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Standard Practice for Selecting Generic Biological Test Methods for Materials and Devices[1]

This standard is issued under the fixed designation F748; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice recommends generic biological test methods for materials and devices according to end-use applications. While chemical testing for extractable additives and residual monomers or residues from processing aids is necessary for most implant materials, such testing is not included as part of this practice. The reader is cautioned that the area of materials biocompatibility testing is a rapidly evolving field, and improved methods are evolving rapidly, so this practice is by necessity only a guideline. A thorough knowledge of current techniques and research is critical to a complete evaluation of new materials.

1.2 These test protocols are intended to apply to materials and medical devices for human application. Biological evaluation of materials and devices, and related subjects such as pyrogen testing, batch testing of production lots, and so on, are also discussed. Tests include those performed on materials, end products, and extracts. Rationale and comments on current state of the art are included for all test procedures described.

1.3 The biocompatibility of materials used in single or multicomponent medical devices for human use depends to a large degree on the particular nature of the end-use application. Biological reactions that are detrimental to the success of a material in one device application may have little or no bearing on the successful use of the material for a different application. It is, therefore, not possible to specify a set of biocompatibility test methods which will be necessary and sufficient to establish biocompatibility for all materials and applications.

1.4 The evaluation of tissue engineered medical products (TEMPs) may, in some cases, involve different or additional testing beyond those suggested for non-tissue-based materials and devices. Where appropriate, these differences are discussed in this practice and additional tests described.

1.5 The ethical use of research animals places the obligation on the individual investigator to determine the most efficient methods for performing the necessary testing without undue use of animals. Where adequate prior data exists to substantiate certain types of safety information, these guidelines should not be interpreted to mean that testing should be unnecessarily repeated.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1262 Guide for Performance of Chinese Hamster Ovary Cell/Hypoxanthine Guanine Phosphoribosyl Transferase Gene Mutation Assay

F619 Practice for Extraction of Medical Plastics

F719 Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation

F720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test

F749 Practice for Evaluating Material Extracts by Intracutaneous Injection in the Rabbit

F750 Practice for Evaluating Material Extracts by Systemic Injection in the Mouse

F756 Practice for Assessment of Hemolytic Properties of Materials

F763 Practice for Short-Term Screening of Implant Materials

F813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone

F1027 Practice for Assessment of Tissue and Cell Compatibility of Orofacial Prosthetic Materials and Devices

[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devicesand is direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

F1408 Practice for Subcutaneous Screening Test for Implant Materials
F1439 Guide for Performance of Lifetime Bioassay for the Tumorigenic Potential of Implant Materials
F1877 Practice for Characterization of Particles
F1903 Practice for Testing For Biological Responses to Particles ***In Vitro***
F1904 Practice for Testing the Biological Responses to Particles ***in vivo***
F1983 Practice for Assessment of Selected Tissue Effects of Absorbable Biomaterials for Implant Applications
F1984 Practice for Testing for Whole Complement Activation in Serum by Solid Materials
F2065 Practice for Testing for Alternative Pathway Complement Activation in Serum by Solid Materials (Withdrawn 2016)[3]
F2147 Practice for Guinea Pig: Split Adjuvant and Closed Patch Testing for Contact Allergens
F2148 Practice for Evaluation of Delayed Contact Hypersensitivity Using the Murine Local Lymph Node Assay (LLNA)
F2382 Test Method for Assessment of Intravascular Medical Device Materials on Partial Thromboplastin Time (PTT)

2.2 *Other Referenced Documents:*
ISO/AAMI/ANSI 10993-1 Biological Testing of Medical and Dental Materials and Devices—Part 1: Evaluation and Testing within a Risk Management Process[4]
EN 10993-1 Biological Testing of Medical and Dental Materials and Devices—Part 1: Evaluation and Testing within a Risk Management Process[4]
General Program Memorandum #G95-1 FDA[5]
Immunotoxicity Testing Guidance-FDA[5]

3. Summary of Practice

3.1 A matrix listing biological endpoints relevant to a biocompatibility evaluation versus materials (devices) and their applications is included in Table 1. The expected duration of use of the device is also considered. Intraoperative is less than 24 h, short-term is up to and including 30 days, and chronic is greater than 30 days. The position of row and column intersection is marked to indicate whether assessment of a biological endpoint is recommended for a material or device for the specific application indicated. The terms relating to device or material type and application are addressed in Section 5. Discussion of applicability, current state of the art, and rationale for individual biological endpoint assessments also appears in that section.

4. Significance and Use

4.1 The objective of this practice is to recommend appropriate biological endpoint assessments (which may or may not require testing) to establish a reasonable level of confidence concerning the biological response to a material or device, while at the same time avoiding unnecessary testing.

4.2 This practice is intended to provide guidance to the materials investigator in selecting the proper procedures to be carried out for the screening of new or modified materials. Because each material and each implant situation involves its own unique circumstances, these recommendations should be modified as necessary and do not constitute the only assessment that will be required for a material. Nor should these guidelines be interpreted as minimum requirements for any particular situation. While an attempt has been made to provide recommendation for different implant circumstances, some of the recommended assessment may not be necessary or reasonable for a specific material or application.

5. Classification of Materials and Devices by End-Use Applications

5.1 *General:*

5.1.1 When new materials are sought for a medical application for use on humans, the material(s) may comprise the whole final device product, or may be one of many component materials in the device. The first step is a thorough literature search for previous use of the material or biocompatibility testing studies to ensure that it has not been known to produce an adverse biological response that exceeds the expected benefit in the use of the device. Note that the final fabricated product may differ chemically, physically, or biologically from the raw materials used to fabricate the product due to processing and this has to be considered when conducting a biocompatibility evaluation and/or designing test protocols. For some devices, if testing is needed, it may be necessary or desirable to take material test samples directly from the final device product. Samples should be fully representative of the finished product in terms of processing, cleaning, packaging, sterilization, and any other procedures that are performed on the materials before the device is used.

5.1.2 At this point, preliminary material screening may be employed, depending on the expertise of the organization(s) evaluating the materials. Since preliminary screening is normally an option to minimize the economic impact of a candidate material failing final biological tests after extensive time and effort, it is not a required procedure. The investigator should be aware that, should an adverse tissue response be observed with a final product, it may be impossible to determine which component or process is responsible without these initial screening tests.

5.1.3 This practice addresses two aspects of tissue-material interactions: duration and tissue type. A third aspect, which should be considered, is the relative size difference between the host and the material, that is, to how much material surface area is the host exposed. The material surface area-to-body weight ratio may become a significant factor for porous materials, and devices of repeated short-term applications (for example, dialysis products). While this practice does not address the issue of "intensity factor" of increased surface area, the biocompatibility testing facility personnel should consider it in their material screening and testing protocol design.

[3] The last approved version of this historical standard is referenced on www.astm.org.
[4] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.
[5] Available from CDRH, 5600 Fishers Ln., Rockville, MD 20857.

TABLE 1 Applicable Biological Endpoints for Biocompatibility Evaluation

Classification of Material or Device and Application	Cell Culture Cytotoxicity	Sensi-tization	Skin Irritation or Intra-cutaneous	Mucous Membrane Irritation	Systemic Toxicity, Acute or Subchronic	Blood Compatibility	Hemolysis	Pyrogenicity	Short-term Implantation	Long-term Implantation	Immune Response	Genotoxicity	Carcinogenicity
External devices													
Intact surfaces (all time periods)	x	x	x										
Breached surfaces													
Intraoperative	x	x	x										
Short-Term	x	x	x		x								
Chronic	x	x	x		x							x	
External Devices Communicating with:													
Intact Natural Channels													
Intraoperative	x	x	x	x									
Short-term	x	x	x	x	x				x				
Chronic	x	x	x	x	x				x		x	x	x
Body Tissues and Fluids													
Intraoperative	x	x	x		x			i[A]					
Short-term	x	x	x		x			i[A]	x		x		
Chronic	x	x	x		x			i[A]	x		x	x	x
Blood Path, indirect													
Intraoperative	x	x	x		x	x	x	x					
Short-term	x	x	x		x	x	x	x					
Chronic	x	x	x		x	x	x	x			x	x	
Blood Path, direct													
Intraoperative	x	x	x		x	x	x	x					
Short-term	x	x	x		x	x	x	x	x		x		
Chronic	x	x	x		x	x	x	x	x		x	x	x
Implanted Devices principally contacting Bone/Tissue/tissue fluid													
Intraoperative	x	x	x		x								
Short-term	x	x	x		x			x	x				
Chronic	x	x	x		x			x	x	x	x	x	x
Blood													
Intraoperative	x	x	x		x	x	x	x					
Short-term	x	x	x		x	x	x	x	x			x	x
Chronic	x	x	x		x	x	x	x	x	x	x	x	x

[A] (i) Pyrogenicity testing may be considered for all devices contacting the central nervous system.

5.1.4 For the purposes of this practice, devices and the materials that comprise them are classified as to end-use human application as outlined in 5.2 – 5.4.

5.1.5 In general, the assessment for tissue engineered medical products (TEMPs) should address the same issues specific to the type, location, and duration of use as other medical devices and products. The selection of additional assessment for compatibility criteria unique to these type of products should be conducted with these recommendations in mind.

5.1.6 When assessing materials that are intended to degrade and/or be metabolized while implanted in the body (both synthetic and TEMPs), consideration should be given to the degradation or metabolic products and appropriate modifications made in test and sample selection so that the compatibility of degradation products as well as ungraded product are tested.

5.2 *External Devices:*

5.2.1 *Devices That Contact Intact Body Surfaces Only*—examples include electrodes, splints, external prostheses, certain dressings, monitors of various types, or ostomy appliances.

5.2.2 *Devices That Contact Breached Body Surfaces*—examples include ulcer, burn, and granulation tissue dressings, or healing devices.

5.3 *Externally Communicating Devices:*

5.3.1 *Devices Communicating with Intact Natural Channels:*

5.3.1.1 *Intraoperative (<24 hours)*—examples include intraintestinal devices (such as sigmoidoscopes, colonoscopes, stomach tubes, or gastroscopes), tracheal tubes, bronchoscopes and any parts of ancillary equipment that are in contact with materials entering the body, and irrigation sets.

5.3.1.2 *Short-term (up to and including 30 days)*—examples include contact lenses, urinary catheters, and intravaginal devices.

5.3.1.3 *Chronic (>30 days)*—examples include urinary catheters for chronic use and intrauterine devices.

5.3.2 *Devices Communicating with Body Tissues and Fluids:*

5.3.2.1 *Intraoperative (<24 hours)*—examples include hypodermic needles, penetrating electrodes, biopsy instruments, arthroscopes, laparoscopes, irrigation equipment, surgical instruments, trochars, and any parts of ancillary equipment that are in contact with materials entering the body.

5.3.2.2 *Short-term (up to and including 30 days)*—examples include cranial calipers, perfusion apparatus, drainage apparatus, stabilizing orthopedic devices, and any parts of ancillary equipment that are in contact with material entering the body.

5.3.2.3 *Chronic (>30 days)*—examples include percutaneous electrodes, active penetrating electrodes, stapedectomy prostheses, partial and total ossicular replacement prostheses, and tympanoplasty ventilation tubes.

5.3.3 *Blood Path, Indirect*—Products contacting blood path at one point (usually less than 24 hours), and that serve as a conduit for fluid entry into the vascular system. Examples include solution administration sets, extension sets, transfer sets, and blood administration sets.

5.3.3.1 Products that are used for >24 hours or that are used repeatedly in the same patient will be considered as chronic usage and should undergo extended testing.

5.3.4 *Blood, Path, Direct*—Single recirculating blood exposure or product that is in the blood path, generally for less than 24 hours. Examples include intravenous catheters, oxygenators, extracorporeal oxygenator tubing and accessories.

5.3.5 *Blood Path, Direct, Short Term, or Chronic, or repeated exposure*—Examples include dialyzers or dialysis tubing and accessories, shunts.

5.4 *Implanted Long-Term Devices:*

5.4.1 *Devices Principally Contacting Bones*—examples include orthopedic pins, screws, replacement joints, bone prostheses, cements, and dental implants.

5.4.2 *Devices Principally Residing in the Subcutaneous Space*—examples include pacemakers, neuromuscular stimulators, facial augmentation devices, tissue expander devices, and breast prostheses.

5.4.3 *Devices Principally Contacting Soft Tissue and Tissue Fluids*—examples include drug supply devices, neuromuscular sensors, replacement tendons, penile, and other implants, cerebrospinal fluid drains, artificial larynx, vas deferens valves, and ligation clips.

5.4.4 *Devices Principally Contacting Blood*—examples include pacemaker leads, artificial arteriovenous fistulae, heart valves, vascular grafts, stents, blood monitors, internal drug delivery catheters, and ventricular assist pumps.

6. Selection of Test Procedures

6.1 *General*—When it is determined that biocompatibility testing is needed, the following should be considered:

6.1.1 Biocompatibility testing involves tests of either the material itself, or an extract from it (see Practice F619), or both, depending on the nature of the end-use application. While this practice does not address specific chemical methods for evaluating the extractable substances or residuals from implant materials, several of the recommended tests (see 6.2, 6.3, 6.6, and 6.7) utilize extracts rather than the original material for testing. If sensitive chemical assay techniques (such as GC, HPLC, and AA) should reveal no detectable substances being extracted into the medium, consideration may be given to deletion of these tests from the test battery. The investigator is cautioned, however, that the detection limit of the analytical chemistry procedures may not be adequate to detect trace extractables that may generate a tissue response. Before analysis of extracts is substituted for actual biocompatibility testing of the extracts, validation procedures to show the relative tissue response to levels of extractable which are slightly above the detection limit may be necessary. It is particularly appropriate that animal testing involving extracts be considered for deletion if there are no detectable substances being extracted.

6.1.2 If the material to be tested is in the form of particles, characterization of the particles in accordance with Practice F1877 should be performed so that the particles can be fully described and their relevance to clinical usage situations evaluated.

6.2 *Cell Culture Cytotoxicity Assays*—This test evaluates *in vitro* toxicity of substrate materials to cultured cells.

6.2.1 The direct relation between results of cytotoxicity testing and biocompatibility of materials has not been documented and there is some controversy as to the value of the testing since some good materials may be excluded and some others that are not biocompatible may pass this test. Cytotoxicity testing is recommended as an early screening test and also to provide information that will aid in the development of cytotoxicity tests predictive of *in vivo* performance.

6.2.2 Several different tests (such as Agar Diffusion, Fluid Medium, Agar Overlay, Flask Dilution, and so forth) are included under this heading. All of these tests emphasize *in vitro* toxicity of either substrate materials or extract solutions to cultured cells. Cellular damage is observed and graded. Two available versions are included in Practice F813 and Test Method F895. An application-specific method is included in Practice F1027. An HIMA/PMA guideline is available from the FDA for a discussion and references on other versions of this test.

6.2.3 Since the biological reaction to particles generated during function may differ from the reaction to soluble products, *in vitro* testing of macrophage/monocyte interaction with representative particles (Practice F1903) may be considered.

6.3 *Sensitization Test*—The guinea pig maximization test (Practice F720) is a procedure whereby the material (or extracts thereof) is mixed with Freund's complete adjuvant and administered to the test animals during a 2-week induction period. After 2 weeks' rest, the guinea pigs are challenged with the test substance and the skin graded for allergic reaction after 24 hours. Other test methods such as the guinea pig split adjuvant and closed patch test (Practice F2147) or the repeated dermal patch may also be used. The mouse local lymph node assay (Practice F2148) should be considered as an alternative to the guinea pig maximization test. Controls are necessary for all tests.

6.3.1 These tests are for sensitization of the cell-mediated type (Type IV). Since there are concerns about materials causing sensitization of the atopic type (Type I), measurement of IgE antibodies in test animals should also be considered. Similarly, measurement of IgE antibodies in humans in clinical trials may be considered.

6.4 *Skin Irritation Assay*—This is a patch test on the skin of rabbits, and after 24 hours the patches are removed and skin graded for erythema and edema. One available version is included in Practice F719.

6.5 *Mucous Membrane Irritation*—The end use of the device product must be considered when deciding what tests to perform. In some circumstances, the mucous membrane should be considered for the testing site. Numerous tests utilizing different mucous membranes and different animals have been reported. There remains some controversy about the applicability of the results of these tests to human clinical use. The material investigator should consider the appropriateness of a particular test site and published discussion of these methods when planning testing. The intracutaneous irritation test (see 6.6) may be the more suitable test.

6.6 *Intracutaneous Injection (Irritation) Assay*—This assay is designed to determine biological response of rabbits to the single-dose intracutaneous injection of appropriate extracts prepared from test samples. All rabbits are observed for signs of erythema (tissue redness) and edema (tissue swelling) at the injection site for periods up to 72 h. Significant reactions are recorded and the test extract is graded. A USP test has been in use for many years, and Practice F749 may be consulted for additional information.

6.7 *Systemic Injection (Acute Toxicity) Assay*—This assay is designed to determine the biological response of animals (mice) to the single-dose intravenous or intraperitoneal injection of extracts prepared from test samples. The preferred extracts are saline, vegetable oil, or other liquids simulating body fluids or the vehicles of pharmaceutical products that may contact and potentially extract the material before reaching the patient. All mice are observed for signs of toxicity immediately after injection and again at specified intervals. Significant responses are recorded, and the test extract is graded. A USP procedure has been in use for many years, and many variations exist, including Practice F750.

6.8 *Blood Compatibility*—Hemolysis and thrombosis are the most obvious examples of blood materials incompatibility, although adverse effects on plasma proteins, enzymes, and formed blood elements can also occur. Thrombogenicity can be studied through specifically designed *in vitro*, or *ex vivo* procedures specific to the type of product being tested. Normally these tests are dynamic, simulated in-use procedures, with each being developed specifically by the organization interested in evaluating the device in question. One such test is described in Test Method F2382. Hemolysis is covered in 6.9.

6.9 *Hemolysis*—While hemolysis testing is frequently performed in combination with other tests for blood compatibility as specified in 6.8, several methods are in use whereby both materials and extracts are utilized for determining hemolysis. Test rods and extracts of the materials are incubated with human or rabbit blood in dynamic and static test tubes. The amount of plasma hemoglobin is measured and compared to reference materials and controls. Practice F756 describes one method for hemolysis testing. In addition, hemolysis may be evaluated in finished devices by means of dynamic *in vitro, in vivo,* or *ex vivo* procedures designed to emphasize the hemolytic effect of the entire device. These tests tend to be proprietary to the various organizations who employ them.

6.10 *Complement Activation*—The interaction of blood with some materials, especially with large surfaces (such as in dialysis membranes), may lead to the activation of the complement cascade, leading to patient morbidity. Testing for activation of the various complement components using *in vitro* systems is available and recommended for blood contacting materials and devices. Two test methods may be found in Practices F1984 and F2065.

6.11 *Pyrogenicity*—Pyrogenic (fever-producing) substances are either components of bacteria (gram-negative predominately) or fungi (rarely) or are chemical in origin. The latter are most commonly known as "material-mediated" pyrogens. The most common causes of pyrogenicity are endotoxins and

lipopolysaccharides (LPS) of gram-negative bacterial cell wall membranes, which can be detected in the Limulus Amebocyte Lysate (LAL) test (USP bacterial endotoxin test). Endotoxins are also detected using the USP rabbit test, which will detect all types of pyrogens, including material-mediated pyrogens. Sterile devices that can be demonstrated as passing either the USP rabbit test or the LAL test are commonly labeled "non-pyrogenic" and each batch of product shall be tested for pyrogenicity (unless a different schedule can be adopted based on historical data, process validation, or controls).

6.11.1 Since removing pyrogens from endotoxin-contaminated devices is difficult, costly, and often impractical, pyrogen testing is sometimes performed on incoming raw materials or components as a screening method. The LAL test should be used for LPS screening purposes before any rabbit test for material-mediated pyrogens. If the identities of possible material-mediated pyrogens are known, every effort should be made to detect material-mediated pyrogens by analytical or other means not involving the USP rabbit test.

6.12 *Implantation Tests*—The end-use application should be considered when choosing the most suitable site for testing.

6.12.1 *Short-Term Subcutaneous Implantation Test*—Since many implants are intended specifically for subcutaneous use, it is important to consider the reaction of this tissue space to implants and materials. The potential for mobility of implants and tissue of the subcutaneous plane makes this site significantly different from other tissue implantation sites. Inflammatory responses may be increased with motion. Practice F1408 provides one method for short-term implant testing in a subcutaneous site.

6.12.2 *Short-Term Intramuscular Implantation Test*—This type of test is designed to evaluate the reaction of living tissue to a sample material that is surgically implanted into animal tissue (preferably the rabbit, but larger animals (such as the dog) may be considered where necessary). At the conclusion of the assay period, the sites of implantation are examined for significant reaction, and the test material is graded. A USP test has been in use for many years and 7- and 30-day evaluation is available in Practice F763.

6.12.3 *Implantation Testing for the Biological Response to Particles*—Practice F1904 is an intermediate-term test to evaluate the unique responses that may occur when materials are introduced in a particulate form or are reduced to particulate form as a result of the mechanical actions of device utilization.

6.12.4 *Long-Term Implant Test*—Practice F981 is a long-term implantation test in muscle and bone for metals, plastics, and ceramics. In the case of absorbable/resorbable implant materials, or TEMPs that degrade or are metabolized over the time of implantation, Practice F1983 should be considered as an alternative to or in addition to Practice F981. Other long-term implant tests may be appropriate for long-term implant applications.

6.13 *Genotoxicity*—A number of tests are available to assess genotoxic potential. The Ames test may be used as a preliminary screening study with materials. A method that has been developed for genotoxicity testing in mammalian cells is included in Guide E1262. Additionally, other tests may be suggested by regulatory agencies for certain implant applications and sites. No single test yet developed can detect all types of mutagens.

6.14 *Carcinogenicity*—Carcinogenicity testing is usually quite specific for the test substance, with no standard procedures available at this time. Guide F1439 provides guidelines for the performance of these types of tests on implant materials. The National Toxicology Program has published a very comprehensive document[6] relating to the conduct of carcinogenicity testing of chemicals. While much of this document may not be applicable to implant materials, many of the recommendations for animal care, selection of model, and methods for ensuring the integrity of data may be applicable. The user of this document should be aware that very little is known about the latency periods for the development of tumors due to implant materials in the human or the relationship between the results of animal testing and the long-term clinical response. The primary measure of the carcinogenic potential of implant materials will be the results of long term clinical use.

6.15 *Immunotoxicity*—Materials may influence the immune system of the host in various ways. There may be toxicity to the cells in the immune system resulting in decreased responsiveness to antigens. There may be stimulation of the immune system resulting in increased immune responses to antigens. There may be stimulation of an immune response to components or extracts of the materials, which may or may not result in patient morbidity or unsatisfactory performance of the device. Testing for immunotoxicity and specific immune responses may be considered, especially for materials of natural origin or materials that are oil, wax, or gel in nature. In the qualification of new tissue-engineered medical products, immunotoxicity may be of particular concern if the tissue is not autologous. Application of these tests or additional testing specifically developed for TEMPs may be necessary as these materials become more commonly used.

6.16 *Batch Testing of Materials and Devices for Biocompatibility*—Biocompatibility testing of materials may, in some circumstances, be done on samples from a batch of material to be used and the methods used for testing depend on the type of industry, product, and manufacturing and quality control operations in use. Periodic biocompatibility audits may be performed, depending on the manufacturer's degree of assurance that the supplier will not change his product or process, intentionally or otherwise. Additional biocompatibility testing must be performed when changes are made in the composition or processing of the materials.

7. Keywords

7.1 animal testing; biocompatibility; *in vivo* testing; laboratory testing; toxicity

[6] General Statement of Work for the Conduct of Toxicology and Carcinogenicity Studies in Laboratory Animals, *National Toxicology Program,* April 1987.

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 Application of any biocompatibility test to a material requires judgment about its appropriateness. No counsel can be given which will be correct under all circumstances. Regulatory agencies may be extremely helpful when available but such guidelines do not exist for all materials or products. It is for such circumstances that a biocompatibility assessment guideline is needed.

X1.2 With time, greater emphasis has been placed on speed and reduced expense in the performance of biocompatibility screening procedures. It is incumbent on the researcher to reduce the numbers of animals used in experimental testing whenever possible. For primary screening, tissue culture testing may satisfy these requirements but no test is universally applicable.

X1.3 Test selection is based upon a stable manufacturing process and for materials that have been characterized chemically. Intended use and duration of use should affect the direction of more extensive testing. Since the results of biological testing may be affected by the cleaning and sterilization processes used, cleaning and sterilization methods that are representative of final processing should be used for test specimens.

X1.4 The rationale for both the practice and the various sections is integrated into the text, since the nature of this practice is such that understanding of the reasoning behind the statement, requirements, and discussion is required as one reads the document.

X1.5 Biocompatibility has traditionally been associated with materials and devices that do not stimulate an adverse biological response. However, there are a growing number of devices that are designed to be bioactive or biointeractive. With these materials and devices, the presence of, or enhancement of, a biological reaction is desirable. Therefore, interpretation of results of biological testing should be done in light of the intended end use of the material and device.

X1.5.1 TEMPs may comprise a special subset of active materials and devices and this document will be revised and updated as additional testing methods become available and as additional criteria for the acceptability of these types of materials and devices are developed.

X1.6 Since this practice was originally written, the International Standards Organization has prepared a document with similar intent and content. (See ISO 10993-1.) This ISO standard has been adopted as a European standard, EN 10993-1, and as an American National Standard. The FDA has enacted a document, General Program Memorandum #G95-1, with guidelines and a table of tests for consideration for evaluation of biocompatibility. Manufacturers and other investigators may want to consult these and other documents to ensure that any differences are addressed in the planning of tests.

X1.7 The user of this practice and the methods that are recommended should be aware that these methods reflect the best available knowledge concerning the assessment of possible physiological effects of materials and their components. No test can guarantee the biocompatibility of a material.

X1.7.1 *In vitro* testing and animal testing are only models of the human clinical environment. The actual clinical experience with a material will only be determined after a period of clinical use. It has been suggested that a clinical use period of several years in a carefully controlled trial with adequate follow-up will be necessary for reasonable assurance of biocompatibility.

X1.7.2 The latency period for the appearance of malignant tumors in response to carcinogenic agents may be 20 years or more in the human. It is unknown what the relationship between the latency period in animals and in the human will be for undiscovered tumor-causing materials.

X1.8 In 2016, this practice was revised to reflect a change in focus from testing to a focus on endpoints for incorporation in a biocompatibility evaluation.