Instructions for use – EX

REF 1081EU – EX UDI 59998629921081EU_1VW

Intended purpose

CE 2265 IVD **The EX is a ready to use reagent for in-vitro diagnostic professional use, intended for examination of the extrinsic coagulation system in citrated blood during viscoelastometry analysis.**

CAUTION: A use of the device outside of its intended purpose, may lead to the test results being incorrectly interpreted by the user.

Indications for use

Indicated to be used when an alteration of the extrinsic coagulation system is suspected.

Contra-indications for use

None identified.

Intended users

- trained healthcare professionals,
- trained laboratory professionals.

Environment of use

Indoors in a typical setting of a laboratory, equipped and designed to ensure standard electrical connections, adequate lighting as well as standard environment settings regarding temperature, humidity and pressure to ensure the functionality of typical electrical devices like electrical medical devices and personal computers.

Intended patient population

Adult patients where an examination of coagulation activation, clot formation and/or clot stability is desired.

Principle of the assay

In the EX assay whole blood coagulation is activated by a combination of recombinant tissue factor (TF), calcium chloride and polybrene as a heparin antagonist.

The clot formation is then detected using viscoelastometry [1-2]. Viscoelastometry allows for the detection of whole blood formation in whole blood and thus detects coagulation initiation (by the clotting time, CT), blood clot firmness (by the maximum clot firmness, MCF, or related parameters, such as the A20, amplitude 20 minutes after CT) and clot stability or fibrinolysis (by the maximum lysis, ML). In viscoelastometry the clot formation depends on the fibrinogen and platelet content of the sample, as well as the process of blood clot polymerization. The coagulation activation can be prolonged when there are clotting factor deficiencies of the extrinsic pathway or non-heparin anticoagulants present in the sample [3].

The use of a combination of recombinant tissue factor, calcium chloride and polybrene is commonly used in viscoelastometry tests such as the ex-tem® or EX-test assays [1-2] (tem® is a registered trademark by CA Casyso, Switzerland). Such assays allow to determine coagulation activation, clot formation and clot stability or fibrinolysis in whole blood, which can support the patient management during coagulopathy, and is often used together with other viscoelastometry assays (ellagic-acid triggered viscoelastometry, viscoelastometry with platelet inhibition) [3].

Materials provided

10 sealed single-use pouches containing one pipet tip with reagent each, providing a dry chemistry reagent composed of recombinant tissue factor, polybrene, calcium chloride, buffer and stabilizers. Each pouch contains one desiccant bag.

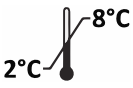
Additional materials and devices required

- Viscoelastometry analyzer and receptacles (Cups & Pins),
- Electronic pipette for 340 µL with 3 sec aspiration / dispensing cycles,
- Blood collection tube (3.2% sodium citrate) for coagulation testing.

Reagent preparation

The reagent is ready to use.

Storage and stability



Store at +2 to +8 °C. The unopened reagent tips are stable until the expiration date stated on the pouch label. Unopened pouches may be stored at room temperature for up to 1 month. Opened pouches are for immediate use within 1 minute after opening the pouch.

CAUTION: Incorrect storage conditions may affect reagent stability and lead to wrong test results.

Warnings and precautions

For professional use by trained personnel.

CAUTION: Do not use tips from defective pouches or from pouches missing the desiccant pack.

CAUTION: Intended for single use - do not reuse.

CAUTION: Any serious incident that has occurred as a result of the use of the device has to be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

CAUTION: Failure to comply with these instructions for use may result in device handling errors leading to wrong test results.

CAUTION: Human blood samples should be handled with care, following general precautions recommended for bio-hazardous materials [4].

CAUTION: General precautions (e.g., wear gloves and minimize skin exposure to specimens and reagents) should be followed when handling all materials.

NOTE: Dispose of waste according to local regulations.

NOTE: A material safety data sheet is available upon request.

Residual risks, undesirable side-effects, and information for the patient

The following residual risks were identified during the risk management activities for the device:

- In case of an off-label use of the product, test results may be incorrectly interpreted by the user.
- In case of device handling errors, patient's coagulation may be incorrectly reflected.
- In case of the use of the expired product, patient's coagulation may be incorrectly reflected.
- In case of unacceptable transport and storage conditions, patient's coagulation may be incorrectly reflected.

Warnings related to the residual risks are provided throughout the document.

No undesirable side-effects were identified during the post-market activities for the device.

No information for the patient is required to be provided for the device.

Sample collection

CAUTION: Collect a venous blood sample according to the recommended procedures [5-6] using a blood collection tube with 3.2% sodium citrate. Samples should be analyzed within 3 hours from blood collection. Store the blood at room temperature. Always ensure blood collection tubes are filled to the indicated fill volume to avoid excessive citrate levels.

Test procedure

1. Check the expiry date of the device. The expiry date format is yyyy-mm-dd.

   CAUTION: Do not use the expired product. The use of the expired product may lead to wrong test results.

2. Allow the reagent tip pouch to reach room temperature.
3. If the sample is cold (< 22°C) it is advised to allow the sample to warm up for 5 min on the heated position of the viscoelastometry analyzer. In evaluations on the effect of pre-warming blood tubes which had room temperature little to no effect was observed vs. tubes which were not pre-warmed.
4. Create the test in the software of the viscoelastometry analyzer according to the analyzer manual.
5. Place the Cup and Pin into the analyzer according to the analyzer manual.
6. Tear open the reagent tip pouch, attach the reagent tip to the electronic pipette and aspirate 340 µL sample from the blood tube.
7. Dispense the blood sample into the Cup.
8. Aspirate and dispense the sample once again to facilitate thorough mixing of the reagents with the blood sample. Ensure sample pipetting is performed without interruption of the process.
9. Start the test as described in the analyzer manual.
10. The test will stop, or you can stop the test as described in the analyzer manual.
11. Remove the Cup & Pin and dispose according to local regulations.

Quality control

Plasma-based quality control materials can be used to confirm the stability of test results determined with the EX assay over time.

Result interpretation and expected values

The coagulation activation is detected via the clotting time parameter (CT, defined as the time from the start of the test until an amplitude of 2 mm is detected). The clot formation is detected via the A5 (amplitude 5 minutes after clotting time - CT), A10 (amplitude 10 minutes after CT), A20 (amplitude 20 minutes after CT), MCF and ML (maximum lysis) parameters.

Reference range

The reference range was determined in a clinical study including 121 healthy individuals, aged 19 - 79.3 years, 54.5% female and 45.5% male, and calculated of the 95% central interval:

Parameter	2.5th percentile – 97.5th percentile	Mean ± SD
CT (sec)	37 – 65	48.3 ± 10.2
A5 (mm)	40 – 57	47.6 ± 4.7
A10 (mm)	48 – 63	55.2 ± 4.2
A20 (mm)	53 – 66	59.6 ± 3.7
MCF (mm)	53 – 67	60.2 ± 3.7
ML (%)	2 – 12	5.9 ± 2.6

Detection of clotting factor deficiencies using the EX assay clotting time (CT)

The sensitivity and specificity of the EX assay clotting time for the detection of a factor deficiency of the extrinsic pathway was tested in a clinical study including patients suspected of a factor deficiency of the extrinsic pathway, due to the intake of vitamin K antagonists or a liver dysfunction.

The prothrombin time assay was used as a reference for the detection of a factor deficiency of the extrinsic pathway. A cut-off of INR >= 1.5 was applied to detect a clinically relevant clotting factor deficiency, which is supported by international guidelines and recommendations [7-9].

The upper limit of the EX CT reference range was defined as the cut-off value for indicating an INR > 1.5, i.e. an EX-CT>65 sec.

Based on the measurements of 105 patients with suspected clotting factor deficiencies and a control group (n=104, intensive care patients and healthy individuals) the following sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive and negative likelihood ratio were determined:

Sensitivity	93%
Specificity	91%
Positive predictive value (PPV)	78%
Negative predictive value (NPV)	97%
Positive likelihood ratio (LR+)	10.25
Negative likelihood ratio (LR–)	0.08

54 patients had an INR>1.5. Their EX assay CT values were as follows:

2.5th perc – 97.5th perc	Mean ± SD
54 - 158	107.8 ± 28.4

Accuracy of the clot firmness parameters

In order to confirm the accuracy of the clot firmness parameters of the EX assay, its results were compared with a reference test, the EX-test assay by enicor [2].

Based on the parallel measurements of 103 samples from intensive care patients and healthy individuals, the slope, bias and correlation were calculated for the EX assay parameters (A5, A10, A20, MCF, ML) vs. the reference test (EX-test by enicor) according to CLSI EP09 (EP09c).

In the following table the Deming regression slope and bias (intercept) and, as a descriptive parameter, the Spearman correlation are shown. EXa: EX assay (Apiro), EXe: EX-test (Enicor)

Parameter	Slope	Bias	Spearman ρ
EXa-A5 vs EXe-A5	1.063	−3.583	0.923
EXa-A10 vs EXe-A10	1.057	−3.471	0.936
EXa-A20 vs EXe-A20	1.056	−3.480	0.94
EXa-MCF vs EXe-MCF	1.068	−4.280	0.947
EXa-ML vs EXe-ML	1.011	0.177	0.9

The determined agreement of the EX assay to the reference method compares very well with recent literature comparing different generations of viscoelastometry assays [10-11].

Detection of fibrinolysis

The ability of the EX assay to detect fibrinolysis was tested by the addition of increasing doses of t-PA to citrated blood. 4-fold determinations were performed. Means and standard deviations (SD) for the maximum lysis (ML) and maximum clot firmness (MCF) are shown below:

	t-PA (ng/mL)					
	0	100	200	300	400	600
ML (%, mean ± SD)	3.25 ± 0.5	3.75 ± 0.5	97 ± 0	96 ± 0	96 ± 0	95 ± 0
MCF (mm, mean ± SD)	56.8 ± 1	56.5 ± 1	48.8 ± 1.3	39.8 ± 0.5	34.3 ± 0.5	28.8 ± 1

This experiment shows that fibrinolysis is effectively detected using the EX assay. The dose-response detected matches well published viscoelastometry results [12].

Precision

In a precision study citrated blood with and without hemodilution (50% using HES 6% in saline with 3.2% citrate) was tested in 3 runs, on three analyzers, three operations and including 3 EX reagent lots (54 determinations per sample). The resulting mean, standard deviation (SD) and coefficient of variation (CV) for the CT and A20 were as follows:

		citrated blood	hemodiluted blood
CT (sec)	mean ± SD	51.9 ± 4.7	114.5 ± 14.8
	CV	9.0%	12.9%
A20 (mm)	mean ± SD	58.3 ± 1.5	34.9 ± 2.2
	CV	2.5%	6.4%

Limitations and interferences

Every in vitro diagnostic method can deliver wrong results under certain circumstances. It is therefore advisable to use certain precautions to avoid misinterpretations.

Measurements with noisy curves or irregular shapes should be discarded and repeated. The clinical context and other laboratory values (when available) should be considered when interpreting the EX assay. Generally, consider repeating a measurement that gives a very surprising or otherwise implausible result.

Interference by the following substances was tested using citrated blood with the in vitro addition of aspirin, cangrelor (ADP receptor antagonist), unfractionated heparin (UFH), low molecular weight heparin (LMWH) and apixaban (Eliquis®, direct FXa antagonist). In addition, hemodilution was tested using 40% of saline 0.9%, sodium citrate 3.2%. Means, standard deviations (SD) and the change vs. the control for the CT and A20 parameters is shown (6-fold determinations):

	CT (sec)		
	mean	SD	Change
Citrated blood (control)	61	4.9	n/a
+ Aspirin 0.1 mg/mL	63.7	6.8	2.7
+ Cangrelor 100 ng/mL	64	3.5	3
+ UFH 3 IU/mL	64.3	3.7	3.3
+ UFH 5 IU/mL	69.5	3.3	8.5
+ LMWH 1 IU/mL	64.7	3.7	3.7
+ Apixaban 100 ng/mL	80.8	3.5	19.8
+ Apixaban 300 ng/mL	96.2	6.2	35.2
40% hemodilution (saline)	65.2	1.5	4.2

One can see that the in vitro addition of aspirin, cangrelor, 3 U/mL UFH, LMWH and the hemodilution using saline resulted in minor prolongations of the CT. 5 U/mL of UFH resulted in a 14% prolongation of the CT (+8.5 sec). Apixaban led to a marked prolongation of the CT.

	A20 (mm)		
	mean	SD	Change
Citrated blood (control)	54.3	0.8	n/a
+ Aspirin 0.1 mg/mL	52.2	1.2	-2.1
+ Cangrelor 100 ng/mL	54	0.6	-0.3
+ UFH 3 IU/mL	53.8	1	-0.5
+ UFH 5 IU/mL	52	0.9	-2.3
+ LMWH 1 IU/mL	53.8	1	-0.5
+ Apixaban 100 ng/mL	54	0.6	-0.3
+ Apixaban 300 ng/mL	54.3	0.5	0
40% hemodilution (saline)	45.3	0.5	-9

One can see that the clot firmness detection in the EX assay did not show any relevant changes in the presence of aspirin, cangrelor, UFH, LMWH or apixaban, while hemodilution led to a marked decline of the clot firmness.

The detected interferences are expected for the EX assay as a functional parameter.

Summary of safety and performance

Summary of safety and performance is provided in electronic format and is available for download on www.apiro.eu/eIFU

Manufacturer

APIRO Diagnostics Kft.
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Symbols

Symbol	Meaning
	Manufacturer
	Batch code

Symbol	Meaning
	Use-by date
	Catalogue number

Symbol	Meaning
	Country of manufacture
	Temperature limit
	Consult instructions for use or electronic instructions for use
	Contains human blood or plasma derivatives
	Not intended for near-patient testing
	Unique device identifier
	In vitro diagnostic medical device

Symbol	Meaning
	Do not use if package is damaged and consult instructions for use
	Do not re-use
	Contains sufficient for <n> tests
	Caution / Warning
	CE marking of conformity
	Biological risks

References

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[2] Heubner L, Mirus M, Vicent O, Güldner A, Tiebel O, Beyer-Westendorf J, Fries D, Spieth PM. Point of care coagulation management in anesthesiology and critical care. Minerva Anestesiol. 2022 Jul-Aug;88(7-8):615-628.

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[4] Biosafety in microbiological and biomedical laboratories; U.S. Department of Health and Human Services, Washington, 5th Edition.

[5] CLSI/NCCLS H03-A6; Procedures for the collection of diagnostic blood specimens by venipuncture.

[6] CLSI H21-A5 Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays.

[7] Nahirniak et al. Recommendations for Use of Prothrombin Complex Concentrates in Canada (National Advisory Committee on Blood and Blood Products, 2022).

[8] National Clinical Guideline Centre (UK). Blood Transfusion (NICE Guideline NG24, 2015).

[9] Kerebel D, Joly LM, Honnart D, Schmidt J, Galanaud D, Negrier C, Kursten F, Coriat P; Lex206 Investigator Group. A French multicenter randomised trial comparing two dose-regimens of prothrombin complex concentrates in urgent anticoagulation reversal. Crit Care. 2013 Jan 10;17(1):R4.

[10] van Haeren MMT et al. Comparison of ROTEM® Delta and ROTEM® Sigma transfusion algorithm performance in thoracic aortic surgery: a single-centre prospective observational cohort study. Br J Anaesth. 2025 Feb;134(2):317-327.

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Version history of these instructions for use

Date	Version	Change description
2025-10-29	1	Initial version