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TECHNOCHROM® anti-Xa

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DE

REF 5340250 TECHNOCHROM® anti-Xa 80 T.

symbols key / Symbolschlüssel / interpretazione dei simboli / explicación de símbolos / explicação dos símbolos / clé des symboles / Symbolnyckel / symbolforklaring / Tegnforklaring / Κλειδί συμβόλων / Използвани символи / символы / Klíčova slova / Značenje simbola			
	manufacturer / Hersteller / fabbricante / fabricante / fabricante / fabricant / Tillverkaren / Fabrikanten / Produsent / Κατασκευαστής / Производител / Производитель / výrobce / Proizvođač		expiry date / Verfallsdatum / data di scadenza / fecha de caducidad / data de validade / date d'expriration / utgångsdatum / udløbsdato / Utløpsdato / Ημερομηνία λήξης / срок на годност / datum expirace/ срок годности / datum exspirace / Rok trajanja
	storage temperature / Lagertemperatur / temperatura di conservazione / temperatura de conservación / temperatura de conservação / température de stockage / lagringstemperatur / opbevaringstemperatur / Oppbevaringstemperatur / θερμοκρασία αποθήκευσης / съхранение на / teplota skladování / температура хранения / teplota skladování / Temperatura lagerovanja		consult instructions for use / Gebrauchsanweisung beachten / consultare le istruzioni per l´uso / consulte las instrucciones de uso / consultar o manual de instruções / instruction d'utilisation / se användarinstruktioner / følg brugsvejledning / Følg bruksanvisningen / συμβουλευθείτε τις οδηγίες για τη χρήση / прочетете инструкцията за работа / potřeba řídit se instrukcemi / перед использованием читайте инструкцию / sledujte návod k použití / Pročitaj upustvo pre upotrebe
CE	CE-mark / CE-Kennzeichnung / marchio CE / marca de CE / Simbolo da CE / marquage CE / CE-märkning / CE-mærket / CE-merke / CE-σημάδι / CE марка / CE-označení / маркировка CE / značka CE / CE-marka		determinations / Bestimmungen / determinazioni / determinaciones / determinações / déterminations / bestämningar / bestemmelser / Bestemmelser / προσδιορισμοί / брой тестове / stanovení / определний / počet stanovení / Definicija
AQUA	distilled water / destilliertes Wasser / acqua distillata / agua destilada / água destilada / eau distillée / destillerat vatten / destilleret vand / Destillert vann / απεσταγμένο νερό / дестилирана вода / destilovaná voda / дистиллированная вода / destilovaná voda / Serija	LOT	lot / Charge / lotto / lote / lote / lot / sats / serie / Parti / ηαρτία / партида номер / šarže / лот / šarže / in vitro dijagnostika
BUF	Reaction buffer / Reaktionspuffer / tampone di reazione / tampón de reacción / Tampão de reacção / tampon de réaction / Reaktionsbuffert / Reaktionsbuffer / Reaksjonsbuffer / διάλυμα αντίδρασης / Реакционен буфер / Рабочий буферный раствор / Reakční pufr / Reakcioni pufer	MTP	microtiter plate / Mikrotiterplatte / placa microtiter / microplaca / microplaca / microplaques sensibilisées / Mikrotiterplatta / Mikrotiterplade / mikrotiterplate / πλάκα μικροτιλοδότησης / Микротитърна плака / Микропланшет / Mikrotitrační destička / Mikrotitracione ploče
CAL	Calibrator / Kalibrator / Calibratore / calibrador / calibrador / calibrateur / Kalibrator / Kalibrator / Kalibrator / Βαθμονομητής / Калибратор / калибратор / kalibrátor / Kalibrator	REF	catalogue number / Katalognummer / numero di catalogo / numéro de catálogo / número de referência / réf. de catalogue / katalognummer / Katalognummer / αριθμός καταλόγων / каталожен номер / katalogové číslo / каталожный номер / katalogové číslo / Kataloški broj
CONJ	Conjugate / Konjugat / Coniugato / conjugado / conjugado / conjugaté / Konjugerad / Konjugat / Konjugat / συνδετικό / Конюгат / Конъюгат / Konjugát / Konjugat	RTU	ready to use / gebrauchsfertig / pronto all'uso / listo para usar / pronto a usar / prêt à l´emploi / färdig att användas / færdig til brug / klar til bruk/ έτοιμο προς χρήση / Готов за употреба / готов к использованию / k přímému použití / Razrediti ili rastvoriti
CONT	Control / Kontrolle / controllo / control / control / contrôle / Kontroll / Kontroll / Kontroll / διάλυμα ελέγχου / Контрол / Контрольный образец / Kontrola / Kontrola	STOP	stop solution / Stopplösung / Soluzione di arresto / solución de parada / solução de paragem / solution d´árrêt / Stopplösning / Stop-opløsning / Stoppløsning / διάλυμα παύσης / Стоп разтвор / Стоп-раствор / Zastavovací roztok / Stop solucija
DIL	dilute or disolve in / verdünnen oder lösen in / diluire o dissolvere in / diluir o disolver / diluir ou dissolver em / diluer ou dissoudre dans / späd eller upplös i / fortyndes eller opløses i / Fortyndes eller oppløses i / αραωση ή διάλνση σε / разтворете или разредете с / zředit anebo rozpustit v / разбавить или растворить в / nařeďte nebo rozpusťte v / razrediti ili rastvoriti u	SUB	substrate / Substrat / substrato / substrato / substrato / substrat / Substrat / Substrat / Substrat / υπόστρωμα / Субстрат / Субстрат / Substrát / Substrat
INC	incubation buffer / Inkubationspuffer / tampone di incubazione / tampón de incubatción / tampão de incubação / tampon d'incubation / Inkubationsbuffert/ Inkubationsbuffer/ Vaskebufferkonsentrat / διάλυμα επώασης / Инкубационен буфер / Буфер для инкубации / Inkubační pufr / Inkubacioni pufer	WASH	washing solution concentrate / Waschlösungskonzentrat / concentrado de solución de lavado / solución de lavado concentrada / tampão de lavagem concentrado / Tampon de lavage concentré / Vattenlösningskoncentrat / Vaskeopløsningskoncentrat / vaskeløsningskonsentrat / συμπυκνωμένο διάλυμα πλύσης / Концентриран миещ разтвор / Концентрат промывочного раствора / Koncentrát promývacího roztoku / Koncetrat solucije za ispiranje
RUO	in vitro research use only		

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TECHNOCHROM® anti-Xa

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PRODUCT DESCRIPTION

INTENDED USE – RESEARCH USE ONLY

TECHNOCHROM® anti-Xa is a system of reagents for the chromogenic determination of direct and indirect Xa inhibitors in human citrated plasma. The assay is based on the inhibition of activated factor X (FXa) by Antithrombin (AT) in the presence of heparin as measured by a chromogenic FXa substrate.

PRINCIPLE

[Xa-Inhibitor] + Xa (excess) ⟶ [Xa-Inhibitor - Xa] + Xa (residual)

Xa (residual) + Substrate ⟶ pNA

The amount of pNA, released by enzymatic hydrolysis, is measured at 405 nm. There is an inverse relationship between the amount of heparin present in the sample and colour development.

COMPOSITION

mL	Reagents	Other data
1 x 20mL	Reagent 1 **(R1)**: anti-Xa buffer :	TRIS-EDTA buffer; pH 8.4; contains sodium azide (<1g/L) as a preservative
1 x 4mL	Reagent 2 **(R2)**:	Bovine Factor Xa, lyophilized; 1 vial; about 24 nkat
1 x 4mL	Reagent 3 **(R3)**:	Chromogenic substrate, lyophilized; 1 vial; 4.8 mg

MATERIAL REQUIRED (not provided with the kit)

- Distilled water
- Acetic acid (20%) or 2% citric acid (end point method)
- **Plasma Calibrators** with a known concentration of Fondaparinux (Arixtra®) (REF 5090010), LMWH (REF 5090040), UFH (REF 5090070), Danaparoïd (Orgaran®) (REF 5090110), Rivaroxaban (REF 5090170) or Apixaban (REF 5090269)
- **Control plasmas** for Fondaparinux (Arixtra®) (REF 5090012, 5090014), LMWH (REF 5090042, 5090044, 5090046), UFH (REF 5090072, 5090072), Danaparoïd (Orgaran®) (REF 5090112, 5090114), Rivaroxaban (REF 5090172, 5090173, 5090174) or Apixaban (REF 5090270, 5090271)
- Common clinical laboratory equipment (spectrophotometer set at 405nm, water-bath set at 37°C, stop-watch, test tubes, pipettes,…)

WARNING AND PRECAUTIONS

- RUO for *in vitro* research use only
- All blood and plasma samples and products have to be regarded as potentially infectious and handled with appropriate care and in compliance with the biosafety regulations in force and must be disposed of in the same way as hospital waste.
- The reagents sometimes contain preserving agents (sodium azide). Beware of swallowing! Avoid contact with skin or mucous membranes!

LIMITATION OF THE TEST

Incubation times indicated have to be followed strictly, as enzymatic reactions in this test are very rapid. Due to the fact that no Antithrombin is added to the test system, the patients' Antithrombin level can influence the test result. No interference could be observed for Hemoglobin (up to 17 g/L) and Billirubin (up to 3.3 mg/L).

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at + 2…8 °C.

Stability after reconstitution:

Reagent	RT *	2…8 °C
Reagent 1 (R1)	2 weeks	4 months
Reagent 2 (R2)	2 weeks	4 months
Reagent 3 (R3)	2 weeks	4 months

* RT = room temperature (18…25°C)

TEST PROCEDURE

PREPARATION OF PLASMA SAMPLES

Mix 9 parts of venous blood and 1 part sodium citrate solution (0.11mol/L) with great care, in order to avoid activation and PF4 release. Sampling must be performed through a net venipuncture, and the first drops must be discarded. Specific collection tubes for heparin testing, such as the CTAD (Citrate, Theophylline, Adenosine and Dipyridanole) tubes, can be used. They improve specimen stability. Centrifuge within 1 hour for 15 minutes at a RCF of at least 2500 (corresponding to DIN 58905) at 18°C. The plasma sample may be stored at room temperature for up to 2 hours; otherwise the sample has to be frozen immediately after centrifugation. Stability at –20°C: 1 month (before use, thaw for 15 min. in a water bath at 37°C).

PREPARATION OF REAGENT

All reagents including distilled water should have reached room temperature before use.
Reagent 1 **(R1)**: ready to use
Reagent 2 **(R2)**: reconstitute in 4mL distilled water
Reagent 3 **(R3)**: reconstitute in 4mL distilled water

Allow the reagents to reconstitute at room temperature for 30 minutes while shaking the vials from time to time.

Then mix the vials gently to obtain homogeneous solutions (vortex mixer). Avoid foam formation!

PERFORMANCE OF THE TEST

Calibration Curve:

- For monitoring of Fondaparinux (Arixtra®) therapy, a calibration curve is constructed using TECHNOVIEW Arixtra® CAL (REF 5090010),
- For monitoring of LMW Heparin therapy, a calibration curve is constructed using TECHNOVIEW LMW CAL (REF 5090040),
- For monitoring of UFH therapy, a calibration curve is constructed using TECHNOVIEW UFH (REF 5090070),
- For monitoring Danaparoïd (Orgaran®) therapy, a calibration curve is constructed using TECHNOVIEW Orgaran® (REF 5090110),
- For monitoring Rivaroxaban therapy, a calibration curve is constructed using TECHNOVIEW Rivaroxaban (REF 5090170)

CEVERON® alpha

Technoclone provides application sheets for Ceveron® alpha. The application sheets contain analyser/assay specific handling and performance information which may differ from that provided in this instruction for use. In this case the information contained in the application sheets supersedes the information in this instruction for use. Please consult the instruction manual of the Ceveron® alpha.

MANUAL METHOD

Test samples and controls

For **Fondaparinux, LMW, UFH** and **Danaparoïd**, samples, calibrators and controls are assayed in a **1:10 dilution** in the standard protocol.

For **Rivaroxaban** concentrations ≤150ng/mL, samples, calibrators and controls are assayed in a **1:5 dilution** in the standard protocol. For **Rivaroxaban concentrations** ≥150ng/mL and **Apixaban**, samples, calibrators and controls are assayed in a **1:20 dilution**.

Table 1: sample dilution with R1:

Sample material	**UFH; Danaparoïd LMW; Fondaparinux,**	**Rivaroxaban conc ≤ 150ng/mL**	**High Rivaroxaban conc ≥150ng/mL, Apixaban**
Sample dilution	1:10	1:5	1:20
Example of dilution	0.025 mL sample + 0.225 mL anti-Xa buffer	0.050 mL sample + 0.200 mL anti-Xa buffer	0.025 mL sample + 0.475 mL anti-Xa buffer

Performance

Preheat reagent 2 (R2) and reagent 3 (R3) (bovine Factor Xa and Substrate) to 37°C.
Dilute calibrators / controls / plasma samples according to table 1. Keep the plasma samples at room temperature. Measurements are done at 37°C.

Into a well of a microtiter plate or a test tube, **incubated at 37°C**, introduce:

	microtiter plate	**Test tube**
Diluted Sample (according to table 1)	50 µL	200 µL
R2 bovine Factor Xa, preheated	50 µL	200 µL
Mix and incubate at **37°C** for exactly **60 sec**.		
R3 Xa Substrate, preheated	50 µL	200 µL
Mix and incubate at **37°C** for exactly **30 sec.**		
2% citric or 20% acetic acid	50 µL	200 µL
Mix and measure absorbance **at 405nm** with **620nm** reference wavelength if available		

A sample blank can be obtained by mixing the reagents in the reverse order from that of the test.

AUTOMATION

Application sheets for auto analysers are available on request.

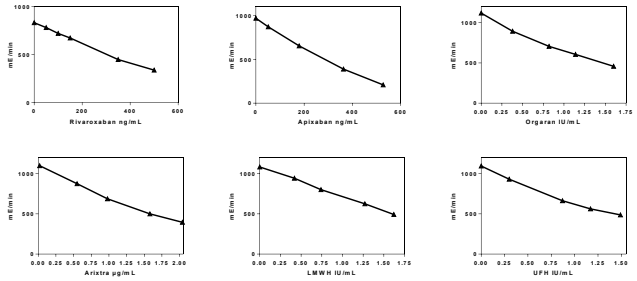
ANALYSES RESULTS

REFERENCE RANGE

For obtaining the right efficacy along with the lowest bleeding risk, anticoagulant dosage must be within the therapeutic range recommended by each drug manufacturer and for each specific indication.

EVALUATION USING A REFERENCE CURVE (example manual method)

Setting up a reference curve:
X axis: Concentration IU/ml (Orgaran®, UFH, LMWH), µg/mL (Arixtra®), ng/mL (Rivaroxaban)
Y axis: Extinction
Draw the best-fit calibration line with linear axis
Example calibartion curves (Ceveron):



Measuring concentration of samples

All samples diluted according to table 1 can be read off directly from the appropriate reference curve. For dilutions other than the one stated in table 1, the value read off from the calibration curve has to be multiplied by the additional dilution factor.

It is recommended to run controls with every test in order to ensure accuracy and reproducibility of the results.

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

PRECISION

Reproducibility was determined with different samples (in series and day to day).
The following results were obtained:

	Intra assay			Inter assay	
	CV%	N		CV%	N
LMW level 1 (1.37 U/mL)	1.6 %	24	LMW level 1 (1.36 U/mL)	1.5 %	6
LMW level 2 (0.52 U/mL)	2.5 %	24	LMW level 2 (0.37 U/mL)	2.5 %	6
UFH level 1 (1.26 U/mL)	0.7 %	24	UFH level 1 (1.45 U/mL)	2.6 %	6
UFH level 2 (0.42 U/mL)	2.1 %	24	UFH level 2 (0.06 U/mL)	5.7 %	6

COMPARISON OF METHODS OR CORRELATION

Following correlation (%) was obtained in comparing STA Rotachrom® Heparin (Diagnostica Stago) on a Thrombolyzer XRC (Behnk Elektronik) coagulation analyzer with the TECHNOCHROM® anti Xa (AT-) test:

y = 0.998x + 0.000 R = 0.98 (n=54)

	ASSAY RANGE	DETECTION LIMIT
Fondaparinux (Arixtra®)	0.07 – 2.00 µg/mL	0.07 µg/mL
LMWH	0.05 – 2.00 IU/mL	0.05 IU/mL
UFH	0.06 – 1.50 IU/mL	0.06 IU/mL
Danaparoïd (Orgaran®)	0.08 – 1.60 IU/mL	0.08 U/mL
Rivaroxaban	10 – 700 ng/mL	10 ng/mL
Apixaban	20 – 700 ng/mL	20 ng/mL

LITERATURE

Please contact Technoclone or your local distributor.