Prothrombin Time Determination At Ambient Room Temperature.

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Introduction: The aim was to explore the possibility of wet-chemistry PT determination at ambient room temperature. This was to accomplish rapid point och care, POC, determinations (no temperature equilibration time) with a minimally complex, and hence inexpensive, instrumentation. The approach was to adhere to the international sensitivity index (ISI)/normal clotting time (NCT) formalism and to view these calibration constants as functions of temperature. The goal was PT analysis on finger puncture blood that agreed with PT analysis at 37ºC on plasma. The acceptability of the approach was checked by 1) the correlation between results obtained at different temperature within the ambient temperature range of 17 to 45ºC and 2) the correlation between an ambient temperature method on capillary blood and a routine method on the same.

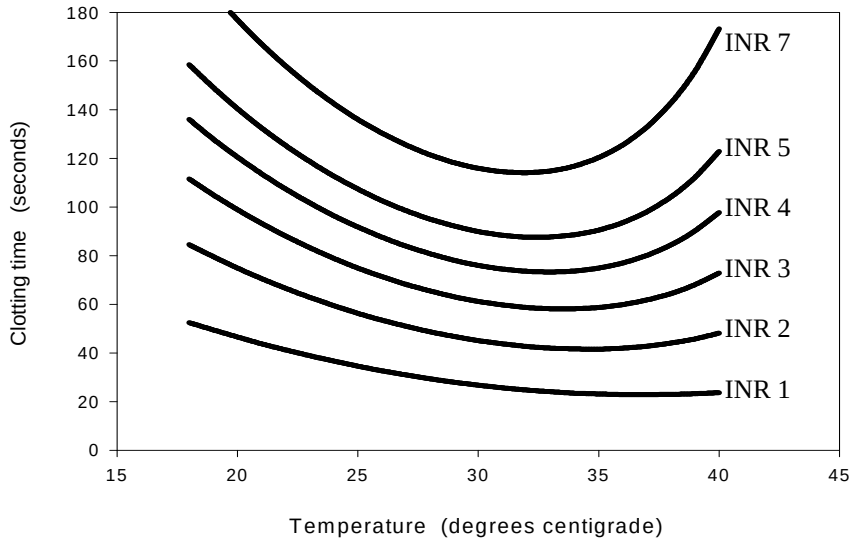
Material and methods: The ISI and NCT were determined at eleven temperatures by a two point calibration method. Calibrators were a normal and an abnormal control plasma, NKP 162 and OKP 167, both from MediRox, Sweden, and both with known INR. Of these, 20 µL were mixed with 400 µL PT reagent GHI 131. Clot detection was manual.

Temperature	CT INR 1.14	CT INR 2.82		NCT	ISI	
18°C		57	109		51.9	1.40
20°C		52	97		47.5	1.45
22°C		45	83		41.2	1.48
24°C		40	75		36.5	1.44
26°C		35	68		31.8	1.36
28°C		33	63		30.1	1.40
30°C		30	61		27.1	1.28
32°C		27	60		24.1	1.13
34°C		26	57		23.2	1.15
36°C		26	57		23.2	1.15
38°C		26	63		22.9	1.02

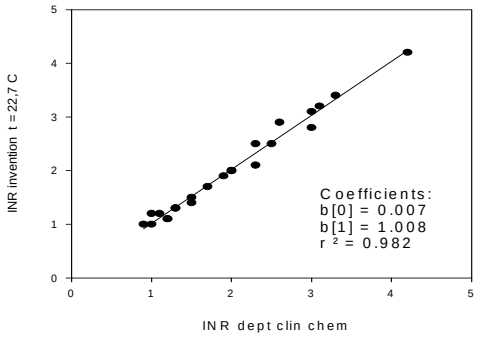
The temperature was controlled by a water bath and checked by a certified thermometer. The reagent mixture, in a polystyrene tube, was tilted about once every second out of the bath and inspected for clot. The ISI and NCT were calcu lated from the clotting times and the known INR of the calibrators:

$ISI = (\ln INR2 - \ln INR1) / (\ln CT2 - \ln CT1)$
$NCT = \exp(\ln CT1 - (\ln INR1)/ ISI)$

The data were fitted to second degree polynomial functions to give:
$NCT(t) = 136.37 – 6.1651t + 0.0837t^2$
$ISI(t) = 1.0584 + 0.0420t - 0.0011t^2$

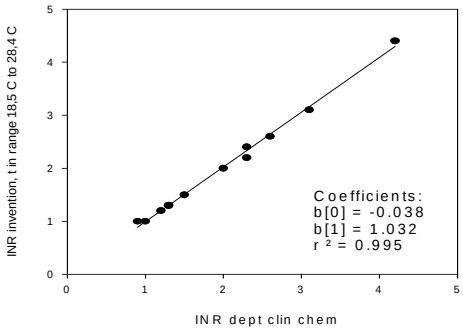


temperatures to be computed for any given INR value. This was of interest because it showed that there is an optimal temperature for PT determinations at about 33ºC, and that e.g. 17ºC is similarly acceptable as 37ºC. An advantage of the selected approach is the fact that temperature is measured with a far higher precision than it is controlled.



Soon to be discarded patient plasma samples with known INR values by a routine method were obtained from the clinical chemistry laboratory of the University Hospital, Linköping, Sweden. These 25 samples were analysed by the manual method at 22,7ºC. The INR was determined from the clotting times and the ISI

and NCT generated by the polynomials.

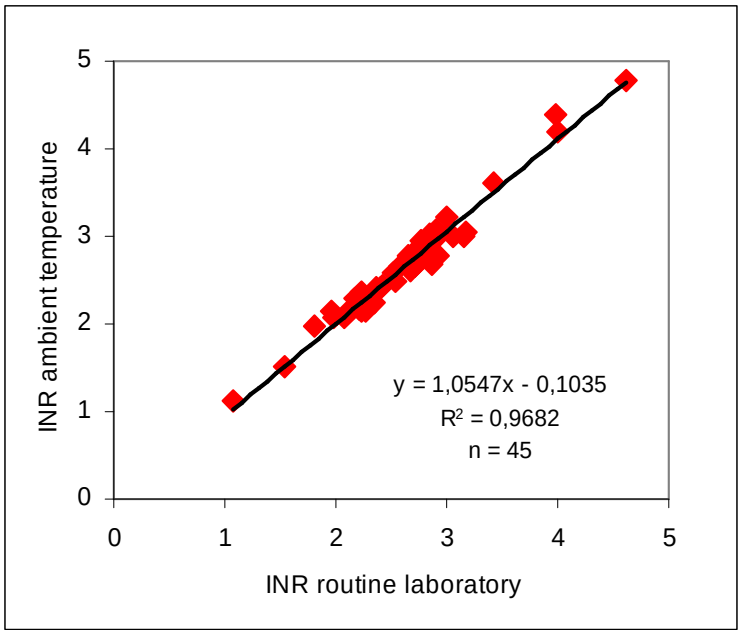


Of the 25 samples, 12 were randomly selected and analysed also at 18,6 and 26,4ºC. The mean of the three determinations was calculated and plotted against the INR values by the routine clinical laboratory procedure.

As obvious from the plots, analysis at ambient room temperature inflicts no serious bias in the PT determination.

Encouraged by the results of manual determinations, a simple reader was constructed around a 4 MHz one-chip computer connected to an optical gap in which a polystyrene tube could be placed. The optics were an 940 nm LED and a light diod. Information on the temperature was from a 10 kohm thermistor. The optics allowed the clotting time to be determined by repeated measurement of the light passing through the mixture of blood and PT reagent. The clotting time and the temperature were fed into the processor which also stored the parameters necessary to obtain the ISI and NCT to allow computation of the INR. The transmitted near IR light also allowed the hematocrit to be estimated and a compensation of its effect to be introduced (see poster no P0706 for details). Armed with the constructed reader, we visited an oral coagulation centre of the mentioned university hospital and received consent to collect 20µL blood from the finger puncture already made on 45 patients for determine PT for routine monitoring purposes. The INR values obtained by

the hospital and with our battery powered reader were compared.



Conclusion: Ambient room temperature, wet-chemistry, POC PT on native whole blood appears to function satisfactorily.