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**Calibration of the proposed 4th International Standard for Thromboplastin,
human, recombinant, plain (08/144)**

Armando Tripodi¹, Veena Chantarangkul¹, Anton M.H.P. van den Besselaar^{2,3}

*¹Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, Department of Internal
Medicine, University and Foundation IRCCS Ospedale Maggiore Policlinico,
Mangiagalli e Regina Elena, Milano, Italy*

*²Department of Thrombosis and Haemostasis, Leiden University Medical Center,
Leiden, The Netherlands*

³Corresponding author

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Summary

Background and aim

Stocks of the current 3rd International Reference Preparation for Thromboplastin, human, recombinant, plain (rTF/95) are nearly exhausted. The aim of the present study was to calibrate two human thromboplastin preparations, coded 07/314 and 08/144, as potential replacement materials.

Methods and results

The preparations were calibrated in an international collaborative study organized and carried out *under the auspices of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH)*. The study involved 21 laboratories from 13 countries from Asia, Europe, North America and South America. The calibration was performed against the existing WHO International Standards (ISs), i.e. rTF/95 and RBT/05. The two candidates were compared based on predetermined criteria which included (i) the within-laboratory precision of calibration, assessed as the coefficient of variation (CV) for the estimation of the slope; (ii) the between-laboratory precision of the calibration, assessed as the CV of the International Sensitivity Index (ISI) and (iii) the conformity to the calibration model, assessed as the percentage of calibrations with important deviations of normals from patients line, according to the WHO guidelines (1).

Twenty of 21 participants submitted data for statistical analysis. Within-laboratory precision of the calibration was below 3% in 87.5% of the cases for 07/314 versus 92.5% for 08/144. The mean International Sensitivity Index (ISI) value of the candidate 07/314 calibrated against the two existing ISs was 1.084 and the between-laboratory CV of the ISI was 5.7%. The corresponding values for the candidate 08/144 were 1.082 and 4.2%. Finally, the percentages of calibrations with important deviations of normals from patients line were 20% and 8.7% for 07/314 and 08/144. Overall, candidate 08/144 gave the lowest mean values of intra- and inter-laboratory variation of the ISI. Furthermore, candidate 08/144 gave the lowest number of calibrations with important deviations from the WHO calibration model.

Stability of the candidate reference preparations was assessed through an accelerated degradation study after storage of ampoules at elevated temperatures (+4, +30, +37, +45 °C) for different time intervals. For both candidates, storage at 30 °C or higher did slightly affect the ISI value. No significant change, however, was observed after storage at 4 °C, suggesting that both preparations display suitable stability when stored at -20 °C.

Proposal

Of the two candidates, preparation 08/144 gave the lowest intra- and inter-laboratory variation of the ISI, and also the lowest number of calibrations with important deviations from the WHO calibration model. **It is therefore proposed that the preparation coded 08/144 be accepted as the WHO 4th International Standard for Thromboplastin, human, recombinant, plain, with an assigned International Sensitivity Index (ISI) value of 1.082.**

Introduction

According to the recommendation issued by the World Health Organization (WHO), working thromboplastins used in the prothrombin time (PT) test for the laboratory control of oral anticoagulant treatment must be calibrated against International Reference preparations or Standards (ISs) to determine the International Sensitivity Index (ISI) necessary to convert PT

results into International Normalized Ratio (INR) (1). The observation that the calibration of a given thromboplastin is in general more precise when it is performed against an IS of similar composition and from the same species, supports the recommendation made by the WHO Expert Committee on Biological Standardization (ECBS) that like vs. like calibration should be performed and is one of the reasons to maintain ISs from different species (1). Another reason to maintain more than one IS is that it permits to assess periodically the stability of the ISs (2, 3). The first IS named 67/40, was a human brain extract to which it was added adsorbed bovine plasma (combined reagent). In 1984, 67/40 was replaced by BCT/253 (4), a human brain extract (plain reagent). This was in turn replaced in 1996 by rTF/95 (human recombinant, plain) (5). Until recently, there were two additional IS available from WHO: OBT/79 (bovine, combined) (6) and RBT/90 (rabbit, plain) (7). Both were recently discontinued, but only RBT/90 was replaced by RBT/05 (8). Reference preparations for thromboplastins are also available from other agencies: CRM 149S (rabbit, plain) and CRM 148 (bovine, combined) both from the European Union (IRMM) and EUTHR-01 (rabbit, plain) from the European Action on Anticoagulation (EAA). Each of the above European secondary references is characterized by a value of the ISI, which is the slope of relationship of the PT values derived directly or indirectly against the primary IS 67/40.

Stocks of the WHO IS from human origin coded rTF/95 (5) are limited and must be replaced to maintain continuity of the human route. The present report deals with the results of an international collaborative study organized *under the auspices of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH)* for the calibration of the replacement candidate.

Design of the collaborative study for ISI value assignment

The candidate materials (07/314 and 08/144) and the current ISs rTF/95 (human) and RBT/05 (rabbit) were tested in each laboratory by the same expert operator with the manual (tilt tube) technique. Test plasmas were freshly prepared from healthy subjects and patients stabilized on long term anticoagulant therapy. Mean prothrombin times for the healthy subjects are given in Table 1. Participants were instructed to select patient plasmas with PT corresponding to an interval of INR from 1.5 to 4.5. Also included in the series of measurements were two lyophilized control plasmas. To account for the effect of inter-day variation, PT measurements were performed in each laboratory on 10 different days (not necessarily consecutive). Participants were instructed to include on each day plasmas from 2 healthy individuals and 6 anticoagulated patients. Healthy individuals and patients had to be different on each working day. To minimize the effect of plasma instability on the relationship between the thromboplastins, the order of testing was changed each day. Plasmas were tested on each day according to the order specified in the data-collection form.

Details of Candidate Materials

Characteristic	07/314	08/144
Presentation	sealed glass 5ml DIN ampoules	sealed glass 2ml ampoules
Excipients/additives	Contains relipidated recombinant human tissue factor and bovine albumin; buffer composition confidential	Contains relipidated recombinant human tissue factor and stabilisers; buffer composition confidential
Liquid filling weight (g)	Mean 0.5052 g (range 0.5020 g – 0.5100 g)	Mean 0.2725 g
Coefficient of variation of the liquid fill (%)	0.301 % based on 368 check-weight ampoules	2.5 % based on 156 check-weight ampoules
Residual moisture after lyophilisation (%)	Mean 0.13 %, CV 45.2% (n = 16)	Mean 3.2 %
Dry weight (mg)	Mean 71.3 mg, CV 1.69% (n = 6)	
Headspace oxygen (%)	Mean 0.20 %, CV 15.6% (n = 12)	Mean 0.4 % (range 0.2 – 0.7 %)
Reconstitution volume and fluid	1.0 ml distilled water	1.0 ml of diluent (08/146) containing calcium chloride and other additives
Number of ampoules available	9,100	10,000
Manufacturing site	NIBSC, Potters Bar, UK	Reagent manufacturer (USA)
Custodian	NIBSC, Potters Bar, UK	Reagent manufacturer (USA) (will be transferred to NIBSC subject to establishment)
Storage temperature	-20 °C	-20 °C (Diluent 2 – 8 °C)

Materials provided for the collaborative study

Participants received the following materials:

1. Study protocol with detailed instructions on how to collect and store fresh plasmas, to reconstitute lyophilized plasmas and thromboplastins and to do actual testing.
2. Lyophilized plasmas (coded A and B).
3. All the ISs presently available from WHO and belonging to the two different species, i.e. rTF/95 (human) and RBT/05 (rabbit)
4. Candidate replacement thromboplastins provisionally coded as 07/314 and 08/144.
5. Vacuum tubes for blood collection containing 0.109 M Sodium citrate.
6. Appropriate diluents to reconstitute rTF/95 (coded as 07/284), RBT/05 (coded as 04/210) and candidate 08/144 (coded as 08/146).
7. Sterile redistilled water to reconstitute candidate 07/314 and lyophilized plasmas.

8. Sterile 25 mM Calcium Chloride to recalcify plasma/thromboplastin mixtures for RBT/05.

Statistical methods

ISI determination

The statistical methods were those employed for the calibration of previous ISs (4-8) and recommended by WHO (1) and from the literature published more recently on this topic (9). In particular, the following steps were accomplished:

- (i) The slope of the relationship between the log-transformed PT obtained with each of the candidate versus each of the IS was estimated by orthogonal regression by including all the available paired-data.
- (ii) Values exceeding the interval 1.5-4.5 INR as measured with the ISs and the candidates were excluded.
- (iii) Outliers were then identified and rejected if they were located at a perpendicular distance of more than 3 times the standard deviation (SD) from the regression line calculated with all data-points (after step ii) included and with the patients-only regression line.
- (iv) Finally, ISI values were calculated as the product of the slope of the orthogonal regression line and the ISI value of the IS.
- (v) ISI values within each route of calibration and each IS were calculated as the mean of the separate regression lines calculated for the different laboratories. The final ISI value assigned to the candidates as well as their standard errors of the mean (SE) were calculated from the mean ISI obtained from the two ISs after exclusion of outliers. These were detected within each route of calibration by the algorithm described previously (9) and used for the calibration of rTF/95 (5) and RBT/05 (8).

Within-laboratory precision of the calibrations

CV values for the slope of each and every calibration plot obtained during the collaborative exercise were calculated as the SE of the slope divided by the slope and multiplied by 100.

Between-laboratory precision of the calibration

CV values for each and every ISI value obtained in the collaborative exercise were calculated as the SD of the ISI divided by the mean ISI and multiplied by 100.

Assessment of compliance with the WHO model

This was achieved by the calculation recommended by the WHO guidelines (1) which call for the following steps.

- (i) For each and every calibration plot obtained in the collaborative exercise PT values corresponding to an INR of 2.0 or 4.5 (INR1) with the ISs were calculated.
- (ii) These PT values were then used to calculate the correspondent candidate PT values using the patient-only regression line. These PT values were eventually converted into INR using the candidate ISI (INR2).
- (iii) The percentage deviation $[(INR2-INR1)/INR1 \times 100]$ was eventually calculated. It is recommended that INR deviation should not be greater than $\pm 10\%$ (1).

INR values for fresh patients' samples

These were calculated by dividing mean PT from patients by the geometric mean PT of normals and raising this ratio to the ISI value of the reagent (1).

Results of ISI value assignment

The study included all the currently available WHO ISs. Following recommendations issued by the SSC of the ISTH (10) and WHO (1) the ISI of the candidate replacement has been calculated as the average value of the calibrations against the existing WHO ISs. Potential candidates to replace rTF/95 were all the available human tissue extracts and those preparations made from relipidated recombinant tissue factor. Additional requirements were that the material should be provided in glass sealed ampoules and a suitable number of them (at least 10,000) donated to WHO after the evaluation was completed and the final choice of the most suitable candidate was made. Two candidates that met the above requirements were submitted for evaluation. One of the two candidates (07/314) was lyophilized and ampouled at the National Institute for Biological Standards and Control (NIBSC, Potters Bar, Hertfordshire, UK) following instructions from the manufacturers. The other (08/144) was lyophilized and ampouled by the manufacturer. Suitable numbers of ampoules of the two candidates were coded as 07/314 and 08/144, at the sites of manufacture, by people neither involved in the calibration exercise nor in the statistical analysis, and calibrated in an international collaborative study.

Twenty-one laboratories from Europe, North America, South America and Asia were invited to participate in the study and results were returned from 20 laboratories (Appendix 1). The vast majority of them had already participated in the collaborative exercise to calibrate rTF/95 (5) and RBT/05 (8), and had experience with the manual (tilt tube) technique for PT testing.

The criteria used to judge the calibration of the two candidates were, the within- and between-laboratory precision of the calibration and the compliance with the model of calibration. They were derived from the procedure recommended by WHO (1) and the literature that appeared on this topic over the last 25 years (4-8). The within-laboratory precision of calibration was better for 08/144 than for 07/314 (Tables 2a and 2b). The percentage of laboratories which scored a CV value below the recommended 3% (1) were 92.5% vs. 87.5% (Tables 2a, 2b and 8). The between-laboratory precision was better for 08/144 than for 07/314, with CV values of 4.2% vs. 5.7% (Tables 3a, 3b, 6-8). Finally, 08/144 was more adequate than 07/314 to fulfil the requirements of the calibration model (Tables 4 and 8). These require that in a given calibration the overall regression line describes patient and normal data points adequately. In cases of marked deviation the assignment of the ISI would not be meaningful. Statistical methods to test deviations from the above assumption have been described (11). However, for practical purposes the WHO guidelines (1) state that the assignment of an ISI is acceptable if the INRs calculated with the ISI derived from the overall regression line (i.e., patients' plus healthy subjects) do not differ by more than 10% in the range of INR 2.0-4.5 from the INRs calculated with the equation describing the regression line for patients only. We tested this assumption (i.e. INR deviation not greater than 10% at INR 2.0 and at INR 4.5) in all calibration plots generated with the two candidates and found that 08/144 calibrations did not comply with the assumption in 8.7% of the cases, whereas 07/314 calibrations did not comply in 20% of the cases (Table 8).

Table 5 shows the means of the patients' INR values for rTF/95, RBT/05 and the two candidates. The differences between the means of all laboratories were 3.1% or less.

Overall, candidate 08/144 gave the lowest mean values of intra- and inter-laboratory variation of the ISI. Furthermore, candidate 08/144 gave the lowest number of calibrations with important deviations from the WHO calibration model. These data are in favour of candidate 08/144 being proposed as the 4th IS, with an assigned ISI value of 1.082.

Stability of the proposed WHO 4th IS for Thromboplastin, human, recombinant plain

Accelerated degradation study

Tissue factor is a glycoprotein that needs the association with phospholipids for full expression of its procoagulant activity. Lyophilized tissue factor reagents (thromboplastins) for prothrombin time (PT) assays usually contain many other components such as residual water which may affect the stability of the reagent. In general, the long-term stability of lyophilized tissue factor from human or animal brain stored at low temperature is excellent. The stability of biological materials may be predicted from accelerated degradation tests. The purpose of an accelerated degradation test is to measure the relative rates of potency loss at several temperatures and to extrapolate the rate to the desired temperature of storage. Only few investigators have attempted to predict the stability of tissue factor from accelerated degradation studies. One reason for this may be that thromboplastins are not easily assayed for potency in the usual sense. Furthermore, complex kinetics of the deterioration process are expected for tissue factor as it is a lipoprotein. Although the accelerated degradation test may not be used to predict the stability of lyophilized tissue factor at low temperature, it may be useful to assess the relative stability under transportation conditions at various temperatures. The accelerated degradation test is a standard procedure to check the stability of thromboplastins [12].

Following shipment of both candidates (coded 07/314 and 08/144) to Leiden, they were stored at minus 20°C. The reconstitution fluid for candidate 08/144 (coded 08/146) was stored at 4°C. For the accelerated degradation test, a number of ampoules were stored at 4°C, 30°C, 37°C, and 45°C, for different time intervals. The reconstitution fluid for candidate 08/144 was stored at the same temperatures as the dry material. At 45°C, the storage time intervals were 1, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70 days. At 4°C, 30°C, 37°C, the candidates were stored for various time intervals up to 154 days. In fact, the ampoules were placed in the incubators at different dates, so that they all could be analyzed in the same session at the end of the different incubation time intervals. After storage of the candidates at these temperatures, they were reconstituted and tested with two deep-frozen pooled plasmas (one normal, coded NP030416, and one coumarin plasma obtained from patients treated with vitamin K antagonists, coded AP090406). The tests were performed with a semi-automatic, electro-mechanical coagulometer according to Schnitger & Gross manufactured by Amelung GmbH (Lemgo, Germany). All ampoules stored at a given temperature were tested in the same session. In addition, six ampoules stored at -20°C were tested in each session. The results obtained with the -20°C ampoules were considered as the initial value at zero storage time, assuming that deterioration at -20°C was negligible. The time between reconstitution of each ampoule and testing of the ampoule was 2 hours. For each storage temperature and time, 3 ampoules were used. Each ampoule was tested in single PT determination. The mean values of the 3 PTs were used for statistical analysis. For each storage temperature and time, a clotting time ratio (PT ratio) was calculated as the mean PT of the coumarin plasma (AP090406) divided by the mean PT of the normal plasma (NP030416). Correlation coefficients according to Spearman were used to test the change of PT or PT-ratio with incubation time. A significance level of 5% was used.

PT's as a function of storage time at various temperatures are shown in Tables 9 and 10. For candidate 07/314, a slight decrease of the PT of abnormal plasma was observed after storage at 30°, 37°, and 45°C (Table 9, Figures 1a and 1b). For candidate 08/144, a relatively rapid decrease of the PT of abnormal plasma was observed which reached a plateau after 14 days of storage

at 30°, 37°, and 45°C (Table 10, Figures 2a and 2b). There was no significant change of PT after storage at 4°C.

PT ratios as a function of storage time are shown in Tables 9-10 and Figures 3a-3b. A decrease of PT ratio was observed at 30°, 37°C and 45°C for both candidates. The rate and extent of the change was greater for candidate 08/144 than for candidate 07/314.

The accelerated degradation test showed that there was a non-linear decrease of the PT of abnormal (coumarin) plasma after storage of the candidates at elevated temperatures. It seems therefore impossible to extrapolate these results to the rate of change at -20°C. In contrast to the results with coumarin plasma, there was no change of the PT of pooled normal plasma. Therefore, a significant non-linear decrease of the PT ratio of abnormal (coumarin) plasma to normal plasma was observed. The observed change in the PT ratio indicates that there is a change in the ISI of the candidate standards. The rate and extent of the PT ratio change was greater for candidate 2 (08/144 + 08/146) than for candidate 1 (07/314). As such, the properties of the candidates appear to be different from the current International Standard rTF/95 whose PT ratio appeared to be stable in an accelerated degradation experiment [13].

Real time stability monitoring of the International Standard is to be performed by designated laboratories [14].

In conclusion, the PT ratio and hence ISI of both candidates may change after storage at 30°, 37° and 45°C. In contrast, no significant change was observed after storage at 4°C for 150 days, demonstrating that both preparations are stable during storage at -20 °C or shipment at 4 °C. For shipment at ambient temperature, however, certain precautions may be advised. The shipment duration should be limited to a few days and cooling packs may be included in order to eliminate any deleterious effects of elevated temperature.

Stability after reconstitution

According to the instructions for use of the current international standards for thromboplastin (i.e. rTF/95 and RBT/05), the reconstituted standards should be kept at room temperature and used within 2 hours of reconstitution.

After reconstitution of the candidates, they were kept at room temperature for various time intervals and then used in a PT test. The time intervals were 4, 3, 2, 1, and 0.5 hours, respectively. For each time interval, three ampules of each candidate were used. Two deep-frozen pooled plasmas (one normal, coded NP030416, and one coumarin plasma obtained from patients treated with vitamin K antagonists, coded AP090406) were used for the PT tests. The tests were performed in duplicate with a semi-automatic, electro-mechanical coagulometer according to Schnitger & Gross manufactured by Amelung GmbH (Lemgo, Germany). Correlation coefficients according to Spearman were used to test the change of PT with incubation time. A significance level of 5% was used.

PT's as a function of time interval between reconstitution and testing are shown in Tables 11-12 and Figure 4. There was no significant change of the PT with time at the 5% significance level. This indicates that both candidates may be used between 0.5 and 4 hours after reconstitution if they are stored at room temperature. In consideration of the possible local variability in room temperature the Instructions for Use will recommend that the International Standard is used within 2 hours of reconstitution.

Instructions for use

Draft instructions for use are provided in Appendix 2.

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- **Filling and Coding of Candidates**
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Table 1: Mean prothrombin times (seconds) of the healthy subjects.

Laboratory	rTF/95	RBT/05	07/314	08/144
1	12.434	17.767	10.683	12.099
2	14.556	18.678	12.523	14.642
3	12.074	15.672	10.037	12.033
4	13.967	16.533	11.132	14.183
5	13.385	16.837	10.834	13.869
6	12.794	16.651	9.959	13.071
7	14.306	17.191	12.557	14.353
8	15.027	19.068	12.822	14.735
9	16.203	17.931	14.703	15.768
10	14.045	17.037	11.691	14.082
11	14.494	17.703	12.570	14.532
12	13.199	15.507	11.233	13.100
13	12.908	16.173	11.399	13.227
14	15.318	18.018	13.493	14.513
15	13.924	16.948	11.182	14.267
16	14.696	16.584	12.637	13.926
17	14.548	17.990	11.189	15.022
18	14.176	16.845	11.242	13.891
19	13.290	16.474	10.583	13.202
20	14.896	16.098	12.053	14.568
Overall Mean	14.012	17.085	11.726	13.954
Min	12.074	15.507	9.959	12.033
Max	16.203	19.068	14.703	15.768

Table 2a: Slopes and coefficients of variation (CV) for calibration of 07/314 vs. ISs.

Laboratory	Vs. rTF/95		Vs. RBT/05	
	Slope	CV	Slope	CV
1	1.106	1.7	0.956	1.9
2	1.154	1.7	0.988	2.1
3	1.171	1.7	0.937	2.3
4	1.080	1.6	0.826	2.2
5	1.162	1.1	0.932	2.1
6	1.053	1.5	0.824	2.1
7	1.203	1.4	1.026	1.7
8	1.181	2.7	0.921	3.9
9	1.186	2.2	0.990	3.0
10	1.097	2.6	0.956	2.8
11	1.232	2.4	1.025	3.0
12	1.199	1.8	1.068	2.4
13	1.153	1.9	0.952	2.3
14	1.028	1.9	0.931	2.4
15	1.134	1.5	0.867	3.3
16	1.160	2.1	0.957	2.3
17	1.167	2.7	0.979	3.5
18	1.090	2.3	0.931	2.9
19	1.155	1.8	0.987	1.8
20	1.115	1.8	0.992	2.2

Table 2b: Slopes and coefficients of variation (CV) for calibration of 08/144 vs. ISs.

Laboratory	Vs. rTF/95		Vs. RBT/05	
	Slope	CV	Slope	CV
1	1.169	1.7	1.009	2.1
2	1.157	1.3	0.990	1.7
3	1.174	1.8	0.951	1.9
4	1.136	1.5	0.856	2.3
5	1.188	1.3	0.959	1.9
6	1.140	1.4	0.894	1.9
7	1.131	1.3	0.969	1.6
8	1.174	2.9	0.919	4.1
9	1.094	1.9	0.911	2.4
10	1.104	1.7	0.981	2.5
11	1.131	1.8	0.946	2.3
12	1.156	1.6	1.030	2.1
13	1.189	1.6	0.982	1.6
14	1.049	1.9	0.950	2.4
15	1.171	1.5	0.903	3.4
16	1.080	2.1	0.934	2.9
17	1.192	2.4	0.984	3.1
18	1.100	2.3	0.936	2.4
19	1.099	1.3	0.929	1.5
20	1.128	1.7	1.005	2.0

Table 3a: ISI values for 07/314 vs. ISs. No outlying ISI values were detected

Laboratory	rTF/95	RBT/05
1	1.040	1.099
2	1.085	1.136
3	1.101	1.077
4	1.015	0.950
5	1.092	1.071
6	0.990	0.947
7	1.131	1.180
8	1.111	1.059
9	1.115	1.139
10	1.031	1.099
11	1.158	1.179
12	1.127	1.228
13	1.084	1.094
14	0.966	1.071
15	1.066	0.997
16	1.090	1.100
17	1.097	1.126
18	1.025	1.070
19	1.086	1.135
20	1.048	1.140
Mean	1.073	1.095
N.	20	20
SD	0.050	0.071
CV	4.6	6.5

Table 3b: ISI values for 08/144 vs. ISs. No outlying ISI values were detected

Laboratory	rTF/95	RBT/05
1	1.098	1.161
2	1.088	1.139
3	1.103	1.094
4	1.068	0.984
5	1.117	1.103
6	1.072	1.028
7	1.064	1.114
8	1.104	1.057
9	1.028	1.048
10	1.037	1.128
11	1.063	1.088
12	1.087	1.185
13	1.117	1.130
14	0.986	1.093
15	1.100	1.038
16	1.015	1.074
17	1.120	1.131
18	1.034	1.076
19	1.033	1.068
20	1.061	1.156
Mean	1.070	1.095
N.	20	20
SD	0.038	0.050
CV	3.5	4.6

Table 4: Assessment of the adequacy of the WHO model. Percentage deviation from an assigned INR as calculated with the ISs (patients plus normal regression line) versus the INR calculated with the candidates using the patient-only regression line (see text for more details). It is recommended that INR deviation should not be greater than $\pm 10\%$.

	07/314 (Candidate 1)				08/144 (Candidate 2)			
	rTF/95		RBT/05		rTF/95		RBT/05	
Laboratory	INR 2.0	INR 4.5	INR 2.0	INR 4.5	INR 2.0	INR 4.5	INR 2.0	INR 4.5
1	-10.7	5.1	-8.3	3.2	-6.7	3.2	-3.8	1.3
2	-10.6	6.9	-15.5	8.0	-6.5	4.0	-10.4	5.2
3	-6.6	4.7	-6.7	6.2	-5.5	3.9	-4.1	4.0
4	-6.5	2.5	-12.8	5.8	-9.8	3.5	-16.7	7.6
5	-7.1	4.6	-9.9	7.1	-6.5	4.5	-8.3	6.6
6	-1.9	1.0	-2.7	1.5	-1.1	0.4	-1.0	0.4
7	-9.2	8.9	-10.5	7.9	-7.5	7.2	-7.5	5.4
8	-5.7	7.7	-13.6	35.7	-4.5	5.1	-13.7	31.7
9	-6.1	5.1	-13.4	11.8	-5.6	4.2	-8.1	7.3
10	-10.9	6.5	-9.6	4.1	-7.0	4.2	-5.7	3.0
11	-10.0	7.2	-9.9	6.8	-6.1	3.7	-4.2	2.7
12	-2.8	6.2	-7.8	12.1	-2.1	4.2	-6.4	9.4
13	-13.8	5.9	-13.4	5.2	-9.5	4.0	-8.3	3.2
14	-5.5	7.1	-4.3	2.7	-7.0	8.8	-6.1	3.9
15	-5.2	2.7	-4.5	3.0	-5.2	2.8	-6.0	4.2
16	-6.6	2.5	-11.7	4.8	-7.7	2.6	-11.4	3.3
17	-2.9	2.9	-11.1	20.3	-4.0	4.5	-10.9	15.8
18	-5.5	6.7	-7.5	7.8	-4.8	5.5	-6.3	5.6
19	-6.8	2.9	-6.5	2.3	-6.6	2.6	-7.6	2.5
20	-6.4	4.3	-3.0	1.3	-7.7	5.7	-2.9	1.5
Mean INR Deviation	-7.0	5.1	-9.1	7.9	-6.1	4.2	-7.5	6.2
Total numbers of laboratories exceeding $\pm 10\%$	4	0	8	4	0	0	5	2

Table 5: Means of the patients' INR values.

Laboratory	rTF/95	RBT/05	07/314	08/144
1	2.940	3.057	2.982	2.839
2	2.738	2.902	2.634	2.663
3	2.622	2.568	2.521	2.524
4	2.811	2.792	2.944	2.775
5	2.813	2.806	2.741	2.678
6	2.753	2.698	3.026	2.805
7	2.669	2.769	2.497	2.652
8	2.373	2.321	2.266	2.234
9	2.486	2.563	2.360	2.553
10	2.800	2.952	2.843	2.892
11	2.738	2.776	2.490	2.744
12	2.205	2.392	2.111	2.162
13	3.051	3.079	2.946	2.887
14	2.510	2.750	2.756	2.675
15	2.801	2.647	2.810	2.693
16	2.889	2.886	2.815	3.001
17	2.369	2.416	2.317	2.287
18	2.332	2.454	2.375	2.367
19	2.779	2.870	2.707	2.867
20	2.730	2.886	2.769	2.746
Overall Mean	2.670	2.729	2.646	2.652
Min	2.205	2.321	2.111	2.162
Max	3.051	3.079	3.026	3.001

Table 6: ISI assignment to candidate 07/314

International Standard (IS)	N	Mean ISI	Between-lab SD	Between-lab CV	SE
rTF/95	20	1.073	0.050	4.6	
RBT/05	20	1.095	0.071	6.5	
Overall Calibration	40	1.084*		5.7	0.0097

*Mean of 2 ISI values obtained with different IS. No outlying ISI values were detected.

Table 7: ISI assignment to candidate 08/144

International Standard	N	Mean ISI	Between-lab SD	Between-lab CV	SE
rTF/95	20	1.070	0.038	3.5	
RBT/05	20	1.095	0.050	4.6	
Overall Calibration	40	1.082*		4.2	0.0072

*Mean of 2 ISI values obtained with different IS. No outlying ISI values were detected.

Table 8: Comparative performance for the two candidates reference materials.

Material	Within-lab Precision of the Calibration	Between-lab precision of the Calibration	Inadequacy of the WHO model		
	% of Calibrations with CV of the slope <3%*	CV (%) value of the ISI**	Numbers (%) of Calibrations with deviations from the adequacy of the WHO model**		
			INR 2.0	INR 4.5	INR 2.0 plus INR 4.5
07/314	87.5	5.7	12 (30)	4 (10)	16 (20)
08/144	92.5	4.2	5 (12.5)	2 (5.0)	7 (8.7)

*the higher the better

**the lower the better

Table 9: Mean PT of candidate **07/314** after storage at various temperatures

+45°C	mean PT		PT ratio
	time (d)	NP030416	AP090406 AP/NP
0	10.2	28.2	2.765
1	9.6	27.7	2.885
7	9.8	27.3	2.786
14	9.6	25.9	2.698
21	10.0	25.5	2.550
28	9.6	25.4	2.646
35	10.0	25.1	2.510
42	9.9	25.0	2.525
49	10.0	25.1	2.510
56	9.6	25.8	2.688
63	9.8	25.2	2.571
70	10.0	24.7	2.470

+37°C	mean PT		PT ratio
	time (d)	NP030416	AP090406 AP/NP
0	10.3	28.7	2.802
2	10.2	28.5	2.803
14	10.2	27.9	2.741
28	9.8	27.9	2.850
42	10.0	27.3	2.733
56	10.0	27.4	2.740
69	10.0	27.0	2.706
84	10.0	27.3	2.730
98	10.0	26.8	2.686
112	9.8	26.7	2.728
125	9.8	26.9	2.745
140	9.8	26.8	2.722
154	10.1	26.4	2.626

+30°C	mean PT		PT ratio
	time (d)	NP030416	AP090406 AP/NP
0	9.9	28.4	2.867
1	10.0	28.2	2.826
13	10.2	28.1	2.767
27	10.0	27.5	2.750
41	9.8	27.5	2.793
55	10.1	27.6	2.724
68	10.5	27.7	2.641
83	10.2	27.3	2.682
97	10.1	27.5	2.711
111	10.0	27.4	2.743
124	10.0	27.4	2.743
139	10.2	27.6	2.715
153	9.5	27.2	2.870

+4°C	mean PT		PT ratio
	time (d)	NP030416	AP090406 AP/NP
0	10.0	27.9	2.788
12	9.83	28.1	2.861
26	10.3	28.5	2.755
40	10.1	28.5	2.818
54	10.0	27.9	2.790
67	9.83	27.7	2.814
82	9.80	27.5	2.810
96	9.97	27.8	2.786
110	9.80	28.6	2.915
123	10.2	28.3	2.787
138	10.1	28.2	2.801
152	10.2	28.6	2.810

Remark: t=0 (mean of 6 points) of -20°C ampoules

Table 10: Mean PT of candidate **08/144** after storage at various temperatures

+45°C	mean PT		PT ratio
	time (d)	NP030416	AP090406
0	12.4	36.8	2.968
1	12.5	34.6	2.768
7	12.5	32.2	2.576
14	12.5	31.7	2.536
21	12.5	31.8	2.544
28	12.7	32.0	2.520
35	11.7	31.8	2.718
42	12.5	31.8	2.544
49	12.5	32.5	2.600
56	12.5	32.3	2.584
63	12.3	32.4	2.634
70			

+37°C	mean PT		PT ratio
	time (d)	NP030416	AP090406
0	12.6	37.3	2.969
2	12.8	35.8	2.794
14	13.0	33.9	2.617
28	13.1	34.2	2.604
42	12.8	34.0	2.649
56	13.0	33.9	2.608
69	13.3	33.5	2.513
84	13.0	34.0	2.625
98	13.5	33.8	2.506
112	13.2	34.1	2.581
125	13.0	34.5	2.654
140	13.1	34.1	2.596
154	13.3	34.3	2.573

+30°C	mean PT		PT ratio
	time (d)	NP030416	AP090406
0	12.9	38.5	2.985
1	12.5	38.0	3.040
13	12.7	35.7	2.816
27	12.8	36.1	2.823
41	12.8	34.8	2.714
55	12.6	35.1	2.788
68	13.0	35.5	2.728
83	13.0	35.3	2.715
97	13.1	35.5	2.701
111	13.0	35.5	2.728
124	13.0	35.1	2.710
139	13.0	35.6	2.741
153	13.0	35.3	2.715

+4°C	mean PT		PT ratio
	time (d)	NP030416	AP090406
0	12.6	37.1	2.959
12	12.5	37.7	3.019
26	12.3	37.5	3.038
40	12.5	37.8	3.032
54	12.5	37.1	2.968
67	12.8	37.1	2.898
82	12.7	37.2	2.934
96	12.5	37.6	3.019
110	12.5	37.9	3.043
123	12.6	37.7	2.982
138	12.5	37.3	2.981
152	12.6	37.8	2.995

Remark: t=0 (mean of 6 points) of -20°C ampoules

Table 11: PT at various times after reconstitution of 07/314

Time (h)	First ampoule						
	NP 030416			PT (s)	AP 090406		PT (s)
	1e	2e	Mean	1e	2e	Mean	
4	10.0	10.5	10.3	29.2	28.8	29.0	
3	10.3	10.4	10.4	28.5	29.2	28.9	
2	10.5	10.6	10.6	29.3	29.2	29.3	
1	10.0	10.4	10.2	28.5	28.4	28.5	
0.5	10.4	10.5	10.5	30.2	29.5	29.9	

Time (h)	Second ampoule					
	1e	2e	Mean	1e	2e	Mean
4	10.4	10.4	10.4	29.2	29.5	29.4
3	10.4	9.4	9.9	28.5	28.7	28.6
2	10.6	10.2	10.4	28.8	28.4	28.6
1	10.6	10.2	10.4	29.5	29.3	29.4
0.5	10.5	10.5	10.5	30.1	29.9	30.0

Time (h)	Third ampoule					
	1e	2e	Mean	1e	2e	Mean
4	9.9	10.4	10.2	28.9	28.7	28.8
3	10.5	10.0	10.3	29.1	29.5	29.3
2	10.4	9.9	10.2	28.6	28.9	28.8
1	10.5	9.9	10.2	28.6	29.1	28.9
0.5	10.0	10.0	10.0	29.1	29.5	29.3

Time (h)	Overall mean n=6	
	NP030416	AP090406
	PT (s)	PT (s)
4	10.3	29.1
3	10.2	28.9
2	10.4	28.9
1	10.3	28.9
0.5	10.3	29.7

Table 12: PT at various times after reconstitution of 08/144

Time (h)	First ampoule						
	NP 030416			PT (s)	AP 090406		PT (s)
	1e	2e	Mean	1e	2e	Mean	
4	12.7	12.7	<i>12.7</i>	37.8	37.2	<i>37.5</i>	
3	13.3	12.5	<i>12.9</i>	38.7	37.7	<i>38.2</i>	
2	12.8	12.7	<i>12.8</i>	37.1	37.1	<i>37.1</i>	
1	12.8	12.8	<i>12.8</i>	37.5	37.2	<i>37.4</i>	
0.5	12.8	12.5	<i>12.7</i>	37.4	37.6	<i>37.5</i>	

Time (h)	Second ampoule					
	1e	2e	Mean	1e	2e	Mean
4	13.2	13.2	<i>13.2</i>	39.0	38.6	<i>38.8</i>
3	13.3	13.3	<i>13.3</i>	37.9	37.4	<i>37.7</i>
2	12.8	12.8	<i>12.8</i>	38.5	38.0	<i>38.3</i>
1	13.0	13.0	<i>13.0</i>	38.0	38.0	<i>38.0</i>
0.5	13.0	13.0	<i>13.0</i>	37.6	37.1	<i>37.4</i>

Time (h)	Third ampoule					
	1e	2e	Mean	1e	2e	Mean
4	13.4	13.3	<i>13.4</i>	37.2	36.6	<i>36.9</i>
3	12.9	12.8	<i>12.9</i>	38.1	37.9	<i>38.0</i>
2	12.8	12.9	<i>12.9</i>	37.2	36.6	<i>36.9</i>
1	12.8	12.8	<i>12.8</i>	37.2	37.5	<i>37.4</i>
0.5	13.2	13.1	<i>13.2</i>	33.0	33.4	<i>33.2</i>

Time (h)	Overall mean n=6	
	NP030416	AP090406
	PT (s)	PT (s)
4	13.1	37.7
3	13.0	38.0
2	12.8	37.4
1	12.9	37.6
0.5	12.9	36.0

Figure 1a: Mean PT normal plasma of candidate 07/314 after storage at various temperatures

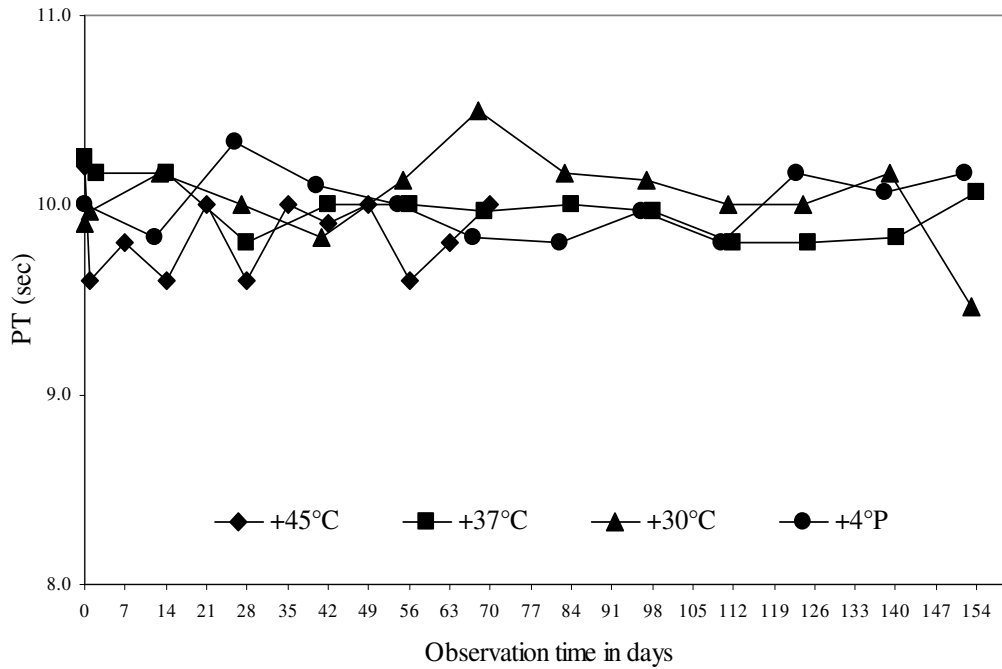


Figure 1b: Mean PT coumarin plasma of candidate 07/314 after storage at various temperatures

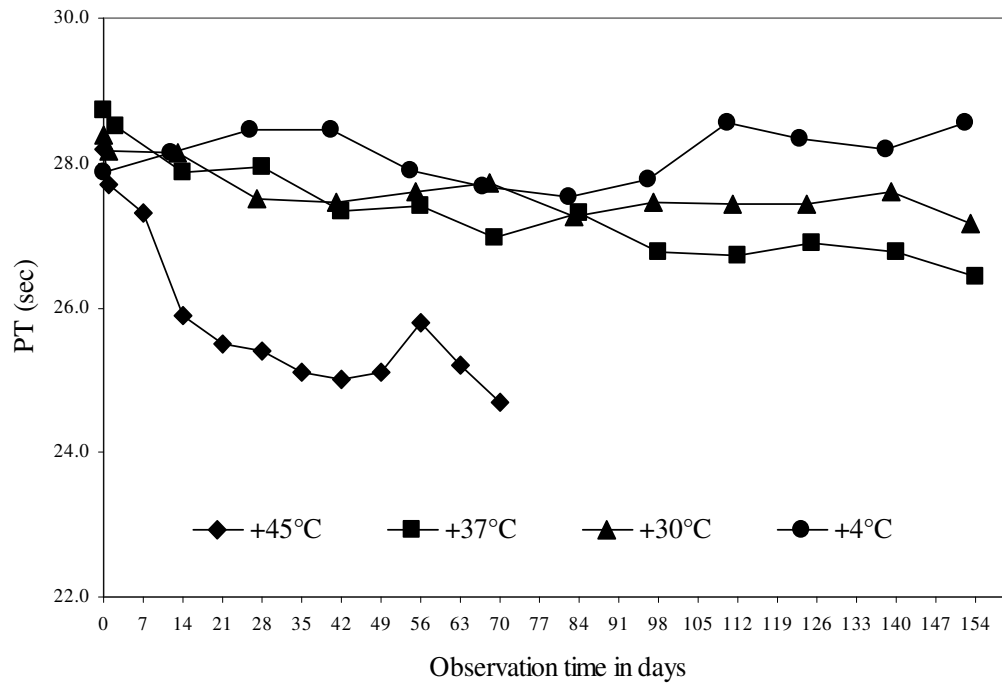


Figure 2a: Mean PT normal plasma of candidate **08/144** after storage at various temperatures

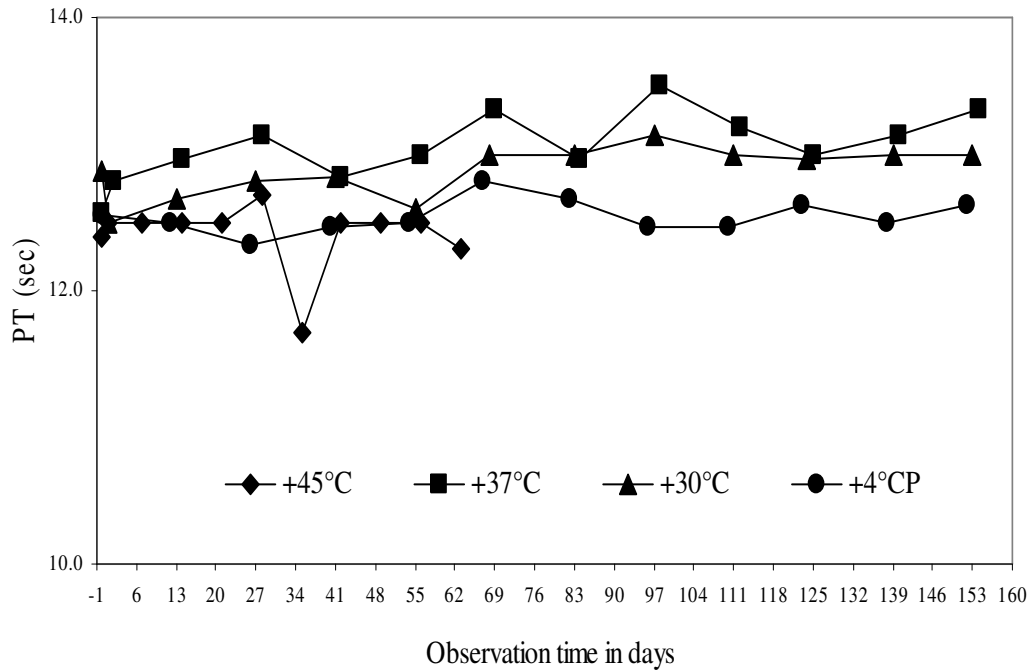


Figure 2b: Mean PT coumarin plasma of candidate **08/144** after storage at various temperatures

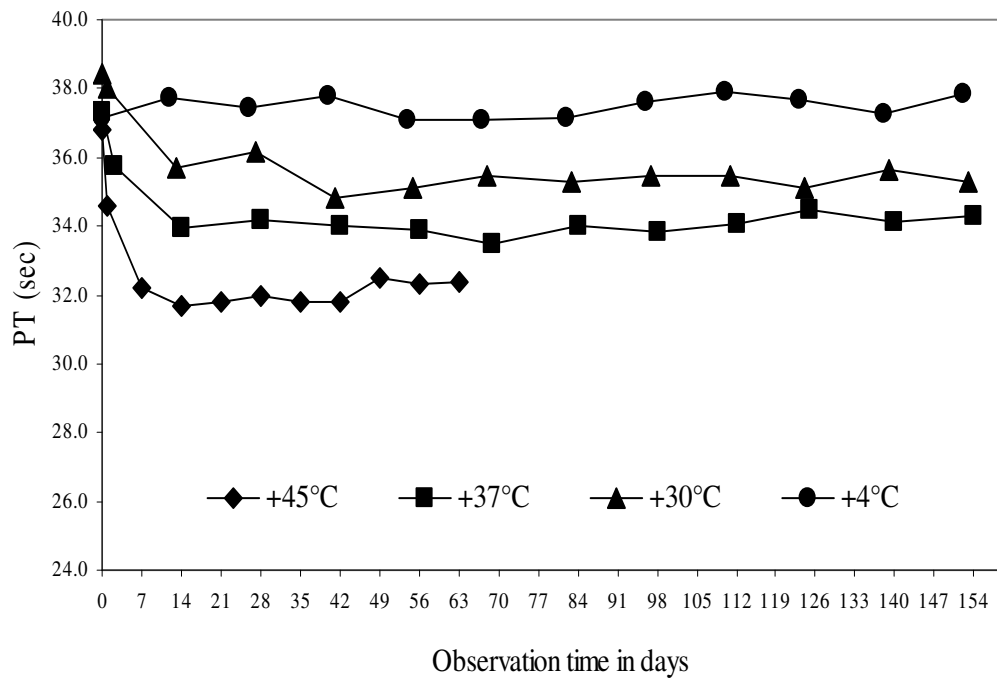


Figure 3a: PT ratio coumarin plasma/normal plasma of candidate 07/314 after storage at various temperatures

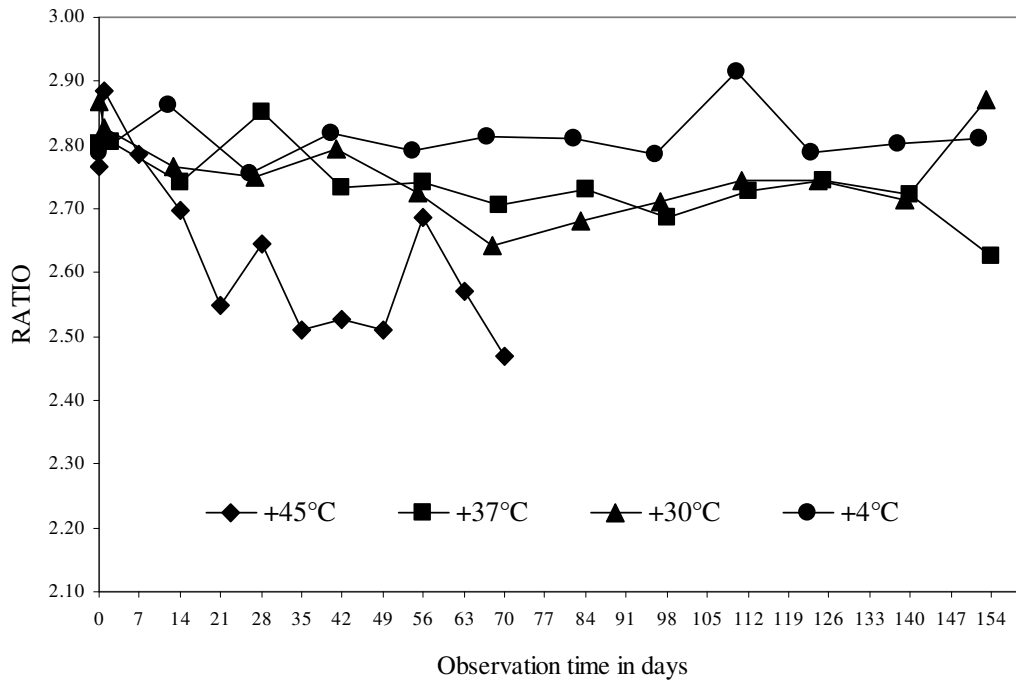


Figure 3b: PT ratio coumarin plasma/normal plasma of candidate 08/144 after storage at various temperatures

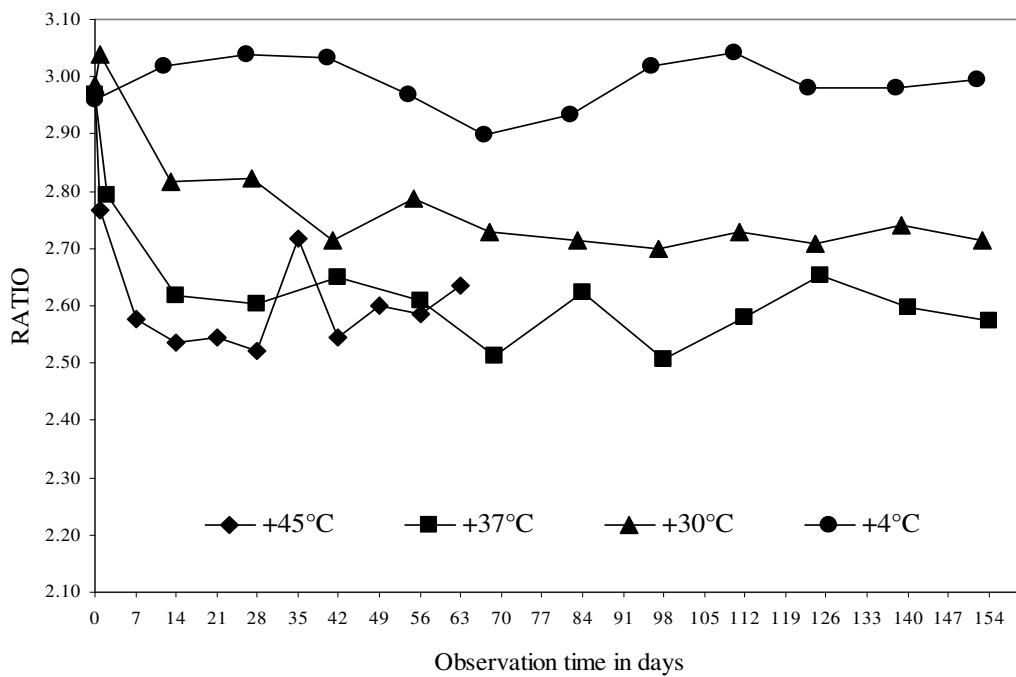
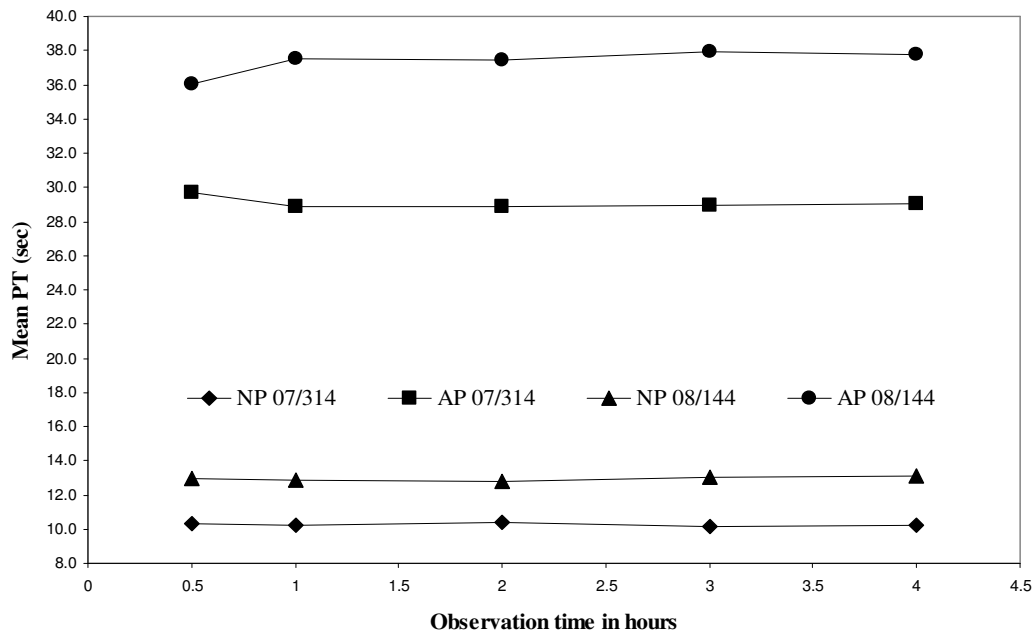


Figure 4: PT at various times after reconstitution of 07/314 and 08/144



Appendix 1

Participants (in alphabetical order) of the international collaborative study for the calibration of a proposed reference preparation for thromboplastin, human, recombinant, plain.

- P. Angchaisuksiri/K. Arurachai. Division of Hematology, Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.
- I. Bodo. Department of Hematology and Stem Cell Transplantation. St. László Hospital Budapest, Hungary.
- V. Chantarangkul/A. Tripodi. Hemophilia and Thrombosis Center Angelo Bianchi Bonomi, Department of Internal Medicine, University and IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena Foundation, Milano, Italy.
- S Reed. Thame Thrombosis and Haemostasis Research Foundation, Thame, Oxon, UK.
- T. Gago. Serviço Patologia Clínica, Hospital de Santa Cruz, Carnaxide, Portugal.
- M. Johnston. Hemostasis Reference Laboratory, Henderson Research Centre, Hamilton, Canada.
- K. Kynde. Department of Clinical Biochemistry, Roskilde University Hospital, Denmark.
- S. Kitchen. Dept of Coagulation, Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Sheffield, UK.
- L. Ippolito/G. Bianchi. U.O. Diagnostica Emato-chimica, Dipartimento di Patologia e Medicina di Laboratorio, Azienda Ospedaliero-Universitaria di Parma, Parma, Italy.
- C. Legnani. U.O. Angiologia e Malattie della Coagulazione "Marino Golinelli", Azienda Ospedaliero-Universitaria di Bologna, Policlinico S. Orsola - Malpighi, Bologna, Italy.
- T. Lindahl. Department of Clinical Chemistry, University Hospital, Linköping, Sweden.
- R. Manning. Laboratory of Haematology, Department of Coagulation, Hammersmith Pathology Centre, Hammersmith Hospital, London, UK.
- M. Martinuzzo. Hematología, Laboratorio de Hemostasia y Trombosis, Fundación Favaloro, Buenos Aires, Argentina.
- D. Mezzano. Laboratorio de Hemostasia y Trombosis, Escuela de Medicina, Univ. Católica de Chile, Santiago, Chile.
- N. Opartkiattikul. Department of Clinical Pathology, Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand.
- K. Overgaard/J Jespersen. Department of Clinical Biochemistry, Hospital of South West Denmark, Esbjerg, Denmark.

- V. Pengo/E. Bison. Ospedale Ex-Busonera, Servizio di Prevenzione e Terapia della Trombosi-Cardiologia, Università di Padova, Padova, Italy.
- W. Plesch. Roche Diagnostics, Mannheim, Germany.
- R. Redaelli. Laboratorio Emostasi e Trombosi, Divisione di Ematologia Talamona, Ospedale Niguarda Cà Granda, Milano, Italy

A.M.H.P. van den Besselaar/E. Witteveen. Thrombosis and Hemostasis Research Center, Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands

Appendix 2

**DRAFT INSTRUCTIONS FOR USE FOR THE PROPOSED WHO 4th
INTERNATIONAL STANDARD, THROMBOPLASTIN, HUMAN, RECOMBINANT,
PLAIN (08/144)**



WHO International Standard
**WHO 4th International Standard Thromboplastin, Human,
Recombinant, Plain**
NIBSC code: 08/144
Instructions for use
(Version 1.00, Dated)

1. INTENDED USE

This material was established as the 4th International Standard (IS) for Thromboplastin Human, Recombinant, Plain by the WHO Expert Committee on Biological Standardization (ECBS) in 2009 and consists of ampoules containing freeze-dried recombinant tissue factor (coded 08/144). Details of the collaborative study can be found in document WHO/BS/09.****.

2. CAUTION

This preparation is not for administration to humans.

This preparation does not contain any material of human origin (see Section 4 for detailed description of contents).

As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

INTERNATIONAL SENSITIVITY INDEX (ISI) AND COLLABORATIVE STUDY

The International Standard has been assigned an ISI value of 1.082

The ISI value was determined in a collaborative study against the WHO International Reference Preparations of tissue factor from human (rTF/95) and rabbit origin (RBT/05). The study involved 20 laboratories from Europe, North America, South America and Asia. The 4th International Standard, 08/144, and the WHO International Reference Preparations of human and rabbit origin (ie. rTF/95 and RBT/05) were tested in each laboratory by the same expert operator using the manual tilt tube technique. Test plasmas were freshly prepared from healthy subjects and patients on long term anticoagulant therapy. Participants selected patient plasmas with prothrombin times (PT) corresponding to an interval of International Normalized Ratios (INR) from 1.5 to 4.5. To account for the effect of inter-day variation, PT measurements were performed in each laboratory on ten different days (not necessarily consecutive). Participants included on each day plasmas from 2 healthy individuals and 6 anticoagulated patients, using plasmas of different healthy subjects and patients on each working day. To minimize the effect of possible plasma instability on the prothrombin times, the order of testing was changed each day. Each plasma was to be tested with each thromboplastin before proceeding to the next. Plasmas were tested on each day according to the following order:
- normal plasma 1, patient plasma 1 through 6 and normal plasma 2.

4. CONTENTS

Country of origin of biological material: United States of America.
THROMBOPLASTIN (lyophilized portion 08/144), the residue of a solution containing:

Tissue Factor (TF). A human recombinant membrane-spanning protein, expressed in a baculovirus expression vector and purified using ion exchange and size exclusion chromatography.

Mixed Phospholipids. Individual phospholipid components were prepared synthetically and are >99.9% pure. An antioxidant is included in the final lipid blend to prevent oxidation.

Stabilizers. A sugar is used as a stabilizer of the lyophilized product.

Preservatives. Sodium Azide (0.04 %) is used as preservative in the lyophilized product

RECONSTITUTION FLUID (code 08/146) A liquid preparation which contains:
Calcium chloride (12.24 mmol/L) and preservative.

5. STORAGE

Unopened ampoules of 08/144 should be stored in the dark at -20 °C or below. Store reconstitution fluid, 08/146, at 2 - 8 °C.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body.

Tap the ampoule gently to collect the material at the bottom (labeled) end. Ensure that the disposable ampoule safety breaker provided is pushed down on the stem of the ampoule and against the shoulder of the ampoule body. Hold the body of the ampoule in one hand and the disposable ampoule breaker covering the ampoule stem between the thumb and first finger of the other hand. Apply a bending force to open the ampoule at the coloured stress point, primarily using the hand holding the plastic collar.

Care should be taken to avoid cuts and projectile glass fragments that might enter the eyes, for example, by the use of suitable gloves and an eye shield. Take care that no material is lost from the ampoule and no glass falls into the ampoule. Within the ampoule is dry nitrogen gas at slightly less than atmospheric pressure. A new disposable ampoule breaker is provided with each DIN ampoule.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

Equilibrate ampoules at room temperature for at least 15 minutes before reconstitution.

Each ampoule of the freeze-dried material is to be reconstituted with exactly 1.0 ml of the provided reconstitution fluid (08/146).

Do not attempt to mix the contents by placing the thumb over the open end of the ampoule.

Leave the ampoule undisturbed for 20 minutes at room temperature and then swirl gently to dissolve the contents. Ensure that the entire freeze-dried residue is dissolved. Pool the contents of ampoules if more than one is needed to complete any one calibration session. Leave the reconstituted thromboplastin at room temperature and use within 2 hours of reconstitution. Unused material should be discarded.

CALIBRATION PROCEDURE TO BE USED WITH rTF/95

According to the WHO Guidelines (1) calibration of thromboplastins should be performed on plasmas from 20 healthy subjects and 60 patients on stabilized oral anticoagulant therapy. The whole calibration procedure can be conveniently split into ten working sessions, not necessarily consecutive. Schedule of one-day calibration During the first 2 hours collect the blood, centrifuge and separate the platelet-poor plasma, and reconstitute thromboplastins according to the instructions. During the next 2-3 hours perform the actual testing of plasmas according to the design provided (see below).

Selection of healthy subjects and patients

Healthy subjects must be ambulant adults (females taking oral contraceptives can be included). On each working day use one male and one female (if it is possible) and select a different pair each day.

Patients must be different on each day and chosen among those who are in good health (outpatients) and have been stabilized for at least 6 weeks in the range of treatment between 1.5 and 4.5 INR, according to the routine





reagent of the laboratory. Select patients covering the whole range of anticoagulation from 1.5 to 4.5. To avoid bias all results obtained with the chosen patients' plasmas must be recorded.

Blood collection and plasma preparation

At the beginning of each working day collect blood from 2 healthy subjects and 6 patients stabilized on oral anticoagulant treatment. Blood will be collected by clean venipuncture in a plastic (or glass siliconized vacuum) tube containing trisodium citrate solution within the range of concentration 105-109 mmol/L (9 volumes of blood/1 volume of sodium citrate anticoagulant). The tube must be inverted several times to ensure complete mixing of blood and anticoagulant. Citrated blood will be centrifuged immediately after collection at least 2,500g for 10 minutes at a controlled room temperature. Platelet-poor plasmas are transferred into plastic tubes and stored capped at room temperature, until tested.

Preparation of thromboplastins

On each working day:

Equilibrate a suitable number of ampoules of 08/144 and the provided reconstitution fluid at room temperature for at least 15 minutes before reconstitution.

Equilibrate a suitable number of ampoules of thromboplastin to be calibrated and its reconstitution fluid (if any) at room temperature for at least 15 minutes before reconstitution.

Reconstitute ampoules of 08/144 following instruction (see sections 6 and 7 above).

Reconstitute ampoules of thromboplastin to be calibrated following instructions. Discard the remaining reconstituted thromboplastins at the end of each working day.

Testing procedure

Test the 8 plasma samples (2 normal and 6 patients stabilized on oral anticoagulant therapy) with the two thromboplastins according to the design given below. Testing must be done as single determinations. Calibrations using 08/144 must be performed exclusively by using manual (either tilt tube or Kollie-Hook) technique, whereas a coagulometer may be used for testing with the thromboplastin to be calibrated with a secondary standard as appropriate. With the tilt tube technique, test tubes must be immersed in the water as deeply as possible to ensure optimal temperature control. Tilt the tubes back and forth at regular intervals. To avoid prolonged removal of tubes from the water, the use of an illuminated water-bath is recommended. The order of testing normal and patient plasmas will be random and must reflect the order of blood collection if this is considered random. As an example collect first the normal 1 (which will be tested first) then the 6 patients on oral anticoagulant treatment and finally the normal 2 (which will be tested last). In any case the order of testing should not be related to the prolongation of the clotting time of the patient plasma. The order of testing on each working day shall be as follows:

Normal 1 Patient 1 Patient 2 Patient 3 Patient 4 Patient 5
Patient 6 Normal 2

Each plasma shall be tested with both thromboplastins before proceeding to the next if both are used with the manual technique. If the prothrombin time system to be calibrated involves the use of an automated instrument, it is not practical to test each plasma with both thromboplastins before proceeding to the next. In that case, all plasmas can be tested with each thromboplastin consecutively and more or less simultaneously with both thromboplastins. The same expert operator shall be in charge to carry out the whole calibration.

Actual testing with 08/144

Place glass test tubes in the water bath and wait at least 5 minutes to reach 37°C.

Pipette 0.2 ml of 08/144 and incubate for at least 2 minutes to reach 37°C.
Pipette 0.1 ml not pre-warmed test plasma and start a stopwatch immediately.
Shake to mix the content and tilt the tube regularly back and forth until clot forms.
Record the clotting time in seconds and 1/10 seconds.

Equipment

Calibrated pipettes to reconstitute thromboplastins and to deliver thromboplastin and plasma samples for actual testing. If automated micro-pipettes are used, tips must be changed for each test.

Non-contact tubes with non-contact stoppers (no rubber) to store blood and plasma.

Non-contact pipettes to transfer plasmas for storage and to dispense plasmas for testing.

Glass tubes for testing

Water-bath thermostatted at 37° C ± 0.5.

Stopwatches

Statistical analysis and ISI determination

For statistical analysis and ISI determination refer to the WHO Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy (1). These requirements are available on request from the Biologicals Unit, WHO, CH-1211 Geneva 27, Switzerland.

8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

1 WHO Expert Committee on Biological Standardization. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy. WHO Technical Report Series 1999; No. 889: 64-93.

10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the participants in the collaborative study. This study was organized and carried out under the auspices of the Scientific and Standardization Committee (SSC) (Subcommittee on Control of Anticoagulation), of the International Society on Thrombosis and Haemostasis (ISTH). Grateful acknowledgements are also due to Instrumentation Laboratory (Orangeburg, NY) and Siemens Healthcare Diagnostics Products GmbH (Marburg, Germany), who donated the candidate materials for the collaborative study.

11. FURTHER INFORMATION

Further information can be obtained as follows;

This material: enquiries@nibsc.hpa.org.uk

WHO Biological Standards: <http://www.who.int/biologicals/en/>

JCTLM Higher order reference materials: <http://www.bipm.org/jctlm>

Derivation of International Units:

<http://www.nibsc.ac.uk/products/faq.asp>

Ordering standards from NIBSC:

<http://www.nibsc.ac.uk/products/faq.asp>

NIBSC Terms & Conditions: <http://www.nibsc.ac.uk/terms.html>

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.hpa.org.uk

**13. CITATION**

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Physical and Chemical properties	
Physical appearance: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): preservative (azide)	Contains recombinant protein, stabilizers and
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

15. LIABILITY AND LOSS

Information provided by the Institute is given after the exercise of all reasonable care and skill in its compilation, preparation and issue, but it is provided without liability to the Recipient in its application and use.

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If any of the Goods supplied by the Institute should prove not to meet their specification when stored and used correctly (and provided that the Recipient has returned the Goods to the Institute together with written notification of such alleged defect within seven days of the time when the Recipient discovers or ought to have discovered the defect), the Institute shall either replace the Goods or, at its sole option, refund the handling charge provided that performance of either one of the above options shall constitute an entire discharge of the Institute's liability under this Condition.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom
* Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.100 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No