INTRODUCTION

Dysfibrinogenemia (type II fibrinogen deficiency) is suspected when the Clauss fibrinogen assay result is significantly lower than the prothrombin time-derived fibrinogen or fibrinogen antigen results.

The Clauss assay determines fibrinogen level by determining the rate of fibrin formation after addition of a high concentration of thrombin, whereas the derived assay measures optical density/light scatter through a formed fibrin clot after clot formation is complete.

The fibrinogen antigen assay detects dysfibrinogenemia as low activity by Clauss assay in the presence of a normal antigen level.

A fibrinogenemia may be associated with bleeding due to defective plasma coagulation and platelet function. A multitude of defects cause dysfibrinogenemia, which may be characterized by impaired release of fibrinopeptide A/B and impaired fibrin monomer polymerization. Type II defects may be asymptomatic, but some variants have been associated with predisposition to bleeding or thrombosis. A patient’s bleeding or thrombotic tendency may be unrelated to dysfibrinogenemia, which may be a chance finding.

MATERIALS

NOR-Partigen Fibrinogen R.I.D. Plate

Manufacturer: Dade Behring

Each plate has 12 wells.

REAGENTS

- A calibration plasma with known fibrinogen concentration that is traceable back to a WHO International Standard for fibrinogen.

- Test Plasma: Citrated plasma is normally tested undiluted, or diluted in saline to give a level of around 2.5 g/l if the level is anticipated to be greater than 5 g/l. Results of less than 0.5 g/l are reported as <0.5 g/l (lower limit of sensitivity).
METHOD

1. Allow the RID plate to warm up to room temperature, then remove the cover and leave open for 5 minutes to evaporate excess moisture.

2. Standard plasma is tested neat and at 75%, 50%, and 25% dilutions in saline.

3. Add exactly 5µl dilutions of standard, quality control, and patient plasma (preferably in duplicate) to the wells. An accurate pipette is essential for the small volumes required.

4. As soon as the samples have diffused into the gel (no more than five minutes after application), replace the cover and place the plate flat in a wet box at room temperature for approximately 48 hours.

5. Results can be estimated after 18 hours, as long as no diameter exceeds 5.5 mm. Final results must be reported after two days, unless a diameter is greater than 8 mm, in which case the plate is left for a further day to allow diffusion to complete. A diameter of >9.3 mm indicates that the fibrinogen level is greater than the level of sensitivity of the assay, in which case the sample must be diluted in saline and re-tested.

6. Measure precipitation diameters using the Behring plate reading device (available with the plates). Measure to nearest 0.5 mm and read twice (diameters at 90° to each other).

7. Plot the standard curve: diameter² against concentration on linear graph paper.

8. Read off quality control and patient levels from this curve.

9. Report results in g/l if quality control is within target range.