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Title: Prekallikrein Activator Assay	

# Prekallikrein Activator Assay

## Sysmex Automated Coagulation Analysis System (SCS)

### Principle of Assay

Prekallikrein activator (PKA), also known as activated FXII or activated Hageman Factor, is a contaminant in human albumin solutions and immunoglobulin solutions prepared from fractionated plasma. Adverse events, including vasodilation and hypotension, may occur where albumin or immunoglobulin solutions are contaminated with significant levels of PKA and are infused into patients. This is of concern since albumin may be administered as a plasma expander to counter hypovolaemia, hence the treatment may exacerbate the existing problem.

1. Prekallikrein Activator sample + Prekallikrein excess  $\Rightarrow$  Kallikrein  
 $\Downarrow$
2. Kallikrein + H-D-Pro-Phe-Arg-pNA $\cdot$ 2HCl  $\Rightarrow$  H-D-Pro-Phe-Arg $\cdot$ OH + pNA405nm

#### Figure 1 Prekallikrein Activator assay principle.

The sample is incubated with prekallikrein derived from human plasma. The prekallikrein activator in the sample converts prekallikrein to kallikrein. The kallikrein cleaves a substrate in a kinetic test measuring the increase in absorbance at 405 nm (Figure 1). The measured kallikrein activity is directly proportional to the activity of prekallikrein activator in the sample. Some samples may contain endogenous kallikrein and proteases that can cleave the used substrate as well. This endogenous activity is measured by including a sample blank for each sample tested. The sample blank assay does not include prekallikrein, instead, the sample is incubated directly with the substrate to measure only the endogenous activity (Figure 2). The assay blank absorbance is then subtracted from the assay absorbance to give the actual prekallikrein activator potency of each sample.



#### Figure 2 Prekallikrein Activator assay blank.

## Purpose and Scope

This document is to provide a method to measure the prekallikrein activator in therapeutic products such as immunoglobulins like Octagam® and Intragam® and Albumins like Albumex®.

## Uncertainty of Measurement (UoM)

For details regarding UoM, refer to Bio-BEE-SOP-26 – Blood Assays Testing Procedure.

## References

- European Union Guidelines ADOPTED in Australia, Blood/Plasma Guidelines
- Certified Product Details (CPD), various human immunoglobulin products
- European Pharmacopoeia 5.02, Biological tests 2.6.15 Prekallikrein Activator. 01-2005:20615 corrected 5.0

## Materials/Equipment

### Equipment

Sysmex Coagulation System (SCS) - wavelength is 405 nm.

Other equipment as detailed in Bio-BEE-SOP-26 – Blood Assays Testing Procedure.

### Standards

#### *2nd International Standard (IS) for Prekallikrein Activator*

NIBSC – Cat# 02/168 - (Also available as Prekallikrein Activator in Albumin, British Reference Preparation (BRP) Batch 1 (Y 0000263) from EDQM).

- Assigned potency = 29 IU/vial
- Stored at -20 °C
- Bring the lyophilised standard to room temperature before reconstitution
- Reconstitute the contents of each vial with 1.0 mL of ddH<sub>2</sub>O. Swirl gently
- Complete dissolution may take up to 30 minutes
- Once reconstituted, the PKA solution should be stored on ice and used within 1 day
- International Standard can be aliquotted and stored and kept at -80 °C for stability study

#### *Prekallikrein Activator 8th In-House Standard (IHS)*

Use IHS in first instance, except when validating against International Standard.

Privigen ® IVIG 10% (Baxter, 20 g/200 ml B# 4347000004, LIMS# 1511004136-R1) diluted 1/1.67 with Buffer B – Privigen Ig Buffer. Then 1st BRP for Prekallikrein Activator (NIBSC - Cat#79/572) was diluted 1/2 with Privigen Ig Buffer. Calibrated against the 2nd IS for Prekallikrein Activator, 02/168.

- Assigned potency = 39.2 IU/mL
- Acceptance range = 35.28 – 43.12 IU/mL
- Stored at -80 °C
- Rapidly thaw in a 37 °C water bath immediately before assay, store at room temperature, use within 2 hours
- Unused thawed control is to be discarded. Do not re-freeze
- Note: The Prekallikrein IHS must be assayed against IS at least once per year for stability review

***Prekallikrein Activator 8th In-House Control (IHC)***

The 8th In House Std (IHS) was diluted 1/2 with Privigen Ig Buffer. Calibrated against the 8th IHS for Prekallikrein Activator. LIMS# 1802000561.

- Assigned potency = 19.5 IU/mL
- Acceptance range = 17.55 – 21.45 IU/mL
- Stored at -80 °C
- Rapidly thaw in a 37 °C water bath immediately before assay, store at room temperature, use within 2 hours
- Unused thawed control is to be discarded. Do not re-freeze
- Note: The Prekallikrein IHC must be assayed at least once per year for stability review

**Samples**

Refer to Bio-BEE-SOP-26 – Blood Assays Testing Procedure. Reconstitute samples as per manufacturer's instructions, then further prepare the samples as detailed in Bio-BEE-Form-23 – Worksheet PKA.

**Note:**

**For Immunoglobulin samples only – dilute to 30 g/L (3%) with Buffer B before assay**

**Albumin samples - to be tested neat**

**Reagents**

For common sysmex reagents refer to Bio-BEE-SOP-26 – Blood Assays Testing Procedure.

All lyophilised reagents should be brought to room temperature before reconstitution.

Details of Reagents, such as batch number and expiry, should be recorded on Bio-BEE-Form-23.

***Prekallikrein Substrate (PK Fraction)***

CoaChrom Diagnostica - Cat#COA0022.

***Preparation of the reagent:***

- Reconstitute the contents of each vial with 2.0 mL of ddH<sub>2</sub>O
- Leave for 30 minutes at +15° C to +25 °C. Mix gently once more before use

- Dilute 1/5 with Buffer B immediately before use

*Storage and stability:*

- Store unopened at +2 °C to +8 °C until the expiry date
- Stability after reconstitution (when stored in the original vial):
  - 24 hours at room temperature
  - 48 hours at +2°C to +8°C
  - 2 months at -20°C
- Rapidly thaw the frozen reagent in a 37°C water bath immediately before assay. If there are particles in the reagent (mostly lipoproteins), allow the vial to rest for 15min at room temperature and use the supernatant or centrifuge the reagent in plastic or siliconised tubes at 2000xg for 10min. Do not re-freeze

***Substrate S-2302™***

Chromogenix - Cat# CX000 82034039.

- Each vial contains chromogenic substrate S-2302 (H-D-Pro-Phe-Arg-pNA·2HCl) 25 mg and mannitol (bulking agent) 60 mg
- Reconstitute the contents of each vial with 10.2mL Buffer B (4mM)
- Dilute 1/2 with Buffer B immediately before use (2mM). Store unopened at +2 °C to +8 °C until expiry date
- After reconstitution, the 4 mM solution is stable for 6 months at +2 °C to +8 °C

***Buffer B (Ph. Eur.)***

(Also used as “Buffer B Reag” for ‘blank’ tests on the sysmex)

- Dissolve 3.03 g Tris(hydroxymethyl)aminomethane and 4.39g sodium chloride in 450 mL of ddH<sub>2</sub>O. Adjust to pH 8.0 with 2 M hydrochloric acid. Bring the final volume to 500mL
- Stability: 4 weeks at +2 °C to +8 °C

## Performing the Assay

- Follow the procedures and methods outlined within the ‘Associated Documents’
- Follow procedure outlined in Bio-BEE-SOP-26 – Blood Assays Testing Procedure with the following variations
  - Both ‘test’ and ‘blank’ protocols need to be selected for each standard/control/sample
  - The standard is tested using a 5-point std curve (neat, 1 in 2, 1 in 4, 1 in 8 and 1 in 16)
  - All samples and control are tested neat (for Immunoglobulin neat will be at 30 g/L)
  - Three replicates each of standard/control/sample should be assayed to obtain a valid UoM range

## Data Analysis

- Enter the raw data results into Bio-BEE-Form-24 - PKA Assay Spreadsheet from the QMS. Save this spreadsheet in the relevant testing folder in Trim
- The correlation coefficient value must be above 0.99. If not, the assay is invalid – discuss with supervisor
- The spreadsheet only uses absorbance values that fall into the range of the standard curve for calculation.

## Conclusions & Recording Results

Follow procedures outlined in Bio-BEE-SOP-26 – Blood Assays Testing Procedure.

## Associated Documents

- Bio-BEE-SOP-26 – Blood Assays Testing Procedure
- Bio-BEE-Method-9 – Performing a Sysmex Assay
- Bio-BEE-Form-23 – Worksheet - PKA
- Bio-BEE-Form-24 – Spreadsheet – PKA Lims# 30002