HemoCue WBC

A system for measurement of the concentration of leukocytes in blood manufactured by HemoCue AB, Sweden

A report from an evaluation organised by SKUP

Evaluated at the request of HemoCue AB, Sweden

SKUP/2010/73

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The organisation of SKUP

Scandinavian evaluation of laboratory equipment for primary health care, *SKUP*, is a co-operative commitment of NOKLUS[1] in Norway, 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

[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).

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Attachment 7 is just included in the copy to the company requesting the report.

1 Summary

The HemoCue® WBC system (HemoCue WBC) measures the "number concentration" of leukocytes in blood (B—Leukocytes) and is intended for use in primary health care. HemoCue WBC is manufactured by HemoCue AB, Sweden. The HemoCue subsidiary companies are the agents for the system in the Scandinavian countries. HemoCue AB in Sweden ordered this evaluation.

The HemoCue WBC system consists of the HemoCue WBC Analyzer and the HemoCue WBC Microcuvettes. The measurements can be done on whole blood from a capillary finger prick or a venous sample. The sample volume, about 10 µL, is achieved by filling the microcuvette. After the placement of the filled microcuvette in the instrument, the procedure is automatic. HemoCue WBC counts the number of stained leukocytes in the microcuvette by image analysis. The result is displayed on the screen at the end of the test. The measuring range is 0,3 to 30,0 × 10^9/L. Measurement duration is 3 minutes.

This evaluation is a complete SKUP evaluation. In a hospital laboratory, experienced biomedical scientists carried out HemoCue WBC measurements on venous samples. At two primary care centres, the measurements were carried out on venous and capillary samples from the same patients. These measurements were in one centre performed by nurse assistants and in the other centre by a biomedical scientist.

The comparison method was performed with an Advia 2120 cell counter supplied by Siemens Diagnostics at the Department of Clinical Chemistry, Södra Älvsborgs Sjukhus (SÄS) hospital, Borås, Sweden. The method is accredited by Swedac.

The quality goal for total error set by SKUP was that 95% of the HemoCue WBC results should not deviate more than ±18% from the comparison method results. The theoretical limits of ±15% had then been widened with ±3% considering the analytical quality of the comparison method.

Results

Venous samples in the hospital laboratory

According to quality goals set up by SKUP, the imprecision of HemoCue WBC should not exceed 5,5% in CV. The estimated CV obtained with all venous samples was 3,1%.
The between-days imprecision for patient sample results was 4,4% and the same imprecision was found for control blood results. This precision of HemoCue WBC with venous blood in the hospital laboratory fulfilled the quality goal.

According to the quality goals set up by SKUP, the bias of HemoCue WBC should not exceed ±6,0%. HemoCue WBC showed different bias depending on the level of B—Leukocytes. The results were sorted according to the concentration and divided into three level groups. The results in the low and high level groups showed almost no bias. The results in the medium level group (3,8 to 7,7 × 10^9/L) showed a negative bias of −6,6%. The HemoCue WBC results with venous samples in the hospital almost fulfilled the SKUP quality goal for bias.

Twenty samples containing atypical leukocytes according the Advia cell counter was selected to check the ability of HemoCue WBC to measure such samples correctly. Almost all venous samples showed good agreement between the HemoCue WBC and the Advia results. One sample containing erythroblasts gave, as expected, false high WBC results.

According to the quality goal for total error, 95% of the HemoCue WBC results should not deviate more than ±18% from the comparison method results. In the hospital 95% were inside the limits. With venous samples the HemoCue WBC results fulfilled the SKUP quality goal for total error.

Venous samples at the primary care centres
The imprecision was similar to the imprecision in the hospital laboratory. This precision was good and fulfilled the quality goal.

The bias of HemoCue WBC was estimated for the results divided into two concentration level groups. The bias for the low level group at the two primary care centres was −16,0% and −12,1% respectively, and for the high level group −6,1% and −5,4% respectively. The quality goal for bias was not fulfilled for the low level group but for the high level group. In contrast to the hospital laboratory evaluation the samples in the primary care evaluation were measured directly after sampling with HemoCue WBC and stored different time before measured with the comparison method. In the first primary care centre the mean storing time was 11,5 hours and in the second 4 hours. This may have influenced the bias.

Capillary samples at the primary care centres
The analytical quality of capillary HemoCue WBC results was evaluated by comparing them with results from the venous comparison method. There was no good agreement between capillary and venous results. The imprecision was 13,4% and 14,1% respectively at the two primary care centres. The quality goal for bias was fulfilled although the uncertainties in the estimates are large. The storing time before the measurements with the comparison method may have influenced the bias as described for venous samples.

Seventy-seven percent (77%) of the capillary results were inside the limits for total error. With capillary samples, the HemoCue WBC results did not fulfil the SKUP quality goal for total error.

User-friendliness
The evaluators' general opinion was that HemoCue WBC was user-friendly and easy to handle. The short shelf life for internal quality control materials already when unopened is a drawback. The mean error code frequency for all measurements in the evaluation was 1,6%. Thus the quality goal of less than 2% error codes was fulfilled, although the frequency was significantly higher than 2% on one of the used instruments.

Conclusion
For venous samples the analytical quality of HemoCue WBC was good and fulfilled the quality goals. However, for capillary samples the quality goals were not fulfilled. HemoCue WBC was easy to handle.

Comments from the manufacturer
For comments from HemoCue AB, please see Attachment 5.

2 Analytical quality goals for B—Leukocytes tests

There are no generally recognised analytical quality goals for B—Leukocytes determinations. Various ways of setting analytical quality goals are discussed below.

2.1 Comparing capillary and venous results

For e.g. glucose there is a well known and systematic difference between the concentrations in venous and capillary blood. Capillary glucose measurement results should therefore only be evaluated by comparisons with results from capillary samples. For B—Leukocytes few and conflicting data are published regarding differences between capillary and venous concentrations [1, 2, 3, 4, 5, 6]. In this evaluation the capillary HemoCue WBC results will be compared with results from the venous comparison method as there is a general opinion to rely on venous results and most experience e.g. the reference interval is achieved with venous samples. As B—Leukocytes in this evaluation will not be measured in the same sample with the two methods, larger random differences are expected than if the measurements had been performed on split capillary samples.

2.2 Discussion about alternative quality goals

2.2.1 Quality goals based on biological variation

Setting quality goals on the basis of biological variation is an acknowledged method [7]. Ricos et al. [8] writes “desirable specifications” when listing figures for imprecision, bias and total error. The word ”desirable” must not be misunderstood. Imprecision, bias and total error are never desirable, but should be as low as possible, and are “desirable” if they are as low as, or lower than, specified. The word allowable is preferred below.

The term ”total error” is used for the combined effects of imprecision and bias.

Abbreviations:

CV_{bw} biological variation within healthy individuals, also called the intra-individual biological variation

CV_{bb} biological variation between healthy individuals, also called the inter-individual biological variation

CV_a analytical imprecision expressed as coefficient of variation usually in percent (CV%).

Calculation of quality goals:

”Allowable imprecision” $\leq \frac{1}{2} CV_{bw}$

”Allowable bias”(without sign) $\leq \frac{1}{4}\sqrt{CV_{bw}^2 + CV_{bb}^2}$

”Allowable total error”(without sign) =
”Allowable bias”(without sign) + 1,65 × ”Allowable imprecision”

In terms of B—Leukocytes, Ricos et al. [8] provide a number of references, of which the most recent are [9, 10, 11, 12, 13, 14, 15]. The biological variation is stated to be CV_{bw} =10,9% and CV_{bb} = 19,6%. From this follows that
Allowable imprecision ≤5,5%, Allowable bias ≤±5,6% and Allowable total error ≤±14,6%.

The Nordic Reference Interval Project (NORIP) presents the reference interval for B—Leukocytes as 3,3 — 8,8 10^9/L, this correspond to CV_{bb} = 21,5% [16]
If this number is combined with Ricos number for CV_{bw} the
Allowable imprecision ≤5,5%, Allowable bias ≤±6,0% and Allowable total error ≤±15,0%.

In principle, quality goals based on biological variation do not take into account clinical requirements. Another limitation of these calculated quality goals is that they are based on the biological variation figures for healthy persons, while the test is most often used on sick patients.

2.2.2 Quality goals based on recommendations from professionals/experts

Van Blerk [17] has compared how B—Leukocytes results are evaluated in different, mostly European, external quality assurance programmes. In some programmes, the maximal allowable deviations are specified in standard deviations, but in eight of them the limits are set in percent: ±6%, ±8%, ±10%, ±10%, ±15%, ±15%, ±18% and ±25%.

The CLIA law in the US, "… sets forth the conditions that all laboratories must meet to be certified to perform testing on human specimens under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). ….. also applies to laboratories seeking payment under the Medicare and Medicaid programs. The requirements are the same for Medicare approval as for CLIA certification." [18]. According to the CLIA law, the minimum acceptable performance for B—Leukocytes measurements is a total error of ≤±15%.

SKUP has in an earlier SKUP evaluation specified the following analytical quality goals for B—Leukocytes: Allowable imprecision ≤5,5 CV%), Allowable bias ≤±6,6% and Allowable total error ≤±16%. The limits were applied for the concentration interval 3,0 to 30,0 10^9/L.

2.2.3 Quality goals based on "state-of-the-art"

In the "Nordic Reference Interval Project" (NORIP) common reference intervals were determined for common serum analysis as well as haematology parameters [16]. The NORIP report states that based on published data [8] on representative CV_{within} and $CV_{between}$, the maximal allowable bias was consequently estimated to be approximately ≤±6% for B—Leukocytes. This figure was by NORIP suggested to be the maximal allowable bias from the overall mean for an instrument group, under which circumstances common reference interval can be applied.

2.3 SKUP's quality goals

2.3.1 SKUP's theoretical quality goals

Based on the above discussion, SKUP has decided the following analytical quality goals for B—Leukocytes:

Table 1. SKUP's theoretical quality goals for B—Leukocytes

Allowable imprecision ($CV_{a)}$):	≤5,5 CV%
Allowable systematic deviation from true values (Bias):	≤±6,0%
Allowable total error: The highest limit is valid. 95% of the results should fall within the limits.	≤±15% or ≤±0,4 10^9/L

2.3.2 Applying the quality goals in practice

When the theoretical quality goals are used in practice in an evaluation, the bias and imprecision of the comparison method also have to be considered.

The repeatability of the Comparison Method in this evaluation has been calculated to 2,5%. See Attachment 2, Section 2.1.

As there is no available reference method and no certified reference materials for B—Leukocytes, the most reliable values are the consensus mean of many cell counters. In this evaluation the bias of the Comparison Method estimated by comparison with the consensus mean in the Swedish EQA scheme organised by Equalis. See Attachment 2, Section 2.2.

The bias of the Comparison Method was calculated to +0,5% with a 95% confidence interval from −2,6% to +3,6%. Zero bias was thus included in the confidence interval and it was decided to make no correction of the Comparison Method results in the present SKUP evaluation. On the other hand, the deviation varied a great deal from sample to sample in the EQA scheme. The variation of the deviation values was calculated to 4,7 CV%. This figure is at the same time a measure of the total variation in the Comparison Method results caused both by method repeatability and imprecision due to matrix effects. This CV value was therefore used in the calculation of allowable tolerance limits for imprecision and total error below.

The Allowable Total Error (TE) for the Tested Method is in practice a function of the Allowable Bias of the Tested Method, the possible Bias of the Comparison Method, Allowable Imprecision of the Tested Method and the Imprecision of the Comparison Method:

$$|TE| = |Bias_{Tested\ Method}| + |Bias_{Comparison\ Method}| + z \times \sqrt{CV^2_{Tested\ Method} + CV^2_{Comparison\ Method}}$$

$$|TE| = 6 + 1{,}65 \times \sqrt{5{,}5 \times 5{,}5 \quad + \quad 4{,}7 \times 4{,}7}$$

$$|TE| = 6 + 12$$

$$|TE| \sim 18 \quad \text{or} \quad \text{Allowable Total Error} \sim \pm 18\%$$

2.3.3 SKUP's applicable quality goals for this evaluation

Based on the above discussion, SKUP has decided to assess the results from HemoCue WBC against the following quality goals:

Table 2. SKUP's applicable quality goals for this evaluation

Allowable imprecision ($CV_{a)}$:	≤5,5 CV%
Allowable systematic deviation from the Comparison Method (Bias):	≤±6,0%
Allowable total error: The highest limit is valid. 95% of the results should fall in inside the limits.	≤±18% or ≤±0,4 10^9/L
Proportion useable results	≥98%

The user-friendliness of the evaluated equipment is assessed as satisfactory just if the equipment has achieved the assessment "2 points" / "satisfactory" for all the evaluated areas of properties: information as in the manual, time factors (time for preparing and performing the analysis), quality control possibilities and operation facilities.

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.

3 Materials and methods

3.1 Definition of the B—Leukocytes test

The Scientific Division of IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) together with IUPAC (International Union of Pure and Applied Chemistry) co-operate in the committee "Nomenclature, Properties and Units (C-NPU)". This committee has defined most diagnostic tests in the NPU database [19], which contains the recommended name of the test, which property is measured and which unit the result should be expressed in.

Table 3. The NPU definition

NPU code	Name of the test according to NPU	Unit
NPU02593	Blood—Leukocytes; number concentration	10^9/L

3.2 The evaluated measurement system: HemoCue WBC

The HemoCue WBC® system (HemoCue WBC) measures leukocytes in human whole blood from capillary or venous samples. HemoCue WBC is a point of care testing system intended for use by health care personnel in primary health care.

The HemoCue WBC system for B—Leukocytes consists of the instrument HemoCue WBC™ Analyzer and disposable HemoCue WBC Microcuvettes.

Figure 2. Picture of HemoCue WBC Analyzer

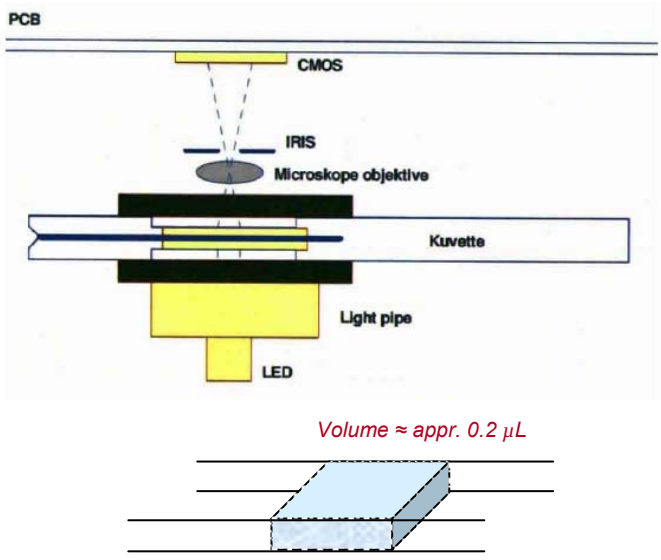
3.2.1 Analysing a capillary or venous patient sample with HemoCue WBC

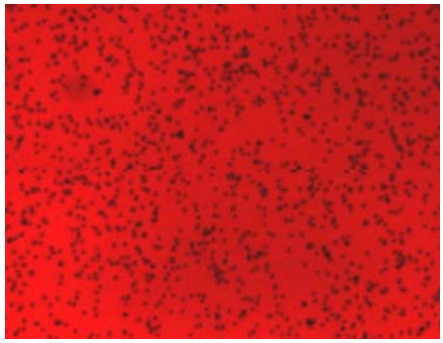
A guide description from the HemoCue WBC manual is reprinted in Attachment 1. The instructions in the guide were followed during the evaluation.

3.2.2 The working principle of the HemoCue WBC

HemoCue WBC is an optic assay for the determination of the concentration of leukocytes in human capillary or venous whole blood.

The blood sample is collected with the microcuvette, before the microcuvette is placed in a cuvette holder on the cuvette moving arm on the instrument. The microcuvette is for single-use. A blood sample of approximately 10 µL is drawn into the cavity by capillary action. The microcuvette serves as the sample container and reaction chamber. In the microcuvette, the blood sample is mixed with the haemolysing agent that lyses the red cells and with a staining agent that colours the white cells. An image is taken of the microcuvette and the number of stained white cells is counted by image analysis. The pictures and text below is a detailed explanation of the working principle according to HemoCue AB.





The volume is specified by having a specific measurement area of the microcuvette and tight tolerances of the cavity depth in the microcuvette ±2µm.

Disposable microcuvette with a fixed thickness and a digital photo give a fixed volume for enumerations of dyed cells.

Figure 3. Illustration of the working principle of HemoCue WBC Analyzer

The number of leukocytes is presented as number of cells per liter, i.e. the number of cells per sample volume. Therefore, the measurement performance is dependent on the surface area (size) of the digital photo as well as the depth of the microcuvette cavity which together corresponds to the sample volume. For a blood sample with a leukocyte count of 5,0 10^9/L, about 1000 cells are counted, which corresponds to a sample volume of about 0,2 µL.

During the calibration procedure, the image area and the number of pixels covered in the digital photo is fixated. The same fixed surface area is thereby measured on every sample. The microcuvettes are manufactured tolerating the cavity depth to vary maximum 2 µm. The surface area and the microcuvette depth correspond to the sample volume.

The B—Leukocytes result is displayed on the screen of the instrument within 3 minutes. The system is factory calibrated and needs no further calibration.

3.2.3 Self test

The HemoCue WBC Analyzer has an internal quality control, the "self test". Every time the analyzer is turned on, the measurement performance is automatically verified. When passing the self test, the display will show the HemoCue symbol and three flashing dashes, indicating that the analyzer is ready to perform a measurement. An error code will be displayed if the self test fails. Another part of the built in self test (QC) is performed for each measurement, including a check of the HemoCue WBC Analyzer, but also several condition checks of the HemoCue WBC Microcuvette and the sample itself. The operator's ability to handle the microcuvette and apply the sample correctly is also included in these self tests.

3.2.4 Intended use of HemoCue WBC

According to HemoCue AB the intended use is as follows.
"The HemoCue WBC system is indicated for use for quantitative determination of white blood cell (WBC) count in capillary or venous whole blood. The HemoCue WBC system is for In Vitro Diagnostic use only. The HemoCue WBC Analyzer is only to be used with HemoCue WBC Microcuvettes. The HemoCue WBC system is indicated for use in clinical laboratories and for point-of-care settings."

In primary health care samples for B—Leukocytes measurements are collected mainly at suspicion of infection, and the expected result is elevated leukocyte concentration. The SKUP evaluation therefore has focus on such situations.

A side-effect of many medicines, especially cytostatic drugs, is leukopoenia. The suspicion of leukopoenia is therefore another common condition, entailing collection of a sample for B—Leukocytes. The SKUP evaluation is not specifically designed to examine the use of HemoCue WBC for this condition.

It is known that samples containing a high proportion of nucleated red cells, deviating forms of leukocytes at acute or chronic leukaemia may produce deviating results with HemoCue WBC. These conditions are not common in samples in the primary care. To get an idea about how frequent and how serious these deviations are, about 20 samples with atypical leukocyte scatter diagrams with the Advia cell counter will also be included in the hospital part of this evaluation.

Osei-Bimpong [20] has measured B—Leukocytes in about 300 patients with abnormal blood pictures with HemoCue WBC and with a five part Sysmex cell counter. Patients with pronounced thrombocytosis (1000×10^9/L), iron deficiency, lymphoma and myeloma produced correct results with HemoCue WBC. The B—Leukocytes with HemoCue WBC were a little higher than the reference method at sickle cell anaemia, thalassaemia major with high number of normoblasts (>2%) and reticulocytosis ($>100 \times 10^9$/L). These results can be explained by the fact that HemoCue WBC includes nucleated red cells in the counted result. HemoCue WBC did not show any error flags for these deviating results.

3.2.5 Specifications and basic facts about the HemoCue WBC system

Table 4. Basic facts about the measurement system

Name of the measurement system:	HemoCue WBC system
Components of the measurement system:	HemoCue WBC Analyzer and disposable HemoCue WBC Microcuvettes
Measurand:	Blood—Leukocytes; number concentration
Sample material:	Capillary or venous blood
Sample volume:	About 10 μL The sample volume is measured by filling the disposable HemoCue WBC Microcuvette.
Measuring principle:	The number of stained white cells in the microcuvette is counted automatically by image analysis.
Traceability:	According to the manufacturer the system is designed and developed to establish agreement with the manual light microscopy method for white blood cell count.
Calibration:	The system is calibrated by the manufacturer and is not constructed to be calibrated by the user.
Measuring range:	0,3 — 30,0 × 10^9/L
Linearity:	Within 0,2 × 10^9/L difference in the measuring interval 0,3 to 3,5 × 10^9/L and within 6% difference in the measuring interval 3,6 to 30,0 × 10^9/L.
Measurement duration:	3 minutes
Electronic self check:	Automatic at the daily start-up. Described in section 3.2.3 of the present report.
Operating conditions:	+15 to +35 ºC, <90% non-condensing humidity. The Analyzer and the Microcuvettes shall reach these conditions before use.

Table 5. The instrument for the HemoCue WBC system

Name of the instrument:	HemoCue WBC Analyzer
Dimensions:	Width: 133 mm Depth: 185 mm Height: 120 mm
Weight:	600 g (with 6 AA batteries)
Electrical power supply:	The specified AC to DC mains adapter or 6 AA batteries, 1,5 V
Software version	107
Is input of patient identification number possible?:	No
Can the instrument be connected to a bar-code reader:	No
Can the instrument be connected to a printer?:	Yes. ASCII printer to a RS-232 connection
What can be printed?	Only the last measurement result
Can the instrument be connected to a computer:	No
What is stored in the memory of the instrument?	Only the last measurement result
Recommended regular maintenance:	Daily: The cuvette holder should be cleaned after each day of use as described in the manual.
Package contents:	• HemoCue WBC Analyzer • AC adapter • HemoCue WBC Operating Manual • HemoCue WBC Quick Reference Guide • Instruction CD • HemoCue Cleaner
Necessary equipment not included in package:	• HemoCue WBC Microcuvettes • Sampling equipment: Equipment for capillary sampling: Lancets (HemoCue or other brands) and others and/or equipment for venous sampling • Six batteries, type AA (necessary only for using the system without connection with the mains)

Table 6. The microcuvettes for the HemoCue WBC system

Name of the microcuvettes:	HemoCue WBC Microcuvettes
Stability in unopened sealed vial:	Until expiration date if kept in room temperature, at +15 to +35 ºC, <90% non-condensing humidity. Up to 4 weeks if kept outside the specified conditions, at ±0 to +50 º, <90% non-condensing humidity C
Stability in opened vial:	Up to 3 months if kept in room temperature, at +15 to +35 ºC, <90% non-condensing humidity All unused Microcuvettes should be kept in the re-capped vial.
Package contents:	40 disposable HemoCue WBC Microcuvettes Package insert

Table 7. The quality control for the HemoCue WBC system

Name of recommended check materials*:	HC WBC Control
Levels:	Three levels Approximate concentrations: 3,0, 8,0 and 23,0 10^9/L
Stability in unopened sealed vial:	105 days
Stability in opened vial:	14 days
Package contents:	Three levels each in a 2,0 mL plastic dropper vial

* According to HemoCue the liquid control materials are not necessary to verify HemoCue WBC, as the automatic self test, described in section 3.2.3, is so comprehensive. The regulation or opinion by the customer may make it necessary to use liquid control materials.

Table 8. Marketing information for the HemoCue WBC system

Manufacturer:	HemoCue AB Box 1204 SE-262 23 Ängelholm Sweden Internet: www.HemoCue.com Phone: +46 431 48 12 00
Retailers in Scandinavia:	Denmark: HemoCue Danmark Bygstubben 5 DK-2950 Vedbaek Denmark Phone: +45 45 66 13 20 E-mail: info@hemocue.dk Norway: HemoCue Norge Postboks 6744 Etterstad N-0609 Oslo Norway Phone: +47 23 37 16 00 E-mail: info@hemocue.no Sweden: HemoCue AB For address see manufacturer.
In which countries is the system marketed?:	Globally
In which Scandinavian languages is the manual available?:	Danish, Norwegian and Swedish

3.2.6 Product details for HemoCue WBC

3.2.6.1 Analyzers and software

Seven HemoCue WBC Analyzers were originally available for the evaluation, but only six were used. The serial numbers of used instruments used at each evaluation site are shown in Attachment 1, Table 4.

The software in the HemoCue WBC Analyzers used in the evaluation had the version number 107.

3.2.6.2 Microcuvettes

At the check of agreement between different HemoCue WBC Analyzers before the evaluation the used microcuvettes had the lot number: 8100029 with expiry date: 2009-07-02.

Three different lots of microcuvettes were used in the evaluation:
lot 8090026 with expiry date 2009-05-02,
lot 8100027 with expiry date 2009-05-03 and
lot 8100029 with expiry date 2009-07-02.
Approximately one third of the measurements were performed with each lot at each evaluation site. The two measurements in each duplicate were performed with the same lot.

3.2.6.3 Material for internal quality control

The lyophilised human control blood material used for HemoCue WBC during the evaluation was supplied by HemoCue AB and manufactured by R & D Systems, Inc, 614 Mc Kinley Place N.E., Minneapolis, MN 55413, USA. Agent for Europe is Eurocell Diagnostics, 27 Rue du village de la Métairie, 35131 Chartres de Bretagne, France:

In the beginning of the evaluation these lots were used in the hospital laboratory only:
HC WBC Control, low level, lot number: HC0981, expiry date: 2008-12-05
HC WBC Control, normal level lot number: HC0982, expiry date: 2008-12-05
HC WBC Control, high level, lot number: HC0983, expiry date: 2008-12-05

In the rest of the evaluation these lots were used:
HC WBC Control, low level, lot number: HC1281, expiry date: 2009-03-05
HC WBC Control, normal level lot number: HC1282, expiry date: 2009-03-05
HC WBC Control, high level, lot number: HC1283, expiry date: 2009-03-05

3.3 The Comparison Method

The designated Comparison Method is this evaluation was the B—Leukocytes method on the Siemens Advia 2120 Hematology System (Advia 2120). The method is put in practice completely according to the instructions from Siemens. It was main routine methods for B—Leukocytes measurements in the Department of Clinical Chemistry in Borås.
The Comparison Method and the verification of it, is described in Attachment 2.

3.3.1 Verification of the Comparison Method

The verification of the Comparison Method is described in Attachment 2. In summary the verification showed that:

- The imprecision of the Comparison Method, calculated from the duplicate measurements on patient samples, was about 2,5 CV%. The CV for the internal quality control results was slightly poorer. The imprecision figures of the Comparison Method are considered to be normal for a hospital laboratory method for B—Leukocytes.
- The bias of the Comparison Method was calculated to +0,5% with a 95% confidence interval from −2,6% to +3,6%. Zero bias was thus included in the confidence interval and it was decided to make no correction of the Comparison Method results in the present SKUP evaluation. On the other hand, the deviation varied much from sample to sample in the EQA scheme. The variation of the deviation values was calculated to 4,7 CV%. This figure is at the same time a measure of the total variation in the Comparison Method results caused both by method repeatability and imprecision due to matrix effects. This CV value was therefore used in the calculation of allowable tolerance limits for imprecision and total error in this evaluation. See Section 2.2.2.

3.4 Planning of the evaluation

In autumn 2007, SKUP in Sweden received the first request for an evaluation of the HemoCue WBC measuring system from Stellan Lindberg, representative of the manufacturer HemoCue AB in Sweden. At the time of the request, a limited number of the system had already been sold on the Scandinavian market.

The protocol for the evaluation was drawn up during autumn of 2008, based on the guidelines: *"Evaluation of analytic instruments. Guidelines particularly designed for evaluation of instruments in primary health care"* [21]. The measurements in the evaluation were carried out during December 2008 and January 2009. The evaluation is a complete evaluation according to the SKUP guidelines.

The evaluation comprised the following studies:

In a hospital laboratory:

- Repeatability
- Reproducibility
- Comparison of venous sample results from HemoCue WBC with venous sample results from the designated Comparison Method
- Practical viewpoints from the users

In two primary care centres:

- Repeatability
- Comparison of both capillary and venous sample results from HemoCue WBC with venous sample results from the designated Comparison Method
- Practical viewpoints from the users

After an inquiry from SKUP, Mona Prytz Carlsson at the administrative office for the primary health care in the Borås area accepted to locally coordinate the evaluation in Borås.

The Department of Clinical Chemistry at the hospital Södra Älvsborgs Sjukhus (SÄS), in Borås, Sweden, accepted to make the hospital part of the evaluation.

The evaluations in primary care were carried out at two primary care centres in the Borås area, Floda and Fristad.

Before the evaluation, Arne Mårtensson from EQUALIS drafted the preliminary protocol in co-operation with co-workers within SKUP, Stellan Lindberg and Monica Menschik from HemoCue AB and the involved persons in Borås.

At the start-up meeting on 2008-11-10, at the Department of Clinical Chemistry in Borås, , the protocol was also thoroughly discussed and finally agreed upon. Mona Prytz Carlsson, Barbro Thurgren, Eva Grandin, Monica Menschik and Arne Mårtensson participated in that meeting.

Contracts were made between SKUP and the Department of Clinical Chemistry in Borås, between SKUP and the administrative office for the primary health care centres around Borås and between SKUP and HemoCue AB in Sweden.

Arne Mårtensson has compiled this report. A preliminary report has been sent to co-workers at EQUALIS, SKUP in Denmark and Norway and Monica Menschik at HemoCue. They have all discussed and commented on the preliminary report and influenced this final report.

3.4.1 Evaluation sites and persons involved

HemoCue WBC is recommended for use by primary health care personnel. According to the SKUP model for evaluations of equipment for the primary care, this evaluation was carried out both in a hospital laboratory by an experienced biomedical scientist (medical laboratory technologist) under conditions when it is most likely to perform well and under real-life conditions in the hands of the intended users at two primary care centres (one of which had limited laboratory experience). This evaluation of HemoCue WBC is thus a complete SKUP evaluation.

The SÄS hospitals are in fact three hospitals, one big in Borås with 452 beds, one small in Skene with 108 beds and another small hospital in Alingsås serving together about 220 000 inhabitants. In the SÄS hospital in Borås, the laboratory measures most of the routine haematology samples on two automated cell counters Advia 2120. The Advia 2120 method in Borås was selected as the Comparison Method in this evaluation. The laboratory also runs a cell counter KX21N manufactured by Sysmex. The laboratory is organised in sections – one is the haematology section. The section is lead by the section leader which is an experienced biomedical scientist. Altogether four biomedical scientists did all the measurements at HemoCue WBC in the hospital laboratory evaluation.

At the Primary Care Centre in Floda there are seven general practitioners, five nurses, four assistant nurses and five medical secretaries. The laboratory work is carried out by the assistant nurses who participated in the evaluation and performed the measurements with the HemoCue WBC in this evaluation. When this centre demands a B—Leukocytes result, the sample is normally sent to the Department of Clinical Chemistry in the SÄS hospital in Alingsås for measurement.

At Primary Care Centre in Fristad there are six general practitioners, one medical intern, one resident physician (registrar), nine nurses, two assistant nurses, one biomedical scientist and four medical secretaries. The laboratory work is performed by the biomedical scientist or by the assistant nurses. The biomedical scientist did all the measurements at HemoCue WBC in this evaluation. When this centre demands a B—Leukocytes result, the sample is normally sent to the Department of Clinical Chemistry in the SÄS hospital in Borås for measurement.

Table 9 below contains an overview of the persons involved in the evaluation, and their respective responsibility.

Table 9. Persons responsible for various parts of this evaluation

Mona Prytz Carlsson	Biomedical Scientist and Quality Manager	Local leader of the evaluation. Working at the administrative office for the primary health care, Primärvårdskansliet, in Borås.
Britt-Inger Anvell	Biomedical Scientist and Quality Manager	Substitute for Mona Prytz Carlsson if necessary. Working at the administrative office for primary health care, Primärvårdskansliet, in Borås.
Rosa-Lill Johansson	Biomedical Scientist	Responsible for the measurements with the Comparison Method in the Department of Clinical Chemistry in the hospital Södra Älvsborgs Sjukhus, in Borås. Together with the other biomedical scientists at the site, Rosa-Lill carried out the measurements on HemoCue WBC in the hospital laboratory during the evaluation.
Barbro Thurgren	Assistant nurse	Contact person for the evaluation at the Primary Care Centre Floda east of Gothenburg. Together with the other assistant nurses at the centre, Barbro carried out the measurements on HemoCue WBC at the centre during the evaluation.
Yvonne Svensson	Biomedical Scientist	Contact person for the evaluation at the Primary Care Centre in Fristad north of Borås. Carried out all the measurements on HemoCue WBC at the centre during the evaluation.
Eva Grandin	Sales Representative	Regional Sales Representative for the HemoCue AB. Gave instructions to the evaluators at the start-up meetings.
Monica Menschik	Research and Development Validation Manager	Contact person at HemoCue AB before and during the evaluation.
Stellan Lindberg	Director of Research and Development	Partner in the discussion of the protocol for the evaluation. Representative for HemoCue AB.
Linda Sundell Therese Lifvendahl Ann-Marie Rönn	Administrative staff	Responsible for input of the raw data into Excel worksheets at the EQUALIS office.
Arne Mårtensson	Clinical Biochemist	Organiser of the evaluation. Author of this report. At EQUALIS the co-ordinator of SKUP in Sweden

3.5 Product details

3.5.1 Blood sampling devices

The capillary punctures at the primary care centres were made with the following lancets: Haemolance Plus® Max Flow, blade style safety lancets, penetration depth 1,6 mm, product number 7591, manufactured and supplied by HaeMedic Sweden AB, Företagaregatan 18, Munka Ljungby, Sweden, E-mail: info@haemedic.se

Both primary care centres used lancets with lot no.: M40D829A7

3.5.2 The Comparison Method

The product details for the Comparison method are presented in Attachment 2.

3.5.3 HemoCue WBC

The product details for HemoCue WBC are presented in the section 3.2.6.

3.6 Evaluation procedure

3.6.1 Training

HemoCue AB in Sweden was responsible for training in usage of HemoCue WBC. Training was provided by Mrs Eva Grandin for those who were going to do the hands-on work with HemoCue WBC. Mrs Monica Menschik was involved as a technical consultant. The training session was similar to what is normally done when the system is sold to a new customer. The duration of the session was less than one hour. When the evaluation began, the evaluators managed the instruments single-handedly, without any supervision or correction from the retailer/manufacturer.

3.6.2 Evaluation procedure in the hospital laboratory

3.6.2.1 Internal quality control

The electronics and the optics of the instruments were checked automatically every day during the evaluation, as described 3.2.3.

Daily internal quality control measurements were carried out throughout the evaluation period. Control blood materials for HemoCue WBC supplied by HemoCue AB were used. On each day of analysis controls on two levels were analysed on each instrument.

3.6.2.2 Selection of specimens

About 100 venous specimens were used in this part of the evaluation. The specimens were selected from the routine samples sent to the hospital laboratory. B—Leukocytes had already been measured routinely with the Advia 2120. Specimens were selected to cover the entire concentration range as evenly as possible. Specimens with extremely low and extremely high B—Leukocytes concentrations were included in the evaluation. A maximum of five specimens with concentrations below 0,3 10^9/L were selected for this evaluation. Similarly, a maximum of five specimens with concentrations above 30,0 10^9/L, were selected. The HemoCue WBC instruments has a measuring range between 0,3 and 30,0 10^9/L. Results below is designated LLL and those above HHH. These samples are rarely encountered, at least in the primary health care.

In addition 20 samples with atypical scatter diagram on Advia 2120 were also included in the evaluation. Those were samples with deviations from normal diagrams likely to affect the leukocyte cell count, such as blasts or leukocytes with low peroxidase activity. As standard procedure, the laboratory in Borås usually examines such samples by microscopy.

3.6.2.3 Handling of specimens and measurements

Since the venous specimens selected for the evaluation were measured as routine specimens, they were stored at room temperature. Either on the same day or the next day B—Leukocytes were measured twice with HemoCue WBC and twice with the Comparison Method. To save time, two specimens were measured simultaneously on two separate HemoCue WBC Analyzers. Three persons made the measurements with HemoCue WBC. For each duplicate the same operator performed one HemoCue WBC measurement on each of the two instruments. All results were listed with sampling date, measurement date and measurement time and signed by the operator.

According to the specification of HemoCue, samples intended for HemoCue WBC are stable 48 h when stored either in refrigerator or at room temperature. However, HemoCue recommend in the first place storage at room temperature. According to the specification of Siemens, samples intended for Advia 2120 are stable 36 h when stored at room temperature and 56 h when stored in refrigerator. The samples in this evaluation were stored at room

temperature and they were at most 36 h old when they were measured. This is valid also for the measurements made for calculation of between-days imprecision.

About five specimens were selected for the evaluation each day, and the measurements were performed on 23 different days during a period of 9 weeks, starting on 2008-12-01 and finishing on 2009-01-26.

The Borås laboratory sent the results to SKUP when the first 10 specimens were analysed. SKUP evaluated the results and contacted HemoCue AB/HemoCue AB to let them decide whether to continue the evaluation or not.

The lot of microcuvettes was changed when one third, as well as when two thirds of the samples had been measured. Each evaluation day the same lot of microcuvettes were used for both the internal controls and the patient samples.

To examine the between-days imprecision a third measurement was also performed on 30 of the venous specimens. As the B—Leukocytes are stable only for 36 h the duplicate measurements had to be performed on the collection day and the third measurement on the day after the duplicate measurements. The between-days imprecision was calculated from the differences between the first and the third measurement. The formula used by SKUP for calculation of imprecision requires that the results are approximately at the same level. Therefore the 30 samples were selected within the limited interval of 5,0 to 8,0 10^9/L.

3.6.2.4 Evaluation of user-friendliness

The user-friendliness was evaluated during and immediately after the practical work, using a questionnaire drafted by SKUP. The questionnaire was translated into Swedish and was adapted to this evaluation before being used.

3.6.3 Evaluation procedure at the two primary care centres

3.6.3.1 Quality control

The electronics and the optics of the instruments were checked automatically every day during the evaluation as described 3.2.3.

Daily internal quality control measurements were also carried out throughout the evaluation period. Control blood materials for HemoCue WBC supplied by HemoCue AB were used for that purpose. On each day of analysis controls on two levels were analysed on each instrument.

3.6.3.2 Recruitment of patients and sampling

Specimens were collected from about 40 patients at each of the two primary care centres. Duplicate measurements with HemoCue WBC were done both on venous and capillary samples on these patients. Patients visiting the care centres and scheduled to have a cell counter sample taken were asked if they were willing to have two extra blood samples taken for HemoCue WBC. It was explained to them that participation was voluntary. Verbal consent was considered to be sufficient. From each patient, two capillary samples were drawn from the same capillary puncture. The capillary punctures were made by ”high flow” lancets. Each sample was drawn directly from the puncture site into the microcuvette. After the capillary puncture, the first three drops of blood were dried off and the measurements were performed on the fourth and fifth drop. Venous samples intended for measurements both with HemoCue WBC and with the Comparison Method were collected at the same time.

At each primary care centre a maximum of eight specimens were selected randomly for the evaluation each day. The measurements were performed on 14 different days at Floda and 12

different days at Fristad during a total period of 6 weeks, starting on 2008-12-10 and completing on 2009-01-13.

The lot of microcuvettes was changed when one third, as well as when two thirds of the patients had been measured. For each patient the capillary samples and the venous samples were measured with the same lot of microcuvettes. The daily measurements of the internal controls were performed with the same lot of microcuvettes as the measurements on the patient samples.

3.6.3.3 Handling of specimens and measurements

After the sampling of the two capillary specimens from each patient, measurements on HemoCue WBC were made immediately or within 40 s. To save time the two measurements in each duplicate were performed simultaneously on two separate HemoCue WBC Analyzers. In each duplicate the same operator performed the two HemoCue WBC measurements. All results were listed with measurement date and measurement time and signed by the operator.

The venous specimens collected from each patient were measured in duplicate at the respective primary care centres and then sent at room temperature by ordinary sample transport to the hospital laboratory in Borås. Each specimen was measured in duplicate with the Comparison Method the day of sampling or one day after the sampling.

3.6.3.4 Evaluation of user-friendliness

The care centre staff who did the measurements on HemoCue WBC also evaluated the user-friendliness questionnaire. The evaluation was done during and immediately after the practical work in the evaluation of HemoCue WBC and in accordance with the questionnaire drafted by SKUP. The questionnaire was translated into Swedish and was adapted to this evaluation before being used.

3.7 Statistical expressions and calculations

See Attachment 3.

4 Results and discussion

4.1 Agreement between HemoCue WBC Analyzers and between lots of microcuvettes

4.1.1 Agreement between different HemoCue WBC Analyzers

The parallel evaluation in the hospital laboratory and at the primary care centres required six HemoCue WBC Analyzers. One extra instrument was included as back-up. Calibration agreement between the used instruments was documented before the evaluation. The seven instruments were placed next to each other in the hospital laboratory in Borås. Two patient samples, one with low and one with high B—Leukocytes concentration were selected from the routine samples. The two samples were analysed six times on each HemoCue WBC Analyzer. The results and calculations of the agreement check are presented in Attachment 1, Section 2.

4.1.1.1 Assessment of the agreement between different HemoCue WBC Analyzers

As can be seen in Attachment 1, Table 2, the means and CVs of all instruments agreed well. The requirements defined by SKUP for agreement between instruments were fulfilled by a comfortable margin. The results are also in agreement with the specifications from HemoCue.

Attachment 1, Table 4 shows which instruments that were used at each site. The conclusion was that the mean results from all instruments used at each site showed good agreement.

4.1.2 Agreement between different lots of microcuvettes

Three different lots of microcuvettes were used in the evaluation. About one third of the measurements were performed with each lot at each evaluation site. Both duplicate measurements on each sample were performed with the same lot.

The number of tests in this SKUP evaluation is not sufficient to perform a statistical comparison between the different lots, as each patient sample is measured just with one lot and not with all the three lots.

The agreement between the different lots used is thus done by visual inspection of the difference plot for the venous samples in the hospital laboratory. See section 4.2.4.1 Figure 4.

4.1.2.1 Assessment of the agreement between different lots of microcuvettes

The difference plot, Figure 4 in section 4.2.4.1, shows no calibration differences between the different lots of microcuvettes used in this evaluation.

4.2 Analytical quality with venous samples in the hospital laboratory

4.2.1 Missing and excluded results and check calculations

See Attachment 4 Table 10A, 10B, 10C and 11B. The numbering of the tables in the attachment follows the numbering of the tables in the report.

4.2.2 Imprecision evaluated in the hospital laboratory

4.2.2.1 Repeatability with venous patient sample results in the hospital laboratory

Results from 94 venous patient samples were first sorted according to duplicate mean concentrations of the Comparison method and then divided into three level groups. The repeatability was calculated from the duplicate HemoCue WBC results in the level groups and for all results together. See Table 10.

Table 10. Repeatability of HemoCue WBC with venous patient samples in the hospital laboratory

Level	Comparison method interval (10^9/L)	Excluded results	n	HemoCue WBC mean (10^9/L)	CV* (95% confidence interval) (%)
Low	1,4 — 6,2	1	30	4,4	3,8 (3,0 — 5,1)
Medium	6,3 — 9,3	0	33	7,3	3,3 (2,6 — 4,3)
High	9,3 — 29,3	0	31	15,3	2,6 (2,0 — 3,4)
All	1,4 — 29,3	1	94	9,0	3,1 (2,7 — 3,7)

* The calculated CV values are practically measures of repeatability, but they also include some additional variance components arising from changes in conditions during the collection of measurement data: two different instruments used between the two measurements on each sample, differences in the batches of microcuvettes (3 different) used, the different levels of leukocyte counts, different matrix in the samples and different days between measuring the different samples.

4.2.2.2 Between-days imprecision with patient sample results in the hospital laboratory

The duplicate measurements of HemoCue WBC for calculation of imprecision were performed on two separate instruments. On 37 of the venous specimens, an additional third measurement was performed with the same instrument as the first measurement, the day after the duplicate measurement. The results were sorted according to duplicate mean concentrations of the Comparison method and then divided into three concentration level groups. The between-days imprecision was calculated from the differences between the first and the third HemoCue WBC results in the level groups and for all results together. See Table 11. The intervals for the level groups are the same as in Table 10 to facilitate a comparison.

Table 11. Between-days imprecision with venous patient samples in the hospital laboratory

Level	Comparison method interval (10^9/L)	Number of excluded results	n	HemoCue WBC mean (10^9/L)	CV* (95% confidence interval) (%)
Low	4,1 — 6,2	0	17	5,1	5,4 (4,0 — 8,2)
Medium	6,3 — 9,3	0	18	6,8	3,8 (2,8 — 5,7)
High	>9,3	0	1	15,5	–
All	4,1 — 15,0	0	36	6,3	4,4 (3,5 — 5,7)

* The calculated CV values are practically measures of between-days imprecision, but they also include some additional variance components arising from changes in conditions during the collection of measurement data: three different microcuvettes lots, different levels of leukocyte counts and different matrix in the samples and different days between measuring the different samples.

4.2.2.3 Between-days imprecision with internal quality control blood in the hospital laboratory

The daily internal quality control results were used for calculation of the Between-days imprecision. See Table 12.

Table 12. Between-days imprecision with internal quality control blood in the hospital laboratory

HemoCue WBC Control	B—Leukocytes assigned value (interval)* (10^9/L)	Number of excluded results	n	HemoCue WBC mean (min. — max.) (10^9/L)	CV# (95% confidence interval) (%)
Control lot HC098.					
Low	3,0 (2,2 — 3,8)	0	13	3,2 (3,0 — 3,3)	2,7 (1,9 — 4,5)
Normal	8,3 (7,1 — 9,5)	0	13	8,3 (7,9 — 8,6)	3,6 (2,5 — 5,9)
High	23,6 (21,1 — 26,1)	0	12	22,7 (21,9 — 23,5)	3,4 (2,4 — 5,8)
Control lot HC128.					
Low	3,1 (2,3 — 3,9)	0	28	3,1 (2,8 — 3,4)	4,6 (3,6 — 6,2)
Normal	8,5 (7,3 — 9,7)	0	27	8,5 (8,1 — 8,9)	3,3 (2,6 — 4,5)
High	22,5 (20,0 — 25,0)	0	27	21,8 (21,2 — 22,6)	2,4 (1,9 — 3,3)

* The assigned values and the acceptable intervals are set by the manufacturer

\# The calculated CV values are practically measures of between-days imprecision, but they also include some additional variance components arising from changes in conditions during the collection of measurement data: two different instruments and three different batches of microcuvettes

4.2.2.4 Assessment of the imprecision with venous patient samples in the hospital laboratory

According to quality goals set up by SKUP, the imprecision of HemoCue WBC should not exceed 5,5% in CV.

For all venous samples the estimated CV was 3,1%. When the results were divided into three separate concentration level groups the CV-values were similar for all groups.

The between-days imprecision CV calculated on patient sample results was 4,4%.

The between-days imprecision was also estimated from measurements on control blood materials. The between-days imprecision was 2,4% to 4,6% in CV, which was similar to the results with patient blood samples.

The precision of HemoCue WBC with venous blood in the hospital laboratory was assessed as good and fulfilled the quality goal.

4.2.3 Bias evaluated in the hospital laboratory

4.2.3.1 Bias with venous patient samples in the hospital laboratory

Results from 93 venous patient samples were first sorted according to duplicate mean concentrations of the Comparison method and then divided into three level groups. The bias was calculated from the means of the duplicate sample results of HemoCue WBC compared with the means of the duplicate determinations with the Comparison Method in the level groups and for all results together.

Two grouping alternatives are presented in Table 13 below. In grouping alternative A the groups were made equal in size. In grouping alternative B, the borders between the level groups were set after visual examination of the difference plot. The borders were set where the bias seemed to change. A medium level group with a more negative bias could be distinguished from the other results. The bias values were calculated for both grouping alternatives and the results are shown in Table 13.

Table 13. HemoCue WBC bias with venous patient samples in the hospital laboratory

Level group	Comparison method interval (10^9/L)	Number of excluded results	n	Comparison method mean (10^9/L)	Bias (95% confidence interval) (10^9/L)	Bias (95% confidence interval) (%)
Grouping alternative A:						
Low	1,4 — 6,2	1	29	4,37	−0,33 (−0,43 —−0,22)	−7,5 (−9,9 — −5,1)
Medium	6,3 — 9,3	0	33	7,25	−0,23 (−0,39 — −0,07)	−3,2 (−5,4 — −0,9)
High	9,3 — 29,3	0	31	15,29	−0,13 (−0,40 — +0,14)	−0,9 (−2,6 — +0,9)
All	1,4 — 29,3	1	93	8,98	−0,23 (−0,34 — −0,12)	−2,5(−3,8 — −1,3)
Grouping alternative B:						
Low	1,4 — 2,4	0	4	1,87	+0,02 (−0,17 — +0,22)	+1,2(−9,3 — +11,7)
Medium	3,8 — 7,7	2	47	5,68	−0,37 (−0,46 — −0,29)	−6,6 (−8,1 — −5,0)
High	8,1 — 29,3	0	41	13,62	−0,12 (−0,33 — +0,09)	−0,9 (−2,4 — +0,7)
All	1,4 — 29,3	2	92	9,04	By purpose not calculated	

The control materials provided by HemoCue AB for internal quality control were measured in parallel with the patient samples and showed very small bias compared to the assigned values during the study period. See section 4.2.2.3.

4.2.3.2 Assessment of the bias with venous samples in the hospital laboratory

According to quality goals set up by SKUP, the bias for HemoCue WBC should not exceed ±6,0%.

In Table 13 the bias was calculated with two grouping alternatives. Alternative B shows concentration level groups with homogenous deviations from the Comparison Method and the results will here only be assessed according to that alternative. HemoCue WBC showed different bias depending on the B—Leukocytes level:

The results in the low and high level groups had almost no bias.

The results in the medium level group had a negative bias of −6,6%.

The medium level group, with B—Leukocytes concentrations between 3,8 and 7,7 × 10^9/L, is clinically interesting because this concentration range contains a majority of all results. When HemoCue WBC is used in primary care, about 50% of the results are in this concentration interval, as seen in the evaluation at the primary care centres. The upper end of the medium level group is also clinically interesting because it is close to the upper limit of the reference interval.

HemoCue WBC results with venous samples almost fulfilled the SKUP quality goal for bias.

4.2.4 Total error evaluated in the hospital laboratory

4.2.4.1 Total error with venous samples in the hospital laboratory

The agreement between HemoCue WBC with venous samples and the Comparison Method is illustrated in a difference plot, Figure 4. In the plot the x-axis represents the mean result of the duplicate measurements with the Comparison Method. The y-axis shows the deviation of the first measurement on HemoCue WBC from the mean value of the duplicate results of the Comparison Method. The difference plot illustrates both random and systematic deviations and reflects the total error of HemoCue WBC.

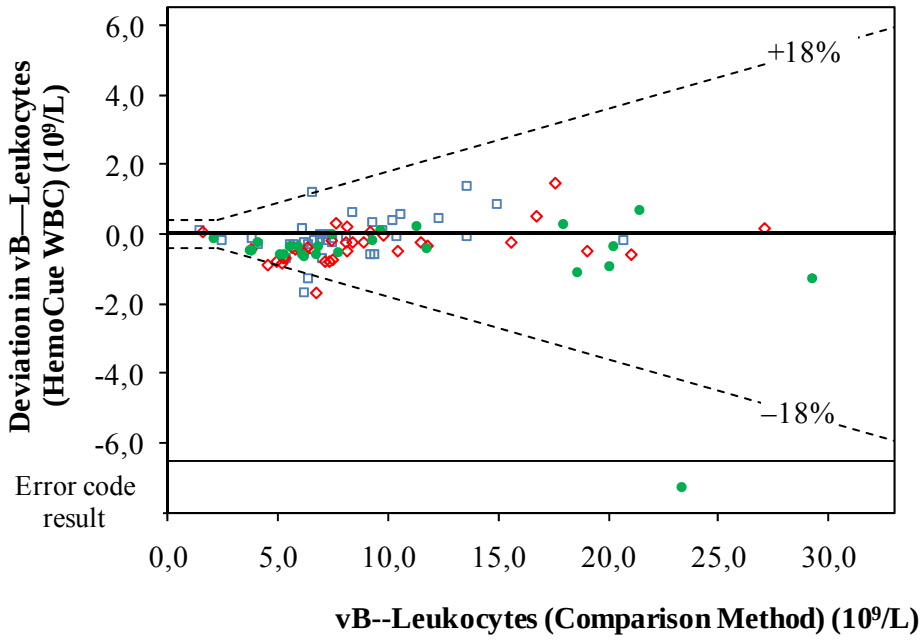


Figure 4. Difference plot, venous samples in the hospital laboratory

The deviations of the venous HemoCue WBC results from the venous Comparison Method results are shown for 95 venous patient samples. Three non-numerical results are not shown. They are correct result codes. One error code result is shown below −6,5 10^9/L. Stippled lines represent the tolerance limits ±18%.

Results are shown with different symbols depending on used lot of microcuvettes:

□ blue squares lot 8090026 ◇ red rotated squares lot 8100027 ● filled green circles lot 8100029.

There are five results outside the tolerance limits, four of them below the lower limit and one above the high limit.

There were 98 venous sample results used for the estimation of total error. Four results fell below the lower limit and one above the high limit. Three results were result codes. Two samples showed the result code LLL, which means that the result is <0,3 10^9/L, and one sample showed the result code HHH, which means that the result is >30,0 10^9/L. These result codes are confirmed as correct by the Comparison Method results. These results are shown in the plot in the field below −6,5 10^9/L. They are counted as successful measurements in the evaluation. One result was an error code. The error code result is excluded when calculating the fraction of results inside the tolerance limits in the difference plot.

4.2.4.2 Assessment of the total error with venous patient samples in the hospital laboratory

According to quality goals set up by SKUP, 95% of HemoCue WBC results should not deviate more than ±18% from the Comparison Method results.

In the hospital 92 out of 97 results or 95%, were inside the limits. With venous samples the HemoCue WBC results thus fulfilled the SKUP quality goal for total error.

4.2.5 Results of samples with atypical scatter diagram on the Advia cell counter

In the manual of HemoCue WBC, HemoCue informs about a limitation of the method: Studies have shown that patient samples with >2% nucleated red blood cells (NRBCs) may give falsely elevated white blood cell count. This has been confirmed by Osei-Bimpong [20] who measured B—Leukocytes in about 300 patients with abnormal blood pictures both with HemoCue WBC and with a five part Sysmex cell counter. HemoCue WBC did not show any error flags for these deviating results.

To get an idea about this limitation of HemoCue WBC, 20 samples which showed atypical scatter diagram on the Advia cell counter, were included in the present evaluation.
The samples were selected from the routine samples in the hospital laboratory if they showed deviations likely to influence on the number of leukocytes; containing a high proportion of nucleated red cells or deviating forms of leukocytes at acute or chronic leukaemia.
These conditions are not common in samples in the primary care. The results of this small investigation are shown in Table 14 and Figure 5 below.

Table 14. HemoCue WBC results of samples with atypical scatter diagram on the Advia cell counter compared with the Comparison Method results

Sample no	Comparison Method mean (10^9/L)	HemoCue WBC mean (10^9/L)	Difference HemoCue WBC minus Comparison Method		Classification of the deviating samples
			(10^9/L)	(%)	
1	0,34	Err01	−	−	Unknown haematological diagnosis
2	0,74	0,80	+0,06	+8	Acute Lymphatic Leukemia (ALL)
3	0,79	1,05	+0,27	+34	Unknown haematological diagnosis
4	1,30	1,35	+0,05	+4	Myelofibrosis (Eosinofiliak)
5	1,30	1,45	+0,15	+12	Adult pre-Acute Lymphatic Leukemia
6	1,50	1,40	−0,10	−7	Lymphoma
7	1,63	2,25	+0,62	+38	MyeloDysplastic Syndrome (MDS)
8	2,3	2,1	−0,3	−11	Blast cells
9	3,2	3,1	−0,1	−3	MyeloDysplastic Syndrome (MDS)
10	4,9	4,3	−0,7	−13	Lack of myeloperoxidase
11	7,4	7,8	+0,4	+5	Lack of myeloperoxidase
12	10,6	10,9	+0,3	+3	Variant lymphocytes
13	11,8	11,1	−0,7	−6	Acute Myeloid Leukemia (AML) type5
14	13,4	14,6	+1,2	+9	Variant lymphocytes (81%)
15	13,6	21,7	+8,0	+59	Erytroblasts
16	24,2	24,4	+0,3	+1	Chronic Lymphatic Leukemia
17	26,6	27,5	+0,9	+3	Chronic Myeloid Leukemia
18	27,0	28,1	+1,1	+4	Neutrofilia
19	52,8	HHH	−	−	Cancer treatment
20	64,6	HHH	−	−	Chronic Myeloid Leukemia
21	150,9	HHH	−	−	Chronic Myeloid Leukemia

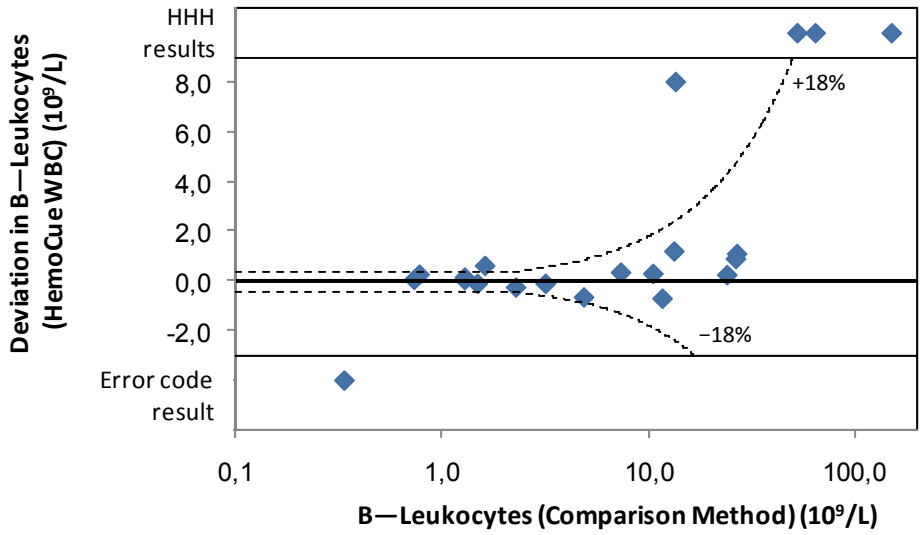


Figure 5. HemoCue WBC results of samples with atypical scatter diagram on the Advia cell counter

The deviations of the venous HemoCue WBC results from the venous Comparison Method results are shown for 21 venous patient samples with atypical scatter diagram on the Advia cell counter. Stippled lines represent the tolerance limits ±18%.

4.2.5.1 Assessment of the results for samples with atypical scatter diagram on the Advia cell counter

As can be seen in Table 14 and Figure 5 HemoCue WBC showed good agreement with the Comparison Method for almost all venous patient samples which had shown atypical leukocyte counts with to the Advia cell counter. HemoCue WBC showed the error code Err01 for one sample and the result code HHH for three samples. For some samples with very low B—Leukocytes results the deviations are low in absolute values but above the quality goal in percentage. These deviations are clinically acceptable. Only one sample showed an obvious false result; sample number 15 containing erythroblasts. This is a confirmation of the known limitation of the HemoCue WBC method; samples with high number of nucleated cells produce high false results.

4.3 Analytical quality with venous samples at two primary care centres

4.3.1 Missing and excluded results and check calculations

See Attachment 4 Table 15A, 15B and 15C. The numbering of the tables in the attachments follows the numbering of the tables in the report.

4.3.2 Imprecision with venous samples at the primary care centres

4.3.2.1 Repeatability with venous patient samples at two primary care centres

For at each of the two primary care centres the results from about 40 venous patient samples were first sorted according to duplicate mean concentrations of the Comparison method and then divided into two level groups. The repeatability was calculated from the duplicate HemoCue WBC results at each primary care centre in the level groups and for all results together. See Table 15.

Table 15. Repeatability of HemoCue WBC with venous patient samples at two primary care centres

Level	Comparison method interval (10^9/L)	Number of excluded results	n	HemoCue WBC mean (10^9/L)	CV* (95% confidence interval) (%)
Primary Care Centre Floda:					
Low	4,4 — 6,8	0	18	5,2	3,5 (2,6 — 5,3)
High	7,1 — 16,3	0	21	8,8	2,2 (1,7 — 3,2)
All	4,4 — 16,3	0	39	7,1	2,6 (2,2 — 3,4)
Primary Care Centre Fristad:					
Low	3,6 — 7,0	0	20	5,1	2,7 (2,1 — 3,9)
High	7,1 — 12,7	0	21	8,2	3,1 (2,3 — 4,4)
All	3,6 — 12,7	0	41	6,7	3,1 (2,5 — 3,9)

* The calculated CV values are practically measures of repeatability, but they also include some additional variance components arising from changes in conditions during the collection of measurement data: different instruments in the two measurements on each sample, three different lots of microcuvettes, different levels in the samples and different matrix in the samples and different days between the different measurements.

4.3.2.2 Between-days imprecision with internal quality control blood at the primary care centres

The daily internal quality control results were used for calculation of the Between-days imprecision. See Table 16.

Table 16. Between-days imprecision with internal quality control blood at the primary care centres

HemoCue WBC Control[1]	B—Leukocytes assigned value (interval)[2] (10^9/L)	Number of excluded results	n	HemoCue WBC mean (min. — max.) (10^9/L)	CV[3] (95% confidence interval) (%)
Floda primary care centre:					
Low	3,1 (2,3 — 3,9)	0	26	3,0 (2,8 — 3,2)	4,0 (3,1 — 5,5)
Normal	8,5 (7,3 — 9,7)	0	24	8,4 (8,1 — 8,8)	3,7 (2,8 — 5,1)
High	22,5 (20,0 — 25,0)	0	22	21,2 (20,0 — 22,6)	4,8 (3,7 — 6,9)
Fristad primary care centre:					
Low	3,1 (2,3 — 3,9)	0	16	3,0 (2,9 — 3,2)	3,3 (2,5 — 5,2)
Normal	8,5 (7,3 — 9,7)	0	16	8,4 (8,1 — 8,7)	3,4 (2,5 — 5,3)
High	22,5 (20,0 — 25,0)	4[4]	16	22,6 (20,2 — 25,0)	8,0 (5,9 — 12,4)

[1] Lot number HC128.
[2] The assigned values and the acceptable intervals are set by the manufacturer.
[3] In addition to the pure repeatability CV, the calculated CV values include some variance components arising from changes in conditions during the collection of measurement data: two different instruments, different days between the measurements on the same sample and three different batches of microcuvettes.
[4] Four values 13,6, 13,9, 13,9 and 14,6 has been excluded. The Burnett outlier test did not identify these outliers because of the low number of results. They are excluded on a personal judgement only. The same day results within the acceptance limits were obtained with a fresh bottle of control blood.

4.3.2.3 Assessment of the imprecision with venous patient samples at the primary care centres

According to quality goals set up by SKUP, the imprecision of HemoCue WBC should not exceed 5,5% CV.

For all venous samples measured at the two primary care centres, the CV was 2,6% and 3,1% respectively.

When the results were divided into two separate concentration level groups the CV values were approximately the same in the two level groups.

The Between-days imprecision was calculated from the measurements on the control blood. The imprecision for control blood results were worse than for the patient blood results but inside the analytical quality goal for imprecision except for the high level in one of the primary care centres.

The precision of HemoCue WBC with venous blood at the primary care centres was assessed as good and it fulfilled the quality goal.

4.3.3 Bias with venous patient samples at the primary care centres

Bias was calculated from the measurement results with venous samples from about 40 patients visiting each primary care centre. The results from each centre were sorted according to duplicate mean concentrations of the Comparison method and then divided into two level groups. The bias was calculated from the means of the duplicate sample results of HemoCue WBC compared with the means of the duplicate determinations with the Comparison Method in the level groups. The bias values for all results together were by purpose not calculated as there were considerable differences in bias between the level groups. See Table 17.

Table 17. Bias of HemoCue WBC with venous patient samples at the primary care centres

Level group	Comparison method interval (10^9/L)	Number of excluded results	n	Comparison method mean (10^9/L)	Bias (95% confidence interval) (10^9/L)	Bias (95% confidence interval) (%)
Primary Care Centre Floda:						
Low	4,4 — 6,8	0	18	5,2	−0,8 (−0,9 — −0,7)	−16,0 (−18,1 — −13,9)
High	7,1 — 16,3	0	21	8,9	−0,5 (−0,7 — −0,4)	−6,1 (−7,9 — −4,3)
All	4,4 — 16,3	0	39	7,2	By purpose not calculated	
Primary Care Centre Fristad:						
Low	3,6 — 7,0	0	20	5,1	−0,6 (−0,7 — −0,5)	−12,1 (−13,9 — −10,3)
High	7,1 — 12,7	0	21	8,2	−0,4 (−0,6 — −0,3)	−5,4 (−7,6 — −3,3)
All	3,6 — 12,7	0	41	6,7	By purpose not calculated	

It should be noted that at the same time as the bias above was observed, the human control blood provided by HemoCue AB as internal quality control materials showed almost no bias compared to the assigned values. See section 4.3.2.2, Table 16.

4.3.3.1 Assessment of the bias with venous patient samples at the primary care centres

According to quality goals set up by SKUP, the bias of HemoCue WBC should not exceed ±6,0%.

HemoCue WBC showed different bias depending on the B—Leukocytes concentration level. When the results were divided into two level groups the bias was as follows:

The low level group showed a bias of −16,0% respectively −12,1% at the two primary care centres.

The high level group showed a bias of −6,1% respectively −5,4% at the two primary care centres.

To sum up, the venous sample results measured with HemoCue WBC at the primary care centres showed negative bias around −14% for the low level group and around −6% for the high level group. The quality goal for bias was not fulfilled for the low level group, but was fulfilled for the high level group.

4.3.4 Total error with venous patient samples at the primary care centres

The agreement between HemoCue WBC results with venous samples measured at the primary care centres and the Comparison Method results is illustrated in a difference plot, Figure 6. In the plot the x-axis represents the mean value of the duplicate results at the Comparison Method. The y-axis shows the deviation of the first measurement of HemoCue WBC from the mean value of the duplicate results of the Comparison Method. The difference plot illustrates both random and systematic deviations and reflects the total error of HemoCue WBC.

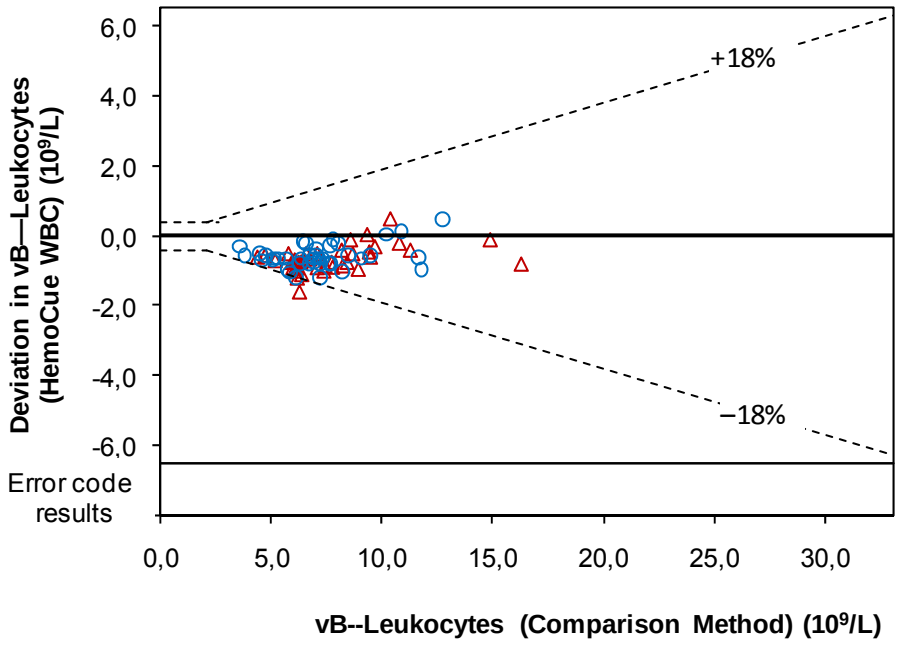


Figure 6. Difference plot, venous samples in primary care
The deviations of the HemoCue WBC results from the Comparison Method results are shown for 80 venous patient samples. Stippled lines represent the tolerance limits ±18%.
The symbols show which primary care centre the results derive from:
- o blue circles Primary Care Centre Floda and
- △ red triangles Primary Care Centre Fristad.

Total error was assessed with 80 venous sample results. Three results were outside the tolerance limits, all three below the lower limit. There was no result code and no error code.

4.3.4.1 Assessment of the total error with venous patient samples at the primary care centres

According to quality goals set up by SKUP, 95% of the HemoCue WBC results should not deviate more than ±18% from the Comparison Method results.

In the primary care centres 77 out of 80 venous results or 96%, were inside the limits. With venous samples the HemoCue WBC results fulfilled the SKUP quality goal for total error.

4.4 Analytical quality with capillary samples at two primary care centres

4.4.1 Missing and excluded results and check calculations

See Attachment 4 Table 15A, 15B and 18C. The numbering of the tables in the attachment follows the numbering of the tables in the report.

4.4.2 Imprecision with capillary patient samples

4.4.2.1 Repeatability with capillary patient samples

For at each of the two primary care centres the results from about 40 capillary patient samples were first sorted according to duplicate mean concentrations of the Comparison method and then divided into two level groups. The repeatability was calculated from the duplicate HemoCue WBC results at each primary care centre in the level groups and for all results together. See Table 18.

Table 18. Repeatability of HemoCue WBC with capillary patient samples at the primary care centres

Level	Comparison method interval (10^9/L)	Number of excluded results	n	HemoCue WBC mean (10^9/L)	CV* (95% confidence interval) (%)
Primary Care Centre Floda:					
Low	4,4 — 6,8	0	16	5,7	12,5 (9,2 — 19,3)
High	7,1 — 16,3	0	17	9,1	13,3 (9,9 — 20,3)
All	4,4 — 16,3	0	33	7,5	13,4 (10,8 — 17,7)
Primary Care Centre Fristad:					
Low	3,6 — 7,0	0	19	5,5	18,2 (13,8 — 26,9)
High	7,1 — 12,7	0	21	8,5	11,7 (9,0 — 16,9)
All	3,6 — 12,7	0	40	7,1	14,1 (11,5 — 18,1)

* The calculated CV values are practically measures of repeatability, but they also include some additional variance components arising from changes in conditions during the collection of measurement data: different instruments in the two measurements on each sample, three different lots of microcuvettes, different levels in the samples and different matrix in the samples and the different samples were measured on different days.

4.4.2.2 Assessment of the imprecision with capillary samples at the primary care centres

According to quality goals set up by SKUP, the imprecision of HemoCue WBC should not exceed 5,5% CV. For capillary samples measured at the two primary care centres, the CV was 13,4% and 14,1% respectively. When the results were divided into two separate concentration level groups the CV values were approximately the same in the two level groups.

The precision of HemoCue WBC with capillary blood at the primary care centres did not fulfil the quality goal.

4.4.3 Bias with capillary samples at the primary care centres

Bias was calculated from the measurements of 33 + 40 capillary samples from patients visiting the primary care centres. The results were sorted according to duplicate mean concentrations of the Comparison method and then divided into two level groups. The bias was calculated from the means of the duplicate capillary sample results of HemoCue WBC compared with the means of the duplicate venous sample results of the Comparison Method in the level groups and for all results together. See Table 19.

Table 19. Bias of HemoCue WBC with capillary patient samples at the primary care centres

Level group	Comparison method interval (10^9/L)	Number of excluded results	n	Comparison method mean (10^9/L)	Bias (95% confidence interval) (10^9/L)	Bias (95% confidence interval) (%)
Primary Care Centre Floda:						
Low	4,4 — 6,8	0	16	5,7	−0,3 (−0,7 — ±0,0)	−5,8 (−12,2 — +0,6)
High	7,1 — 16,3	0	17	8,9	−0,3 (−1,2 — +0,6)	−3,6 (−13,7 — +6,4)
All	4,4 — 16,3	0	33	7,4	−0,3 (−0,8 — +0,1)	−4,4 (−10,8 — +1,9)
Primary Care Centre Fristad:						
Low	3,6 — 7,0	0	19	5,5	+0,3 (−0,4 — +0,9)	+4,7 (−7,7 — +17,0)
High	7,1 — 12,7	0	21	8,5	−0,1 (−0,7 — +0,6)	−0,6 (−8,3 — +7,1)
All	3,6 — 12,7	0	40	7,1	+0,1 (−0,4 — +0,6)	+1,3 (−5,1 — +7,8)

It should be noted that at the same time as the data above was assembled, the human control blood provided by HemoCue AB as internal quality control materials showed almost no bias compared to the assigned values. See section 4.3.2.2, Table 16.

4.4.3.1 Assessment of the bias with capillary samples at the primary care centres

According to quality goals set up by SKUP, the bias of HemoCue WBC should not exceed ±6,0%.

The capillary sample results measured with HemoCue WBC showed a negative bias of −4,4% at one of the primary care centres and a small positive bias of +1,3% at the other primary care centre. The quality goal for bias was fulfilled although the uncertainties in the estimates are large.

4.4.4 Total error with capillary samples at the primary care centres

The agreement between HemoCue WBC results with capillary samples measured at the primary care centres and the venous Comparison Method results is illustrated in a difference plot, Figure 7. In the plot the x-axis represents the mean value of the duplicate results at the Comparison Method. The y-axis shows the deviation of the first measurement of HemoCue WBC from the mean value of the duplicate results of the Comparison Method. The difference plot illustrates both random and systematic deviations and reflects the total error of HemoCue WBC. The tolerance limits in the plot are according to quality goals set up by SKUP and are specified in Section 2.3.

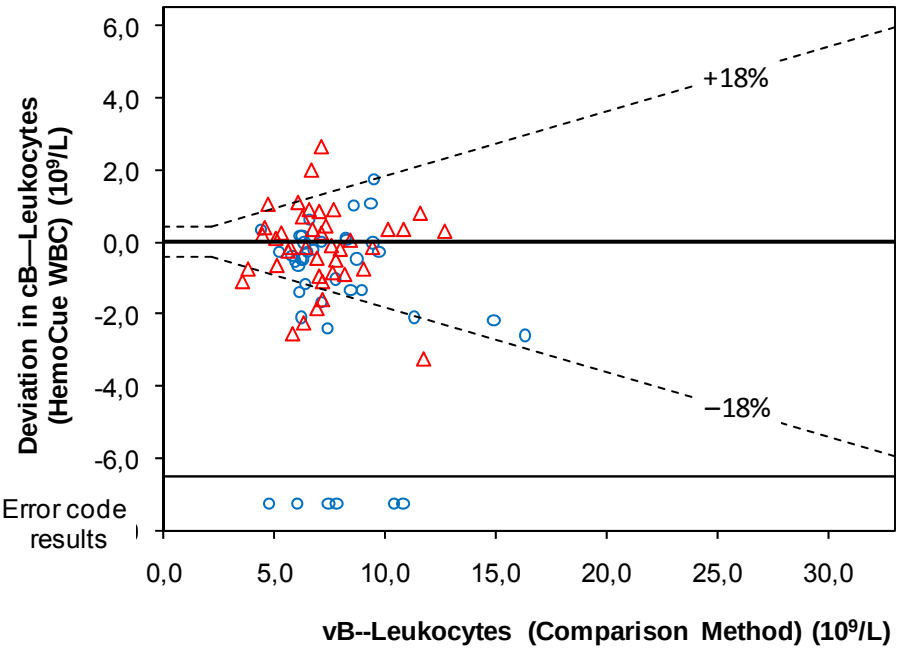


Figure 7. Difference plot, capillary samples in primary care

The deviations of the HemoCue WBC results from the Comparison Method results are shown for 80 capillary patient samples. Stippled lines represent the tolerance limits ±18%.

The symbols show which primary care centre the results derive from:

- o blue circles Primary Care Centre Floda and
- △ red triangles Primary Care Centre Fristad.

The results which were error codes on HemoCue WBC are shown below −6,5 10^9/L.

The total error was assessed with 80 capillary sample results. There were 17 results outside the tolerance limits, 13 of them below the lower limit and 4 above the higher limit. There were also 6 error code results on HemoCue WBC. They are shown in the field below $-6{,}5\ 10^9$/L in the plot. Six of totally eight error codes with capillary samples were shown by the same instrument. The error codes are excluded when calculating the fraction of results inside the tolerance limits in the difference plot.

4.4.4.1 Assessment of the total error with capillary samples at the primary care centres

According to quality goals set up by SKUP, 95% of the HemoCue WBC results should not deviate more than ±18% from the Comparison Method results. The total error of the HemoCue WBC capillary results was evaluated by comparing with venous Comparison Method results.

Seventy-seven percent (77%) of the capillary results were inside the limits for total error. With capillary samples, the HemoCue WBC results did not fulfil the SKUP quality goal for total error.

4.5 Some experiences from all evaluation sites

4.5.1 Different HemoCue WBC bias with venous samples at the different evaluation sites

The concentration intervals of the low level group at the primary care centres and of the medium level group in the hospital part of the present evaluation are similar. Therefore it is interesting to compare the bias of these level groups. The primary care results are considerable lower and have a more negative bias than the hospital results. The most probable explanation of these divergent results is the different time factors at the different evaluation sites. Table 20 below is presented to show the differences in elapsed time between sample collections and measurements together with the different observed bias values.

Table 20. Different bias and different time factors at the different evaluation sites.

	Evaluation in the hospital laboratory	**Evaluation at the Primary Care Centre Floda**	**Evaluation at the Primary Care Centre Fristad**
B—Leukocytes, concentration interval (10^9/L):	3,8 — 7,7	4,4 — 6,8	3,6 — 7,0
B—Leukocytes, mean concentration (10^9/L):	5,7	5,2	5,1
Average elapsed time between sample collection and measurements with the Comparison Method:	5 hours	11,5 hours	4 hours
Average elapsed time between sample collection and measurements with HemoCue WBC:	6 hours	0 hours	0 hours
Bias (%):	−6,6	−16,0	−12,1

There is no evident explanation of the results in Table 20. In a hypothesis the results could be explained as follows. Assumption 1: The HemoCue WBC bias would be −7,6% if the measurements with HemoCue WBC and the Comparison Method had been done simultaneously. Assumption 2; The B—Leukocytes results increased about 1% per hour for both methods the first hours after the sample collection. With these assumptions the expected bias values would be −6,6, −18,1 and −11,6% respectively at the three evaluation sites, which are close to the found bias shown on the bottom row in Table 20. If this hypothesis is true, the more negative bias at the primary care centres was just an effect of the differences in the elapsed time between the sample collection and the measurements.

4.5.2 Error codes instead of B—Leukocytes results

4.5.2.1 The error codes explained

The HemoCue WBC system has an elaborate system to warn the operator if the measurement is not reliable. In such cases the HemoCue WBC Analyzers show an error code instead of the B—Leukocytes concentration.

During the evaluation it never occurred that HemoCue WBC missed to show an error code when error code should have been shown.

Table 21 lists the error codes shown by each instrument during the evaluation. The error codes shown during the evaluation of instrument agreement and when the atypical leukocytes were measured are not included. When an error code was shown, the measurement was repeated once on the same instrument.

4.5.2.2 Error code frequency

Table 21 also shows the frequency of error codes at the instruments used at the different evaluation sites. The frequency of error codes is calculated by dividing the number of error codes among the first measurements at the same instrument with the total number of measurements.

4.5.2.3 Assessment of the error code systems

A good system which warns the operator of errors, for example error in handling of the sample, error handling of the instrument or error in the measurement in the instrument, could be a valuable feature of a safe measurement system.

According to the quality goals set up by SKUP, the frequency error codes should be less than 2%. The mean error code frequency for all measurements in the evaluation was 1,6% so the quality goal was fulfilled. Two of the six used instruments had higher frequency than the 2% limit, 2,2% and 5,7% respectively.

Early during the evaluation a high frequency of error codes was noticed by the users on one of the instruments at Floda Primary Care Centre. The suspicion was then that the optics of that instrument had become dirty. Therefore it was agreed that the HemoCue sales representative should do a service visit to Floda to clean that instrument on December 19 2008. Approximately the same numbers of the samples were measured before and after the service visit. However, two error codes were shown before the service visit and five after.

The error code frequency of 5,7% (95% confidence interval: 2,5 – 11,9%) is significantly higher than for the other instruments together (95% confidence interval: 0,4 – 2,1%) and also significantly higher than the 2% limit so that instrument deviated from the other and did not fulfil the quality goal. It is from this evaluation impossible to know if one failing instrument out of six is just an accidental occurrence or typical for all HemoCue WBC instruments.

Table 21. Error codes during the evaluation

Error code	Explanation of the error code according to the manual	Number of error code results							Likely cause
		Instr. 08203 50008 **Borås**	**Instr.** 08203 50010 **Borås**	**Instr.** 08203 50018 **Floda**	**Instr.** 08203 50019 **Floda**	**Instr.** 08203 50007 **Fristad**	**Instr.** 08203 50011 **Fristad**	**All instr.**	
Err01	A portion of the image area is unable to be analyzed. 1. Due to measurement error. 2. Due to abnormal sample.	0	Ven.2	Cap.1 Contr.1	0	0	0	Cap.1 Ven.2 Contr.1	An instrument fault or a user fault?
Err02	Uneven spatial distribution of detected cells.	0	0	Cap.1	0	0	Cap.1	Cap.2	An instrument fault or a user fault?
Err03	Image, or part of the image area is detected as out-of-focus.	0	0	Cap.3	0	0	0	Cap.3	An instrument fault or a user fault?
Err30	1. Optical parts dirty. 2. Optical parts wet after cleaning.	Ven.1						Ven.1	A user fault?
Err33	Empty microcuvette, not filled with sample.	0	Contr.2	0	Cap.1	0	0	Cap.1 Contr.2	An instrument fault
Err60	General hardware error.	0	0	Cap.1	0	0	0	Cap.1	An instrument fault
	Number of error codes:	1	4	7	1	0	1	14	
	Number of measurements	208	181	122	122	110	110	853	
	Percentage of error codes:	0,5	2,2	5,7	0,8	0,0	0,9	1,6	

Explanations:
"Contr.2" means that this error code was shown twice on control blood samples.
"Cap. 3" means that this error code was shown three times on capillary samples.
"Ven.1" means that this error code was shown once on a venous samples.

4.5.3 Variation of capillary B—Leukocytes results

In section 4.4.4 the total error of capillary HemoCue WBC results is assessed as SKUP always do with single values from the evaluated measurement system: "Seventy-seven percent (77%) of the capillary results were inside the limits for total error. With capillary samples, the HemoCue WBC results did not fulfil the SKUP quality goal for total error."

However, only in a try to explain the results we have also calculated the outcome with the mean values of the duplicate capillary results: Eighty-nine (89%) of the mean values were inside the limits! – The mean values of the duplicates thus deviate much less from the venous comparison method results than the single values do.

The result of the calculation indicates that the variation and deviations in leukocyte concentrations arose at the capillary sample collections and not when measured in the instrument.

4.6 Evaluation of the user-friendliness of HemoCue WBC

At the end of the evaluation period, the users filled in a questionnaire about the user-friendliness of the HemoCue WBC. The questionnaire and expressed opinions are presented in Table 22 to 25. The first column explains the evaluated properties. The second column shows the expressed opinions by the users. The first row shows the opinions of the four biomedical scientists in the Borås hospital laboratory. The second row shows first the collected opinion of the four assistant nurses at the Primary Care Centre in Floda, and then the opinion of the biomedical scientist at the Primary Care Centre in Fristad. The third to fifth column show the rating options the evaluators had. The cells with the overall ratings from all three evaluating sites are marked by thicker frames and bold text. The last row in each table summarises the ratings in that table.

The total rating of each row is not determined by the arithmetic mean of the individual ratings in the row. In the same way, the total rating of each table is not determined by the arithmetic mean of the individual ratings on the rows above. The total ratings are more an overall assessment of the property described on the row or in the headline of the table. A single bad rating can justify an overall bad rating if that property seriously influences on the user-friendliness of the system.

Table 22. Assessment of the information in the manual / insert

Information in manual / insert about:	**Ratings**	**Overall rating**		
		0 point	**1 point**	**2 points**
General impression	2 2 2 2 1 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Table of content	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Preparations / pre-analytical procedures	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Specimen collection	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Measurement / reading	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Measurement principle	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Sources of error	2 2 2 2* 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Fault-tracing / troubleshooting	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Index	– – – – – –[#]	**Un-satisfactory**	Less satisfactory	Satisfactory
Readability / clarity of presentation	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Available in Danish, Norwegian, Swedish	2 2 2 2 2 2	No	In part	**Yes**
Others comments about information in the manual / insert (please specify)	– 2 2 2 2 –	Un-satisfactory	Less satisfactory	Satisfactory
Rating for information in manual / insert	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**

The evaluators made the following additional comments concerning the information in the manual / insert:

Positive comments:
No comments given

Negative comments:

* Borås: Information on sources of error is not collected under one headline.

[#] There is no index in the manual.

Table 23. Assessment of the time factors

Time factors	Ratings	Overall rating		
		0 point	**1 point**	**2 points**
Time for preparations / pre-analytical time	2 2 2 2 2 2	>10 min	6 — 10 min	**<5 min**
Analytical time	2 2 2 2 2 2	>10 min	6 — 10 min	**<5 min**
Others comments about time factors (please specify)	– – – – – –	Un-satisfactory	Less satisfactory	Satisfactory
Rating for time factors	2 2 2 2 2 2			**Satisfactory**

The evaluators made the following additional comments concerning the time factors:

Positive comments:

Fristad: We think it is good that the measuring duration is short.

Negative comments:

Floda: Three minutes is a little long time to wait if you have several patients waiting and also has to take care to start the measuring on the microcuvettes within 40 s after filling them.

Table 24. Assessment of the quality control possibilities

Quality Control, possibilities to perform:	**Ratings**	**Overall rating**		
		0 point	**1 point**	**2 points**
Internal quality control	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
External quality control	– – – – – –	Un-satisfactory	**Less satisfactory**	Satisfactory
Stability of the quality control materials, unopened	– – – –* – 2	**<3 months**	**3 to 5 months**	>5 months
Stability of the quality control materials, opened	2 2 2 2 2 2	<1 day	<1 week	**>1 week**
Storage conditions for quality control materials	– – – –# 2 2		–20 °C	**+2 — +30 °C**
Others comments about quality control (please specify).	– – – – – –	Un-satisfactory	Less satisfactory	Satisfactory
Rating for quality control	2 2 2 – 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**

The evaluators made the following additional comments concerning the quality control possibilities:

Positive comments:
No comments given

Neutral comments:

* Borås: The unopened quality control materials are stable until the expiry date. We can't rate the shelf life time as we don't know the normal time interval between delivery date and expiry date.
SKUP comments:
According to HemoCue the customers will receive the control materials one to four months before expiry date.
According to HemoCue the liquid control materials are not necessary to verify HemoCue WBC as the automatic self test, described in section 3.2.4, is so comprehensive. The regulation or opinion by the customer may make it necessary to use liquid control materials and then the recommended check materials can be used.

The actual prescribed storage conditions for quality control materials is +2 to +8 °C.

Negative comments:

Borås: A suitable external quality control scheme for B—Leukocytes is not available.
SKUP comment:
A suitable external quality control scheme is so far not available. To start such a scheme is not the responsibility of HemoCue but of the external quality assurance organisers. When or if the HemoCue WBC system will be common in the primary health care such schemes will be started.

Borås: In spite of correct handling, we got the HHH code result twice with the high quality control blood. Then we used a new bottle of control and got correct results.

Floda: Due to unclear symbols it was hard to distinguish between the different quality control bottles, low, normal and high.
The bottle-neck of the quality control bottle was made of soft material, so the bottle-neck bent and the bottle was impractical to handle.

Table 25. Assessment of the operation facilities

Operation facilities	Rating	Overall rating		
		0 point	1 point	2 points
Content of the test kit. Complete?	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Preparations / pre-analytical procedures	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory***
Application of specimen	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Specimen volume	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Number of procedure steps	2 2 2 2 1 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Instrument and microcuvettes	1 1 2 1 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Reading	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Sources of error	2 2 2 1 0 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Cleaning/maintenance	2 2 2 2 0 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Hygiene, when using the test	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Stability of microcuvettes, unopened package	2 – – –* 0 2	<3 months	3 to 5 months	**>5 month**
Stability of microcuvettes, opened package	2 2 2 2 2 2	<14 days	<1 month	**>1 month**
Storage conditions for micro-cuvettes, unopened package	– 2 – –# 2 2		–20 °C	**+2 to +30 °C***
Environmental aspects: waste handling	2 2 2 2 2 2		Sorted waste	**Infectious waste or none**
Educational requirements	2 2 2 2 2 2		Biomedical scientist	**Laboratory experience or none**
Required training time	2 2 2 2 2 2	Days	>2 hours	**0 to 2 hours**
Size and weight of packages	2 2 2 2 2 2	Un-satisfactory	Less satisfactory	**Satisfactory**
Others comments about operation: Frequent error codes.	– – – 2 2 –	Un-satisfactory	Less satisfactory	Satisfactory
Rating for operation facility	2 2 2 2 2 2			**Satisfactory**

The evaluators made the following additional comments concerning the operation facilities. See the comments on the next page.

Positive comments:

Floda: The small size of the instrument is good.

Fristad: We think it is good that the different HemoCue measurement systems (for B—Haemoglobin, P—Glucose and B—Leukocytes) have the same concept and are used in a similar manner.

Negative comments:

* Borås: In unopened package the microcuvettes are stable until the expiry date. We can't rate the shelf life time as we don't know the normal time interval between delivery date and expiry date. See comment by SKUP below.
SKUP comment:
According to HemoCue the customers will receive the microcuvettes ten to five months before expiry date.

Borås: The actual prescribed storage condition for the microcuvettes is +15 to +35 °C.

Borås: The carrier arm for the microcuvette is a little hard to handle. It seems to be spring-loaded as it closes with a snap. We tried to slow down the movement to avoid the risk of the microcuvette flying off the carrier arm.

Borås: We got the error code which means that the microcuvette is empty even it was filled.

Floda: Easy measurement routine = good.

Floda: We got many error codes – for 9 out of 40 patient samples.

Floda: We are of the opinion that the microcuvettes are leaking both in the instrument and on the finger of the patient.

Floda: We think there were big differences between the capillary and venous results. The instrument gave an unstable impression.

Floda: There were big differences between the first and the second results.

4.6.1 Assessment of the user-friendliness

For all the items in the questionnaire, except two, HemoCue WBC got the best assessment "Satisfactory". For one item about index in the manual HemoCue WBC got the assessment "Not satisfactory" as there is no index in the manual. For one item about the external quality control possibilities HemoCue WBC got the assessment "Less satisfactory": Suitable external quality control schemes are not yet available. To start such schemes is not the responsibility of HemoCue.

One evaluation site had negative comments concerning the high frequency error codes on one of the instruments. The most likely cause is that there was something wrong with that single instrument.

The overall opinion was that the evaluators liked the system and thought it was easy to handle.

4.6.2

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Attachment 1.
The HemoCue WBC Method

Table of content

1 The HemoCue WBC Method manual

Some parts of the HemoCue WBC manual is here reprinted to make it possible for the reader of the SKUP report to know how the measurements are performed on the HemoCue WBC system. These instructions were followed during the present evaluation.

1.1 Analysing a capillary patient sample

The following guide description is printed in the HemoCue WBC manual.

1. To perform a test, the cuvette moving arm should be in its loading position. The display will show three flashing dashes and the HemoCue symbol. Take a HemoCue WBC Microcuvette from the vial.
2. Make sure the patient's hand is warm and relaxed. Use only the middle or ring finger for sampling. Avoid fingers with rings on.
3. Clean fingertip with disinfectant and allow it to dry completely or wipe off with a dry, lint-free wipe.
4. Using your thumb, lightly press the finger from the top of the knuckle towards the tip.
5. Sample at the side of the fingertip.
6. While applying light pressure towards the fingertip, puncture the finger using a lancet. (Spring loaded lancets with a puncture depth of at least 2 mm are preferred to produce a sufficient blood flow.)
7. Wipe away the first two or three drops of blood.
8. Re-apply light pressure towards the fingertip until another drop of blood appears.
9. When the blood drop is large enough, fill the microcuvette in one continuous process. Do NOT refill!
   NOTE: Make sure that the microcuvette is filled from the tip, placed at about a 45 degree angle towards the blood drop according to the picture on page 18.
10. Wipe off excess blood from the outside of the microcuvette with a clean, lint-free wipe. Do not touch the open end of the microcuvette.
11. Look for air bubbles in the filled microcuvette. If present, discard the microcuvette and fill a new microcuvette from a new drop of blood. Small bubbles around the edge can be ignored.
    NOTE: Make sure that the microcuvette is filled according to picture in the manual since an improper filling angle might cause air bubbles to be introduced.
    NOTE: If a second sample is to be taken, it is important that this is done after the measurement of the first sample is complete. Wipe away the remains of the drop of blood and fill the second microcuvette from a new drop of blood as per steps 7–11 above.
12. Place the filled microcuvette in the cuvette holder within 40 seconds after filling.
13. Gently push the cuvette moving arm towards the measuring position. It will automatically slide to the measuring position and the measurement starts.
14. During the measurement, a "sandglass symbol", three fixed dashes and the HemoCue symbol, will be shown.
15. After approximately 3 minutes, the WBC value is displayed. The result will remain on the display as long as the cuvette moving arm is in the measuring position. Do not re-measure the filled microcuvette.
16. Always handle blood specimens with care, as they might be infectious. Consult local environmental authorities for proper disposal.

1.2 Analysing a venous patient sample

Most of the above instructions for capillary samples apply also for venous samples.
In addition the following should be observed:

EDTA anticoagulant may be used, preferably in solid form to avoid dilution effects.

Mix all specimen tube thoroughly on a mechanical mixer for at least 2 minutes or invert the tube 10 — 20 times by hand. The specimen can be stored at room temperature,
at +15 — +35 °C or in a refrigerator at +2 — +8 °C for 48 hours. If the specimen has been stored in a refrigerator, it will be viscous and the blood should be allowed to warm up to room temperature before mixing. Alle disse detaljene er unødvendige.

2 Check of the agreement between instruments

HemoCue AB specifies the within-series-imprecision and the total imprecision. See Table 1 below. They have determined the within-series-imprecision and the total imprecision according to the CLSI document EP05-A2. The results in the table are derived from four lots of HemoCue WBC Microcuvettes and five HemoCue WBC Analyzers. Commercial control materials at three levels were used. The concentrations of B—Leukocytes were measured in duplicate twice a day, in the morning and in the afternoon, during 20 consecutive days.

Table 1. Imprecision specifications for HemoCue WBC

Level	N	B—Leukocytes (10^9/L)	Within-series-imprecision (CV %)	Total imprecision (CV %)
1	400	2,5	4,06	5,4
2	400	7,2	2,92	3,5
3	400	19,0	1,63	1,9

SKUP uses the rule of thumb that the CV is allowed to be 30% higher as a maximum, when the total imprecision of the results from all instruments is compared with the mean imprecision within several individual instruments. If the total CV is higher, the instruments do not fulfil the agreement requirement. In that case, the instrument(s) with deviating mean value or deviating CV should be identified and excluded from the evaluation. The manufacturer should, in such a case, be contacted for exchange of the deviating instrument. This model for assessing the agreement has been used also in this evaluation.

Calibration agreement between the HemoCue WBC instruments used in the present evaluation was checked by placing the seven instruments next to each other in the hospital laboratory in Borås. Two patient samples, one with low and one with high B—Leukocytes concentration were selected from the routine samples. The two samples were analysed six times on each HemoCue WBC Analyzer. The results of the agreement check are shown in the Tables 2, 3 and 4 below.

Table 2. Agreement between different HemoCue WBC Analyzers

	Instrument serial number						
	082035 0010	082035 0008	082035 0019	082035 0011	082035 0018	081835 0019	082035 0007
Sample no. 1 (n = 6)							
B—Leukocytes mean (10^9/L)	4,82	4,77	4,78	4,65	4,67	4,68	4,68
CV (%)	2,0	3,2	2,4	2,6	2,2	4,1	3,1
Sample no. 2 (n = 6)							
B—Leukocytes mean (10^9/L)	19,08	18,95	19,05	19,32	19,12	19,28	19,15
CV (%)	0,9	1,4	1,6	2,5	0,8	2,1	2,4
Both samples							
B—Leukocytes mean (10^9/L)	12,0	11,9	11,9	12,0	11,9	12,0	11,9
CV (%)	1,5	2,3	2,0	2,6	1,5	3,1	2,8

Table 3. ANOVA calculations of agreement between different HemoCue WBC Analyzers

Sample no.	**B—Leukocytes mean (10^9/L)**	**Within-instrument CV component (%)**	**Between-instruments CV component (%)**	**Total CV (%)**	**Increase of CV (%)**
1	4,72	2,90	0,74	2,99	3
2	19,14	1,81	0,00	1,78	0

"Within-instrument CV component" refers to the mean contribution to the "Total CV" originating from the within instrument imprecision.
"Between-instruments CV component" refers to the mean contribution to the "Total CV" originating from the between instruments imprecision. The within-instrument imprecision is not included in this figure.

"Increase of CV" refers to the increase in percent of the CV from "Within-instrument CV component" to "Total CV".

As can be seen in Table 2 the means and CVs of all instruments agreed well. The requirements defined by SKUP for agreement between instruments were fulfilled by a comfortable margin. The results are also in agreement with HemoCue's own specifications.

Six instruments were planned to be used in the evaluation, and the seventh instrument was a back-up instrument. The instrument with serial number 0818350019 was thus put aside as a back-up instrument. HemoCue AB was informed of these results as soon as they were ready. HemoCue AB accepted the decision.

Table 4. List of instruments used at each evaluation site and check of calibration differences between the evaluation sites

Evaluation site	**Instrument serial number**	**B—Leukocytes mean (10^9/L) n = 6 for each mean**	
Borås hospital laboratory	0820350008	4,77	18,95
	0820350010	4,82	19,08
	All at the site	**4,80**	**19,02**
Floda Primary Care Centre	0820350018	4,67	19,12
	0820350019	4,68	19,28
	All at the site	**4,68**	**19,20**
Fristad Primary Care Centre	0820350007	4,68	19,15
	0820350011	4,65	19,32
	All at the site	**4,66**	**19,24**

The "B—Leukocytes mean" in the right columns are for each instrument the mean of six determinations on the low level sample respectively the high level sample. The bold figures show the means of the means for each evaluation site.

Table 4 shows which instruments that were used at each site. In each duplicate, the first result was measured with one of the instruments and the second result was measured with the other instrument at the same site. The two columns to the right in the table show that there were just negligible calibration differences between the HemoCue WBC Analyzers at the different evaluation sites.

Attachment 2.
The Comparison Method

Table of content

The Comparison Method

The standard protocol for evaluations organised by SKUP includes a comparison of the results of the evaluated measurement system with the results from a well established hospital method. The hospital method used in this evaluation of HemoCue WBC is the routine method at SÄS County Hospital in Borås. It is below called "the Comparison Method".

1 Description of the Comparison Method

1.1 The measuring principles of the Comparison Method

The following information about the Advia 2120 is taken from Siemens Internet pages [1].

The Advia 2120 uses a combination of light scatter, cytochemical staining, and nuclear density on two independent channels to measure the total and differential white cell counts. The technology utilizes peroxidase staining for differential testing.

Cells are analyzed using the principles of flow cytometry whereby the Advia 2120 sheath/rinse reagent encases the sample stream. This results in a single cell stream, which minimizes coincidence. Measurement of total white cell count and differential cluster analysis identify each cell according to its size and light absorption properties. The exact number of cells within each cluster is counted. The positions of thresholds for the clusters are automatically adjusted in order to adapt to changes in cell characteristics in the individual sample.

1.1.1 The Basophile/Lobularity Method/Channel

The basophile method provides the primary total white cell count on the Advia 2120. The BASO reagent lyses the red cells, platelets, and the cytoplasm of all white cell types except basophiles. The BASO diagram uses cluster analysis to identify and count the cells and the nuclei in each population based on their position, area, and density. High-angle light scatter, which reflects the nuclear configuration or number of segments in the nucleus, is plotted on the x axis. Low-angle light scatter, which reflects the cell size, and is plotted on the y axis.

The following clusters are identified in the diagram:

1. Noise
2. Blast cell nuclei
3. Mononuclear WBCs (Monocyte and Lymphocyte nuclei)
4. Basophiles
5. Baso Suspect
6. Saturation
7. Polymorphonuclear WBCs (Neutrophil and Eosinophil nuclei)

The following White Cell Morphology Flags can be shown:

ATYPS – Atypical Lymphocytes

NRBC – Nucleated Red Blood Cells

Blasts – Suspected Blasts

LS – Left Shift

IG – Immature Granulocytes

1.1.2 The Peroxidase Method/Channel

This is the primary differential method on the Advia 2120, and additionally provides a secondary total white cell count. This back-up white cell count acts as an internal QC check to monitor sample integrity.

The cytochemical reaction is a two-stage chemistry method utilizing the intracellular myeloperoxidase enzyme to differentiate cells using stain and size characteristics. The cells are analyzed by the addition of the peroxidase enzyme substrate. Absorbance of the white light from the tungsten light source is a measure of the intensity of the peroxidase reaction: neutrophils, monocytes, and eosinophils are peroxidase positive, whereas lymphocytes and basophiles are peroxidase negative. Peroxidase reactivity is plotted on the x axis and cell volume is plotted on the y axis.

In addition to the standard five-population differential, the Advia 2120 reports an additional population called large unstained cells (LUCs). These cells are usually virally activated lymphocytes, plasma cells, hairy cells, paediatric lymphocytes, or peroxidase negative blasts.

The following clusters are identified in the diagram:

1. Noise
2. Nucleated Red Blood Cells
3. Platelet Clumps
4. Lymphocytes and Basophiles
5. Large Unstained Cells
6. Monocytes
7. Neutrophils
8. Eosinophils

Cells identified as mature red blood cells are shown in red, while cells identified as reticulocytes are coloured blue. The map shows reticulocytes as cells that are larger than mature red cells with lower haemoglobin concentration.

1.2 Method manual

Selected parts of the method manual [2] used for the Comparison Method in the Borås laboratory are reproduced below. Only the parts valid for B—Leukocytes are quoted here. *Some comments in italics have been entered by SKUP.*

1.2.1 Measurement principle

The method is based on a discovery by Cremins that the cytoplasm of basophilic leukocytes is especially resistant to decompose at influence of acid in combination with detergent. Whole blood is mixed with Baso Diluent, which haemolyses the erythrocytes and thrombocytes. At the same time the cytoplasms of all leukocytes, except the basophilic leukocytes, are dissolved. The result is that only cell nuclei remain of all leukocytes, except of the basophilic cells that are intact. The reaction is accelerated by warming the sample mixture to 30ºC. The reaction mixture passes in laminar flow the same type of flow cell that is used for the erythrocyte count. The reflected laser light is measured at two different angle intervals 2 to 3 degrees and 5 to 15 degrees. The measurement at low angle separates the basophilic leukocytes from the other leukocytes and the measurement at high angle classifies the nuclei of the leukocytes in and polynuclear also warns about suspected blast cells. An index of lobularity is calculated as the proportion between the polynuclear and mononuclear populations and also detects the presence of leukocytes with band formed nucleus.

1.2.2 Apparatus and accessories

Two instruments, Advia 2120 from Siemens, with automatic sampler for closed sampling.

1.2.3 Sample collection and sample handling

3 mL venous blood is collected in vacuum tubes with di-potassium-EDTA as additive. The samples are stored at room temperature but if the measurement can not be performed within 8 hours the sample is stored in refrigerator at 2 to 8ºC. The samples are for B—Leukocytes measurements stable at least 48 hours in refrigerator. The sample must reach room temperature before the measurement.
The used vacuum tubes have the brand name Vacuette and is manufactured by Greiner Bio-One, Bad Haller Straße 32, A-4550 Kremsmünster, Austria.
Product number: 454411, intended filling volume of blood: 3mL, cap colour: lavender black, dimensions 13 x 75mm, additive: 1,8 mg dry K2-EDTA per mL intended filling volume, label: transparent

1.2.4 Reagents

All reagents are supplied by Siemens.

1.2.5 Calibration

Calibrator: Advia 120 SETpoint Hematology Calibrator, product no T03-3685-52

Calibration is performed after Siemens service and after exchange of the whole or parts of the block or the sample valve. The calibration is performed according to the Siemens Operators Manual for Advia 2120.

1.2.5.1 Traceability

With the used calibrator the B—Leukocytes results are comparable with the results of the reference method according to the Coulter principle.

1.2.6 Quality control procedures

1.2.6.1 External quality control

The Borås laboratory participates with the Comparison Method in the EQUALIS External Quality Assurance (EQA) Scheme for "Haematology, including cell counter classification of leukocytes".

Control samples from EQUALIS are analysed ten times a year. Each material consists of whole blood mainly from one donor. Around 20% of each material usually derives from a second donor. The samples are incubated to room temperature before the measurement. The result from a single determination is reported with two decimals.
For results see below in Section 2.2.1, Table 3

1.2.6.2 Internal quality control

A. With commercial control materials

Advia 3 in TESTpoint Hematology Control Abnormal 1, product no T03-4417-54
Advia 3 in TESTpoint Hematology Control Normal, product no T03-4416-54
Advia 3 in TESTpoint Hematology Control Abnormal 2, product no T03-4418-54

Fixed SD limits are used for maximum allowed deviation from assigned values.
The limits for B—Leukocytes:

Abnormal 1:	±2 SD = ±0,30 10^9/L,	±3 SD = ±0,45 10^9/L
Normal:	±2 SD = ±0,60 10^9/L,	±3 SD = ±0,90 10^9/L
Abnormal 2:	±2 SD = ±1,6 10^9/L,	±3 SD = ±2,4 10^9/L

Batches of analysed patient samples are approved if the internal controls fall within ±2 SD.
Batches may also be approved if a single internal control falls outside ±2 SD and within ±3 SD.
Batches are never approved if several consecutive controls are outside ±2 SD or any of the internal controls fall outside ±3 SD.
For results see below in Section 2.2.1, Table 2.

B. With a fresh patient sample

During each weekday a fresh whole blood sample is used as stability control. Between two and five measurements are in the morning performed on each Advia instrument and the sample thereby get an assigned value. The same control rules as for the Normal commercial control are then applied during the rest of the day.

C. With patient mean

As the commercial control materials just have a stability over 12 weeks the patient mean is checked regularly.

1.2.7 Reporting

B—Leukocytes are reported with two decimals and in the unit 10^9/L.

The first time it is noted that a patient has B—Leukocytes <1,0 10^9/L the result shall be reported on telephone to make sure that it observed by the personnel responsible for the care of the patient.

1.2.8 Measuring range

B—Leukocytes: 0,02 to 409 10^9/L

1.2.9 Reference interval

B—Leukocytes: 3,5 to 8,8 10^9/L (valid for adult women and men)

1.2.10 Interferences

B—Leukocytes (baso method):

- The haemolysis of basophilic leukocytes may in some patient samples be incomplete, i.e. in samples from patient with leukaemia and high concentrations of leukocytes. This may produce falsely elevated B—Leukocytes results.
- Samples with erythroblasts may produce falsely elevated B—Leukocytes results.
- Micromegakaryocytes may be counted as leukocytes.

1.2.11 Verification

Measurements to check the within-series imprecision were done in February and March 2005. At each instrument a patient sample was measured 20 times and the following imprecision figures were achieved:
Advia 2120, serial no 368: B—Leukocytes level (baso): 6,37 10^9/L, Repeatability: 1,8 CV%
Advia 2120, serial no 377: B—Leukocytes level (baso): 4,85 10^9/L, Repeatability: 1,8 CV%

Comparisons of results with the old instruments Advia 120 and the new instruments Advia 2120 were done in February and March 2005. The following figures were achieved:
B—Leukocytes (baso): n = 64, mean (minimum to maximum) value of the patient samples: 9,12 (0,31 to 57,0) 10^9/L, coefficient of correlation: 0,9970.

2 Verification of the Comparison Method

2.1 Imprecision of the Comparison Method

2.1.1 Missing and excluded results and check calculations

See Attachment 4, Section 3, Table 1B.

2.1.2 Imprecision of the Comparison Method in the HemoCue WBC evaluation

For each patient in the hospital evaluation the same venous sample tube was used for measurements with HemoCue WBC and the Comparison method. Both methods measured in duplicates.

For each patient in the evaluation at the primary care centres the same venous sample tube was used for measurements with HemoCue WBC and the Comparison method. In addition a duplicate capillary samples were collected from each patient at the primary care centres.

Table 1 show the imprecision of the Comparison Method with the venous patient samples used in the evaluation for comparison with HemoCue WBC. The imprecision of the Comparison Method is calculated on the duplicate results.

Table 1. Repeatability of the Comparison Method with venous patient samples used for comparison with HemoCue WBC.

Level[#]	Comparison Method interval (HbA1c %)[#]	Number of excluded results*	n	Comparison Method mean (HbA1c %)[#]	CV (%) (95 % confidence interval)
Hospital laboratory in Borås:					
Low	1,4 — 6,2	1	33	4,50	2,3 (1,9 — 3,1)
Medium	6,3 — 9,3	0	33	7,48	1,9 (1,6 — 2,6)
High	9,3 — 29,3	1	32	15,88	1,6 (1,3 — 2,2)
All	1,4 — 29,3	2	98	9,22	2,0 (1,7 — 2,3)
Primary Care Centre Floda:					
Low	4,4 — 6,8	0	20	5,97	1,8 (1,4 — 2,7)
High	7,1 — 16,3	0	19	9,41	1,8 (1,4 — 2,7)
All	4,4 — 16,3	0	39	7,82	1,9 (1,5 — 2,4)
Primary Care Centre Fristad:					
Low	3,6 — 7,0	0	20	5,67	1,6 (1,2 — 2,3)
High	7,1 — 12,7	0	21	8,64	2,7 (2,1 — 3,9)
All	3,6 — 12,7	0	41	7,19	2,5 (2,0 — 3,2)

[#] The results are divided into concentration subgroups according to the Comparison Method results to enable a comparison of the results in Table 1 in this Attachment, with the results in Table 10, Table 15 and Table 18 in the HemoCue WBC report. The tables in the report contain the corresponding HemoCue WBC results. Note: However, the calculated imprecision in the tables are of different kinds; in Table 1, Table 10 and Table 15 the imprecision is calculated from duplicate determinations on the same sample and in Table 18 it is calculated from determinations of dupli-cate samples from the same finger puncture measured on two different HemoCue WBC instruments.

* Please refer to the text in Attachment 3 about applied test for exclusion of results.

2.1.3 Internal quality control results

Table 2 contains the internal quality control results obtained with the Comparison Method during the evaluation period. Each level of the internal quality control material have been measured about once per day.

Table 2. Internal quality control results during the evaluation

Quality control level	B—Leukocytes (Comparison Method) (10^9/L)			n	CV % (95 % conf. interval)
	Assigned value	Found average	Difference Found–Assigned		
Level 1	3,51	3,48	−0,03	53	4,3 (3,6 — 5,4)
Level 2	7,19	7,12	−0,07	64	3,0 (2,6 — 3,7)
Level 3	16,26	16,49	+0,23	50	2,4 (2,0 — 3,0)

2.1.4 Assessment of the imprecision of the Comparison Method

The CV, calculated from the duplicate measurements on patient samples was about 2,5%. The CV for the internal quality control results was a little worse. The imprecision figures of the Comparison Method are considered to be normal for a hospital method.

2.2 Trueness of the Comparison Method

2.2.1 The Comparison Method in the EQUALIS EQA scheme

All the EQA results from the Comparison Method from the period before and during the SKUP evaluation of HemoCue WBC, February 2008 to February 2009, are shown in Table 3.

Table 3. The Comparison Method results compared with the results of all participants in the EQUALIS EQA scheme.

Date of measurement	Total number of participants	B—Leukocytes, mean of all participants (10^9/L)	SD between-laboratories (10^9/L)	CV between-laboratories (CV%)	B—Leukocytes, Comparison Method (10^9/L)	Deviation of the Comparison Method		
						(10^9/L)	(%)	Number of SD
2008-02-27	288	6,24	0,24	3,9	6,11	−0,13	−2,1	−0,5
2008-04-02	288	7,35	0,26	3,5	7,36	+0,01	+0,1	±0,0
2008-05-14	269	5,78	0,24	4,2	5,31	−0,47	−8,1	−1,9
2008-06-25	279	4,94	0,21	4,3	5,29	+0,35	+7,0	+1,6
2008-08-20	277	5,03	0,21	4,2	5,16	+0,13	+2,5	+0,6
2008-09-10	285	4,74	0,18	3,7	4,73	−0,01	−0,1	±0,0
2008-10-08	279	3,54	0,22	6,1	3,39	−0,15	−4,3	−0,7
2008-11-12	286	6,96	0,32	4,6	7,53	+0,57	+8,2	+1,8
2008-12-10	285	5,88	0,24	4,1	6,01	+0,13	+2,2	+0,5
2009-01-21	286	4,75	0,18	3,7	4,79	+0,04	+0,7	+0,2
2009-02-18	284	7,45	0,30	4,0	7,40	−0,05	−0,6	−0,2
Mean	**282**	**5,70**	**0,24**	**4,2**	**5,73**	**+0,04**	**+0,5**	**+0,1**

As there is no available reference method and no certified reference materials for B—Leukocytes, the most reliable values are the consensus mean of many cell counters. The results in Table 3 above derive from Equalis EQA samples measured about nine months before and three months during the SKUP evaluation.

The table shows that

- the deviations of the Comparison Method from the national mean values were normal for a method in an hospital laboratory. This is obvious when looking at the deviations expressed in standard deviations. The standard deviations are measures of the between-laboratories variation in Sweden.
- the calculated bias of the Comparison Method was small +0,5%.
- the deviations of the Comparison Method from the national mean values varied from sample to sample. The 95% confidence interval of the bias was from −2,6 to +3,6%.

2.2.2 Assessment of the trueness of the Comparison Method

The bias of the Comparison Method was calculated to +0,5% with the 95% confidence interval from −2,6 to +3,6%. Zero bias was thus included in the confidence interval and it was decided to make no correction of the Comparison Method results in the present SKUP evaluation. On the other hand, the deviation varied much from sample to sample in the EQA scheme. The variation of the deviation values in Table 3 was calculated to 4,7 CV%. This figure is at the same time a measure of the total variation in the Comparison Method results caused both by method repeatability and imprecision due to matrix effects. This CV value was therefore used in the calculation of allowable tolerance limits for imprecision and total error in this evaluation. See the main report, Section 2.2.2.

3 References

1. Internet address: http://diagnostics.siemens.com/webapp/wcs/stores/servlet/PSGenericDisplay~q_catalogId~e_-111~a_langId~e_-111~a_pageId~e_78670~a_storeId~e_10001.htm
2. Document name: B-Haematology status including cytochemical differential count of leukocytes, Advia 2120, document number-version: 2685-1, valid from 2009-02-03.

Attachment 3.
Statistical expressions and calculations

Table of contents

1 Introduction

In this document we want to explain the statistics in the SKUP-reports;

- the meaning of statistical terms
- criteria for exclusion of outliers
- how missing and excluded values are shown
- how the calculations are done and which formulas are used
- how the results are illustrated in diagrams

Some parts of the text are direct quotations from the ISO/IEC Guide 99:2007, International vocabulary of metrology — Basic and general concepts and associated terms [1]. The document is also called "VIM, 3rd edition". The quotations from ISO/IEC Guide 99:2007 (ISO G99) are here printed in different style exactly as they are in the guide including the paragraph number. When an expression is printed with bold letters in the guide, it means that the expression is defined in another paragraph of the guide.

2 Precision measured as imprecision

Quotation from ISO G99:

"**2.41 measurement precision / precision**

closeness of agreement between **measured quantity values** obtained by replicate **measurements** on the same or similar objects under stated specified conditions

NOTES

1. Measurement precision is usually expressed numerically by measures of imprecision, such as standard deviation, variance, or coefficient of variation under the specified conditions of measurement.
2. The specified conditions can be **repeatability conditions of measurement, intermediate precision conditions of measurement, or reproducibility conditions of measurement**.
3. Measurement precision is used to define **measurement repeatability, intermediate measurement precision, and measurement reproducibility**." [1]

Precision is a descriptive general term connected to words like good, acceptable and poor. The lack of precision is measured as imprecision. Imprecision is often expressed as standard deviation (SD) or coefficient of variation (CV). SD is reported in the same unit as the analytical result and CV is usually reported in percent (CV%). Good precision produces low imprecision figures and vice versa.

Quotation from ISO G99:

"**2.42 repeatability condition of measurement / repeatability condition**

condition of **measurement** in the set of conditions that includes the same **measurement procedure**, same operators, same **measuring system**, same operating conditions and same location, and replicated measurements on the same or similar objects over a short period of time

NOTE

In chemistry, the term 'intra-serial precision condition of measurement' is sometimes used to designate this concept." [1]

The imprecision can be measured in many ways. One estimate of the imprecision is called repeatability. See definition above. Different estimates of the imprecision give different values and repeatability is the smallest value. All other imprecision measures are the agreement between the results carried out under more or less changing measuring conditions. The changing conditions could for example be varying laboratories, varying measuring days, varying operators, varying lots of reagents, varying calibrators, varying instruments and even varying methods. The repeatability value is a part of other imprecision values.

SKUP uses the term "repeatability" for the imprecision calculated from duplicate measurements on patient samples with similar concentration – within the same level group (similar objects). The measurements within the duplicates are measured by the same operator, with the same instrument, with the same lot of reagent and within a short time. However, the different replicates are often measured by different operators, with different lots of reagent and on different days.

SKUP uses the term "between-days-imprecision" when the two measurements in each duplicate are made on different days. The imprecision is calculated from patient sample results which have similar concentration – within the same level group. The measurements within the duplicates are usually measured by the same operator, always with the same instrument, always with the same lot of reagent. However, the different duplicates are often measured by different operators, with different lots of reagent and on different days.

The conditions for repeatability are well defined, but for all other measures of imprecision, the conditions during the measurements have to be described. A term like between-series imprecision or between-days imprecision is not defined by the expression, but has to be described. The imprecision results in this report will be summarised in tables and under each table the conditions during collection of the data are described.

3 Trueness measured as bias

Quotation from ISO G99:

"**2.27 measurement trueness / trueness of measurement / trueness**
closeness of agreement between the average of an infinite number of replicate **measured quantity values** and a **true value** of the **measurand**

NOTES

1. Measurement trueness cannot be expressed numerically.
2. Measurement trueness is inversely related to only **systematic measurement error**.
3. Measurement trueness should not be used for **measurement accuracy**." [1]

Trueness is descriptive in general terms (good, poor). The lack of trueness is measured as bias (mean deviation or systematic error). The bias is reported in the same unit as the analytical result and/or in percent. Good trueness produces low bias figures and vice versa.

In the SKUP-reports the bias at different concentration levels are shown in tables.

4 Accuracy measured as total error

Quotation from ISO G99:

"2.11 measurement accuracy / accuracy of measurement / accuracy

closeness of agreement between a **measured quantity value** and a **true value** of the **measurand**

NOTES

1. Measurement accuracy cannot be expressed numerically.
2. Measurement accuracy is inversely related to both **systematic measurement error** and **random measurement error**.
3. The term 'measurement accuracy' should not be used for **measurement trueness** and the term measurement precision should not be used for 'measurement accuracy'.
4. Sometimes 'measurement accuracy' is considered, in a qualitative sense, to be inversely related to measurement uncertainty." [1]

When SKUP uses the term "total error" the "true values" are produced by the Comparison Method. The statement that the Comparison Method produces "true values" can naturally be called in question to some extent.

Accuracy is a descriptive general term connected to words like good, acceptable and poor. The term inaccuracy is used to describe the degree of lack of accuracy. "Total error" is a measure of inaccuracy used by SKUP [2]. The total error of a method is a measure of many single measurement result's deviations from the true values. Total error is the result of the combined effect of random and systematic errors (analytical imprecision and bias). Total error doesn't distinguish between random and systematic errors in the measuring system. The total errors of the Evaluated Measuring Systems are in the SKUP-reports illustrated by difference plots with quality goals for the total error shown as deviation limits in percent.

Uncertainty is an alternative measure of inaccuracy. The term uncertainty is a part of the model of thinking called the "error propagation model". The idea is that all error components should be correctly identified and properly summed up to the uncertainty. SKUP normally don't evaluate the uncertainty.

5 Check for and exclusion of statistical outliers

The sample results are first sorted with the concentrations in ascending order. The mean result of each duplicate with the Comparison Method decided the sorting order. Then the results are divided into three level groups. The outlier test described below is then applied to the data in each level group separately. The following differences are tested for outliers:

- The differences between the two measurements with the Comparison Method.
- The differences between the two measurements with the Evaluated Measuring System.
- The differences between the mean value of the two measurements with the Evaluated Measuring System and the mean value of the two measurements with the Comparison Method. These differences include an element of bias as described below.

Statistical outliers are treated differently in the calculations and in the diagram. The purpose of the calculations of imprecision and bias is to give estimates valid for typical patient sample results. The results with too big differences between the two duplicate results with the Evaluated Method, according to the routine described below, are considered to be outliers and are thus excluded in these calculations. The total error diagram, on the other hand, should

show both systematic and random errors. Therefore results are excluded in the diagram only if the Comparison Method result is uncertain. According to the routine described below the Comparison Method results with statistically too big difference between the two duplicate results is determined.

Before the duplicate results are used for the calculation of imprecision they have been checked for the occurrence of outliers. For each duplicate determination the difference between the two results is calculated. Burnett's rule [3] is applied to all differences in the same level group. First, preliminary mean value and SD are calculated for all differences within the level group. If any difference differs by more than approximately ±3 SD from the mean of all differences in the level group, that duplicate pair is excluded.

The model takes into consideration the number of observations together with the statistical significance level for the test. The exact numbers of standard deviations for exclusion thus varies depending on the number of values the calculation is carried out for. For instance, for n = 20, the limit is set at ±3,02 SD, for n = 30 at ±3,14 SD, and for n = 100 at ±3,47 SD. As described by Burnett, the outlier test is repeated in the necessary number of steps until no value differs more than allowed. The significance level is often set to 5 %, which is also the case in each step of this outlier test used by SKUP. The numbers of found and excluded outliers are presented in each table and under the diagram. In th tables the number of results remaining after exclusion of outliers is specified as n, this number is also the number of results included in the calculation.

In a similar way, before calculation of bias, Burnett's rule has been applied to the differences between the tested method and the Comparison Method.

6 Missing results

Besides the statistical outliers, some results are missing or excluded for other reasons in the calculations. An overview of both missing and excluded values is shown in Attachment 4.

7 Calculations of imprecision

The duplicate measurements on the Evaluated Measuring System are used for calculation of the imprecision.

On some of the venous specimens in the hospital laboratory, a third measurement is also performed. On half of the specimens the third measurement is performed on the day after the duplicate and on the remaining half of specimens the third measurement is performed two days after the first measurement. Between-days imprecision is calculated from the differences between the first and the third measurements. These two measurements are always performed with the same instrument.

The imprecision is calculated with the following formula:

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

The imprecision may also be calculated with the following formula:

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

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

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

Even if these formulas are based on the differences between duplicate measurements, the SD/CV is still a measure of the imprecision of single values, and completely comparable with the more commonly used calculation based on repeated measurements of only one sample (common repeatability measurements).

The assumption, when using the formula for imprecision calculations, is that no systematic difference of importance exists, between the two series of first measurements on the two instruments. To check that the assumption is valid; the mean difference with confidence intervals is calculated and presented in the SKUP-report for each level group of results.

In some SKUP-evaluations, the two results in each duplicate of the Evaluated Measuring System are performed on two separate instruments. Already before such evaluation starts, the between-instrument variance is checked to be acceptable. In spite of this precautionary measure, the calculated imprecision in such evaluations includes a small variance component arising from the fact that two separate instruments of the Evaluated Measuring System are used for the two measurements in each duplicate.

8 Calculation of bias

The bias is the mean deviation of the Evaluated Measuring System results from the Comparison Method results. The means of the duplicate results are used for both methods when calculating the bias. The bias can be either a positive or a negative value. It is calculated in the same unit as the results and in percent (%) of the mean result.

In the SKUP reports, the bias of the Evaluated Measuring System is calculated at different concentration levels. The results obtained in the hospital laboratory are divided into three concentration intervals and the results from the primary care centres are divided into two concentration intervals, respectively.

The bias is calculated by use of the formula:

$Bias = \Sigma d/n$

d = deviation: the mean of the duplicate results with the Evaluated Measuring System minus the corresponding mean of the duplicate results with the Comparison Method,
n = number of deviations

The bias is also calculated in percent by use of the following formula:

*Bias (%) = Bias (units) / Mean value of the Comparison Method results (units) * 100*

The standard deviation of all the deviations used for the bias calculation is calculated by use of the following formula:

$$SD_{deviations} = \sqrt{\frac{\sum (d - Bias)^2}{n-1}}$$

9 Illustration and calculation of total error

To evaluate the accuracy of the results at the Evaluated Measuring System, the agreement between results with the Evaluated Measuring System and results with the Comparison Method is illustrated in difference plots. In the plots the x-axis represents the mean value of the duplicate results at the Comparison Method. The y-axis shows the difference between the first measurement result at the Evaluated Measuring System and the mean value of the duplicate results at the Comparison Method for a total of three lots. The number of and the percentage of results inside the quality goals are written in the text under the diagram.

10 Terms in English, Danish, Norwegian and Swedish

English	Danish	Norwegian	Swedish
accuracy	**akkuratesse**	**nøyaktighet**	**noggrannhet**
between-days imprecision	**dag til dag imprecision**	**mellom-dags-imprecision**	**mellandags-imprecision**
bias Synonym of “mean deviation” and for “systematic error”	**bias** Synonym for ”gennemsnitssaf-vigelse” og for “systematisk fejl”	**bias** Synonym for ”gjennomsnittsavvik” og for ”systematisk feil”	**bias** Synonym för ”medelavvikelse” och för “systematiskt fel”
coefficient of variation	**variationskoefficient**	**variasjonskoeffisient**	**variationskoefficient**
condition of measurement	**målebetingelser**	**målebetingelser**	**mätförhållanden**
CV/CV% Abbreviation for “coefficient of variation”	**CV/CV%** Forkortelse for “variationskoefficient”	**CV/CV%** Forkortelse for “variasjonskoeffisient	**VK/CV/VK%/CV%** Förkortning för “variationskoefficient”
imprecision	**impræcision**	**upresishet**	**imprecision**
inaccuracy	**unøjagtighed**	**unøyaktighet**	**onoggrannhet**

English	Danish	Norwegian	Swedish
intermediate precision Can be used for all different measures of precision except “repeatability”. Used in the quoted part of ISO G99 but not used by SKUP.	**intermedier præcision** Kan anvendes for alle forskellige former for præcision bortset fra ”repeterbarhed”. Anvendes i ISO G99 (citeret del) SKUP anvender ikke længere udtrykket.	**intermediær presisjon** Kan anvendes for alle mål på presisjon bortsett fra ”repeterbarhet”. Anvendes i sitered del av ISO G99, men ikke av SKUP.	**mellanliggande precision** Kan användas för alla mått på precision utom ”repeterbarhet”. Används i citerad del av ISO G99 men används inte av SKUP.
intra-serial imprecision Mentioned in the quoted part of ISO G99 as a synonym of “repeatability”. Not used by SKUP	**intra-serie præcision** ISO G99 anvender intra-serie præcision som synonym for ”repeterbarhet”. SKUP anvender ikke længere udtrykket.	**innen-serie presisjon** Nevnes i sitered del av ISO G99 som synonym for ”repeterbarhet”. Anvendes ikke av SKUP.	**inomserieimprecision** Nämns i citerad del av ISO G99 som en synonym för ”repeter-barhet”. Används inte av SKUP.
mean deviation See bias	**gennemsnits afvigelse** Se bias	**gjennomsnittsavvik** Se bias	**medelavvikelse** Se bias
measurand	**kvantitet** eller ”egenskab som ønskes målt”	**det som måles**	**egenskap som man vill mäta**
measured quantity value	**måleresultat** eller ”opnået værdi”	**måleverdi** eller ”oppmålt verdi”	**mätvärde** eller ”uppmätt värde”
measurement procedure Used in the quoted part of ISO G99 but not used by SKUP	**målemetode/ procedure** Anvendes i ISO G99 (citeret del) SKUP anvender ikke udtrykket.	**måleprosedyre** Anvendes i sitered del av ISO G99, men ikke av SKUP.	**mätrutin** Används i citerad del av ISO G99 men används inte av SKUP.
measuring system	**målesystem**	**målesystem**	**mätsystem**
measurement uncertainty Mentioned in the quoted part of ISO G99 as being inversely related to “accuracy”. Not used by SKUP	**måleusikkerhed** ISO G99 anvender udtrykket i citeret del som omvendt relateret til ”akkuratesse”.. SKUP anvender ikke udtrykket.	**måleusikkerhet** Nevnes i sitered del av ISO G99 som omvendt relatert til ”nøyaktighet”. Anvendes ikke av SKUP.	**mätosäkerhet** Nämns i citerad del av ISO G99 som omvänt relaterat till ”noggrannhet”. Används inte av SKUP.

English	Danish	Norwegian	Swedish
outlier	**outlier** eller ”afviger”	**slenger** eller ”outlier”	**extremvärde** eller ”outlier” eller ”avvikande värde”
precision	**præcision**	**presisjon**	**precision**
random measurement error	**tilfældig målefejl**	**tilfeldig målefeil**	**slumpfel vid mätningen**
repeatability	**repeterbarhed**	**repeterbarhet**	**repeterbarhet**
reproducibility	**reproducerbarhed**	**reproduserbarhet**	**reproducerbarhet**
SD Abbreviation for ”standard deviation”	**SD** Forkortelse for ”standarddeviation”	**SD** Forkortelse for ”standardavvik”	**SD** Förkortning för ”standardavvikelse”
standard deviation	**standarddeviation**	**standardavvik**	**standardavvikelse** eller ” standard-deviation”
systematic error See bias	**systematisk fejl** Se bias	**systematisk feil** Se bias	**systematiskt fel** Se bias
total error	**totalfejl**	**totalfeil**	**totalfel**
true value	**sand værdi**	**sann verdi**	**sant värde**
trueness	**rigtighed**	**riktighet**	**riktighet**
variance Used in the quoted part of ISO G99 but not used by SKUP	**varians** ISO G99 anvender udtrykket i citeret del. SKUP anvender ikke udtrykket.	**varians** Anvendes i sitered del av ISO G99, men ikke av SKUP.	**varians** Används i citerad del av ISO G99 men används inte av SKUP
variation	**variation**	**variasjon**	**variation** eller “spridning”

The comment “Used in the quoted part of ISO G99” means that SKUP in this document has quoted a part of ISO G99 that contains the expression.

11 References

1. ISO/IEC Guide 99:2007, International vocabulary of metrology — Basic and general concepts and associated terms, also called VIM, 3rd edition, JCGM 200:2008.

   The document is produced by Working Group 2 of the Joint Committee for Guides in Metrology (JCGM/WG 2), 2008. The document will perhaps be published by all the JCGM member organizations, BIPM, IEC, IFCC, ILAC, ISO, IUPAC and OILM.
   It is available free of charge on the following web address:
   http://www.bipm.org/en/publications/guides/vim.html

   In this document the quotations from the guide are printed exactly as they are in the guide. When an expression is printed with **bold letters** in the guide, this means that the expression is defined in another paragraph of the guide.

2. Hyltoft Petersen, P., D. Stöckl, et al. (2001). "Models for Combining Random and Systematic Errors. Assumptions and Consequences for different Models." Clin Chem Lab Med 39(7): 589-595.

3. Burnett RW. Accurate Estimation of Standard Deviations for Quantitative Methods Used in Clinical Chemistry. Clinical Chemistry 1975; 21 (13): 1935 – 1938.

Attachment 4.
Missing or excluded results and check calculations

Table of content

1 Explanation of the content in this attachment

For some samples some results are missing. For other samples some results are excluded as statistical outliers. All missing or excluded results are explained in this attachment to show that the raw data has been treated correct and that exclusion of data has been done in a consequent manner.

How the calculations of imprecision from duplicates have been done is explained in Attachment 3. The used formula will not produce correct CV values if there is big difference between the means of the first and the second measurements. All the result groups are therefore tested for such differences. The tables in this attachment show the mean differences with confidence intervals. There is no systematic difference between the first and the second measurements in the duplicates if the confidence interval of the difference includes zero. The conclusion is thus that the CV calculations are valid.

2 The Comparison Method

2.1 Missing or excluded results in the imprecision calculation for the Comparison Method

In Attachment 3, Section 4, a detailed explanation of applied test for exclusion of statistical outliers is given. Why and where some results are missing or excluded in he imprecision calculation for the Comparison Method are shown in Table 10A and Table 15A below. The number of results remaining in respective calculation and in the diagram is shown in Table 10B and 15 B below.

2.2 Check of the imprecision calculation for the Comparison Method

The calculation of imprecision of the Comparison Method is presented in Table 1 in Attachment 2 to the HemoCue WBC report. The Table 1B below shows the check of differences between first and the second measurements in the same results group. The confidence intervals of the differences for eight out of ten result groups include 0,00 10^9/L. For the total set of data the conclusion is that there is no systematic difference between the first and the second measurements in the duplicates. The calculated CV values in Table 1 are thus valid.

Table 1B. Differences between the 1st and the 2nd measurements with the Comparison Method

Level	**1. – 2. mean difference (95 % confidence interval)** (10^9/L)
Samples used in the evaluation in the Hospital laboratory in Borås:	
Low	−0,17 (−0,23 — −0,11)
Medium	−0,07 (−0,19 — +0,04)
High	+0,10 (−0,09 — +0,29)
All	−0,05 (−0,13 — +0,03)
Samples used in the evaluation at the Primary Care Centre Floda:	
Low	+0,10 (+0,04 — +0,15)
High	−0,02 (−0,12 — +0,09)
All	+0,04 (−0,03 — +0,10)
Samples used in the evaluation at the Primary Care Centre Fristad:	
Low	−0,03 (−0,09 — +0,03)
High	−0,05 (−0,19 — +0,10)
All	−0,04 (−0,12 — +0,04)

3 The hospital evaluation

3.1 Missing or excluded results in the hospital evaluation

There were a total of 100 patient results. In Attachment 3, Section 4, a detailed explanation of applied test for exclusion of statistical outliers is given. Why and where some results are missing or excluded are shown in Table 10A. The number of results remaining in respective calculation and in the diagram is shown in table 10B.

Table 10A. Missing or excluded sample results in the hospital laboratory

Sample no.	B—Leukocytes (10^9/L)			Missing/ excluded due to	Excluded in calculation of
	Comparison Method 1	Comparison Method 2	Difference causing exclusion		
97	5,6	5,0	0,6	Comp.1 ≠ Comp.2	All calculations
82	31,6	30,1	1,5		
74	6,0	6,2	0,9	HC1 ≠ HC2	HemoCue WBC bias
83	0,02	0,05	-	HemoCue WBC result code, incomplete duplicate	HemoCue WBC repeatability, between-days imprecision and bias calculations
94	0,02	0,01	-	HemoCue WBC result code, incomplete duplicate	HemoCue WBC repeatability and bias calculations
93	30,1	30,0	-		
34	6,2	6,1	−1,55	Comp.mean ≠ HC mean	HemoCue WBC bias
35*	6,5	6,5	+1,20		

"Comp. 1 ≠ Comp. 2" means that the difference between these two duplicate Comparison method results was so big that the result was considered to be outliers.

“HC1 ≠ HC2” means that the difference between these two duplicate HemoCue WBC results was so large that the result was considered to be outliers.

“HemoCue WBC result code” means that the HemoCue WBC result was not a number but a code, which can not be included in the calculations. The code was either LLL which means that the result was less than 0,3 10^9/L or HHH which means that the result was more than 30,0 10^9/L. As can be seen when comparing with the Comparison Method result the codes were all correct results.

“Comp.mean ≠ HC mean” means that the difference between the Comparison method mean and the HemoCue WBC mean is so large that the result is considered to be outliers.

*Sample no 35 was identified as outlier only in the grouping alternative B. See table 13.

Table 10B. Patient sample results in the hospital laboratory included in calculations and diagram

Calculation/diagram	Number of results included
Imprecision of the Comparison method	98
Imprecision of HemoCue WBC	94
HemoCue WBC between-days imprecision	36
Bias of HemoCue WBC	93/92*
Total error of HemoCue WBC	98 including three result codes

*Sample no 35 was identified as outlier only in the grouping alternative B.

3.2 Check of the repeatability calculation for HemoCue WBC with venous samples in the hospital evaluation

The calculation of repeatability for HemoCue WBC with venous samples in the hospital evaluation is presented in Table 10 in the report. The Table 10C below shows the check of differences between first and the second measurements in the same results group. The confidence intervals of the differences for three out of four result groups include 0,00 10^9/L. For the total set of data the conclusion is that there is no systematic difference between the first and the second measurements in the duplicates. The calculated CV values in Table 10 are thus valid.

Table 10C. Differences between the 1st and the 2nd measurements

Level	1. – 2. mean difference (95 % confidence interval) (10^9/L)
Low	−0,17 (−0,23 — −0,11)
Medium	−0,07 (−0,19 — +0,04)
High	+0,10 (−0,09 — +0,29)
All	−0,05 (−0,13 — +0,03)

3.3 Check of the between-days imprecision calculation for HemoCue WBC with venous samples in the hospital evaluation

The calculation of between-days imprecision for HemoCue WBC with venous samples in the hospital evaluation is presented in Table 11 in the report. The Table 11B below shows the check of differences between first and the second measurements in the same result group. The confidence intervals of the differences for all three result groups include 0,00 10^9/L. For the total set of data the conclusion is that there is no systematic difference between the first and the second measurements in the duplicates. The calculated CV values in Table 11 are thus valid.

Table 11B. Differences between the 1st and the 2nd measurements

Level	**1. – 2. mean difference (95 % confidence interval)** (10^9/L)
Low	−0,02 (−0,21 — +0,17)
Medium	−0,02 (−0,20 — +0,15)
High	–
All	−0,01 (−0,13 — +0,12)

4 The evaluation at the primary care centres

4.1 Missing or excluded results in the evaluation at the primary care centres

Totally there were 43 + 41 patient results. In the calculations and in the diagram some results are missing or excluded. The reasons for why they are missing or excluded are shown in Table 15A. In Attachment 3, Section 4, a detailed explanation of the applied test for exclusion of statistical outliers is given. The numbers of results remaining in the calculations and in the diagram are shown in table 15B.

Table 15A. Excluded/missing results at the primary care centres

Sample no.	B—Leukocytes (10^9/L)			Missing/ excluded due to	Excluded in calculation of
	Comparison Method 1	Comparison Method 2	Difference causing exclusion		
Primary Care Centre Floda:					
22	-	-		Missing Comp Method results	All calculations
23	-	-			
26	-	-			
43	-	-		Missing HemoCue WBC first results	All calculations
4	4,8	4,7		HemoCue WBC error code result on at least one of the first capillary measurements	HemoCue WBC capillary imprecision and bias
40	6,0	6,0			
27	7,5	7,4			
8	7,9	7,7			
25	10,4	10,4			
32	10,6	11,0			
Primary Care Centre Fristad:					
9	3,6	3,6		HemoCue WBC error code result on the second capillary measurements	HemoCue WBC capillary imprecision and bias

Table 15B. Patient sample results at the primary care centres included in calculations/diagram

Calculation/diagram	Number of results included
Venous samples:	
Imprecision of the Comparison method	39 + 41
Imprecision of HemoCue WBC	39 + 41
Bias of HemoCue WBC	39 + 41
Total error of HemoCue WBC	39 + 41
Capillary samples:	
Imprecision of HemoCue WBC	33 + 40
Bias of HemoCue WBC	33 + 40
Total error of HemoCue WBC	39 + 41 including 6 error code results

4.2 Check of the repeatability calculation for HemoCue WBC with venous samples in the evaluation at the primary care centres

The calculation of repeatability for HemoCue WBC with venous samples in the evaluation at the primary care centres is presented in Table 15 in the report. The Table 15C below shows the check of differences between first and the second measurements in the same results group. The confidence intervals of the differences for five out of six result groups include 0,00 10^9/L. For the total set of data the conclusion is that there is no systematic difference between the first and the second measurements in the duplicates. The calculated CV values in Table 15 are thus valid.

Table 15C. Differences between the 1st and the 2nd measurements

Level	**1. – 2. mean difference (95 % confidence interval)** (10^9/L)
Primary Care Centre Floda:	
Low	+0,02 (−0,10 — +0,14)
High	+0,06 (−0,06 — +0,18)
All	+0,04 (−0,04 — +0,13)
Primary Care Centre Fristad:	
Low	−0,07 (−0,16 — +0,01)
High	−0,12 (−0,27 — +0,02)
All	−0,10 (−0,18 — −0,02)

4.3 Check of the imprecision calculation for HemoCue WBC with capillary samples in the evaluation at the primary care centres

The calculation of repeatability for HemoCue WBC with capillary samples in the evaluation at the primary care centres is presented in Table 18 in the report. The Table 18C below shows the check of differences between first and the second measurements in the same results group. The confidence intervals of the differences for all six result groups include 0,00 10^9/L. For the total set of data the conclusion is that there is no systematic difference between the first and the second measurements in the duplicates. The calculated CV values in Table 18 are thus valid.

Table 18C. **Differences between the 1st and the 2nd measurements**

Level	**1. – 2. mean difference (95 % confidence interval)** (10^9/L)
Primary Care Centre Floda:	
Low	−0,21 (−0,71 — +0,29)
High	−0,32 (−1,15 — +0,51)
All	−0,27 (−0,75 — +0,22)
Primary Care Centre Fristad:	
Low	+0,26 (−0,38 — +0,90)
High	−0,05 (−0,67 — +0,56)
All	+0,10 (−0,35 — +0,54)

Ängelholm
April 26, 2010

Comments from HemoCue AB

The HemoCue® WBC system is a unique point-of-care testing system for the determination of white blood cell count. Based on HemoCue's proven and reliable cuvette technology and ease of use, lab quality results are obtained within minutes, making the system optimal for hospital primary care settings.

HemoCue AB would like to thank SKUP for performing this thorough independent evaluation of the HemoCue WBC system. It is with proud and pleasure that HemoCue acknowledges that the operators participating in this study rated the system "Satisfactory" –the highest level, and also considering it practical and easy to operate. We are proud to offer the HemoCue WBC system for use in hospitals (e.g. emergency room), primary care settings and other care settings where quick WBC results are of value, given the excellent precision and safety this system offers.

The evaluation brings out a couple of issues we would like to address:
Hematology comparison studies are complex and challenging to perform accurately. The stability of blood samples are limited, the break down on certain cells starts already immediately after sampling and it is therefore extremely important to analyze samples with both methods as soon as possible. In this SKUP study, the HemoCue WBC system fulfills all the quality goals for venous samples. The primary care centers samples however, show a slight bias which is probably related to the differences in the elapsed time between sample collection and measurements, causing a more negative false bias compared to the hospital laboratory results, but still fulfills the requirements (see section 4.5.1).

Calibration difference on cell counters is common. The HemoCue WBC system has a traceability to manual microscope counting and the calibration level is very equal to different Sysmex instruments. In this SKUP study the comparison method (ADVIA) has a bias, a relatively big variation and drift, which is evident in the EQUALIS EQA scheme (see attachment 2, table 3).

Hematology parameters are also affected by biological factors such as body position, body activity, stress etc and taking samples from different sites (venous vs. capillary) further increases the variation if different sample material are used for the two methods. According to our knowledge, there are no established quality goals for comparison between capillary and venous results. In this SKUP study at the primary care centers, the capillary samples analyzed with the HemoCue system were compared with venous samples analyzed with the comparison method, against HemoCue's recommendation. None of the biological pre-analytical factors mentioned above were taking into consideration and the same strict quality goals were applied as for split samples.

The inability to fulfill all the quality goals for capillary samples used in the primary care centers, is a combination of instability of venous samples, calibration bias between ADVIA and HemoCue WBC together with the biological pre-analytical factors comparing venous and capillary sampling. Those factors are not only valid for HemoCue WBC, but for all instruments using capillary samples for measuring leukocytes in blood. SKUP does acknowledge this issue.

In order to demonstrate how well the HemoCue WBC system performs with capillary blood, when reducing pre-analytical factors on the comparison method, see references noted 1) and 2) below. Please contact your HemoCue representative if you are interested in a copy of the below references, and/or if you have any additional questions.

1) J.R. Casey et al, A comparison of 2 white cell count devices to aid judicious antibiotics prescribing, Clinical Pediatrics Vol 48; No 3; April 2009; 291 -294.
2) HemoCue comparison vs Sysmex 1000i, HemoCue internal data.

Attachment 6. List of previous SKUP evaluations

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

SKUP evaluations from number 38 and further

Evaluation no.	Component	Instrument/testkit	Producer
SKUP/2009/75	Glucose	Contour	Bayer HealthCare
SKUP/2009/74	Glucose[1]	Accu-Chek Mobile	Roche Diagnostics
SKUP/2008/73	B–Leukocytes	HemoCue WBC	HemoCue AB
SKUP/2008/72	Glucose[1]	*Confidential*	
SKUP/2009/71	Glucose[1]	GlucoMen LX	A. Menarini Diagnostics
SKUP/2008/69*	Strep A	Diaquick Strep A test	Dialab GmbH
SKUP/2008/66	Glucose[1]	DANA DiabeCare IISG	SOOIL Developement co. Ltd
SKUP/2008/65	HbA1c	Afinion HbA1c	Axis-Shield PoC AS
SKUP/2007/64	Glucose[1]	FreeStyle Lite	Abbott Laboratories
SKUP/2007/63	Glucose[1]	*Confidential*	
SKUP/2007/62*	Strep A	QuikRead	Orion Diagnostica Oy
SKUP/2008/61	CRP	i-CHROMA	BodiTech Med. Inc.
SKUP/2007/60	Glucose[1]	*Confidential*	
SKUP/2007/59	Glucose[1]	Ascensia BREEZE2	Bayer HealthCare
SKUP/2006/58	HbA1c	*Confidential*	
SKUP/2007/57*	PT (INR)	Simple Simon PT	Zafena AB
SKUP/2007/56*	PT (INR)	*Confidential*	
SKUP/2007/55	PT (INR)	CoaguChek XS	Roche Diagnostics
SKUP/2007/54*	Mononucleosis	*Confidential*	
SKUP/2006/53*	Strep A	*Confidential*	
SKUP/2005/52*	Strep A	Clearview Exact Strep A Dipstick	Applied Biotech, Inc.
SKUP/2005/51*	Glucose[1]	FreeStyle	Abbott Laboratories
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 evaluations from number 1 — 37

Evaluation no.	Component	Instrument/test kit	Producer
SKUP/2004/37*	u-hCG	Quick response u-hCG	Wondsfo Biotech
SKUP/2004/36*	Strep A	Dtec Strep A testcard	UltiMed
SKUP/2004/35*	u-hCG	QuickVue u-hCG	Quidel Corporation
SKUP/2004/34*	u-hCG	RapidVue u-hCG	Quidel Corporation
SKUP/2004/33	PT (INR)	Hemochron Jr. Signature	ITC International Technidyne
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

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

[1] Including a user-evaluation among diabetes patients.

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

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