



AXIS-SHIELD RAPID CAPILLARY THROMBOPLASTIN

Introduction

The reagent 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 and X and has been designed specifically for use as a **capillary reagent in the control of anticoagulant therapy. It may also be used for citrated whole blood or plasma.**

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 gently 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.*

Collection of Specimens

Venous Blood or Plasma: Blood 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: This is obtained directly from the finger into a clean dry pipette and tested using reagent reconstituted in distilled water.

Technique for Manual, Semi-automated or Automated methods. 50 μ l of capillary blood, citrated whole blood or plasma is added to 250 μ l 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™ (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.

Notes

- 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 containing the 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 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.

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 \pm < 3 %).

The tables show clotting times and the corresponding International Normalised Ratio. The latter is the ratio which would have been obtained had the Primary Reference Preparation been used, and provides a common scale of measurement at all centres. The average normal clotting time for capillary blood has been determined as 15.0 seconds by extensive replication. Random normal samples will vary between 13.4 and 16.4 seconds. The INR's have been calculated on a normal clotting time of 15.0 seconds.

Packaging 6 x 5 ml

Stability

Stored at 4^oC or below, the lyophilised reagent in the unopened vial is stable for 2 years.

After reconstitution:

at 2 – 8 ^oC, the reagent is stable for 3 days.

at 20 ^oC, the reagent is stable for 8 hours.

at 37 ^oC, the reagent is stable for 1 hour.

Influence of Heparin

LMW or Unfractionated Heparin at concentrations within the therapeutic range has little or no effect 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.

References

- 1) Biggs, R & 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.

