Scottish Trial. FVIII:Ag (FVIII:Ag) Assay Method (CARDIFF)

The FVIII:Ag assay used in Cardiff is a two-site, single antibody, solid phase IRMA carried out in polystyrene tubes.

Principle.

i) 

\[ \text{Saturate Ab (carboxylic acid purified whole IgG fraction of high titre human inhibitor plasma in bicarbonate buffer pH 9.6, 4°C overnight.} \]

Wash 3 barbitol buffer + Bovine serum albumin + then distilled water.

\[ \text{Antigens (diluted in barbitol buffer + 5% BSA, 37°C overnight.} \]

Wash 3 x 1  \text{barbitol buffer + 0.1% BSA.} \]

ii) 

\[ \text{Radiolabelled immunopurified inhibitor IgG as above (¹²⁵I, 4,000 counts/male). 37°C for 2hrs.} \]

Wash 3 distilled water. Count radioactivity.

Freeze dried 50 donor B.T. S. pool used as source of normal plasma. Diluted 1/5, 1/10, 1/20, 1/40, 1/80, 1/160, 1/320, 1/640, 1/1280.

In duplicate.

Test samples diluted according to expected FVIII:Ag levels. 5 dilutions in duplicate.

Standardised against X11th Std.