

Coagulation Factors VIII, IX, XI, XII

Factor VIII is a key coagulation factor of the intrinsic pathway; activity levels are based on correction of clotting time for plasma deficient of the factor of interest and reported as a percent

Materials

1. ST4 semi-automated mechanical coagulation instrument (Diagnostica Stago, NJ)
2. 4-well cuvettes
3. Magnetic mixing ball
4. Citrated plasma samples
5. HN/BSA
6. aPPT reagent
7. 25 mM CaCl₂
8. Citrated plasma deficient of factor VIII[#]
9. Normal mouse plasma (NMP) BL/6 pool

Procedure

1. Instrument, cuvettes, and mixing balls are pre-warmed to 37°C
2. Citrated plasma samples are diluted 1/20 in HN/BSA
3. 30 µL of sample dilutions, in duplicate, are added to each well, followed by 30 µL of citrated plasma deficient of factor VIII[#] and then incubated at 37°C for 5 minutes
4. 30 µL of CaCl₂ is added to each well to initiate clotting
5. Time until clot formation is measured in seconds
6. Time is interpolated on a standard curve based on NMP serial dilutions and reported as %BL/6

[#] For Factor IX, Factor XI, and Factor XII: follow the factor VIII method, using plasma deficient of the specific factor being measured in place of factor VIII deficient plasma.