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

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ε[1] NOTE—Editorial corrections were made to 4.1 and 4.2.1 in May 2021.

INTRODUCTION

The goal of blood pump development is to replace or supplement the function of the human heart to circulate blood. In practice, blood pumps are commonly used in cardiopulmonary bypass during routine cardiac surgery, and for ventricular assist, percutaneous cardiopulmonary support, and extracorporeal membrane oxygenation applications.

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 and can occur from mechanical, thermal, or chemical sources in these devices. Dynamic *in vitro* hemolysis testing is an essential component of the assessment of devices, as it evaluates the biological response during operation of the device under worst-case clinical use conditions. Directly comparing the reported results of *in vitro* hemolysis testing between laboratories is of limited utility, however, due to the lack of uniformity of the test methods employed, and variability in the fragility of the test blood and the measurement assays used to assess hemolysis. Thus, it is necessary to provide standardization of the methods for performing and reporting dynamic *in vitro* hemolysis tests in the evaluation of potential clinical blood pumps. As there is a diverse range of device technologies and clinical pump applications, this standard proposes methodology for evaluating a blood pump under its simulated clinical use conditions and in relation to a relevant comparator device.

1. Scope

1.1 This practice covers a protocol for the assessment of the hemolytic properties of continuous, intermittent, and pulsatile flow blood pumps used in circulatory assist, including extracorporeal, percutaneous, and implantable devices. An assessment is made based on the pump's effects on the erythrocytes over a certain period of time. Adopting current practices for this assessment, a 6-hour *in vitro* test is performed on a pump placed in a device-specific recirculating blood loop that mimics the pressure and flow conditions of the expected worst-case clinical use of the device. If the ultimate goal of the testing is to evaluate the blood damage potential of a pump for clinical use, it is suggested that paired testing between the subject blood pump and a legally marketed comparator device be conducted using the same blood pool in a matched blood test loop so that a relative hemolysis comparison can be made.

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the*

[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 on Cardiovascular Standards.

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Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

F1830 Practice for Collection and Preparation of Blood for Dynamic ***in vitro*** Evaluation of Hemolysis in Blood Pumps

2.2 *ISO Standards:*

ISO 14708-5 Implants for surgery—Active implantable medical devices—Part 5: Circulatory support devices

3. Terminology

3.1 *Definitions:*

3.1.1 *comparator device*—a legally marketed blood pump intended for a similar application and flow rate range as the subject device (e.g. adult implantable blood pump providing full circulatory support), which can be tested in parallel with the subject device using the same blood pool so that a relative hemolysis comparison can be made between the devices.

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

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

3.1.4 *subject device*—a blood pump to be evaluated for its hemolysis potential prior to clinical use.

3.2 *Index of Hemolysis:*

3.2.1 *normalized index of hemolysis (NIH)*—increase in 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.

3.2.2 *modified index of hemolysis (MIH)*—mass of hemoglobin released into plasma normalized by the total mass of hemoglobin pumped through the loop (multiplied by 10^6).

4. Formulas (see Appendix X2 for units and sample calculations)

4.1 *Normalized Index of Hemolysis (NIH)* **(1, 2, 3, 4)**[3]:

$$\text{NIH (g/100 L)} = \frac{\Delta PfH * V * \frac{(100 - Hct)}{100}}{Q * \Delta T * 1000} \quad (1)$$

where:

ΔPfH = change in plasma free hemoglobin concentration (mg/dL) over the sampling time interval,
V = blood volume in the loop (mL),
Q = flow rate (L/min),
Hct = hematocrit (%), and
ΔT = sampling time interval (min).

4.2 *Modified Index of Hemolysis (MIH):*

4.2.1 Modified index of hemolysis (MIH) **(5, 6)** can be written with no units or as (mg of hemoglobin released into plasma / mg of total hemoglobin pumped through the loop) × 10^6 factor. The 10^6 factor is introduced to reduce the number of decimal places **(5)** and is accounted for in the following equation when the appropriate parameter units are used (see Appendix X2):

$$\text{MIH} = \frac{\Delta PfH * V * \frac{(100 - Hct)}{100}}{Q * \Delta T * Hgb} \quad (2)$$

where:

Hgb = total blood hemoglobin concentration at time zero (g/dL).

4.3 *Differences Between NIH and MIH*—While the NIH value has historically been reported for blood pumps because of its simplicity, this index is limited as it does not account for the total hemoglobin concentration of the blood **(6)**. For this reason, the MIH equation is often used as it considers both blood hematocrit and hemoglobin concentration directly **(5)**. Thus, along with the PfH values, it is recommended that both the NIH and MIH indices be reported to express the degree of hemolysis caused by a blood pump tested in a recirculating flow system. Example calculations for NIH and MIH are included in Appendix X2.

5. Summary of Practice

5.1 The hemolytic potential of a subject blood pump is assessed by operating it in a device-specific recirculating blood loop that mimics the pressure and flow conditions of the expected clinical use of the device. Blood is recirculated for 6 h and the hemolysis is assessed by measuring the plasma free hemoglobin concentration (PfH) at periodic time points. If the goal of the testing is to evaluate the blood damage potential of a pump intended for clinical use, it is suggested that paired testing between the finalized subject blood pump and a legally marketed comparator device be concurrently conducted using the same blood pool in a matched blood test loop so that a relative hemolysis comparison can be made. This standard provides methodology for preparing blood, designing a recirculating flow loop, conducting replicate pump testing, and evaluating and reporting the results (see Table in Appendix X4).

6. Significance and Use

6.1 The objective of this practice is to standardize the evaluation method for assessing the hemolytic effect of a blood pump used in extracorporeal circulation and/or circulatory assistance. By comparing the hemolysis results between a subject device and a comparator device through paired testing, a relative evaluation of hemolysis for the subject device can be made.

7. Preparation of Hemolysis Test

7.1 *Blood for Testing*—See Practice F1830 for details on obtaining and preparing blood for testing. Briefly, the blood is obtained from animals or human volunteers having afebrile

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

body temperatures, no physical signs of disease, and an acceptable range of hematological values. Human, bovine, porcine, and ovine blood have been used as the primary sources of blood in the evaluation of pumps. Because the level of trauma-induced hemolysis may be different based on the source of blood, it is necessary to identify the source of blood when reporting the indices of hemolysis. It is preferable that animal blood be collected by controlled venipuncture to minimize the risk of contamination with debris or fluids other than blood. The blood from an abattoir can be used if it is obtained in such a way as to minimize contamination and excessive trauma to the blood (see Practice F1830). Commonly used blood anticoagulants include heparin (4000 to 6000 USP units per liter of collected blood), ACD-A (anticoagulant citrate dextrose solution A; volume ratio of ACD-A to blood is typically 1:5 to 1:8), and CPDA-1 (citrate phosphate dextrose adenine anticoagulant solution; volume ratio of CPDA-1 to blood is typically 1:7). Blood obtained for device testing is generally used within 48 h of blood draw. As a quality control measure, an initial plasma free hemoglobin concentration should generally be less than 50 mg/dL. 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 of 35 ± 2 % by hemodilution (with phosphate buffered saline) or hemoconcentration (via minimal centrifugation). Alternate hematocrit values may be used if clinically relevant (e.g. lower for diluted blood, or higher for pediatric application). Blood temperature during storage is typically kept between 2 and 8 °C, and warmed to testing temperature prior to use. It is recommended that acceptable values for physiological blood parameters (e.g. pH, glucose) be maintained prior to and during testing, as appropriate **(5)**.

7.2 *Recirculating Blood Test Loop:*

7.2.1 *Loop Configuration*—As there is a diverse range of device technologies and clinical pump applications, a device-specific flow loop should be used to test the blood pump under conditions which simulate its worst-case clinical use. For illustrative purposes, Figure 1 shows the components of a typical flow loop for testing an extracorporeal blood pump that may be used for cardiopulmonary bypass procedures, or a pump which may be used for ventricular assist. For other types of blood pump designs or placements, the test loop should be appropriate to mimic the upstream and downstream geometries, flows, and pressures experienced by the pump during clinical use. The example test loop in Fig. 1 consists of polyvinylchloride tubing, a blood reservoir, the pump, a water bath or heat exchanger to maintain a target blood temperature, and equipment to measure the blood flow rate, temperature, and pre- and post-pump pressures. The primed blood volume should be minimized to increase the sensitivity of the hemolysis test results while being sufficient to prevent air-blood mixing or collapse of any of the loop components due to suction. Blood volumes used in test loops for pump testing are typically less than 500 mL.

A screw clamp, positioned at the outlet side of the pump, is applied to produce the required post-pump pressure conditions for the clinical application. For example, a 100 mmHg pressure head may be appropriate for an adult left heart assist application. For cardiopulmonary support applications (i.e. extracorporeal or percutaneous), a pressure head in the range of 325 to 500 mmHg may be appropriate **(7)**.

An ultrasonic or electromagnetic flow probe is typically used to monitor the blood flow rate. The flow meter should be calibrated using blood at the proper hematocrit and temperature. As pumping may cause a temperature rise in the blood, thermal control should be considered in the form of a water bath or heat exchanger to maintain an appropriate and constant blood temperature during the experiments. Blood temperature should be monitored and recorded throughout the testing. As local thermal heating within pumps can damage blood, testing should be performed at 37 ± 2 °C for pump applications in which the patient is expected to be normothermic. For cardiopulmonary bypass applications, it may be appropriate to maintain the blood during the testing at 23 ± 2 °C.

All necessary blood-contacting components and provided accessories to be used with the subject device (e.g. a device-specific cannula) should be included in the testing loop as they could impact the hemolysis results. Moreover, pumps being evaluated for clinical use may be subjected to pre-conditioning such as sterilization and aging (if sensitive to possible effects). See ISO 14708-5 for details on pre-conditioning.

7.2.2 *Preparing the Loop for Blood Testing*—Since all test runs are of a 6-h duration, sterility is generally considered not necessary. Prior to testing with blood, the loop should be filled with phosphate buffered saline (PBS) that is recirculated for approximately 5 to 10 min to rinse and wet all the blood-contacting surfaces.

7.3 *Pump Conditions*—The pump flow rate for the testing should be determined based on the expected clinical use conditions of the subject device. Testing should be conducted at the proposed maximum limit for the pump (i.e. worst-case clinical use condition) by considering the maximum flow rate and pump speed at a clinically-relevant pressure head. Additional testing may also include the "nominal" and minimum flow rates for pump operation as appropriate.

7.4 *Concurrent Testing of Subject and Comparator Devices:*

7.4.1 *Paired Device Testing*—If the goal of the testing is to evaluate the blood damage potential of a pump intended for clinical use, it is recommended that paired testing between the subject blood pump and a comparator device be conducted concurrently using the same blood pool in matched blood test loops. Paired testing allows for a relative hemolysis comparison to be made by accounting for the variability in the blood due to different donors and handling on each test day.

7.4.2 *Number of Replicate Paired Pump Tests*—At least five replicate paired pump tests should be conducted to determine the variability across devices; this requires five separate subject devices and five comparator devices. Typically, washing and re-using the comparator devices is not recommended.

8. Procedure

8.1 The PBS which was recirculated through the loop (as noted in 7.2.2) is usually drained completely from the loop prior to filling it with blood at the appropriate test temperature to minimize any dilution effects. An alternate technique is to gently introduce the blood into the loop to displace the PBS,