

LupoTek KCT

For the detection of circulating lupus-like anticoagulants



INTENDED USE

The LupoTek kaolin clotting time (KCT) is a kaolin activated partial thromboplastin reagent without phospholipid. The LupoTek KCT is a qualitative in vitro coagulation screening assay for use in professional laboratories as an aid in the detection of circulating lupus-like anticoagulants in citrated human plasma.

SUMMARY

The Kaolin Clotting Time (PTTK), an activated partial thromboplastin time test which uses kaolin as the activator, was first described in 1958 (1). The Kaolin Clotting Time formulated without any added phospholipid (KCT) was first used to study lupus anticoagulants (LA) in 1978 (2).

The KCT test has been reported to be the most sensitive test for the detection of circulating anticoagulants such as LA (3), and is one of the tests recommended by the ISTH for use in the diagnosis of LA (4).

PRINCIPLE

The LupoTek KCT is an activated partial thromboplastin time reagent without any added phospholipids and with kaolin as the contact activator. In the absence of added phospholipid the assay only poorly accelerates the clotting cascade and is sensitive to coagulopathies in the contact and common pathways.

REAGENTS

For In Vitro Diagnostic Use Only

LupoTek KCT

Catalog Number 87-305:

5 x 5 mL vials of KCT reagent

5 x 5 mL vials of 0.025M Calcium Chloride reagent

1. KCT Reagent

Ingredients: Each vial of LupoTek KCT contains 5 mL of a low turbidity, slow-settling kaolin formulated with enhanced colloidal stability, suitable for automated KCT tests. The reagent contains no phospholipid.

Preparation for Use: LupoTek KCT is provided as a liquid suspension, which can settle upon storage. Ensure the reagent is completely re-suspended and thoroughly mixed before and during use.

2. Calcium Chloride Reagent

Ingredients: Each vial contains 5 mL 0.025M Calcium Chloride reagent stabilized with 0.02% Sodium Azide.

Preparation for Use: The Calcium Chloride reagent is packaged ready for use.

Storage and stability of both reagents: Unopened vials are stable until the expiration date printed on the labels when stored at 2-8°C. Open-vials stability is 24 hours when stored at room temperature.

WARNING: SODIUM AZIDE. Sodium azide can form highly explosive metal azides if exposed to lead or copper in plumbing. Any such materials should be discarded into a sink only with large volumes of water to minimize such a risk.

Materials and Equipment Required but not Provided:

Semi-automated or automated coagulation analyzer
Common clinical laboratory equipment and materials such as centrifuges, test tubes, pipettes, and distilled water.

Control Plasmas:

- PlasmaCon N
- PlasmaCon LA

SPECIMEN COLLECTION AND HANDLING

Plasma is obtained from whole blood anti-coagulated with 1 part 3.2% sodium citrate to 9 parts whole blood. Process the collected whole blood, double centrifuge the plasma, and handle the plasma according to the CLSI guideline H21-A5 (or superseding edition) (7).

ASSAY PROCEDURE

Please contact r2 Diagnostics for validated applications.

MIXING TESTS

Mixing tests are recommended for use with the LupoTek KCT reagent in order to distinguish between factor deficiencies and antibody related inhibitors in samples testing outside the normal range (2). r^2 recommends the 1:1 mix of patient plasma and normal pooled plasma for most testing purposes (6).

Normal plasma pools (NPP) used for the mixing studies should be a carefully prepared pool of normal donors. The donor plasma should be prepared in a similar fashion to the patient samples. All plasmas should be double spun or filtered to remove platelet fragments. The normal pool should be well characterized to ensure at least 75% activity of all coagulation factors. The normal pool may be aliquoted and stored at < -50°C to provide some measure of standardization.

QUALITY CONTROL

Quality control of coagulation tests involves multiple components. Each laboratory should establish a quality control program that includes both normal and abnormal control plasmas.

EXPRESSION OF RESULTS

The KCT of neat patient sample and mixes of patient sample and NPP may be expressed as the raw clotting time in seconds.

INTERPRETATION OF RESULTS

An abnormal KCT time is only generally indicative of a defect in coagulability of the neat sample. Mixing tests with NPP are usually performed to distinguish between factor deficiencies and antibody inhibitors. 1:1 mixes that recover within the normal range of the KCT assay are often indicative of factor deficiencies, whereas those that remain abnormal are often indicative of antibody-related inhibitors.

Further workup with Factor assays, phospholipid-dependent Lupus Anticoagulant assays, or antibody affected assays such as the Bethesda assay are required to determine the final diagnosis (6, 9). No single assay is definitive for Lupus anticoagulants. The diagnosis of LA requires an algorithm that includes multiple assays (4).

LIMITATIONS:

Heparin interferes with the KCT. The KCT test is not suitable for patients undergoing heparin. Samples either known to contain heparin or suspected of heparin contamination should be further analyzed with either the Thrombin Time and Reptilase Time tests, or treated with heparinase, according to generally accepted laboratory protocols (9).

The KCT may be prolonged due to a variety of clinical conditions, such as factor deficiencies or oral anticoagulant therapy. Mixing studies are recommended to differentiate between these conditions and antibody-like inhibition (6, 9).

Platelet contamination can cause problems in kaolin-based APTT reagents and KCT reagents and can shorten the clot times, particularly with freeze-thawed plasma (10). Plasmas should be double centrifuged, especially prior to freezing.

The KCT has not been evaluated for patients undergoing Direct Thrombin Inhibitor therapy. The KCT has not been evaluated in pediatric populations.

The performance characteristics of the LupoTek KCT have been determined using an automated analyzer with a mechanical detection system (the Stago Compact). Please contact r2 Diagnostics for validated instrument applications.

PERFORMANCE CHARACTERISTICS

Precision: Precision estimates of three lots of LupoTek KCT were determined in a two run per day, twenty day exercise using normal and abnormal (LA positive) QC plasmas as described in the CLSI guideline EP5-A2 “Evaluation of Precision Performance of Quantitative Measurement Methods” (11) on a Stago STA Compact. The average precision results as %CV of the clotting times were:

Plasma	Mean Time (sec)	Repeatability	Total
Normal	61.8	1.2%	2.2%
Abnormal	266.3	3.1%	6.6%

Interferences: Interference studies of LupoTek KCT were determined on the Stago STA Compact. Interferant was spiked into pooled normal plasma and a dilution series prepared. The maximum concentration tolerated in the assay was defined as the highest concentration of interferant wherein any consistent shift relative to the recovered value of the base PNP clotting time was less than 10%. The maximum concentrations were:

Interferant class	Added interferant	Maximum concentration tested	Maximum tolerated concentration
Hemolysis	Hemoglobin	500 mg/dL	500 mg/dL
Icterus	Unconjugated bilirubin	20 mg/dL	20 mg/dL
Lipemia	IntraLipid®	2,000 mg triglyceride/dL	2,000 mg/dL
Heparin	Heparin	2.0 Unit/mL	<0.1 Unit/mL

Intralipid® is a registered trademark of Fresenius Kabi.

Normal Reference Range: One hundred thirty-one normal donors were analyzed on the STA Compact. The geometric mean and standard deviation of the clotting times were calculated, and the range was calculated as the mean +/- 2 standard deviations. The calculated normal range was 49.2 - 90.7 seconds.

These values should be considered illustrative only. Each laboratory should establish its own normal reference range on its own instrument(s).

Method Comparison: A total of one hundred eighty patient samples of known clinical status, including known LA patients, were analyzed in three laboratories with a test lot of KCT and with Phospholin ES, an LA sensitive APTT reagent. Each sample was scored as abnormal for either the KCT or the APTT according to their raw clotting times. Percent positive, percent negative, and overall percent agreement with the clinical status were calculated according the FDA guidance document 1620,

“Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests”. The results were:

Three site study	LupoTek KCT	APTT
Percent Positive Agreement (for LA)	100%	87%
Percent Negative Agreement (for LA)	59%	15%
Overall Percent Agreement	79%	50%

The performance of any assay is influenced by the instrumentation on which it is run and the practices of the laboratory. Each assay should be reviewed for the individual analyzer(s) in use in each laboratory according to the CLSI guideline EP15-A2 “User Verification of Performance for Precision and Trueness” (12) or to a similar guideline.

REFERENCES

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