Comparative Haematology International (1999) 9:92–106

COMPARATIVE HAEMATOLOGY INTERNATIONAL

Original Article

Evaluation of the CELL-DYN® 3500 Haematology Instrument for the Analysis of the Mouse and Rat Blood

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Abstract. The objective of this study was to evaluate the performance of the CELL-DYN® 3500 for rat and mouse blood analysis in a routine environment. The WBC (white blood cells), RBC (red blood cells), PLT (platelets) counts and the WBC differential were determined. In addition, the following aspects were studied: within-run precision, day-to-day precision, bias-free paired difference precision; extended ranges of linearity for RBC, HCT (haematocrit), WBC, PLT; carry-over, the effect of blood ageing, cell stability with different anticoagulants; and the normal ranges, the out of range flagging and some typical pathology cases.

The CELL-DYN® 3500 is a multiparameter flow cytometer which counts and differentiates WBC, based on the principle of multi-angle polarised light scatter separation. RBC and PLT are determined by the impedance method. The WBC count is evaluated by both, optical and impedance methods. Reference methods used were according to the ICSH recommendations on blood cell analysis, including manual counts of WBC and platelets, a centrifugal microhaematocrit method and a haemoglobin measurement by spectrophotometry using the WHO haemoglobin standard. All cell counts were compared with the results obtained by our routine blood cell analyser (Contraves AL820), and the WBC differential was compared with the manual microscopic differentiation of the 400 WBC (200 cells differentiated by two technicians).

The following coefficients of vartiation were obtained: within-run precision was 1.2% and 2.7% for WBC; 1.0% and 1.0% for RBC; 1.3% and 0.9% for haematocrit; 2.1% and 2.7% for platelets (rats and mice respectively). Day-to-day precision was performed using human tri-level control blood, and the CVs were found to be <1.7% for WBC, <1.4% for RBC, <1.2% for haemoglobin and <6.3% for platelets.

The following ranges of measurement were found to be linear in the rat: WBC: $0.10–20.20 \times 10^3/\mu$l; RBC: $0.016–14.3 \times 10^6/\mu$l; haemoglobin: 0.08–26.8 g/dl; haematocrit: 5.0%–77%; platelets: $14.0–1670.0 \times 10^3/\mu$l. Equal ranges were observed for mouse blood. Carry-over in rat blood was found to be 0.12% for WBC, 0.05% for RBC, 0.15% for haemoglobin and 0.46% for platelets. In mice, similar carry-over results were obtained. The correlation coefficients (Pearson, correlation coefficient) between the CELL-DYN® 3500 and Contraves AL 820 using linear regression analysis were as follows: 0.988 and 0.997 for WBC; 0.986 and 0.920 for RBC; 0.995 and 0.984 for haemoglobin; 0.958 and 0.85 for haematocrit; 0.958 and 0.963 for platelets, for rats and mice, respectively. Correlation coefficients between the CELL-DYN® 3500 and the manual differential of NEU (neutrophils) and LYM (lymphocytes) were higher than 0.8 in rats and higher than 0.9 in mice. Due to the relatively low absolute counts of MONO (monocytes), EOS (eosinophils) and BASO (basophils), only moderate correlation of methods was found.

The CELL-DYN® 3500 was judged to be reliable, accurate and easy-to-use for counting and identifying normal and most of the pathological blood specimens obtained from mice and rats. By using the CELL-DYN® 3500, the time for blood sample analysis can be shortened significantly and provides extensive opportunities to characterise pathological samples.

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Keywords: Blood cell differentiation; Cell-Dyn® 3500; Flow cytometry; Impedance measurement; Mouse and rat haematology; Reference values

Introduction

Haematological parameters are routinely required in the context of rodent studies in research. However, most automated cell counters were developed for human blood samples and do not provide multiple species settings. The CELL-DYN® 3500 has been developed for laboratories which analyse blood samples from various animal species concomitantly. It provides hard- and software suitable for a multiple species analysis environment and the programmed settings can be stored for each species separately. Performance with rodent blood samples is examined in this paper, and reference values of Sprague–Dawley rats and Zur:ICR mice are provided.

Materials and Methods

Study Design

This study consisted of two phases. In the preliminary phase (phase 1) optimal settings of the instrument were determined for rat and mouse blood. This was achieved by comparing results obtained from each of 20 young (7 weeks of age) and 20 adult (15–20 weeks) rats and mice with those obtained by reference methods. Based on these results, appropriate adjustment were made to the CELL-DYN® 3500.

The main study (phase 2) was performed with the settings determined in phase 1 on 40 Sprague–Dawley rats, 10 Wistar rats and 40 ICR mice.

The study goals were:

1. To evaluate the overall performance of the CELL-DYN® 3500 on rat and mouse blood;
2. To determine the accuracy of the instrument by comparing the results with those of established reference methods;
3. To verify instrument precision;
4. To evaluate linearity and carry-over;
5. To verify stability of red blood cells (RBC) and white blood cells (WBC).

Animals and Blood Samples

The animals used in this study were obtained from the Institute of Laboratory Animal Science, University of Zurich, Switzerland. For the main study, the blood samples of three groups of healthy animals were used:

1. Forty Sprague–Dawley rats, outbred, strain:Zur: SIV; 7–26 weeks old; 20 males and 20 females bred under specified pathogen-free (SPF) conditions;
2. Forty mice, outbred, strain: Zur:ICR; 8–26 weeks old; 20 males and 20 females bred under SPF conditions;
3. Ten Wistar rats, outbred, strain: Hanlbm:Wist; 7–26 weeks old; five males and five females; purchased from the SPF-breeding unit of the Biological Research Laboratories Ltd., Füllinsdorf, Switzerland.

All animals were bred under barrier conditions and the breeding units were regularly checked for the absence of pathogenic organisms (bacteria, viruses, fungi and parasites) according to FELASA (1994) recommendations. Animals were kept under optimal hygienic conditions (OHC) and had at least 8 days adaptation period before the experiment started. They were housed in macrolon type IV (rats) or type III (mice) cages on sterilised softwood chips in groups of five animals by sex. The animals were provided with food (autoclaved standard lab chow, N 850, Nafag AG, Gossau, Switzerland) and pasteurised chlorinated tap water ad libitum.

The animal rooms were temperature controlled ($22 \pm 1^{\circ}C$) and had a regulated humidity of $55 \pm 5\%$ with 15 air changes per hour. Animals were exposed to artificial light from 6 a.m. to 8 p.m. with a twilight transition.

The 7-week-old rats and 8-week-old mice were bled consecutively every four weeks for 5 months (August 1993–December 1993), thus covering the adolescence phase of the animals, and providing us with a large range of haematological data. This procedure allowed us to define reference values for the rats under study at the age of 7, 11, 15 and 19 weeks and for the mice at 8, 12, 16 and 20 weeks. The blood samples were collected between 7 and 10 a.m. and analysed within 4 h. All samples were uniquely identified by age and sex. Samples with microclots, haemolysis or lipaemia were excluded from the study.

Blood was taken by puncture of the retro-orbital venous plexus under Metofane (metoxyflurane) anaesthesia with heparinised capillaries into K_3-EDTA anticoagulated tubes (Sarstedt AG, Sevelen, SG, Switzerland). The size of the tubes was 2 ml for rats and 1.3 ml for mouse blood samples. At least 1 ml of blood for rats and 0.5 ml for mice was obtained. Between collection and analysis samples were stored at room temperature.

In addition to the above samples collected from healthy animals, pathology samples were analysed. These were kindly provided by Dr R. Zinkernagel, Institute of Experimental Immunology and Dr J. Eckert, Institute of Parasitology, University of Zurich.

CELL-DYN® 3500 System

The CELL-DYN® 3500 is an electronic cell counter based on the impedance method pioneered by Coulter (1956) and a flow cytometer built within one instrument. The pinciple of electronic resistance, with volumetric metering, is used to count and size red blood cells and platelets (PLT). Haemoglobin is measured by a modified

haemoglobin cyanide absorbency method with automatic adjustment to the reagent absorption.

A laser-based flow cytometer is employed to count, size and classify leucocytes. Simultaneously, during the measurement cycle, each cell is counted and individually characterised by four specific angles of light scatter for the differential classification of the lymphocytes, basophils, monocytes, neutrophils and eosinophils. This is referred to as multi-angle polarised scatter separation (MAPSS) by the manufacturer.

The erythrocyte and platelet pulses are collected and presented in numbers per litre of whole blood. In addition, they are displayed in a volume distribution curve. Simultaneously, the following indices are calculated: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), the red cell distribution width (RDW), the mean platelet volume (MPV) and the platelet distribution width (PDW).

The leucocyte differential data can be routinely viewed and displayed in the form of six two-dimensional scattergrams. The instrument requires 130 μl of whole blood; 10 000 leucocytes are counted and differentiated in each blood sample.

Calibration and Quality Control

The CELL-DYN® 3500 was calibrated at the factory prior to shipment. During instrument installation this calibration was confirmed. Only the directly measured parameters WBC, RBC, HGB, MCV, PLT and MPV needed to be calibrated. On-board quality control programmes are designed to provide continual monitoring and verification of instrument calibration. These quality control programmes are designated as follows.

X-B Analysis: This programme calculates the average of the RBC indices and some WBC differential values, analysed per session, and allows the display of these values over time. It corresponds to the procedure of Bull (1975).

QC Files: There is space on the hard disk to store up to 20 quality control (QC) files. This allows the statistical and graphical analysis of the data in each file for calcuation of the mean, standard deviation and coefficient of variation and to generate Levey–Jennings graphics.

Westguard rules: This programme tests the control result against control limits to determine whether the instrument shows acceptable accuracy and precision. The limits are derived from the mean and standard deviation of control measurements.

The calibration verification of the CELL-DYN® 3500 was performed daily according to the manufacturer's guidelines. This verification included measurement of three commercial human control blood samples of abnormally low, normal and abnormally high blood cell counts and parameters, and on a regular basis, the use of fresh whole blood from the animals under investigation. When instrument calibration was required, the CELL-DYN Calibrator (P/N:99120-01) was used.

Reagent System

The reagent system was formulated specifically for the CELL-DYN 3000 series instrument flow systems in order to provide optimal system performance.

Diluent (L/N 99231-01) was formulated to act as diluent for the WBC (for the impedence count only), RBC, PLT and haemoglobin and to stabilise the volume of each red cell and platelet during the counting and sizing process.

WIC/HGB lyse (L/N 99431-01) was formulated to rapidly lyse the red blood cells and minimise the resultant stroma and to strip the white cell cytoplasm leaving the nuclear membrane intact so that the white cell nuclei can be enumerated. In addition, this reagent converts haemoglobin into a modified haemoglobincyanide complex that is measurable at 540 nm.

Detergent (L/N 99321-01) was formulated to provide an optically clear solution that is needed to obtain the zero reference during the haemoglobin measurement cycle, to provide proper meniscus formation in the WIC and RBC/PLT metering tubes and to maintain it during each run cycle, and to rinse the WIC[1] counting chamber, the RBC/PLT counting chamber, the RBC/PLT metering tube and the HGB flow cell with minimal bubble formation.

Sheath reagent (L/N 99311-01) was formulated to osmotically lyse the red cells, to maintain the light-scattering properties of the WBC for the duration of the measurement period, to serve as a sheath fluid for the hydrodynamic focusing process and to provide sufficient wetting action to prevent accumulation of air bubbles in the flow system.

Shear valve lubricant (L/N 99630-01) (lubricates the inner surface of the shear valve) and enzymatic cleaner (L/N 99644-01) (removes protein build-up within the instrument) were also used regularly.

Reference Methods

As a reference method for the determination of RBC and haematocrit, an electronic cell counter that operates according to the impedance method was used (Contraves AL 820, AVL AG, Schaffhausen, Switzerland; Winkler et al. 1995). For each sample the haematocrit was determined electronically by both instruments and by a haematocrit centrifuge (Hettich AG, Bäch, Switzerland) according to the ICSH Protocol H-20 (ICSH 1984). A total of 204 rat samples and 150 mouse samples were compared. In addition, for 30 rat and 30 mouse samples, manual WBC and manual PLT counts were compared with the values obtained by the instruments. Cross-

[1]WIC: WBC impedance channel.

calibration of the instruments was performed using fresh whole blood. The automated five-part differential data obtained by the CELL-DYN® 3500 were compared to the manual differential cell counts. Two smears were made from each sample prior to analysis and stained within one hour with an automated slide-stainer (Hema-Tek Ames Company, Division Miles Lab. Inc., Elkhart, Indiana, USA) using Wright stain. A light microscope (Leitz Laborlux S, Wild Leitz AG, Switzerland, magnification 10 × 100) was used for WBC differentiation. A total of 200 WBC were counted and differentiated by two trained technicians, thereby producing a 400-cell count on each specimen. Each technologist also recorded the morphological abnormalities seen in the leucocyte and/or erythrocyte population. The slides were stored for future review. The HGB values of the CELL-DYN® 3500 were compared to the WHO recommended reference method (cyanmethaemoglobin method, ICSH 1984).

Precision

Three approaches were selected to determine the precision of the CELL-DYN® 3500:

1. Within-run precision was determined by measuring a sample 31 times consecutively. A pooled blood sample was made by mixing blood specimens collected from four Sprague-Dawley rats (males, 10-weeks old). Prior to pooling, aliquots of the four samples were mixed and checked for the absence of agglutination, haemolysis and lipaemia. Results of all measured parameters were stored as a QC-file in the QC log of the CELL-DYN® 3500. The mean, standard deviation (SD) and coefficient of variation (CV) were calcuated by the instrument.
2. Precision of paired duplicates was determined by testing all samples in replicates during the study period. This allowed the calculation of the sample precision in the low, normal, and high range of cell concentrations (Bland 1991).
3. The day-to-day precision was determined by analysing tri-level commercial control blood samples (stabilised human blood, Abbott, Santa Clara, California, USA). The tri-level controls were analysed daily over a period of 30 working days. The data were stored in a day-to-day QC-file which allowed the calculation of the day-to-day precision.

Range of Linearity

The range of linearity was assessed according to the ICSH protocol H-20 of 1984 on rat and mice samples in the low, normal and high ranges. The following parameters were included: WBC, RBC, HGB, HCT and PLT.

In order to increase the range in which linearity was determined, the cellular content of different blood samples was increased by centrifugation and removal of some of the plasma. After mixing, linear dilutions were made using the plasma previously removed. The average of two duplicate specimens was plotted against the expected value of each dilution precentile.

Carry-over

Carry-over was determined according to the procedure described by Broughton et al. (1974), using a normal blood sample run in triplicate, immediately followed by three background cycles.

The carry-over percentage was calculated using the following equation:

$$\frac{\mathrm{L1} - \mathrm{L3}}{\mathrm{H3} - \mathrm{L3}} \times 100\%$$

where L1 and L3 are the results of the first and third measurements of the sample with a low analyte concentration and H3 is the third measurement of the sample with a high analyte concentration.

Effect of Anticoagulants and Ageing

To evaluate the effect of anticoagulants we compared results obtained from blood samples collected from each of four rats in K_3-EDTA anticoagulant with those obtained from blood samples from the same animals collected in a tube with lithium heparin (Sarstedt AG, Sevelen, SG, Switzerland). The values obtained with the two different anticoagulants were statistically compared using the *u*-test of Mann and Whitney (1947).

To study the effect of blood ageing in K_3-EDTA, blood was collected from five normal rats and seven normal mice. The samples were analysed immediately after collection and after 1, 2, 4, 8 and 16 h.

Statistical Methods

Accuracy of the CELL-DYN® 3500 was examined by comparison with reference methods and/or routine methods used in our laboratory (Van Assendelft and England 1992). Linear regressions of the form $y = a + bx$ and the correlation coefficients were calculated by personal computer software (StatView). The calculation of the correlation coefficient was based on the least squares method. In addition, linear regressions were calculated according to Bablok and Passing (1985) with the software kindly provided by W. Bablok (version 2.0x).

For the anticoagulant study the *u*-test by Mann and Whitney (1947) was used. The effect of aging on haematological parameters and reference values was examined by the paired student's t-test (Bland 1991).

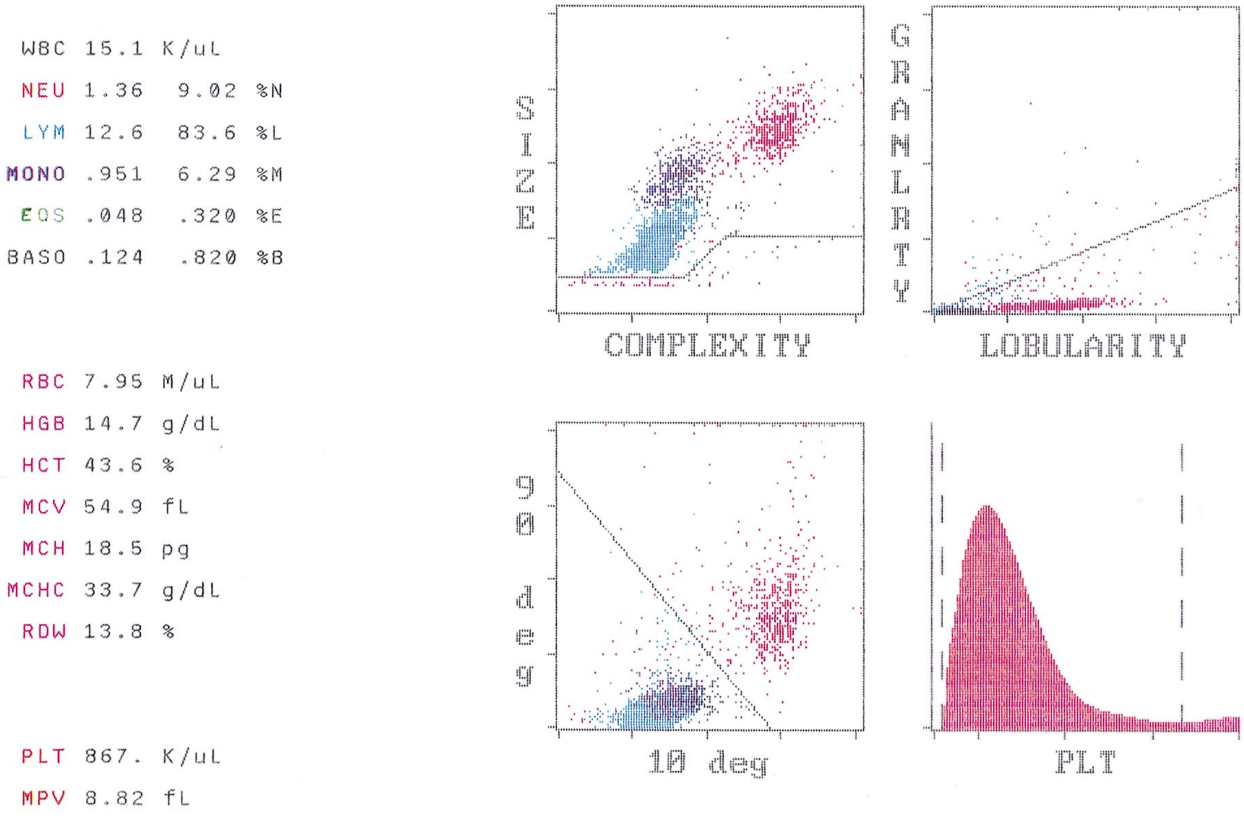

Fig. 1. A printout of a normal rat specimen.

Results

Settings of the CELL-DYN® 3500 Instrument and Printout of Results

The data produced by the CELL-DYN® 3500 are displayed and printed as shown in Fig. 1. This is a typical report for a normal rat blood sample. The computer graphics allow visualisation of several distinct cell population. Up to 24 individual parameters and up to six scatterplots and/or five histograms can be displayed in four designated fields: the screen and the printout are divided into two sections (left and right). Individual parameters are listed in the left section and the scatterplots and histograms are listed in the right section. The demographic information on the sample (ID-number, species, sex, date of birth) is displayed at the top of the screen and printout form. The operator can choose between the following scatterplots:

1. 0°/10° is used to separate lymphocytes, monocytes and basophils;
2. 90°D[2]/90° is used to separate eosinophils from the neutrophils;
3. 90°/10° is used to separate the mononuclear from the polymorphonuclear cells;
4. 90°/0° provides information on the presence of suspected blast cells and left shift.

The following histograms can be displayed:

1. Histogram of mononuclear and polymorphonuclear cell size distribution data derived from WOC (WBC optical channel) mode;
2. Histogram of lymphocytes, basophils and monocytes cell size distribution data derived from WOC mode;
3. Histogram of WBC derived from WIC (WBC impedance channel) mode;
4. Histogram of the red cells (A) and platelets (B) separated by means of a dynamic threshold. This histogram is derived from the impedance mode.
5. Histogram of the platelet size derived from the impedance mode. The platelets are separated from the background and the red cells by means of a lower and upper floating threshold.

The instrument settings were determined according to the manufacturer's manual. Identical settings were used for Wistar and Sprague–Dawley rats of different age groups (data not shown). Moreover the rat conversion factors were transferable to another lab for blood cell analysis of PVS rats. Optimal cluster separation on mice blood required different settings. The conversion factors

[2]Depolarized light

link the species-specific settings to the human default settings of the instrument.

Within-run Precision

For almost all parameters the coefficients of variation (CVs) in rats were lower than those specified by the manufacturer (Table 1). The CVs of WBC, RBC, HGB, HCT and MCV values derived from rat blood were about 1% or lower. The CVs of platelets (2.1%) and lymphocytes (2.1%) were only slightly higher than those of red cell parameters. The CVs shown for neutrophils (NEU) (7%), monocytes (MONO) (36%), eosinophils (EOS) (55%) and basophils (BASO) (44.5%) were highest due to the lower absolute numbers obtained for these cell populations.

For mouse blood, the CVs of RBC, HCT and PLT were somewhat higher (5.8%, 6% and 6.7%, respectively) (Table 2). However, paired difference precision data on mice blood (Table 3) are in agreement with the good precision data obtained for rats.

Day-to-day Precision

The CELL-DYN® 3500 showed little variation over time as exhibited by good day-to-day precision for all parameters of the three levels of control blood samples on which this precision study was based (Table 4). The CVs for WBC varied from 1.7% for high to 2.7% for low control blood, CVs for RBC, HGB, HCT were below 1.5% for all levels. Even for WBC differential counts, which showed the greatest variation, the CVs were for NEU (2.1%, 2.8% and 3.1% for low, normal and high, respectively), lymphocytes (LYM) (12.3%, 10.4% and 2.7%), MONO (13.8%, 9.2% and 4.6%) and EOS (12.3%, 9.5% and 9.7%).

Table 3. Paired difference precision of mouse haemogram

Parameter	CV (%)
WBC	2.3
RBC	1.8
HGB	0.9
HCT	1.6
MCV	1.4
PLT	3.9

n = 93, paired samples of ICR mice. For abbreviations see Table 1.

Table 3 shows the results for paired difference precision of mouse haemogram. The CV varied from 0.9% (HGB) to 2.3% (PLT).

Range of Linearity

In order to scrutinise the linear relationship of measurements over an extended range, we diluted packed cells in autologous platelet-poor plasma at different concentrations. For each parameter studied a separate dilution was made. Figure 2 shows the linearity range for eight parameters of rat blood. Linear results were obtained over the low, normal and high ranges. Equal linearity was found for similar ranges in mouse blood (not shown). Linearity extending to very low

Table 1. Within-run precision for rats. Blood was collected from four healthy male 10-week-old, Sprague–Dawley rats, pooled and measured 31 times

Parameter	WBC ($10^3/\mu l$)	NEU ($10^3/\mu l$)	LYM ($10^3/\mu l$)	MON ($10^3/\mu l$)	EOS ($10^3/\mu l$)	BASO ($10^3/\mu l$)	RBC ($10^6/\mu l$)	HGB (g/dl)	HCT (%)	MCV (fl)	PLT ($10^3/\mu l$)
Mean	14.1	0.875	12.6	0.423	0.063	0.085	7.18	14.6	41.4	57.7	870
CV%	1.2	7.0	2.1	36	55	44.5	1.0	0.4	1.3	1.1	2.1
CV% (m.s.)[a]	1.9	n/a[b]	n/a	n/a	n/a	n/a	1.0	0.7	n/a	0.8	3.1

[a] Manufacturer's specification. [b] Not applicable. WBC, white blood cells; NEU, neutrophils, LYM, lymphocytes; MON, monocytes; EOS, eosinophils; BASO, basophils; RBC, red blood cells; HGB, haemoglobin; MCV, mean cell volume; PLT, platelets; HCT; haematocrit.

Table 2. Within-run precision for mice. Blood was collected from seven healthy male 6-month-old, ICR mice, pooled and run 18 times

Parameter	WBC ($10^3/\mu l$)	NEU ($10^3/\mu l$)	LYM ($10^3/\mu l$)	MON ($10^3/\mu l$)	EOS ($10^3/\mu l$)	BASO ($10^3/\mu l$)	RBC ($10^6/\mu l$)	HGB (g/dl)	HCT (%)	MCV (fl)	PLT ($10^3/\mu l$)
Mean	6.06	0.777	5.08	0.88	0.081	0.033	7.4	14.1	36	48.6	1270
CV%	2.7	52.3	8.5	11.9	68.9	n/a	5.8	1.0	6.0	0.7	6.7
CV% (m.s.)[a]	1.9	n/a[b]	n/a	n/a	n/a	n/a	1.0	0.7	n/a	0.8	3.1

[a] Manufacturer's specification. [b] Not applicable. For abbreviations see Table 1.

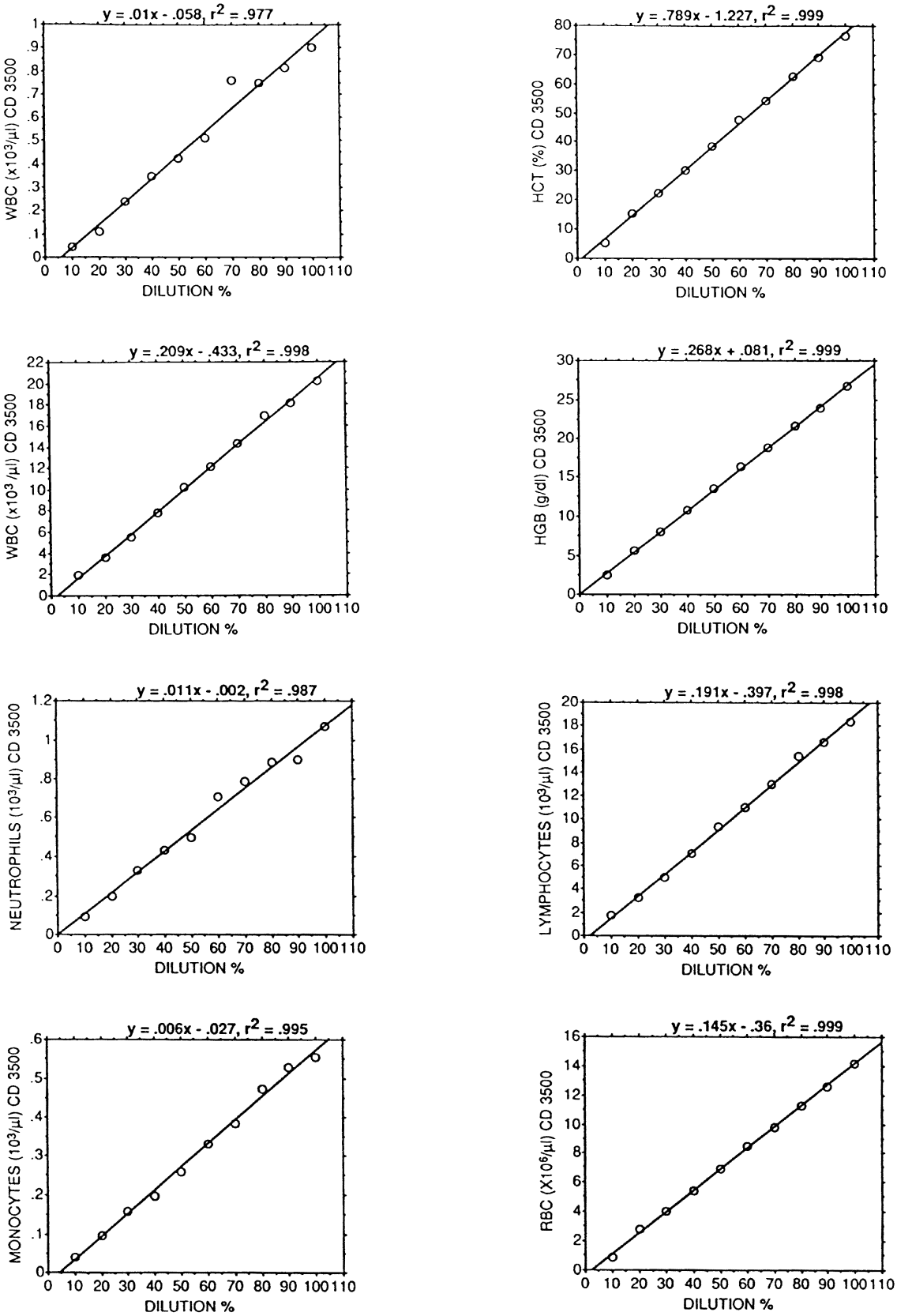

Fig. 2. Determination of range of linearity for rat white blood cells (WBC) (normal and pathologically low values), neutrophils (NEU), lymphocytes (LYM), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT) and platelets (PLT).

Table 4. Day-to-day precision (human blood). Commercial control blood was measured daily within on month. Control samples included low, normal and high values of WBC, RBC and PLT

Parameter		WBC ($10^3/\mu l$)	NEU ($10^3/\mu l$)	LYM ($10^3/\mu l$)	MON ($10^3/\mu l$)	EOS ($10^3/\mu l$)	BASO ($10^3/\mu l$)	RBC ($10^6/\mu l$)	HGB (g/dl)	HCT (%)	MCV (fl)	PLT ($10^3/\mu l$)
N	low	27	27	27	27	27	27	27	27	27	27	27
	normal	25	25	25	25	25	25	25	25	25	25	25
	high	23	23	23	23	23	23	23	23	23	23	23
Mean	low	2.14	1.66	0.252	0.156	0.052	0.026	2.53	6.90	20.4	80.6	48.8
	normal	7.64	4.93	1.69	0.683	0.182	0.160	4.28	12.9	38.4	89.7	211
	high	16.4	9.52	4.77	1.54	0.295	0.321	4.65	14.9	44.0	94.6	449
Target	low	2.10	1.70	0.450	0.200	0.100	0.300	2.53	6.90	20.4	80.6	49.0
	normal	7.60	4.90	1.70	0.700	0.200	0.200	4.28	12.9	38.4	89.7	211
	high	16.4	9.50	4.80	1.50	0.300	0.300	4.65	14.9	44.0	94.6	449
CV%	low	2.7	3.1	12.3	9.2	12.3	48.6	1.4	1.2	1.4	0.2	6.3
	normal	2.5	2.8	10.4	13.8	9.5	59.3	1.2	0.5	1.6	0.9	3.7
	high	1.7	2.1	2.7	4.6	9.7	14.7	1.1	0.4	1.5	0.9	1.9

For abbreviations see Table 1.

Table 5. Range of linearity for rat and mouse blood

Parameter	Rats		Mice	
	Lower limit	Upper limit	Lower limit	Upper limit
WBC ($10^3/\mu l$)	0.100	20.2	0.600	19.4
RBC ($10^6/\mu l$)	0.016	14.3	2.700	11.1
HGB (g/dl)	0.081	26.8	5.100	20.9
HCT (%)	5.000	77.0	14.000	55.3
PLT ($10^3/\mu l$)	14.000	1670.0	49.000	1690.0

For abbreviations see Table 1.

Table 6. Carry-over for rat and mouse blood

Parameter	WBC	RBC	HGB	PLT
Rats	0.12%	0.05%	0.15%	0.46%
Mice	0.027%	0.041%	0.156%	0.81%
Manufacturer specifications	<0.05%	<0.05%	<0.05%	<0.1%

For abbreviations see Table 1.

values is shown in Table 5, as is also the linearity for very high values (as demonstrated by PLT and HCT).

Carry-over

No significant carry-over was noted for all directly measured parameters (Table 6). For WBC, RBC and HGB carry-over was far below manufacturer specifications. For PLT, carry-over was above the specification for both mice and rats, although they were comparable to those of other parameters.

Accuracy of the CELL-DYN® 3500

To determine accuracy of the instrument, the different parameters were measured by the instrument and concomitantly by reference methods. Results were statistically compared by linear regression and the Passing–Bablok (Passing and Bablok 1983) methods. The results for rat and mouse blood are shown in Tables 7 and 8. Figure 3 shows the intercept and slope of the linear regression curves for WBC, RBC, HGB, HCT, PLT, NEU and LYM counts.

Correlation of WBC, RBC, HGB, HCT and PLT in rats was linear when compared to the Contraves AL 820 results, as judged by visual inspection of the plots. Correlation of the HGB and HCT in rats compared with the reference methods was also linear. Linear correlation with manual differential counts (NEU, LYM, MONO, EOS, BASO) was characterised by a higher degree of variation.

Cell Stability in Different Anticoagulants

We compared the effect of different anticoagulants (K_3-EDTA and lithium heparin) in four blood samples. Care was taken to ensure that the blood-to-anticoagulant ratio and the time between blood collection and measurement (2 h) was similar. No significant differences were seen with the two anticoagulants for the parameters studied (RBC, WBC, HGB, HCT and PLT) (Table 9).

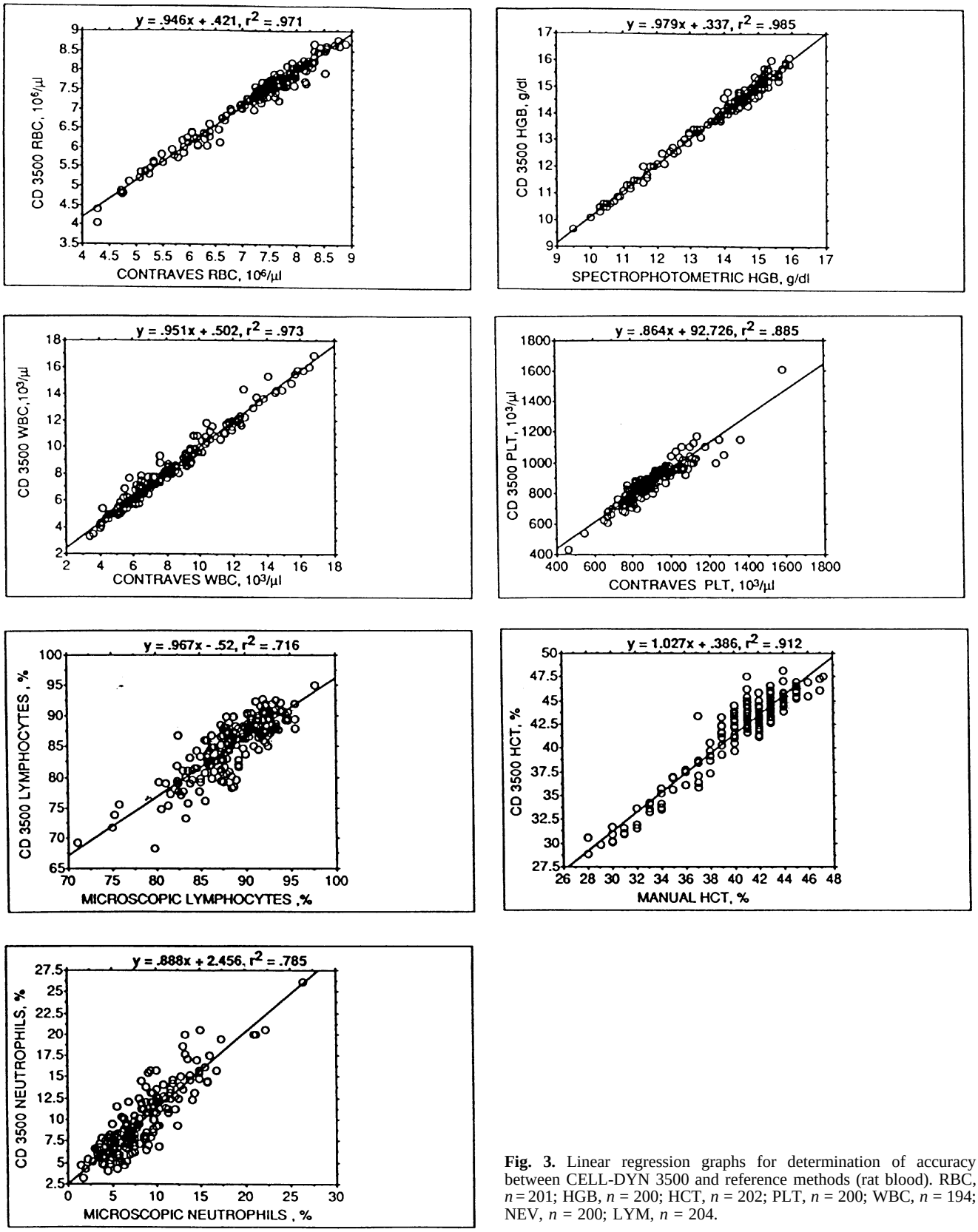

Fig. 3. Linear regression graphs for determination of accuracy between CELL-DYN 3500 and reference methods (rat blood). RBC, $n = 201$; HGB, $n = 200$; HCT, $n = 202$; PLT, $n = 200$; WBC, $n = 194$; NEV, $n = 200$; LYM, $n = 204$.

Table 7. Correlation between CELL-DYN 3500 and reference methods for rats

Parameter	Least squares method		Passing–Bablok method	
	Contraves AL 820	Manual	Contraves AL 820	Manual
WBC	$r = 0.986$ $a = 0.502$ $b = 0.951$	n/a[a]	$r = 0.986$ $a = -0.365$ $b = 1.043$	n/a
NEU	n/a	$r = 0.886$ $a = 2.456$ $b = 0.888$	n/a	$r = 0.886$ $a = -1.611$ $b = 1.002$
LYM	n/a	$r = 0.767$ $a = 1.535$ $b = 0.942$	n/a	$r = 0.767$ $a = 16.243$ $b = 0.848$
MONO	n/a	$r = 0.431$ $a = 2.399$ $b = 0.494$	n/a	$r = 0.431$ $a = -1.148$ $b = 0.934$
EOS	n/a	$r = 0.441$ $a = 0.316$ $b = 0.543$	n/a	$r = 0.441$ $a = -0.096$ $b = 1.196$
BASO	n/a	$r = 0.305$ $a = 0.761$ $b = 0.418$	n/a	$r = 0.304$ $a = -0.065$ $b = 0.147$
RBC	$r = 0.986$ $a = 0.421$ $b = 0.946$	n/a	$r = 0.986$ $a = -0.402$ $b = 1.050$	n/a
HGB	$r = 0.995$ $a = 0.098$ $b = 0.990$	$r = 0.993$ $a = 0.280$ $b = 0.982$	$r = 0.995$ $a = 0.000$ $b = 1.000$	$r = 0.993$ $a = 0.000$ $b = 1.000$
HCT	$r = 0.995$ $a = 3.166$ $b = 0.920$	$r = 0.955$ $a = 0.386$ $b = 1.027$	$r = 0.955$ $a = -3.787$ $b = 1.096$	$r = 0.955$ $a = 0.667$ $b = 0.952$
MCV	$r = 0.845$ $a = 19.818$ $b = 0.647$	n/a	$r = 0.845$ $a = -13.625$ $b = 1.250$	n/a
PLT	$r = 0.941$ $a = 4.719$ $b = 0.981$	n/a	$r = 0.941$ $a = -57.979$ $b = 1.096$	n/a

[a] Not applicable. For abbreviations see Table 1.

Effects of Ageing

The parameters RBC, WBC, HGB, HCT, PLT, LYM, NEU, EOS, MONO, BASO remained unchanged during the observation period ($p>0.05$, paired *t*-test). The only exception was MCV which increased by 4% during the 16 h of the study ($p = 0.0013$; data not shown).

Reference Ranges

Reference ranges, generated for 7-, 11-, 15- and 19-week-old healthy Sprague–Dawley rats and 8-, 12-, 16- and 20-week-old ICR mice, separated by sex, are shown in Tables 10 and 11. For each age group data were obtained from 20 male and 20 female animals. The data of Tables 10 and 11 are now stored in the animal catalogue of the CELL-DYN® 3500, and can be used as reference values with each new sample measured. Four sets of limits can be defined for each species. Whenever a parameter result exceeds an entered limit, the result is displayed in colour on the screen to alert the operator. Results displayed in yellow are below the limits and results displayed in purple are above the limits.

Discussion

The CELL-DYN® 3500 uses a unique form of flow-cytometry called multi-angle polarised scatter separation which allows the differentiation of the white blood cells in their natural state. The simulataneous measurement of WBC by two independent procedures (WIC and WOC) in the same instrument is an entirely new development offering significant diagnostic advantages, since it eliminates the limitations of each methodology therefore increasing the number of samples that can be analysed without review.

Table 8. Correlation between CELL-DYN 3500 and reference methods for mice

Parameter	Least squares method		Passing–Bablok method	
	Contraves AL 820	Manual	Contraves AL 820	Manual
WBC	$r = 0.997$ $a = -0.116$ $b = 0.991$	n/a[a]	$r = 0.997$ $a = 0.095$ $b = 1.013$	n/a
NEU	n/a	$r = 0.959$ $a = 0.644$ $b = 0.993$	n/a	$r = 0.959$ $a = 0.161$ $b = 0.987$
LYM	n/a	$r = 0.925$ $a = -3.104$ $b = 1.047$	n/a	$r = 0.925$ $a = 9.461$ $b = 0.869$
MONO	n/a	$r = 0.727$ $a = 0.719$ $b = 1.228$	n/a	$r = 0.727$ $a = 1.097$ $b = 0.654$
EOS	n/a	$r = 0.217$ $a = 0.058$ $b = 0.077$	n/a	$r = 0.216$ $a = 0.321$ $b = 11.905$
BASO	n/a	$r = -0.22$ $a = 0.661$ $b = -0.227$	n/a	$r = -0.22$ $a = 0.000$ $b = 0.000$
RBC	$r = 0.920$ $a = 1.232$ $b = 0.853$	n/a	$r = 0.920$ $a = 1.232$ $b = 0.853$	n/a
HGB	$r = 0.984$ $a = 0.260$ $b = 0.978$	$r = 0.964$ $a = -0.059$ $b = 1.009$	$r = 0.984$ $a = 0.100$ $b = 1.000$	$r = 0.964$ $a = -0.100$ $b = 1.000$
HCT	$r = 0.850$ $a = 4.990$ $b = 0.856$	$r = 0.858$ $a = 0.775$ $b = 1.010$	$r = 0.850$ $a = 4.036$ $b = 0.922$	$r = 0.858$ $a = 7.000$ $b = 0.833$
MCV	$r = 0.857$ $a = 4.762$ $b = 0.877$	n/a	$r = 0.859$ $a = 9.062$ $b = 0.968$	n/a
PLT	$r = 0.971$ $a = 30.535$ $b = 0.930$	n/a	$r = 0.971$ $a = -16.708$ $b = 1.058$	n/a

[a] Not applicable. For abbreviations see Table 1.

Table 9. Comparison of raw data for rat blood taken in K_3 EDTA and heparin anticoagulants, respectively

Sample no.	Anticoagulant	WBC	RBC	HGB	HCT	PLT
1	EDTA	5.02	6.97	13.9	40.7	797
	Heparin	5.34	7.03	14.0	40.1	712
2	EDTA	7.83	7.24	13.6	41.7	590
	Heparin	6.46	6.94	13.4	39.2	486
3	EDTA	6.36	7.32	14.5	42.3	733
	Heparin	6.02	7.27	14.5	41.3	697
4	EDTA	7.78	6.83	13.6	40.3	763
	Heparin	7.77	6.97	13.7	39.7	715

For abbreviations see Table 1.

The instrument was evaluated over a period of one year. Although the instrument looked rather complex when introduced to the lab, its maintenance and operation became very easy. The CELL-DYN® 3500 graphically displays coloured clusters of the different cell types, which makes it easier to identify normal and abnormal cells. This instrument helps reduce time for analysis and it provides objective and quantitative

Table 10. Reference ranges generated for rat WBC

Distribution	Male			Female		
	3%	50%	97%	3%	50%	97%
WBC 7 weeks	7.863	11.65	18.61	7.583	11.15	15.99
11 weeks	5.927	10.82	15.97	5.246	7.955	13.47
15 weeks	4.781	9.88	15.25	5.048	7.245	12.06
19 weeks	3.649	8.39	12.18	4.379	7.315	10.691
NEU (abs) 7 weeks	0.433	0.557	0.739	0.237	0.541	1.106
11 weeks	0.335	0.798	1.672	0.226	0.502	1.149
15 weeks	0.520	0.890	1.708	0.364	0.619	1.064
19 weeks	0.255	0.786	1.369	0.202	0.473	1.07
LYM (abs) 7 weeks	7.135	10.829	17.353	6.592	10.209	14.853
11 weeks	4.920	9.541	14.771	4.902	9.101	11.256
15 weeks	3.906	9.08	13.385	4.405	6.208	10.598
19 weeks	3.159	7.403	10.577	3.782	6.324	9.288
MON (abs) 7 weeks	0.019	0.287	0.586	0.124	0.381	0.708
11 weeks	0.179	0.327	0.674	0.125	0.329	0.471
15 weeks	0.166	0.319	0.761	0.017	0.231	0.554
19 weeks	0.121	0.327	0.709	0.053	0.235	0.382
EOS (abs) 7 weeks	0.013	0.027	0.082	0.012	0.024	0.109
11 weeks	0.015	0.039	0.098	0.012	0.024	0.063
15 weeks	0.018	0.03	0.073	0.012	0.029	0.064
19 weeks	0.005	0.024	0.112	0.013	0.028	0.123
BASO (abs) 7 weeks	0.005	0.034	0.094	0.008	0.037	0.062
11 weeks	0.019	0.067	0.165	0.014	0.044	0.124
15 weeks	0.029	0.089	0.246	0.001	0.055	0.172
19 weeks	0.013	0.088	0.265	0.046	0.092	0.165
NEU (%) 7 weeks	3.745	4.825	7.855	2.273	4.9	8.779
11 weeks	5.087	7.325	11.25	3.976	6.09	14.267
15 weeks	5.143	7.935	18.86	5.779	7.665	12.88
19 weeks	4.718	8.875	14.34	3.352	7.515	14.94
LYM (%) 7 weeks	88.05	91.7	95.2	86.59	91.05	93.5
11 weeks	83.06	88.15	92.49	82.24	89.85	92.74
15 weeks	77.48	86.85	91.9	82.81	88.2	90.6
19 weeks	78.2	86.7	91.87	77.36	87.4	94.7
MON (%) 7 weeks	0.199	2.485	5.821	1.274	3.665	7.217
11 weeks	1.348	3.515	4.765	2.252	2.95	5.46
15 weeks	1.435	3.285	5.024	0.284	3.05	4.906
19 weeks	2.187	3.615	6.179	0.851	3.255	6.439
EOS (%) 7 weeks	0.105	0.203	0.769	0.102	0.21	1.054
11 weeks	0.125	0.321	1.31	0.131	0.307	1.049
15 weeks	0.145	0.318	0.629	0.101	0.413	0.754
19 weeks	0.135	0.279	1.05	0.185	0.425	1.622
BASO (%) 7 weeks	0.051	0.262	0.556	0.055	0.313	0.729
11 weeks	0.221	0.55	1.537	0.176	0.559	1.448
15 weeks	0.389	0.86	3.257	0.004	0.82	1.466
19 weeks	0.157	1.11	2.497	0.646	1.15	2.382
RBC 7 weeks	5.544	6.045	6.888	5.269	6.325	7.014
11 weeks	6.733	7.465	7.896	7.085	7.545	8.289
15 weeks	7.315	8.025	8.659	7.189	7.605	8.1
19 weeks	6.831	8.175	8.751	7.021	7.66	8.55
HGB 7 weeks	12.33	13.2	14.3	12.14	13.3	14.28
11 weeks	14.02	15	15.5	13.82	14.55	15.37
15 weeks	14.4	14.95	15.97	13.7	14.3	15.18
19 weeks	13.71	15.05	15.89	13.47	14.35	15.2
HCT 7 weeks	33.42	36.05	40.32	33.87	37.45	40.56
11 weeks	41.18	44	45.95	40.49	43.65	46.73
15 weeks	42.68	44.65	48.14	40.59	43	46.92
19 weeks	39.84	44.75	47.22	40.39	43.15	46.07
MCV 7 weeks	57.4	59.95	61.87	56.48	59.35	64.38
11 weeks	57.52	58.45	61.29	55.09	58.3	60.79
15 weeks	54	56.3	58.85	56.33	56.55	58.18
19 weeks	53.25	55.35	58.3	54.43	56.5	58.18
PLT 7 weeks	600	863.5	992.7	779.8	979.5	1197.8
11 weeks	711.4	826.5	949.5	737.9	892.5	1068.2
15 weeks	624.2	828.5	948.2	711.8	877.5	1090.5
19 weeks	731	847	942.8	442.6	872.5	1113.9

For abbreviations see Table 1.

Table 11. Reference ranges generated for mice WBC

Distribution	Male			Female		
	3%	50%	97%	3%	50%	97%
WBC 8 weeks	1.848	4.44	7.162	2.741	4.31	7.015
12 weeks	3.068	5.09	7.619	3.265	5.65	8.36
16 weeks	3.13	6.32	7.645	3.44	4.85	7.458
20 weeks	2.739	3.75	6.762	3.294	4.615	8.023
NEU (abs) 8 weeks	0.414	0.685	1.324	0.324	0.759	1.133
12 weeks	0.553	0.795	1.099	0.509	0.798	1.254
16 weeks	0.648	1.008	2.616	0.403	0.676	1.632
20 weeks	0.552	0.858	1.517	0.475	0.735	1.622
LYM (abs) 8 weeks	1.361	3.617	5.755	2.253	3.58	5.879
12 weeks	2.198	4.255	6.743	2.37	4.683	7.322
16 weeks	2.391	5.018	6.483	2.74	4.294	6.731
20 weeks	1.924	2.92	5.558	2.429	3.764	6.993
MON (abs) 8 weeks	0.029	0.062	0.342	0.006	0.033	0.082
12 weeks	0.02	0.072	0.25	0.009	0.069	0.193
16 weeks	0.007	0.062	0.543	0.009	0.053	0.125
20 weeks	0.006	0.061	0.206	0.02	0.06	0.159
EOS (abs) 8 weeks	0.001	0.006	0.049	0	0.004	0.066
12 weeks	0	0.003	0.061	0.001	0.003	0.078
16 weeks	0.001	0.003	0.057	0	0.004	0.042
20 weeks	0.002	0.007	0.072	0.001	0.004	0.014
BASO (abs) 8 weeks	0.002	0.012	0.107	0.001	0.012	0.099
12 weeks	0.002	0.012	0.124	0.003	0.021	0.082
16 weeks	0.006	0.036	0.138	0.001	0.013	0.085
20 weeks	0.002	0.024	0.076	0.004	0.032	0.094
NEU (%) 8 weeks	11.09	19.3	27.69	6.571	17.25	26.01
12 weeks	9.573	15.5	27.75	6.855	12.5	24.22
16 weeks	11.44	17.2	36.78	8.226	13.4	34.39
20 weeks	14.44	20.5	34.3	11.13	15.25	28.6
LYM (%) 8 weeks	69.25	79.3	86.26	71.76	81.75	92.55
12 weeks	67.5	81.2	89.47	72.22	84.5	92.08
16 weeks	54.04	81.1	87.68	63.20	84.6	90.77
20 weeks	59.03	76.45	84.75	69.94	82.6	87.3
MON (%) 8 weeks	1.104	1.82	6.119	0.163	0.756	2.084
12 weeks	0.394	1.82	3.987	0.172	1.29	4.037
16 weeks	0.103	1.35	7.623	0.233	0.904	2.982
20 weeks	0.174	1.255	5.213	0.325	1.12	3.658
EOS (%) 8 weeks	0.002	0.145	1.112	0	0.076	1.187
12 weeks	0	0.057	1.061	0.001	0.058	1.47
16 weeks	0.013	0.062	0.904	0	0.072	0.73
20 weeks	0.061	0.154	1.776	0.016	0.069	0.338
BASO (%) 8 weeks	0.069	0.418	1.918	0.03	0.219	3.232
12 weeks	0.033	0.271	2.133	0.074	0.378	1.317
16 weeks	0.117	0.499	2.413	0.002	0.253	1.475
20 weeks	0.057	0.528	2.221	0.077	0.677	2.026
RBC 8 weeks	7.725	8.02	8.362	7.196	8.1	8.806
12 weeks	7.781	8.18	8.91	7.627	8.21	8.634
16 weeks	7.662	8.3	8.808	7.694	8.37	8.726
20 weeks	7.702	8.13	8.745	7.886	8.37	9.102
HGB 8 weeks	13.27	14	14.5	13.32	14.6	15.48
12 weeks	13.31	14.2	14.99	13.73	14.6	15.86
16 weeks	13.22	14.5	14.89	13.6	14.6	15.37
20 weeks	12.84	14.15	14.9	14.11	14.8	15.87
HCT 8 weeks	38.11	41.2	42.53	38.6	43.15	47.5
12 weeks	36.24	39.8	42.66	38.04	40.2	43.29
16 weeks	35.15	40.1	42.19	37.69	41.1	43.67
20 weeks	35.47	39.1	41.66	38.81	40.7	44.38
MCV 8 weeks	49.1	51.5	53.99	50.03	53.65	55.3
12 weeks	45.91	48.2	49.87	47.56	49.4	50.97
16 weeks	45.84	48.2	50.57	47.39	49	51.62
20 weeks	46.03	47.85	50.07	47.03	48.7	50.27
PLT 8 weeks	931.5	1039	1183	634.2	912.5	1071
12 weeks	986.6	1164	1300	837.9	1022	1179
16 weeks	1084	1183	1394	672.43	937	1137
20 weeks	861.2	1177	1327	821	1110	1336

For abbreviations see Table 1.

information about blood samples. Intuitive software, simple button switch between animal species, comprehensive QC package, high throughput (100 samples/h) made the instrument very user-friendly. The instrument is also very stable and did not require frequent recalibration.

The CELL-DYN® 3500 was found to perform very satisfactorily in virtually all areas. The results from precision, linearity, accuracy and carry-over studies were all remarkably good, when compared with reference instrumentation (see below).

Linearity

A linear relationship was found between expected and observed values of the WBC count, RBC count, HGB, HCT and PLT parameters measured by the CELL-DYN® 3500. The instrument exhibited an excellent performance in the following ranges (for rats): WBC $0.10–20.2 \times 10^3/\mu l$; RBC $0.016–14.3 \times 10^6/\mu l$; HGB 0.08–26.8 g/dl; HCT 5–77%; PLT $14.0–1670 \times 10^3/\mu l$; similar ranges were observed for mouse blood. The range of linearity for the measurements of RBC, HCT, HGB was quite remarkable, allowing accurate determination even at the very low and/or high RBC counts that can be expected in anaemic or polycythaemic blood samples. An extended range of linear measurements for WBC was found between 100 and 20 000/μl. Hence, correct WBC counts on leukopenic samples in the toxicology lab became possible. The linear range determination for WBC counts was not extended beyond 20 000/μl. This would have required the use of a higher number of animals, which we wanted to avoid for ethical reasons. However, from our observation with blood samples from other species with WBC values above 100 000/μl (data not shown) and the linearity range for human blood samples up to 100 000/μl, there is no doubt that WBC values up to 100 000/μl can also be determined in mouse and rat samples.

Accuracy

To determine the accuracy of the instrument, comparative measurements were performed by the CELL-DYN® 3500 versus the reference methods. The analysis of the coefficients of correlation showed a good agreement between the CELL-DYN® 3500 instrument and the reference method for NEU and for LYM. For MONO the correlation was modest ($r = 0.431$ for rats and $r = 0.727$ for mice). The lower r value for MONO can be explained by the smaller numbers of these cells present in the examined sample or by the fact that monocytes are sometimes difficult to distinguish from activated lymphocytes. The correlation for BASO and EOS was not so satisfactory in the rat and even less satisfactory in mouse blood. This is probably due to their very low numbers present in the blood samples, inducing a higher statistical variation. In addition, in mice, eosinophils appear to be recognised by the instrument less clearly than in rats. An explanation for this observation may lie in the fact that the granules of mouse EOS are finer than those of rats. In two cases of rats with microscopical eosinophilia (values of above 10%), the instrument precisely determined the percentage of EOS. Unfortunately, no samples from mice with hypereosinophilia were available, so proof that eosinophilia in mouse blood can be determined reliably remains open as yet.

Hence, this study shows that the instrument is capable of performing leucocyte differential counts on normal specimens and on those with leucocyte distribution abnormalities, with good accuracy for NEU, LYM, and in many cases for MONO, but less satisfactory results for low EOS and BASO.

Precision

The within-run precision for the parameters RBC, HGB, HCT, MCV, PLT and WBC were within the specifications stated by the manufacturer and compare very favourably with CV values of other instruments (Bollinger et al. 1987; Devreese et al. 1991). The CVs for NEU and LYM were very good for rats and mice. The CVs for MONO were somewhat higher, and even higher for EOS and BASO. These observations are explained on statistical grounds, since a decreasing number of cellular events are counted for these cell populations. However, these within-run precision data are better than has currently been reported on other instruments (Weingard et al. 1990; Davies and Fisher 1991).

To determine day-to-day precision, we relied on the stabilised human control blood samples provided by the manufacturer. Again, observed CVs for WBC, RBC, HGB, HCT, MCV, NEU and PLT were very low and exceeded the specification of the manufacturer. As with rats and mice, human blood CVs for LYM, MONO, EOS and BASO were higher according to the lower numbers present of these cell types. Generally, the low control blood sample exhibited higher CVs than the normal or high control blood sample, as had been expected for statistical reasons. An interesting observation was a low CV found for the determination of MONO in the ‘low’ control blood. This illustrates the strong performance of this instrument on leukopenic samples, but dilution studies will further support this hypothesis.

Ideally, precision studies should be made over the entire pathological range, but, as only a few pathological blood samples were available, it was impossible to determine precision in the low and high ends of the range with fresh whole blood. But day-to-day precision, which was performed with commercial controls, showed good results, proving the high quality of the instrument as well as the high quality of the control products.

Blood Ageing

Blood collection is associated with several pre-analytical variables, including the correct ratio of anticoagulant to blood volume, storage time in the anticoagulant and temperature of the blood specimen. The effect of ageing was determined in five rat and seven mouse samples for a period of up to 16 h. The parameters remained remarkably stable over the observation period with the exception of the MCV, which increased by 4% in 16 h. This latter observation reflects natural ageing of RBC and is an agreement with others. Thus, analysis of blood samples can be performed up to 16 h after collection without significant loss of precision and accuracy.

Carry-over

Carry-over from high to low samples was less than 1% for all parameters, and is excellent and better than that of most other instruments (Bollinger et al. 1987; van Leewen et al. 1991).

Pathological Samples

With some experience, the operator can readily recognise from the screen whether the RBC are normal in size and in distribution. From the PLT volume distribution curve it can be estimated visually whether the PLT number and distribution are adequate. Most important, by observing the scattergram of the leucocyte clusters, it can readily be concluded whether the cell differentiation is normal or pathological. The CELL-DYN® 3500 can differentiate most pathological samples. Our experience has shown that the system can recognise such pathological states as left shift, presence of lymphoblasts, monocytosis, elevated and decreased neutrophil and lymphocyte counts. In some pathological cases, when the instrument could not distinguish different leucocytes, default settings were chosen, which gives an indication that the sample has to be manually differentiated. It is particularly noteworthy that the instrument can count nucleated red cells, corresponding to the difference between the WOC and WIC counts, since the WIC counts all nucleated cells, whereas the WOC counts only leucocytes.

To summarise, we have found the CELL-DYN® 3500 to be a reliable and accurate differential cell counter for veterinary haematology in rats and mice. Routine use in the clinical laboratory of the CELL-DYN® 3500, which uses a small amount of blood (130 μl per analysis) and has a high throughput (100 samples per hour), permits a reduction in turnaround time and costs compared to the microscopic count. In addition, this allows the operators to direct greater efforts to those samples which have counting or distribution abnormalities. Therefore, it has potential to change the way the routine haematology laboratory operates.

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