

Overview of *In Vitro* Thrombogenicity Testing—FDA/CDRH research update and regulatory considerations

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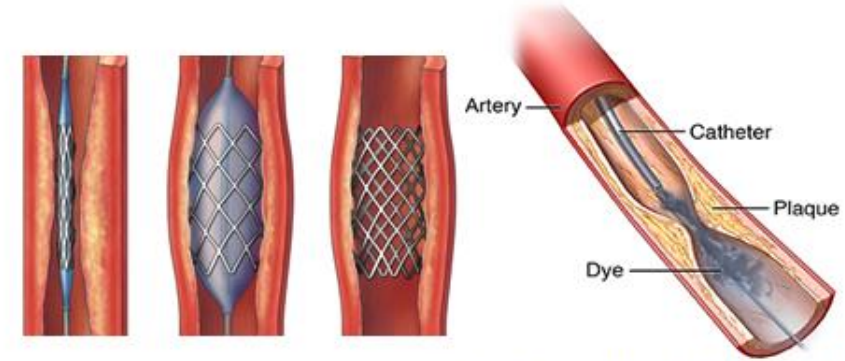
[SOT-MDCPSS Webinar, March 30, 2021](#)

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Thrombosis Associated with Medical Devices

- Blood-contacting devices are susceptible to thrombosis and thromboembolic events
 - **For both short-term and long-term use devices**
 - **Clinical consequences and prevalence vary among devices**
- More robust pre-clinical thrombogenicity testing could:
 - **Improve material selection and device design**
 - **Provide a more reliable prediction of device safety**

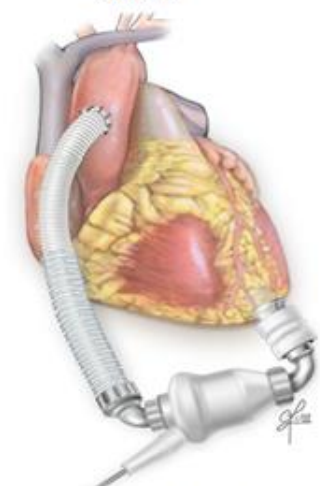


<http://centralgaheart.com/need-know-heart-stent/>

http://www.hopkinsmedicine.org/healthlibrary/test_procedures/cardiovascular/cardiac_catheterization_procedure_92,07964/



<https://www.cancer.gov/publications/dictionaries/cancer-terms?cdrid=45962>



<https://my.clevelandclinic.org/health/articles/lv-ad-devices>

Major Factors That Affect Device Thrombogenicity

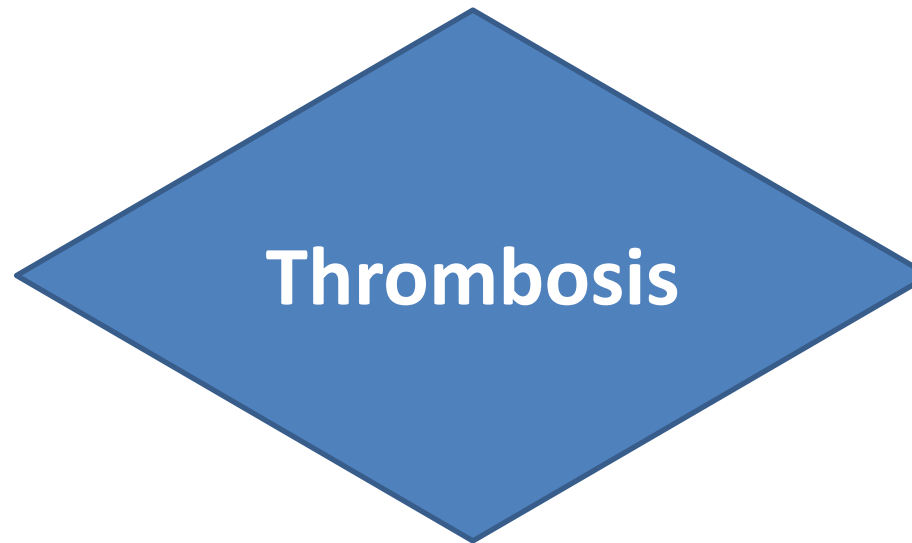


Blood Flow Through and Around the Device

(Geometry of the device, implantation site)

Device's Blood Contacting Surfaces

(Material compositions, surface
morphology)



Patient's Blood Coagulability

(Anticoagulation conditions)

Blood Contacting Duration

General FDA/CDRH Recommendations for Thrombogenicity Evaluation—See FDA’s Biocompatibility Guidance

- Tests may be waived based on appropriate risk assessment
 - e.g., compare materials, geometry, surface characteristics, and manufacturing processes of the new device to a legally US-marketed device
- *In vivo* testing in a suitable animal model
 - Preferably as part of a safety or functional animal study
 - May be recommended for long-term implants
- *In vitro* assessments (static/dynamic)
 - Panel of assays recommended to evaluate different thrombosis contributing factors

<https://www.fda.gov/media/85865/download>; Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process" - Guidance for Industry and Food and Drug Administration Staff (fda.gov);

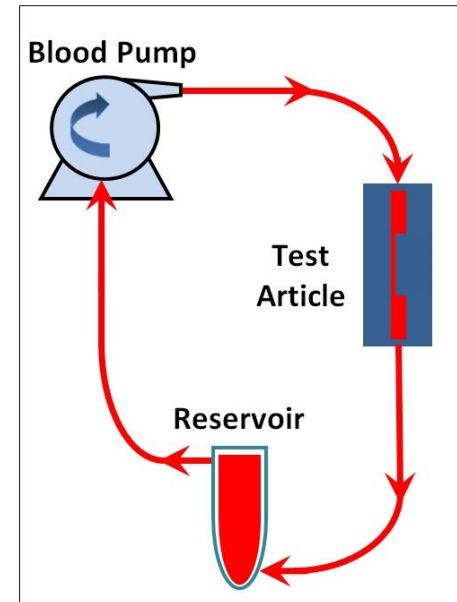
Thrombogenicity Research at the FDA/OSEL Blood Damage Lab

Overall Goal: Develop, qualify, and standardize a panel of *in vitro* methods to enhance thrombogenicity testing of medical devices and biomaterials

(1) Static Testing

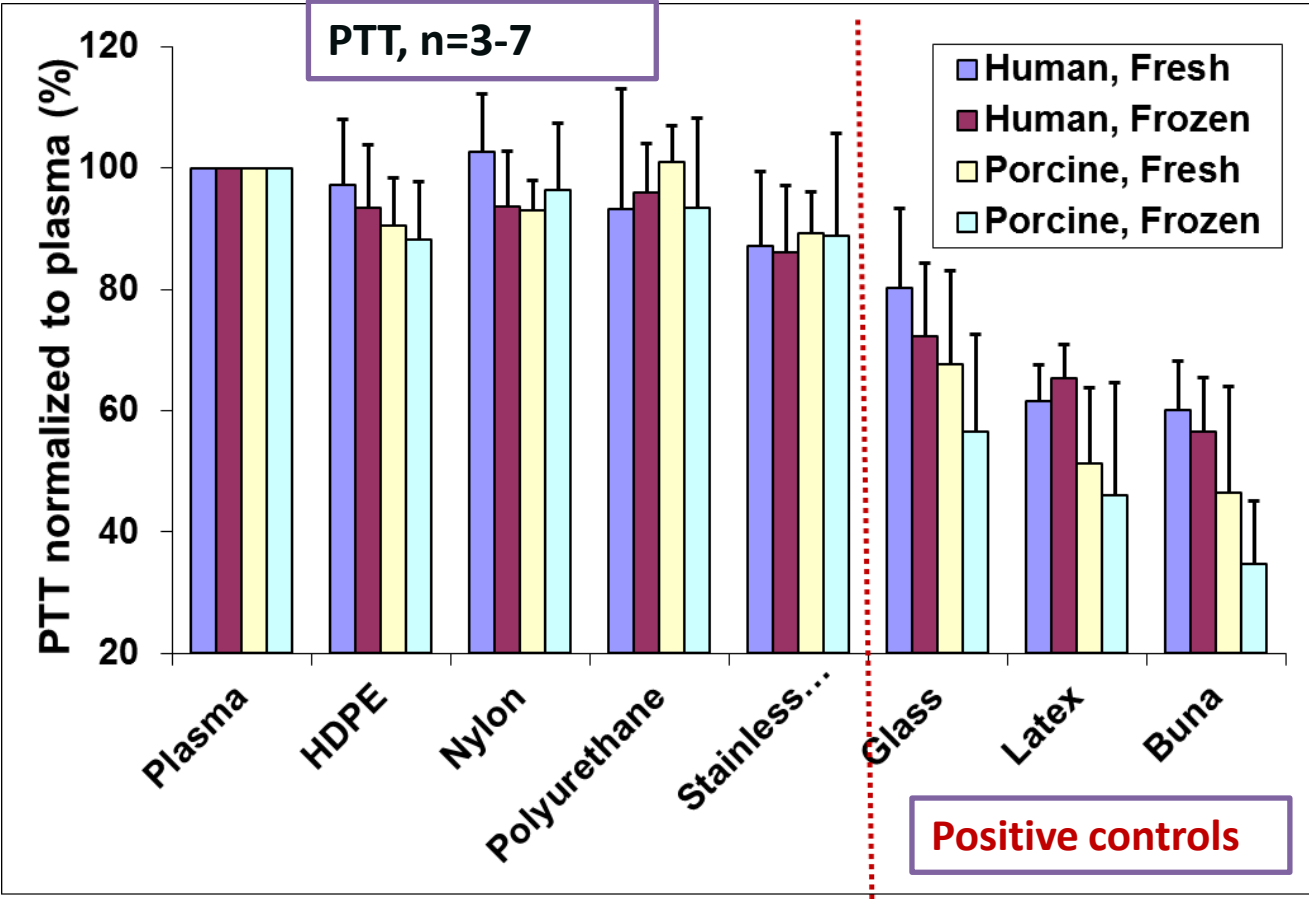
- Coagulation tests
-e.g. PTT, TAT
- Platelet tests
- e.g. platelet counts,
platelet activation markers

(2) Dynamic Testing



Blood flow loop

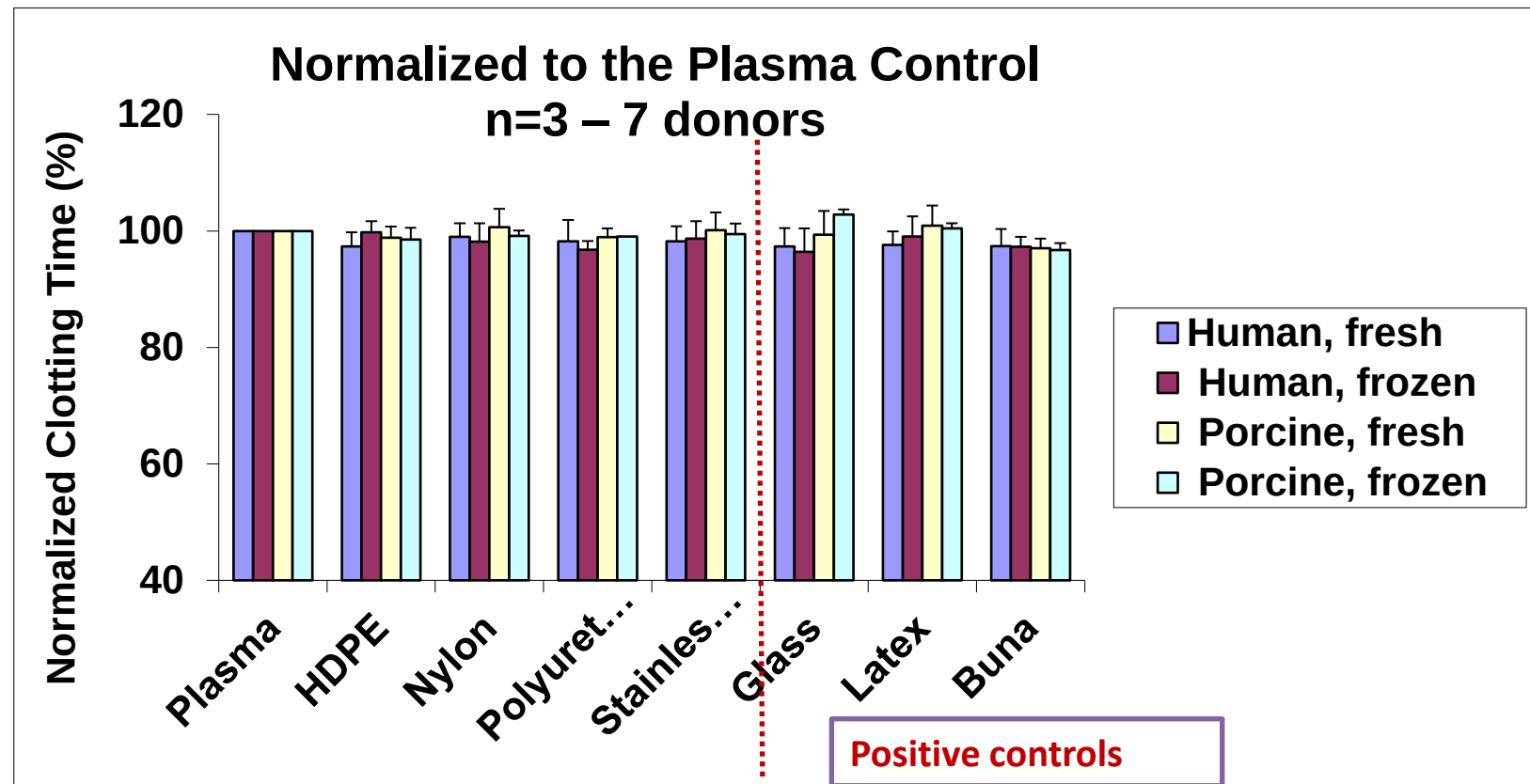
Partial Thromboplastin Time (PTT) Assay



- PTT test has the potential to differentiate thrombogenic positive control materials from commonly used biomaterials
- %PTT (relative to the plasma control) could sometimes be >50% for positive controls using human plasma

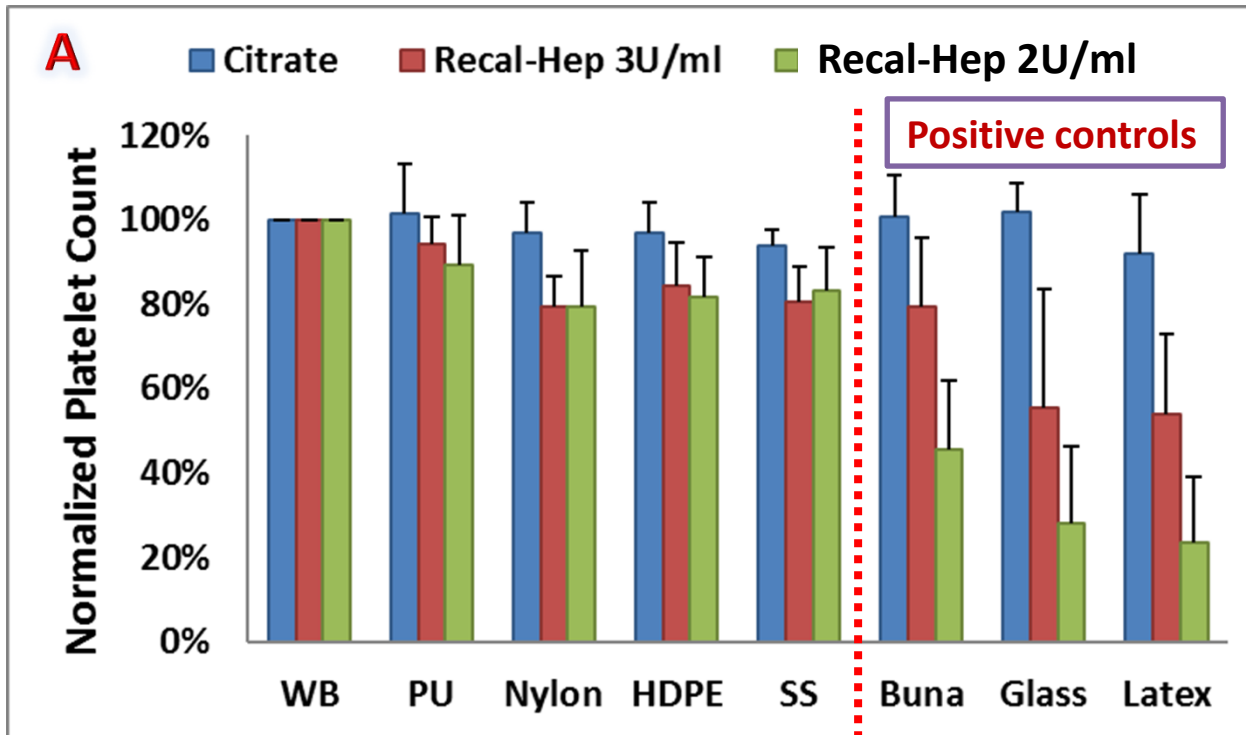
ASTM F2382-04 for PTT assay was revised in 2018 and subsequently fully recognized by FDA

Prothrombin Time (PT) Assay

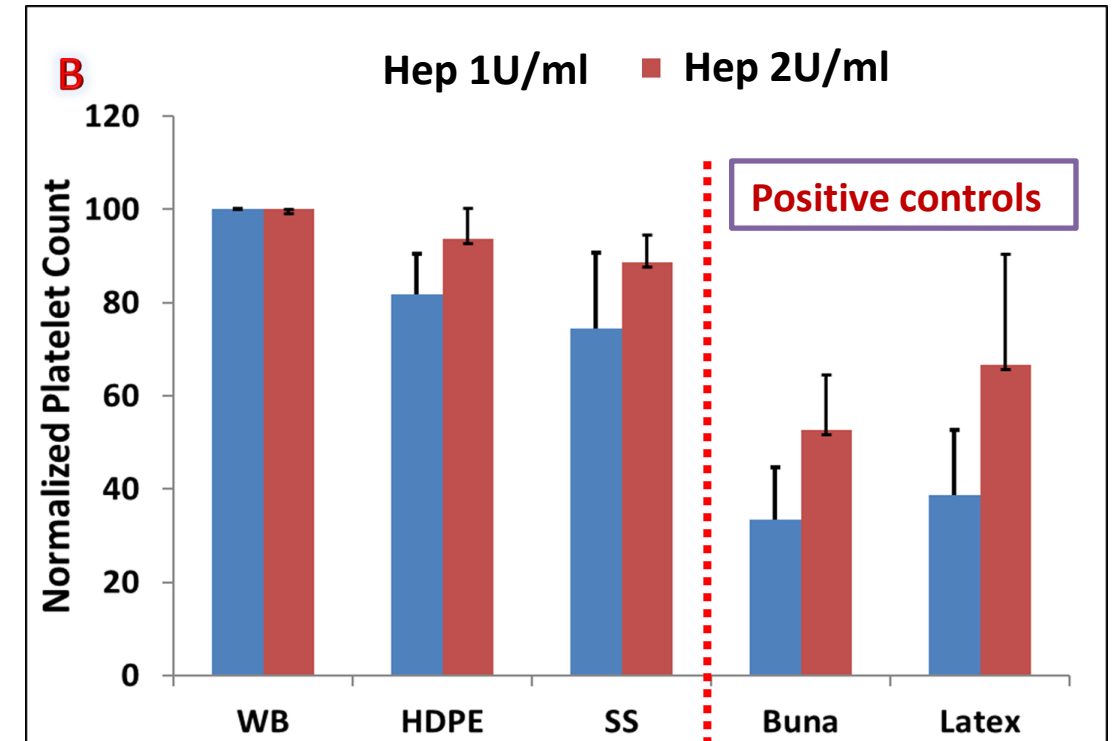


- PT assay could not differentiate the positive controls from the other materials; no longer recommended as one of the commonly used test methods in ISO 10993-4 (2017).

Platelet and Leukocyte Count Assay



A) Testing with sodium citrate blood with or without recalcification and heparinization



B) Testing with directly heparinized blood

- The use of sodium citrate anticoagulation as stipulated in the ASTM F2888-13 standard could not differentiate materials with different thrombogenic potentials
- The revised ASTM F2888-19 recommends heparinization at 2 U/ml for recalcified blood and 1 U/ml for direct heparinization

When is Material-Mediated Thrombogenicity Testing Most Likely to be Appropriate?



- For blood-contacting material changes that do not affect the geometry of the device
 - change in material (e.g., base material, supplier, formulation, coating), manufacturing, or sterilization processes
 - Excludes novel materials (May need additional tests)
 - The material change should not dramatically affect the mechanical properties of the device (e.g., stiff stainless steel to a soft silicone)
- As a supplement to an in vivo animal study or other dynamic thrombogenicity assessments, if the results are ambiguous
- Other situations based on case-by-case thrombogenicity risk assessments
 - E.g., certain devices with simple geometries, short blood contacting durations (<15 min), and used with anticoagulation

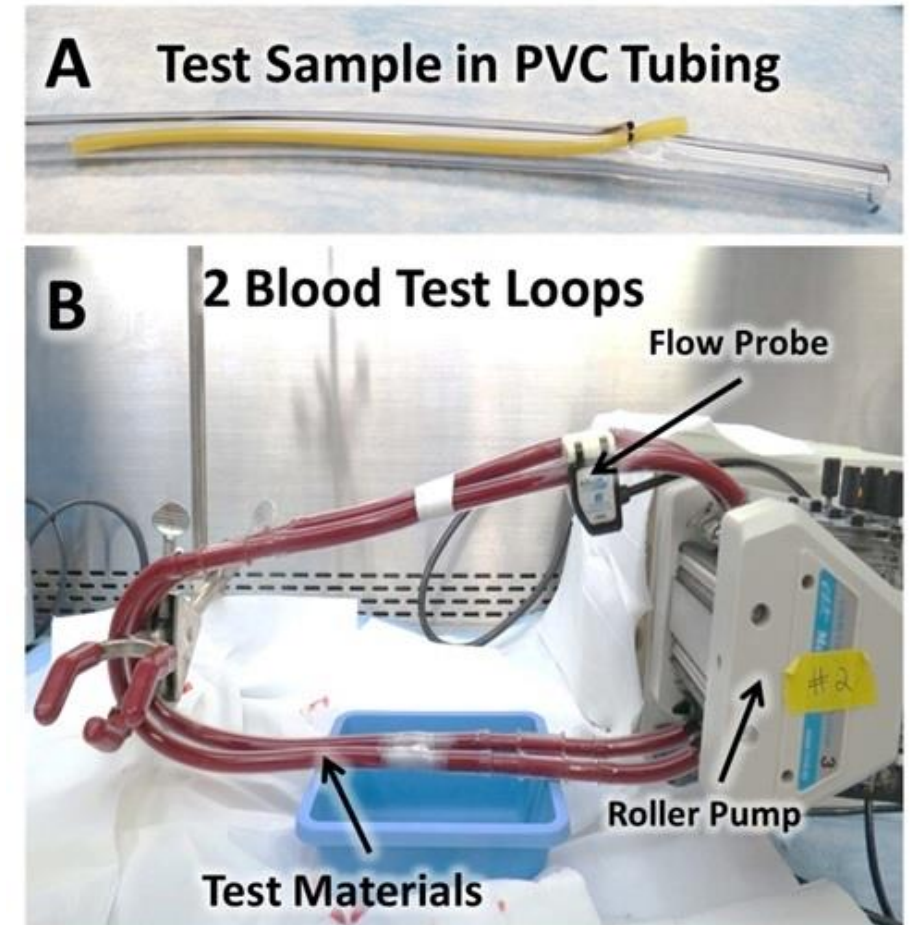
Typical Test Panel for Material-Mediated Thrombogenicity Evaluation



- An assessment of the coagulation system (e.g., Thrombin-Antithrombin Complex (TAT), Partial Thromboplastin Time (PTT, ASTM F2382-18))
- An Assessment of platelets (e.g., Platelet and Leukocyte count assay, ASTM F2888-19)
- Surface assessment
 - e.g., 40X optical microscopy or 40X SEM images at representative locations (including junctions) are often recommended to demonstrate that the surfaces of the subject devices are comparable to a reference device
 - Do not contain features (e.g., defects, protruding fibers, rough surfaces) that could increase the risk of thrombosis.

In Vitro Dynamic Flow Loop Test System

- Aim to replace or reduce the use of an acute animal study — the traditional 4-hr venous implantation model
- Flow Loop
 - 6.4 mm ID PVC tubing
 - Test materials: OD 2.1-3.2 mm
- Blood anticoagulation: heparin added to recalcified citrate blood
- Blood circulated at 200 ml/min for 1 hr at room temperature
- Thrombogenicity markers measured
 - Thrombus surface coverage, thrombus weight, and platelet count reduction

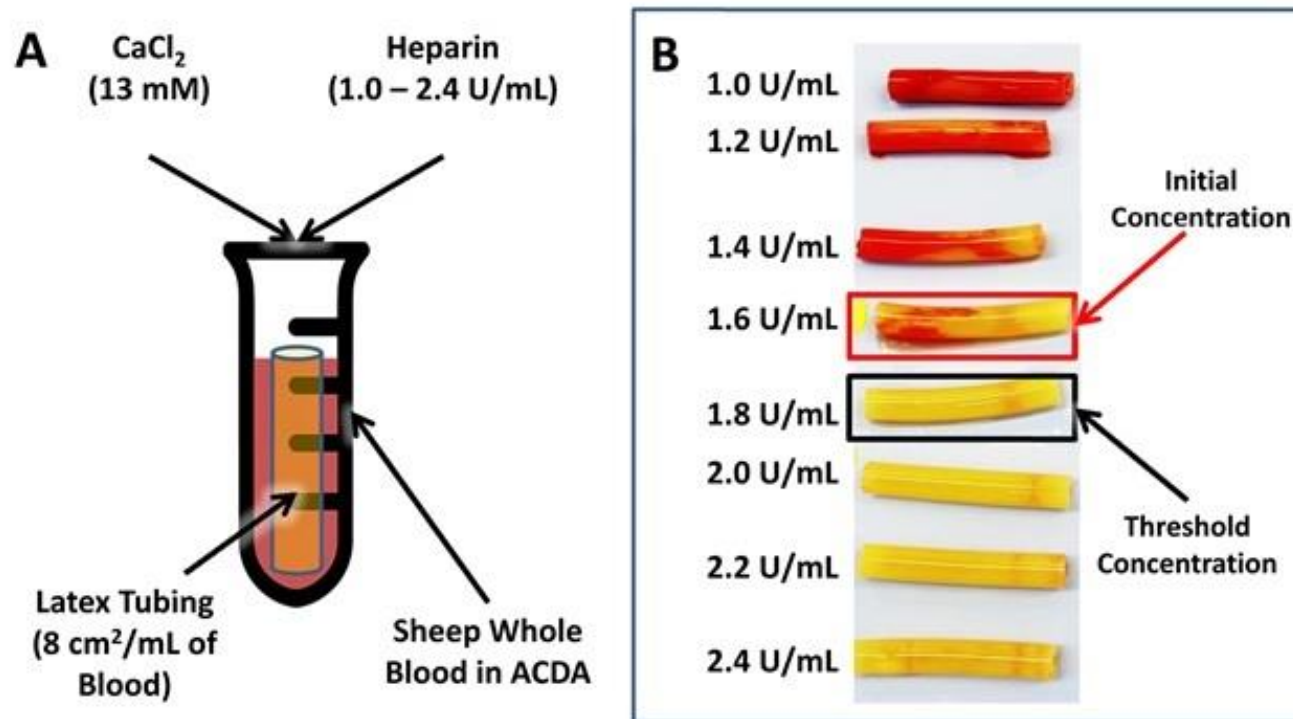


Donor-specific Heparin Concentration Determination



- Static pre-test with latex tube incubation in re-calcified blood with a series of heparin concentrations for 30 mins to estimate an initial concentration
- Final heparin concentration confirmed by pilot dynamic testing

Static Pre-test



Pilot dynamic flow loop Test

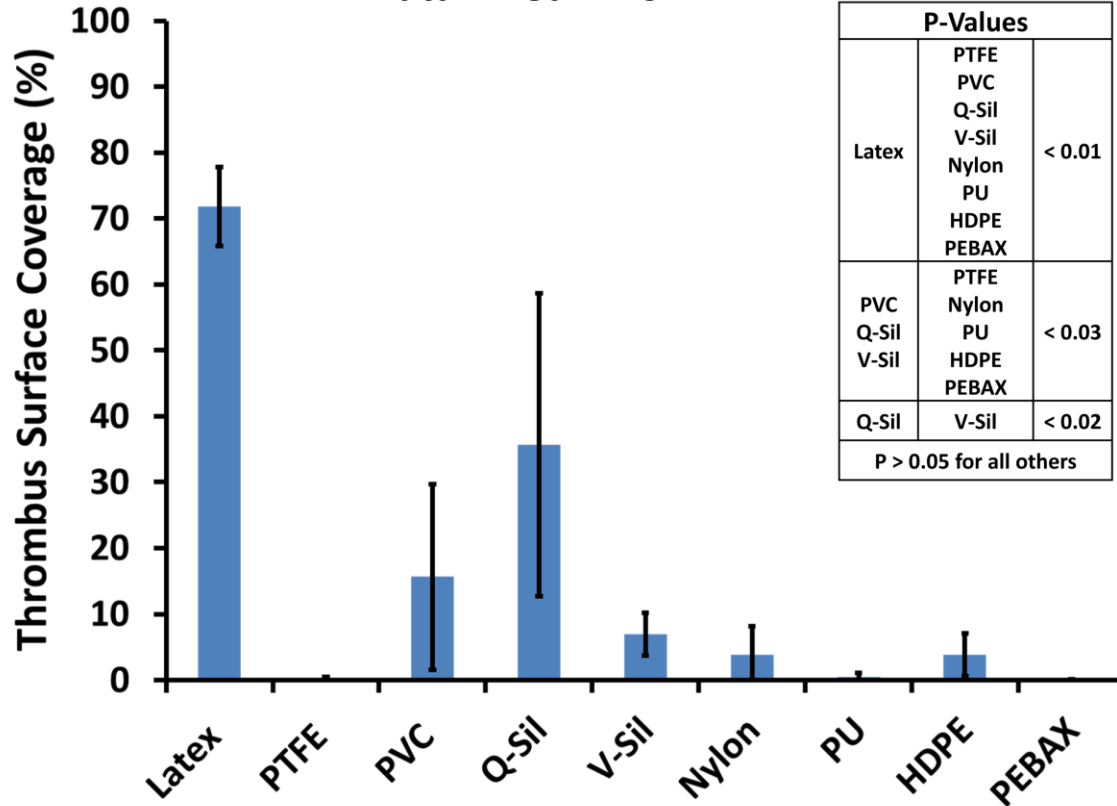


Heparin concentration confirmed if thrombus surface area is < 10% for PTFE and > 50% for latex

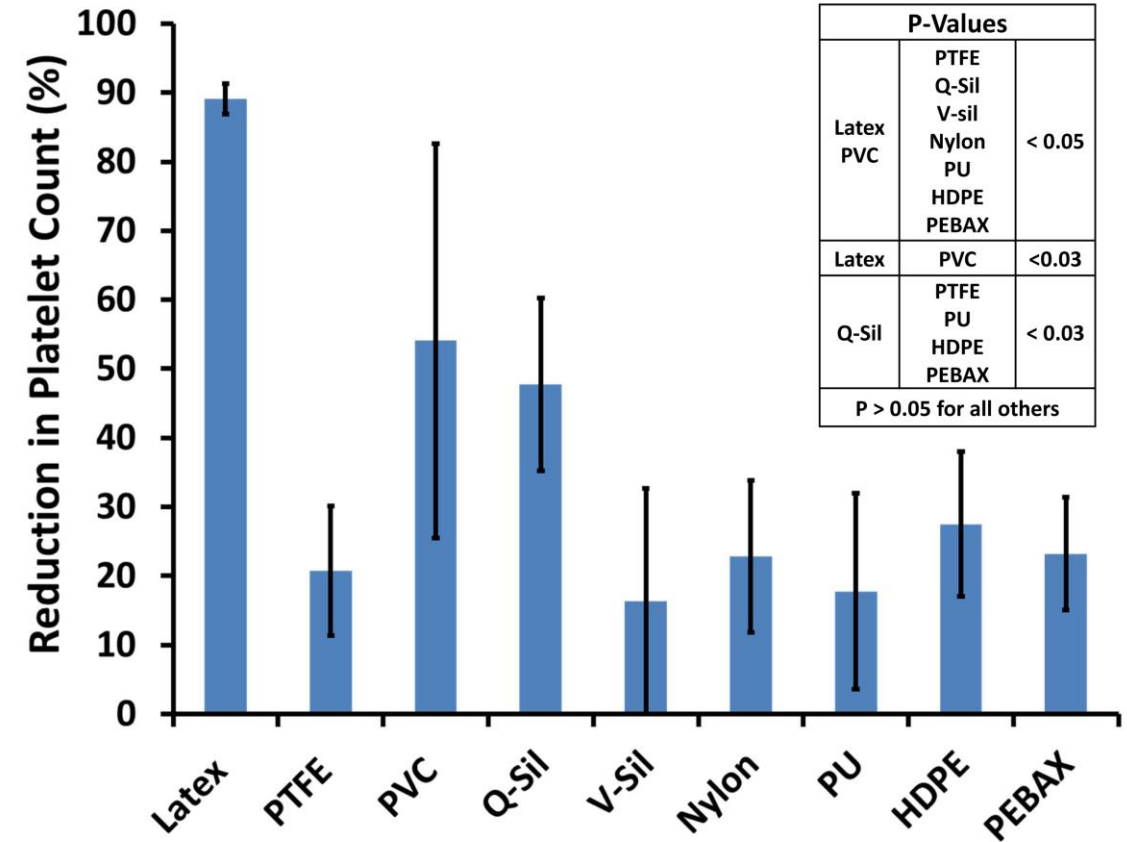
Ovine Blood Testing on Various Materials



N=6 sheep donors
Data: Mean $\pm$ SD



P-Values		
Latex	PTFE	< 0.01
	PVC	
	Q-Sil	
	V-Sil	
	Nylon	
	PU	
	HDPE	
PVC	PTFE	< 0.03
	Nylon	
	PU	
	HDPE	
	PEBAX	
Q-Sil	V-Sil	< 0.02
P > 0.05 for all others		



P-Values		
Latex PVC	PTFE Q-Sil V-sil Nylon PU HDPE PEBAX	< 0.05
Latex	PVC	<0.03
Q-Sil	PTFE PU HDPE PEBAX	< 0.03
P > 0.05 for all others		

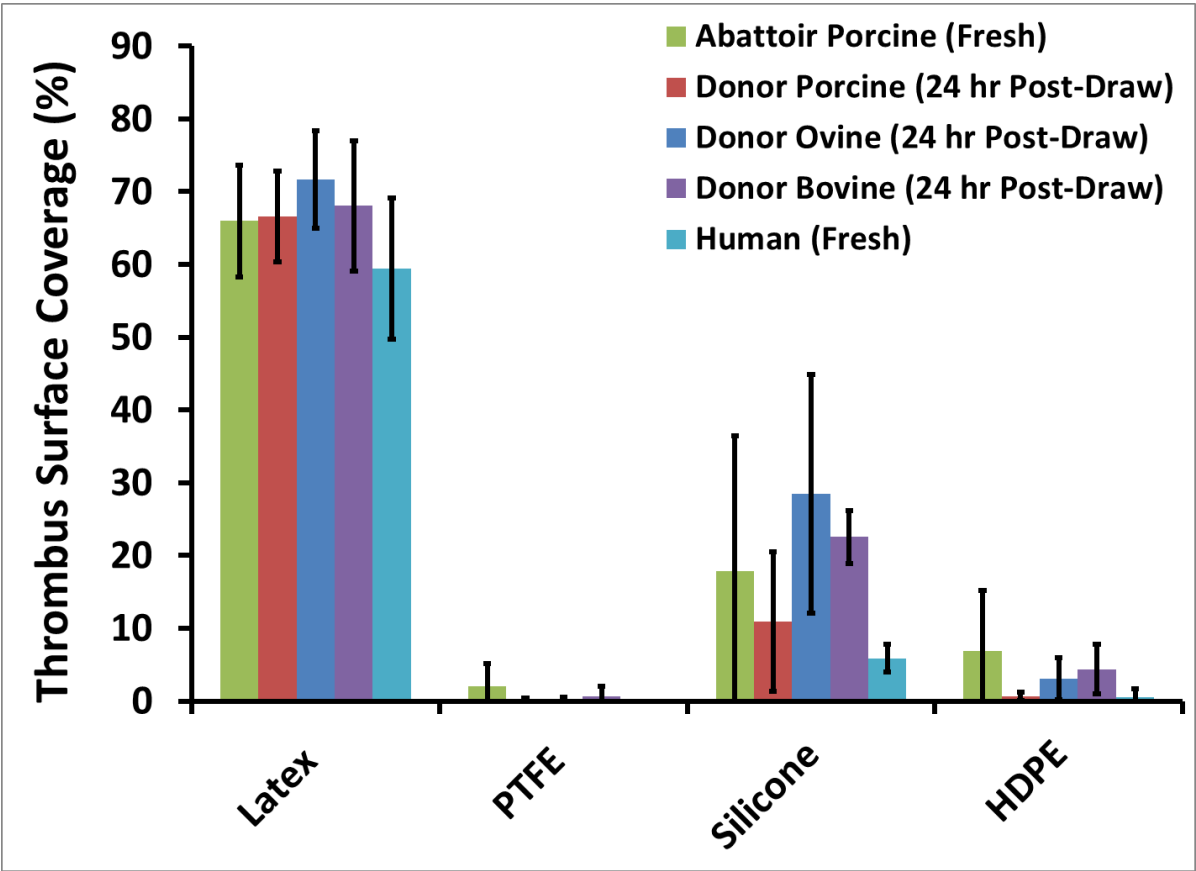
- The test system was effective in comparing relative thrombogenicity of different materials: latex > Q-Sil $\geq$ PVC > remaining biomaterials $\geq$ PTFE.

Jamiolkowski et al., ASAIO Journal 2020; 66:183–189.

Effects of Blood Species and Collection Methods



N=4 for human blood, n=5 for animal blood

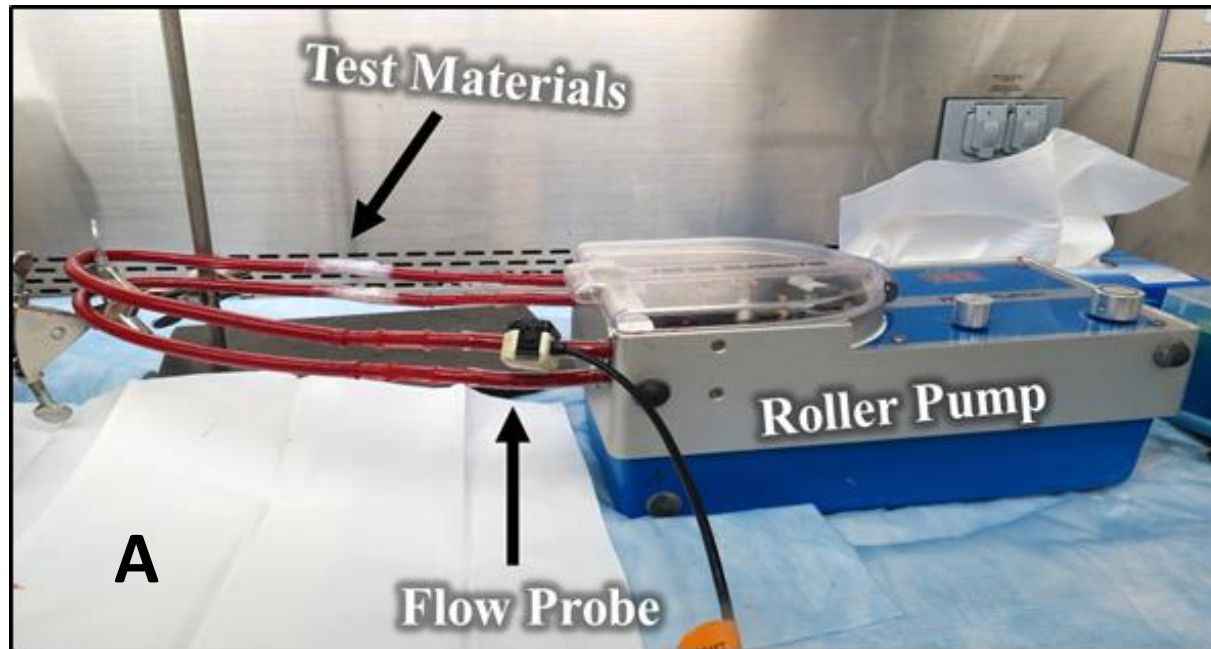


Blood Sources and Conditions

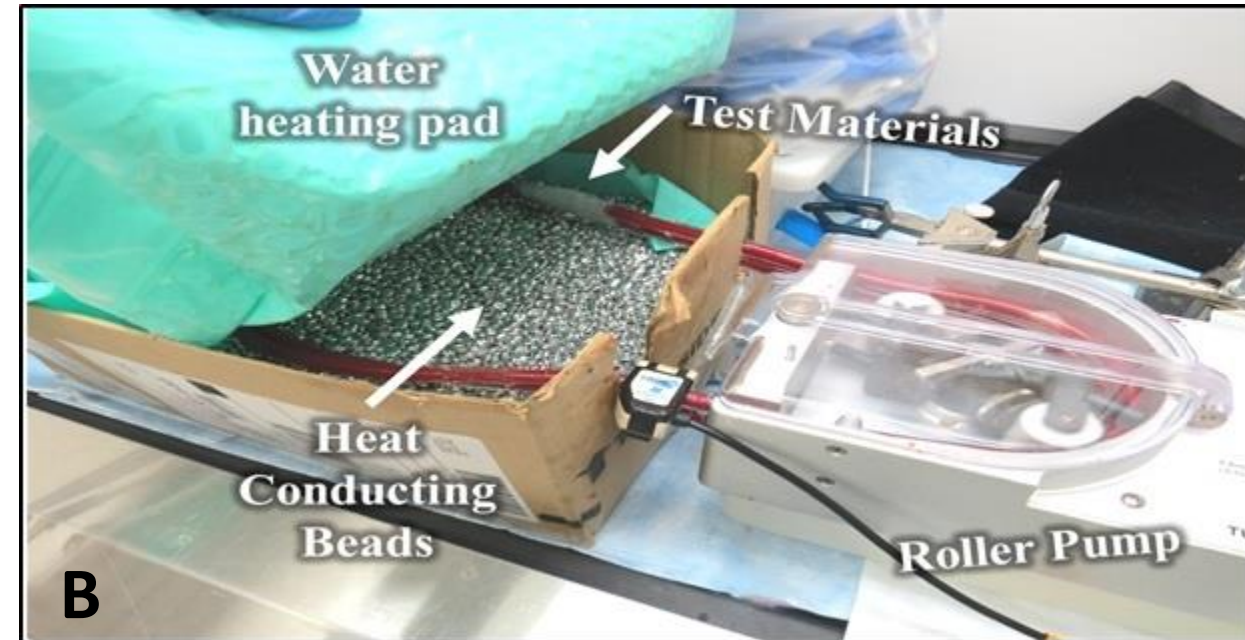
Blood source	Blood storage time before use (hr)	Heparin concentration for dynamic test (U/ml)
Human	2 - 6	1.8 - 2.8
Donor ovine	24 - 36	1.4 - 1.8
Donor bovine		0.6 - 1.4
Donor porcine		3.5 - 7.0
Abattoir porcine	2 - 12	3.0 - 4.5

- All tested blood sources allowed differentiation between thrombogenic and thrombo-resistant materials; slight differences in test sensitivity.
- Donor-specific anticoagulation levels were dependent on the animal blood source.

Flow Loop Set-up for Test Temperature Comparison



A) Set-up for room temperature test. Bovine blood circulated for 1 hour at 200 mL/min.



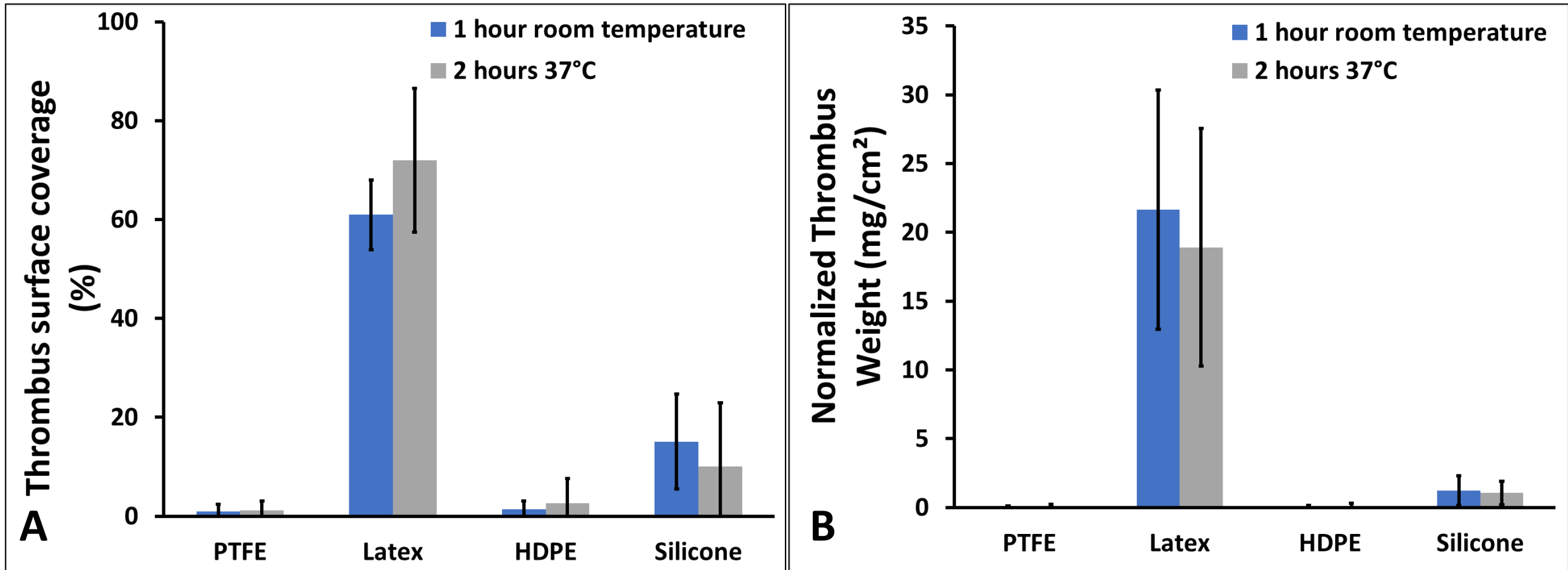
B) Set-up for 37°C test. Bovine blood circulated for 2 hours at 200 mL/min.

Note: Water circulation pump (not shown in the picture) is needed to warm up and maintain the temperature of the water heating pad.

Results of Test Temperature Comparison



Tested with donor bovine blood. N=6



- Relative material thrombogenicity was similar at both temperatures: Latex>Silicone >=HDPE>=PTFE
- At 37°C, the blood circulation time had to be increased from 1 to 2 hours and the heparin concentration decreased by approximately 0.2 U/ml to produce acceptable amounts of thrombus deposition on the positive and negative controls as compared to the room temperature.

Summary of Dynamic Flow Loop Test

- Using donor-specific anticoagulation, the in vitro flow system can effectively differentiate from thrombogenic and thromboresistant materials using various blood species
- Room temperature test with bovine blood seems to accelerate in vitro thrombus formation and allow for the comparison of different biomaterials in shorter durations compared to 37°C testing.
- Future work towards standardization:
 - Additional testing to verify temperature effects with other species
 - Evaluate the effects of device geometry
 - Evaluate different blood anticoagulation strategies
 - Develop more quantitative methods for determining donor specific anticoagulation
 - More importantly, interlaboratory study (round-robin) is needed

Call for Participants for Round Robin Testing



- FDA is looking for industry and academic labs to participate in a round-robin study on dynamic flow thrombogenicity testing to meet our goal to convert research results into regulatory tools for evaluating medical devices and materials
- Please contact Qijin.lu@fda.hhs.gov for more details if interested

Acknowledgment

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Thank You!

Questions and comments?

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