



Standard Practice for Testing for Alternative Pathway Complement Activation in Serum by Solid Materials¹

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^{€1} NOTE—Editorial corrections were made throughout this standard in June 2001.

1. Scope

1.1 This practice provides a protocol for rapid, in vitro screening for alternative pathway 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 alternative pathway complement activating properties of solid materials intended for use in contact with blood. For this practice, “serum” is synonymous with “complement.”

1.3 This practice consists of two procedural parts. Procedure A describes exposure of solid materials to a standard lot of C4-deficient guinea pig serum [C4(-)GPS], using 0.1-mL serum per 13 × 100-mm disposable glass test tubes. Sepharose[™] CL-4B is used as an example of test materials. Procedure B describes assaying the exposed serum for significant functional alternative pathway complement depletion as compared to control samples. The endpoint in procedure B is lysis of rabbit RBC in buffer containing EGTA and excess Mg⁺⁺.

1.4 This practice does not address function, elaboration, or depletion of individual complement components except as optional additional confirmatory information that can be acquired using human serum as the complement source. This practice does not 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 F 748 may provide guidance for the selection of appropriate methods for testing materials for other aspects of biocompatibility. Practice F 1984 provides guidance for testing solid materials for whole complement activation in human serum, but does not discriminate between the classical or alternative pathway of activation.

2. Referenced Documents

2.1 ASTM Standards:

F 748 Practice for Selecting Generic Biological Test Meth-

ods for Materials and Devices³

F 1984 Practice for Testing for Whole Complement Activation in Serum by Solid Materials³

2.2 Other Document:

ISO 10993-4: Biological Evaluation of Medical Devices. Part 4: Selection of Tests for Interactions with Blood⁴

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *water*—distilled, endotoxin-free.

3.2 *Abbreviations*: Abbreviations:

3.2.1 *Ab*—antibody (hemolysin)

3.2.2 *BBS*—barbital buffered saline

3.2.3 *BBS-G*—barbital buffered saline – gelatin

3.2.4 *BBS-G-EGTA/Mg (Mg Buffer)*—barbital buffered saline – gelatin EGTA Mg⁺⁺

3.2.5 *BBS-GM (Ca Buffer)*—barbital buffered saline – gelatin metals

3.2.6 *C'*—complement

3.2.7 *C4(-)GPS*—C4-deficient guinea pig serum [serum from guinea pigs genetically incapable of producing C4, the fourth component of complement]

3.2.8 *EDTA*—ethylenediaminetetraacetic acid, disodium salt: dihydrate

3.2.9 *EGTA*—ethylene glyco-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, tetrasodium salt

3.2.10 *HAGG*—heat aggregated gamma globulin

3.2.11 *HS*—human serum

3.2.12 *I*—control tube with serum but no material, kept on ice.

3.2.13 *M*—tube containing serum plus a test material

3.2.14 *NM*—tube containing serum but no material

3.2.15 *PVDF*—polyvinylidene fluoride

3.2.16 *RBC*—red blood cell(s)

4. Summary of Practice

4.1 Solid material specimens are exposed to a standard lot of C4(-)GPS complement under defined conditions, in parallel to appropriate controls (Procedure A). If the alternative

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 Sepharose[™] is a registered trademark of Pharmacia, Inc.

³ *Annual Book of ASTM Standards*, Vol 13.01.

⁴ Available from American National Standards Institute, 11 W. 42nd St., 13th Floor, New York, NY 10036.