

Neuville sur Oise, July 24th, 2017

Dear Customers,

To adjust the Heparin Anti-Xa (2 stages) and Heparin Anti-IIa (2 stages) assays to the new pharmacopeias' evolution (USP, EP) we introduced slight modifications of presentations, and they concern mainly dilution buffers proposed in our BIOPHEN Heparin Anti-Xa (2 stages) (# 221010) and BIOPHEN HEPARIN Anti-IIa (2 stages) (# 221025) reagents.

We therefore changed both kit presentations, in order to comply with each pharmacopeia (USP 40:2017 and EP 9th Edition 2017).

The new modified kits will replace the existing ones. Kit performances and applications are maintained.

Only the dilution buffers for LMWH measurements have been modified, as per the USP/EP changes. In addition, these dilution buffers for LMWH are different in recommendations from USP or EP. This is why the different buffers are now proposed separately from the kit core, which contains AT, enzymes (Xa or IIa), and FXa or FIIa specific substrates, and which is unchanged.

We then confirm that enzymes (FXa or Thrombin), Antithrombin and substrates are offered in one kit for each anti-Xa or anti-IIa (2-stage) assays. Presentations of these basic components in both kits are unchanged.

Briefly, discontinued presentations are:

BIOPHEN Heparin Anti-Xa (2 stages) (# 221010) and BIOPHEN HEPARIN Anti-IIa (2 stages) (# 221025).

New presentations are:

- **BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay)** (# 221005): contains AT, FXa and FXa-substrate (identical to the former presentation # 221010)

and

- **BIOPHEN™ ANTI-IIA (2 Stages Heparin Assay)** (# 220005): contains AT, Thrombin and Thrombin-substrate (identical to the former presentation # 221025).

The new kit presentations are detailed here below:

Kit presentation for Anti-Xa Assay (# 221005)	Kit presentation for Anti-IIa Assay (# 220005)
Reagent 1: 2x1 mL: Human Antithrombin (ATIII), lyophilized vial containing about 5 IU/mL.	Reagent 1: 2x1 mL: Human Antithrombin (ATIII), lyophilized vial containing about 1.25 IU/mL.
Reagent 2 : 2x1 mL: Purified bovine Factor Xa, lyophilized vial containing about 40 µg	Reagent 2 : 2x1 mL: Purified human Thrombin, mainly in the α form, lyophilized vial containing about 120 NIH.
Reagent 3 : 2x1 mL: Chromogenic substrate specific for Factor Xa (CS-11(65)), vial of about 4 mg (about 6 µmol)	Reagent 3 : 2x1 mL: Chromogenic substrate specific for Thrombin (CS-01(38)), vial of about 6.25 µmol.

Buffers presented separately:

Product Name	Reference
- Tris-EDTA-NaCl-PEG, pH 8.40	# AR030K
Or - Tris-EDTA-NaCl-BSA, pH 8.40	# AR031K
- Tris-NaCl-BSA, pH 7.40	# AR005L
- Tris-NaCl, pH 7.40	# AR028K
- Tris-EDTA-NaCl, pH 8.40	# AR029K

For the anti-Xa 2-stage assay, for performing the method in compliance with the new USP or EP guidances, the needs are summarized in the here below table, for LMWH or UFH:

Anti-Xa 2-stage (# 221005)	Volume of buffer (for 1 mL of reagent)		Buffer used	
	Heparin measured	LMWH	UFH	UFH
Sample			# ARO05L (EP) # ARO28K (USP)	# ARO30K (USP and EP)
R1	4mL	4mL	# ARO05L (USP and EP)	# ARO30K (USP and EP)
R2	4mL	4mL	# ARO05L (USP and EP)	# ARO30K (USP and EP)
R3	4mL		# ARO29K (USP and EP)	

For the anti-IIa 2-stage assay, for performing the assay in compliance with the new USP or EP guidances, the needs are summarized in the here below table:

Anti-IIa 2-stage (# 220005)	Volume of buffer (for 1 mL of reagent)		Buffer used	
	Heparin measured	LMWH	UFH	UFH
Sample			# ARO05L (EP) # ARO28K (USP)	# ARO30K (USP and EP) or # ARO31K (USP)
R1	4mL	4mL	# ARO05L (USP and EP)	# ARO30K (USP and EP) or # ARO31K (USP)
R2	4mL	4mL	# ARO05L (USP and EP)	# ARO30K (USP and EP) or # ARO31K (USP)
R3	4mL		# ARO29K (USP and EP)	

By this modification we always challenge to offer you the most appropriate tools for complying with USP and EP recommendations and to support our customers.

In addition, we took this opportunity for developing some instrument applications for these assays and more specifically for the UFH/LMWH assay with BIOPHEN™ ANTI-Xa (2 stages Heparin assay) (# 221005) and BIOPHEN™ ANTI-IIa (2 Stages Heparin Assay) (# 220005) on STA-R® and Sysmex CS-5100 analyzers.

Do not hesitate to contact us or your local distributor for any support that you may need.

NB: For those who want to strictly stay with the old working protocols, without switching to the newly published USP or EP recommendations, please use in place of # 221010 or # 221025 the following reagents:

Discontinued Reagents		New kit presentation		Additional buffer
BIOPHEN Heparin Anti-Xa (2 stages) (# 221010)	➔	BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay) (# 221005)	+	Tris-EDTA-NaCl-PEG, pH 8.40 (# ARO30K)
BIOPHEN Heparin Anti-IIa (2 stages) (# 221025)	➔	BIOPHEN™ ANTI-IIA (2 Stages Heparin Assay) (# 220005)	+	Tris-EDTA-NaCl-BSA, pH 8.40 (# ARO31K)

Yours sincerely,

Anne Marie VISSAC
President
HYPHEN BioMed

