



## Standard Practice for Assessment of Hemolytic Properties of Materials<sup>1</sup>

This standard is issued under the fixed designation F 756; 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 the assessment of hemolytic properties of materials used in the fabrication of medical devices that will contact blood.

1.2 This practice is intended to evaluate the acute *in vitro* hemolytic properties of materials intended for use in contact with blood.

1.3 This practice consists of a protocol for a hemolysis test under static conditions with either an extract of the material or direct contact of the material with blood.

1.4 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 a specific application.

1.5 *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.*

1.6 *Identification of a supplier of materials or reagents is for the convenience of the user and does not imply single source. Appropriate materials and reagents may be obtained from many commercial supply houses.*

### 2. Referenced Documents

#### 2.1 ASTM Standards:

F 619 Practice for Extraction of Medical Plastics<sup>2</sup>

F 748 Practice for Selecting Generic Biological Test Methods for Materials and Devices<sup>2</sup>

### 3. Terminology

#### 3.1 Definitions:

3.1.1 *plasma hemoglobin*—the amount of hemoglobin in the plasma.

3.1.2 *% hemolysis*—the quotient of the free plasma hemoglobin (mg/ml) released as a result of contact with test material or extract divided by the total hemoglobin (mg/ml) present in the blood solution multiplied by 100. (This is synonymous with hemolytic index).

3.1.3 *comparative hemolysis*—comparison of the hemolytic index produced by a test material compared with that produced by a standard reference material such as polyethylene at the same test conditions.

3.1.4 *direct contact test*—a test for the hemolytic property performed with the test material in direct contact with the blood.

3.1.5 *extract test*—a test for the hemolytic property performed with an isotonic extract of the test material, as described in F 619, in contact with the blood.

3.1.6 *hemolysis*—destruction of erythrocytes resulting in the liberation of hemoglobin into the plasma or suspension medium.

3.1.7 *negative control*—a material, such as a polyethylene, that produces little or no hemolysis (<2 % after subtraction of the blank) in the test procedure. It is desirable that the control specimens have the same configuration as the test samples.

3.1.8 *positive control*—a material capable of consistently producing a hemolysis of at least 8 % after subtraction of the results of the blank. Suggested materials include, but are not limited to, BUNA N (Aero Rubber and other suppliers), and plastisol (Plasti-Coat, Watertown CT.).

3.1.9 *cyanmethemoglobin reagent*—a reagent to which whole blood, plasma, or test supernatant is added that quickly converts most of the forms of hemoglobin to the single cyanmethemoglobin form for quantification at its 540 nm spectrophotometric peak. The reagent (based on that by van Kampen and Zijlstra (1), pH 7.0-7.4), is made with 0.14 g potassium phosphate, 0.05 g potassium cyanide, 0.2 g potassium ferricyanide, and 0.5-1 ml of nonionic detergent diluted to 1 L with distilled water. The conversion time of this reagent is 3-5 minutes. This reagent is recommended by the National Commission for Clinical Laboratory Studies (NCCLS) and may be made from the chemicals or purchased from supply houses.

The first cyanmethemoglobin reagent used to measure total blood hemoglobin concentration was Drabkin's Reagent (1 g of sodium bicarbonate, 0.05g of potassium cyanide, 0.2 g of potassium ferricyanide and diluted with distilled water to 1 L). The disadvantages of using the Drabkin's reagent versus the NCCLS cyanmethemoglobin reagent are that it has a conversion time of 15 minutes, and pH of 8.6 which may cause

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<sup>2</sup> *Annual Book of ASTM Standards*, Vol 13.01.