BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay)

Technical File

Product Ref. N°	A221005
Kit presentation	Chromogenic method, R1: ATIII (human); R2:FXa (bovine); R3: Substrate
Status	RUO

Intended use:

Two stages Anti-Xa chromogenic assay for Heparin (UFH or LMWH) activity measurement on human citrated plasma, or purified medium, with manual or automated methods, according to Pharmacopeias (USP, EP).

Assay principle:

Heparin binding to Anti-thrombin (AT) induces a conformational change, thereby increasing inactivation of factor Xa. The residual factor Xa, not inhibited by the Heparin–AT complex, is quantified with a chromogenic substrate. The amount of pNA released (measured at 405 nm) is then dependent on the residual factor Xa. There is an inverse relationship between the concentration of heparin and color development.

Heparin + AT ——————> [AT Hep.]

[AT Hep.] + [FXa (excess)] ————> [FXa-AT-Hep.] + [residual FXa]

[residual FXa] + Substrate ————> Peptide + pNA

Anti-Xa assay in practice

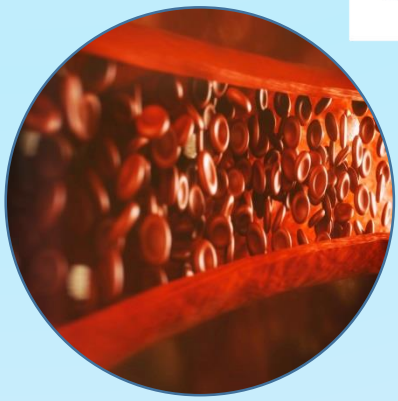
- **Pharmaceutical Applications:**
  - AT dependant assay developed to ensure the specificity of the method for measuring the anticoagulant activity of heparin. Heparin potentiates AT to inactivate factor Xa.
  - Used in combination with the anti-IIa assay, the anti-factor Xa to anti-factor IIa ratio can be measured.
  - This assay is compliant with pharmacopeias.
- **Market requirements & challenges:**
  - Dynamic range compliant with pharmacopeias, manual or automated methods available, easy to use.

Key Characteristics & Advantages:

Complete solution compliant with Pharmacopeias

Easy and fast :
- Lyophilised and ready to use reagents, rapid (total assay time < 3 min with automated methods and <6 min with manual methods)
- Easy to use on major coagulation analyzers, microplate or with basic equipment
Method applications: Sysmex CS-series, STA-R® series

Optimized consumption with good stability
- After reconstitution: 15 days at 2-8°C, 7 days at 18-25°C, 2 months frozen
- On board: 18 hours (CS series and STA-R® series)
- Shelf life: 30 months



Extended measurement range useful to explore various contexts
- **CS series: 0.086 – 1.250 IU/mL for UFH and 0.090 – 1.250 IU/mL for LMWH**
- **STA-R® series: 0.12 – 1 IU/mL for UFH and 0.15 – 1 IU/mL for LMWH**

Excellent Precision with low CVs:
- CS series: Within-run < 3 % (UFH / LMWH)
- STA-R® series: Within-run < 5 % (LMWH) and < 10% (UFH)

Specific and reliable assay, reduced interferences related to sample content
(Hemoglobin, Bilirubin, Intralipids, Dabigatran, Apixaban)

Safe and standardized method for optimal testing
- Separate packaging for buffers to be compliant with each parmacopeia
- High quality raw materials used
- Calibrators/controls traceable to WHO International Standards

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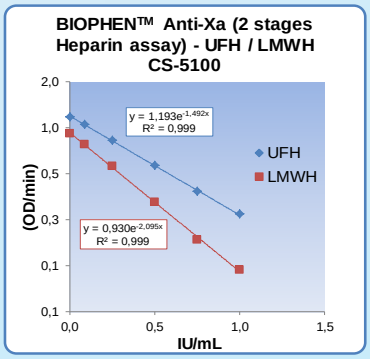
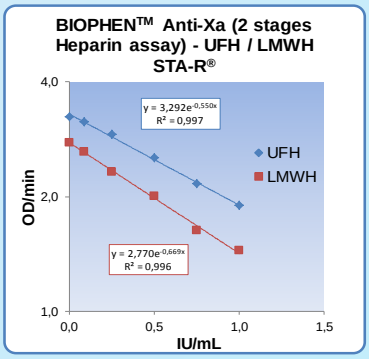
Assay Characteristics*:
Standardized method for manual or automated use, compliant with pharmacopeias

Calibration curves:





Precision (Intra-series):

CS-5100 (n ≈ 10 determinations)

- CV% 2.9% (UFH, 0.17 IU/mL)
- CV% 1.2% (UFH, 0.42 IU/mL)
- CV% 2.0% (LMWH, 0.28 IU/mL)
- CV% 1.6% (LMWH, 0.51 IU/mL)

STA-R® (n ≈ 10 determinations)

- CV% 9.5% (UFH, 0.23 IU/mL)
- CV% 7.5% (UFH, 0.49 IU/mL)
- CV% 3.5% (LMWH, 0.35 IU/mL)
- CV% 4.9% (LMWH, 0.56 IU/mL)

Interferences:

CS-5100: No interferences up to….

- 500 mg/dL Hemoglobin, 30 mg/dL Bilirubin, 1000 mg/dL Intralipids, 200 ng/mL Dabigatran, 50 ng/mL Apixaban

STA-R®: No interferences up to….

- 250 mg/dL Hemoglobin, 60 mg/dL Bilirubin, 1000 mg/dL Intralipids, 400 ng/ mL Dabigatran, 50 ng/mL Apixaban

Reagents preparation:

After reconstitution of each reagent by 1 mL of distilled water, just before use, dilute 1/5 in the appropriate buffer according to the Heparin to be assayed:

	Dilution of reagent		Buffer(1)	
Heparin	LMWH	UFH	LMWH	UFH
R1	1/5	1/5	AAR005L	AAR030K**
R2	1/5	1/5	AAR005L	AAR030K**
R3	1/5	1/5	AAR029K	Distilled water

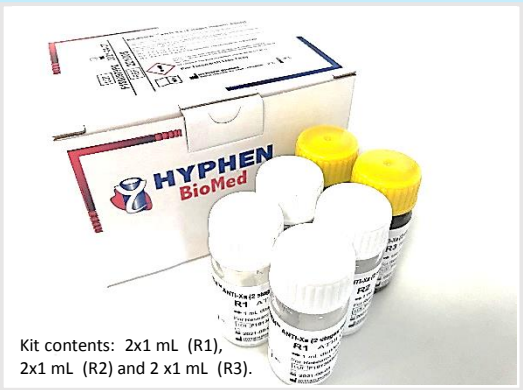
Calibrators, samples and controls preparation:

Just before use, dilute 1/15 in the appropriate buffer according to the Heparin to be assayed and the pharmacopeia followed:

Heparin	Dilution	Buffer(1)
LMWH	1/15	AAR005L (EP) or AAR028K (USP)
UFH	1/15	AAR030K**

Volumes and incubation times are harmonized for easier handling and automation. Final reactional concentration are compliant with Pharmacopeia.

Kit presentation:



Kit contents: 2x1 mL (R1), 2x1 mL (R2) and 2 x1 mL (R3).

Related products:

Needed	Product name	Ref N°:
Calibrators / Controls	BIOPHEN™ UFH Control Plasma BIOPHEN™ LMWH Control Plasma BIOPHEN™ LMWH Control Low BIOPHEN™ Heparin Calibrator BIOPHEN™ UFH Calibrator	A223101 / A224101 / A223901 A223001 / A223801 / A224201 A223701 / A224301 / A224401 A222001 A222301
(1) Buffers	Tris-EDTA-NaCl-PEG, pH 8.40 Tris-NaCl-EDTA-BSA, pH 8.40 (optional) Tris-NaCl-BSA, PH 7.40 Tris-NaCl, pH 7.40 Tris-EDTA-NaCl, pH 8.40	AAR030K AAR031K AAR005L AAR028K AAR029K

Related products	Product name	Ref N°:
Reagents	BIOPHEN™ Heparin LRT BIOPHEN™ Heparin BIOPHEN™ Anti-IIa (2 Stages Heparin Assay)	A221011-RUO/A221013-RUO/ A221015-RUO A221003 / A221006 A220005

* The values cited for the performance characteristics of the assay represent example of typical results and are not to be regarded as specifications for the test.
** AAR031K buffer can be used instead of AAR030K buffer.

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