Simple Simon® PT

A system for measurement of prothrombin time [P—PT (INR)]
manufactured by Zafena AB, Sweden

Report from an evaluation
in a hospital laboratory
organised by SKUP

SKUP/2007/57*

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The organisation of SKUP

Scandinavian evaluation of laboratory equipment for primary health care, *SKUP*, is a co-operative commitment of NOKLUS [1] in Norway, "Afdeling BFG"[2] in Odense, 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 aim of SKUP is to produce reliable, objective and independent information about analytical quality and user-friendliness of laboratory equipment for primary healthcare. 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 of blood glucose. 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 summaries are distributed to physicians' offices, councils for laboratory medicine, laboratory instructors and healthcare authorities.

For a detailed list of previous SKUP evaluations, please see attachment 6.

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[1] NOKLUS (Norwegian Quality Improvement of Primary Care Laboratories) is an organisation funded by Kvalitetssikringsfond 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, in Bergen, Norway.

[2] "Afdeling for Biokemi, Farmakologi og Genetic" (Afdeling BFG) is the Department for Clinical Chemistry at the University Hospital in Odense, Denmark. "Afdeling BFG" in Odense and the national "Fagligt Udvalg vedrørende Almen Praksis" (Professional Committee for General Practice) have through an agreement created "the SKUP-division in Denmark". "Fagligt Udvalg vedrørende Almen Praksis"is a joint committee for "PLO", "Praktiserende Lægers Organisation" (General Practioners Organisation) and "Sygesikringens Forhandlingsudvalg" (Committee for Negotiations within the General Health Insurance System).

[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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1. Summary

Background
The Simple Simon® PT System is a measurement system for prothrombin time (PT), designed for near-patient testing. Simple Simon PT is a wet chemistry analysis procedure based on the Owren method. The Owren method is used in Scandinavian hospital laboratories. Simple Simon measures the activity of the vitamin-K dependent factors II, VII and X. The reagent comes freeze dried and is reconstituted in a buffer. The clot is detected optically. The sample is citrate anti-coagulated plasma or blood, or native whole blood. The sample volume is 10 µL. The measuring time is typically 60 seconds. The measuring range for PT (INR) is from 0,8 to 8,0.
A calibrated Simple Simon Reader, reagent components, tubes, stoppers, pipettes and pipette tips are delivered as a package deal product. When 1200 tests have been performed, a new lot of the complete product is put into use and the exhausted reader with its pipettes is returned for service.

The aim of the evaluation
The aim of the evaluation of Simple Simon PT is to assess the analytical quality achievable under standardised and optimal conditions by experienced laboratory trained personnel. Simple Simon PT was not evaluated under primary care conditions, as indicated by the asterisk behind the evaluation number.

Materials and methods
Blood samples of 73 outpatients on long-term oral anticoagulation therapy were collected in evacuated plastic tubes containing citrate anticoagulant. Of these patients, 23 also contributed a second sample at a second occasion, giving a total of 96 patient samples. The blood of the samples were analysed in duplicate on Simple Simon, the corresponding plasmas in duplicate on a comparison method. The first 29 samples were analysed on a lot of Simple Simon calibrated with samples of in-patients at one hospital laboratory, the remaining 67 samples on a lot calibrated with samples of out-patients at eight hospital laboratories. All data was used in assessing precision, but only those of the second lot in assessing bias and accuracy. The designated comparison method was a PT method with SPA reagent on a STA Compact instrument, both from Stago, calibrated with calibrators from EQUALIS.
The analytical quality goal of SKUP for PT is: Repeatability CV <5 % and a total error <±20 %.

Results
The precision of Simple Simon PT was good, with a repeatability CV of approximately 3 % for INR values >2, slightly higher at INR values <2. Simple Simon showed a small positive bias relative to the comparison method. The bias (in INR) was approximately +0,1 in the therapeutic range. The accuracy was good, in spite of the small bias. The analytical quality goals of SKUP were attained.

Conclusion
The analytical quality of Simple Simon PT is good, as demonstrated by skilled laboratory personnel under optimal conditions. The analytical quality goals of SKUP are attained. The user-friendliness is good, but the system requires some training to attain optimal analytical quality. The performance of Simple Simon PT in the hands of primary care users was not examined.

Comments from the manufacturer
For comments from Zafena AB, please see attachment 5.

2. Analytical quality goals

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

At present, there are no generally recognised analytical quality goals for the determination of prothrombin time (PT), and no international (Gold) Standard for evaluation of Point of Care test instruments for the PT measurement in primary health care.
The new ISO-standard for anticoagulant therapy self-testing [1] is still under development. According to SKUP, the coming ISO-standard has too tolerant quality goals. In our 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 then voted no to the final suggestion.

Setting quality goals on the basis of biological variation is an acknowledged method [2, 3].
It is recommended that analytical imprecision should be less than, or equal to, half the intra-individual biological variation. Ricos et al. [4] state the biological variation for PT (INR) as 4 % (CV_{bw}) and 6,8 % (CV_{bb}). According to Kjeldsen, Lassen et al. [5], the "in-treatment within-subject biological variation" of PT (INR) is 10,1 % (CV_{bw}). For systems used for monitoring, the analytical performance should aim at low imprecision compared with the within-subject biological variation ($CV_a \leq 1/2\ CV_{bw}$) [6].

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 PT (INR) [7]:
Bias ≤±6 % and reproducibility ≤5 % (CV) for instruments used in primary health care, and bias ≤±3 % and reproducibility ≤3 % (CV) 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 PT (INR), and the fact that anticoagulant devices are designed for *monitoring* PT (INR), SKUP recommends that these instruments should achieve repeatability below 5 % (CV). SKUP has not taken out a separate goal for the bias, but on the other hand sets 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 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 the PT (INR) level 2,5. Accordingly, the bias should not exceed ±5 % at the same PT-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 various comparison methods are not likely to give exactly the same PT-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 to the total error to a CV of 3 %.

When comparing two different PT (INR) methods, either both methods use Owren-based reagents, or especially when one of the methods is a ”Quick-method”, there is often a certain “interference” or matrix-effect which will manifest itself. When comparing PT-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 put the probable matrix effect in 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 % CV).

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

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

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

The analytical quality goals of SKUP for PT (INR) are

Repeatability, CV_a: <5 %
Total error: <±20 %

It is accepted that up to 5 % of the results can deviate more than ±20 %. Only 1 % of the results should deviate more than ±25 %. The results achieved with Simple Simon PT will be discussed and evaluated in proportion to these quality goals.

3. Materials and methods

3.1. The prothrombin time test [P—PT (INR]

The Scientific Division of IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) together with IUPAC (International Union of Pure and Applied Chemistry) cooperate in the committee "Nomenclature, Properties and Units (C-NPU)". The committee has defined most diagnostic tests in the NPU database. The prothrombin time test [P—PT (INR)] is internationally performed according to two different method principles, namely the Owren method and the Quick method. The Scandinavian hospital laboratories use wet chemistry analysis procedures based on the Owren method. In other parts of the world the PT method according to Quick is dominating. The main difference between Owren and Quick methods is the extent of sample dilution and the sensitivity towards Factor V and fibrinogen. The final plasma dilution in the Owren method is 1:21, whereas the authentic Quick method has a sample dilution of 1:3. The Owren method gives a measure of the activity in plasma of the vitamin-K dependent coagulation factors II, VII and X, whereas the Quick method is sensitive for Factor II, V, VII and X and fibrinogen (Factor I).
The NPU data base defines the prothrombin time test [P—PT (INR)] according to Owren and Quick as follows:

Method	Formal full name of test	NPU code
Owren	P—Coagulation, tissue factor-induced; relative time (actual/normal; INR; IRP 67/40; procedure)	NPU01685
Quick	P—Coagulation, tissue factor-induced; relative time (actual/normal; INR; IRP 67/40; II+V+VII+X)	NPU21717

3.2. The product Simple Simon® PT

3.2.1. *Description of Simple Simon® PT*

Simple Simon® PT (SSPT) is intended for near patient-testing of prothrombin time (PT) in smaller hospital laboratories, primary health care centres and doctors' offices. Simple Simon specifically measures the activity of the K-vitamin dependent coagulation factors II, VII and X, and is suited for monitoring of anticoagulation treatment with K-vitamin antagonists such as warfarin. SSPT is a wet chemistry analysis procedure analysing PT according to the method of Owren. The thromboplastin of the reagent comes from rabbit brain, and the fibrinogen and factor V from bovine plasma. The reagent is freeze dried and is reconstituted by adding a pre-portioned volume of buffer. The SSPT analysis is always performed with 10 µL of sample and 200 µL of reagent, i.e. a final sample dilution of 1:21. The sample may interchangeably be citrated anti-coagulated plasma, citrate anti-coagulated blood or native whole blood. Freeze dried control plasmas as well as blood or plasma controls are well suited as control materials for SSPT.

The portable Simple Simon Reader is battery-operated and will, maintenance-free, perform 1200 tests. The reader automatically determines the nature of the sample - blood or plasma. If the sample is blood the fraction of red cells, the EVF, is automatically estimated. The reader determines the coagulation time and the temperature at which the reaction is performed. At the

clotting point, the microprocessor of the reader calculates the PT result from the clotting time, the temperature and the EVF. The results are displayed in International Normalized Ratio, INR.
The Simple Simon PT product is calibrated against authentic patient samples with PT (INR) values determined at Scandinavian hospital laboratories, where equipment is calibrated against materials from EQUALIS, the External Quality Assurance in Laboratory Medicine in Sweden, or DEKS, the Danish Institute for External Quality Assurance for Hospital Laboratories. More information about the calibration procedures is found in attachment 5, comments from the producer.

A calibrated Simple Simon Reader, reagent components, reaction tubes and stoppers, pipettes and pipette tips are delivered as a package deal product. The product and its components have the same lot number and expiry date. When 1200 tests have been performed on a reader, a freshly serviced reader with new pipettes is put into use. The exhausted reader with its pipettes is returned for service. A change to a new lot of reagent, equivalent to a new lot of product, always constitutes a change to a fresh reader. The intention is to provide the users with the same lot of reagent for about a year.

3.2.2. *Product information, Simple Simon PT*

SSPT is manufactured by:

Zafena AB	Phone: +46 141 40520
Husbyvägen 16	Mobil: +46 736 22 94 84
590 31 Borensberg	e-mail: mats@zafena.se
Sweden	Internet: www.zafena.se

SSPT is represented in Scandinavia by:

Medic Danmark	Phone: +45 3692 8300, +45 6140 5140
Tune Erhvervspark	Fax: +45 3692 8330
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DK-4000 Roskilde	Internet: www.medic24.dk
Medic Norge as	Phone: +47 35 50 48 60
Hagebyveien 39, Kjørbekk	Fax: +47 35 50 48 61
P.O.Box 2513	e-mail: pia.virik.moldestad@medic24.no
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Kuskvägen 8	E-mail: info@ils-laboratories.se
191 92 Sollentuna	Internet: www.ils-laboratories.se
Sweden	

SSPT instruments
Lot number G024M1, Reader number 62, used from 09.05.2006 until 20.06.2006
Lot number G024M1, Reader number 67 (reserve)

Lot number G185M1, reader number 72, used from 28.06.2006 and trough out the evaluation period
Lot number G185M1, reader number 74 (reserve)

Reagent and diluent
SSPT reagent, Lot G024M1, expiry date 10-2007, used from 09.05.2006 until 20.06.2006
SSPT diluent, Lot G024M1, expiry date 10-2007
SSPT reagent, Lot G185M1, expiry date 11-2007, used from 28.06.2006 and trough out the evaluation period
SSPT diluent, Lot G185M1, expiry date 11-2007

Pipettes
SSPT 10 µL, SSPT 200 µL

Quality Control materials from MediRox AB
NKP, Normal Control Plasma Coagulation, GHI 162. Lot F251N, expiry date 06-2007
OKP, Abnormal Control Plasma Coagulation, GHI 167B. Lot E461A, expiry date 11-2006

3.2.3. *Technical data*
Technical data from the manufacturer is shown in Table 1.

Table 1. Technical data from the manufacturer.

TECHNICAL DATA FOR SIMPLE SIMON PT	
Working temperature	+17 — +45 °C
Sample materials	Citrate anti-coagulated plasma or blood, native whole blood
Blood sample size	10 µL
Units	INR (a ratio without units), seconds, EVF and ^{o}C
Measuring time	Typically 60 seconds (blood INR 2,5, 24 ^{o}C)
Measuring range	PT (INR) 0,8 — 8,0
International sensitivity index, ISI	Typical ISI is 1,25 (blood at 24 ^{o}C)
Thromboplastin	Rabbit brain
Memory	The latest results are stored in the memory of the reader (the results can be transferred to a computer through a USB connection)
Power supply	Three AAA batteries
Operating time with battery	Approximately 1200 tests
Meter size	Length x width x height: 145 x 100 x 75mm
LCD Size	Width x height: 65 x 13mm
Weight	640g

3.3. The designated comparison method

3.3.1. *Definition*

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.

3.3.2. *Description of the designated comparison method in this evaluation*

The automated laboratory instrument STA Compact® (Diagnostica Stago, France) using the SPA-reagent (Diagnostica Stago, France) was assigned to be the comparison method in this evaluation. This method is the routine method for the determination of PT (INR) in the laboratory at Haraldsplass Diaconal Hospital (HDS), and the laboratory leader and the staff agreed to take the responsibility for the practical work connected with the evaluation.
The SPA reagent is a combined rabbit brain thromboplastin. The final dilution of the citrate plasma is 1:21. The method is sensitive for decreased activity of Factor II, VII and X. The method is calibrated with calibrators from EQUALIS, traceable to the reference thromboplastin RBT/90 from WHO. The comparison method is an Owren method, and the most used method at Norwegian hospitals for measurement of PT (INR). Setting Thrombotest aside, all hospital methods in Norway are calibrated with the EQUALIS INR calibrators.

3.3.3. *Procedures at the laboratory at HDS*

Fresh SPA reagent is made every morning. Possible reagent leftovers from the night are disregarded. When the instrument needs calibration, freshly made reagent is kept at room temperature for four hours in advance, according to the guidelines. The daily internal quality control is performed with Scandinorm and Scandipath from Stago. To monitor the stability of the SPA reagent during the day, and the stability of the PT level over time, a human plasma pool produced in the laboratory at HDS is continuously analysed day and night. The pool is made of freshly frozen citrate plasma with a PT (INR) at approximately 3. An aliquot of the control is thawed every morning and is placed in the STA Compact instrument for the next 24 hours. The control results slightly changes trough the day, giving a poorer CV than with freshly thawed controls, or if results achieved at the same time of each day are compared. The plasma pool results are primarily used to reveal any systematic shift in PT-level over time.
During the evaluation period, two lots of reagent were used. The system was calibrated in April, before the evaluation started, and recalibrated 17.08.06 when the reagent lot was changed.

3.3.4. *Product information, the comparison method*

Instrument

STA Compact® from Diagnostica Stago, France. Serial no. 6120561.

Reagent

STA-SPA 50 reagent from Diagnostica Stago, France, for the determination of the combined Factors II-VII-X on STA Compact instruments.
Lot no. 50281 was used from the evaluation started in May until 17.08.2006. This lot of reagent was calibrated at the instrument 04.04.2006 with calibrators from EQUALIS.
Lot no. 51751, with expiry date 2007-06, was used from 17.08.2006 and throughout the rest of the evaluation period. This lot of reagent was calibrated 17.08.2006.

Calibrators

EQUALIS calibration kit for P—PT (INR) according to Owren

Calibrator Low, lot no. 13, expiry date 2006-11
Certified PT (INR) value: 1,096 ±0,093 (95 % CI)

Calibrator High, lot no. 14, expiry date 2006-11
Certified PT (INR) value: 3,63 ±0,45 (95 % CI)

Control, lot no. 15, expiry date 2006-11
Certified PT (INR) value: 2,95 ±0,34 (95 % CI)

Internal quality control
Scandinorm
Producer: Diagnostica Stago, France. Lot no. 50187 was used from the start of the evaluation until11.08.06. Expiry date 2007-01. Stated PT (INR) value from producer: 1,00. Internal PT (INR) target value at the laboratory at HDS: 0,98 ±0,078.
Lot no. 52501 was used from 11.08.2006 and throughout the rest of the evaluation period. Stated PT (INR) value from producer: 0,95. Preliminary internal PT (INR) target value in the laboratory at HDS: 0,93 ±0,074. From 03.10.2006 the internal PT (INR) target value was adjusted to 0,967.

Scandipath
Producer: Stago, France. Lot no. 50811 was used from the start of the evaluation until 05.09.2006. Expiry date 2007-03. Stated PT (INR) value from producer: 2,85.
PT (INR) internal target value at the laboratory at HDS: 2,55 ±0,204.
Lot no. 60103 was used from 05.09.06 and throughout the rest of the evaluation period. Expiry date: 2008-01. Stated PT (INR) value from producer: 3,05. Preliminary internal target value in the laboratory at HDS; PT (INR) = 2,75 ±0,220. From 03.10.06 the PT (INR) internal target value was adjusted to 2,852 ±0,228.

Patient control/Stability control
The stability control is a patient plasma pool, freshly frozen after sampling.
Lot no. 1/2006 was used from May until 09.07.06. PT (INR) = 3,59 ±0,359.
Lot no. 2/2006 was used from 09.07.06 until 05.10.06. PT (INR) = 3,00 ±0,300.
Lot no. 3/2006 was used from 05.10.06. PT (INR) = 3,12 ±0,312.

Coagulation sodium citrate tubes
Vacuette, evacuated 2 mL 3,2 % (0,105 mol/L) Sodium Citrate tubes from Greiner.

3.3.5. *The analytical quality of the comparison method*
The analytical quality of the comparison method is demonstrated by means of the patient samples in the evaluation, together with different control and calibrating materials.
Repeatability is shown by means of 96 patient samples. Each patient sample in the evaluation was analyzed in duplicate.
Daily internal quality control is performed with Scandinorm and Scandipath from Diagnostica Stago. Scandinorm is freeze-dried, citrated normal human plasma. Scandipath is freeze-dried, citrated abnormal human plasma. The laboratory at HDS sets up their own target values for the

two controls. As well as monitoring the daily analytical quality, the results achieved over time with the two control materials can give a picture of the reproducibility of the method.
To monitor the stability of the reagent during the day, and the stability of the PT level over time, a human plasma pool produced at the laboratory at HDS is continuously analysed day and night. The pool is made of freshly frozen plasma and has a PT (INR) value at approximately 3.

There is no Gold Standard or a real true value for PT (INR). The PT values will depend both on the choice of reagent, the calibrators and the instrument. The designated comparison method at the laboratory at HDS is the most used PT (INR) system in Norway, with SPA reagent at a Stago instrument, calibrated with calibrators from EQUALIS. The following materials have been analyzed to demonstrate the PT (INR) level of the comparison method:

PT (INR) calibrators from EQUALIS
The calibration kit from EQUALIS consists of two PT (INR) calibrators and one control.
The three materials are manufactured by MediRox AB. Each material is a pool of citrated anti-coagulated freeze-dried plasma of human origin (Swedish donors), supplied in a siliconised glass bottle sealed with a rubber stopper and an outer plastic screw cap. The certified values are traceable to an internationally agreed reference measurement procedure (WHO's manual tilt tube technique) and the reference thromboplastin WHO RBT/90 [8, 9]. The procedures used to assign values are described in several publications and documents [10, 11, 12].

PT (INR) calibrators from DEKS, the Danish Institute for External Quality Assurance for Hospital Laboratories
The calibration materials from DEKS are freshly frozen pooled citrate-plasmas which serve as national reference plasmas. The assigned value of the so called ISI calibrator is the mean value obtained by testing with manual tilt tube technique against international reference preparations of thromboplastins; BCT/099 (human plain), OBT/79 (bovine combined), RBT/79 (rabbit plain) and CRM 149R (rabbit plain). The value of later calibrators is compared with the previous calibrator. The normal calibrator was assigned a "consensus" value of PT (INR) of 1,0. Today, the ISI calibrator has been replaced by two frozen pools of plasmas, one at the therapeutic level of PT (INR) between 2 and 3 and one at a higher PT (INR) level about 4.

PT (INR) Controls produced at NOKLUS
NOKLUS produces control materials at regular intervals for the Norwegian external quality assessment scheme. The materials are freshly frozen pooled citrate-plasma from Norwegian donors. The NOKLUS controls "White" and "Blue" were available for SKUP in this evaluation. Control batch white 20904 has been used in eight different surveys and control batch blue 20805 has been used in three surveys. The INR-value of the controls used by NOKLUS in the surveys is the overall method-mean achieved in the external quality assessment scheme. In addition, method-mean values are calculated separately according to different types of reagent in use. More than 40 laboratories participate in the "SPA-group", and 20 hospital laboratories form the part using Nycotest PT reagent.

3.4. Planning of the evaluation

3.4.1. *Background for the evaluation*

Simple Simon PT is a measurement system for the prothrombin time (PT), designed for near-patient testing. SSPT is a wet chemistry analysis procedure based on the Owren method, which is the method used in the Scandinavian hospital laboratories. SSPT measures the activity of the vitamin-K dependent factors II, VII and X, and is suited for monitoring of anticoagulation treatment with K-vitamin antagonists such as warfarin.
Zafena is launching the new instrument into the Scandinavian market and wanted to demonstrate the analytical quality and user friendliness of the system in a SKUP evaluation.

3.4.2. *Arrangements about the evaluation*

In February 2006 SKUP contacted Zafena AB by letter informing about two PT (INR) evaluation projects under the direction of SKUP. Zafena was invited to join the two approaching evaluations. Zafena accepted the invitation in March and an informal agreement about the evaluation was made shortly after. A meeting was held at NOKLUS Centre in the end of March, where the evaluation was discussed and prepared and the practical training with SSPT was done. The equipment necessary for the evaluation was supplied from Medic in April. A preliminary protocol for the evaluation was sent to Zafena in May. The protocol was agreed upon after a period with professional discussions. The equipment necessary for the evaluation was received at NOKLUS Centre in April. The contract for the evaluation was set up in May, and the first patients enrolled in the study at the same time. The contract was signed later in the summer.

3.4.3. *Evaluation sites and persons involved*

According to the SKUP model for evaluations of laboratory equipment for primary care, an evaluation should be made under standardised and optimal conditions in a hospital laboratory by qualified laboratory-educated personnel, as well as under real-life conditions in the hands of the intended users at primary care centres. Generally, at least two primary care centres participate in the evaluation. After some discussion, Zafena decided that the evaluation should be carried out without the participation of the end users (see attachment 5, comments from Zafena AB). The basic aim of Zafena was to get a comparison of the PT (INR) level of the new instrument with a "typical" Norwegian hospital PT (INR) level. The evaluation of SSPT was performed at the laboratory of Haraldsplass Diaconal Hospital (HDS) in Bergen in the period May – November 2006.
A survey of the persons responsible for the various parts of the evaluation is given in table 2 on the next page.

Table 2. Persons responsible for various parts of the evaluation

Marte Hammersland Eli Vik Skare Kjersti Østrem	Biomedical laboratory scientists at the laboratory, HDS	Collected the patient blood samples in the laboratory at HDS
Solveig Heimark	Secretary at the laboratory, HDS	Coordinator of the patient consultations and the sampling at the laboratory at HDS
Anne Elisabeth Solsvik	Biomedical laboratory scientist at the laboratory, HDS	Quality manager in the laboratory at HDS and responsible for the evaluation in the laboratory
Grete Monsen	Biomedical laboratory scientist, Project manager for SKUP	Responsible for the evaluation. Carried out the measurements on SSPT at NOKLUS Centre Author of this report
Arne Mårtensson	Clinical Biochemist, coordinator for SKUP in Sweden	Carried out the statistical calculations of the SSPT data
Una Sølvik	Associate professor	Carried out the measurements at SSPT at NOKLUS Centre
Marie Danielsson	Quality assurance, Zafena AB	Demonstrated SSPT and trained the evaluators
Mats Rånby	VD, Zafena AB	Ordered the evaluation. Demonstrated SSPT and trained the evaluators
Kjell Myrseth Pia Virik Moldestad	Marketing manager, Medic Norge as Product specialist, Medic Norge as	Suppliers of SSPT in Norway. Delivered the equipment necessary for the evaluation

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3.4.4. *Recruitment of patients*

The SKUP evaluation model describes an evaluation involving a total of 100 patient samples. The plan was to enrol 100 outpatients attending laboratory PT monitoring, and ensure that all of them were on long-term, stabilized oral anticoagulant treatment (OAT). It soon became clear, however, that these patients, in a much larger extent than only a few years ago, were not attending the hospital outpatient clinics any more, but get their PT monitored at the primary care centres by their general practitioner (GP). Unfortunately, the recruitment of patients did not progress as fast as hoped for. In addition, the summer holiday period lay ahead for laboratory staff, patients and coordinators. Under these circumstances, a letter was sent to some GPs having an agreement to collaborate with the laboratory at HDS asking for help to recruit some of their

OAT patients. At the same time, an advertisement was composed for the daily press. In the newspaper announcement the patients in the primary health care were asked to volunteer for the evaluation study. Samples from 15 hospitalized patients were also collected and included at that time, to add to the total number of samples. The results from the hospitalized patients were rejected later on, and are not part of the final data set.
The letter did not pay off, but approximately 25 extra patients were recruited as a result of the advertisement in the daily press. The intensive recruiting efforts resulted in a total of 73 patients. 23 of these patients showed up twice in the hospital outpatient clinic during the evaluation period, and were allowed to participate for a second time. The 23 results from the second consultation are included in the calculations of the imprecision, but are excluded with regard to the calculation of accuracy and trueness, to avoid the potential risk of an influence in double dose of matrix effects of single patients.

3.5. Evaluation procedure

3.5.1. *Training*

Mats Rånby and Marie Danielsson from Zafena AB in Sweden came to NOKLUS Centre at the end of March to demonstrate SSPT and train the evaluators. Present at the demonstration and training were Grete Monsen and Una Sølvik from NOKLUS Centre, the quality leader of the laboratory at HDS, Anne Elisabeth Solsvik, and Eli Vik Skare and Kjersti Østreim, two biomedical laboratory scientists designated to collect all the blood samples and perhaps also do some of the measurements on SSPT.

3.5.2. *Evaluation procedure in the hospital laboratory (standardised and optimal conditions)*

For practical reasons, SSPT was placed at the laboratory at NOKLUS Centre during the evaluation, and the practical work was performed at NOKLUS by two biomedical laboratory scientists/laboratory educated personnel at NOKLUS (Una Sølvik and Grete Monsen) who had previously received thorough training. The evaluation was done in exact accordance to the protocol and user manual. All possible disturbances of, and interferences with the measurements were tried kept at a minimum. The evaluation under standardised and optimal conditions documents the quality of the system under as good conditions as possible.

3.5.3. *Sampling and sample-handling*

The patients who enrolled in the evaluation were on long-term, stabilized oral anticoagulant treatment. The collection of the samples was made in the outpatient clinic at the laboratory at HDS. For the comparison method, venous blood was drawn in an evacuated tube (3,2 % sodium citrate). A similar tube was collected for SSPT. The sampling as well as further treatment of the samples followed the internal routines of the laboratory in detail. Continuously after the sampling, and always within two hours, the samples for the comparison method were centrifuged for 15 minutes at 2500 g. Plasma was separated and placed in the instrument directly. The samples were included among the routine PT-analysis at the laboratory. Unlike the routine samples, the samples for the evaluation were measured in duplicate, simply by ordering a rerun for these series. As a rule, the PT-results from the comparison method were available within two hours after the sampling had taken place. The venous samples for Simple Simon were picked up at the laboratory and brought to NOKLUS Centre two floors up within 15 minutes, where the

measurements were performed with citrated whole-blood on Simple Simon within approximately 30 minutes, and always within two hours.

3.5.4. *Quality control, SSPT*

To monitor the quality of the measurements on SSPT during the evaluation period, two control materials from MediRox, supplied by Zafena and Medic, were used. The two materials are freeze-dried plasmas stored in siliconized glass bottles. The NKP control is citrated plasma with normal PT (INR) and the OKP control is citrated plasma with abnormal PT (INR). The substance is reconstituted with 1 ml of high quality water. Approximately 15 minutes after the reconstitution the control plasma is ready for use. According to the package insert, the controls are stable for 12 hours after reconstitution, when stored at room temperature. The two controls were analysed continuously during the evaluation period and every time new reagent vials were reconstituted (see section 5.2.2).

4. Statistical expressions and calculations

4.1. Statistical terms and expressions

4.1.1. *Precision*

The often used terms within-series imprecision and between-series imprecision are often misinterpreted. Especially the terms between-series and between-day imprecision are often not precisely defined. In this report, the terms are replaced by *repeatability and reproducibility*. 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. The two terms are measured as imprecision. Precision is descriptive in general terms (good, acceptable and poor e.g.), whereas imprecision is expressed by means of the standard deviation (SD) or coefficient of variation (CV). The standard deviation is reported in the same unit as the analytical result and CV is usually reported in percent. The imprecision will be summarised in tables.

4.1.2. *Accuracy*

Accuracy is the closeness of agreement between the result of one measurement and the true value. Inaccuracy is a measure of the deviation of a single measurements from a 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 will be illustrated in a difference-plot with quality goals for the total error shown as deviation limits in percent.

4.1.3. *Trueness*

Trueness is the agreement between an average value obtained from a large number of measuring results and a true value. Trueness is measured as bias (systematic errors). Trueness is descriptive in general terms (good, acceptable and poor e.g.), whereas bias is the estimate, reported in the same unit as the analytical result or in %. The bias at different PT (INR) levels will be summarised in tables.

4.2. Statistical calculations

4.2.1. *Number of samples*

Samples from 73 different outpatients were collected in the hospital outpatient clinic. Of these patients, 23 showed up twice during the evaluation period and were allowed to donate a second sample for the evaluation. As an outset, this gives a total number of samples of 96. For details about the recruitment of an adequate number of patients, see section 3.4.4.

When the first 29 results were examined closer, the SSPT product lot number was changed due to a positive bias compared to the comparison method. The evaluation then proceeded and 67 samples were measured with the new lot of reagent, including the 23 patients that showed up for a second time. This gives the following number of results for the different statistical calculations, before possible outliers are excluded:

Precision
96 duplicate results for the calculation of the imprecision of SSPT.

Trueness/bias
The calculation of bias is based on 44 duplicate results (67 minus 23) on SSPT and 44 corresponding duplicate results on the comparison method. Only the results achieved with the second lot of SSPT are included in these calculations. The 23 results from patients that showed up twice are not included in the calculation.

Accuracy/total error
67 results from the second lot are shown in the figure showing accuracy/total error. 23 of these results are from the second consultation, marked with a differentiating symbol and not included in the counting for the quality goal.

4.2.2. *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 %, so also in this evaluation. Where the results are classified according to different PT (INR) levels, the outlier-testing is done at each level separately. Statistical outliers are excluded from the calculations. Possible outliers will be commented below each table.

4.2.3. *Missing or excluded results*

One outlier is excluded in the calculation of imprecision. Only the results achieved with the new lot of SSPT are included in the assessment of trueness and accuracy. No further results are missing or excluded.

4.2.4. *Calculations of imprecision based on duplicate results*

The imprecision was calculated by use of paired measurements, based on the following formula:

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

Even if this formula is based on the differences between the two measurements of every duplicate, the calculated standard deviation is still a measure of the imprecision of single values, and completely comparable with the more generally used calculation based on repeated measurements of only one sample. The assumption for using this formula is that no systematic difference between the 1st and the 2nd measurements of the duplicates is acceptable.
Table 3 shows that there is no systematic difference in PT values between the 1st and the 2nd measurements on SSPT in this evaluation.

Table 3. Comparison of the 1st and the 2nd measurement. T-test for paired values.

	PT (INR) level	**PT (INR) mean 1st measurement**	**PT (INR) mean 2nd measurement**	**PT (INR) mean difference 2nd – 1st measurement (95 % CI)**	**n**
Simple Simon PT	<2,0	1,695	1,718	+0,023 (–0,007 — +0,053)	10
	2,0 — 3,5	2,755	2,761	+0,006 (–0,022 — +0,034)	69
	>3,5	4,040	4,018	–0,023 (–0,067 — +0,022)	16

4.2.5. *Calculation of trueness*

To measure the trueness of the results on SSPT, the average bias at three levels of PT (INR) is calculated based on the results obtained under standardised and optimal measuring conditions. A paired t-test is used with the mean values of the duplicate results on the comparison method and the mean values on SSPT. The mean difference is shown with a 95 % confidential interval.

4.2.6. *Calculation of accuracy*

To evaluate the accuracy of the results on SSPT, the agreement between SSPT and the comparison method is illustrated in a difference plot. In the plot 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 by SSPT and the mean value of the duplicate results at the comparison method.

5. Results and discussion

5.1. Analytical quality of the designated comparison method

5.1.1. *The precision of the comparison method*

The repeatability of the comparison method is demonstrated by means of the patient samples in the evaluation. Each of the 96 samples was analysed in duplicate. The results are divided in three groups according to the PT level, and the calculation of the repeatability is done for each level separately. There are only four samples with PT (INR) >3,5 at the comparison method. To be able to differentiate between the repeatability at the therapeutic level and a higher PT level, the cut off value between the two levels is set at PT (INR) = 3,0.
The reproducibility is demonstrated by means of the internal controls Scandinorm and Scandipath.

Internal quality control with Scandinorm and Scandipath
Scandinorm and Scandipath were always analysed on the comparison method together with the samples from the evaluation. In addition, the two internal control materials were analysed by routine at the laboratory several times during the day, giving a considerably number of control results. Only the results connected to the evaluation are included in the calculations of the reproducibility.

The reproducibility of the comparison method can be calculated from the results of the patient control produced at the laboratory for monitoring the stability of the method. As discussed in section 3.3.3, the variation with the patient control covers more than the actually method reproducibility, and gives a higher CV than with freshly thawed materials.

The repeatability of the comparison method is shown in Table 4 on the next page.
The raw data is shown in attachment 1.

Internal quality control results and reproducibility with Scandinorm and Scandipath are shown in Table 5.
Raw data is shown in attachment 2.

The reproducibility of the comparison method with the patient control material is shown in Table 6.

Table 4. Repeatability with patient samples, the comparison method.

PT (INR) level	PT (INR) average (range)	CV % (95 % C.I.)	n	Outliers
<2,0	1,7 (1,1 — 2,0)	1,2 (0,9 — 1,9)	17	0
2,0 — 3,0	2,5 (2,0 — 3,0)	1,5 (1,3 — 1,8)	63	0
>3,0	3,4 (3,0 — 4,6)	1,4 (1,0 — 2,1)	16	0

Table 5. Reproducibility with freeze dried control materials, the comparison method.

Control	PT (INR) target value from producer	Period	PT (INR) achieved value	CV % (95 % C.I.)	n
Scandinorm	1,00	09.05.06 — 10.08.06	0,97	1,6 (1,3 — 1,9)	64
	0,95	21.08.06 — 15.11.06	0,96	2,5 (2,1 — 3,1)	53
Scandipath	2,85	09.05.06 — 21.08.06	2,53	2,7 (2,3 — 3,2)	75
	3,05	21.08.06 —15.11.06	2,81	4,4 (3,7 — 5,4)	53

Table 6. Reproducibility with patient control material, the comparison method.

Period	Lot no.	PT (INR) internal target value	CV % (95 % C.I.)	n	Outliers
May - July	1/2006	3,59 ±0,359	4,2 (4,0 — 4,6)	390	0
August - October	2/2006	3,00 ±0,300	4,2 (3,8 — 4,6)	225	0
October - November	3/2006	3,12 ±0,312	4,6 (4,2 — 4,9)	311	0

Discussion

The precision of the comparison method is good. The repeatability CV is between 1,0 and 1,5 %. With freeze dried control materials the reproducibility CV is between 2 and 4 %. All internal control results were within the stated limits for the controls.

The CV achieved with the patient control material is approximately 4,5 %. The CV achieved with the patient control would be considerably lower if the control was freshly thawed each time, but this is not the purpose of this control (se section 3.3.3).

The trueness of the comparison method
To demonstrate the trueness of the comparison method, the calibrators from EQUALIS were analysed as anonymous samples at three different occasions in the evaluation period. The Danish calibrators from DEKS and NOKLUS control materials have also been analysed.
The results achieved with EQUALIS calibrators are shown in Table 7.
The results with DEKS calibrators and the control materials from NOKLUS are shown in table 8.

Table 7. EQUALIS calibrators measured on the comparison method.

Material	PT (INR) Certified value	Date	PT (INR) Comparison method, average value	n
EQUALIS INR calibrator Low	1,096 ±0,093	22.05.06	1,07	3
		08.09.06	1,10	3
		07.11.06	1,08	2
EQUALIS INR calibrator High	3,63 ±0,45	22.05.06	3,46	3
		08.09.06	3,45	3
		07.11.06	3,28	2
EQUALIS INR control	2,95 ±0,34	22.05.06	2,81	3
		08.09.06	2,87	3
		07.11.06	2,72	2

Table 8. DEKS calibrators and NOKLUS Control materials on the comparison method.

Material	PT (INR) assigned value	Date	PT (INR) comparison method, average value	n
DEKS INR calibrator Normal	0,96 ±0,026	19.05.06	0,96	3
		08.09.06	1,01	1
		07.11.06	0,95	2
DEKS INR calibrator Therapeutic	2,30 ±0,09	19.05.06	2,16	3
		08.09.06	2,23	2
		07.11.06	2,06	2
DEKS INR calibrator High	3,92 ±0,22	19.05.06	3,49	3
		08.09.06	3,45	2
		07.11.06	3,24	2
NOKLUS control White*	2,0** 2,1***	08.09.06	1,88	2
		07.11.06	1,80	2
NOKLUS control Blue*	3,0** 3,2***	08.09.06	2,80	2
		07.11.06	2,67	2

* The PT values of the NOKLUS controls "White" and "Blue" result from the NOKLUS external quality assessment scheme.
** Overall mean for the hospital laboratories using SPA-reagent and INR-instrument from Stago. More than 40 laboratories participate in this group and the group represents the majority of the Norwegian hospital laboratories.
*** Overall mean for the 20 hospital laboratories using Nycotest PT reagent on Thrombolyzer or Thrombotrack.

Discussion

Table 7 shows that the comparison method agrees well with the EQUALIS calibrator with certified PT (INR) value at approximately 1,0. It is clear, however, that the comparison method has a small negative bias compared to the EQUALIS calibrator at the high PT level [PT (INR) = 3,6]. The achieved values are still within the uncertainty limits of the calibrator. The negative bias also appears in the therapeutic range, as shown with the EQUALIS Control, but is not as distinguished as for the higher PT values.

The results in Table 8 achieved with the NOKLUS Control materials confirm the small negative bias of the comparison method. The significant differences between the "SPA/Stago group" and the "NycoTest PT group" have been shown repeatedly during the last years in Norway. If this evaluation had been performed with Nycotest PT reagent, the results on the comparison method most probably would have been slightly higher. Still this would not influence the conclusions in this report.

Table 8 also shows that the negative bias of the comparison method tends to get more distinct when compared to the Danish DEKS Calibrators. The calibrating systems from EQUALIS and DEKS are quite different, with respect to the production of the materials as well as the way the calibrators get the certified PT values. For high PT values, the discrepancy between the two calibrating systems has been shown before by others. EQUALIS, as well as the Expert Group for Coagulation appointed by EQUALIS are looking deeper into this matter.

Due to the present bias of the comparison method, it was decided that all the results from the comparison method should be adjusted to meet with the target values for the two EQUALIS calibrators and the EQUALIS control. The adjustment was done by means of the following regression equation ($R^2 = 1,0$):

$$\mathbf{y = 1,0866x – 0,0864}$$

Further on in this report, whenever SSPT results are compared with the comparison method (trueness and accuracy), the results from the comparison method have already been adjusted according to this equation.

5.2. Analytical quality of Simple Simon PT used in a hospital laboratory

5.2.1. *The precision of Simple Simon PT under standardised and optimal conditions*

The repeatability of the SSPT is demonstrated by means of 96 patient samples analysed in duplicate. All results with the two lots of SSPT are included in the calculation. The results are divided in three groups according to the PT (INR) level, and the calculation of the repeatability is made for each level separately.

The repeatability is shown in Table 9.
The raw data is shown in attachment 3.

Table 9. Repeatability, Simple Simon PT. Results achieved under standardised and optimal test conditions

PT (INR) level	**PT (INR), average (range)**	**CV % (95 % C.I.)**	**n**	**Outliers**
<2,0	1,7 (1,2 — 1,9)	5,0 (3,4 — 9,1)	10	0
2,0 – 3,5	2,8 (2,0 — 3,5)	2,9 (2,5 — 3,5)	69	1*
>3,5	4,0 (3,6 — 5,5)	3,2 (2,3 — 4,9)	16	0

* The outlier is the pair of results from patient number 13. The duplicate measurements on SSPT gave the results 3,15 and 2,72 PT (INR). The tests were carried out as usual and without apparent mistakes, and the results were displayed without any error messages. The difference between the two results was just outside the limit for the outliers according to Burnett. The result is excluded from the calculation of imprecision.

Discussion

The precision of the PT (INR) measurements on Simple Simon PT is good. The CV is approximately 5 % at the low level and approximately 3 % at the therapeutic level and at the high level. The quality goal of SKUP is attained.

5.2.2. *Internal quality control, Simple Simon PT*

The results with NKP and OKP Control plasmas from MediRox on SSPT are shown in Table 10.
Raw data is shown in attachment 4.

Table 10. Reproducibility, internal control NKP and OKP on Simple Simon PT

QC material	Target value PT (INR)	Simple Simon meter no.	Simple Simon PT (INR) average (range)	CV % (95 % CI)	n	Outliers
NKP	0,90 — 1,20	62 and 67	0,99 (0,90 — 1,13)	6,8 (4,8 — 11,2)	13	0
		72	1,01 (0,96 — 1,05)	2,5 (1,8 — 3,9)	16	0
OKP	2,3 — 2,9	62 and 67	3,11 (2,93 — 3,47)	4,8 (3,7 — 6,8)	23	0
		72	2,87 (2,60 — 3,14)	4,7 (3,5 — 7,0)	18	0

Discussion
The reproducibility achieved with two freeze dried control materials is acceptable, with an average CV of approximately 4,6 % in the normal range and 4,8 % in the therapeutic range. With SSPT of lot G024M1, the results on the OKP control were above the range indicated by the manufacturer of the control, while those of lot G185M1 were at, or occasionally above, the upper limit of this range.

5.2.3. *The trueness of Simple Simon PT under standardised and optimal conditions*
The trueness of SSPT is calculated from 44 results achieved by two biomedical laboratory scientists at NOKLUS Centre. The results are achieved with the lot G185M1 of the product. The reduction from 67 to 44 results is because 23 of the patients donated samples at two occasions, of which the latter was excluded. The exclusion is to prevent the bias from being influenced by a double dose of a possible individual matrix effect. Inclusion of the 23 results only influences the estimate marginally.

The bias of SSPT relative to the comparison method is shown in Table 11.
Raw data is shown in attachment 3.

Table 11. Bias. Mean difference between Simple Simon PT and the comparison method, based on the mean of each duplicate at both methods. Results achieved under standardised and optimal conditions. N = 44

PT (INR) level group	**PT (INR) Simple Simon mean**	**PT (INR) mean deviation from the comparison method (95 % CI)**	**n**	**Number of outliers**
<2,0	1,76	+0,06 (–0,24 — +0,37)	3	0
2,0 — 3,5	2,68	+0,11 (+0,05 — +0,16)	33	0
>3,5	4,13	+0,24 (–0,20 — +0,68)	8	0
All	2,88	+0,13 (+0,05 — +0,21)	44	0

Discussion
Simple Simon PT has a small positive bias when compared with the comparison method. The bias expressed as INR is approximately 0,1 in the therapeutic range. The low number of results in the low and high level group makes these estimations of the bias more uncertain.

5.2.4. *The accuracy of Simple Simon PT under standardised and optimal conditions*
To evaluate the accuracy of the results at SSPT, the agreement between SSPT and the comparison method is illustrated in a difference plot. The plot shows the deviation of single results at SSPT from the true value. The plot gives a picture of both random and systematic deviation and reflects the total measuring error.
The total error is demonstrated for the first measurement of each paired result. Only the 44 results achieved with lot G185M1 of SSPT are included in the assessment. The results with the second samples of the 23 patients who donated samples at two occasions are also shown in the figure, but in a differentiating symbol. Possible patient matrix effects are thus not allowed to influence the results in double doses.
The limits in the plot are based on the quality goals discussed in chapter 2 in this report.

The accuracy of SSPT is shown in Figure 1.

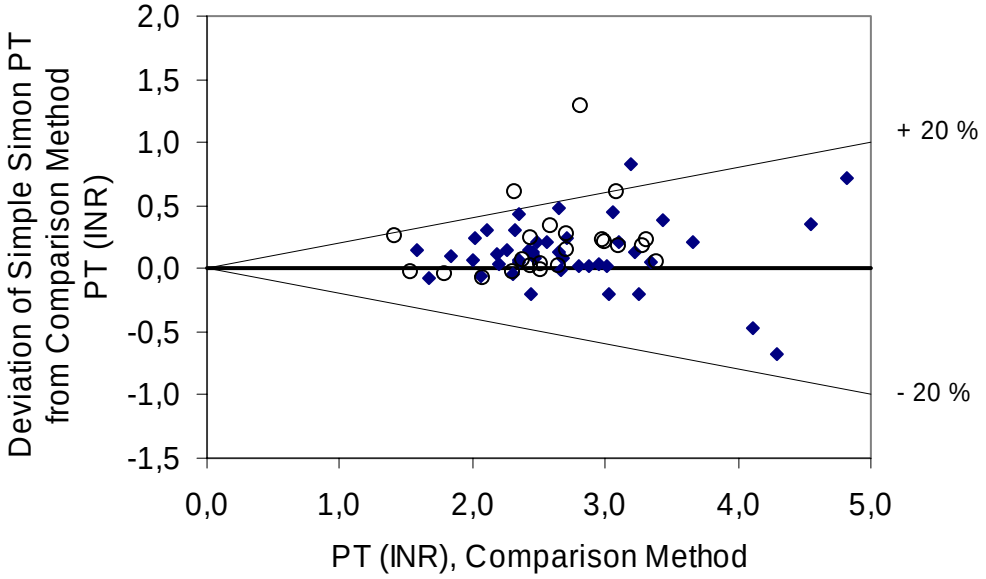

Figure 1. The accuracy of Simple Simon PT as achieved with lot G185M1 of the product. The filled diamonds are the results of one sample of each of 44 different patients of an outpatient clinic. The open circles are results of a second sample of 23 of these patients.

Discussion
The assessment of the accuracy of the measurements on Simple Simon PT is based on a limited number of results. The main impression of the accuracy of Simple Simon PT measurements is good. A small positive systematic difference between the measurement results at Simple Simon PT and at the comparison method results comes forward. This assessment is valid for the results from the total group of patients, including the patients that participated twice in the evaluation. The clear outlier from the second consultation is unexplained. One result of the 44 first patient sample results deviates more than 20 %. Simple Simon PT fulfils the analytical quality goal set by SKUP, but the assessment is based on a limited number of results.

5.3. Analytical quality of Simple Simon used in primary health care

An evaluation in primary health care is not part of this evaluation. See comments from Zafena AB, attachment 5.

5.4. Evaluation of user-friendliness

5.4.1. *Evaluation of user-friendliness by the users in primary health care*

The most important response regarding user-friendliness of any laboratory equipment intended for the users in primary health care must come from the users themselves. The end-users often emphasize other aspects than those pointed out by more extensively trained laboratory personnel. An evaluation in primary health care is not part of this evaluation.

5.4.2. *Evaluation of user-friendliness by laboratory educated persons*

The practical work with SSPT in this evaluation was performed by two laboratory educated persons at NOKLUS Centre. Their observations and opinions are summarized below:

Advantages

- The system is easy to operate – Simple Simon "tells you"
- The reconstituted reagent can be stored in a refrigerator. No need of a freezer
- The user can chose between three different sample materials

Possible improvements or issues that must be paid attention to

- One bottle of reconstituted reagent can be used for 30 days and gives 40 tests. For small primary care units with few oral anticoagulant treatment patients, most probably this will cause some reagent waste. The producer or supplier should consider giving the users an option of a smaller volume as well
- The precision of the measurements at SSPT depends to a great extent on good pipette technique. The system requires some training before the precision gets acceptable

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Attachments

Attachment 1. Raw data, STA Compact, results from patient samples

Attachment 2. Raw data, STA Compact, results from internal quality controls

Attachment 3. Raw data, results from patient samples, Simple Simon PT

Attachment 4. Raw data, Simple Simon PT, internal quality control results

Attachment 5. Comments from Zafena AB

Attachment 6. Evaluations under the direction of SKUP

Attachments with raw data are included only in the report to Zafena AB, Sweden.

Attachment 5

SKUP evaluation of SSPT

Comments of the manufacturer, Zafena AB
Simple Simon® PT (SSPT) is a conceptually new, wet-chemistry, point-of-care PT (INR) product. By the novelties, SSPT determines PT at ambient room temperature and performs a concomitant EVF estimate. At the clotting point, the PT (INR) is automatically computed from the determined clotting time, temperature and EVF. In other respects, the analysis is according to the method of Owren, including a final sample dilution of 1:21.

Field testing of SSPT commenced in the fall of 2005, and by February 2006 some eight evaluations had been performed. These evaluations included comparing the results obtained by SSPT in point-of-care milieu with results on the same samples remitted to Scandinavian hospital laboratories for routine PT analysis.

At that time, February 2006, a suggestion was made by SKUP to have SSPT subjected to an impartial evaluation by this organization. Prior to the decision of apply for such an evaluation, representatives of Zafena visited the SKUP facilities at Haraldsplass Diakonale Sykehus (HDS), Bergen, Norway, on March 27, 2006. At that visit, Zafena personnel analysed nine citrated blood samples provided by SKUP.

The PT (INR) of the samples was known to SKUP by routine plasma PT determinations by the hospital laboratory of HDS, but was unknown to Zafena personnel performing the analysis. The first phase of the exercise was analysis of restored citrated blood. The restoration was by repeated head-over-end mixing of the sample tubes to disperse the blood cells pelleted at the bottom of the sample tubes. The pelleting, by centrifugation, was from the first step of the HDS PT determination, which is performed on plasma. Three SSPT readers, #56, #53 and #52, and all SSPT materials were of lot G024M1. Each blood sample was analysed on all thee readers with the following results.

Sample#	HDS 1&2	S#56	S#53	S#52	INRm	CV
1	2,2 & 2,16	2,51	2,48	2,58	2,52	2,0%
2	2,3 & 2,30	2,89	2,96	2,84	2,90	2,1%
3	2,0 & 1,97	2,22	2,27	2,21	2,23	1,4%
4	2,1 & 2,14	2,38	2,54	2,51	2,48	3,4%
5	1,0 & 1,00	0,98	0,98	1,03	1,00	2,9%
6	1,1 & 1,02	1,03	1,11	1,05	1,06	3,9%
7	1,0 & 1,02	1,06	1,07	1,05	1,06	0,9%
8	3,9 & 3,64	4,56	4,38	4,54	4,49	2,2%
9	1,3 & 1,26	1,33	1,32	1,31	1,32	0,8%

Apart from the results with each SSPT reader, the mean and the CV of the three determinations are given. The mean of the nine within-series CV estimates computes to 2.2% with a 95% confidence limits of 0 and 4,3%. For comparison, two HDS results are given for each sample, HDS 1 & 2. The first of these was by the routine PT analysis performed a few hours prior to the SSPT analysis. The second was obtained afterwards. For this, the blood samples were again remitted to the HDS laboratory where they again were centrifuged, and the PT (INR) again determined on the plasmas. The second HDS determination was within an hour of the SSPT determination. The first HDS result is rounded off to the nearest tenth of an INR, as this is the resolution with which HDS results are routinely reported. The second value is to the nearest hundredth, as is the resolution given by the automatic analyser of HDS.

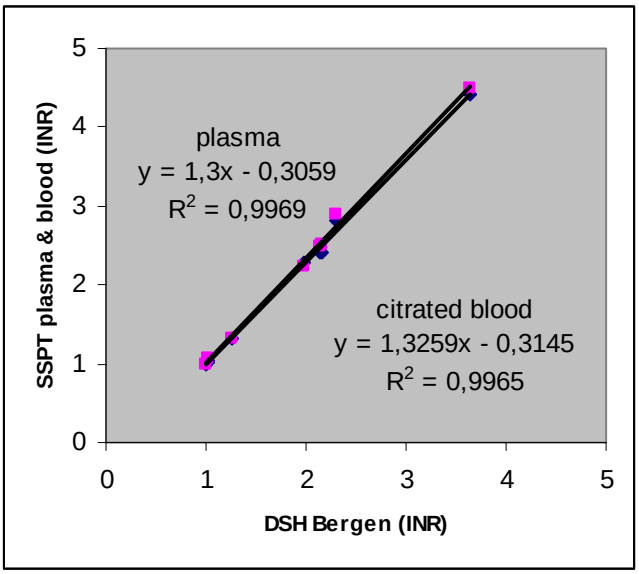
When the nine citrated blood plasmas returned from the HDS laboratory upon the second PT (INR) determination, the blood cells remained pelleted at the bottom of the sample tubes. This allowed Zafena personnel to perform SSPT analysis on the plasma. These results were:

Sample#	HDS 1&2	S#56	S#53	S#52	INRm	CV
1	2,2 & 2,16	2,39	2,40	2,47	2,42	1,8%
2	2,3 & 2,30	2,89	2,80	2,77	2,82	2,2%
3	2,0 & 1,97	2,23	2,23	2,37	2,28	3,6%
4	2,1 & 2,14	2,41	2,39	2,39	2,40	0,5%
5	1,0 & 1,00	0,99	0,99	0,99	0,99	0,0%
6	1,1 & 1,02	1,03	1,02	1,00	1,02	1,5%
7	1,0 & 1,02	1,05	1,04	1,03	1,04	1,0%
8	3,9 & 3,64	4,43	4,38	4,45	4,42	0,8%
9	1,3 & 1,26	1,34	1,31	1,30	1,32	1,6%

The mean CV of the SSPT plasma analysis was 1.4%.

All SSPT determinations referred to above were performed at the SKUP laboratory facilities of HDS under the supervision of SKUP personnel.

To compare the results, the mean of the SSPT blood analysis and plasma analysis were plotted against the results of the second HDS determination, see below. The second determinations were chosen because they were obtained in closer temporal proximity to the SSPT determinations, and because the sequence of analysis was deemed more relevant.



The above regression analysis demonstrates a near perfect correlation between the results of the HDS plasma PT-INR analysis and the results of both SSPT determinations, that on restored citrated blood and on plasma alike. The correlation was thus very much on, but the calibration off. At the low end, at INR 1, the values were the same within 4%, but at the high end, e.g. at INR 2.5, the SSPT values were about 18 % up. Viewed within the ISI/INR formalism, the difference resided mainly in the ISI values. To achieve good harmonization, apart for a small adjustment of the normal clotting time, the ISI of SSPT needed to be reduced by about 14%, or the ISI of DSH correspondingly increased.

As stated, the lot of SSPT used in the above exercise was G042M1 which was calibrated against authentic citrated blood samples analysed at a Scandinavian hospital laboratory that

calibrates with materials from EQUALIS. The fact, that HDS also calibrates with materials from EQUALIS only served to increase the surprise at finding a calibration mismatch. There was here a mystery of some sort.

A first step in in understanding the nature of the disclosed calibration mismatch was do a check the SSPT calibration with samples analysed at the original hospital laboratory. This was done on April 5, 2006, a week after the DSH exercise, and revealed nothing - the calibration checked out. However, it was consciously noted, that the samples were of inpatients whereas the HDS samples were of outpatients. Still, in the minds of Zafena personnel, it appeared that the laboratories of the hospital and of HDS, at this given point in time (March - April 2006), were not delivering fully congruent PT(INR) results, those of DSH being some 18 % low (or the other hospital's high) at INR 2.5, the middle of the therapeutic range.

In spite of the realization of the existence of a calibration imperfection, Zafena decided to submit the SSPT product lot G024M to SKUP in Bergen for their scrutiny. The idea was to allow SKUP personnel to acquaint themselves with the SSPT procedure and to promote activities to unravel the source of the calibration imperfection.

The SKUP results with SSPT lot G024M contributes with 29 data points in the present evaluation. Zafena was presented with the first fifteen of these, and could acknowledge that the results were about as before, an about 18 % difference at INR 2.5. At this time, Zafena could present to SKUP some SSPT product of lot G185M1. This lot was aligned to results on 241 samples of outpatients analysed by both SSPT and one of eight Scandinavian hospital laboratories. This lot thus had strengthened claim to generated PT INR values typical of Scandinavian hospital laboratories. The remaining 65 data points of the present evaluation were generated with lot G185M1 of SSPT. Compared to lot G024M1, lot G185M1 generated PT (INR) values that were 6 % lower at INR 2.5.

At SKUP, the calibration of the HDS equipment was scrutinized. When an larger set of EQUALIS calibrator materials was considered, it was found that the HDS equipment generated PT INR levels that were 6 % low at INR 2.5.

To summarize: Zafena's approach to calibrate lot G185M1 against an average results of eight Scandinavian hospital laboratories using materials from either EQUALIS or DEKS closed the gap between initial SSPT, and HDS results by about 6% at INR 2.5. SKUP decision to adjust the HDS results against an average of several EQUALIS calibrators and controls closed the gap by another 6%. These 12 % and the persisting difference of 6% reported on in the present evaluation thus accounted for the 18 % difference originally found between the results of HDS and SSPT.

Zafena stands fast in its ambition to provide PT INR levels that represent those of a typical Scandinavian hospital laboratory. To accomplish this Zafena will continue to employ an indirect, multi-centre approach that employs authentic blood samples from primary care centres at which PT (INR) is routinely determine by SSPT. According to details in the process, tentatively calibrated SSPT material is submitted to centres with the request that the tentatively calibrated material be used in parallel with regular on anti-coagulated blood samples, and that the samples subsequently are remitted to a nearby hospital laboratory for PT (INR) for the reference determination. A requirement is that the hospital laboratories employ PT (INR) equipment calibrated against calibration materials from either EQUALIS or DEKS.

Upon request, Zafena will inform on how a given lot of SSPT has been calibrated and how the calibration has been verified.

At the present point in time, February 2007, SSPT is routinely used at about 20 primary care centres (vårdcentraler) in Sweden and at about 20 (legekontor) in Norway. Because of this abundance of information is available on how the system performs in the hands of the intended users of the product. An impartial evaluation by SKUP of such performance therefore appeared less pertinent to Zafena. This point of view could well change, since it is realized, that the information retrieved by the expert evaluators of SKUP could prove more tangible and better prioritized, than that otherwise available. Such improved information may be of great importance in modifying the use of present product and in designing its next generation offspring.

The following information on SSPT by its users has been obtained: 1) The precision is satisfactory. 2) The results agree well with those of hospital laboratories. 3) Compared to methods previously used, much labour time is saved. And, 4) Training is necessary in order to obtain optimal analytical results.

In particular, Zafena is indebted to the organisation Laboratory Medical Centre (LMC) of the County Council of Östergötland, Sweden. In the past year Simple Simon PT has been introduced at some 20 primary care centres of this organization. The personnel operating the equipment have been given good training, both individually and at specially organized courses. In addition, the analytical performance of SSPT is continuously monitored. The monitoring is by two protocols. At each primary care centre, control plasma with PT level in the therapeutic range (INR in the range of 2 to 3) is analysed daily and required to show levels with specified limits. Similarly, a control plasma with PT (INR) in the normal range (INR about 1) is analysed weekly. In addition, once every month and always upon change of reader or lot of disposables, samples of anti-coagulated blood are analysed at the centres and then submitted to the laboratory of the University Hospital of Linköping. At the hospital, the samples are analysed on the one piece of automated equipment with reference status, the "mentor", which, to ensure agreement with PT (INR) results of laboratories of western Europe, participates in the external quality control programs of EQUALIS, ECAT, and INSTAND.

All results of LMC's internal quality control routines are recorded in a data base, the contents of which, in part, has kindly been made available to Zafena. During the past six months the data has comprised the results of some 3000 analysis of plasma controls and some 200 analysis of authentic blood samples. The results are from about 30 SSPT readers, and indicate an over-all reproducibility CV of about 6 %, which is in line what was revealed in the present SKUP evaluation. The trueness of the results is also in line with this. Zafena is deeply indebted to LMC for its great, continuous contribution in upholding and advancing the quality of SSPT performance in primary care laboratory milieu.

For support in making the decision to apply for a SKUP evaluation, and for contributing to the financials, we thank our collaborators at MEDIC Norway and ILS Sweden. For the good planning and rigorous execution of the present evaluation, and for the fair reporting on the results, we thank the competent personnel of SKUP.

Zafena AB, Borensberg, Sweden, February 6, 2007, Mats Rånby

Attachment 6

List of evaluations organised by SKUP

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

Evaluations performed in 2004 – 2007

Evaluation no.	Component	Instrument/testkit	Producer
SKUP/2007/57*	PT (INR)	Simple Simon PT	Zafena AB
SKUP/2007/55	PT (INR)	CoaguChek XS	Roche Diagnostics
SKUP/2005/52*	Strep A	Clearview Exact Strep A Dipstick	Applied Biotech, Inc.
SKUP/2005/51*	Glucose[1]	FreeStyle	Abbott Laboratories
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/2005/41*	HbA1c	*Confidential*	
SKUP/2005/40	Glucose[1]	OneTouch GlucoTouch	LifeScan, Johnson & Johnson
SKUP/2005/39	Glucose[1]	OneTouch Ultra	LifeScan, Johnson & 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	QuickVue u-hCG	Quidel Corporation
SKUP/2004/34*	u-hCG	RapidVue u-hCG	Quidel Corporation
SKUP/2004/33	PT (INR)	Hemochron Jr. Signature	ITC International Technidyne Corp
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

*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 diabetic patients.

Evaluations performed in 1999 - 2003

Evaluation no.	Component	Instrument/test kit	Producer
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

* 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 an user-evaluation among diabetic patients.

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