CE

Usage
Diagnostic
In Vitro

Abliance

APTT-TCA

REF AP02: R1 (5 x 2 mL) + R2 (1 x 12 mL)
REF AP05: R1 (10 x 5 mL) + R2 (1 x 60 mL)

Made in France Revision: 10/10/2018

Reagent for determination of activated partial thromboplastin time (APTT) in human plasma

PRINCIPLE OF THE METHOD [3] [4]

In presence of standardized amount of phospholipids (Cephalin), calcium chloride, and activator (kaolin), the factors of intrinsic coagulation system in citrated plasma are activated. The clotting time is measured.

CLINICAL SIGNIFICANCE [6] [7]

The measure of APTT is a common coagulation test used for investigation of intrinsic coagulation pathway (factors VIII, IX, XI, XII, V, X, II and I). It is commonly used to monitor heparin therapy.
Abnormal APTT may require further investigations related to congenital or acquired deficiencies and should not be made a single test.

REAGENTS

R1: APTT-TCA REAG1
Cephalin (rabbit cerebral tissues)
Activator (Kaolin)

Once reconstituted: Working reagent R1 is not classified as dangerous

R2: $CaCl_2$ REAG2

Calcium chloride 0.025M

According to 1272/2008 regulation, this reagent is not classified as dangerous

SAFETY CAUTIONS

ABLIANCE reagent kits are designated for professional in vitro diagnostic use.
Good Laboratory Practices must be applied during use of reagents, reference or control plasmas, and human samples (to manipulate as potentially infectious)
For further information, Material Safety datasheet is available upon request.
Waste disposal: Respect legislations in place in the country

PREPARATION OF REAGENTS

R1: Freeze dried reagent.
Open a vial of R1. Add promptly to the contents of the vial the amount of distilled water indicated on the label.
Cap the vial and mix gently until complete dissolution.
R2: Ready for use.

STABILITY AND STORAGE

Unopened vials stored at 2-8°C are stable until the expiry date stated on the label.
R1: after reconstitution the working reagent is 30 days at 2-8°C.
R2: once opened, if stored at 2-8°C and free from contamination, R2 content is stable until the expiry date stated on the label. Discard any cloudy reagent.
Discard any reagent:
- after expiry date
- if quality controls values are out of the range

SAMPLES COLLECTION AND HANDLING [1] [8]

Plasma from careful venipuncture with anticoagulant ratio of 1/10 (trisodium citrate solution 0.109M).
Mix immediately the blood with anticoagulant.
Avoid drawing with a syringe that could result in the formation of micro-clots.
Centrifuge 10 minutes at 2500g.
The specimen is stable 3 hours after collection, at room temperature (15-25°C).
Patients under heparin anticoagulant therapy: run the assay within 1 hour following blood collection.

LIMITS [2] [4] [5]

Heparin, depending on its origin and composition (calcium or sodium salt) has a different influence on the sensitivity of the reagent.
Mishrahi et al. indicate an easy procedure to determine the sensitivity of the method used in each laboratory and to inform the clinician optimize the dose.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

ADDITIONAL EQUIPEMENT

General laboratory equipment
Coagulation analyzer or semi-automated analyzer
Distilled or demineralised water for reconstitution of reagent

EXPECTED VALUES [1]

Normal values (usually < 35 sec) may vary with local conditions.
It is advised to each laboratory to establish its own reference range of expected values

QUALITY CONTROL

At least once a run, when changing reagent vial or after maintenance of the analyzer, it is advised to use 2 levels of control plasmas:
REF NP01: Normal and Pathological plasmas
If the controls results are out the defined range, perform consecutively until correction: repetition of the test with fresh control plasma.
If no solution is found, contact your local supplier or Abliance technical support.

PROCEDURE

Manual method on semi-automated systems:

- Plasma: 100 µL
- R1: APTT-TCA (mix before use): 100 µL

Mix and incubate for 180 sec at 37 °C.

- R2: CaCl2 (37°C): 100 µL

The automatic Countdown timer will start immediately after Reagent R2 addition and stop when the clot is formed.

Automated method on Thrombolyzer series
Refer to the full detailed application specific to the automated system
Note:

- Performances and stability data have been validated on Thrombolyzer Compact X (available on request).
- With manual procedure and on other automated coagulation analyzer, performances and stability data must be validated by user.

Other validated applications or proposal applications are available on request.

CALIBRATION

Results are expressed in seconds or ratio. Take special care to temperature and time measurement which ensure precision of the measurement.

CALCULATION [5]

APTT may be expressed directly in seconds or in ratio using the following formula:

Ratio = Patient APTT (sec) /Normal plasma APTT (sec)

It is advised to each laboratory to determine its own normal plasma APTT

PERFORMANCES

The within run and between run studies were performed with normal and abnormal plasma on Thrombolyzer Compact X:

Within run ***N = 20***	Normal Plasma	High Plasma
Mean (sec)	34.8	65.7
S.D. (sec)	0.44	0.77
C.V. %	1.25	1.18

Between run ***N = 20***	Normal Plasma	High Plasma
Mean (sec)	36.6	62.4
S.D. (sec)	0.92	2.00
C.V. %	2.50	3.21

Comparison with commercially available reagent:
192 plasmas located between 21.6 sec and 68.6 sec were tested:
y = 0.8515 x + 3.498 r= 0.9424

Interferences:

Total bilirubin	Positive interference from 133 µmol/L
Turbidity	No interference up to 731 mg/dL of triglycerides
Hemoglobin	No interference up to 261 µmol/L

Other substances may interfere with the results (see § Limits)

On board stability: at least 7 days

REFERENCES

(1) *Clinical Guide to Laboratory Test, 4th Ed., N.W. TIETZ (2006) p.46-47*
(2) *YOUNG D.S., Effect of Drugs on Clinical Laboratory Tests, 4th Ed. (1995) p.3-447 à 3-448*
(3) *Bell W.N., Alton H.G., Nature, 1954, **174**, 880-881.*
(4) *Struver G.P., Bittner D.L. Am. J. Clin. Path. 1962, **38**, 473-481).*
(5) *Misrahi N., Manet L., Conard J., Samama M., Act. Pharm. Biol. Clin. 1981, **1**, 81-85.*
(6) *Langdell R.D., WAGNER R.H., BRINKHOUS K.M.: "Effects of antihemophilic factor on one-stage clotting tests". J. Lab. Clin. Med., **41**, 637-647(1953)*
(7) *ITALIAN C.I.S.M.E.L. Study Group: "Activated partial thromboplastin time: a multicenter evaluation of commercial reagents in the diagnosis of mild factor VIII deficiency and other coagulation disorders" in "International symposium on Standardization and Quality Control of coagulation tests", Roma, 27-28 March, 1980*
(8) *"Etude des différents paramètres intervenant dans les variables préanalytiques (revue de littérature) ». Sang Thromb. Vaiss., **10**, p.5-18 (1998)*

Manufacturer | Use by | IVD In vitro diagnostic | Temperature limitation
REF Catalogue number | See insert | LOT Batch number | → Dilute with
REAG Reagent | | H2O Distilled water |

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