Simple Simon® PT

A system for measurement of P—Prothrombin time (INR)
manufactured by Zafena AB, Sweden

MixxoCap®

A device for collection and handling of capillary samples
manufactured by Zafena AB, Sweden

Report from an evaluation of
the capillary samples device MixxoCap®
performed in primary health care
and organised by SKUP

The evaluation was ordered by
Zafena AB and Medic24, Norway

SKUP in Norway, NOKLUS, Box 6165, 5892 Bergen, Phone +47 55 97 95 02, www.SKUP.nu

SKUP/2011/84*

The report was written by SKUP, May 2011.
Main author was Camilla Eide Jacobsen, SKUP in Norway.

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. In addition, SKUP reports are published at www.skup.dk, where they are rated according to the national Danish quality demands for near patient instruments used in primary health care. SKUP as an organisation has no responsibility for 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 in Denmark 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).

To make contact with SKUP

SKUP secretariat
Grete Monsen
+47 55 97 95 02
grete.monsen@noklus.no

SKUP in Denmark
Esther Jensen
Stine B. Weber
Hillerød Hospital
Klinisk Biokemisk Afdeling
Dyrehavevej 29, indgang 16A
DK-3400 Hillerød
+45 48 29 41 76
esj@hih.regionh.dk
sbwe@hih.regionh.dk

SKUP in Norway
Grete Monsen
Camilla Eide Jacobsen
Marianne Risa
Sverre Sandberg
NOKLUS
Boks 6165
NO-5892 Bergen
+47 55 97 95 02
grete.monsen@noklus.no
camilla.jacobsen@noklus.no
marianne.risa@noklus.no
sverre.sandberg@isf.uib.no

SKUP in Sweden
Arne Mårtensson
Gunnar Nordin
Lena Morgan
EQUALIS
Box 977
SE-751 09 Uppsala
+46 18 69 31 64
arne.martensson@equalis.se
lena.morgan@equalis.se
gunnar.nordin@equalis.se

www.SKUP.nu

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 Zafena AB and Medic24.

1. Summary

Background
Simple Simon PT was evaluated by SKUP in 2006 under standardised and optimal conditions in a hospital laboratory by experienced laboratory personnel, SKUP/2007/57*. Medic24 and Zafena AB applied to SKUP in October 2010 for an evaluation of MixxoCap; the new Zafena device for collection and handling of capillary samples for Simple Simon PT. SKUP agreed to evaluate the user-friendliness of MixxoCap and the precision of capillary PT (INR) results using Simple Simon PT and MixxoCap. The evaluation was carried out at two primary health care centres where the staff was experienced users of Simple Simon PT.

The aim of the evaluation
- Determination of the repeatability precision when using capillary patient samples collected with MixxoCap
- A comparison of the repeatability achieved with MixxoCap and the repeatability achieved with the primary health care centres' routine methods for measurement of PT (INR)
    - Routine method at Arna Legekontor: venous citrate whole-blood samples analysed on Simple Simon PT
    - Routine method at Legekontoret Kleppestø Senter: capillary samples analysed at Simple Simon PT using the ordinary Simple Simon pipette for collection and handling of the samples
- An evaluation of the user-friendliness of MixxoCap
- An evaluation of the user-friendliness of Simple Simon PT

Materials and methods
The two primary health care centres were chosen in the light of the aim of this evaluation; testing the use of MixxoCap for capillary samples. Both centres had Simple Simon PT as their routine method for measurements of PT (INR). Arna Legekontor uses venous citrate whole blood samples for measurement of PT (INR). Legekontoret Kleppestø Senter uses capillary samples with use of the ordinary Simple Simon pipette. A total of 74 patients (84 measurements) were included in the evaluation. Capillary blood sampling with duplicate measurements on the Simple Simon PT systems was performed.

Results
When already familiar with capillary sampling technique for PT (INR), the precision obtained with capillary samples and MixxoCap under real-life conditions was good (CV 3,6%), and the recommended quality goal for precision was obtained. The same precision as achieved with venous samples using the ordinary Simple Simon pipette was obtained with capillary samples using MixxoCap on Simple Simon PT (CV approximately 3,6%). When not familiar with capillary sampling technique, the precision seems to get poorer when changing from venous samples to capillary samples and Mixxocap. Still the precision was good for results <2,5 INR. For results ≥2,5 INR, the precision was intermediate (CV 5,7%), but affected especially by one atypical duplicate. The two primary health care centres found the MixxoCap device easy to use, and they were satisfied with the device, as well as with the Simple Simon PT system.

Conclusion

Training and practise with the capillary sampling technique seem to be important for achieving good precision on PT (INR) results on Simple Simon PT. The new MixxoCap device seems to make the capillary blood sampling easier. When already familiar with capillary sampling technique, the precision obtained with capillary samples and MixxoCap under real-life conditions was good, with a CV <5%. When not familiar with capillary sampling technique, the precision was good for results <2,5 INR and intermediate for results ≥2,5 INR (CV 5,7%). The primary health care centres were satisfied with the MixxoCap device and the Simple Simon PT system.

Comments from the manufacturer

A letter with comments and additional information from the manufacturer 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 analytical quality as well as satisfactory user-friendliness.

2.1. Analytical quality goals

Currently, 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. Unfortunately, there is no performance criterion for imprecision in the standard.

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 PT (INR) as 4% (CV_{bw}). According to Kjeldsen, Lassen et al. [8], 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 ($CV_a \leq 1/2\ CV_{bw}$) [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

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) [10] and recommends a reproducibility of ≤5% (CV) for instruments used in primary health care and ≤3% (CV) for hospital instruments.

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 a repeatability CV below 5%.

2.2. Evaluation of user-friendliness

The evaluation includes an evaluation of the user-friendliness of MixxoCap as well as an evaluation of the user-friendliness of the Simple Simon PT (SSPT) system.

MixxoCap
MixxoCap is a disposable plastic capillary for blood collection, handling and mixing of samples. The user-friendliness of MixxoCap was evaluated by means of a special designed questionnaire worked out by SKUP and approved by the manufacturer and distributor. For questionnaire, see attachment 1.

Simple Simon PT
The evaluation of user-friendliness of SSPT was carried out by asking each of the evaluation sites to fill in a questionnaire worked out by SKUP, see section 5.3.

SKUP's standardised questionnaire divides the user-friendliness into four sub-areas:
- Rating of the information in the manual and insert*
- Rating of time factors for the 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 rated with the following points:
"0 point" Unsatisfactory
"l point" Intermediate
"2 points" Satisfactory

To achieve the overall rating "satisfactory", the tested equipment must reach the total rating of "satisfactory" in all four sub-areas of characteristics mentioned above.

*Rating of the information in the manual and insert for the SSPT system is not performed in this evaluation. The two primary health care centres, participating in the evaluation, are experienced users of the SSPT system. They know the method and instrument well, and are no longer depending on the information given in the manual. This could most likely influence their opinion about the manual. The main intention with this evaluation was to focus on the new MixxoCap device.

2.3. SKUP´s quality goal in this evaluation

Based on the discussion about analytical quality goals, SKUP decided to assess the results from the evaluation of SSPT and MixxoCap against the following goals:

Repeatability CV ..... <5%
Fraction of technical errors ..... <2%

User-friendliness of SSPT: To achieve the overall rating "satisfactory", the tested equipment must reach the total rating of "satisfactory" in all sub-areas of characteristics mentioned in table 8 to 10 in section 5.3.2.

3. Materials and methods

3.1. Definition of the measurand

The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and the International Union of Pure and Applied Chemistry (IUPAC) work in a joint Committee on Nomenclature, Properties and Units (C-NPU). The descriptions of clinical laboratory tests are listed in the "NPU database" [11]. The recommended name for the measurand is given, together with which unit the result should be reported in. In this report, the measurand is referred to as PT (INR).

Regarding the P—Prothrombin time (INR), two measurands have been defined; one for measurements according to the Owren method and one for measurements according to the Quick method, shown in table 1.

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

NPU code	Full name of test according to NPU	Method	Unit
NPU01685	P—Coagulation, tissue factor-induced; relative time (actual/normal; INR; IRP 67/40; procedure)	Owren	Unit 1, but usually given without unit
NPU21717	P—Coagulation, tissue factor-induced; relative time (actual/normal; INR; IRP 67/40; II+V+VII+X)	Quick	Unit 1, but usually given without unit

The main difference between the two PT (INR) methods is the extent of sample dilution and the sensitivity towards factor (F) 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 FII, FVII and FX, whereas the Quick method is sensitive for FII, FV, FVII and FX and fibrinogen (FI).

3.2. Simple Simon PT and MixxoCap

The text in section 3.2.1 and 3.2.2 is derived mainly from the manufacturer's information material.

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. SSPT 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. The

measuring range is 0,7 – 8,0 INR. The measuring time is typically 60 sec. Freeze-dried control plasmas as well as blood or plasma controls are well suited as control materials for SSPT.

The portable SSPT Reader is battery-operated and will, without maintenance, perform 4000 tests. The reader automatically determines the nature of the sample; blood or plasma. If the sample is whole blood, the fraction of red cells, the EVF, is automatically estimated. The reader determines the coagulation time and the temperature at which the reaction takes place. 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 as an International Normalized Ratio (INR) value.
The SSPT product is calibrated against authentic patient samples with PT (INR) values determined at Scandinavian hospital laboratories, where the 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.

A calibrated SSPT 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 4000 tests have been performed on a reader, a freshly serviced reader with new pipettes is put into use. The used 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 an updated reader. Readers intended for use with the MixxoCap are calibrated together with MixxoCaps of a certain lot.

3.2.2. *The MixxoCap device*

MixxoCap is produced by Zafena AB. It is intended to facilitate capillary blood sampling in determination of PT (INR) by SSPT. MixxoCap is an all-plastic disposable device for collection and handling of blood and other biological fluids.
MixxoCap consists of three parts; rubber “hat”, clear plastic “body” and clear plastic end-to-end capillary. The “body” and the capillary are delivered in one piece, and are for one time use. The “hat” is reused several times; one hat is delivered with every 100 pieces of the disposable part. In conjunction to use, the smaller end of the rubber “hat” is inserted into the socket of the “body” and, with a twist, fitted to its bottom. MixxoCap is used to transfer 10 µL of capillary blood from the finger to the reaction cup of the SSPT reader, and to mix the blood with the reagent.

3.2.3. *Product information*

The SSPT system is manufactured by Zafena AB. Technical data from the manufacturer is shown in table 2. For name of suppliers in the Scandinavian countries and more details about SSPT, see attachment 2.

Simple Simon PT instruments
Lot K142MPN
Serial no. instrument A: 465, instrument B: 428 (Legekontoret Kleppestø Senter)
Serial no. instrument A: 775, instrument B: 112 (Arna Legekontor)

Reagent and diluents
Lot reagent K142M expiry 05-2012
Lot diluent K142M expiry 05-2012

MixxoCap
Lot K142M expiry 05-2012

Quality Control materials
ZAP (Zafena abnormal plasma) lot K373M expiry 03-2013
Buffer lot K373E-1 expiry 03-2013

Sampling devices
Accu-Chek Safe-T-Proplus penetration depth setting: 1,3 mm, 1,8 mm and 2,3 mm
(Arna legekontor) lot 1033005 expiry 06-2014

Haemolance, green penetration depth: 1,8 mm
(Legekontoret Kleppestø Senter)

Table 2. Technical data from the manufacturer

TECHNICAL DATA FOR SIMPLE SIMON PT	
Optimal operating temperature	+17 - +40° C
Humidity	<85%
Sample material	Capillary blood, venous blood or citrated plasma
Blood sample volume	10 µL
Measuring time	Approximately 60 sec
Measuring range	0,7 – 8,0 INR
Hematocrit	0,2 – 0,7
Storage capacity	The internal memory of the reader stores the latest analytical result*.
Electrical power supply	Three AA batteries
Operating time	Approximately 1200 tests
Dimensions	145 mm (L) x 100 mm (D) x 65 mm (H)
Weight	720 g

*When the reader is connected to the it product Zafena Connector, all data, including supplementary data like patient/sample ID, reader ID and sample type, from about 40 thousand analyses is stored on one SD-card. A new SD-card including software updates is periodically supplied.

3.3. Planning of the evaluation

Background for the evaluation
Simple Simon PT was evaluated by SKUP in 2006 with focus on analytical quality for measurements with venous citrate whole blood. The evaluation was performed by experienced laboratory personnel under standardised and optimal conditions in a hospital laboratory, see report SKUP/2007/57*. In 2010, Zafena introduced MixxoCap as a new device intended to facilitate capillary blood sampling. Zafena asked SKUP to evaluate the user-friendliness and analytical quality for measurements of capillary PT (INR) samples on SSPT using the new Zafena device MixxoCap. It was pointed out that the evaluation should be carried out under real-life conditions in the hands of the intended users in primary health care.

Inquiry about an evaluation
Pia Virik Moldestad, Medic24, and Mats Rånby, Zafena, applied to SKUP in October 2010 for an evaluation of SSPT in two primary health care centres using the new device for collection and handling of capillary samples; MixxoCap. SKUP accepted to carry out this evaluation on behalf of Medic24 and Zafena AB.

Protocol, arrangements and contract
The protocol for the evaluation was approved in February 2011. Medic24, Zafena AB and SKUP signed a contract about the evaluation in the end of February. The primary health care centres Arna Legekontor (hereafter called PHCC1) and Legekontoret Kleppestø Senter (hereafter called PHCC2) in Bergen agreed to carry out the analytical part of the evaluation.

Preparations, training program and practical work
SKUP started the preparation for the evaluation in January 2011. Advisory biomedical laboratory scientist Stein Binder, NOKLUS, contacted the two primary health care centres, and supported the staff during the evaluation. SKUP went through the evaluation procedure with Stein Binder in March 2011. Since both primary health care centres had SSPT as their routine method for measurements of PT (INR), it was only necessary to give training in use of MixxoCap and general guidance in capillary sampling. In the middle of March, Pia Virik Moldestad from Medic24 trained the staff at the two primary health care centres for the practical work by. At the same time, the centres also received all equipment needed for the evaluation. The practical work was carried out within four weeks in March and April 2011.

3.3.1. *Evaluation sites and persons involved*
PHCC1 has four physicians, three health secretaries and one medical secretary.
PHCC2 is a small primary health care centre with two physicians and two medical secretaries.

An overview of the persons responsible for the various parts of the evaluation is given in table 3.

Table 3. Persons responsible for various parts of the evaluations

Name	**Title**	**Responsibility**
Mats Rånby	Managing director, Zafena AB	Ordered the evaluation
Pia Virik Moldestad	Product Manager, Medic24	Suppliers of SSPT in Norway. Ordered the evaluation. Delivered the equipment and trained the staff at both primary health care centres
Grete Monsen	Biomedical laboratory scientist, Section leader of SKUP, NOKLUS	Responsible for the evaluation
Camilla Eide Jacobsen	Biomedical laboratory scientist, M.S., SKUP/NOKLUS	Carried out the preparations, statistical calculations and the report writing
Stein Binder	Advisory biomedical laboratory scientist, NOKLUS	Guided and supported the two primary health care centres
Linda Bratland	Health secretary	Responsible for the practical work with the evaluation at PHCC1
Linda Solberg	Health secretary	
Lene Holdhus	Health secretary	
Renate Johannessen	Medical secretary	
Hildegunn Normann Iversen	Medical secretaries	Responsible for the practical work with the evaluation at PHCC2
Anne-Sissel Ingvaldsen		

3.4. The evaluation procedure

3.4.1. *The evaluation model*

The model of the SKUP evaluations is based on the guidelines in the book "*Evaluation of analytical instruments. A guide particularly designed for evaluations of instruments in primary health care*" [12].

The evaluation of SSPT comprises the following:

- Specification and basic facts about the instrument, see attachment 2
- Determination of repeatability precision when using capillary patient samples collected with MixxoCap
- A comparison of the repeatability achieved with MixxoCap and the repeatability achieved with the primary health care centres' routine methods for measurement of PT (INR):
  - Routine method at PHCC1: venous citrate whole-blood samples analysed on SSPT
  - Routine method at PHCC2: capillary samples analysed on SSPT. The ordinary Simple Simon pipette is used for collection and handling of the capillary samples
- An evaluation of the user-friendliness of MixxoCap
- An evaluation of the user-friendliness of SSPT

Blood sampling

All samples for the evaluation of SSPT were collected from finger capillary punctures using two different sampling devices with penetration depth from 1,3 mm to 2,3 mm. Medic24 approved this. MixxoCap was used to collect, transfer and mix the capillary blood with SSPT reagent. The primary health care centres' routine method for measurements of PT (INR) was carried out according to the centres' own procedure, and did not include the use of MixxoCap.

3.4.2. *Evaluations procedure in primary health care*

The primary health care centres were chosen in the light of the aim of this evaluation; testing the use of MixxoCap for capillary samples. Both primary health care centres had SSPT as their routine method for measurements of PT (INR). PHCC1 uses venous citrate whole blood as routine samples for measurement of PT (INR). PHCC2 uses capillary samples as routine samples with use of the ordinary Simple Simon pipette.

To serve the two primary health care centres with free PT (INR) reagent for the extra routine measurements during the evaluation, both centres received two SSPT instruments (called A and B), all four systems with the same lot number. As described in section 3.2.1., the SSPT system is delivered as a complete package, consisting of the Reader, reagents, tubes, stoppers, pipettes and pipette tips. The lot number is assigned for the whole package.

All samples included in the study (capillary and routine samples) were to be analysed at both instrument A and B, representing duplicates.

Measurement procedure

The three following steps were identical for the two primary health care centres:

1. SSPT instrument A and B were prepared for analysing PT (INR)
2. The routine sample for measurement of PT (INR) was collected and measured according to the primary care centres' procedures. The measurements were performed on instrument A first and then on instrument B, representing duplicates
3. SSPT instrument A and B were again prepared for analysing PT (INR)

Further procedure for PHCC1:

4. A sufficient puncture in a finger (approximately 1,8 mm) was performed
5. The first blood drop was wiped off and the second blood drop was used for measurement of PT (INR) on instrument A. The MixxoCap device was used for collection, transfer and mixing of the blood with the PT reagent
6. A new sufficient puncture in a new finger (approximately 1,8 mm) was performed
7. The first blood drop was wiped off and the second blood drop was used for measurement of PT (INR) on instrument B. The MixxoCap device was used for collection, transfer and mixing of the blood with the PT reagent

Further procedure for PHCC2:

4. A sufficient puncture in a finger (approximately 1,8 mm) was performed
5. The first blood drop was wiped off and the second blood drop was used for measurement of PT (INR) on instrument A. The MixxoCap device was used for collection, transfer and mixing of the blood with the PT reagent
6. The third blood drop was wiped off and the fourth blood drop was used for measurement of PT (INR) on instrument B. The MixxoCap device was used for collection, transfer and mixing of the blood with the PT reagent

Since PHCC1 uses venous samples for their routine method for measurement of PT (INR), it was decided to do two finger punctures for their capillary sampling.
The patients recruited from PHCC2 had already been punctured in a finger for the routine method for measurement of PT (INR), and therefore it was decided to take the duplicate capillary samples for the evaluation from only one new finger puncture. According to the manufacturer Zafena AB, it is of no importance whether the blood drop used for measurement of PT (INR) on SSPT is the second, third or fourth blood drop taken from the same finger puncture. The manufacturer also recommends that the time from sampling to analysing PT (INR) ought not to exceed two minutes.

Training

Pia Virik Moldestad from Medic24 was responsible for training the staff at the primary health care centres in handling the MixxoCap when analysing capillary samples on SSPT. Stein Binder from NOKLUS was responsible for explaining the measurement procedure the staff was to follow in the evaluation. Training was given to those who were going to perform the measurements on SSPT. During the evaluation, the manufacturer Zafena and the distributor Medic24 were not allowed to contact or supervise the primary health care centres.

Internal analytical quality control

To monitor the quality of the measurements on SSPT during the evaluation period, the control material ZAP, supplied by Zafena and Medic24, was used. The instruments were checked by

means of the control solution every second day they were in use; instrument A was checked the first day, instrument B was checked the second day, instrument A was checked the third day and so on.

Recruitment of patients
Each primary health care centre was supposed to recruit 40 patients. Patients, who got their PT monitored at the two primary health care centres, were asked to participate in the evaluation study. Participation was voluntary, and verbal consent was considered sufficient. If a patient showed up for a new appointment during the evaluation period, he/she was allowed to participate again.

Handling of specimens and measurements
The measurements at PHCC1 were carried out over six different days. PHCC2 used 15 days to complete the practical work with the evaluation. There was no demand to the PT (INR) levels in the samples. The evaluation was carried out in accordance to the protocol.

Recording of results
All results were registered consecutively on a registration form prepared by SKUP. The SSPT instruments were not connected to a printer during the evaluation. All measurement data, mistakes and errors were reported. The persons performing the practical work with SSPT signed all results.

Evaluation of user-friendliness
After the practical work was completed, the staff at the two primary health care centres evaluated the user-friendliness of MixxoCap by means of a separate questionnaire, see attachment 1 and section 5.3.1.
The user-friendliness of the SSPT system was evaluated in a standardised questionnaire worked out by SKUP. Rating of time factors, quality control and operation facilities was required, see section 5.3.2. Rating of information in the manual/insert was not performed, see the explanation in section 2.2.

4. Statistical expressions and calculations

This chapter with standardised text deals with the statistical expressions and calculations used by SKUP. The statistical calculations will change according to the type of evaluation. The descriptions in section 4.2 are valid for evaluations of quantitative methods with results on the ratio scale.

4.1. Statistical terms and expressions

The definitions in this section come from the ISO/IEC Guide 99; International Vocabulary of Metrology, VIM [13].

4.1.1. *Precision*

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

Precision is measured as imprecision. Precision is descriptive in general terms (good, intermediate, poor e.g.), whereas the 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. CV is usually reported in percent.

To be able to interpret an assessment of precision, the precision conditions must be defined. Repeatability is the precision of consecutive measurements of the same component carried out under identical measuring conditions (within the measuring series).

Reproducibility is the precision of discontinuous measurements of the same component carried out under changing measuring conditions over time.

4.1.2. *Trueness*

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 inversely related to systematic measurement error. Trueness is measured as bias. Trueness is descriptive in general terms (good, intermediate, poor e.g.), whereas the bias is reported in the same unit as the analytical result or in percent.

4.1.3. *Accuracy*

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

Accuracy is not a quantity and cannot be expressed numerically. A measurement is said to be more accurate when it offers a smaller measurement error.
Accuracy can be illustrated in a difference-plot. Accuracy is descriptive in general terms (good, intermediate, poor e.g.).

4.2. Statistical calculations

4.2.1. *Statistical outliers*

The criterion promoted by Burnett [14] is used for the detection of outliers. The model takes into consideration the number of observations together with the statistical significance level for the test. The significance level is set to 5%. The segregation of outliers is made with repeated truncations, and all results are checked. Where the results are classified according to different concentration levels, the outlier testing is carried out at each level separately. Statistical outliers are excluded from the calculations.

4.2.2. *Calculations of imprecision based on duplicate results*

The precision of the field method is assessed by use of paired measurements of genuine patient sample material. The results are often divided into three concentration levels, and the estimate of imprecision is calculated for each level separately, using the following formula [15,16]:

$$SD = \sqrt{\frac{\sum d^2}{2n}}$$

d = difference between paired measurements (formula 1)
n = number of differences

This formula is used when the standard deviation can be assumed reasonable constant across the concentration interval.
If the coefficient of variation is more constant across the concentration interval, the following formula is preferred:

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

d = difference between paired measurements (formula 2)
m = mean of paired measurements
n = number of differences

The two formulas are based on the differences between paired measurements. The calculated standard deviation or CV is still a measure of the imprecision of single values. The assumption for using the formulas is that there is no systematic difference between the 1st and the 2nd measurement of the pairs.

4.2.3. *Calculation of bias*

The mean deviation (bias) at different concentration levels is calculated based on results achieved under 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 of the duplicate results on the field method. The mean difference is shown with a 95% confidence interval.

4.2.4. *Assessment of accuracy*

The agreement between the field method and the comparison method is illustrated in a difference-plot. The x-axis represents the mean value of the duplicate results on the comparison method. The y-axis shows the difference between the first measurement on the field method and the mean value of the duplicate results on the comparison method.

5. Results and discussion

Four SSPT instruments were required for the evaluation. Medic24 was responsible for the concordance between these instruments.

5.1. Number of samples

PHCC1 made 40 measurements in duplicate on SSPT from 40 patients. PHCC2 made 44 measurements in duplicate from 34 patients (eight patients participated twice and one participated three times).

5.1.1. *Excluded results*

- One sample was excluded as a statistical outlier according to Burnett's model. This applied for ID 22, duplicate measurements with the routine method at PHCC1.
- For ID 29 at PHCC2, the capillary measurement with use of MixxoCap showed the two results 2,91 and >8,0 INR. Results outside the measuring range (here >8,0) cannot be included in the calculations, neither for exclusion of outliers according to Burnett, nor for the calculation of imprecision. But the duplicate result was clearly an outlier (visual inspection), and was excluded. The result is counted as an outlier in table 5 and 6. New capillary samples were collected from ID 29, and the new results were included in the calculation of imprecision.

5.1.2. *Failed measurements*

PHCC2 reported one measurement that failed due to technical measurement error. This applied for ID 32, capillary measurement with use of MixxoCap on instrument B. The result from this patient is counted as a failed measurement, and the result is not included in the total number of samples used for statistical calculations in Table 5 and 6.

5.2. Precision of Simple Simon PT using MixxoCap in primary health care

5.2.1. *Internal quality control*

The four SSPT systems used in the evaluation were checked with the manufacturer's control solution ZAP every second day they were in use (see table 4). All results were within the control range given by the manufacturer.

The raw data is shown in attachment 3.

Table 4. Reproducibility, Simple Simon PT. Results achieved with the control solution ZAP

SSPT	n	Excluded results	Target value PT (INR)	Mean value (range)	CV % (95% confidence interval)
ZAP	21	0	2,45	2,44 (2,22 – 2,63)	4,8 (3,7 – 7,0)

Comments

The reproducibility CV achieved with the control solution was <5%.

5.2.2. *Comparison of the 1st and 2nd measurement*

Four samples were taken of each patient for measurements on SSPT instrument A and B at each primary health care centre. All results have been checked to meet the assumption in 4.2.2. No systematic difference was pointed out between the paired measurements (T-test for paired values). Table 5 shows the results from this comparison.

Table 5. Comparison of measurement performed on instrument A and B

SSPT	PT (INR) level	n	Mean measurement Instrument A	Mean measurement Instrument B	Mean difference B – A	95% CI for the mean difference
PHCC1 Routine samples	<2,5	19	2,17	2,17	0,00	-0,06 – +0,05
	≥2,5	21*	2,79	2,80	+0,01	-0,06 – +0,08
PHCC1 MixxoCap samples	<2,5	19	2,12	2,14	+0,02	-0,04 – +0,09
	≥2,5	21	2,67	2,61	-0,06	-0,16 – +0,04
PHCC2 Routine samples	<2,5	27	2,13	2,10	-0,03	-0,07 – +0,01
	≥2,5	15	3,01	2,95	-0,06	-0,18 – +0,06
PHCC2 MixxoCap samples	<2,5	27	2,06	2,06	0,00	-0,04 – +0,04
	≥2,5	16**	2,84	2,87	+0,02	-0,06 – +0,10

The given numbers of results (n) are counted before exclusion of outliers. The calculations are performed after exclusion of outliers.

*One statistical outlier (ID 22) according to Burnett's model

**One outlier (ID 29) after visual inspection, see section 5.1.1.

Comments

PHCC1 used the second blood drop for the duplicate measurements on SSPT with use of MixxoCap (two different finger punctures). PHCC2 used the second blood drop for measurement on instrument A and the fourth blood drop on instrument B with use of MixxoCap (same finger puncture). The sampling and analysing sequences were carried out within the recommended time. None of the calculations in table 5 show systematic difference between the 1st and the 2nd measurement. Therefore, it seems to be of no importance whether the blood drop used for measurement of PT (INR) on SSPT is the second or the fourth blood drop taken from the same finger puncture. This corresponds to the information given by the manufacturer, see section 3.4.2.

5.2.3. *The precision of Simple Simon PT*

The repeatability obtained under real-life conditions in the hands of the intended users in primary health care, is shown in table 6 and in figure 1 and 2. The repeatability is demonstrated by means of 40 sample results at PHCC1 and 43 sample results at PHCC2. Both centres' routine methods for measurements of PT (INR), and the PT (INR) measurements performed with MixxoCap, are analysed in duplicate. The results are sorted according to the PT (INR) concentration of the centres' routine methods mean and divided into two PT (INR) levels. The calculations of repeatability were carried out using formula 1 in section 4.2.2. The CV values in this set of data change only slightly if formula 2 is used.
The PT (INR) sample range for PHCC1 was 1,7 – 3,2 INR, and for PHCC2 1,2 – 4,1 INR. The same lot of SSPT instrument, reagent, diluents and MixxoCap was used for all measurements.

The raw data is shown in attachment 4.

Table 6. Repeatability, Simple Simon PT. Results achieved by the primary health care centres

SSPT	PT (INR) level	n	Excluded results	Mean value PT (INR)	CV % (95% confidence interval)
PHCC1 routine, venous samples	<2,5	19	0	2,17	3,6 (2,7 – 5,3)
	≥2,5	21	1*	2,80	3,9 (3,0 – 5,8)
PHCC1 MixxoCap, capillary samples	<2,5	19	0	2,13	4,4 (3,3 – 6,5)
	≥2,5	21	0	2,64	5,7 (4,4 – 8,4)#
PHCC2 routine, capillary samples	<2,5	27	0	2,11	3,4 (2,7 – 4,7)
	≥2,5	15	0	2,98	5,1 (3,7 – 8,1)
PHCC2 MixxoCap, capillary samples	<2,5	27	0	2,06	3,6 (2,8 – 4,9)#
	≥2,5	16	1**	2,85	3,6 (2,6 – 5,7)

The given numbers of results (n) are counted before exclusion of outliers. Mean and CV are calculated after exclusion of outliers.
*One statistical outlier (ID 22) according to Burnett's model
**One outlier (ID 29) after visual inspection, see section 5.1.1.

After visual inspection of the results from PHCC1 and PHCC2, it was detected that the difference in two of the duplicate measurements with capillary samples using MixxoCap was atypical and very close to being excluded as outliers according to Burnett. This applied for ID 4 (3,14 and 2,45 INR) at PHCC1, and ID 14 (2,10 and 2,43 INR) at PHCC2. See the discussion in section 5.2.4.

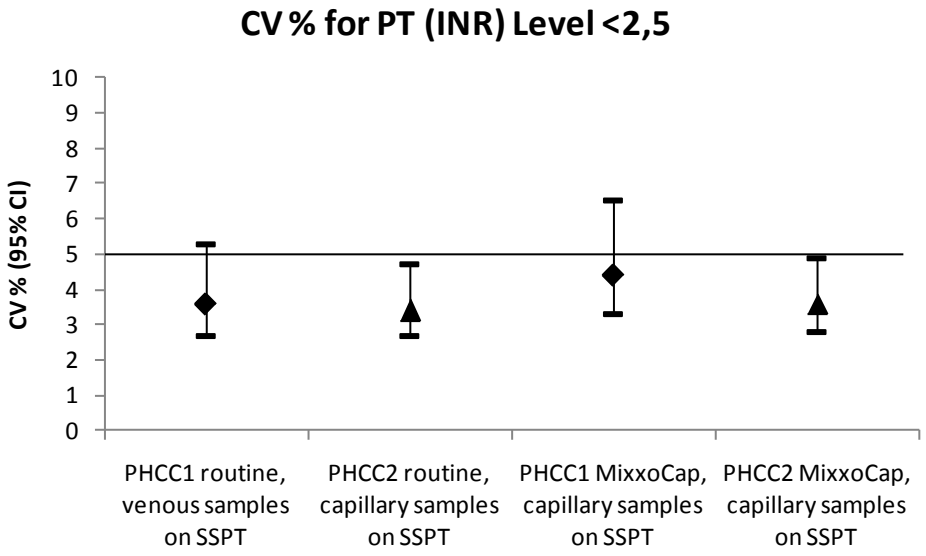


Figure 1. CV % with 95% confidence interval for PT (INR) level <2,5 for Simple Simon PT at two primary health care centres (PHCC). The diamond symbol represents PHCC1 and the triangle symbol represents PHCC2. CV is shown for the centers' routine method and for capillary samples using the MixxoCap device. SKUPs quality goal for repeatability is marked at CV 5%.

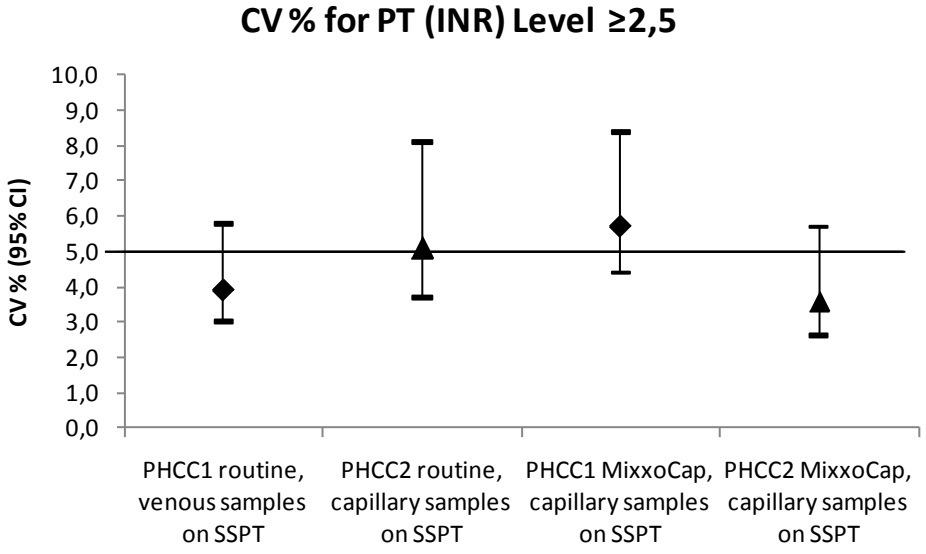


Figure 2. CV % with 95% confidence interval for PT (INR) level ≥2,5 for Simple Simon PT at two primary health care centres (PHCC). The diamond symbol represents PHCC1 and the triangle symbol represents PHCC2. CV is shown for the centers' routine method and for capillary samples using the MixxoCap device. SKUPs quality goal for repeatability is marked at CV 5%.

5.2.4. *Discussion*

The results show that it is possible to obtain good precision with capillary samples using MixxoCap on Simple Simon PT. The precision achieved with capillary samples using MixxoCap is quite comparable with the precision achieved with venous samples using the ordinary Simple Simon pipette (PHCC1 routine).

PHCC1 uses venous samples for their routine PT (INR) method on Simple Simon PT. For capillary results <2,5 INR with MixxoCap, the precision was good with a CV = 4,4% and the quality goal (CV<5%) was fulfilled. For results ≥2,5 INR with MixxoCap, the precision was intermediate with a CV = 5,7%. PHCC1 is not used to the capillary sampling technique for PT (INR), and seems to get a poorer precision when changing from venous to capillary samples and MixxoCap (not statistically significant).
After visual inspections of the PHCC1 measurement results ≥2,5 INR, it was detected that ID 4 had a duplicate with an atypical difference, close to the outlier-limit, calculated as suggested by Burnett [14]. There was no error code or obvious procedure error connected to this measurement. If this duplicate had been excluded from the calculation of repeatability, the precision had improved to CV = 4,2% and the recommended quality goal set by SKUP had been fulfilled.

PHCC2 uses capillary samples and the ordinary Simple Simon pipette for their routine PT (INR) method on Simple Simon PT. For results <2,5 INR, they seem to keep their good precision when using MixxoCap (CV = 3,6%). For results ≥2,5 INR, PHCC2 seems to improve the precision when using the MixxoCap device (F-test, p = 0,08). With their routine capillary method, the precision was intermediate for PT (INR) measurements ≥2,5 INR. With MixxoCap, the precision was good, with a CV = 3,6%. The MixxoCap results reached the quality goal set by SKUP.
After visual inspections of the PHCC2 measurement results <2,5 INR, it was detected that ID 14 had a duplicate with an atypical difference, close to the outlier-limit. There was no error code or obvious procedure error connected to this measurement either. If this duplicate had been excluded from the calculation of repeatability, the precision for results <2,5 INR had improved to CV = 2,9%.

The new MixxoCap device seems to make the handling of capillary blood sampling easier. The advantages of knowing capillary sampling technique have positive effect on the quality of capillary PT (INR) measurements.

The quality goal set for fraction of technical errors <2% was fulfilled.

5.3. Evaluation of user-friendliness

The most important response regarding user-friendliness comes from the users themselves. The end-users often emphasize other aspects than those pointed out by more extensively trained laboratory personnel. At the end of the evaluation period, both evaluation sites filled in questionnaires about the user-friendliness of MixxoCap and of SSPT.

5.3.1. *Questionnaire about MixxoCap*

The questionnaire was made up of nine questions concerning the use of MixxoCap, see attachment 1. Table 7 summarizes five questions where the evaluators were asked to rate the answers on a scale from 1 to 6, where 1 is difficult and 6 is simple. The last column in table 7 shows the two evaluation sites' ratings.

Table 7. Questions about MixxoCap

Question		Rating
How will you rate your answer to the following questions on a scale from 1 to 6, where 1 is difficult and 6 is simple	To make MixxoCap ready for use	6 — 6
	To collect blood using MixxoCap	6 — 6
	To transfer and mix blood with reagent using MixxoCap	5 — 6
	To work hygienically using MixxoCap	5 — 6
	All in all, to operate MixxoCap	6 — 6

The ratings in table 7 show that both primary health care centres thought that MixxoCap was easy to use and they were satisfied with the device.

None of the primary care centres reported any problems caused by the use of MixxoCap during the evaluation. They were also satisfied with the training and guidance they received from Medic24.

The two primary health care centres were also asked if they had any positive and/or negative comments about MixxoCap.

Positive comments

- Avoid air bubbles in the sample
- Easier to fill with blood than the ordinary SSPT pipette
- Easier to mix the sample with the SSPT reagent

No negative comments about MixxoCap were reported.

Other comments about the device

"We enjoyed using MixxoCap from the first day."
"We wish to start using MixxoCap in our routine as soon as possible."

5.3.2. *Questionnaire about the Simple Simon PT system*

The questionnaire and the expressed opinions about user-friendliness of SSPT are presented in table 8 to 10. The first column shows what is up for consideration. The second column shows the rating by the two evaluation sites. The third to fifth column show the rating options. Coloured frames mark the cells with the overall ratings from the evaluating sites. The last row in each table summarises the rating in the table. The total rating is an overall assessment of the described property, and not necessarily the arithmetic mean of the rating in the row. Consequently, a single poor rating can justify an overall poor rating, if this property seriously influences on the user-friendliness of the system.
Unsatisfactory and intermediate ratings will be marked with an asterisk and explained below the table. The two primary health care centres were given a Norwegian version of the questionnaire.

Table 8. Assessment of time factors

Time factors	**Ratings**	**Overall rating**		
		0 point	**1 point**	**2 point**
Time for preparations / Pre-analytical time	2,2	>10 min	6 to 10 min.	<6 min.
Analytic time	2,2	>20 min	10 to 20 min.	<10 min.
Required training time	2,2	>8 hours	2 to 8 hours	<2 hours
Stability of test (reagent), unopened package	2,2	<3 months	3 to 5 months	>5 months
Stability of test (reagent), opened package	2,2	<14 days	14 to 30 days	>30 days
Other comments about time factors (please specify)		Un-satisfactory	Intermediate	Satisfactory
Rating of time factors				**Satisfactory**

Positive comments: -

Negative comments: -

Table 9. Assessment of quality control possibilities

Quality Control	**Ratings**	**Overall rating**		
		0 point	**1 point**	**2 point**
Internal quality control	2,2	Un-satisfactory	Intermediate	Satisfactory
External quality control	2,2	Un-satisfactory	Intermediate	Satisfactory
Stability of quality control material, unopened	2,2	<3 months	3 to5 months	>5 months
Stability of quality control material, opened	2,2	≤1 day	2 to 6 days	>6 days or disposable
Storage conditions for quality control materials, unopened	1,1	–20°C	+2 to +8°C	+15 to +30°C
Storage conditions for quality control materials, opened	1,1	–20°C	+2 to +8°C	+15 to +30°C
Usefulness of the quality control	2,2	Un-satisfactory	Intermediate	Satisfactory
Other comments about quality control (please specify)		Un-satisfactory	Intermediate	Satisfactory
Rating of quality control				**Satisfactory**

Positive comments: -

Negative comments: -

Table 10. Assessment of the operation facilities

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

Comments:
*One of the primary health care centres reported that the sampling device must be handled as sorted waste.

5.3.3. *Assessment of the user-friendliness*

The two primary health care centres agreed that the MixxoCap device was easy to use, and they were most satisfied with the device.

The two primary health care centres regarded the user-friendliness of Simple Simon PT as satisfactory, based on the assessment of time factors, quality control possibilities and the operation facilities. The staff in both primary health care centres was experienced users of the Simple Simon PT system.

References

1. ISO/DIS 17593; Clinical laboratory testing and in vitro diagnostic test systems – In vitro monitoring systems for anticoagulation therapy self-testing.
2. Fraser, CG & Hyltoft Petersen P. "Quality goals in external quality assessment are best based on biologi", Scand J Clin Lab Invest 1993; 53 suppl. 212. Chapter I. Quality planning.
3. Petersen, P. H., C. G. Fraser, et al. (2002). "Combination of analytical quality specifications based on biological within- and between-subject variation." Ann Clin Biochem 39 (Pt 6): 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 P-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. Stöckl D, Baadenhuijsen H, Fraser CG, Libeer JC, Petersen PH, Ricos C. "Desirable Routine Analytical Goals for Quantities Assayed in serum". Eur J Clin Chem Biochem 1995; 33 (3): 157 – 69.).
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. http://www.sst.dk/English/NPULaboratoryTerminology.aspx
12. Christensen, N.G, Monsen G, Sandberg S, *Utprøving av analyseinstrumenter*. 1997: Alma Mater Forlag.
13. International vocabulary of metrology – Basic and general concepts and associated terms, VIM, 3rd edition, JCGM 200:2008.
14. Burnett RW, "Accurate Estimation of Standard Deviations for Quantitative Methods Used in Clinical Chemistry". Clinical Chemistry 1975; 21 (13): 1935 – 1938.
15. Saunders, E. Tietz textbook of clinical chemistry and molecular diagnostics. 2006. Chapter 14, Linnet, K., Boyd, J. "Selection and analytical evaluation of methods – with statistical techniques", ISBN 0-7216-0189-8.
16. Fraser, C.G. Biological variation: From principles to practice. 2006. Chapter 1 "The Nature of Biological Variation". AACC Press. ISBN 1-890883-49-2.

Attachments

1. Questionnaire, user-friendliness of MixxoCap (in Norwegian)
2. Specifications and basic facts about Simple Simon PT
3. Raw data PT (INR), internal quality control, Simple Simon PT
4. Raw data PT (INR), results from two primary health care centres, Simple Simon PT
5. "SKUP-info". Summary for primary health care (in Norwegian)
6. List of previous SKUP evaluations
7. Comments from Zafena AB

Attachments with raw data are included only in the report to Zafena AB and Medic24.

Navn legekontor: _____

Simple Simon PT

Spørreskjema om MixxoCap; et redskap for oppsugning og overføring av 10 µL kapillærblod

Hvordan vil du rangere følgende på en skala fra 1 til 6, der 1 er *vanskelig* og 6 er *enkelt*:

1. Å gjøre klar MixxoCap til bruk

Vanskelig *Enkelt*

1 **2** **3** **4** **5** **6**

☐ ☐ ☐ ☐ ☐ ☐

2. Å bruke MixxoCap til å suge opp blod

Vanskelig *Enkelt*

1 **2** **3** **4** **5** **6**

☐ ☐ ☐ ☐ ☐ ☐

3. Å bruke MixxoCap til overføring og blanding av blod med reagenset

Vanskelig *Enkelt*

1 **2** **3** **4** **5** **6**

☐ ☐ ☐ ☐ ☐ ☐

4. Å arbeide hygienisk med MixxoCap

Vanskelig *Enkelt*

1 **2** **3** **4** **5** **6**

☐ ☐ ☐ ☐ ☐ ☐

5. Å betjene MixxoCap, totalt sett

Vanskelig *Enkelt*

1 **2** **3** **4** **5** **6**

☐ ☐ ☐ ☐ ☐ ☐

Snu arket

6. **Oppstod det problemer som skyldtes bruk av MixxoCap?** ☐ **Ja** ☐ **Nei**

   Hvis ja, kan du beskrive problemet/ene:_____

   _____

   _____

7. **Fikk du/dere tilstrekkelig opplæring/veiledning i bruk av MixxoCap?** ☐ **Ja** ☐ **Nei**

   Hvis nei, kan du beskrive hva som manglet: _____

   _____

   _____

8. **Synes du det er noen fordeler med MixxoCap?**

- _____
- _____
- _____

9. **Synes du det er noen ulemper med MixxoCap?**

- _____
- _____
- _____

Evt. andre kommentarer:_____

_____

_____

_____

Specifications and basic facts about Simple Simon PT

Parts of this form are filled in by the producer.

Table 1. Facts about the measurement system

Name of the measurement system:	Simple Simon® PT
Components of the measurement system:	Simple Simon® PT reader, reagents, Zafena Abnormal Plasma (ZAP), pipette, Mixxocap and plastic consumables
Measurand:	Prothrombin time, PT (INR)
Sample material:	Capillary blood, venous blood and citrated plasma
Sample volume:	10µL
Measuring principle:	Optical
Traceability:	EQUALIS calibrators, and International Reference Preparation (IRP) RBT/05
Calibration:	By manufacturer, lot (batch) specific
Measuring range:	INR 0.7 to INR 8.0 (by upper limit adjustable if desired, currently only INR 8.0)
Linearity:	INR 0.7 to INR 8.0 (uncertainty about linearity above INR 8)
Measurement duration:	Plasma (22°C): INR1,0 ~ 33 seconds, INR 2,5 ~ 65 seconds Blood (22°C): INR 1,0 ~ 36 seconds, INR 2,5 ~ 78 seconds
Operating conditions:	Operable between 17°C and 40°C, operation outside of range is automatically prohibited

Table 2. Facts about the instrument

Dimensions:	Width: 100 mm, Depth:145 mm, Height: 65 mm
Weight:	720 g
Electrical power supply:	3 AA batteries
Is input of patient identification number possible?	Yes, via the IT-product Zafena Connector
Can the instrument be connected to a bar-code reader?	Yes, via the IT-product Zafena Connector
Can the instrument be connected to a printer?	Yes, via the IT-product Zafena Connector
What can be printed?	Testresults, Sample ID (PID or LID), reader ID, reader number, operator ID, time and date, etc.
Can the instrument be connected to a computer?	Yes
What is the storage capacity of the instrument and what is stored in the instrument?	Via the IT-product Zafena Connector approximately 50.000 results, including progression tracings of the reaction. When used together with other POC-devices, ones that do not deliver reaction

	graphs, millions of results can be stored.
Recommended regular maintenance:	None, apart from replacement of batteries about every 1500 tests. The reader will be replaced with one that has undergone factory service and quality assurance in connection with introduction of new lot, about once every 15 months. The IT-product Zafena Connector receives updated program once every year.
Package contents:	Reader, pipette
Necessary equipment not included in the package:	None, reader and pipette are renewed by manufacturer with change of lot (batch)

Table 3. Facts about the reagent/test strips/test cassettes of the measurement system

Name of the reagent/test strips/test cassettes:	Simple Simon®PT reagent and buffer
Stability in unopened sealed vial:	24 months stored at 2-8°C, typically 18 months open stability at delivery
Stability in opened vial:	3 weeks (21 days) when handled according to instructions
Package contents:	20 vials of freeze dried reagent, 20 vials of buffer (refrigerated storage). Plastic consumables for 400 tests are included (room temperature storage)

Table 4. Facts about quality control for the measurement system

Electronic self check:	No
Recommended check materials and volume:	Zafena Abnormal plasma, ZAP, 400µL (other plasma controls may be used)
Stability in unopened sealed vial:	30 months when stored at 2-8°C
Stability in opened vial:	2 weeks (14 days) when handled according to instructions
Package contents:	10 vials of lyophilized control plasma, 1 vial of buffer

Table 5. Marketing information about the measurement system

Manufacturer:	Zafena AB, visiting, and postal address: Husbyvägen 16, 590 31 Borensberg, Sweden
Retailers in Scandinavia:	Denmark: Medic24 Norway:Medic24 Sweden:Zafena and Medic24
In which countries is the system marketed:	Globally ☐ Scandinavia x Europe ☐
Date for start of marketing the system in Scandinavia:	July 2005
Date for CE-marking:	February 7, 2005
In which Scandinavian languages is the manual available:	Swedish

Attachment 3

Raw data PT (INR), internal quality control, Simple Simon PT

Simple Simon PT Control	Lot	Expiry	PT (INR) level
ZAP (Zafena Abnormal Plasma)	K373M	03-2013	2,45 (2,20 – 2,70)
Buffer	K373E-1	03-2013	-

Simple Simon PT Control ZAP analysed on the two primary health care centres, instrument A and B

Date	Legekontoret Kleppestø Senter Control Zap, PT (INR)
	Instrument A and B
16.mar	2,27
17.mar	2,50
18.mar	2,48
21.mar	2,59
22.mar	2,40
23.mar	2,36
24.mar	2,36
25.mar	2,63
28.mar	2,22
29.mar	2,50
30.mar	2,57
31.mar	2,43
01.apr	2,37
04.apr	2,40
05.apr	2,60

Date	Arna Legekontor Control Zap, PT (INR)
	Instrument A and B
16.mar	2,34
17.mar	2,62
21.mar	2,50
22.mar	2,30
23.mar	2,39
24.mar	2,42

SKUP-info

MixxoCap; et redskap til bruk ved kapillær prøvetaking og analysering av PT-INR på Simple Simon PT
Produsent: Zafena AB
Forhandler i Norge: Medic24

Sammendrag fra en utprøving i regi av SKUP

Konklusjon
Et legekontor som hadde erfaring med kapillært prøvemateriale til analyse av PT-INR fikk god presisjonen med MixxoCap på Simple Simon (CV 3,6 %). Kvalitetskravet (CV < 5 %) ble oppfylt.
Et legekontor som ikke hadde erfaring med kapillære prøver til PT-INR fikk god presisjon i PT-nivå < 2,5 INR. Dette legekontoret fikk litt dårligere presisjon når INR var over 2,5.
MixxoCap ser ut til å gjøre håndteringen av kapillært prøvemateriale enklere. Begge legekontorene var fornøyde med det nye kapillærredskapet MixxoCap og med Simple Simon PT.

MixxoCap er et redskap som kan benyttes til oppsugning, overføring og blanding av kapillærblod ved analysering av kapillære prøver på Simple Simon PT (SSPT).
Simple Simon PT er beregnet for pasientnær testing av protrombintid (PT) i primærhelsetjenesten. Analysen utføres med 10 µL kapillærblod, venøst blod eller citratplasma og 200 µL reagens. Måleområdet er fra 0,7 til 8,0 INR.
SSPT ble utprøvd av SKUP på et sykehuslaboratorium i 2006. Rapporten (SKUP/2007/57*) finnes på www.skup.nu.

Utprøvingen av MixxoCap ble utført på to norske legekontor. De hadde SSPT instrument fra før. Til rutinemåling av PT-INR benyttet det ene legekontoret venøse prøver, mens det andre analyserte PT-INR i kapillærblod ved hjelp av den vanlige SSPT pipetten. Til utprøvingen av MixxoCap ble det tatt prøver av 74 personer med til sammen 84 kapillære prøver i duplikat.

Resultater
Legekontoret som hadde erfaring med analysering av kapillære prøver til PT-INR fikk god presisjon med MixxoCap, med CV på ca. 3,6 %. Kvalitetsmålet ble oppfylt.
Legekontoret som ikke hadde erfaring med kapillære prøver til PT-INR, fikk god presisjon i PT-nivå under 2,5 INR. De fikk litt høyere CV (5,7 %) når INR var over 2,5. Det var spesielt *en* dårlig dobbeltmåling som gjorde at denne presisjonen ikke ble like god.
Praktisk trening med kapillær prøvetakingsteknikk ser ut til å være av betydning for å oppnå best mulig presisjon på målinger av PT-INR på Simple Simon PT. Det nye MixxoCap-redskapet ser ut til å gjøre håndteringen av kapillært prøvemateriale enklere.

Brukervennlighet
Begge legekontorene syntes MixxoCap var enkelt å bruke, og de var fornøyde med både kapillærredskapet og med Simple Simon PT.

Tilleggsinformasjon
Den fullstendige rapporten fra utprøvingen av MixxoCap og Simple Simon PT, SKUP/2011/84*, finnes på SKUPs nettside www.skup.nu. Kommentarer fra produsenten Zafena ligger som et vedlegg til rapporten. Opplysninger om pris fås ved å kontakte leverandør.
Laboratoriekonsulentene i NOKLUS kan gi nyttige råd om analysering av PT-INR på legekontor. De kan også orientere om det som finnes av alternative metoder/utstyr.

List of previous SKUP evaluations

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

SKUP evaluations from number 51 and further

Evaluation no.	Component	Instrument/testkit	Producer
SKUP/2010/89*	Glucose	FreeStyle Lite	Abbott Laboratories
SKUP/2010/88*	HbA1c	*Confidential*	
SKUP/2011/86	Glucose[1]	OneTouch Verio	LifeScan, Johnson & Johnson
SKUP/2011/84*	PT (INR)	Simple Simon PT and MixxoCap	Zafena AB
SKUP/2010/83*	Glucose	*Confidential*	
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/2010/80	PT (INR)	INRatio2	Alere Inc.
SKUP/2010/79*	Glucose, protein, blood, leukocytes, nitrite	CombiScreen 5SYS Plus urine test strip and CombiScan 100 urine analyser	Analyticon Biotechnologies AG
SKUP/2010/78	HbA1c	In2it	Bio-Rad
SKUP/2011/77	CRP	*Confidential*	
SKUP/2009/76*	HbA1c	*Confidential*	
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/2011/70*	CRP	smartCRP system	Eurolyser Diagnostica GmbH
SKUP/2008/69*	Strep A	Diaquick Strep A test	Dialab GmbH
SKUP/2010/67	Allergens	*Confidential*	
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 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.

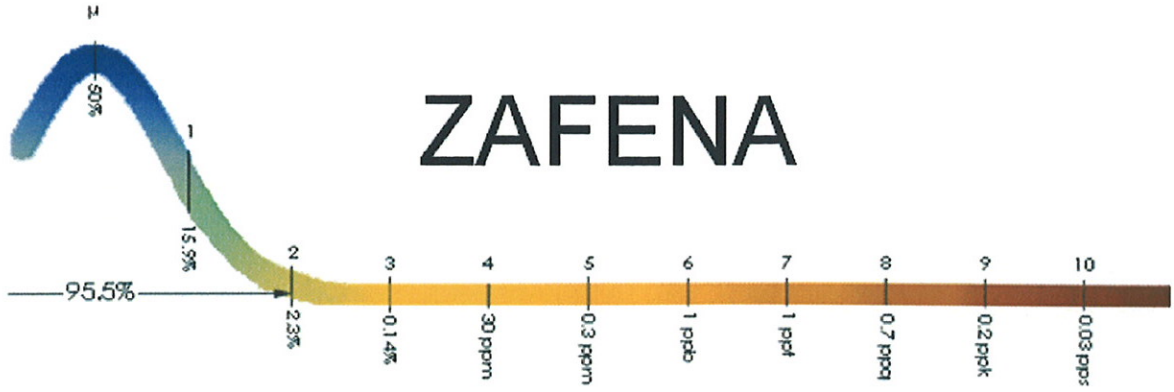
[1] Including a user-evaluation among diabetes patients

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 & 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	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 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
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.



Comments from the manufacturer

The present is a well designed and well executed clinical evaluation of a near patient laboratory diagnostic equipment, Simple Simon® PT, and of a disposable capillary device MixxoCap for collecting and handling fingertip blood.

Simple Simon PT fulfilled the quality goals of SKUP; the repeatability CV was <5%, and the frequency of technical errors was <2%.

The quality goals of SKUP were fulfilled also when fingertip blood, collected by MixxoCap, was analyzed.

The medical quality of the anticoagulation treatment was excellent at the two primary health care centers engaged in the study. At both centers Simple Simon PT has been used for years to control the intensity of anticoagulation treatment with warfarin (Marevan®), with the goal of maintain the INR within the "therapeutic" range, i.e. between 2.0 and 3.0. The study enrolled, at random, essentially sequentially, 86 patients, and according to the routine INR procedure of the center, the fraction of patients in-range was 77%. According to the experimental INR procedures of the evaluation, i.e. with MixxoCap instead of pipette, and for PHCC1, fingertip blood instead of venous blood, the fraction of patients in-range was 82%. It is important to note, that the medical achievement at both primary care centers was excellent. In an international perspective, an in-range of 80% is remarkably good, and is, in a sense, a main medical goal of the anticoagulation treatment. As a manufacturer of laboratory diagnostic equipment, Zafena is proud to have been involved.

The evaluation punctured rumors, three of them, as far as Simple Simon is concerned:

1. The Simple Simon PT device, which employs a wet-chemistry procedure, is rumored to be operable only by personnel with formal laboratory training. In the present evaluation, six operators were involved, three health secretaries and two medical secretaries (none with formal medical training). The personnel were well acquainted with Simple Simon and found it practical and convenient. The present evaluation showed that the personnel reached the analytical quality goals of SKUP, and, most important, accomplished excellent medical results.

2. It is rumored that blood by venous puncture ("glas") gives superior INR results compared to blood by fingertip puncture (native blood). The present evaluation does <u>not</u> support this rumor. By Simple Simon PT, using MixxoCap for blood collection, the analytical quality of INR determined on fingertip blood was as good as that

determined on venous blood. This is an important finding; a venous puncture is a more invasive than a fingertip puncture, and if unnecessary should be avoided.

3. It is rumored that only the first drop of fingertip blood is suited for INR determination. The present evaluation does not support this rumor. At PHCC2, INR results from the second drop of blood was compared to the fourth drop (the first and third drop were wiped away), no difference was found. It is assumed, that less than about two minutes elapse between the puncture and the collection, and use, of the fourth drop. The evaluation showed that procedural details in collecting of fingertip are not a critical. Regardless of how, within reason, the blood from a finger puncture is collected with MixxoCap, and analyzed for INR, by Simple Simon, the results will be good.

The Zafena Connector:
The reader of Simple Simon PT can be connected to an IT-device, the Connector, made up of a small computer box, a small USB screen, and a bar code reader.

The Connector is a new product that during the last eight months has been installed at about 40 Swedish primary care centers. The Connector was considered for use in the present evaluation, but from this it was refrained because the Connector was then not yet in routine use in Norway. If it had been used, the Connector could have ruled in, or ruled out, deviant duplicates as transcription errors, et cetera.

Borensberg, September 5, 2011

Mats Rånby
Zafena AB