



# ECAA PT/INR LINE PLASMA SET

For the Derivation of Local INR

## Introduction

The Prothrombin Time/International Normalised Ratio (PT/INR) Line is a recently described simple method which provides dependable local INR [1,2]. There is no need for local manual prothrombin time (PT) testing using WHO thromboplastin international reference preparations (IRP) required in the conventional WHO method for international sensitivity index (ISI) calibration in order to obtain International Normalised Ratios (INR) [3]. The PT/INR Line is a development and extension of previous concepts, principally the 'Direct INR' method of Houbouyan and Goguel [4-6] in external quality control studies and the contribution to PT/INR derivation by Adcock and Johnston using frozen certified plasmas with their PT/INR ratio [7].

The WHO ISI calibration scheme has been limited in application because of its heavy demands such as requirement for multicentre testing with the now discarded manual (tilt tube) PT of 60 patients and 20 normals. There is also a requirement for local availability of the relevant species WHO thromboplastin and the need to perform orthogonal regression analysis to derive local ISI.

## Principle

The PT/INR Line is derived using a set of 5 ECAA certified plasmas with stated INR derived by manual PT testing using the thromboplastin IRP of human or rabbit origin by manual testing at experienced certifying centres. PT results of plasmas with the local technique and reagents are then plotted against their certified values on a natural logarithm scale. The PT/INR Line is then determined using linear regression and local INR is derived directly from the estimated PT/INR Line. In WHO ISI calibrations, the more complex orthogonal regression analysis is required. It has been previously shown that using the simple linear regression to determine the PT/INR Line gave similar INR results [1,2].

## Materials Supplied

5 x 0.5ml Lyophilised Human Plasma  
1 x 3.0ml Reagent Grade Water

## Materials required but not Supplied

Pipette and tips capable of reconstituting each plasma with 0.5mL water.  
Thromboplastin Reagent.  
INR Quality Control Plasmas

## Procedure

### a) Manual Procedure

1. Reconstitute each vial of control plasma with 0.5ml of the Reagent Grade Water provided.
2. Allow each vial to stand for 10 minutes at room temperature, and no longer than 30 minutes.
3. Swirl each vial to mix the contents.
4. Measure the PT time of each ECAA plasma in duplicate according to the reagent manufacturers instruction.
5. Plot Log (certified INR) vs Log (Mean PT Time) and determine the line of best fit by linear regression. Ensure the Certified Values appropriate for the thromboplastin in use are used in the calibration.
6. Measure the PT time of patient samples and directly interpolate the INR from the calibration curve.

### b) Automated Procedure

Refer to the Instrument Manufacturers instruction manual for detailed instructions on test set up.

Set up a new test for PT-INR

Test Calibration:

x-axis – Certified INR

y-axis – Measured PT Time

Line of best fit – linear regression

1. Follow Steps 1-3 of the Manual Procedure to reconstitute the ECAA Plasmas
2. Run the calibration curve on the analyser according to the manufacturers instructions.
3. Ensure the Certified Values appropriate for the thromboplastin in use are used in the calibration.
4. Check the calibration using INR quality control plasmas.

### Acceptable Results

The linear correlation coefficient for the calibration line should be >0.9 for an acceptable calibration.

The measured INR of the Quality Control plasmas should be within the manufacturers assigned values.

### References

1 Poller L, Ibrahim S, Keown M, et al. Simplified method for International Normalised Ratio (INR) derivation based on the prothrombin time/INR Line: An International study. Clin Chem 2010;56:1608-17.

2 Poller L, Ibrahim S, Keown M, et al. The Prothrombin Time/International Normalised Ratio (PT/INR) Line: derivation of local INR with commercial thromboplastins and coagulometers - two independent studies. J Thromb Haemost in press.

3 WHO Expert Committee on Biological Standardisation. 33rd Report, WHO Technical Series 1983;687:81-105.

4 Houbouyan LL, Goguel AF. Long-term French experience in INR standardization by a procedure using plasma calibrants. Am J Clin Pathol 1997; 108: 83-9.

5 van den Besselaar AMHP, Barrowcliffe TW, Houbouyan- Réveillard LL, et al. Guidelines on preparation, certification and use of certified plasmas for ISI calibration and INR determination. J Thromb Haemost 2004; 2: 1946-53.

6 Clinical and Laboratory Standards Institute. Procedures for Validation of INR and Local Calibration of PT/INR Systems; Approved Guideline. Volume 25 Number 23. 2005.

7 Adcock, D.M and Johnston, M. Evaluation of frozen plasma calibrants for enhanced standardisation of the international normalised ratio (INR): a multi-center study. Thromb. Haemost., 2002; 87: 74-79.

## Certification of ECAA plasmas (batch number B-1461-1)

### *in terms of WHO human and rabbit IRP*

CERTIFIED VALUES	CERTIFIED INR
<b>Human Recombinant IRP (RTF95)</b>	
Plasma 1	2.2
Plasma 2	3.1
Plasma 3	1.8
Plasma 4	4.2
Plasma 5	2.7
<b>Rabbit Plain IRP (RBT05)</b>	
Plasma 1	2.2
Plasma 2	2.7
Plasma 3	1.9
Plasma 4	3.5
Plasma 5	2.4