J. Dairy Sci. 97:1–7
http://dx.doi.org/10.3168/jds.2013-7736

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

L. Roland,* M. Drillich,* B. Fidlschuster,* I. Schwendenwein,† and M. Iwersen*[1]
*Clinical Unit for Herd Health Management for Ruminants, University Clinic for Ruminants, Department for Farm Animals and Veterinary Public Health, and
†Central Diagnostic Unit, Department for Pathobiology, University of Veterinary Medicine Vienna, 1210 Vienna, Austria

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. Intra-assay 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 ≥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 granulocytes, 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 ref-

Received November 19, 2013.
Accepted May 27, 2014.
[1]Corresponding author: michael.iwersen@vetmeduni.ac.at

erence ranges, which were calculated by Mindray using 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 cornsilage, 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 intra-assay 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 intra-assay precision, 2 samples were randomly selected using the Excel random function and measured 10 times each. A CV of less than