CE

Usage
Diagnostic
In Vitro

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: 18/12/2014

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
Cephalin (rabbit cerebral tissues)
Activator (Kaolin)

R2 : $CaCl_2$
Calcium chloride

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, avoiding foam.
R2 : Ready for use.
Bring to 37°C the necessary volume of R2 for 15 minutes before work.

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 stable 21 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.

INTERFERENCES [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 in order to optimize the dose.

Interferent	Results
Haemoglobin	No interference up to 327 µmol/L
Turbidity	Negative interference above 0.2 %
Bilirubin	Positive interference above 60 µmol/L

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 stopwatch and bath (37°C+/-0.5)
Distilled or demineralised water for reconstitution of reagent
Control plasmas

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

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 includes normal and pathological plasmas
or any other assayed control plasmas referring to the same method.

If the controls results are out the defined range, perform consecutively until correction: repetition of the test with fresh control plasma, calibration with a new vial of reagent, use of a new vial of reference plasma. If no solution is found, contact your local supplier or Abliance technical support.

EXPECTED VALUES [1]

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

CALIBRATION

Results are expressed in seconds or ratio, do not need any calibration. Take special care to temperature and time measurement which ensure precision of the measurement.

PERFORMANCES

Results of Performances Assays on Thrombolyzer Compact X:

Repeatability (within run):

Mean PT (sec): 32.2	CV%: 0.9	n= 32
Mean PT (sec): 42.2	CV%: 0.75	n= 32
Mean PT (sec): 51.2	CV%: 0.67	n= 32

Reproducibility (run to run):

Mean PT (sec): 33.3	CV%: 2	n= 18
Mean PT (sec): 41.4	CV%: 1.8	n= 18

PROCEDURE

Semi-automated and Automated method:
Refer to the appropriate instrument Operator's manual for detailed instructions.
Manual method:
Pre-incubate the R2 reagent to reach a temperature of 37°C.

- Plasma: 100µL

Mix gently the reagent R1 before pipetting.

- R1 Reagent : 100µL

Mix and incubate for 3 min. at 37°C.

- R2 Reagent : 100µL

Start a stopwatch immediately, and record the time required for clot formation in seconds.

CALCULATION [5]

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

Ratio: Patient APTT/Normal plasma APTT

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

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

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