

Intended Use

For establishing laboratory, instrument and reagent specific International Sensitivity Index (ISI); required for calculating the International Normalised Ratio (INR) for patients receiving Vitamin K antagonist (VKA) therapy.

Introduction

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 performed by testing a number of plasma samples from patients on anticoagulant therapy, together with normal plasma samples. The log prothrombin times (PTs) 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⁾ Diagen Calcium Rabbit Brain Thromboplastin reagent has been manually calibrated against a house standard preparation which has in turn been calibrated against the International Reference Preparation RBT90 or CRM149s and the International Sensitivity Indices are printed in the batch specific instructions for use (IFU).

Summary and Principle

Diagen Calibration Plasmas have been specifically designed for use with the Diagen Calcium Rabbit Brain Thromboplastin reagent to ensure that the correct instrument specific ISI is being used for calculating patient INRs. It is well documented⁽³⁾ that coagulation instruments can alter the manually defined ISI of Thromboplastin reagents and this altered or corrected ISI can be established by use of calibration plasma as described below. The INR Calibration Plasmas have been defined using the Diagen house standard Thromboplastin reagent by multiple testing using the manual technique.

Reagent

INR Calibration Plasma Set

6 vials

Lyophilised, buffered human plasma. For reconstitution, remove the cap and rubber stopper and add 0.5 mL of distilled water. Swirl gently and allow 5 - 10 minutes for complete solution.

Warnings and precautions

POTENTIAL BIOHAZARD MATERIAL.

Diagen Calibration INR Plasma is of human origin. All donor units used in production of this product have been found negative for anti HIV, anti HCV, HBsAg and Syphilis by approved methods. However, all plasma of human origin should be considered as potentially infectious and handled appropriately. Please refer to the Human Plasma MSDS Sheet (provided on request) for handling and safety procedures. Dispose of all waste materials according to the national and local regulations.

Procedure

Materials Provided

Cat. No.

CALS090 – INR Calibration Plasma set (6 x 0.5 mL)

Materials and equipment required, but not provided:

1. General routine laboratory coagulation equipment.
2. Reaction cups or test tubes (12 x 75 mm).
3. Pipette delivering between 0.1 and 0.5 mL.
4. Distilled water.

Technique –Manual Method

1. 200µl of Diagen Calcium Rabbit Brain Thromboplastin is pre-warmed at 37°C in a tube or cuvette (see instrument manuals for detail).
2. 100µl of calibration plasma is added and the timer started.
3. The clotting time is determined and the **INR is derived from the Thromboplastin manual stated ISI and the laboratory derived Mean Normal Prothrombin Time (MNPT):**

$$INR = (\text{Calibration plasma clotting time} / \text{MNPT})^{ISI}$$

4. Each calibration plasma should be tested in quadruplet and the mean value for the clotting time taken. It is recommended that the calibration procedure should be performed on more than one occasion and for each new Thromboplastin lot.

5. For photo-optical and mechanical instruments, always follow the manufacturer's instructions for PT testing.

System ISI Calibration

If the mean, calculated calibration plasma INR is within an acceptable value of the defined calibration plasma INR, then no ISI correction is necessary – see *WHO guidelines for acceptable level & clinical relevance* – and the provided chart ISI can be used. If the difference is outside an acceptable range, then a system ISI should be calculated. The System ISI is calculated by plotting the Log10 of clotting time (y-axis) against the Log10 of the defined INR (x-axis). **The regression line is defined by the equation:**

$$\text{Log}_{10} \text{ Clotting time (y)} = \text{Slope} \times \text{Log}_{10} \text{ INR (x)} + \text{Intercept}$$

Where the inverse of the slope (1/slope) is the **system ISI** and the antilog10 of the Intercept is the system Mean Normal Prothrombin Time (**MNPT**).

(See Figure 1 below).

Calibration Plasma Lot B19	Defined INR	FOUND Clotting Time	Log10 Defined INR	Log10 Clotting Time
C1				
C2				
C3				
C4				
C5				
C6				

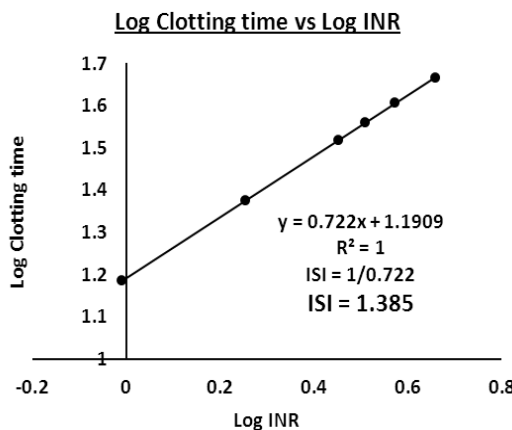


Figure 1. Graph showing the Log10 of clotting time against the Log10 of the assigned INR for plasmas C1-C6 and the associated equation used to adjust the reagent ISI.

The defined INR value of each calibration plasma is printed on the vial label. Clotting time results along with Calibration Plasma Lot number can be emailed to calibration@diagen.co.uk and a corrected ISI will be sent by return.

Limitations

Diagen INR Calibration Plasmas are only applicable to Diagen Calcium Rabbit Brain Thromboplastin and may not be correct for different species of Thromboplastin or other manufacturer's reagents.

Storage and stability

The unopened freeze dried vials are **best stored deep frozen**, but may be stored for up to 3 years at 2 - 8°C without deterioration. Once reconstituted the contents of the vial are then stable for up to 4 hours when held at 2 - 8°C









Packaging

6 x 0.5 mL

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) Poggio M, van den Besselaar AMHP, van der Velde EA, Bertina RM. The effect of some instruments for prothrombin time testing on the ISI of two rabbit thromboplastin reagents. Throm Haemostasis 1989; 62: 868-874

Key guide to symbols

 REF	Manufacturers catalogue number.		Consult instructions for use.
 LOT	Manufacturers batch number.	 Recon.	Requires reconstitution.
 IVD	For <i>in vitro</i> diagnostic use only.		Product expiry date.
	Biological risks.		Store at 4°C or below. Best stored deep frozen.

 Manufacturer.

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