Designation: F1984 – 99(Reapproved 2008)

Standard Practice for Testing for Whole Complement Activation in Serum by Solid Materials[1]

This standard is issued under the fixed designation F1984; 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 provides a protocol for rapid, *in vitro* screening for whole complement activating properties of solid materials used in the fabrication of medical devices that will contact blood.

1.2 This practice is intended to evaluate the acute *in vitro* whole complement activating properties of solid materials intended for use in contact with blood. For this practice, the words "serum" and "complement" are used interchangeably (most biological supply houses use these words synonymously in reference to serum used as a source of complement).

1.3 This practice consists of two procedural parts. Procedure A describes exposure of solid materials to a standard lot of human serum, using a 0.1-mL serum/13 x 100-mm disposable test tube. Cellulose acetate powders and fibers are used as examples of test materials. Procedure B describes assaying the exposed serum for significant functional whole complement depletion as compared to control samples.

1.4 This practice does not address function, elaboration, or depletion of individual complement components, nor does it address the use of plasma as a source of complement.

1.5 This practice is one of several developed for the assessment of the biocompatibility of materials. Practice F748 may provide guidance for the selection of appropriate methods for testing materials for other aspects of biocompatibility.

1.6 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

2.2 *ISO Document:*

ISO 10993-4: Biological Evaluation of Medical Devices, Part 4: Selection of Tests for Interactions with Blood[3]

3. Terminology

3.1 *Abbreviations:*

3.1.1 *Ab*—antibody (hemolysin).

3.1.2 *BBS*—barbital buffered saline.

3.1.3 *BBS-G*—barbital buffered saline—gelatin.

3.1.4 *BBS-GM*—barbital buffered saline—gelatin metals.

3.1.5 *C′*—complement.

3.1.6 *EDTA*—ethylenediaminetetraacetic acid, disodium salt: dihydrate.

3.1.7 *HS*—human serum.

3.1.8 *PVDF*—polyvinylidene fluoride.

3.1.9 *RBC*—red blood cell(s).

4. Summary of Practice

4.1 Solid material specimens are exposed to contact with a standard lot of complement under defined conditions (Procedure A). Exposed serum then is tested for significant functional complement depletion compared to controls under identical conditions (Procedure B).

5. Significance and Use

5.1 Inappropriate activation of complement by blood-contacting medical devices may have serious acute or chronic effects on the host. This practice is useful as a simple, inexpensive screening method for determining functional whole complement activation by solid materials *in vitro*.

5.2 This practice is composed of two parts. In Part A (Section 11), human serum is exposed to a solid material. Complement may be depleted by the classical or alternative pathways. In principle, nonspecific binding of certain complement components also may occur. The alternative pathway can

[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.

Current edition approved Aug. 1, 2008. Published August 2008. Originally approved in 1999. Last previous edition approved in 2003 as F1984 – 99 (2003). DOI: 10.1520/F1984-99R08.

[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.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

deplete later acting components common to both pathways, that is components other than C1, C4, and C3 (**1**).[4] In Part B (Section 12), complement activity remaining in the serum after exposure to the test material is assayed by classical pathway-mediated lysis of sensitized RBC.

5.3 Assessment of *in vitro* whole complement activation, as described here, provides one method for predicting potential complement activation by medical materials intended for clinical application in humans when the material contacts the blood. Other test methods for complement activation are available, including assays for specific complement components and their split products (see X1.3 and X1.4).

5.4 This *in vitro* test method is suitable for adoption in specifications and standards for screening solid materials for use in the construction of medical devices intended to be implanted in the human body or placed in contact with human blood.

6. Preparation of Buffers

6.1 *Buffers,* are prepared according to detailed protocol(**2**). "Water" refers throughout to distilled, endotoxin-free water. The use of barbital (veronal) buffer is recommended. Barbital is a class IV regulated substance and requires a DEA (**3**) license for purchase. The use of other buffer systems, such as, TRIS, is permissible if they have been demonstrated not to activate complement(**4**).

6.2 *5X Stock BBS (barbital-buffered saline),* is prepared by adding 20.75 g NaCl plus 2.545 g sodium barbital (sodium-5, 5-diethyl barbiturate) to about 400 mL water. The pH is adjusted to 7.35 with 1 N HCl, then brought to a final volume of 500 mL in a volumetric flask.

6.3 *Metals Solution,* is prepared by making a 2.0 M solution of $MgCl_2$ (40.66 g $MgCl_2$• 6 H_2O into 100 mL distilled endotoxin-free water), and a 0.3 M solution of $CaCl_2$ (4.41 g $CaCl_2$• 2 H_2O into 100 mL distilled endotoxin-free water), and combining the two solutions 1:1 (v:v). These solutions are stable one month at 4°C.

6.4 *BBS-GM Working Solution,* is prepared daily, by dissolving 0.25 g gelatin in 50 mL endotoxin-free distilled water that is gently heated and stirred. The gelatin solution is added to 50 mL 5X stock BBS plus 0.25 mL metals solution, brought up to about 200 mL, then adjusted to pH 7.35 (with 1 N HCl or 1 N NaOH) before bringing the final volume to 250 mL in a volumetric flask.

6.5 *BBS-G Working Solution,* is prepared the same way, but the addition of the metals solution is omitted.

6.6 *10X Stock EDTA (0.1 M disodium dihydrate EDTA),* is prepared by adding 7.44 g disodium EDTA•2 H_2O to about 160 mL water, adjusting the pH to 7.65 (with 1 N NaOH or 1 N HCl), then bringing the volume to 200 mL in a volumetric flask.

6.7 *BBS-G-EDTA (to be used in preparing RBC before being washed out),* is prepared by adding 10 mL of stock 10X EDTA to 90 mL of BBS-G in a volumetric flask.

7. Preparation of Sheep RBC

7.1 Commercially-obtained sheep red blood cells (RBC) preserved in Alsever's solution are stored at 4°C. The cells are discarded after eight weeks or when the supernatant from the second wash contains hemoglobin by visual inspection.

NOTE 1—All centrifugations are at 4°C. Except where indicated, all reagents, tubes, and cell preparations are kept on ice.

7.2 Five mL of sheep RBC are centrifuged at 1 000 x g for 10 min.

7.3 The cell pellet is resuspended in 10 mL of cold BBS-G-EDTA and incubated for 10 min at 37°C. The cells are centrifuged, and the pellet resuspended in 10 mL of BBS-G-EDTA.

7.4 The cells are centrifuged, the supernatant discarded (first wash), and the pellet resuspended in 10 mL of cold BBS-GM. Repeat twice (total of three washes).

7.5 Adjust cell count spectrophotometrically (where an absorbance of 0.56 corresponds to 1.5 x 10^8 sheep RBC/mL, at a wavelength of 412 nm and a 1.0-cm light path for 1 volume of cells in BBS-GM plus 24 volumes of water) or count with a hemocytometer, preparing 10 mL of 1.5 x 10^8 cells/mL in cold BBS-GM.

7.6 The washed, diluted RBC can be held on ice and used for at least 12 h.

8. Absorption of Serum (Complement)

8.1 The use of human complement is required since there are species differences in the efficiency of complement activation and the test materials are to be used in humans. Human serum suitable as a source of complement may be purchased from biological supply houses, and generally, is labeled as reagent-grade complement.

8.2 Human serum may be absorbed with sheep RBC in order to remove naturally-occurring anti-sheep RBC hemolytic antibodies, though for most purposes, the amount of heterophile antibody in human serum is not enough to influence the reaction assuming the cells are optimally sensitized with hemolysin. The procedure is detailed in 8.3-8.8.

8.3 Fresh human serum or a commercial lot of human serum is obtained and stored at −70°C. Fresh serum is preferred as lyophilized complement often is not as active as fresh serum.

8.4 The serum is thawed on ice or reconstituted (if lyophilized) with ice-cold (4°C) distilled endotoxin-free water.

8.5 All manipulations are done on ice, with ice cold reagents and cells; centrifugations are carried out at 1000 x g at 4°C. It is critical that this entire procedure be done in the cold to avoid activation of complement in this step.

8.6 Cold serum and cold, packed, washed sheep RBC are mixed, 0.1 mL RBC/2.5 mL serum, incubated for 10 min on ice, then centrifuged at 1 000 x g for 10 min at 4°C. The supernatant is transferred carefully to a new container on ice.

[4] The boldface numbers in parentheses refer to the list of references at the end of this specification.