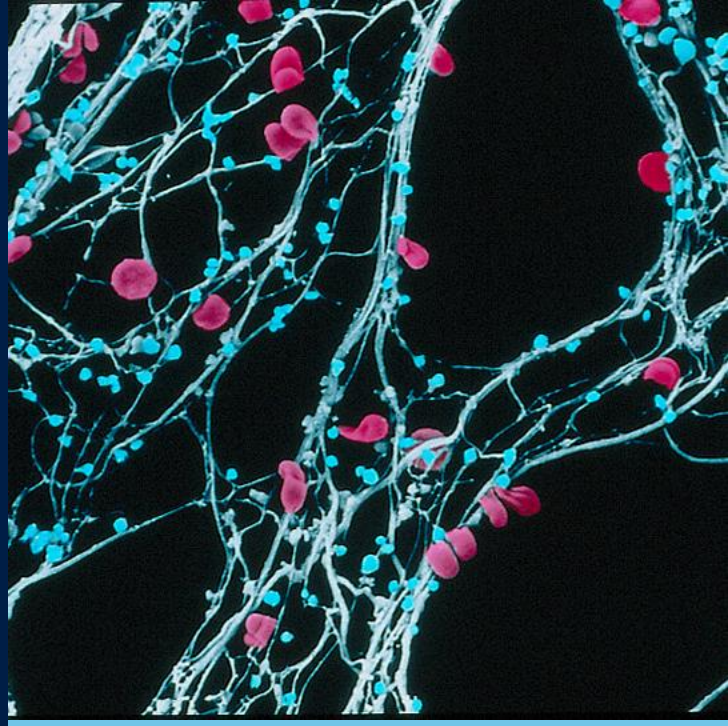
ISO10993-4:
Biological Evaluation of Medical Devices: Selection of Tests for Interactions with Blood:
anticipated changes in the standard based upon the 2016 final draft international standard



Michael F. Wolf

Medtronic Inc.
Core Technologies Group, Minneapolis Minnesota

SOT MDCPSS Webinar

Medtronic
Further, Together

ISO10994-4 FDIS Contributors:

Experts from:

- **Regulatory Agencies** (FDA, Health Canada, European Regulatory Bodies [DE, NL], CFDA, MHLW)
- **Medical Device Manufaturers** (Boston Scientific, Cook Medical, Medtronic, St. Jude Medical, Terumo Medical Corp, WL Gore)
- **Commercial Test Labs** (NAMSA, Nelson Labs, Toxikon, WuXi AppTec)

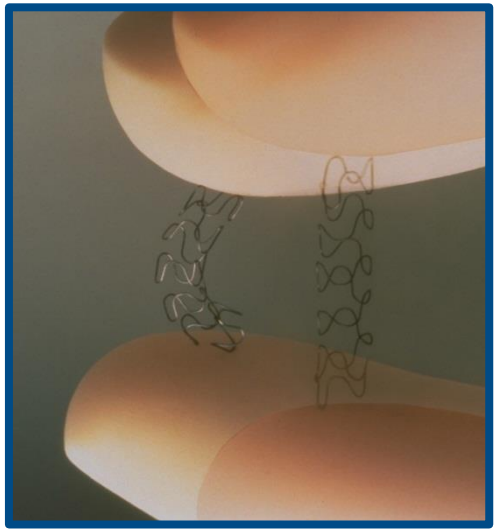
Applies to:

- New cardiovascular devices
- New device materials
- Change in an existing device

When,

the application involves:

- Direct blood contact, or
- Indirect blood contact

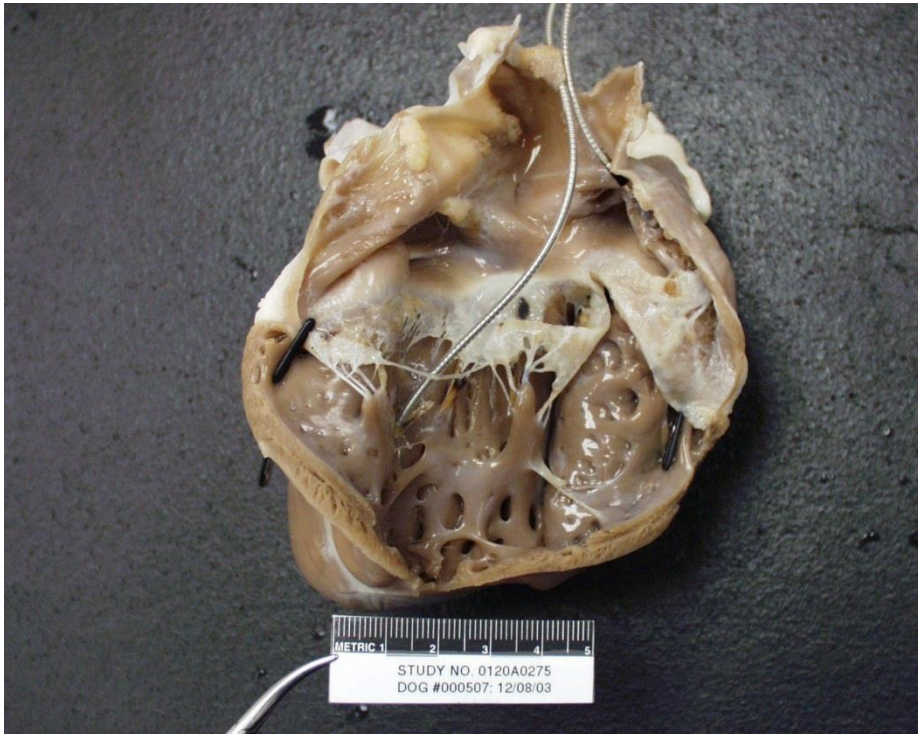


"The dose makes the poison"
- Paracelsus view of chemicals

~1 cm^2

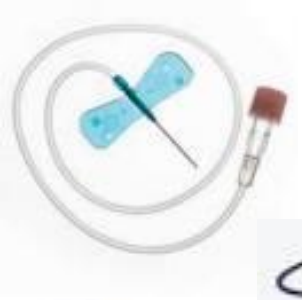
≥ 25,000 cm^2

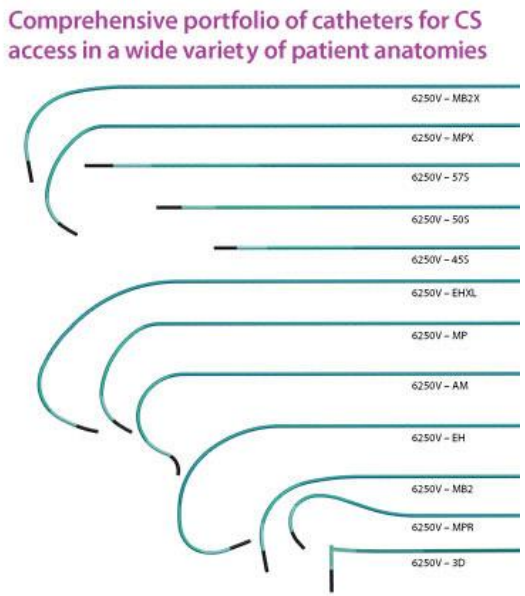
Surface areas vary tremendously

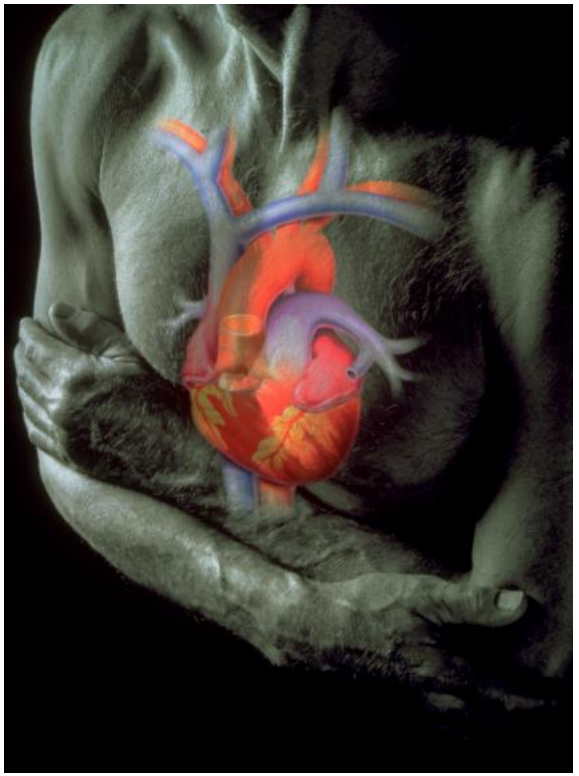


Materials vary = biologics, ceramics, metals, polymers

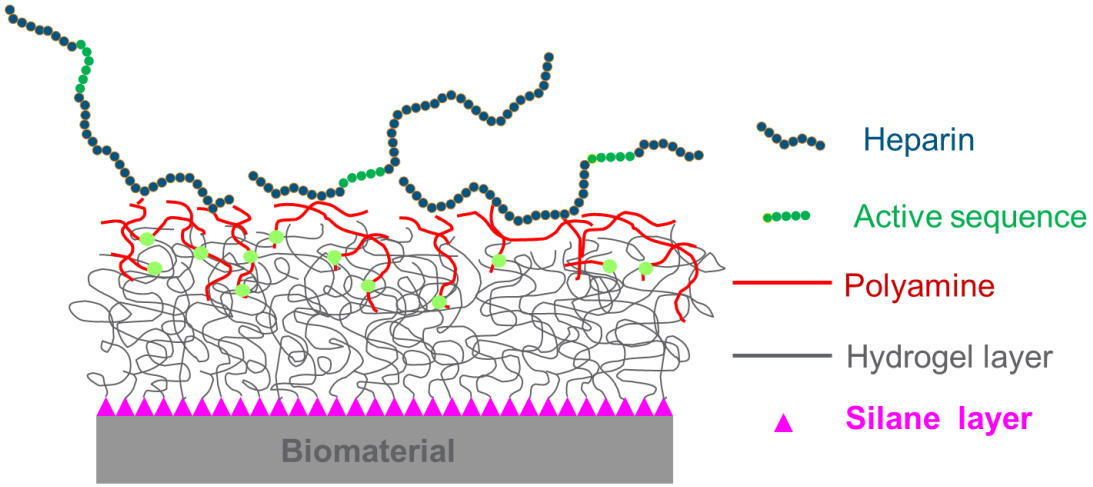
Exposure durations = seconds, minutes, hours, and permanent







Surface of Device X ≠ *Surface of Device X + Surface Modification*



Surface modified vs. non-modified

Heparin

Anticoagulants

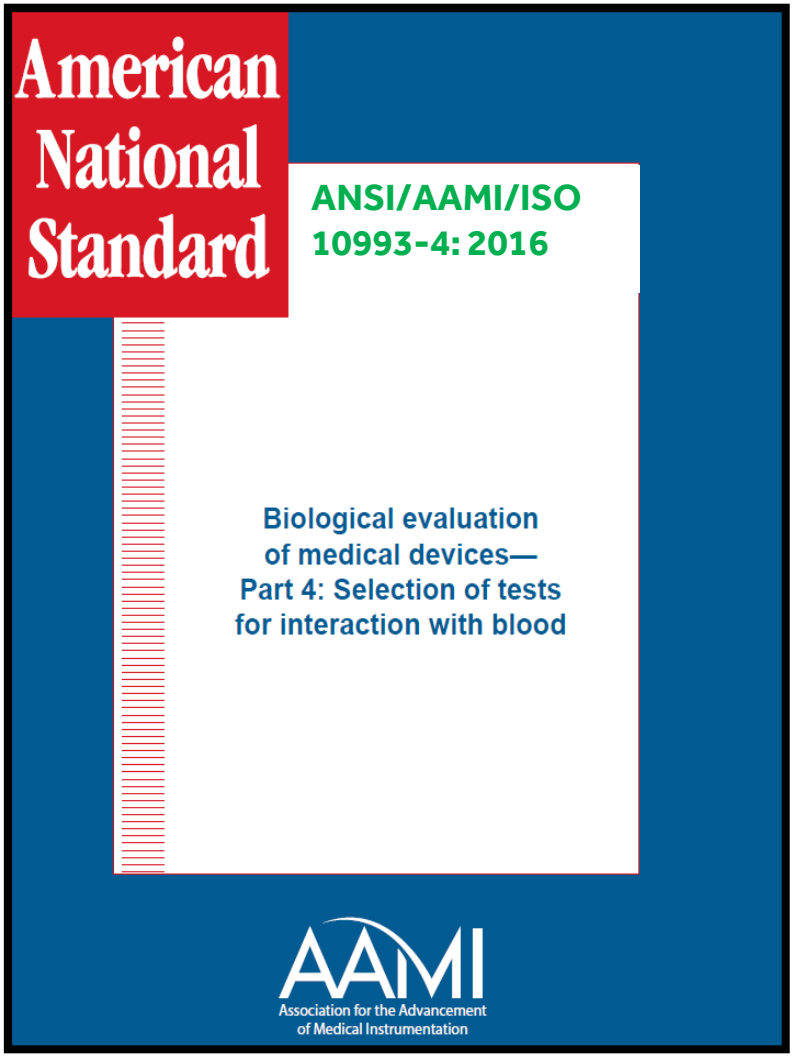
Device/Biomaterial

Device Performance w. Anticoagulants
≠
Device Performance w/o Anticoagulants

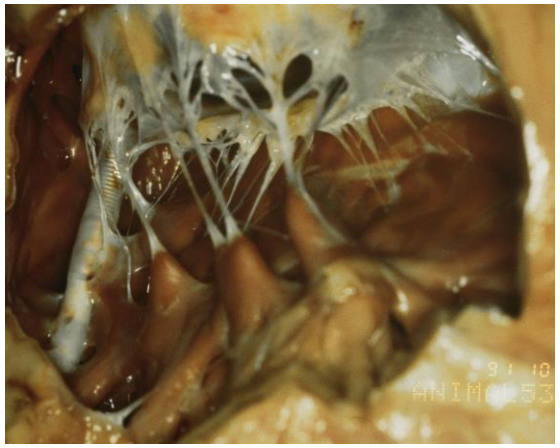
Device/Biomaterial

no anticoagulants present

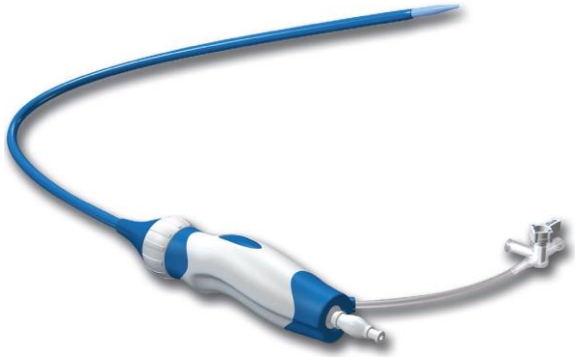
Anticoagulants + other drugs = present or not present clinically?

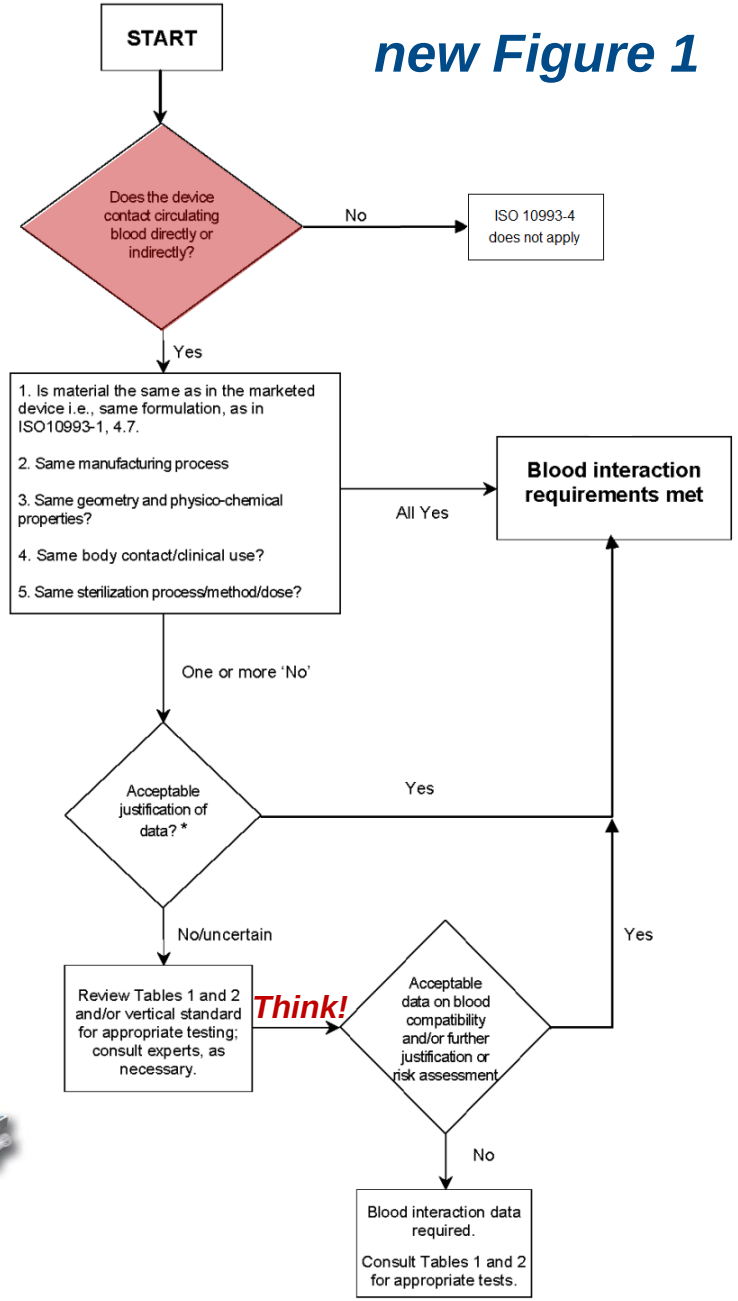

American National Standard
ANSI/AAMI/ISO 10993-4: 2016
Biological evaluation of medical devices— Part 4: Selection of tests for interaction with blood
AAMI
Association for the Advancement of Medical Instrumentation

new Figure 1




eSVS™ = external
saphenous vein stent




START
Does the device contact circulating blood directly or indirectly?
No
ISO 10993-4 does not apply
Yes
1. Is material the same as in the marketed device i.e., same formulation, as in ISO10993-1, 4.7.
2. Same manufacturing process
3. Same geometry and physico-chemical properties?
4. Same body contact/clinical use?
5. Same sterilization process/method/dose?
All Yes
Blood interaction requirements met
One or more 'No'
Acceptable justification of data? *
Yes
No/uncertain
Review Tables 1 and 2 and/or vertical standard for appropriate testing; consult experts, as necessary.
Think!
Acceptable data on blood compatibility and/or further justification or risk assessment
Yes
No
Blood interaction data required.
Consult Tables 1 and 2 for appropriate tests.

updated terms and new definitions

clause	Term	Definition
3.1	**anticoagulant**	agent which prevents or delays blood coagulation EXAMPLES Heparin, ethylenediaminetetraacetic acid (EDTA), sodium citrate
3.2	**blood/device interaction**	any interaction between blood or a blood component and a device
3.3	**coagulation**	phenomenon that results from activation of the clotting (coagulation) factor cascade Note 1 to entry: Factors of the coagulation cascade and fibrinolytic systems can be measured following exposure to devices either *in vitro* or *in vivo*.
3.4	**colloidal osmotic pressure**	total influence of the proteins or other large molecular mass substances on the osmotic activity of plasma
3.5	**complement system**	part of the innate immune system consisting of over 30 distinct plasma proteins, including enzymes, cofactors, and cellular receptors which may be involved in the promotion of thrombosis
3.6	**direct blood contact**	term used when the device or device material comes into physical contact with blood or blood constituents
3.7	**embolization**	process whereby a blood thrombus, or foreign object, is carried in the bloodstream and which may become lodged and cause obstructed blood flow downstream
3.8	***ex vivo* test system**	term applied to a test system that shunts blood directly from a human subject or test animal into a test chamber located outside the body
3.9	**haematology**	study of blood that includes quantification of cellular and plasma components of the blood
3.10	**haematocrit**	ratio of the volume of erythrocytes to that of whole blood in a given sample
3.11	**hemolysis**	liberation of haemoglobin from erythrocytes, either by destruction or through a partially damaged but intact cell membrane
3.12	**hemocompatible**	term applied to a device or device material wherein contact with blood does not cause any appreciable clinically-significant adverse reactions such as thrombosis, haemolysis, platelet, leukocyte, and complement activation, and/or other blood-associated adverse event
3.13	**indirect blood contact**	term used in reference to devices that contact the patient's blood path at one point and serve as conduit for entry into the vascular system; e.g., drug and parenteral nutrition solution delivery devices.
3.14	**legally-marketed comparator device (LMCD)**	an approved, long-established, and recognized-to-be-safe medical device used as a reference control in an in vitro or in vivo safety evaluation of a test device of similar design, material(s), and clinical use
3.15	**non-blood-contact**	term used in reference to the nature of the device or material contact with the patient's body where the device or potentially extracted material does not have direct or indirect contact with blood
3.16	**platelets**	anuclear, cellular bodies which include platelets and platelet-derived microparticles that are present in blood and contribute to the blood coagulation process by adhering to surfaces, releasing factors, and/or aggregating to form a haemostatic plug
3.17	**platelet adherent**	adjective describing a material or device that tends to allow or promote platelets to attach to its surface relative to a negative control, positive control, and/or LMCD upon blood contact due to its surface properties (Note: platelet adherent does not necessarily mean platelet activating i.e., platelets on a surface may or may not be activated)
3.18	**thrombin generating**	adjective describing a material or device that due to its surface properties tends to promote or show increased thrombin formation relative to a negative control, positive control, and/or LMCD upon blood contact
3.19	**thrombogenic**	adjective describing a material or device that due to its surface properties tends to form or promote thrombus formation relative to a negative control, positive control, and/or LMCD upon blood contact
3.20	**thrombo-embolisation**	process where a dislodged thrombus is carried downstream where it adheres and may cause subsequent vascular blockage or occlusion
3.21	**thrombus**	coagulated mixture of red cells, aggregated platelets, fibrin and other cellular elements
3.22	**thrombosis**	formation of a thrombus under in vivo, ex vivo, or in vitro simulated conditions, caused by activation of the coagulation system and platelets in flowing whole blood
3.23	**whole blood**	unfractionated blood drawn from a selected donor; the blood may be non-anticoagulated or anticoagulated e.g., contain sodium citrate or heparin as an anticoagulant

Table 1 – Circulating blood-contacting devices or device components and the categories of appropriate testing – External communicating and implant devices

new Table 1

Device examples	Test category						
	Haemolysis		Thrombosis				
			In vitro				
	Material-induced	Mechanically-induced	Coagulation	Platelet Activation	Complement [d]	Haematology	*In vivo / Ex vivo* [a]
External communicating devices							
Blood monitors (temporary/*ex vivo*) [b]	X		X	X		X	
Blood storage and administration equipment (e.g., infusion/transfusion sets), blood collection devices, extension sets	X		X	X		X	
Catheters in place for less than 24 h (e.g., atherectomy devices, intravascular ultrasound catheters, antegrade/retrograde coronary perfusion catheters, guide wires); cannulae	X		X [c]	X [c]		X [c]	X [c]
Catheters in place for more than 24 h (e.g., parenteral nutrition catheters, central venous catheters); cannulae	X		X [c]	X [c]		X [c]	X [c]
Cell savers [b]	X		X	X			
Devices for adsorption of specific substances from blood [b]	X	X	X	X	X		
Donor and therapeutic aphaeresis equipment and cell separation systems [b]	X	X	X	X	X		
Cardiopulmonary bypass system [b]	X	X	X [c]	X [c]	X	X [c]	X [c]
Haemodialysis/haemofiltration equipment [b]	X	X	X [c]	X [c]	X	X [c]	X [c]
Leukocyte removal filter [b]	X		X [c]	X [c]	X	X [c]	X [c]
Percutaneous circulatory support devices [b]	X	X	X [c]	X [c]	X	X [c]	X [c]
Implant devices							
Annuloplasty rings, mechanical heart valves	X	X					X
Embolization devices	X						X
Endovascular grafts	X						X
Implantable defibrillator and cardioverter leads	X						X
Intra-aortic balloon pumps [b]	X	X					X
Pacemaker leads	X						X
Prosthetic (synthetic) vascular grafts and patches, including arteriovenous shunts	X						X
Stents (vascular)	X						X
Tissue heart valves, vascular grafts and patches, and AV shunts	X						X
Total artificial hearts	X	X					X
Vena cava filters	X						X
Ventricular-assist devices [b]	X	X					X

[a] Thrombosis is an *in vivo* or *ex vivo* phenomenon, but can be simulated with *in vitro* conditions. *In vivo or Ex-vivo* testing may not be necessary if clinically relevant *in-vitro* thrombosis testing is performed.

[b] Direct or indirect blood-contacting components only. For components that have only indirect blood contact, in-vivo thrombogenesis and mechanical haemolysis or complement activation may not be necessary.

[c] It is recognized that coagulation, platelet, and leucocyte responses are primarily involved in the process of thrombosis. Therefore, it is up to the manufacturer to decide if specific testing in the coagulation, platelet, and haematology test categories is appropriate as an alternate to in-vivo testing.

[d] See also ISO/TS 10993-20 for information on when complement activation should be considered for other endpoints such as anaphylaxis.

new Table 2

Table 2 — Common tests used to assess interaction with blood

Tests by categories	
Haemolysis	Material-induced (e.g., ASTM, NIH, MHLW)
	Mechanical-induced
Thrombosis (*in vivo*, *ex vivo*)	Gross analysis [a], percentage occlusion, light microscopy, SEM
***In vitro* thrombosis**	
Coagulation	Thrombin (e.g., TAT, F1.2), fibrin (e.g., FPA) assays, PTT assay
Platelet Activation	Platelet count (% loss) and some indicator of activation (e.g., platelet release products such as βTG,PF4, TxB2), or SEM (platelet morphology)
Haematology	Complete blood count (CBC), leucocyte activation
Complement system	SC5b-9 (C3a optional)

[a] Included in all animal studies (see B.2.1 and ISO 10993-6).

Not all tests are needed for each category, and testing in each category may not be equivalent.

Where did all the old tests go?

Trash?

new Annex F: Less common laboratory tests

Table F.1 — Less common tests used to assess interactions with blood

Tests by categories [a]	
Thrombosis	Flow reduction, gravimetric analysis, pressure drop across device, adsorbed protein analysis, imaging techniques
***In vitro* thrombosis**	
Coagulation	thrombin generation assay using chromogenic substrates, fibrinogen and fibrin degradation products (FDP), D-dimer
Platelets	platelet adhesion assessments, flow cytometry analysis of platelet activation, platelet microparticle formation, gamma imaging of radiolabelled platelets, platelet aggregometry
Haematology	Leucocyte activation by flow cytometry, blood cell adhesion assessments, platelet-leucocyte complexes (PLCs)
Complement system	Bb, C3bBb, C5a

[a] Because of biological variability and technical limitations, the accuracy and predictivity of many of these tests, which are most commonly used for research purposes, requires careful attention to methodology and caution in interpretation of results.

new Annex G: Tests which are not recommended

Table G.1 — Tests not used in preclinical assessment of medical device safety

Tests by categories	
In vitro Thrombosis	
Coagulation	APTT, PT and TT
Platelets	template bleeding time, platelet lifespan (survival)
Haematology	reticulocyte count
Complement system	CH-50, C3 convertase, C5 convertase

Annex B updated new Figure B.1*

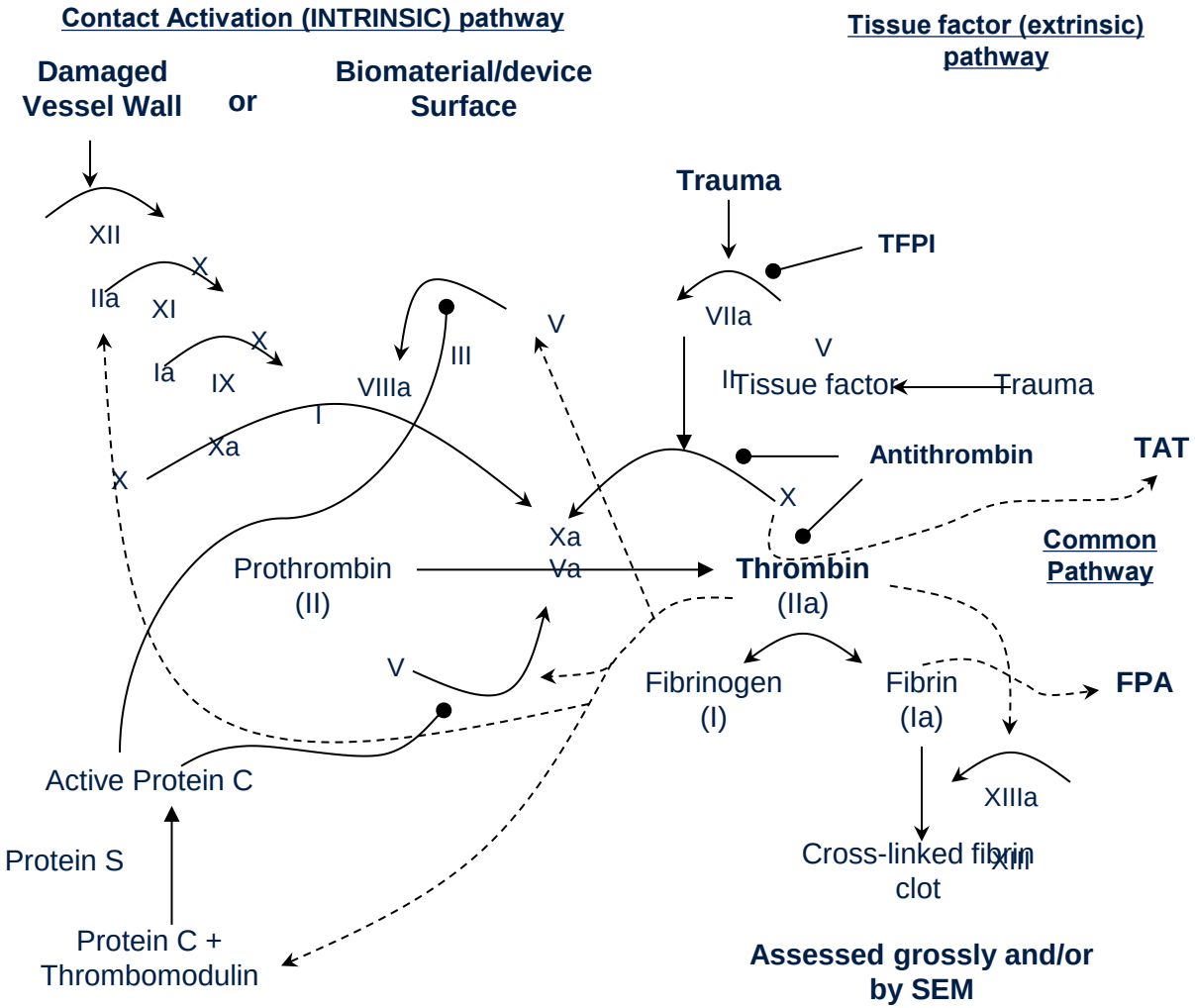
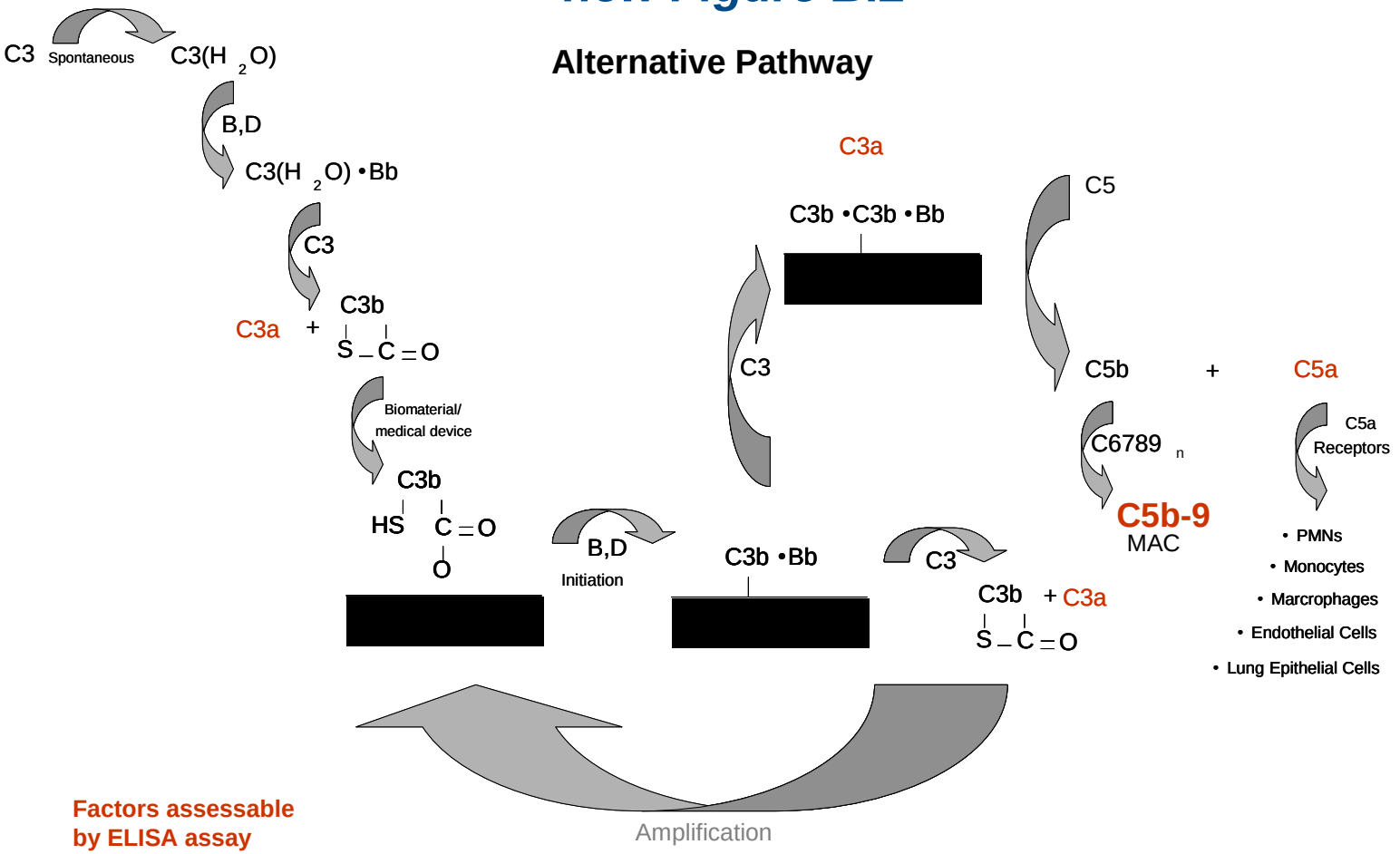


Figure B.1 – The coagulation cascade

*** Annex A (informative) Preclinical evaluation of cardiovascular devices and prostheses – mainly language refinements**

new Figure B.2



Factors that are shown in red are measurable by commercially available assay kits.

Figure B.2 — The alternative complement pathway

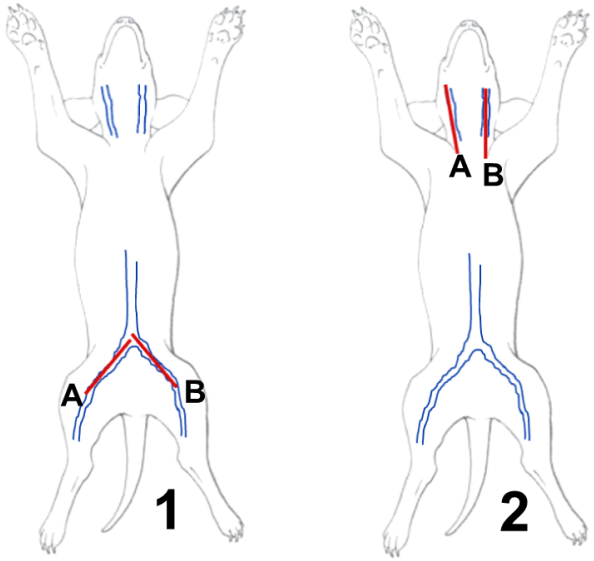
What is the #1 best method?

C.2: Test the device in an in vivo implant model that reflects, as best possible, the intended clinical application

- **implant site**
- **implant method**
- **implant sizing**
- **clinical drugs**
- **implant duration**
- **consult vertical standards**
- **etc..**

Include in the protocol the details for how thrombosis will be assessed

New Annex C: Thrombosis – Methods for in vivo testing
Method C.3 NAVI and AVI Testing

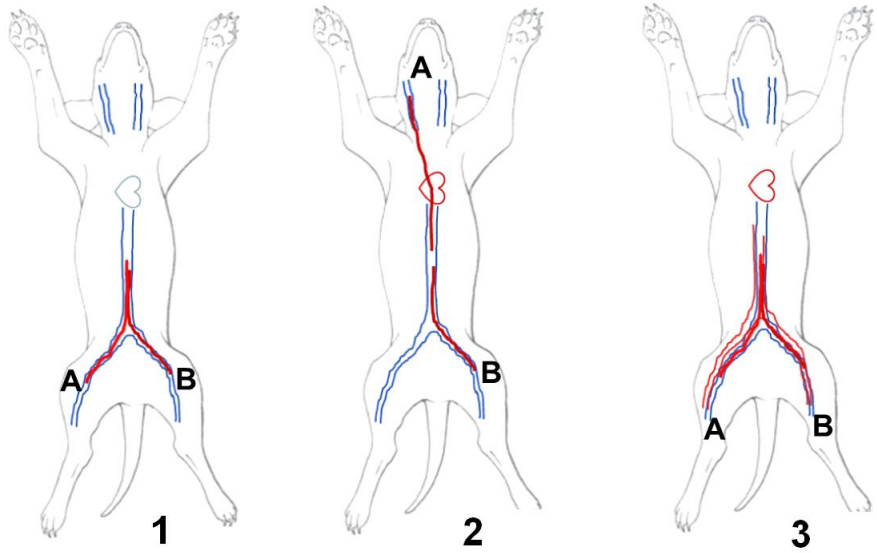


Key
1 Femoral
2 Jugular

Figure C.1 — Main implant positions used in the NAVI/AVI model

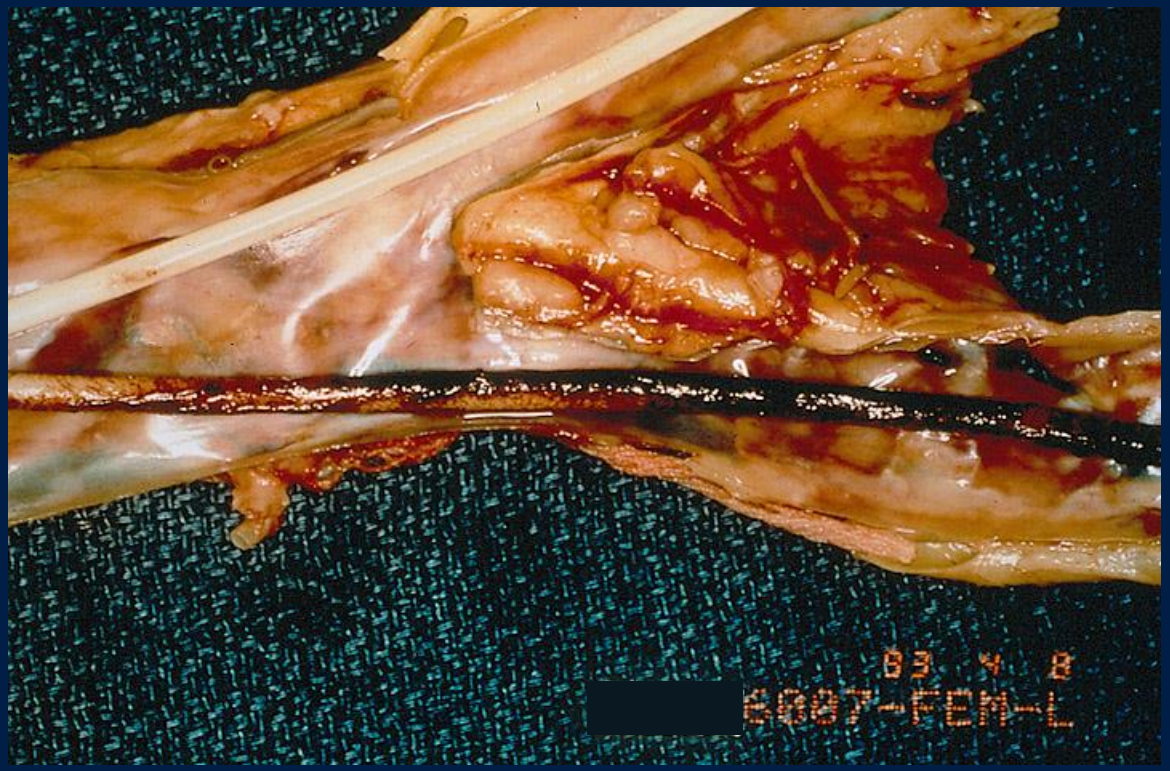
NAVI Model — non-anticoagulated venous implant

AVI Model — anticoagulated venous implant



Key
1 IVC-IVC
2 SVC-IVC
3 IVC-AA

Figure C.2 — Other less-frequently used NAVI and AVI implant positions



Heparin coated CVC
vs.
LMCD (non-coated)

Canine femoral vein NAVI model, [H] = 0; 60 minutes

Pebax ablation catheter

Canine jugular vein NAVI model, [H] = 0; 60 minutes

SUMMARY OF CHANGES IN ISO10993-4 (2016)

- **Revised and new definitions**
- **Original Tables 1 and 2 = new Table 1** (now includes material and mechanical-induced haemolysis testing and in vitro and in vivo testing thrombosis)
- **Original Tables 3 and 4 = new Table 2** (simplified to most common tests)
- **New Figure 1** (simplified)
- **Annex B updated** (covers only the most common practiced)
- **New Annex C** (covers the topic of in vivo thrombosis and methods for testing)
- **Annex D (previously Annex C on hemolysis testing) updated** (includes info on mechanically-induced haemolysis)
- **New Annex E** (covers the topic of Complement testing and best test method practices)
- **New Annexes F and Annex G** (present the less common and not used tests)
- **Language refinements** (found throughout the revised document)
- **Expanded and reorganized Bibliography**

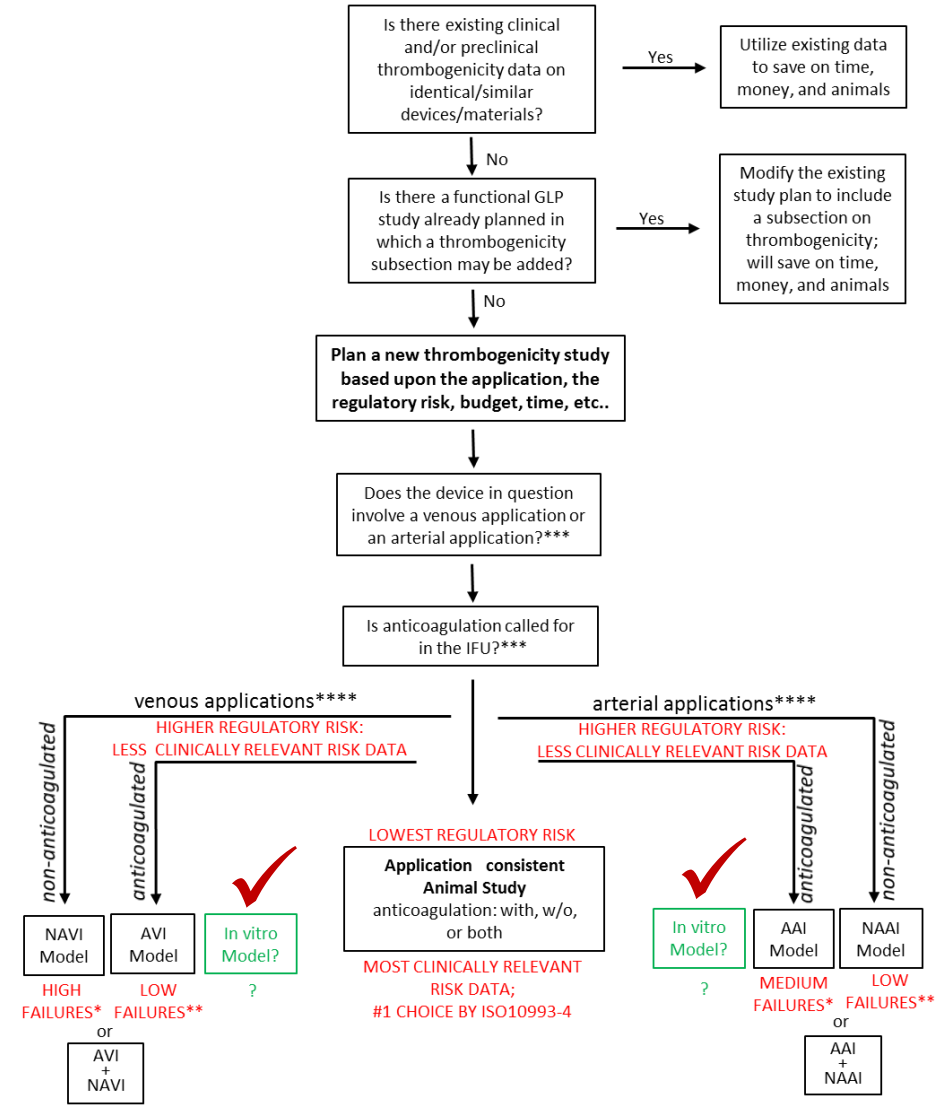
Example: typical question from FDA:

You have not performed in vivo thrombogenicity testing on the subject device, but have instead performed partial thromboplastin time (PTT) testing. FDA does not agree that PTT testing is sufficient to address the thrombogenicity of your device. In particular, PTT is a static coagulation test, which does not take into account platelet activation or any physiologic flow effects of thrombus formation. To address thrombogenicity using in vitro testing, FDA recommends that coagulation (e.g., unactivated PTT) and platelets be assessed. In addition, we recommend that geometry-mediated thrombogenicity potential in physiologic flow be considered. As you have conducted only a PTT test, please address the following. Please note that in the event that your response is considered inadequate, an in vivo thrombogenic test will be requested.

a) Please provide additional in vitro studies to address the effect of your device materials on platelets and need for assessment of thrombogenicity in response to physiological flow. If in vitro testing is planned, validation data may be needed, and we strongly recommend that protocols and validation data be discussed prior to study initiation using FDA's presubmission process.

b) Alternatively, please provide results from canine in vivo thrombogenicity testing

ISO10993-4 decision tree for testing for thrombosis



* due to blood flow and material properties
** due to presence of anticoagulant
*** devices which experience both venous and arterial exposure , or, see use with and without anticoagulation, may require a combination of tests
**** testing of devices intended for arterial application in the venous system, and vice versa, is NOT RECOMMENDED and has very high regulatory risk
NOTE: NAVI, AVI, NAAI, and AAI study designs must be balanced in terms of equal number of test and control devices in each implant site, and a test and control device in each animal, whenever possible.

new Table 2

Table 2 — Common tests used to assess interaction with blood

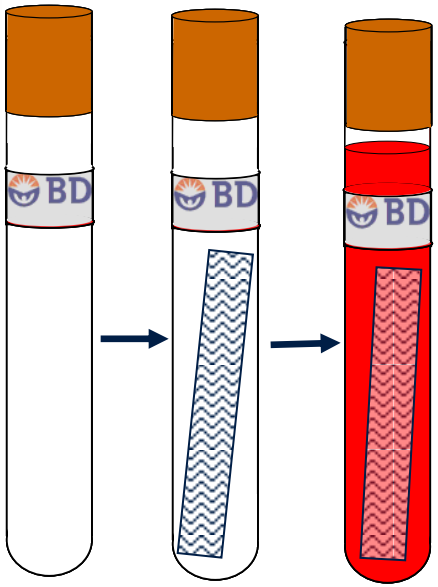
Tests by categories	
Haemolysis	Material-induced (e.g., ASTM, NIH, MHLW)
	Mechanical-induced
Thrombosis (*in vivo*, *ex vivo*)	Gross analysis [a], percentage occlusion, light microscopy, SEM
***In vitro* thrombosis**	
Coagulation	Thrombin (e.g., TAT, F1.2), fibrin (e.g., FPA) assays, PTT assay
Platelet Activation	Platelet count (% loss) and some indicator of activation (e.g., platelet release products such as βTG,PF4, TxB2), or SEM (platelet morphology)
Haematology	Complete blood count (CBC), leucocyte activation
Complement system	SC5b-9 (C3a optional)

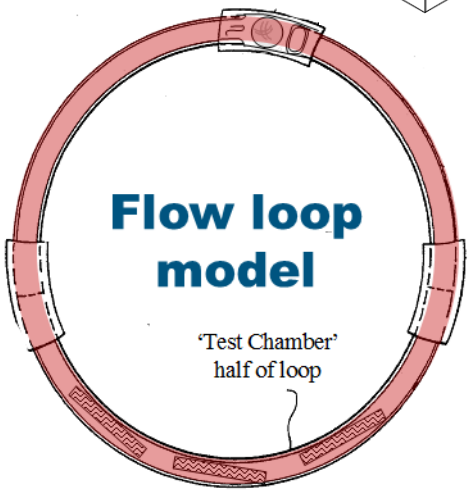
[a] Included in all animal studies (see B.2.1 and ISO 10993-6).

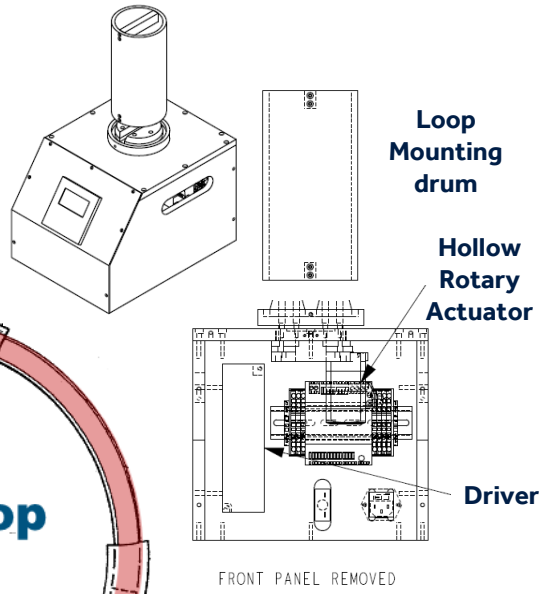
Not all tests are needed for each category, and testing in each category may not be equivalent.

In vitro models

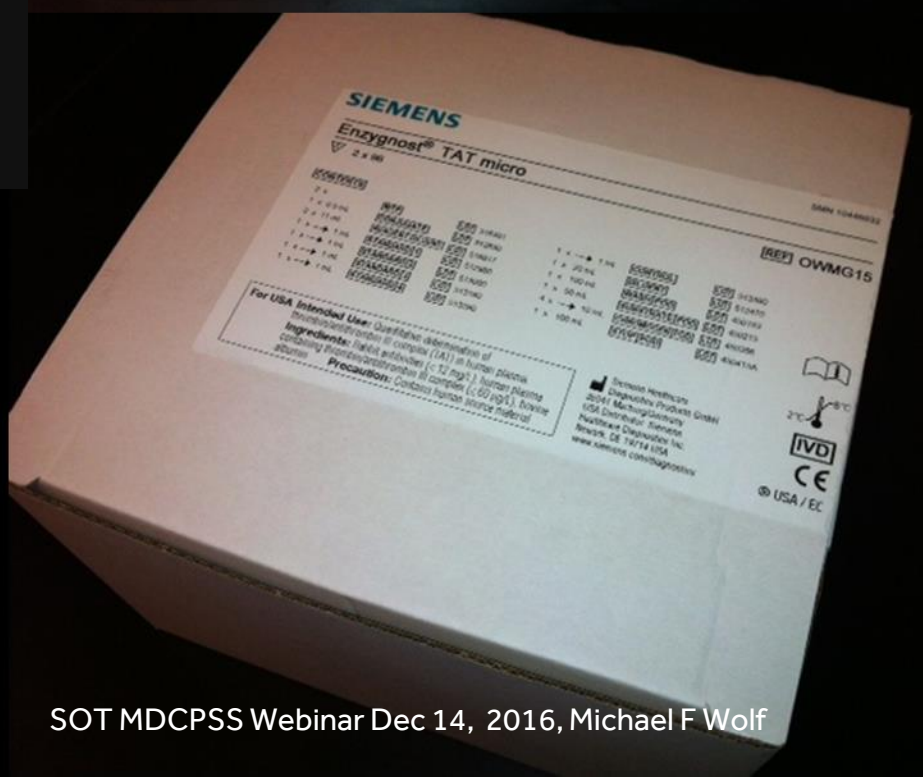
Test Tube Model







- ✓Models are simple
- ✓Human blood = human responses
- ✓Controlled blood exposures (e.g., SA_{TM}, [H], +/- physiological flow)
- ✓Small volumes = higher replicates (DOEs) +*more materials tested at a time*
- ✓Donor variability - can be studied
- ✓Multiple protein indicators of thrombosis
- ✓Low cost
- ✓Fast turnaround (~ 1 week vs. 1-2 months for NAVI or AVI)
- ✓*No animals required*



Cost:

- **$1000/kit**
- **50-100 samples/kit**
- **1-2 days/analysis**

IN VITRO TAT (COAGULATION) INTERPRETATION (PROPOSAL):

Results	In vitro Score	Interpretation
TAT_{test} < empty	-1	Anti-thrombogenic
TAT_{test} = empty	0	Non-thrombogenic
empty < TAT_{test} < LMCD	1	Low thrombogenicity
TAT_{test} = LCMD	2	Predicate-consistent thrombogenicity
LCMD < TAT_{test} < glass	3	Moderate thrombogenicity
TAT_{test} ≥ glass	4	High thrombogenicity


Human Blood Study Data Sheet
Date: 12/9/2016
Time: am pm
Venipuncturist initials:
Donor initials: BT
Model: Tube (buffer displacement) Tube (vacuum) X Loop Other:
Added details:
Experiment Name: Thromboresistivity RR #2
Material SA:blood volume ratio: 27 cm²/3.0 mL 18 cm²/3.0 mL X Other:
Replication: Duplicates X Other
Blood draw volume per each sample: 3.0 mL: 2.0 mL: X Other:
Anticoagulant Information:
Anticoagulant level*** (U Heparin /mL blood): 0.6 ; 1.0 X ; 2.0 ; Other
Anticoagulant Product Name: Hospira Lot #: 53152AB Expiration date: May 2017
Diluent PlasmaLyte-A Lot#: 68524J2 Expiration date: Nov 2016
CTAD Tubes: Lot #: 613-052AB Expiration date: 4/2017
*** show calculations on back of this sheet, if necessary
Draw Start Time: Draw End Time:
Sample # | Empty Weight (gr) | Weight with Blood (gr) | Fill Volume (gr/mL) | Draw Order* | Material** | Comments (observations worth noting)
Particulates? (if present)
1 Glass 1
2 Glass 1
3 Glass 2
4 Glass 2
5 PE
6 PE
7 PEV
8 PEV
9 PEU + CRAS
10 PEU + CRAS
11 P-Low
12 P-Low
13 Empty
14 Empty
15
16
* Randomized sample draw order
** always add Bi tube (initial blood CTAD tube) and Bf tube (final blood CTAD tube
Person filling out this form:

Thrombogenicity Round Robin Study Design

Models: tube,

Measured factors: TAT, BTG, [Plt], others?

Blood: fresh human

Heparin level(s): 0.6 (low) and 1.0 (high) IU/mL

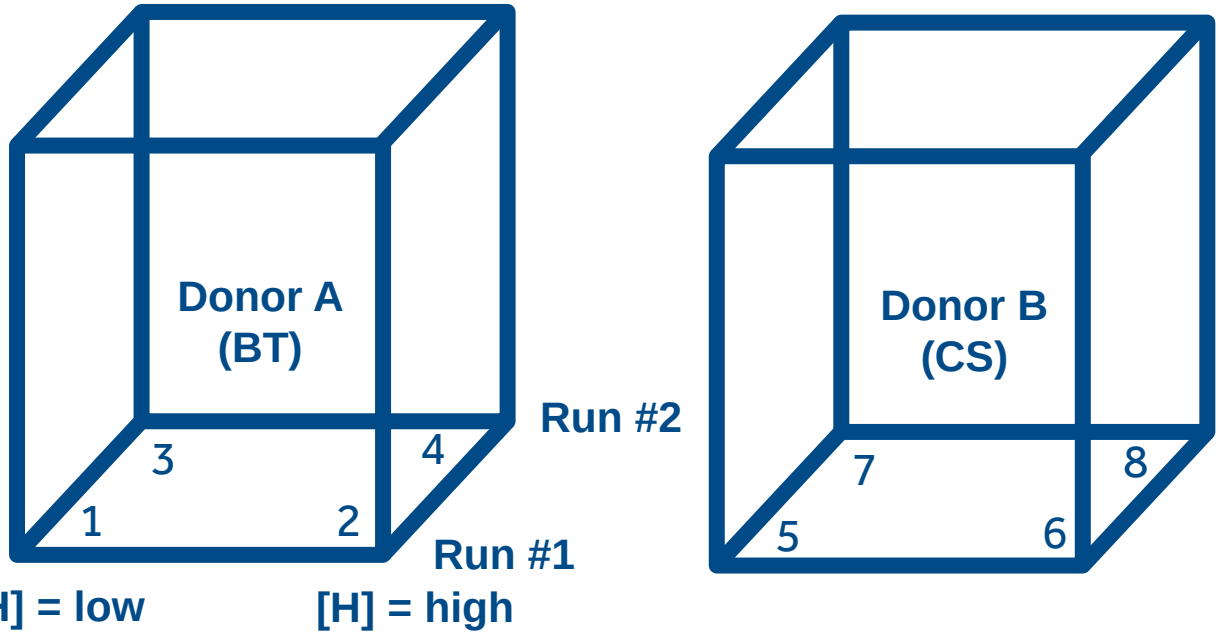
Exposure ratio: 9 cm^2/mL WB

n: 2 per condition

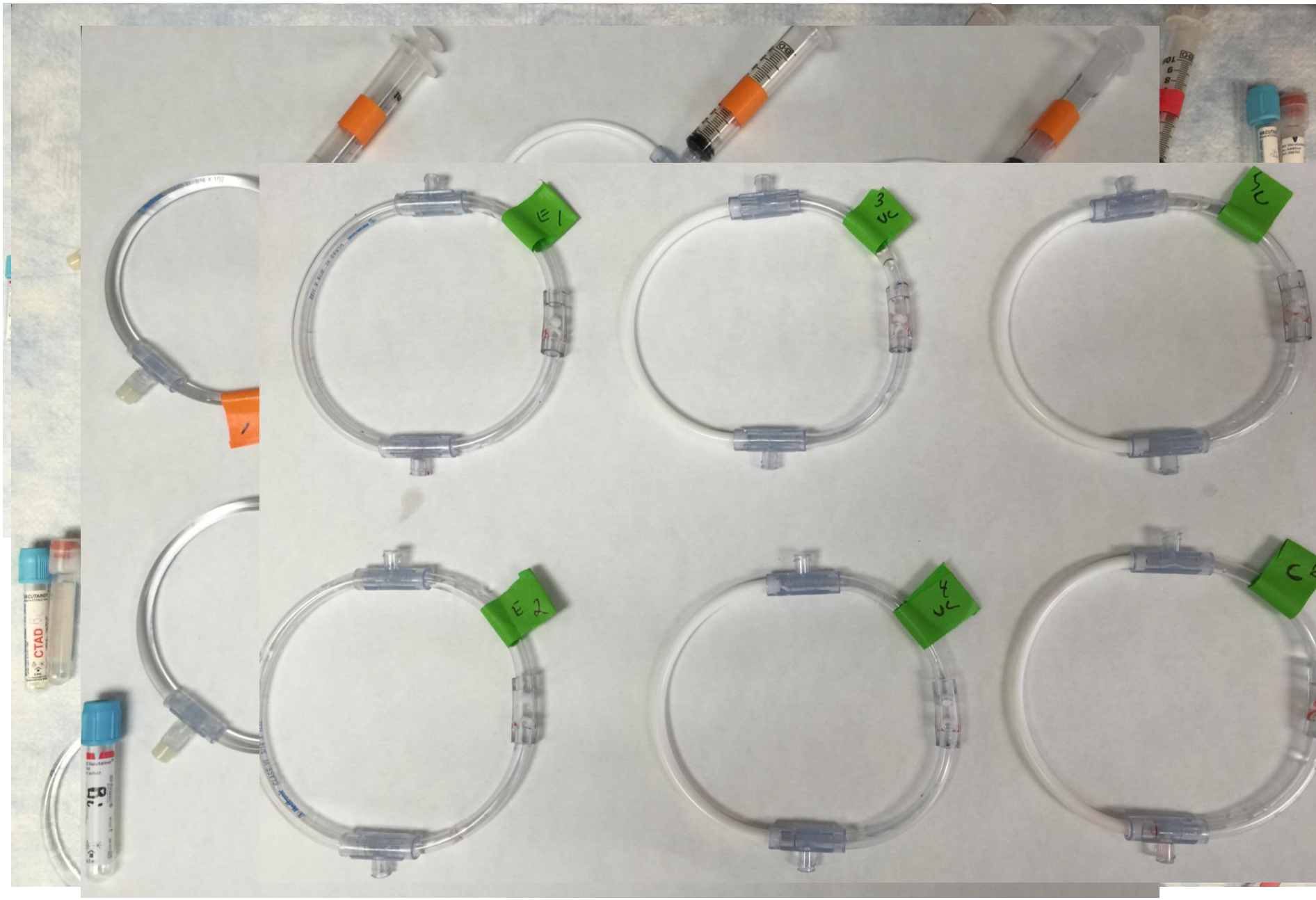
Statistics: ANOVA + Tukey Kramer

Materials

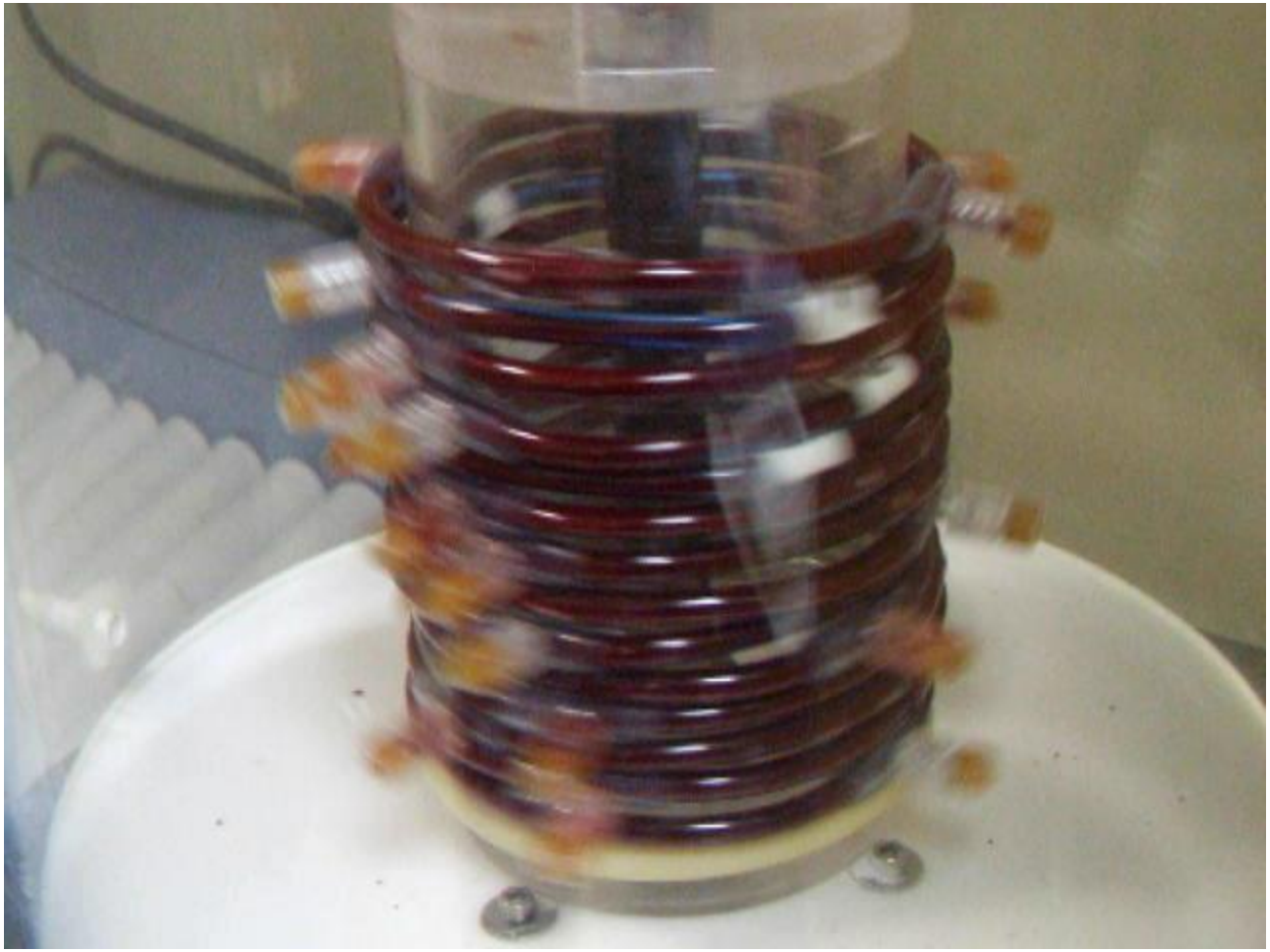
Bi
1. Glass 1
2. Glass 1
3. Glass 2
4. Glass 2
5. PE
6. PE
7. PEU
8. PEU
9. PEU+CBAS
10. PEU+CBAS
11. Pebax
12. Pebax
13. Empty
14. Empty
Bf




37°C
incubator closet


E 1
3 VC
E 2
4 VC
CTAD

SIMULATED PULSATILE BLOOD FLOW



Practical considerations of tube and loop models

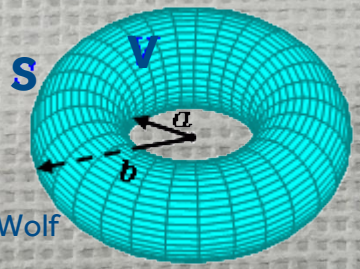
ID ≈ 4.8 mm (3/16")
V ≈ 6 mL
SA ≈ 52 cm²

ID ≈ 6.4 mm (1/4")
V ≈ 11 mL
SA ≈ 70 cm²

ID ≈ 9.5 mm (3/8")
V ≈ 27 mL
SA ≈ 112 cm²

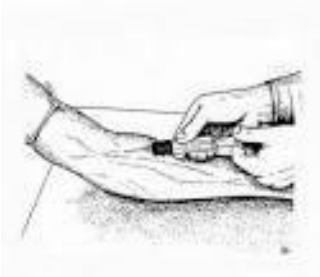
V ≈ 3-5 mL

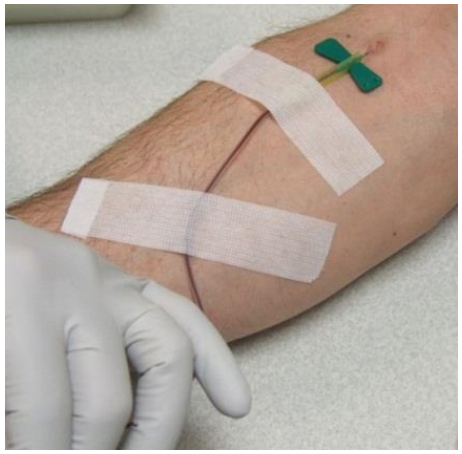
V ≈ 1-2 mL



Torus
volume (V) = ¼ π² (a+b)(b-a)²
surface area (SA) = π² (b²-a²)

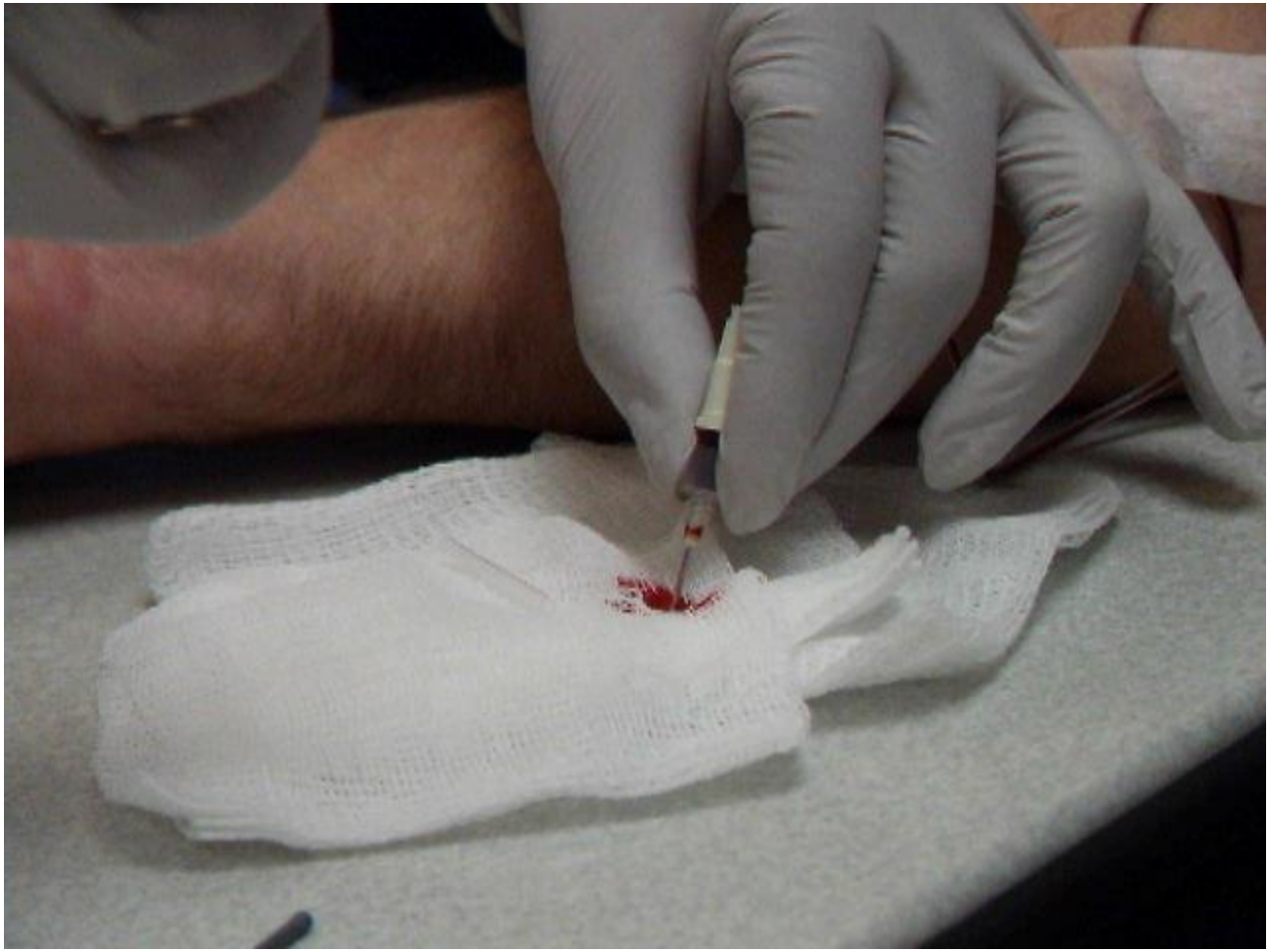
BLOOD ACQUISITION/EXPOSURE – MANY METHODS





Method A = Method B = Method C?

BLOOD FILLING – VACUUM BLOOD DRAW



BLOOD FILLING – SALINE DISPLACEMENT BLOOD DRAW

Thank you

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