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# Design Considerations for Mechanical Hemolysis Testing

# Disclaimer

- Employed by Nelson Labs
- No Conflicts of Interest

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# Types of Hemolysis

Hemolysis - rupture of the erythrocyte membrane

ISO 10993-4: Annex D - Haematology/haemolysis — Methods for testing

Three types of hemolysis: material mediated, osmotic pressure induced, and mechanically induced

Mechanical forces which can induce hemolysis include:

- flow rates
- shear forces
- turbulence
- impact

The more complex the flow path of the blood, the greater the risk of hemolysis

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# When Should Mechanical Hemolysis Be Assessed?

- Table 1 of ISO 10993-4 provides guidance as to what type of devices require mechanical hemolysis testing
- Direct blood contacting devices and implants:
  - Hemodialyzers
  - Blood Pumps
  - Ventricular Assist Devices (VAD)
  - Mechanical heart valves
- Other devices that may need hemolysis consideration:
  - Catheters
  - Blood warmers
- Dependent upon the Regulatory Body

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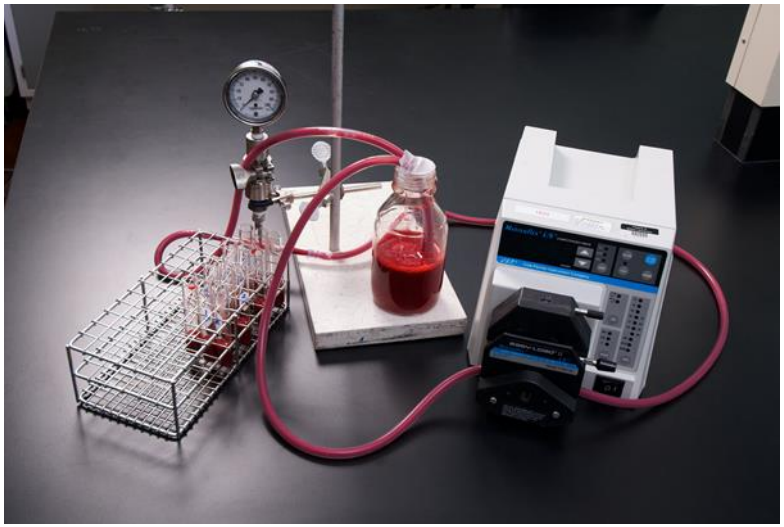
# Initial Considerations for Testing

- Test Design Should Reflect Clinical Use
- Blood Types (Species) Used for Testing
  - Human always preferable
  - May not always be possible depending on volume required
  - Bovine and Porcine commonly used
- Anticoagulants Used
  - Sodium citrate vs Heparin
  - Amount used
- Predicate Device Comparison?
- Initial Levels of Plasma Free Hemoglobin (fHb)
  - Blood containing an initial concentration above 20 mg/dL should not be used (ASTM F1841-97 (2013))

# Dynamic Test Set up for Mechanical Hemolysis Testing

## Single Pass Testing

- Paired Test Set Up – Test vs Predicate Device
- Five Replicates
- Baseline samples taken before testing is started
- The same volume of blood used for both test and predicate testing
  - Volume of blood should be minimized to increase the sensitivity of the assay
- Maximum flow rate of device should be used to produce the maximum of hemolysis possible



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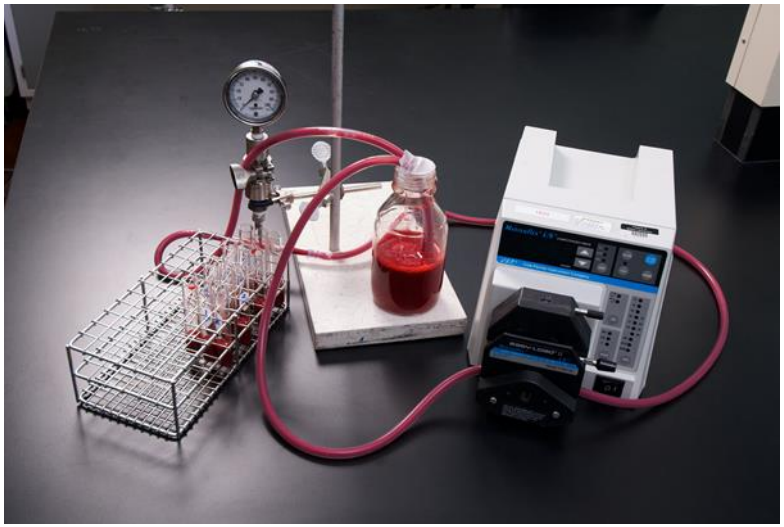
# Dynamic Test Set up for Mechanical Hemolysis Testing

## Recirculated Blood Testing

- Paired Test Set Up – Test vs Predicate Device
- Five Replicates
- Baseline samples taken before testing is started
- The same volume of blood used for both test and predicate testing
  - Volume of blood should be minimized to increase the sensitivity of the assay
- Maximum flow rate of device should be used to produce the maximum of hemolysis possible
- Recommended recirculation time is 6 hours (F1841-97(2013)), any other recirculation time must be justified



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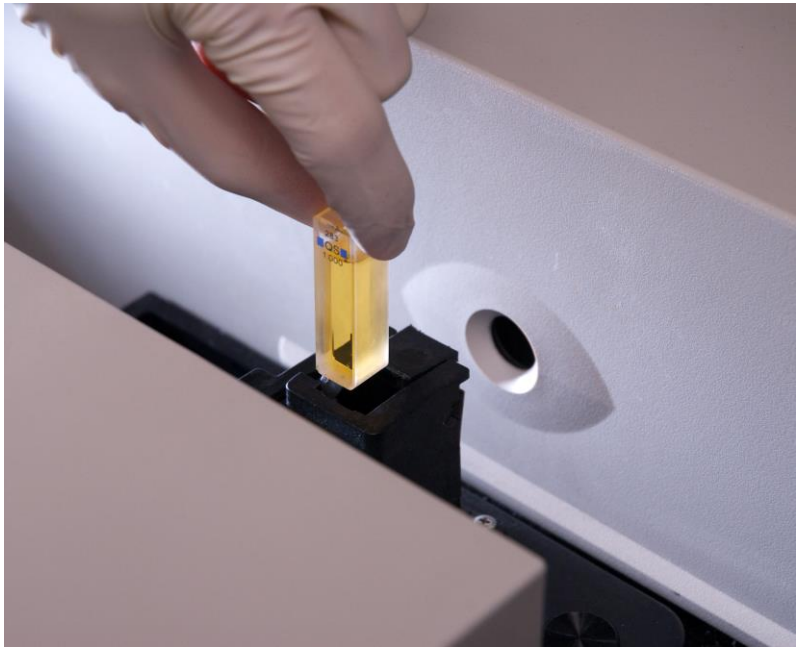
# Analysis of Hemolytic Samples

## Validated Methods

- Total hemoglobin levels
- ASTM F756 - use of the cyanomethemoglobin method
  - Pros: simple set up, accepted by FDA
  - Cons: Uses toxic reagents which are difficult to dispose of properly
- Direct Oxyhemoglobin methods (Cripps)
  - Pros: Does not use toxic reagents
  - Cons: Requires more initial instrumentation validation



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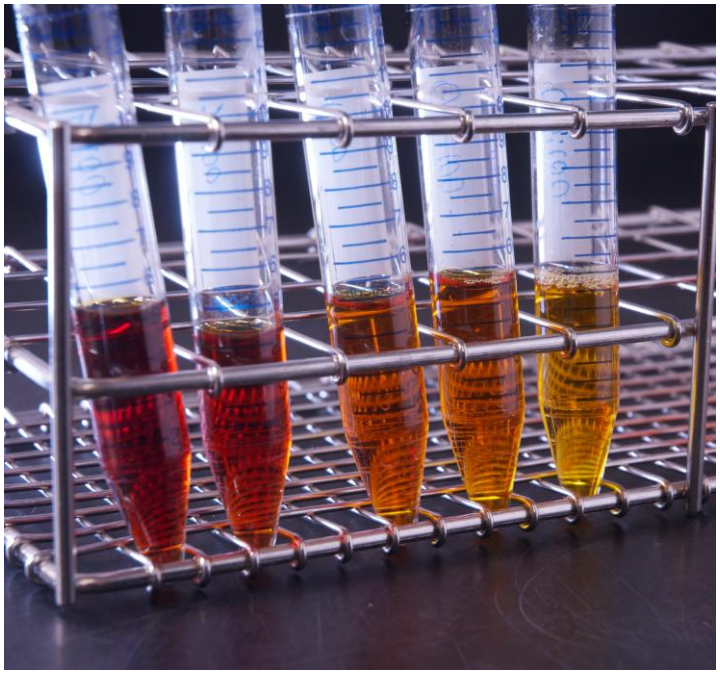
# Analysis of Collected Data

- Hemolytic Index (% Hemolysis)
  - Appropriate for static or single pass samples
  - HI = (supernatant fHb / total hemoglobin) x 100%
- Normalized Index of Hemolysis (NIH)
  - Corrects for volume, hematocrit, flow rate, time
  - Calculates fHb in g/L
- Modified Index of Hemolysis (MIH)
  - Corrects for volume, hematocrit, flow rate, time, and total hemoglobin concentration
  - Unitless calculation
  - Recommended when measuring recirculating blood systems (F1841-97(2013))
- There is no established acceptance criteria for hemoylsis so test sample must be compared to predicate to determine hemolytic effects



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# Conclusions

- Mechanical Hemolysis is due to the physical flow and movement of the blood against the device
- Mechanically induced hemolysis must be assessed for most blood contacting devices and many indirect blood contacting devices
- Predicate device selection is crucial
- Test setup should be reflective of clinical use

# References


- International Standards Organization . (2017a). ISO 10993-4. Biological Evaluation of Medical Devices—Part 4: Selection of Tests for Interactions with Blood, 3rd ed. (2017-4). International Standards Organization, Geneva, Switzerland.
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- Cripps, C M. “Rapid method for the estimation of plasma haemoglobin levels.” *Journal of clinical pathology* vol. 21,1 (1968): 110-2. doi:10.1136/jcp.21.1.110


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# Thank You

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