Afinion 2™ Analyzer

A system for measurement of HbA1c, CRP, ACR and Lipid Panel manufactured by Abbott Diagnostics Technologies AS

An evaluation of the measurement of HbA1c

Report from the evaluation SKUP/2021/126

organised by SKUP at the request of Abbott Rapid Diagnostics GmbH in Germany

SKUP in Denmark, DEKS, Rigshospitalet-Glostrup, DK-2600 Glostrup, Phone +45 38634406, www.skup.org
SKUP in Norway, Noklus, Box 6165, NO-5892 Bergen, Phone +47 480 15 282, www.skup.org
SKUP in Sweden, Equalis, Box 977, SE-751 09 Uppsala, Phone: +46 18 490 31 44, www.skup.org

SKUP secretariat
Anne Christin Breivik
+47 480 15 282
anne.breivik@noklus.no

SKUP in Denmark
Dår Kristian Kur
Gitte M. Henriksen
DEKS
Rigshospitalet – Glostrup
Valdemar Hansens vej 1-23
DK-2600 Glostrup
+45 38 63 44 06
daar.kur@deks.dk
gitte.m.henriksen@deks.dk

SKUP in Norway
Anne Christin Breivik
Hege Alvheim
Mette Christophersen Tollånes Sverre Sandberg
Noklus
Boks 6165
NO-5892 Bergen
+47 480 15 282
anne.breivik@noklus.no
hege.alvheim@noklus.no
mette.tollanes@noklus.no
sverre.sandberg@noklus.no

SKUP in Sweden
Elisabet Eriksson Boija
Gunnar Nordin
Equalis AB
P.O. Box 977
SE-751 09 Uppsala
+46 18 490 31 44
elisabet.eriksson.boija@equalis.se
gunnar.nordin@equalis.se

www.SKUP.org

SKUP would like to acknowledge with thanks those who contributed to the practical work with this evaluation including Pernille Gjengedal in Kerteminde primary health care centre; *Charlotte Loft Reuter and Karin Slyk Jørgensen in Glamsbjerg primary health care centre; Catharina Désirée Strange and Barbara Ella Toftanes in the department of Clinical Biochemistry and Pharmacology, Odense University Hospital.*

Table of contents

ATTACHMENTS

Attachments with raw data are included only in the copy to Abbott Rapid Diagnostics GmbH

1. Summary

Background
The Afinion 2 Test System is an in vitro diagnostic device for quantitative measurement of Haemoglobin A1c (HbA1c), C-reactive protein (CRP), albumin/creatinine ratio (ACR) and Lipid Panel. The product is intended for professional use. The sample material is fresh capillary whole blood and venous whole blood with an anticoagulant. The system is produced by Abbott Diagnostics Technologies AS and was launched into the Scandinavian market in 2017. A predecessor of the system, the Afinion AS100 Analyzer, is still on the market. The SKUP evaluation was carried out May to June 2021 at the request of Abbott Rapid Diagnostics GmbH in Germany.

The aim of the evaluation
The aim of the evaluation was to assess the analytical quality and user-friendliness of the Afinion 2 Analyzer for measurement of HbA1c, both when used under optimal conditions by experienced laboratory personnel and under real-life conditions by intended users in primary health care centres (PHCCs).

Materials and methods
Under optimal conditions fresh capillary whole blood samples from 100 patients were measured on the Afinion 2 Analyzer. Under real-life conditions, in two PHCCs, fresh capillary whole blood samples from a total of 97 patients were measured on the Afinion 2 Analyzer. Venous whole blood samples from the same patients were analysed on a comparison method (Tosoh Automated Glycohemoglobin Analyzer HLC-723 G11, Tosoh Bioscience, Inc.). The trueness of the comparison method was verified with fresh frozen venous samples with certified values. The analytical results and user-friendliness were assessed according to pre-set quality goals. The quality goal for precision was a repeatability (CV) ≤3,0 %. For accuracy ≥95 % of the results should be within ±3,0 mmol/mol from the results of the comparison method in HbA1c concentration <35,3 mmol/mol and within ±8,5 % at HbA1c concentration ≥35,3 mmol/mol. The user-friendliness was assessed using a questionnaire with three given ratings; satisfactory, intermediate and unsatisfactory, and with the quality goal of a total rating of "satisfactory".

Results
The CV achieved under optimal conditions varied between 1,2 and 1,8 % depending on the concentration level. The CV achieved by intended users varied between 1,2 and 1,7 %. Under optimal conditions, the bias between the Afinion 2 Analyzer and the comparison method was 0,70 and 2,00 mmol/mol at level 2 and level 3, respectively. There were no bias shown at the PHCCs. Under optimal conditions, 95 % of the results were within the allowable deviation limits and when handled by intended users, 97 % of the results were within the limits for accuracy. The user-friendliness was rated as satisfactory.

Conclusion
The quality goal for repeatability was fulfilled under optimal conditions and by intended users. The quality goal for accuracy was fulfilled both under optimal conditions and by intended users. The quality goal for user-friendliness was fulfilled.

Comments from Abbott Rapid Diagnostics GmbH
A letter with comments from Abbott Rapid Diagnostics GmbH is attached to the report.

This summary is also published in Danish, Norwegian and Swedish at www.skup.org.

2. Abbreviations and Acronyms

ACR	Albumin/Creatinine Ratio
BLS	Biomedical Laboratory Scientist
C-NPU	Committee on Nomenclature, Properties and Units
CI	Confidence Interval
CRP	C-reactive protein
CV	Coefficient of Variation
DANAK	The Danish Accreditation Fund
DCCT	Diabetes Control and Complications Trial
DEKS	Danish Institute of External Quality Assurance for Laboratories in the Health Sector
DSKB	The Danish Society of Clinical Chemistry
EDTA	Ethylenediaminetetraacetic acid
EQA	External Quality Assessment
Equalis	External quality assessment in laboratory medicine in Sweden
HbA1c	Haemoglobin A1c
HPLC	High performance liquid chromatography
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
KBF-OUH	Clinical Biochemistry and Pharmacology, Odense University Hospital
LC/MS	Liquid Chromatography / Mass Spectrometry
NGSP	National Glycohaemoglobin Standardization Program
Noklus	Norwegian Organization for Quality Improvement of Laboratory Examinations
PHCC	Primary Health Care Centre
SD	Standard Deviation
SKUP	Scandinavian evaluation of laboratory equipment for point of care testing
VUK	Videnskabeligt Udvalg for Kvalitetssikring (The Danish Scientific Committee for Quality Assurance)

3. Introduction

The purpose of Scandinavian evaluation of laboratory equipment for point of care testing (SKUP) is to improve the quality of near patient testing in Scandinavia by providing objective information about analytical quality and user-friendliness of laboratory equipment. This information is generated by organising SKUP evaluations in point of care settings.

3.1. The concept of SKUP evaluations

SKUP evaluations follow common guidelines and the results from various evaluations are comparable[1]. The evaluation set-up and details are described in an evaluation protocol and agreed upon in advance. The analytical results and user-friendliness are assessed according to pre-set quality goals. To fully demonstrate the quality of a product, the end-users should be involved in the evaluation. If possible, SKUP evaluations are carried out using three lot numbers of test cartridges from separate and time-spread productions. Some evaluation codes are followed by an asterisk (*), indicating an evaluation with a more specific objective. The asterisk is explained on the front page of these protocols and reports.

3.2. Background for the evaluation

The Afinion 2 Test System is an in vitro diagnostic device for the quantitative measurement of Haemoglobin A1c (HbA1c), C-reactive protein (CRP), Albumin/Creatinine Ratio (ACR) and Lipid Panel. The product is intended for professional use. The sample material is fresh capillary whole blood or venous ethylenediaminetetraacetic acid (EDTA) whole blood. The Afinion 2 Analyzer is an upgraded version of the Afinion AS100 Analyzer, which was evaluated by SKUP in 2008. Both systems are still on the market. The Afinion 2 Test System is produced by Abbott Diagnostics Technologies AS and was launched into the Scandinavian market in 2017. The SKUP evaluation was carried out May 2021 to June 2021 at the request of Abbott Rapid Diagnostics GmbH in Germany.

3.3. The aim of the evaluation

The aim of the evaluation was to assess the analytical quality and user-friendliness of the Afinion 2 Analyzer for measurement of HbA1c, both when used under optimal conditions by experienced laboratory personnel and when used under real-life conditions by intended users in primary health care.

3.4. The model for the evaluation of the Afinion 2 Analyzer

SKUP evaluations for quantitative methods are based upon the fundamental guidelines in a book concerning evaluations of laboratory equipment in primary health care [1].
This evaluation consisted of two parts (figure 1). One part of the evaluation was carried out under optimal conditions by experienced laboratory personnel. This part documents the quality of the system under conditions as favourable as possible for achieving good analytical quality. The other part of the evaluation was carried out by intended users in two primary health care centres (PHCCs). This part documents the quality of the system under real-life conditions.

[1]SKUP evaluations are under continuous development. In some cases, it may be difficult to compare earlier protocols, results and reports with more recent ones.

The evaluation of the Afinion 2 Analyzer for measurement of HbA1c in fresh capillary whole blood samples included:

- Examination of the analytical quality (precision and accuracy) under optimal conditions
- Examination of the analytical quality (precision and accuracy) in the hands of intended users
- Evaluation of the user-friendliness of the Afinion 2 Analyzer and its manual by intended users

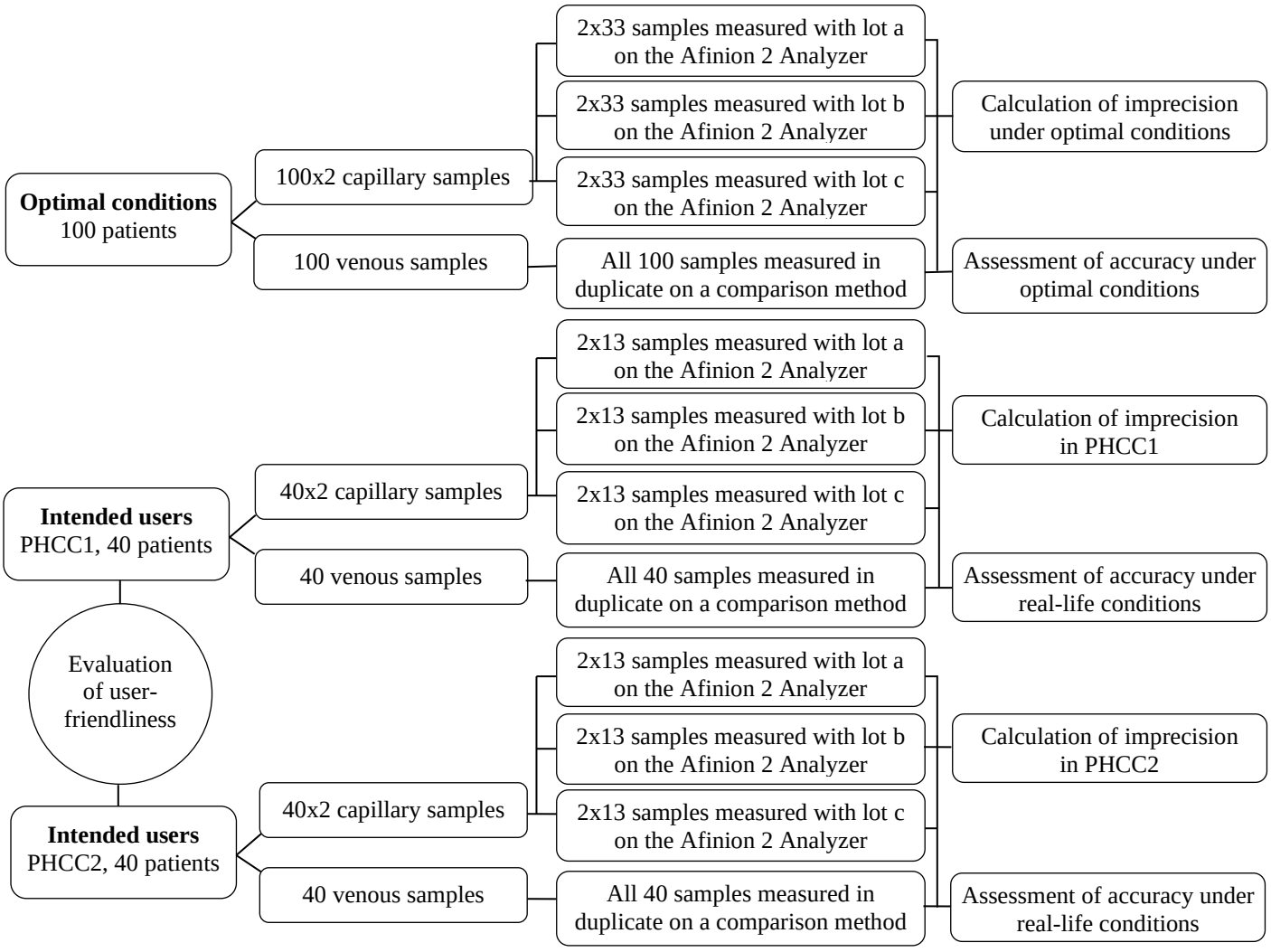

Figure 1. Flowchart illustrating the model for the evaluation of the Afinion 2 Analyzer for measurement of HbA1c.

4. Quality goals

4.1. Analytical quality

The quality goals in this evaluation are based on HbA1c results expressed in mmol/mol (IFCC units; International Federation of Clinical Chemistry and Laboratory Medicine). Quality goals specified for HbA1c results in mmol/mol must be recalculated to quality goals for results expressed in National Glycohaemoglobin Standardization Program (NGSP) units. Weycamp *et al.* [2] have explained why the numerical values for analytical goals for HbA1c measurement in mmol/mol and Diabetes Control and Complications Trial (DCCT) % are different.

The Danish Society of Clinical Chemistry (DSKB) has a scientific committee for quality assurance Videnskabeligt Udvalg for Kvalitetssikring (VUK). In 2011, the committee specified the following quality goals for HbA1c mmol/mol when used for diagnosis and monitoring of diabetes in Denmark [3]:
Maximum allowable imprecision CV (coefficient of variation): 2,8 %
Maximum allowable bias at HbA1c level 48 mmol/mol: ± 2,8 %
Maximum allowable deviation at HbA1c level 48 mmol/mol: ± 7,3 % (requirement for deviation from true target).

The Norwegian Directorate of Health specified quality goals for diagnostic use of HbA1c.
The HbA1c method must be traceable to the IFCC reference method, and a deviation <±7,4 % (in IFCC units) from reference target at a level of 48 mmol/mol and a CV <3 % must be documented [4, 5].

In Sweden, the national analytical quality goals are set up by External quality assessment in laboratory medicine in Sweden's (Equalis) advisory group for protein analysis and were approved by the Swedish Association for Clinical Chemistry in 2010 [6].
Maximum bias: ± 1,5 mmol/mol
Between-laboratories-variation (CV): 2,5 %
Allowable deviation: bias + 1,65 × standard deviation (SD) ~ bias + 1,65 × 0,025 × HbA1c level
Thus, the allowable deviation at 48 mmol/mol is $\leq$±3,5 mmol/mol.

SKUP has chosen to use a quality goal of 3,0 CV % for repeatability. To fulfil the accuracy goal at least 95 % of the individual HbA1c results shall fall within ±3,0 mmol/mol of the average measured values of the comparison method at HbA1c concentrations <35,3 mmol/mol, or within ±8,5 % at HbA1c concentrations ≥35,3 mmol/mol. The quality goals are based on SKUPs own estimations and the national recommendations from Denmark, Norway and Sweden [7]. SKUP's quality goals for HbA1c in this evaluation are as presented in section 4.4.

4.2. User-friendliness

The evaluation of user-friendliness was carried out by asking the evaluating persons in the PHCCs to fill in a questionnaire, see section 6.5. The system must reach a total rating of "satisfactory" to fulfil the quality goal.

Technical errors

SKUP recommends that the fraction of tests wasted due to technical errors should not exceed 2 %.

4.3. Principles for the assessments

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.

4.3.1. Assessment of the analytical quality

The analytical results were assessed according to pre-set quality goals.

Precision

The decision whether the achieved CV fulfils the quality goal or not, is made on a 5 % significance level (one-tailed test). The distinction between the ratings, and the assessment of precision according to the quality goal, are shown in table 1. Based on the results from each evaluation site, an overall conclusion will be drawn in the summary of the report.

Table 1. The rating of precision

Distinction between the ratings	Assessment according to the quality goal
The CV is equal or lower than the quality goal (statistically significant)	The quality goal is fulfilled
The CV is equal or lower than the quality goal (not statistically significant)	Most likely the quality goal is fulfilled
The CV is higher than the quality goal (not statistically significant)	Most likely the quality goal is not fulfilled
The CV is higher than the quality goal (statistically significant)	The quality goal is not fulfilled

Bias

SKUP does not set separate quality goals for bias. The confidence interval (CI) of the measured bias is used for deciding if a difference between the evaluated method and the comparison method is statistically significant (two-tailed test, 5 % significance level). The bias will also be discussed in connection with the accuracy. Proven systematic deviation of the results achieved by intended users will be discussed in relation to the bias found under optimal conditions.

Bias with three lots of test cartridges

Separate lot calculations were not performed. The results achieved with the three lots are included in the assessment of accuracy in the difference plot. If distinct differences between the lots appear, this will be pointed out and discussed.

Accuracy
The accuracy was illustrated in a difference plot with limits for the allowable deviation according to the quality goal. The fraction of results within the limits is counted. The accuracy was assessed as either fulfilling the quality goal or not fulfilling the quality goal.

4.3.2. Assessment of the user-friendliness
The user-friendliness was assessed according to the answers and comments given in the questionnaire (see section 6.5). For each question, the evaluator could choose between three given ratings; satisfactory, intermediate and unsatisfactory. A written guidance with examples was available. The responses from the evaluators was reviewed and summed up. To achieve the overall rating "satisfactory", the tested equipment must reach a total rating of "satisfactory" in all four subareas of characteristics described in section 6.5.

Technical errors
The evaluating persons registered error codes, technical errors and failed measurements during the evaluation. The fraction of tests wasted due to technical errors was calculated and taken into account in connection with the assessment of the user-friendliness. Possible technical errors included errors regarding reading of data matrix, errors in the detection of the test cartridges and electronic errors. User errors were not included in the calculation.

4.4. SKUP's quality goals in this evaluation
As agreed upon when the protocol was drawn up, the results from the evaluation of the Afinion 2 Analyzer were assessed against the following quality goals:

Repeatability (CV) ..... ≤3,0 %
Allowable deviation of the individual result from the comparison method result
for HbA1c concentrations <35,3 mmol/mol ..... ≤±3,0 mmol/mol
and for HbA1c concentrations ≥35,3 mmol/mol ..... ≤±8,5 %
Required percentage of individual results
within the allowable deviations……..... ≥95 %
User-friendliness, overall rating..... Satisfactory

The results in this evaluation will only be presented in mmol/mol.
Results can be recalculated between the two units with the following equations:
HbA1c (IFCC, mmol/mol) = 10,93 × HbA1c (NGSP, %) – 23,54
HbA1c (NGSP, %) = 0,0915 × HbA1c (IFCC, mmol/mol) + 2,153

5. Materials and methods

5.1. Definition of the measurand

The measurement systems intend to measure the substance fraction of glycated haemoglobin per mol haemoglobin measured in whole blood. For the evaluated system the sample material in this evaluation is fresh capillary whole blood, and for the comparison method the sample material is venous EDTA blood. The results are traceable to the IFCC Reference method and are expressed in the unit mmol/mol. The Committee on Nomenclature, Properties and Units (C-NPU) systematically describes clinical laboratory measurands in a database [8]. The NPU code related to the measurand in this evaluation is NPU27300. Some parts of the world only accept HbA1c results in NGSP unit (%), which is specified in NPU03835. In this report the term HbA1c will be used for the measurand.

5.2. The evaluated measurement system the Afinion 2 Analyzer

The information in this section derives from the company's information material.

The Afinion 2 Analyzer (figure 2) is intended for professional use in clinical laboratory settings or locations for near-patient testing, such as in PHCCs. For the Afinion 2 Test System, Afinion HbA1c, CRP, ACR and Lipid Panel test kits are available. The Afinion 2 Analyzer for measurement of HbA1c includes:

- The Afinion 2 Analyzer
- Afinion HbA1c test kit

Figure 2. The Afinion 2 Analyzer.

The Afinion 2 Analyzer for measurement of HbA1c is an in vitro diagnostic test system designed to quantitatively determine HbA1c in human capillary whole blood and venous whole blood treated with anticoagulant (K_2 or K_3-EDTA, lithium or sodium heparin, sodium citrate or sodium fluoride/potassium oxalate).

The measurement principle is boronate affinity. After placing the test cartridge, containing the blood sample, into the Afinion 2 Analyzer the sample is automatically diluted and mixed with a solution that releases haemoglobin from the erythrocytes. The haemoglobin precipitates, and blue boronate acid conjugate in the solution binds to the unique cis-diol configuration of HbA1c to form a stable blue boronate-haemoglobin conjugate. The reaction mixture is soaked through a filter membrane and all the precipitated haemoglobin (i.e. glycated and non-glycated haemoglobin) are collected on the membrane. Any excess of conjugate is removed with a washing reagent. The concentration of HbA1c is calculated by measuring the ratio between the blue (conjugated haemoglobin) and the red (haemoglobin) color intensities reflected in the precipitate on the membrane.

The blood sample is collected using the integrated transfer capillary on the test cartridge. The sample volume is 1,5 µL and the measurement time is approximately 3 minutes. The results are presented in mmol/mol or NGSP unit (%). The Afinion 2 Analyzer can store up to 500 patient results and 500 control results. When the Afinion HbA1c test cartridge enters the instrument, the integrated camera automatically reads the lot-specific calibration data stored in the barcode label on the test cartridge, eliminating the need for calibration by the user. Each lot of the Afinion

HbA1c test cartridge is traceable to the IFCC reference method [9]. Each Afinion HbA1c Control kit contains two levels of liquid quality control samples (Cont. I and Cont. II); normal and abnormal.

For technical details about the Afinion 2 Analyzer, see table 2. For more information about the Afinion 2 Analyzer, and name of the manufacturer and the suppliers in the Scandinavian countries, see attachment 2 and 3. For product specifications in this evaluation, see attachment 4.

Table 2. Technical details from the manufacturer.

Technical details for the Afinion 2 Analyzer	
Sample material	Fresh capillary or venous blood with K_2/K_3-EDTA, lithium or sodium heparin, sodium citrate or sodium fluoride/potassium oxalate.
Sample volume	1,5 µL
Measuring time	Approx. 120 seconds
Measuring range	20 – 140 mmol/mol
Storage capacity	500 patient and control results
Electrical power supply	100 – 240 V

5.3. The selected comparison method

A selected comparison method is a fully specified method which, in the absence of a Reference method, serves as a common basis for the comparison of the evaluated method.

5.3.1. The selected comparison method in this evaluation

The selected comparison method in this evaluation was the routine method for HbA1c; Tosoh Automated Glycohemoglobin Analyzer HLC-723 G11 at the department of Clinical Biochemistry and Pharmacology, Odense University Hospital (KBF-OUH), Denmark hereafter called "the comparison method". The same instrument was used throughout the evaluation. The method is accredited according to DS/EN ISO 15189 by The Danish Accreditation Fund (DANAK).

Principle:	High performance liquid chromatography (HPLC)
Column:	TSK gel G11 Variant
Reagent:	G11 Elution buffer HSi Variant No.1, No.2, No.3, haemolyzing solution and wash solution
Calibrators:	HbA1c calibrators in three levels from Danish Institute of External Quality Assurance for Laboratories in the Health Sector (DEKS). Traceable to IFCC method and reference materials developed by IFCC Working group on Standardization of HbA1c [9]
Reportable range:	20 – 195 mmol/mol

Internal analytical quality control
Internal analytical quality control samples, two levels (Lyphochek Diabetes Control, normal and abnormal, Bio-Rad), were measured each evaluation day on the comparison method.

External analytical quality control
The hospital laboratory participates in Labquality's external quality assessment (EQA) scheme for HbA1c with two levels in six rounds per year. The EQA control material is fresh human whole blood. The assigned value for HbA1c is traceable to the IFCC reference method.

5.3.2. Verification of the analytical quality of the comparison method

Precision
The repeatability (CV) of the comparison method was calculated from duplicate measurements of the venous patient sample collected under optimal conditions.

Trueness
To document the trueness of the comparison method, fresh frozen venous K_2-EDTA patient samples with certified values assigned from the IFCC liquid chromatography / mass spectrometry (LC/MS) reference measurement procedure at INSTAND, Germany were used [10]. The samples consist of four patient samples with HbA1c concentrations at different levels with given uncertainties. The target value is given with an expanded uncertainty of <2 % (k=2). If necessary, the comparison method's results are adjusted according to the certified INSTAND targets. The adjustment is carried out by means of inverse calibration [11,12]. The trueness of the comparison method was also verified with EQA results for a period circumventing the evaluation period.

5.4. The evaluation

5.4.1. Planning of the evaluation

Inquiry about an evaluation

Abbott Rapid Diagnostics GmbH via Susanne Emmerich, EMEA Medical Director, applied to SKUP in October 2020 for an evaluation of the Afinion 2 Analyzer.

Protocol, arrangements and contract

In April 2021, the protocol for the evaluation was approved, and Abbott Rapid Diagnostics GmbH and SKUP signed a contract for the evaluation. Biomedical laboratory scientists (BLSs) at the department of KBF-OUH were assigned to do the practical work with the Afinion 2 Analyzer under optimal conditions and analysing the samples on the comparison method. Two PHCCs, Morten Toftagers lægepraksis and Glamsbjerglægerne from Funen county agreed to represent the intended users in this evaluation.

Training

Birgit Lybye and Marianne Schreiner from Abbott Rapid Diagnostics, the local supplier, demonstrated the Afinion 2 Analyzer for the hospital laboratory and for the PHCCs. The training reflected the training usually given to the end-users. Abbott Rapid Diagnostics was not allowed to contact or supervise the evaluators during the evaluation period.

5.4.2. Evaluation sites and persons involved

The practical work was carried out during May 2021 to June 2021 under optimal conditions in the hospital laboratory and under real-life conditions by intended users in the PHCCs. BLSs at KBF-OUH were responsible for the comparison method as well as the practical work with the evaluation of the Afinion 2 Analyzer under optimal conditions. One BLS was involved in the practical work with the comparison method and one BLS performed measurements with the Afinion 2 Analyzer.

From PHCC1, one BLS and five nurses participated in the evaluation. From PHCC2 one BLS and three nurses participated in the evaluation. One BLS from PHCC1 and one BLS and one nurse from PHCC2 performed the sampling and measurements with the Afinion 2 Analyzer, the rest helped with patient recruitment. Both PHCCs use venous sampling as their routine procedure, but the samples are transported to the hospital laboratory for HbA1c measurement.

5.4.3. The evaluation procedure

Internal analytical quality control

Internal analytical quality control samples for the Afinion 2 Analyzer (Afinion HbA1c controls, Cont. I and Cont. II, Abbott Rapid Diagnostics GmbH), were measured each evaluation day on the Afinion 2 Analyzer under optimal conditions, and one level per day alternating between the two levels in the PHCCs. The reproducibility (CV) as achieved with the quality control material was calculated.

Recruitment of patients
Patients, 18 years or older coming into the hospital laboratory or PHCC for HbA1c measurements, were asked if they were willing to donate two capillary and one venous blood sample for the evaluation. The patients were selected to cover a wide range of HbA1c concentrations. Patients with known haemoglobinopathies, haemoglobin F >10%, haemophilia, chronic hepatic or renal disease, iron deficiency, haemolytic anaemia, pregnancy, those known to take glucocorticoids or nicotinic acid medications, or had been transfused or received chemotherapy within the previous 3 weeks were not recruited. Participation was voluntary and verbal informed consent was considered sufficient.

Handling of the samples and measurements
Fresh capillary whole blood samples were used for measurement with the Afinion 2 Analyzer. All measurements were performed in duplicate, i.e. two separate finger pricks. The puncture site was disinfected with alcohol pads, and the area was dried completely before sampling.

For capillary sampling, disposable lancing devices (Vitrex Flex 3 26G, Vitrex Medical) with adjustable penetrating depth (1,3-2,3 mm) were used. A drop of capillary blood was allowed to form before using the integrated capillary to transfer the blood (1,5 µL) to the Afinion HbA1c test cartridge. The sample was measured immediately on the Afinion 2 Analyzer and in accordance with the instructions from the manufacturer. The complete sampling and measurement procedure were repeated immediately for the second measurement on the Afinion 2 Analyzer. In case of error codes, the test was repeated if possible until a result was obtained. Three lot numbers of test cartridges were used at each site during the course of the evaluation.

The venous samples for the comparison method were obtained from venous puncture and collected into 2 mL vacutainer tubes with K_2-EDTA (BD Medical). The tubes were inverted 10 times to ensure thorough mixing and kept in room temperature, before transported to the hospital laboratory to be measured later the same day or the day after. The samples were measured in duplicate for HbA1c on the comparison method within 72 hours after sampling. Confirmed specimen stability was 72 hours at room temperature and six days in refrigerator.

6. Results and discussion

Statistical expressions and calculations used by SKUP are shown in attachment 5.

6.1. Number of samples

Scheduled number of samples in this evaluation was 100 patient samples measured in duplicate under optimal conditions and 80 patient samples measured in duplicate by intended users in the PHCCs. At the end of the evaluation, a total of 197 patients were enrolled.
Under optimal conditions, 100 patients were recruited (SKUP ID 501 – 600). PHCC1 recruited 56 patients (SKUP ID 101 – 156) and PHCC2 recruited 41 patients (SKUP ID 301 – 341). The results from the comparison method covered the HbA1c interval 31 – 127 mmol/mol. The evaluation was carried out using three lot numbers of test cartridges, and each evaluation site was alternating between the lot numbers. An account of the number of samples not included in the calculations, is given below.

Missing results

- On four occasions the internal analytical quality control results were missing or the lot of the Afinion HbA1c test cartridge, used for the internal analytical quality control, was different to the one used for patient samples. The results from the patient samples were still included in the calculations.
- ID 531; only a single result on the Afinion 2 Analyzer, due to an error code. The single value was included in the calculation of bias and the assessment of accuracy, but not in repeatability.
- ID 102, 103 and 144; no results from the comparison method as the sample never arrived the clinical laboratory. The results from the Afinion 2 Analyzer were included in the calculation of repeatability but not in the calculation of bias and the assessment of accuracy.
- ID 106 and 111; not measured on the Afinion 2 Analyzer, because of a mismatch between patient samples. The results from the comparison method were included in the calculation of repeatability of the comparison method, but not included in the calculation of bias and the assessment of accuracy of the Afinion 2 Analyzer.

Omitted results

- ID 101 was measured on the comparison method >72 hours after sampling. The results were not included in the calculation of bias and the assessment of accuracy but results from the Afinion 2 Analyzer were included in the calculation of repeatability.
- ID 321, the comparison method detected the presence of an HbA1c variant in the sample. The variant was not known by the PHCC, and due to probable interference, the ID was excluded from all calculations.

Excluded results (statistical outliers)

Statistical outliers in SKUP evaluations are detected by the criterion promoted by Burnett [13]. No statistical outliers were found.

Recorded error codes, technical errors and failed measurements
Five errors were reported. One was deemed a preanalytical or handling error (error code 201). The other four were deemed as technical errors; three related to failure in the test cartridge or the instrument (error code 214) and one related to the start-up of the instrument (error code 29). There were 390 original samples and 4 technical errors. This amounts to 1 % technical errors. The SKUP recommendation of a fraction of ≤2 % tests wasted due to technical errors was achieved.

6.2. Analytical quality of the selected comparison method

6.2.1. Internal analytical quality control
All results from the internal analytical quality control (Lyphochek Diabetes Control, Bio-Rad), two levels, were within the allowable control limits (data not shown).

6.2.2. The precision of the comparison method
Duplicate measurements of venous samples from the patients participating under optimal conditions and in the PHCCs were performed on the comparison methods. The results were checked to meet the imposed condition for using formula 1 in attachment 5. There was no systematic difference between the paired measurements (data not shown) in the comparison method.

The precision is presented as repeatability (CV). The CV with a 90 % CI is shown in table 3. The results were sorted and divided into three concentration levels according to the mean of the results. Raw data is attached for the requesting company only, see attachment 6.

Table 3. Repeatability (CV) of the comparison method for HbA1c measured in venous whole blood samples.

Level	n	Excluded results (statistical outliers)	Mean value HbA1c, mmol/mol	CV (90% CI), %
1	23	0	35,8	0,7 (0,6 – 1,0)
2	42	0	50,9	0,6 (0,5 – 0,8)
3	34	0	75,3	0,5 (0,4 – 0,6)

An account of the number of samples is given in section 6.1.

Discussion
The CV for the comparison method was between 0,5 and 0,7 % for the three concentration levels.

6.2.3. The trueness of the comparison method
In order to demonstrate the trueness of the comparison method, four levels of venous fresh frozen K_2-EDTA patient samples with assigned values from a reference method at INSTAND were analysed. The analyses were performed in triplicate on three occasions during the evaluation period. The agreement between the comparison method and the samples with assigned reference values is shown in table 4.

Table 4. Samples with assigned reference values measured on the comparison method.

Material	Date	Assigned HbA1c values INSTAND (k=2) mmol/mol	n	Mean value HbA1c Tosoh G11	Deviation from target value, %
Level 1	2021-05-20	**29,1** (28,7 – 29,5)	3	30,83	+6,0
	2021-06-07		3	31,00	+6,5
	2021-06-30		3	30,93	+6,3
	Total		**9**	**30,92**	**+6,3**
Level 2	2021-05-20	**48,4** (47,7 – 49,1)	3	50,33	+4,0
	2021-06-07		3	50,30	+3,9
	2021-06-30		3	50,70	+4,8
	Total		**9**	**50,44**	**+4,2**
Level 3	2021-05-20	**57,2** (56,3 – 58,1)	3	59,27	+3,6
	2021-06-07		3	59,93	+4,8
	2021-06-30		3	59,90	+4,7
	Total		**9**	**59,70**	**+4,4**
Level 4	2021-05-20	**79,6** (78,4 – 80,8)	3	82,20	+3,3
	2021-06-07		3	82,83	+4,1
	2021-06-30		3	83,53	+4,9
	Total		**9**	**82,86**	**+4,1**

Comments

Table 4 shows that the HbA1c results for the reference samples were above the upper uncertainty limit for all levels. All results from the comparison method were therefore adjusted according to the assigned values from INSTAND. The adjustment was carried out by means of inverse calibration [11, 12] by the following regression equation: **y = 0,9715x − 0,8076**.
Further on in the report, whenever a result from the comparison method is presented, the result has already been adjusted according to this.

To verify the trueness of the adjusted comparison method results, external quality controls produced by Labquality were analysed in a period circumventing the evaluation. The agreement between the comparison method and the target values from the European Reference laboratory in Holland is shown in table 5.

Table 5. Trueness of the comparison method.

Control	Date	Target value* HbA1c, (±8 % acceptance limits) mmol/mol	n	Adjusted value HbA1c, mmol/mol
S001	2021-04-15	**59,3** (54,6 – 64,0)	1	57,4
S002	2021-04-15	**34,8** (32,0 – 37,6)	1	32,7
S001	2021-08-19	**37,7** (34,7 – 40,7)	1	38,1
S002	2021-08-19	**53,0** (48,8 – 57,2)	1	51,4

*Determined by the European Reference Laboratory for Glycohemoglobin

Discussion
The trueness of the comparison method after adjustment was confirmed by the results in the national EQA programme for HbA1c.

6.3. Analytical quality of the Afinion 2 Analyzer under optimal conditions

The results below reflect the analytical quality of the Afinion 2 Analyzer under optimal conditions. The results document the quality of the system under conditions as favourable as possible for achieving good analytical quality.

6.3.1. Internal analytical quality control

All results from the internal analytical quality control, two levels (Afinion HbA1c Control, Cont. I and Cont. II, Abbott Rapid Diagnostics GmbH), were within the allowable control limits (data not shown). The reproducibility (CV) achieved with the internal analytical quality control samples were 1,7 % for Cont. I (n=18) and 2,1 % for Cont. II (n=18). Raw data is attached for the requesting company only, see attachment 7.

6.3.2. The precision of the Afinion 2 Analyzer

Duplicate measurements of fresh capillary whole blood from each patient were performed on the Afinion 2 Analyzer. The results were checked visually to meet the imposed condition for using formula 1 in attachment 5. There were no systematic differences pointed out between the paired measurements (data not shown).

The precision is presented as repeatability (CV). The CV with a 90 % CI is shown in table 6. The results were sorted and divided into three concentration levels according to the mean of the results on the Afinion 2 Analyzer. Raw data is attached for the requesting company only, see attachment 8.

Table 6. Repeatability (CV) of the Afinion 2 Analyzer for HbA1c measured in capillary whole blood samples. Results achieved under optimal conditions.

Level	n	Excluded results (statistical outliers)	Mean value HbA1c, mmol/mol	CV (90% CI), %
1	25	0	34,1	1,8 (1,5 – 2,4)
2	42	0	50,2	1,7 (1,4 – 2,1)
3	32	0	75,3	1,2 (1,0 – 1,5)

An account of the number of samples is given in section 6.1.

Discussion
The CV achieved under optimal conditions varied between 1,2 and 1,8 % depending on the concentration level. The CV was statistically significant lower than the quality goal for the three concentration levels.

Conclusion
Under optimal conditions the quality goal for repeatability (CV ≤3,0 %) was fulfilled.

6.3.3. The bias of Afinion 2 Analyzer

The mean deviation (bias) of the Afinion 2 results from the comparison method was calculated. The bias is presented with a 95 % CI in table 7. The results were sorted and divided into three concentration levels according to the mean results of the comparison method. Raw data is attached for the requesting company only, see attachment 6 and 8.

Table 7. Bias of the Afinion 2 Analyzer for HbA1c measured in capillary samples. Results achieved under optimal conditions.

Level	n	Excluded results (statistical outliers)	Mean HbA1c value comparison method, mmol/mol	Mean HbA1c value Afinion 2, mmol/mol	Bias (95 % CI), mmol/mol	Bias, %
1	30	0	35,3	35,2	-0,09 (-0,64 – 0,46)	-0,3
2	41	0	51,1	51,8	0,70 (0,19 – 1,20)	1,4
3	29	0	74,9	76,9	2,00 (1,18 – 2,83)	2,7

An account of the number of samples is given in section 6.1.

Discussion

The Afinion 2 Analyzer did not give a statistically significant bias at the lowest level. It gave systematically higher results than the comparison method for HbA1c level 2 and level 3. The positive bias was statistically significant in those levels.

6.3.4. The accuracy of the Afinion 2 Analyzer

To evaluate the accuracy of HbA1c results on the Afinion 2 Analyzer, the agreement between the Afinion 2 Analyzer and the comparison method is illustrated in a difference plot (figure 3). The limits for the allowable deviation according to the quality goal (within ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol), are shown with stippled lines. All the first measurements from the Afinion 2 Analyzer are included in the plot. The plot illustrates both random and systematic errors, reflecting the total measuring error in the results on the Afinion 2 Analyzer. Raw data is attached for the requesting company only, see attachment 6 and 8.

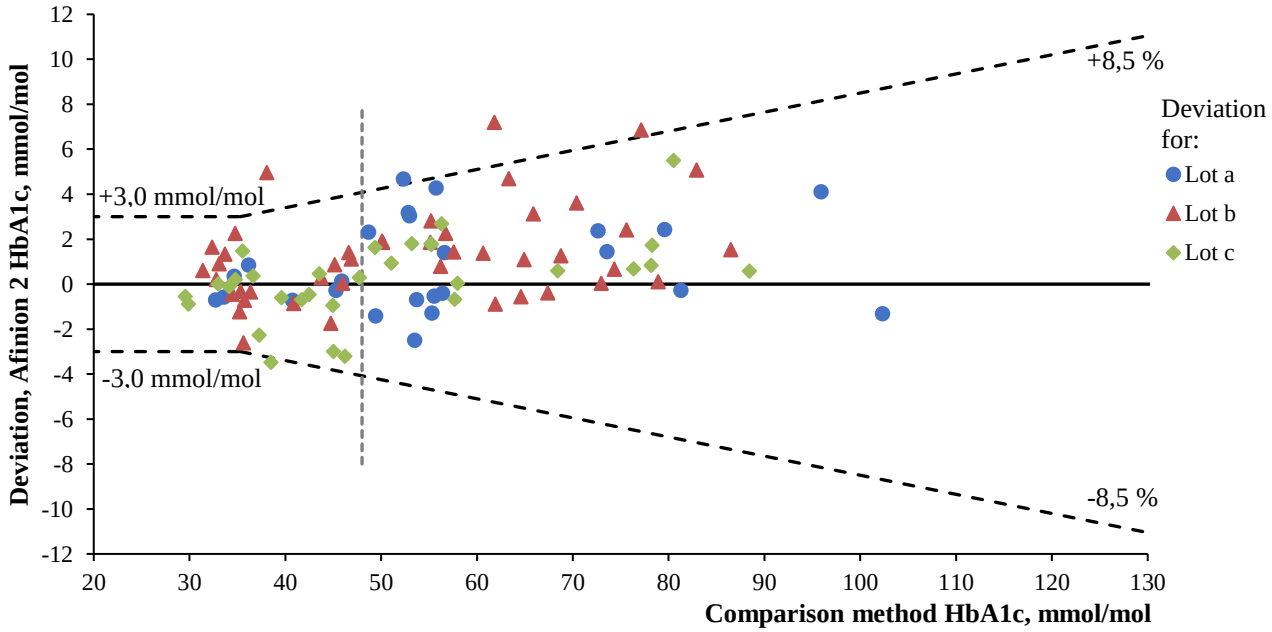

Figure 3. Accuracy of HbA1c results on the Afinion 2 Analyzer under optimal conditions. The x-axis represents the mean HbA1c result of the comparison method. The y-axis represents the HbA1c deviation in mmol/mol of the first capillary measurement on the Afinion 2 Analyzer from the mean result of the corresponding sample of the comparison method. The vertical line at 48 mmol/mol HbA1c illustrates the diagnostic threshold value for diabetes. The different lots of test cartridges are illustrated with the symbols ● (Lot a, 10209602), ▲ (Lot b, 10208561) and ♦ (Lot c, 10209195). Stippled lines represent the allowable deviation limits of the quality goal (within ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol). Number of results (n) = 100. An account of the number of samples is given in section 6.1.

Discussion

As shown in figure 3, the results in HbA1c level 2 and level 3 tend to be higher than the results from the comparison method, which is consistent with the calculated bias. Out of the 100 results, 95 were inside the limits for allowable deviation of ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol, corresponding to 95 % within the limits.

Conclusion

When measurements were performed under optimal conditions the quality goal for accuracy was fulfilled.

6.4. Analytical quality of the Afinion 2 Analyzer achieved by intended users

The results below reflect the analytical quality of the Afinion 2 Analyzer under real-life conditions in the hands of intended users in PHCCs. The results may deviate from the results achieved under optimal conditions.

6.4.1. Internal analytical quality control

All results from the internal analytical quality control, two levels (Afinion HbA1c Control, Cont. I and Cont. II, Abbott Rapid Diagnostics GmbH), were within the allowable control limits (data not shown). The reproducibility (CV) achieved with the internal analytical quality control samples were 3,0 % for Cont. I (n=15) and 3,3 % for Cont. II (n=12). Raw data is attached for the requesting company only, see attachment 9.

6.4.2. The precision of the Afinion 2 Analyzer

Duplicate measurements of fresh capillary whole blood from each patient were performed on the Afinion 2 Analyzer. The results were checked visually to meet the imposed condition for using formula 1 in attachment 5. There were no systematic differences pointed out between the paired measurements (data not shown).

The precision is presented as repeatability (CV). The CV with a 90 % CI is shown in table 8. The results were sorted and divided into three concentration levels according to the mean of the results on the Afinion 2 Analyzer. The CV achieved by intended users in the two PHCCs varied between 1,0 and 1,8 % depending on the concentration level and was statistically significant lower than the quality goal (not shown). Since the variances between the two PHCCs was not significantly different (F-test, 5 % significance level) the results from the two PHCCs were merged before the calculation of CV. Raw data is attached for the requesting company only, see attachment 10.

Table 8. Repeatability (CV) of the Afinion 2 Analyzer for HbA1c measured in capillary whole blood sample. Results achieved by intended users.

Place	Level	n	Excluded results (statistical outliers)	Mean value HbA1c, mmol/mol	CV (90 % CI) %
PHCC1	1	23	0	38,6	1,5 (1,2 – 2,0)
+	2	45	0	49,3	1,7 (1,4 – 2,1)
PHCC2	3	26	0	76,1	1,2 (1,0 – 1,6)

An account of the number of samples is given in section 6.1.

Discussion

The CV achieved by intended users varied between 1,2 and 1,7 % depending on the concentration level. The CV was statistically significant lower than the quality goal for the three concentration levels.

Conclusion

When measurements were performed by the intended users the quality goal for repeatability (CV ≤3 %) was fulfilled.

6.4.3. The bias of the Afinion 2 Analyzer

The mean deviation (bias) of the Afinion 2 results from the comparison method was calculated. The bias is presented with a 95 % CI in table 9. The results were sorted and divided into three concentration levels according to the mean results of the comparison method. Raw data is attached for the requesting company only, see attachment 6 and 10.

Table 9. Bias of the Afinion 2 Analyzer for HbA1c measured in capillary whole blood samples. Results achieved by intended users.

Place	Level	n	Excluded results (statistical outliers)	Mean HbA1c value comparison method, mmol/mol	Mean HbA1c Afinion 2, mmol/mol	Bias (95 % CI), mmol/mol	Bias, %
PHCC1	1	9	0	38,6	38,5	-0,12 (-0,73 – 0,49)	-0,3
	2	26	0	48,1	47,9	-0,13 (-0,89 – 0,63)	-0,3
	3	15	0	80,9	82,5	1,59 (-0,36 – 3,55)	2,0
PHCC2	1	11	0	37,8	38,5	0,73 (0,01 – 1,45)	1,9
	2	21	0	51,3	51,4	0,10 (-0,72 – 0,93)	0,2
	3	8	0	65,8	67,9	2,14 (-0,91 – 5,19)	3,2

An account of the number of samples is given in section 6.1.

Discussion

For the three concentration levels no statistically significant bias was pointed out between the Afinion 2 Analyzer and the comparison method for PHCC1. The bias was statistically significant for PHCC2 at HbA1c level 1, but not at levels 2 and level 3. At the lowest level, the Afinion 2 Analyzer at PHCC2 showed systematically higher values than the comparison method, as opposed to measurements in PHCC1 and under optimal conditions.

6.4.4. The accuracy of the Afinion 2 Analyzer

To evaluate the accuracy of HbA1c results on the Afinion 2 Analyzer, the agreement between the Afinion 2 Analyzer and the comparison method is illustrated in a difference plot (figure 4). The limits for the allowable deviation according to the quality goal (within ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol), are shown with stippled lines. All the first measurements from the Afinion 2 Analyzer are included in the plot. The plot illustrates both random and systematic errors, reflecting the total measuring error in the results on the Afinion 2 Analyzer. Raw data is attached for the requesting company only, see attachment 6 and 10.

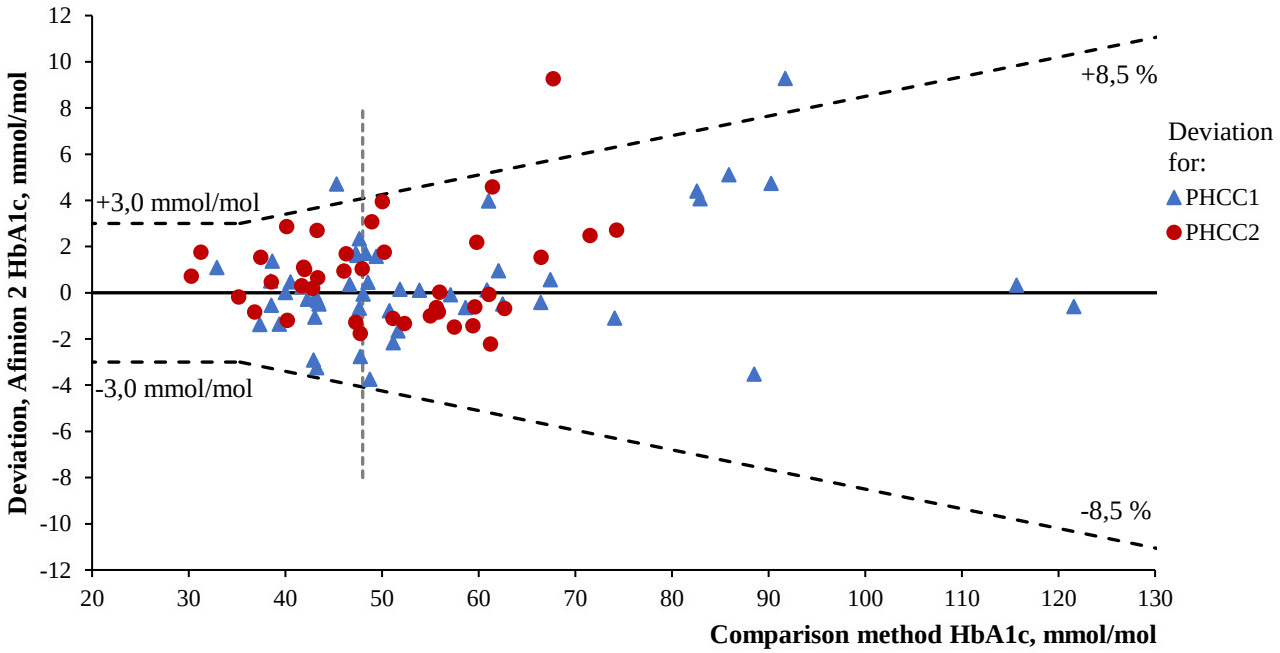

Figure 4. Accuracy of HbA1c results on the Afinion 2 Analyzer achieved by intended users. The x-axis represents the mean HbA1c result of the comparison method. The y-axis represents the HbA1c deviation in mmol/mol of the first capillary measurement on the Afinion 2 Analyzer from the mean result of the corresponding sample of the comparison method. The vertical line at 48 mmol/mol HbA1c illustrates the diagnostic threshold value for diabetes. The different PHCCs are illustrated with the symbols ▲ (PHCC1) and ● (PHCC2). Stippled lines represent the allowable deviation limits of the quality goal (within ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol). Number of results totally (n) = 91. Number of results included in the calculation of accuracy (n) = 90. An account of the number of samples is given in section 6.1.

Discussion

As shown in figure 4, the HbA1c results on the Afinion 2 Analyzer tend to be more evenly spread below and above zero, compared to the results achieved under optimal conditions. For PHCC2 there is a more distinct tendency of results above zero than below, which corresponds to the calculated biases for PHCC2.

Out of the 90 results, 87 were inside the limits for allowable deviation of ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol, corresponding to 97 % within the limits.

Conclusion

When measurements were performed by the intended users the quality goal for accuracy was fulfilled.

6.5. Evaluation of user-friendliness

6.5.1. Questionnaire to the evaluators

The most important response regarding user-friendliness comes from the intended users themselves. The end-users often emphasise other aspects than those pointed out by more extensively trained laboratory personnel.

At the end of the evaluation period, the intended users filled in a questionnaire about the user-friendliness of the measurement system. SKUP has prepared detailed instructions for this.

The questionnaire is divided into four subareas:

Table A) Rating of operation facilities. Is the system easy to handle?
Table B) Rating of the information in the manual / insert / quick guide
Table C) Rating of time factors for the preparation and the measurement
Table D) Rating of performing internal and external analytical quality control

The intended users filled in table A and B. SKUP filled in table C and D and in addition, topics marked with grey colour in table A and B.

In the tables, the first column shows what is up for consideration. The second column in table A and B shows the rating by the users at the evaluation sites. The rest of the columns show the rating options. The overall ratings from all the evaluating sites are marked in coloured and bold text. The total rating is an overall assessment by SKUP of the described property, and not necessarily the arithmetic mean of the rating in the rows. 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 are marked with a number and explained below the tables. The intermediate category covers neutral ratings assessed as neither good nor bad.

An assessment of the user-friendliness is subjective, and the topics in the questionnaire may be emphasised differently by different users. The assessment can therefore vary between different persons and between the countries. This will be discussed and taken into account in the overall assessment of the user-friendliness.

Comment
In this evaluation, the user-friendliness was assessed by one BLS from PHCC1, and one BLS and one nurse from PHCC2.

Table A. Rating of operation facilities

Topic	Rating	Rating	Rating	Rating	Option
To prepare the test / instrument	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
To prepare the sample	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Application of specimen	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Specimen volume	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Number of procedure step	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Instrument / test design	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Reading of the test result	**E, E**	**Easy**	Intermediate	Difficult	No opinion
Sources of errors	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Cleaning / Maintenance	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Hygiene, when using the test	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Size and weight of system	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Storage conditions for tests, unopened package		**+15 to +30°C***	+2 to +8°C	–20°C	
Storage conditions for tests, opened package		**+15 to +30°C or disposable**	+2 to +8°C	–20°C	
Environmental aspects: waste handling		**No precautions**	Sorted waste	Special precautions	
Intended users		**Health care personnel or patients**	Laboratory experience	Biomedical laboratory scientists	
Total rating by SKUP		**Satisfactory**			

* According to the package insert of the test cartridge, the unopened test cartridges can be stored between 15 to 25°C for 90 days or stored refrigerated until the expiry date.

Additional positive comments (Table A): The instrument is easy to learn and simple to use. The results are clearly displayed on the screen. Filling the capillary directly from the control vial makes it easy to perform internal quality control. The small blood volume needed for the test and the capillary make it easy to apply the sample.

Additional negative comments (Table A): If you choose a wrong sample mode, you can't go back and change it. A lot of waste after each test.

Table B. Rating of the information in the manual and quick guide

Topic	Rating	Rating	Rating	Rating	Option
Table of contents/Index	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Preparations/Pre-analytic procedure	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Specimen collection	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Measurement procedure	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Reading of result	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Description of the sources of error	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Help for troubleshooting	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Readability / Clarity of presentation	**U[1], I[1]**	Satisfactory	**Intermediate**	**Unsatisfactory**	No opinion
General impression	**S, S**	**Satisfactory**	Intermediate	Unsatisfactory	No opinion
Measurement principle		**Satisfactory**	Intermediate	Unsatisfactory	
Available insert in Danish, Norwegian, Swedish		**Satisfactory**	Intermediate	Unsatisfactory	
Total rating by SKUP		**Satisfactory**			

[1] The size of the text is too small in the manual.

Additional positive comments (Table B): The quick guide is easy to read and easily understood. The explanations and illustrations are good.

Table C. Rating of time factors (filled in by SKUP)

Topic	Rating	Rating	Rating
Required training time	**<2 hours**	2 to 8 hours	>8 hours
Durations of preparations / Pre-analytical time	**<6 min.**	6 to 10 min.	>10 min.
Duration of analysis	**<10 min.**	10 to 20 min.	>20 min.
Stability of test, unopened package	**>5 months**	3 to 5 months	<3 months
Stability of test, opened package	**>30 day or disposable***	14 to 30 days	<14 days
Stability of quality control material, unopened	**>5 months**	3 to 5 months	<3 months
Stability of quality control material, opened	**>6 days or disposable**	2 to 6 days	≤1 day
Total rating by SKUP	**Satisfactory**		

* The test cartridge must be used within 10 minutes after opening the foil pouch.

Table D. Rating of analytical quality control (filled in by SKUP)

Topic	Rating	Rating	Rating
Reading of the internal quality control	**Satisfactory**	Intermediate	Unsatisfactory
Usefulness of the internal quality control	**Satisfactory**	Intermediate	Unsatisfactory
External quality control	**Satisfactory**	Intermediate	Unsatisfactory
Total rating by SKUP	**Satisfactory**		

6.5.2. Assessment of the user-friendliness

Assessment of the operation facilities (table A)
The operation facilities were in total assessed as satisfactory. Both PHCCs had positive comments about the design of the instrument, including the integrated capillary in the test cartridge that makes it easy to apply the control or blood sample into the Afinion HbA1c test cartridge, and an intuitive software that makes it easy to learn and use the system. One of the PHCCs had a negative comment about that the sample mode (control or patient mode) could not be changed after being selected. There was also a comment about the amount of waste generated because each of the test cartridges are packaged separately in foil pouches.

Assessment of the information in the manual (table B)
The manual was assessed as satisfactory but there were one unsatisfactory and one intermediate rating regarding the small size of letters in the manual. The letters in the quick guide were of good size and the description of the procedure steps were good with self-explanatory illustrations, which is important for the user-friendliness.

Assessment of time factors (table C)
The time factors were assessed as satisfactory.

Assessment of analytical quality control possibilities (table D)
The analytical quality control possibilities were assessed as satisfactory.

Conclusion
In all, the user-friendliness of the Afinion 2 Analyzer and its manual was rated as satisfactory, although there is improvement potential pointed out. The quality goal for user-friendliness was fulfilled.

7. References

1. Christensen NG., Monsen G. & Sandberg S. Utprøving av analyseinstrumenter, 1997. Alma Mater Forlag ISBN 82-419-0230-1.

2. Weykamp CW. *et al.* The analytical goals for hemoglobin A1c measurement in IFCC units and national glycohemoglobin standardization program units are different. Clin Chem 2011; **57**: 1204 – 1206.

3. DSKB. Rapport fra VUK om HbA1c. Analysekvalitetskrav til HbA1c ved brug til diagnostik og monitorering af diabetes (2011). https://dskb.dk/wp-content/uploads/2020/11/VUK-HbA1c.pdf (accessed date 2021-09-22)

4. The Norwegian Directorate of Health. https://www.helsedirektoratet.no/tema/diabetes#diagnostiske-kriterier-for-diabetessterk-anbefaling (accessed date 2021-09-22)

5. Noklus. https://www.noklus.no/media/lexh2sbo/innsnevring-av-kvalitetskrav-for-hba1c_2019.pdf (accessed 2021-09-22).

6. Equalis. https://www.equalis.se/sv/produkter-tjanster/kunskapsstod/rekommendationer/kvalitetsmal-for-hba1c-metoder-vid-diagnostik-av-typ-2-diabetes-s006 (accessed date 2021-09-22)

7. DSKB-nyt 2/2021. Analysekrav til HbA1c på POCT-udstyr ved SKUP-afprøvning. https://dskb.dk/wp-content/uploads/2021/06/202102_DSKBnyt_web.pdf (accessed date 2021-09-22)

8. The IFCC – IUPAC terminology for properties and units. http://www.ifcc.org/ifcc-scientific-division/sd-committees/c-npu/npusearch/ (accessed date 2022-09-22)

9. Jeppsson JO. *et al.* Approved IFCC reference method for the measurement of HbA1c in human blood. Clin Chem Lab Med 2002; **40** (1): 78 – 89.

10. Kaiser P. *et al.* Modified HPLC-electrospray ionization/mass spectrometry method for HbA1c based on IFCC reference measurement procedure. Clin Chem 2008; **54** (6): 1018 – 1022.

11. Krutchkoff RG. Classical and inverse regression methods of calibration. Technometrics 1967; **9** (3): 425 – 439.

12. Tellinghuisen J. Inverse vs. classical calibration for small data sets. Fresenius J. Anal. Chem. 2000; **368** (6): 585 – 588.

13. Burnett RW. Accurate estimation of standard deviations for quantitative methods used in clinical chemistry. Clin Chem 1975; **21** (13): 1935 – 1938.

Attachments

1. The organisation of SKUP
2. Facts about the Afinion 2 Analyzer
3. Information about manufacturer, retailers and marketing
4. Product specifications for this evaluation, Afinion 2 Analyzer
5. Statistical expressions and calculations
6. Raw data HbA1c, results from the comparison method
7. Raw data HbA1c, internal analytical quality control results, Afinion 2 Analyzer, optimal conditions
8. Raw data HbA1c, Afinion 2 Analyzer results, optimal conditions
9. Raw data HbA1c, internal analytical quality control results, Afinion 2 Analyzer, intended users
10. Raw data HbA1c, Afinion 2 Analyzer results, intended users
11. Comments from Abbott Rapid Diagnostics GmbH

Attachments with raw data are included only in the copy to Abbott Rapid Diagnostics GmbH.

The organisation of SKUP

Scandinavian evaluation of laboratory equipment for point of care testing, SKUP, is a co-operative commitment of Noklus[1] in Norway, DEKS[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 about analytical quality and user-friendliness of laboratory equipment. This information is generated by organising SKUP *evaluations*.

SKUP offers manufacturers and suppliers evaluations of laboratory equipment for point of care testing. 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. The analytical results are assessed according to *pre-set quality goals*. To fully demonstrate the quality of a product, the *end-users* should be involved in the evaluations.

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 the report was completed and a serial number. A report code, followed by an asterisk (*), indicates an evaluation with a more specific objective. The asterisk is explained on the front page of these protocols and reports.

SKUP reports are published at www.skup.org.

[1] Noklus (Norwegian Organization for Quality Improvement of Laboratory Examinations) is a national not for profit organisation offering activities for quality improvement to all medical laboratory services in Norway. Noklus was established in 1992 and is governed by a management committee consisting of representatives from the Norwegian Government, the Norwegian Medical Association and the Norwegian Society of Medical Biochemistry, with the Norwegian Association of Local and Regional Authorities (KS) as observer.

[2] DEKS (Danish Institute for External Quality Assurance for Laboratories in the Health Sector) is a non-profit organisation owned by the Capital Region of Denmark on behalf of all other Regions in Denmark.

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

Facts about the Afinion 2 Analyzer

This form is filled in by Abbott Rapid Diagnostics AS

Table 1. Basic facts

Name of the measurement system	Afinion 2
Dimensions and weight	Width: 200 mm Depth: 328 mm Height: 186 mm Weight: 3.4 kg
Components of the measurement system	The Afinion 2 Package unit includes: • Afinion 2 Analyzer • Power cable • Power supply • User manual
Measurand	HbA1c
Sample material	Capillary and venous whole blood sample material
Sample volume	1.5 µL
Measuring principle	Boronate affinity assay
Traceability	Afinion HbA1c is traceable to the IFCC Reference Method for Measurement HbA1c Jeppsson, JO et al., Approved IFCC Reference Method for the Measurement of HbA1c in Human Blood, Clin Chem Lab Med 2002; 40(1):78-89
Calibration	Factory calibration of analyzer and test; no operator intervention
Measuring range	20-140 mmol/mol, 4-15% HbA1c
Haematocrit range	N/A
Measurement time	3 minutes
Operating conditions	The Afinion HbA1c Test Cartridge: Operating temperature of 18-30°C Analyzer: Operating temperature 15-32°C
Electrical power supply	Power supply, 24 VDC
Recommended regular maintenance	No maintenance of the Afinion 2 Analyzer is required other than cleaning the exterior and cartridge chamber
Package contents	Afinion 2 components (as above). Afinion HbA1c kit contents: 15 test cartridges packaged separately in foil pouches with a desiccant bag + 1 Package insert.
Necessary equipment not included in the package	Afinion HbA1c Control Standard blood collection equipment

Table 2. Post analytical traceability

Is input of patient identification possible?	Yes, The Afinion 2 patient ID functionality will, if configured, allow up to four patient ID fields to be entered. The Patient ID will be stored with each patient test result in the result records.
Is input of operator identification possible?	Yes. The Afinion 2 operator functionality will, if configured, require the operators to login before testing. The functionality may also prevent unauthorized operators to login, perform tests and configuration. The operator ID will be stored with each test result in the result records.
Can the instrument be connected to a bar-code reader?	Yes, an external barcode reader can be connected.
Can the instrument be connected to a printer?	Yes, a printer can be connected.
What can be printed?	The following parameters: Date and time, run number, patient ID/control ID, operator ID, lot number of test cartridge and the test result.
Can the instrument be connected to a PC?	Results records can be exported to connected USB flash. The results will be stored on the USB in a .txt file for each assay. These files may be opened in e.g. Microsoft Excel for further processing.
Can the instrument communicate with LIS (Laboratory Information System)? If yes, is the communication bidirectional?	Yes, the Afinion 2 Analyser connectivity automatically transfers patient and control results to a connected LIS/HIS/EMR system via TCP/IP networking using the protocols POCT1-A (bidirectional), HL7, ASTM 1381-85 (low level) or ASTM 1394-97 (high level), selectable by configuration.
What is the storage capacity of the instrument and what is stored in the instrument?	The patient and control results are stored in the memory of the Afinion 2 Analyzer. The last 500 patient results and the last 500 control results are saved in separate records. When exceeding the capacity of 500 results, the oldest result will be deleted. The following parameters are listed for each run: Date and time, run number, patient ID/control ID, operator ID, lot number of test cartridge and the test result.
Is it possible to trace/search for measurement results?	Manual search by scrolling up/down in the results records is possible. You must export the results to search for specific results.

Table 3. Facts about the reagent/test strips/test cassettes

Name of the reagent/test strips/test cassettes	Afinion HbA1c Test Cartridges.
Stability in unopened sealed vial	The Afinion HbA1c Test Cartridges are stable until the expiry date when stored refrigerated in sealed foil pouches. Can be stored in unopened foil pouches at room temperature (15-25 °C) for 90 days.
Stability in opened vial	The test cartridge must be used within 10 minutes after opening the foil pouch.
Package contents	One test kit contains 15 test cartridges packaged separately in foil pouches with a desiccant bag.

Table 4. Quality control

Electronic self-check	A self-test is performed during start-up of the analyzer to ensure that the instrument is operating according to established specifications. If the self-test fails at any point, the red LED will start flashing and an information code will be displayed on the touch screen. When the analyzer is switched on for a longer period, it will automatically restart once a day to ensure that a self-test is done regularly. This procedure does not interrupt any analysis of the test cartridge.
Recommended control materials and volume	Afinion HbA1c Control, contains liquid preparations of stabilized porcine whole blood (Control C I) and human whole blood (Control C II), respectively. Kit contents: • 1 x 0.5 mL Afinion HbA1c Control C I. • 1 x 0.5 mL Afinion HbA1c Control C II.
Stability in unopened sealed vial	Unopened control vials are stable until expiry date indicated on the vial label when stored refrigerated (2-8°C).
Stability in opened vial	Opened control vials are stable for 60 days when stored refrigerated (2-8°C).
Package contents	1 x 0.5 mL Afinion HbA1c Control C I. 1 x 0.5 mL Afinion HbA1c Control C II. Package insert.

Information about manufacturer, retailers and marketing

This form is filled in by Abbott Rapid Diagnostics AS

Table 1. Marketing information

Manufacturer	Abbott Diagnostics Technologies AS
Retailers in Scandinavia	Denmark: Abbott Rapid Diagnostics A/S (Distributor: Abena A/S) Norway: Abbott Rapid Diagnostics AS Sweden: Abbott Rapid Diagnostics AB
In which countries is the system marketed	Globally ☒ Scandinavia ☐ Europe ☐
Date for start of marketing the system in Scandinavia	Already on the market (Afinion AS100 since 2005)
Date for CE-marking	Afinion 2 was CE marked in 2017
In which Scandinavian languages is the manual available	All Scandinavian languages. (Norwegian, Swedish and Danish)

Product specifications for this evaluation, Afinion 2 Analyzer

Afinion 2 Analyzer serial numbers

Serial number	Used by
AF20033467	Optimal conditions
AF20033848	PHCC1
AF20033845	PHCC2
AF20034184	Spare

Afinion HbA1c test kits

Lot no	Alias	Expiry date	Used by
10209602	Lot a	OCT. 2022	All evaluation sites
10208561	Lot b	JUN. 2022	All evaluation sites
10209195	Lot c	SEP. 2022	All evaluation sites

Allowable range of the Afinion HbA1c Controls

Control	Lot no	Expiry date	Allowable range	Used by
Cont. I	10210953	2022-11	38 – 51 (mmol/mol) 5.6 – 6.8 (%)	Hospital laboratory and PHCC1
Cont. II			57 – 75 (mmol/mol) 7.4 – 9.0 (%)	
Cont. I	10211314	2022-11	38 – 51 (mmol/mol) 5.6 – 6.8 (%)	PHCC2
Cont. II			57 – 75 (mmol/mol) 7.4 – 9.0 (%)	

Other equipment used in the evaluation

Equipment	Penetrating depth (mm)	Expiry date	Supplier	Article no	Used by
Barcode scanner			Zebex Industries Inc.	Z-3100-U-B	All evaluation sites
Vitrex Flex 3 lancet	Adjustable (1,3-2,3 mm)		Vitrex Medical A/S	396813 L	All evaluation sites*
Alcohol Pads		NOV. 2025	Vitrex Medical A/S	520213 L	All evaluation sites

*Evaluation sites may have used their own lancing device due to problems with the shipment.

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 this document are valid for evaluations of quantitative methods with results on the ratio scale.

Statistical terms and expressions

The definitions in this section come from the International Vocabulary of Metrology - Basic and general concepts and associated terms; VIM [a].

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, 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.

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, poor e.g.), whereas the bias is reported in the same unit as the analytical result or in percent.

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. Accuracy is descriptive in general terms (good, poor e.g.). A measurement is said to be more accurate when it offers a smaller measurement error. Accuracy can be illustrated in a difference plot.

a. International vocabulary of metrology – Basic and general concepts and associated terms, VIM, 3rd edition, JCGM 200;2012. www.bipm.org

Statistical calculations

Statistical outliers

The criterion promoted by Burnett [b] 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.

Calculation of imprecision

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

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

d = difference between two 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:

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

m = mean of paired measurements (formula 2)

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 imposed condition for using the formulas is that there is no systematic difference between the 1st and the 2nd measurement of the pairs. The CV is given with a 90 % confidence interval.

Calculation of bias

The mean deviation (bias) at different concentration levels is calculated. 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 evaluated method. The mean difference is shown with a 95 % confidence interval.

Assessment of accuracy

The agreement between the evaluated 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 evaluated method and the mean value of the duplicate results on the comparison method. The number of results within the quality goal limits is counted and assessed.

b. Burnett RW. Accurate estimation of standard deviations for quantitative methods used in clinical chemistry. *Clin-Chem* 1975; **21** (13): 1935 – 1938.
c. Dahlberg G. Statistical methods for medical and biological students, 1940. Chapter 12, Errors of estimation. George Allen & Unwin Ltd.
d. 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. Elsevier Saunders ISBN 0-7216-0189-8.
e. Fraser C.G. Biological variation: From principles to practice, 2006. Chapter 1, The Nature of Biological Variation. AACC Press ISBN 1-890883-49-2.

Raw data HbA1c, results from the comparison method

Shown to the requesting company only.

Raw data HbA1c, internal analytical quality control results, Afinion 2 Analyzer, optimal conditions

Shown to the requesting company only.

Raw data HbA1c, results from the Afinion 2 Analyzer, optimal conditions

Shown to the requesting company only.

Raw data HbA1c, internal analytical quality control results, Afinion 2 Analyzer, intended users

Shown to the requesting company only.

Raw data HbA1c, results from the Afinion 2 Analyzer, intended users

Shown to the requesting company only.

Comments from Abbott Rapid Diagnostics GmbH

Abbott Diagnostics Technologies AS
Kjelsåsveien 161
P.O. Box 6863 Rodeløkka
NO-0504 Oslo
Norway

T: +47 24 05 6000
F: +47 24 05 6010

Enterprise No. NO 981 363 019 MVA

October 26, 2021

SKUP
DEKS Rigshospitalet
Glostrup Valdemar Hansens Vej 1-23
Indgang 8, 1. sal
2600 Glostrup
Denmark

Dear Sir or Madame,

Comments on the SKUP evaluation on Afinion™ HbA1c on AFINION 2™ Analyzer; SKUP/2021/126

Abbott would like to thank SKUP for its thorough evaluation of the Afinion HbA1c assay on the Afinion 2 Analyzer.

The Afinion Test System is designed to be easy to use and provide highly accurate results. We are very happy that all quality goals for repeatability, accuracy, and user-friendliness were fulfilled under both optimal conditions and, most importantly, by the intended users.

Recognizing the extraordinary times under which this evaluation was performed, we would also like to thank SKUP for an excellent collaboration and expeditious completion of the study.

Sincerely,

DocuSigned by:
Susanne Emmerich
Name des Unterzeichners: Susanne Emmerich
Signiergrund: Ich bin der Verfasser dieses Dokuments
Signierzeit: October 28, 2021 | 4:26:16 AM CDT
DC80B8794FDD4DB0ACCD7738CB25B1FC

Dr. Susanne Emmerich
Ass. Director Medical Affairs EMEA
Abbott Rapid Diagnostics Germany GmbH

www.abbott.com/poct