Evaluation of haematology analyzer CELL-DYN 3700 SL

Enver Suljevi}, Muhamed Fazli}, Jozo]ori} i Emina Kiseljakovi}
Institute for Clinical Chemistry and Biochemistry, Clinical Centre of University of Sarajevo
Institute for Physiology and Biochemistry, Department of Biochemistry, Faculty of Medicine, Sarajevo

Abstract

Research on the parameters of full blood count and differential white blood count is included in the program of all medical laboratories of primary, secondary and tertiary health care levels. Today, all haematological tests are exclusively performed on the haematology analyzers. Automation of haematology laboratories is a result of the huge requires for haematological test performing, timely issuing of the haematological findings, and possibility of the usage of modern techniques.

This work is an evaluation of laser haematology analyzer Cell-Dyn 3700 SL. It investigates the reliability of test results throughout the following parameters: precision, accuracy, sensitivity and specificity of determination methods. It also explores the influence of sample transferring and correlation with haematology analyzer MAXM Retti. Haematology parameters that have been investigated are: white blood cell (WBC), neutrophils (NEU), lymphocytes (LXM), monocytes (MONO), eosinophils (EOS), basophils (BASO), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCHC) red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), plateletocrit (PCT), and platelet distribution width (PDW).

The results confirms that precision of analyzer fulfils the reproducibility of testing parameters: WBC, RBC, HGB, MCV, MCH, MCHC, and PLT. Correlation coefficient values (r) gained throughout the statistical analysis, that is linear regression results obtained throughout the comparison of two analyzers are adequate except for MCHC (r = 0.64), what is in accordance with literature data.

Accuracy is tested by haematology analyzer method and microscopic differentiating method. Correlation coefficient results for granulocytes, lymphocytes and monocytes point the accuracy of methods. Sensitivity and specificity parameters fulfil the analytical criteria.
It is confirmed that haematology analyzer Cell-Dyn 3700 SL is reliable for the determination of full blood count in everyday work. Analyzer and its program for differential white blood count can be used for the research and separation of normal and pathological blood counts with addition of microscopic methods confirming distribution or morphologic changes of leukocytes.

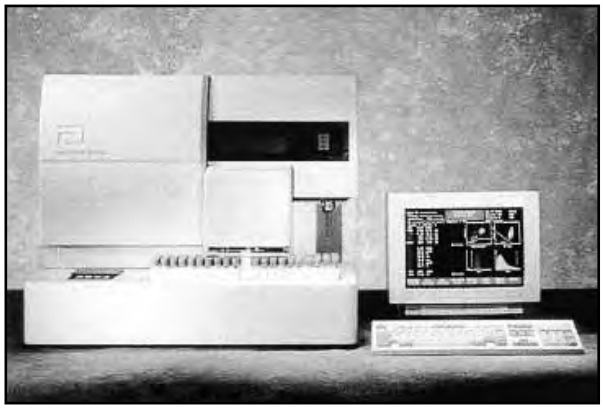
Introduction

Laboratory investigation of the count, concentration and relative relation of haematological parameters is extremely important in clinical and other researches. Determination of the full blood count and differential white blood count is included in the program of all medical laboratories of primary, secondary, and tertiary health care levels (1).
Manual techniques have been performed in haematological laboratories for a long time. These techniques are slow and strenuous while their subjectively based test estimation and work precision like white blood cells count on haemocytometer are not on the acceptable level. Today, haematological tests are being performed on haematology analyzers. Started in 1960's, automation in haematology laboratories has been developed very fast during the last 20 years. The reasons for that are increasing demands established in haematological laboratories; timely issuing of the large number of haematological findings and attaining a high-level of the accuracy and precision in laboratory work.
Microprocessors and computers, as integral part of haematological counter, induced a sudden development of automation and an expansion of different types of haematological tests (2). New generation of haematology analyzers enable fast and reliable obtaining of the full blood count data and differential white blood count information screening. Data on abnormalities in distribution, that is changes in relations between normal leukocyte types helps in diagnosing and following up of different disorders such as haematological and infectious disease processes.

The basic goal of determination of the differential white blood count on haematology analyzer is a decrement in usage of manual microscopic differentiation in routine work. The usage of instruments provides a proper estimation of whether there is a normal or susceptive pathological differential white blood count, which needs to be accurately examined later on.

Cell-Dyn 3700 SL is haematology analyzer manufactured by Abbott-USA. It measures 17 blood parameters including differential white blood count. This work investigates the precision, accuracy, sample transferring, sensitivity, and specificity of haematology analyzer Cell-Dyn 3700 SL and evaluates its usage and reliability in everyday work.

Figure 1 Haematology analyzer CELL-DYN 3700 SL



Material and methods

Instrument

Haematology analyzer Cell-Dyn 3700 SL measures 17 blood parameters at the same time: white blood cell (WBC), number and percentage of neutrophils (NEU), number and percentage of lymphocytes (LXM), number and percentage of monocytes (MONO), number and percentage of eosinophils (EOS), number and percentage of basophils (BASO), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCHC) red cell distribution width (RDW), platelet (PLT), mean platelet volume (MPV), plateletocrit (PCT), platelet distribution width (PDW).

Analyzer works according to two principles - volumetric impedance and optical detection. There are 4 independent measurements: leukocyte counting and differentiation performed by optical principle (diffraction light usage), leukocyte counting performed by impedance principle in the current canal (measurement of single cell electrical resistance), erythrocyte and platelet counting performed in the independent canal and haemoglobin measurement performed by spectrophotometer. White blood cells (WBC), red blood cells (RBC), haemoglobin (HGB), and platelets (PLT) are measured directly, but other parameters (HTC, MCV, MCH, MCHC, RDW, and PDW) are calculated automatically from the measured parameter data. Haemoglobin is measured by spectrophotometer according to cyanmethemoglobin method at wavelength of 540 nm.
Principle of the five-parts differential white blood count is based on the erythrocyte membrane lysis done by haemolysing reagents and followed by the differentiation of leukocyte types (NEU, LYM, MONO, EOS and BASO) according to size of the cell or nucleus. These leukocyte types are detected by peaks and size of histograms and are distributed into 5 groups.

For the full blood cell count analysis on Cell-Dyn 3700 SL on Open Mode procedure it's necessary to collect 130 L of the full blood while 240 L of the full blood for Close Mode procedure. Apparatus receives previously mixed blood and during the routine analyzing procedure is possible to analyze an urgent sample, as well. These operations are completely automatic. Ten stands with ten test tubes of blood each - 100 samples in each series can be placed in the porter (mixer) of samples. Instrument can store up to 10 000 sample (patient) data. Besides numerical data, analyzer provides histograms of the leukocyte, erythrocyte and platelet distribution. Its informative system has a quality control program that provides monitoring process of the apparatus accuracy and reproducibility in everyday work (4).

Chemicals

The original regent sets and control blood from manufacturer Abbott are used:

- Diluent Cell-Dyn Reagent, 99231,
- Sheath Reagent, 99311,
- Detergent Cell-Dyn Dif Screen Reagent, 99321,
- Lyse Reagent, 99431,
- Control Blood, Tri Level Control, 22PA.

Blood sample

Venous blood of the patients from Clinics of Clinical Centre of University of Sarajevo, (N= 219) have been sampled by vacutainer blood tubes (Becton Dickenson Vacutainer Systems) of 3.0 mL containing K3EDTA as an anticoagulant (5). All samples have been collected from ambulance patients and patients with different diseases hospitalised in Clinical Centre. Samples have been stored at room temperature.

Precision

The precision inside series has been tested by consecutive measurements (N=25) of the same blood sample according to haematological parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT.
The precision between series for the same parameters has been tested by analysing of the series of samples (N =20) during the different time periods. First series of samples has been tested after the blood collection and 2 hours later, second series has been tested after the blood collection and 5 hours later, and third series has been tested after the blood collection and 10 hours later.

Sample transferring

Sample transferring has been performed according to method introduced by Broughton and associates (3). A

sample with high level of analytes has been consequently measured for three times (a1, a2, a3) and after that a sample with low level of analytes has been consequently measured for three times (b1, b2, b3). Percentage of the transferring of each sample analyte has been calculated by formula:

$$\frac{b_1 \times b_3}{a_3 \times b_3} \times 100$$

Accuracy

The accuracy of measurements has been tested by parallel measuring of 65 full blood samples according to haematological parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT on haematology analyzers Cell-Dyn 3700 SL Abbott and MAXM Retti Coulter. Both analyzers have been previously calibrated by the same universal calibrator and checked by the same control.
Parallel measuring of 93 blood samples on haematology analyzer Cell-Dyn 3700 SL Abbott and manual microscopic leukocyte differentiating has been performed in order to investigate the accuracy of measurements of the differential white blood count parameters. Microscopic differentiating of blood smear coloured by Pappenheim method has been performed on 100 cells (6).

Sensitivity and specificity

Sensitivity and specificity of haematology analyzer Cell-Dyn 3700 SL have been tested by comparison of the differential white blood count results (N=93) gained on analyzer and throughout microscopic differentiating.

Statistics

Results have been statistically evaluated and expressed by means of standard deviation (SD), mean value (X), and coefficient of variation (CV). Congruency of results has been investigated by analysis of the linear regression and expressed as a coefficient of correlation (r).

Results and discussion

Precision testing

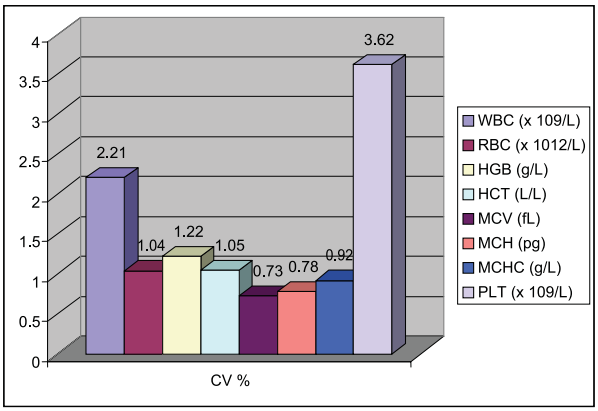
Measurement of the the same sample (N=25) according to haematological parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT has been performed for the precision testing. Mean value (X), standard deviation (SD), and coefficient of variation (CV) have been calculated for every parameter. Statistical parameters are shown in Table 1.

Table 1 Results of precision testing

Parameter	$\bar{X}$	SD	CV %
WBC (x 10^9/L)	8. 17	0.18	2.21
RBC (x 10^{12}/L)	5. 47	0.05	1.04
HGB (g/L)	162	1.84	1.22
HCT (L/L)	0.46	0.01	1.05
MCV (fL)	83.9	0.53	0.73
MCH (pg)	29.6	0.22	0.78
MCHC (g/L)	353	3.10	0.92
PLT (x 10^9/L)	26 6	9.70	3.62

Coefficient of variation data ranging from 0.73 for MCV to 3.62 for PLT points the acceptable reproducibility for every tested parameter. They are within the span recommended by Cell-Dyn 3700 SL manufacturer. Figure 2 presents a graphic survey of the coefficient of variation values for the tested parameters.

Figure 2 Graphic survey of the coefficient of variation values for the tested parameters



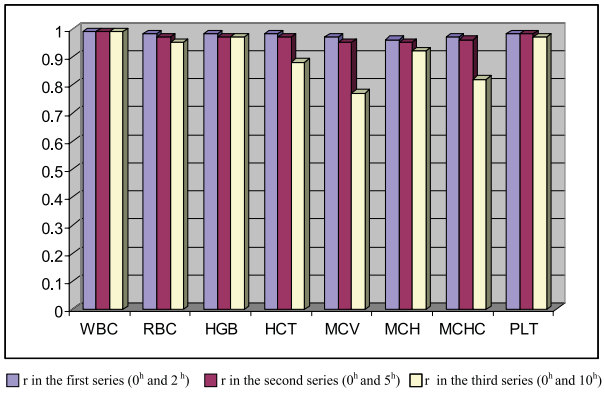
Precision testing results between three series (N=20) for parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT are expressed as a coefficient of correlation (r) and shown in Table 2. Graphic survey of these parameters is presented in Figure 3.

All coefficient of correlation-results for tested parameters (in the function of time - 0h and 2h, 0h and 5h and 0h and 10h) are acceptable except for MCV (r = 0.77), probably as a consequence of erythrocyte swelling after the sample 10-hours storage at room temperature.

Table 2 Precision testing results - coefficient of correlation (r) between series

Time	WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT
0^h and 2^h	0.99	0.98	0.98	0.98	0.97	0.96	0.97	0.98
0^h and 5^h	0.99	0.97	0.97	0.97	0.95	0.95	0.96	0.98
0^h and 10^h	0.99	0.95	0.97	0.88	0.77	0.92	0.82	0.97

Figure 3 Graphic survey of precision - coefficient of correlation (r) between series



Sample transferring testing

The results of the sample transferring influences during the continuous measurement of haematological parameters WBC, RBC, HGB, PLT, GR and LYM are shown in Table 3.
Percentage data on sample transferring are below 1% what means that sample transferring is insignificant for all tested parameters.

Accuracy testing

For accuracy testing of the blood count parameters, 65 full blood samples have been simultaneously tested on Cell-Dyn 3700 SL and MAXM Retti analyzers. Correlation coefficient values (r) obtained throughout statistical analysis and linear regression comparative results (between two analyzers) are shown in Figure 4.

Correlation coefficient values for the tested parameters are satisfactory except for MCHC (r = 0.64), what is in accordance with literature data (7). Low correlation coefficient values for MCHC (up to r = 0,15(3)) could be found in numerous scientific publications. Authors have utilized different haematology counters and analyzers. Some authors explained MCHC low results as a consequence of the non-existence of two identical analyzers with equal measurement fissures resulting with the different values of indirect, calculated parameters. It is important to mention that correct calculation of the MCHC value requires values of erythrocyte number, haemoglobin concentration, and haematocrit result.

For accuracy testing of the differential white blood count parameters, 93 full blood samples have been tested on Cell-Dyn 3700 SL analyzer and by microscopic differentiating method. In this case, we have statistically processed three leukocyte series: absolute number of granulocytes (NEU + EOS + BASO), lymphocytes and monocytes (because we have considered that 93 blood samples are not sufficient for the statistical calculation of five-part differential white blood count (NEU, LYM, MONO, EOS and BASO). Linear regression two-method analysis for three, above-mentioned parameters is shown in Figure 5.

Linear regression data have acceptable correlation coefficient values for granulocytes (r = 0.977), lymphocytes (r = 0.973) and monocytes (r = 869), what is in accordance with literature data(8).

Table 3 The sensitivity and specificity of CEA determinations in breast cancer patients.

Parameter	High level	Low level	Transferring (%)
WBC (x 10^9/L)	13.7	3.05	0.91
RBC (x 10^{12}/L)	6.54	1.80	0.64
HGB (g/L)	195	34	0.85
PLT (x 10^9/L)	806	59	0.55
GR (%)	83	10	0.41
LYM (%)	57	6	0.26

Figure 4 linear regression comparative results between two analyzers Cell Dyn 3700 SL and MAXM Retti according to parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC, and PLT.

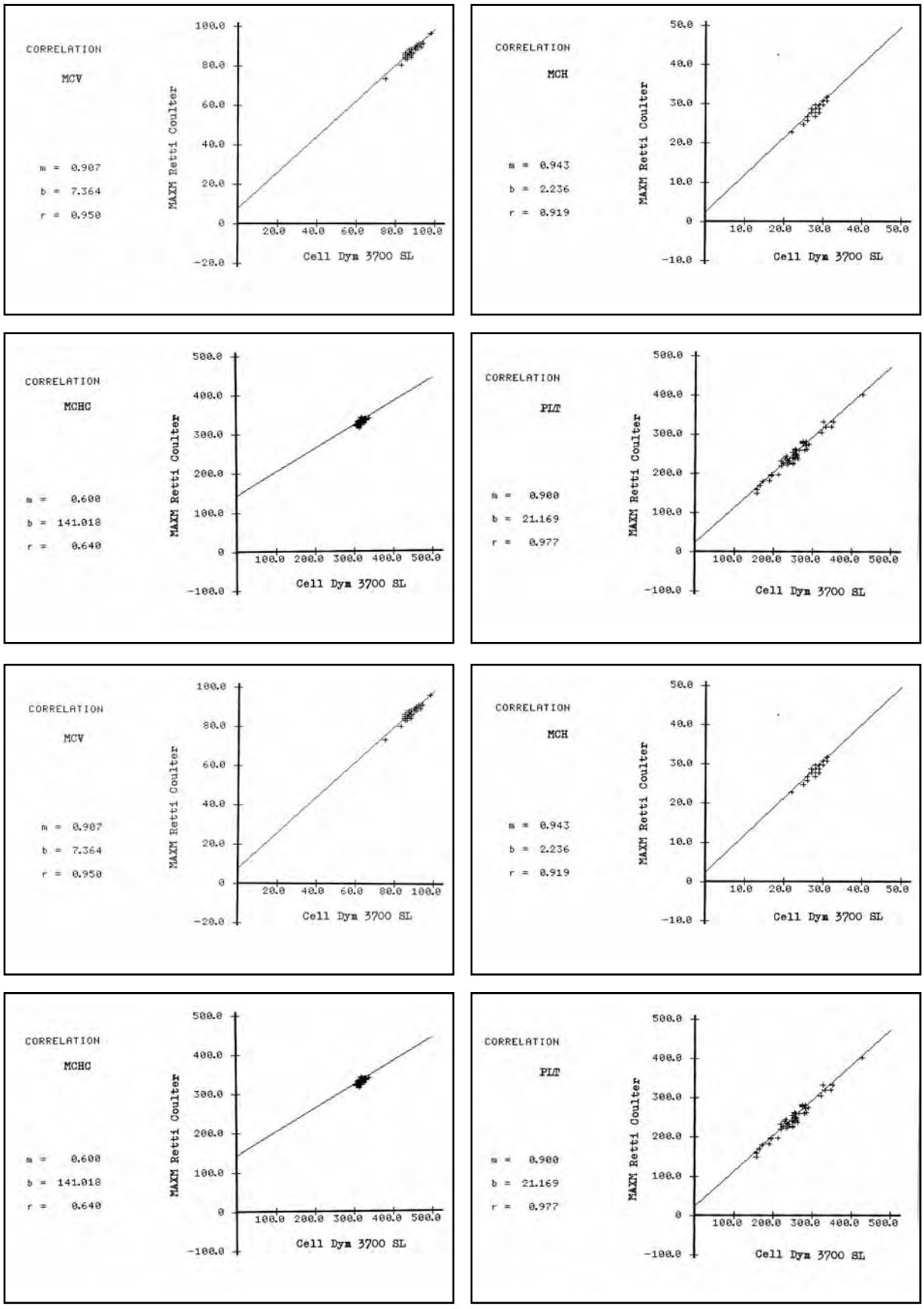
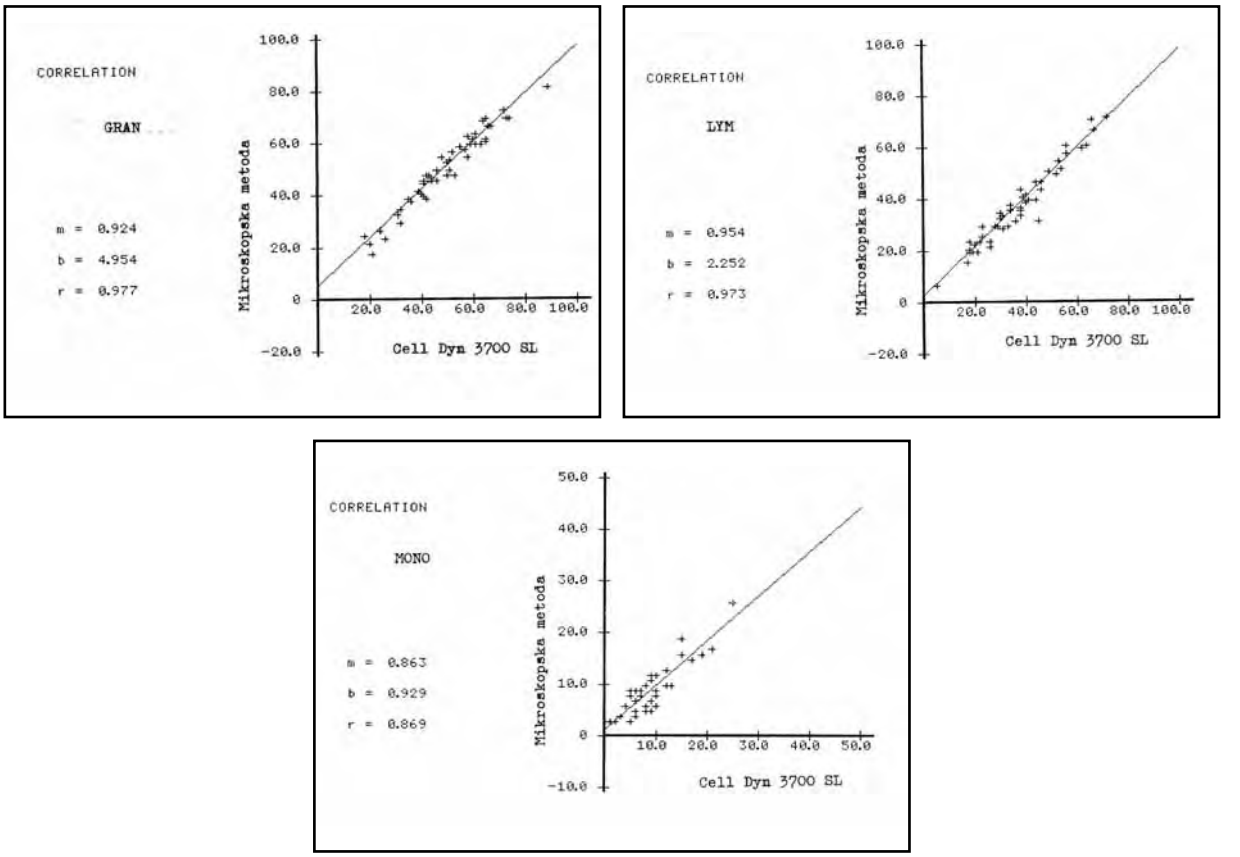


Figure 5 Analysis of the linear regression comparative results of differential white blood count on Cell-Dyn 3700 SL and by manual differentiating method.



Sensitivity and specificity

Sensitivity and specificity of haematology analyzer Cell-Dyn 3700 SL according to the differential white blood count parameters have been performed by differentiation analysis on apparatus and microscopically. Same blood samples used for accuracy testing have been also used for the testing of reliability parameters. Truly negative result (TN) and truly positive result (TP) have been calculated according to relation for sensitivity TP/(TP+FN) x 100 and relation for specificity TN/(TN+FP) x 100. Sensitivity and specificity testing results are shown in Table 4.

Table 4 Testing parameters for the sensitivity and specificity of method

Parameter	Cell Dyn 3700 SL	
	Negative	Positive
Negative	62	1
Positive	2	28

Truly negative result (TN) means a non-pathological differential white blood count result having a good differentiation correlation on analyzer and microscopically. We have obtained 62 (66.6%) truly negative results throughout comparison of the differential white blood counts.

Truly positive result (TP) means a pathological differential white blood count result having a good differentiation correlation on analyzer and microscopically. We have obtained 28 (30.1%) truly positive results meaning the presence of immature cells (myelocyte, metamyelocyte and lymphoblast).

False negative result (FN) means a differential white blood count result not marked as a pathological on analyzer while microscopic sample result was different. Only 1 sample (1.07%) out of 93 tested samples had a false negative result.

False negative result (FP) means a differential white blood count result marked as a pathological on analyzer while microscopic sample result was different. Two samples (2.15%) out of 93 tested samples had false positive results.

In comparison with literature data, our correlation for the differential white blood count are better. Especially good is correlation between manual differentiation and analyzer differentiation with samples having no morphologic or distributive abnormalities of leukocytes.

Results of this evaluation indicate that this type of samples can be differentiated with confidence on analyzer in

everyday work. On the contrary, when analyzer indicates any kind of abnormalities in blood cell composition or analyzer warning system is on, a sample has to be microscopically tested. When it is already known or the analyzer "warns" on the pathological morphology of blood cells, a differential white blood count result obtained on the analyzer needs to be processed microscopically, particularly in the cases of acute infectious processes, haematological diseases and allergy accompanied with haematology parameter changes. In all cases, only microscopic differentiation provides the correct differential white blood count findings. In addition, all cases of the impossibility of proper recognition of the leukocyte morphological abnormalities (the presence of immature leukocytopoiesis cells, lithoplasma and granulation changes, and the presence of toxic granulations) demand microscopic blood sample differentiation. During the process of differentiation of differential white blood count on analyzer 7 warning cases (7.5%) have been found and later processed microscopically.

Conclusion

Automatic laser haematology analyzer Cell-Dyn 3700 SL is a reliable haematology analyzer for the determination of full blood count in everyday work. Analyzer and its program for differential white blood count could be used for the research and separation of normal from pathological blood counts. For that reason, it is possible to decrease significantly everyday manual differentiation, particularly differentiation of the blood samples from healthy persons who have no morphological or distributive abnormalities in leukocytes. Together with the interpretation principle of results obtained on analyzer and respecting of analyzer "warning" system, it is still necessary to perform the microscopic method of leukocyte differentiating, particularly in detection of new haematological diseases and control of the previously detected and treated cases.

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