Hemochron® Jr. Signature
Whole Blood Microcoagulation System
manufactured by
ITC , US

A test for Prothrombine Time, PT (INR)

Report from an evaluation
organised by SKUP

Evaluated at the request of the Norwegian supplier
Medimport A/S

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Evaluation of Hemochron®Jr. Signature Whole Blood Microcoagulation Systems

CONTENT

Summary

Background The Norwegian supplier Medimport AS ordered a SKUP evaluation of the Hemochron® Jr. Signature Whole Blood Microcoagulation Systems (Hemochron) manufactured by ITC US. Hemochron is intended for measurement of Prothrombine Time (PT) in the primary health care. The PT analysis is used for monitoring of patients in vitamin K antagonist treatment to prevent thrombosis.

Hemochron The measurement principle is whole blood clot time measured after optical detection of the change in movement of the mixture in the cuvette. The clotting time is defined as the time from the mixing of blood and reagents until the blood movements of the mixture decreases below a predetermined rate. The system is based on the Quick method for Prothrombine Time (PT), (factor II, V, VII, X and fibrinogen). From the whole blood measurement the equivalent plasma PT is calculated based on regression analyses performed across multiple centres. The result is given in the scale INR (International Normalised Ratio). The International Sensitivity Index (ISI) is approximately 1.0. A high number in the INR-result reflects a high anticoagulation effect. Both capillary and venous blood samples can be measured, but with two different kinds of cuvettes.

Results. The analytical quality and the user friendliness are regarded equally important.

Analytical quality

	Type of sample	N	CV % (within) (95 % CI)	Bias (%) At 3 or 2 levels	Total Error, fulfilment of goal
Quality goals (SKUP)			≤ 5 %		> 95% < ±20% deviation
Quality goals (Denmark[1])			≤ 5 %	≤ ± 6%	
Hospital laboratory	Venous	100	8.5 (7.4 - 9.8)	1.5, -2.2, -10.2	84.0 %
	Capillary	46	7.9 (6.5 – 9.9)	1.5, -14.5	73.9 %
Primary care	Venous	40 39	7.4 (6.1 – 9.4) 7.7 (6.3 – 9.8)		
	Capillary	40	9.1 (7.5 – 11.7)		

User friendliness evaluated with venous samples: The ratings of the 'Information in Manual', 'Time factors' and 'Operation' were all 'satisfactory' both in the hospital laboratory and in the primary care. 'Quality control' was not 'satisfactory'; see comments in the report.

Conclusion Hemochron does not fulfil the quality goals set up by SKUP (or the Danish 'Laboratorieudvalget'[1]) for analytical imprecision (CV_{within}) and total error in this evaluation, neither with venous nor with capillary samples. The within-series imprecision was > 5 % for both venous and capillary samples. The Total Error was < 20 % for only 84 % of the results with venous samples. The user friendliness of 'Manual', 'Time factors' and 'Operation' for venous samples were regarded as satisfactory, while 'Quality control' was not.

1. Planning of the evaluation

1.1. Background

A SKUP evaluation is usually performed in a hospital laboratory and by two general practitioners. At least one of the general practitioners has a staff without a laboratory technologist.
The Analytical quality and user friendliness are evaluated both in the hospital laboratory and among the general practitioners. It has been a wish from the general practitioners in Denmark that analytical quality and User friendliness are weighted equally in the SKUP evaluations.

The aim of the hospital laboratory evaluation is to investigate the analytical performance and the user friendliness under standardised and optimal conditions. The performance of the system in the hospital laboratory is considered the best the system can achieve. The evaluation in primary health care reveals the 'daily day user' quality and pitfalls and is considered the achievable quality under 'real' conditions.

In March 2004 SKUP in Norway was asked to make a complete evaluation of Hemochron®Jr. Signatur Whole Blood Microcoagulation Systems for MEDimport, Norway. In April the evaluation became urgent, because the system should replace Rapid Point Coag instruments from Bayer. Rapid Point was used in Norway and Denmark and was withdrawn from the market at that time.
SKUP in Denmark could start the evaluation immediately after writing the protocol. A protocol was written in Danish and given to Kjell Myrseth in a meeting in Lyngby, where Hans Inge Søvik, MEDimport, also was present. The first evaluation began in May 2004 and was interrupted in June due to technical problems.

Esther Jensen, M. Sc. Per Hyltoft Petersen, Cand. Pharm Karin Kynde and General Practitioner Per Grinsted have written the protocol. The protocol was approved by the supplier MEDimport A/S, Norway and Ron Korona, ITC, Italy. At request of the supplier and the manufacturer (ITC), parts of the protocol were translated into English. Ron Konora, ITC, Kjell Myrseth, MEDimport, and Esther Jensen and Nina Brøgger, SKUP, had a meeting in July, where the technical problems were solved and the evaluation was re-started.

The hospital laboratory evaluation was performed in the Department of Clinical Biochemistry, Odense University Hospital (OUH), Denmark and in the Department of Clinical Biochemistry, Amtssygehuset in Roskilde. The Danish Institute for External Quality Assurance for Laboratories in Health Care (DEKS) uses the Roskilde laboratory as a reference laboratory for the prothrombin analysis in Denmark. One Danish and one Norwegian general practitioner accepted to participate in

the evaluation of the Hemochron . The two general practitioners had no laboratory technologists employed.

Esther Jensen had the main responsibility for this evaluation. The evaluation in the hospital laboratory was done by the laboratory technologists Nina Brøgger and Ann Jepsen. In the primary care centre in Denmark, two nurses performed the tests, and at the centre in Norway the evaluation was carried out by two medical secretaries. Cand. Pharm. Karin Kynde was responsible for the testing in the reference laboratory at Roskilde Amtssygehus in Roskilde.

The supplier Medimport A/S signed a contract with SKUP 15.th of July 2004. Medimport A/S has supplied SKUP with the equipment necessary for the evaluation. After the second protocol was approved, the personnel performing the evaluation were taught during 30 minutes how to perform the test.

Esther Jensen and Per Hyltoft Petersen have made the statistical calculations and written this evaluation report. Karin Kynde and SKUP have approved the report. After the first round it was also sent to the supplier. They all got the opportunity to discuss and comment the report. The report will be published on Internet by SKUP (www.skup.nu and www.SKUP.dk), if the system is in use in the Scandinavian market. SKUP and the manufacturer can use the results from the report in publications.

1.2. Addresses

Producer
International Technidyne Corportation (ITC),
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NJ 08820, USA
Telephone: +1 732 548 5700
FAX: +1 732 632 9299.
E-mail: ronkorona@compuserve.com

Supplier
Medimport A/S
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00 47 3591 3738
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Co-workers
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DEKS (Danish Institute for External Quality Assurance for Laboratories in Health Care)
Reference laboratory
Karin Kynde
E-mail rskak@ra.dk

Primary care centres
Lægerne Holsedore, Odense, Denmark
Bakklandet legekontor, Norway

2. Material and method

2.1. The Hemochron system

The measurement principle is whole blood clot time measured after optical detection of the change in movement of the mixture in the cuvette. The clotting time is defined as the time from the mixing of blood and reagents until the blood movements of the mixture decreases below a predetermined rate. The system is based on the Quick method for Prothrombine Time (PT), and the results are therefore influenced by the concentrations of factor II, V, VII, X and fibrinogen. From the whole blood measurement the equivalent plasma PT is calculated based on regression analyses performed across multiple centres. The result is given on the international scale INR (International Normalised Ratio). The International Sensitivity Index (ISI) is approximately 1.0. A high number in the INR-result reflects a high anticoagulation effect.

Both capillary blood and citrated whole blood can be measured, but with two different kinds of cuvettes: Hemochron Jr. Prothrombin Time (PT, J201) and Citrate Prothrombin Time (PT, J201C) tests are microcoagulation assays intended for use in performing quantitative, one stage prothrombin times. Both assays require whole blood samples, either fresh (J201) or anticoagulated with sodium citrate (J201C). The thromboplastin reagents in these cuvettes are highly sensitive, low ISI reagents.

The plasma equivalent PT is calculated from the INR based upon an ISI of 1.0. This differs significantly from the routine laboratory calculation of INR, which first requires calculating the ratio of the patient's PT and a local mean normal PT, then raising this ratio to the power of the ISI. The decision to calculate the INR at Hemochron directly from the whole blood clotting time was made by the manufacturer to minimise the imprecision introduced by employing several extra mathematical steps. Since whole blood clotting times are longer than plasma clotting times, use of the traditional equation in a whole blood system would first require the conversion of the whole blood clotting time to a plasma equivalent value. This value could then be used for the standard INR equation. Mathematically, imprecision is introduced into a system with each calculation performed; therefore, the more direct conversion of whole blood clotting time to INR is preferred.

Local adjustment of the PT mean normal is not possible when using the Hemochron instrument. The mean normal PT programmed into the system is only used to calculate the plasma equivalent clotting time from the INR, not vice versa.

The information above is all according to ITC.

Traceability: none, see enclosed technical report, Lot to lot reproducibility, (Enclosure B)

Producer: International Technidyne Corportation, (ITC), 8 Olsen Avenue, Edison, NJ 08820, USA
E-mail: ronkorona@compuserve.com

Agent in Denmark: Jepsen Import, Udviklingspark Øresund, Korskildelund 6, 2670 Greve

Agent in Norway: Medimport A/S, Postbox 2513, N-3702 Skien, +47 9131 4241,
E-Mail: kjell.myrseth@medimport.no

Agent in Sweden: none

The Hemochron instrument

Size: 5 x 19 x 9 cm (h x w x d)

Weight: 0,340 kg

No of test chambers: 1

Incubation time: 30-90 seconds

Use of instrument without reloading: 2-3 hours, 49 tests ~ 150 seconds /test
17 test ~ > 500 seconds/test

Battery: 500 re-loadings

Type of Battery: Nickel Cadmium

Reloading: 230 V

Allowed room temperature for the use of the instrument: 15-30^{0}C

Sample volume: 100 µl

Measuring time: dependent of result. 1 INR ~ 1 minute and 5 INR ~ 2 min

QC in two levels

Temperature QC

2.2. Quality control

The analytical quality of the Hemochron was documented by means of the internal quality controls throughout the evaluation period.

Internal Quality Control, possibilities

1) Electronic System Verification Cartridges, 2 levels

2) Temperature (Temperature Verification Cartridges)

3) Normal control, whole blood, lyophilised

4) High level control, whole blood, lyophilised

Two controls in the therapeutic level (Normal and High) can be bought separately from the supplier. There are separate control products for the two cuvette types.

External Quality Control, possibilities

Denmark: For external Quality Control only parallel analysing[1] is used. However it has been a wish from the laboratory consultants to get a tool for error detection when they visit general practice.

Norway For external Quality Control lyophilised whole blood, normal and abnormal level controls are used.

Sweden Measurement of PT-INR with the Quick method is not recommended.

2.3. Time schedule

The first evaluation period:

Hospital laboratory	May to June 2004

The second evaluation period:

Hospital laboratory	29th of July to 23rd of September 2004
Primary Care, Denmark	August to October 2004
Primary care, Norway	August to November 2004

Writing of Report: October 2004 to January 2005

2.4. Materials

Four Hemochron instruments, No S 3626, S 3624, S 3605 and S 3630. The last one was not used.

Cuvettes for citrated venous whole blood: Ref J201C Lot F4CPT020

Expiration date June 2005

Cuvettes for fresh capillary whole blood: Ref J201 Lot F4JPT032

Expiration date September 2005

Control normal lot no F4DNC005

Control abnormal lot no E4DAC003

Evaluation site	Number of test cassettes and controls used
Hospital laboratory (1 instrument and 1 as back-up)	
To get familiar with test (2 persons)	10 x 2 x 2 = 40
Intra-assay -/ Inter-assay variation	
Venous samples (Citrate)	119 x 2 = 238
Capillary blood	57 x 2 = 114
Control	40 x 2 = 80
Two general practitioners (2 instruments)	
To get familiar with test (4 persons)	10 x 2 x 2 x 2 = 80
Intra-assay -/ Inter-assay variation	
Venous samples	40 x 2 x 2 =160
Capillary samples	40 x 2 = 80

2.5. Reference laboratory

Instrument: ACL Futura, ILS Laboratories Scandinavia.

Reagent: PT Nycotest, Medinor A/S.
Principle: Owren method, rabbit brain thromboplastin and adsorbed bovine plasma.

Calibrators: 3 point calibration with:
Koagulationskalibrator Normal, DEKS, lot. 061099. Code: 2004 Value: 1.00 INR
INR Kalibrator Terapeutisk, DEKS, lot. 03-09. Value: 2.30 INR,
Uncertainty = 0.09 INR (k=2.1).
INR Kalibrator Høj, DEKS, lot. 03-02. Value: 3.92 INR
Uncertainty = 0.22 INR (k=2.2).

Traceability: 2nd IRP, Bovine, Combined, coded OBT/79. Manual tilting technique.

Control samples: 2 fresh frozen plasma pools from DEKS, normal and abnormal (AK-level).

Test samples: Frozen samples are thawed in water 37 °C in 5 minutes, analysed within 30 minutes in duplicate.

Quality demands: All calibrators are analysed 6 times (3 vials of each) in the series of patient samples. DEKS controls are analysed for each 20 samples.
CV < 3.0 % in duplicates.

Comparison samples 1) Equalis calibrators, normal and abnormal (AK-level).
2) Bioclin calibrators 1, 2 and 3.

2.6. Hospital Laboratory

For comparison of fresh and frozen samples in the Department of Clinical Biochemistry, OUH, the STAClot from Stago was used.

2.7. Materials and subjects

Hospital laboratory. Evaluation under standardised and optimal conditions

Blood samples were collected from 100 individuals in treatment with vitamin-K antagonist (vKa) during at least 20 days. In total, 100 venous samples and 46 capillary tests were analysed in duplicates.

Demands to the INR-results:

Only results between 1.5 INR to 5.0 INR in the Hemochron instrument were used.

10 results have to be between 1.5 and 1.9 INR, and

15 results have to be between 3.0 and 5.0 INR

2.7.1. *Preparation of tests used in the hospital laboratory*

In total three tubes were taken in one venous puncture. The first sample was analysed as in usual routine. The second sample was immediately analysed in the Hemochron instrument in duplicate. It was then centrifuged and the plasma was frozen. Later, after collecting all samples, the second sample was analysed in duplicate in the reference laboratory as described above. The plasma from the third sample was also frozen. Later on it was analysed in the local laboratory and compared with the fresh sample result, This was done according to the protocol, but is not part of the Hemochron evaluation.

A volume of 100 µl fresh Citrated whole Blood (3.2% Citrate, three tubes, one skin perforation) was applied into the sample well of the PT test cuvette. The well was filled from the bottom to prevent air bubbles into the blood sample (see enclosure A).

The Hemochron instrument displays “Sample too large” or “Sample too small” if an excessive or inadequate blood sample volume has been provided. If an appropriate amount is provided the measured result in INR with one decimal, is seen in the display.

46 patients also had capillary tests performed. Blood from a finger stick was filled directly into a cuvette designed for fresh capillary whole blood. Duplicate measurements were performed (two skin perforations).

The tubes were centrifuged within 30 minutes and the plasma was frozen at – 80 ºC within 2 hours.

2.7.2. *Preparation of tests used in the evaluation in General Practice*

40 patients in treatment with vitamin-K antagonist had two venous samples taken. The first sample was treated as normal in the routine and the second was analysed in duplicate in the Hemochron instrument. The duplicate measurements give the CV_{within}.

In the Danish primary care centre the 40 patients also had two skin perforations for capillary measurements in duplicate from a finger stick.

Evaluation of bias and Total Error in Primary care is not part of this evaluation.
However, the Total Error in primary care was compared to the hospital laboratory using the routine reagents (Stago, STAClot instrument).

Goals for analytical quality and user friendliness

There is no international (Golden) Standard for evaluation of Point of Care Test instruments for the prothrombine time measurement for primary health care.

The quality goals for PT-INR according to SKUP:

CV: < *5 %*

Total error: < ± *20 %*

Where Total Error (TE) = bias + $z \times \sqrt{CV^2_{testmethod} + CV^2_{comparisonmethod} + CV^2_{betweenlaboratories} + CV^2_{matrx}}$

TE = 5 % + 1.65 x $\sqrt{25+9+9+25}$ = 5 + 13.6 ~ 20 %

It is accepted that up to 5 % of the results can deviate more than ± 20 %. Only 1 % of the results must deviate more than ± 25 %.

A Danish committee appointed by the National Ministry of Health has specified the demands to analytical quality[1] for PT-INR: Bias ≤ 6% and CV_{total} ≤ 5% for instruments used in primary health care. In the Danish goals, there is no demand to the total error. The goals for hospital instruments are Bias ≤ 3%, CV_{total} ≤ 3%.

The analytical quality and the user friendliness are regarded equally important in the SKUP evaluation. Each area is subdivided and each subdivision has the following possible outcome:

unsatisfactory

less satisfactory

satisfactory

very satisfactory

Each of the sub-areas within Analytical quality and User friendliness has to achieve ‘satisfactory’.

User friendliness. Parameters evaluated

- manual /insert
- time factors

- quality control
- operation of the test

3. Statistical formulas

$$SD_{total} = \sqrt{\frac{\sum \left(x_i - \overline{x_i}\right)^2}{n-1}} \qquad CV_{total} = \frac{SD_{total}}{\overline{x_n}} \cdot 100\%$$

$$CV_{within} = \sqrt{\frac{\sum \left(\frac{\Delta_i}{\overline{x_i}}\right)^2}{2n}}$$

Where $\sum\Delta$ = the sum of the differences, and Δ_i is the difference between duplicates, and $\overline{\chi}_i$ is mean of duplicates for each sample

Bias: Systematic deviation from the reference method

Total Error = the first measurement on Hemochron should deviate < ± 20 % from the duplicate result at the reference method.

95 % Confidence Interval for CV: calculated from inverse Chi^2 -distribution

4. Results - first investigation

According to the protocol the four different instruments were tested to assure that they gave agreeing results. Before the evaluation started, two experienced laboratory technologists analysed the same sample in duplicate in two of the four instruments each. They measured the sample every hour for 4 hours. Analytical imprecision (CV_{within}) was about 8.5 %.

One of the technologists got error warnings from the instrument for > 20 % of the measurements.. Most of these errors were 'sample too small'. It was observed that the blood did not reach the measuring point. She sometimes had five errors in a row and had to give up analysing duplicates. The performance of the system was not better the following days. It was therefore tested if the errors were related to only one instrument, which they were not. The pipettes were checked and the pipetting technique was discussed. Both laboratory technologists used techniques where air bubbles were avoided. The laboratory technologist with the highest percentage of errors is left-handed and was mentioning that she should turn the instrument – and then she could not read the display – or she should hold the pipette. in an angle of 90 º to the instrument while the right-handed technician held it in an angle of 45 º. The general practitioners in Denmark have specified that they will not accept more that 2 % of invalid test in any test.

The evaluation was stopped because of the problems. The analytical imprecision (CV_{within}) = 8.5 % and problems with an unexpected high number of errors were discussed at a SKUP meeting in Odense June 9 2004. MEDimport was contacted and it was decided that MEDimport and ITC should discuss and suggest possible improvements of the system. It was already clear that without any change in instructions, how to handle the test, the SKUP report would end with unsatisfying user friendliness.

The English manual tells that 15 µl blood in the measuring channel is enough but 35 µl is recommended. In the first Danish manual 50 µl was required. 200 µl gave an invalid test due to too much blood in the waste channel.

According to new instructions from the manufacturer, the sample volume was increased from 50 µl to 100 µl and the number of invalid tests was decreased to < 1%.

5. Results and discussion- second investigation

5.1. Results of Hemochron in hospital Laboratory

Table I: Number of samples at each level. N ≥100.

INR Level	Hemochron Venous	Hemochron Capillary	Reference laboratory Venous
< 1.5	4 #	1 + 2 #	2
1.5-1.9	13	9	9
2.0-3.0	61	29	59
3.1-5.0	26	7	29
> 5.0	2 #	1	1
Re-participants No extra sample	12 * 1**	8 *	
In total	119	57	100

All measurements are registered. Only results within the interval 1.5 -5.0 INR were used.

excluded, result not in the range 1.5 to 5.0 INR. * excluded due to re-participating

** no second sample

5.1.1. *Analytical quality*

CV_{within}, Bias and Total Error are calculated for three subgroups: the highest INR-values, the lowest and the middle level of INR.

Table II: Hemochron. Analytical imprecision (CV_{within}) and Bias, venous samples.

INR interval Reference laboratory	N	Average INR	Bias %	95 % CI	CV_{within} %	95 % CI
1.2-2.35	33	2.02	+1.5	(-3.4) – (6.4)	9.5	7.6 – 12.4
2.35- 2.87	34	2.58	-2.2	(-6.5) – (-2.2)	7.9	6.3 – 10.3
2.93-5.41	33	3.06	-10.2	(-13.9) – (-6.5)	8.1	6.5 – 10.6
all	100	2.56	-3.6		8.4	7.4 – 9.8

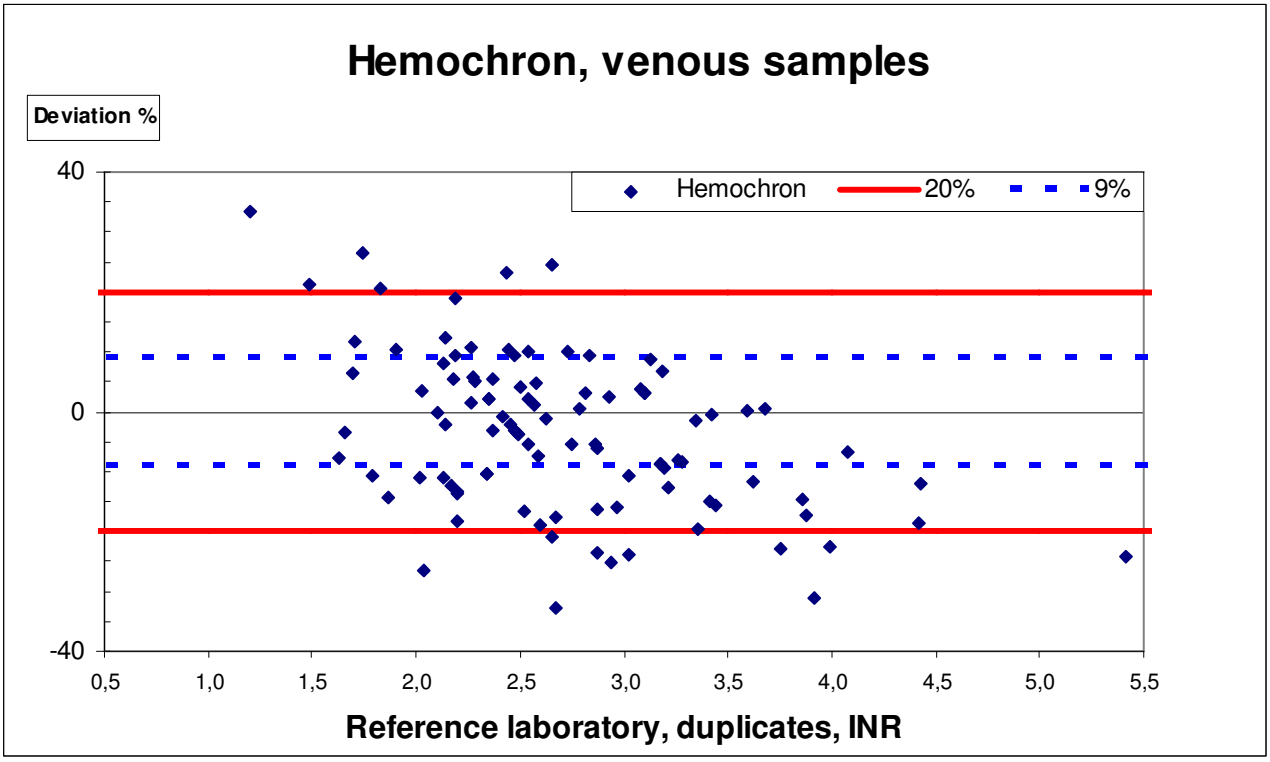
Table III: Hemochron. Total Error, venous samples

INR	N	< 9% n	9-20% n	> 20% n	> 25% n
1.2-2.35	33	12	16	5	3
2.35- 2.87	34	20	9	5	1
2.93-5.41	33	16	11	6	2

Table IV: Hemochron. Analytical imprecision (CV_{within}) and Bias, capillary samples

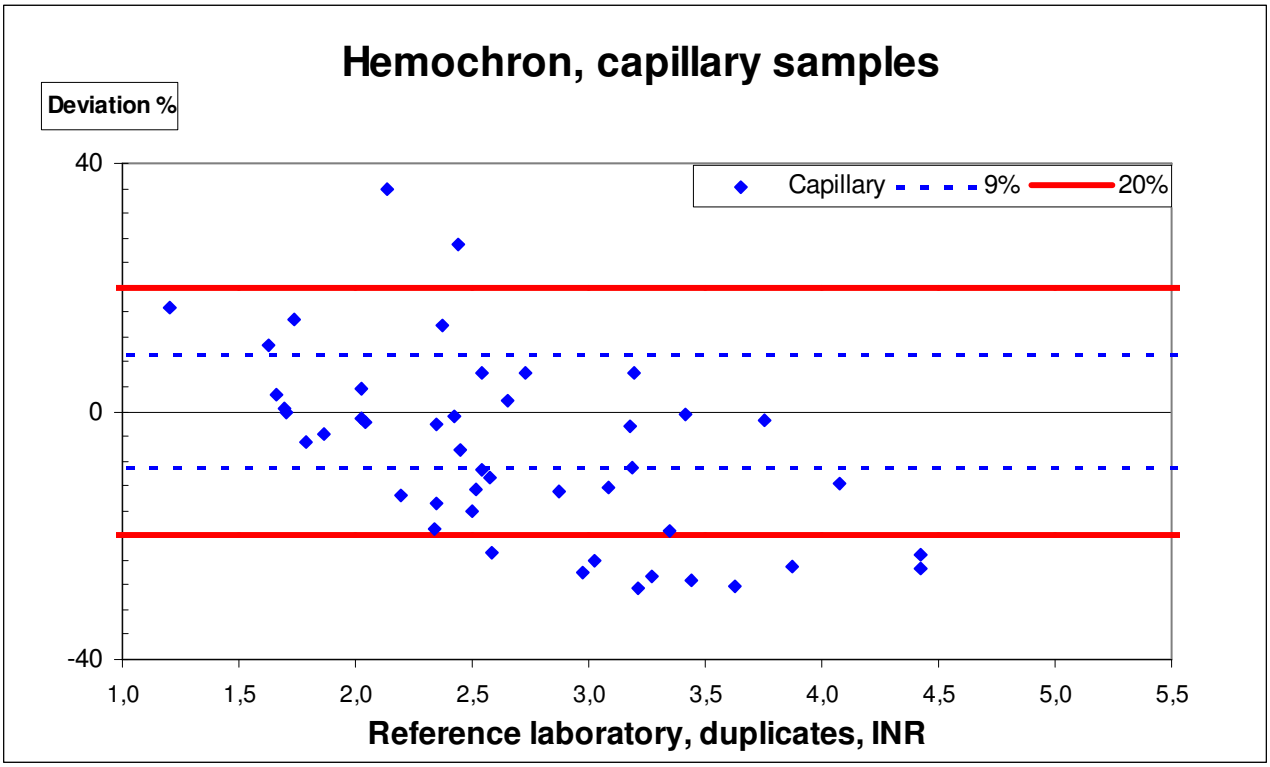
INR interval Reference laboratory	N	Average INR Hemochron	Bias %	95 % CI	CV_{within} %	95 % CI
1.2-2.54	23	2.11	+1.5	(-4.2) - (7.2)	6.8	(5.1-10.2)
2.54 - 5.41	23	2.76	-14.5	(-19.4) - (-9.5)	8.8	(6.1-11.1)
all	46	2.43	- 6.5		7.9	(6.5-9.9)

Figure 1. Total Error. Venous samples in hospital laboratory



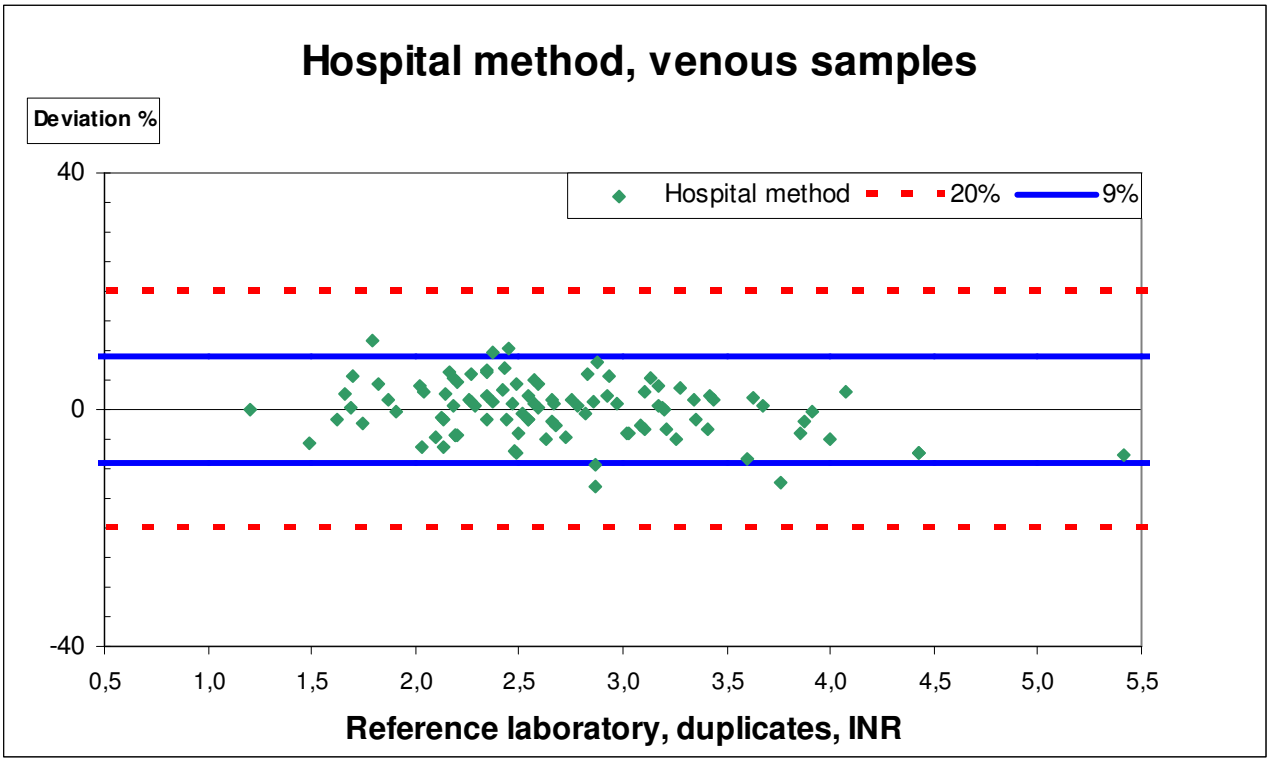
The diagram shows the deviations of the Hemochron results with venous samples. X-axis = mean of reference method duplicate results and Y-axis = ((first Hemochron result– mean of reference method, duplicate results)/mean of reference method, duplicate results) x 100. Acceptance limits for Hemochron is ± 20 %. 95 % of the results should be within the acceptance limits. It is considered as acceptable that 1 % of the results deviate > ± 25 % from the reference laboratory.. Acceptance limits for the hospital laboratory is ± 9 %. 95 % of the results should be within the acceptance limits.

Figure 2. Total Error. Capillary samples in hospital laboratory



This figure can be explained as figure 1, but this is the Hemochron results of capillary samples.

Figure 3. Total Error. Routine method in the hospital laboratory



The diagram shows the deviations of the hospital laboratory method results with venous samples. X-axis = mean of duplicate results with the reference method and Y-axis = ((first hospital laboratory result – mean value of reference method, duplicates)/mean of reference method duplicates) x 100.
Acceptance limits for Hemochron results are ± 20 %. 95 % of the results should be within the acceptance limits.
Acceptance limits for the hospital laboratory results are ± 9 %. 95 % of the results should be within the acceptance limits. It can be seen, that the hospital laboratory fulfils the goals.

5.1.2. *Analytical Quality Controls*

Table V

Three patient samples with different levels of PT were each analysed 12 times within 1 hour

Test no	PT result (INR)		
	Sample1	Sample 2	Sample 3
1	1.9	2.2	3.6
2	2.1	2.9	3.8
3	2.2	2.4	3.4
4	2.2	3.0	3.2
5	2.2	2.6	3.6
6	2.1	2.7	3.5
7	2.1	2.4	3.6
8	1.9	2.3	3.3
9	1.9	2.7	3.5
10	2.1	2.4	3.6
11	1.7	2.6	3.6
12	1.9	2.7	3.4
$\overline{X}$	2.025	2.575	3.508
CV %	7.9	9.4	4.6

Table VI

High and low control samples were analysed twice a day every second day during the evaluation.

Date	PT result (INR)			
	low 1	low 2	High 1	High 2
29-07-2004	1.9	1.7		
30-07-2004			4.5	4.4
09-08-2004	1.4	1.7		
12-08-2004			4.2	4.5
16-08-2004	1.7	1.6		
18-08-2004			4.7	4.5
19-08-2004	1.3	1.6		
23-08-2004			4.5	4.3
24-08-2004	1.6	2		
25-08-2004			4.5	4.5
26-08-2004	(0.9*) 1.9	2.2		
27-08-2004			4.4	4.9
30-08-2004	1.5	1.7		
31-08-2004			4.9	4.1
01-09-2004	1.7	1.7		
13-09-2004			5.3	4.4
14-09-2004	1.4	1.7		
15-09-2004			5	4.5
16-09-2004	1.7	1.6		
17-09-2004			4.7	4.7
20-09-2004	1.7	1.9		
21-09-2004			4.8	4.5
23-09-2004	1.5	1.7		
CV % without outlier*	11.8 %		6.0 %	

Instrumental checks

During the 27 days the evaluation lasted, the following instrumental check values were read:

temperature	EVQnormal	EVQabnormal
37.0	30.0	299.0

The values did never change.

5.1.3. *Evaluation of user friendliness*

The ratings of the staff that performed the evaluation are marked with coloured fields. At the evaluations in the general practices, only the white fields are filled in. At the evaluation in the hospital laboratory, the blue fields are also filled in. Any free comments belonging to the four sub-areas will be placed under the table concerning the area.

An average rating is made for each of the four sub-areas: Insert, Time factors, Quality Control and Operation. The summary of the user friendliness is based on the rating of all sub-areas. 2 or 3 points fulfil the expectations, 0 or 1 point does not fulfil the expectations. If 0 or 1 point is given the reason is explained in the text.

Table VII. User friendliness estimated in the hospital laboratory

Information in the manual / insert about:	0 point	1 point	2 point	3 point
Content, clearness in presentation	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Specimen collection	Unsatisfactory	Less satisfactory*	Satisfactory	Very satisfactory
Materials required, provided/not provided	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Pre-analytic/test procedure	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Interpretation of the results	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Measurement principle	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Error sources	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Troubleshooting	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Insert available in Danish, Norwegian, Swedish	No	Partly	Yes	English + Scandinavian
Easy to read?	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Rating of the manual / insert			Satisfactory	

*) How to handle the capillary samples should be explained better

Time factors	0 point	1 point	2 point	3 point
Pre-analytic time	>10 min	6 to 10 min.	3 to 5 min.	≤ 2 min.
Analytic time	>10 min	6 to 10 min.	3 to 5 min.	≤ 2 min.
Training / Education	Very difficult	Difficult	Easy	Very easy
Stability of test, unopened, (no/package)	≤ 3 months	3 — 5 months	6 — 12 months	> 12 months
Stability of control material	≤ 3 months	3 — 5 months	6 — 12 months	> 12 months
Storage conditions of tests, unopened	-20^{0}C	2 — 8 ^{0}C	15 — 30^{0}C	2 — 30^{0}C
Storage conditions of control material	-20^{0}C	2 — 8 ^{0}C	15 — 30^{0}C	2 — 30^{0}C
Rating of time factors			Satisfactory	

Quality Control, venous	0 point	1 point	2 point	3 point
Internal quality control	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
External quality control**	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Interpretation of the Quality Control ***	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Rating of quality control				

**) External quality control results are easy to read and practicability is reasonable.

For capillary samples, the external quality control was not tested.

***) Venous samples: The CV % for the control samples in the low therapeutic range is so high (11.8 %) that the control cannot be used for troubleshooting. The CV % for control samples in the high range is 6 %, which is 'less satisfactory'. (According to ITC the CV% are within the expected range of < 14%).

Operation	0 point	1 point	2 point	3 point
To prepare the test / instrument	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
To prepare the sample	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Application of sample	Unsatisfactory	Less satisfactory#	Satisfactory	Very satisfactory
Amount of sample	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Procedure step	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Interpretation of the test	Very difficult	Difficult	Easy	Very easy
Sources of errors	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Cleaning/maintenance	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Hygiene, using the test	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Environmental requirements	Poison	Special arrangement	Biohazard	Daily renovation
Demands to education	Lab technician	Course	GP personal	None
Demands to training	days	> 2 hours	½-2 hours	0-30 minutes
Size and weight of package	Unsatisfactory	Less satisfactory	Satisfactory	Very satisfactory
Rating of operation			Satisfactory	

Comments: #) The place for application of the blood is so close to the instrument, that it is difficult to fill the well with a capillary sample. (The laboratory technologists found that the application of the capillary sample sometimes could be awkward, while the nurses in the primary care found it often awkward.)

Summary of the user friendliness

The ratings of the Information in Manual / Insert, Time factors and Operation were 'satisfactory' for venous samples. For capillary samples, see also comments.

The Quality Control was evaluated for venous samples, where it was 'unsatisfactory' for the low therapeutic range, since the CV % was so high that the control cannot be used for troubleshooting. The CV % for control samples in the high range was 6 %, which is 'less satisfactory'. According to ITC the CV% are within the expected range of < 14%.

The Quality Control was not evaluated for capillary samples.

5.1.4. *Evaluation of analytical quality and user friendliness in the hospital*

Analytical imprecision, Bias, Total Error and number of invalid tests

1. CV < 5%.

   As seen in the tables II and IV the CV_{within} does not fulfil the requirements at any level.

2. Bias < 6 %.

   Is fulfilled for the low values of INR, but not for the highest results, which had a Bias of

   –10.2 %.

3. Total Error: 95 % of the tests should deviate < 20 %.

   Only 84 % of the results fulfil the requirement.

4. Invalid tests: < 1 %.

   Is fulfilled.

Controls

The CV values are lower in the high samples, independently of the material, (artificial, venous, capillary) and independently of education of the person performing the test (laboratory technologist, GP or nurse).

The results of the 'low therapeutic value' control have a CV of 11.8 %. This means, that it cannot be recommend as a tool for control or troubleshooting. The 'high therapeutic value' control had a CV of 6.0 %, which is also too high.

User friendliness

- The ratings of the Information in Manual / Insert, Time factors and Operation were 'satisfactory' for venous samples.
- The manual should describe the specimen collection of capillary samples better.
- The laboratory technologists found that the application of the capillary sample sometimes could be awkward, while the nurses in the primary care found it often awkward.
- The Quality Control was not satisfactory, because the CV was 11.9 % for the 'low therapeutic value' and 6.0 % for the 'high therapeutic value'.
- For capillary samples, Quality control was not tested

5.2. Interferences and cross reactions

Not investigated.

5.3. Evaluation of Hemochron at the two primary care centres

I: All measurement results are used in the calculations. One GP measured both venous and capillary samples on the Hemochron and sent a sample to the Department of Clinical chemistry at Odense University Hospital. The other GP just measured venous samples in duplicate on the Hemochron.

Table VIII: Number and of measurement results at different levels from the two primary care centres.

INR level	GP 1 Venous	GP 2 Venous	GP 1 Capillary
< 1.5	1	4	1
1.5-1.9	4	8	4
2.0-3.0	29	22	30
3.1-5.0	5	6	5
> 5.0	0	0	0

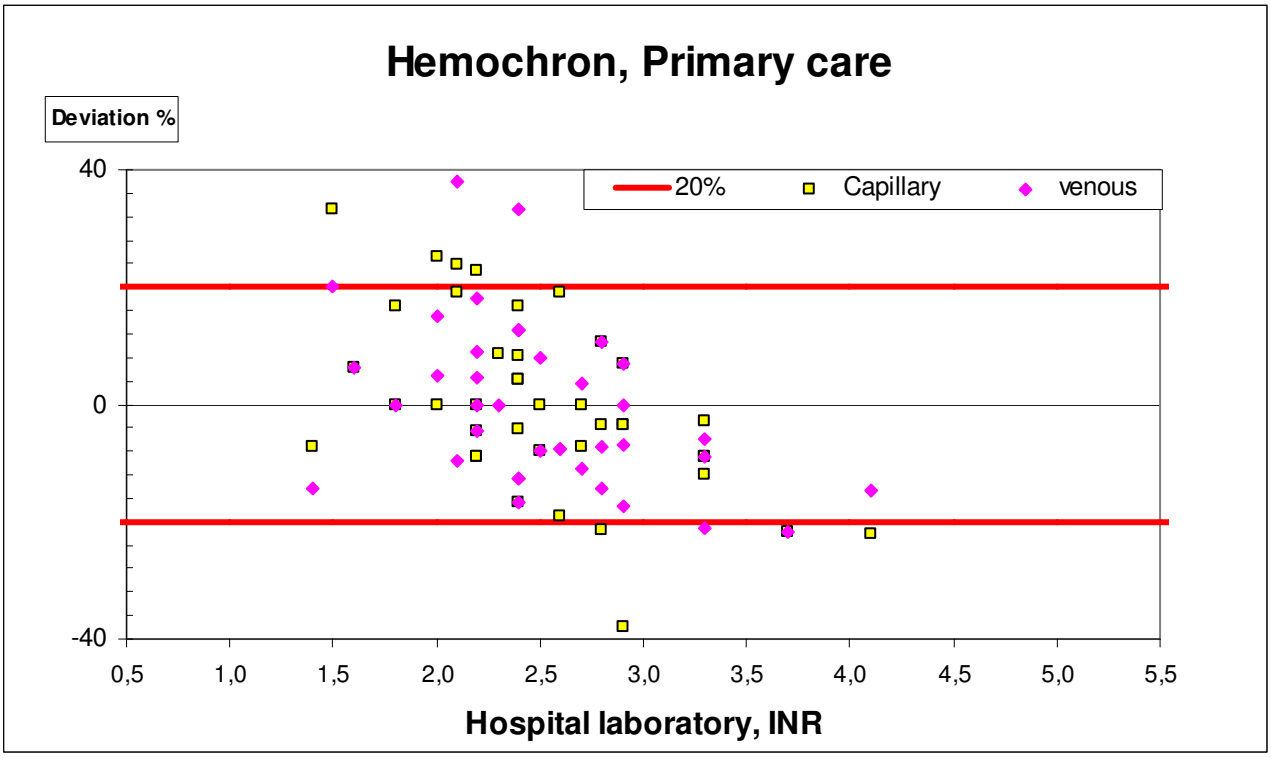
5.3.1. *Analytical quality*

Table IX: Analytical imprecision (CV_{within})

GP Sample type	N	INR range	INR mean	CV_{within} % (95 % CI)
GP 1				
Venous	19	1.20-2.45	2.17	8.7*(7.2-11.2)
Venous	20	2.50-3.35	2.83	6.5 (5.3- 8.3)
Venous	39	all	2.42	7.7*(6.3- 9.8)
Capillary	20	1.30-2.40	2.08	10.0 (7.7-14.5)
Capillary	20	2.40-3.65	2.83	8.1 (6.2-11.7)
Capillary	40	all	2.45	9.1 (7.5-11.7)
GP 2				
Venous	20	0.95-2.20	1.79	7.1 (5.4-10.3)
Venous	20	2.25-4.45	2.78	7.6 (5.8-11.0)
Venous	40	all	2.28	7.4 (6.1- 9.4)

* One outlier excluded. The first value was 1.1, the next was 2.8 and the third 2.9 INR.

Figure 4 Total Error in the primary care



The figure shows the deviations of the first venous and the first capillary Hemochron results compared to the single results of the hospital laboratory method. X-axe = result of the hospital laboratory method. Y-axe = ((Hemochron result –hospital laboratory method result)/ hospital laboratory method result) x 100). It can be seen that the Hemochron bias and the total error are similar when comparing with the hospital laboratory method and when comparing with the rreference laboratory method (Figure 1 and figure 2)

Evaluation of bias and total error for primary care is not part of this evaluation.

The comparison of results from fresh and frozen samples should demonstrate that the results do not change when samples are frozen. In figure 2 the results from the fresh single samples are shown against the reference laboratory (frozen samples) and in figure 4 the venous and capillary result from GP1 are shown against the local laboratory (fresh samples).

It should be noted that the Quality demands[1] for the comparison method in the hospital laboratory is fulfilled with CV 0.9 %, Bias 2.75 % and a TE< 9 % for 95 % of the results. Data not shown.

5.3.2. *Evaluation of Hemochron in primary care*

Analytical imprecision As seen in the table IX, GP 1 and GP2 got CV values above 5 % with venous samples at all levels. The measurements with capillary samples gave similar results.

User friendliness evaluated in the primary care gave similar results as in the hospital laboratory and is therefore mentioned earlier.

6. Conclusion

The evaluation was done with 100 venous samples and with 46 capillary samples under standardised conditions in the hospital laboratory. In addition Hemochron was evaluated in one primary care centre in Denmark and one in Norway. Hemochron does not fulfil the analytical quality goals for Analytical imprecision (CV_{within}) and Total Error in this evaluation. Analytical imprecision was > 5 % for both venous and capillary samples. Total Error < 20 % was fulfilled for only 84 % of the results. The goal for Bias in the Danish Quality Control system < ± 6 %, was exceeded for the high values. The CV for the Control samples was 6.0 % for the high values and 11.8 % for the low therapeutic values. The Quality Control for capillary samples was not tested. The user friendliness of the 'Manual', 'Time factors' and 'Operation' were 'satisfactory' in the hospital laboratory and so it was for the venous samples in the primary care. For capillary samples in primary care it was mentioned, that it could be a bit awkward to fill the well.

7. References

1) Kvalitetskrav og kvalitetsvurdering for hyppigt udførte klinisk biokemiske og klinisk mikrobiologiske analyser i almen praksis. Konsensus dokument udarbejdet af Laboratorieudvalget under Sygesikringens og PLO´s Faglige Udvalg vedr. Almen Praksis i samarbejde med DEKS og Dansk Selskab for Klinisk Biokemi's Videnskabelige udvalg. Nov 2003.

2) Lassen JF, Kjeldsen J, Antonsen S, Petersen PH, Brandslund I. Interpretation of Serial Measurements of International Normalized Ratio for Prothrombin Times in Monitoring Oral Anticoagulant Therapy. Clinical Chemistry, Vol. 41, No. 8 1995, 1171-1176.

3). Lassen JF, Brandslund I, Antonsen S. International Normalized Ratio for Pro-thrombin Times in Patients Taking Oral Anticoagulants: Critical Difference and Probability of Significant Change in Consecutive Measurements. Clinical Chemistry, Vol. 41, No. 3, 1995, 444-447.

4). Kjeldsen J, Lassen JF, Petersen PH, Brandslund I. Biological variation of International Normalized Ratio for prothrombin times, and consequences in monitoring oral anticoagulant therapy: computer simulation of serial measurements with goalsetting for analytical quality. Clin Chem 1997; 43(11):2175-82.

5). Geigy Scientific Tables. Volume 2. Eight, revised and enlarged edition. CIBA-GEIGY

Enclosure A

The figure shows the Hemochron instrument and how to apply a venous sample.
To handle the sample is a little more awkward for a left handed person than for a right handed person if the left handed person also want to read the display.

Enclosure B

Technical Service Report
HEMOCHRON® Jr. PT / Citrate PT
INR Calculations and Lot to Lot Reproducibility

INR Calculation

The Hemochron Jr. Prothrombin Time (PT, J201) and Citrate Prothrombin Time (PT, J201C) tests are microcoagulation assays intended for use in performing quantitative, one stage prothrombin times. Both assays require whole blood samples, either fresh (J201) or anticoagulated with sodium citrate (J201C). The thromboplastin reagents in these cuvettes are highly sensitive, low ISI (approximately 1.0) reagents providing optimal sensitivity for Vitamin K dependent clotting factors.

The Hemochron Jr series instruments calculate the INR of the sample directly from the whole blood clotting time based on regression analyses performed across multiple centers during the assay development. The plasma equivalent PT is calculated from the INR based upon an ISI of 1.0. This differs significantly from the routine laboratory calculation of INR which first requires calculating the ratio of the patient's PT and a local mean normal PT, then raising this ratio to the power of the ISI.

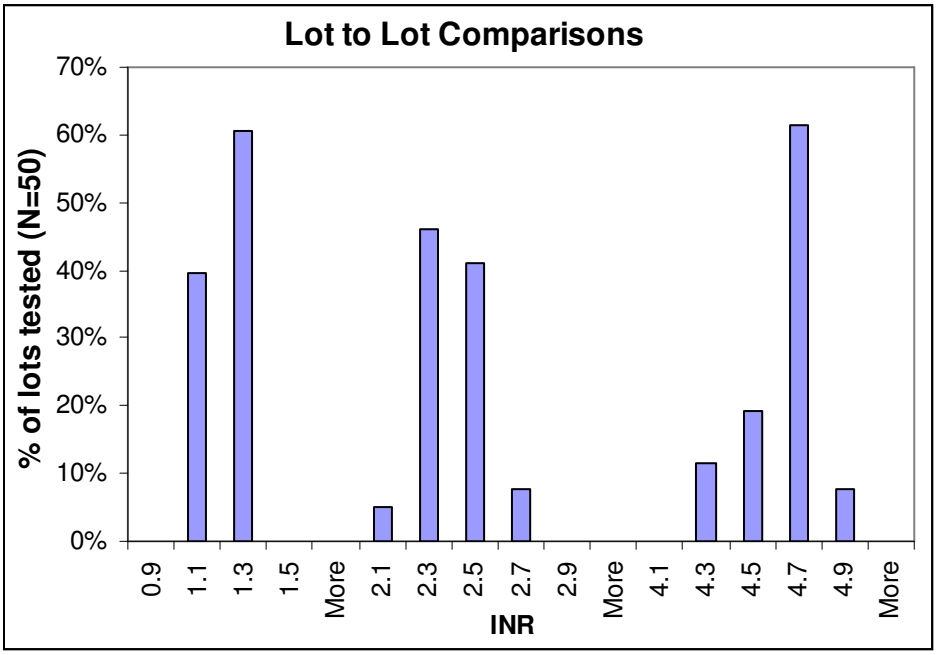
The decision to directly calculate the INR from the whole blood clotting time was made to minimize the imprecision introduced by employing several extra mathematical steps. Since whole blood clotting times are, by their nature, longer than plasma clotting times, use of the traditional equation in a whole blood system would first require the conversion of the whole blood clotting time to a plasma equivalent value. This value could then be used for the standard INR equation. Mathematically, imprecision is introduced into a system with each calculation performed, therefore, the more direct conversion of whole blood clotting time to INR is preferred.

Local adjustment of the PT mean normal is therefore unnecessary when using any Hemochron Jr series instrument. The mean normal PT programmed into the system is only used to calculate the plasma equivalent clotting time from the INR, not vice versa.

Lot to lot reproducibility

When employing any Hemochron® Jr PT assay, it is not possible to alter either the mean normal PT nor the reagent ISI. This places the onus on ITC, as the manufacturer of these tests, to ensure the consistency of the results obtained between cuvette lots. This has been accomplished through the implementation of substantial procedures for the characterization of the thromboplastin employed as well as extensive Quality Control testing of each lot of cuvettes prior to release for sale.

The procedures employed in the characterization of the thromboplastin preparations employed have been comprehensively reviewed by the US FDA as part of their review of the PT assays. These protocols ensure that each batch of thromboplastin used to manufacture cuvettes retains an ISI close to 1.0. This ISI assignment has been independently challenged and verified by Gosselin and colleagues (Thromb Haemostas, 2000, 83: 698 – 703).
Prior to release for sale, each lot of cuvettes is challenged with defined substrates in the normal (INR < 1.5), therapeutic (INR between 2.0 and 3.0) and supratherapeutic (INR between 4.0 and 5.0) ranges. The graph below shows the mean values obtained during this testing for a sequence of 50 lots of cuvettes spanning two independent thromboplastin preparations.



The percent of lots displaying each value is plotted against this mean INR. Clearly, there is very little lot to lot variability observed during this testing. Between lot comparisons of the INR results at each level show neither clinical nor statistical differences between any of the lots examined.

Enclosure C

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HEMOCHRON® Signature System

Sample Collection and Handling: Fresh Whole Blood or Citrated Blood

Fresh Whole Blood- ACT+, ACT-LR, PT, APTT Cuvettes

1. Collect a minimum of 0.2 ml of fresh whole blood with a syringe or by fingerstick (PT cuvettes only).
2. Immediately dispense 1 drop of blood into the sample well of the test cuvette- filling the well from the bottom of the well up- this will avoid addition of air bubbles into the blood sample.
3. A sufficient quantity of blood must be added directly to the center sample well to fill it flush to the top.
4. Should a large drop of blood extend above the center sample well on the cuvette, push any excess blood over into the outer sample well on the cuvette.

Citrated Whole Blood- Citrated PT and Citrated APTT Cuvettes

1. Collect blood in an evacuated tube containing sodium citrate (3.2% or 3.8%). Mix gently.
2. Before testing, invert the test tube at least four times to ensure complete mixing of sample.
3. Dispense 1 drop of citrated blood into the sample well of the test cuvette- filling the well from the bottom of the well up- this will avoid addition of air bubbles into the blood sample.
4. A sufficient quantity of blood must be added directly to the center sample well to fill it flush to the top.
5. Should a large drop of blood extend above the center sample well on the cuvette, push any excess blood over into the outer sample well on the cuvette.

NOTES:

1. A minimum volume of 50 ul blood sample is required for the HEMOCHRON Signature cuvette system.
2. The Signature instrument displays “Sample too large” or “Sample too small” if an excessive or inadequate blood sample volume has been provided.

APRIL 2004

February 16, 2005

Esther Jensen M. Sc.
Scandinavian Evaluation of Laboratory Equipment for Primary Health Care
Danmark, Afdeling KKA
Odense Universitets hospital
5000 Odense C, tlf. 65412865

Dear Ms. Jensen,

Thank you for providing the opportunity to review and comment upon the SKUP report conducted for our point-of-care testing (POCT) device, the Hemochron Jr. Signature Prothrombin Time test. We appreciate the vast amount of work and effort which went into this evaluation as requested by our distributor, Medimport A/S, and appreciate your attention to detail in the conduct of the described studies. As we peruse the report, we find a number of your observations are consistent with our published performance expectations as well as being in line with the existing state-of-the art for PT(INR) testing. Furthermore we also observed that a number of the SKUP criteria for acceptable performance are inconsistent with the guidelines of the international ISO committee on INR standardization and thus your summary, while appropriate based upon your pre-test criteria and observations may be non-applicable for any POCT-INR system evaluation.

Analytical Precision: On this very critical issue, the ISO Committee Draft (CD/DIS 17593) for PT-INR for self-testing specifically states that the appropriate measure of clinical agreement of the POCT system to the reference lab standard is that 95% of values less than an INR of 2.0 should agree within 0.5 INR, and 95% of values between 2.0-4.5 INR should agree within 30%. In your observations of venous samples the bias % average is -3.6% with a range, across all levels of -10.2 – 1.5%. Applying the ISO recommendation, all samples across the specified ranges fall well within this standard. For total error, the data cannot be compared to the ISO standard as there is no distinction >25 %; However, across all levels only 6% of samples are beyond 25%, half of these in the <2.35 INR range. In the capillary specimens, the bias % average is -6.5% with a range, across all levels of -14.5 – 1.5%. Again using the ISO recommendation in the 2 – 4.5 INR range; all samples fall well within this standard. Upon further review, it is also unfortunate that the bias tabulation was not performed for the data collected in the Primary Care centers. From the graph, it appears that in this setting, when comparing to a standard system (Stago analyzer with STAClot reagent) the point of care system meets the SKUP requirements for bias.

Imprecision Testing: We note your evaluation of imprecision using both patient samples replicates and control material all are within 10% which is consistent with our package labeling. The exception is the normal external control which yielded an 11% CV, still within our published 14% when using external material, which naturally has greater variability than fresh blood.

It is noteworthy that the SKUP guidelines which were applied in this evaluation have never to date been applied to a POCT system. All prior SKUP evaluations and reports were conducted under more generous acceptance guidelines. While we recognize the need for continued improvement of diagnostic testing results, the background for the ISO recommendations is the recognition of inherent variability of INR depending upon specific reagent and instrument system, a well published observation in the world-wide literature. It is universally recognized that the INR system is imperfect despite the application of the ISI for reagent sensitivity. This is a critical consideration when assessing bias between any two systems, such as that which you observed. Thus the most important consideration in any PT system is agreement within clinical standards in which the delivery of therapy is not adversely effected by imprecision of the test system. In this regard the Hemochron Jr. Signature Prothrombin Time performs as well as any other POCT system and is comparable to laboratory assays for the purpose of clinical intervention decisions.

Again, thank you for the opportunity to review and comment on your report.

Sincerely yours,

Marcia L. Zucker, Ph.D.
Director of Clinical Affairs

Frank M. LaDuca, Ph.D.
Vice President,
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