Short communication: Evaluation of an automated in-house hematology analyzer for bovine blood

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ABSTRACT

The objective of this study was to evaluate the suitability of the V-Sight hematology analyzer (A. Menarini Pharma GmbH, Vienna, Austria) for bovine blood by a comparison with a reference device (Advia 2120i, Siemens AG, Erlangen, Germany). In total, 97 blood samples were obtained from 75 dairy cows. Analyzed parameters included counts of white blood cells (WBC), lymphocytes, monocytes, granulocytes, red blood cells (RBC), and platelets (PLT), as well as hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), mean platelet volume (MPV), and plateletcrit (PCT). Based on Passing-Bablok regression, the V-Sight provided accurate and precise results for MCH and MCHC only. The PCT results were comparable to the reference method, but precision was inconclusive. Significant proportional differences were detected for monocytes, granulocytes, HCT, and PLT. For all other analytes, significant proportional and systemic differences were observed. The WBC and lymphocyte results from the V-Sight were characterized by poor accuracy, poor precision, and a high number of false positive outliers. Bland-Altman analysis indicated negative biases for all WBC parameters, the erythrocyte indices, and PLT. Positive biases were observed for RBC, HGB, HCT, MPV, and PCT. Correlation coefficients of >0.9 between the V-Sight and the reference method were found only for RBC, HGB, HCT, and MPV. Intraassay precision of the V-Sight analyzer was acceptable (coefficient of variation $<5\%$) for granulocytes, the erythrocyte indices, and MPV. It was unacceptable (coefficient of variation $\geq 5\%$) for WBC, lymphocytes, monocytes, as well as RBC, and inconclusive for HGB, HCT, PLT, and PCT. Sensitivity was high for all RBC counts and indices as well as PLT, but low for monocytes, granulocytes, and MPV. Specificity was high for monocytes and granu-

locytes, but low for RBC, HCT, MCH, and MCHC. With accurate and precise results for only 2 out of 13 parameters, the V-Sight cannot be recommended for analysis of bovine blood.

Key words: cattle, blood, hematology analyzer, method validation

Short Communication

Hematologic analysis is not only relevant for diagnosing disorders of the hematologic system, but also helpful in the diagnosis, surveillance, and prognosis of many other diseases. In particular, hematologic analyses may contribute to the timely identification of diseases in the periparturient period. The aim of the current study was to evaluate the suitability of the A. Menarini V-Sight hematology analyzer (A. Menarini Pharma GmbH, Vienna, Austria) for the analysis of bovine blood. To our knowledge, ours is the first study evaluating an in-house hematology analyzer specifically for use in dairy cows.

Analyzers and Sampling

The V-Sight is a fully automatic in-house hematology analyzer providing up to 18 blood parameters for up to 16 animal species. The analyzer conducts a 3-part differential white blood cell (**WBC**) count and reports 3 histograms plotting cell distribution widths. Total leukocytes, erythrocytes, platelets, and hemoglobin concentration are directly measured. The measurement methods employed by the V-Sight are an impedance method for determining WBC, red blood cells (**RBC**), and platelets (**PLT**), and a colorimetric method for determining hemoglobin concentration (**HGB**). The percentages for the leukocyte subpopulations, as well as mean corpuscular volume (**MCV**), red cell distribution width, mean platelet volume (**MPV**), and platelet distribution width, are derived from the histograms (Shenzhen Mindray Bio-Medical Electronics Co. Ltd., 2009). The analyzer is programmed with default reference ranges, which were calculated by Mindray us-

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ing 120 sample subjects according to the guidelines established by the Clinical and Laboratory Standards Institute (2008). Only reagent solutions specific for the V-Sight were used. The analyzer was calibrated by A. Menarini staff using the auto calibration program and commercial calibrator reagents. The quality control program was run before each blood analysis.

As a reference method, the samples were analyzed with the hematology analyzer Advia 2120i (Siemens AG, Erlangen, Germany) in the Central Diagnostic Unit (CDU) of the Veterinary University of Vienna (Austria). The CDU laboratory is certified under EN ISO 9001:2008 (ISO, 2008). Blood smears were prepared using the Bayer Hematek Stain Pak (Bayer AG, Leverkusen, Germany) and examined microscopically for blood samples exhibiting parameters with deviations of more than 25% from the reference range. The Advia analyzer is based on flow cytometry and uses light scatter, differential lysis, and staining. These analyzers provide a complete blood cell count, including a 5-part WBC differential (Moritz and Becker, 2010). The predecessor model of the Advia 2120i, the Advia 120, was evaluated and approved to be suitable for routine veterinary diagnostics for several species including cattle by Moritz (2002). The Advia used in the current study is calibrated semiannually. Accurate analyzer function was tested daily via measurement of 3 levels (normal, low, and high) of commercial quality control samples (Testpoint 3 in 1 Control, Siemens AG). Coefficient of variation, percentage of bias, and total allowable error were calculated and required to lie within predefined target values.

Blood samples were obtained from 75 dairy cows located at the Teaching and Research Farm of the University of Veterinary Medicine (Vienna, Austria). The use of animals for sampling purposes was discussed and approved by the institutional ethics committee in accordance with good scientific practice guidelines and national legislation. The sample size was chosen based on comparable studies reported in the literature (Bienzle et al., 2000; Bauer and Moritz, 2008; Goldmann et al., 2013). The sample population consisted of Simmental ($n = 50$), Brown Swiss ($n = 7$), and Holstein Friesian ($n = 18$) cows in their first to sixth lactation (median = third) and at different stages of lactation, from 73 d antepartum to 400 d postpartum (median = 19 d postpartum). Cows were housed in large groups in a freestall barn with access to outside paddocks. They were fed a TMR consisting of grass- and corn-silage, hay, and concentrates and had free access to water. The ration was balanced by a dairy nutritionist to meet the energy and nutrient requirements for dairy cows as recommended by the German Society on Nutrition Physiology (GfE, 2001). The herd average

ECM production was 8,082 kg, based on 4.0% butterfat and 3.4% protein. The animals were predominantly healthy; however, during the sampling period, 8 cows were diagnosed with mastitis, 5 with endometritis, 4 with lameness, 3 with fertility disorders, and 5 with other diseases. These cows received appropriate medical treatment. A total of 75 blood samples were collected from individual animals at 3 dates in 2012 [August 22 ($n = 27$), August 29 ($n = 21$), and September 4 ($n = 27$)] for direct comparison of results from the V-Sight and Advia analyzers. A further 22 samples were collected on November 15 from animals previously sampled to investigate intraassay precision and carryover of the V-Sight analyzer. The sampling took place in the morning hours. Blood was collected by puncture of a coccygeal vessel with 20-gauge, single-use drawing needles (0.90×38 mm, Greiner Bio-One, Kremsmünster, Austria) into 9-mL K_3 -EDTA-coated vacuum tubes (Vacuette, Greiner Bio-One). All 75 samples were analyzed with the V-Sight at the Teaching and Research Farm within 2 h after collection. The samples were stored at room temperature and were mixed thoroughly by hand before analysis. Afterward, they were transported in a cooled box to the CDU, where they were analyzed with the Advia within 8 h after collection. The 22 samples collected at the fourth date were analyzed with the V-Sight only for intraassay precision and carryover assessment.

All data sets ($n = 97$) were compiled from the V-Sight and Advia printouts into the Microsoft Excel program (Microsoft Excel for Mac 2011, version 14.2.2, Microsoft Co., Montrouge, France). Because the Advia measures segmented cells, bands, eosinophils, and basophils, these subpopulations were added to create an equivalent to the category "granulocytes" used by the V-Sight. Descriptive statistic parameters and Pearson correlation coefficients were calculated for the first 3 data sets ($n = 75$) using PASW for Windows (version 17.02, IBM, Armonk, NY).

Method Validation

The evaluation of the V-Sight was conducted by assessing accuracy or agreement, precision, carryover, sensitivity, and specificity (Shinton et al., 1982; Krimer, 2011). Accuracy is a measure of how well test results reflect the true value of a variable, whereas agreement or bias refers to comparability with a reference method. As indicators of precision, variance, SD, CI around mean values, and the CV were calculated. For the determination of intraassay precision, 2 samples were randomly selected using the Excel random function and measured 10 times each. A CV of less than 5% was considered acceptable (Campbell et al., 2007;

Krimer, 2011). Bland-Altman analysis (Bland and Altman, 1986) and Passing-Bablok regression (Passing and Bablok, 1983) were used for assessing agreement between the test method and the reference method, as well as precision. Bland-Altman analysis was conducted with BiAS software (version 10.0, Epsilon-Verlag, Frankfurt, Germany). Passing and Bablok regression was performed with MedCalc software (version 12.3.0, MedCalc, Mariakerke, Belgium).

Carryover was calculated for WBC, RBC, and PLT by testing a sample with a high concentration of a certain analyte thrice (H_1 , H_2 , H_3), followed immediately by testing a sample with a low concentration thrice (L_1 , L_2 , L_3). Carryover was calculated according to the method of Broughton et al. (1969), as explained by Shinton et al. (1982), as $(L_1 - L_3)/(H_3 - L_3) \times 100\%$.

Sensitivity provides information about the probability of a device recognizing a result outside of the reference range. Specificity informs about the probability of a device to identify results within the reference range (Krimer, 2011; Lundorff Jensen and Kjelgaard-Hansen, 2011). Four outcomes are possible: correct outcomes include the events that a V-Sight result lies outside [true positive (TP)] or inside [true negative (TN)] of the reference interval and this outcome is confirmed by the Advia result; incorrect outcomes occur if the V-Sight, but not the Advia result, lies inside of the reference range [false negative (FN)], or vice versa, if the V-Sight, but not the Advia result, lies outside of the reference range [false positive (FP)]. Sensitivity was calculated as $TP/(TP + FN) \times 100\%$ and specificity as $TN/(FP + TN) \times 100\%$ (Beaglehole et al., 1997; Krimer, 2011).

WBC Results

The total WBC and lymphocyte measurement results of the V-Sight were characterized by a larger range and SD than observed using the Advia analyzer. For total WBC counts, SD of the V-Sight (7,561 cells/ μ L) was 5.3 times as large as that of the Advia (1,437 cells/ μ L). Several measurements resulted in abnormally high results. The 4 most extreme results could not be confirmed by repeated measurements with the V-Sight or by measurement with the Advia, and were thus regarded as outliers. After exclusion of outliers and recalculation, the SD of the V-Sight WBC results decreased, but was still greater by a factor of 2.3 than the Advia results. The same trend was observed for lymphocytes. For monocytes and granulocytes, range, mean, median, and SD only changed minimally after excluding outliers, suggesting that the outliers originated from the lymphocyte population. Range and SD of

monocytes and granulocytes were moderately larger for the V-Sight than for the Advia. Weak correlation coefficients between the V-Sight and the Advia results were found for total WBC, including and excluding outliers, lymphocytes, monocytes, and granulocytes (Table 1).

Bland-Altman analysis calculated a negative bias (mean of differences, d) for all WBC parameters (Table 1). For total WBC counts, including outliers, a bias of $-3,327$ cells/ μ L, an SD of differences (s) of $7,624$ cells/ μ L, and limits of agreement ($d \pm 1.96 s$) from $-18,270$ to $11,617$ cells/ μ L were calculated (Figure 1). After excluding outliers, bias, s , and limits of agreement were reduced but still large, thus suggesting that the V-Sight analyzer is of limited use for clinical purposes. The Bland-Altman plot of lymphocytes resembles the pattern observed for total WBC to a large extent, whereas results for monocytes and granulocytes were more dispersed. Passing-Bablok regression detected significant proportional and systematic difference for total WBC (including and excluding outliers) and lymphocytes with regard to the reference method (Table 1). For WBC including outliers, the 95% CI for the intercept (a) was 983 to 2,675 and the interval for the slope (b) 0.49 to 0.73. After excluding outliers, the CI were 635 to 1,900 for a and 0.60 to 0.78 for b . Both CI for a did not include 0 and the CI for b did not include 1. This indicated that significant proportional and systematic differences were observed between methods for these analytes and that the results cannot be regarded as comparable. Passing-Bablok regression showed no systematic, but proportional differences for monocytes and granulocytes.

Intraassay precision of the V-Sight analyzer was acceptable (CV $<5\%$) for granulocytes, but not (CV $\geq 5\%$) for WBC, lymphocytes, or monocytes. Carryover of the V-Sight analyzer was calculated to be -11.11% for WBC. A negative result did not provide useful information and was assumed to be caused by the large variability observed in results. Sensitivity and specificity were intermediate (between 50 and 90%) for WBC and lymphocytes. Sensitivity was low ($<50\%$) and specificity was high ($>90\%$) for monocytes and granulocytes (Table 1).

Overall, we concluded that the V-Sight did not deliver comparable results to the reference method when analyzing WBC and lymphocytes. Accuracy and precision were poor. The clinical usefulness of results for WBC is questionable due to large variation, poor precision, and the number of FP outliers observed. Results were more accurate for monocytes and granulocytes; however, proportional differences were observed relative to the reference method. Precision was deemed not acceptable for monocyte results.

Table 1. Pearson correlation coefficients, test performance, and differences between the V-Sight¹ and the Advia² hematology analyzers as calculated using the Bland-Altman analysis method and Passing-Bablok regression analysis

Parameter	r	Sensitivity (%)	Specificity (%)	Bland-Altman analysis ^{3,4}		Passing-Bablok regression analysis ^{3,4}				Intraassay CV	
				Bias (<i>d</i>)	SD of <i>d</i>	Intercept (<i>a</i>)	95% CI for <i>a</i>	Slope (<i>b</i>)	95% CI for <i>b</i>	Sample 1 ⁵	Sample 2 ⁵
WBC including outliers	0.05	67	90	−3,327	7,624	1,623	983 to 2,675	0.63	0.49 to 0.73	14.9	5.9
WBC excluding outliers	0.55	62	93	−1,704	2,747	1,168	635 to 1,900	0.71	0.60 to 0.78	—	—
Total lymphocytes	0.07	50	83	−2,174	6,651	1,076	523 to 1,528	0.51	0.33 to 0.66	62.3	17.9
Total monocytes	0.28	6	100	−295	222	71	−6 to 162	0.43	0.27 to 0.60	16.7	9.6
Total granulocytes	0.68	40	100	−875	1,120	−16	−285 to 347	0.87	0.77 to 0.95	4.8	4.2
Red blood cells	0.96	100	38	1.11	0.22	−0.53	−1.00 to −0.09	1.33	1.24 to 1.43	9.6	6.4
Hemoglobin	0.96	100	85	0.94	0.26	−0.98	−1.77 to −0.16	1.20	1.11 to 1.29	9.2	1.8
Hematocrit	0.94	100	12	4.1	0.9	−0.84	−2.90 to 1.27	1.20	1.12 to 1.29	10.4	1.2
Mean corpuscular volume	0.95	100	78	−2.6	1.3	3.49	0.65 to 6.55	0.89	0.82 to 0.94	1.1	0.5
Mean corpuscular hemoglobin	0.86	100	34	−2.04	0.67	−0.72	−3.23 to 1.78	0.94	0.81 to 1.07	1.2	1.4
Mean corpuscular hemoglobin concentration	0.18	100	0	−2.3	1.3	2.66	−17.02 to 14.14	0.88	0.58 to 1.38	2.0	1.6
Platelets	0.82	100	88	−82	71	−7	−62 to 41	0.85	0.75 to 0.97	16.0	3.2
Mean platelet volume	0.24	33	68	2.0	1.2	−10	−21 to −5	3.38	2.31 to 5.50	2.2	1.3
Plateletcrit	0.63	NA ⁶	NA	0.03	0.06	−0.0015	−0.10 to 0.05	1.15	0.92 to 1.54	16.9	3.7

¹A. Menarini V-Sight hematology analyzer (A. Menarini Pharma GmbH, Vienna, Austria).²Siemens Advia 2120i hematology analyzer (Siemens AG, Erlangen, Germany).³*d* = mean of differences between the V-Sight and the Advia results; *a* = intercept of the regression line (measure of systematic error); *b* = slope of regression line (measure of proportional error).⁴*n* = 75, except WBC excluding outliers where *n* = 71.⁵Randomly chosen.⁶NA = not applicable (no reference limits available for V-Sight).

Figure 1. Bland-Altman plots of white blood cell (WBC) counts including (top left, $n = 75$) and excluding (top right, $n = 71$) outliers, red blood cell counts (RBC, bottom left, $n = 75$), and platelet counts (PLT, bottom right, $n = 75$). The solid line in the middle represents the mean; the upper and lower dotted lines represent the mean ± 2 SD.

RBC Results

The V-Sight results for total RBC counts had an SD of 0.5×10^6 cells/ μ L. The V-Sight provided slightly lower mean and associated median, range, and SD values for RBC, HGB, and HCT than the Advia. Both, the Advia and the V-Sight results for RBC were concentrated at or below the lower end of the reference interval. For the erythrocyte indices MCV, MCH, and MCHC, range and SD results were slightly larger for the V-Sight than the Advia results, whereas the mean and median were lower. The correlation coefficients for RBC, HGB, HCT, and MCV were good ($r > 0.9$), whereas MCH and especially MCHC were less well correlated, with r values of 0.86 and 0.18, respectively.

Bland-Altman analysis demonstrated a positive bias for RBC, HGB, and HCT and a negative bias

for MCV, MCH, and MCHC (Table 1 and Figure 1). Several results fell outside of the limits of agreement. Passing-Bablok regression detected significant systematic and proportional differences between the V-Sight and the Advia results for RBC, HGB, and MCV and proportional differences for HCT (Table 1). No significant proportional and systemic differences for MCH and MCHC were observed, indicating that the V-Sight and the Advia delivered comparable results for these 2 parameters.

Intraassay precision was not acceptable for RBC, but was acceptable for MCV, MCH, and MCHC. With regard to HGB and HCT, the results were inconclusive: the CV were not acceptable for sample 1, but were acceptable for the same parameters of sample 2. Carryover for RBC was 1.52%, which exceeds the V-Sight specifications of $\leq 0.5\%$ (Shenzhen Mindray Bio-Medi-

cal Electronics Co. Ltd., 2009). Sensitivity was high for all RBC counts and indices. However, a large number of FP outcomes were noted. All FP outcomes for RBC and HCT were results that fell short of the reference interval, whereas the FP results for MCV, MCH, and MCHC exceeded the reference range. Specificity was intermediate for HGB and MCV and low for RBC, HCT, MCH, and MCHC.

Overall, accuracy and precision for RBC, HGB, and HCT of the V-Sight were not comparable to that of the Advia. The V-Sight results for MCH and MCHC were characterized by a high accuracy and precision and could therefore be considered as comparable to those of the Advia. The V-Sight results for MCV were precise, but did not agree with the reference method.

PLT Results

Range and SD of the V-Sight were larger for PLT and PCT, but smaller for MPV compared with the Advia. The correlation coefficients for platelet parameters were intermediate for PLT and PCT and low for MPV (Table 1).

Bland-Altman analysis calculated a negative bias for PLT and positive biases for MPV and PCT (Table 1 and Figure 1). Passing-Bablok regression indicated proportional, but no systematic differences between the V-Sight and the Advia results for PLT. For MPV, significant systematic and proportional differences as well as a deviation from linearity were detected. Neither systemic nor proportional differences were observed for PCT.

Intraassay precision was acceptable for MPV and inconclusive for PLT and PCT. Carryover was -0.91% for PLT. Sensitivity was high for PLT and low for MPV. Specificity was intermediate for both analytes. For MPV, a large number of FP outcomes were observed and some FN outcomes were detected.

In summary, PLT measures from the V-Sight were characterized by proportional differences with reference to the Advia and precision was inconclusive. Although precise, the MPV results exhibited low comparability to the reference method. The PCT results were accurate, but precision was inconclusive.

Discussion and Conclusion

The V-Sight provided accurate and precise results for only 2 out of 13 parameters: MCH and MCHC. Proportional differences were detected for monocytes, granulocytes, HCT, and PLT. For all remaining analytes, significant proportional and systemic differences were observed. In particular, WBC and lymphocyte results were characterized by large variation, poor precision,

and large numbers of FP outcomes. Hence, the use of the V-Sight analyzer cannot be recommended for the analysis of bovine blood.

Causes for the significantly higher leukocyte counts reported by the V-Sight analyzer may include abnormally large or clumped platelets, nucleated red blood cells, insufficient lyses of erythrocytes or platelets, or excessive Heinz bodies (Webb and Latimer, 2011). Blood smears from samples with abnormally large WBC counts were evaluated microscopically, but no evidence of any of these conditions was found. Potential further causes might include inadequate counting and calculation algorithms in the V-Sight software. If abnormally high WBC or lymphocytes are flagged using the V-Sight, repeated measurements should be undertaken and a microscopic blood film evaluation should be carried out for verification of the results.

Extremely variable results observed using the V-Sight within a healthy cow population may result in the significant number of FP results observed. Falsely flagged leukocytosis may contribute to the incorrect diagnosis of several conditions associated with elevated leukocytes, such as infectious diseases, inflammatory conditions (e.g., puerperal disease, acute bacterial mastitis, foreign body peritonitis), intoxication, endocrine conditions, central nervous disorders, anaphylactic shock, leukemia, or leukocyte adhesion deficiencies (Webb and Latimer, 2011).

A large number of RBC and HCT results from both analyzers fell at or below the lower end of the reference range, and MCH and MCHC results fell at the upper end or above the reference range. Therefore, further studies should evaluate if the reference intervals of both analyzers are suitable for the modern Austrian cow population. As George et al. (2010) demonstrated, several hematological parameters have changed significantly within the past 50 yr in North American dairy cows. The changes were primarily attributed to genetic, environmental, and management factors, as well as disease prevalence. Alternatively, elevated MCH and MCHC might be caused by hemolysis (Brockus, 2011).

A potential weakness of the current study is the sample size and composition of the sample set, which was composed of samples collected from predominantly healthy dairy cows, yielding mainly normal physiological samples. However, the outcome of the present study is not expected to change substantially by selecting a more diverse sample population due to the poor performance of the V-Sight in the limited sample set tested. Sample size calculation was based on comparable studies reported in the literature. A more sophisticated study design should consider a sample size calculation for each tested hematological parameter.

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