



MICRONISED SILICA APTT REAGENT

INTRODUCTION

The activated partial thromboplastin (APTT) or kaolin cephalin clotting (KCCT) has developed from the partial thromboplastin time, the recalcification time and the whole blood clotting time and measures the same clotting factors as these tests. In this test system micronised silica replaces kaolin as the choice of activator which makes the reagent more suitable for photo-optical instruments. It represents the ultimate refinement in which platelet activity is standardized by the use of platelet substitute and contact activation is standardized by preincubation of the plasma with the platelet substitute/silica mixture for a standard time before recalcification¹. At 5 minutes preincubation the normal range for this test is 28-43 seconds (40-150% factor VIIIc). Plasma samples completely deficient in factors VIII or IX give clotting time in the region of 120 seconds, and minor deficiencies of these factors should result in a silica cephalin clotting time prolonged beyond the normal range. Because of the "broad spectrum" nature of this test, it will reflect deficiency of any of the factors concerned in intrinsic blood clotting. It is therefore a valuable ancillary screening test. It should be remembered, however, that a normal result obtained with this test may not exclude minor deficiencies of any of these factors. It is equally true, however, that when used as a screening test prior to surgery, uncontrollable bleeding should not be encountered in patient giving a clotting time within the normal range of this test in the absence of other abnormalities.

RECONSTITUTION

Remove metal cap and rubber bung and add the required volume of distilled water to the content of the vial. The vial now contains a suspension of silica in platelet substitute.

COLLECTION OF BLOOD SAMPLES

Blood (9 vols) is collected into 1 part of 3.2% trisodium citrate and the plasma obtained by centrifugation at 2500 g for 15 minutes. The plasma should be stored in stoppered tubes. The use of 3.2% citrate containing 5% HEPES buffer improves the stability of both fresh and deep frozen plasma.

TECHNIQUE

0.1ml of silica/platelet is placed in a clotting tube and 0.1 ml of plasma added and the tube gently tilted at intervals for exactly 5 minutes. 0.1ml of 0.025M calcium chloride is then added and a stop watch started. The tube is tilted at 3-5 second intervals and the clotting time recorded. The test is carried out in duplicate for both a normal control and the patient's sample, and the mean value obtained. The clotting time of the normal sample should be 28-43 seconds. A prolonged time is obtained when there are reduced values of any or all of the following factors:- I, II, V, VIII, IX and XII.

For photo-optical and mechanical instruments, follow the manufacturers instructions.

NORMAL RANGE

The normal range should be determined locally in each laboratory, especially where photo-optical or mechanical instruments are used. This may be obtained cumulatively by testing individual fresh normal plasma samples at the same time keeping the method "in control" by the use of freeze dried plasma control as a test of reagent, water bath temperature, calcium chloride etc. The normal range quoted is that obtained using a photo-optical instrument.

PERFORMANCE AND SENSITIVITY²

Precision of replicate clotting times

20 replicate determinations on each of a normal and abnormal sample gave the following %CV's

	<u>No.</u>	<u>Manual</u>	<u>Photo-Optical</u>
Normal	20	1.2	1.1
Abnormal	20	1.6	1.6
4 Hours Later			
Normal	20	1.6	1.4
Abnormal	20	1.9	1.8

Between-day precision

A normal lyophilized plasma was tested daily for 20 days and gave the following % CV's

<u>Manual</u>	<u>Photo-Optical</u>
1.8	2.0

Sensitivity to Heparin

At therapeutic levels of 0.2 to 0.5 u/ml of heparin the clotting time ratio of $\frac{\text{Patient plasma clotting time}}{\text{Normal plasma clotting time}}$ is 2.0-3.5

Sensitivity to lupus inhibitors

For prolonged clotting time the following method is useful.

Reconstitute 1 vial of silica reagent in half the recommended volume in distilled water, (x2 strength).

Reconstitute a 2nd vial at normal strength in distilled water. Alternatively dilute a known volume of the x2 strength reagent 1 in 2 with distilled water (x1 strength).

The following pattern of clotting times are obtained:-

	<u>Vial 1</u> <u>(x2 strength)</u>	<u>Vial 2</u> <u>(x1 strength)</u>
Normal Plasma	35 seconds	35 seconds
Factor deficiency	76 seconds	69 seconds
Ratio Abn/Norm	2.17	1.97
Lupus Inhibitor	100 seconds	136 seconds
Ratio Abn/Norm	2.85	3.89

Factor deficiency gives little or slight increase in ratio at x2 strength and lupus inhibitors give a decrease in ratio at x2 strength.

STORAGE AND SABILITY

The freeze dried material in unopened vial can be stored at 4°C for two years without any deterioration. After reconstitution, the suspension is stable for at least 2 weeks at 4°C. A normal control should be include with each series of tests.

Normal control plasma is available for this test in packs of 6x0.5 ml vials. Please specify “for use in the silica/cephaline clotting time” when ordering

References

1. Deson, K.W.E. (1976) in “Human Blood Coagulation, Haemostasis and Thrombosis”. (Ed. R. Biggs). Blackwell Scientific Publication, Oxford, London, Edinburgh and Melbourne.
2. Koepke, J.A. (1986) ICSH Panel on the PTT. Thrombosis and Haemostasis 55(1) 143-144.

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