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Evaluation of the LaserCyte® : an in-house haematology analyzer for dogs and cats

Wenger-Riggenbach, Bettina

Abstract: Das Ziel der Studie war, den LaserCyte®, der erste Flow Zytometer für die Bestimmung der hämatologischen Parameter von Katzen- und Hundeblutproben in der Privattierarztpraxis, zu evaluieren. Die Präzision wurde mittels Berechnung der Variationskoeffizenten (CV) bestimmt: Erythrozyten 3.9%, Retikulozyten 14.9% - 102%, Gesamtleukozytenzahl 3% - 9.5%, Neutrophile 3.9% - 6.5%, Lymphozyten 7% - 17.9%, Monozyten 4.9% - 13.1%, Eosinophile 10.4% - 32.1%, Basophile 7.8% - 32%, Thrombozyten 3.1% - 13.2% und Thrombozytenindizes 0% - 28.2%. Die Linearität ist auch ausserhalb des Referenzbereiches gegeben. Die Korrelationskoeffizienten (r) als Mass der Richtigkeit ergeben folgende Werte: 0.96 (Erythrozyten), 0.94 (Hämatokrit), 0.96 (Hämoglobin), 0.95 (MCV), 0.94 (Leukozyten), 0.93 (Neutrophile), 0.77 (Lymphozyten), 0.77 (Monozyten), 0.29 (Eosinophile), 0.03 (Basophile), 0.13 (Retikulozyten), und 0.86 (Thrombozyten). In Anbetracht der klinischen Relevanz wurden Proben mit Lymphozytenpenien in 51 von 89 Fällen, Monozytopenien in 1 von 11 Fällen und die Retikulozytenzahlen in 85 von 149 Fällen korrekt wiedergegeben. Zuverlässige Resultate werden für das rote Blutbild mit Ausnahme der Retikulozytenbestimmung, der Leukozytengesamtzahl und der Neutrophilenanzahl bei beiden Tierarten sowie der Thrombozyten beim Hund erhalten. Zur Erkennung von pathologischen Zellmorphologien, Vorläuferzellen und Blutparasiten ist die mikroskopische Untersuchung nach wie vor unerlässlich. In the present study, the LaserCyte®1 instrument, an automated flow cytometer for use in veterinary practice, was evaluated for dogs and cats. Precision (coefficient of variation, CV) for red blood cell (RBC) parameters was 3.9%, for reticulocytes between 14.9% and 102%, for white blood cells (WBC) between 3% and 9.5%, for neutrophils between 3.9% and 6.5%, for lymphocytes between 7% and 17.9%, for monocytes between 4.9% and 13.1%, for eosinophils between 10.4% and 32.1%, for basophils between 7.8% and 32%, for platelets between 3.1% and 13.2%, and for platelet indices between 0% and 28.2%. The range of linearity extended the reference ranges. The agreement with reference methods (coefficient of correlation, r) were 0.96 (RBC), 0.94 (haematocrit), 0.96 (haemoglobin), 0.95 (mean corpuscular volume), 0.94 (WBC), 0.93 (neutrophils), 0.77 (lymphocytes), 0.77 (monocytes), 0.29 (eosinophils), 0.03 (basophils), 0.13 (reticulocytes), and 0.86 (platelets). The LaserCyte® allowed the correct assessment of RBC and WBC parameters with respect to clinical relevance in the majority of samples. Lymphocytopenia was detected in only 51 out of 89 cases and monocytopenia in 1 out of 11 cases. The reticulocyte counts were correctly estimated in 85 out of 149 cases. It was concluded that the LaserCyte® allowed reliable determination of the RBC parameters, WBCs, neutrophils in both species and platelets in dogs. Based on its capability to reliably determine feline platelets and of the parameters mentioned above, this instrument is considered a useful analyzer for the veterinary practice. Qualitative microscopic assessment of blood smears is still necessary for detecting abnormal cell morphologies, cell precursors and blood parasites.

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Evaluation of the LaserCyte®: an in-house haematology analyzer for dogs and cats

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vorgelegt von

Bettina Wenger-Riggenbach

Tierärztin
von Rünenberg BL und Blumenstein BE, Schweiz

genehmigt auf Antrag von

Prof. Dr. med. vet. Hans Lutz, Referent
Prof. Dr. med. vet. Thomas Lutz, Korreferent

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Contents:

Evaluation of the LaserCyte®: an in-house haematology analyzer for dogs and cats

Bettina Wenger-Riggenbach, med. vet.[1]; Mike Hässig, PD Dr. med. vet. MPH FVH ECBHM ECVPH[2]; Regina Hofmann-Lehmann, Prof. Dr. med. vet, FVH[1]; Hans Lutz, Prof. Dr. med. vet. FVH FAMH[1]

Affiliations and addresses of the authors:

[1]Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland, [2] Department of Farm Animals, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland

Name of corresponding author:

Hans Lutz, Clinical Laboratory, Vetsuisse Faculty, University of Zurich

Winterthurerstr. 260, CH-8057 Zurich, Switzerland

Tel. +41-44-635-83-12, Fax. +41-44-635 89 06

Email: hlutz@vetclinics.unizh.ch

URL: http://www.vetlabor.unizh.ch

Abstract:

In the present study, the LaserCyte®[1] instrument, an automated flow cytometer for use in veterinary practice, was evaluated for dogs and cats. Precision (coefficient of variation, CV) for red blood cell (RBC) parameters was ≤ 3.9%, for reticulocytes between 14.9% and 102%, for white blood cells (WBC) between 3% and 9.5%, for neutrophils between 3.9% and 6.5%, for lymphocytes between 7% and 17.9%, for monocytes between 4.9% and 13.1%, for eosinophils between 10.4% and 32.1%, for basophils between 7.8% and 32%, for platelets between 3.1% and 13.2%, and for platelet indices between 0% and 28.2%. The range of linearity extended the reference ranges. The agreement with reference methods (coefficient of correlation, r) were ≥ 0.96 (RBC), ≥ 0.94 (haematocrit), ≥ 0.96 (haemoglobin), ≥ 0.95 (mean corpuscular volume), ≥ 0.94 (WBC), ≥ 0.93 (neutrophils), ≥ 0.77 (lymphocytes), ≥ 0.77 (monocytes), ≥ 0.29 (eosinophils), ≥ 0.03 (basophils), ≥ 0.13 (reticulocytes), and ≥ 0.86 (platelets). The LaserCyte® allowed the correct assessment of RBC and WBC parameters with respect to clinical relevance in the majority of samples. Lymphocytopenia was detected in only 51 out of 89 cases and monocytopenia in 1 out of 11 cases. The reticulocyte counts were correctly estimated in 85 out of 149 cases. It was concluded that the LaserCyte® allowed reliable determination of the RBC parameters, WBCs, neutrophils in both species and platelets in dogs. Based on its capability to reliably determine feline platelets and of the parameters mentioned above, this instrument is considered a useful analyzer for the veterinary practice. Qualitative microscopic assessment of blood smears is still necessary for detecting abnormal cell morphologies, cell precursors and blood parasites.

Keywords:

Haematology, flow cytometry, blood cell counting, leukocyte differentiation, evaluation, dogs, cats.

Introduction:

Haematology results provide important information on the patient's state of health, disease history and response to treatment. Blood cell counts and characteristics of cells can change quickly in drawn samples; rapid analysis of these parameters is therefore vital for clinicians. The invention of the Coulter cell counter and cell volume analyser in 1956 highly reduced time-consuming manual work by automating the counting and sizing of cells (Fulwyler, 1980). Today, instruments based on Coulter's impedance principle are widely distributed in veterinary practices. Another method to determine differential blood cell numbers is used by the centrifugal haematology analyzer (QBC® VetAutoread Haematology system[1]), which applies the principle of quantitative buffy coat analysis (Hofmann-Lehmann et al., 1998; Bienzle et al., 2000). Haematology instruments based on laser flow cytometry can provide additional diagnostic information, such as WBC differentiation or reticulocyte counts, but have typically been found only in reference laboratories and high-volume clinics due to their size and costs. While haemoglobin concentrations are usually measured by means of spectrometry, the QBC® VetAutoread Haematology system reliably deduces the haemoglobin concentration from the depth of submergence of the float in the RBCs (Hofmann-Lehmann et al., 1998).

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The LaserCyte® is the first in-house haematology analyser based on the principle of flow cytometry adapted for the needs of veterinary medicine in the smaller practice setting. Only little hands-on-time is necessary for sample analysis and for maintenance; the analysis time is 13 minutes per sample, which seems acceptable for veterinary practitioners. The aim of this study was to evaluate the LaserCyte[®] on feline and canine blood samples and to test the instrument with respect to the clinical relevance of its results.

Material and methods:

Blood samples:

Blood samples, were collected by venipuncture from 137 and 176 healthy and ill dogs and cats, respectively, regardless of sex, age or breed, during the routine clinical work at the Small Animal Clinic, Vetsuisse Faculty, University of Zurich. Samples were used to asses the precision, linearity and accuracy of the instrument. The samples were collected in tubes containing K3-EDTA[2]. After arrival at the laboratory, 500μl of the blood sample were transferred to special plain tubes[3] that fit the sample holder of the LaserCyte®. The remaining sample volume was subjected to the analysis by reference methods simultaneously to the analysis by LaserCyte®. All samples were analysed within four hours of collection with the exception of the determination of the precision which lasted up to six hours.

Instruments and methods used:

LaserCyte®:

The LaserCyte® provides results for 18 haematological parameters: counts of white blood cells (WBC), lymphocytes (LYM), monocytes (MONO), neutrophils (NEU), eosinophils (EOS), basophils (BASO), red blood cells (RBC), reticulocytes (RETI) and platelets (PLT) are determined directly in the flow cytometer; haemoglobin (HGB) is evaluated spectrophotometrically by a proprietary technique (see below), while haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), red cell distribution width (RDW), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW) are calculated. For the analysis of each sample a separate test tube is used. The test tube (CBC5R tube) contains New Methylene Blue, Purfac-A-39-Pric, buffers, preservatives, and latex beads. The latter are particles with a diameter of 4 µm and present in the tube in a known quantity; they are used for quality assurance within each sample. In the flow cytometer, a stream containing the cells is transported through a flow chamber, where each cell passes through a beam of laser light. The incident light is absorbed and scattered by the blood cells. The size and granularity of the cells are measured and the resulting extinction (EXT), the direction of scattered light (low angle forward light scatter (FSL), right angle scatter (RAS), high angle forward light scatter (FSH), the amount of scattered light and the time, during which a cell is exposed to laser light (time of flight (TOF)) are used to characterize each cell passing the device. The LaserCyte® performs two successive measurement steps. In the first step, the sample is analyzed to determine RBCs, RETI, PLT, MCV, and MPV. In addition, the amounts of scattered and absorbed light generated by the latex beads present in the

test tube are determined. They are used as an internal control of the gate settings, which in turn are relevant to assign the individual signals to populations of identical or similar cells. The Purafac-A-39-Pric in the test tube causes the RBCs to become spherical. The quantity of light scatter generated by the spherical RBCs is depending on the cell size, but not on the orientation of the cells. The RETIs are stained with the New Methylene Blue present in the test tube. They are differentiated by the instrument from mature RBCs by the different extinction and light scatter caused by the stained RNA fragments in the RETI. In the second measurement step, the RBCs are osmotically lysed by the addition of a hypotonic sheath solution and the WBC counts determined and the WBC differentiation is made. The haemoglobin concentration is determined during both measurement steps by spectrometry. In the first measurement step, the haemoglobin concentration is measured on the basis of the absorbance of intact, Methylene Blue stained RBCs. The specific absorbance spectrum associated with this staining solution is measured at four wave lengths. During the second measurement step, the RBCs are lysed. This facilitates the measurement of the free haemoglobin concentration in solution. As intact red cell membranes scatter light, the haemoglobin concentrations from the first measurement step is not as accurate as those of the second measurement step. However, comparison of the results of the two measurements serves as an internal control. If the two independent haemoglobin measurements and the dye ratio between the two solutions match, the correctness of the dilutions is confirmed. The sample preparation takes just a few seconds; the analysis itself takes 13 minutes using the software version 1.15, which was used in this study. To expedite the study, two instruments were made available. One instrument (DXBP 001484) was used only for the accuracy of the canine blood sample while the

other instrument (DXBP001412) was used for the remaining analysis (accuracy of feline blood, precision, linearity). This enabled us to run two samples in parallel. Quality control of the instrument is provided with each measurement by assessing the number and position of the latex beads on the dot plot and by the evaluation of the ratio of the two haemoglobin measurements mentioned above. No additional control measurements are necessary.

Reference methods:

The CellDyn 3500®[4] was used as the reference instrument (Kieffer J. et al., 1999). This haematology analyzer is based on the combination of the impedance method and flow cytometry. The white blood cells are counted by two separate channels, the electrical impedance channel (white blood cell impedance count = WIC) and the optical flow channel (white blood cell optical count = WOC). Differentiation of the WBC is done in the WOC channel. The haemoglobin concentration is measured spectrophotometrically on the basis of a hemiglobinhydroxylamine method. The indices of the red blood cells and platelets were calculated. The following parameters generated by the CellDyn 3500® were used as reference values: the counts of WBCs, RBCs of both species, and canine PLTs, the HGB, HCT, MCV, RDW, MCHC, MCH, MPV, PCT, and PDW. Microscopic methods were used to determine differential WBC and RETI counts in blood samples from dogs and cats and to investigate platelet counts in blood samples from cats. For the WBC differentials, blood smears were stained automatically using an automated staining instrument [5]. Two technicians with > 10 years of experience in veterinary haematology differentiated 100 cells per smear each. The mean of the 200 cells was used to calculate the percentage distribution of the WBC differentials.

Absolute values of leucocytes differentials were obtained by multiplying the absolute WBC counts of the CellDyn 3500® by the microscopically determined percentage of each WBC subpopulation, i.e. neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Manual RETI counts were performed by enumerating the RETI as a percentage of 1'000 mature RBC using a standard method based on Brillant Cresyl Blue stained blood smears. In blood samples from cats, only the aggregated reticulocytes were counted, which represent the more immature cells and are characterized by large clumps or strands of precipitated nucleoprotein (Perkins, Grindem, and Cullins, 1995). Absolute values of reticulocytes were calculated by multiplication of the microscopically determined fraction of RETI with the RBC counts of the CellDyn 3500®. The feline PLT counts were determined using a Neubauer haemocytometer [6]. This was necessary because feline platelets cannot be determined by the Coulter principle as large platelets overlap in volume with small erythrocytes. The blood was diluted at a ratio of 1:100 in phosphate buffered 1% ammoniumoxalat with Unopettes [7], which led to a lysis of the RBCs but not the PLT, WBC and RETI. PLTs were enumerated microscopically by two technicians experienced in veterinary haematology. The mean of the two counts were used as the reference value of PLT count.

Precision:

When the within-run precision of an instrument is determined, the reproducibility of the results is tested on the basis of repeated analyses of the same blood sample. The size of random errors is thus determined by calculation of the coefficient of variation (CV) (Knoll and Rowell, 1996). The precision of the LaserCyte® was determined on the basis

of 12 and in one blood sample of 16 analyses of fresh K3-EDTA-anticoagulated blood

samples. The blood samples had been collected from one dog with intermediate (17.03

x 10^3/µl), one dog with a low (4.53 x 10^3/µl), one dog with a high (82.59 x 10^3/µl), and

from one cat with an intermediate (9.26 x 10^3/µl) WBC count. For these measurements

at least 2 ml of blood were used. As the determination of the precision required a

relatively large amount of blood, precision was determined once only with cat blood in

order not to collect too much blood from an already sick cat. The blood samples were

stored at room temperature during testing. The precision was assessed for the counting

of WBCs, LYMs, MONOs, NEUs, EOSs, BASOs, RBCs, RETIs, and PLTs, and the

measurements of HGB, HCT, HCV, MCH, MCHC, RDW, PCT, and PDW. The

assessment of precision took between 3½ and six hours. During this time, cell aging

can occur. To compensate for these processes, which could interfere with the precision

measurement, the blood samples were analyzed simultaneously using the LaserCyte®

and the reference methods. In addition, uninterrupted analyses could cause the

LaserCyte® to overheat. To determine the effect of any instrument warming on the

blood cells, a 100-minute cooling-down phase was introduced after each eight analyses.

Linearity:

The linearity of the measurement range was assessed to determine whether blood

values outside of the reference interval can be measured correctly. Cell counts could be

underestimated when with increasing cell counts the probability of multiple cells passing

simultaneously through the orifice of the laser beam increases. The linearity of the

measurement range was assessed using two K3-EDTA anticoagulated canine blood

samples. One blood sample was used to assess the linearity of the measurement range

of WBC, LYM, MONO, NEU, HCT, RBC, HGB, and RETI. A second blood sample with especially high PLT count (673 x 10^3/µl) was used to determine the linearity of the PLT measurement. To obtain haematological values above and below the reference range, the two canine blood samples were centrifuged at 400 x g for ten minutes[8]. Some plasma was removed, and the concentrated HCT and blood cells were resuspended by careful mixing. The samples were then diluted with isotonic phosphate buffered saline (PBS[9]) in 10% increments from 100% (= undiluted samples) down to 10%. Measured values were plotted versus calculated vales in an x-y scattergram; the regression was calculated using the least square method and the equation of the resulting line was described as $y = a + bx$. The range of linearity was determined by visual inspection of the plots.

Accuracy:

The accuracy was measured by comparison of the LaserCyte® results with those of the reference methods. A total of 132 blood samples from dogs and 175 blood samples from cats were included. Around 90% of the blood samples were analysed within two, 10% within four hours after collection.

Evaluation of the clinical relevance of results obtained by LaserCyte®:

For each sample, the numeric data obtained by the LaserCyte ® were compared with accepted reference ranges for dogs and cats (Table 1). The results obtained from the canine and feline samples were interpreted to be either within the reference range or below or above the reference range. The corresponding results obtained by the reference methods were analyzed identically. The two resulting interpretations obtained

for each sample and each parameter determined by the two methods were compiled and compared to each other.

Statistical methods:

All data were compiled using the Excel program[10]. Precision was determined by calculation of the coefficients of variation (CV). The variation determined included not only the technical variation but also variation due to aging processes of the cells; the latter was assessed by calculating a regression line of the measured values over time and the aging effect was eliminated by calculating the CV of the mean plus the residual for each time point using a PC-based statistical program[11] (Altman, 1994). To determine whether changes of the values over time were significant, the p values of the regression coefficients were calculated; p values of ≤ 0.05 were considered significant. Linearity and accuracy were calculated on the basis of determination of the regression line of the form y = a + bx and calculation of the coefficient of correlation (r) using the Excel program[10]. Accuracy was calculated by method comparison using the Passing Bablok method (Passing, Bablok, and Glocke, 1981; Passing and Bablok, 1983; Eisenwiener et al., 1984; Passing and Bablok, 1984; Bablok and Passing, 1985; Bablok et al., 1988). In addition, method comparison was also done by the difference plots described by Jensen (Jensen, 2000). To test whether the differences between the methods deviated significantly from zero the Wilcoxon Signed rank test was used (Jensen, 2000).

Message Codes:

Message codes are displayed by the LaserCyte[®] when the instrument's system for quality assurance is not able to assign certain cells to a population or when certain limit

values are exceeded. The messages draw the user's attention to abnormal blood samples or technical problems. When results were displayed with a message code, samples were analysed a second time. If the second analysis of a blood sample yielded again a message code, the results of the entire run were excluded from the determination of the accuracy with the exception of the samples that had been marked with a MPV-flag. The latter message code ("MPV out of reportable range") is displayed if the MPV is supposedly outside the 2 – 30 fl range. For samples with a MPV message code, only the MPV values were disregarded; all other parameters were included in the analyses as they should not have been affected. In samples displaying a message code twice the reason for the message code was evaluated by additional examination of the dot plots and the smear; the results were compiled separately in a table.

Results

Display of measured values:

After each measurement, the LaserCyte® displays the results of the 18 parameters analyzed on a report form together with reference values for juvenile, adult and senile dogs and cats. In addition, the dot plots can be printed out. An experienced user can thus check the measurement visually. The dot plots for the first measurement step, relating to RBCs, reticulocytes and platelets are shown in figure 1 A and the dot plot for the second step, relating to WBC differentiation, in figure 1 B. The dot plots of the feline and canine blood samples in principle are quite similar. The cell populations and the latex beads are represented by coloured clouds and the gates set by the instrument to discriminate the different cell populations are indicated by black lines. The correct differentiation of the cells can be estimated on the basis of the dot plots. In 25 feline and

in two canine blood samples, a shift to the upper left of cell population representing the neutrophils was observed (figure 1 C). This shift indicates the presence of stab neutrophils. In 12 out of these 25 feline blood samples and in both of the canine blood samples, a left shift of the neutrophils could also be found microscopically. An additional two feline and 16 canine blood samples, stab neutrophils were found microscopically but no left shift of the neutrophils was seen on the dot plots.

Precision:

The precision of instrument no.DXBP001412 was determined using software version 1.15. The mean values and coefficients of variation for within-run precision are summarized in Table 2. The following message codes were obtained during the assessment of the precision: in the canine blood sample with a mean WBC count of 4.53×10^3/µl: four times "DECAY" standing for a significant number of WBC which decayed during the analysis; in the canine blood sample with a WBC count of 17.03×10^3/µl: two times "differential algorithm issues" concerning problems with the differentiation of lymphocytes and monocytes; in the canine blood samples with a WBC count of 82.54×10^3/µl: "differential algorithm issues" concerning the differentiation of neutrophils and monocytes in every run. In the feline sample, three times the message code "MPV out of reportable ranges" was shown. Measurements associated with a message code were excluded from the study.

During the precision studies it was found that the results changed with time. Therefore, the results were analyzed for systematic changes. The influence of cell ageing and instrument warming was examined. Figures 2 A and 2 B show the analysis of cell

ageing and possible effects of instrument warming. The cooling break did not result in a

visible alteration of the precision in all measured parameters. The changes of the results

caused by the sample ageing process are presented in Table 3. To prevent this

biological process of cells from having a negative impact on measurement precision, the

CV was calculated by means of analysis of residues.

Linearity

The parameters and ranges for which linearity was tested are compiled in Table 4. In

addition, the linearity for RBC measurements is shown in figure 3. For basophils and

eosinophils, the linearity was not assessed as the cell numbers were too small. It

became evident that the measurement of all parameters evaluated by the LaserCyte®

was linear at least within the tested ranges.

Accuracy:

The results of the comparison of methods are listed in Table 5; one example (WBC) of

the comparison is graphically displayed in figure 4. With the CellDyn 3500®, the platelet

indices MPV, PCT and PDW are only displayed if a certain species–specific limit value

for platelets is exceeded. Only 59 out of 116 canine blood samples and 19 out of 129

feline blood samples exceeded this limit value. In view of the relatively low clinical

significance of the platelet indices, these were excluded from the comparative study.

The evaluation of the accuracy by visual inspection revealed values which were clearly

separated from the regression line and were therefore considered "outliers". A total of

12 outliers were identified, namely for monocytes (one dog), basophils (one cat, one

dog), eosinophils (three cats, three dogs), HCT (one cat, one dog), and reticulocytes (one dog).

Evaluation of the clinical relevance of results obtained by LaserCyte®:

The evaluation of the LaserCyte® results with respect to their clinical relevance in comparison to the results of the reference methods are compiled in Tables 6A and 6B. Every LaserCyte® result that may have led to a clinical conclusion different from that of the reference method, was individually judged with respect to the degree of the deviation and two categories were difined: if the discrepancy was minor e.g. the MCHC of the reference method was 33 (reference values 32 – 36 g/l) and the LaserCyte® had 31.7g/l the discrepancy was considered minor. Minor deviations were usually << 10% of the lower or upper reference values. Major deviations were defined as discrepancies that would have led to a severely different clinical interpretation e.g. > 10% of the lower and upper reference value.

Message codes:

The distribution of the various message codes in terms of numbers is summarized in Table 7.

One or more message codes were displayed for 16 out of 137 canine blood samples (11.7%) and 97 out of 176 feline blood samples (55%). A total of 38 samples (ten canine and 28 feline) were marked with "Differential algorithm issues" or "Possible rate analysis issue" because of difficulties in assigning individual cells to a certain group. As recommended for each of these samples, the blood smears were inspected microscopically. In the only canine blood sample with the message code concerning the

differentiation of the monocytes and lymphocytes, a moderate left shift was observed by microscopy. In the two feline blood samples no obvious reason for the message code "differential algorithm issues" was found. In six canine and in eight feline blood samples no explanation was found for the message code "differential algorithm issues" regarding the separation the monocytes from the neutrophils. In three out of nine canine blood samples and in two out of 12 feline blood samples the message code "differential algorithm issues" was associated with a left shift. In one feline and one canine blood sample an increased number of normoblasts was seen (9/100 WBC, 56/100 WBC respectively) and in one cat suffering from a lymphoma some atypical cells were found which might have caused the message code. In the microscopic examination of the 14 feline blood samples with the message code "Possible rate analysis issue" no hint as to the cause of this message code was found. In 81 out of 113 message codes observed in feline blood samples the message codes were related to feline platelets (69 "MPV out of reportable range", 12 "PLT Aggregations"). In the 12 feline samples and the one canine sample displaying the message code "PLT Aggregation", platelets were inspected microscopically. In seven feline blood samples the message code could be confirmed while in the six remaining samples no obvious aggregation was found on the blood smears. One of nine samples flagged with the message code "MCHC out of reportable range", was hyperlipaemic. Three samples marked with the message code "Too many RBC fragments" could be explained by haemolytic anaemia.

Discussion:

General feasibility of the LaserCyte®:

The LaserCyte® is the first flow cytometer designed for haematology in private veterinary practice. It is an extremely user-friendly instrument and can be used after approximately one hour of training. It took approximately 13 minutes to analyse one blood sample. (According to the manufacturers information the current software version should need about ten minutes for analyzing a blood sample.) The instrument was evaluated over a period of nine months. Towards the end of the evaluation, one instrument became clogged, requiring the intervention of a technician.

Representation of the measured values:

There was a strong association between the left shift of neutrophils in the blood smears and an appearance of a neutrophil population extending to the upper left in the dot plot (12 out of 14 cats with left shift). It was concluded that appearance of this extension to the upper left of neutrophils present in the dot plot is a criterion for a left shift in cats. The same phenomenon can be observed in the dot plots of the CellDyn 3500®. In 12 out of 14 feline blood samples of this study, the shift of the neutrophils to the upper left region of the dot plots correlated clearly with a left shift detected microscopically. In 13 feline samples were the LaserCyte® detected a shift of the neutrophils to the upper left region of the dot plots, no left shift was seen in the blood smear. A possible explanation for this discrepancy between the LaserCyte® and the microscopical findings could be offered by an increased sensitivity of the LaserCyte® to detect immature neutrophils in cats. However, two samples with a microscopically confirmed left shift were not recognized by the LaserCyte®. It is suggested that samples with a shift of neutrophils to

the upper left region of the dot plot are microscopically checked for presence of stab neutrophils. In canine blood samples, there was no correlation between a shift of the neutrophils to the upper left region of the dot plot and the microscopic detection of stab neutrophils. In only two out of 16 samples with microscopically detected stab neutrophils a shift to the upper left region of the dot plot was observed. In order not to miss left shifts in canine blood samples, it is therefore recommended to qualitatively evaluate each blood smear by microscopy.

Precision:

Usually, CVs within series of up to 3% are considered good and up to 5% acceptable for cell counts (Bollinger et al., 1987; Winkler et al., 1995; Hofmann-Lehmann et al., 1998). For statistical reasons CV may be higher if the absolute number of counted cells is small. Good to acceptable precision was achieved for the measurement of the RBC parameters, with the exception of reticulocytes in both species, and platelets in dogs. The precision for reticulocyte counts for the feline blood sample and the canine sample with high WBC was in the range of the microscopy, which was 12.9% (data not shown). The two other canine samples gave very high CVs of up to 102%. A valid explanation for these unacceptable high CVs can not be offered. It is speculated that the differentiation between mature red cells and reticulocytes on the basis of different extinction and light scatter caused by ribosomal fragments of the reticulocytes by the LaserCyte® is insufficient. For the WBC, the precision of the measurements in the canine blood samples with average and high WBC counts was considered good; in the feline and the canine blood samples with low WBC counts the precision was moderate. The precision of the monocyte and neutrophil measurements were good in the canine

blood sample with average WBC counts; it was moderate in the other blood samples. The CV for lymphocytes of between 7 – 17.9% was in the same range as that of microscopy (16.2%, data not shown). Lymphocyte precision has been a focal point of algorithm advancements in the software of the LaserCyte®. Subsequent software releases may improve the separation of the cell populations. The high CVs for eosinophils and basophils can be explained by the low mean values and cannot be attributed solely to the measurement system. Therefore, the precision of the instrument for the eosinophils and basophils cannot be specified. The relatively high CV of 13.2% found for the measurement of feline platelets can be explained by the fact, that feline platelets are relatively large and often have a tendency to form aggregates, which break up over time resulting in increasing numbers (Norman et al., 2001). However, the high precision of the PDW measurements (CV 2.2 – 3%) contradicts the general observation that platelets form aggregates; no explanation for this discrepancy can be offered. The precision of the MPV and PCT measurements can be rated as good in dogs with low and normal WBC counts. The higher CV for the MPV and PCT measurements in a canine blood sample with leucocytosis (28.2 % and 19.8 %, respectively) and a feline blood sample (6.1 % and 18.2%, respectively) may be explained by the pathologically high variability in platelet size in these two patients.

The advantage of an electronic cell counter over manual differentials is the high number of counted cells. This results in a better statistical distribution and a higher precision of measurements than can be achieved with any microscopic method (Pohland, 1989). During the determination of the within run precision, we observed systematic changes over time for some parameters (Table 3). In order to determine whether these systematic changes may be due to the instrument warming, measurements were

interrupted to let the instrument cool. From the observation, that the systematic changes continued it was concluded that the phenomenon was caused by ageing of the samples and not by the instrument warm-up. Although the change over time in absolute values was not very large (with the possible exception of monocytes and platelets in the feline samples) some of these changes were significant. Similar ageing effects were also seen in the reference method CellDyn 3500 (data not shown) and therefore cannot be attributed to the LaserCyte®. Still, these changes appear to be higher than in an other report (Sachse and Henkel, 1996) where - in contrast to our study - normal healthy subjects were used for blood collections to be used in a ageing study. We therefore conclude that especially in ill animals in some of the parameters an ageing effect has to be considered already in the first few hours after collection. In this context it is important to note, that the cat used for the precision study was FeLV infected. FeLV is known to replicate among others in megacaryocytes and leukocytes, which may explain the accelerated loss of these cells during storage.

Linearity of the measurement range:

From the observation that all parameters measured showed no deviation of linearity over the range tested, which was reflected by coefficients of correlation range from 0.849 to 0.998, we concluded that the linearity was excellent. The linearity of basophils and eosinophils measurements was not calculated because of the very low cell counts even in the undiluted blood sample (basophils 0.22 x10^3/µl and eosinophils 0.39 x10^3/µl).

Accuracy:

The correlation coefficient (r) is a measure of the comparability of measurement methods. Under linear conditions, an r of 1 or -1 reflects total consistency between the results of both methods. An r of >0.95 can be rated as very good and an r of >0.8 as acceptable (Tisdall, 1985; Bollinger et al., 1987; Tvedten and Wilkins, 1988; Winkler et al., 1995). In addition to the determination of r, the intercept and the slope have to been considered (Tvedten and Korcal, 1996). If the intercept and the slope deviate from zero and one, respectively, systematic errors have to be suspected. In order to characterize the method comparisons in the present study, we have not only evaluated the coefficient of correlation but also the intercept and the slope. In addition, to determine presence of absolute and proportional inaccuracies, difference plots were carried out (Jensen, 2000). Good to very good correlation between the LaserCyte® results and those of the reference methods could be observed for WBCs, NEU, RBCs, HCT, HGB and MCV in both species and for platelets in dogs. Acceptable correlation was obtained for lymphocytes and monocytes in dogs and platelets and MCH in cats. The correlation for feline lymphocytes and monocytes was less satisfactory. This was probably attributable to the wide scatter of lymphocyte counts around the regression lines in the presence of only moderate precision. In ten out of 12 samples the reference method (manual differential in percent times the optical white cell count) found a lymphocytopenia, which was not readily detected by the LaserCyte®. This became especially evident when difference plots were evaluated (data not shown). It was not determined to what degree this discrepancy was caused by the reference methods or the LaserCyte®. The numbers of monocytes in both species were generally higher in the LaserCyte®. Again, this could be explained by difficulties of the instrument to gate

monocytes clearly from the other white blood cells, or by the fact that white blood cells, especially monocytes are not evenly distributed on blood smears (Tvedten and Wilkins, 1988). In the presence of good to very good precision, the fact that the correlation for RDW and MCHC in both species and MCH in dogs was only moderate is attributable to the narrow biological range of scatter associated with these parameters (cloud phenomenon). In addition to the cloud phenomenon the distribution plots of canine MCHC revealed a slight systematic underestimation of MCHC of the LaserCyte® in the lower range and an overestimation of the values in the higher ranges. It was not determined to what degree the two methods contributed to this discrepancy. The correlation was poor for eosinophils, basophils and reticulocytes. In case of eosinophils and basophils, the insufficient accuracy can be explained by a low precision of both methods and the lack of a sufficient number of samples with significant counts of these cell types. No explanation can be offered for the low correlation of the LaserCyte® with the reference methods for the reticulocytes. Of the 12 outliers, nine could be explained by a discrepancy between the LaserCyte® and the reference method concerning the differential, suggesting that the outlier is explained by the uneven distribution on the smear or the low cell counts. One of the outliers regarding the reticulocytes was explained by presence of a high number of normoblasts (60/100 WBCs). This outlier was evident from the dot plots showing an overlap of reticulocytes and RBC. No explanation could be offered for the two outliers that concerned the haematocrit.

Message codes:

Message codes are displayed when the LaserCyte's® system for quality assurance is not able to verify the results of a particular parameter. The message code "differential

algorithm issues" and "possible rate analysis issue" are displayed when there are difficulties assigning the leukocytes to the respective populations or when a large number of WBCs decompose during the analysis process. In four out of ten canine blood samples and in four out of 28 feline blood samples, a left shift of the neutrophils or an increased number of normoblasts might have been the reason for these message codes. In order not to miss these clinically important parameters, we recommend to confirm the WBC differential with a blood smear under the microscope. In some cases, platelet aggregates, which occur frequently in cats for biological reasons (Norman et al., 2001), are difficult to distinguish from WBC populations. Occurrence of possible platelet aggregates is indicated by the LaserCyte® with the message code "platelet aggregation". The aggregates could be confirmed by microscopy in seven out of 13 flagged samples. One canine and 69 feline samples had a message code regarding the MPV. These results couldn't be further evaluated as the CellDyn 3500® usually does not yield MPV-values for feline and canine samples. The MPV results outside the 2-30 fl range and MCHC results outside the 24.5-39.5 g/dl range are flagged with the messages "MPV out of reportable range" or "MCHC out of reportable range", respectively. In the presence of fragile RBCs, the message "too many RBC fragments" indicates that fragile RBCs may interfere with the platelet count. The samples flagged by this message originated from canine haemolytic blood samples. If the message "too many RBC fragments" is set by the instrument, it is recommended to estimate the platelet and the erythrocyte counts on a blood smear. The message "internal QA failure", which appeared three times during the study, indicates inadequate detection of the latex beads. This means that the internal quality control is not assured and the analysis must be repeated. Other message codes not discussed here may also be

displayed. The instrument also informs the user of the measures to be taken when these message codes show up.

Evaluation of the clinical relevance of results obtained by LaserCyte®:

The vast majority of all results obtained by the LaserCyte® would have led to the same clinical interpretation as the results obtained by the reference methods. In the following parameters the discrepancy between the results of the LaserCyte® and the reference methods were minor and wouldn't have led to different clinical conculsions: RBC, MCV, HCT, and HGB in both species. In WBC and NEU in 18 out of 28 and 13 out of 26 blood samples respectively, the discrepancy would have led to different clinical consequences. In addition, ten of 11 canine samples with low monocyte counts were not detected by the LaserCytes®. This however was of no clinical relevance and could be explained by lack of detection of low monocyte counts in the blood smear. There were three parameters where the LaserCyte® values were in serious discrepancy with the results of the reference methods: lymphocytes, and reticulocytes of both species and platelets in cats. The LaserCyte® did not detect 26 of 34 canine samples with lymphocytopenia, and 20 of 23 dogs with increased reticulocyte counts. In the cat, 12 of 55 lymphocytopenias were not detected. In the reticulocytes 12 of 50 feline samples that were within the reference range by the reference method had increased reticulocyte counts by LaserCytes®. In feline samples with low platelet counts the LaserCyte® would have missed 18 of 67 thrombocytopenias.

Thus, as a consequence of the analysis of the clinical relevance it became evident that the LaserCyte® has some weaknesses in detecting lymphocytopenia in dogs and cats, thrombocytopenias in some of the cats, and is unable to correctly judge increased

numbers of reticulocytes in the dog and normal reticulocyte counts in the cat. These problems could be solved by the determination of reference values for dogs and cats by the LaserCyte® and/or by adapting the software which processes the signals created by each cell in order to have a clearer separation between the different cell populations.

Conclusion: The instrument is very suitable for use in private veterinary practice. With the LaserCyte®, the results of haematological analysis are obtained with little effort within 13 minutes. Results for WBC, NEU, RBC, HCT, HGB, MCV in both animal species and platelet measurements in dogs are obtained with a high degree of reliability. According to the evaluation of the clinical relevance of results obtained by the LaserCyte® the user has to be aware of the possibility to miss lymphocytopenias in both species, reticulocytosis in dogs and - despite the noteworthy good correlation for the automated feline platelet counts - thrombocytopenias in cats. In cases where no clear evaluation is possible, this is indicated by appropriate message codes. The dot plot evidence of a left shift in cats is also very useful. In the dot plots of canine blood samples, however, left shifts can not be identified. The ability to recognize abnormal cell morphologies and blood cell precursors is limited with all types of automatic cell counting. In order to recognize blood samples with such abnormalities, it is suggested to evaluate all the samples qualitatively by microscopy in addition to measurement by the instrument.

Footnotes

[1] IDEXX Laboratories, Westbrook, Maine 04092, USA

[2] Micro tube K3-EDTA, Sarstedt, D-51588 Nümbrecht

[3] BD Vacutainer, No Additive, Franklin Lakes, NJ 07417-1885

[4] Abbott AG, Baar, Switzerland

[5] Hema Tek 1000, Bayer AG, Zürich, Switzerland

[6] Assistant Germany, Karl Hecht AG, D-97647 Sandheim

[7] Becton Dickinson and Company, Ranklin Lakes NJ 07414-1885, USA

[8] Hettich Centrifuge, Rotanta 460 S, 8806 Bäch, Switzerland

[9] Dulbecco's Phosphate Buffered Saline, SIGMA-Aldrich Company, LTD Ir vine,

Ayrshire KA 12 8NB, UK

[10] Excel 2003, Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-6399,

USA

[11] Stat View software, Version 5, SAS Campus Drive, Cary, NC 27513-2414, USA

Legend to the figures:

Figure 1 A: Scattergram presentation of normal feline red cells, reticulocytes and platelets. x-axis: right angle scatter, y-axis: extinction of every cell passing the laser. a = RBC, b = RBC-Doublets, c = PLT, d = RETI.

Figure 1 B: Scattergram presentation of normal feline white cells. x-axis: right angle scatter, y-axis: extinction of every cell passing the laser. a = NEU, b = MONO, c = LYM, d = BASO, e = EOS, f = Cell Debris, g = Latex Beads

Figure 1 C: Representative scattergram presentation of a shift of the neutrophil population to the upper left region indicating an increase of stab neutrophils in a feline blood sample. x-axis: right angle scatter, y-axis: extinction of every cell passing the laser. Black arrow = usual direction of the neutrophil population; Red arrow = shift of the neutrophil population to the upper left region.

Figure 2: Aging effect on RBC values over an observation time of six hours. A canine blood sample was aliquoted into 16 tubes, which were stored at 20°C and analyzed by the two instruments in 20 minute intervals. To determine the effect of instrument warm-up on the blood cells, a 100 minute cooling-down phase was introduced after the eighth analysis. Red arrow 100 minute cooling-down phase.
A: results obtained by the LaserCyte®; y = 0.04x + 5.15, B: results obtained by the CellDyn 3500®, y = 0.02x + 5.12.

Figure 3: Linearity of the measurement range for RBC over a range of 0.25 x10^6 –

11x10^6 RBC/µl. x-axis: values measured by LaserCyte®, y-axis: RBC values calculated

for the respective dilutions, y = 0.89x + 0.87.

Figure 4: Method comparison of WBC counts determined by CD 3500® (x-axis) and

LaserCyte® (y-axis). The linear regression line and coefficient of correlation were

calculated according to Passing and Bablock[4-6]; n = 116, r = 0.98, y = 0.93x + 0.56,

Table 1: Reference values of haematological parameters for dogs and cats used in this study[a].

Parameter:	Dogs:	Cats:
WBC (x10^3/µl)	6 – 17	5.5 – 19.5
LYM (x10^3/µl)	1 – 4.8	1.5 – 7
MONO (x10^3/µl)	0.15 – 1.35	0 – 0.85
NEU (x10^3/µl)	3 – 11.5	2.5 – 12.5
EOS (x10^3/µl)	0.1 – 1.25	0 – 1.5
BASO	Rare	Rare
PCV (%)	37 – 55	24 – 45
RBC (x10^6/µl)	5.5 – 8.5	5 – 10
HGB (g/dl)	12 – 18	8 – 15
MCV (fl)	60 – 77	39 – 55
MCHC (g/dl)	32 – 36	31 – 35
RETI (%)	0 – 1.5	0 – 0.4
PLT (x10^3/ul)	200 – 500	300 – 800

[a] According to Feldman et al "Schalm's Veterinary Hematology" (Feldman, Zinkl, and Jain, 2000), fifth edition, pages 1058 and 1065.

Table 2: Within-run precision: Mean value and coefficient of variation for blood samples from dogs with either low, normal or high total WBC counts and a cat with a normal WBC count.

Parameter:	Canine: WBC 4.53x10^3/µl N = 12		Canine: WBC 17.03x10^3/µl n = 12		Canine: WBC 82.59x10^3/µl n = 16		Feline: WBC 9.26x10^3/µl n = 12	
	mean	CV %	mean	CV %	mean	CV %	mean	CV %
WBC	4.53	7.9	17.03	3.4	82.59	3	9.27	9.5
LYM	1.01	16.6	1.14	7	2.35	9.1	4.77	17.9
MONO	0.43	13.1	2.04	4.9	**	**	0.57	7.8
NEU	3.10	6.4	13.58	3.9	**	**	3.44	6.5
EOS	0.06	32.1	0.26	18.5	1.60	10.4	0.42	11.4
BASO	0.03	11.3	0.06	32	0.47	7.8	0.07	16
HCT	43.27	3.1	34.63	2.3	38.30	2.3	44.23	2.6
RBC	5.97	3.1	4.93	2.3	5.49	2.3	9.54	2.5
HGB	14.88	1.6	11.14	1.5	12.39	1.3	14.40	0.8
RETI	86.86	102	22.38	42.7	17.72	14.9	48.58	18.2
MCV	72.48	0.4	70.27	0.3	69.80	0.3	46.34	0.3
RDW	15.67	0.4	15.68	0.5	16.74	0.6	18.73	1.1
MCHC	34.39	3.9	32.23	3.2	32.42	2.4	32.60	2.7
MCH	24.92	3.9	23.87	3.1	22.60	2.5	15.12	2.7
PLT	308.17	5	202.85	3.1	103.25	4.4	365.92	13.2
MPV	12.74	4	16.77	4.9	21.30	28.2	28.37	6.1
PCT	0.40	0	0.35	7.3	0.24	19.8	1.08	18.2
PDW	19.62	2.2	20.86	2.5	25.68	2.4	20.26	3

** Message codes in every analyses.

Table 3: Age-related changes in measured values observed in two canine blood samples containing 17.08 x10^3/ul, and 82.59x10^3/µl WBC respectively, and in a feline blood sample with a WBC count of 9.26 x10^3/ul.

Age-related change	Parameter	% Deviation after 4-6 hours of aging		
Parameters with significant increase of values ($p < 0.05$)		Canine blood sample; WBC 17.08 x10^3/µl	Canine blood sample; WBC 82.59x10^3/µl	Feline Blood sample; WBC 9.26x10^3/µl
	HGB	+ 4%	+ 9%	-
	MCV	+ 3%	+ 2%	-
	HCT	-	+ 9%	-
	RBC	-	+ 10%	-
	RETI	-	+ 30%	-
	PDW	+ 6%	+ 5%	+ 10%
	MONO	-	-	+ 23%
Parameters with significant decrease of values ($p < 0.05$)				
	MPV	- 20%	-	-
	PLT	- 18%	-	- 60%
	PCT	- 30%	-	-
	LYM	-	- 8%	- 30%
	WBC	-	-	- 16%

Table 4: Linearity of the measurement range of blood samples from two dogs with coefficient of correlation, regression line, and the range of the tested parameters.

Parameter	Coefficient of correlation r	Regression line		Range tested for linearity	Reference range according to the LaserCyte® for adult dogs
		Intercept a	Slope b		
WBC	0.997	7.11	0.89	2.74 – 75.07 x10^3/ul	5.5 – 16.9 x10^3/μl
LYM	0.943	0.05	0.78	0.16 – 1.66 x10^3/ul	0.7 – 4.9 x10^3/μl
MONO	0.899	0.53	1	0.05 – 1.93 x10^3/ul	9.1 – 1.4 x10^3/μl
NEU	0.997	6.88	1.12	2.39 – 70.9 x10^3/ul	2 – 12 x10^3/μl
HCT	0.998	6.07	0.89	1.9 – 77.4 %	37 – 55 %
RBC	0.997	0.87	0.89	0.25 – 11.02 x10^6/ul	5.5 – 8.5 x10^6/μl
HGB	0.998	0.23	0.83	3.3 – 22.3 g/dl	12 – 18 g/dl
RETI	0.849	7.05	0.90	27.3 – 68.6 x10^3/ul	None
PLT	0.995	-53.33	1.03	131 – 950 x10^3/ul	175 – 500 x10^3/μl

Table 5: Accuracy of the LaserCyte®, determined by comparison of the results with those obtained by reference methods. In addition, the significances of the differences of methods were calculated according to the Wilcoxon Signed Test.

Parameter	Species	Sample number n	Coefficient of correlation r	Inter-cept a	Slope b	Significance of differences of methods [b]
WBC	Dog	116	0.983	0.564	0.926	0.0765
	Cat	129	0.944	-0.871	1.084	0.3231
LYM absolute	Dog	116	0.798	0.482	0.938	**<0.0001**
	Cat	123	0.769	0.168	0.915	0.1921
MONO absolute	Dog	116	0.809	0.401	1.146	**<0.0001**
	Cat	123	0.767	0.210	2.025	**<0.0001**
NEU absolute	Dog	116	0.983	0.206	0.849	**<0.0001**
	Cat	123	0.934	-0.863	1.007	**0.0035**
EOS absolute	Dog	116	0.289	0.171	0.158	**<0.0001**
	Cat	123	0.632	0.251	0.769	**<0.0001**
BASO absolute	Dog	116	0.032	-0.336	31.097	**<0.0001**
	Cat	123	0.084	0.042	1.489	**<0.0001**
HCT	Dog	116	0.960	-1.756	1.036	0.1196
	Cat	129	0.944	-1.437	1.120	**<0.0001**
RBC	Dog	116	0.961	0.032	0.978	**<0.0001**
	Cat	129	0.968	0.053	0.986	0.5872
HGB	Dog	116	0.962	0.443	0.960	0.0536
	Cat	129	0.985	-0.039	1.023	**<0.0001**
RETI absolute	Dog	91	0.360	21.982	-0.240	**0.0003**
	Cat	58	0.128	-179.541	15.612	**<0.0001**
MCV	Dog	116	0.951	4.690	0.941	**<0.0001**
	Cat	129	0.953	1.799	1.041	**<0.0001**
RDW	Dog	116	0.777	8.077	0.503	0.0052
	Cat	129	0.754	9.525	0.517	0.9916
MCHC	Dog	116	0.094	-638.49	19.605	0.9637
	Cat	129	0.276	-158.9	5.638	**<0.0001**
MCH	Dog	116	0.712	-4.009	1.181	0.0866
	Cat	129	0.845	-4.050	1.309	**<0.0001**
PLT	Dog	116	0.946	35.202	0.793	**<0.0001**
	Cat	79	0.862	-4.805	1.520	**<0.0001**

[b] difference plots evaluated by Wilcoxon Signed Rank Test (Petrie and Watson, 1999)

Table 6 A and B: Clinical relevance of results obtained by LaserCyte® discrepant from those obtained by reference methods:

Table 6A: Canine samples

Parameter	n total	Correctly recognized canine samples /evaluated samples			n not correctly recognized canine samples		
		< reference range	Within reference range	> reference range	Total	Minor deviations (definition see text)	Major deviations (definition see text)
RBC	116	23/24	84/88	3/4	6	6	-
MCV	116	1/2	107/108	5/6	3	3	-
HCT	116	21/23	87/88	4/5	4	4	-
HGB	116	14/15	85/92	4/9	13	13	-
MCHC	116	1/2	81/113	0/1	34	23	11
PLT	116	13/16	63/71	13/29	27	15	12
RETI	91	None	63/68	3/23	25	4	21
WBC	116	13/15	79/85	14/16	10	8	2
LYM	116	8/34	78/80	1/2	29	2	27
MONO	116	1/11	74/93	12/12	29	4	25
NEU	116	6/6	82/88	18/22	10	5	5
EOS	116	3/26	74/87	0/3	39	15	24

Table 6B: Feline samples

Parameter	n total	Correctly recognized feline samples /evaluated samples			n not correctly recognized feline samples		
		< reference range	Within reference range	> reference range	Total	Minor deviations (definition see text)	Major deviations (definition see text)
RBC	129	8/8	109/111	7/10	5	5	-
MCV	129	2/17	110/112	None	17	15	2
HCT	129	7/12	108/115	2/2	12	8	4
HGB	129	8/10	115/117	2/2	4	4	-
MCHC	129	None	81/127	1/2	47	38	9
PLT	79	49/67	9/11	1/1	20	5	15
RETI	58	None	12/50	7/8	39	8	31
WBC	129	14/18	93/102	6/9	16	10	6
LYM	123	43/55	56/68	None	24	6	18
MONO	123	None	59/112	11/11	53	5	48
NEU	123	9/10	82/92	16/21	16	8	8
EOS	123	None	115/119	2/4	6	2	4

Table 7: Message codes set by the LaserCyte® during analyses of 137 canine and 176 feline blood samples

Message codes	Flagged samples: 16 out of 137 canine blood samples	message code confirmed by observation	Flagged samples: 97 out of 176 feline blood samples	Message code confirmed by observation
"Differential algorithm issues. Confirm with blood smear": Concerning lymphocytes and monocytes.	1	stab neutrophils (1.5%)	2	0
"Differential algorithm issues. Confirm with blood smear": Concerning monocytes and neutrophils.	9	2 samples with stab neutrophils (16.5%, 1%) 1 sample with stab neutrophils (4%) and normoblasts (56/100WBC) 6 normal blood samples	12	2 blood samples with stab neutrophils (7%, 25.5%), 1 blood sample with normoblasts (9/100 WBC), 1 blood sample with atypical cells (lymphoma), 8 normal blood samples.
"Possible rate analysis issue. Confirm differential with blood smear and WBC":	0		14	14 normal blood samples
"MPV out of reportable range":	1	Not evaluated	69	Not evaluated
"Too many RBC fragments. Confirm PLT value with blood smear":	3	Haemolytic blood samples	0	
"MCHC out of reportable range":	5	1 lipaemic blood samples, 4 normal blood samples	4	4 normal blood samples
"PLT Aggregation.	1	Normal blood sample	12	7 blood samples with confirmed PLT

Confirm differential with blood smear":				aggregates, 5 normal blood samples
"Internal QA failure; qualiBeads not recovered"	3	Technical problems	0	

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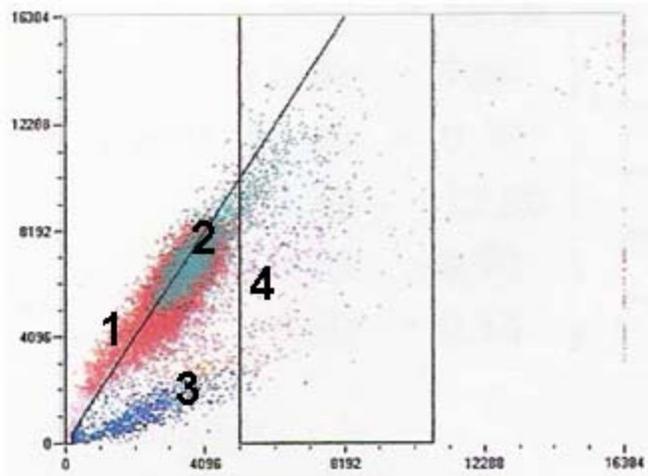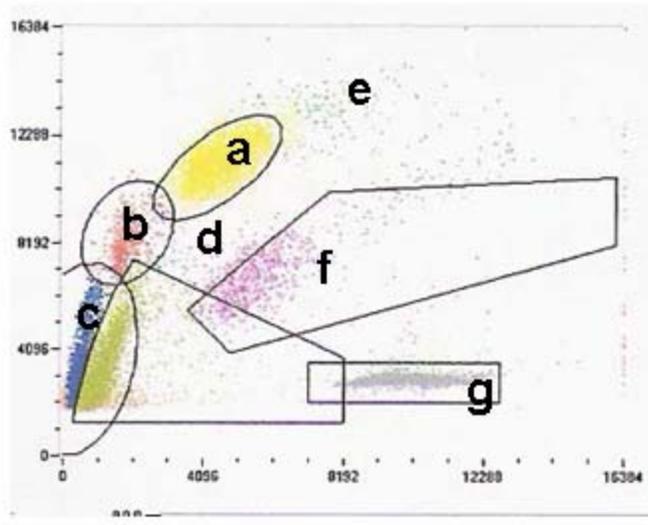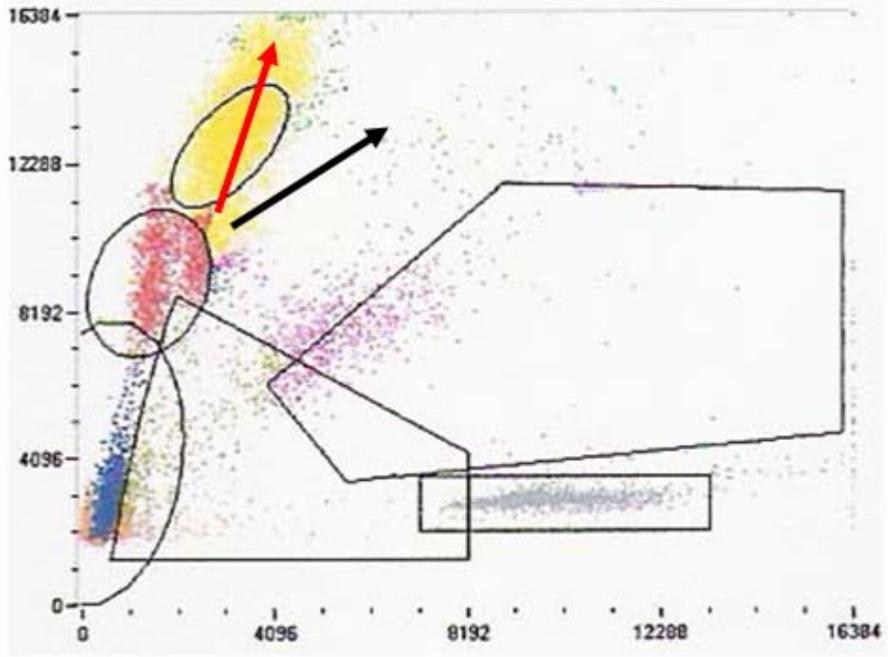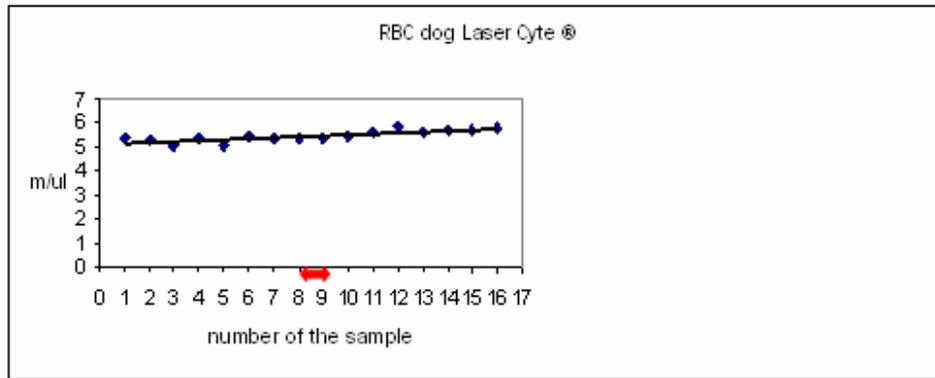
Fig. 1a

Fig. 1b

Fig. 1c

RBC dog Laser Cyte ®

Fig. 2 a
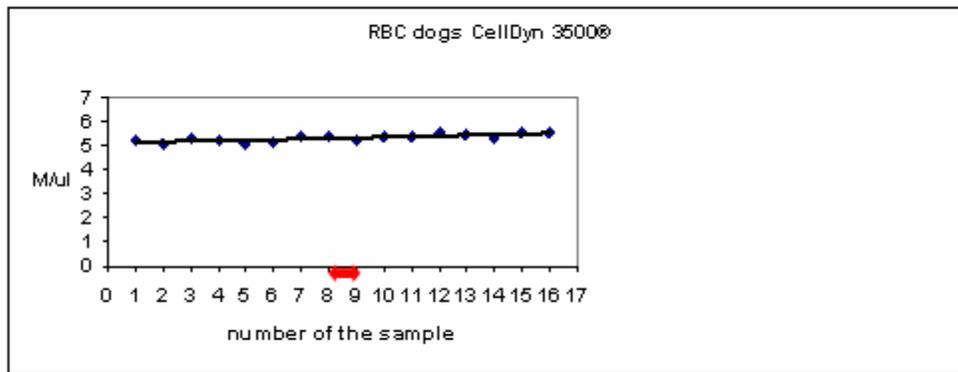
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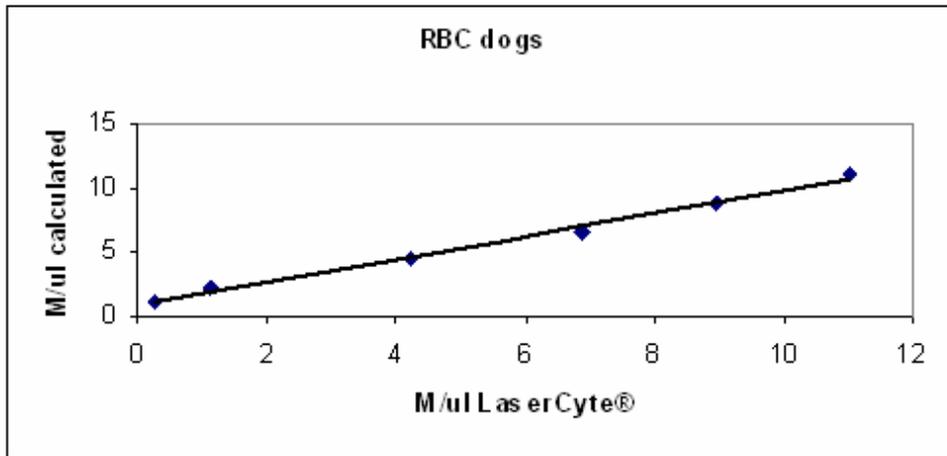
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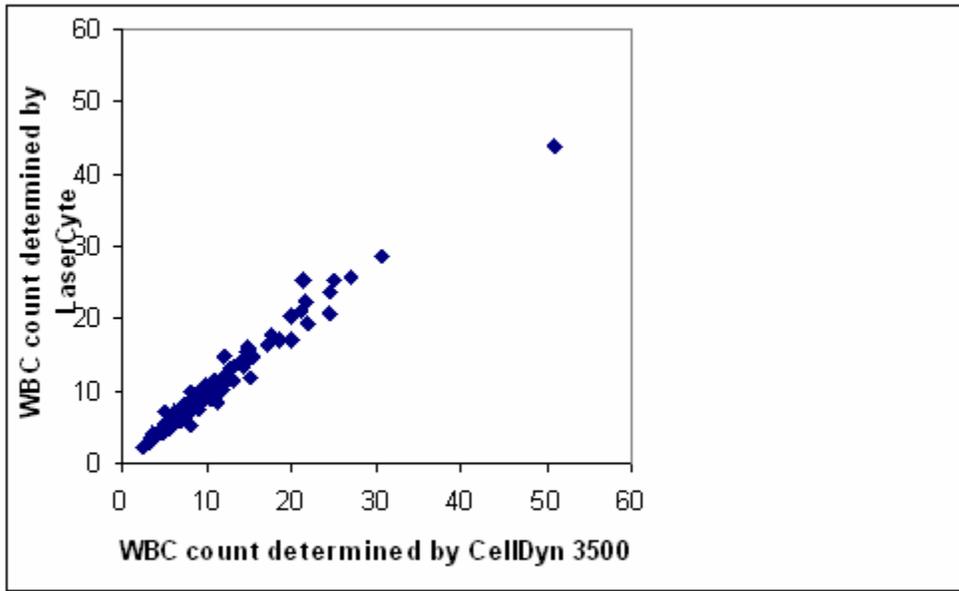
Fig. 4

Lebenslauf

Name:	Bettina, Wenger-Riggenbach
Geburtsdatum:	10. August 1967
Geburtsort:	Frauenfeld
Nationalität:	Schweiz
Heimatort:	Rünenberg BL, Blumenstein BE
1985 – 1988	Thurgauisch-Schaffhauserische Schule für allgemeine Krankenpflege in Frauenfeld TG Diplom 19. Oktober 1988
Dezember 1988 – Februar 1989	Krankenschwester in der Chirurgischen Akutabteilung im Spital Wil SG
März 1989 – September 1990	Krankenschwester in der Angiologischen Abteilung der Medizinischen Poliklinik am Universitätsspital Zürich ZH
Oktober 1990 – September 1996	Krankenschwester im Onkologisch/Medizinischen Ambulatorium am Kantonsspital Frauenfeld TG
1993 – 1996	Thurgauisch-Schaffhauserische Maturitätsschule für Erwachsene in Frauenfeld TG Matura Typus E, Juli 1996
1996 – 2002	Studium der Veterinärmedizin an der Universität Zürich, Schweiz
15. November 2002	Abschlussprüfung an der Universität Zürich, Schweiz
Februar 2003 – April 2004	Doktorandin bei Prof. Dr. Hans Lutz, Veterinärmedizinisches Labor, Vetsuisse-Fakultät der Universität Zürich ZH
Mai 2004 – Juni 2005	Assistentin und Stellvertreterin des Laborleiters für das Klinische Labor, Veterinärmedizinisches Labor, Vetsuisse-Fakultät der Universität Zürich ZH
Seit Juli 2005	Assistenz-Tierärztin an der Vetsuisse-Fakultät, Departement für Kleintiere, Klinik für Kleintiermedizin

Bettina Wenger-Riggenbach
11.06.2006