DIAGNOS APTT

Haemostasis Reagent for APTT Determination

EXPLANATION OF THE TEST

DIAGNOS APTT is a ready to use activated Cephaloplastin reagent for use in the in vitro testing of Activated Partial Thromboplastin Time by mechanical clot detection system. The test is used for monitoring heparin therapy, for diagnosing congenital deficiency of factor VIII, IX, XI and XII in presurgery screening.

PRINCIPLE

DIAGNOS APTT is a liquid Cephaloplastin reagent activated with Ellagic acid. The method is responsive to coagulation factors of the intrinsic path way in plasma in the presence of calcium ions. The deficiency of one or the more clotting factors of the intrinsic path way and also the presence of heparin (coagulation inhibitor) prolongs Activated Partial Thromboplastin Time (APTT) of the plasma. The time taken for the clot to form is measured and used to determine the Anticoagulant status of the patient.

MATERIALS PROVIDED

1. Diagnos APTT Reagent
2. Calcium Chloride (0.025 mol / litre)
3. TSC Solution (3.2% buffered Tri-sodium citrate solution)

MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette for 100μl
2. Waterbath at 37°C or other coagulometer
3. Stop Watch
4. Test Tubes

KIT PRESENTATION

2 ml

STORAGE AND STABILITY

DIAGNOS APTT kit should be stored at 2-8°C in the coolest & driest area available. The kit has a shelf life of 24 months from the date of manufacturing.

Do not freeze the Reagent.

PRECAUTIONS

In order to obtain reproducible results, the following instructions must be followed:

1. For *in vitro* diagnostic use only.
2. Do not use the kit beyond the expiry date.
3. Do not eat or smoke while handling specimens.
4. Do not combine reagents from different batches as they are optimized for individual batch to give best results.
5. Clean up spills thoroughly using an appropriate disinfectant.
6. The 37°C incubator should be calibrated daily since the Activated Partial Time Test functions accurately only at 37 $\pm$ 1°C.
7. The kit reagent and $CaCl_2$ should be properly pre-warmed at 37°C before use.
8. **For best results, follow the given test procedure and storage instructions strictly.**
9. Do not exchange the cap of APTT & $CaCl_2$ vials as it will lead to erroneous results.

Important : The test tubes and microtips used for performing the test should be clean & dry otherwise erroneous results may be obtained.

SAMPLE / SPECIMEN COLLECTION AND STORAGE

Collect 9 parts of venous whole blood in a clean container / tube having 1 part of TSC Solution (3.2% Tri-sodium citrate solution) and immediately mix the blood with anticoagulant avoiding the foam formation. Centrifuge the sample for 15 minutes at approximately 2000g (3000 rpm) and collect the plasma in a separate tube. Fresh plasma is preferred for testing as it performs best when tested immediately after collection. However, if testing is delayed, sample may be tested within 2 hours at 25-30°C and within 3 hour at 2-8°C. Do not use haemolysed, lipaemic, turbid samples to avoid erroneous result.

FNP Collection

Prepare a plasma pool FNP of freshly collected blood from atleast five normal healthy individuals and process as above.

Important : Plasma must be tested within 3 hours of blood collection.

TEST PROCEDURE

1. Bring the kit & sample to room temperature before use.
2. Always mix **DIAGNOS APTT** reagent by gently swirling before use.
3. Pipette 100μl each of **DIAGNOS APTT** and 0.025 mol/L Calcium Chloride reagent in separate test tubes and incubate in water bath at 37°C for 3 min.
4. Add 100μl plasma sample to the tube containing pre-warmed APTT reagent and incubate in water bath at 37°C for 3 minutes.
5. After 3 minutes, add 100μl of well mixed Calcium Chloride reagent pre-warmed to 37°C to the tube containing **DIAGNOS APTT** reagent and plasma and simultaneously start the stop watch. Mix the contents of the tube gently and keep for 20 seconds at 37°C in water bath. Following 20 seconds incubation, remove the tube from 37°C and tilt the test tube back & forth and stop the stopwatch as soon as fibrin strand is visible which initiates gel clot formation.
6. Measure the time taken for clot formation to the nearest 0.1 seconds. This is the Activated Partial Test Time (APTT) in seconds.
7. Repeat steps 2-6 for the same sample & calculate the mean APTT.

For use with automated or semi-automated coagulometers, follow the manufacturer instructions.

CALCULATION OF RESULTS

The results may be interpreted directly in terms of APTT of the test plasma in seconds (refer step 6 in test procedure) or as a ratio 'R'.

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

Normal Values

22-32 seconds (at 3 minutes activation time)

It is recommended that each laboratory must establish their own normal values for photo optical systems as it depends on method used, activation time and instrument used.

HEPARIN CONCENTRATION

For determination of Heparin Concentration, a calibration curve has to be made as following :

1. Prepare a pool of fresh normal plasma from atleast 5 Normal Healthy individuals (FNP).
2. Dilute Heparin (as used for treatment) with 0.9% normal saline to a concentration of 10 U/ml.
3. Mix 0.2 ml of diluted 10 U/ml Heparin with 1.8 ml of FNP as to give Heparin concentration of 1 U/ml.
4. Dilute the 1 U/ml Heparin standards with FNP as follows :

Test Tubes	1	2	3	4	5	6	7
Heparin Standard 1 U/ml	0.5ml	0.4ml	0.3ml	0.2ml	0.1ml	0.1ml	-
FNP	-	0.1ml	0.2ml	0.3ml	0.4ml	0.9ml	0.5ml
Heparin Dilution	1 U	0.8 U	0.6 U	0.4 U	0.2 U	0.1 U	0 U

5. Pipette 100μl each of the 7 Heparin dilutions into separate test tubes.
6. Add 100μl Diagnos APTT reagent pre-warmed to 37°C to each test tube. Mix well and incubate at 37°C for 3 minutes.
7. Add 100μl Calcium Chloride pre-warmed to 37°C to each test tube, one by one whilst simultaneously start the stop watch.
8. Mix the reagent properly & tilt the test tube back & forth and stop the stop watch as soon as fibrin strand is visible which indicates gel clot formation.
9. Repeat steps 5 to 8 for each dilution for duplicate reading and calculate the mean test values.
10. Plot mean test values in seconds against each heparin concentration on heparin graph paper.
11. Connect the points on the graph and plot clotting time (seconds) of the test specimen on the calibration curve.
12. Extrapolate the heparin concentration of the sample (U/ml) from the graph.

LIMITATIONS OF THE PROCEDURE

1. Improper mixing of blood sample with sodium citrate solution, use of contaminated reagents / test tubes / tip, incorrect heparin dilution, incorrect prewarming time of $CaCl_2$ & reagent are potential source of error and may give erratic results.
2. It is recommended to run both normal and abnormal control simultaneously with each test series to validate the test procedure.
3. Every lab should establish their own normal and abnormal range. The labs using automated equipment should strictly follow the manufacturer instructions.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction-manual, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer's liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed. The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

REFERENCES

1. Biggs, R., ed.: Human Blood Coagulation, Haemostasis and Thrombosis, Blackwell, Scientific Publications Oxford, England, 1972.
2. Hoffmann, J.J.M.L. and Neulendijk P.N.: Thrombos. Haemostas. (Stuttgrat) 39 640 (1978).
3. CRC Handbook Series in Clinical Laboratory, Science, Section I: Hematology, Volume III, 1980. CRC Press, Inc. Boca Raton, Florida.

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in vitro diagnostic reagent, not for medicinal use

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