**PRINCIPLE**

Thrombin and calcium are required to activate FXIII such that it will cross-link fibrin into a stable form. In this method, despite using citrated plasma, sufficient calcium ions are still available for FXIII activation.

A normal ethylenediaminetetraacetic acid (EDTA) anticoagulated plasma is used for a control. In this plasma, EDTA results in a complete chelation of calcium ions, which means that the FXIII is not able to crosslink fibrin. Addition of 2% acetic acid or 5M urea results in the lysis of non-cross-linked clots, whereas citrated plasma with greater than 10 U/dl of FXIII activity has an insoluble clot. The test is generally more sensitive if acetic acid (rather than urea) is employed, since the clot will dissolve at higher levels of FXIII in the presence of acetic acid (Jennings et al. 2003).

**MATERIALS/REAGENTS**

- 75 × 10 mm glass tubes
- 0.9% saline
- 30 U/ml thrombin
- Normal EDTA plasma
- 2% acetic acid

**METHOD**

1. Add 0.2 ml test citrated plasma to 0.2 ml 0.9% saline in a glass tube. For a positive control, repeat with 0.2 ml EDTA plasma. For a negative control, repeat with 0.2 ml normal citrated plasma.

2. Add 0.1 ml of 30 U/ml thrombin. Mix.

3. Leave for 30 minutes at 37°C.

4. Flick tubes to loosen clot from sides.

5. Add 5 ml 2% acetic acid and stopper the tube. Leave at room temperature for 12 hours.
RESULTS
• EDTA plasma should have no visible clot.
• Normal citrated plasma should have an intact, visible clot.
• If clot is not visible, the subject has FXIII deficiency.

NORMAL RANGE
Normal subjects have a visible clot after 12 hours in 2% acetic acid.

NOTES
• 5M urea can be used in place of 2% acetic acid. The incubation time for clot dissolution is then 18 hours. This method is less sensitive than employing acetic acid (described above).
• Clotting with calcium and lysis with urea produces abnormal results only when levels of FXIII are below 5 U/dl. By comparison, clotting with 30 U/ml thrombin followed by lysis with 2% acetic acid produces abnormal results at levels below 10 U/dl (Jennings et al. 2003).
• Occasionally, patients with FXIII levels above 5 U/dl may bleed (see Bolton-Maggs et al. 2004 for review).

REFERENCES