INRatio2

A system for measurement of P—PT (INR)
manufactured by
Alere Inc.

Report from an evaluation organised by SKUP

Evaluated at the request of TRIOLAB in Denmark

SKUP/2010/80

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This report was written by SKUP, July 2010.
The main author was Esther A. Jensen, SKUP in Denmark.

The organisation of SKUP

Scandinavian evaluation of laboratory equipment for primary health care, *SKUP*, is a co-operative commitment of NOKLUS[1] in Norway, DAK-E[2] in Denmark, and EQUALIS[3] in Sweden. SKUP was established in 1997 at the initiative of laboratory medicine professionals in the three countries. SKUP is led by a Scandinavian *steering committee* and the secretariat is located at NOKLUS in Bergen, Norway.

The purpose of SKUP is to improve the quality of near patient testing in Scandinavia by providing objective and supplier-independent information on analytical quality and user-friendliness of laboratory equipment. This information is generated by organising SKUP *evaluations*.

SKUP offers manufacturers and suppliers evaluations of equipment for primary healthcare and also of devices for self-monitoring. Provided the equipment is not launched onto the Scandinavian market, it is possible to have a confidential pre-marketing evaluation. The company requesting the evaluation pays the actual testing costs and receives in return an impartial evaluation.

There are *general guidelines* for all SKUP evaluations and for each evaluation a specific *SKUP protocol* is worked out in co-operation with the manufacturer or their representatives. SKUP signs *contracts* with the requesting company and the evaluating laboratories. A *complete evaluation* requires one part performed by experienced laboratory personnel as well as one part performed by the intended users.

Each evaluation is presented in a *SKUP report* to which a unique *report code* is assigned. The code is composed of the acronym SKUP, the year and a serial number. A report code, followed by an asterisk (*), indicates a special evaluation, not complete according to the guidelines, e.g. the part performed by the intended users was not included in the protocol. If suppliers use the SKUP name in marketing, they have to refer to www.skup.nu and to the report code in question. For this purpose the company can use a logotype available from SKUP containing the report code.

SKUP reports are published at www.skup.nu and www.skup.dk

[1] NOKLUS (Norwegian Quality Improvement of Primary Care Laboratories) is an organisation founded by Kvalitetsforbedringsfond III (Quality Improvement Fund III), which is established by The Norwegian Medical Association and the Norwegian Government. NOKLUS is professionally linked to “Seksjon for Allmennmedisin” (Section for General Practice) at the University of Bergen, Norway.

[2] SKUP in Denmark is placed in Hillerød Hospital. SKUP reports to DAK-E (Danish Quality Unit of General Practice), an organisation that is supported by KIF (Foundation for Quality and Informatics) and Faglig udvalg (professional Committee), which both are supported by DR (The Danish Regions) and PLO (The Organisation of General Practitioners in Denmark).

[3] EQUALIS AB (External quality assurance in laboratory medicine in Sweden) is a limited company in Uppsala, Sweden, owned by “Sveriges Kommuner och Landsting” (Swedish Association of Local Authorities and Regions), “Svenska Läkaresällskapet” (Swedish Society of Medicine) and IBL (Swedish Institute of Biomedical Laboratory Science).

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Table of contents

A detailed list of previous SKUP evaluations is included in the attachments.
Attachments with raw data are included only in the copy to Triolab.

1. Summary

Background Triolab turned to SKUP in 2009 for an evaluation of INRatio2, an instrument for determination of P—PT (INR) in capillary samples only. The evaluation was performed in the hospital laboratory in Hillerød and in two primary health care centres.

The aim of the evaluation

- Determination of the within-series-imprecision with samples from 101 patients in the hospital laboratory and 40 patients in each primary care centre
- Comparison of the INRatio2 hospital laboratory results with results from frozen plasma samples measured with the comparison method in the Roskilde hospital laboratory
- Comparison of the INRatio2 primary care results with results from fresh plasma samples measured with the comparison method in the Hillerød hospital laboratory
- Evaluation of the user-friendliness
- Investigation of the influence on the result from haematocrit

Materials and methods The comparison method for the hospital laboratory evaluation was run on an Instrumentation Laboratory ACL TOP instrument in the department of Clinical Biochemistry, Roskilde. For the primary health care the comparison method was run on a Sysmex CA 7000 in the department of Clinical Biochemistry, Hillerød. Four INRatio2 instruments and three lot numbers were used for the analysing of samples from totally 181 patients.

Results The imprecision of INRatio in the hospital laboratory was 6,3%. The mean imprecision for the lowest tertile was 5,9%, 5,6% for the middle, and 7,4% for the highest tertile. The bias of INRatio was less than 6% in all concentration levels and the total bias was +2,3% (-2,2 to +5,7%) compared to Roskilde.
In total four of the 101 hospital results deviated more than ±20% from the comparison method results, one deviated >50% in both duplicates. Haematocrit did not seem to influence on the measurements.
In the primary care evaluation one practitioner had a CV of 5,1%, the other had a CV of 8,6%. Significant differences of imprecision were observed due to used lot numbers. The total error goal (<±20%) was fulfilled in the hospital laboratory evaluation and in primary care centres. The user friendliness was satisfying. It was noticed that it was difficult to know when the test strip was properly filled with blood. External quality assurance is not possible with INRatio2 but two built-in controls are included in each strip. Eight measurements out of 362 failed of different reasons.

Conclusion The quality goal for total error goal (<±20,0%) was fulfilled in all evaluation sites. The quality goal for imprecision (<5,0% CV) was not fulfilled neither in the hospital laboratory nor in the two primary care centres. Bias was less than 6%. The frequency of failed measurements in the evaluation was 2,2%. Thus the quality goal of less than 2,0% failed measurements was almost fulfilled. The user friendliness was satisfying; however, there were comments about the application of the sample.

Comments from Alere A letter with comments from Alere is attached to the report.

2. Quality goals

To qualify for an overall good assessment in a SKUP evaluation, the measuring system must show satisfactory quality as well as satisfactory user-friendliness.

There are no generally recognised analytical quality goals for P—PT (INR) determinations. Various ways of setting goals for analytical quality are presented below.

2.1. Analytical quality goals

2.1.1. *Analytical quality goals based on recommendations from professionals/experts*

For the present, there are no generally recognised analytical quality goals for the determination of prothrombine time in plasma (P—PT (INR)), and no international (Gold) Standard for the evaluation of Point of Care test instruments for the P—PT (INR) measurements in primary health care.
The new ISO-standard for anticoagulant therapy self-testing (1) is under development. According to SKUP, the ISO-standard has too tolerant quality goals. According to SKUP's opinion, the submitted claim for minimum acceptable system accuracy (total error) of ±30% for 90% of the results is too tolerant. Unfortunately, there is no performance criterion for imprecision in the standard. In the international consultative round and following voting over the draft standard, Sweden and Norway commented on the draft standard, and voted no to the final suggestion.

Setting quality goals on the basis of biological variation is an acknowledged method (2-5).
It is recommended that analytical imprecision should be less than, or equal to, half the intra-individual biological variation. Ricos et al. (6-7) state the biological variation for P—PT (INR) as 4% (CV_{bw}) and 6,8% (CV_{bb}). According to Kjeldsen, Lassen et al. (8), the "in-treatment within-subject biological variation" of P—PT (INR) is 10,1%. For systems used for monitoring, the analytical performance should aim at low imprecision compared with the within-subject biological variation (9).

CV_a The analytical imprecision expressed as coefficient of variation in percent (CV%). This imprecision is called repeatability in the result part of this report.
CV_{bw} The biological variation within healthy individuals, also called the intra-individual biological variation.
CV_{bb} The biological variation between healthy individuals, also called the inter-individual biological variation.

In principle, quality goals based on biological variation do not take into account clinical requirements.

A committee appointed by the National Ministry of Health in Denmark has specified the demands to analytical quality for P—PT (INR) (10):
Bias ≤6% and reproducibility ≤5% for instruments used in primary health care, and bias ≤3% and reproducibility ≤3% for hospital instruments. There is no separate goal for the total error in the Danish specifications.

Based on the given data on biological variation for P—PT (INR), and the fact that anticoagulant devices are designed for *monitoring* P—PT (INR), SKUP recommends that these instruments should achieve repeatability below 5%. SKUP has not taken out a separate goal for the bias, but sets on the other hand out a quality goal for the total measuring error. The term total error is used for the combined effects of imprecision and bias. An acceptable bias can be calculated as 1/16 of the therapeutic interval for P—PT (INR), while a minimum goal can be calculated as 1/8 of the therapeutic interval. This gives an acceptable bias at approximately 2,5% at level 2,5 INR. Accordingly, the bias should not exceed 5% at the same INR-level. SKUP has used a bias of 5% in the calculation of the total error.

In method evaluation and method comparisons, one has to take the imprecision of the comparison method into account. SKUP allows an imprecision of the comparison method up to 3%. In addition different comparison methods are not likely to give the exact same INR-results. The differences should be regarded as an inter-laboratory variation and should be taken into the calculation of the total error as imprecision. SKUP has estimated the contribution of the inter-laboratory variation of the total error to 3%.

When comparing two P—PT (INR) methods based on Owren and Quick, there is always a certain "interference" or matrix-effect. When comparing INR-results from a Quick-method and an Owren-method, this effect is a result of real method differences. It can be discussed whether one should incorporate this effect in the total error quality goal itself or not. As an alternative, one can accept more results outside the quality limits when it comes to the final evaluation. SKUP has chosen to add a matrix effect to the calculation. Under given conditions the real matrix effect can be calculated. SKUP has set the contribution of matrix effect at the same magnitude as the imprecision (5%).

The quality goal of SKUP for the total error (TE) was calculated as follows:

$$\text{TE} = \text{bias } 5\% + 1{,}65 \times \sqrt{CV^2_{testmethod} + CV^2{}_{comparisonmethod} + CV^2_{betweenlab} + CV^2{}_{matrix}}$$

$$= 5\% + 1{,}65 \times \sqrt{25+9+9+25} = 5 + 13{,}6 \approx 19\%$$

It is accepted that up to 5% of the results can deviate more than ±20% from the comparison method. Only 1% of the results should deviate more than ±25%. The results achieved with INRatio2 will be discussed and evaluated in proportion to this quality goal.

In Denmark an additional quality goals is that bias (systematic deviation from the Comparison Method) is <±6%.

2.2. Evaluation of user-friendliness

The evaluation of user-friendliness was done by asking each of the evaluators to fill in a questionnaire.

The quality of the tested equipment in the user-friendliness questionnaires is separated in four sub-areas:

- Rating of information in manuals and inserts
- Rating of time factors of both measurement and preparation
- Rating of performing internal and external quality control
- Rating of operation facilities. Is the system easy to handle?

Evaluation of user-friendliness is divided into the following points:
”0 points” stands for un-satisfactory
”l point” stands for less satisfactory
”2 points” stands for satisfactory

The tested equipment must reach the total rating of ”2 points” in all four sub-areas of characteristics mentioned above, to achieve the overall rating ”satisfactory”.

2.3. SKUP’s quality goal in this evaluation

Based on the discussion about quality goals above, SKUP has decided to assess the results from the evaluation of INRatio2 against the following quality goals:

Total Error (allowable deviation)..... <±20%
Repeatability CV_a (within-series imprecision INRatio2)..... <5%
Fraction of technical errors should be..... <2%

3. Materials and methods

3.1. Definition of P—PT (INR)

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and International Union of Pure and Applied Chemistry (IUPAC) joint Committee on Nomenclature, Properties and Units (C-NPU) describes quantities, or measurands, for clinical laboratory tests. Regarding prothrombin complex two measurands have been defined which express results as 'International Ratio', one for measurements according to Owren (NPU01685) and one for measurements according to Quick (NPU21717) as shown in table 1.

Table 1. Name, codes and units for P—PT (INR) tests according to C-NPU

NPU code	Descriptive name	Unit
NPU01685	P—Coagulation, tissue factor-induced; rel.time(actual/norm; INR; IRP 67/40; proc.)	Unit 1, but usually given without unit
NPU21717	P—Coagulation, tissue factor-induced; rel.time(actual/norm; INR; IRP 67/40; II+V+VII+X)	Unit 1, but usually given without unit

For P—PT (INR), Owren's method (NPU01685), the coagulation factors II, VII and X are measured.
For P—PT (INR), Quick's method (NPU21717), fibrinogen and the coagulations factor II, V, VII and X are measured.

3.2. The INRatio2

3.2.1. *Description of the instrument*

Figure 1. INRatio2

The INRatio2 is a diagnostic Near Patient Test system manufactured by Alere Inc. The system provides Prothrombin Time (PT) and International Normalized Ratio (INR) results using fresh

capillary whole blood from a finger stick.
Only capillary whole blood can be used and the sample volume is 15 µL. The measuring range is 0,7 – 7,5 INR and the test results are displayed after approximately 60 seconds as an INR only or in both PT seconds and INR.
Each lot of test strips has a unique code, which provides the instrument with lot specific calibration data (MNPT = Mean Normal Protrombine Time and ISI = International Sensitivity Index) and the interval for the built in quality controls. When changing lot the user has to enter the new code manually. The INRatio2 test strips are packed individually in foil and must be used within ten minutes after they are taken out of the foil. The sample application point at the INRatio2 test strip has top dosing.
Immediately (15 seconds) after pricking the finger, the sample should be placed on the test strip directly from the finger or it may be transferred to the test strip by means of a micro-safe capillary collection tube/small pipette. The micro-safe capillary tube is supplied to be used whenever more than one individual is using the analyser. The INRatio2 test strips can be stored at temperature between 2 and 32 °C until they expire.

The INRatio2 instrument automatically performs electronic system self-tests for battery condition, proper heating range, temperature and memory on each test assuring that the device is working properly. The system is self-maintaining. It has also a built-in RS232 port for electronic communication.
The disposable INRatio2 test strip consists of co-laminated layers of transparent plastic. The design features a sample well where blood is applied, three channels through which the blood sample flows to reach the testing areas, reagents to start the coagulation process and electrodes that interface with the INRatio2 instrument.

Built-in quality controls
The instrument uses the test strips three channel technology to perform the PT test and two quality control tests (normal and therapeutic) simultaneously, and determines whether the controls are within preset limits. If they are, strip integrity is verified and the instrument reports the PT test result. If they are not, the instrument displays an ERROR message. Possible causes for control out of range messages include improper storage, deterioration of strips and incorrect strip code input. The quality control test is performed alongside the actual test sample. The test channel contains only thromboplastin but the control channels contain additional reagents to yield low and high PT values irrespective of the actual PT of the sample. Liquid quality controls have not been examined in this evaluation.

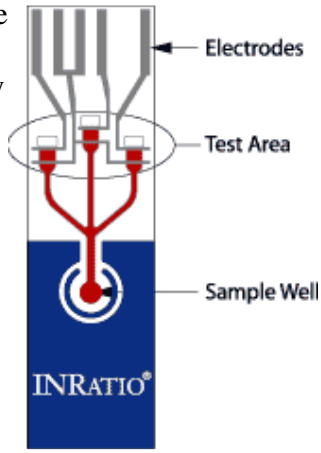
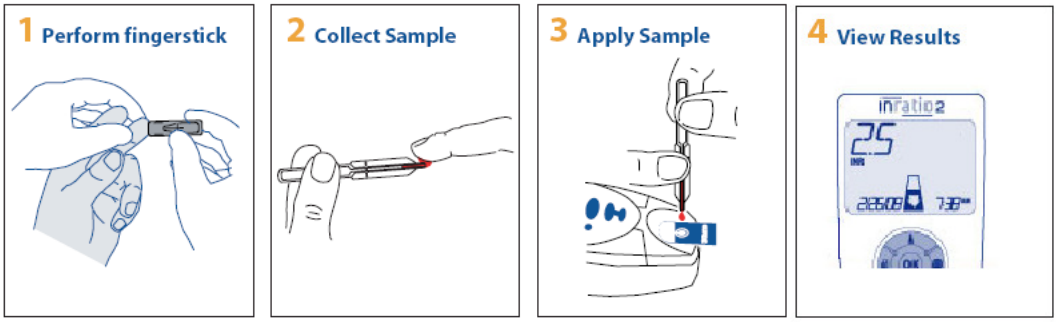


Figure 2. The test strip

3.2.2. *Analyzing a patients sample*
The procedure for analyzing capillary blood samples on the instrument is shown in figure 3. The illustration is made by combining figures from www.medline.com and Alere's (Hemosense) own web-page.



Perform finger prick.

Collect 15 µl sample using the micro-safe tube.

Apply the sample by holding the micro-safe tube in a 90 degree angle to the sample well, cover the small air vent holes in the tube and the drop will leave the sample collector.

After applying the sample the procedure is automatic and the result will appear on-screen in less than two minutes.

Figure 3. Analysing with INRatio2

The procedure can also be done without the micro-safe tube, thereby applying a large drop of blood directly from the finger. One of the primary care centres chose to do so, and Triolab accepted this. The other primary care centre and the hospital laboratory chose to use the micro-safe tube.

3.2.3. *Analytical principle*

INRatio2 performs a modified version of the one-stage P—PT (INR) test using a recombinant human thromboplastin reagent and calculates the INR value. As blood clots, the meter detects a change in electrical impedance between the electrodes. This is used, in conjunction with pre-assigned calibration information contained within the strip code, to calculate PT and INR. INRatio2 is based on the Quick method, while the methods used in Scandinavian hospitals are based on the Owren method.

Technical data from the manufacturer is shown in table 2. More facts about the instrument are shown in attachment 1.

Table 2. Technical data from the manufacturer

TECHNICAL DATA FOR INRATIO2	
Working temperature	+10 - +32^{o}C
Sample	capillary blood
Sample volume	15 µL
Units	INR or PT and INR
Measuring time	1 minute
Measuring range	0,7 – 7,5 INR
Memory	60 results
Power supply	Input: 240 VAC, output: 7,5 VDC or 4 AA batteries
Operating time with battery	app. 200 tests
Dimension	151 x 74 x 46 mm
Weight	263 g (with batteries)

3.2.4. *Product information*

INRatio2 instrument

Serial no.	085130605	hereafter referred to as instrument 1
	085130517	hereafter referred to as instrument 2
	085130631	hereafter referred to as instrument 3
	085130579	hereafter referred to as instrument 4

INRatio2 Test Strips

Lot A	lot no. 213040A	Exp. 2010/06
Lot B	lot no. 214540	Exp. 2010/06
Lot C	lot no. 216976	Exp. 2010/05

3.2.5. *Manufacturer of the INRatio2 system*

Alere, Inc.
9975 Summers Ridge Road
San Diego
CA 92121 USA
Internet: www.alere.com

3.2.6. Supplier*s of INRatio2 in the Scandinavian countries*

Triolab AS Vallensbækvej 35 DK-2605 Brøndby Denmark Phone: +45 4396 0012 Fax: +45 4396 4312 www.triolab.dk	Triolab AB Åbäcksgatan 6 Box 2109 SE-431 02 Mölndal Sweden Phone: 031-81 72 00 Fax: 031-81 72 19 www.triolab.se	BERGMAN DIAGNOSTIKA AS Jogstadveien 21 N-2007 Kjeller Norway Phone.: +47 6383 5750 Fax: +47 6383 5740 www.bergmandiag.no

3.3. The designated comparison methods

A designated comparison method is a fully specified method which, in the absence of a reference method, serves as the common basis for the comparison of a field method.

3.3.1. *Abbreviations:*

DEKS	Danish Institute of External Quality Assurance for Laboratories in Health Care
EQUALIS	External quality assurance in laboratory medicine in Sweden
EQUALM	European Committee for External Quality Assurance Programmes in Laboratory Medicine. European reference material for P—PT (INR) (11)

3.3.2. *The comparison method in the hospital laboratory in Roskilde*

Laboratory: Department of clinical biochemistry, Roskilde, which is responsible for the DEKS calibrations and the quality control system for coagulation methods in Denmark.

Instrument: Instrumentation Laboratory ACL TOP supplied by ILS Laboratories Scandinavia ApS

Reagent: Nycotest PT, Axis-Shield
Principle: Owren's method, with rabbit brain thromboplastine and adsorbed bovine plasma.

Calibrators: 3 point calibration with:
INR calibrator Normal, DEKS, lot. 04-09, value 0.96 INR (U=0.026)
INR calibrator Therapeutic, DEKS, lot. 03-09, value 2.30 INR (U=0.02)
INR calibrator High, DEKS, lot. 09-01, value 3.68 INR (U=0.20)
All calibrators were analyzed six times using two different vials of each.

Traceability: 2nd IRP, Bovine, Combined, coded OBT/79 using manual tilt tube technique.

Control samples: 3 fresh frozen venous plasma pools from DEKS, one normal and two abnormal (AK) levels.
4 EQUALM (European control with target value) controls and 3 EQUALIS controls. The controls were analyzed for every 20 samples.

Test samples: Frozen samples were thawed in water at 37°C in 5 minutes, and analyzed within 30 minutes in duplicate. CV<3,0% in duplicates.

Quality demands: bias <3,0% for a comparison method for primary health care centres.
CV <3,0% for a comparison method for primary health care centres (10).

3.3.3. *The comparison method in the hospital laboratory in Hillerød*

Instrument: Sysmex CA7000 system, the Sysmex coagulation instruments were supplied by Siemens Healthcare Diagnostics Inc.

Reagent: Nycotest PT, Axis-Shield
Principe: Owren's method, rabbit brain thromboplastine and adsorbed bovine plasma.

Calibrators: DEKS calibrators in 3 levels with assigned values, see 3.3.2.

Quality Assurance: External quality control: Controls from DEKS
Internal quality control: Control Plasma P (CPP), run every 3 hours.

Traceability: DEKS (2nd IRP, Bovine, Combined, coded OBT/79).

Quality demands: bias <3,0% for a comparison method for primary health care centres.
CV <3,0% for a comparison method for primary health care centres (10).

3.3.4. *Verification of the Comparison methods*

The bias of the two comparison methods was verified during the evaluation.
The imprecision in daily routine of the comparison method in Roskilde were <3,0% for PT-% and for P—PT (INR) therapeutic concentration about 4%. For the present evaluation the CV% was 1,2% in patient samples and 1,1 in the control samples.
The imprecision in daily routine of the comparison method in an commersial control material at the concentration of 1,8 INR was 3,5%. See also text, tables and figures in chapter 6.3.

3.4. Planning of the evaluation

Background for the evaluation
Triolab considered selling the INRatio2 instrument in Scandinavia. Therefore Triolab asked SKUP in Denmark how evaluations were performed, and a meeting in Triolab, Brøndby 25th of March 2009 was arranged.

3.4.1. *Meetings, protocol and contract*

Meeting in Brøndby 25th of March 2009
Participants:

- Richard Hughes, International Product Manager, Alere International
- Jutta Kraenke, Business Development Manager, Alere distribution business
- Bue Svensen, Sales Manager, Triolab
- Esther Jensen, SKUP in Denmark

A protocol for the evaluation was not sent to Triolab before the meeting. However; the participants were recommended to read the reports SKUP/2007/56* and SKUP/2004/33 which are previous SKUP evaluations of P—PT (INR). The latest version of the protocol in English was given to the participants in the meeting.

Several issues were discussed:
Richard Hughes was concerned that a possible bias of one laboratory in Denmark was able to misjudge the INRatio2. It was also a concern that the Nycotest PT-reagent was used in both Hillerød and Roskilde; in previous testings INRatio2 had been compared with Quick-tests or Stago reagents.
It was agreed the protocol was OK since the samples in the comparison method was analysed fresh in duplicates in Hillerød and after termination of the evaluation in duplicates in Roskilde. In case of difference between Hillerød and Roskilde, the samples could be re-analysed in Hillerød to assure that the freezing had no importance.
To make sure that bias in one hospital should not ruin the evaluation, samples with target values from DEKS (Danish), EQUALIS (Swedish and Norwegian) and EQUALM (European) were run for every 20 samples.
Compared to a standardised SKUP evaluation, that includes one hospital and a minimum of two primary care units, this protocol deals with an extra hospital, Roskilde. The coagulation unit of DEKS is located in Roskilde and about 95% of the Danish laboratories use the calibrators from DEKS for measuring P—PT (INR).
It was agreed to proceed with the protocol. Triolab should forward data from other evaluations, an instrument, a manual, plus all technical reports about the instrument to SKUP before the next meeting. The protocol was to be approved in SKUP and Triolab.

Meeting in Hillerød 16th of June 2009
Participants:

- Jutta Kraenke, Business Development Manager, Alere distribution business
- Petra Bleibtreu-Partie, Alere GmbH
- Gert Pynt Andersen, Product Manager, Triolab

- Esben Smith, Product Specialist, POCT, Triolab
- Vivian Larsen, Biomedical Laboratory Scientist, Department of Clinical Biochemistry, Hillerød Hospital
- Kirsten Stilling, Biomedical Laboratory Scientist, Department of Clinical Biochemistry, Hillerød Hospital
- Conny Pedersen, Biomedical Laboratory Scientist, Department of Clinical Biochemistry, Hillerød Hospital
- Esther Jensen, Physician, SKUP in Denmark

Issues before final protocol were
- The samples were centrifuged in room temperature as in daily routine.
- A full SKUP evaluation is a hospital + two primary care centres
- In Denmark SKUP always compare the samples from primary care centres with the hospital. It is possible because we have a good transport system for samples from primary care to the hospital.
- Three lot numbers are standard if available at the time of the evaluation.

At the meeting INRatio2 was demonstrated and four instruments and two lot numbers was tested. Alere (previous Hemosense) assured that the four instruments were measuring correct. Kirsten and Vivian from the department of clinical biochemistry learned to handle the instrument and their performance was accepted by Hemosense and Triolab.
Two ways of sampling were tested. Kirsten and Vivian preferred to use the designated pipette for the testing. Kirsten produced documents for data registration. It was demonstrated how data was transferred from instruments to a computer (not part of the evaluation). The final protocol in paper was given to the participants.

The protocol was circulated and accepted by Triolab, Alere (previous Hemosense) and SKUP. The protocol was signed September 2009 and evaluation began.

Meeting 7th of January 2010, Department of clinical biochemistry, Hillerød Hospital
- Gert Pynt Andersen, Product Manager, Triolab
- Esben Smith, Product Specialist, POCT, Triolab
- Vivian Larsen, Department of clinical biochemistry
- Stine B Weber, SKUP in Denmark
- Esther Jensen, SKUP in Denmark

Stine B Weber was going to take over the testing and was trained in using the instrument. Gert Pynt Andersen and Esben Smith, Triolab, accepted Stines performance.

Meeting 7th of April 2010, Department of clinical biochemistry, Hillerød Hospital
- Richard Hughes, International Product Manager, Alere International Sarl, Inverness medical
- Jutta Kraenke, Business Development Manager, Alere distribution business
- Vigdis Breen: (Bergman Diagnostics) Sales Manager, Norway
- Lodewijk Blokker: Alere Area Sales Manager EME
- Gert Pynt Andersen, Product Manager, Triolab

- Esben Smith, Product Specialist, POCT, Triolab
- Stine B Weber, SKUP in Denmark
- Esther Jensen, SKUP in Denmark

After the first 20 samples of the hospital evaluation, the imprecision was calculated. The CV% was below 5,0%. And all deviations calculated from the comparison method in Hillerød and from the comparison method in Roskilde was less than 20%. In February and March the duplicates had a higher variation. Therefore the results were discussed even though the evaluation was not complete.

3.4.2. *Evaluation sites and persons involved*

The hospital evaluation took place in Hillerød Hospital, Department of Clinical Biochemistry. Stine Beenfeldt Weber, SKUP/Hillerød, Vivian Larsen, and Kirsten Stilling, both Department of Clinical Biochemistry, did the practical work including collecting capillary and venous samples for the evaluation.

The primary care evaluation took place in centres that normally use capillary samples to analyse P—PT (INR). Laboratory consultant Inge Lykke Pedersen was contact person to the primary care centres.

Primary Care Centre 1 (GP1): Helsinge Lægecenter, Frederiksborgvej 18-1, 3200 Helsinge. At this primary care centre there are six doctors, five nurses, one secretary, and one Biomedical Laboratory Scientist. The Biomedical Laboratory Scientist does all the laboratory work, and therefore alone handled the samples for the evaluation.
Primary Care Centre 2 (GP2): Lægehuset Nivå, Gammel Strandvej 9, 2990 Nivå. At the primary care centre there are three general practitioners, one secretary, and two nurses. The nurses both do laboratory work and therefore both handled the samples for the evaluation.

The statistical calculations were made by Esther Jensen, who also wrote most of the report.

Table 3. Evaluation sites and persons involved

Place	**Person**	**Title**	**Task**
Hillerød Hospital	Esther A Jensen	Physician	Author of report
Hillerød Hospital	Vivian Larsen	BLS	Hospital laboratory testing
Hillerød Hospital	Kirsten Stilling	BLS	Hospital laboratory testing
Hillerød Hospital	Conny Pedersen	BLS	Responsible for comparison method
Hillerød Hospital	Stine B. Weber	Biochemist MSc.	Hospital laboratory testing
Hillerød Hospital	Inge Lykke Pedersen	BLS	Primary care testing
General practice	Karina Lundin	BLS	Primary care testing
General practice	Gitte Weeke, Heidi Dyrberg	Nurse Nurse	Primary care testing
Roskilde Hospital	Karin Kynde	Pharmacologist MSc.	Responsible for comparison method

BLS = Biomedical Laboratory Scientist

3.4.3. *Blood sampling devices*

The capillary punctures were performed with the lancet Owen Mumford, Unistik®3 Extra, Gauge 21G (0,81mm) Depth 2,0 mm. Pipettes used to collect the capillary blood were Alere™ INRatio® Microsafe Capillary Tubes (15 µl).The lancets and pipettes were supplied by Triolab, Denmark.

Venous blood for P—PT (INR) measurements was drawn into 3,5 mL Vacuette Greiner Bio-One from Greiner containing 9NC Coagulation sodium citrate 3,2%.

Venous blood for measuring heamatochrit was drawn in 3mL Vacuette Greiner Bio-One from Greiner containing K_3EDTA.

4. Evaluation procedure

4.1. The evaluation model

The evaluation in the hospital laboratory and two primary care centres deals with:

- Within-series-precision with 100 + 40 + 40 capillary patient samples (hospital laboratory + two primary care centres)
- Comparison with an established venous method for P—PT (INR), the comparison method in Roskilde
- Investigation of interferences of haematocrit
- Evaluation of user-friendliness

The hospital laboratory evaluation was performed in the Department of Clinical Biochemistry in Hillerød. Samples were measured in duplicates with INRatio2 in Hillerød and with the comparison method in Roskilde. The capillary samples from one patient were measured on the same INRatio2 instrument, using the same lot number.
Venous plasma was frozen at -80^{o}C in tubes, one millilitre in each. Two tubes were sent to Roskilde and analysed as 'true duplicates' with Roskilde comparison method.
According to the protocol, the first 20 samples were analysed within two hours after sampling in duplicates on the comparison method in Hillerød to achieve the best mean comparison result. The 20 x 2 frozen samples were then analysed in Roskilde.

4.2. Evaluation procedure in the hospital laboratory

All data, specimen collection, days of analyses, lot numbers on test results, internal controls etc. were reported. The lot numbers were used box by box at random; Lot A. was used for 47 patients, lot B for 28 patients, and lot C for 26 patients.
The samples from the patients were measured on one INRatio2 instrument and three lot numbers of test strips.

4.2.1. *Training*

The supplier in Denmark was responsible for the training with INRatio2. Training was given by Jutta Kraenke and Petra Bleibtreu-Partie from Alere GmbH to Vivian Larsen and Kirsten Stilling in the department of clinical biochemistry. The training was performed 16th of June 2009.
January 7th 2010, training was also given by Triolab to Stine B Weber in Hillerød Hospital.
At the training some of the staff volunteered to have samples taken.

4.2.2. *Internal quality control*

An internal system check is made automatically turning on the instrument. An Internal control is carried out on the strip by means of a built in two level control as described in section 3.2.1.

4.2.3. *Recruitment of the patients*

100 out-patients taking vitamin K antagonist, marevan (warfarin) or marcoumar (phenprocoumon), agreed to participate in the hospital evaluation and had two skin penetrations in separate fingers for capillary P—PT (INR) (duplicate measurements) as well as a measurement of haematocrit using venous EDTA blood. The patients were in steady state after at least four

weeks of treatment. First all 100 individuals had duplicate capillary test performed. Then venous samples (three tubes of 3.2% Sodium Citrate and one EDTA tube in one skin perforation) were drawn. The tubes were inverted 8-10 times to ensure thorough mixing.
At least 10 sample-results should be below 2,0 (1,5 to 1,9 INR), and 15 had to be >3,0 INR.
The samples in the evaluation were collected in a time span of at least 20 days.

4.2.4. *Handling of specimens and measurements*

Blood samples were collected from 100 individuals in steady state. Most of them had been in treatment for years and had their P—PT (INR) measured in the out-patient clinic regularly. In total, 100 capillary tests were analysed in duplicates on INRatio (two skin perforations in two separate fingers). Blood from a finger prick was filled into a cuvette designed for fresh capillary whole blood, see section 3.2.2. Following, they had a total of three citrate tubes and one EDTA tube taken in one venous puncture. The first sample was analysed in routine. The other samples were immediately centrifuged and plasma was frozen at -80^{o}C. All venous blood samples were drawn in continuance of the capillary samples and therefore were well within one hour from the testing of the capillary samples.

The venous samples:
Venopuncture was performed to collect four tubes of blood: three citrate tubes for P—PT (INR) measurements and one EDTA tube for measurement of hematocrit at Sysmex.
The citrate samples were centrifuged using normal procedure. The plasma was pooled and aliquotes of 1mL was frozen at -80^{o}C (a total of six tubes were frozen). From drawing the blood until the aliqoutes were frozen a maximum of one hour went by.

Analysing:
One fresh plasma sample was analyzed for P—PT (INR) on one of the Sysmex CA7000 Systems as a routine sample in Department of Clinical Biochemistry, Hillerød. The measurement was then repeated, using the same sample, on the other CA7000.

After collecting all the samples, the frozen samples were thawed and analysed in Roskilde in duplicates. If differences between Hillerøds fresh sample measurements and Roskildes frozen sample measurements occurred, the frozen samples were to be reanalysed in duplicates at Hillerød Hospital. Measuring fresh and frozen samples in Hillerød Hospital should demonstrate that nothing happens to the samples while frozen.
The results from Roskilde Hospital Laboratory were used as comparison method results in the hospital laboratory evaluation.

The capillary samples for the evaluation were measured in duplicate on INRatio2. The samples had to be applied to the test strip within 15 seconds from sampling. The duplicate measurements on samples from one patient were measured on the same INRatio2 instrument. In the hospital evaluation one instruments was used – instrument 4, and one (instrument 3) was a back-up system, that never was used. Test cartridges with three different lot numbers were used.

Stine B Weber, Vivian Larsen, and Kirsten Stilling at Department of Clinical Biochemistry took care of practical things. They also decided how to store the results from the evaluation safely.

The samples were within two hours from the measurement on INRatio2 also measured with the Hillerød comparison method. The haematocrit of the EDTA whole blood was also measured.

All results were registered by the evaluators doing the practical work. If an instrument showed an error while analysing a sample, a new measurement was made on the same instrument. The errors were recorded. All results were signed by the person doing the practical work. Data was recorded in Excel.

Twice in the evaluation samples were sent to Roskilde; when 20 samples had been included and when the evaluation was finalised.

4.2.5. *Evaluation of user-friendliness*

Vivian Larsen, Kirsten Stilling and Stine Weber evaluated the user friendliness immediately after testing had ended. They used the evaluation form with the four categories; manual, time factors, quality control, and operation facilities.

4.3. Evaluation procedure in Primary Care Centres

40 patients in treatment with warfarin in two primary care centres were asked to participate in the evaluation.

4.3.1. *Evaluation procedure in the primary health care*

40 patients, having their P—PT (INR) measured routinely, agreed to participate in the evaluation in two separate primary care centres. They each had two capillary samples measured on the INRatio2. All results were registered in the same way as in the hospital laboratory.

The samples from the 40 patients were measured on one instrument and using test cartridges with two different lot numbers in each primary care centre.

GP1 did the measurements during 21 days.
GP2 did the measurements during 61 days.

4.3.2. *The evaluation – model*

- Within-series-precision with 40 capillary patient samples in duplicate in each primary care centre
- Comparison with the CA7000 in Hillerød
- Evaluation of user-friendliness

4.3.3. *Training*

The supplier was responsible for training on the INRatio2. Training was given by Triolab to the staff in the two primary care centres. When the evaluation began, the evaluators had to handle INRatio2 on their own, without any supervision or correction from the manufacturer/supplier. If there were questions they were addressed to SKUP.

Training Primary care centre Helsinge16th of February 2010

- Esben Smith, Product Specialist, POCT, Triolab
- Karina Lundin, Biomedical Laboratory Scientist
- Stine B Weber, SKUP
- Esther Jensen, SKUP in Denmark

Training Primary care centre Nivå 9th of March 2010

- Gert Pynt Andersen, Product Manager, Triolab
- Esben Smith, Product Specialist, POCT, Triolab
- Gitte Weeke, nurse
- Heidi Dyrberg, nurse
- Esther Jensen, SKUP in Denmark

4.3.4. *Evaluation procedure in the primary care centres*
All data, specimen collection, dates of analyses, lot numbers on test cartridges and controls, results etc. were reported.

4.3.5. *Built-in quality control*
When running a sample, QC low and QC high are run automatically in the instrument (built-in-controls), see figure 2.

4.3.6. *Recruitment of the patients*
40 patients taking marevan (warfarin) or marcoumar (phenprocoumon) agreed to participate in the evaluation in each primary care centre.

4.3.7. *Handling of specimens and measurements*
Blood from a finger prick was filled into a cuvette designed for fresh capillary whole blood. A volume of 15 µl whole blood was applied into the test strip. The patients had a citrate tube taken in one venous puncture. The sample was sent to Hillerød by ordinary sample transport at room temperature. In the Department of Clinical Biochemistry P—PT (INR) was analysed with the comparison method in Hillerød.

The samples were measured within 24 hours with the comparison method in Hillerød and on the INRatio2. One INRatio2 instrument and two lots of tests were used in each primary care centre.

All results were registered and signed by the evaluator doing the practical work. If an instrument showed an error code while analysing a sample, a new measurement was performed. The error codes were recorded. Data was recorded in a form produced by Stine B Weber.

4.3.8. *Evaluation of user-friendliness*

The evaluators filled in the user-friendliness questionnaire after completing the testing. They were also questioned verbally about their opinion on the four categories; manual, time factors, quality control, and operating facilities.

5. Statistical expressions and calculations

The definitions in this section come from the International Vocabulary of Metrology, VIM (12).

5.1. Statistical terms and expressions

5.1.1. *Precision*

Definition: Precision is the closeness of agreement between measured quantity values obtained by replicate measurements on the same or similar objects during stated specified conditions.

Precision is descriptive in general terms (good or poor e.g.) and measured as imprecision. Imprecision is expressed by means of the standard deviation (SD) or coefficient of variation (CV). SD is reported in the same unit as the analytical result and CV is usually reported in percent.

Repeatability is the agreement between the results of consecutive measurements of the same component carried out under identical measuring conditions (within the measuring series). Reproducibility is the agreement between the results of discontinuous measurements of the same component carried out under changing measuring conditions over time. The reproducibility includes the repeatability.

To be able to interpret an assessment of precision, the precision conditions must be defined. The "specified conditions" can be, for example, repeatability, intermediate precision, or reproducibility conditions of measurement. The precision conditions in this evaluation are close to the defined *repeatability* and *reproducibility* conditions, and the imprecision is expressed as repeatability CV and reproducibility CV. The imprecision is summarised in tables.

5.1.2. *Accuracy*

Definition: Accuracy is the closeness of agreement between a measured quantity value and the true quantity value of a measurand.

Inaccuracy is a measure of the deviation of a single measurement from the true value, and implies a combination of random and systematic error (analytical imprecision and bias). Inaccuracy, as defined by a single measurement, is not sufficient to distinguish between random and systematic errors in the measuring system. Inaccuracy can be expressed as **total error**. The inaccuracy is illustrated by difference-plots with quality goals for the total error shown as deviation limits in percent.

5.1.3. *Trueness (bias)*

Definition: Trueness is the closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.

Trueness is measured as bias (systematic errors). Bias: Systematic deviation from the reference method. Trueness is descriptive in general terms (good, poor), whereas bias is the estimate, reported in the same unit as the analytical result or in %. The bias at concentration levels (high, medium, low) is summarised in tables.

5.2. Statistical calculations

5.2.1. *Statistical outliers*

All the results are checked for outliers according to Burnett (13), with repeated truncations. The model takes into consideration the number of observations together with the statistical significance level for the test. The significance level is often set to 5%, therefore also in this evaluation. Where the results are classified according to different concentration levels, the outlier-testing is done at each level separately. Statistical outliers are excluded from the calculations. Possible outliers will be commented on under each table.

5.2.2. *Calculations of imprecision*

The imprecision was calculated by use of the following formula:

$SD = \sqrt{\frac{\sum d^2}{2n}}$, d = difference between duplicate measurements, n = number of differences

The imprecision may also be calculated with the following formula:

$CV = \sqrt{\frac{\sum (d/m)^2}{2n}}$

d = difference between duplicate measurements
m = mean of the duplicate measurements
n = number of differences

This formula is preferred when estimating CV over a large concentration interval within which the CV is assumed to be reasonable constant.

5.2.3. *Calculation of trueness*

To measure the trueness of the results on the INRatio2, the average bias at three concentration levels is calculated based on the results obtained under standardised and optimal measuring conditions.

5.2.4. *Assessment of accuracy*

To evaluate the accuracy of the results on the INRatio2, the agreement between INRatio2 results and the comparison method results is illustrated in difference plots. In the plots the x-axis represents the mean value of the duplicate results at the comparison method. The y-axis shows the difference between the first measurement at INRatio2 with three lots and the mean value of the duplicate results at the comparison method.

6. Results and discussion

It was not a part of the evaluation to investigate if the instruments were in agreement with each other. The supplier guaranteed that the lot numbers were measuring similar, a difference of 0,4 INR was accepted.

6.1. Outliers and missing results

One sample in hospital was not measured in duplicate on INRatio2 because the patient was not feeling well. One duplicate sample in hospital measured on INRatio2 had an error code twice. One result in hospital from INRatio2 was excluded as an outlier (4,6 and 3,0 INR with INRatio2. One sample was a true outlier (4,55 in INRatio2 and 3,0 INR in Roskilde and 3,05 in Hillerød) Three samples from the primary care centre 2 did not have enough sample volume for the comparison method in Hillerød.

6.2. Failed measurements

Table 4. List of failed measurements with INRatio2

Remarks
Strip with "green colour" not used
Error 144 (error for high and low QC)
Error 114 (error for high QC control)
Error 114 twice on the same patient (error for high QC control)
Blood does not flow into the test strip. when put on table: blood flows in
Bubbles in two chambers in the test strip
Air bubbles in a test strip (primary care centre)

In total eight measurements failed due to errors which probably not are caused by user mistakes. Seven of the failed measurements were seen in hospital laboratory and one in one of the primary care centres.
In one additional strip no error message occured even if the blood flowed very slowly into the test strip. This sample is not counted as an error.
The frequency of failed measurements was 2,2% (8/362 x 100).

6.3. Analytical quality of the designated comparison methods

6.3.1. *The precision of the comparison methods*

The comparison of methods was carried out with fresh samples from the 101 venous specimens measured in duplicates on both the comparison method in Dept of Clinical Chemistry at Hillerød Hospital, and frozen samples measured in Roskilde Hospital. The results are presented in a difference plot. The mean deviation (bias) with confidence interval is calculated for all results, and for the results divided into three measuring ranges.

Table 5. Patient sample results with the comparison methods in Roskilde and Hillerød

Level	Roskilde comparison method interval (INR)	n	Excluded results	Roskilde comparison method P—PT (INR)		Hillerød comparison method P—PT (INR)	
				mean	CV% (95% CI)	mean	CV% (95% CI)
Low	1,04 — 2,03	33	0	1,72	0,9 (0,8 — 1,2)	1,7	2,4 (1,9 — 3,2)
Medium	2,03 — 2,55	34	0	2,27	0,8 (0,7 — 1,1)	2,3	2,4 (2,0 — 3,3)
High	2,55 — 5,96	34	0	3,19	1,6 (0,7 — 1,1)	3,2*	2,7 (2,2 — 3,6)
All	1,04 — 5,96	101	0	2,40	1,2 (1,3 — 2,1)	2,4	2,5 (2,2 — 2,9)

* For two samples there is only a single result with the Hillerød comparison method. The results in Roskilde originates from duplicates measurements on the same instrument within a short timespan. In Hillerød the results originates from two instruments.

The calculated CV values are practical measures of repeatability, but they also include some additional variance components: The measurements with the comparison method in Hillerød were performed during seven months. The first and the second measurement in each duplicate were measured, with maximum a few hours time difference and each one on the two instruments.

6.3.2. *The trueness of the comparison method in Roskilde*

The trueness of the comparison method in Roskilde was determined by DEKS and EQUALIS calibrators and control materials, plus four reference control materials from EQUALM, see tables 6-8 and figure 4 below.

6.3.3. *External quality control for the comparison method in Roskilde*

In Roskilde controls and calibrators with target values was analysed for every 20 patient samples. Table 6 shows the results of the EQUALM control 1-4.

Table 6. The Roskilde comparison method checked with EQUALM controls

EQUALM material	EQUALM n=	Target	n	P—PT (INR) measured
Control	605	2,12	4	1,99
Control	605	2,12	4	2,08
Control	605	2,12	2	2,04
Control	603	2,55	4	2,44
Control	603	2,55	4	2,64
Control	603	2,55	2	2,58
Control	599	3,09	4	2,87
Control	599	3,09	4	3,00
Control	599	3,09	2	-
Control	602	3,27	4	3,16
Control	602	3,27	4	3,27
Control	602	3,27	2	3,10

Comments

Table 6 shows the results of the four EQUALM controls with the comparison method in Roskilde. The controls were run after every 20 comparison sample. Target is given by about 600 European laboratories. The results are also visualised in figure 4. The controls were analysed in December 2009 and May 2010.

Table 7. The Roskilde comparison method checked with EQUALIS calibrators and controls

EQUALIS material	EQUALIS description	P—PT (INR) target	n	P—PT (INR) measured
EQUALIS Calibrator	Normal	1,04	4	1,08
EQUALIS Calibrator	Normal	1,04	4	1,09
EQUALIS Calibrator	Normal	1,04	5	1,06
EQUALIS Calibrator	High	3,03	4	3,11
EQUALIS Calibrator	High	3,03	4	3,19
EQUALIS Calibrator	High	3,03	5	3,00
EQUALIS Control	2,39 INR	2,39	4	2,43
EQUALIS Control	2,39 INR	2,39	4	2,47
EQUALIS Control	2,39 INR	2,39	5	2,39

Comments

Table 7 demonstrates the bias in Roskilde when using the EQUALIS control materials and calibrators for every 20 comparison samples. The results in Roskilde are a little higher than the EQUALIS target. The results are also visualised in figure 4. The controls and calibrators were analysed in December 2009 and May 2010.

Table 8. The Roskilde comparison method checked with DEKS calibrators and controls

DEKS material	DEKS description	P—PT (INR) target	n	P—PT (INR) measured
DEKS Calibrator	Normal	0,96	6	0,96
DEKS Calibrator	Normal	0,96	4	0,97
DEKS Calibrator	Terapeutic	2,30	6	2,28
DEKS Calibrator	Terapeutic	2,30	4	2,23
DEKS Calibrator	High	3,68	6	3,70
DEKS Calibrator	High	3,68	4	3,74
DEKS Control	DEKS 111091 n=53	0,93	2	0,91
DEKS Control	DEKS 111091 n=53	0,93	2	0,92
DEKS Control	DEKS 111091 n=53	0,93	2	0,93
DEKS Control	DEKS 111091 n=53	0,93	4	0,93
DEKS Control	DEKS 111091 n=53	0,93	4	0,95
DEKS Control	DEKS 203082 n=63	2,34	2	2,27
DEKS Control	DEKS 203082 n=63	2,34	2	2,31
DEKS Control	DEKS 203082 n=63	2,34	2	2,31
DEKS Control	DEKS 211063 n=61	4,14	4	3,97
DEKS Control	DEKS 211063 n=61	4,14	4	3,95

Comments

Table 8 demonstrates the bias in Roskilde when using the DEKS control materials and calibrators. The results are also visualised in figure 4. The controls and calibrators were analysed in December 2009 and May 2010.

6.3.4. *The Roskilde comparison method checked with calibrators and controls from Denmark, Scandinavia and Europe*

During the evaluation controls and calibrators used in Denmark, Norway, Sweden and the rest of Europe were analysed on the comparison method in Roskilde at Roskilde Hospital. The results are seen in figure 4.

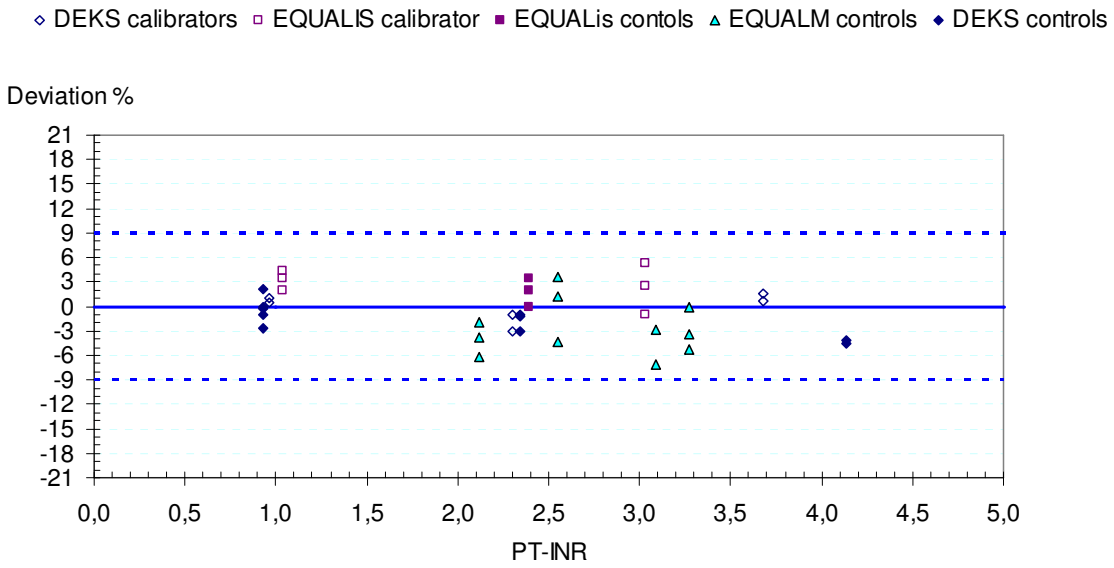


Figure 4. The figure has the target value of each sample on the x-axis. The y-axis shows the deviation in percent of the mean measurements of P—PT (INR) measured in Roskilde. Symbols not filled with any colour are calibrators and symbols filled with colour are control materials. The dottet blue line indicate maximal allowable deviation, Bias ± 1,96 × CV% ~9%

Comments
The bias is for DEKS, EQUALIS and EQUALM is less than ±3%.
EQUALIS seems to have a positive bias and EQUALM seems to have a negative bias compared to DEKS.

6.3.5. *The trueness of the comparison method in Hillerød*

The trueness of the comparison method in Hillerød was determined by DEKS control materials, sent five times per year, see tables 9.A and 9.B below.

6.3.6. *External quality control for the comparison method in Hillerød*

The Hillerød comparison method, CA7000 was checked with DEKS controls in 2009 and 2010. The control materials (four samples) were sent from department of clinical biochemistry, DEKS, Roskilde five times per year. The results are seen in Table 9.A.

Table 9.A The Hillerød comparison method checked with control material from DEKS

DEKS Survey 2009-10	February	Maj	June	September	November	February			May				June				mean
Deks no	201091	203084	201092	201093	211063	1-1	1-2	1-3	1-4	2-1	2-2	2-3	2-4	3-1	3-2	3-3	3-4	%
Hillerød CA-1	1,7	2,3	1,8	1,8		3,3	0,9	0,9	1,3	2,4	0,9	1	1,2	0,9	2,4	1,2	0,9	
Hillerød CA-2	1,8	2,5			4,1	3,2	0,9	0,9	1,3	2,4	0,9	1	1,2					
Mean (Denmark)	1,70	2,36	1,80	1,72	4,10	3,29	0,92	0,94	1,30	2,32	0,94	0,95	1,17	0,92	2,47	1,17	0,95	
Bias (%)	2,9	1,7	0,0	4,7	0,0		-2,03				1,75				-1,85			0,9

In 2009 only the samples >1,5 INR was used. In 2010 all four samples was used. The bias of the Hillerød comparison method was in average +0,9% (range -2,0 to +4,7%) during 2009 and 2010 compared to the rest of Denmark. The goal was a bias less than 3,0%.

The results from Hillerød (table 5) were also in good agreement with the results in Roskilde.

6.3.7. *Internal quality control with the comparison method in Hillerød*

The imprecision of the comparison method in Hillerød calculated from the internal control sample results are shown in table 9.B. The samples were run every 3 hours on both instruments during the testing period.

Table 9.B The comparison method in Hillerød checked with internal quality control samples

			2009					2010		
Month	August	September	Oktober	November	December	Januar	Februar	Marts	April	Maj
					Mean INR					
CA-1	2,0	1,9	1,9	1,7	1,7	1,8	1,8	1,8	1,8	1,8
CA-2	1,9	1,9	1,8	1,8	1,8	1,7	1,7	1,7	1,8	1,8
					Mean CV%					
CA-1	3,5	2,6	4,3	2,9	4,0	4,0	2,8	2,7	2,7	4,9
CA-2	1,6	2,6	4,0	4,0	5,5	4,0	2,9	2,9	4,6	3,9
					Lot numbers					
CPP (control)	512623c	512623c	509911A	509911A	509911A	509911A	509911A	509911A	509911A	509911A
Nycotest	10139886	10139886	10139886	10139886	10139886	10139886	10139886	10139886	10139886	10139886

Comments

The imprecision goal was a CV less than 3,0% however the mean CV was 3,5% at the concentration 1,8 INR. This is significantly higher than the CV% of the genuine samples measured in two different instruments, (table 5). The reason for the difference is not known.

The comparison method in Hillerød was during the evaluation period adjusted to give the same results as in the hospital laboratories in Helsingør and Frederikssund.

6.4. Analytical quality of INRatio2 used in a hospital laboratory

6.4.1. *Built-in self-check*

A System Check is run within each test strip to check that the optical and operating systems were working correctly. If the system does not work correct, an error occurs.

Table 10. Repeatbility and reproducibility achieved with INRatio2 in the internal built-in quality control QC1 and QC2

	n	**QC1 ~ normal INR**	**QC2 ~ therapeutic concentration**
Repeatbility		**CV% (CI 95%)**	**CV% (CI 95%)**
lot 214540	26	5,4 (4,3—7,5)	5,7 (4,6—7,9)
lot 216976	26	5,2 (4,1—7,2)	5,8 (4,6—8,0)
lot 213040A	45	4,7 (4,0—6,0)	5,2 (4,3—6,5)
mean	97	5,1 (4,5—6,0)	5,5 (4,9—6,4)
Reproducibility			
CV% total	199	5,8	10,6
mean (seconds)	199	12,1	22,5

Comments

The low internal built-in controls have a repeatability CV between of 5,1% and the high built-in control have a CV of 5,5%. The total CV was 5,8% and 10,6%; respectably. The CV% of the high and low control does not reflect the CV of the genuine samples and the CV% of the three lots do not differ from each other with mean CV between 4,7-5,4% in the low control and 5,2-5,7% in the high controls.

All the QC results were within the range set by the manufacturer. According to the manufacturer the built-in controls are more sensitive to for example temperature than the genuine samples. The raw data is shown in an attachment.

6.4.2. *Comparison of the 1st and 2nd measurements*

Two capillary samples were taken from 100 individuals for measurements on INRatio2. The results are checked to meet the assumption that there is no difference between the first and the second measurement. Table 11 shows that no systematic difference was pointed out between the paired measurements.

Table 11. Comparison of the 1st and 2nd measurements on INRatio2

	n	Mean P—PT 1st measurement (INR)	Mean P—PT 2nd measurement (INR)	Mean difference 2nd – 1st measurement (INR)	95% CI for the mean difference, (INR)
capillary	100	2,44	2,43	0,01	-0,03 - +0,07

Comments
It is observed, that there are no differences between first and second measurements.

6.4.3. *The precision of INRatio2*

Repeatability under standardised and optimal measuring conditions in a hospital laboratory was obtained with capillary blood samples (table 12) the raw data is shown in attachment for Triolab. Repeatability was calculated for three subgroups: the highest P—PT (INR)-values (n=34), the lowest (n=33) and the middle level of P—PT (INR) (n=34). The three groups are chosen according to their concentration with the comparison method.

Table 12. Repeatability of INRatio2 in the hospital laboratory

Level	Roskilde comparison method interval (INR)	n*	Excluded results	INRatio2 mean (INR)	CV% (95% CI)
Low	1,04 — 2,03	33	1	1,8	5,9 (4,8 — 7,9)
Medium	2,03 — 2,55	34	0	2,3	5,6 (4,6 — 7,4)
High	2,55 — 5,96	34	2	3,3	7,4 (6,0 — 9,8)
All	1,04 — 5,96	101	3	2,4	6,3 (5,6 — 7,4)

*The given numbers of results (n) are counted before exclusion of outliers. Mean and CV are calculated after exclusion of outliers: One sample was not measured in duplicate on INRatio2, one sample had duplicate error with INRatio2 and one sample had 4,6 and 3,0 INR on INRatio2.

The calculated CV values are practical measures of repeatability, but they also include some additional variance components arising from changes in conditions during the collection of measurement data: Three lots of test strips were used.

Discussion
The quality goal for CV, less than 5%, was not fulfilled in any concentration level. The imprecision seems to be the same at all concentration levels.

6.4.4. *External quality control*

There are no external quality control materials available for INRatio2.

6.4.5. *The trueness of INRatio2*

Trueness was calculated against the values obtained with the Roskilde comparison method, where the calibrators and controls from Scandinavia and Europe were run in between the Roskilde comparison method results used in the evaluation.

Bias was calculated for the 101 patient sample results divided in three subgroups according to the P—PT (INR) level with the comparison method.

Table 13. Bias of INRatio2 in the hospital laboratory

Level	Roskilde comparison method interval (INR)	n	Excluded results	INRatio2 mean (INR)	Bias % (95% CI)
Low	1,04 — 2,03	33	1*	1,8	+2,1 (−0,9 — +5,2)
Medium	2,04 —2,55	34	0	2,3	+1,1 (−2,4 — +4,2)
High	2,56 —5,96	34	3**	3,3	+3,6 (+0,2 — +6,8)
All	1,04 —5,96	101	4	2,4	+2,2 (+0,2 — +4,0)

* one sample was not measured in duplicate on INRatio2 ** one sample produced duplicate error with INRatio2. One sample was 4,6 and 3,0 INR with INRatio2. One sample was 4,7 and 4,4 INR with INRatio2 and 2,99 and 3,01 INR with the Roskilde comparison method and 3,0 and 3,1 with the Hillerød comparison method.

Discussion

The bias was between 1,1% and 2,7%. Therefore the INRatio2 results fulfil the Danish quality goal for bias, less than ±6,0%, in the hospital laboratory evaluation.
One patient sample duplicate result was a true outlier compared to the all duplicates. The reason was not investigated. No further samples were collected from that patient. In Denmark it has been found that large deviations (more than 1 INR) between the Owren and the Quick results is seen for about 1:250 individuals in warfarin treatment (personal communication).

6.4.6. *The accuracy of INRatio2*

To evaluate the accuracy of the results on the INRatio2, the agreement between INRatio2 and the Roskilde comparison method is illustrated in a difference-plot. The plot shows the deviation of single measurement results on INRatio2 from the true value, and gives a picture of both random and systematic deviation, reflecting the total error for measurements on INRatio2. The total error is demonstrated for the first measurements of the paired results only. Under standardised and optimal conditions three different lots of test strips were used. The allowable deviation in this evaluation was ±20%.

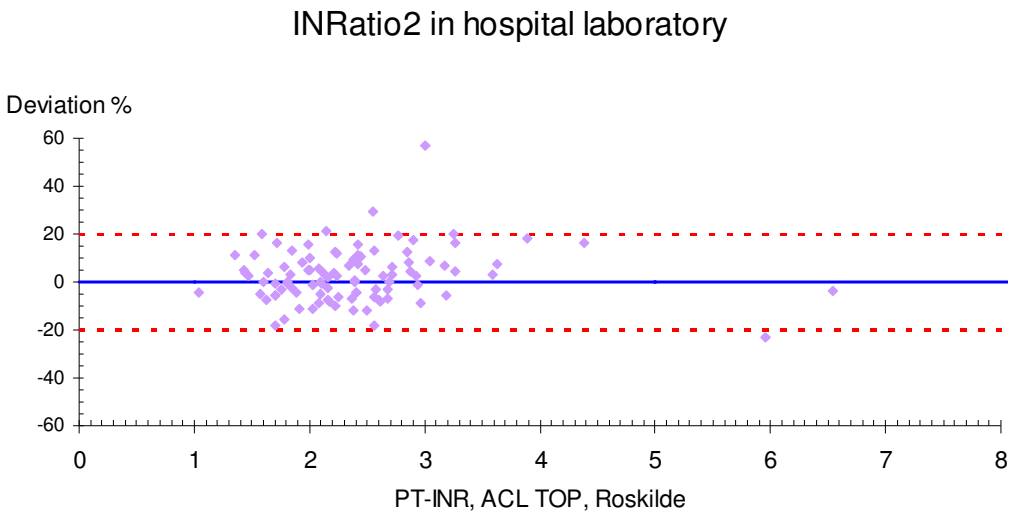


Figure 5. Difference plot. INRatio2 results with capillary samples in the hospital laboratory.
The diagram shows the deviations of the capillary INRatio2 P—PT (INR) results from the Roskilde venous comparison method results for 100 patient samples. X-axis = mean of the Roskilde comparison method duplicate results and Y-axis = ((the first INRatio2 result– mean of the duplicate results with the Roskilde comparison method,)/ mean of the duplicate results with the reference method) x 100. Stippled lines represent the tolerance limits ±20%.

Discussion
95% of the results should be within the tolerance limit to fulfil the goal for Total Error of ±20% and 99% should be within ±25%.
Only four of 100 results exceed the maximal allowable deviation of ±20%. Two results deviate more than 25%. In the hospital laboratory the Total Error for capillary sample results fulfils the quality goals.

6.4.7. *Interference from haematocrit*
A possible interference from haematocrit is checked by plotting the haematocrit-values on the X-axis and the deviations from the Roskilde Comparison Method on the Y-axis in a diagram. In case of a deviation >20% it is investigated if the deviation was caused by an abnormal haematocrit in the sample.

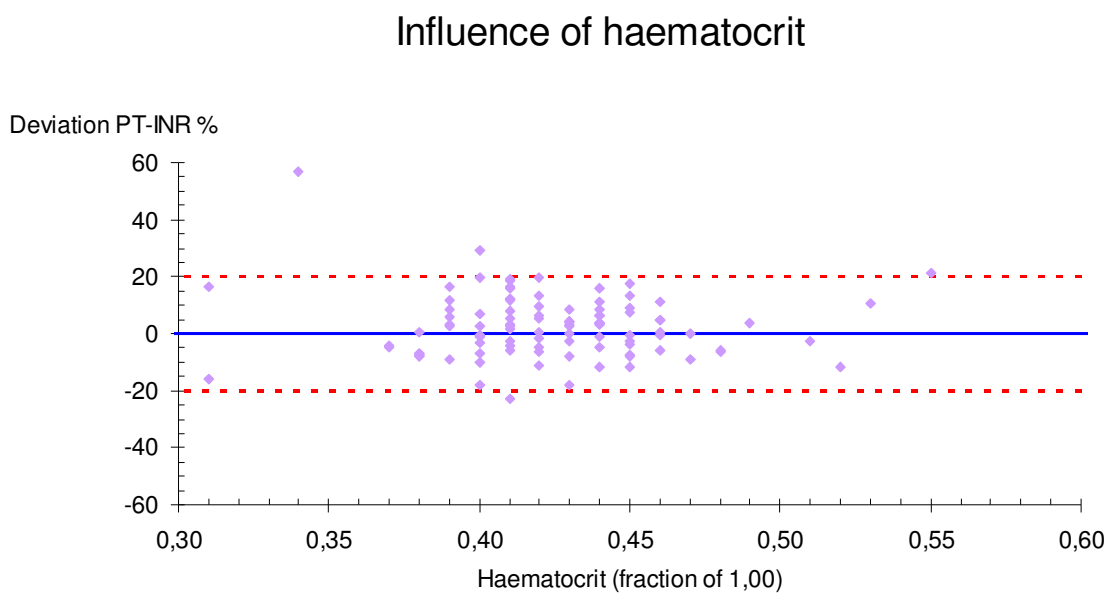


Figure 6. Difference plot. Haematocrit (fraction) and deviation of INRatio2. The diagram shows the deviations of the capillary INRatio2 P—PT (INR) results as a function of haematocrit in the samples for 100 patient samples. X-axis = haematocrit in the sample. Y-axis = ((the first INRatio2 result– mean of the duplicate results with the Roskilde comparison method,)/ mean of the duplicate results with the reference method) x 100. Stippled lines represent the tolerance limits ±20%.

Discussion
The figure demonstrates that the haematocrit, EVF between 0,40 to 0,50 does not seem to influence the results of the samples.

6.4.8. *Matrix-differences*
One patient had a duplicate result in the INRatio2 that differed significantly from the comparison method results in Roskilde and Hillerød. It is known that some few patients always can have a huge difference between results from the Owren and the Quick method. No further investigation was made.

6.5. Analytical quality of INRatio2 used in primary health care

6.5.1. *Built-in self-check*

A System Check is run within each test strip to check that the optical and operating systems are working correctly. If the system does not work correct, an error code is shown, see also 6.4.1.

6.5.2. *The precision of INRatio2*

The imprecision of the duplicate measurements on the INRatio2 in the primary care centres was calculated according to 5.2.2. The results are shown below for the two centres. The sampling was done in 21 days in primary care centre 1 and during 62 days in primary care centre 2. The analysing was performed by a Biomedical Laboratory Scientist in primary care centre 1 and by two nurses in primary care centre 2.

Table 14. Repeatability of INRatio2 in the primary care centres

Level	**Hillerød comparison method interval (INR)**	**n**	**Excluded results**	**INRatio2 mean INR**	**CV% (95% CI)**
Primary care centre 1					
Low	1,6 — 2,5	20	0	2,2	6,9 (5,3 — 10,0)
High	2,5 — 4,0	20	0	3,1	10,0 (7,7 — 14,6)
All	1,6 — 4,0	40	0	2,6	8,6 (7,1 — 11,1)
Primary care centre 2					
Low	1,6 — 2,5	20	0	2,2	6,0 (4,7 — 8,8)
High	2,5 — 5,5	20	0	3,3	4,1 (3,1 — 5,9)
All	1,6 — 5,5	40	0	2,7	5,2 (4,3 — 6,6)

The calculated CV values are practically measures of repeatability, but they also include some additional variance components arising from changes in conditions during the collection of measurement data as each primary care centre used two lots of test strips.

Comments

The imprecision in Primary care centre 1 was higher than 5,0% for both high and low concentrations. The 40 measurements were performed within 61 days.

The imprecision in Primary care centre 2 were carried out during 21 days. The CV was close to the quality goal of <5,0%. Because of the large differences in imprecision between the primary care centres, the possible difference depending on used lot numbers were further investigated.

Table 15. Repeatability achieved with INRatio2 self-test control QC1 and QC2

	GP1		GP2	
	QC1	QC2	QC1	QC2
n	80	80	80	80
mean (seconds)	12,5	22,8	12,2	23,1
CV%	5,9	10,1	6,5	11,6

Comments
The CV achieved with the self-test system in INRatio2 was almost the same in primary care as in the hospital laboratory evaluation, see also table 10.

6.5.3. *The trueness of INRatio2 in primary care*

Bias was calculated for the 40 patients divided in two subgroups of P—PT (INR)-values. The groups were chosen according to the concentration measured with the Hillerød comparison method. Bias was calculated against the values obtained in Hillerød.

Table 16. Bias of INRatio2 P—PT (INR) with patient samples at the primary care centres

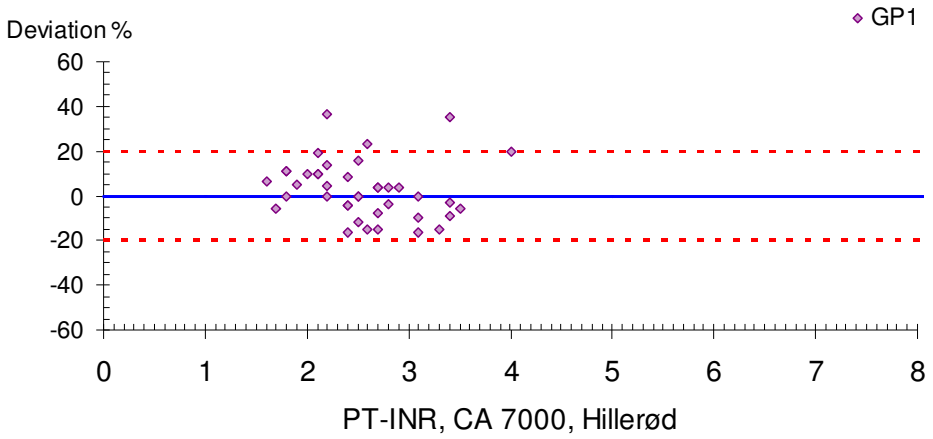
Level group	**Hillerød comparison method interval (INR)**	**n***	**Excluded results**	**Hillerød comparison method mean (INR)**	**Bias % (95% CI)**
GP1					
Low	1,6 — 2,5	20	0	2,2	+5,2 (+0,4 — +9,9)
High	2,5 — 4,0	20	0	3,1	−0,2 (−6,5 — +6,0)
All	1,6 — 4,0	40	0	2,6	+3,5 (+0,2 — +6,8)
GP2					
Low	1,6 — 2,5	20	2	2,2	+4,3 (+1,1 — +7,6)
High	2,5 — 5,5	20	1	3,3	+3,7 (−0,3 — +7,7)
All	1,6 — 5,5	40	0	2,7	+4,0 (+1,5 — +6,4)

*The given numbers of results (n) are counted before exclusion of outliers. Mean and CV are calculated after exclusion of outliers. Three samples were not measured in the comparison method in Hillerød in Hillerød

Comments
The results in primary care 1 and 2 fulfil the quality goal, less than ±6% for bias in all concentration intervals.

6.5.4. *The accuracy of INRatio2*

The accuracy in primary care results (two lots of test strips) is shown in figure 7. The accuracy was evaluated against the values obtained in Hillerød



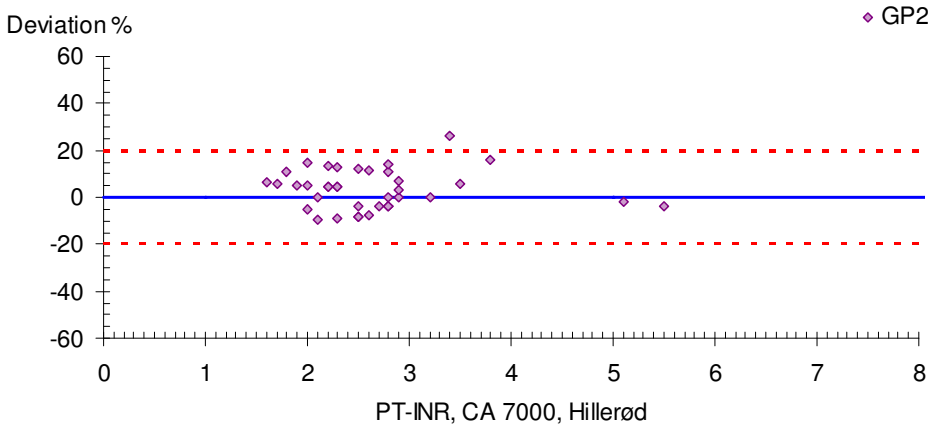


Figure 7. Difference plot, samples in the primary care centre 1 and 2.
The diagram shows the deviations of the capillary INRatio2 results from the Hillerød venous comparison method results for 40 patients. X-axis = Hillerød comparison method result and Y-axis = ((first INRatio2 result– Hillerød comparison method result)/ Hillerød comparison method result) x 100. Stippled lines represent the tolerance limits ±20%.

Comments
95% of the results should be within the tolerance limits to fulfil the quality goal for total error of ±20%.
In primary care centre 1, where a Biomedical Laboratory Scientist took care of the analysing, there are three of 40 results outside the tolerance limits.
In primary care centre 2, where two nurses took care of the analysing, there is one result outside the tolerance limits.
It was investigated how much influence the lot number of the test strips had, see section 6.7.

6.6. Influence of lot numbers

Some results were hard to explain in the evaluation and it appeared that it could depend on the lot numbers used. Therefore imprecision and bias were calculated for the lot numbers used in hospital laboratory as well as primary care.

6.6.1. *Repeatability of three test strips lots*

Table 17. Repeatability of INRatio2 with three lots of test strips

lot	n	CV% (95% CI)	n	CV% (95% CI)	n	CV% (95% CI)
	Hospital laboratory		**Primary care 1**		**Primary care 2**	
213040A	46	4,8 (4,0 — 6,1)	19	5,1 (3,9 — 7,4)	20	6,2 (4,8 — 9,1)
214540	26	6,0 (4,7 — 8,2)	-	-	20	3,8 (2,9 — 5,4)
216976	26	8,7 (6,9 —12,0)	21	10,9 (8,4 —15,6)	-	-
in total	98	6,3	40	8,6	40	5,2

Comments

The results in table 17 demonstrate that the repeatability is significant different in lot 213040A and in lot 216976 in both the hospital evaluation and the primary care centre 1.

The analysing was performed of an experienced Biomedical Laboratory Scientist working in the primary care centre. It is also seen that the lot 216976 performed poorly in hospital laboratory as well as in primary care.

6.6.2. *Bias with three test strips lots*

Table 18. Bias of INRatio2 in three lots of test strips

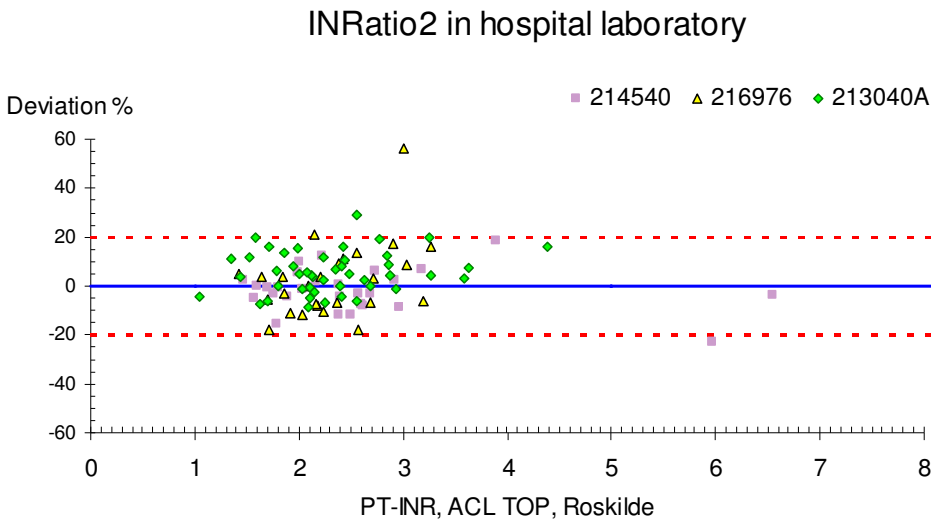
lot	n	Bias % (95% CI)	n	Bias % (95% CI)	n	Bias % (95% CI)
	Hospital laboratory		**Primary care 1**		**Primary care 2**	
213040A	46	+5,7 (+3,3 — +8,1)	19	+1,9 (-3,2 — +7,0)	20	+7,3 (+3,4 — +11,1)
214540	26	+1,3 (-4,5 — +7,1)	-	-	20	+1,3 (-1,6 — +4,2)
216976	26	-2,2 (-5,8 — +1,4)	21	+5,0 (+0,34 — +9,6)	-	-
in total	98	+2,3	40	+3,5	40	+4,0

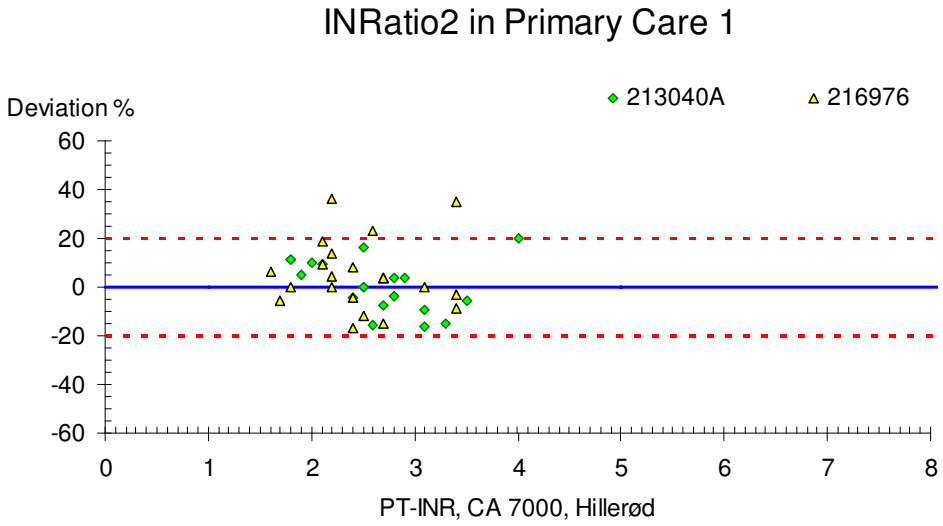
Comments

The bias results show large confidence intervals due to the poor imprecision in measurement results. In the in hospital laboratory evaluation there is significantly higher bias in the 46 results with lot 213040A than in the 26 results with lot 216976.

6.6.3. *Total Error for the three lots of test strips*

The three lots of test strips used in the hospital evaluation and in the primary care centres are shown in the figure 8a, 8b and 8c.


INRatio2 in hospital laboratory
Deviation %
214540
216976
213040A
60
40
20
0
-20
-40
-60
0
1
2
3
4
5
6
7
8
PT-INR, ACL TOP, Roskilde


INRatio2 in Primary Care 1
Deviation %
213040A
216976
60
40
20
0
-20
-40
-60
0
1
2
3
4
5
6
7
8
PT-INR, CA 7000, Hillerød

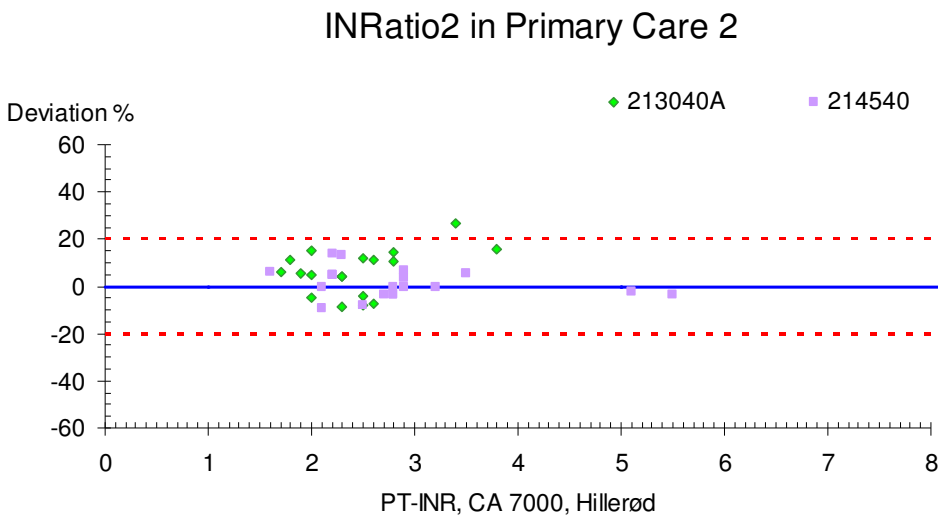


Figure 8a, 8b, 8c. Difference plot, for the evaluation in the hospital laboratory and in primary care centre 1 and 2 respectively. The diagrams show the deviations of the INRatio2 results from the venous comparison method results for 100 samples in hospital laboratory and 40 patient samples in each primary care centre. X-axis = comparison method and Y-axis = ((first INRatio2 result–comparison method)/ comparison method x 100)). For the evaluation in the hospital laboratory the results from the Roskilde comparison method are used and in the primary care centre evaluations the results from the Hillerød comparison method are used. The outliers are included in figure 8a.

Comments

Figure 8a-c demonstrates the deviation of the three lots of test strips. None of the lots had both high bias and high imprecision

6.7. Evaluation of user-friendliness

6.7.1. *Evaluation of user-friendliness*

Each evaluating personal evaluated the user-friendliness and filled in the form. Indicating 0 and 1 point had to be followed by an explanation. Any free comments belonging to the four sub-areas were placed under the table concerning the area, or after all the tables if more suitable. The total rating of each row was not determined by the arithmetic mean of the individual ratings in the row. In the same way, the total rating of each table was not determined by the arithmetic mean of the individual ratings on the rows above. The total ratings were more an overall assessment of the property described in the row or in the headline of the table. Thus could a single bad rating justify an overall bad rating if that property seriously influences on the user-friendliness of the system.

Table 19. Assessment of the information in the manual / insert

Information in manual / insert about:	**0 point**	**1 point**	**2 point**
General impression	Un-satisfactory	Less satisfactory	Satisfactory
Table of contents	Un-satisfactory	Less satisfactory	Satisfactory
Preparations / Pre-analytic procedures	Un-satisfactory	Less satisfactory	Satisfactory
Specimen collection	Un-satisfactory	Less satisfactory	Satisfactory
Measurement / Reading	Un-satisfactory	Less satisfactory	Satisfactory
Measurement principle	Un-satisfactory	Less satisfactory	Satisfactory
Sources of error	Un-satisfactory	Less satisfactory	Satisfactory
Fault-tracing / Troubleshooting	Un-satisfactory	Less satisfactory	Satisfactory
Index	Un-satisfactory	Less satisfactory	Satisfactory
Readability / Clarity of presentation	Un-satisfactory	Less satisfactory	Satisfactory
Available in Danish, Norwegian and Swedish	Un-satisfactory	Less satisfactory	Satisfactory
Rating for information in manual			Satisfactory

Comments: Primary care centres were given a Danish short version.

Table 20. Assessment of Time factors

Time factors	**0 point**	**1 point**	**2 point**
Duration of preparations / Pre-analytical time	>10 min.	6 to 10 min.	<6 min.
Duration of analysis	>20 min.	10 to 20 min.	<10 min.
Required training time	>8 hours	2 to 8 hours	<2 hours
Stability of test, unopened package	<3 months	3 to 5 months	>5 months
Stability of test, opened package	<14 days	14 to 30 days	>30 days
Rating of time factors			Satisfactory

Comments: none

Table 21. Assessment of Quality control possibilities

Quality Control	**0 point**	**1 point**	**2 point**
Built-in self-test*	Un-satisfactory	Less satisfactory	Satisfactory
Internal quality control**	Un-satisfactory	Less satisfactory	Satisfactory
External quality control**	Un-satisfactory	Less satisfactory	Satisfactory
Stability of quality control material, unopened	<3 months	3 to 5 months	>5 months
Stability of quality control material, opened	≤1 days	2 to 6 days	>6 days or disposable
Storage conditions for control material, unopened	–20°C	+2 to +8°C	+15 to +30°C
Storage conditions for control material, opened	–20°C	+2 to +8°C	+15 to +30°C
Usefulness of the Quality Control	Un-satisfactory	Less satisfactory	Satisfactory
Rating of Quality Control**			

* The importance and outcome of built-in controls compared to usual internal and external analytical quality control are being discussed in SKUP for the moment. SKUP will probably rate built-in controls lower than usual internal QC in later corresponding evaluations. The evaluation form will be modified to better capture the difference in the various control possibilities.
** No control materials are available, therefore stability and storage of the quality control materials are not evaluated in the table. A possibility for Quality Control is parallel analysing with a comparison laboratory.

Table 22. Assessment of Operation facilities

Operation facilities	**0 point**	**1 point**	**2 point**
To prepare the test / instrument	Un-satisfactory	Less satisfactory	Satisfactory
To prepare the sample	Un-satisfactory	Less satisfactory	Satisfactory
Application of specimen	Un-satisfactory	Less satisfactory	Satisfactory
Specimen volume	Un-satisfactory	Less satisfactory	Satisfactory
Number of procedure step	Un-satisfactory	Less satisfactory	Satisfactory
Instrument / test design	Un-satisfactory	Less satisfactory	Satisfactory
Reading / Interpretation of the test result	Un-satisfactory	Less satisfactory	Satisfactory
Sources of errors	Un-satisfactory	Less satisfactory	Satisfactory
Cleaning / Maintenance	Un-satisfactory	Less satisfactory	Satisfactory
Hygiene, when using the test	Un-satisfactory	Less satisfactory	Satisfactory
Storage conditions for tests, unopened package	–20°C	+2 to +8°C	+15 to +30°C
Storage conditions for tests, opened package	–20°C	+2 to +8°C	+15 to +30°C
Environmental aspects: waste handling	Special precautions	Sorted waste	No precautions
Intended users	Biomedical scientists	Laboratory experienced	GP personnel or patients
Size and weight of package	Un-satisfactory	Less satisfactory	Satisfactory
Rating of operation facilities			Satisfactory

Comments:

- It would be nice if the instrument made a sound to indicate sufficient amount of sample material.
- One has to press the button many times to run a sample. This button has to be pushed rather hard. However the instruments can be turned on automatically by strip insertion.

The user-friendliness form and comments to these are shared between users in hospital laboratory and primary care.

7. References

1. ISO/FDIS 17593; Clinical laboratory testing and in vitro diagnostic test systems – In vitro monitoring systems for anticoagulation therapy self-testing.
2. Fraser, C.G. Biological variation: From principles to practice. 2006. Chapter 1 "The Nature of Biological Variation". AACC Press. ISBN 1-890883-49-2.
3. Petersen, P. H., Fraser C.G. et al. "Combination of analytical quality specifications based on biological within- and between-subject variation." Ann Clin Biochem 2002;**39:**543 – 50.
4. 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.
5. 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.
6. http://www.westgard.com/guest21.htm
   Biological Variation Database & Desirable Quality Specifications
   Carmen Ricos et al.Analytical Quality Commission of the Spanish Society of Clinical Chemistry and Molecular Pathology (SEQC). The 2001 Updates for PT-INR is referring to the next reference.
7. Ricos, C., V. Alvarez, et al. (1999)."Current databases on biological variation: pros, cons and progress." Scand J Clin Lab Invest **66** (4): 337 – 49.
8. 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.
9. Fraser C.G. Hyltoft Petersen P, Quality goals in external quality assessment are best based on biology, Scand J Clin Lab Invest 1993; 53 suppl 212. Chapter I. Quality planning.
10. 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.
11. Karin Kynde, Tim Woods, Piet Meijer, Ton v.d. Besselaar Report International INR Project European Committee for External Quality Assurance Programmes in Laboratory Medicine. DEKS, UK NEQAS for blood Coagulation, ECAT Foundation
12. International vocabulary of metrology – Basic and general concepts and associated terms, VIM, 3rd edition, JCGM 200:2008.
13. Burnett RW, "Accurate Estimation of Standard Deviations for Quantitative Methods Used in Clinical Chemistry". Clinical Chemistry 1975; 21 (13): 1935 – 1938.

Attachments

Attachment 1 Facts about the system
Attachment 2 Raw data, INRatio2, Hospital laboratory
Attachment 3 Raw data, INRatio2, Primary Care Centre
Attachment 4 Raw data, INRatio2, Primary Care Centre
Attachment 5 List of evaluations organised by SKUP
Attechment 6 Comments from Alere

Attachment 1 Facts about the system

a) Name of the analyser	***Alere INRatio®2 PT/INR Measurement System***

Physical dimensions	Length: 5.9 inches (15.1 cm) Width: 2.9 inches (7.4 cm) Height: 1.8 inches (4.6 cm) Weight: 9.3 oz. (263 g) with batteries
Manufacturer (with address)	Alere San Diego 9975 Summers Ridge Road San Diego, California 92121
Distributor (with address)	Triolab AS Vallensbækvej 35 DK-2605 Brøndby Phone +45 4396 0012 Fax +45 4396 4312 www.triolab.dk
	BERGMAN DIAGNOSTIKA AS Jogstadveien 21 N-2007 Kjeller, Norway www.bergmandiag.no Phone.: +47 6383 5750 Fax: +47 6383 5740
	Triolab AB Åbäcksgatan 6 Box 2109 SE-431 02 Mölndal, Sweden www.triolab.se Phone: 031-81 72 00 Fax: 031-81 72 19
	Triolab OY Lemminkäisenkatu 20 B FIN-20520 Turku, Finland www.triolab.fi Phone: +35 8201 226600 Fax: +35 8201 226601

b) Analysis menu, sample materials and sample volume

Component	Sample materials	Sample volume
PT/INR	*Whole Blood*	*15 uL*

c) Analysis principles (reference to the instruction manual)

Parameter	Principle
PT/INR	*Monitors the formation of the blood clot by measuring the electrical impedance.*

d) Measuring range

Component	Measuring range	Unit
PT/INR	*0,7 – 7,5 INR*	*INR*

e) Time for analysis per component (precisely stated)

Component	Pre-analysis time (with an explanation)	Measuring time
PT/INR	10-30 seconds warm up	~60 seconds

f) Calibration

Is calibration possible?	*No*
How often is calibration recommended?	*N/A*
Number of standards	*N/A*
Who should carry out calibration?	*N/A*

g) Recommended maintenance

Maintenance	How often?
None	

h) Control materials

Is control material available (from the producer or other companies)?	*No, there is not control materials available; however there is a built-in self-check in each test strip at two concentration levels. These are measured at the same time as the patient sample.*

i) Marketing

In which country is the analyser marketed?	*Worldwide*
When did the analyser first appear on the Scandinavian market?	*2010*
When did the analyser receive CE approval?	*2007*

j) Language

In which Scandinavian language is the manual?	*Danish, Norwegian, Swedish*

k) Memory

What is the storage capacity of the analyser and what is stored?	*120 results, time, date, lot number, Patient INR/PT and QC1/2 values*
Is it possible to identify patients?	*Only by using external hardware and software*
If yes, describe this:	

a) Name of the analyser	**Alere INRatio®2 PT/INR Measurement System**

l) Power supply

Electric network connection	*Yes*
Battery	*Yes*
If yes, which type and how many batteries	*4 x AA*

m) Electronic communication

Can a printer be connected to the analyser?	*Not currently but new INRatio®2 has printer function*
Can a barcode reader be connected to the analyser?	*no*
Interface	*yes*
If yes, which port is required?	*RS232*
Communication method	*RS232 Serial Communication*
Transfer mode	*RS232 Serial Communication*
Transfer protocol	*Alere Proprietary Protocol Data Access Interface Specification*

n) Standards and controls

	Standard	Control
Name	*N/A*	*N/A*
Volume		
Shelf life unopened		
Shelf life opened		
Any comments:		

o) Reagents

Component	Time and temperature, unopened	Time and temperature, opened
Test Strip	*12 Months from Manufacture 2-32^{o}C*	*10 minuts 10-32^{o}C*

p) Additional information

Attachment 5. List of previous SKUP evaluations

Summaries and complete reports from the evaluations are found at www.skup.nu and www.skup.dk

SKUP evaluations from number 51 and further

Evaluation no.	Component	Instrument/testkit	Producer
SKUP/2010/82*	Glucose, protein, blood, leukocytes, nitrite	Medi-Test URYXXON Stick 10 urine test strip and URYXXON Relax urine analyser	Macherey-Nagel GmBH & Co. KG
SKUP/2010/81*	Glucose	mylife PURA	Bionime Corporation
SKUP/2010/79*	Glucose, protein, blood, leukocytes, nitrite	CombiScreen 5SYS Plus urine test strip and CombiScan 100 urine analyser	Analyticon Biotechnologies AG
SKUP/2009/75	Glucose	Contour	Bayer HealthCare
SKUP/2009/74	Glucose[1]	Accu-Chec Mobile	Roche Diagnostics
SKUP/2010/73	Leukocytes	HemoCue WBC	HemoCue AB
SKUP/2008/72	Glucose[1]	*Confidential*	
SKUP/2009/71	Glucose[1]	GlucoMen LX	A. Menarini Diagnostics
SKUP/2008/69*	Strep A	Diaquick Strep A test	Dialab GmbH
SKUP/2008/66	Glucose[1]	DANA DiabeCare IISG	SOOIL Developement co. Ltd
SKUP/2008/65	HbA1c	Afinion HbA1c	Axis-Shield PoC AS
SKUP/2007/64	Glucose[1]	FreeStyle Lite	Abbott Laboratories
SKUP/2007/63	Glucose[1]	*Confidential*	
SKUP/2007/62*	Strep A	QuikRead	Orion Diagnostica Oy
SKUP/2008/61	CRP	i-CHROMA	BodiTech Med. Inc.
SKUP/2007/60	Glucose[1]	*Confidential*	
SKUP/2007/59	Glucose[1]	Ascensia BREEZE2	Bayer HealthCare
SKUP/2006/58	HbA1c	*Confidential*	
SKUP/2007/57*	PT (INR)	Simple Simon PT	Zafena AB
SKUP/2007/56*	PT (INR)	*Confidential*	
SKUP/2007/55	PT (INR)	CoaguChek XS	Roche Diagnostics
SKUP/2007/54*	Mononucleosis	*Confidential*	
SKUP/2006/53*	Strep A	*Confidential*	
SKUP/2005/52*	Strep A	Clearview Exact Strep A Dipstick	Applied Biotech, Inc.
SKUP/2005/51*	Glucose[1]	FreeStyle	Abbott Laboratories

*A report code followed by an asterisk, indicates that the evaluation for instance is a pre-marketing evaluation, and thereby confidential. A pre-marketing evaluation can result in a decision by the supplier not to launch the instrument onto the Scandinavian marked. If so, the evaluation remains confidential. The asterisk can also mark evaluations at special request from the supplier or evaluations that are not complete according to SKUP guidelines, e.g. the part performed by the intended users was not included in the protocol.

[1] Including a user-evaluation among diabetes patients

Grey area – The instrument is not in the Scandinavian market any more

SKUP evaluations from number 1 — 50

Evaluation no.	Component	Instrument/test kit	Producer
SKUP/2006/50	Glucose[1]	Glucocard X-Meter	Arkray, Inc.
SKUP/2006/49	Glucose[1]	Precision Xtra Plus	Abbott Laboratories
SKUP/2006/48	Glucose[1]	Accu-Chek Sensor	Roche Diagnostic
SKUP/2006/47	Haematology	Chempaq XBC	Chempaq
SKUP/2005/46*	PT (INR)	*Confidential*	
SKUP/2006/45	Glucose[1]	HemoCue Monitor	HemoCue AB
SKUP/2005/44	Glucose[1]	Accu-Chek Aviva	Roche Diagnostics
SKUP/2005/43	Glucose[1]	Accu-Chek Compact Plus	Roche Diagnostics
SKUP/2005/42*	Strep A	Twister Quick-Check Strep A	ACON laboratories, Inc.
SKUP/2006/41*	HbA1c	*Confidential*	
SKUP/2005/40	Glucose[1]	OneTouch GlucoTouch	LifeScan, Johnson &
SKUP/2005/39	Glucose[1]	OneTouch Ultra	LifeScan, Johnson &
SKUP/2004/38*	Glucose	GlucoSure Plus	Apex Biotechnology Corp.
SKUP/2004/37*	u-hCG	Quick response u-hCG	Wondsfo Biotech
SKUP/2004/36*	Strep A	Dtec Strep A testcard	UltiMed
SKUP/2004/35*	u-hCG	RapidVue u-hCG	Quidel Corporation
SKUP/2004/34*	u-hCG	QuickVue u-hCG	Quidel Corporation
SKUP/2004/33	PT (INR)	Hemochron Jr. Signature	ITC International
SKUP/2004/32*	Strep A	QuickVue In-Line Strep A test	Quidel Corporation
SKUP/2004/31*	PT (INR)	*Confidential*	
SKUP/2004/30	Glucose[1]	Ascensia Contour	Bayer Healthcare
SKUP/2004/29	Haemoglobin	Hemo_Control	EKF-diagnostic
SKUP/2003/28*	Strep A	QuickVue In-Line Strep A test	Quidel Corporation
SKUP/2003/27*	Strep A	QuickVue Dipstick Strep A test	Quidel Corporation
SKUP/2003/26*	HbA1c	*Confidential*	
SKUP/2003/25*	HbA1c	*Confidential*	
SKUP/2003/24*	Strep A	OSOM Strep A test	GenZyme, General Diag.
SKUP/2002/23*	Haematology with CRP	ABX Micros CRP	ABX Diagnostics
SKUP/2002/22	Glucose[1]	GlucoMen Glycó	Menarini Diagnostics
SKUP/2002/21	Glucose[1]	FreeStyle	TheraSense Inc.
SKUP/2002/20	Glucose	HemoCue 201	HemoCue AB
SKUP/2002/19*	PT(INR)	Reagents and calibrators	
SKUP/2002/18	Urine–Albumin	HemoCue	HemoCue AB
SKUP/2001/17	Haemoglobin	Biotest Hb	Biotest Medizin-technik GmbH
SKUP/2001/16*	Urine test strip	Aution Sticks and PocketChem UA	Arkray Factory Inc.
SKUP/2001/15*	Glucose	GlucoSure	Apex Biotechnology Corp.
SKUP/2001/14	Glucose	Precision Xtra	Medisense
SKUP/2001/13	SR	Microsed SR-system	ELECTA-LAB
SKUP/2001/12	CRP	QuikRead CRP	Orion
SKUP/2000/11	PT(INR)	ProTime	ITC International Technidyne Corp
SKUP/2000/10	PT(INR)	AvoSure PT	Avocet Medical Inc.
SKUP/2000/9	PT(INR)	Rapidpoint Coag	
SKUP/2000/8*	PT(INR)	Thrombotest/Thrombotrack	Axis-Shield

SKUP/2000/7	PT(INR)	CoaguChek S	Roche Diagnostics
SKUP/2000/6	Haematology	Sysmex KX-21	Sysmex Medical Electronics Co
SKUP/2000/5	Glucose	Accu-Chek Plus	Roche Diagnostics
SKUP/1999/4	HbA1c	DCA 2000	Bayer
SKUP/1999/3	HbA1c	NycoCard HbA1c	Axis-Shield PoC AS
SKUP/1999/2*	Glucose	Precision QID/Precision Plus Electrode, whole blood calibration	Medisense
SKUP/1999/1	Glucose	Precision G/Precision Plus Electrode, plasma calibration	Medisense

For comments regarding the evaluations, please see the indications on the first page

Attechment 6 Comments from Alere

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December 20, 2010

Comment to the SKUP Evaluation Report on INRatio®2

Dear Ladies and Gentlemen,

Thank you for taking the time to evaluate the Alere INRatio®2 PT/INR Monitoring System.

We are generally happy with your findings that the majority of the goals set in the protocol were fulfilled, although the overall imprecision was slightly higher than the 5% goal set by SKUP. We have noticed that the higher CV at one of the GP sites is mainly caused by high deviation on one specific day. What caused this we can only speculate, but pre-analytical factors could well have influenced the results. It is noted that CVs on subsequent days were seen to be much improved.

It is highly important to us, that SKUP judges the user-friendliness of the INRatio®2 system as completely satisfactory in primary care settings. The only comments by users were that the button was hard to push, which in the meantime has been remedied by redesign of the instruments with hard solenoid buttons. The comment that it was a bit difficult to know when the test strip was properly filled with blood is not a comment we see frequently, since the sample volume (15 µl) is generally easy to provide, if the patient is properly prepared (warm hands). As an option the 15 µl micro tubes can be used and will guarantee that the right blood amount to be applied to the strip.

Best regards
Alere GmbH

Dr. Wolfgang Teckentrup
Director Distribution Business EME

Jutta Kraenke
Business Development Manager EME