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Designation: F 1906 – 98

Standard Practice for Evaluation of Immune Responses In Biocompatibility Testing Using ELISA Tests, Lymphocyte Proliferation, and Cell Migration[1]

This standard is issued under the fixed designation F 1906; 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 a foreign substance into mammalian body that may induce the formation of an immune response. The immune response may lead to inadvertent tissue damage and be an undesirable event. In the standard protocols for biocompatibility testing, various studies in animals are done. These animals or their blood and tissues could be used to determine if immune responses have occurred and what types have occurred. At the current time, the immunologic testing in biocompatibility protocols is very limited. Techniques can be developed in the future which are simple, reliable, and sensitive.

1.2 It is the purpose of this practice to delineate some possible test methods. It must be remembered that these are protocols for use in biocompatibility testing, they are not diagnostic tests for evaluation of human conditions. Diagnostic tests for use on humans must go through evaluation at the regulatory agencies. The tests described here are clearly adaptable for use in humans and can be used for research purposes and provide data in clinical trials, but are not necessarily cleared for diagnostic purposes. This practice present selected methods. Other validated methods may be equally applicable.

1.3 The values stated in SI units are to be regarded as the standard.

1.4 *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:*

F 619 Practice for Extraction of Medical Plastics[2]

F 719 Practice for Testing Biomaterials in Rabbits for Primary Skin Irritation[2]

F 720 Practice for Testing Guinea Pigs for Contact Allergens: Guinea Pig Maximization Test[2]

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices[2]

F 763 Practice for Short-Term Screening of Implant Materials[2]

F 981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone[2]

3. Summary of Practice

3.1 Immunologic 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. Blood or biopsies from patients in a clinical trial may also be used. Animals (rabbits or mice) are also immunized with various antigens in this practice. Humans may be immunized with an approved vaccine.

3.2 Immunologic testing is done using materials, known components of the materials, or extracts prepared according to Practice F 619. These materials, components, or extracts may be used for *in vivo* tests or for the *in vitro* tests.

4. Significance and Use

4.1 This practice is to be used to help evaluate the biocompatibility of materials used in medical devices in terms of the immune response.

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

4.3 The testing suggestions in Practice F 748 and in the matrices of recommended tests issued by regulatory agencies may be considered before proceeding with these tests.

4.4 These tests require the use of blood. Procedures for obtaining whole blood or serum should follow the recommendations of the animal research committee of the institution

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[1] This practice is under the jurisdiction of ASTM Committee F-4 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] *Annual Book of ASTM Standards*, Vol 13.01.

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responsible for the animals. In general serum and plasma behave the same in these tests, but it should be noted which was used.

4.5 *The Testing Protocols*—These will be divided into the two specific areas of humoral immunity and cell mediated immunity, and subdivided from there. The tests for the humoral immune responses will be based on solid phase immunoassays for use with enzyme linked immunoassays (ELISA) techniques.

4.6 *Abbreviations*:

4.6.1 *RPMI 1640*—Specific growth medium (Roswell Park Memorial Institute).

4.6.2 *FCS (FBS)*—Fetal Calf Serum (Fetal Bovine Serum).

4.6.3 *NCS*—Newborn Calf Serum.

4.6.4 *MTT*—(3-[4,5-Dimethylthiazol-2-yL]-2,5-diphenyltetrazolium bromide: Thiazolyl blue.

4.6.5 *LIF*—Leukocyte Migration Inhibition Factor.

4.6.6 *Ig*—Immunoglobulin.

4.6.7 *MIF*—Macrophage Migration Inhibition Factor.

4.6.8 *PEC*—Peritoneal Exudate Cells.

4.6.9 *PMNS (POLYS)*—Polymorphonuclear Leukocytes.

4.6.10 *PHA*—Phytohemagglutinin.

4.6.11 *ConA*—Conconavalin A.

4.6.12 *PBS*—Phosphate Buffered Saline.

4.6.13 *PBS/T*—Phosphate Buffered Saline with Tween 20.

4.6.14 *MW*—Molecular Weight.

4.6.15 *MED 199*—Medium 199, a specfic growth medium.

5. Tests for Production of Humoral Immune Responses

5.1 These tests are generally done with serum or plasma. The use of peritoneal or ascites fluid is also permissible.

5.1.1 *Response to Immunogenic Substances*—Proteins and most carbohydrates will adhere to polystyrene, especially when dissolved in buffer at pH 8 to 9. Other materials also usually will adhere. Solid materials may be used as the solid support substrate with appropriate controls for nonspecific binding.

5.1.2 The use of polystyrene 96-well microtiter plates is recommended. There are several manufacturers (for example, Costar, Falcon, Nunc) and they are available from standard supply houses (for example, Fisher, VWR, Thomas). No specific source is recommended, however the choice should be documented in the report.

5.1.2.1 The configuration of the testing protocol is up to the evaluator but control wells must be included in the matrix. It is recommended that 8 wells receive no antigen and serve as reagent controls. A negative control using serum from matched animals not receiving the material should be used in at least four wells. If known positive antisera is available, this should be used in at least four wells. If quantitation is desired, standard solutions of at least three different concentrations should be run in triplicate and a buffer control run in triplicate. Each specimen to be tested should be run at two different dilutions and each in triplicate.

5.1.3 Dissolve the substance in an aqueous based solution. Adjust the pH to between 8.5 and 9.5 or as high as possible before it precipitates. A phosphate buffer is recommended. The final concentration of the substance should be 0.5 mg/mL. (Any extract prepared according to Practice F 619 may be used in this protocol. The pH should be adjusted to pH 8.5 to 9.5 where possible without precipitation of the extract.

5.1.4 Add 100 uL of this to each of the wells except the control wells. Incubate the plates in a humid environment at room temperature (18 to 22°C) or 37°C for 1 h, place in the cold (4 to 8°C) overnight. Wash the plates with phosphate buffered saline with Tween 20 (PBS/T) at least three times. Wash at least twice with coating buffer that is the PBS/T with a protein source such as 1 % gelatin, egg albumin, or serum. This blocks other combining sites on the plates and reduces the background. The plates may be used immediately or stored in the cold until used.

5.1.5 Add 100 uL of the appropriate serum samples or buffer control to the wells. The test serum samples should be run in at least two dilutions. It is recommended that eight wells receive only buffer and serve as an antigen-second antibody control. Incubate at room temperature or 37°C for 1 to 2 h. Wash well with PBS/T.

5.1.6 Add 100 uL of the appropriate antiserum to all of the wells. This second antibody should be labeled with an enzyme (horse radish peroxidase or alkaline phosphatase are recommended). These antisera can be purchased from scientific supply houses and should be used at the dilution recommended by the manufacturer. It is recommended that polyvalent antisera directed against IgG, IgM, IgA of the appropriate species of the animal be used and that IgE specific antisera be used separately. The plates should be incubated at room temperature or 37°C for 1 to 2 h. They should then be washed well with PBS/T.

5.1.7 Add 100 uL of the appropriate substrate to all wells. The substrate specific for the enzyme label and at the correct concentration should be used. This information should be supplied by the supplier of the antisera. Incubate at room temperature (usually in the dark) for the recommended time (usually 20 to 30 min). Read the optical density at the appropriate wave length for the substrate.

5.1.8 *Analysis of the Data*—The results of the optical density achieved with the test serum samples should be compared to the control samples. Significant elevations or depressions would be signified by values outside two standard deviations of the control. If known standards were included, the results can be expressed from comparison with the standard curve.

6. Response to Haptenic Substances

6.1 The immune response to haptens (low molecular weight materials) is similar to the immune response already described. However, this response will be dealt with separately here since there are substantial differences in testing methodologies.

6.1.1 The production of an immune response to low molecular weight degradation, wear, or elution products from materials is an important issue in biocompatibility. These low molecular weight substances may bind to host tissue or protein and become immunogenic. It is not apparent that determination of responses to haptenic materials can be determined using a simple modification of the procedure in Section 5. It is necessary to first coat the plates with a carrier to bind the hapten. For most haptens, albumin serves as an appropriate carrier.