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Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps[1]

This standard is issued under the fixed designation F1841; 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 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 either SI units or inch-pound units are to be regarded separately as standard. The values stated in each system may not be exact equivalents; therefore, each system shall be used independently of the other. Combining values from the two systems may result in non-conformance with the standard.

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:*[2]

F1830 Practice for Selection of Blood for ***in vitro*** Evaluation of Blood Pumps

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.

[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 F04.30 onCardiovascular Standards.

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[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.

3.1.4.3 *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**)[3]:

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

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

where:

V = circuit volume (L),
Q = flow rate (L/min),
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 hemotocrit. 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 F1830). 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 phospate 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 ± 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 ± 0.25 L/min at the circulating blood temperature of 37 ± 1°C. The total pressure head is set at 100 ± 3 mm Hg for the left heart assist application and 500 ± 15 mm Hg for cardiopulmonary bypass application. However, additional testing temperatures can be chosen from 0 to 42°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

[3] The boldface numbers given in parentheses refer to a list of references at the end of the text.

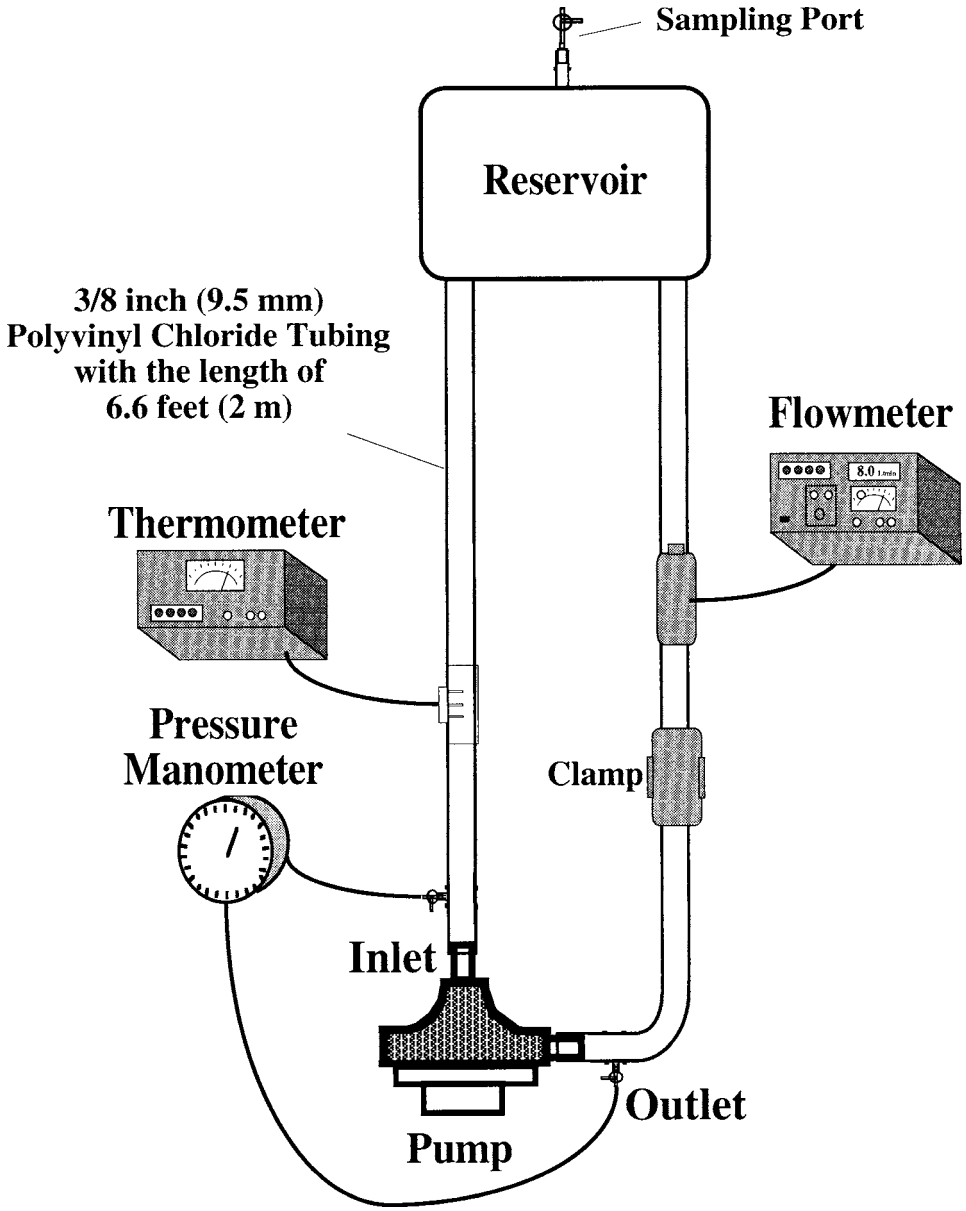

FIG. 1 Test Loop

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° 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 F1830). 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 ± 2 % by hemodilution (with phospate buffered saline) or hemoconcentration (via minimal centrifugation). Proper and acceptable ranges of the physiological blood parameters should be maintained prior to and during testing (for example, pH, base excess, glucose concentration).

7.2 *Test Loop (See* Fig. 1*)*—The closed test loop contains a total of 6.6 ft [2 m] of 3/8 in. [9.5 mm] ID polyvinylchloride tubing, a reservoir with a sampling port, an ultrasonic or electromagnetic flow probe and its corresponding flowmeter, a thermistor and its corresponding thermometer, and a blood pump. The loop should be filled with phosphate buffered saline that is recirculated for approximately 10 to 20 min to rinse and wet all of the blood-contacting surfaces. The phosphate buffered saline is drained completely from the loop prior to filling it with blood. After being washed with phosphate buffered saline, the circuit is primed with 450 ± 45 mL of fresh blood into the reservoir bag. Air collected in the reservoir should be eliminated and no air interface left in the reservoir. A screw

clamp, that is applied to produce the required condition of pressure head, is positioned at the outlet side of the pump. 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 screw clamp and the reservoir to monitor the flow rate.

7.3 *Pump Conditions*—The flow meter should be calibrated using blood at the proper hematocrit and temperature. The pump revolution rate is adjusted to provide 5 ± 0.25 L/min flow rate as determined by the in-line flow meter, and all experiments are conducted at a 37 ± 1°C environment that is achieved through submerging portions of the loop into a water bath. However, additional tests conducted in temperatures ranging from 0 to 42°C can be performed according to the intended clinical use of the pump. Since all test runs are of a 6-h duration, sterility is generally considered not necessary.

7.4 *Evaluations*—Blood samples of 1 to 2 mL (preferably 1 mL) are drawn from the reservoir before pumping and at every hour of pumping. It would be preferable to withdraw at least two blood samples at each sample time. At each sampling, the first sample of 1 mL should be discarded because it may contain blood that was stagnant in the sampling port. The second sampling of 1 mL should be used for measurement of plasma free hemoglobin. If the saline is drained completely from the test loop prior to testing, the initial total blood hemoglobin concentration, plasma hemoglobin concentration, and hematocrit can be determined from the pre-pumping control blood. Preferably, these time zero measurements are obtained from blood that has circulated through the loop for approximately 5 min to ensure complete mixing and dilution.

7.5 *Static Blood Controls*—The control blood is kept in a blood bag at the same temperature environment as that of the testing blood. For sampling, the sampling procedures as those for testing blood are required (see 8.5).

8. Procedure

8.1 Figure 1 describes a standard closed loop for hemolysis testing, that consists of the blood pump subjected to the test, a reservoir with a sampling port, inlet and outlet tubings with a pressure monitoring port at each segment of the tubing, pressure transducers or a differencial pressure manometer, a thermistor, and a flow probe. A screw clamp is also included in this figure.

8.2 The blood warmed to 37°C (or other appropriate temperature) should be infused by gravity into the test loop through a sampling port of the blood bag.

8.3 After the test loop is operated for approximately 5 min and air bubbles are eliminated from the test loop through the sampling port, the first blood sample is taken as the pre-pumping control.

8.4 The blood pump is started and adjusted at the flow rate of 5 ± 0.25 L/min.

8.5 The test duration recommended is 6 h, and one blood sample is taken before pumping, and six blood samples are taken at every hour of the test. This recommended sampling schedule provides a sufficient number of test samples for statistical evaluation. The free plasma hemoglobin is determined by a clinically accepted assay method. To ensure proper samplings, gentle massaging of the reservoir and discarding 1 mL of the blood from the sampling ports are recommended prior to blood sampling.

8.6 For general testing, the circulating blood temperature should be maintained at 37 ± 1°C during the entire test duration, although testing at other appropriate use temperatures may be necessary.

9. Report

9.1 At first, the results should be reported as the time dependent hemolysis data displayed graphically for each of the five test devices and static blood controls. The regression coefficient of these device plasma hemoglobin plots should be greater than 0.95. Then, the report should be given in the form of an index of hemolysis which is defined as milligram of plasma free hemoglobin per 100 L blood pumped (mg N.I.H.) and as an M.I.H. value.

9.2 At least five such tests should be conducted to confirm the reproducibility of the tests. The individual index of hemolysis values (both mg N.I.H. and M.I.H.) should be reported for each of the five tests, along with the mean value ± standard deviation. The designated index should be the highest of the five tests.

9.3 The clinically accepted assay methods are referred to in the Appendix(**7**, **8**).

9.4 The modified index of hemolysis (M.I.H.) is recommended as the most appropriate measurement index in evaluating the degree of hemolysis caused by a blood pump in a recirculating system. Although the mg NIH equation has been typically used by researchers, it does not correct for differences in hematocrit or hemoglobin content of the pumped blood (**5**, **6**).

9.5 The blood donor source (for example human, bovine, porcine) should be specified. Test temperature and pressure head should also be reported.

10. Keywords

10.1 blood pump; blood trauma; index of hemolysis; modified index of hemolysis (M.I.H.); normalized index of hemolysis (N.I.H.)

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 Even though blood trauma imposed on platelets and leukocytes by blood pumps should be studied, the hemolysis generated by a blood pump is the most significant blood trauma. Thus, this practice was generated.

X2. CITRATE PHOSPHATE DEXTROSE ADENINE (CPDA) SOLUTION USP

X2.1 A CPDAI solution USP of 63 mL is added for collection of 450 mL blood.

X2.2 Each 63 mL of CPDAI contains 2 g of dextrose (monohydrate) USP, 1.66 g sodium citrate (dihydrate) USP, 188 mg citric acid (anhydrous) USP, 140 mg monobasic sodium phosphate (monohydrate) USP, and 17.3 mg adenine USP.

X2.3 The pH of the solution may have been adjusted with sodium hydroxide.

X3. HEPARIN

X3.1 500 mL of blood containing 2000 to 3000 USP units of heparin is utilized.

X4. CLINICALLY ACCEPTED ASSAY METHODS (7, 8)

X4.1 These are colorimetric assays, direct spectrophotometric assays, and, derivative spectrophotometric assays.

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