Bio/Data Corporation

Cephalinex®

Activated PTT REAGENT
(Silica Activated)

For use in the Activated Partial Thromboplastin Time Test

PRODUCT DESCRIPTION

Cephalinex® is a lyophilized preparation of rabbit brain cephalin and particulate (microsilica) activator. Cephalinex supplies the phospholipid platelet substitute and negatively charged surface required for plasma coagulation by way of the "intrinsic" hemostatic pathway.

INTENDED USE

Cephalinex is for use in performing an activated partial thromboplastin time (APTT) and coagulation factor assays which are based on a modified APTT.

The APTT is the most widely used method for monitoring intravenous heparin anticoagulation therapy.[1,2] It is also a fundamental screening test for deficiencies or abnormalities of the intrinsic coagulation factors: VIII, IX, XI, XII, and factors common to both the intrinsic and extrinsic pathways: I (fibrinogen), II, V, X.[3] When used in conjunction with deficient substrate plasma, the APTT provides the basis for the quantitation of specific coagulation factors.

PRINCIPLE

The intrinsic capability of blood to form a fibrin clot requires coagulation factors XII (Hageman), XI (plasma thromboplastin antecedent), IX (Christmas), VIII (anti-hemophilic), X (Stuart-Prower), V (proaccelerin), II (prothrombin), I (fibrinogen), platelet lipid, and calcium.[4]

Historically, intrinsic coagulation was measured by timing fibrin clot formation upon recalcification of citrated, anticoagulated, platelet rich plasma. Measurement with platelet rich plasma, however, relied on the platelets as a source of phospholipid to the extent that variables such as centrifugation and patient platelet count had a significant bearing on the test results.[5]

The partial thromboplastin time (PTT), a modification of the plasma recalcification time, introduces a platelet substitute which eliminates test variability due to the availability of platelet phospholipid. By adding a substance to activate factors XII and XI, the contact factors, the partial thromboplastin time becomes the "activated" partial thromboplastin time (APTT). Because coagulation endpoints are shorter and sharper than with the PTT, the APTT has proven to be a simple and highly reliable measurement of the intrinsic coagulation mechanism.[5]

PRECAUTIONS

Cephalinex is FOR IN VITRO DIAGNOSTIC USE ONLY and is NOT FOR INGESTION OR INJECTION.

MATERIALS PROVIDED

Cephalinex, 20 x 3.0mL, or 15 x 10.0mL. Store at 2° - 8° C prior to reconstitution.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Purified water, pH 5.3 - 7.2 (distilled, deionized or reagent grade)
2. Pipettes (10.0mL, 3.0mL, and 0.1mL volume capacities)
3. Citrated control plasmas (see PRODUCT AVAILABILITY)
4. Calcium Chloride, 0.025 M (see PRODUCT AVAILABILITY)

INSTRUMENTATION

Activated partial thromboplastin time endpoints may be detected by manual methods or with any automated or semi-automated coagulation analyzer. Follow the manufacturer's instructions for operating the analyzer in use.

COLLECTION AND PREPARATION OF TEST PLASMA

Test plasma for the APTT should be prepared from citrated whole blood.

1. Blood Collection
   Blood collection for the APTT, as for any coagulation test, should be performed with care to avoid hemolysis or contamination by tissue fluids.
   a. Syringe Technique:
      Draw blood. Mix immediately with 0.11M sodium citrate by adding 9 parts whole blood to 1 part anticoagulant. Invert gently to mix.
   b. Vacuum blood collection tube technique:
      Draw blood using tubes containing sodium citrate anticoagulant. Invert gently to mix.
2. Centrifuge blood at 2500 x g for 15 minutes.
3. Remove plasma from cells, being careful not to disturb the buffy coat. Plasma should be free of red cells and platelets.
4. If testing is delayed, refrigerate the plasma at 2° - 8° C for a maximum of 2 hours.

NOTE: *THE FIRST TUBE DRAWN SHOULD NEVER BE USED FOR COAGULATION TESTS.*

RECONSTITUTION

Warm vial to room temperature prior to reconstitution.

1. Tap the vial to dislodge material adhering to the stopper.
2. Remove the aluminum seal by lifting the plastic safety cap.
3. Remove the stopper and reconstitute the vial contents with the volume of purified water specified on the label.
4. Replace the stopper and invert the vial to thoroughly disperse the contents. Let stand for no less than 15 minutes prior to use to assure complete rehydration of the contents.

Once reconstituted, Cephalinex is stable for seven days when stored in the tightly capped original container at 2° - 8° C.

TEST PROCEDURE

NOTE: *FOR BEST RESULTS SUSPENSION OF THE REAGENT SHOULD BE MAINTAINED BY MAGNETIC STIRRING OR INVERSION IMMEDIATELY PRIOR TO USE.*

1. Pipette 0.1mL test or control plasma into a test cuvette and incubate plasma at 37°C for 2 minutes.
2. Preincubate 0.025M Calcium Chloride to 37°C.
3. Pipette 0.1mL of reconstituted Cephalinex into a test cuvette with the plasma.
4. Incubate at 37°C for exactly 5 minutes.
5. Add 0.1mL preincubated Calcium Chloride, simultaneously starting the timer.
6. Record the clotting time.
7. Repeat steps 1 through 6 for duplicate sample. These results should correlate within ± five percent (5%).

NOTE: *THIS TEST PROCEDURE IS FOR MANUAL OR SEMI-AUTOMATED COAGULATION SYSTEMS. FOR USE WITH AUTOMATED COAGULATION SYSTEMS, FOLLOW THE INSTRUCTIONS IN THE OPERATOR'S MANUAL PROVIDED WITH THE INSTRUMENT.*

QUALITY CONTROL

The precision and accuracy of APTT test results may be affected by a number of factors. Significant interlaboratory differences arise from the variety of coagulation systems used to measure clotting endpoints.[6,7] Intralaboratory variables which may impact APTT results include: pH of purified water used for reagent reconstitution, pipetting technique, incubation temperatures, incubation time, reagent contamination, and change in reagent lot number.[8] Periodic quality control procedures, performed on a regular basis, will help to identify the occurrence of any deviations which may lead to erroneous test results.

Each laboratory should establish acceptance limits for each lot of control plasma and APTT reagents by performing replicate studies, in accordance with established laboratory procedures.[8]

Four varieties of control plasmas are available for performing quality assurance tests with Cephalinex® (see PRODUCT AVAILABILITY). When used as test specimens, these controls represent four significant levels of the APTT test: normal results, two levels of abnormal results, and therapeutic (heparin anticoagulant) results.

RESULTS

The APTT result is reported as the clotting time endpoint in seconds. In addition to the clotting time, a predetermined normal range should be reported as APTT ranges may vary substantially from laboratory to laboratory.[7-10]

EXPECTED VALUES

The results obtained with Cephalinex are influenced by the method of coagulation endpoint detection. In general, expected results for APTTs performed with Cephalinex on a photo optical coagulation analyzer will be in the range of 26 to 38 seconds. When a mechanical based coagulation analyzer is used, expected values are generally in the range of 30 to 42 seconds. Each laboratory should established its own reference range. For manual methods, expected results range from 30 to 42 seconds. Due to this variability it is essential that each laboratory determine the normal range based on the coagulation analyzer to be used and the patient population to be assessed.

When Cephalinex is used to evaluate the integrity of the intrinsic coagulation mechanism, results outside of the reference range could be indicative of single or multiple factor deficiencies in the intrinsic coagulation pathway or the presence of a circulating inhibitor.

When Cephalinex is used for monitoring heparin therapy, results should be evaluated relative to the therapeutic range established by the institution.

LIMITATIONS

Grossly lipemic or hemolyzed specimens may produce erroneous test results. Incorrect blood to anticoagulant ratios may result in spurious test results. The quantity of sodium citrate added to blood must be proportionally decreased in patients with hematocrit values above 53 and increased in patients with hematocrit values below 25.[11]

Delay in testing, difficulty in specimen collection, or venipuncture above the site of a heparin lock may result in a falsely prolonged APTT value. When results are not within the expected limits the possibility of improper specimen collection or handling should be investigated.[9]

PERFORMANCE CHARACTERISTICS

Cephalinex has been tested with photo optical, mechanical, and manual endpoint detection systems. Its precision and sensitivity are sufficient to provide a reliable method of evaluating mild to severe abnormalities in intrinsic coagulation as well as monitoring anticoagulation with heparin.[12]

REFERENCES

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PRODUCT AVAILABILITY

PRODUCT	NET CONTENTS	CATALOG NUMBER
Cephalinex®, APTT	20 x 3.0mL	101162
Reagent *(Silica Activated)*	15 x 10.0mL	102677
ALSO AVAILABLE		
Plastinex®	20 x 4.0mL	101158
Thromboplastin Reagent	15 x 10.0mL	102672
Coagulation Control Plasma		
Citrex® I (Normal)	20 x 1.0mL	101166
Citrex II (Abnormal)	20 x 1.0mL	101170
Citrex III (Abnormal)	20 x 1.0mL	101174
Citrex H (Heparin Control)	20 x 1.0mL	102682
Thrombinex®		
(Bovine Thrombin)	20 x 2.0mL	101628
Calcium Chloride Solution		
0.025M	1 x 473mL	100989

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