One-Stage Intrinsic Assay of Prekallikrein (PKK) and High Molecular Weight Kininogen (HMWK)

**PRINCIPLE**

HMWK (Fitzgerald factor) and PKK (Fletcher factor) are coagulation factors found in the contact pathway. A reduction in either causes a greatly prolonged APTT with most APTT reagents, depending on the activator used. In the complete absence of HMWK or PKK (<1 U/dl), the APTT is normally >200 seconds and is longer than the APTT with the same test system in the presence of complete deficiency of factors VIII or IX. APTT reagents that utilize ellagic acid as activator (for example, Actin FS) will give completely normal results with either deficiency. Therefore, such reagents cannot be used in the one-stage assay of these factors.

The dose-response curve in the one-stage assay of these factors is steeper for some reagents than others. The method below describes use of one particular reagent for which the dose-response curve is particularly steep, leading to more accurate and precise assay results.

**REAGENTS**

- Dapttin APTT reagent (Technoclone, Vienna, Austria)
  Store at 2°C–8°C, as per manufacturer’s instructions
- 25mM CaCl₂
  Store at 2°C–8°C
- Owren’s Veronal buffer
  Store at 2°C–8°C
- Prekallikrein (Fletcher factor)-deficient plasma
  Example: Freeze-dried (Technoclone, Vienna, Austria)
  Store at 2°C–8°C
- HMWK (Fitzgerald factor)-deficient plasma
  Example: Freeze-dried (Technoclone, Vienna, Austria)
  Store at 2°C–8°C
- Reference plasma (for example, pooled normal plasma, see Section 7)
- Internal quality control sample
METHOD

1 Assay design is as for one-stage assays of FVIII described in Section 23 (i.e. 3 dilutions of standard, 3 dilutions of test plasma).

2 Dilutions are made using Owren’s buffer.

3 The most suitable assay dilutions are typically higher than those used in one-stage FVIII or FIX assays previously described.

4 Analysis and construction of calibration curve, as well as calculation of test results, are as described under the one-stage FVIII assays (Section 23).

Normal ranges from the literature are as follows:
HMWK: 0.70–1.20 U/ml (70–120 U/dl)
PKK: 0.70–1.20 U/ml

At the time of writing, there are no International Standards for HMWK or PKK.