



## CALCIUM - RABBIT BRAIN THROMBOPLASTIN

A freeze dried extract of rabbit brain thromboplastin combined with calcium chloride and buffer, of low ISI, suitable for use in the one-stage prothrombin time, and for the control of oral anticoagulant therapy.

### **Reconstitution**

Add the appropriate quantity of distilled water and warm for 5-10 minutes at 37°C.

### **Collection of Blood Samples**

Blood (9 vols) is added to 1 volume of 3.3% sodium citrate in a plastic tube and the plasma obtained by centrifugation at 2500 g for 15 minutes.

### **Technique**

0.2ml of thromboplastin is placed in a clotting tube in a water bath at 37°C and left for two to three minutes to reach 37°C. 0.1 ml of plasma is then added and a stop watch started. The tube is gently tilted at 2 - 3 second intervals (returning to the water bath between tilting) and the time for the formation of a clot recorded. This is known as the prothrombin time.

- Notes:
1. Tubes should be new and scrupulously clean.
  2. Water bath temperature should be 37°C.
  3. Our freeze dried normal plasma can be used as a normal control and day to day QC.

### **Interpretation**

Deficiency of factors II, V, VII, X or fibrinogen will result in a prolonged clotting time.

### **Reporting Results**

The method recommended is to report the ratio of the patients time divided by the normal control time and then to convert this to an International Normalised Ratio (INR).

### **Control of Anticoagulant Therapy and International Calibration of Thromboplastins**

Because of differing sensitivities of the methods used to control anticoagulant therapy, there is difficulty in comparing the level of anticoagulation at different centres. An approach to this problem was made by Biggs and Denson in 1967<sup>(1)</sup> 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 using the clotting time ratio method. The calibration is now performed by testing a number of samples from patients on stabilised anticoagulant therapy, together with normal plasma samples. The log clotting times for the test preparation are then plotted against those for the reference preparation and the best line obtained by orthogonal regression analysis.

The slope of this line is then termed the International Sensitivity Index (ISI), and using this slope, any clotting time ratio can be converted to the equivalent clotting time ratio for the Primary International Reference Preparation. The latter is termed the International Normalised Ratio (INR) and is the ratio that would 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 were 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.<sup>(2,3)</sup>. Further reference preparations of rabbit brain thromboplastin have since been made. Calcium-Rabbit Brain Thromboplastin has been calibrated against a secondary reference preparation which has in turn been calibrated against the IRP RBT/90.

$$\text{INR} = \text{PT ratio}^{\text{ISI}} \text{ e.g. for a PT ratio of 2.0 and an ISI of 1.2} \qquad \text{INR} = 2.0^{1.2} = 2.30$$

It is now well established that coagulometers may alter the ISI from the value obtained manually, and for this reason each batch of reagent has a stated ISI for the manual method and for the commonly used coagulometers.

### **Stability**

Stored at 4°C, unopened vials are stable for 2 years. After reconstitution, 37°C - 3 hours, 20 - 25°C - 8 hours, 2 - 8°C - 7 days.

### **Packaging**

2ml, 5ml and x 10 ml lyophilized, and equivalent volumes of buffer.

### **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. Loeliger, E.A., van den Besselaar, A. M. H. P., Hermans, J. and van der Velde, E.A. The Certification of Three Reference Materials for Thromboplastins. BCR Information. Commission of the European Communities 1984.

## **NORMAL REFERENCE/CONTRO. HUMAN PLASMA**

A multipurpose reference plasma set in the normal range for assaying Factor VIIIc, Factor IX, antithrombin III and fibrinogen. It also serves as a normal control plasma for use with phenolised and freeze dried rabbit brain thromboplastin, Bell and Alton platelet substitute, Kaolin/platelet substitute mixture and for the thrombin clotting time. Our own values for these parameters are shown below.

Fibrinogen (Claus)	2.6 g/l
Factor II	88 iu/dl
Factor V	95 u/dl

Factor VII	103 iu/dl
Factor VIIIc	88 iu/dl
Factor IX	90 iu/dl
Factor X	98 iu/dl
Factor XI	95u/dl
Factor XII	98 u/dl
vWF Antigen	83 u/dl
Ristocetin co-factor	66 u/dl
Protein C Activity	97 u/dl
Protein S Total	82 u/dl
Antithrombin (Amidolytic)	84 u/dl

One-stage P.T.

Freeze-dried (manual)	14.5 $\pm$ 2sec
Calcium Thromboplastin (manual)	16.4 $\pm$ 2sec

**A.P.T.T**

Bell and Alton Platelet Substitute (2 min activation)	44.5 $\pm$ 2sec
Kaolin/Platelet Substitute Mixture (2 min activation)	42.3 $\pm$ 2sec
Micronised Silica/Platelet Substitute (5 min activation)	37.5 $\pm$ 2sec
Thrombin time (7.0 u/ml) 0.1ml thrombin +0.2ml plasma	12-15 sec

**Reconstitution** Add the appropriate volume of distilled water, mix gently and leave for five minutes.

**Storage & Stability** Store at 4°C. The expiry date is printed on the label. In the dried state this is at least one to two years. After reconstitution the stability is at least three hour at room temperature.

**Packaging** 6 x 0.5ml and 6 x 1ml vials per pack.

Normal and abnormal controls are also provided for the P.T., A.P.T.T. and rabbit brain capillary (finger prick) reagent. All human blood donations used in the preparation of our products have been tested individually and found to be negative for HbsAg, HCV and HIV antibody. However, we recommend that all material of human origin should be considered as potentially hazardous and be handled with appropriate care.

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