

# LupoTek KCT

For the detection of circulating anti-coagulants



## FOR INVESTIGATIONAL USE ONLY

### INTENDED USE

LupoTek KCT is intended for use in the detection of circulating anticoagulants.

### SUMMARY

The Kaolin Clotting Time (KCT)<sup>1</sup> was first used to study lupus anticoagulants (LA) in 1978<sup>2</sup>. The KCT test has been reported to be the most sensitive test for the detection of circulating anticoagulants such as LA<sup>3</sup>. Platelet contamination can cause problems in kaolin-based APTT reagents and KCT tests<sup>4</sup>. The APTT and KCT tests detect all classes of inhibitors, including those directed against factor VIII and contact activation as well as heparin. However, in many clinical situations (studies of LA in pregnancy), it is often desirable to retain a broad screening method such as the LupoTek KCT for anticoagulants. Use of the Rosner Index (RI)<sup>6</sup> to report KCT results increases the specificity of the test for LA. The LupoTek KCT suitable for use both as a manual technique and most coagulation analyzers.

### PRINCIPLE

The LupoTek KCT is a modified activated partial thromboplastin time (APTT) reagent without any added phospholipids. Phospholipids are believed to be a major target of anti-phospholipid antibodies that are often associated with lupus anticoagulants. When these antibodies are present, the test is prolonged.

### REAGENTS

#### WARNING: FOR INVESTIGATIONAL USE ONLY.

#### 1. KCT Reagent

**Ingredients:** Each vial of LupoTek KCT contains 5 mL of a low turbidity, slow-settling kaolin specifically intended for automated KCT tests.

**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 use.

**Storage and Stability:** The reagent should be stored at 2-8°C and is stable until the date printed on the vial.

#### 2. 0.025M Calcium Chloride Reagent

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

**Preparation for Use:** The reagent is packaged ready for use.

**Storage and Stability:** The reagent should be stored at 2-8°C and is stable until the date printed on the label.

### SPECIMEN COLLECTION

#### Specimen Collection And Handling

**NOTE:** After initial whole blood collection, during testing all test tubes, syringes and pipettes should be plastic

**Specimen:** Plasma obtained from whole blood anti-coagulated with 0.1 M sodium citrate.

**Specimen Collection:** Nine parts freshly collected whole blood should be immediately added to one part anticoagulant.

**Specimen Preparation:** Centrifuge the whole blood specimen at 1500 x g for 15 minutes (NCCLS H21-A2,1991). Immediately separate the plasma from the red blood cells (if necessary) using

a plastic pipette and place it in a plastic test tube at 2-8°C until assayed. Perform the test within 4 hours.

**Storage and Stability:** Before and during testing, the plasma sample should be maintained in the plastic tubes at 2-8°C to ensure stability of the factors. If testing is delayed for more than 2 hours, the plasma may be stored at -20°C for two weeks or at -70°C for up to one month. Frozen samples should be thawed rapidly at 37°C before testing.

**Materials Provided:** Materials needed for LupoTek KCT assays are provided in the following packaging configuration.

#### LupoTek KCT

**KCT Reagent, 5 x 5 mL vials**

**0.025M Calcium Chloride Reagent, 5 x 5 mL vials**

#### Materials and Equipment Required but not Provided:

Coagulation Instrument or 37°C water bath and timer

Reaction Cups or plastic test tubes

Pipettes to deliver 0.5 and 0.1 mL

Centrifuge

Distilled or deionized water

Control Plasmas:

PlasmaCon N

PlasmaCon L-2

### PROCEDURE

#### 1. CLOTTING TEST:

A simplified LupoTek KCT technique identical to that for an Activated Partial Thromboplastin Time (APTT) is now recommended for automation. Any instrument protocol for performing an APTT can be used for determining the Kaolin Clotting Time by substituting LupoTek KCT for the APTT reagent.

This method is to be used as a guideline and should be adapted to suit individual instruments

Technical assistance can be obtained from R2 Diagnostics.

- Pipette 100µL of test plasma into a plastic test tube.
- Add 100µL LupoTek KCT reagent and incubate for 3-5 minutes at 37°C.
- Add 100µL 0.025M calcium chloride.
- Time and record clotting end-point (in seconds).

#### 2. MIXING TESTS:

Mixing tests are recommended for use with the LupoTek KCT reagent in order to reduce the effect of non-LA related clotting abnormalities. To make LupoTek KCT more specific for circulating inhibitors the test should be carried out on mixtures of patient and normal plasma<sup>2</sup>. Both 1:1 and 1:4 (patient: normal) proportions have been recommended for screening purposes<sup>5</sup>. R<sup>2</sup> recommends the 1:1 mix for most testing purposes. Normal plasma pools 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 donor plasmas should be filtered to remove platelet fragments prior to being added to the pool. 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.

To prepare samples, mix 50 µL patient plasma with 50 µL normal plasma for a 50:50 (1:1) or 20 µL patient plasma with 80 µL normal plasma for a 20:80 (1:4) mix and carry out the KCT with the resulting 100µL of test plasma exactly as described for the clotting test above. Larger volumes can be prepared for duplicate testing.

### QUALITY CONTROL

The KCT of normal quality control plasmas is largely dependant on the level of platelet contamination. For adequate sensitivity to LA, baseline KCT results should be in the range 80-120 sec. Commercial QC normal plasmas yielding such results should be tested with each batch of product. Filtered normal plasmas should yield 100-150 sec.

Commercial QC normal and abnormal plasmas should be tested with each new lot of KCT. A known LA positive sample (if available) should also be tested.

### EXPRESSION OF RESULTS

KCT results should be expressed as follows:

**Rosner Index (RI)**<sup>8</sup> Testing a 1:1 mix (patient:normal pool), the patient plasma and the normal pool.

$$RI = \frac{[KCT (mix) - KCT (normal pool)]}{KCT (patient)}$$

This expression reduces the effect of any coagulation factor deficiencies within the patient plasma.

Alternatively, the results may also be expressed as follows:

- a) Raw results, preferably in a mix, relative to a normal reference range established by the laboratory.
- b) As a ratio of delta KCT: the difference between the KCT in a 20:80 mix and the KCT of normal plasma, to the result on normal plasma alone.

### INTERPRETATION OF RESULTS

A prolonged KCT is likely to be a result of LA when:

**RI > 0.16**

or if the delta KCT ratio is > 0.1.

### LIMITATIONS:

Note that HEPARIN and DIRECT THROMBIN INHIBITORS interfere with the KCT. The KCT test is not suitable for patients undergoing heparin or Direct Thrombin Inhibitor therapy. Note that fresh plasma or filtered frozen plasma is recommended for testing. Platelet contamination may shorten the KCT.

### REFERENCES

1. Margolis J. The kaolin clotting time. A rapid one-stage method for diagnosis of coagulation defects. J.Clin.Pathol. 1958. 11:406-409.
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3. Lesperance B., David M., Rauch J., et al. Relative sensitivity of different tests in the detection of low titre lupus anticoagulants. Thromb. Haemostasis. 1988. 60: 217-219.
4. McGlasson DL, Brey RL, Strickland DM, Patterson WR. Differences in kaolin clotting times and platelet counts resulting from variations in specimen processing. Clin.Lab.Sci. 1989. 2:109-110.
5. Gibson J, Starling E, Date L, et al. Simplified screening procedure for detecting lupus inhibitors. J.Clin. Pathol. 1988. 41:225-231.
6. Rosner R, Pauzner R, Lusky A. Detection and quantitative evaluation of lupus anticoagulant activity. Thromb.Haemostas. 1987. 57:144-147.
7. Exner T, Papadopoulos G, Sahman N. Solvent extraction of test plasmas for improved recovery of lupus anticoagulant activity. Thromb. Haemostas. 1990. 4: 121 -123.

