In Vitro Dynamic Hemolysis Testing of Blood Pumps: Updating the ASTM F1841 Testing Standard

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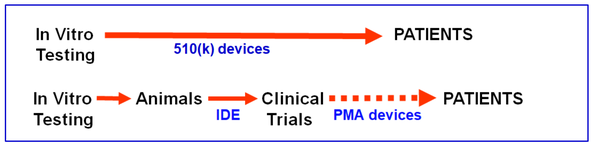
Synopsis

During the safety evaluation of new circulatory support devices, benchtop testing with blood is a critical tool to ensure that pumps do not cause excessive damage to blood cells. To account for ongoing advances in blood pump technologies, FDA led the effort to revise the ASTM F1841-97 (2017) blood testing standard (*Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps*) originally published in 1997. The revised ASTM F1841-19 standard was published in Dec. 2019 and formally recognized by the FDA for use in regulatory submissions in July 2020.

Introduction

Background: In the safety assessment of new and modified blood pumps intended for circulatory support, benchtop testing using animal blood is one of the first evaluation steps and a critical tool to ensure that devices do not excessively damage blood cells (Figure 1). To enable uniformity in testing, the ASTM F1841 standard was originally published in 1997 to characterize mechanical hemolysis (i.e., damage to red blood cells resulting in the release of toxic hemoglobin into the plasma) caused by blood pumps under fixed test conditions (e.g., blood flow rate of 5 L/min, specific flow loop tubing configuration, and 450 mL blood loop volume).

Figure 1: General Regulatory Pathways Utilizing In Vitro Hemolysis Testing



Purpose: As blood pump technology and clinical applications have significantly advanced over the last 20 years, FDA led a consensus effort to update the ASTM F1841 *in vitro* hemolysis testing standard to allow greater flexibility for industry to assess their circulatory support devices under relevant clinical use conditions for regulatory submissions to the FDA.

Important Changes to the ASTM F1841 Hemolysis Testing Standard

Issue	Original Version (1997-2017)	Revision (Dec. 2019)
Scope	Characterize blood pump hemolysis under uniform (one size fits all) testing conditions	Characterize pump hemolysis under worst-case clinical use conditions of a subject device relative to a comparator device
Regulatory Impact	Often required modifications for FDA submissions	Applicable for all FDA submissions for blood pumps
Devices	Continuous flow blood pumps, including roller and centrifugal pumps, used in extracorporeal circulation and circulatory assistance	Continuous, intermittent, and pulsatile flow blood pumps used in circulatory assist, including extracorporeal, percutaneous, and implantable devices

Materials and Methods

Researchers from CDRH/OSEL led the efforts in revising the ASTM F1841-97(2017) standard to address three aspects of the testing:

1. Blood Preparation
2. Testing Conditions
3. Evaluation of Hemolysis – Plasma free Hemoglobin (PfH), Normalized Index of Hemolysis (NIH), Modified Index of Hemolysis (MIH)

1. Blood Preparation	Recommendation
Species	Human, porcine, bovine, ovine
Anticoagulation and collection	Heparin, ACD-A, or CPDA-1 (refer to ASTM F1830*)
Hematocrit	35 +/- 2% or clinically relevant value
Blood volume in loop	Minimize the blood volume (typically < 500 mL)
Total hemoglobin concentration	Measure and report (needed to calculate MIH)
Post-draw blood age	Refer to ASTM F1830* (generally < 48 hrs)
Initial plasma free hemoglobin	< 50 mg/dL (as a quality control measure)
Physiological parameters to maintain	pH, glucose, pO2
Antibiotic in blood	Refer to ASTM F1830*

* ASTM F1830-19: *Standard Practice for Selection of Blood for in vitro Evaluation of Blood Pumps*

2. Testing Conditions	Recommendation
Device configuraton	Perform tests on all necessary blood-contacting components of the subject test device
Flow rate	Maximum flow rate for the intended clinical use
Pre-pump pressure	Appropriate value per clinical use of the subject device
Post-pump pressure	Use tubing clamp to set to clinically-relevant value
Blood temperature	Appropriate value per clinical use of the subject device
Test duration	6 hours
Sampling schedule	0, 1, 2, 3, 4, 5, 6 hours
Number of paired tests	n = 5 replicate paired tests (use the same blood pool for concurrent testing of the subject and comparator devices)
Comparator device	Legally marketed blood pump with similar use indications
Re-use of devices	Not recommended

3. Evaluation of Hemolysis	Recommendation
Plasma free Hemoglobin (PfH) concentration	Measure PfH concentration using a validated assay. Plot PfH curves as a function of test time for each individual device tested. Using a least squares fit to the data, determine the regression coefficient for each device test. In general, the plots are linear with $r^2 > 0.95$. (see Subsection 9.2)
Hemolysis Index values (NIH, MIH)	Provide calculations of each hemolysis index per Subsections 4 and 9.3 of ASTM F1841-19.
Relative hemolysis between comparator and subject device	Perform statistical testing (paired t-test) to determine whether there is a statistical difference between the comparator and subject devices with respect to PfH, NIH, and MIH.

Results

Revisions to the ASTM F1841 standard aid blood pump developers in preparing an acceptable device submission to the FDA by using:

- A device-specific flow loop, higher hematocrit to increase test sensitivity, reporting of hemolysis values (PfH, NIH, MIH), testing at expected maximum clinical flow rate and relevant pressures (worst-case operating point with regards to hemolysis), paired hemolysis testing for comparison to a comparator clinical device.

Figure 2. Typical blood flow loop for testing extracorporeal or implantable blood pumps.

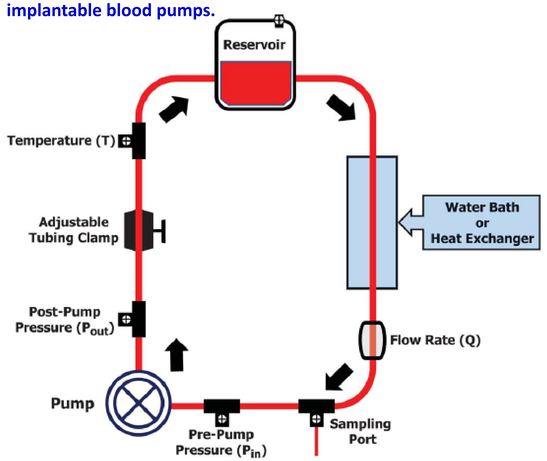


Figure 3. Increasing PfH concentration as hemoglobin is released from damaged red blood cells (i.e., hemolysis).

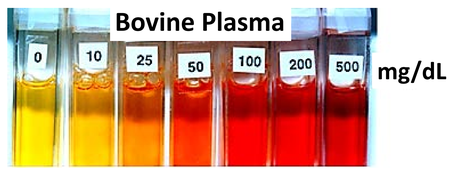
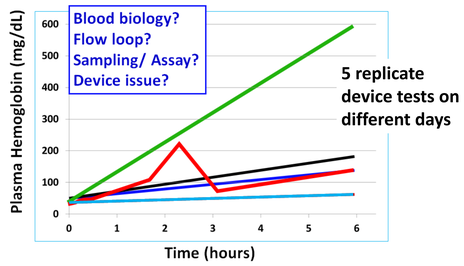


Figure 4. Example hemolysis results for 5 pump tests. Methods in the revised standard can help to identify possible issues during the testing (e.g., explanation for variability in the PfH vs time plots below).



Discussion

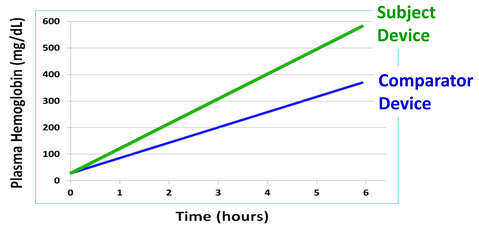
Regulatory Considerations for Device Hemolysis Testing

Was the testing conducted at the expected worst-case clinical use conditions in terms of flow rate, pump speed, and pressures?

Were the results acceptable on each test day (reproducible, linear)?

Were the paired hemolysis levels statistically similar between the subject and comparator devices tested on each test day (Figure 5)?

Figure 5. Example paired hemolysis test results for a subject and comparator blood pump.



If blood parameters (hematocrit, flow rate, blood volume) were similar during the paired testing between the subject and comparator devices, then the PfH values can be directly compared. If changes between the test loops occurred (e.g., use of purge fluid with a pump, or differences in blood conditions on different test days) then the Modified Index of Hemolysis (MIH) can be used to normalize the results as follows:

$$MIH = \frac{\Delta PfH * V * (100\text{-}Hct)/100}{Q * \Delta T * Hgb} \quad (x\ 10^6)$$

where ΔPfH = change in plasma hemoglobin level, V = blood volume, Hct = hematocrit, Q = flow rate, ΔT = time between blood samples, Hgb = total hemoglobin concentration

Conclusions and Regulatory Impact

The ASTM F1841-97(2017) version of the *in vitro* hemolysis testing standard had fixed conditions (e.g., adult blood flow rate of 5 L/min, specific flow loop tubing configuration, 450 mL blood loop volume) for characterizing pump hemolysis which were not amenable to regulatory evaluation of new and innovative devices that often have very different clinical use conditions.

The revised version of the ASTM F1841-19 standard acts as a flexible guideline for testing modified, new and innovative devices (such as pediatric blood pumps, pumps which use purge fluid to wash blood-contacting bearings, pumps with artificial pulsatility, and percutaneous pumps for use in different locations in the vasculature).

To assist industry and FDA in the safety evaluation of new/ modified blood pumps for years to come, the revised ASTM F1841-19 standard details how to perform and assess paired hemolysis testing of a subject device relative to a legally marketed comparator device, so that a regulatory decision for clinical acceptability of the subject device can be made.