Scottish Trial: EVIII RAg (VWF Ag) Assay Method (CAPD196)

VWF Ag Assay - Larwell Immunoimmunoelectrophoresis

Reagents & Materials:
Glass plates 160 x 80 mm.
1% Agarose: Miles Medium electrophoresis, 25mls.
Buffer: Tris/Tricine pH 8.6.

per 4 litres:
Tris 39.2g
Ca lactate 0.424g
Tricine 17.2g
Thioglycolic 0.4g
Distilled water 964 litres.
Adjust pH to 10 N HCL.

Antibody: BR 8173: Home produced Rabbit anti-human VWF Ag. Used at concentration of 1/500 ml of agarose.

Method:
Normal plasma: Freeze dried pool of 30 B.T.S. donors.
Assume VWF Ag level of one unit per ml.
Diluted 1/2, 1/5, 1/10, 1/20 in electrophoresis buffer.

Test plasma, diluted 1/5 & 1/10 in electrophoresis buffer tested in duplicate.

10 μl antigen applied per well.
Run at 150 volts overnight on cooling plate.

Roeed heights measured from centre of well.
Plotted on double log graph paper.