



Designation: F 1841 – 97

## Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps<sup>1</sup>

This standard is issued under the fixed designation F 1841; 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.

### INTRODUCTION

The goal of blood pump development is to replace or supplement the function of the human heart. As a result, continuous flow blood pumps, including roller pumps and centrifugal pumps, are commonly used in clinical extracorporeal circulation. They are used not only for cardiopulmonary bypass in routine cardiac surgery but also for ventricular assist, percutaneous cardiopulmonary support, and extracorporeal membrane oxygenation.

Many investigators have attempted to develop an atraumatic blood pump. Hemolysis is one of the most important parameters of blood trauma induced by blood pumps. However, comparative in vitro evaluation of the reported results of hemolysis are difficult due to the lack of uniformity of the test methods employed. Thus, it is necessary to standardize the method of performing in vitro hemolysis tests for the evaluation of continuous flow blood pumps.

### 1. Scope

1.1 This recommended practice covers a protocol for the assessment of the hemolytic properties of continuous flow blood pumps used in extracorporeal or implantable circulatory assist. An assessment is made based on the pump's effects on the erythrocytes over a certain period of time. For this assessment, a recirculation test is performed with a pump for 6 h.

1.2 The values stated in both inch - pound and SI units are to be regarded as the standard. The SI units given in parentheses are for information only.

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:*

F 1830 Practice for Selection of Blood for In Vitro Evaluation of Blood Pumps<sup>2</sup>

### 3. Terminology

3.1 *Definitions:*

3.1.1 *continuous flow blood pump*—a blood pump that produces continuous blood flow due to its rotary motion.

3.1.2 *free plasma hemoglobin*—the amount of hemoglobin (iron or heme-containing protein) in plasma.

3.1.3 *hemolysis*—damage to erythrocytes resulting in the liberation of hemoglobin into the plasma.

3.1.4 *Index of Hemolysis*

3.1.4.1 *normalized index of hemolysis*—added grams of plasma free hemoglobin per 100 l of blood pumped, corrected for plasma volume using hematocrit and normalized by flow rate and circulation time.

3.1.4.2 *normalized milligram index of hemolysis*—normalized index of hemolysis expressed by milligram value of free plasma hemoglobin.

3.1.4.3 *modified index of hemolysis*—mass of hemoglobin released into plasma normalized by the total amount of hemoglobin pumped through the loop.

### 4. Formulas

4.1 *Normalized Index of Hemolysis (N.I.H.) (1,2,3,4)<sup>3</sup>:*

$$N.I.H. \text{ g/100l} = \Delta_{\text{free Hb}} \times V \times \frac{100 - Ht}{100} \times \frac{100}{Q \times T} \quad (1)$$

$\Delta_{\text{free Hb}}$  = increase of plasma free hemoglobin concentration (g/L) over the sampling time interval,

<sup>1</sup> 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 F04.30 on Cardiovascular Standards.

Current edition approved Dec.10, 1997. Published June 1998.

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

<sup>3</sup> The boldface numbers given in parentheses refer to a list of references at the end of the text.

where:

$V$  = circuit volume (L),

$Q$  = flow rate (L/min), and

$Ht$  = hematocrit (%), and

$T$  = sampling time interval (min).

4.2 *Normalized Milligram Index of Hemolysis. (mg.N.I.H.) (2,3,4):*

$$-mg.N.I.H./mg/100l = \Delta freeHb \times V \times \frac{100 - Ht}{100} \times \frac{100}{Q \times T} \quad (2)$$

4.3 *Modified Index of Hemolysis (M.I.H.):*

4.3.1 Modified index of hemolysis (M.I.H.) (5,6) that can be written with no units or as (milligram of hemoglobin released into plasma/mg of total hemoglobin pumped through the loop):

$$M.I.H. = \Delta freeHb \times V \times \frac{100 - Ht}{100} \times \frac{10^6}{Q \times T \times Hb} \quad (3)$$

where:

$Hb$  = total blood hemoglobin concentration at time zero (mg/L), and

$\Delta free Hb$  = increase of plasma free hemoglobin concentration (mg/L) over the sampling time interval.

4.3.2 Among these indices, M.I.H. is recommended as an index to express the degree of hemolysis caused by a blood pump in a recirculating system. N.I.H. was proposed to account for the plasma volume based on the hematocrit. Recent development of less hemolytic blood pumps has since made it convenient to use mg. N.I.H. rather than N.I.H. However, both the N.I.H. and the mg N.I.H. vary with hematocrit of the blood (6). M.I.H. is the recommended index to express the degree of hemolysis caused by a blood pump in a recirculating system. The M.I.H. equation corrects for differences in blood hemoglobin concentration and hematocrit directly (5).

4.4 *Testing Blood*— Because the level of trauma-induced hemolysis is different based on the source of blood, it is necessary to identify the source of blood and its respective index of hemolysis. Human, bovine, or porcine blood are recommended as the primary sources of testing blood (see Practice F 1830). It is preferable that the blood collected at a standard slaughter house not be used due to the risk of being contaminated with fluids other than blood, unless the blood is obtained by controlled venipuncture. Although animal blood is used in the development stage of a pump, it is suggested that pre-clinical evaluation tests be repeated with human blood.

## 5. Summary of Practice

5.1 *Blood*—The blood is obtained from human volunteers, cattle or pigs having normal body temperatures, no physical signs of disease, including diarrhea or rhinorrhea, and an acceptable range of hemotological profiles. The blood should be collected by vascular puncture using a needle (14G or larger) and collected into the standard 500–2000 mL bags containing citrate phosphate dextrose adenine (CPDA-1) anticoagulant solution (See Appendix X2) or heparin sulfate (See Appendix X3). The blood from a slaughterhouse can typically be used if it is obtained by controlled venipuncture.

5.2 *Test Loop (4)* (See Fig. 1)—The test loop consists of a total of 6.6 ft (2 m) of 3/8 in. (9.5 mm) ID polyvinylchloride tubing and a reservoir (typically, 13 by 13 cm) with a sampling

port. The primed blood volume is  $450 \pm 45$  mL. A screw clamp, that is positioned at the outlet side, is applied to produce the required conditions for the left heart assist application (5 L/min against 100 mm Hg pressure head (that is, with the pressure sampling ports at the same vertical height, the pressure in the outlet line of the pump is 100 mm Hg greater than in the inlet line)) and for the cardiopulmonary bypass application (5 L/min against 500 mm Hg pressure head). (Optional testing at 350 or 700 mm Hg is also advisable.) To monitor such pressure heads, the pressure monitoring lines are incorporated into the test loop both at the inlet and outlet tubes. An ultrasonic or electromagnetic flow probe is placed at the outlet side of the pump between the clamp and the reservoir to monitor the flow rate. A thermistor is connected to the loop, and the blood temperature is measured using a corresponding thermometer.

5.3 *Pump Conditions*— Pump flow rate is set at  $5 \pm 0.25$  L/min at the circulating blood temperature of  $37 \pm 1^\circ\text{C}$ . The total pressure head is set at  $100 \pm 3$  mm Hg for the left heart assist application and  $500 \pm 15$  mm Hg for cardiopulmonary bypass application. However, additional testing temperatures can be chosen from 0 to  $42^\circ\text{C}$  according to the intended clinical use of the pump (for example, cardiopulmonary bypass may include cooling and warming during surgery.)

5.4 *Evaluation*—The free plasma hemoglobin is determined by a clinically approved assay method (see 9.3). The free plasma hemoglobin is standardized by calculating the M.I.H.

## 6. Significance and Use

6.1 The objective of this practice is to standardize the evaluation method for detecting the hemolytic effect of a continuous flow blood pump used in extracorporeal circulation and circulatory assistance.

## 7. Preparation of Hemolysis Test

7.1 *Blood*—The blood is obtained from human volunteers having normal body temperature, exhibiting no physical signs of disease and having hematological profiles in the normal acceptable range. (Donors are subjected to standard blood donor screening procedures.) The donor should be fasted for 8 h or more to avoid additional hemolysis due to a high concentration of lipids in the blood. The delay in the collection of the blood and the hemolysis test should not exceed 48 h of refrigerated storage with the blood temperature kept between 2 and  $8^\circ\text{C}$  or more than 2 h at ambient condition. As an alternative source of blood, animal blood can be used, but it is necessary that the source of blood is identified. The preferred animal blood is bovine and porcine (See Practice F 1830). Since the use of completely fasted animals is impractical, it is recommended that the animals be subjected to a 12-h fasting. As a quality control measure, any blood having free plasma hemoglobin of more than 20 mg/dL should not be used for this test. In order to standardize the blood trauma testing, the blood subjected to the test should have the hematocrit value adjusted to be within the range  $30 \pm 2\%$  by hemodilution (with phosphate buffered saline) or hemoconcentration (via minimal