Swelab Alfa instrument

Clinical and performance test procedures

2021-01-28ThO

Purpose

The purpose of this document is to verify that the Swelab Alfa instrument from Boule Medical AB fulfils the quality demands as specified for the instrument in the Boule Medical quality documentation.

For basic calculations and instrument verification procedures, see Doc I-1040 as well as the referenced international standards.

This document refers to clinical and performance characteristics. For electrical testing refer to the certificates on the low voltage directive and the directive on EMC emission and immunity.

The Swelab Alfa instrument have been run on the reagents given below after calibration and control with the material indicated below.

Reagents/Controls:

Diluent:	Swelab Alfa Diluent
Lyse:	Swelab Alfa Lyse
Calibrator:	Boule Cal
Control:	Boule Con-Diff Normal
	Boule Con-Diff Low
	Boule Con-Diff High

Definitions

Clinical test procedure
A procedure using clinical samples (human blood) to test the performance of the reagents in a haematology analyzer in comparison to a haematology analyzer reference system.

Perfomance test procedure
A procedure using clinical samples (human blood), or solutions mimicking specific properties of blood samples, to test the performance of the reagents in a haematology analyzer or to test properties of the reagents of importance for function of the analysis.

Parameter
The recorded analytical result recorded for one type of blood cell after the completion of an analysis of a blood sample. For both test procedures the parameters are limited to the actually measured parameters (primary parameters) of RBC, MCV, HGB, PLT, WBC, lymphocytes and granulocytes. MPV and mid-cells are also directly recoded but are not investigated since MPV follows MCV and mid-cells is a small fraction of the WBC with known relatively poor correlation to e.g. monocytes.

Precision
The precision is defined as the coefficient of variation (CV %) on the repeated analysis of a blood sample within the normal range of analytical parameters.

Carry-over
The contamination from one sample to the following sample and expressed as % influence.

Correlation
Correlation between a measured parameter from the test system (instrument/reagent) and a reference system (standard) expressed by the correlation coefficient.

Linearity
Linearity is defined as the ability of an instrument to measure and present data, from samples with known and varied concentrations, so that the pair of data from test and reference measurements form a straight line when presented in a graph

Turbidity
Turbidity is defined as the influence of particles in the sample cuvette on a measured parameter with a photometric method (HGB).

Calibrator
Material with properties similar to a blood sample and with assigned parameter values that are traceable to reference methodology according to international quality standards. The calibrator is used to calibrate the instrument system.

Control
Material with properties similar to a blood sample and assigned parameter values linked to the calibrator. The control is used to monitor instrument performance and to demonstrate the continued integrity of the calibration status.

Tests

Test procedures for the instrument system are performed on a essential parameters to ensure that critical qualities of the instrument system are within the instrument specifications.

The Swelab Alfa system was calibrated and controlled according to the instrument manual as given in the following sections.

These studies are generally conducted in collaboration with external partners for the main clinical evaluation (clinical tests) and as in-house studies for the performance evaluations.

Linearity

The linearity is tested by the measurement of solutions (samples) with know but varied parameter concentrations covering as much a possible of the measurable range for the parameter in the Swelab Alfa instrument. The samples are prepared according to the following procedure. The method used is based on fit of a polynomial to a number of data points, the method used is described in EP6-A.[1]

The results are a part of the Technical File (Doc # 02242) for the Swelab Alfa system.

a) The sample with the highest concentration is tested using the Swelab Alfa instrument so as to be close to the upper measurable limit. The concentration does not have to be known on beforehand. The blood may have to be slightly manipulated e.g. for high RBC/HGB the sample can be enriched by centrifugation and for high PLT a trombocyte concentrate may be used.
b) A dilution series of at least 7 concentrations is prepared, including the highest concentration. The concentrations do not have to be at equidistance and the lowest concentration may be at zero (diluent).
c) The test is performed in duplicates for each concentration
d) The results are plotted using the actual measurement for each parameter against the calculated (or relative) concentration for each data pair.

Linearity RBC
Test Doc# 02242

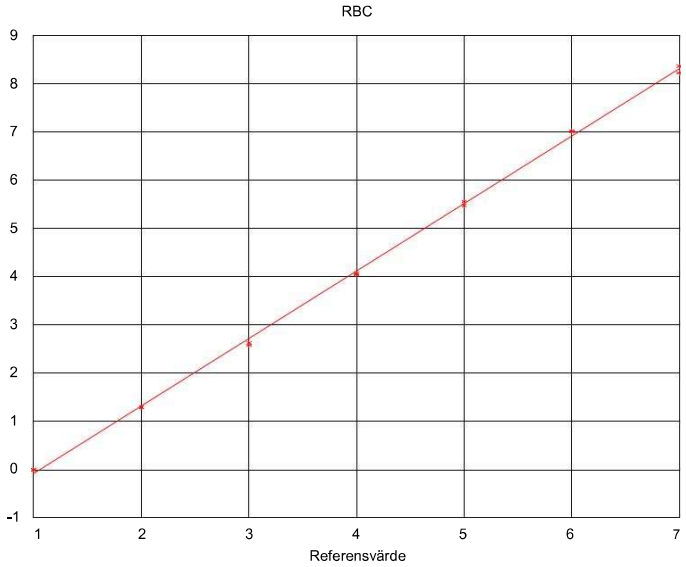

Figure 1. The linearity for RBC between reference (calculated value) and test (actual value).

[1] EP6-AEvaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, volume 23 of EP6-A, 2003. ISBN 1-56238-498-8.

Linearity HGB

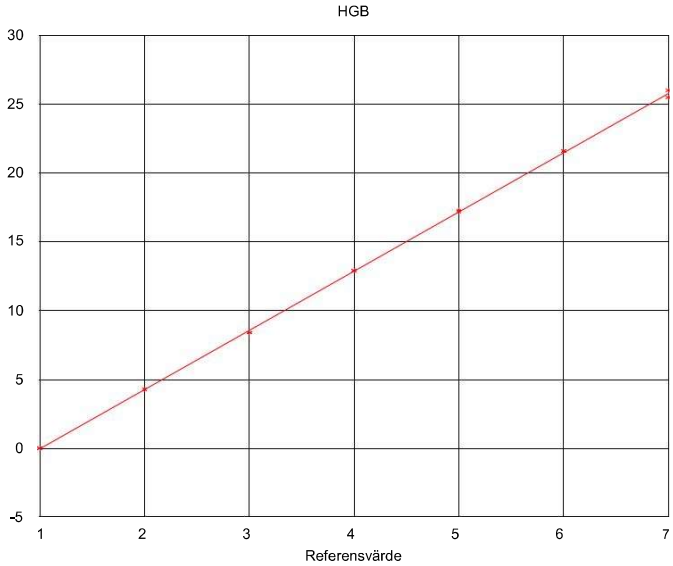

Figure 2. The linearity for HGB between reference (calculated value) and test (actual value).

Linearity PLT

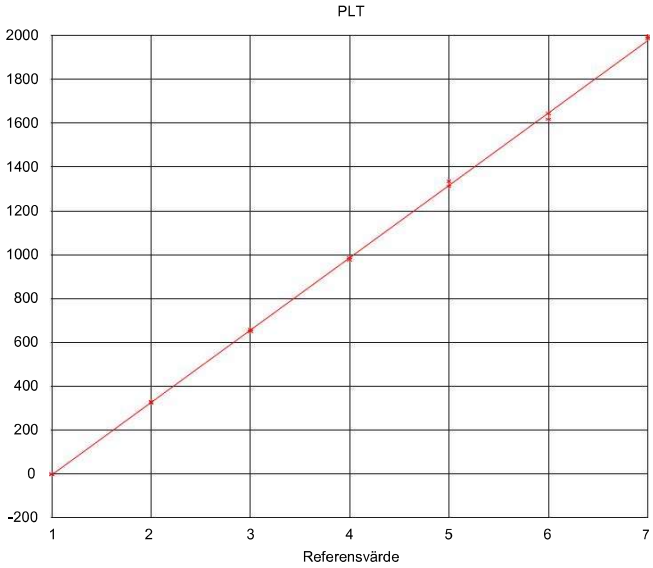

Figure 3. The linearity for PLT between reference (calculated value) and test (actual value)

Linearity WBC

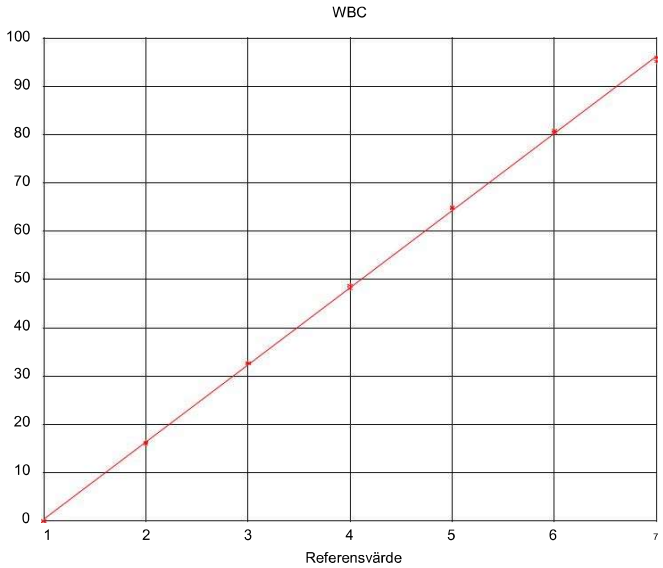

Figure 4. The linearity for WBC between reference (calculated value) and test (actual value).

Results linearity

Parameter	Range	Limit
RBC	0,00 – 7,00 x 10^{12}/L	Within ± 2%
HGB	0,0 – 25,0 g/dL	Within ± 3%
PLT	0 – 1800 x 10^{9}/L	--
WBC	0,0 – 80,0 x 10^{9}/L	Within ± 3%

Table 1Specification according to Doc # 00003

All parameters were found to be linear according to the above specification[2]

[2] Doc No 0003 rev 05 BM800 System Requirement Specification

Precision

The precision with the Swelab Alfa system, expressed as the $CV\%_{tot}$, is checked by repeat analysis of three different normal human blood sample against the instrument specification (n=10) according to the following procedure. The results are a part of the Technical File (Doc # 02242) for the Swelab Alfa system.

1) Repeat the testing of each human blood 10 times
2) Calculate the mean (X) and the standard deviation (SD) of each series
3) Calculate the precision of each run by the coefficient of variation (CV %) as (X/SD x 100).
4) Calculate a common CV% for all three series, $CV\%_{tot}$, :

$$CV\%_{tot} = \sqrt{\frac{CV\%_1^2 + CV\%_2^2 + CV\%_3^2}{3}}$$

5) Display the results ($CV\%_{tot}$) for RBC, MCV, PLT, HGB and WBC in comparison to the values specified for the Medonic M-series instrument.

Precision for Swelab Alfa

The below data is a part of the Swelab Alfa Technical File, the following material has been used in the evaluation.

Hematology Analyzer Swelab Alfa, part no 1400016
Diluting reagent Swelab Alfa Diluent, 20 L, part no 1504124
Lytic reagent Swelab Alfa Lyse 5 L, part no 1504125

Clinical samples
The evaluation is made from venous samples (EDTA tubes) from healthy test persons, i.e. having test results within normal range for the respective parameters. Samples in micro capillary (20µL) have been drawn from the EDTA sample tubes.

The evaluation is performed according to internal test method (I-1040) that, among other things, is based on parts of guidance from the Clinical and Laboratory Standards Institute (CSLI former NCCLS).

Results precision

The tabel below shows results using open-tube (OT) sample inlet respective micro capillary (via micro capillary inlet , MCI), $CV\%_{tot}$ calculated as shown above, using a 95% confidence interval.

Parameter	Precision OT		Precision MCI	
	(CV %)	Limit %	(CV %)	Limit %
RBC	1,6	≤ 1,8	2,1	≤ 2,7
MCV	1,3	≤ 1,5	0,7	≤ 1,5
PLT	4,1	≤ 5,2*	4,8	≤5,2*
HGB	1,2	≤ 1,5	1,7	≤ 2,4
WBC	2,7	≤ 3,5	2,6	≤ 3,6

Table 2. Doc # 02242 Precision (CV%) for Swelab Alfa system, humanblod * updated to agree with current instrument specification Doc # 0003 ed 12

The precision with the Swelab Alfa reagents was within the given limits for all the parameters.

HGB absorbance spectrum

The Swelab Alfa reagents are based on a cyanide free formulation to avoid potentially hazards with cyanide containing waste etc. The HGB determination is based on the relatively broad absorbance peak, around 535 nm, of HGB in complex with components from the lytic reagent. The emission spectrum of the LED is dependent on both the feed current and the temperature. The maximum emission is expected to occur around 540 nm for the conditions employed in the Boule instruments (e.g. I_F= 2 mA). Thus, there is a very good match with an absorbance maximum of 535 nm of the HGB. The results are a part of the Technical File (Doc # 02656) for the Swelab Alfa system.

Testing is done by measuring the absorbance spectrum in a reference spectrophotometer (glass cuvettes, 10 mm lightpath, 2 nm bandwidth) according to the following procedure.

a) Prepare both the test and reference cuvette with a 1:1 mixture of diluent and lyse
b) Add normal human blood to a dilution of 1:400 to the reference cuvette and mix
c) Initiate the recoding of the absorbance spectrum within 10 seconds from mixing
d) Display the spectrum between 500 and 600 nm

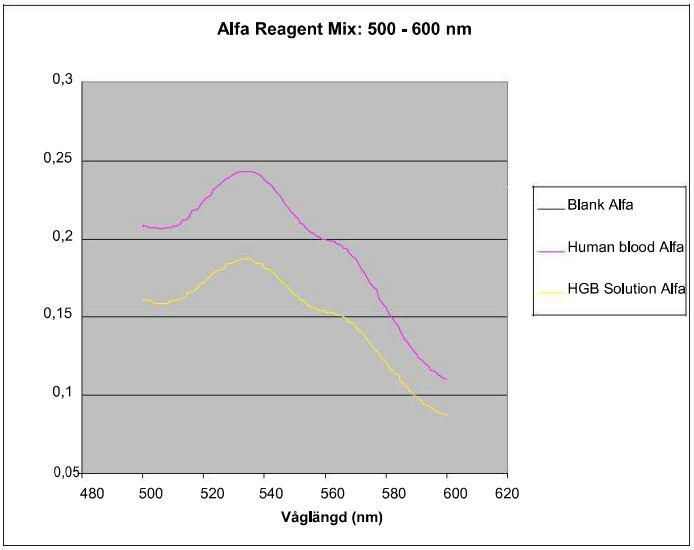

Figure 5. Absorbance spectrum HGB for the Swelab Alfa reagents using human blood (147 g/L) or purified HGB (only displayed for comparative purposes)

The absorbance peak corresponds very well with the filter/lamp combination.

Turbidity

The effect of turbidity within the HGB photometer is checked with latex particles with a volume within the range 100-400 fL (target 180 fL) so as to represent the WBC populations (e.g. granulocytes, lymphocytes) after lysing. Test should be done up to a particle concentration > 60 x 10^9 cells/L. The results are a part of the Technical File (Doc # 01720) for the Swelab Alfa system.

Testing is done in the Swelab Alfa instrument by the following procedure (see also I-1040).

a) Prepare a base solution of latex particles in diluent at a calculated concentration > 60 x 10^9 cells/L.
b) Make an analysis of the solution in the pre-dilute inlet so as to ensure that the WBC (particle) concentration is sufficient
c) Make a serial dilution of the base so as to represent 75, 50 and 25 % of the original content. The content of each solution should be enough for three assays
d) Run the solutions as samples through the pre-dilute inlet and record WBC and HGB values.
e) Repeat the procedure three times and calculate the mean (mean values given in the table below).

WBC value	74.7	55.6	37.4	18.7
HGB recorded	0.3	0.2	0.1	0.0

Table 3 Results from turbidity test

The instrument specifications state that the interference from particles (WBC) shell be < 0.5 g/dl at a level up to 60 x 10^9 cells/L. The results were within specifications.

Carry-over

The carry over is checked by determination of the difference by a low sample and the same low sample run immediately after a high sample for RBC and WBC. For reagents the following procedure is done

Test method is described in I-1040, the definition of carry over according to the below formula is identical to the one published in an article in ICS94, Appendix 4[3].

a) Run the high sample three times
b) Run the low sample three times
c) Calculate the difference between the first low sample and the last low sample (l_1-l_3)
d) Calculate the difference between the last high sample and the last low sample (h_3-l_3)
e) Calculate the % carry-over as:

$$carry-over\% = h^{1} \frac{l-l}{-l} {}^{3} {}_{3} * 100\% \leq CO_{\max}\ \%$$

f) Repeat 3 times, highest value reported as carry-over.

Carry-over för Swelab Alfa

The below data is a part of the Swelab Alfa Technical File, the following material has been used in the evaluation.

Hematology Analyzer Swelab Alfa, part no 1400016
Diluting reagent Swelab Alfa Diluent, 20 L, part no 1504124
Lytic reagent Swelab Alfa Lyse 5 L, part no 1504125

Samples

The test was performed using Boule Con-Diff High and Boule Con-Diff Low.

The evaluation is performed according to internal test method (I-1040) that, among other things, is based on parts of guidance from International Council for Standardization in Haematology (ICSH).

[3] ICSH. Guidelines for the evaluation of blood cell analyzer… *Clinical and laboratory haematology, (16):157-174, 1994.*

Result carry-over

The table below shows results from open tube inlet (OT), micro capillary (via micro capillary inlet, MCI) and pre-diluted sample (PD).

Carry-over	Inlet	RBC	PLT	HGB	WBC	Results
Limit CO%	*OT*	*1*	*2*	*1*	*1*	
Alfa 10103	OT	0.5	1.2	0.3	0.3	OK
Limit CO%	*MCI*	*2*	*3.5*	*2*	*2*	
Alfa 10103	MCI	1.0	1.0	0.7	0.3	
Limit CO%	*PD*	*2*	*3.5*	*2*	*2*	
Alfa 10040	PD	1.5	3.0	1.3	1.4	OK

Table 4. Specification according to Doc # 00003.
Results from Doc # 02242 Carry over (%) for Swelab Alfa

The carry over was confirmed to be within the instrument specifications.

Calibrators and Control

General

The purpose of this section is to define a secure and practical method to calibrate and control the Swelab Alfa instrument during normal operation.

Introduction

Within hematology a general problem is found in standardizing the calibration method(s). Because of obvious reasons, a standard as known within the clinical-chemistry field is not possible as the number of cells cannot be standardized. Blood controls are therefore called "controls" and not "standards".

A general control of the used analyzer should therefore be preformed in comparison with the well known and established microscope method. However, in such a case, the comparison will be completely dependent on the skill of the microscope operator. This is, however, the only reliable method in checking automatic hematology analyzers.

To simplify the calibration procedure at the end-user, certain hematology parameters can be determined with reasonable accuracy by using commercial calibrators with assigned values traceable to reference methods. Traceability matrix for Boule Cal is described in Doc no 2246[4].

Limitations

WBC differentials

Cell differentials cannot be standardized as white blood cell cannot act as real white blood cells in a control blood. As the cells within a commercial control are fixed, there is no check whether the reagents used are reliable or not.

RDW

The Red Cell Distribution Width (RDW) is one of the most sensitive parameters with hematology. Prolonged waiting time as well as high temperatures of the specimen will heavily effect this parameter. As the RDW is a not common defined parameter (differences between different brands/analyzers), this parameter is not "standardized" within this document. However, most of the commercial available analyzers today, as well as Swelab Alfa, are based upon a CV analysis of a portion of the RBC size distribution and the RDW is therefore expressed in %.

Calibration procedure

The following describes in short the calibration process, detailed description is found in the User's Manual, section 7[5]

- Always use calibrator having values assigned to the instrument you are using, e.g. Boule Cal
- Handle and prepare the calibrator in accordance to calibrator package insert.

[4] Doc No 2246 Certificate of Compliance
[5] Swelab Alfa User's Manual part no 1504170 November 2006

- In the Swelab Alfa instrument the following parameters can be calibrated:
  RBC, MCV, PLT, MPV, HGB and WBC. It is not recommended that the end-user change the preset calibration factors for RDW%, RDWa, and PDW.
- Use the installed barcode reader to scan the Calibrator ID from the calibrator label
- To perform calibration, it is recommended that five calibration analysis be performed in consecutive order
- Calibration analysis must be the last analysis performed on instrument for parameter value to be shown in calibration menus
- Scroll through parameter screen and verify that the CVs for the following parameters are within limits: RBC, MCV, PLT, HGB, WBC. If CV values are not within range operator will be unable to perform calibration
- Calibration can be performed in three ways:
  1. automatic calculation of factors by using the [USE CAL] button
  2. enter target value from calibrator
  3. manually calculate and enter calibration factors
- It is recommended to run controls after calibration to verify that all parameters have been calibrated correctly

Control samples

It is advisable that the performance of the Swelab Alfa system is checked daily with certified blood controls authorized by Boule, e.g. Boule Con-Diff or Boule Con. Comparing the analyzer results to the known values on the Boule control assay sheet is a good assurance that the system is functioning properly.

- Handle and prepare controls in accordance to control package insert.
- Never use an open vial longer than recommended by the manufacturer or subject any vial to excessive heat or agitation.
- Wipe the aspiration needle with a clean, dry tissue before each control run. Not following this discipline might lead to decreasing parameter values

Clinical tests

The performance of the test system (Swelab Alfa system) is compared against a reference method. The Swelab Alfa system, including the Alfa reagents, was calibrated and controlled according to the instrument manual.

The study is generally conducted by an external partner, but could also be done as an in-house study.

Clinical Study # 1, Swelab Alfa vs ADVIA

Introduction

The first study was done to ensure that the linearity and correlation agreed with the results from an independent haematology system. The results are a part of the Technical File (Doc # 01919) for the Swelab Alfa system.

The evaluation was performed as given below.

Clinical samples

The evaluation has been done in collaboration with The Karolinska University Hospital, Dept. Clinical Chemistry with reference values from a Bayer Advia 120 system, and according to the standard SS-EN 13612 for compliance with the demands in the European IVD-directive (98/79/EC).

The correlation studies are based on in total 247 samples taken randomly from the normal routine. Reference values have been available to varying extent depending on the studied parameter (number of complete data pairs (n) are given in the figures). The analysis has been performed in the open tube (OT) mode for the test system and the assay has been completed within 4 hours from the analysis in the reference system.

Result of the correlation studies

Erythrocytes, concentration (red blood cells, RBC)

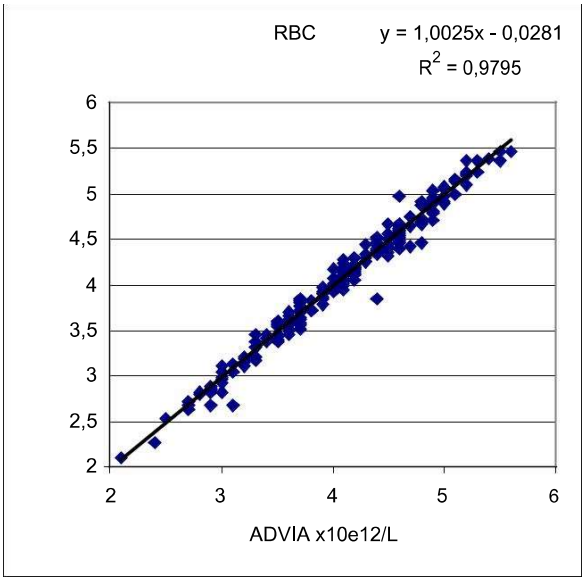

Figure 6. Correlation RBC between test system and reference system (n=219).

Erythrocytes, mean cell volume (MCV)

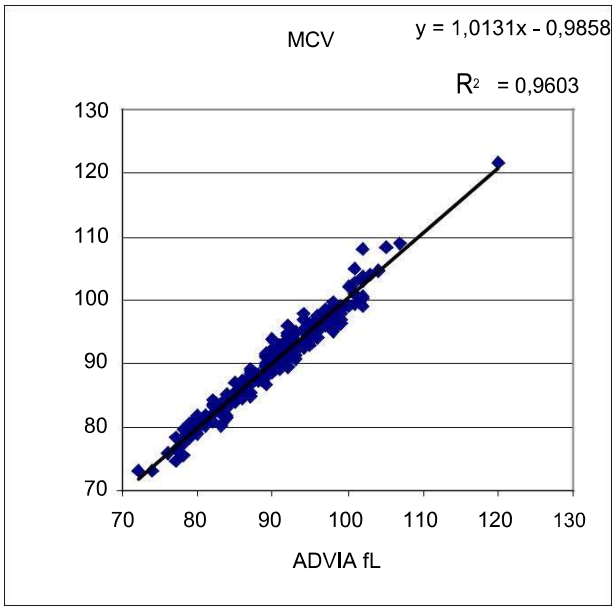

Figure 7. Correlation MCV between test system and reference system (n=218)

Thrombocytes, concentration (platelets, PLT)

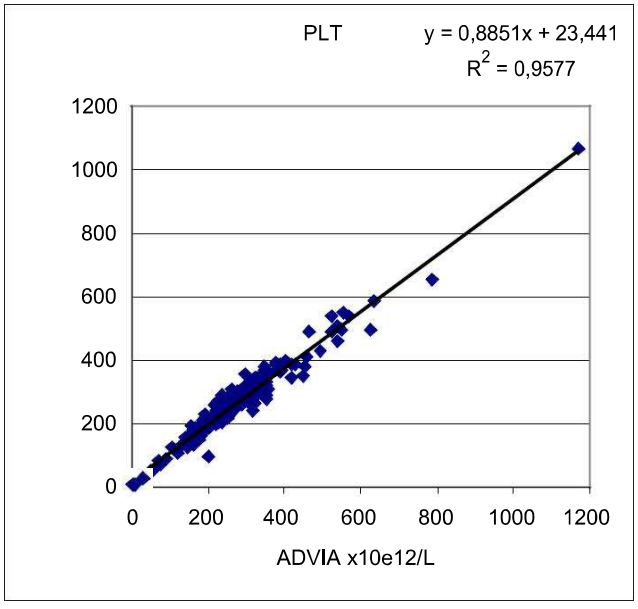

Figure 8. Correlation PLT between test system and reference system (n=219) .

Hemoglobin, concentration (HGB)

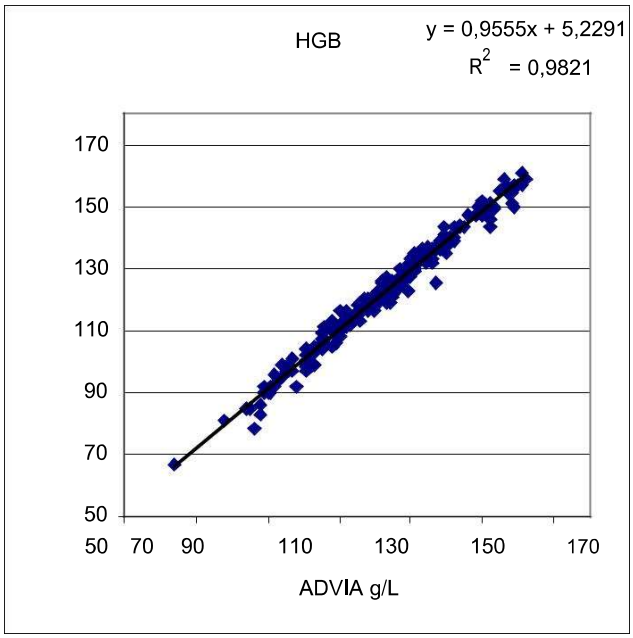

Figure 9. Correlation HGB between test system and reference system (n=236) .

Leucocytes, concentration (white blood cells, WBC)

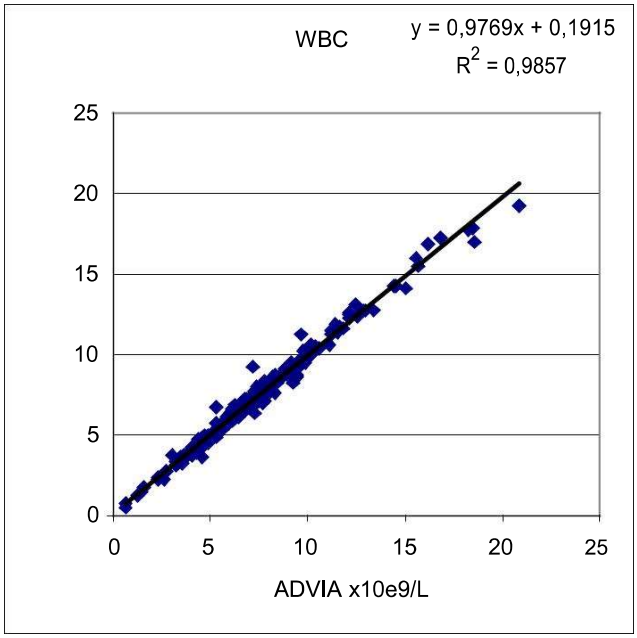

Figure 10. Correlation WBC between test system and reference system (n=483) [1)].

Lymphocytes, concentration (Lymph)

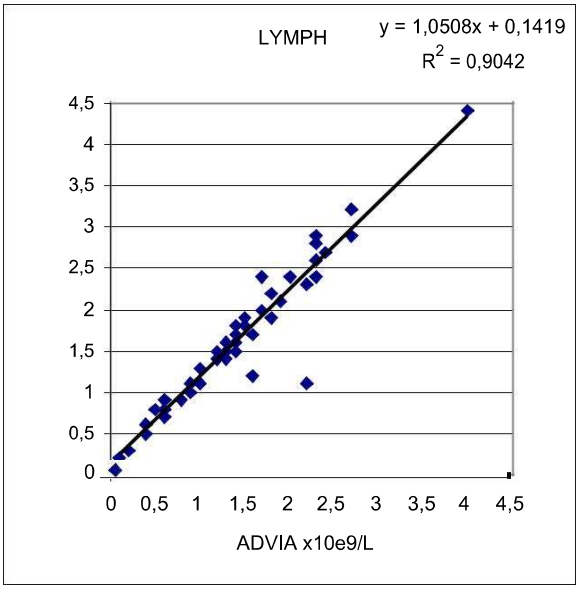

Figure 11. Correlation lymphocytes between test system and reference system (n=49) [1)].

Granulocytes, concentration (Gran)

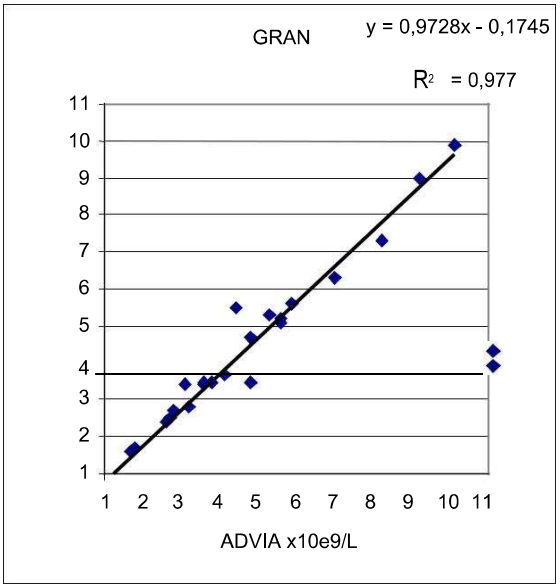

Figure 12. Correlation granulocytes between test system and reference system (n=49) [1)]. Neutrophilic, basophilic and eosinophilic cells have been summarized as granulocytes for the Advia results.

Additional notes to the results:

As a conclusion the correlation results were excellent meeting the specifications for linear correlations (R) to the reference system (Advia) as given below.

	RBC	**MCV**	**PLT**	**HGB**	**WBC**
Study	0.99	0.98	0.98	0.99	0.99
Specification	≥ 0.98	≥ 0.98	≥ 0.95	≥ 0.98	≥ 0.97

Table 5. Specification from Doc # 00003, R calculated from given - R^2 values.

Clinical Study # 2, Swelab Alfa vs ADVIA

Introduction

The second study was done to ensure that the linearity and correlation agreed with the results from an independent haematology system using an alternative operator (and to complement with additional lymphocyte and granulocyte values). The results are a part of the technical File (Doc # 02279) for the Swelab Alfa system.

The evaluation was performed as given below.

Clinical samples

The evaluation has been done in collaboration with The Karolinska University Hospital, Dept. Clinical Chemistry with reference values from a Bayer Advia 120 system.

The correlation studies are based on in total 100 samples taken randomly from the normal routine. Reference values have been available to varying extent depending on the studied parameter (number of complete data pairs (n) are given in the figures). The analysis has been performed in the open tube (OT) mode for the test system and the assay has been completed within 6 hours from the analysis in the reference system.

Result of the correlation studies

Erythrocytes, concentration (red blood cells, RBC)

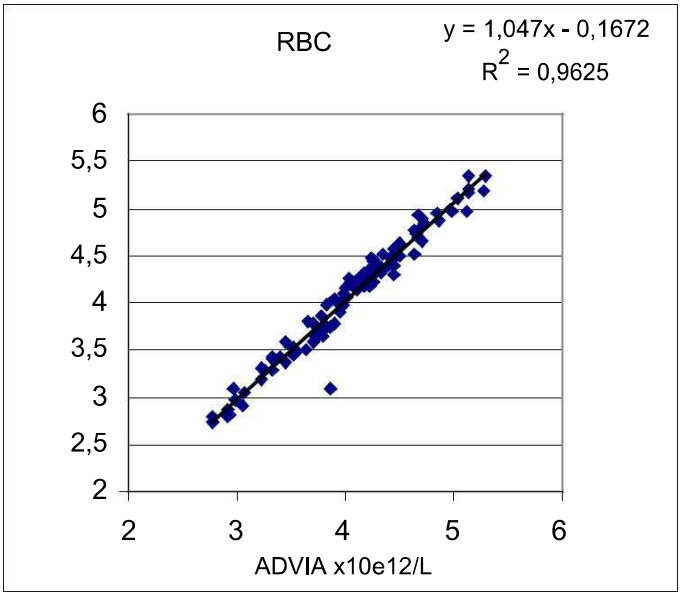

Figure 13. Correlation RBC between test system and reference system (n=8100).

Erythrocytes, mean cell volume (MCV)

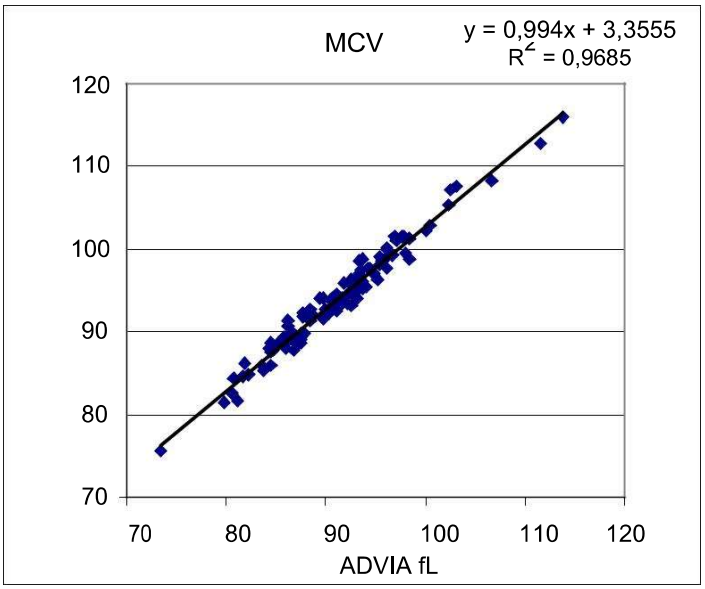

Figure 14. Correlation MCV between test system and reference system (n=100).

Thrombocytes, concentration (platelets, PLT)

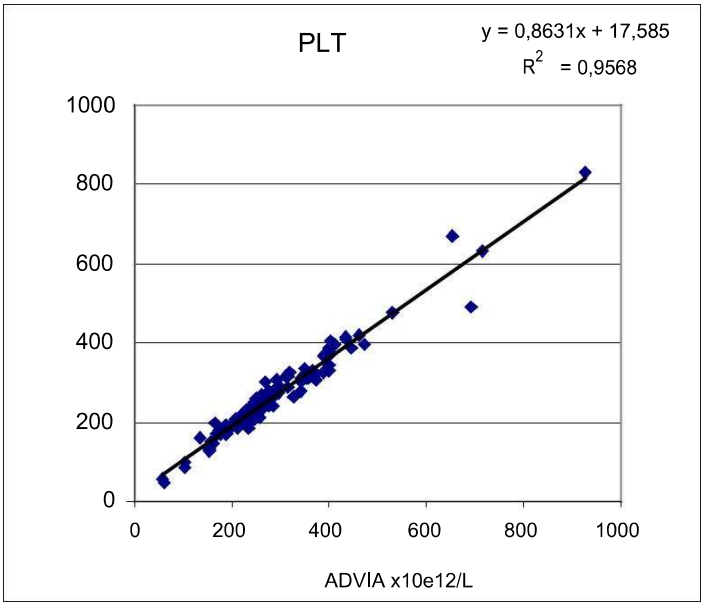

Figure 15. Correlation PLT between test system and reference system (n=100).

Hemoglobin, concentration (HGB)

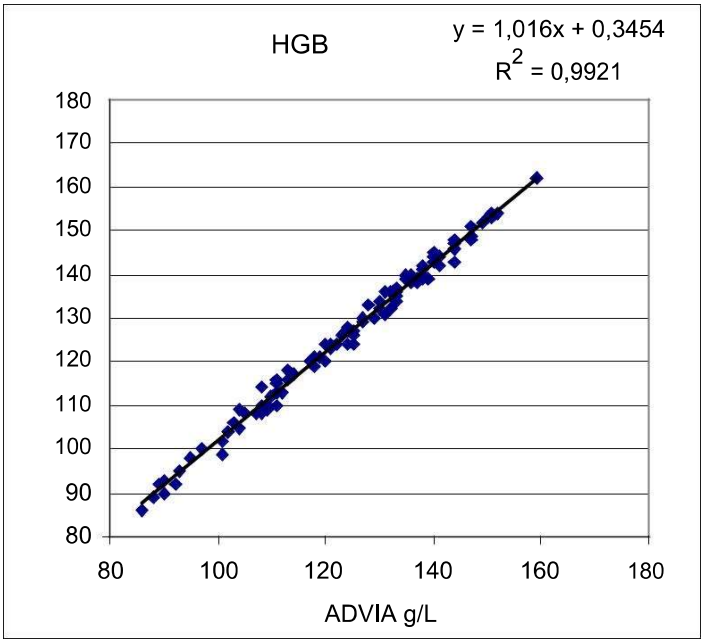

Figure 16. Correlation HGB between test system and reference system (n=100).

Leucocytes, concentration (white blood cells, WBC)

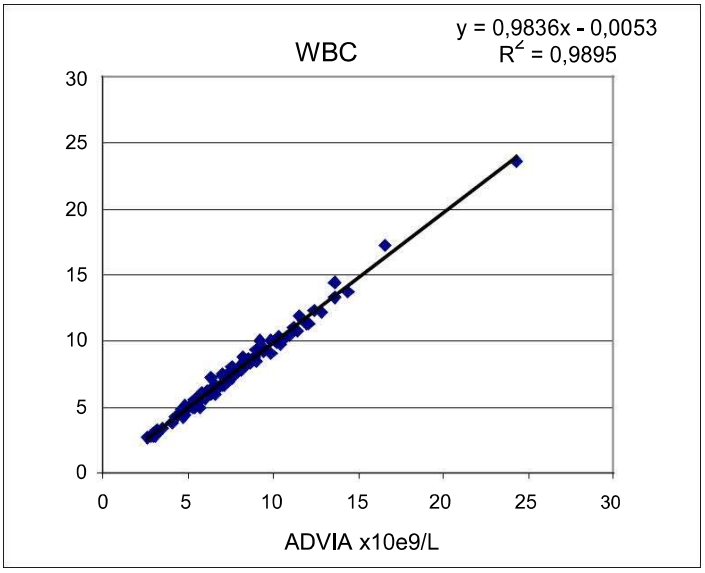

Figure 17. Correlation WBC between test system and reference system (n=100).

Lymphocytes, concentration (Lymph)

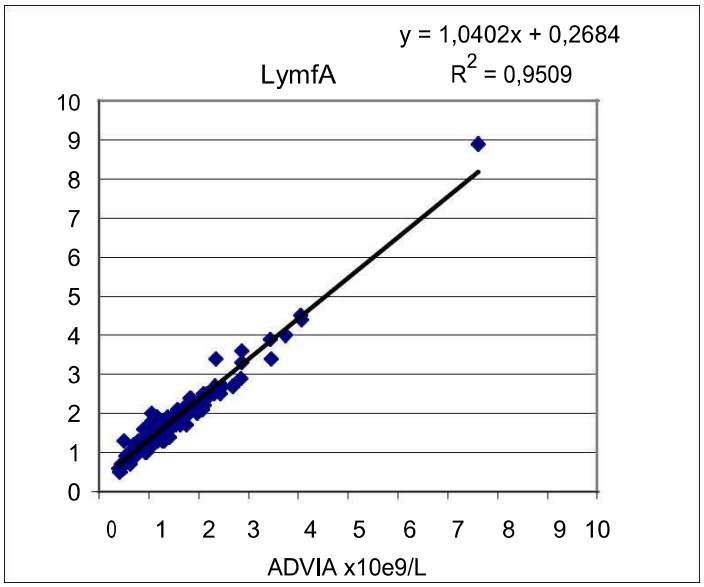

Figure 18. Correlation lymphocytes between test system and reference system (n=100).

Granulocytes, concentration (Gran)

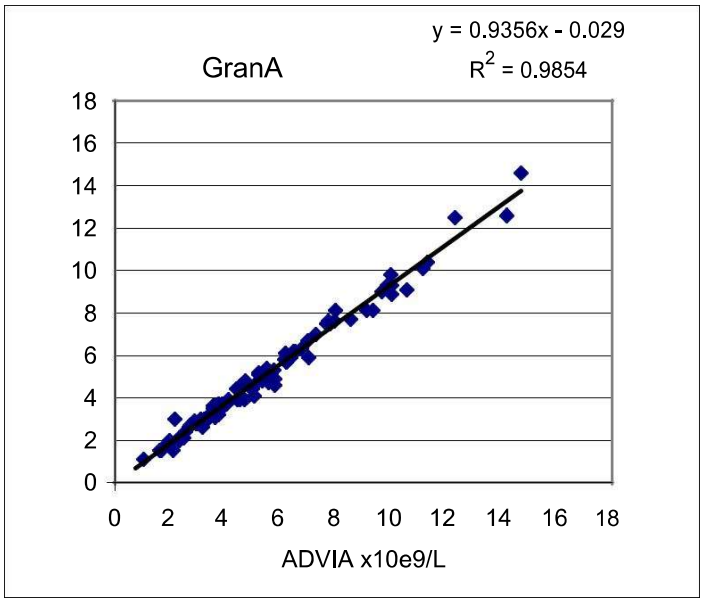

Figure 19. Correlation granulocytes between test system and reference system (n=100). Neutrophilic, basophilic and eosinophilic cells have been summarized as granulocytes for the Advia results.

Additional notes to the results

1) One sample gave a significantly lower RBC result with the Swelab Alfa in comparison to the ADVIA reference. This sample was tested also on two other Alfa instruments with similar results. Thus, partial hemolysis during transport etc may have affected results in comparison to the original reference measurement.
2) Two sample gave a significantly different PLT results with the Swelab Alfa in comparison to the ADVIA reference. One sample had a PLT:DE flag. Both samples were tested also on two other Alfa instruments with similar results. Thus, changes during transport etc may have affected results in comparison to the original reference measurement
3) 3) The correlation between the test system (Swelab Alfa) and the reference system (ADVIA) was excellent in all other respects.