BIOMATERIAL & BIOCOMPATIBILITY TESTING LABORATORY

World Health Organization
COUNTRY OFFICE FOR India

NHSRC

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MESSAGE

Medical Implants form an important and a distinct category within the medical devices, from dental filling to pacemakers, medical implants encompass a board and complex variety of technologies that have been developed over decades and which are continuously evolving and becoming better. Complexity of implants increases further as these remain inside the body of the patient for many years, unlike most other medical devices that come in contact with the body either once or only a few times. In order to comply with all safety requirement, sets of universal standards and norms have been prescribed for biomaterials and biocompatibility. The utility in terms of safety, effectiveness and performance for such compliance can be established through testing. The product testing, therefore, is the first level of assessment of appropriateness of an implant.

Implants have a very broad range and so do the testing requirements to establish their safety. However, common set of prescribed tests and standards can be used for most Implants. This report essentially brings out the basic requirements, work flow, infrastructure and human resources required for establishing medical devices testing laboratory/facility for testing of biomaterials and Implants.

With the current emphasis on make in India and medical devices sector emerging in the country, this technical concept note is both timely and encouraging. I congratulate National Health System Resource Centre, a technical institution under the Ministry of Health and Family Welfare, Government of India for formulating this report. This would serve the need of government agencies, laboratories and research institutions, as well as medical devices manufactures in a highly complex and technical area of work.

Place : New Delhi
January 2015

(K.L. Sharma)

Foreword

Within the ambit of medical devices, implants require special considerations of safety and accuracy. From dental fillings to pacemakers, most implants remain in-vivo for the entire duration of a patient's life. This obligates stake holders including manufacturers, suppliers, healthcare professionals and patients to attach highest degree of caution in dealing with medical and surgical implants. Nation's infrastructure and process capabilities towards testing of implants are of paramount importance. With growing medical devices industry and Government's focus on manufacturing sector, a proportionate effort is crucial towards building laboratories for implant testing. This report is therefore both timely and needful. The technical support received from DS Nagesh and S Balram from the Biomedical Technology Wing Sri Chitra Tirunal Institute of Medical Sciences & Technology (SCTIMST, Trivandrum) in content development and editing of this report deserve special mention. I also thank World Health Organization Country Office for India for providing technical support. I congratulate the team from Healthcare Technology Division of NHSRC including Anurag, Jitendar Sharma, Mohammad Ameel and Prabhat Arora for bringing out the report in a complex domain of implant and biomaterial testing. I sincerely hope that this report would be immensely useful for establishment of biomaterial and biocompatibility testing laboratory, shall contribute in improving safety of health products and be an enabler of domestic manufacturing sector.

Dr. Sanjiv Kumar
Executive Director

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1.1 Introduction

Biomaterials can be derived either from nature or synthesized in the laboratory using a variety of chemical approaches utilizing metallic components,polymers, ceramics or composite materials. They are often used and/or adopted for a medical application, and thus performs, augments, or replaces a natural function. Such functions may be benign, like being used for a heart valve, or may be bioactive with a more interactive functionality such as coated hip implants. Biomaterials are also used every day in dental applications, surgery, and drug delivery. For example, a construct with impregnated pharmaceutical products can be placed into the body, which permits the prolonged release of a drug over an extended period of time. A biomaterial may also be an autograft, allograft or xenograft used as a transplant material.

1.2 Biocompatibility

Biocompatibility is, by definition, a measurement of how compatible a device is with a biological system. The purpose of performing biocompatibility testing is to determine the fitness of a device for human use, and to see whether use of the device can have any potentially harmful physiological effects.

Typically, material characterization and analysis of a device's components are conducted prior to any biological testing. This involves extracting leachable materials from the device or components at an elevated temperature, and analyzing the leachable extracts for potentially harmful chemicals or cytotoxicity.

Once in vitro testing has been completed, in vivo biological testing can be done based upon the device's intended use. This testing can range from skin irritation testing to hemocompatibility and implantation testing. Turnaround time for tests can range from three weeks to greater than several months, depending on the specific test data needed. Subchronic or chronic implantation testing can last even longer. The two ways to test the biocompatibility in vivo implant are "Tissue culture test" and "Blood and contact tests".

Table 1.1: Examples of Biomaterials application in medical devices

S.No.	Biomaterials Application
1	Joint replacements
2	Bone plates
3	Bone cement
4	Artificial ligaments and tendons
5	Dental implants for tooth fixation
6	Blood vessel prostheses
7	Heart valves
8	Skin repair devices (artificial tissue)
9	Cochlear replacement
10	Contact lenses
11	Breast implants
12	Drug delivery mechanisms
13	Sustainable materials
14	Vascular grafts
15	Stents
16	Nerve Conduits

Some commonly used Biomaterials are given below:

Material categorizes	Applications
Silicone rubber	Catheters, tubing
Dacron	Vascular grafts
Cellulose	Dialysis membranes
Poly(methyl methacrylate)	Intraocular lenses, bone cement
Polyurethanes	Catheters, pacemaker leads
Hydogels	Ophthalmological devices, Drug Delivery
Stainless steel	Orthopedic devices, stents

Titanium	Orthopedic and dental devices
Alumina	Orthopedic and dental devices
Hydroxyapatite	Orthopedic and dental devices
Collagen (reprocessed)	Opthalmologic applications, wound dressings

Biocompatibility testing includes several specialized processes, some of them are explain below:

1.2.1. Toxicology

A biomaterial should not be toxic, unless it is specifically engineered for such requirements (for example, a "smart bomb" drug delivery system that targets cancer cells and destroys them). Since the nontoxic requirement is the norm, toxicology for biomaterials has evolved into a sophisticated science. It deals with the substances that migrate out of biomaterials. For example, for polymers, many low-molecular-weight "leachables" exhibit some level of physiologic activity and cell toxicity. It is reasonable to say that a biomaterial should not give off anything from its mass unless it is specifically designed to do so. Toxicology also deals with methods to evaluate how well this design criterion is met when a new biomaterial is under development.

1.2.2.Biocompatibility

Biocompatibility is determined as the ability of a material to co-exist and perform with a natural substance in a specific biological application. Since the material should be non toxic to perform with an appropriate host response, which having a biomaterial interface with human body are required to perform a particular physiological function such as that of stent, knee replacement or pacemaker. Biomaterials incorporated into medical devices are implanted into tissues and organs. Therefore, the key principles governing the structure of normal and abnormal cells, tissues and organs, the techniques by which the structure and function of normal and abnormal tissue are studied, and the fundamental mechanisms of disease processes are critical considerations. Special processes are invoked when a material or device heals in the body. Injury to tissue will stimulate the well-defined inflammatory reaction sequence that leads to healing. Where a foreign body (e.g., an implant) is present in the wound site (surgical incision), the reaction sequence is referred to as the "foreign body reaction." The normal response of the body will be modulated because of the solid implant. Furthermore, this reaction will differ in intensity and duration depending upon the anatomical site involved. An understanding of how a foreign object alters the normal inflammatory reaction sequence remain an important concern.

1.2.3. Mechanical and Performance Requirements

An intraocular lens may go into the lens capsule or the anterior chamber of the eye. A hip joint will be implanted in bone across an articulating joint space. A heart valve will be sutured into cardiac muscle and will contact both soft tissue and blood. A catheter may be placed in an artery, a vein or the urinary tract. Each of these sites challenges the biomedical device designer with special requirements for geometry, size, mechanical properties, and bioresponses.

Biomaterials and devices have mechanical and performance requirements that originate from the physical and /or eletro-chemcial properties of the material.

Such requirements varies in mechanical properties for example :

Hip prosthesis must be strong and rigid, tendon material must be strong and flexible, heart valve leaflet must be flexible and tough, dialysis membrane must be strong and flexible, but not elastomeric, articular cartilage substitute must be soft and elastomeric.

Then, mechanical performance varies based on a diverse range of requirements. Similarly the duration of contact also varies , for example :-

A catheter may be required for just 3 days, bone plate may fulfill its function in 6 months or longer, leaflet in a heart valve must flex 60 times per minute without tearing for the lifetime of the patient (realistically, at least for 10 or more years), hip joint must not fail under heavy loads for more than 10 years and so on. There are also other biophysical properties and other aspects of performance. The dialysis membrane has a specified permeability, the articular cup of the hip joint must have high lubricity, and the intraocular lens has clarity and refraction requirements. To meet these requirements, design principles from physics, chemistry, mechanical engineering, chemical engineering, and materials science are to be accurately integrated.

1.2.4. Regulation

Patient care demands safe medical devices. To prevent inadequately tested devices and materials from coming on the market. Most nations of the world have medical device regulatory bodies. In addition the International Standards Organization (ISO) has introduced international standards for the world community. The costs to comply with the standards and to implement materials, biological, and clinical testing are enormous. As per regulartory requirement testing and other factors involved in biomaterials can be catergorized as below :

Table1.2: Requirements of Implants

Requirements of implants

Compatibility

- Tissue reactions
- Change in properties
  a. Mechanical
  b. Physical
  c. Chemical
  d. Electronics
  e. Electrophysical

Mechanical properties

- Elasticity
- Yield stress
- Ductility
- Toughness
- Time dependent deformation
- Creep
- Ultimate strength
- Fatigue strength
- Hardness
- Wear resistance

Manufacturing

- Fabrication methods
- Consistency and conformity to all requirements
- Quality of raw materials
- Superior techniques to obtain excellent surface finish or texture
- Capability of material to get safe and efficient sterilization
- Cost of product

1.2.5 Biomaterials associated infection

Biomaterials associated infection (BAI) is one of the most common complications associated with implantation of any biomaterial regardless of form or function. These infections usually involve bacterial colonization and biofilm formation on the biomaterial itself, rendering the infection impervious to antimicrobials and host defenses. In addition, it is becoming increasingly clear that infection of the surrounding tissues also plays an important role in BAI, and that the infection may be influenced by the composition and design of the implanted biomaterial. Advantages and disadvantages of biomaterials in some substances commonly used in implants is tabled below:

Table 1.3: Advantages and disadvantages of biomaterials

Biomaterials	Advantages	Disadvantages	Types of Biomaterials
Polymeric	- Easy to make complicated items - Tailorable physical & mechanical properties - Surface modification -Immobilize cell etc. -Biodegradable	- Leachable compounds - Absorb water & proteins etc. - Surface contamination - Wear & breakdown - Biodegradation - Difficult to sterilize	- PMMA - PVC - PLA/PGA - PE - PP - PA - PTFE - PET - PUR - Silicones
Bioceramic	- High compression strength - Wear & corrosion resistance - Can be highly polished - Bioactive/inert	- High modulus (mismatched with bone) - Low strength in tension - Low fracture toughness - Difficult to fabricate	- Alumina - Zirconia(partially stabilized) - Silicate glass - Calcium phosphate (apatite) - Calcium carbonate
Metallic	- High strength - Fatigue resistance - Wear resistance - Easy fabrication - Easy to sterilize - Shape memory	- High moduls - Corrosion - Metal ion sensitivity and toxicity - Metallic looking	- Stainless steel (316L) - Co-Cr alloys - Ti Al V 6 4 - Au-Ag-Cu-Pd alloys - Amalgam (AgSnCuZnHg) - Ni-Ti - Titanium

Figure 1.1

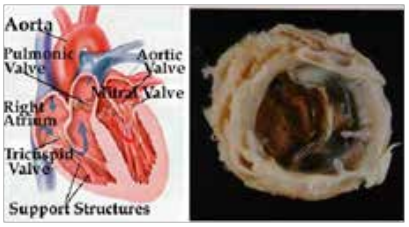


Figure 1.2

1.3 Standards for biomaterials and biocompatibility testing

The starting point for understanding biocompatibility requirements is ISO Standard 10993, Biological Evaluation of Medical Devices. Part 1 of the standard is the Guidance on Selection of Tests, Part 2 covers animal welfare requirements, and Parts 3 through 19 are guidelines for specific test procedures or other testing-related issues. (A list of the individual sections of ISO 10993 can be found in table 1.4 and other relevant standards are given in table 1.5)

The core of the ISO Standard is confirmation of the fitness of the device for its intended use. Although ISO develope policy and publishes standards related to conformity assessment, it does not perform conformity assessment activities.

ISO 17025 state the general requirement for the competence of testing and calibration laboratories. This is the main ISO standard used by testing and calibration laboratories.

Table1.4 List of standards for biocompatibility tests

S.No	Standards	Title	Description
1	ISO 10993	Biocompatibility	This standard gives the basic guidelines of bio-compatibility
2	ISO 10993-1:2009	Evaluation and testing in the risk of management process	-The general principle governing the biological evaluation of medical devices within a risk management process; -The general categorization of devices based on the nature and duration of their contact with the body; -The evaluation of exiting relevant data from all sources; -The identification of gaps in the available data set on the basis of a risk analysis; -The identification of additional data sets necessary to analyze the biological safety of the medical device; -The assessment of the biological safety of the medical device;
3	ISO 10993-2	Animal welfare requirements	The standard specifies the minimum requirements to be satisfied to ensure and demonstrate that proper provision has been made for the welfare of animals used in animal tests to assess the biocompatibility of materials used in medical devices.

S.No	Standards	Title	Description
4	ISO 10993-3	Tests for genotoxicity, carcinogenicity and reproductive toxicity	The standard specifies strategies for hazard identification and tests on medical devices for the following biological aspects: genotoxicity, carcinogenicity, and reproductive and developmental toxicity
5	ISO 10993-4	Selections of tests for interactions with blood	The standard provide general requirement for evaluation the intersections of medical devices with blood. It describes: -A classification of medical and dental devices that are intended for use in contact with blood, based on the intended use and duration of contact as defined in ISO 10993-1; -The fundamental principle governing the evaluation of the interaction of devices with blood; -The rationale for structured selection of tests according to specific categories, together with the principle and scientific basis of these tests. Detailed requirements for testing cannot be specified because of limitations in the knowledge and precision of tests for interactions of devices with blood. ISO 10993-4:2002 describes biological evaluation in general terms and may not necessarily provide sufficient guidance for tests methods for a specific device.
6	ISO 10993-5	Tests for in vitro Cytocompatibility evaluation	The standard describes test methods to assess the in vitro Cytocompatibility evaluation of medical devices. These methods specify the incubation of cultures cells in contact with a device and/or extracts of a device either directly or through diffusion. These methods are designed to determine the biological response of mammalian cells in vitro using appropriate parameters.

S.No	Standards	Title	Description
7	ISO 10993-6	Tests for local effects after implantation	The standard specifies tests methods for the assessment of the local effects after implantation of biomaterials intended for use in medical devices. ISO 10993-6:2007 applies to materials that are - Solid and non-biodegradable; - Degradable and/or reserbable; - Non-solid, such as porous materials, liquids, pastes and particulates. ISO 10993-6:2007 may also be applied to medical devices that are intended to be used topically in clinical indications where the surface or lining may have been breached, in order to evaluate local tissue responses.
8	ISO 10993-7	Ethylene oxide sterilization residuals	The standard specifies allowable limits for residual ethylene oxide (EO) and ethylene chlorohydrin (ECH) in individual EO-sterilized medical devices, procedures for the measurement of EO and ECH, and methods for determining compliance so that devices may be released. Additional background, including guidance and a flowchart showing how the standard is applied are also included in informative annexes. EO-sterilized devices that have no patient contact (e.g., in vitro diagnostic devices) are not covered by ISO 10993-7:2008.
9	ISO 10993-8	Selection of reference materials	The standard gives guidance on selection and qualification of reference materials for biological test (usually sent by the client).
10	ISO 10993-9	Framework for identification and quantification of potential degradation products	The standard provides general principle for the systematic evaluation of the potential and observed biodegradation of medical devices and for the design and performance of biodegradation studies. ISO 10993-9: 2008 consider both non- resorbable and resorbable materials

S.No	Standards	Title	Description
11	ISO 10993-10	Tests for irritation and delayed-type hypersensitivity	The standard describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitization. ISO 10993-10:2010 includes: -Pretest considerations for irritation in silico and in vitro methods for dermal exposure; -Details of in vivo (irritation and sensitization) test procedures; -Key factors for the interpretation of the results. Instructions are given for the preparation of materials specifically in relation to the above tests and several special irritation tests are described for application of medical devices in areas other than skin.
12	ISO 10993-11	Tests for systemic toxicity	The standard specifies requirements and gives guidance on procedures to be followed in the evaluation of the potential for medical device materials to cause adverse systemic reactions.
13	ISO 10993-12	Sample preparation and reference materials	The standard specifies requirements and gives on the procedures to be followed in the preparation of samples and the selection of reference materials for medical device testing in biological systems in accordance with one or more parts of ISO 10993. Specifically, ISO 10993-12:2012 addressers the following: -Test sample selection; -Selection of representative portions from a device; -Test sample preparation; -Experimental controls; -Selection of, and requirements for, reference materials; -Preparation of extracts. ISO 10993-12:2012 in not applicable to live cells, but can be relevant to the material or device components of combination products containing live cells.

S.No	Standards	Title	Description
14	ISO 10993-13	Identification and quantification of degradation products from polymeric medical devices	ISO 1099313:2010 provides general requirements for the design of tests in a simulated environment for identifying and quantifying degradation products from finished polymeric medical devices ready for clinical use.
15	ISO 10993-14	Identification and quantification of degradation products from ceramics	Biological evaluation of medical devices Part 14: Identification and quantification of degradation products from ceramics
16	ISO 10993-15	Identification and quantification of degradation products from metals and alloys	Biological evaluation of medical devices Part 15: Identification and quantification of degradation products from metals and alloys
17	ISO 10993-16	Toxicokinetic study design for degradation products and leachables	Biological evaluation of medical devices Part 16: Toxicokinetic study design for degradation products and leachables
18	ISO 10993-17	Establishment of allowable limits for leachable substances	Biological evaluation of medical devices Part 17: Establishment of allowable limits for leachable substances
19	ISO 10993-18	Chemical characterization of materials	Biological evaluation of medical devices Part 18: Chemical characterization of materials
20	ISO 10993-19	Physico-chemical,morphological and topographical characterization of materials	Biological evaluation of medical devices Part 19: Physico-chemical, morphological and topographical characterization of materials
21	ISO 10993-20	Principles and methods for immunotoxicology testing of medical devices	Biological evaluation of medical devices Part 20: Principles and methods for immunotoxicology testing of medical devices

Table1.5 List of standards for biomaterials testing

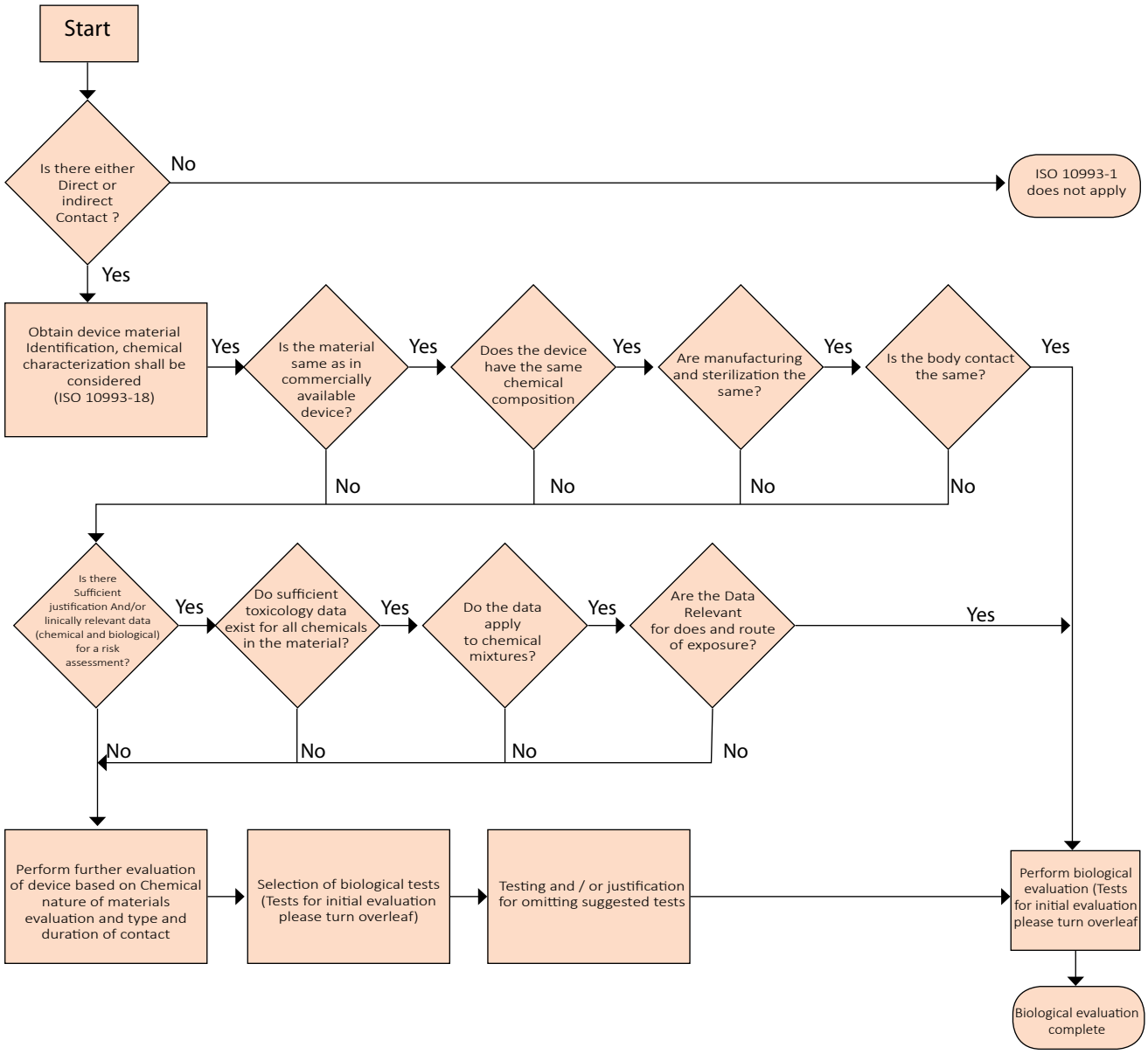
S.No	Standards	
1	Particle testing and corrosion testing	See Annexure 1
2	Mechanical testing lab for ageing, Package validation, Wear and Tear testing	See Annexure 2
3	Fluid flow testing to measure functionality of device in presence of body fluids	See Annexure 3
4	Spine stimulator test (for orthopaedic implants)	See Annexure 4
5	Total knee wear test(for orthopaedic implants)	See Annexure 5
6	MRI compatibility test	See Annexure 6

2.1. Selection of tests for Biocompatibility

Biocompatibility testing and evaluation of medical devices is performed to determine the potential toxicity resulting from contact of the device with body. The device materials should not-either directly or though the release of their material constituents-produce any local or systemic adverse effects, be carcinogenic, or produce adverse reproductive and/or development effects.

The biological evaluation of any material or medical device intended for use in humans forms part of a structured biological evaluation programme within a risk management process in accordance with ISO 14971 (Application of risk management to medical devices) as set out in the figure 2.1 below

Figure2.1: Flow chart on systematic approach to biological evaluation of medical devices as part of a risk management process



As per ISO 10993 the choice of tests and the data required in a biological evaluation and their interpretation shall take into account the chemical composition of the materials, including the conditions of exposure as well as the nature, degree, frequency and duration of exposure of the medical devices or its constituents to the body, enabling the categorization of devices to facilitate the selection of appropriate tests.

The biological evaluation performed based on ISO 10993 categorizes medical devices according to:

- Nature of body contact- Surface device, External communicating device and implant device

- Contact duration - Limited (<24 h), Prolonged (24 h to 30 days) and Permanent (>30 h)

Table2.1: for initial evaluation - extracted from ISO 10993 - Biological evaluation of medical devices

Medical device categorization by			BIOLOGICAL EFFECTS							
Nature of body contact		Contact duration A-Limited (<24 h) B-Prolonged (24 h to 30 days) C-Permanent (>30 days)	Cytocompatibility evaluation	Sensitization	Irritation or Intracutaneous reactivity	Systemic toxicity (Acute)	Subacute and subchronic toxicity	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
Surface Device	Skin	A	X	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
External Communicating device	Blood path, indirect	A	X	X	X	X				X
		B	X	X	X	X				X
		C	X	X		X	X	X		X
	Tissue/bone/dentin	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Circulating blood	A	X	X	X	X				X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X
Implant device	Tissue/bone	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Blood	A	X	X	X	X	X		X	X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X

2.2. Requirements of a Laboratory

Folling major component are required in a laboratory for testing of Biomaterials and Biocompatibility:

1. Cytocompatibility evaluation Testing (Tissue Culture Laboratory)
2. Tissue compatibility evaluation laboratory
3. Hemocompatibility evaluation laboratory
4. Sterility evaluation laboratory
5. Histopathology evaluation laboratory
6. Accelerated Aging laboratory
7. Physiochemcial evaluation laboratory
8. Package validation laboratory

2.2.1 Cytocompatibility evaluation laboratory

The Cytocompatibility tests involve the exposure of substances extracted from test material to cell culture lines. Cell cultures are extremely sensitive to minute quantities of leachable chemicals and readily display characteristic signs of toxicity in the presence of potentially harmful leachables. The tests are frequently used during product planning stages to qualify the use of a material and as a periodic check for routinely used materials to ensure that no shift in quality has occurred. Cytocompatibility in vitro testing is also required in testing the biocompatibility of materials. Typical testing programs will utilize the ISO test method to meet international regulatory requirements. The screening test method can be performed to characterize materials or to evaluate new materials against established ones.

There are three Cytocompatibility evaluation tests commonly used for medical devices.

(a) The Direct Contact procedure is recommended for low density materials, such as contact lens polymers. In this method, a piece of test material is placed directly onto cells growing on culture medium. The cells are then incubated. During incubation, leachable chemicals in the test material can diffuse into the culture medium and contact the cell layer. Reactivity of the test sample is indicated by malformation, degeneration and lysis of cells around the test material.

(b) The Agar Diffusion assay is appropriate for high density materials, such as elastomeric closures. In this method, a thin layer of nutrient-supplemented agar is placed over the cultured cells. The test material (or an extract of the test material dried on filter paper) is placed on top of the agar layer, and the cells are incubated. A zone of malformed, degenerative or lysed cells under and around the test material indicates cytotoxicity.

(c) The MEM Elution assay uses different extracting media and extraction conditions to test devices according to actual use conditions or to exaggerate those conditions. Extracts can be titrated to yield a semi-quantitative measurement of cytotoxicity. After preparation, the extracts are transferred onto a layer of cells and incubated. Following incubation, the cells are examined microscopically for malformation, degeneration and lysis of the cells.

There also exists specific quantitative tests. Two quantitative Cytocompatibility evaluation tests have been internationally recognised for chemicals and medical devices:

(a) The MTT Cytocompatibility Test measures the viability of cells by spectrophotometric methods. This measures the reduction of the yellow, water-soluble MTT (3-4,5 dimethyl- thiazol-2-yl) – (2,5-diphenyl tetrazolium bromide) by mitochondrial succinate dehydrogenase. A minimum of four concentrations of the test material are

tested. This biochemical reaction is only catalyzed by living cells.

(b) The Colony Formation Cytocompatibility Test enumerates the number of colonies formed after exposing them to the test material at different concentrations. This is a very sensitive test since the colony formation is assessed while the cells are in a state of proliferation (logarithmic phase), and thus more susceptible to toxic effects. A concentration-dependence curve evaluating the induced inhibition of the test material can be created, and the IC50 value (concentration of the test material that provides 50% inhibition) can be calculated. The quantitative tests can be performed on extracts and by direct contact.

At least one type of Cytocompatibility test, qualitative or quantitative, should be performed on each component of a device. List of cytotoxicity tests and their relevant standards are mentioned in table 2.2 and min required laboratory equipment list is given in table 2.3.

Table2.2:List of Cytocompatibility evaluation tests

LAB Category	Test Name	Test method/Standard
Cytocompatibility evaluation Testing (Tissue Culture Laboratory)	1. In Vitro Cytocompatibility test 1.1 Direct contact 1.2 Indirect contact 1.3 Test on extract 1.4 MTT assay 2. Cell Adhesion test	1.ISO 10993-5 2. Approved protocol

Table2.3 : List of Cytocompatibility evaluation laboratory equipment

SNo.	Equipment Name	Definition
1	Laminar Flow Bench	A laminar flow cabinet or laminar flow closet or tissue culture hood is a carefully enclosed bench designed to prevent contamination of semiconductor wafers, biological samples, or any particle sensitive device. Air is drawn through a HEPA filter and blown in a very smooth, laminar flow towards the user. The cabinet is usually made of stainless steel with no gaps or joints where spores might collect.
2	CO2 Incubator	An incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO_2) and oxygen content of the atmosphere inside.
3	Microscope-binocular	A microscope is an instrument used to see objects that are too small for the naked eye. The science of investigating small objects using such an instrument is called microscopy. Microscopic means invisible to the eye unless aided by a microscope

SNo.	Equipment Name	Definition
4	Autoclave	An autoclave is a device used to sterilize equipment and supplies by subjecting them to high pressure saturated steam at 121 °C for around 15–20 minutes depending on the size of the load and the contents
5	ETO Sterilization	Ethylene Oxide (EtO) sterilization is mainly used to sterilize medical and pharmaceutical products that cannot support conventional high temperature steam sterilization - such as devices that incorporate electronic components, plastic packaging or plastic containers.
6	Pipette (all sizes)	A pipette, pipet, pipettor or chemical dropper is a laboratory tool commonly used in chemistry, biology and medicine to transport a measured volume of liquid, often as a media dispenser
7	Laboratory water bath	A water bath is a piece of equipment used in science and industry to heat materials gently and gradually to fixed temperatures, or to keep materials warm over a period of time.
8	Cryogenic storage vessel	Cryogenic storage vessels are specialized types of vacuum flask used for storing cryogens (such as liquid nitrogen or liquid helium), whose boiling points are much lower than room temperature.
9	Basic laboratory glassware/ plasticware	Laboratory Glassware products are an important part of scientific laboratories wherever the highest quality is required. An ideal lab glassware must comprise of borosilicate glass with a low coefficient of expansion, for resistance to heat, and a very high resistance to chemical attack.
10	Ultraviolet lamp-Laboratory purpose	Ultraviolet radiation is widely used for killing bacteria or producing fluorescence.
11	Air Monitoring system	Measure the laboratory air temp, humidity, etc.

2.2.2 Tissue compatibility evaluation laboratory

These tests estimate the local irritation potential of devices, materials or extracts, using sites such as skin or mucous membranes, usually in an animal model. The route of exposure (skin, eye, mucosa) and duration of contact should be analogous to the anticipated clinical use of the device, but it is often prudent to exaggerate exposure conditions to establish a margin of safety for patients.

In the Intracutaneous Test, extracts of the test material and blanks are injected intradermally. The injection sites are scored for erythema and edema (redness and swelling). This procedure is recommended for devices that will have internal contact with the body or body fluids. It reliably detects the potential for local irritation due to chemicals that may get extracted from a biomaterial.

The Primary Skin Irritation test should be considered for topical devices that have external contact with intact or breached skin.

Mucous Membrane Irritation Tests are recommended for devices that will have contact with natural channels or tissues. These studies often use extracts rather than the material itself. Some common procedures include vaginal, cheek pouch and eye irritation studies.

List of Tissue compatibility evaluation tests and their relevant standards are mentioned in table 2.4 and required laboratory equipment list shown in table 2.5.

Table2.4: List of Tissue compatibility evaluation tests

LAB Category	Test Name	Test Standard
Tissue compatibility evaluation Laboratory	3. Irritation test	3. ISO10993-10 /USP<88>
	3.1. Intracutaneous irritation test (Rabbit) on cotton seed oil and normal saline extract	3.1.ISO10993-10 /USP<88>
	3.2 Skin Irritation test	3.2.ISO10993-10
	3.3 Vaginal irritation test	3.3.ISO10993-10
	3.4 Penile irritation test	3.4.ISO10993-10
	4. Acute Systemic Toxicity (Mice) Intravenous/ Intraperitoneal on cotton seed oil and normal saline extract	4. ISO10993-11 /USP<88>
	5. Sensitization	5. ISO10993-10
	5.1.Closed patch method-Guinea Pig	5.1. ISO10993-10
	5.2. Maximization method-Guinea Pig	5.2. ISO10993-10
	6. Rabbit Pyrogen Test	6. ISO10993-11
	7. Hemolytic property (Rabbit Blood)	7. ASTM 756
	8. Implantation test	8. ISO 10993-6
	9. Genotoxocity test	9. ISO 10993-3
	10.Mucous membrane irritancy test in rabbit (Acute eye irritation test in rabbit/corrosion)	

Table2.5: List of Tissue compatibility evaluation laboratory equipment

SNo.	Equipment Name
1	Laminar Flow Bench
2	CO2 Incubator
3	Microscope-binocular
4	Autoclave
5	ETO Sterilization
6	Pipette (all sizes)
7	Laboratory water bath
8	Cryogenic storage vessel
9	Thermometer
10	Basic laboratory glassware/plasticware
11	Ultraviolet lamp-Laboratory purpose
12	General Laboratory Instruments
13	Chemical Testing Instruments
14	Basic OT Equipment

2.2.3 Hemocompatibility evaluation laboratory

This laboratory carries out the evaluation of material - blood interaction. The tests used for evaluation of blood compatibility are classified into five categories according to the process or system being tested such as Thrombosis, Coagulation, Platelets and platelet function, hematology and immunology. In addition, quantitative estimation of protein absorption and endothelial cell adhesion and proliferation on surfaces with ability to resist shear stress under, which are important indicators of blood compatibility are also being carried out. Other activities include the development of new reagents and tests for evaluation of blood - material interaction, development of haemostatic bioadhesives for clinical applications, studies on modification of blood contacting biomaterials to reduce thrombogenicity by tissue engineering, in combination with bioadhesives.

Some of the Partial thromboplastin time (PTT) is a blood test that looks at how long it takes for blood to clot. It can help tell if there is any bleeding or clotting problems. Prothrombin (PT) is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to check for bleeding problems. PT is also used to check whether medicine to prevent blood clots is working. A fibrinogen activity test is used to determine the level of fibrinogen in blood.

List of thrombosis tests and their relevant standards are mentioned in table 2.6 and min required laboratory equipment list shown in table 2.7.

Table2.6: List of thrombosis research tests

LAB Category	Test Name	Test Standard
Hemocompatibility evaluation laboratory	11.Percentage hemolysis 12.Partial Thromboplastin time (PTT) 13.Prothrombin Time (PT) 14.Fibrinogen Assay	ISO 10993-4

Table2.7: List of Hemocompatibility evaluation laboratory equipment

S. No	Equipment Name
1	Laminar Flow Bench
2	Incubator
3	Thermal Incubator
4	Water bath
5	Spectrophotometer (UV Based)
6	Biochemistry Analyzer
7	Hematology Analyzer
8	Coagulameter
9	Agrigometer
10	Elisa reader
11	Flow cytometer
12	Anti coagulated tube
13	Microscope-binocular
14	Multiplate photometer
15	Pipette (all sizes)
17	Cryogenic storage vessel
18	Thermometer
19	Basic laboratory glassware/plasticware
20	Ultraviolet lamp-Laboratory purpose
21	General Laboratory Instruments
22	Chemical Testing Instruments

2.2.4 Sterility evaluation laboratory

The Sterility evaluation laboratory is a complete diagnostic laboratory that performs testing for a full range of human pathogens including aerobic and anaerobic bacteria; yeasts and molds; blood, tissue, enteric parasites; and mycobacteria.

Once isolated and identified, antibiotic susceptibility testing is performed on bacteria and antifungal susceptibility testing on yeast. Additional activities include reference microbiology, support to epidemiology and infection control programs, surveillance cultures for the detection and monitoring of outbreak investigations, supporting control and prevention of health care associated infections, and applied research.

The test is applied to substances or preparations which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating Microorganism has been found in the sample examined in the conditions of the test. List of microbiology tests and their relevant standards are mentioned in table 2.8 and min required laboratory equipment list shown in table 2.9.

Table2.8 : List of Microbiology tests

LAB Category	Test Name	Test Standard
Microbiology Laboratory	15. Microbiological Sterility Testing	
	15.1. As per USP without antimicrobial activity	15.1.USP 71
	15.2. As per USP with antimicrobial activity	15.2.USP 71
	15.3. As per ISO-sterilization validation- without antimicrobial activity	15.3. Approved protocol based on ISO 11737
	15.4.As per ISO-For samole containing anti microbial property	15.4. USP 71
	16. Invitro bacterial reverse mutation assay (Ames test)	16. ISO 10993-3
	16.1.5 strains, single concentration, one extractant	
	16.2. 5 strains, single concentration, two extractants	
	16.2. 5 strains, five concentrations, two extractants	
	17.Microbiological testing-General Analysis	
	17.1. Anti Microbial Activity testing	
	17.1.1. Anti Microbial Activity testing by Agar Diffusion Method for two strains	7.1.1 Approved protocol
	17.2. Anti Microbial Activity testing by parallel streak method	

Table2.9 : List of Microbiology laboratory equipment

S. No.		Equipment Name
Sterility evaluation laboratory area	1	Laminar Flow Bench Type 2A-Biological safety Cabinet
	2	Incubator
	3	Ultra low Freezer
	4	Microscope-binocular
	5	Basic laboratory glassware/plasticware
	6	ETO Sterilization
	7	Heating Block
	8	Versatile Mixing
	9	Refrigerator - Laboratory use
Decontamination area	10	Autoclave
	11	Hot air oven
	12	Sink- 3 different type
	13	Room fumigator
	14	Refrigerator - Laboratory use
	15	Water can
	16	Basic laboratory glassware/plasticware
Sterilization area	17	Autoclave- Horizontal
	18	Weighing balance
	19	PH meter with flat probe
	20	Conductive meter
	21	Microwave
Media/Chemical storage area	22	Storage rack
	23	Data weighing system
	24	Room Air monitoring system
Controlled environment	25	Refrigerator - Laboratory use
	26	Laminar Flow Bench Type 2A-Biological safety Cabinet
	27	Incubator-Cooled
	28	Incubator-Ambient
	29	Media sterilizer

2.2.5 Histopathology evaluation laboratory

An important measure of hemocompatibility is the hemolysis test, which measures the ability of a material or material extract to cause rupture of red blood cells. Hemolysis testing should be performed on all materials directly contacting the bloodstream, or any materials used to form a fluid conduit to the bloodstream. The following tests are derived from well-established studies/ standards and are useful in evaluating a variety of materials intended to contact blood or fluids entering the circulatory system. ASTM methods are accepted by regulatory agencies, as preferred method for compliance.

List of histopathological evaluation tests and their relevant standards are mentioned in table 2.10 and min required laboratory equipment list shown in table 2.11.

Table2.10: List of Histopathology tests

LAB Category	Test Name	Test method /Standard
Histopathology Laboratory	18.Gross and Histopathological evaluation - soft tissue	18.ISO 10993-6
	19. Gross and Histopathological evaluation - hard tissue	19.ISO 10993-6

Table2.11: List of Histopathology laboratory equipment

	S No.	Equipment Name
Processing area	1	Laminar Flow Bench
	2	Macro Digital Imaging System
	3	Instrument for chemical handling
	4	Swart tissue processor
Embedding area	5	Tissue Embedding
Staining area	6	Auto Stainer
	7	Coverslipper
Sectioning area-Soft tissue	8	Fully Automated Rotary Microtome-sectioning equipment
	9	Water Bath
Sectioning area-Hard tissue	10	Precision saw
	11	Grinder
Analyzing area	12	Trinocular Fluorescence Microscope
	13	Microscope
	19	Basic laboratory glassware/plasticware
	20	Ultraviolet lamp-Laboratory purpose
	21	General Laboratory Instruments
	22	Chemical Testing Instruments

2.2.6 Physiochemcial evaluation laboratory

Physiochemcial evaluation laboratory lab focuses on polymers, polymeric formulations, composites and devices suitable for different biomedical applications. The laboratory is equipped with facilities for compounding/ mixing, moulding, extrusion and electrospinning facilities. The laboratory is also equipped with equipment for static testing and dynamic mechanical analysis of polymeric materials. Facilities for polymer synthesis are also in place.

List of polymer analysis/processing tests and their relevant standards are mentioned in table 2.12 and min required laboratory equipment list shown in table 2.13. List of physiochemical material procedures mentioned in table 2.14.

Table2.12: List of Phsiochemical tests

LAB Category	Test Name	Test method/Standard
Physiochemcial evaluation laboratory	22.X-ray Diffraction Spectrum	22.Approved Protocol
	23.Vicker's Mirco Hardness Testing	23.Approved Protocol
	24.Mechanical Testing	24.Approved Protocol based on ASTM
	25.Dynamic Mechanical Analysis	25.Approved Protocol
	26.Mechanical Testing Using UTM	26.Approved Protocol based on ASTM
	27.FTIR Spectroscopy	27.Approved Protocol based on ASTM
	28.FT Raman Spectroscopy	28.Approved Protocol
	29.Thermocycler	29.Approved Protocol
	30.Micro CT Imaging	30.Approved Protocol
	31.Water sorption & Solubility	31.As per ISO
	32.Thermal Analysis	
	13.1.DSC in O2/Nitrogen Atmosphere	32.ASTM E1356-03
	13.2.DTA (RT to 1400 degree C)	33.Approved Protocol
	13.3.TGA(RT to 1400 degree C)	34.ASTM 1131-03
	33.GPC/HPLC/GC	33.ASTM D 5296/ANSI
	34.FTIR Spectroscopy (ATR/KBr)	34.Approved Protocol based on ASTM
	35.UV-VISIBLE light Spectroscopy	35.Approved Protocol
	36.Transmission Electron Microscopy (TEM) Analysis	36.Approved Protocol
	37.SEM Analysis	37.Approved Protocol
	38.Confocal Microscopy	38.Approved Protocol

Table2.13: List of Physiochemcial evaluation laboratory equipment

S. No.	Material Characterization
	Test name
1	Dynamic Mechanical Tester
2	FTIR spectroscope
3	FT Raman spectroscope
4	Thermocycler
5	Micro CT imager
6	Vicker's micro hardness tester
7	X-ray diffraction spectrum analyser
8	Gel Permeation Chromatography (GPC) High pressure liquid Chromatography (HPLC) Gas Chromatography (GC)
9	UV-Vis spectroscope
10	Life/surface profilometer
11	Transmission Electron Microscope
12	SEM/SEM-EDS Analyser
13	Confocal microscope

Table2.14: List of physiochemical material procedures

S. No.	
TESTS PROCEDURES FOR EXTRACTABLE MATERIAL	
1	UV/Visible Spectroscopy
2	Gas Chromatography
3	Liquid Chromatography
4	Infrared Spectroscopy (IR)
5	Mass Spectrometry
6	Residual Solvents
7	Atomic Absorption Spectroscopy (AAS)
8	Inductively-coupled Plasma Spectroscopy (ICP)
BULK MATERIAL CHARACTERIZATION	
9	Infrared Spectroscopy Analysis for Identity and Estimation of Gross Composition
10	Reflectance Spectroscopy
11	Transmission Spectroscopy
12	Atomic Absorption Spectroscopy (AAS)
13	Inductively-coupled Plasma Spectroscopy (ICP)
14	Thermal Analysis
SURFACE CHARACTERIZATION	
15	IR Reflectance Spectroscopy
16	Scanning Electron Microscopy (SEM)
17	Energy-dispersive X-ray Analysis (EDX)

2.2.7 Accelerated Aging laboratory

Since, evaluating the long term operating life of a product or system under real conditions would require years of time; hence there is need for tests that would include accelerated aging. Once levels of accelerated aging is measured, it is easier to then extend the concept to the lifetime of implantable device.

List of accelerated aging tests and the required laboratory equipment list is given in table 2.15.

Table2.15: List of accelerated aging laboratory equipment

S. No.	Biomaterials Testing Equipment	Description
1	Multi-Turn torsion testers	Provides dependable torsion, axial-torsion and low torque testing capabilities
2	Model 3345	Single column system performing a peel test
3	Optical profilometer	
4	Servo-Hydraulic Testing Machine	-Used to examine the physical strength of biological materials. -Can produce computer-controlled displacements or forces of almost any desired shape and duration. -Capable of producing 50ms sinewave load and position-controlled impacts of 13.0 kN. -Data can be analyzed to produce force-time and force-displacement curves, as well as calculate stiffness, Young's modulus and yield strength
5	Torque Testing Machine	-Provides torque moment or rotational movement to an object using load cell of either 25 inch/lbs or 200 inch/lbs Uses optical rotary encoder to measure rotary motions. -Used to evaluate surgical equipment and procedures, as well as bone repair methods and materials. -Used to determine failure torque, energy and stiffness of these materials
6	High-Speed Pneumatic Impacter	-Can produce an impact of up to 35 mph to simulate a car crash or other high speed impact injury. -Uses high-pressure nitrogen to fire a piston, accelerating an impact mass toward the target object. -Initial velocity of the impact mass is measured with an optically triggered speed trap. The impact forces are measured with an inertially compensated load cell. -Used to test the response of tissues and bones to high-speed impacts. -Results are used to design safety equipment.

S. No.	Biomaterials Testing Equipment	Description
7	Hyperextension Fixture	-Custom designed to test behavior of specimens under loads while allowing rotation. -Used in conjunction with the XY table to simulate tensile or compressive stresses -Used in research to analyze the mechanical behavior of knee joints when forces similar to those experienced in nature are applied.
8	Stainless Steel XY Table	-Allows researcher to exert tensile or compressive forces in Z-direction while allowing free movement in the X and Y directions. -Can be mounted on the Instron testing machine, where data can be captured and the XY displacement data from the table can be synchronized. -Table's position can be measured to 1/1000 of a millimeter and can be read at 10,000 samples per second. -Y Axis can be fixed in order to measure any loads in Y direction.
9	Aluminum XY Table	-Allows researcher to exert tensile or compressive forces in Z-direction while allowing free movement in the X and Y directions. -Can be mounted on the Instron testing machine, where data can be captured and the XY displacement data from the table can be synchronized. -Table's position can be measured to 1/1000 of a millimeter and can be read at 10,000 samples per second. -Smaller, designed for lighter loads than the stainless steel XY Table
10	Rotary Encoders	-Available with a resolution of 0.02 degrees, sampled at up to 10,000 samples per second. -Data from encoders, XY Table and Instron can be synchronized. -Used to measure angular motions -Experiment shown on the right uses two encoders to calculate the bending moment at the joint.

informed design decisions.

2.2.8 Package Validation Laboratory

It provides package testing services that evaluate the strength and integrity characteristics of a packaging system before and after simulating anticipated distribution and storage conditions that the system may undergo. A combination of simulation and evaluation is used to validate package compliance to ASTM, ISO, ISTA and other accepted industry standards.

Vibration, physical shock, thermal shock, friction, flow rate, force to operate, leakage and compression testing are among the many tests that are carried out. Custom test development and protocol creation can also be provided to support specific requirement.

To complement its packaging testing, Product & Materials Division takes design understanding to a deeper level by testing and evaluating the materials that make up the package itself. From tensile and tear testing of poly films and trays to applying compression and tension to packaging components materials testing can increase a packaging engineer's understanding of available options, allowing for more

3.1 Laboratory Setup :

Resources required for setting up a laboratory primarily includes

3.1.1 Human Resources

3.1.2 Infrastructural Layout

3.1.3 Equipment and Instruments (2.2.1 to 2.2.8)

3.1.1 Human Resources

For the laboratory, the number of personnel and their educational and experience levels depends on the type of tests to be offered, the methods chosen and the expected sample output. The first step is to create an organized structure for the test laboratory and to define the activities that would take place in the laboratory, as illustrated in figure 3.1

The laboratory's human resource pool are grouped into different sections depending on the type of EMI/EMC tests it intends to perform. Similar types of tests are usually grouped into one section. Ideally, a senior scientist is responsible for each area, with 2 or 3 technicians to rotate around the various tests within their 'section'. This enables an overall knowledge to be gained of the working requirements for each area of the laboratory. It is important to keep the job interesting and challenging for staff and to avoid monotonous, repetitive work where ever possible. The vertical division of the laboratory reflects the different positions and responsibilities within the organization. As a guide, some typical positions can be identified as follows:

i. The Laboratory Manager is responsible for the whole laboratory and the development of its strategic plan. A key work of this position is the external communication with clients and potential clients, as well as full responsibility for results reported to clients. Management systems must be put in place to ensure reliable data are produced and that the reporting of this data is thoroughly checked prior to releasing reports.

ii. The Quality Assurance (QA) Manager is responsible for quality assurance within the laboratory, and should have an independent position. The QA Manager may also have responsibility for Health and Safety Management, Environmental Management within the laboratory.

iii. The Section Head or Senior Technician is responsible for the daily organization of the analytical process, ensuring that daily and weekly deadlines within their section are met; quality control for each batch of testing meets requirements and is recorded; staff training is up-to-date; and that there are sufficient staff to meet the workload requirements. Maintaining stocks of the necessary chemicals and consumables are also the responsibility of the senior technician, who should inform the Laboratory Manager in sufficient time to enable ordering and delivery prior to stocks running low. The Section Head or Senior Technician is also responsible for specific equipment and methods, especially trouble-shooting and maintenance as well as continuing training of junior staff.

iv. Junior technician(s) are responsible for performing analytical work following Standard Operating Procedures (SOPs), under the direction of the Section Head or Senior Technician.

This organization reflects an 'ideal' situation in a matured laboratory. In a new laboratory, however, the structure may initially be quite different. From the outset, the laboratory should have all expertise needed to perform all the methods it offers. In practice this means recruiting senior technicians with the background needed for the methods. This group should be regarded as the backbone of the laboratory that trains additional personnel in case of an increasing

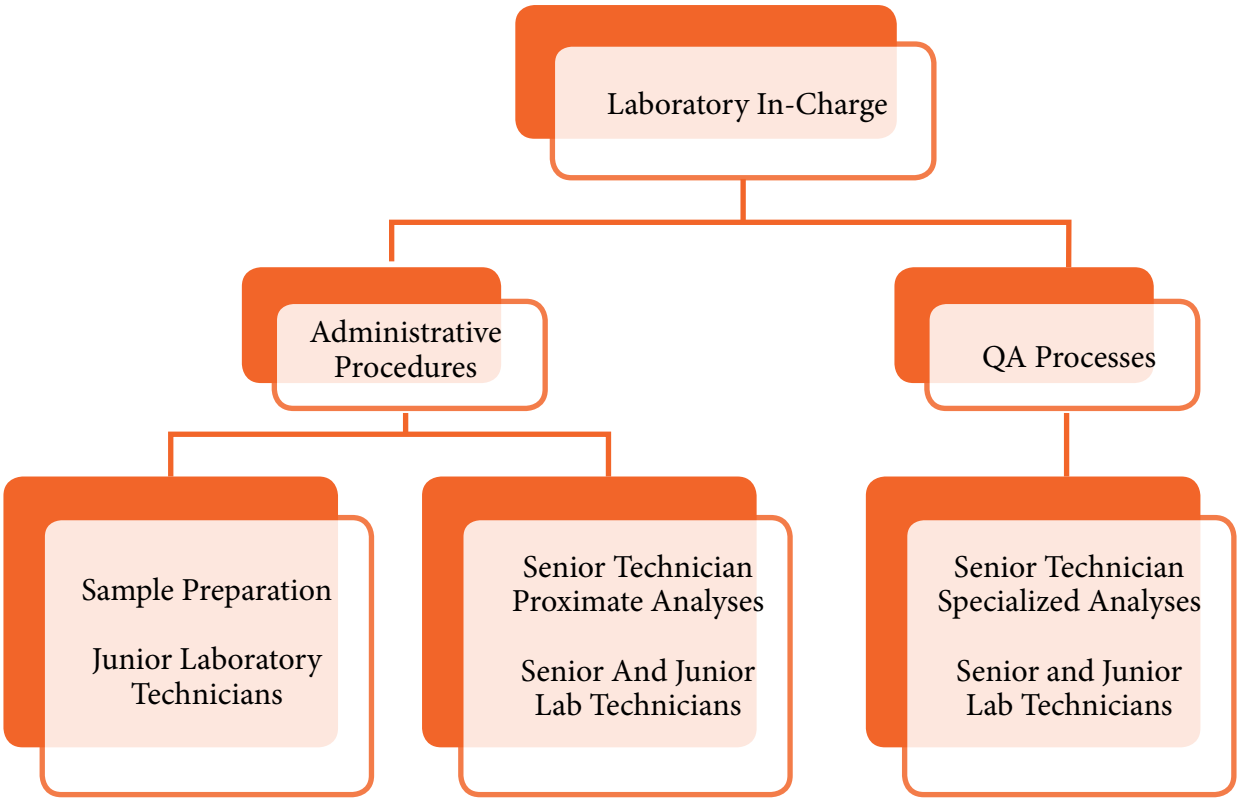


Figure 3.1: Laboratory Organizational Structure

volume of work and as a back-up for each assay. If the laboratory is part of a larger organization, such as a research organization, its position and relationship with the other units should also be clearly described within its structure. The laboratory has a responsibility to ensure that the quality and credibility of its results are of the utmost quality.

Besides the physical completion of the laboratory, procedures such as SOPs, quality control program, participation in proficiency (both internal and external) programs and use of reference materials are needed to be put in place to ensure that laboratory consistently produces results of a high accuracy. These procedures should guarantee that all aspects of the analytical process are performed efficiently and are traceable. This should be documented in a set of SOPs that form the basis of the Quality Management System (QMS).

Procedures should ensure that the laboratory can prioritize and organize its workload and guarantee the quality of results produced. In the initial phase, however, the focus should be on aspects that directly influence the quality of the results. These includes:

- Acceptance criteria for samples.
- Sample preparation.
- Description of methods, including validation of results.
- Quality control.
- Maintenance and calibration records of equipment.
- Job descriptions, including responsibilities and continuing competence of individual technical staff.
- Training records of technical staff, covering which methods they can perform, level of training, whether they can perform a method independently or under supervision, and their ability to train others, etc.
- Traceability and storage of raw data.
- Cleaning procedures for the laboratory.

The presence and implementation of these procedures from the initiation of the laboratory will positively affect the quality of the results, and can be used as a starting point for the implementation of a comprehensive quality system.

3.1.2 Layout and Controls

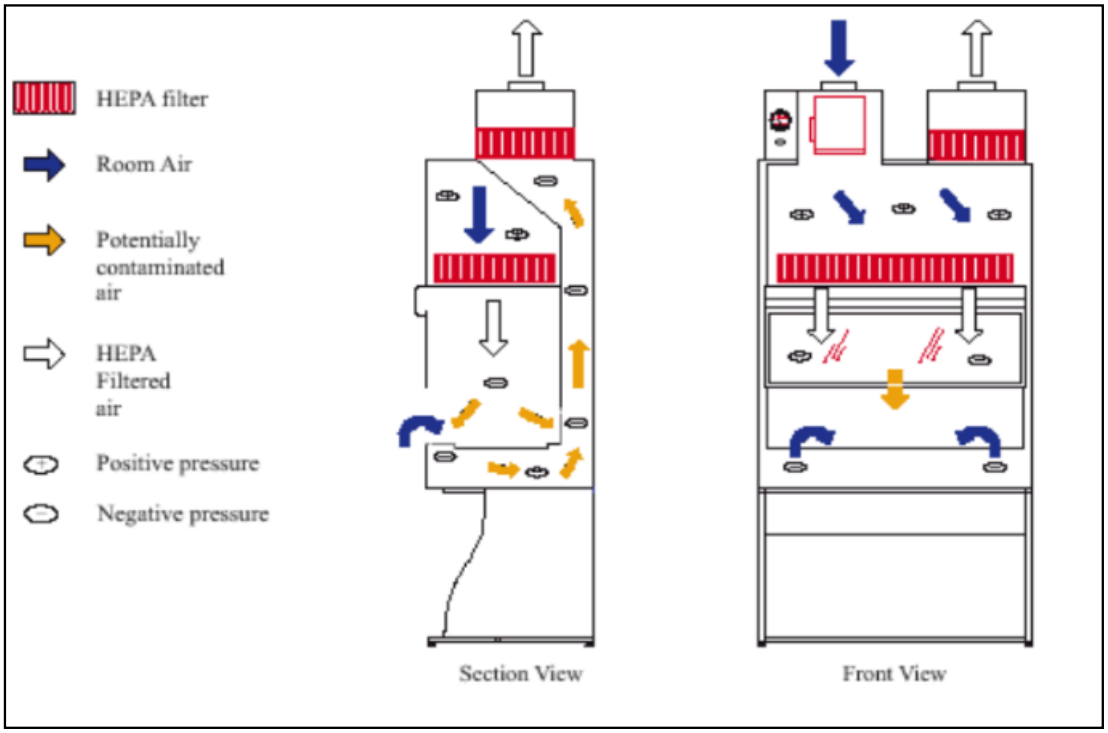
In the hierarchy of controls, the highest level of control is directed at the source. From an occupational health perspective, the highest level of control may be immunization of workers who may come in direct contact with infected samples, good controls such as vaccines, proper ventilation, needleless systems, safety engineered sharps, biological safety cabinets, and effective biological waste containment contribute to minimizing the transmission of infectious agents. Engineering controls, once designed and implemented, are not under the control of the worker, but are directed at the source of the hazard.

The main health and safety issues for laboratory technicians are:

- Toxic, corrosive, and flammable chemical hazards.
- Exposure to compressed gases.
- Exposure to blood, body fluids, and tissues which may contain infectious agents.
- Needle-stick injuries.
- Fires from flammable materials and electronic equipment.
- Physical hazards such as radioactive materials, ultraviolet light sterilizers and lasers.
- Cryogenic (ultra-cold) materials, e.g., liquid nitrogen and dry ice (solid carbon dioxide).
- Working in awkward positions.
- Standing for long periods of time, creating risks of back and arm injuries.
- Repetitive motion injuries.
- Electrical hazards from electrical equipment and instruments.
- Slips, trips and falls from spilled liquids and congested work areas.
- Cuts and lacerations from broken glass.
- Burns and scalds from hot equipment

Biological Safety cabinets are specialized local exhaust ventilation devices often used in laboratories. Class II cabinets are the most common type used in biomedical laboratories as they provide both health care worker (HCW) and sample, protection from contamination. Several types of Class II cabinets are available. These types vary as per the percentage of air recirculated to the cabinet, and by the type of exhaust (hard-ducted to the exterior or exhausted into the laboratory air). The following figure 3.2 depicts how the ventilation works in a Class II cabinet (Type B2), making the laboratory safe and hazards free.

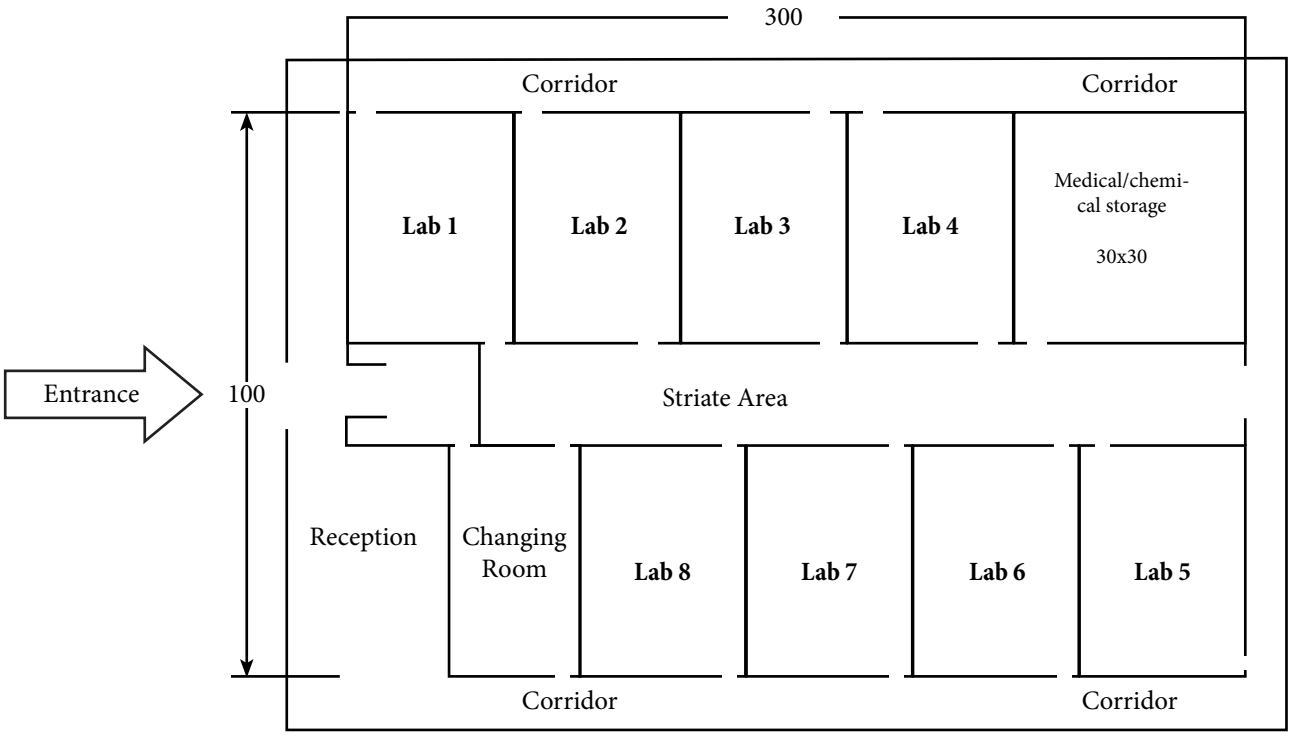
Figure 3.2: Air circulation system in a BSC Class II cabinet (Type B2)



Biomaterials and biocompatibility testing laboratories building should have 3,000-4,000 square foot facility with all and laboratories, reception area, staff changing room, storage room, surgery suite, necropsy lab, radiation lab, procedure rooms, and support areas. The laboratory should have a HEPA-filtered air supply and dedicated procedure space. Animal facilities and critical equipment are to be monitored 24/7. Emergency power is supplied by an on-site generator.

As shown in figure 3.3, Laboratory1 - Laboratory 8 should have one way movement for both staff and sample with maintaining sterility of the area.

Figure 3.3: Layout of Biomaterials and biocompatibility testing laboratories



3.2 CONCLUSION

Biomaterials have found growing application in healthcare technology industry including drug delivery systems, active substance encapsulation, tissue scaffolds, wound care, implants, cosmetics and diagnostics.

There is a growing interest in the processing of biomaterials as developers find new applications. Novel methods of processing have also given rise to materials with controlled physical properties (e.g. porosity).

Biomaterial and Biocompatibility Test Laboratory aims to provide Biomaterials Testing Solutions and gain extensive knowledge on how these materials behave under a variety of different mechanical and environmental stresses throughout their life-cycle, including manufacturing, sterilization and in-vivo functioning. The laboratory may also cater to the experiments which study the properties of the various biological materials. The Laboratory may also capacity to understand the biosafety of a growing number of biomaterials utilized in healthcare products through physical, chemical, mechanical and microbiological testing.

Annexure 1

STANDARDS FOR PARTICLE TESTING AND CORROSION TESTING

S. No.	Standards	Title	Description
1	ASTM F 1089	Standard Test Method for Corrosion of Surgical Instruments	This test method covers general test procedures and evaluation criteria for the corrosion resistance of surgical instruments intended for reuse in surgery and fabricated from stainless steel such as, but not limited to, those listed in Specification F899 (Standard Specification for Wrought Stainless Steels for Surgical Instruments).
2	ISO 10271:2011:	Dental metallic materials - Corrosion test methods for metallic materials.	ISO 10271:2011 provides test methods and procedures to determine the corrosion behaviour of metallic materials used in the oral cavity. It is intended that the test methods and procedures in ISO 10271:2011 be referred to in the individual International Standards specifying such metallic materials. This is not applicable to instruments and dental amalgam and appliances for orthodontics.
3	ISO 13402:1995:	Surgical and dental hand instruments -- Determination of resistance against autoclaving, corrosion and thermal exposure	Surgical and dental hand instruments - Determination of resistance against autoclaving, corrosion and thermal exposure
4	ISO 16428:2005:	Implants for surgery -- Test solutions and environmental conditions for static and dynamic corrosion tests on implantable materials and medical devices	ISO 16428:2005 specifies standard environmental conditions for the testing of metallic materials intended for implantation, surgical implants, and medical devices. The testing conditions described simulate physiological conditions in a simplified manner controlling the test solution, the temperature, the gaseous atmosphere and the proportions of sample size and volume of solution.
5	ISO 16429:2004:	Implantable materials and medical devices over extended time periods.	Implants for surgery -- Measurements of open-circuit potential to assess corrosion behaviour of metallic ISO 16429:2004 specifies a test method for measurements over extended time periods of the open-circuit potential of implant materials and surgically implantable devices immersed in a test environment related to body fluid, using a standard corrosion test cell to study the electrochemical corrosion properties of the devices.

S. No.	Standards	Title	Description
6	ASTM F746-04:	Standard test method for pitting or crevice corrosion of metallic surgical implant materials	This test method covers the determination of resistance to either pitting or crevice corrosion of metals and alloys from which surgical implants will be produced. It is a modified version of an established test and is used as a screening test to rank surgical implant alloys in order of their resistance to localized corrosion.
7	ASTM F897-02 (2007):	Standard Test Method for Measuring Fretting Corrosion of Osteosynthesis Plates and Screws	This test method provides a screening test for determining the amount of metal loss from plates and screws used for osteosynthesis (internal fixation of broken bones) due to fretting corrosion in the contact area between the screw head and the plate hole countersink area. The implants are used in the form they would be used clinically. The machine described generates a relative motion between plates and screws which simulates one type of motion pattern that can occur when these devices are used clinically.
8	ASTM F1801-97 (2004)	Standard Practice for Corrosion Fatigue Testing of Metallic Implant Materials	This practice covers the procedure for performing corrosion fatigue tests to obtain S-N fatigue curves or statistically derived fatigue strength values, or both, for metallic implant materials. This practice describes the testing of axially loaded fatigue specimens subjected to constant amplitude, periodic forcing function in saline solution at 37°C and in air at room temperature. The environmental test method for implant materials may be adapted to other modes of fatigue loading such as bending or torsion. While this practice is not intended to apply to fatigue tests on implantable components or devices, it does provide guidelines for fatigue tests with standard specimens in an environment related to physiological conditions.
9	ASTM F2129:	Standard Test Method for Conducting Cyclic Potentiodynamic Polarization Measurements to Determine the Corrosion Susceptibility of Small Implant Devices	This test method assesses the corrosion susceptibility of small, metallic, implant medical devices, or components thereof, using cyclic (forward and reverse) potentiodynamic polarization. Examples of device types that may be evaluated by this test method include, but are not limited to, vascular stents, ureteral stents (specification F 1828), filters, support segments of endovascular grafts, cardiac occluders, aneurysm or ligation clips, staples, and so forth.

S. No.	Standards	Title	Description
10	G71: ASTM G71-81(2009)	Standard Guide for Conducting and Evaluating Galvanic Corrosion Tests in Electrolytes	This guide covers conducting and evaluating galvanic corrosion tests to characterize the behavior of two dissimilar metals in electrical contact in an electrolyte under low-flow conditions. It can be adapted to wrought or cast metals and alloys.
11	G 61	Test Method for Conducting Cyclic Potentiodynamic Polarization Measurements for Localized Corrosion Susceptibility of Iron-, Nickel-, or Cobalt-Based Alloys	This test method covers a procedure for conducting cyclic potentiodynamic polarization measurements to determine relative susceptibility to localized corrosion (pitting and crevice corrosion) for iron-, nickel-, or cobalt-based alloys in a chloride environment.
12		Stent fatigue testing	GODMANN test - there is no standard for this, this is a curve achieved by applying repeated cycles and hence is one of the methods within the broad engineering scope of fatigue testing.
13	ASTM F2477 - 07(2013):	Standard Test Methods for in vitro Pulsatile Durability Testing of Vascular Stents	This test method covers the procedure for determining the durability of ballon-expandable and self- expanding metal or alloy vascular stents. Tests are performed by exposing specimens to physiologically relevant diametric distention levels using hydrodynamic pulsatile loading. Specimens should have been deployed into a mock or elastically simulated vessel prior to testing. The test methods are valid for determining stent failure due to typical cyclic blood vessel diametric distention and include physiological pressure tests and diameter control tests. These do not address other modes of failure such as dynamic bending, torsion, extension, crushing, or abrasion. Test apparatus include a pressure measurement system, dimensional measurement devices, a cycle counting system, and a temperature control system.

Annexure 2

MECHANICAL TESTING LAB FOR AGEING, PACKAGE VALIDATION, WEAR AND TEAR TESTING

S. No.	Standards	Title	Description
1	ISO 18192-1:2011:	Implants for surgery -- Wear of total intervertebral spinal disc prostheses -- Part 1: Loading and displacement parameters for wear testing and corresponding environmental conditions for test	This standard defines a test procedure for the relative angular movement between articulating components, and specifies the pattern of the applied force, speed and duration of testing, sample configuration and test environment for use for the wear testing of total intervertebral spinal disc prostheses.
2	ISO 18192-2:2010:	Implants for surgery - Wear of total intervertebral spinal disc prostheses, Part 2: Nucleus replacements	This defines a test procedure for spinal nucleus prostheses under the relative angular movement conditions specified by ISO 18192-1. ISO 18192-2:2010 is applicable to both lumbar and cervical prostheses. It is not applicable to total disc replacements and facet joint replacements. The method includes wear and fatigue testing. Additional mechanical tests such as creep tests can be required. ISO 18192-2:2010 does not reproduce the complex in vivo loads and motions. The wear and fatigue data obtained with this test method will enable comparison between different types of implant but can differ from the clinical wear performance.
3	Astm F1980	Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices	This guide provides information for developing accelerated aging protocols to rapidly determine the effects, if any, due to the passage of time on the sterile integrity of the sterile barrier system (SBS) and the physical properties of their component packaging materials

Annexure 3

FLUID FLOW TESTING TO MEASURE FUNCTIONALITY OF DEVICE IN PRESENCE OF BODY FLUIDS

S. No.	Standards	Title	Description
1	ISO 5840:2005:	Cardiovascular implants -- Cardiac valve prostheses	Apart from basic material testing for mechanical, physical, chemical and biocompatibility characteristics, part of ISO 5840 also covers important hydrodynamic and durability characteristics of transcatheter heart valve substitutes and their delivery systems. This part of ISO 5840 does not specify exact test methods for hydrodynamic and durability testing but it offers guidelines for the test apparatus.

Annexure 4

SPINE STIMULATOR TEST (FOR ORTHOPAEDIC IMPLANTS)

S. No.	Standards	Title	Description
1		Spine stimulator test	This is an active implant and not an orthopaedic implant (it is a signalling system which is to solve the pain caused by orthopaedic issues) and consists of a combination of components, hence a test as such does not exist – instead they have to be tested in accordance with ISO 13485 for all relevant electronics and metallic components including ISO 60601-1 & RoHS.

Annexure 5

TOTAL KNEE WEAR TEST(FOR ORTHOPAEDIC IMPLANTS)

S. No.	Standards	Title	Description
1	ISO 14242-1:2012:	Implants for surgery -- Wear of total hip-joint prostheses -- Part 1: Loading and displacement parameters for wear-testing machines and corresponding environmental conditions for test	The test specifies the relative angular movement between articulating components, the pattern of the applied force, the speed and duration of testing, the sample configuration and the test environment to be used for the wear testing of total hip-joint prostheses.
2	ISO 14242-3:2009:	Wear of total hip-joint prostheses -- Part 3: Loading and displacement parameters for orbital bearing type wear testing machines and corresponding environmental conditions for test	ISO 14242-3:2009 specifies relative angular movement between articulating components, the pattern of the applied force, speed and duration of testing, sample configuration and test environment to be used for the orbital bearing type wear testing of total hip joint prostheses.

S. No.	Standards	Title	Description
3	ISO 14243-1:2009 :	Implants for surgery -- Wear of total knee-joint prostheses -- Part 1: Loading and displacement parameters for wear-testing machines with load control and corresponding environmental conditions for test	The test specifies the flexion/extension relative angular movement between articulating components, the pattern of the applied force, speed and duration of testing, sample configuration and test environment to be used for the wear testing of total knee-joint prostheses in wear-testing machines with load control.
4	ISO 14243-2:2009:	Implants for surgery -- Wear of total knee-joint prostheses -- Part 2: Methods of measurement	The test specifies a method of assessment of wear of the tibial component of total knee-joint prostheses using the gravimetric technique for components tested in accordance with ISO 14243-1.
5	ISO 14243-3:2004:	Implants for surgery -- Wear of total knee-joint prostheses -- Part 3: Loading and displacement parameters for wear-testing machines with displacement control and corresponding environmental conditions for test	This standard specifies relative movement between articulating components, the pattern of the applied force, speed and duration of testing, sample configuration and test environment to be used for the wear testing of total knee-joint prostheses in wear-testing machines having axial load control, flexion/extension angular motion control, AP displacement control and tibial rotation control.
6	ISO/CD 14243-4:	Implants for surgery -- Wear of total knee prostheses -- Part 4: Wear of the patella-femoral joint -- Loading and displacement parameters for wear-testing machines and corresponding environmental conditions for test	This test focuses on loading and displacing parameters and the impact it makes to the environment.
7	ISO 17853:2011:	Wear of implant materials -- Polymer and metal wear particles -- Isolation and characterization	This test specifies methods for sampling wear particles generated by joint replacement implants in humans and in joint simulators. It specifies the apparatus, reagents and test methods to isolate and characterize both polymer and metal wear particles from samples of tissues excised from around the joint replacement implant, obtained at revision surgery or post mortem, and from samples of joint simulator test fluids.

Annexure 6

MRI COMPATIBILITY TEST

S. No.	Standards	Title	Description
1	ISO/TS 10974:2012:	Assessment of the safety of magnetic resonance imaging for patients with an active implantable medical device	ISO/TS 10974:2012 is applicable to implantable parts of active implantable medical devices (AIMDs) intended to be used in patients who might undergo a magnetic resonance scan in 1,5T, cylindrical bore, whole body MR scanners for imaging the hydrogen nucleus. The tests that are specified in ISO/TS 10974:2012 are type tests intended to be carried out on samples of a device to characterize interactions with the magnetic and electromagnetic fields associated with an MR scanner.
2	IEC 60601-2-33:	Medical electrical equipment - Part 2-33: Particular requirements for the basic safety and essential performance of magnetic resonance equipment for medical diagnosis	IEC 60601-2-33:2010 establishes particular basic safety and essential performance requirements for magnetic resonance equipment to provide protection for the patient and the magnetic resonance worker
3	ASTM F2182 - 11a :	Standard Test Method for Measurement of Radio Frequency Induced Heating On or Near Passive Implants During Magnetic Resonance Imaging	This test method covers measurement of radio frequency (RF) induced heating on or near a passive medical implant and its surroundings during magnetic resonance imaging (MRI)
4	ASTM F2119-07(2013):	Standard Test Method for Evaluation of MR Image Artifacts from Passive Implants	This test method characterizes the distortion and signal loss artifacts produced in a magnetic resonance (MR) image by a passive implant (implant that functions without the supply of electrical or external power). Anything not established to be MR-Safe or MR-Conditional is excluded.
5	ASTM F2503-13:	Standard Practice for Marking Medical Devices and Other Items for Safety in the Magnetic Resonance Environment	This international standard applies to the practice of marking of items that might be used in the magnetic resonance (MR) environment.

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