Stability of Autologous Clotting Factor Produced at the Point-of-Care

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Statement of Purpose:

In the United States, bovine thrombin remains the primary hemostatic agent used in surgical procedures. Although generally safe, there have been clinical concerns associated with its use, including allergic reactions, potential transmission of bovine spongiform encephalopathy (BSE), and emergence of anticoagulopathies upon secondary exposure.[1]

A second potential source of thrombin is derived from pooled human sources. However, these sources can transmit infections such as HIV, hepatitis, and other viral diseases.[2]

To mitigate these risks, devices have recently been developed to create an autologous clotting factor solution (ACFS) from a patient's own blood at the point-of-care. One such system is the TPD™ system developed by Thermogenesis Corp. (Rancho Cordova, CA). Another system, the Clotalyst™ device, was co-developed as a second generation system by Biomet Biologics (Warsaw, IN) and Thermogenesis. Both systems operate by using a $CaCl_2$ -EtOH reagent and an inert, negatively charged surface to initiate a coagulation reaction to produce an autologous clotting factor solution from the donor's blood. The Clotalyst™ differs from the TPD™ in physical configuration and the addition of a 25°C temperature control during blood processing.

The purpose of this study was to determine if the stability of autologous clotting factor produced from these two systems is sufficient to be used in major surgical procedures. Both systems were used to process 12ml of fresh anticoagulated blood from 10 donors, producing approximately 6 ml of ACFS. Their ability to clot a standard fibrinogen solution was measured at various timepoints up to five-hours after production.

Methods:

Twenty-seven milliliters of whole blood were drawn from each of 10 donors into a 30ml syringe containing 3ml of the anticoagulant ACD-A. The sample was placed into a room temperature water bath (approx. 25°C) for a minimum of 25 minutes to equilibrate the temperature of the input blood. After equilibration to room temperature, 12ml aliquots were processed in each of the Clotalyst™ and TPD™ devices per manufacturer's instructions for use.

After preparation of the ACFS outputs, one-half of the sample was maintained at room temperature and the other at 4°C. The clot times of both aliquots were determined using a standard human fibrinogen solution, 1mg/mL concentration (Enzyme Research Laboratories, South Bend, IN), in a Start 4 machine (Diagnostica Stago, Parsippany, NJ). The samples were evaluated at 0, 30, 60, 90, 120, 180, 240, and 300 minutes after processing. Five minutes prior to the testing, each specimen at 4°C was sampled and warmed to room temperature using a 25°C water bath. Statistical significance was determined using a Student's t-test (α=0.05). In addition to a t-test, a coefficient of variance was calculated to demonstrate variability of the output from the two devices.

Results:

The clotting times of the Clotalyst™ and TPD™ outputs at time points up to five hours after production are listed in Table 1.

Time Post Production	Time (Seconds)			
	Clotalyst		TPD	
	RT	4°C	RT	4°C
0 Hours	9.8725 ± 1.77 n=10 (CV% 0.18)	10.03 ± 1.86 n=10 (CV% 0.19)	17.6775 ± 7.68 n=10 (CV% 0.43)	18.565 ± 9.09 n-=10 (CV% 0.49)
0.5 Hours	10.27 ± 2.00 n=10 (CV% 0.19)	11.2275 ± 2.06 n=10 (CV% 0.18)	31.7575 ± 26.74 n=10 (CV% 0.84)	24.7975 ± 14.73 n=10 (CV% 0.59)
1.0 Hours	10.8375 ± 2.05 n=10 (CV% 0.19)	11.9025 ± 2.15 n=10 (CV% 0.18)	36.025 ± 24.97 n=9* (CV% 0.69)	30.305 ± 26.26 n=10 (CV% 0.87)
1.5 Hours	11.785 ± 2.54 n=10 (CV% 0.22)	11.5275 ± 2.19 n=10 (CV% 0.19)	56.6444 ± 50.90 n=9* (CV% 0.90)	33.595 ± 28.19 n=10 (CV% 0.84)
2.0 Hours	11.3075 ± 2.04 n=10 (CV% 0.18)	11.3525 ± 2.07 n=10 (CV% 0.18)	53.7938 ± 40.75 n=8* (CV% 0.76)	32.97 ± 23.10 n=10 (CV% 0.70)
3.0 Hours	11.645 ± 2.65 n=10 (CV% 0.23)	11.3175 ± 2.40 n=10 (CV% 0.21)	70.679 ± 81.90 n=6* (CV% 1.16)	34.1306 ± 26.15 n=9* (CV% 0.77)
4.0 Hours	13.455 ± 3.26 n=10 (CV% 0.24)	12.8375 ± 3.30 n=10 (CV% 0.26)	73.935 ± 85.24 n=5* (CV% 1.15)	49.0472 ± 34.00 n=9* (CV% 0.69)
5.0 Hours	13.42± 4.06 n=10 (CV% 0.25)	12.06 ± 4.00 n=10 (CV% 0.29)	50.30 ± 61.81 n=4* (CV% 1.23)	45.7167 ± 37.17 n=9* (CV% 0.81)

Table 1- Average clotting times (seconds)
*Samples not available because no clot formed

Five hours after production, the clotting time of the room temperature TPD™ samples increased by approximately 162 seconds whereas the room temperature Clotalyst™ samples increased by approximately 4 seconds. The difference between products stored at either 4°C or room temperature was not significant. Even though the small sample size prevented determination of statistical significance, maintaining the TPD™ output at 4°C appeared to maintain its clotting ability for a longer duration.

Conclusions:

This analysis demonstrated that the Clotalyst™ device produces a more stable product that can be used effectively up to five hours after production. The TPD™ output degrades quickly and may only be effective up to one hour after production unless refrigerated. Future work should be done with a greater sample size to account for the variability of individual donor blood samples and reduce the high CV% found in this study.

References

1. Ortel TL *et al.* Immunologic Impact and Clinical Outcomes After Exposure to Bovine Thrombin. Ann Surg 2001;223(1):88-96.
2. Kawamura M *et al.* Frequency of Transmission of Human Parvovirus B19 Infection by Fibrin Sealant Used During Thoracic Surgery. Ann Thorac Surg 2002;73:1098-1100.