Diagnostic Reagents Limited

UK CA

Rapid Capillary Thromboplastin

Batch Number: **CA**
Catalogue Number: **RCTA580**

For *in vitro* diagnostic use only.

Intended Use
Rapid Capillary Thromboplastin is an *in vitro* combined PT reagent designed for use in the control of oral anticoagulant therapy.

Summary and Principle
Rapid Capillary Thromboplastin is a stable freeze-dried mixture of rabbit brain thromboplastin and adsorbed ox plasma, both devoid of factors II, VII, IX, and X, together with Calcium chloride and buffer. The reagent gives an accurate measure of the combined depression of factors II, VII & X and has been designed specifically for use as a capillary reagent in the control of oral anticoagulant therapy. It may also be used for citrated whole blood or plasma.

Collection of Blood Samples
Venous blood or Plasma is collected into 3.2% sodium citrate dihydrate in the ratio of 9 volumes of blood to 1 volume of sodium citrate in a **plastic** tube calibrated at 10 volumes. The whole citrated blood may then be tested directly using reagent reconstituted in 4 mM Calcium chloride solution, or alternatively the plasma may be separated by centrifuging and tested using reagent reconstituted in 6 mM Calcium chloride solution.
Capillary Blood is obtained directly from the finger into a clean, dry pipette (see warnings and precautions) and tested using reagent reconstituted in distilled water.

Reconstitution
For reconstitution, remove the cap and rubber stopper and add 5.0 mL of the appropriate **room temperature diluent,*** distilled water for testing capillary (finger-prick) blood, 6 mM Calcium chloride for testing **undiluted** plasma and 4 mM Calcium chloride for testing **citrated whole blood.** The reagent should be mixed vigorously and allowed to equilibrate for 15 minutes before use. ***Room temperature reconstitution is important to avoid the precipitation of pre-fibrin, which may cause a shortening of clotting times.***

Procedure
Materials Provided:
Materials needed for capillary blood testing is shown below:
Catalogue No.
RCTA580 - Rapid Capillary Thromboplastin (6 x 5.0 mL vials).

Materials and equipment required, but not provided:
1. General routine laboratory coagulation equipment.
2. Pipettes delivering 50 µL, 250 µL & 5.0 mL.
3. Distilled water, 4 mM or 6 mM Calcium chloride solution (see reconstitution).
4. Diagen Finger Prick Control Plasmas: CFPN110 (Normal) and CFPA120 (Abnormal).

Technique
Manual, Semi-automated or Automated methods
50 µL of capillary blood, citrated whole blood or plasma is added to 250 µL of pre-warmed reagent in tube or cuvette (see instrument manual for detail). The clotting time is determined and the INR is derived from the calibration charts provided. For instruments other than the Thrombotrack™ (pink chart provided), the plasma ISI can be determined by use of calibration plasmas, but for capillary & whole blood, the ISI should be determined by calibration against the plasma method, as there are no calibration plasmas that accurately reflect the properties of capillary & whole blood.

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Warnings and Precautions
1. When using capillary blood, it is essential to obtain a deep clean puncture. The pipette should be filled from the first drop of blood and transferred as quickly as possible to the tube or cuvette containing the prewarmed reagent.
2. The variation in haematocrit in a given patient from day to day is usually slight and should not give rise to wide fluctuations although gross alterations in the haematocrit due to polycythaemia or severe anaemia will significantly affect the overall measure of anticoagulation. When either situation occurs, reference should be made to the plasma method.
3. The ISIs and table of INRs for Rapid Capillary Thromboplastin reagent have been calculated from a calibration using plasma samples from patients on oral anticoagulant therapy according to WHO requirements. Although rabbit brain (unlike ox brain) does not measure the inhibitory effect of the sub carboxylated precursor forms of factors II, VII and X, the calibration procedure takes this into account, and results for patients on anticoagulant therapy are the same when either reagent is used.
4. Rapid Capillary Thromboplastin contains material of both Bovine and Lapine origin from animals that have been passed fit for human consumption. However, normal precautions should be taken for handling potentially infectious material. Further details can be obtained from the Rapid Capillary Thromboplastin MSDS (RCT580).
5. Diagen Finger Prick Control Plasmas are designed to mimic capillary blood and are used to Quality Control (QC) instrument and reagent performance. However, it must be stressed, that there is no true substitute for capillary blood. It is strongly recommended that reference be made to a citrated venous plasma sample for initial reagent and instrument validation. It is also advisable when changing reagent lots, after instrument service or with any perceived performance problems.

Calcium chloride
Standardised Calcium chloride solution for reconstituting Rapid Capillary Thromboplastin reagent **must** be accurately titrated to avoid erroneous results. The solutions may be obtained from us.

Control of Anticoagulant Therapy and International Calibration of Thromboplastin Preparations
Because of differing sensitivities of the methods used to control anticoagulant therapy, there is difficulty in comparing the level of anticoagulation at different centres within any one country or at different centres throughout the world. An approach to this problem was made by Biggs and Denson 1967[(1)] who showed that it is possible to calibrate thromboplastin preparations in terms of their sensitivity to the anticoagulant defect and to compare the sensitivity of any preparation against a selected reference material. The calibration is done by testing a number of plasma samples from patients on anticoagulant therapy, together with normal plasma samples. The log prothrombin times for the test preparation are plotted against those for the reference preparation and the best line obtained by orthogonal regression analysis. The slope of this line is termed - the International Sensitivity Index (ISI) and using this slope, any clotting time ratio obtained with the given preparation can be converted to an equivalent clotting time ratio for the Primary International Reference Preparation. The latter is termed the International Normalised Ratio (INR) and is the ratio that should have been obtained had the primary reference preparation been used for the patient's sample. A reference material coded 67/40 was prepared in 1967 and this was established by W.H.O. in 1976 as the first International Reference Preparation of thromboplastin. Three secondary reference preparations of rabbit brain, ox brain and human brain have been calibrated against 67/40 under the auspices of the Community Bureau of Reference of the E.E.C., W.H.O., I.C.S.H. and I.C.T.H. [(2)] Rapid Capillary Thromboplastin reagent has been calibrated against the reference material RBT/79 and the International Sensitivity Indices are printed on the tables converting clotting times to INRs. All production batches are tested to conform to this index (CV ≤ 3%).

The table (Figure 1.) shows manual clotting times and the corresponding International Normalised Ratio (INR). The pink table (Figure 2.) shows the clotting times obtained using the Thrombotrack™ and the corresponding INRs

The average normal clotting time for capillary blood has been determined by extensive replication (Figure 1). Random normal samples will vary between 13.4 and 16.4 seconds. The INRs have been calculated using this normal clotting time.

Precision
A coefficient of variation (CV) of less than 3% (within runs) is observed for Rapid Capillary Thromboplastin on the Thrombotrack™ with Diagen Finger Prick Control Plasma (CFPA120).

Measuring Range
Calibration charts have been prepared to give INR values of up to 9.0. However, it must be stressed that only INRs in the therapeutic range (up to 4.5) have been used in the calibration procedure and caution should be used in interpreting values above this.

Interpretation of results
Normal range: See page 2
Therapeutic range: Based on long term experience, the therapeutic range for patients receiving oral anticoagulant treatment is INR 2.0 – 4.5 (using Rapid Capillary Thromboplastin). However, the degree of anticoagulation is determined by the reason for anticoagulant therapy and the patients' general condition [3]. This decision should be made only by the clinician treating the patient.

Influence of Heparin
LMW or Unfractionated Heparin at concentrations within the therapeutic range has little or no affect on the reagent clotting time. In our hands, any variation is below the WHO guidelines on clinical relevance. For Heparin levels greater than this, neutralisation with Protamine Sulphate is recommended.

Troubleshooting
1. Always use the first drop of capillary blood. Do not use cotton wool before sampling as it may initiate coagulation.
2. Use only clean pipettes and tubes.
3. Always reconstitute the reagent with correct diluent at room temperature.
4. Always check that the calibration INR chart has lot number corresponding to the reagent lot number.
5. Check that the appropriate ISI is being used.

Quality Control
The testing of patient samples should be performed in conjunction with the use of control plasma specifically designed to ensure the validity of the test system used. For capillary blood testing, the Diagen Finger prick control plasma should be used – see materials not provided.

Limitations
1. Only citrate should be used as an anticoagulant in sample collection (neither EDTA nor heparin should be used).
2. For haematocrit variations see warnings and precautions.
3. Activation of the coagulation system should be avoided as this can considerably shorten the clotting time and give erroneous patient results.
4. For sample collection, always ensure that tubes are made from "non-activating material" and that the correct citrate concentration is used.
5. Avoid cold activation of plasma samples.
6. Ensure that all testing is performed at 37°C.

Stability and Storage
Stored at 4°C or below, the lyophilised reagent in the unopened vial is stable for 2 years.
After reconstitution: at 2 - 8°C, the reagent is stable for 3 days.
at 20°C, the reagent is stable for 8 hours.
at 37°C, the reagent is stable for 1 hour.
at -20°C, the reagent is stable for 14 days*.

*Frozen reagent should be thawed at 37°C for at least 10 minutes in a water bath or 15 minutes in a heating block.

Packaging
6 x 5 mL (120 tests).

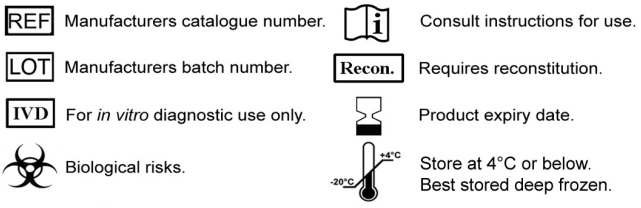
References
1. Biggs, R and Denson, K.W.E. Standardisation of the one-stage prothrombin time for the control of Anticoagulant Therapy. Brit. Med. J. 1967, 1.84.
2. W.H.O. Expert Committee on Biological Standardisation. 33rd Report, W.H.O. Tech. Rep. Ser. 1983.
3. Poller L. Therapeutic ranges for oral anticoagulation in different thromboembolic disorders. Ann. Hematol. 64, 1992

CALIBRATION CHART FOR MANUAL USE - LOT AXS195

PLASMA ISI 1.07			WHOLE CITRATED BLOOD ISI 0.92			CAPILLARY (finger prick) ISI 1.11	
Clotting Time (secs)	INR		Clotting Time (secs)	INR		Clotting Time (secs)	INR
15.9	**1.00**	**MNPT**	**16.9**	**1.00**	**MNPT**	**14.8**	**1.00**
16.5	1.04		17.5	1.03		16.5	1.13
17	1.07		18	1.06		17	1.17
18	1.14		18.5	1.09		18	1.24
19	1.21		19	1.11		19	1.32
20	1.28		20	1.17		20	1.40
21	1.35		21	1.22		21	1.47
22	1.42		22	1.27		22	1.55
23	1.48		23	1.33		23	1.63
24	1.55		24	1.38		24	1.71
25	1.62		25	1.43		25	1.79
26	1.69		26	1.49		26	1.87
27	1.76		27	1.54		27	1.95
28	1.83		28	1.59		28	2.03
29	1.90		29	1.64		29	2.11
30	1.97		30	1.70		30	2.19
31	2.04		31	1.75		31	2.27
32	2.11		32	1.80		32	2.35
33	2.18		33	1.85		33	2.44
34	2.26		34	1.90		34	2.52
35	2.33		35	1.95		35	2.60
36	2.40		36	2.01		36	2.68
37	2.47		37	2.06		37	2.77
38	2.54		38	2.11		38	2.85
39	2.61		39	2.16		39	2.93
40	2.68		40	2.21		40	3.02
41	2.76		41	2.26		41	3.10
42	2.83		42	2.31		42	3.18
43	2.90		43	2.36		43	3.27
44	2.97		44	2.41		44	3.35
45	3.04		45	2.46		45	3.44
46	3.12		46	2.51		46	3.52
47	3.19		47	2.56		47	3.61
48	3.26		48	2.61		48	3.69
49	3.33		49	2.66		49	3.78
50	3.41		50	2.71		50	3.86
51	3.48		51	2.76		51	3.95
52	3.55		52	2.81		52	4.03
53	3.63		53	2.86		53	4.12
54	3.70		54	2.91		54	4.21
55	3.77		55	2.96		55	4.29
56	3.85		56	3.01		56	4.38
57	3.92		57	3.06		57	4.47
58	3.99		58	3.11		58	4.55
59	4.07		59	3.16		59	4.64
60	4.14		60	3.21		60	4.73
65	4.51		65	3.45		65	5.17
70	4.88		70	3.70		70	5.61
75	5.26		75	3.94		75	6.06
80	5.63		80	4.18		80	6.51

Figure 1. Table showing **manual** clotting times using LOT AXS with corresponding INRs. See **pink** sheet for table showing results using the **Thrombotrack™**

Key guide to symbols

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Diagnostic Reagents Limited is a BS EN ISO13485:2016 certified company