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ACTIVATED PARTIAL THROMBOPLASTIN TIME

Diagnostic reagent for the in-vitro quantitative determination of activated partial thromboplastin time (APTT) in human plasma, using ellagic acid as an activator in both automatic and manual systems

REF: BS.1/PTT01.003.0003	30 test	REF: BS.1/PTT01.004.0004	40 test
REF: BS.1/PTT05.003.0015	150 test	REF: BS.1/PTT06.003.0018	180 test
REF: BS.1/PTT05.004.0020	200 test		

CLINICAL SIGNIFICANCE [1]

Formation of a stable fibrin clot in order to arrest bleeding is a complex process that involves sequential interaction of a series of plasma proteins in a highly ordered and complex manner. Activated Partial Thromboplastin Time (APTT) is a basic coagulation screening test useful to investigate the intrinsic coagulation pathway. It is prolonged with a deficiency of coagulation factors of the intrinsic pathway of the human coagulation mechanism such as factor XII, XI, IX, VIII, X, V, II, and Fibrinogen. Determination of APTT helps in estimating abnormality in most of the clotting factors of the intrinsic pathway and is also a sensitive procedure for generating heparin response curves for monitoring heparin therapy. Prolonged APTT may require further investigations related to congenital and acquired deficiencies

METHOD PRINCIPLE [2]

BioScien reagent involves recalcification of normal anticoagulated plasma. Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions, the time to formation of a fibrin clot is then measured. APTT is prolonged if there is a deficiency of factors involved in the coagulation mechanism.

REAGENT COMPOSITION

Reagent (R):	Composition
R1: LS-THROMBOCELIN	It is phospholipids preparation derived from rabbit brain with ellagic acid as an activator
R2: Calcium Chloride	0.025 mol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the APTT reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien LS- Thrombocelin reagent is ready-to-use and is stable until expiration date stated on label when properly stored refrigerated at 2-8°C (do not freeze).
Stability: Till the expiry date on the label at 2-8 ºC,
1 week at 15-25 ºC,
2 days at 37ºC.

Deterioration
The ***BioScien*** LS- Thrombocelin reagent is normally clear, do not use reagent if it is turbid or contaminated.

SPECIMEN COLLECTION AND PRESERVATION [1]

Plasma
Patient should be careful venipuncture and the blood should be transferred into anticoagulated tube containing trisodium citrate (0.11 mol/L, 3.2%). Mix blood with anticoagulant with definite ratio 4.5 ml blood and 0.5 ml sodium citrate (ration 9:1), avoid foam formation during mixing.
For occasional patients with hematocrit less than 20% or greater than 55%, this ratio must be readjusted to ensure valid result. Centrifuge immediately for 15 minutes at 1500 – 3000 rpm (approximately 1500g) on laboratory centrifuge and transfer the plasma into a clean test tube. It should be ensured that the plasma is free from platelets. Cap the test tubes to prevent deterioration of samples. Plasma must be tested immediately. However if the specimens are held at 2 - 4 °C then they may be tested within 3 hours.

Pooled Plasma
Prepare a fresh normal plasma pool (FNP) from at least five normal healthy donors and process as above. Plasma must be tested within 3 hours of blood collection

SYSTEM PARAMETERS

Refer to "***Tridema BioScien***" instrument instructions for test determination parameters or equivalent.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and water bath

ASSAY PROCEDURE

Homogenized LS-Thrombocelin reagent before use and often during use.

Automated instrument procedure
The reagent requires a stirred position on automatic instrument. If there is no mix gently before each series of measurement. Refer to "***Tridema BioScien***" instrument instructions or equivalent instrument manufacturer's instructions.

Manual Procedure
Thromboplastin and calcium chloride reagents amount required for immediate testing in duplicate is aspirated in a clean dry test tube and warmed for 15 minutes at room temperature before using to determine the APTT in Sec.

	Test tube (glass or plastic)
Thrombocelin	100 µl
Specimen	100 µl
Incubate for 3 minutes at 37ºC	
Calcium chloride	100 µl

Simultaneously start a stop watch and shake the tube gently to mix contents. After 20 sec. incubation, gently tilt the tube back and forth and stop the stopwatch as soon as the first fibrin strand is visible and the gel/clot formation begins. Repeat steps for a duplicate test and record the average time in seconds presenting Activated Partial Thromboplastin Time (APTT of patient plasma).
Replace the patient's plasma with the fresh normal plasma and record the APTT values of FNP in duplicate.

CALCULATION [3]

The result of the mean of the double determination of APTT of the test plasma may be reported as:

1. Result in seconds = Patient time and Reference Plasma time
2. Result in ratio = Patient time / Reference Plasma time

Example:

$$R = \frac{\text{APTT of patient plasma (in seconds)}}{\text{APTT of FNP (in seconds)}}$$

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact ***BioScien*** technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean sec	28.2	55.7	34.3	53.4
SD.	0.17	1.22	1.27	1.46
CV. %	0.59	2.19	3.7	2.73

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)

Result obtained from ***BioScien*** APTT reagent compared with commercial reagent of the same methodology performed on 20 human plasma samples give a correlation of 0.979.

INTERFERING SUBSTANCES [2,4]

Heparin, depending on its origin and composition (calcium or sodium salt) has different influence on the sensitivity of reagent.

Haemolysis
No significant interference from haemoglobin up to 327 µmol/L.

Icterus
Significant interference of bilirubin levels above 60 µmol/L.

Others
Turbidity caused negative interference above 0.2%.

EXPECTED VALUES [1,3]

Plasma	***In Sec.***
Adult	28-42
Newborn	APPT time is prolonged and reaches normal values at around 6 months.

Each Laboratory should determine its reference normal time using a pool of normal patient specimens.

DYNAMIC RANGE

Normal APTT In sec. ranging between 28-42.

Remarks

- Clotting time of patients on anticoagulant therapy depends upon the type and dosage of anticoagulant and also the time lag between the specimen collection and the dose.
- Abnormalities of coagulation factor VII, factor XIII, and platelets are not detected by this method.
- Platelet factor IV, a heparin-neutralizing factor can be released due to platelet aggregation or damage. In order to prevent this phenomenon in-vitro the specimen should be collected with a minimum of trauma.
- Decrease in APTT time is observed in males under estrogen therapy and oral contraceptive administration in females.

REFERENCES

1. Clinical Guide to Laboratory Test,4th ED., N.W. TEITZ (2006) p.46-47.
2. Struver G.P., Bittner D.L., Am. J. Clin. Path. 1962, 174, 880-881.
3. Misrahi N., Manet L, Conard J., Samama M., Act. Pharm. Biol. Clin.1981, 1,81-85
4. YOUNG D.S., Effect of Drugs on Clinical laboratory Tests,4th ED (1995) p,3-447-448.

SYMBOLS IN PRODUCT LABELLING

IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		

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CE