User Manual: Large Diameter Flow Loop for In Vitro Dynamic Thrombogenicity Testing

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User Manual for Large Diameter Flow Loop for In Vitro Dynamic Thrombogenicity Testing

This document provides a detailed test protocol to design a 9.5 mm inner diameter (ID) recirculating flow loop test system and perform bench-top thrombogenicity testing of catheter-like blood-contacting medical devices and materials. This methodology also includes a dynamic occlusion setting method to improve pump flow rate consistency. It was adapted from the test method of the research paper by Serna et al.[1] that described the study performed at FDA.

Protocol test

Note: T The specific instruments and suppliers listed are those used in the FDA verification study[1]. Identification of commercial products is for informational purposes only. Alternative suppliers can also be used in this test method if their use is appropriately validated. The mention of any commercial products and/or manufacturers does not imply endorsement by the FDA or the U.S. Department of Health and Human Services.

1. Test materials

1.1 Material for the static latex pre-test

1.1.1 Latex tubing (4.8 mm inner diameter, 8.0 mm outer diameter) for the static-pretest purchased from Fisher Scientific Inc. (catalog # S50616A).

1.2 Materials for dynamic flow loop testing

1.2.1 For this test methodology, the inclusion of a negative control (e.g., PTFE) and a positive control (e.g., latex) is required by the test design to demonstrate test validity and sensitivity[1]. The information for the negative and positive controls used in the verification study is shown in **Table 1** below.

Other materials can be used as negative and positive controls, given you have validation data to demonstrate that these controls exhibit consistent thrombogenic potentials when tested in this flow loop test system. In general, a positive control with a smaller or similar outer diameter to that of the test articles is recommended. For test articles having very small diameters (e.g., =<15% that of the flow loop tubing ID), using a positive control

with the same or smaller diameter as the test article can help verify the system's ability to differentiate thrombogenic from thromboresistant samples at that scale.

1.2.2 Since this test system relies on a comparative assessment, testing a comparison device (e.g., a marketed device with a documented history of clinical use) concurrently with the subject device can facilitate data interpretation.

1.2.3 Three additional test materials, each with an outer diameter (OD) of 3.2 mm, were investigated in the verification study: nitrile rubber (BUNA), silicone, and 3D-printed nylon (**Table 1**). As the flow loop test conditions (e.g., blood sources, equipment, chemical reagents) may vary between different laboratories, it is important to test these additional materials together with the negative and positive controls to verify that your flow loop can differentiate test samples with different thrombogenic potentials (low, intermediate, and high). However, for routine testing after the test system is validated, only the negative and positive controls (Section 1.2.1), and a marketed comparison device (Section 1.2.2) are necessary.

Table 1. Materials tested in the 9.5 mm inner diameter in vitro flow loop verification study.

Test material	Dimensions		Supplier
	Outer diameter (OD)	Inner diameter (ID)	
polytetrafluoroethylene (PTFE) cord (**negative control**)	3.2 mm	N/A	McMaster-Carr (Catalog# 84935K64)
latex tubing (**positive control**)	3.2 mm	1.6 mm	McMaster-Carr (Catalog# 5234K93)
BUNA-N Round Cord	3.2 mm	N/A	W.W. Grainger, Inc. (Catalog# 6VFH7)
silicone tubing	3.2 mm	1.6 mm	Qosina (Catalog# T2009)
PA 2200 nylon 12, White	3.2 mm	N/A	EOS (Article Number: 9012-0014)

2. Equipment

2.1 Hematology analyzer (e.g., Model # Hemavet 950 FS, Drew Scientific Inc., Miami Lakes, FL; Model #ADVIA 2120i, Siemens Healthineers, Erlangen, Germany; VETSCAN® HM5, Zoetis, Parsipanny, NJ) is used to measure complete blood cell count in the blood samples.

2.2 Shaking Water Bath (e.g., Precision Reciprocal Shaking Water Bath, Thermo Fisher Scientific, Waltham, MA) is used to incubate latex samples at 37°C for the static pretest to estimate donor-specific heparin concentration.

2.3 Laser tachometer (e.g., Model # PLT200 by Monarch Instrumentation Corp., purchased from W.W. Grainger, Inc, Catalog # 4YE86) is used for measuring actual RPM of the roller pumps.

2.4 Ultrasonic flow sensor (e.g., Catalog # ME 9PXL or 9HXL, Transonic Systems Inc.) is used to measure blood flow rate inside the flow loops.

2.5 Clinical roller pumps are used to drive blood through the flow loops. Information about the clinical roller pumps (from various manufacturers) used in the verification study is listed below in **Table 2**.

Table 2. Roller pumps from different manufactures were used in the verification study.

	Make	Model/Catalog #
Pump 1	COBE (Cardiovasc Inc., Arvada, CO)	043600-000
Pump 2	BAXTER (Deerfield, IL)	043605-000
Pump 3	STOCKERT (Sorin Group USA, Arvada, CO)	N/A
Pump 4	LivaNova (London, United Kingdom)	S3

3. 9.5 mm (3/8”) ID Tubing Flow Loop System setup

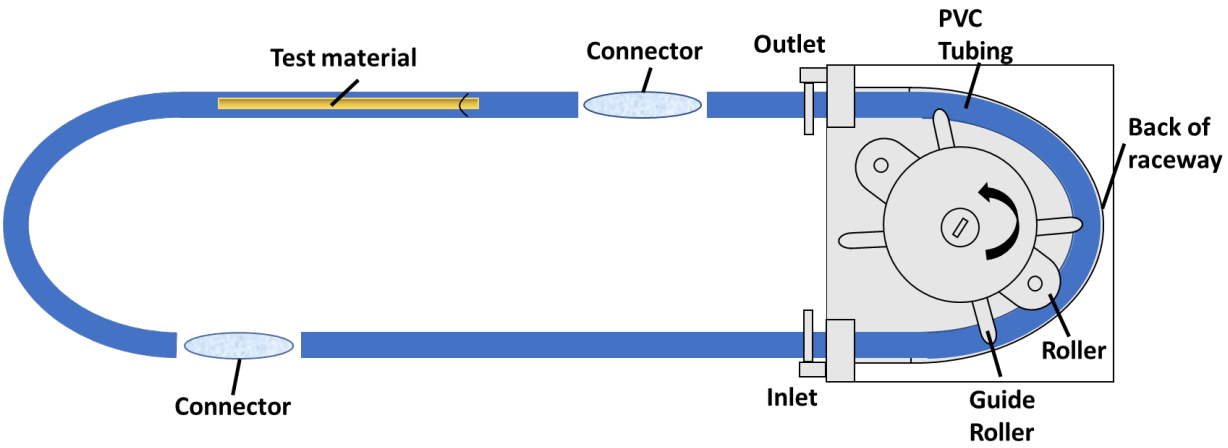

Figure 1: Overview of the flow loop assembly. The dynamic flow loop consisted of a reusable segment of polyvinyl chloride (PVC) tubing (right), which is used for all the tests conducted with the same donor blood on each test day, inserted into a roller pump along the entire length of the raceway and connected to a test material segment (upper left) via straight connectors. One test sample is used in each blood flow loop.

3.1 Reusable Loop Tubing Segment Assembly and Preparation

3.1.1 Cut an 80 cm length of 9.5 mm (3/8") ID polyvinyl chloride (PVC) tubing (reusable segment).

3.1.2 Measure 18, 32, and 72 cm from one end of the tubing and mark these positions (**Figure 2**).

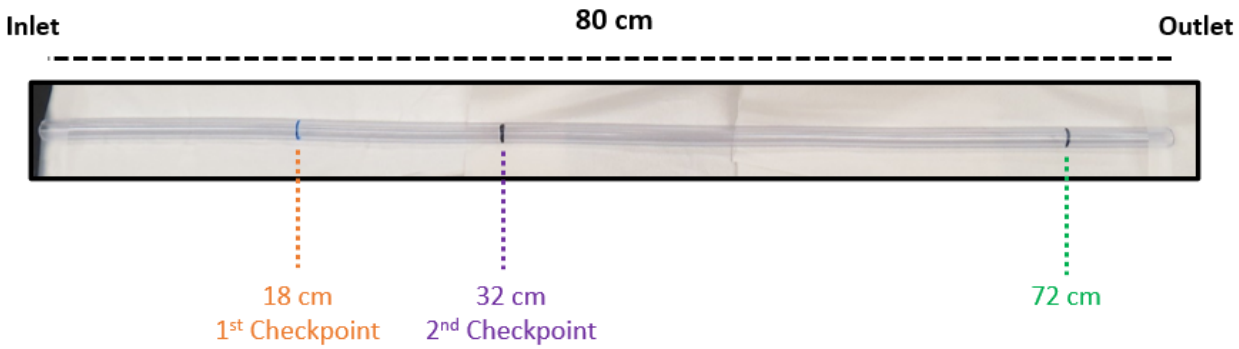

Figure 2: An 80 cm reusable tubing section showing 18 cm mark in orange (1st checkpoint for occlusion setting), 32 cm mark in purple (2nd checkpoint for occlusion setting, inlet gate clamp position), and 72 cm mark in green denoting the outlet gate clamp position.

3.1.3 Insert the tubing into the pump by gently rotating the rollers, ensuring that all tubing is in contact with the raceway walls (Figure 3)

3.1.4 Position the tubing within the pump so that the 32 cm mark is located at the inlet gate and the 72 cm mark is located at the outlet gate (Figure 3)

3.1.5 Make sure these two marks are parallel when clamped down by the inlet/outlet gates

3.1.6 Once the appropriate position has been established, close the gates, and tighten the clamps by rotating the knops on the top of the gates to secure the tubing in place (**Figure 3**)

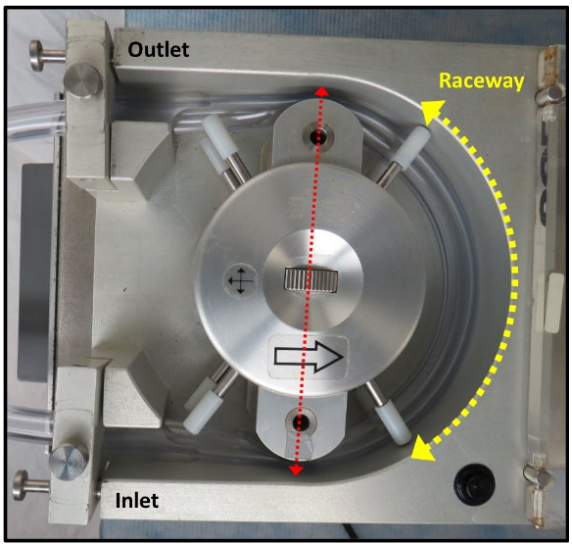

Figure 3: Reference roller position (red) used while placing tubing to make contact along the entire length of the raceway (yellow).

3.1.7 Insert a 9.5 mm (3/8”) OD straight polycarbonate (PC) connector into outlet of the tubing

3.1.8 Set the occlusion pressure using the method described in Section 3.2 below**

3.1.9 Fill the tubing by aspirating 1X phosphate buffered saline (PBS) through the loop at 20 rpm using the roller pump

3.1.10 Stop the pump and raise the PVC tubing segment to make the tubing ends pointing upward. Remove as much air from the loop as possible by tapping the tubing and using a transfer pipet to remove air and replace fluid. Close the loop by connecting the inlet to the outlet via the 9.5 mm (3/8”) OD straight PC connector

3.1.11 To maintain consistent flow rates within the loops throughout the experiments, the reusable segment is preconditioned by circulating phosphate buffered saline (PBS) for 1 hour at 50 rpm prior to the start of each day’s study

3.1.12 After the tubing has been preconditioned, reset the occlusion pressure using the method described in Section 3.2 below**

3.2 Setting occlusion on roller pumps

**(Roller occlusion needs to be set twice: once before tubing preconditioning (Section 3.1.11) and another time after the preconditioning but before filling the loops with blood). If the reusable tubing section needs to be removed for cleaning purposes, the roller occlusion also needs to be reset prior to the start of the next test. **

3.2.1 Adjust the pump speed to 20 RPM and verify the speed with a tachometer (20 ± 0.2 RPM). To reduce measurement background noise, maintain a consistent distance (e.g., 30 cm) between the laser tachometer and the pump throughout the study. Place a container at the base of the pump directly under the inlet gate as shown in **Figure 5**.

3.2.2 Add appropriate amount (e.g., 750 ml in a 1000 ml beaker) of PBS to the container so that the depth of the PBS is 10.3 cm from the bottom of the container to the meniscus.

3.2.3 Turn on the pump to allow the rollers to begin rotating

3.2.4 When a roller passes by designated raceway location (**Figure 4**, left panel), immediately submerge the tubing inlet into the PBS container so that the tip of the tubing is near (1-2 cm) the bottom of the container.

3.2.5 Make note of the aspirated PBS level reached after the roller turns ½ of a rotation.

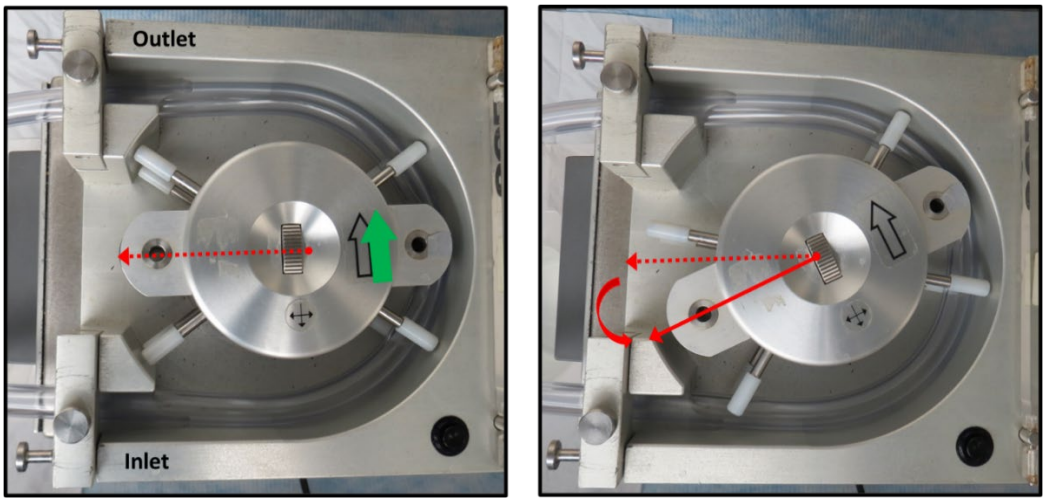

Figure 4: Left image shows the roller position at which the inlet tube will begin to be submerged into the PBS container. Right image shows the roller position at which the inlet tube is fully submerged (the tip of the inlet tube is 1-2 cm from the bottom of the container).

3.2.6 Proper roller occlusion would allow the aspirated PBS to reach between 18 cm to approximately 22 cm on the inlet tube after the roller completes ½ rotation and reaches 32cm to approximately 36 cm after the roller completes the first full rotation (**Figure 5**). Adjust the occlusion accordingly (tighten the occlusion if the aspirated PBS is lower than the above levels or loosen the occlusion if the aspirated PBS level is too high) and repeat the above steps until the aspirated PBS levels fall within the above ranges.

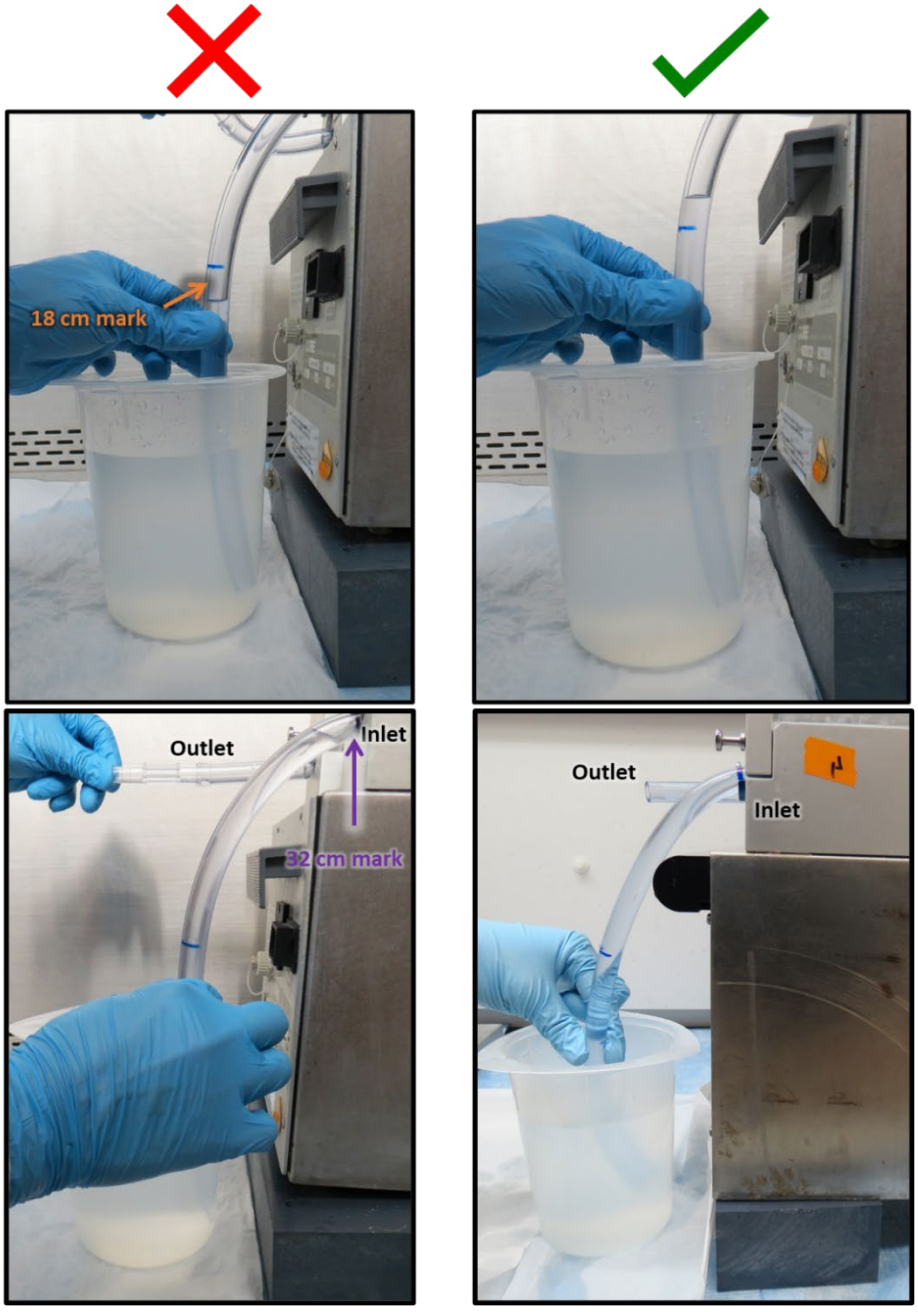

Figure 5: Images demonstrating the first two occlusion setting checkpoints. The top left panel image shows the PBS level is below 18 cm mark, indicating insufficient occlusion. The top right panel image shows the PBS level appropriately reaching the 1st checkpoint at or above the 18 cm mark. The bottom left panel shows the PBS level is below 32 cm mark/inlet gate, indicating an insufficient occlusion setting. The bottom right panel image shows the PBS level appropriately reaching the 2nd checkpoint at or above the 32 cm mark/inlet gate.

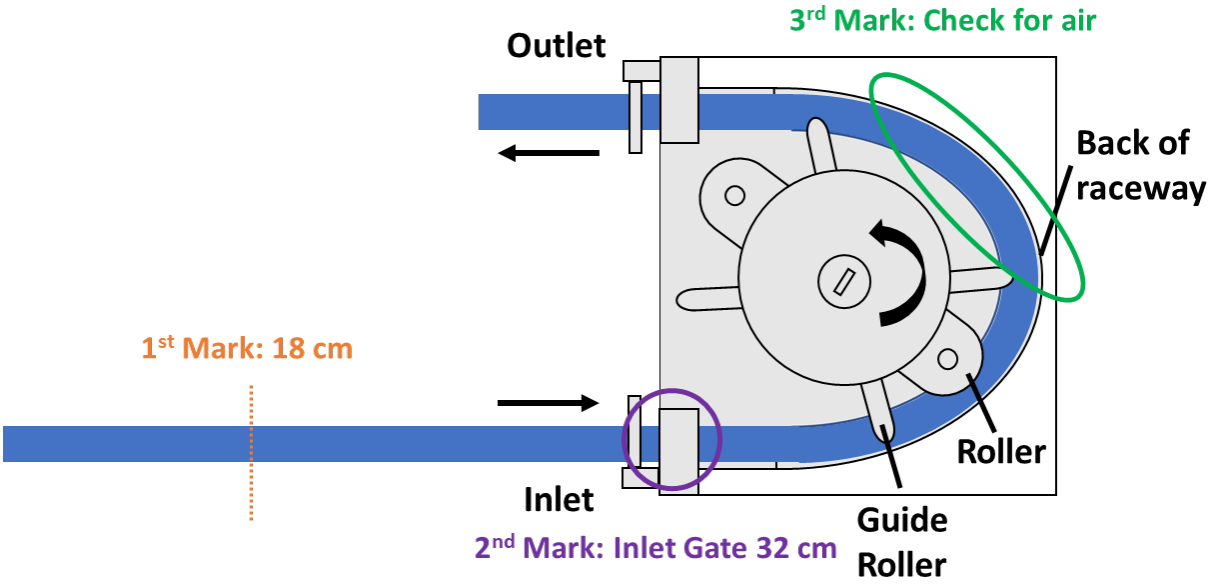

Figure 6: Schematic of roller pump from top view. 1st (orange), 2nd (purple), and 3rd (green) occlusion setting checkpoints are marked.

3.2.7 Using the roller pump set at 20 rpm fill the remaining of loop with PBS

3.2.8 Visually verify there is not any trapped air in the tubing within the back region of the raceway (**Figure 6**, green)

3.2.8.1 If air pockets are observed, try tapping on the tubing segment to dislodge any bubbles formed. If air bubbles remain, increase the occlusion, and repeat steps 3.2.2 to 3.2.7 until the air is removed and the roller occlusion meets the specifications above.

3.2.9 If preconditioning of the loop has not been done, connect the inlet of the reusable segment to the outlet via a 9.5 (3/8") straight PC connector and circulate the PBS for 1 hour at 50 rpm prior to another round of occlusion setting

3.2.10 After preconditioning and proper occlusion setting, empty the loop and get ready for blood recirculation as described below.

3.3 Test Material Segment Assembly and Preparation

3.3.1 Cut a 43 cm length of 9.5 mm (3/8”) ID PVC tubing
3.3.2 measure 8 cm from one end of the tube
3.3.3 Make a small n-shaped incision with a lancet at the 8 cm measurement on the tubing in the direction of blood flow, as shown in Figures **7** and **8**

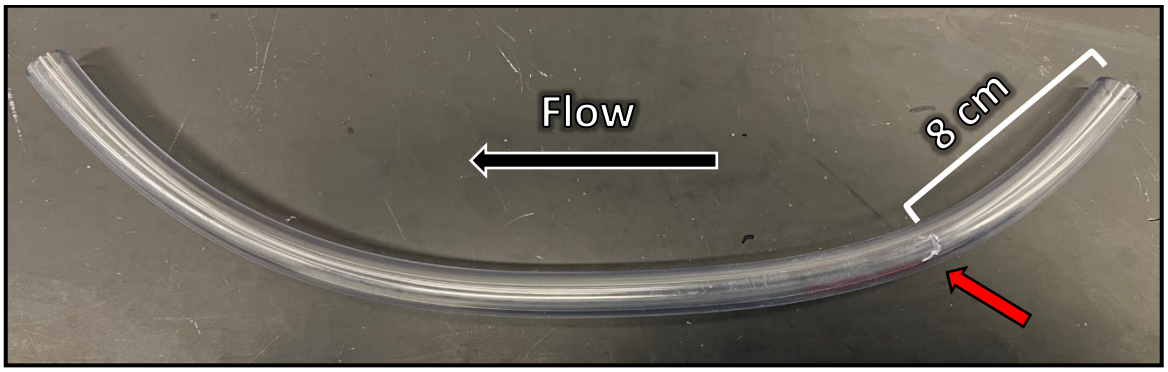

Figure 7: Top view of a 43 cm length material tubing with incision site (shown by red arrow) at the 8 cm measurement. The direction of blood flow is indicated by the black arrow.

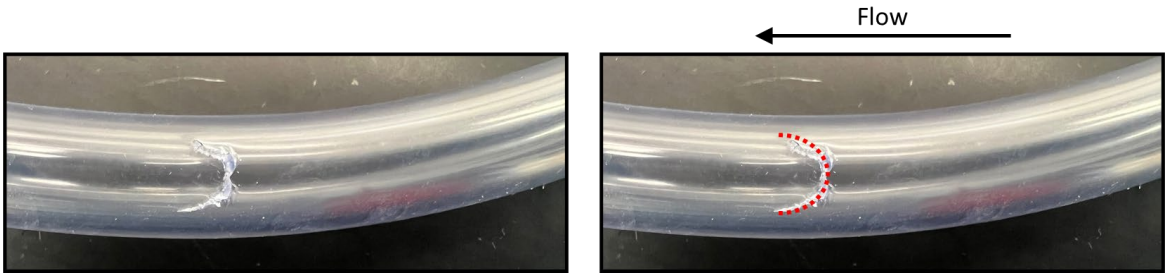

Figure 8: Incision made using a lancet (red) in relation to direction of blood flow (black arrow).

Note: The incision is towards the long end of the tube and the incision is curved. Make the incision through only one side (top side as shown in the picture) of the tubing wall so that the material can be introduced into the lumen of the loop. Do not pierce through both sides of the tube.

3.3.4 Each test material is cut into 13 cm length segments

Note: In the verification study[1], silicone and latex were only available as small-bore tubing, so both ends needed to be plugged with a PTFE cord (0.7 cm length, 2.5 mm OD) to prevent blood flow into the lumen of the tubing

3.3.5 Cut 0.7 cm of a 2.5 mm OD PTFE rod to make the plugs for the small-bore latex and silicone tubes

3.3.6 Fully insert the PTFE plugs into both ends of the latex and silicone tubes so that the ends of the plugs are flush with the ends of the tubes (**Figure 9** and **10**)

3.3.6.1 Alternatively, for easier handling of the PTFE plugs, a section of 2.5 mm OD PTFE rod longer than 0.7 cm can be prepared and inserted only 0.7 cm of the rod into the ends of the latex tube. Then the remaining PTFE rod can be cut along the edge of the latex tube, leaving a 0.7 cm PTFE plug inside the latex tube.

Figure 9: PTFE plugs (0.7 cm) to be inserted into both ends of the small-bore latex tubing (13 cm).

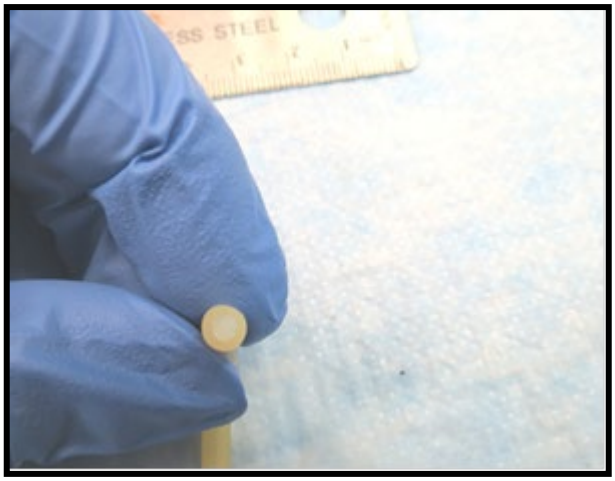

Figure 10: The end of the PTFE plug is flush with the end of the latex tubing.

3.3.7 Insert 12 cm of the test material into the 38 cm length of PVC tubing through the n-shaped incision, leaving 1 cm of the material out of the tube (**Figure 11** and **12**)

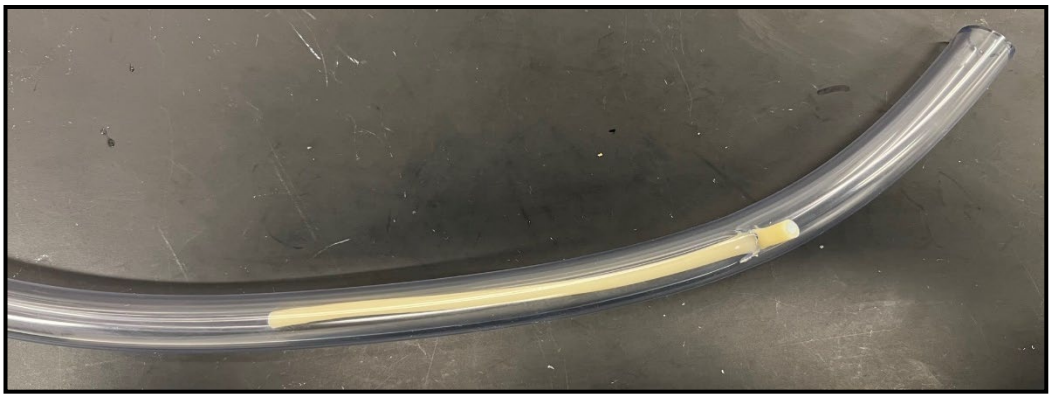

Figure 11: Top view of the 13 cm latex (plugged) in the PVC tubing

Figure 12: Side view of the latex tubing (plugged) inserted into the PVC tubing with 1 cm remaining outside (red).

3.3.8 Wrap Parafilm around the insertion site to prevent blood from leaking out of the loop – stretch the Parafilm and wrap multiple layers to ensure a tight seal.

3.3.9 Connect the PVC tubing test material segment to the outlet of the reusable PVC tubing segment via a 9.5 mm (3/8") OD straight PC connector.

3.3.10 Rinse the loop with PBS prior to blood circulation by aspirating 80 mL of PBS through the loop. Drain the loop so that minimal residual PBS remains within the PVC tubing.

4. Blood Preparation

4.1 Donor bovine blood collected in Anticoagulant Citrate Dextrose Solution A (ACDA), with a volume ratio of ACDA: whole blood = 15: 85, can be obtained from commercial suppliers of biological materials. The verification study used Lampire Biologic Laboratories (Pipersville, PA) as the blood supplier[1].

4.1.1 Blood is shipped overnight at 2-8°C (using ice packs) to the test lab and used within 36 hr. of blood draw as used in the verification study.

4.1.2 To account for blood variability, it is suggested that blood from at least 3 different donors be used for the tests. In the FDA verification study of this

assay[1], the blood from different donors were tested separately on different days and pooled blood was not evaluated. Thus, it may not be appropriate to use blood pooled from multiple animals for this test method.

4.2 Remove the blood bottle from the package and place it in a ventilated hood to allow the blood to warm up to room temperature (approximately 22 ± 2 °C).

4.3 Assess blood usability prior to testing

4.3.1 Use a hematology analyzer to measure complete blood counts in 3 samples to initially screen the usability of the blood. In the FDA verification study (Serna et al., 2024), blood was discarded if:

4.3.1.1 The average platelet count was less than 150 K/µL

4.3.1.2 The average hematocrit was less than 25%

Note: These thresholds are specific to that study using bovine blood. Each laboratory should establish its own specifications through an appropriately designed verification study.

4.4 Heparinize the ACDA bovine blood and then recalcify immediately before starting dynamic flow loop testing

4.4.1 Heparinization: Add appropriate amount of 200 U/ml heparin solution to the blood to obtain a donor-specific heparin concentration (see Section 5 below regarding how to determine donor-specific heparin concentrations).

4.4.2 Recalcification: add appropriate amount of 2 M $CaCl_2$ solution to the heparinized whole blood to obtain a final $CaCl_2$ concentration of 13 mM in the blood

5. Static Pretest to Estimate Donor-Specific Heparin Concentration

5.1 Cut an 8 mm OD latex tubing (1.6 mm wall thickness, 4.8 mm ID, 8 mm OD, Fisher Scientific, Hampton, NH) into eight 4 cm length samples.

5.2 Add 2.146 ml of whole blood to eight 5 mL round bottom PP tubes (approximately 8 cm^2/mL latex surface area/blood volume ratio)

5.3 Add varying amounts of 200 U/ml lithium heparin solution to each tube to obtain the following heparin concentrations in 0.2 U/mL increments (one heparin concentration per tube): 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 U/ml heparin

5.4 Add 14.3 µL of 2M $CaCl_2$ (13 mM final concentration) to each tube

5.5 Mix the tubes thoroughly using a tilted angle rotation (**Figure 13**)

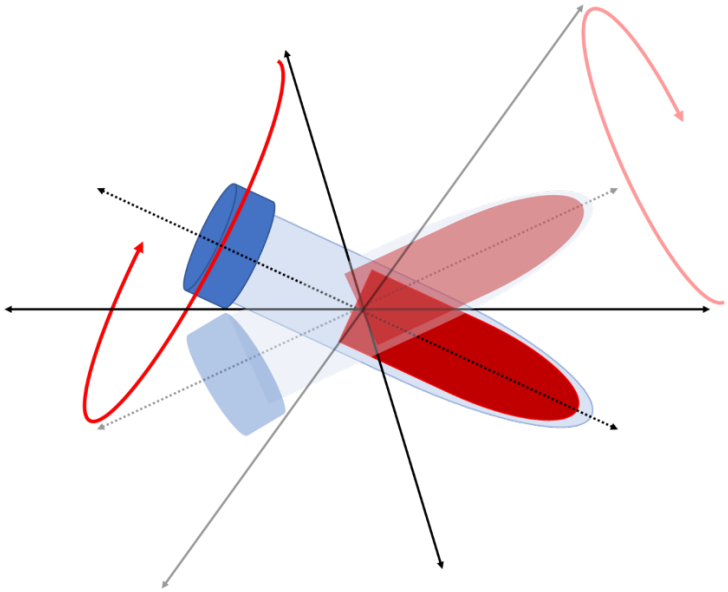

Figure 13: Diagram of how the tubes are mixed using a tilted angle rotation

5.6 Add one piece of the latex samples to each tube

5.7 Incubate the tubes for 15 minutes at 37°C in a shaking water bath with gentle agitation at 60 rpm

5.8 After incubation, gently rinse the samples with PBS and visually inspect the samples for thrombi adhesion to the outer surface of the latex tubes (**Figure 14B**).

5.9 The minimum heparin concentration that produces a thrombus surface coverage of ≤ 10% on latex is defined as the threshold concentration.

5.10 Select the initial heparin concentrations to start dynamic flow loop testing to be 0.6 U/ml and 0.8 U/ml below the threshold concentration. See examples in **Figure 14B**. In the verification study, two heparin concentrations were evaluated in the pilot dynamic flow loop test (described Section 6 below) to increase the likelihood of finding the correct donor-specific concentration during the first round of testing.

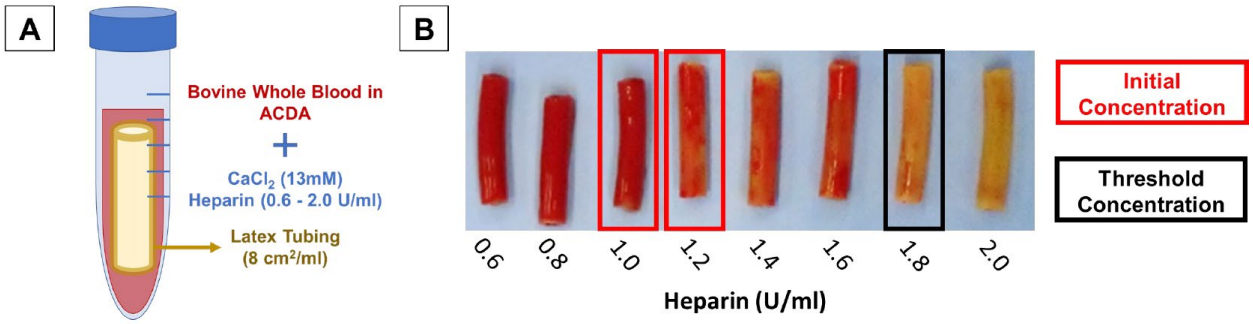

Figure 14. Static latex pre-test performed to predict donor-specific heparin concentrations to be used for the dynamic flow loop. A) Uniform latex tubes were incubated at 37°C for 15 minutes in re-calcified blood with a series of heparin concentrations. B) The minimum heparin concentration resulting in a thrombus surface coverage of <10% was defined as the **Threshold Concentration;** 0.6 U/mL and 0.8 U/ml below this threshold concentration were selected as the **Initial Concentrations** to start the pilot flow loop testing.

6. Pilot Flow Loop Test to Verify Donor-Specific Heparin Concentration

Note: A pilot dynamic flow loop test is performed to verify that the heparin concentration selected from the static pre-test would provide appropriate anticoagulation for each blood pool based on thrombus coverage on a set of control materials (negative control [PTFE] and positive control [latex]). The pilot test is performed according to the General Protocol for Blood Circulation described in Section 7 below.

6.1 Perform the blood circulation procedure described in section 7 on two sets of control materials (PTFE and Latex) using the initial heparin concentrations selected from the static pre-test in four different loops simultaneously (one control material per loop)

6.2 If the Acceptance Criteria below are met with either of the heparin concentrations, then that heparin concentration is used for the remainder of the experiment for that specific donor blood on that day

6.3 If the platelet count reduction in the negative control loop is >30% or the thrombus surface coverage produced on the PTFE material (negative control) is >10%, then increase the heparin concentration by 0.2 U/mL and repeat the pilot study

6.4 If the thrombus surface coverage produced on the latex material (positive control) combined with the surface area of the non-adherent thrombi is < 50%, then decrease the heparin concentration by 0.2 U/mL and repeat the pilot study

Blood acceptance Criteria

- The platelet count reduction in the negative control loop is < 30%
- The thrombus surface coverage produced on the PTFE material (negative control) is <10%
- The thrombus surface coverage produced on the latex material (positive control) is > 50%
- The platelet count reduction in the positive control loop is > 80%

Note: The blood may need to be discarded if an acceptable heparin concentration cannot be identified. The above criteria were established based on the FDA verification study[1] and are specific to this test methodology. Each laboratory should establish its own criteria based on verification data.

7. General Protocol for Blood Circulation

7.1 After blood preparation in Section 4.1 to 4.3 above, aliquot 120 mL of the bovine whole blood into 125 ml PP or HDPE bottles. Keep the blood at room temperature.

7.2 Immediately prior to filling the loop

7.2.1 Add appropriate amount of heparin to the whole blood to obtain the donor-specific heparin concentration (See Section 5 and 6 above)

7.2.2 Add 2M $CaCl_2$ to the whole blood to produce a final concentration of 13 mM (800 µL of 2M $CaCl_2$ in 120 mL of whole blood)

7.2.3 Mix thoroughly by using a tilted angle rotation (**Figure 13**)

7.3 Take two aliquots of the recalcified and heparinized blood (approximately 1 mL each) as the static control

7.3.1 Measure the platelet count using a hematology analyzer at the start of the 1-hour flow loop circulation

7.3.2 Measure the platelet count using a hematology analyzer at the end of the 1-hour flow loop circulation

7.4 Fill the loop with the blood (approximately 80 mL)

7.4.1 Run the pump at 20 rpm to aspirate the blood from the beaker to fill the loop. Allow the blood to cycle through the loop and back into the beaker until all of the air has been removed. The tubing can be tapped lightly to aid in de-airing the loop.

7.4.2 Once there are no bubbles visible within the tubing, stop the pump and carefully raise the flow loop inlet and outlet up to a position higher than the pump raceway.

7.4.3 Use a transfer pipet to completely fill the tubing, making sure to remove visible bubbles (**Figure 15**)

Figure 15: Images showing how to use a transfer pipet to completely fill the loop and remove bubbles

7.4.4 Close the loop by connecting the PC connector on the outlet to the flow loop inlet and start the pump

7.4.5 Check for bubbles – if there are any large bubbles, open the loop by disconnecting the connector and repeat steps b-f (a few small bubbles (< 1 mm) are acceptable)

7.4.6 Take a blood sample (approximately 1 mL) from the blood container used to fill the loop and measure the platelet count using a hematology analyzer. This is the Initial Platelet Count.

7.5 Position the flow loop using laboratory clamps/stands to minimize bending or twisting of the test sample (**Figure 16**)

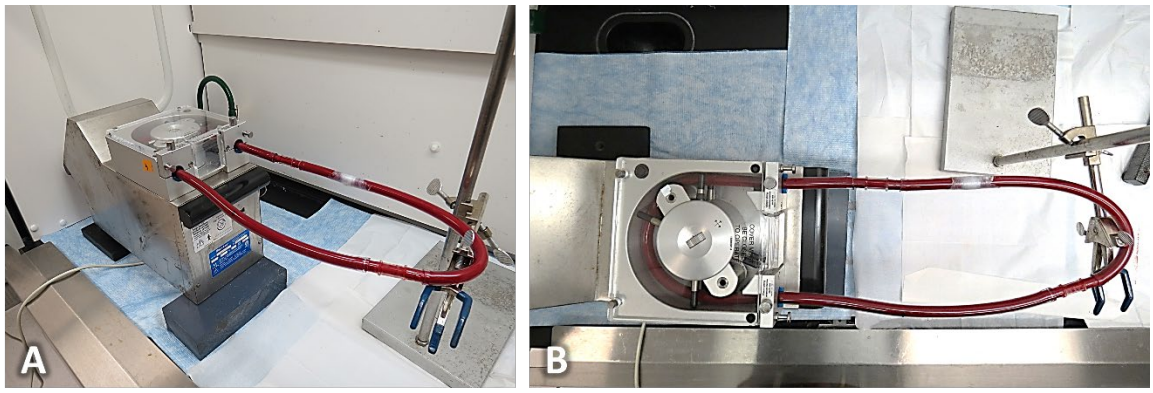

Figure 16: Example of how laboratory clamps/stands are set up to minimize the bending or twisting of the test sample during blood circulation. A) Side view. B) Top view

7.6 Circulate the blood at room temperature (21 ± 2°C) for 1 hour at 20 rpm (flow rate around 500-530 ml/min). This flow rate was selected to produce a physiological venous shear rate of approximately $100s^{-1}$

7.7 After the circulation, disconnect the PC connector and drain the blood from the loop into a clean PP or HDPE container

7.8 Take a sample of the blood from the container and measure the platelet count using a hematology analyzer

7.9 The remaining collected blood is filtered through a sieve (e.g., 100µm filter mesh) to collect any visible non-adherent thrombi. The thrombi are left in fume hood overnight (≥12 hrs.) to dry at room temperature.

7.10 Aspirate 120 ml of PBS through the loop at a decreased pump rotation speed of 10 rpm to gently rinse the blood from the loop while avoiding thrombus dislodgement from the test sample

7.11 Detach the test material segment from the reusable segment of the loop and carefully remove the test sample from the tubing by cutting the tubing wall using surgical scissors or a cutting blade

7.12 After removal, gently rinse the sample with PBS to remove any blood residue but avoid disrupting the thrombi on the sample

7.13 Photograph all sides of the test sample

7.14 Visually inspect the test sample for thrombus deposition and perform data collection as described in Section 8 below

7.15 Before starting the next test:

7.15.1 Rinse the reusable segment of the loop three times by recirculating PBS at 20 rpm through the tubing

7.15.2 Use a swab applicator to remove any visible thrombi that are attached to the inner lumen of the PVC tubing

7.15.3 After using the swab, rinse the tubing again with PBS and verify that all visible clots have been removed

8. Data Collection

8.1 To assess the relative thrombogenicity of all test samples, measure and calculate the percent thrombus surface coverage, thrombus weight, and platelet count reduction as described in Table 1 below.

8.2 Dry the non-adherent thrombi collected in the sieve after the flow loop recirculation overnight, weigh with an analytic balance, and add the non-adherent thrombus weight to the total thrombus weight of the sample.

8.3 Comparing the test sample results to that of the comparison article and positive and negative controls can be used to evaluate the relative thrombogenic potential of the test article.

Table 1 Methods to quantify the thrombogenicity of the test samples

Thrombogenicity Marker	Measurement Method
Thrombus Surface Coverage (%)	From the opposite end of the incision site, measure 11 cm length, measure and estimate the thrombi coverage in this 11 cm segment. The lengths of the thrombi adhered to the test sample surface are measured using a ruler and their widths are determined by estimating the fraction of the material's circumference covered by the thrombi. Then, the percent thrombus surface area coverage is calculated by the following equation: $\%\ Thrombus\ surface\ coverage = \frac{\sum Thrombus\ \ Area}{Test\ \ Material\ \ Surface\ \ Area}\ x\ 100\ \%$
Thrombus weight (mg)	The test materials and non-adherent thrombi are dried overnight (≥12 hours at room temperature) in a fume hood and their weights are measured using an analytical balance. Due to the possibility of disrupted flow conditions at the insertion site, any thrombus formed within 1 cm of the insertion site was excluded from this measurement. The original test material weight, that was collected prior to

	insertion in the flow loop, is subtracted from the post-test measurement to calculate the dry thrombus weight.
Platelet Count Reduction (%)	Blood platelet counts are measured before and after the 1 hr. circulation in the flow loop using a hematology analyzer. Then the percentage of platelet count reduction is determined by the following equation: $\%\ Platelet\ count\ reduction = \frac{Initial\ Platelet\ Count - Final\ Platelet\ Count}{Initial\ Platelet\ Count}\ X\ 100\ \%$

References:

1. Serna et al. Development of a large diameter in vitro flow loop thrombogenicity test system. Artif Organs. 2024. doi: 10.1111/aor.14852.