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

Richard Malinauskas, Luke Herbertson, Jean Rinaldi, Megan Jamiołkowski, Qijin Lu
FDA/ Center for Devices and Radiological Health (CDRH)/ Office of Science and Engineering Laboratories (OSEL)

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. A 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).

Key Changes to the ASTM F1841 Hemolysis Testing Standard


Materials and Methods

Researchers from CDRH/OSellan 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 (PH), Normalized Index of Hemolysis (NIH), Modified Index of Hemolysis (MIH)

Testing Methods

1. Blood Preparation

- Species: Human, porcine, bovine, ovine
- Anticoagulation and collection: Heparin, ACD-A, or CPDA-1
- Hematocrit: 36% ± 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®

2. Testing Conditions

- Device configuration: Perform tests on all necessary blood-contacting components of the subject test device
- Flow rate: Maximum flow rate for the intended clinical use
- Post-pump pressure: Appropriate value per clinical use of the subject device
- Post-pump pressure: Using tube clamping 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

- Plasma free Hemoglobin (PH) concentration
- Measure PH concentration using a validated assay. Plot PH 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. In general, the plots are linear with r² > 0.95. (See Subsection 9.2)

- Hemolysis Index values (NIH, MIH)
- Provide calculations of each hemolysis index per subject and comparator devices.

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 (PH, 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.

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)?

Conclusions and Regulatory Impact

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.

Figure 3. Increasing PH 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 NIH vs time plots below).

Table 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 relative to their clinical use conditions for regulatory submissions to the FDA.

Important Changes to the ASTM F1841 Hemolysis Testing Standard

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<tr>
<td><strong>Scope</strong></td>
<td>Characterize blood pump hemolysis under uniform testing conditions</td>
<td>Characterize blood pump hemolysis under worst-case clinical use conditions of a subject device relative to a comparator device</td>
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<td><strong>Regulatory Impact</strong></td>
<td>Often required modifications for FDA submissions</td>
<td>Applicable for all FDA submissions for blood pumps</td>
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<td><strong>Devices</strong></td>
<td>Continuous flow blood pumps, including roller and centrifugal pumps, used in extracorporeal circulation and extracorporeal and caval circuits</td>
<td>Continuous, intermittent, and pulsatile flow blood pumps used in circulatory assist, including testing for a percutaneous, extracorporeal, and implantable devices</td>
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Table 2: Testing Conditions Recommendation

| Device configuration | Perform tests on all necessary blood-contacting components of the subject test device |
| Flow rate | Maximum flow rate for the intended clinical use |
| Post-pump pressure | Appropriate value per clinical use of the subject device |
| Test duration | 6 hours |

Table 3: Evaluation of Hemolysis Recommendation

| Plasma free Hemoglobin (PH) concentration | Measure PH concentration using a validated assay. Plot PH 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. In general, the plots are linear with r² > 0.95. (See Subsection 9.2) |
| Hemolysis Index values (NIH, MIH) | Provide calculations of each hemolysis index per subject and comparator devices. |

Table 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 NIH vs time plots below).