Designation: F 1905 – 98 (Reapproved 2003)

Standard Practice For Selecting Tests for Determining the Propensity of Materials to Cause Immunotoxicity[1]

This standard is issued under the fixed designation F 1905; 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 covers the introduction of foreign materials into the body that may have an impact on the immune system. One possible effect is that the immune system will be depressed or certain cell types may be affected. Immunotoxicity may be determined with blood and tissue samples from the animals used in the other biocompatibility test procedures such as implantation and blood contact test protocols. It is also possible to use these techniques with blood samples from human patients in a clinical trial. Any procedures with human subjects should follow the appropriate rules of the local institutional review board and the appropriate regulatory agencies. This document may serve as an annex to Practice F 748.

1.2 The material may affect the humoral immune response, the cell mediated response, or both.

1.3 *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]

F 619 Practice for Extraction of Medical Plastics

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

3. Terminology

3.1 *Definitions:*

3.1.1 *antigens*—these are substances that stimulate the host to produce an immune response

3.1.2 *cell mediated immunity (CMI)*—some antigens stimulate the production of lymphocytes that react specifically with the antigen. These cells do not circulate widely in the host and are generally located at the site of antigen deposition. The use of living lymphocytes is required to test for CMI to an antigen.

3.1.3 *complement*—this is a complex system of circulating proteins (enzymes, pro-enzymes, and co-factors) found in the blood. This system is usually activated by antigen-antibody reactions and is a reflection of humoral immunity. However it is apparent that other factors can activate the complement system. These include large polysaccharides and various materials and tissues. Activation of complement can affect the immune system, inflammation, and vascular activity with fever and shock as a consequence of complement activation in the host.

3.1.4 *humoral immunity*—some antigens stimulate the host to produce antibodies (immunoglobulins) which are specific for the antigen and react with the antigen. Antibodies circulate in the blood and tissue fluids. The antibodies produced can be detected using blood from the host.

3.1.5 *inflammatory factors*—various soluble substances may be produced by lymphocytes in response to an antigen. This may occur in humoral immune responses or in CMI. These substances may influence the function of other cells and are called cytokines. Many of these act on various white cells and are called interleukins. They are reflection of antigenic stimulation of the host.

4. Summary of Practice

4.1 Immunotoxicity testing is done using specimens from animals being tested according to the Practice F 748 matrix for irritation and sensitivity, or for implantation. Blood, organs, or tissues from the animals may be used. In predicting biocompatibility, tests using animals are recommended even though the material will eventually be used in humans. The use of human material is also feasible in this testing protocol for those laboratories having approval for such studies.

4.2 Immunologic testing is done using materials or extracts according to Practice F 619. These materials or extracts may be used for *in vivo* tests or for the *in vitro* tests.

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[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the 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.

5. Significance and Use

5.1 This practice is to be used to help assess the biocompatibility of materials used in medical devices. It is designed to test the immunotoxicity of such materials.

5.2 The appropriateness of the methods should be carefully considered by the user since not all materials or applications need be tested by this practice.

5.3 The testing suggestions in Practice F 748 and in the matrices of recommended tests issued by regulatory agencies such as the FDA Immunotoxicity Testing Guide may be considered before proceeding with these tests.

5.4 *Abbreviations*:

5.4.1 *BSA*—Bovine Serum Albumin.

5.4.2 *IgG, IgA, IgM, IgE, IgD*—Immunoglobulin Class G, Class A, Class M, Class E, Class D.

5.4.3 *ELISA*—Enzyme Linked Immunosorbent Assays

5.4.4 *EIA*—Enzyme Immunoassays.

5.4.5 *RIA*—Radio Immunoassays.

5.4.6 *PHA*—Phytohemagglutinin.

5.4.7 *ConA*—Concanavalin A.

5.4.8 *IL*—Interleukin (identified by numbers).

5.4.9 *C*—Complement (components are numbered or lettered).

5.4.10 *SRBC*—Sheep Red Blood Cells.

5.4.11 *CD*—Cluster of Differentiation (antigenic markers on cells).

5.4.12 *CNS*—Central Nervous System.

6. In Vitro Tests for Immunotoxicity

6.1 *Effects on the Humoral Response*—The following determinations are important in assessing the ability of a material to depress the immune response.

6.1.1 *Total Immunoglobulins*—Commercially available kits are recommended for measuring immunoglobulin Class specific (IgG, IgM, IgA, IgD, IgE) immunoglobulins may also be measured and commercially available kits are recommended.

NOTE 1—Changes in total immunoglobulins are seen only after major disturbance of the immune system.

6.1.2 *Effect on Specific Immune Response*:

6.1.2.1 The use of BSA as the antigen is recommended in animal studies. Tetanus toxoid or another approved vaccine is recommended in human clinical trials. The immune response in those receiving the material and the antigen and the immune response in those receiving only the antigen should be determined and compared. A standard ELISA or RIA assay for the immune response is recommended.

6.1.2.2 The use of SRBC is also recommended in animal studies. The assay for plaque forming cells using single cell suspensions from spleens harvested four days after immunization is a sensitive test for IgM antibody.

6.1.2.3 The use of a known adjuvant such as Complete Freund's Adjuvant or Titer Max is recommended. The use of physiologic saline or phosphate buffered saline as a negative control is recommended.

6.1.3 *Effect on B Cell Numbers*—This provides direct information on the effect of the material on the B cell proliferation and killing. This should be done with commercially available, labeled anti-species IgM or IgD, or with an appropriate anti CD antibody and with the use of a cell sorter for enumeration.

6.2 *Effects on the cell Mediated System*:

6.2.1 *Enumeration of Lymphocytes*:

6.2.1.1 *Total T Cell Counts*—This should be done using species specific labeled anti T cell CD antigens, preferably anti CD2 or anti CD3 and a cell sorter.

6.2.2 *Enumeration of T Cell Types*—This should be done using species specific labeled anti CD4 and anti CD8.

6.2.3 *Responses of T Cells*—T cells have receptors on their surfaces for plant lectins such as PHA, ConA and they will divide and proliferate in response. T cells are incubated with PHA or ConA in tissue culture for four days. The level of response can be determined by cell counts or by the uptake of tritiated thymidine.

6.3 *Effects on Inflammatory Factors*:

6.3.1 *Interleukins*—The incubation of lymphocytes in culture with the material may lead to the stimulation of the production of cytokines. The most common, and the ones that are readily detectable and quantifiable, are the interleukins, IL-1 and IL-2 are the recommended ones.

6.4 *Complement*—Materials, especially those in devices that are blood contacting, or in contact with the CNS or large extracorporeal circuits, may affect complement which is a series of proteins important in inflammation and the immune response. The usual method is to determine levels of a component of complement. Commercially available kits are recommended. It is essential to follow the instructions since serum and plasma may not be interchangeable in these tests. The use of a known nonstimulatory biomaterial and a known stimulant such as cobra venom factor are recommended as controls. (NB: measurable effects on the complement system generally occur only after major alterations in the immune system.)

6.4.1 To determine the effect on the Classical Pathway, measurement of C2 is recommended or the split products of C4.

6.4.2 To determine the effect on the Alternate Pathway, measurement of factor B, Bb, or D are recommended.

6.4.3 To determine the effect on the total complement system, measurement of C3 is recommended. Measurement of the split products of C3 or C5 is highly recommended.

6.4.4 The activity of the complement system is best determined with a hemolysis assay or by assay for the terminal complex (Sc5b-9). The standard 50 % hemolytic assay (CH 50) is still a method of choice and is described in the cited "other documents".

7. Data Analysis and Report

7.1 The data obtained should be compared to the results in control animals not receiving the material. In tests where actual numbers are obtained these should be reported as mean and standard deviation or standard error. In some cases, the report of presence or absence of response or estimation of the magnitude of the responses will suffice.

8. Keywords

8.1 B cells; biocompatibility; cell mediated immunity; complement; humoral immunity; immunotoxicity; T cells

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 The primary purpose of this practice is to describe methodologies to determine the propensity of materials to affect the immune response.

X1.2 It is well recognized that the immune response is an important defense mechanism of the host. The interaction of materials with host tissue might alter this response and it is important to understand what materials affect which parts of the immune system.

X1.3 The nature of the immune response and features of immunotoxicity have been an active research area for many years. However, not many studies have been done with medical materials. Many investigators have developed procedures for doing immunotoxicity studies. This document is intended to delineate the information necessary for selection of tests to be done.

X1.4 The interaction of the immunological system with materials could lead to the production of various responses. It is unknown at this time whether immunotoxicity which may be caused by the materials is unfavorable to the host. Immunotoxicity studies using medical materials are important so that this information can be obtained.

X2. ADDITIONAL REFERENCES

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