Evaluation of the pocH-100iV DIFF hematology analyzer for use in horses and cattle

Evaluatie van de pocH-100iV DIFF-hematologie analyzer voor paarden- en runderbloed

P. Deprez, C. Bauwens, K. Van Schandevijl, L. Lefère, H. Nollet, D. De Clercq, G. van Loon

Department of Large Animal Internal Medicine
Faculty of Veterinary Medicine
Ghent University
Salisburylaan 133, 9820 Merelbeke, Belgium

Piet.deprez@ugent.be

ABSTRACT

The results of the analysis of equine and bovine blood samples with the automated pocH-100iV DIFF hematology analyzer were compared with the results obtained with reference methods or other analyzers (Vet ABC, Coulter Counter ZF, Coulter LH 750 analyzer). For equine blood and most parameters in bovine blood good to excellent correlations between methods and analyzers were obtained. For bovine blood good to poor correlations and significant differences were obtained between the pocH-100iV DIFF and other methods or analyzers mainly for hematocrit and hemoglobin determinations and platelet counts. Overall the pocH-100iV DIFF seems to be a reliable and user-friendly analyzer.

SAMENVATTING

De resultaten van het hematologisch onderzoek van paarden- en runderbloed met de pocH-100iV DIFF-analyzer werden vergeleken met de resultaten bekomen met referentiemethoden of andere toestellen (Vet ABC, Coulter Counter ZF, Coulter LH 750 analyser). Bij de analyse van paardenbloed werden goede tot zeer goede correlaties en overeenkomsten gevonden tussen de verschillende methoden en toestellen. De analyse van runderbloed leverde eveneens goede tot zeer goede correlaties op voor de meeste parameters, maar bij de bepaling van hematocriet, hemoglobine en bloedplaatjes op runderbloed werden een matige correlatie en een significant verschil gevonden tussen de resultaten bekomen met de pocH-100iV DIFF en de andere toestellen. In het dagelijkse gebruik bleek de pocH-100iV DIFF-analyzer een betrouwbaar en gebruiksvriendelijk toestel te zijn.

INTRODUCTION

In large animal veterinary medicine blood analyzes are traditionally processed by specialized clinical laboratories. These laboratories try to communicate the results within one or two days, but there is an increasing demand for a 24-hour access to laboratory results. This is only feasible with in-house laboratory facilities for “point-of-care-testing” (POCT). At this moment several systems for POCT are available for the veterinary profession. These systems include biochemistry analyzers, mostly based on the dry chemistry technology (Sontag, 1993), and hematology analyzers. Recently a new veterinary hematology analyzer was introduced on the Belgian market, namely the pocH-100iV DIFF (Sysmex Europe GmbH, Hamburg, Germany). This article describes the evaluation of this analyzer for use with horse and cattle blood with regard to the analytical and the handling characteristics of the analyzer.

MATERIAL AND METHODS

Analyzer

The pocH-100iV DIFF automatically determines the number of white (WBC) and red (RBC) blood cells, the number of platelets and the hemoglobin (Hb) concentration. Furthermore the analyzer makes a WBC differentiation by determining the number of lymphocytes and the number of “other” WBC (granulocytes and monocytes). For feline and canine blood, the number of eosinophils is also determined, for other species the eosinophils are included in the number of “other” WBC.

The technology for the enumeration and differentiation of cells is based on the passage of individual blood cells through an aperture between two electrodes (hydrodynamic focusing with direct current detection) resulting in a change in electrical resistance across the aperture in function of the volume of the cells. The Hb

analysis is done photometric after lysis of the red blood cells and conversion of the hemoglobin into acid methemoglobin.

Based on these results hematocrit (Hct), mean erythrocyte volume (MCV), mean hemoglobin amount per RBC (MCH) and mean corpuscular hemoglobin concentration (MCHC) is calculated. Other, less frequently used parameters in large animal medicine are calculated and reported: red blood cell distribution width standard deviation and coefficient of variation (RDW-SD, RDW-CV), mean platelet volume (MPV), platelet distribution width (PDW) and the ratio of large (> 12 fl) versus total number of platelets (P-LCR).

The analyzer uses 15 µl of an EDTA blood sample and the results are available after approximately 148 seconds. The results include both the numerical values as well as distribution histograms of RBC, WBC and platelets.

Protocol

Blood from horses and cattle presented to the faculty of veterinary medicine was collected in EDTA containing tubes (Venoject ®, Terumo ®, Leuven, Belgium) and analyzed with the current techniques and analyzers in the lab as well as with the pocH-100iV DIFF.

Parameters

The following parameters were examined in this evaluation (England *et al.*, 1994):

Comparability

The WBC count from the pocH-100iV DIFF was compared with the results of the Coulter Counter (Model ZF, Coulter Electronics LTD, Harpenden Herts, England) (41 equine and 35 bovine blood samples) and the VET ABC (ABX Diagnostics, Montpellier, France) (24 equine samples and 25 bovine samples).

The WBC differentiation of the pocH-100iV DIFF was compared with the manual cell differentiation (examination of 400 WBC on a blood smear) on 41 equine and 35 bovine samples.

Results of the RBC count, platelet count, Hct and Hb obtained from the pocH-100iV DIFF were compared with those obtained with the Vet ABC (24 equine samples and 25 bovine samples). Additionally 10 bovine samples were used to compare the results of the Hct, Hb and platelet count obtained from the pocH-100iV DIFF with the results from the Coulter LH 750 Analyzer (Beckman Coulter)

The Hct determination of the pocH-100iV DIFF was compared with the determination obtained with a capillary centrifuge (ALC Centrifugette 4203, Thermo Electron Industries, Chateau Gontier, France, 15 000 g, 6 minutes) on 15 equine and 25 bovine samples.

Stability

Ten equine and bovine blood samples were stored at both 4°C and at room temperature (25°C). At 0, 24 and 48 hours after sampling the number of WBC, RBC and platelets, the Hct and a cell differentiation were determined.

Reproducibility

Seven equine and bovine samples with high, low or normal RBC, WBC and platelet counts were analyzed five times successively.

Linearity

On seven equine and five bovine samples the linearity of the analysis for RBC, WBC and platelets was determined by analyzing the original samples and samples diluted with autologous plasma to 75%, 50%, 33%, 25%, 10% and 5% of the original volume.

Carry over

Carry over was determined by analyzing a sample with a high count of either WBC, RBC or platelets on three consecutive times, immediately followed by three consecutive runs with a sample with respectively a low WBC, RBC or platelet count and the percentage of carry over was calculated.

Handling characteristics

During the test period the time needed for training of a new user, the overall appreciation of the ease of handling and the number of malfunctions were recorded.

Statistical analysis

The results obtained with the different analyzers on identical blood samples were compared with a paired t-test. For the evaluation of the reproducibility the coefficient of variation was determined. All analyzes were performed using the Analyze-it® software (Microsoft Excel add-in, Analyze-it Software Ltd., Leeds, UK).

RESULTS

Comparability

WBC counts on equine blood samples obtained with the pocH-100iV DIFF, the Coulter Counter ZF and the Vet ABC showed a high correlation (0.98) and no significant difference. The comparison of bovine WBC counts with the pocH-100iV DIFF and the Vet ABC yielded an excellent correlation (0.97) and no significant difference. There was also a high correlation between the results of the WBC count in bovine blood obtained with the pocH-100iV DIFF compared

with the Coulter Counter ZF (0.98), but with a mean difference of 0.8 x 10^9 WBC/l ($p<0.001$).

The differentiation of the WBC showed a good correlation (0.89) between the pocH-100iV DIFF and the manual method both on equine and bovine samples, with a moderate underestimation of the number of lymphocytes by the pocH-100iV DIFF (on average – 6.5%) and an overestimation of the number of "other" cells (on average + 7.2 %).

The determination of the RBC count and Hct in equine samples showed no significant differences between the pocH-100iV DIFF, the Vet ABC and the reference method for Hct determination (capillary centrifugation). With bovine samples there was no significant difference between the determination of the number of RBC with the pocH-100iV DIFF and the Vet ABC, but the Hct determination differed significantly ($p<0.001$) between the capillary centrifugation and both the pocH-100iV DIFF and the Vet ABC. The pocH-100iV DIFF (mean value 308 ml/l) and the Vet ABC (mean value 312 ml/l) underestimated the Hct compared to the reference method (mean value ALC centrifugette: 340 ml/l). 10 additional samples were run on the pocH-100iV DIFF and the Coulter LH 750 analyzer, showing a high correlation (0.93) and no significant difference.

In equine blood samples Hb concentrations showed an excellent correlation between the pocH-100iV DIFF and the Vet ABC (0.99) and no significant difference. With bovine blood however there was a slightly lower correlation (0.92) and a significant difference ($p< 0.001$) between the two analyzers with a lower mean Hb concentration found with the Vet ABC (6.5 mmol/l versus 6.9 mmol/l). Comparison of 10 additional bovine samples with the pocH-100iV DIFF and the Coulter LH 750 analyzer resulted in an excellent correlation (0.99) and again a significant difference ($p< 0.001$) with a lower mean Hb concentration found with the pocH-100iV DIFF compared to the Coulter LH 750 analyzer (5.7 mmol/l versus 5.9 mmol/l).

The platelet count in equine blood showed a fair correlation (0.75) between the pocH-100iV DIFF and the Vet ABC, with the pocH-100iV DIFF producing slightly lower mean results (158 x 10^9/l versus 181 x 10^9/l) ($p<0.05$). In cattle samples there was a poor correlation between the two analyzers (0.34): the mean platelet count with the pocH-100iV DIFF was 345 x 10^9/l and the mean count with the Vet ABC was 427 x 10^9/l ($p<0.01$). The comparison of 10 additional bovine samples with the pocH-100iV DIFF and the Coulter LH 750 analyzer showed a fair correlation (0.78) and no significant difference (Table 1 and 2).

Stability

Preservation of equine blood at 4°C or at room temperature induced no significant changes in the results for RBC, WBC and platelet count, Hct determination

Table 1. Comparability of analyzes of equine hematological parameters between the pocH 100iV DIFF hematology analyzer and other methods or analyzers.

Parameter	Method / analyzer	Number of samples	Correlation coefficient	P value
WBC count	Coulter Counter ZF	41	0.98	> 0.05
WBC count	Vet ABC	24	0.98	> 0.05
WBC differentiation	Manual differentiation	41	0.89	< 0.01
RBC count	Vet ABC	24	0.85	> 0.05
Hct	Vet ABC	24	0.99	> 0.05
Hct	ALC centrifugette	15	0.99	> 0.05
Hb	Vet ABC	24	0.99	> 0.05
Platelets	Vet ABC	24	0.75	< 0.05

Table 2. Comparability of analyzes of bovine hematological parameters between the pocH-100iV DIFF hematology analyzer and other methods or analyzers.

Parameter	Method / analyzer	Number of samples	Correlation coefficient	P value
WBC count	Coulter Counter ZF	35	0.98	< 0.001
WBC count	Vet ABC	25	0.97	> 0.05
WBC differentiation	Manual differentiation	35	0.89	< 0.05
RBC count	Vet ABC	25	0.98	> 0.05
Hct	Vet ABC	25	0.94	< 0.001
Hct	ALC centrifugette	25	0.90	< 0.001
Hct	Coulter LH 750	10	0.93	> 0.05
Hb	Vet ABC	25	0.92	< 0.001
Hb	Coulter LH 750	10	0.99	< 0.001
Platelets	Vet ABC	25	0.34	< 0.01
Platelets	Coulter LH 750	10	0.78	> 0.05

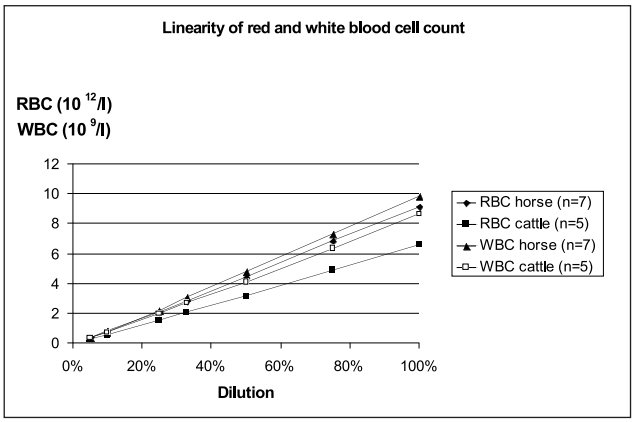

Figure 1. Linearity of the red and white blood cell counts in equine and bovine blood samples.

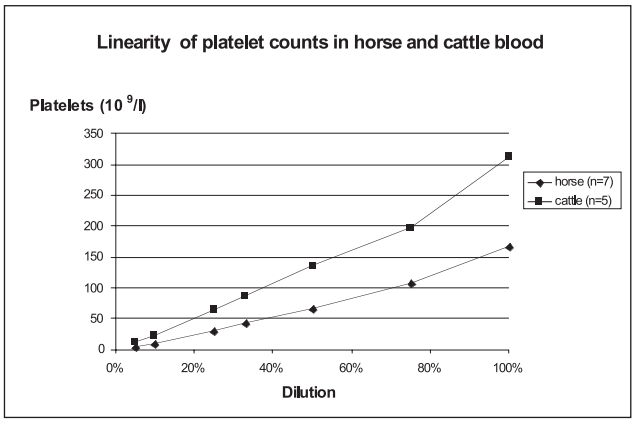

Figure 2. Linearity of platelet counts in equine and bovine blood.

and WBC differentiation for at least 24 hours after sampling.

In bovine blood, significant differences already occurred in the number of RBC after storage of the blood for 24 hours at room temperature, in Hct after storage of the blood for 24 hours at 4°C and in the platelet count after preservation both at 4°C and at room temperature.

Reproducibility

The reproducibility characteristics of the various determinations fell within acceptable range, with coefficients of variation lower than 5 % for most parameters (Table 3 and 4). Only in cattle samples with low numbers of platelets or low lymphocyte counts, the coefficients of variation exceeded 10%.

Linearity

A good linearity was obtained for the RBC and WBC counts during increasing dilution (Figure 1). Regarding the platelet counts some of the linearity was lost in the higher range of platelets in cattle samples (Figure 2).

Carry over

The carry-over percentage was very low, varying from 0.48% for the RBC count to 1.4 % for the WBC counts.

Handling characteristics

All staff members could routinely use of the analyzer

Table 3. Reproducibility of the results obtained with the pocH-100iV DIFF hematology analyzer, determined with 5 repeated analyzes on seven equine blood samples with low, normal or high values for the different parameters.

Parameter	Mean range	Range of the SD	Range of the CV (%)
WBC (x 10^9/l)	1.16 – 15.4	0.05 – 0.15	0.74 – 5.16
RBC (X 10^{12}/l)	2.89 -11.32	0.06 – 0.05	0.50 – 2.98
Hct (%)	13.9 – 50	0.24 – 0.61	0.66 – 2.43
Hb (mmol/l)	2.4 – 10.38	0 – 0.08	0 – 1.85
Platelets (x 10^9/l)	48 – 259	2.4 – 10.63	4.09 – 6.76
% lymphocytes	8 -61	0.44 – 2.73	0 – 5.08
% « other » cells	39 - 91	2.7 – 0.44	0 – 7.02

Table 4. Reproducibility of the results obtained with the pocH-100iV DIFF hematology analyzer, determined with 5 repeated analyzes on seven bovine blood samples with low, normal or high values for the different parameters.

Parameter	Mean range	Range of the SD	Range of the CV (%)
WBC (x 10^9/l)	5.94 -32.4	0.16 – 0.48	0.66 – 2.81
RBC (X 10^{12}/l)	5.12 – 10.42	0.08 – 0.07	0.59 – 1.62
Hct (%)	22.9 – 45.6	0.13 – 0.32	0.51 – 1.13
Hb (mmol/l)	5 – 9.2	0 – 0.08	0 – 1.34
Platelets (x 10^9/l)	121 – 1104	13 -12	0.97 – 11.16
% lymphocytes	5.4 – 59.4	0.54 – 1.34	1.61 – 10.14
% « other » cells	40.6 – 94.6	1.34 – 0.54	0.57 – 3.30

after one or two demonstrations. The interpretation of the flags and histograms required more time and access to the manual remained necessary for infrequent users. No major malfunctioning occurred during the test period.

DISCUSSION

The pocH-100iV DIFF compares well to other analyzers or reference methods on the level of accuracy, especially with equine blood samples. The results for parameters such as reproducibility, linearity and carry-over indicate that the technology used in the pocH-100iV DIFF has reliable analytical characteristics.

Blood cell differentiation with the pocH-100iV DIFF has a good correlation with the manual reference method with a slight underestimation of the lymphocyte percentage, probably because of the incorporation of monocytes and eosinophils in the group of "other" cells.

In contrast to equine samples, the results for bovine samples showed some differences between methods or analyzers. A consistent lower Hct value was obtained both on the pocH-100iV DIFF and the VetABC compared to the reference method (capillary centrifugation). Hct determination on a top-range analyzer (Coulter LH 750) confirmed this tendency: the absence of a significant difference with the pocH-100iV DIFF indicates that indeed lower values are obtained with automatic analyzers. This difference could be explained by trapping of plasma in the RBC column with the microhematocrit centrifugation: approximately 3% of the red cell column in normal humans, up to 6% in anemic patients, may consist of trapped plasma (England *et al.*, 1972). Therefore the absence of significant difference between the analyzers and the centrifugation method in horses compared to the observed difference in cattle might perhaps be explained by the higher Hct present in horses. Similar results were obtained when blood from dogs, cats, horses and cattle was analyzed with the centrifugal CBC analyzer and a combined impedance light-scatter analyzer: in this report, it was also with bovine blood that significant differences with the reference method were found, especially with low Hct values (Bienzle *et al.*, 2000).

Hb determination in bovine blood resulted in significant differences between the analyzers. The pocH-100iV DIFF positioned itself between the VetABC and the Coulter LH 750. In other comparisons between automated hematology analyzers, bovine hemoglobin determination also seemed to be a parameter that was more prone to variation between analyzers (Dawson *et al.*, 2000).

Platelet counts are notoriously difficult to standardize. Platelet activation and aggregation is easily provoked during blood sampling and the differences between species in platelet size and ease of activation and aggregation make it difficult for automated analyzers to generate reliable results for different animal species (van Leeuwen and Teske, 1999). The comparison between analyzers for equine platelet counts resulted in a fair correlation but with a significant difference, indicating that special attention to flags and the histogram on the report is required. One must bear in mind that especially abnormal results need to be checked on a smear or confirmed by a manual platelet count. Probably due to the smaller size and the wider range of normal numbers of bovine platelets a poor correlation was found between the pocH-100iV DIFF and the Vet ABC. The correlation between the pocH-100iV DIFF and the top-range Coulter LH 750 analyzer was better but no definitive conclusions can be made because no manual count of platelets was performed.

To conclude, the pocH-100iV DIFF hematology analyzer has good analytical characteristics, allows performing hematological examinations at any time of the day and is easy to use. It gives a quick and reliable overview of the main hematological parameters of equine and bovine blood, but two considerations should be kept in mind: the differentiation of white blood cells is only a two-way differentiation and especially for bovine blood Hct and Hb determinations and platelet counts may differ from other analyzers or methods. At all times the histograms and possible flags on the report have to be checked and, in case of doubt, examination of a blood smear is advisable.

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