Harran Üniv Vet Fak Derg, 2022; 11 (1): 093-099 *Research Article*
DOI:10.31196/huvfd.1096972

Comparison of the Results of Automatic Blood Analyser and Manual Peripheral Smear Method in Total and Differential Leukocyte Count in Goats

Gülten Emek TUNA[1,a,*]

[1]University of Aydın Adnan Menderes, Faculty of Veterinary Medicine, Department of Internal Medicine, Aydın, Turkey.
[a]ORCID: 0000-0002-9729-8813

Geliş Tarihi: 01.04.2022 **Kabul Tarihi: 11.05.2022**

Abstract: The Abacus Vet 5 (AV5) is a volumetric impedance-based automated haematology analyser that offers a total and 3-part differential (lymphocytes, neutrophils and monocytes) white blood cell (WBC) count in goats. This study aimed to compare the total and differential leukocyte counts (absolute and relative) measured with the AV5 haematology analyser with the results obtained by the manual method from blood smears in goats. It is also aimed to compare the compatibility between the two methods. The blood samples of 40 hair goats (9 healthy and 31 diseased) were analysed by both methods. The relationships between the values were evaluated with a correlation coefficient, and the agreements between the methods were assessed with the Bland-Altman method. The correlation between two methods were excellent for total WBC counts (rho = 0.963, p = 0.000), absolute neutrophils (rho = 0.964, p = 0.000), lymphocytes (rho = 0.928, p = 0.000), a good for the neutrophil percentages (rho = 0.824, p = 0.000), and a weak for absolute monocytes (rho = 0.426, p = 0.006). Although the bias for lymphocyte (−8.25 %) and neutrophil (10.02 %) percentages was relatively significant and the confidence intervals were wide, the agreement for all parameters was acceptable between the two methods in the Bland atman method. In conclusion, the AV5 haematology analyser performed well for total, and differential WBCs counts in goats. However, abnormal results should still be confirmed by a blood smear examination. In addition, instrument performance evaluations, including increased sample sizes, should be performed in further studies.
Keywords: *Blood smear, Goat, Haematology analyser, Manual method, White blood cell.*

Keçilerde Total ve Diferansiyel Lökosit Sayımlarında Otomatik Kan Analizörü ve Manuel Periferik Yayma Yöntemi Sonuçlarının Karşılaştırılması

Özet: Abacus Vet 5 (AV5), keçilerde toplam ve 3 parçalı diferansiyel (lenfositler, nötrofiller ve monositler) lökosit (WBC) sayısı sunan hacimsel empedans tabanlı otomatik bir hematoloji analizörüdür. Bu çalışmada, keçilerde AV5 hematoloji analizörü ile ölçülen toplam ve diferansiyel lökosit sayılarının (mutlak ve bağıl), kan yaymalarından manuel yöntemle elde edilen sonuçlarla karşılaştırılması amaçlandı. Ayrıca iki yöntemin uyumluluğunun karşılaştırılması da amaçlanmaktadır. Kırk kıl keçisinin (9 sağlıklı ve 31 hasta) kan örnekleri her iki yöntemle de analiz edildi. Değerler arasındaki ilişkiler korelasyon katsayısı ile, yöntemler arasındaki uyumlar ise Bland-Altman yöntemi ile değerlendirildi. İki yöntem arasındaki korelasyon, toplam WBC sayıları (rho = 0.963, p = 0.000), mutlak nötrofiller (rho = 0.964, p = 0.000) ve lenfositler (rho = 0.928, p = 0.000) için çok iyi, nötrofil yüzdeleri için iyi (rho = 0.824, p = 0.000) ve mutlak monositler için zayıftı (rho = 0.426, p = 0.006). Lenfosit (% -8,25) ve nötrofil (%10,02) yüzdeleri için yanlılık göreceli olarak önemli ve güven aralıkları geniş olmasına rağmen, Bland-Altman yönteminde iki yöntem arasında tüm parametreler için uyum kabul edilebilirdi. Sonuç olarak, AV5 hematoloji analizörü keçilerde toplam ve diferansiyel WBC sayıları için iyi performans göstermiştir. Bununla birlikte, anormal sonuçlar yine de bir kan frotisi incelemesi ile doğrulanmalıdır.
Anahtar Kelimeler: *Hematoloji analiz cihazı, Kan frotisi, Keçi, Lökosit, Manuel yöntem.*

Introduction

The complete blood count (CBC) is a fast, inexpensive diagnostic test that helps the responsible veterinarian with various problems. Getting the results fast provides a significant advantage to both physicians and patients. CBC provides a rapid assessment of quantitative and qualitative changes in different blood cells (e.g., erythrocytes, leukocytes and platelets) (Mehain et al., 2019; Rejec et al., 2017). From these blood cells, leukocytes (WBC) (total and differential) are widely used in the clinical process to determine the inflammatory response (Willard and Tvedten, 2012). This relationship between WBC and inflammatory response makes the number of WBCs an important measurement for diagnosing and prognosis of various diseases (Chung et al., 2015). WBC is a group of heterogeneous nucleated cells that can circulate at least once in their lifetime, including neutrophils, eosinophils, basophils, lymphocytes and monocytes. The average WBC count in healthy goats ranges from 4 to 13 × 10^3/µL (Weiss and Wardrop, 2010). The differential count by cell type

is more important than the total WBC count because increases and decreases in individual cell types can co-occur, and the total WBC count may remain unchanged (Jones and Allison, 2007).

The leukocyte count can be performed with automatic devices or manually. Automated methods can count large numbers of cells to provide a statistically more accurate reading of the WBC count. The most important feature of these devices is that they give results quickly compared to manual methods and minimise the number of smears to be examined. Therefore, various brands and models of devices are widely used in human and veterinary medicine. However, it is expensive because it requires special equipment (Stirn et al., 2014). These devices measure according to the number of cells, size, surface area, and properties such as granules inside. Because of these features, they may sometimes not be able to distinguish normal cells from abnormal ones and may cause erroneous counts in case of abnormal cells (Platelet aggregates, giant platelets, normoblasts, erythrocytes resistant to lytic solutions) are present (Putzu and Di Ruberto, 2013). The cells should be checked by doing a peripheral smear to confirm the results of the device and identify morphological abnormalities (Jones and Alison, 2007).

Therefore, this study aimed to compare the total and differential leukocyte counts (absolute and relative) measured with the AV5 haematology analyser with the results obtained by the manual method from blood smears in goats. It is also aimed to compare the compatibility between the two methods.

Materials and Methods

The Animal Research Ethics Committee approved the study of the Aydın Adnan Menders University under protocol number 64583101/2022/016.

The animal material of the study consisted of 40 hair goats of different ages and genders, which were brought to Adnan Menderes University Veterinary Faculty Research Hospital and various veterinary clinics in Aydın for examination, treatment and control. Five millilitres of blood samples were taken from *vena jugularis* into tubes with ethylenediaminetetraacetic acid (EDTA) for laboratory examinations from goats. Total and differential (absolute and relative) leukocytes (neutrophils, lymphocytes and monocytes) counts were determined by performing CBC with the Abacus Vet 5 Hematology Device (AV5) (Abacus Vet, Diatron MI LTD, Hungary) within 4 hours from the blood samples taken. The device used the volumetric impedance method, which counts cells according to their properties, such as size and surface area. Also, at least two Wright-Giemsa stained blood smears were prepared for each goat to determine manually the total and differential WBC counts using the method reported by Vives Corrons et al. (2004). Olympus CX21 microscope was used for the manual method. The WBC counts per area in 20 fields (× 40 objective) in a monolayer of the smear were calculated and averaged. The estimated WBC counts were determined by multiplying the obtained value by 1500 (Bellwood and Andrasik-Catton, 2014; Petanides et al., 2004). The recommended correction factor of 100 to 150 for a ×100 magnification on several blood films was used to determine WBCs accurately (Harvey, 2001; Katsogiannou et al., 2019). A microscope with an x 100objective lens was used for the differential count of WBCs. The morphology of 200 WBCs were carefully evaluated for the manual differential in each blood smear. Neutrophil, lymphocyte, and monocyte counts were determined using the battlement method. Relative (%) and absolute numbers ($x10^3/\mu L$) of cells were then calculated. Two different observers evaluated the same blood smears independently, and the average of the two evaluations was used in the study. Samples with inappropriately filled tubes and poor-quality blood smears were excluded from the study.

All statistical analyses were performed using the software Statistical Package for the Social Sciences (SPSS) 19.0 (IBM Corporation, Armonk, USA) and GraphPad Prism 9 (GraphPad Software Inc. San Diego, USA). The distribution of numerical data was checked with Kolmogorov-Smirnov and Shapiro-Wilk tests. Student T-test was used to compare the normally distributed parameters, and A Mann-Whitney test was used to compare non-normally distributed parameters. The relationships between the values were evaluated with the Pearson correlation coefficient (for normal distribıtion data) or Spearman's correlation coefficient (for non-normal distribution data). An agreement between the methods was assessed with the Bland-Altman method (Bland and Altman, 1986), and the manual method for WBC differentials was taken as the reference method. $P<0.05$ was considered significant in all evaluations.

Results

Forty Turkish hair goats of different ages (1-3 years old) and gender were enrolled in this study. Nine goats were healthy, and 31 goats were diseased. Nine goats were deemed healthy based on history, clinical examination and laboratory results. All 31 goats showed varying pneumonia signs, and various parasites were found in their

Table 1. Descriptive statistics and statistical differences of haematological parameters measured with the manual method and the AV5 haematology device.

Parameters	Method	Mean± SD	Median (IQR)	Min-Max	p
WBC (×10³/µL)	M	11.67 ± 4.84	10.35 (8.41-12.95)	4.65-23.85	0.004
	AV5	12.24 ± 5.77	10.98 (8.07-13.58)	4.67-27.86	
Lymphocytes (×10³/µL)	M	6.61 ± 3.37	5.86 (4.21-7.21)	2.34-15.57	0.000
	AV5	5.71 ± 3.02	4.77 (3.54-6.46)	2.18-14.21	
Lymphocytes (%)	M	56.09 ± 11.35	57 (50.00-65.75)	26-76	0.002
	AV5	46.82 ± 11.46	47.40 (40.92-54.93)	18.00-66.80	
Monocytes (×10³/µL)	M	0.09 ± 0.08	0.08 (0.04-0.11)	0-0.33	0.866
	AV5	0.11 ± 0.08	0.09 (0.05-0.13)	0.02-0.44	
Monocytes (%)	M	0.76 ± 0.47	1 (0.5-1)	0-2.07	0.869
	AV5	0.85 ± 0.43	0.7 (0.6-0.9)	0.5-2.80	
Neutrophils (×10³/µL)	M	5.30 ± 3.33	4.41 (2.88-6.29)	1.44-17.27	0.000
	AV5	6.39 ± 3.59	5.30 (3.97-7.68)	1.96-17.34	
Neutrophils (%)	M	42.25 ± 12.22	41.25 (33-48)	25-73.50	0.000
	AV5	52.27 ± 11.52	51.95 (44.35-58.53)	32.70-80.20	

Abbreviations: AV5, Abacus Vet 5; IQR, interquartile range; M, Manuel method; Min.-Max., minimum-maximum;SD, standard deviation; WBC, White blood cell.

stool. When evaluated by both methods, ten goats had leukocytosis, and the leukocyte values of 21 goats were within the reference ranges.

Descriptive statistics and statistical differences of haematological parameters measured with the manual method and the AV5 haematology device are shown in Table 1. WBC, absolute neutrophil count and neutrophil percentage measured on the automated analyser were significantly higher than in the manual method. In contrast, absolute lymphocyte counts and lymphocyte percentages were significantly higher in the manual method ($p > 0.05$) (Table 1). There were no statistical differences in the monocytes (absolute and relative) between methods ($p > 0.05$). Comparing the manual method and the AV5 automated analyzer results, an excellent correlation was determined for WBC count (rho = 0.963, p = 0.000), absolute neutrophil (rho = 0.964, p = 0.000) and lymphocyte count (rho = 0.928, p=0.000), a good correlation for the neutrophil percentages (rho = 0.824, p = 0.000), and a weak correlation for absolute monocyte count (rho = 0.426, p = 0.006). There was no significant relationship between the lymphocyte (rho = - 0.039, $p > 0.05$) and monocyte percentages (rho = 0.063, $p > 0.05$) between the methods. The Bland-Altman plots are presented in Figure 1 and Figure 2. Although low bias was determined in the WBC, neutrophils, lymphocytes, monocytes and monocytes percentages, the bias was relatively high in the percentage of lymphocytes and neutrophils. In addition, 95% of the differences between the measurement values of the parameters evaluated by the two methods were within the limits of agreement (LOAs). However, LOAs for the percentage of lymphocytes and neutrophils were relatively wide.

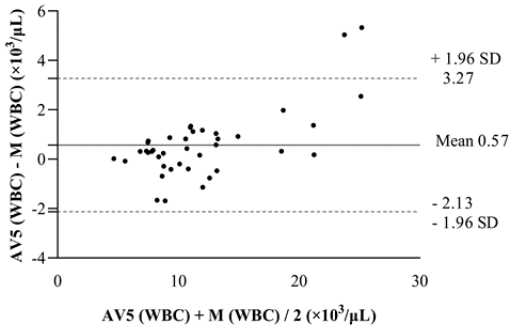

Figure 1. Comparison of WBC obtained from the two methods by Bland-Altman analysis. The solid dark line indicated the bias (mean difference), and dashed dark lines represent the 95% confidence intervals of the bias.
Abbreviations: AV5, Abacus Vet 5; M, Manuel method; SD, standard deviation; WBC, White blood cell.

Discussion and Conclusion

Goats are ruminants that are easy to care for and feed and provide income to their breeders with milk, meat, lint and hair. Better use of bush, rocky and mountainous areas also puts goats in a different place among farm animals. Due to all these features, the interest in goat breeding in animal production increases day by day. In parallel with this increase, an increase is observed in the number of goats brought to veterinary clinics due to

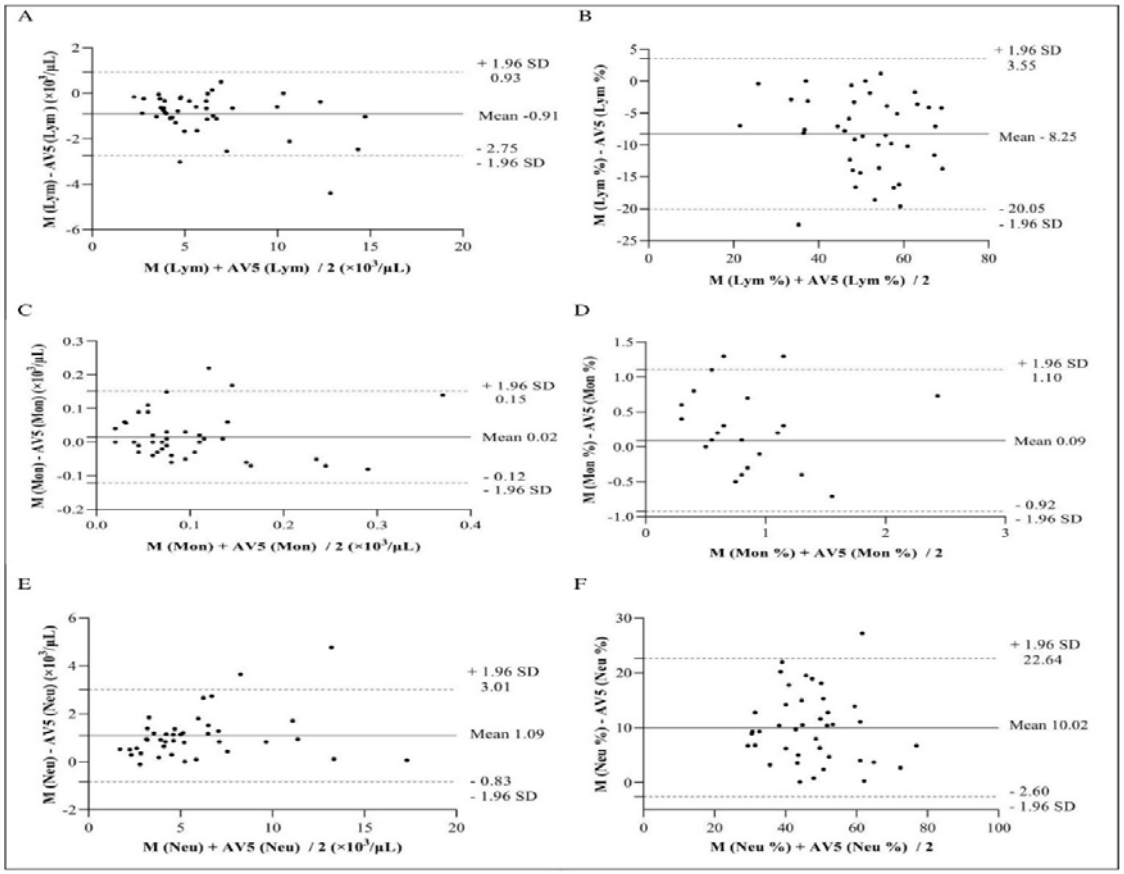

Figure 2. Comparison of lymphocyte counts (A), lymphocyte percentages (B), monocyte counts (C), monocyte percentages (D), neutrophil counts (E), neutrophil percentages (F) obtained from the two methods by Bland-Altman analysis. The solid dark line indicated the bias (mean difference), and dashed dark lines represent the 95% confidence intervals of the bias.
Abbreviations: AV5, Abacus Vet 5; Lym, lymphocyte; M, Manuel method; Mon, monocyte; SD, standard deviation; Neu, neutrophil.

health problems. In ruminants, laboratory evaluation of the CBC is an important extension of the physical examination and has become an important tool in approaching medical cases (Jones and Alison, 2007; Oikonomidis et al., 2020). It can identify certain disease processes and monitor and evaluate animals' health and welfare status. Also, it is beneficial for predicting prognosis. The Abacus Vet 5 is an automated haematology analyser that offers a total and 3-part differential (lymphocytes, neutrophils and monocytes) WBC results on goats. It is widely used in veterinary clinics and hospitals in our country. Therefore, this study aimed to evaluate the effectiveness and agreement of the AV5 automatic analyser compared to the manual method. This is the first study to characterise the analytical performance of the automated analyser for WBC (total and differentiated) measurement in goats.

The agreement of the measurements of the newly developed device with the reference (standard) method is evaluated by method comparison. According to the evaluation result, whether the new device is usable or not can be assessed. For this purpose, comparison of the mean or median of the new (test) and reference method, correlation analysis, linear, Passing Bablok or Deming regression, and Bland-Altman method are statistical methods that are frequently used in determining the agreement between measurement techniques (Giavarina, 2015; Özen, 2018; Van Stralen et al., 2008).

Comparing the mean/median of two methods (new/test-reference) shows the general trend of increasing or decreasing values rather than reflecting the actual difference between them (Bland and Altman, 1986). This statistical method reveals a non-proportional but constant difference between two sets of measurements (Giavarina, 2015). There was a significant difference between WBCs and differentials (absolute and relative) measured with the AV5 automatic analyser and the manual method (Table 1). The automated analyser generated significantly higher WBC, absolute neutrophil count, and neutrophil percentage than the manual method. In contrast, absolute

lymphocyte counts and lymphocyte percentages were significantly higher in the manual method (Table 1). This difference between the two methods can be interpreted as a general trend of increasing or decreasing values rather than inconsistency. This statistical method reveals a disproportionate but constant difference between the two measurement groups (Megahed et al., 2019; Simundic, 2012). The difference between the two methods for WBC counting was an expected result because reference methods such as hemocytometer were not used for WBC counting in this study; only the estimated WBC count and automatic blood count results were compared. Although the manual method is considered the reference method for studies confirming WBC differentials, it is also characterised by significant variability (Oikonomidis et al., 2020). Also, automated devices may not distinguish normal cells from abnormal ones due to abnormal cells, resulting in erroneous results (Putzu and Di Ruberto, 2013). These situations can explain differences in lymphocytes (absolute and relative) and neutrophils (absolute and relative).

The WBC analyses of goats samples for the total and 3-part differential showed good to excellent correlations for WBC count (rho = 0.963, p = 0.000), absolute neutrophil (rho = 0.964, p = 0.000), absolute lymphocyte (rho = 0.928, p=0.000) and neutrophil percentages (rho = 0.824, p = 0.000). Moreover, there was a weak correlation in absolute monocyte (rho = 0.426, p = 0.006), while no significant correlation was found in the lymphocyte (rho = -0.039, p > 0.05) and monocyte percentages (rho = 0.063, p > 0.05). Although correlation coefficients are frequently used to determine the agreement between the two methods, it is also known that this test has potential disadvantages (Giavarina, 2015; Van Stralen et al., 2008). The correlation coefficient primarily depends on the distribution width of the data. Since the total and differential WBC counts were measured in healthy and diseased goats in this study, the parameters covered a wide range of values (Table 1). The wide distribution of the data can explain the excellent to good positive correlations between the values measured by the two methods (Van Stralen et al., 2008). The correlation coefficient can describe the linear relationship between two different data sets; however, it cannot detect whether there is a constant or proportional difference between the two methods (Van Stralen et al., 2008). Therefore, this situation should not mean that the two methods agree. They also report that although the two methods are weakly agreement, they can show high correlation (Bland and Altman, 1986; Chhapola et al., 2015; Jensen and Kjelgaard-Hansen, 2006).

It is emphasised that the Bland and Altman method should be used in determining the agreement of the two methods due to the comparison of mean/median values and various disadvantages of correlation analysis (Giavarina, 2015; Jensen and Kjelgaard-Hansen, 2006; Özen, 2018). The Bland-Altman plots revealed a low bias for WBC (Figure 1), lymphocyte, neutrophil, monocyte and percentage monocyte (Figure 2). The fact that the bias of WBC, lymphocyte, neutrophil, monocyte and percentage monocyte are small and the agreement limits are acceptable shows that the agreement between the two methods for these five parameters are good. However, significant negative (−8.25) and positive (10.02) biases were determined for lymphocyte and neutrophil percentages, respectively (Figure 2). The AV5 haematology device works on the volumetric impedance principle and identifies cells according to their size and surface area. Goats primarily have small and medium-sized lymphocytes (Jones and Alison, 2007). However, small ruminant blood smears may typically contain a low number of large lymphocytes (Oikonomidis et al., 2020). An increased number of large lymphocytes, platelet aggregations, giant platelets and normoblasts may be misclassified by automated devices, affecting the percentage of neutrophils and lymphocytes (Oikonomidis et al., 2020). It should also not be forgotten that the manual method, which is the reference method, is also characterised by significant variability (Rümke, 1977). These conditions can explain the considerable biases between the two methods in lymphocyte and neutrophil percentages. Although significant bias was detected for both parameters and the 95% agreement limits were moderately wide, 95% of the differences between the measurement values of lymphocyte and neutrophil percentages were within LOAs. The Bland-Altman analysis objectively reveals the measurement differences between the two methods. It leaves the interpretation of the acceptability level of the differences to the clinician's opinion (Jensen ve Kjelgaard-Hansen, 2006). Given that the reference ranges for lymphocyte (50-70 %) and neutrophil percentages (30-48 %) in goats were relatively wide and the absolute values of both parameters agreed between methods, this bias was unlikely to affect clinical interpretations. Thus, it was assumed that the agreement between the two methods was also acceptable for these parameters.

There are several limitations to this study. First, the recommended reference method in goats was not used for the total leukocyte count. The agreement between the estimated WBC counts obtained from the peripheral smear and the WBC counts obtained from the automated device was

compared. Second, CBCs were not performed in duplicate as ideally recommended in the ASVCP guidelines (Arnold et al., 2019) for method comparison. Third, the agreements of total and differential WBCs of sick and healthy goats were evaluated together and were not assessed separately. Finally, the sample count was the minimum recommended for method comparisons (Bilic-Zulle, 2011; Jensen & Kjelgaard-Hansen, 2006; Westgard, 2010). A larger number of animals could have increased the power of the tests.

In conclusion, the Abacus Vet 5 haematology analyser appears to perform well for total and differential WBCs compared with the manual method, and it can be used safely for routine laboratory examinations in goats. However, there is a high probability of numerical and morphological abnormalities in total and differential WBCs of sick goats. Therefore, the abnormal results should always be confirmed with a blood smear examination. In addition, the precision, accuracy and instrument performance evaluations, including increased sample sizes, should be performed in further studies.

Acknowledgement

We would like to thank Aleyna DEMİRCİ for their support in this study.

Conflict of Interest

The author stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

This study was approved by the xAydın Adnan Menderes University Animal Experiments Local Ethics Committee (64583101/2022/016 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Similarity Rate

We declare that the similarity rate of the article is 8% as stated in the report uploaded to the system.

Author Contributions

Motivation / concept: GET
Design: GET
Control/supervision: GET
Data collection and/or processing: GET
Analysis and/or Interpretation: GET
Literature review: GET
Writing the article: GET
Critical review: GET

References

Arnold JE, Camus MS, Freeman KP, Giori L, Hooijberg EH, Jeffery U, Korchia J, Meindel MJ, Moore AR, Sisson SC, Vap LM, Cook JR, 2019: ASVCP guidelines: principles of quality assurance and standards for Veterinary Clinical Pathology (version 3.0). *Vet Clin Pathol*, 48 (4), 542-18.

Bellwood B, Andrasik-Catton M, 2013: Veterinary Technician's Handbook of Laboratory Procedures. 1st ed., Wiley-Blackwell Publishing, New York, USA.

Bilić-Zulle L, 2011: Comparison of methods: Passing and Bablok regression. *Biochem Med,* 21 (1), 49-52.

Bland JM, Altman DG, 1986: Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1 (8476), 307-310.

Chhapola V, Kanwal SK, Brar R, 2015: Reporting standards for Bland-Altman agreement analysis in laboratory research: a cross-sectional survey of current practice. *Ann Clin Biochem*, 52 (3), 382-6.

Chung J, Ou X, Kulkarni RP, Yang C, 2015: Counting White Blood Cells from a Blood Smear Using Fourier Ptychographic Microscopy. *PLoS One*, 10 (7), e0133489.

Giavarina D, 2015: Understanding Bland Altman analysis. *Biochem Med*, 25 (2),141-51.

Harvey J (2001). Leukocyte evaluation In: Atlas of Veterinary Hematology: Blood and Bone Marrow of Domestic Animals. Harvey J (Ed), 24-26, Saunders Company, Philadelphia.

Kjelgaard-Hansen M, Jensen AL, 2006: Is the inherent imprecision of manual leukocyte differential counts acceptable for quantitative purposes? *Vet Clin Pathol,* 35 (3), 268-270.

Jones ML, Allison RW 2007: Evaluation of the ruminant complete blood cell count. *Vet Clin North Am Food Anim Pract*, 23 (3), 377-402.

Katsogiannou EG, Athanasiou LV, Katsoulos PD, Polizopoulou ZS, Tzivara A, Christodoulopoulos G, 2020: Estimation of white blood cell and platelet counts in ovine blood smears, and a comparison with the ADVIA 120 hematology analyser. *Vet Clin Pathol*, 49, 222-226.

Megahed AA, Hiew MWH, Grünberg W, Constable PD, 2016: Evaluation of 2 portable ion-selective electrode meters for determining whole blood, plasma, urine, milk, and abomasal fluid potassium concentrations in dairy cattle. *J Dairy Sci*, 99 (9), 7330-7343.

Mehain SO, Haines JM, Lee PM, 2019: Platelet indices as biomarkers for characterisation and determination of severity in canine chronic enteropathy. *Vet J*, 248, 37-41.

Oikonomidis IL, Brozos C, Kiossis E, Tsouloufi TK, Kritsepi-Konstantinou M, 2021: A comparison study between the results of the Siemens ADVIA 120 analyser and

the manual method for differential leukocyte counts in sheep. *Vet Clin Pathol*, 50, 203-208.

Özen D, 2018: Ölçüm teknikleri arasındaki uyumun belirlenmesinde kullanılan grafiksel yöntemlerin ve regresyon modellerinin bir uygulama ile incelenmesi. *Eurasian J Vet Sci*, 34 (4), 265-271.

Petanides TA, Mylonakis ME, Koutinas AF, 2004: Evaluating the leukogram in the dog and cat. *J Hellenic Vet Med Soc*, 55, 130-135.

Putzu L, Di Ruberto C, 2013: White blood cells identification and counting from microscopic blood image. Int. J. Pharm. Biomed. Res, 7 (1), 15-22.

Rejec A, Butinar J, Gawor J, Petelin M, 2017: Evaluation of Complete Blood Count Indices (NLR, PLR, MPV/PLT, and PLCRi) in Healthy Dogs, Dogs With Periodontitis, and Dogs With Oropharyngeal Tumors as Potential Biomarkers of Systemic Inflammatory Response. *J Vet Dent*, 34 (4), 231-240.

Rümke SL, 1977: The statistically expected variability in differential leukocyte counting. In: Differential Leukocyte Counting, Koepke JA (Ed), 39-45, College of American Pathologists, Aspen, USA.

Simundic AM, 2012: Practical recommendations for statistical analysis and data presentation in Biochemia Medica journal. *Biochem. Med,* 22 (1), 15-23.

Stirn M, Moritz A, Bauer N, 2014: Rate of manual leukocyte differentials in dog, cat and horse blood samples using ADVIA 120 cytograms. *BMC Vet Res*, 10, 125.

Van Stralen KJ, Jager KJ, Zoccali C, Dekker FW, 2008: Agreement between methods*. Kidney Int,* 74 (9), 1116-1120.

Vives Corrons JL, Albarède S, Flandrin G, Heller S, Horvath K, Houwen B, Nordin G, Sarkani E, Skitek M, Van Blerk M, Libeer JC, 2004: Haematology Working Group of the European External Committee for External Quality Assurance Programmes in Laboratory Medicine. Guidelines for blood smear preparation and staining procedure for setting up an external quality assessment scheme for blood smear interpretation. Part I: Control material. *Clin Chem Lab Med*, 42 (8), 922-6.

Weiss DJ, Wardrop KJ, 2010: Schalm's Veterinary Hematology, 6th ed., Wiley-Blackwell Publishing, New York, USA.

Westgard JO, 2010: The Comparison of Methods Experiment. http://www.westgard.com/lesson23.htm#10, Erişim tarihi; 18.11.2019.

Willard DM, Tvedten H, 2012: Small Animal Clinical Diagnosis by Laboratory Methods. 5th ed., Elsevier, USA.

*__Yazışma Adresi:__ Gülten Emek TUNA

University of Aydın Adnan Menderes, Faculty of Veterinary Medicine, Department of Internal Medicine, Aydın, Turkey

e-mail: emektuna@adu.edu.tr