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APTT

Activated Partial Thromboplastin Time (APTT) Reagent

REF

Catalog No. S12-745

IVD

For Professional Use Only

INTENDED USE

Immunospec **APTT** reagent is an *in vitro* diagnostic assay intended for use in determining activated partial thromboplastin time (APTT) and coagulation factor assays that are based on a modified APTT.

SUMMARY

The activated partial thromboplastin time (APTT) is used as a general screening test for the detection of coagulation abnormalities in the intrinsic pathway. The APTT is sensitive to deficiencies or abnormalities of factors VIII, IX, XI, XII, X, and II, prekallikrein, high molecular weight kininogen (HMWK), and fibrinogen. APTT is also sensitive to inhibitors of blood coagulation such as lupus inhibitor and fibrin/fibrinogen degradation products [1]. The APTT is the most widely used method for monitoring intravenous heparin anticoagulation therapy [2, 3].

PRINCIPLE

The capacity of blood to form a fibrin clot by way of the intrinsic hemostatic pathway requires coagulation factors I, II, V, VIII, IX, X, XI and XII, platelet lipids and calcium[4]. The assay is performed by the addition of a suspension of rabbit brain cephalin with a surface activator[1]. The APTT has proven to be a simple and highly reliable measurement of the intrinsic coagulation mechanism[5].

REAGENT

Immunospec **APTT** reagent is a preparation of rabbit brain cephalin and ellagic acid activator with buffer, stabilizers and preservatives. The reagent is provided ready to use.

PRECAUTIONS

Do not ingest. Avoid contact with skin, eyes or clothing.

STORAGE AND STABILITY

The Immunospec **APTT** reagent is stable to the expiry date shown on the label when stored in the original container at 2 to 8°C.

SPECIMEN COLLECTION AND PREPARATION

Test plasma should be prepared from citrated whole blood **without** heparin, EDTA or oxalate.

1. Blood Collection using Syringe Method: Draw venous blood into a plastic or siliconized syringe. Immediately transfer 9.0 mL of blood into a tube containing 1.0 mL of 3.2% or 3.8% sodium citrate solution.

2. Blood Collection using an Evacuated Blood Collection Tube: Draw venous blood into a commercial vacuum tube containing 3.2% or 3.8% sodium citrate solution. Insure that a full draw has been obtained since the ratio of 9 parts blood to 1 part citrate is critical. A heparinized lock or transfer line should not be used. It is generally recommended that the second or third tube draw be used for coagulation tests.

3. Plasma Preparation: Mix well by inversion and centrifuge at 2,500 x g for 15 minutes soon after blood collection. Unless samples are to be processed immediately, transfer the plasma into a plastic tube. Plasma that is clearly hemolyzed or contains > 10,000 platelets per cubic milliliter or red cells is not suitable for coagulation testing.

4. Plasma Storage: Plasma samples should be transferred to a plastic tube as soon as possible and stored refrigerated (2 to 8°C). Plasma samples should be tested within 4 hours and should not be incubated at 37°C for more than 5 minutes to avoid loss of factors V and VII.

MATERIALS PROVIDED

Immunospec **APTT** reagent.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Immunospec Calcium Chloride, 0.02M.
2. Immunospec Coagulation Control Plasmas 1, 2 and 3.

PROCEDURE

This procedure pertains to manual or semi-automated coagulation systems. Refer to your instrument manual for more detailed instrument specific instructions.

1. Pre-incubate the Immunospec Calcium Chloride (0.02M) to 37°C for at least 10 minutes.
2. Pipette 100 µL of test or control plasma into a test cuvette. Incubate at 37°C for 1 to 2 minutes.
3. Add 100 µL of the Immunospec **APTT** reagent to the cuvette containing the plasma. Maintain the suspension of the reagent by magnetic stirring or mixing by inversion immediately prior to use.
4. Incubate the mixture at 37°C for 3 minutes.
5. Rapidly add 100 µL of the pre-incubated Immunospec Calcium Chloride (0.02M) and simultaneously start the timer.
6. Record the clotting time in seconds.

QUALITY CONTROL

Reliability of test results should be monitored within each run using Immunospec **Coagulation Control Plasmas 1**, **2** and **3**. Each laboratory should establish a control range to determine the allowable variation in day to day performance of each control plasma.

CALCULATION OF RESULTS

For best results, duplicate samples are recommended. APTT results should be reported as clotting time in seconds. Calculate the mean clotting time of duplicate samples and controls. Differences between duplicate results should be less than 5%. Repeat the test if necessary.

LIMITATIONS

To prevent discrepant results ensure the blood to anticoagulant ratio is 9:1. Grossly lipemic or hemolysed samples may produce erroneous APTT values[6].

Delay in testing, difficulty in specimen collection, or venipucture above the site of a heparin lock may result in falsely prolonged APTT results[7]. The APTT may also be influenced by certain drugs and medications[8]. APTT results can vary with anticoagulation therapy depending upon the type and dosage of anticoagulant, the route of administration and the time of administration of the last dose.

EXPECTED VALUES

APTT results are influenced by the method of clot detection and can vary from laboratory to laboratory. In general an APTT test performed on a photo-optical coagulometer will give clotting time for normal plasma in the range of 24 to 39 seconds. Therapeutic ranges for monitoring oral anticoagulation therapy will vary from laboratory to laboratory, therefore it is essential that each laboratory establish relevant APTT ranges for its respective patient population.

Abnormal results obtained with a plasma from a patient not on anticoagulant therapy may indicate a factor deficiency or the presence on an inhibitor. The result may also be due to the effects of certain drugs and medications. Additional procedures such as the PT test and mixing studies using factor deficient plasma are usually required.

PERFORMANCE CHARACTERISTICS

Precision: Within-run precision was assessed using normal and abnormal plasma controls, on manual (Fibron-1), semi-automated (MLA™ Electra 900C) and fully automated (Instrumentation Laboratory ACL-100) instruments. The results are shown in the following table.

Sample	Fibron-1 (Manual)	Electra 900C (Semi-Automated)	ACL-100 (Automated)
Control Level 1	3.3 %	2.0 %	1.2 %
Control Level 2	1.3 %	2.2 %	0.6 %
Control Level 3	4.2 %	2.3 %	0.8 %

Correlation: Correlation studies were performed against another APTT reagent on the MLA™ Electra 900C and the Instrumentation Laboratory ACL 100 coagulometers. The results are shown in the following table

Instrument	Regression Coefficient	Slope	Intercept
Electra 900C	0.938	0.727	6.96
ACL-100	0.930	0.952	3.76

Factor Sensitivity: APTT times obtained with the Immunospec **APTT** reagent were evaluated on factor-deficient plasmas using the MLA™ Electra 900C. The results are shown in the following table.

Percent Factor	APTT – Clotting Times (in seconds)			
	Factor VIII	Factor IX	Factor XI	Factor XII
100 %	25.4	25.7	25.8	25.9
60 %	28.2	27.8	28.6	29.6
50 %	28.7	28.3	30.2	31.5
40 %	29.6	30.7	32.0	33.2
30 %	33.5	31.1	34.3	35.1
20 %	35.0	32.9	36.5	38.7
10 %	41.4	36.7	42.4	45.7

These values should be used as guidelines only. Each laboratory should establish Factor sensitivity using their instruments, reagents and techniques.

Heparin Sensitivity: The anticoagulant action of heparin depends on many factors. Each laboratory should determine the relative heparin sensitivity by adding known amounts of unfractionated heparin to pooled normal plasma and determining the elevation in clotting time using the Immunospec **APTT** reagent. The following results were obtained using the MLA™ Electra 900C and the Immunospec **APTT** reagent.

Heparin Concentration (units / mL)	APTT – Clotting Time (in seconds)
0.0	23.0
0.1	28.0
0.2	38.1
0.3	59.0
0.4	76.1
0.5	99.4

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