Advances in Cardiovascular Medical Devices

Thrombogenicity Testing for Blood-Contacting Medical Devices in an in vitro Human Blood-Loop

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Thrombogenicity Test

ISO 10993-4 thrombogenicity testing is widely used for meeting regulatory requirements for approval of blood-contacting medical devices.

– Thrombosis deposition
– Thromboembolism
**In vivo** Thrombogenicity

- In vivo implant in clinical relevant location
- In vivo thrombogenicity study
  - NAVI (Non-anticoagulated venous implant)
  - AVI (Anticoagulated venous implant)
  - NAAI (Non-Anticoagulated arterial implant)
  - AAI (Anticoagulated arterial implant)
Limitations of \textit{in vivo} Model

- Acknowledged by only a few regulatory bodies
- Controversy and caveats with the methodology
  - The implant position
  - The implant technique
  - The extent of device-vessel wall contact (Tissue Factor)
  - The explant technique
  - Failure of LMCD
  - Hydrophilic surface
  - Statistical power

In vitro Ovine Blood Loop

Thrombogenicity Testing of Medical Devices in a Minimally Heparinized Ovine Blood Loop

ISO 10993-4 in vivo thrombogenicity testing is frequently performed for regulatory approval of many blood-contacting medical devices and is often a key part of submission packages. Given the current state of in vivo thrombogenicity assays, a more robust and reproducible assay design, including in vitro models, is needed. This study describes an in vitro assay that integrates freshly harvested ovine blood containing minimal heparin in a closed pumped loop. To confirm the reproducibility of this assay, control materials were identified that elicited either a positive or a negative thrombogenic response. These controls demonstrated reproducibility in the resulting thrombogenicity scores with median scores of 5 and 0 for the positive and negative controls, respectively, which also demonstrated a significant difference (p<0.0001). For a direct comparison of the in vitro blood loop assay to the traditional in vivo nonanticoagulated venous implant (NAVI) assay, seven sheep were used as blood donors for the loop and then as subjects for an NAVI assay. In each assay—loop or NAVI—three study articles were used: the positive and negative controls and a marketed, approved catheter. The resulting thrombogenicity scores were similar when comparing the loop to the NAVI results. For each study article, the median thrombogenicity scores were the same in these two different assays, being 0, 1, and 3 for the negative control, the marketed catheter, and the positive control, respectively. These data suggest that the in vitro assay performs similarly to the in vivo NAVI assay. This in vitro blood loop method has the potential to predict a material’s in vivo thrombogenicity, can substantially de-risk the materials or coating selection process, and may eventually be able to replace the in vivo models currently in use.

[DOI: 10.1115/1.4035724]
3RS Award for *in vitro* Alternative Assay

Printed from www.AAALAC.org on December 6, 2017

THE GLOBAL 3RS AWARDS PROGRAM

2017 Award Winners

The Global 3Rs Awards program, a collaboration between AAALAC International and the IQ Consortium, recognizes the following individuals for their significant innovative contributions toward the 3Rs of animal research to advance ethical science in academia or industry in any area of biology. **The 2017 winners are:**

1. **Dr. Mark E. Smith**
   - Chief Scientific Officer at American Preclinical Services (APS) in Minneapolis, Minnesota.
   - He is receiving a Global 3Rs Award for the article, "Thrombogenicity Testing of Medical Devices in a Minimally Heparinized Ovine Blood-Loop," *Journal of Medical Devices* (2017). This work addresses the initial validation and continuing development of a novel test for screening medical devices that are placed in the bloodstream of patients to assess thrombogenicity, their potential to form blood clots. This new test has the potential to replace the commonly used in vivo test -- the Non-Anticoagulated Venous Implant (NAVI) thrombogenicity test.
In vitro Human Blood Loop

Enhanced potential for prediction of clinical risk
In vitro Blood Loop Configuration
Typical Assay Design

Positive Control x 3

LCMD Comparator Article (1-3)

Test Article (1-3)

Negative Control x 3
Blood Collection/Qualification

- Drawn from healthy donors
- Final heparin 0.7 IU per mL
- Time from collection to loading of the loop (~3 hours)
- Baseline ACT (120 – 250 seconds)
- Minimal of three donors
Temperature Control
Study Assessment

• Thrombus formation
  – % surface area coverage

• Non-adherent thrombus
  – Weights of non-adherent thrombus

• Blood characterization
  – Complete blood counts (CBC)
  – Activated clotting time (ACT)
  – Platelet counts
In situ Visual Observation

(a) Neg. Control

Score 0

(b) Pos. Control

Score 5
Quantitative Thrombus Assessment

Cross section View - Example for Calculating Coverage

Insertion Site

3.1 cm

1.0 cm

= test device (Total Length = 10 cm)

= Thrombus formation

Thrombus on segment:
For 50% coverage

\[
(3.1 \text{ cm} \times 50\%) + (1 \text{ cm} \times 100\%) \quad \frac{10 \text{ cm}}{10 \text{ cm}} = 26\% \text{ coverage}
\]

For 100% coverage
Positive and Negative Controls (N=13 studies)
Non-Adherent Thrombus

Weights of recovered non-adherent thrombus from several sets of assays were evaluated and in only one instance (a positive control loop from one individual assay) was any non-adherent thrombus visible. In this single instance, the observed weight of thrombus was <10 mg.
## Complete Blood Count

**Blood CBC analysis before and after circulation in loop**

### Table 1: Summary CBC Data from Blood Loop Validation

<table>
<thead>
<tr>
<th>Blood Parameter</th>
<th>Heparinized blood</th>
<th>After 4 hours ± 30 min (end of run)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neg. Control loop</td>
</tr>
<tr>
<td>RBC count (1000/µl)</td>
<td>5.11 ± 0.53</td>
<td>4.93 ± 0.32</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.9 ± 4.9</td>
<td>43.3 ± 5.2</td>
</tr>
<tr>
<td>WBC (1000/µl)</td>
<td>5.08 ± 1.3</td>
<td>4.47 ± 1.3</td>
</tr>
</tbody>
</table>
Blood ACT Values

ACT (seconds) ± std dev

Baseline  Negative Control  Positive Control

0  100  200  300  400
Blood Platelet Counts

Baseline

Negative Controls

Positive Controls

Platelet Count (1000's per µL)
**In vivo – in vitro Comparison**

- **The implant position**
  - Uniform and consistent blood flow, Controlled tubing diameter
- **The implant technique**
  - Controlled deployment procedure
- **The extent of device-vessel wall contact (Tissue Factor)**
  - Minimal tubing wall contacting, no TF components
- **The explant technique**
  - In situ thrombus observation, non-adherent thrombus quantification
- **Failure of LMCD**
  - ?
  - Positive and negative control
- **Hydrophilic surface**
  - Non-adherent thrombus quantification
- **Statistical power**
  - Minimal of there different donors, up to 3 replicates per loop
Acknowledgement

- Dr. Mark E. Smith
- Dr. Michael L. Conforti
- Sarah E. Howard
- Matthew R. Cunningham
- Abigail C. Beltrame
THANK YOU

Any questions