Thrombin Generation Assay

The Versiti Diagnostic Labs Thrombin Generation Assay (TGA) measures the overall potential of a plasma sample to produce thrombin. TGA is a global assay, and is a more effective method for assessing the potential for a plasma sample to coagulate, as compared to measurements using clotting assay such as PT or aPTT or individual factor assays. TGA uses a fluorescent endpoint that is not heavily influenced by turbidity, allowing for the presence of platelets or clot formation. This allows for a more physiologically relevant measurement of thrombin generation over time.

For research use only. Not for use in diagnostic procedures.

Assay utility:

The Thrombin Generation Assay can be used to determine the amount of thrombin that a plasma sample can generate over time after being activated by a small amount of tissue factor. This makes this test uniquely suited for testing plasma samples for their potential to cause thromboembolic events (TEEs). TGA can also be used to evaluate the effect of new coagulation-directed therapeutics and their thrombogenicity or assessing the potential for off-target hemostatic effects of other drug candidates undergoing clinical trials. Another area of interest is in studies on chronic diseases that involve coagulation abnormalities. Elevated thrombin has been associated with a hypercoagulability state and an increase in thrombotic risk whereas reduced thrombin generation has been correlated to poor outcomes in many hemorrhagic syndromes such as hemophilia.

Test method:

In the Thrombin Generation Assay thrombin production is initiated by the addition of Tissue Factor and phospholipids to a plasma sample. After a short incubation a thrombin specific, synthetic fluorogenic substrate plus calcium is added to initiate the thrombin generation reaction. The resulting fluorescent signal is then collected by measurement in a fluorometer at 20 sec. intervals for 1 hour. The resulting raw data is processed by a software package that eliminates variances due substrate consumption and background due to plasma color and signal loss. The sample data is then compared to a parallel reaction (calibrator reaction) where a thrombin calibrator (fixed amount of thrombin-alpha-2-macroglobulin complex) was added to the same test plasma. The fluorescent signal is created by the cleavage of the synthetic substrate by thrombin's endogenous enzymatic activity releasing the fluorophore. The measured output signal, after correction, is proportional to the amount of thrombin present in the reaction when compared to the reference rate of the calibrator reaction. The result is a curve or thrombogram where the area under the curve is quantified and translated into an Endogenous Thrombin Potential (ETP) expressed as nmol/L thrombin x min.

Assay sensitivity and limitations:

This assay requires using citrated platelet poor plasma (PPP). Use with any other sample material (platelet rich plasma, cryoprecipitate or drug formulation) would require further validation. Versiti has established an ETP range for the assay, running multiple normal samples (½ male and ½ female). A low responding and high responding sample outside of the normal reference range were tested. Both samples were found to react correctly. Inter and intra-assay studies indicated good agreement between all samples with a CV≤ 10%.

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Specimen requirements:

- 0.600 uL citrated plasma (1000 uL preferred) frozen in a plastic tube.
- Follow the manufacturer's instructions for processing the collection tubes.

Recommended Specimen Collection:

- The anticoagulant used for coagulation assays should be 105 to 109 mMol/L, 3.13% to 3.2% (commonly described as 3.2%) of the dihydrate form of trisodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), buffered or nonbuffered (light blue top vacuum tube).
- Follow the guidelines for Hemostasis Processing found in the Diagnostic Labs FAQ: www.versiti.org/faq/diagnostic-lab-faq

SHIP

Shipping requirements:

Ship the specimen(s) on a minimum of 5 pounds of dry ice. Use only plastic tubes and cushion them to protect from breakage during shipment. Please be aware that dry ice is also considered a hazard for shipping and must be packaged in compliance with DOT, IATA, and the requirements of the overnight carrier used. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Hemostasis Laboratory
Versiti – Wisconsin
638 N. 18th St.
Milwaukee, WI 53233

ORDER

Order Information:

Contact the Versiti Clinical Trial Services Office:

1-800-245-3117 x6250

Labinfo@versiti.org

Test Code: 1980

References:

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