Multicentre evaluation of the Thrombotest International Sensitivity Index used with a steel ball coagulometer

A M H P van den Besselaar, R H M Peters

Abstract

Aim—To compare the International Sensitivity Index (ISI) of the Thrombotest reagent used with a steel ball coagulometer (KC) to the ISI of the same reagent used with the manual (tilt tube) technique.

Methods—The study was carried out by eight laboratories using their own KC instrument and method of testing. All laboratories used the same batch of Thrombotest to determine the clotting times of fresh blood samples from 20 local healthy volunteers and 60 patients on long term oral anticoagulant therapy. KC clotting times were plotted against manual clotting times on double logarithmic scales. Orthogonal regression lines were calculated to assess the ISI.

Results—In two laboratories the ISI of the KC method was lower than that of the manual method; these differences, however, were 2% or less. In the other laboratories no clinically important differences were observed between ISI values obtained. However, the clotting times determined with the KC methods were shorter than the manual values.

Conclusions—The ISI of Thrombotest determined with the KC methods was very similar to the manual value. Therefore, use of the ISI value supplied by the manufacturer without adjustment is justified. The mean normal prothrombin time, however, must be determined locally.

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Keywords: international sensitivity index, international normalised ratio, oral anticoagulant control, prothrombin time.

The recommended scale for reporting the prothrombin time in oral anticoagulant control is the international normalised ratio (INR).^{1,2} According to the calibration model adopted by the World Health Organisation, the INR can be calculated as follows:

$$\text{INR} = (\text{PT}/\text{MNPT})^{\text{ISI}} \quad (1)$$

where PT is the patient's prothrombin time, MNPT is the mean normal prothrombin time of fresh individual samples measured using the same method, and ISI is the international sensitivity index of the method used to measure the prothrombin time. The ISI should be provided by the manufacturer of the thrombo-

plastin reagent. Some manufacturers include a conversion table for translating the prothrombin time into the INR, which should be used only if the MNPT of the individual laboratory is the same as that in the manufacturer's table. Several studies have shown that the ISI and MNPT are not a function of thromboplastin alone, but also of the method and coagulometer used.³⁻⁸

In the Netherlands bovine brain thromboplastin combined with adsorbed bovine plasma (Thrombotest) is the most popular reagent used by anticoagulant clinics.⁹ At present, the manual (tilt tube) technique for determining the prothrombin time is used by two laboratories only (2.4% of all laboratories using Thrombotest). The most popular instrument is a semiautomatic coagulometer by which clotting is detected photo-optically.⁸ Another popular instrument is the steel ball coagulometer (KC, manufactured by Amelung, Lemgo, Germany) which is currently used by 16 laboratories (19% of Thrombotest users). External quality assessment (EQA) data accumulated by the Federation of Dutch Thrombosis Centres showed that the Thrombotest clotting times determined by laboratories using KCs were shorter than those measured by other techniques. The manufacturer of Thrombotest provides only one ISI and MNPT value for each batch of reagent, which are based on the manual technique for venous blood samples.

The aim of the present study was to investigate whether there is a significant difference in the Thrombotest ISI used with the manual and KC methods.

Methods

APPARATUS

Four types of steel ball coagulometers were used: KC4, KC4A, KC10, and KC10A. All KC instruments use the same detection mechanism, as described previously.^{10,11} The coagulometers were manufactured by H Amelung GmbH (Lemgo, Germany). KC4 and KC4A have four measurement channels, while KC10 and KC10A have 10. The affix A indicates a more recent version of the instrument equipped with a microprocessor controlled calculation unit to translate clotting times into other units.

REAGENT

Prothrombin times were measured using Thrombotest (manufactured by Nycomed AS, Oslo, Norway). A single batch (number 526)

Leiden University Hospital, Department of Haematology, Haemostasis and Thrombosis Research Centre, PO Box 9600, 2300 RC Leiden, The Netherlands
A M H P van den Besselaar

Hospital "De Tjongerschans", Laboratory for Clinical Chemistry and Haematology, Thialfweg 44, 8441 PW Heerenveen, The Netherlands
R H M Peters

Correspondence to: Dr A M H P van den Besselaar.

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Table 1 Coagulometer methods used by participants

Laboratory number	Coagulometer type	Sample volume (ml)	Reagent volume (ml)	Mode*	Incubation time (minutes)
1	KC10	0.02	0.100	SR	—
2	KC10	0.02	0.100	SR	—
3	KC10	0.02	0.100	SR	—
4	KC10A	0.02	0.100	RS	—
5	KC4A	0.02	0.100	SR	1
6	KC10	0.05	0.250	SR	3
7	KC10	0.05	0.250	SR	3
8a	KC4	0.05	0.250	SR	1
8b	KC4	0.05	0.250	RS	—

* Order of pipetting: RS = reagent, sample; SR = sample, reagent.

Table 2 Geometric mean clotting times (seconds) of fresh samples from patients and normal controls. Manual and KC clotting times were compared using the Student's paired *t* test

Laboratory number	Normal controls				Patients			
	<i>n</i>	Manual	KC	<i>p</i> value	<i>n</i>	Manual	KC	<i>p</i> value
1	20	39.0	34.8	<0.001	60	148.5	130.3	<0.001
2	20	40.1	36.8	<0.001	60	114.8	103.6	<0.001
3	18	38.7	34.8	<0.001	57	124.1	112.8	<0.001
4	20	38.8	37.1	<0.001	60	128.1	122.2	<0.001
5	20	36.9	34.7	<0.001	60	109.3	105.7	<0.001
6	20	36.7	33.0	<0.001	60	141.9	128.7	<0.001
7	20	38.8	35.5	<0.001	59	113.5	104.5	<0.001
8a	20	39.3	35.9	<0.001	60	121.1	110.6	<0.001
8b	20	39.3	38.5	<0.01	60	121.1	120.8	NS

NS = not significant at the 5% level; *n* = number of subjects.

Table 3 Mean (SD) clotting times of three lyophilised control plasmas. Each laboratory provided 30 measurements for each plasma/method combination. The SD is the within laboratory standard deviation

Laboratory number	PN20		Ak110		Lot number 850618A	
	Manual	KC	Manual	KC	Manual	KC
1	37.7 (1.3)	36.2 (1.1)	110 (1.7)	103 (1.1)	124 (2.1)	121 (2.1)
2	38.4 (1.1)	36.9 (0.8)	108 (1.2)	106 (1.2)	123 (2.2)	124 (0.9)
3	39.1 (1.2)	37.5 (1.0)	109 (2.2)	100 (4.1)	124 (4.2)	119 (7.4)
4	37.8 (0.7)	37.4 (0.7)	108 (1.7)	107 (1.5)	124 (2.1)	124 (1.4)
5	38.8 (1.1)	36.7 (0.9)	107 (1.6)	103 (1.2)	121 (1.2)	119 (0.75)
6	39.5 (1.1)	36.9 (0.9)	114 (3.4)	107 (1.3)	128 (2.7)	121 (1.7)
7	40.3 (2.1)	37.6 (1.1)	107 (2.7)	104 (2.2)	126 (4.0)	124 (2.6)
8a	39.1 (1.1)	37.1 (0.6)	112 (2.3)	107 (1.8)	127 (2.4)	125 (2.2)
8b		39.2 (0.8)		112 (1.8)		129 (2.6)
Between laboratory CV (%)	2.2	2.3	2.3	3.3	1.8	2.6

was used throughout the study. The ISI of this batch had been determined using the manual technique by calibration against OBT/79, the International Reference Preparation for thromboplastin, bovine, combined.⁹ The ISI of batch 526 was 0.97. The reagent was reconstituted and used as recommended by the manufacturer.

LYOPHILISED POOLED PLASMAS

The following lyophilised pooled plasmas were used: Control Plasma Normal, batch number PN20 (Nycomed AS); Control Plasma from patients treated with oral anticoagulants, batch number AK110 (Immuno AG, Vienna, Austria); Control Plasma from patients treated with oral anticoagulants, batch number 850618A (Dutch Reference Laboratory for Anticoagulant Control, Leiden, The Netherlands). The plasmas were reconstituted and used as recommended by the manufacturers.

FRESH BLOOD SAMPLES

Blood samples were obtained from healthy subjects (normal controls) and from patients on long term oral anticoagulant treatment.

Nine volumes of blood were collected by venepuncture into siliconised glass or plastic tubes, containing one volume of 0.11–0.13 mol/l trisodium citrate. The blood samples were stored at room temperature.

DETERMINATION OF PROTHROMBIN TIME

The prothrombin time was measured in citrated blood samples using the manual technique and by the KC method. The manual method (as recommended by the manufacturer) is as follows: 0.25 ml Thrombotest reagent is heated in a glass tube in a waterbath at 37°C for at least three and at most 60 minutes. Then 0.05 ml of sample is added and the timer is started. Clotting times were determined by the tilt tube technique.

The volumes of sample and reagent used for the KC method varied from laboratory to laboratory. Five used 0.02 ml sample and 0.1 ml Thrombotest reagent and the remainder used 0.05 ml sample and 0.25 ml reagent. Two different modes for the addition of sample and reagent were used. Two participating laboratories pipetted the reagent first, followed by the sample (RS mode). The remainder pipetted the sample first and then added preheated re-

Table 4 Orthogonal regression line slopes and ISI values for the KC method v the manual method. The SD is the within laboratory standard deviation of the slope

Laboratory number	Slope (SD)	p value (Null hypothesis slope = 1.0)	ISI KC method
1	1.01 (0.02)	NS	0.98
2	1.02 (0.03)	NS	0.99
3	0.99 (0.02)	NS	0.96
4	1.00 (0.02)	NS	0.97
5	0.98 (0.01)	p<0.01	0.95
6	0.99 (0.05)	NS	0.96
7	0.99 (0.02)	NS	0.96
8a	0.99 (0.02)	NS	0.96
8b	0.98 (0.02)	p<0.01	0.95

agent (SR mode). In the latter mode the sample was incubated at 37°C for various times before the reagent was added. One laboratory used both modes. The methods are summarised in table 1.

STUDY DESIGN

Each participant received a set of instructions and forms to report the measurement results. Every day for five days, each participant measured prothrombin times using both the manual and KC methods. The prothrombin times of the three lyophilised control plasmas were measured six times and the blood samples (four from normal controls and 12 from patients on anticoagulant treatment) in duplicate each day. Blood from different patients and controls was tested each day. The order of manual and KC testing alternated from day to day.

CALCULATIONS AND STATISTICAL METHODS

To calculate the ISI of the KC method, calibration lines were fitted by orthogonal regression^{12,13}: $y = a + b \times x$, where y and x are the logarithms of the prothrombin times of normal controls and patients determined by the manual and KC methods, respectively. The standard deviation of the slope b was calculated as described by Van der Velde.¹³ The ISIs for the KC methods were derived by multiplication of the stated ISI value from the manual method with the value of the orthogonal regression slope b. To test whether the slope b was equal to 1.0, we used the method described by Poggio *et al.*³ Student's *t* test was used for paired observations to assess the significance of differences in the prothrombin times measured using both methods. INR equivalents were calculated for the lyophilised plasmas using formula (1) and each laboratory's MNPT and ISI values.

Table 5 INR equivalents for lyophilised plasmas

Laboratory number	PN20		AK110		Lot number 850618A	
	Manual	KC	Manual	KC	Manual	KC
1	0.97	1.04	2.73	2.90	3.07	3.39
2	0.96	1.00	2.61	2.85	2.97	3.33
3	1.01	1.07	2.73	2.75	3.09	3.26
4	0.97	1.01	2.70	2.79	3.09	3.22
5	1.05	1.05	2.81	2.81	3.16	3.22
6	1.07	1.11	3.00	3.09	3.36	3.48
7	1.04	1.06	2.68	2.81	3.13	3.32
8a	1.00	1.03	2.76	2.85	3.12	3.31
8b	—	1.02	—	2.76	—	3.15
Mean	1.01	1.04	2.75	2.85	3.12	3.30
Between laboratory CV (%)	4.1	3.3	4.2	3.6	3.6	3.0

Results

KC reaction conditions varied from laboratory to laboratory. Reagent and sample volumes, and the order in which the reagent and sample were added into the coagulometer cuvette differed, as did the incubation time used (table 1).

The mean clotting times of normal control and patient samples are given in table 2. The clotting times measured using the KC method were shorter than those of the manual technique except in laboratory number 8, which used the RS mode. The mean clotting times of the three lyophilised plasmas are given in table 3. The differences in clotting time between manual and KC methods were greater for the fresh blood samples than for the lyophilised plasma samples.

The slopes of the orthogonal regression lines relating KC to manual determinations are given in table 4. Only two slopes were significantly different from 1.0 at the 1% level of significance.

Laboratory number 8 used both the RS and SR modes of analysis, allowing comparisons to be drawn. The clotting times measured using the SR mode were significantly shorter than those using either the RS mode or the manual method (table 2). The ISI value of the RS mode was significantly lower than that of both the manual technique (table 4) and the SR mode.

INR equivalents for the three lyophilised plasmas are given in table 5. There was a trend to higher values for the KC methods compared with the manual method. The interlaboratory variation in the INR equivalents ranged from 3.0 to 4.2% CV.

Discussion

The purpose of the present study was to compare the ISI of Thrombotest used with KC methods to the ISI of the same reagent used with the manual (tilt tube) technique. Although all of the participating laboratories used very similar KC instruments, the volumes of reagent and sample, mode of adding reagent and sample, and incubation times used differed (table 1). Despite these differences, the range of ISI values for the KC methods was rather narrow (table 4). In fact, the range of ISI values for the KC methods was smaller than that for the Lode micro method (1.00–1.07), as observed by Peters *et al.*⁸ The ISI of the KC

methods was significantly different from the manual ISI in only two out of nine comparisons (table 4). Between laboratory variation in the ISIs for the KC methods is probably the result of variability in both the manual and semiautomated methods as the interlaboratory variation in the clotting times measured using these methods was very similar (table 3).

Most laboratories used the SR mode for the KC method (table 1). The RS mode was used by only two laboratories in this study. One of these (laboratory 8) used both modes. The results suggest that the clotting times obtained with the RS mode are closer to the manual clotting times than those of the SR mode. This may be explained by the fact that the manual method is also carried out in the RS mode. In this mode, the blood sample is at room temperature when it is added to the reagent. In the SR mode, the blood sample is first preheated at 37°C. Therefore, in the manual method and the RS mode of the KC, the initial temperature of the reaction mixture is below 37°C and the initial reaction rate may be slightly lower than that of the SR mode.

We conclude that the ISI of the Thrombotest/KC combination is practically the same as the ISI of the manual Thrombotest determination. Although we investigated only one batch of Thrombotest, we feel that the manual method ISI supplied by the manufacturer can be used with the semiautomated KC method. In contrast, the MNPT determined using the KC method is significantly shorter than that of the manual method and therefore the MNPT should be determined locally by each individual laboratory, as recommended previously.^{14,15}

There is considerable interest in lyophilised plasmas because these are used in EQA schemes^{16,17} and are also proposed as calibrants for local calibration of prothrombin time systems.^{18,19} In both applications, it is assumed that lyophilised plasmas can replace fresh samples without influencing the relations between different prothrombin time systems. There is evidence that this assumption is not always true.²⁰⁻²² In the present study we included three lyophilised plasmas for quality control. INR equivalent values were calculated with method specific MNPT and ISI values. The INR equivalents determined with the KC methods were higher than those obtained with the manual methods (table 5). If INR equivalents assigned to these lyophilised plasmas are based on the manual technique and the KC method is calibrated using these values, the resulting calibration line would be biased and INRs of fresh samples read from this line would be underestimated. Further studies on the possible application of lyophilised calibrants are needed.

Participating laboratories were as follows: Rode Kruis Hospital, Beverwijk (J W Huisman); Hospital De Sionsberg, Dokkum (A P Anker); Sint Anna Hospital, Geldrop (R T P Jansen); Hospital De Tjongerschans, Heerenveen (R H M Peters); University

Hospital, Leiden (A M H P van den Besselaar); University Hospital, Maastricht (K Hamulyák); Sint Franciscus Hospital, Roosendaal (G D van Waveren Hogervorst); Sint Antonius Hospital, Sneek (J W E Visser).

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