AP Assay

Instructions for Use

REF 1051EU | IFU_1051EU_EN Rev.1

1. Intended Purpose

The AP is a ready to use reagent for in-vitro diagnostic professional use, intended for examination of whole blood clot formation with fibrinolysis inhibition 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.

2. Indications for Use

Indicated to be used when an alteration of whole blood clot formation is suspected.

3. Contra-indications for Use

None identified.

4. Intended Users

- trained healthcare professionals
- trained laboratory professionals

5. 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.

6. Intended Patient Population

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

7. Principle of the Assay

In the AP assay whole blood coagulation is activated by a combination of recombinant tissue factor, calcium chloride and polybrene as a heparin antagonist. Concurrently fibrinolysis is blocked by tranexamic acid, which is a synthetic inhibitor of fibrinolysis [1].

The clot formation is then detected using viscoelastometry [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 use of a combination of recombinant tissue factor, calcium chloride, polybrene and a fibrinolysis inhibitor is commonly used in viscoelastometry tests such as the ap-tem® or AP-test assays [2] (tem® is a registered trademark by CA Casyso, Switzerland). Such assays allow to determine clot formation under conditions of fibrinolysis inhibition, which can support the patient management during coagulopathy, and is used typically together with other viscoelastometry assays (tissue-factor triggered viscoelastometry, viscoelastometry with platelet inhibition) [3].

8. 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, tranexamic acid (a fibrinolysis inhibitor), calcium chloride, buffer and stabilizers. Each pouch contains one desiccant bag.

9. 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

10. Reagent Preparation

The reagent is ready to use.

11. 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.

12. 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.

13. Residual Risks, Undesirable Side-Effects, and Information for the Patient

- 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.

14. 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.

15. 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.
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.

16. Quality Control

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

17. Result Interpretation and Expected Values

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 ranges (121 healthy individuals, 19–79.3 years, 95% central interval):

Parameter	2.5th–97.5th percentile	Mean	SD
A5 (mm)	47–63	55.3	4.9
A10 (mm)	39–57	47.4	4.2
A20 (mm)	53–66	59.8	3.7
MCF (mm)	54–67	60.4	3.7
ML (%)	2–11	3.7	2.6

CT process marker:

	2.5th percentile	97.5th percentile	Mean	SD
CT (sec)	28	56	38.3	8.64

Accuracy comparison (AP assay vs AP-test by Enicor, n=103, CLSI EP09):

Parameter	Slope	Bias	Spearman ρ
APa-A5 vs APe-A5	0.964	1.3731	0.9321
APa-A10 vs APe-A10	0.978	1.0067	0.9225
APa-A20 vs APe-A20	0.9969	0.0986	0.9376
APa-MCF vs APe-MCF	1.0067	−0.6235	0.9332
APa-ML vs APe-ML	1.074	0.0683	0.8792

In the study group 15 samples had either a high platelet count (>400 G/L) and/or a high fibrinogen level (>4 g/L). In this group the clot firmness parameters provided on average higher results than the reference range.

Parameter	Reference range (2.5th–97.5th percentile)	High platelet/fibrinogen (2.5th–97.5th percentile)
A5 (mm)	47–63	55.4–70.2

Parameter	Reference range (2.5th–97.5th percentile)	High platelet/fibrinogen (2.5th–97.5th percentile)
A10 (mm)	39–57	46.8–65.7
A20 (mm)	53–66	60.1–72.6
MCF (mm)	54–67	60.4–72.6

Fibrinolysis inhibition (t-PA dose response, means and SDs):

	t-PA [ng/mL blood]: 0	100	200	300	400	600
CT (sec) — Mean	39.5	40.3	38.8	35	38	35.8
CT (sec) — SD	1.9	3.4	1.7	3.9	0.8	2.5
MCF (mm) — Mean	54.8	57	55.5	55.5	55	54.4
MCF (mm) — SD	0.5	0.8	0.5	0.5	0.6	0.6
ML (%) — Mean	3	2.3	3.3	4.3	4	4
ML (%) — SD	0.8	0.6	0.5	0.6	0.8	0.6

This experiment shows that fibrinolysis is effectively blocked in the AP assay.

Precision (3 runs, 3 analyzers, 3 operators, 3 AP reagent lots, 54 determinations):

	Citrated blood A20 (mm)	Hemodiluted citrated blood A20 (mm)
Mean	57.1	36.4
SD	1.3	1.0
CV	2.8%	2.2%

18. 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 AP assay. Generally, consider repeating a measurement that gives a very surprising or otherwise implausible result.

Interference table (A20 parameter, 6-fold determinations):

Substance / Condition	A20 Mean (mm)	SD	Change vs control
Citrated blood (control)	53.0	0.9	—
+ Aspirin 0.1 mg/mL	53.7	0.8	+0.7
+ Cangrelor 100 ng/mL	54.3	0.5	n.a.
+ UFH 3 IU/mL	52.0	1.1	−1.0
+ UFH 5 IU/mL	44.8	0.4	−8.9 (marked)

Substance / Condition	A20 Mean (mm)	SD	Change vs control
+ LMWH 1 IU/mL	53.7	0.6	−0.3
+ Apixaban 100 ng/mL	53.3	0.5	−0.5
+ Apixaban 300 ng/mL	54.2	0.4	0
40% haemodilution (saline)	—	—	Marked decline

The clot firmness detection in the AP 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.

19. 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

20. Manufacturer

APIRO Diagnostics Kft.

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21. Symbols Table

Symbol description	Meaning
Manufacturer	Identifies the manufacturer of the device
Use-by date	Indicates the date after which the device should not be used
Batch code	Indicates the manufacturer's batch code
Catalogue number	Indicates the manufacturer's catalogue number
Country of manufacture	Identifies the country where the device was manufactured
Do not use if package is damaged and consult instructions for use	Device should not be used if the package is damaged; consult IFU
Temperature limit	Indicates the temperature limits to which the device can be safely exposed
Do not re-use	Indicates a medical device that is intended for a single use only
Consult instructions for use or electronic instructions for use	Indicates the need to consult the instructions for use or electronic IFU
Contains sufficient for tests	Indicates the number of tests the device is sufficient for
Contains human blood or plasma derivatives	Indicates that the device contains human blood or plasma derivatives
Caution/Warning	Indicates the need for the user to consult the IFU for cautionary information
Not intended for near-patient testing	Indicates that the device is not intended for near-patient testing

Symbol description	Meaning
CE marking of conformity	Indicates conformity with applicable EU legislation
Unique device identifier	Identifies the device through its distribution and use
Biological risks	Indicates that the device contains potentially biohazardous material
In vitro diagnostic medical device	Indicates the device is intended for use as an in vitro diagnostic device

22. References

[1] Cai J et al. The many roles of tranexamic acid: An overview of the clinical indications for TXA in medical and surgical patients. Eur J Haematol. 2020 Feb;104(2):79-87.

[2] Volod O et al. Viscoelastic Hemostatic Assays: A Primer on Legacy and New Generation Devices. J Clin Med. 2022 Feb 7;11(3):860.

[3] Heubner L et al. Point of care coagulation management in anesthesiology and critical care. Minerva Anestesiol. 2022 Jul-Aug;88(7-8):615-628.

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

[8] Frick R et al. Comparison of 2 thromboelastography methods using patient and control samples. Res Pract Thromb Haemost. 2025 Mar 30;9(3):102843.

23. Version History

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