



# Thrombogenicity Testing

## Kallikrein Activation

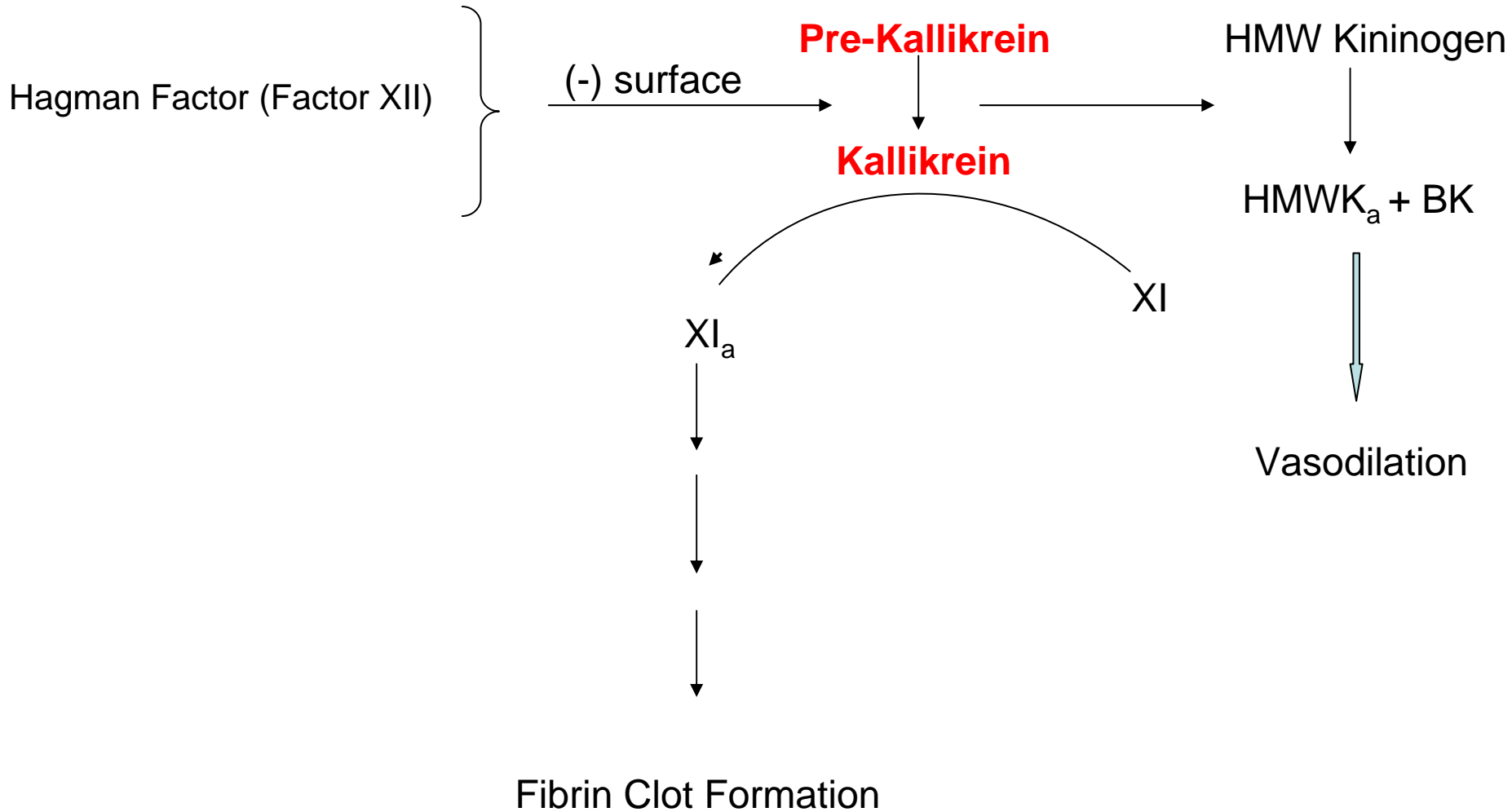
**This is an example of the type of testing Hydromer, Inc. can perform on a contract basis. Topic of this report features the new Hydromer F200 and F202 Thrombo-resistant coating technology.**

# Purpose

- Thrombogenicity refers to the tendency of a material in contact with the blood to produce a thrombus or clot. It not only refers to fixed thrombi but also to emboli, thrombi which have become detached and travel through the bloodstream.
- The biocompatibility of materials used in medical devices and the medical coatings associated with these devices can be evaluated by testing their “thrombogenic potential”. This can easily be accomplished by measuring the *in-vitro* activation of prekallikrein to kallikrein, one of the major proteins involved in initiating surface-mediated blood coagulation.

# Role of Kallikrein in Thrombogenicity

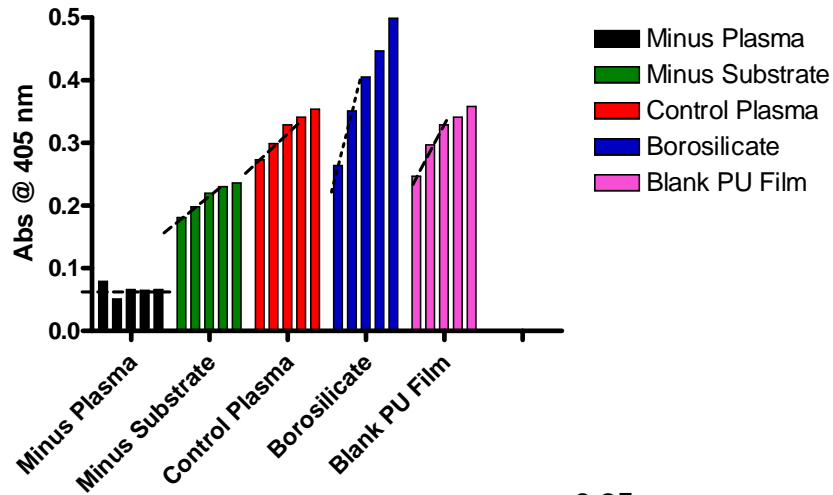
## Contact Activation (Intrinsic) Pathway



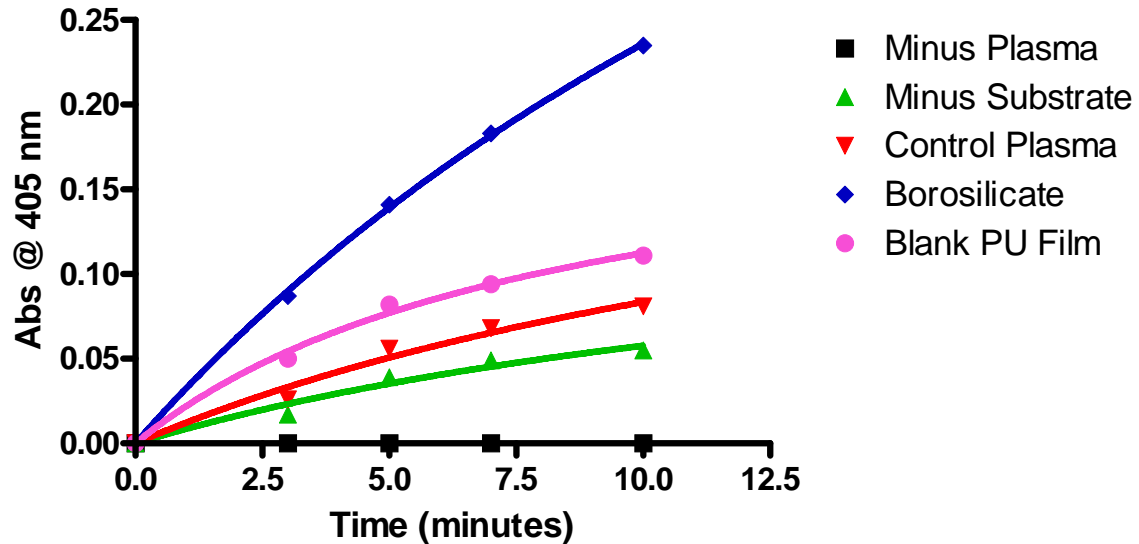
# Assay for Kallikrein Activation

- **Activation Step:** Normal citrated human plasma is incubated for 5 minutes @ 37°C or for 10 minutes @ 10°C with the surface to be tested.
- **Assay protocol:** Add buffer (50 µl of 0.10 M Tris-HCl, pH 7.8).
- Add diluted plasma sample (20 µl of 1:2).
- Start reaction with substrate (30 µl P-Kal reagent, 0.40 mM Final Conc., (American Diagnostica)).
- Monitor absorbance @ 405 nm

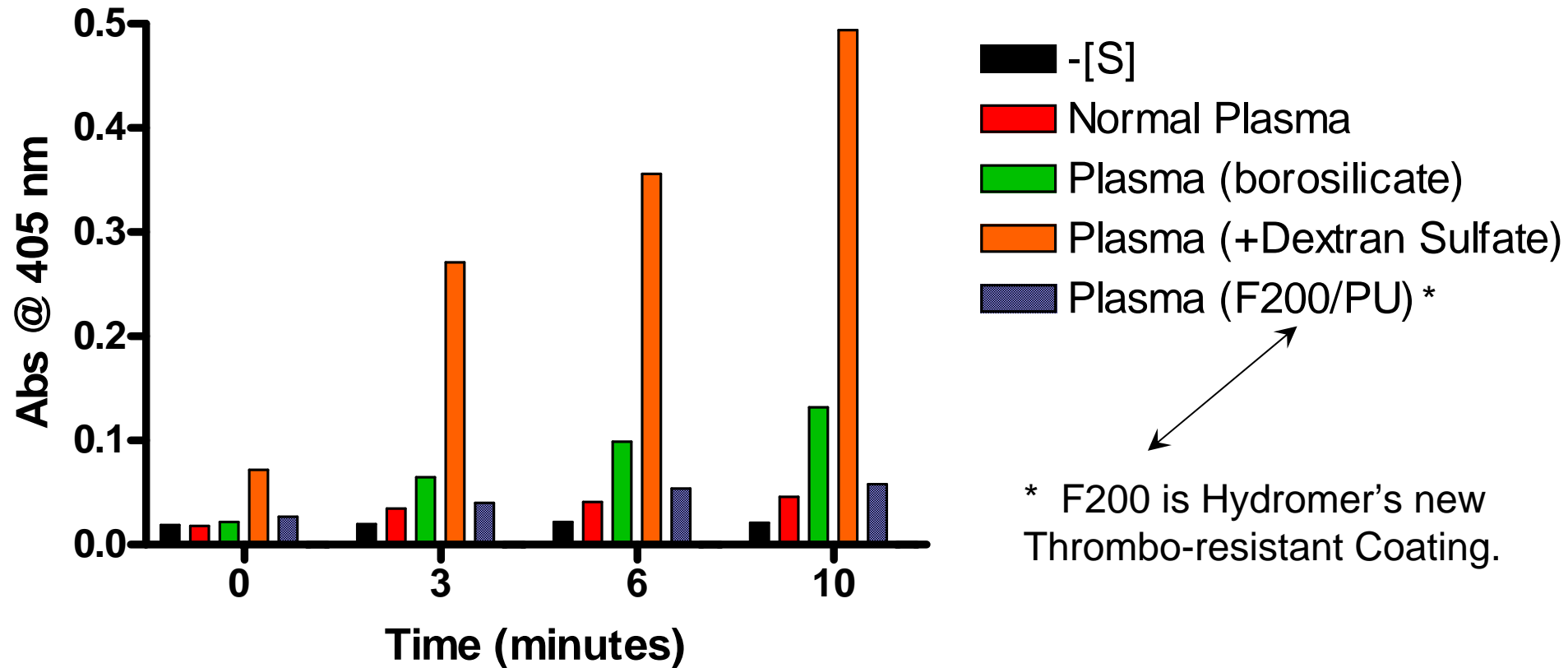
# Assay for Measuring the Rate of Conversion of Prekallikrein to Kallikrein



Time points are: 0, 3, 5, 7 and 10 minutes.

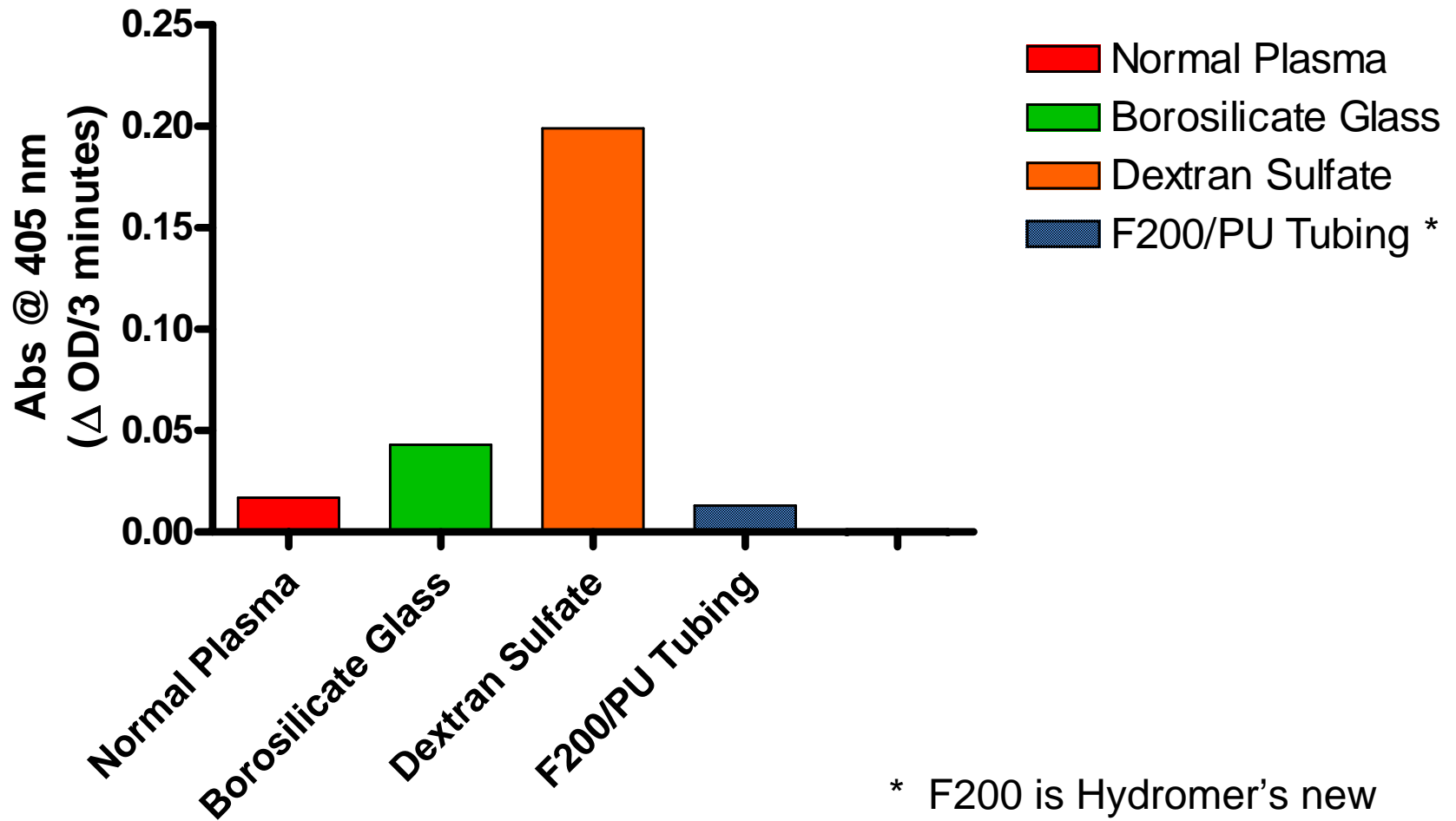


# Time Course of Kallikrein Activation of Normal Human Plasma



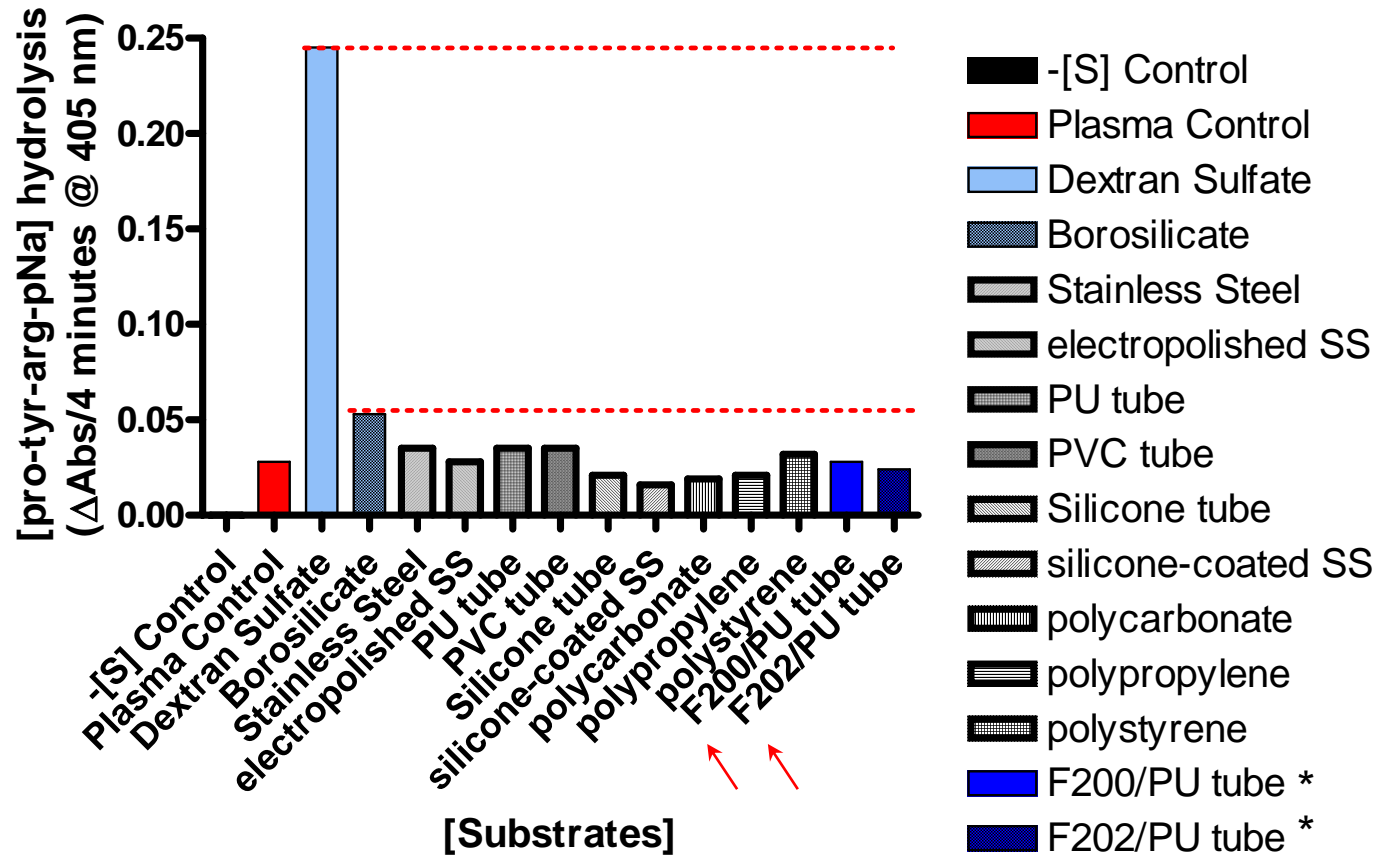
Plasma was diluted 1:2 with PBS and then placed in contact with test substance for 10 minutes @ 10°C. The diluted plasma was then tested for kallikrein activation for up to 10 minutes @ 37°C.

# Initial Rate (3 minutes) of Kallikrein Activation of Normal Human Plasma



\* F200 is Hydromer's new Thrombo-resistant Coating.

# Thrombogenic Potential of Various Substrates



Dextran Sulfate (0.10 mg/ml) and borosilicate glass are reported to be positive controls.

\*F200 & F202 are Hydromer's new Thrombo-resistant Coating.

# Usefulness of Kallikrein Activation Test

- This test has been used in the literature and can be used here at Hydromer to assess the “*Thrombogenic Potential*” of any given surface or coating.
- Our F200 and F202 coatings on polyurethane tubing do not appear to be thrombogenic.