained in the test report. In general, the number of samples is higher for attribute sampling (pass/fail) than for variable sampling (measured value).

For attribute sampling, a standard table with the number of samples based on the required confidence and reliability level is often used. For variable testing, an assumption of the required number of samples can only be made when there is an idea of the average value and standard deviation. The closer the average to the limit, and the wider the standard deviation, the more samples will be required.

Case Study 1: Heated Sleep Apnea Device (Active Device, Class IIa)
Like many medical devices, a continuous positive airway pressure (CPAP) device falls in risk Class IIa. Although Class IIa devices are medium-risk devices, the amount of testing can be extensive, as this example will show.

CPAP devices are intended to deliver air with a slight over-pressure to the user (patient) to keep the throat open during sleep. The device consists of a humidifier (driving-unit) to which the heated tube is connected. At the patient side, the heated tube is connected to a face-mask (left out for this example). The humidifier vaporises water so the air is humidified to prevent the patient’s throat from becoming very dry. The heated tube prevents condensation of the humid air to the wall of the tube.

At first glance, the device seems straightforward and not too complicated. However, there are several product-specific standards with a long list of requirements.

Bench Testing
As stated above, this active device can contact the patient and therefore must comply with the basic safety standard (EN IEC 60601-1) and EMC standard (EN IEC 60601-1-2). As this device is used in the home environment, the standard for home use (EN IEC 60601-1-11)22 also applies. Because sleep apnea devices are used widely, there are specific product standards applicable to this type of device (EN ISO 80601-2-70 and EN ISO 80601-2-74).23 On the positive side, once the device meets all applicable requirements of these standards, the manufacturer can claim that the device is safe and performs as intended.

Detailed knowledge of the standards, test methods and specific test equipment is required. Therefore, these tests are usually outsourced to test-houses specialised in electrical safety testing. Note that the test-houses expect the manufacturer to write the test plans, including the test method.

Today, devices like the humidifier contain software to drive the device and a display to create an appealing user-interface. Although the software is embedded in the device and not a device on its own, it must comply with the medical device software lifecycle standard (EN IEC 62304).

Nonclinical Evaluation: Biocompatibility
Since the device has indirect and possibly direct contact with the patient, it (specifically, the tube) must comply with the EN ISO 10993-1 standard and the applicable parts of the EN ISO 10993-X series. In addition, for sleep apnea devices, a specific series of biocompatibility test requirements are listed in the EN ISO 18562-X series.25

Although the test-house usually writes the test protocol, the manufacturer must carefully review the set-up. For example, the inside of the sleep apnea tube is the specific area to be tested. Thus, the test-house should create a special set-up instead of the whole device being placed in the container with the extraction fluid. These customised tests are expensive.

Usability Testing
Depending on its intended use, the CPAP device may be operated by professional users and/or lay users (home use). Usability tests will have to address all steps involved in using the device, starting with unpacking the device, operating the device, maintaining the device and cleaning the heated tube. Determining whether the user can understand the manual is part of the testing. For lay use, the amount of testing will need to be more extensive than for professional use.

Case Study 2: Preclinical Testing of a Drug-Eluting Stent (Class III Implant and Combination Device)
For products that also contain a medicinal substance, the benefits and risks of this substance need to be evaluated in addition to those of the medical device alone. As an example, the preclinical testing of a drug-eluting stent is discussed.

The combination of a Drug Eluting Stent (DES) and an ancillary medicinal substance creates the potential for both local and systemic effects not seen previously with bare metal stents (BMS). Although it is recognised that the total medicinal product amount incorporated in the DES is substantially lower than used systemically in other clinical applications, local safety aspects are a major point of concern and should be taken into account in the (non-) clinical evaluation program. The medicinal substance’s benefit-risk profile in the context of a DES is linked with the chosen stent platform, the surface coating and drug carrier system (if...
present) and any interaction among these. Evaluating the medicinal substance’s safety and clinical risk-benefit profile in the context of a DES in coronary stenting is complicated by the fact that in case of adverse events (e.g., Major Adverse Cardiac Events (MACE)), the medicinal substance and device component’s influence cannot be separated easily.

**Bench Testing**
The manufacturer is expected to perform a series of bench tests on the integrity of the device component of the investigational combination product. It must be demonstrated that the ancillary medicinal substance and device neither chemically nor physically interact adversely with each other. In addition, it is important for the manufacturer to elucidate how the medicinal substance and drug carrier’s application to the device may affect its fatigue and corrosion properties, coating integrity, durability and any other relevant combination product-specific components.

**Nonclinical Evaluation: Biocompatibility**
The manufacturer must submit biocompatibility testing results of the bare stent platform to support the initiation of a human clinical study. The biocompatibility testing should provide results on all relevant materials, including carrier and stent material.

**Nonclinical Pharmacodynamics (Proof of Concept) Testing**
The manufacturer should elucidate the medicinal product(s)’s mechanism of action, justified by relevant data.

**Nonclinical Pharmacokinetic Testing**
Drug-eluting coronary stents present major challenges for in vivo pharmacokinetic (PK) characterisation. The devices are designed to release the medicinal substance locally, with the intent of maximising or controlling bioavailability within local vascular tissue. The development of a suitable in vivo local pharmacokinetic testing model is complicated by the lack of an animal model equivalent to human atherosclerotic disease. DES pharmacokinetic testing consists of local, regional and systemic assessments.

**Testing Multiple Overlapping Stents**
Treatment of large vascular lesions requires multiple stent implantations. At sites of stent overlap, the load and release of the medicinal substance(s) are increased, and substantial deposition of the medicinal substance(s) could occur because of altered flow. Within the clinical practice context of implanting multiple overlapping stents, the effect of stent overlap on vascular healing should be evaluated.

**Preclinical Toxicity Studies**
Because efficacy prediction from current animal models is not reliable, animal testing is limited primarily to safety evaluation. The proposed clinical medicinal substance dose and release characteristics should be justified by nonclinical data. Preclinical dose range finding studies are strongly recommended, showing effects across ranges from sub-therapeutic to toxic levels, where practicable. A multiple-dose study should be performed in an animal model to establish safety margins and toxicity in choosing a dose for clinical trials. The dosing studies will establish a performance margin between the sub-therapeutic dose and the therapeutic dose, and a safety margin between the therapeutic dose and the toxic dose.

**Testing an Unapproved Medicinal Substance**
Additional animal toxicity studies are expected to be conducted if the medicinal substance is not approved for use in a stent.

**Conclusion**
Preclinical testing is a critical part of the medical device lifecycle, as it is the stage in which the product design is fixed. Preclinical testing is proof the device meets all requirements, except for the clinical evaluation. Preclinical testing is usually not a one-way process but can be conducted in phases in case the device has to be re-tested because a test failed or design changes were made during the development process. Preclinical testing showing the device meets its requirements, together with clinical evaluation, is the basis for a market approval application or a clinical trial application.

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