In Vitro Hemocompatibility Testing: Continuing Development of an Ovine Blood-Loop Assay

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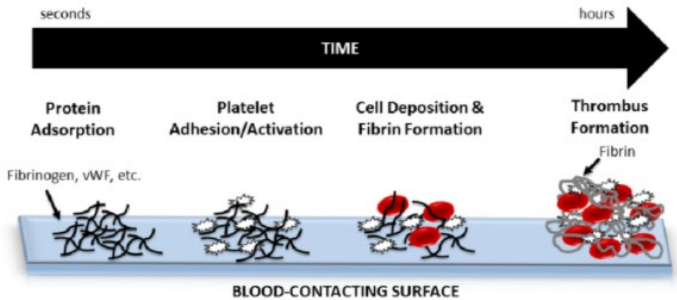
DISCLAIMER

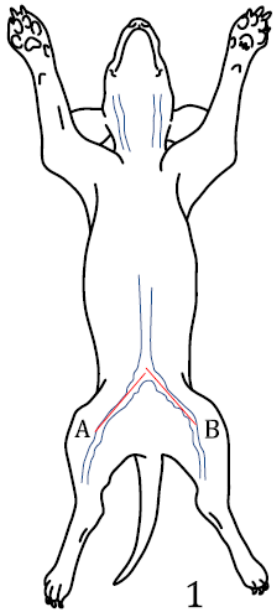
The material presented is for general information sharing only and is solely the opinion of the presenter.

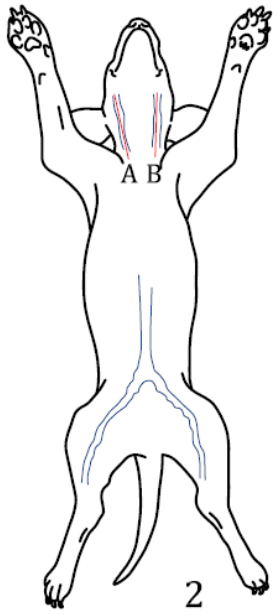
THROMBOGENICITY TEST

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



- In vivo thrombogenicity study
  - NAVI (Non-anticoagulated venous implant)
  - AVI (Anticoagulated venous implant)
  - NAAI (Non-Anticoagulated arterial implant)
  - AAI (Anticoagulated arterial implant)



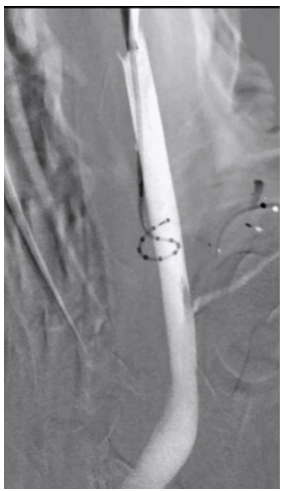


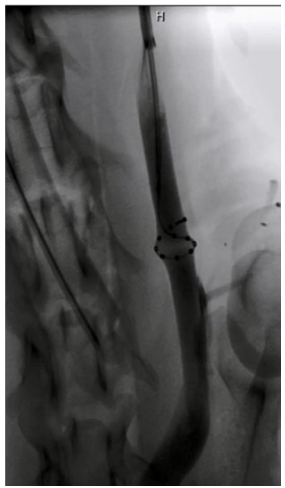
A: Test
B: LMCD (Legally Marketed Comparison Device)
1: Femoral
2: Jugular

ISO 10993-4 Selection of tests for interactions with blood (2017)

TRADITIONAL NAVI TEST FOR THROMBOGENICITY

- Beagle or Mongrel Dogs (N=2-3)
- Non-heparinized Animals
- Evaluation in the Jugular/Femoral vein
- 4 Hour Dwell Time





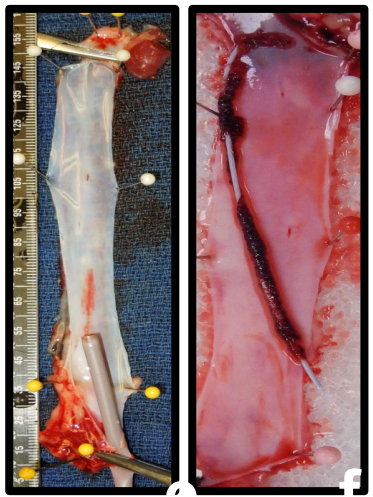


Table C.1 — NAVI/AVI scoring scheme A

Thrombus formation score description	Score
No significant thrombosis (a very small clot is acceptable at insertion).	0
Minimal thrombosis, one location.	1
Minimal thrombosis, multiple locations.	2
Significant thrombosis, > 1/4 to ≤ 1/2 the surface of the implant, vessel patent.	3
Significant thrombosis, > 1/2 the surface of the implant, vessel patent.	4
Vessel completely occluded.	5

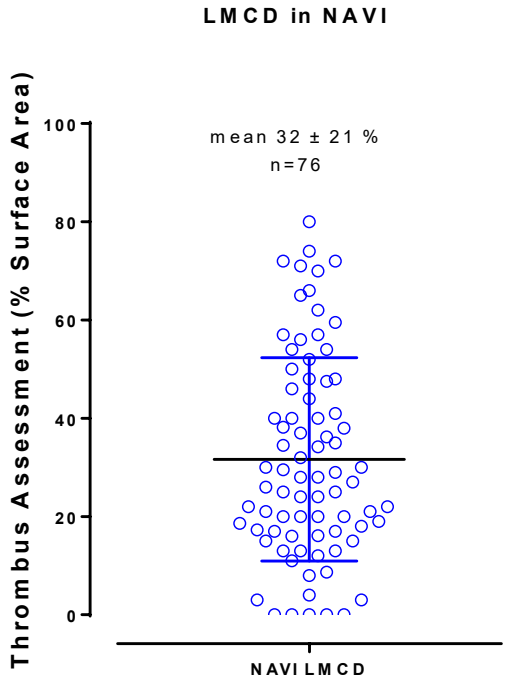
Table C.2 — NAVI/AVI scoring scheme B

Thrombus formation score description	Score
Thrombus non-existent or minimal and, if present, appears to be associated with implant venotomy site.	0
Thrombus minimal, observed to be covering 1 % to 25 % of material surface.	1
Thrombus moderate, observed to be covering 26 % to 50 % of material surface.	2
Thrombus severe, observed to be covering 51 % to 75 % of material surface.	3
Thrombus extensive, covers 76 % to 100 % of material surface.	4

ISO 10993-4 Selection of tests for interactions with blood (2017)

CHALLENGES OF THE IN VIVO MODEL

- Not internationally accepted by regulatory agencies
- Controversy and caveats with the methodology
  - The implant location/anatomy
  - The implant technique
  - The extent of device-vessel wall contact (Tissue Damage)
  - Time/incubation period
  - The explant technique
  - Hydrophilic surface
  - Statistical power
  - Inconsistent data for LMCD (legally marketed comparison device)

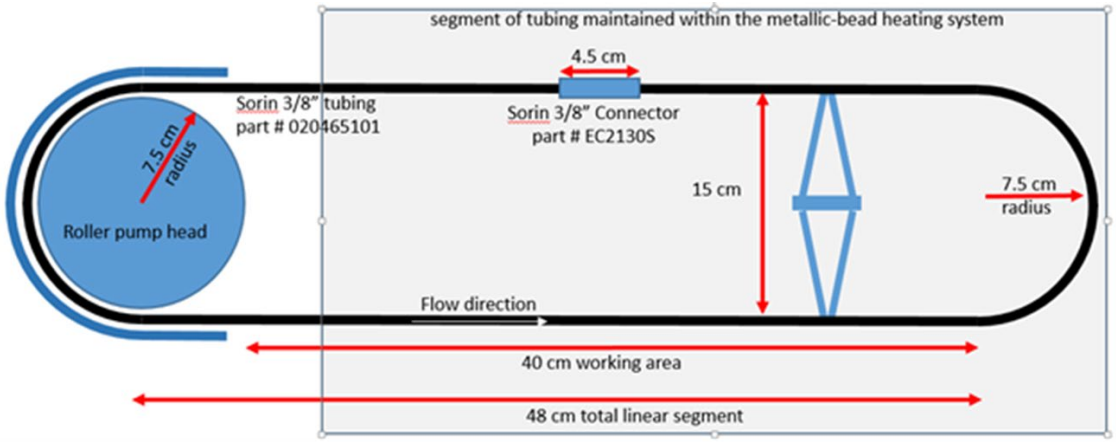


Is the NAVI model clinically relevant?

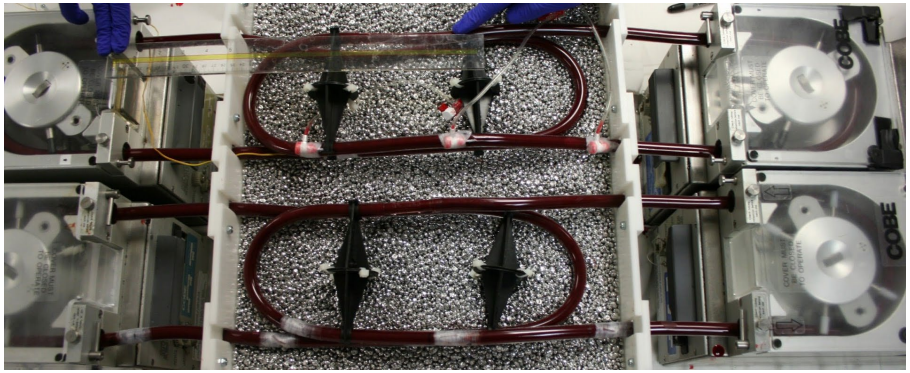
[1]Wolf, M. F., and Anderson, J. M., 2012, "Practical Approach to Blood Compatibility Assessments: General Considerations and Standards," Biocompatibility and Performance of Medical Devices, J.-P. Boutrand, ed., Woodhead Publishing, Oxford, UK.

IN VITRO MODEL – BLOOD LOOP

Loop Configuration



Temperature Control



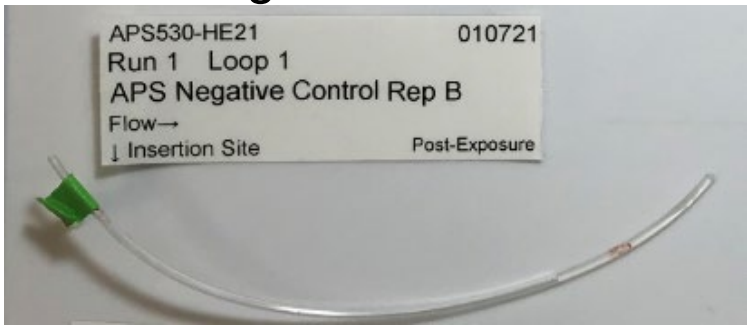
- Positive Control x 3
- LMCD Comparator Article (1-3)
- Test Article (1-3)
- Negative Control x 3

Positive Control: Latex Tubing (Manually Abraded)

Negative Control: PU Catheter (Hydromer Coated)

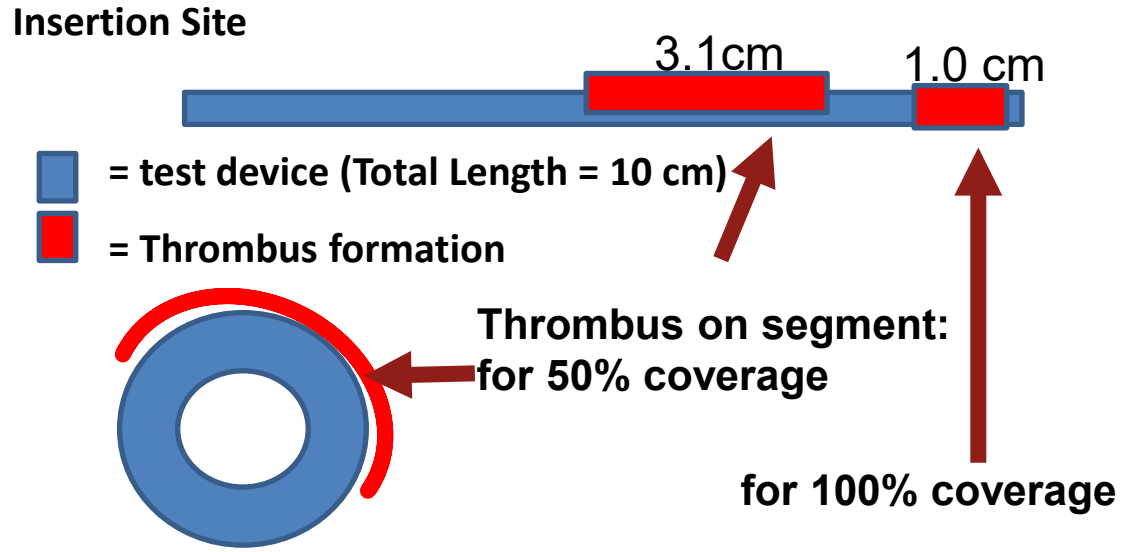
QUANTITATIVE THROMBUS EVALUATION

Negative Control



Positive Control

Cross section View - Example for Calculating Coverage



$$\frac{(3.1cm\ x\ 50\%)+(1cm\ x\ 100\%)}{10cm} = 26\%\ coverage$$

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 VIVO – IN VITRO COMPARISON

	NAVI (in vivo)	Blood Loop (in vitro)
Implant position and technique	• Animal anatomy variation • Experience based implant technique • Hard to check device deployment	• Uniform tubing diameter • Controlled deployment procedure
Device-vessel wall contact	• Tissue Factor	• Minimal tubing wall contacting • No Tissue Factor components
Statistical power	• N=2-3	• N= 3 – 9 • Three donors
Controls	• LMCD	• LMCD • Positive Control • Negative Control
Risk for thromboembolism	• Downstream Organs (Heart and Lung) • Unable to distinguish between test and LMCD	• Non-adherent thrombus quantification • Separate loop to distinguish between test and LMCD

IN VITRO BLOOD LOOP - PUBLICATIONS

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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 5 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 materials' 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.

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

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Background

Thrombogenicity testing continues to be a critical requirement for regulatory approval of blood-contacting medical devices and the ISO guidelines have recently been updates [1]. This new guideline ascribes value to both in vivo and in vitro testing including both the non-anticoagulated venous implant (NAVI) model, and the new methods for in vitro testing. One challenge with the animal-blood-based in vitro assays that have been validated and used for submissions is that they still may not accurately translate to clinical safety or predict the risk for thrombogenic potential in humans. We have previously described a model using minimally heparinized ovine blood and are continuing to improve the overall methodology [2,3]. In addition, we have transferred these methods to a human blood assay which therefore has enhanced potential for prediction of clinical risk. As with the ovine model, the key characteristics of a successful in vitro method include fresh blood, low levels of anticoagulation, flow conditions and minimization of air/blood interfaces. This human model integrates freshly harvested human blood containing minimal levels of heparin with variable flow from a unidirectional peristaltic pump and unlike many of the human blood assays, it can accommodate larger devices and higher flow rates than previously described [1,4]. Control materials which were optimized in the ovine model were also used to reproducibly elicit positive and negative thrombogenic responses. We feel that this model can be used for validation of the ovine model with cross comparisons of a number of legally marketed comparator devices. Alternatively, if the human blood methodology can be streamlined and performed cost effectively on a regular and basis, this assay could supplant the current ovine model and allow a highly predictive preclinical test for thrombogenicity of devices.

Methods

Blood Collection: Fresh human blood was collected from healthy donors (in collaboration with a custom draw and anticoagulation protocol at Memorial Blood Center, St. Paul, MN). Heparin was added to a final concentration ranging from 0.7 to 1.00 IU/ml. Donors were not exposed to any anticoagulant drugs within 7 days of blood draw. Blood collection bags (Baxter, IntraVia Container, Chicago, IL) were pre-loaded with porcine sodium heparin and the collection bag was gently mixed continuously from the start of the blood draw until blood was loaded into the loop. Only heparinized blood having Activated Clotting Time (ACT) <230 seconds was used.

Positive and negative control materials: The control articles consisted of ~15 cm lengths of 5 French medical grade intravascular polyurethane tubing, Heparin CBAS® Coated Polyurethane Catheter for the negative control and uncoated abraded polyurethane tubing for the positive, both obtained from Solomon Scientific, Norfolk, UK [2]. A new positive control material was also utilized, a latex tubing Super-Soft Latex Rubber Semi-Clear Tubing, abraded (OD: 1/8"; ID: 1/16" Wall Thickness: 1/32", McMaster-Carr, Elmhurst, IL). Each control was inserted into the loop; the external 1-2 cm of tubing was folded to prevent retrograde blood flow and the access site sealed with parafilm.

Loop preparation: As in the ovine blood method [2], the loop was constructed from blood-compatible tubing (3/8" inside diameter) and connectors from Sorin Group (Milan, Italy). For each unit of blood obtained (~450 ml), four separate blood loops were prepared. The blood was introduced into each loop (total volume ~100 ml/loop) no more than three hours after the completion of the blood draw. Air was removed from the loop which was then sealed to maintain a closed system. The blood was maintained at 34-37 °C throughout the duration of the assay using a customized heating table.

Thrombus Evaluation: After 4 hours ± 30 min of exposure, each individual loop section containing the test or control material was opened, photographed in situ, then rinsed with saline and evaluated for the presence of thrombus. The thrombus evaluation was performed in the spirit of the new ISO 10993-4 standards for in vivo thrombogenicity testing. The scoring yielded a thrombus formation as % surface area based on an assumption of uniform diameter for the length of the device (Figure1).

Results

Blood Collection: In all cases (13 replicate runs with differing heparin concentrations), blood was obtained from

3R's AWARD FOR *IN VITRO* ALTERNATIVE ASSAY

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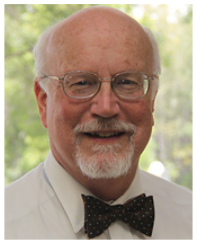
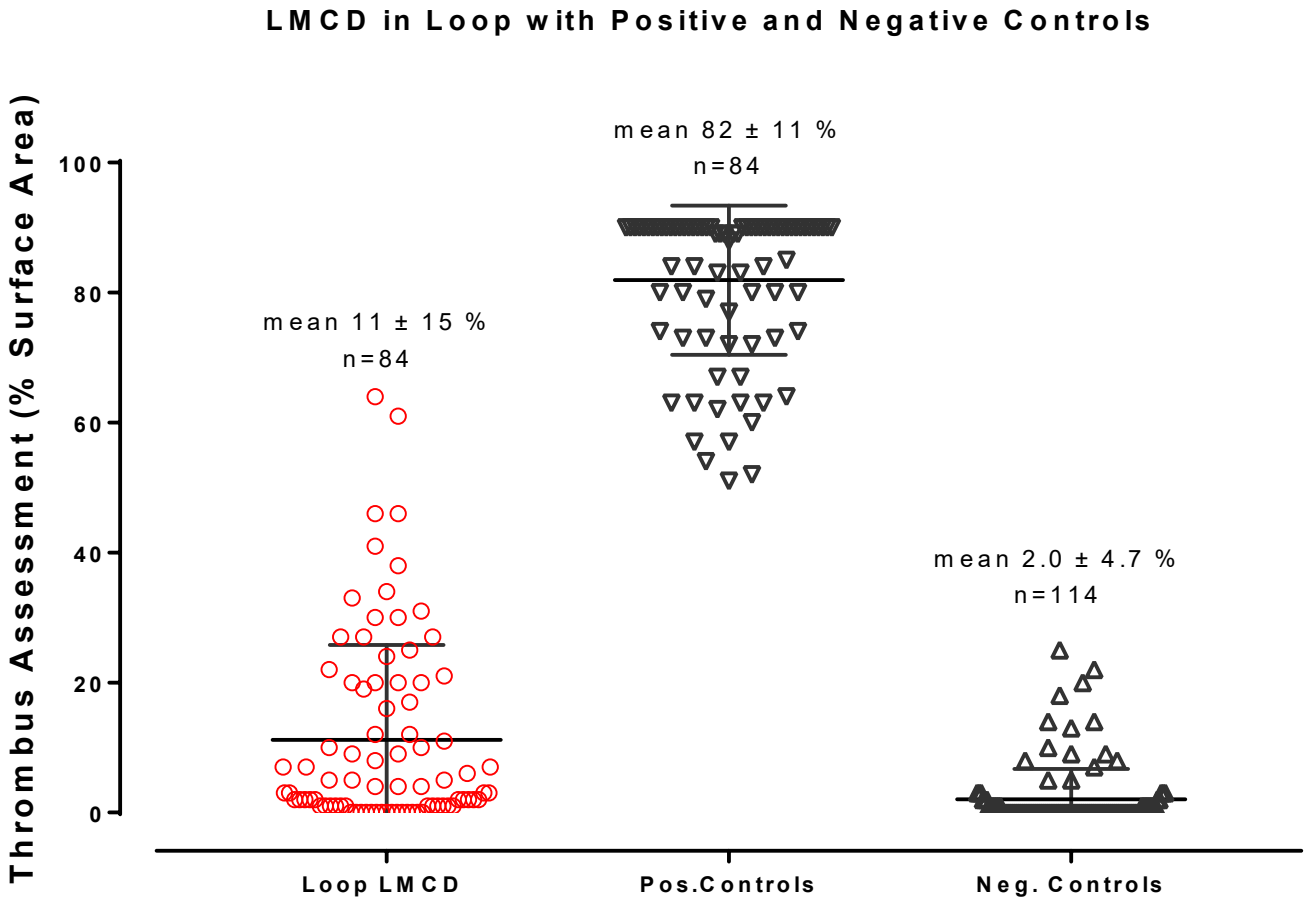
Dr. Mark E. Smith

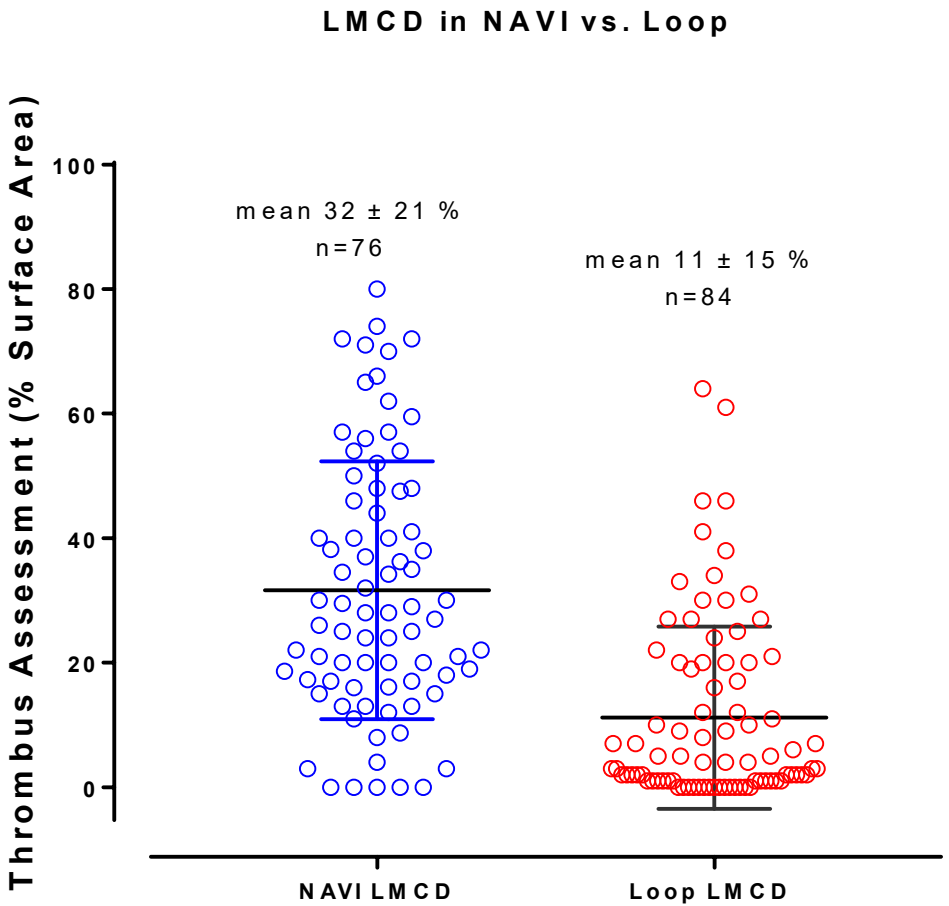


Dr. Mark E. Smith is Chief Scientific Officer at American Preclinical Services (APS) in Minneapolis, Minnesota. He is receiving a Global 3Rs Award for the article, "Thrombogenicity Testing of Medical Devices in a Minimally Heparinized Ovine Blood-Loop," *Journal of Medical Devices* (2017). This work 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.

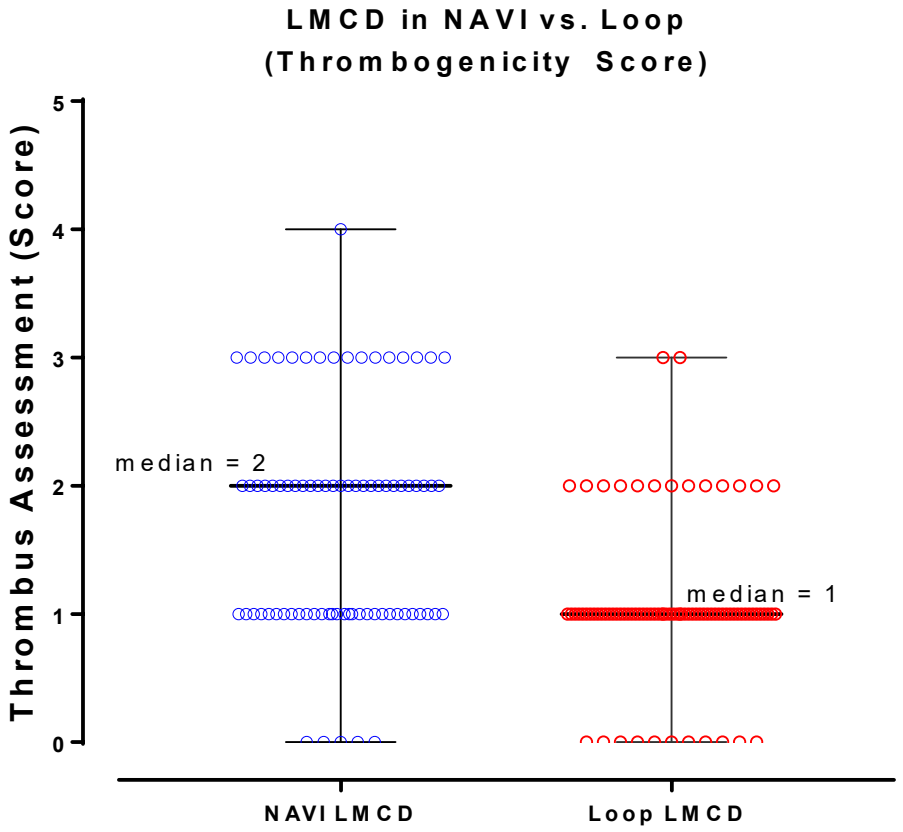
PERFORMANCE OF LMCD IN THE LOOP



RESULTS COMPARISON IN VIVO – IN VITRO



SCORE - PERCENTAGE SURFACE AREA



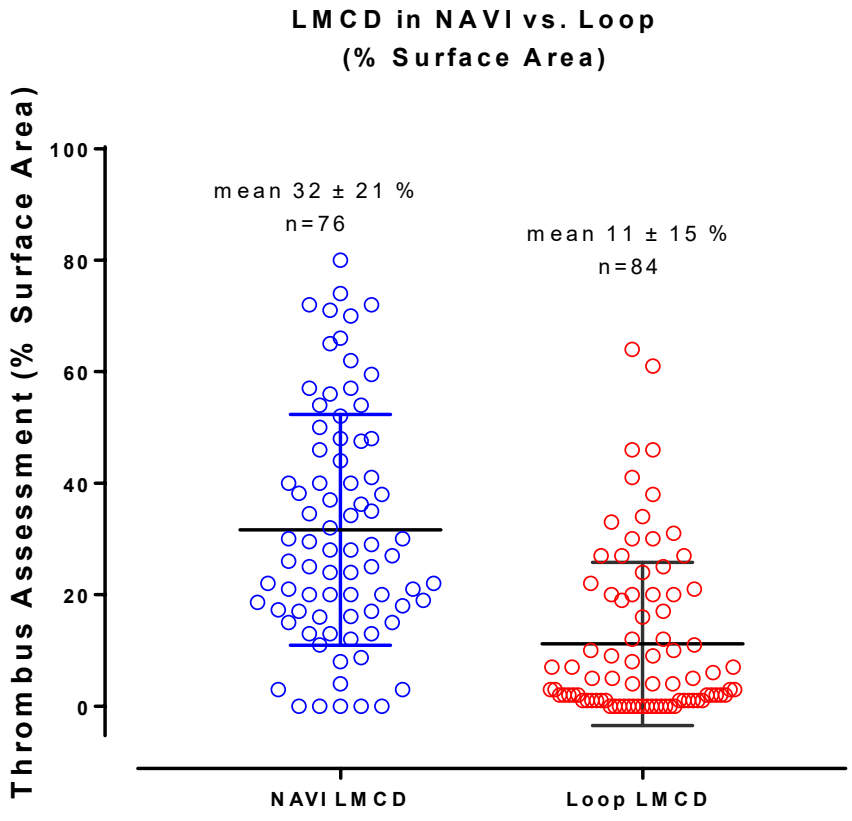


Table C.2 NAVI/AVI Scoring Scheme B (0-4)

CONCLUSIONS

- The high frequency of thrombus formation leading to scores of ≥3 (≥ 51% surface area overage) for LMCDs highlights the deficiency in the performance of NAVI assay.
- In comparison, the in vitro blood loop assay results where assay performance of LMCDs was much more in line with their clinical performance and regulatory history.
- In addition, the blood-loop assays also carries the enhanced support by concurrent use of well-behaved positive and negative controls to address the variability of the in vitro assay.
- Overall, these results strongly supported the inability of the NAVI model to predict clinical risk. Alternative assays need to be developed and the regulatory acceptance is desired.

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APS

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Sarah Howard
Heather Ackerson
Tammy Fossum

QUESTIONS

Thank you!

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