7 Summary

Clinical evaluation of the automatic blood cell counter CA530-VET
by comparison with the CELL-DYN 3500 and standard methods
for canine, feline and equine blood

147 pages; 21 figures; 33 tables; 147 citates

Since 1997 the Cell-Analyser Series 530 (CA530, Boule Medical, Stockholm, Sweden) has been used successfully for human medical laboratory diagnostic. The tested instrument, CA530-VET, represents the veterinary edition and has been available on the market since the year 2000.

The goal of our study was to examine the operational competence of CA530-VET, model ODEN, for the automatic analysis of canine, feline and equine blood. Carried out under clinical conditions, the study investigated the reliability of the results and evaluated the instrument's general practicability for veterinary use. The fully automated low-end-unit hematology analyser (electronic impedance principle) determines 16 parameters, including the differentiation of three leukocyte populations.

Reference instrument for the assessment of the machine's accuracy was the CELL-DYN 3500 (Abbott Laboratories, Illinois, USA) which uses the electronic impedance principle in combination with the measurement of light scattering. For the parameters hematocrit (HCT), the white blood cell differentiation and the platelet count for horses and cats, manual methods such as microcentrifugation, double chamber counting (Thrombo Plus from Sarstedt, Neubauer chamber) and the manual differentiation of 2 blood smears (Pappenheim's stain) each with 100 counted cells, were used as reference. In the Clinic for Small Animals (Klinik und Poliklinik für Kleine Haustiere, Freie Universität Berlin) blood from both healthy and sick dogs (242), cats (166) and horses (144) was examined to check the blood carry-over, precision and accuracy of the instrument. The samples were obtained within the period of one year and each sample was examined within half an hour to four hours after collection. The statistical analysis was carried-out using the statistic program SPSS for Windows version 11.0 (SPSS Inc., Chicago, USA).

The assessment of the results was made based on guidelines for "Quality Assurance for Quantitative Laboratory Medical Examinations" from the BUNDESÄRZTEKAMMER (BÄK, 2001) and using the "performance goals for internal quality control of multichannel hematology analysers" established by KLEE (1990). Carry-over of blood cells and hemoglobin was evaluated by measuring control blood in high and low concentration and calculating the carry-over ratio [K %] according to the formula from BROUGHTON et al. (1969). Short-term stability, within each batch, was obtained by running ten replicate analyses of five different blood samples from each animal species. The precision of results

obtained from repeated analysis was assessed by comparing duplicate analyses of 550 samples collected for the method comparison study. Control blood (Para 12 Plus, Streck Laboratories, La Vista USA) was used to calculate the long-term stability. Precision was evaluated with measurements taken on each study-workday. An evaluation of CA530-VET's accuracy in comparison with CELL-DYN 3500 and the standard manual methods was made according to the modified model from BLAND and ALTMAN (1986). The individual differences of the results between the two methods were calculated and plotted against the value of the reference method. As a final evaluation, the differences between the tested instrument and the reference method were expressed as percentages and compared with the values for maximal allowable inaccuracy as given in the guidelines from the BÄK and the "maximum allowable total bias" (mid range) as demanded by KLEE (1990).

From the total number of patients (n = 552), 48.6 % gave healthy blood samples with values within our own established reference intervals. 5.8 % of all blood samples showed deviations from normal plasma quality. The average time span between sample collection and blood analysis was 1.43 hours. The carry-over ratio [K %], calculated as the mean of 10 single determinations for K, was 0.28 % for erythrocytes (RBC), 0.59 % for platelets (PLT), 0.32 % for white blood cells (WBC) and 0.18 % for hemoglobin (HGB). Therefore the K-values for all four parameters were smaller than 2 % and consequently had no effect on the instrument's precision.

The results of the within-batch precision (coefficients of variation see table 6.1) and the precision of the repeated control blood measurement over time (n = 105) were for the blood of all animal species, except for the parameter PLT when measuring low concentrated control blood (7,2 %), clearly within the demanded limits of both the BÄK and KLEE (see table 5.1) as well. For the duplicate measurements of blood samples the variation coefficients (CV %) were likewise completely within the limits of the BÄK and KLEE. The platelet count for cats (CV 8.7 %) and horses (CV 9.5 %) proved to be the exception, exceeding the BÄK's maximum allowable deviation of 7 % for reliability.

The arithmetic mean and standard deviation for the most important parameters from the method comparison study are shown table 6.2. Compared to the CELL-DYN-3500, the CA530-VET showed excellent accuracy for the parameter WBC for dogs and horses and RBC for horses. Sufficiently accurate values were determined by the instrument for the parameter WBC for cats, RBC for dogs and cats as well as HGB and MCV for all three animal species. The accuracy between the obtained HCT values and the microhematocrit results was excellent only for horses. For cats the value exceeded slightly and for dogs quite clearly the BÄK's maximum allowed inaccuracy. KLEE (1990) does not give a limit for HCT in this respect, however the parameters RBC and MCV were lying within the KLEE's (1990) maximum allowable bias. When determinating platelet numbers with the CA530-VET, the results of all tested animal species were unacceptable for both BÄK and KLEE. The CA530-VET showed insufficient accuracy for the dog in comparison to CELL-DYN 3500 and insufficient accuracy for cats and horses in comparison to the manual platelet count. However extremely thrombocytopenic samples with PLT values below 20 x 10^3/mm^3 were

detected well for all three animal species. It has to be taken into consideration that the applied standards derive from human medicine and were adopted for veterinary use without any modification. In regards to the relative differential count, there are no existing official limits for the maximum allowable deviation from the reference method. For the lymphocyte and above all the midcell population however the deviation of the measurements in percentage was very high. The best detected cell population was the granulocyte population.

The practical experiences with the CA530-VET gained during the study were positive. The analyser worked quickly (results displayed within less than 1 minute), was reliable when serviced regularly and consumed only a small amount of blood (125 µl). The operation of the instrument was simple and had low susceptibility to interferences. One disadvantage however was the temperature-dependent limited durability of the reagents, especially when the daily turnover was low.

Altogether the CA530-VET can be considered as a suitable instrument for cell counting in veterinary medicine providing one takes careful consideration on the platelet count. The automatic differential count represents the weak point of this instrument and most others in this price category. Only the results for the granulocyte population can be accepted. For 21 % of all blood samples examined though, the CA530-VET could not give a differential count. Primarily affected were cat samples. Currently a new software version is available with the CA620-VET model which might allow better differentiation in this respect.

Tab. 6.1: Coefficients of variation (CV %) for the within-batch precision (10 repeated measurements) of the CA530-VET for canine, feline and equine blood samples

Parameters	CV % DOG (n = 5x10)	CV % CAT (n = 5x10)	CV % HORSE (n = 5x10)
RBC (10^6 /mm^3)	1.74	0.98	1.20
HCT (%)	1.81	0.96	1.34
MCV (µm^3)	0.31	0.43	0.51
MCHC (g/dl)	1.45	4.10	1.39
MCH (pg)	1.38	0.82	1.45
RDW (%)	4.23	1.46	1.74
PLT (10^3/mm^3)	5.27	4.53	5.80
MPV (µm^3)	3.06	3.39	2.30
WBC (10^3/mm^3)	1.91	3.51	1.71
HGB (g/dl)	1.24	0.90	1.00
GRAN (%)*	3.71	3.77	5.22
MID (%)*	/	/	8.43
LYMF (%)*	10.02	19.78	13.50
GRAN abs*	4.95	3.67	5.35
MID abs*	/	/	11.93
LYMF abs*	8.06	27.13	15.90

* GRAN (%) / abs = neutr. and eos. granulocytes in % / absolute. MID (%) / abs = monocytes and baso. granulocytes in % / absolute. LYMF (%) / abs = lymphocytes and blasts in % / absolute. For the differential count the case numbers were smaller than given in the head of the table as the CA530-VET did not always succeed in differentiation

Tab. 6.2: Mean differences and standard deviations between the results of the respective reference methods and the CA530-VET

	DOG (n = 210)	CAT (n = 148)	HORSE (n = 125)	reference method
WBC* (10^3/mm^3)	1.11±1.32	-1.19±3.40	0.51±0.52	CELL-DYN 3500
RBC (10^6/mm^6)	0.34±0.20	0.36±0.41	0.28±0.27	CELL-DYN 3500
HGB (g/dl)	0.54±0.42	0.27±0.46	0.30±0.31	CELL-DYN 3500
HCT (%)	2.00±1.84	1.10±1.61	0.74±1.16	microcentrifugation
MCV (µm^3)	-0.35±1.39	-0.15±1.46	0.16±1.54	CELL-DYN 3500
PLT (10^3/mm^3)	116.44±81.73	88.58±79.89	55.83±46.57	CELL-DYN 3500 (Hd) chamber count (Ktz/Pfd)
GRAN (%)	2.97±7.45	-0.44±8.16	-4.44±7.24	blood smear
MID (%)	-7.33±3.09	-6.83±1.91	-6.84±2.37	blood smear
LYM (%)	-2.24±7.44	1.66±8.48	5.98±6.88	blood smear

* For the Parameter WBC the case numbers are higher than given in the head of the table: n = 241 (dog), n = 162 (cat), n = 143 (horse). ** GRAN (granulocytes), MID (midcellpopulation) and LYM (lymphocytes). For the differential count the case numbers were smaller than given in the head of the table as the CA530-VET did not always succeed in differentiation