# Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination – a rebuttal

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*See also* van den Besselaar AMHP, Barrowcliffe TW, Houbouyan-Re veillard LL, Jespersen J, Johnston M, Poller L, Tripodi A on behalf of the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the ISTH. Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination. *J Thromb Haemost* 2005; **2**: 1946–53; van den Besselaar AMHP. Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination – reply to a rebuttal. This issue, pp 2371–2.

In the November issue (2004) of the *Journal of Thrombosis and Haemostasis*, Van den Besselaar *et al*. [1] present guidelines for the use of certified plasmas for ISI calibration and 'direct' INR determination. The guidelines reflect the official standpoint of the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the ISTH and give clear instructions for both manufacturers and users of certified plasma samples. The authors describe two main procedures of using certified plasmas and the guidelines are backed-up from experiences of various well-defined multicentre studies or occasionally full-scale field studies. However, it was with disappointment that we learned that the authors only discussed results concerning plain thromboplastin reagents (Quick-PT type of assay) and left out experiences with combined, Owren PT type of reagents. An alternative procedure for certifying INR calibrant plasmas and direct local INR calibration has been performed in Sweden and Norway for several years [2].

Swedish laboratories employ combined PT reagents of the Owren type based on rabbit brain thromboplastin and adsorbed bovine plasma. This makes the test specific for the vitamin K-dependent factors II, VII and X and independent of the level of fibrinogen and factor V in the patient plasma. The use of a single species thromboplastin makes it easier to standardize the assay. The Owren type PT assay has several advantages over conventional, plain, thromboplastins [3–5]. It can be performed with many types of samples and calibration can be done equally well with artificially depleted plasma samples or plasma from patients on antivitamin K therapy and it is insensitive to the citrate concentration in the blood collection tube. Lyophilized plasma samples can be used without loss of accuracy. Thus, the Owren type PT is a more robust assay and is less affected by preanalytical factors.

In 1997, the Swedish organizer of external quality assessment schemes, EQUALIS, gained acceptance for the new concept of INR calibration. The process involved a standardized calibration procedure for the Owren PT assay as previously described [2,3]. EQUALIS produces plasma calibrators with assigned INR values and issues a certificate for each material, distributes them, and provides support to the users when they perform the local calibration. Due to the established relationship between Owren PT percent and INR according to WHO procedure [3], the certified INR values are traceable to the first international reference preparation. Moreover, as the relationship between Owren PT and manually performed Quick PT only needs to be performed once, it is now possible for manufacturer to obtain new calibrant plasmas in a reproducible fashion only based on collection of normal plasma samples. The trueness and precision of Swedish INR measurements have been investigated for several years using plasma with assigned INR values according to the guidelines presented by Van den Besselaar *et al*. [1] with equivalent results. In a multi-centre exercise, we obtained similar mean INR values but much less variation compared with Quick PT assay users [3], indicating both accuracy and robustness of the chosen local INR calibration procedure for Owren PT users in Sweden and Norway.

In Sweden more than 300 laboratories have, since 1999, performed direct local INR calibration by means of only two plasma calibrators. A 3-year follow-up [2] showed for primary care laboratories ($n = 246$) and larger hospital-based laboratories ($n = 88$) an interlaboratory CV $< 6\%$ in the INR range 2–4. In the INR range $< 2$, the CV was reduced to 4% for all laboratories. The low interlaboratory CV has been sustained over time since 1999.

As the primary aim for the calibration procedures for PT testing is to improve INR reliability, we suggest that the members of the Subcommittee on Control of Anticoagulation of the Scientific and Standardization Committee of the

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ISTH considers the results obtained in Sweden and other countries using the Owren type PT assay. We have shown that there are several preanalytical and analytical advantages of the Owren type PT assay compared with the conventional Quick PT assay. We have also described a reproducible way to characterize INR calibrant plasmas based on collection of normal plasma samples and a very simple procedure for direct local INR calibration with highly competitive results. The current situation with many different PT methods and complicated calibration procedures are clearly not an optimal situation. The Swedish experience indicates that it is time to discuss which PT methods should be used in order to improve the current situation. The Owren PT assay allows simplification of standardization procedures [6]. It should be the task of the Subcommittee to take all facets of oral anticoagulation control into consideration, not only primarily for the benefit of patients but also to give the laboratories the optimal tools in order to achieve the goals of improvement of the quality of PT testing.

## References


1 Van den Besselaar AMHP, Barrowcliffe TW, Houbouyan-Réveillard LL, Jespersen J, Johnston M, Poller L, Tripodi A. Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination. *J Thromb Haemost* 2004; **2**: 1946–53.

2 Hillarp A, Egberg N, Nordin G, Stigendal L, Fagerberg I, Lindahl TL. Local INR calibration of the Owren type prothrombin assay greatly improves the intra- and interlaboratory variation. A three-year follow-up from the Swedish national external quality assessment scheme. *Thromb Haemost* 2004; **91**: 300–7.

3 Lindahl TL, Egberg N, Hillarp A, Ødegaard OR, Edlund B, Svensson J, Sandset PM, Rånby M. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. *Thromb Hemost* 2004; **91**: 1223–31.

4 Horsti J. Agreement of Owren and Quick prothrombin times: effects of citrate and calcium concentrations and international sensitivity index correction. *Clin Chem* 2001; **47**: 940–4.

5 Horsti J. Comparison of Quick and Owren prothrombin time with regard to the harmonisation of the international normalised ratio (INR) system. *Clin Chem Lab Med* 2002; **40**: 399–403.

6 Jackson CM. Monitoring oral anticoagulant therapy – INR values for the Owren prothrombin time. *Thromb Haemost* 2004; **91**: 210–2.


# Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination – reply to a rebuttal


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In their letter to the editor, Egberg *et al*. [1] refer to their recently published method for calibration of so-called Owren-PT-type reagents [2,3]. These reagents are very popular in Scandinavian countries for the control of oral anticoagulant therapy. The Owren-PT-type reagents are similar in composition, i.e., containing rabbit tissue factor and adsorbed bovine plasma. The calibration procedure chosen by the Scandinavian group involved the establishment of a mathematical relation between the Owren PT(%) and INR obtained with the manual Quick method with reagent ISI assigned according to WHO recommendations. The Scandinavian procedure allowed the production of calibrant plasmas with assigned INR values that are traceable to the international reference preparation for thromboplastin. The Scandinavian procedure involved a two-point calibration curve where the local PT in seconds is plotted in a log–log diagram on the *y*-axis and the reference INR values on the *x*-axis.

Egberg *et al*. are disappointed that experiences with Owren-type reagents are not referred to in the 'Guidelines on preparation, certification, and use of certified plasmas for ISI calibration and INR determination' [4] published a few months after the publication of the calibration method by the


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