PTT and PT Assays for Thrombogenicity Evaluation: An FDA Perspective

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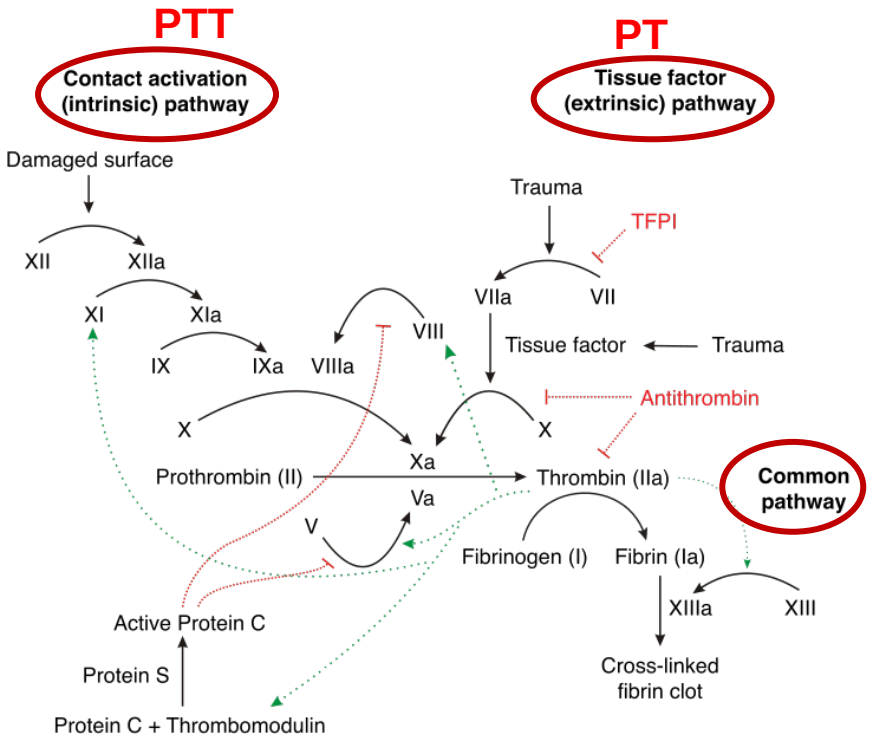
PTT and PT Assays--Clinical Use

- Partial thromboplastin time (PTT) and prothrombin time (PT)
  - monitor anticoagulation therapy or evaluate coagulation-factor deficiencies in patients
  - aPTT (activated partial thromboplastin time) for intrinsic and common pathway
  - PT for extrinsic and common pathway
  - Clotting time lengthened by anticoagulants and/or coagulation factor deficiencies



Adapted from http://en.wikipedia.org

PTT and PT in Hemocompatibility Testing

- Adapted to evaluate blood compatibility using plasma with normal coagulation factors
  - PTT reagent (cephalin) without an activating substance used in clinical aPTT testing; the test material acts as the activator
  - no modifications for PT testing
  - Shortened clotting time indicates activation of the coagulation cascade by the test materials
  - Lengthened clotting time may also occur (e.g. heparin release materials, coagulation factor depletion)

PTT and PT--Methods

- Test procedures:
  - Incubate plasma with test material at 37 ˚C for a certain period of time (15, 30, 60 mins)
  - Measure the clotting time after adding PTT or PT reagents
  - Compare clotting time to the negative and positive controls
- Factors that may affect test outcomes
  - Blood age, incubation time, ratio of material surface to plasma volume, sources of PTT and PT reagents

ASTM Standard for PTT

- ASTM F2382 – 04 (Reapproved 2010): Assessment of Intravascular Medical Device Materials on PTT
  - Plasma: fresh or frozen human plasma
  - Incubation: 15 min in an agitating water bath at 60 rpm
  - Negative control: Untreated plasma
  - Positive controls: Latex or black rubber
  - Acceptance: Pass if PTT > 50% of negative control
- Use of the ASTM method
  - Clinical relevance of the acceptance criteria unknown
  - Not recognized by FDA and not widely used by test labs

Typical PTT Data Submitted to FDA

Parameters	Conditions/Values
Plasma	Fresh or frozen, human, citrated
Incubation time	15, 30, or 60 mins
Material surface to plasma ratio	3, 4, or 6 cm^2/ml
Positive control	Black rubber or glass
Sources of PTT reagents	Vary from lab to lab
PTT Clotting time	Plasma: 60 to >300 sec Black rubber: 60 to 90 sec (20-30% of plasma) Glass: 50 to 100 sec (30-80% of plasma)

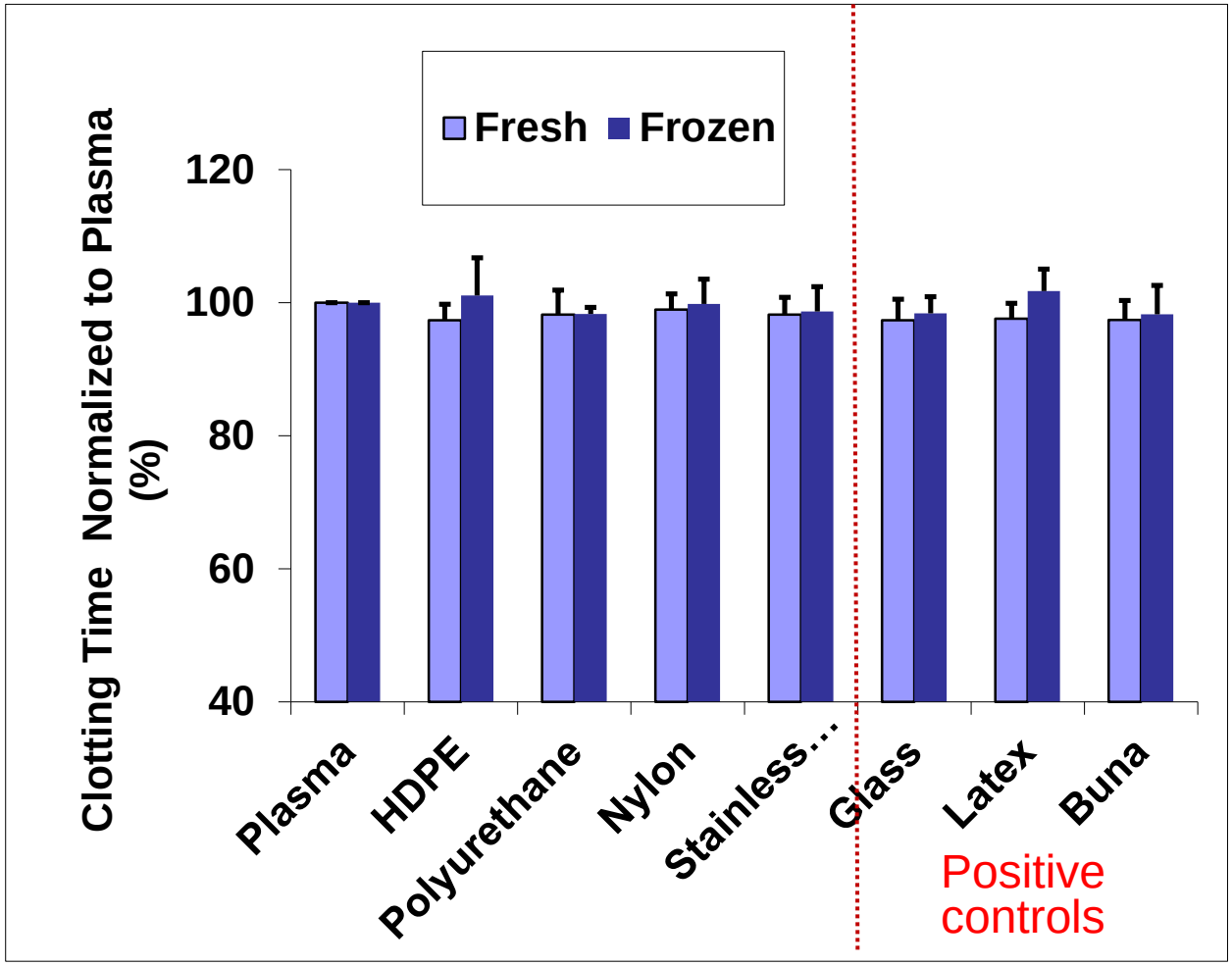
Typical PT Data Submitted to FDA

Parameters	Conditions/Values
Plasma	Fresh or frozen, human, citrated
Incubation time	15, 30, or 60 mins
Material surface to plasma ratio	3, 4, or 6 cm^2/ml
Positive control	No positive control material used
Sources of PT reagents	Vary from lab to lab
PT Clotting time	Range: 10 to 14 seconds; No significant difference between the plasma, test samples and reference materials

Evaluation of PTT and PT Assays at the Blood Damage Assessment Lab in FDA/CDRH

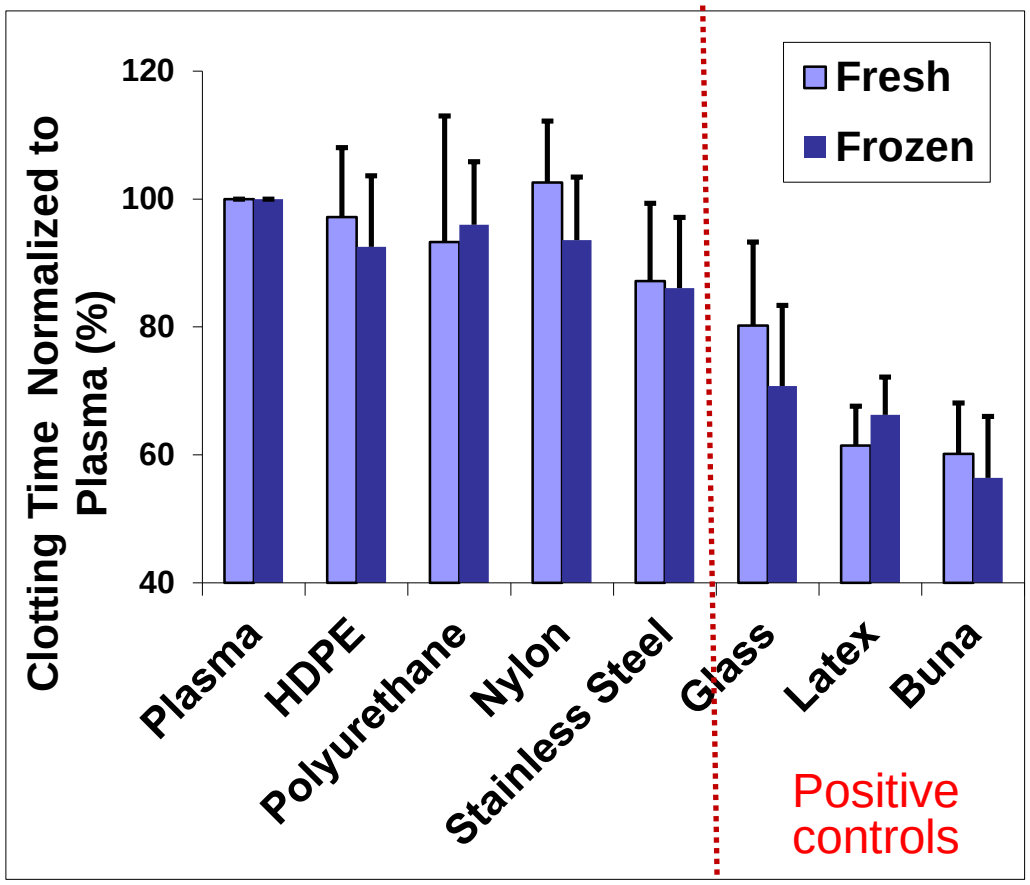
Parameters	Test Conditions
Plasma	Fresh or frozen, human, citrated
Incubation time	15, 30, or 60 mins
Material surface to plasma ratio	4 cm^2/ml (Same as in the ASTM standard)
Positive controls	Buna-N Rubber, Latex, Glass beads
Tested Materials	HDPE, Nylon, Polyurethane, 316L Stainless Steel

PT Test Results (Preliminary data, n=3 to 7)



- PT assay showed no difference between materials

PTT Results (Preliminary data, n=3 to 7)



- Showed the potential to differentiate between materials
- Only used PTT reagent from one source and one instrument for clotting time

Summary

- PTT and PT assays are static, acute tests that only evaluate the effects of materials on the plasma coagulation cascade.
  - Need other tests to evaluate flow/geometry/platelets
- PTT showed the potential to differentiate materials with different thrombogenicity; PT did not.
- Further research is needed to validate PTT test procedures and establish clinically relevant pass/fail criteria.

Thank you

- **Questions, comments, and suggestions?**

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