

CEPHEN 5

Reference ACK515K-RUO

APTT clotting time reagent

8 x 50 tests

Liquid reagent, ready to use

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.



Manufactured By: HYPHEN BioMed

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INTENDED USE:

Liquid reagent, ready to use, for the determination of Activated Partial Thromboplastin Time (APTT) on citrated human plasma, using a manual, semi automated or automated clotting method. This reagent has a low sensitivity to Lupus Anticoagulant. **This assay is for research use only and should not be used for patient diagnosis or treatment.**

ASSAY PRINCIPLE:

Measurement of the plasma recalcification time, in presence of the standardized APTT (Activated Partial Thromboplastin Time) reagent (cephalin and activator), on human citrated plasma, to explore the activity of the intrinsic pathway coagulation factors (II, V, VIII:C, IX, X, XI, XII). This reagent has intentionally less sensitivity to lupus anticoagulants than routine reagents. Otherwise it is similar to Cephens LS reagent.

ASSAY SPECIMEN:

Human plasma obtained from Trisodium Citrate anticoagulated blood.

REAGENTS:

8 vials of 5 ml (liquid reagent, ready to use).

REAGENTS AND MATERIAL REQUIRED, BUT NOT SUPPLIED:

- Pipettes.
- Stop watch.
- Semi-automatic or automatic coagulation instrument, Fibrometer or electromagnetic water bath.
- Distilled water.
- Quality control plasmas (eg: BIOPHEN Normal (#A223201) and Abnormal (#A223301) Control Plasma).
- 0.025M Calcium Chloride (eg : AAR001A/K).

REAGENT PREPARATION AND STABILITY:

In the original package, and before any use, when stored at 2-8°C, the reagent is stable until the expiry date printed on the box.

Preparation: Each vial contains 5 ml of ready to use APTT reagent. Before each use, let the reagent stabilize for 30 min. at room temperature (18-25°C); while shaking the vial from time to time.

Stability: When open, the reagent is stable for at least:

- 7 days at room temperature (18-25°C)
- 3 months at 2-8°C

provided that any contamination or evaporation is avoided.

Note:

Sodium azide (<1g/L), used as preservative, may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink (which is not recommended because of environmental risks). Stability studies for 3 weeks at 30°C show that the reagent can be shipped at room temperature without damage. White sediment may be noticed at 2-8°C, and disappears by homogenizing at RT. This product must be handled with all the required cautions.

OPERATING PROCEDURE:

Sample collection and preparation:

Blood (9 vol.) must be collected preferably into 0.109M or 0.129M trisodium citrate anticoagulant (1 vol.) in a plastic or siliconized glass tube; plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma must be tested within 4 hours when stored at 20°C (or within 2 hours for heparinized samples). Specific collection tubes for heparin testing, such as the CTAD (Citrate, Theophylline, Adenosine and Dipyridamole) tubes, can be used. They improve specimen stability.

Note: Refer to GEHT or NCCLS guidelines for further instructions on specimen collection, handling and storage. Discard any plasma presenting an unusual aspect (haemolysed...).

Tested plasma:

Plasma must be tested **undiluted**.

Note: Clotting times can slightly vary according to the type of citrated anticoagulant used.

Assay:

Mechanical manual method:

Principle: a mechanical coagulation indicator, such as a metal ball or index, or balancing, is used for detecting clotting. The test is performed at 37°C.

Into a small test tube, or in the reactional cuvette of the coagulation instrument, introduce:

- 100 µL of citrated test plasma
- 100 µl of reagent.

Mix and incubate for exactly 3 minutes at 37°C, and then introduce (Starting the stop-watch):

- 100µl of 0.025M Calcium Chloride (pre incubated at 37°C).

Record the exact clotting time (stop of the metal ball or index, or coagulation detected by clot formation...)

Automated method:

Adaptations to the main coagulation analyzers are available upon request.

RESULTS:

As an indication, APTT values obtained on KC10 or STA-R, for normal plasmas, are usually in the range:

28 sec. to 37 sec.

APTT is abnormal if: **> 40 seconds.**

The obtained APTT for the patient must be compared with that of the reference normal range for the laboratory.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

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The clotting times obtained can vary according to the citrated anticoagulant used (0.109 or 0.129M), as well as to the clot detection mode (mechanical or optical). Clotting times are shorter when using 0.109M citrate, and an optical detection mode.

Each laboratory should establish its own usual normal range (mean \pm 2 standard deviations (SD)), which can slightly vary according to the reagent lot and instrument used.

QUALITY CONTROL:

Use of quality control plasmas ensures consistency from run to run, for the same lot of reagent.

The following controls can be used for the test validation:

BIOPHEN Normal Control Plasma (#A223201)

BIOPHEN Abnormal Control Plasma (#A223301)

The clotting time obtained for a repeat test and with the same reagent lot can vary slightly according to the instrument used and the clot detection sensitivity. Each laboratory should establish and validate its own usual range, mean and standard deviation, in its specific test conditions.

PROLONGED APTT:

A prolonged APTT can result from:

- Presence of "anticoagulant" activities induced by therapy (Heparin, Hirudin, Argatroban®, Angiox®, Vitamin K antagonists...).
- Factor deficiencies: II, V, X (<5 to 10%), VIIIc, IX, XI, XII (<20%), including high molecular weight kininogen (<5%).
- Abnormalities or acquired deficiencies due to an excessive consumption of the coagulation factors, hepatic disorders...
- Coagulation inhibitors such as Lupus Anticoagulant or auto-antibodies to coagulation factors. Note that this reagent is intentionally less sensitive to lupus anticoagulants than most other routine APTT reagents.

PERFORMANCES:

- As an example, the "usual" APTT range has been determined for citrated normal human plasmas using:

| Lot 070622B | KC10 | STA-R | ACL 7000 (research software) (optical mode) |
|--------------------|-----------|-----------|---|
| N | 30 | 50 | 30 |
| M (Mean APTT, sec) | 28.8 | 31.4 | 25.1 |
| SD (sec) | 2.01 | 2.66 | 1.77 |
| M \pm 2SD (sec) | 24.8-32.8 | 26.1-36.7 | 21.6-28.6 |
| Min-Max (sec) | 25.6-33.5 | 27.1-39.2 | 22.2-30.0 |

- A good sensitivity is obtained to low concentrations of heparin present in the tested plasma (from 0.1 IU/ml of Unfractionated Heparin (UFH); or from 0.2-0.4 IU/ml of Low Molecular Weight Heparin (LMWH) (prolongation of clotting time in presence of UFH or LMWH can slightly vary from plasma to plasma). As an example, the following clotting times (in seconds) were obtained for UFH or LMWH spiked into a normal citrated human plasma pool, using the STA-R instrument (for a same amount of heparin in plasma, prolongation of APTT is dependent on the plasma used) :

| Lot | IU/ml | Plasma Pool + UFH | | | | Plasma Pool + LMWH | | | |
|---------|------------|-------------------|------|------|-------|--------------------|------|------|------|
| | | 0 | 0,20 | 0,35 | 0,50 | 0 | 0,20 | 0,50 | 1,00 |
| 90201 | aPTT (sec) | 31.1 | 59.3 | 97.9 | 177.0 | 31.7 | 38.4 | 45.5 | 60.8 |
| 080326B | aPTT (sec) | 30.1 | 52.7 | 85.6 | 154.9 | 30.5 | 37.0 | 43.1 | 58.1 |

Cephen is very sensitive to Unfractionated Heparin (UFH) in plasma, and clotting times prolongation is significative from 0.1 IU/ml. This sensitivity is lower for Low Molecular Weight Heparin (LMWH).

Heparin sensitivity is variable for the various APTT reagents marketed. It also can present slight variations from lot to lot for a same reagent. Heparin sensitivity must be checked by the laboratory in the actual conditions of testing, and for the lot used. A same plasma heparin concentration can produce variable prolongations of the APTT, and of the clotting time ratio Patient/Normal Control.

- Good sensitivity to hirudin, using the KC10 instrument, from 0.1 to 0.2 μ g/ml in plasma.

- Accuracy: as an example, the following results were obtained using the STA-R instrument:

| Lot 070622B | APTT (sec) | N | Intra assay CV (%) | Inter assay CV (%) |
|--------------------|------------|----|--------------------|--------------------|
| Normal plasma pool | 33.0 | 10 | 0.38% | 2.22% |
| Heparinized plasma | 67.0 | 10 | 0.53% | 2.26% |

LIMITS:

- Various drugs or therapies can affect APTT results. An additional investigation should be conducted to determine the origin of each unexpected or abnormal result.
- A "repeat" clotting time for a sample even with the same reagent lot can vary slightly according to the instrument used, and the clot detection mode and instrument setting (clot detection sensitivity). Each laboratory should establish and validate its own usual range, mean and standard deviation, in its specific test conditions. In the same way, many variables (eg: different sources of heparin) can affect the results obtained: each laboratory should establish its own acceptable ranges.
- The 3 min. incubation time must be adhered to consistently. If this incubation time must be changed for the needs of an instrument application (for example 4 min.), it must be the same for all the tests performed.
- The reagent offers a good sensitivity for a prekallikrein deficiency <1%, but no sensitivity for concentrations >5%.
- Using the KC10 instrument, there was no significant interference noticed up to <0,25 mg/ml bilirubin, or up to <5 mg/ml haemoglobin added to plasma.
- Any sample presenting an abnormal aspect (eg: lipaemic, haemolysed, partial coagulation...) should be rejected.
- **The results obtained should be for research purposes only and not used for patient diagnosis or treatment.**

REFERENCES:

- "Hémorragies et thromboses – Du diagnostic au traitement", M.M. Samama et coll., Abrégés, Masson, 2004.
- www.geht.org
- NCCLS (CLSI) document H21-A4 on specimen collection, handling and processing.