

# EVALUATION OF THROMBIN PRODUCED FROM A SINGLE UNIT OF PLASMA USING THE THROMBIN ACTIVATION DEVICE

Semple E<sup>1</sup>, Bang A<sup>2</sup>, Kim M<sup>2</sup>, Madsen T<sup>1</sup>, Semple JW<sup>2</sup>.

1. THERMOGENESIS CORP. Rancho Cordova, USA 2. St Michael's Hospital, Toronto, Canada

**Introduction:** Currently, thrombin based clotting products used for fibrin sealant and platelet gels are primarily derived from pooled human plasma or bovine sources. Here we present a device that converts prothrombin to active thrombin using either autologous or allogeneic plasma from a single donor.

**Aim:** This study was designed to evaluate thrombin produced from a single unit of plasma using the Thrombin Activation Device (TAD, THERMOGENESIS CORP.) both in the consistency of thrombin production and activation of platelets. **Figure 1**

## Materials and Methods

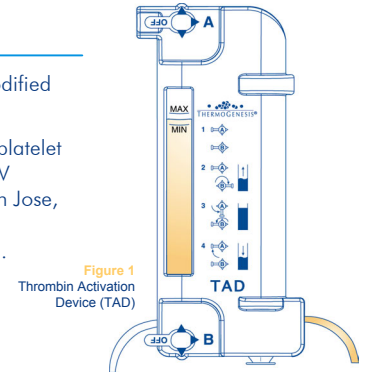
Single donor plasma from either whole blood donations or apheresis was used. As anticoagulant CPD, ACD, CPDA-1, CP2D and sodium-citrate was used.

**Production of thrombin using the TAD:** To 10 ml of plasma, Ca-ions and ethanol (7.2mM and 19% final concentration, respectively) was added. In the presence of a negative surface prothrombin was converted to thrombin in approximately 60 min.

**Figure 1**

**Thrombin activity:** Was determined using a modified Clauss method.

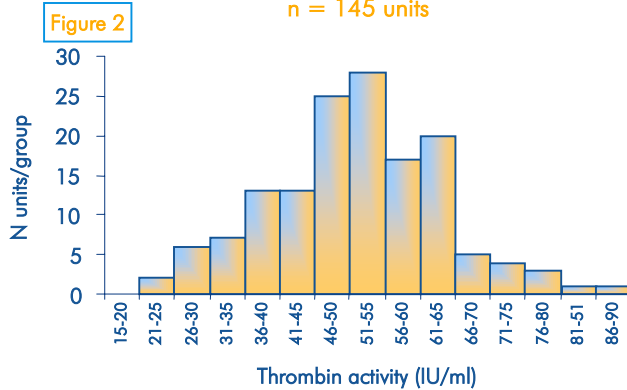
**Activation of platelets:** Was assessed in CPD platelet rich-plasma by anti-CD62 expression and Annexin V binding using flow cytometry (Becton Dickinson, San Jose, CA). TAD produced thrombin was compared with commercially available thrombin (Ortho) and CaCl.



## Results

### Distribution of Thrombin activity

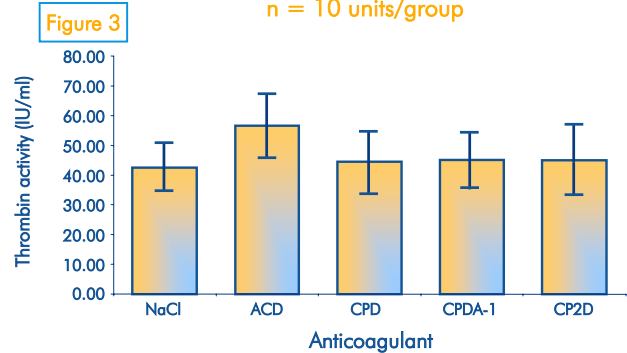
n = 145 units



**Figure 2** The range for thrombin activity in 145 units of CPD anticoagulated plasma was  $51.8 \pm 12.4$  IU/ml (Mean $\pm$ sd). 95% confidence interval: 49.8 - 53.8 IU/ml).

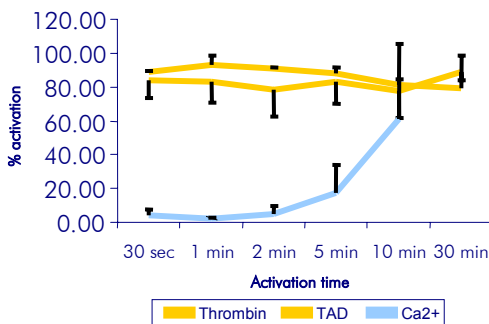
### Comparison of Different Anticoagulants

n = 10 units/group



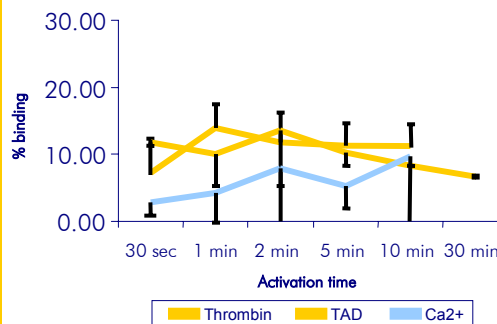
**Figure 3** There was no significant difference in thrombin activity using any of the citrate-based anticoagulants. Mean  $\pm$  sd.

### Platelet expression of CD62



**Figure 4**

### Annexin V binding



**Figure 5**

The ability of the TAD-produced thrombin to activate platelets was assessed by CD62 expression (Figure 4) and Annexin V binding (Figure 5), and found to be equivalent, both in speed and extent, to that of commercially available thrombin (Ortho). Final thrombin concentration: 1IU/ml.

Using Ca ions the activation was slower and reached comparable expression of surface markers as using thrombin after 10 minutes. Final concentration: 10mM.

Mean  $\pm$ sd, n=5/group.

**Conclusion:** These results demonstrate that TAD-produced thrombin from plasma anticoagulated with any citrate-based anticoagulant has consistent thrombin activity. The ability to activate platelets was found to be similar to commercially available thrombin.