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PERFORMANCE EVALUATION OF 3-PART MULTISPECIES HEMATOLOGY ANALYZERS FOR WHITE BLOOD CELL COUNTING IN SHEEP

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Abstract

This study aimed to assess the accuracy and precision of 3-part multispecies hematology analyzers for white blood cell (WBC) counting in comparison to manual quantification in apparently healthy Sipli sheep (n = 60). Blood samples were collected once and analyzed using a hemocytometer with two different dilutions (1:20 and 1:40), referred to as WBC-1 and WBC-2, respectively. Automated WBC counting was performed using two multispecies veterinary hematology analyzers, WBC-R and WBC-B. The mean (± SE) values and reference intervals (RIs) for overall and group-wise data showed that only WBC-R fell within the physiological range for sheep, whereas WBC-1, WBC-2, and WBC-B reported lower values. A weak agreement was observed between the two multispecies analyzers, with a mean bias of -30.97 (upper limit: -14.56, lower limit: -46.77) and a standard deviation of bias of 8.37. The intraclass correlation coefficient (ICC) was also low (0.619), indicating poor consistency. Additionally, Lin's concordance correlation coefficient (LCCC) measured accuracy at 0.086, while precision was determined to be 0.603. In summary, hemocytometer-based manual WBC counting in sheep may lack accuracy. Among the tested analyzers, Rayto RT-7600Vet (China) produced WBC counts closest to the physiological range, making it a more suitable option for clinical use. It is concluded that 3-part hematology analyzers with predefined settings for sheep require calibration with separate set of RIs to ensure accurate analysis of sheep blood.

Key words*: hematological validations, multispecies hematology analyzers, point-of-care-tests.*

INTRODUCTION

The hemocytometer is widely regarded as the "standard manual tool" for blood cell counting. However, it has largely been replaced by highly sensitive and specific hematology analyzers, which offer rapid and automated cell counts along with a broader range of hematological parameters (Huang et al., 2023; Data Bridge Market Research, 2022). Despite these advancements, the hemocytometer remains a key reference tool for validating the accuracy of hematology analyzers.

Over the past two decades, significant achievements in hematology and improved the accessibility and precision of automated analyzers in both human and veterinary medicine (DeNicola, 2011). Sysmex Corporation (Japan) is recognized as a global leader in hematology analyzer development, while other countries, including China, the USA, France, and Germany, have also made substantial contributions to analyzer manufacturing. China, in particular, has been developing these analyzers for nearly 40 years, making it the second-largest market for global research and production (Song & Zhu, 2020; Absar et al., 2024). Since medical diagnoses rely heavily on analyzer-generated results, ensuring analytical validity through systematic verification is crucial before implementing a new hematology analyzer in clinical practice (Farooq et al., 2023a; Vis & Huisman, 2016).

In veterinary medicine, 3-part and 5-part multispecies hematology analyzers are extensively used for white blood cell (WBC) counting and differential leukocyte analysis as point-of-care tests (POCTs). The 5-part analyzers provide more detailed leukocyte differentiation, whereas 3-part analyzers

classify WBCs into three broad categories: lymphocytes, intermediate cells, and granulocytes (Daves et al., 2024; Farooq et al., 2025). This limited classification may compromise the accuracy of WBC counts, particularly in species with unique hematological characteristics, such as sheep. WBC counting in sheep poses additional challenges, especially when using 3-part multispecies hematology analyzers, which may not provide precise results. Previous studies suggest that WBC counts obtained from different analyzers can vary significantly, often producing erroneous values. In Pakistan, multispecies hematology analyzers are becoming increasingly popular for veterinary diagnostics, yet their accuracy, precision, and reliability for WBC counting in sheep have not been systematically evaluated. This study is the first of its kind to assess the accuracy and precision of 3-part multispecies hematology analyzers compared to manual quantification using a hemocytometer with two dilution methods in apparently healthy Sipli sheep.

MATERIALS AND METHODS

Geo-Location of the Study

The current research was carried out concurrently at two locations within The Islamia University of Bahawalpur (IUB), Pakistan: the Livestock Farm under the Faculty of Veterinary and Animal Sciences (FV & AS), and the Postgraduate Laboratory in the Department of Physiology. Both facilities are located near the outskirts of the Cholistan Desert - locally referred to as Rohi - in Bahawalpur, Punjab. The desert is an extension of the Great Indian Desert, which also encompasses the Rajhsatan Desert in India and the Thar Desert in Sindh Province, Pakistan. Distributed through an area of 26000 km², it begins around 30 km from the city of Bahawalpur, Punjab, Pakistan, and is situated between latitudes 27º42´and 29º45´North and longitudes 69º52´and 75º24´East. This region endures an arid subtropical continental climate with extreme temperatures, high rates of evaporation, moderate to minimal rainfall, strong summer winds, and low moisture content. It is acknowledged as one of Pakistan's warmest regions, with an extended summertime that lasts from May to October (Farooq et al., 2010).

Study Animals

The study included Sipli sheep (n = 60) raised at the university farm, all of which appeared clinically healthy. An intensive farming system is being implemented for the animal rearing. They are fed in stalls with a concentrate ration that contains about 15% crude protein and freshly chopped seasonal fodder. In addition, depending on demand, maize silage and wheat straw is also provided. The fresh and pure drinking water is always made available for them. Regular vaccination and deworming are conducted at the farm in addition to routine veterinary inspections. Routine hematological evaluations, combined with thorough physical examinations and input from farm staff, confirmed that all animals involved in the study were clinically healthy and exhibited no indications of illness.

The Sipli is a native sheep breed of Pakistan with somewhat long and thin tail, medium-sized body, with males weighing an average of 32.8 kg and females 29.2 kg. They produce 0.2-0.4 L of milk per day. Its head and ears are light brown or white, with its white body coat. Its ears extend to a length of approximately 15 centimeters, and its medium-sized head features a flat nose. It is primarily raised for the production of mutton and wool by the nomadic herders of Cholistan desert of Southern Punjab, Pakistan (Idris et al., 2024a; 2024b; Sharif et al., 2024; 2025).

Blood Sampling

Blood collection was performed by trained personnel, with each animal gently restrained during the procedure. A total of 5 mL of blood was aseptically drawn from the jugular vein using sterile technique. The samples were collected into EDTA-coated vacutainer tubes (TUBER® Vac EDTA K2, Australia) with purple caps, each containing 0.5 mL of 1% EDTA solution to prevent coagulation. Blood samples were brought to the laboratory in ice box and analyzed within 4 hours.

White Blood Cell Counting

The WBC counting was conducted by following techniques:

a) ***Manual method:*** White blood cell (WBC) counts were determined using a Neubauer hemocytometer (MARIENFELD, Germany), following the standard procedure described by

Berkson et al. (1935), with minor modifications. Turk's solution (SDL Scientific Enterprise, Pakistan) served as the diluting agent. Two dilution ratios, 1:20 and 1:40, were employed for the analysis and designated as WBC-1 and WBC-2, respectively.

b) *Automated method:* Automated WBC counts were conducted using two multispecies veterinary hematology analyzers - Rayto RT-7600Vet and Biobase BK-5000Vet (China) - referred to in this study as WBC-R and WBC-B, respectively. Both devices are 3-part differential analyzers that classify WBCs into lymphocytes, intermediate cells, and granulocytes. These analyzers support hematological evaluation across 13 predefined species (including sheep) and four user-defined species. Given that sheep is among the predefined options, this default species setting was utilized for the current analysis.

Key operational specifications of both analyzers include a minimum whole blood requirement of 9.8 µL, a pre-diluted volume of 20 µL, and a test completion time of approximately one minute. They function optimally within a temperature range of 15-35°C and relative humidity of 10-90%. Each analyzer generates 23 hematological parameters along with histograms for WBCs, red blood cells (RBCs), and platelets (PLTs). The systems employ specific reagents - lysing agents, diluents, and cleaning solutions - supplied by the manufacturers to ensure accurate results, as per their user manuals. Additionally, reference intervals can be manually adjusted for individual species, and any values outside the normal range are automatically flagged in the complete blood count (CBC) report (Dokumen, 2020).

Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics (Version 20) and GraphPad Prism (Version 8.0.1). Outlier detection was performed through visual inspection in conjunction with the Shapiro-Wilk test to evaluate normality. Data were stratified based on sex (females, $n = 43$; males, $n = 17$) and age categories (≤1 year, $n = 10$; 1–2 years, $n = 35$; >2 years, $n = 15$). Reference intervals (RIs), defined as the 25th to 90th percentiles, were established in accordance with the guidelines of the American Society for Veterinary Clinical Pathology (Friedrichs et al., 2012), utilizing the Reference Value Advisor software (version 2.1; available at: http://www.biostat.envt.fr/reference-value-advisor).

Comparisons between manual counts and automated hematology analyzers were assessed using one-way ANOVA followed by Duncan's multiple range post-hoc test, with significance determined at $P \leq 0.05$. Pearson's correlation coefficient was employed to examine the relationships between WBC counts derived from different methods, while linear regression was applied to develop predictive equations.

To evaluate the level of agreement between the two automated analyzers, three complementary methods were used: Bland-Altman analysis (B & A), Cronbach's alpha, and the Intraclass Correlation Coefficient (ICC). Furthermore, Lin's Concordance Correlation Coefficient (LCCC) was utilized to assess both accuracy and precision of the analyzers' measurements.

RESULTS AND DISCUSSIONS

Multispecies automated hematology analyzers are intricate instruments validated by manufacturers under ideal conditions before market release, following national or regional regulatory frameworks such as those of the FDA (USA) and CE Mark (EU). However, their routine analytical and diagnostic performance often shows significant variability. To ensure accurate and reliable results in daily use, international guidelines provided by organizations like American Society for Clinical Veterinary Pathology (ASVCP), the Clinical Laboratory Standards Institute (CLSI), and the International Organization for Standardization (ISO) outline verification criteria, including precision, accuracy, linearity, and comparability. These guidelines also emphasize the importance of performance evaluation, which is at the discretion of clinical laboratories and their personnel (Farooq et al., 2023a; 2023b). This study, is the first of its kind, which incorporated indigenous Sipli breed of sheep of Pakistan, for assessing the performance evaluation of 3-part multispecies hematology analyzer for WBC counting, in comparison to manual quantification methods (through hemocytometer) in a method-comparison analysis.

The results of normality testing in terms of Shapiro-wilk value, kurtosis, skewness and CV% are given in Figure 1.

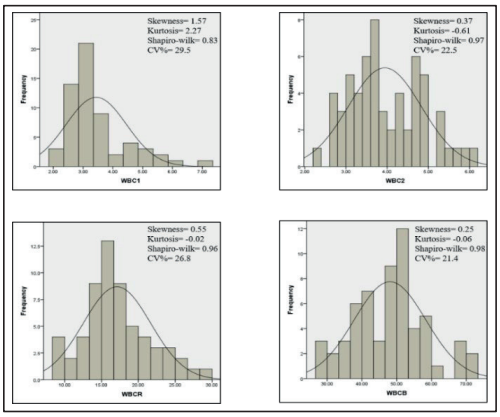

Figure 1. Histograms depicting the distribution of white blood cell (WBC) counts in Sipli sheep (n = 60), obtained through manual counting methods (WBC-1 and WBC-2) and two automated hematology analyzers (WBC-R and WBC-B). The plots illustrate the variability and spread of WBC values across all four methods, enabling visual comparison of data distribution patterns

In the present study, data of WBC count attained through both multispecies veterinary hematology analyzers (WBC-R and WBC-B) had lowest skewness and kurtosis which is in line with previous studies (Daves et al., 2024; Huang et al., 2023; Ma et al., 2023). Manual quantification of white blood cells using hemocytometry is inherently subjective and susceptible to variability due to operator skill level and inconsistencies in dilution techniques. Previous studies have demonstrated that automated hematology analyzers offer superior sensitivity, reliability, and predictive accuracy when compared to traditional manual methods such as hemocytometry (Clark et al., 2019; Lee et al., 2022; Oikonomidis et al., 2024).

The results for overall comparison of WBC count between manual methods (WBC-1 and WBC-2) and through multispecies automated hematology analyzers (WBC-R and WBC-B) are given in Table 1.

Results revealed that the mean (±SE) values for WBC-1 and WBC-2 were non-significantly different from each other whereas, WBC-R and WBC-B were significantly different (P≤0.05) from each other, and also differed significantly from WBC-1 and WBC-2.

Table 1. Descriptive statistics for white blood cell (WBC) counts obtained from apparently healthy Sipli sheep (n = 60) using manual methods (WBC-1 and WBC-2) and automated hematology analyzers (WBC-R and WBC-B). Parameters presented include overall mean ± standard error (SE), median, interquartile range (IQR), minimum and maximum values, reference interval (RI; 25th to 90th percentile), and 95% confidence interval (CI).

Methods	Mean ± SE	Median (IQR)	Range (Min.-Max.)	RI (25^{th}-90^{th})	95% CI
WBC-1	3.44±0.13[a]	3.10(0.98)	4.90 (2.10-7.00)	2.80-5.19	3.17-3.70
WBC-2	3.94±0.11[a]	3.70(1.45)	3.70 (2.30-6.00)	3.22-5.20	3.71-4.17
WBC-R	17.09±0.59[b]	16.5(5.79)	19.92 (9.45-29.37)	13.84-24.60	15.91-18.28
WBC-B	48.07±1.32[c]	48.56(13.15)	44.95 (27.97-72.92)	40.28-60.58	45.41-50.73

[a, b, c] Superscripts indicate the significance at (P≤0.05) for different methods of WBC counting.

Considering the mean (±SE) values and RIs for WBCs of the present study, it was revealed that data of WBC-R was within the physiological range for sheep blood given elsewhere (Mostaghni et al., 2005; Rishniw & Pion, 2016). Similar results were attained for gender-based and age-based groups as well as shown in Tables 2 and 3, respectively. The WBC-R for overall and group-wise data was significantly (P≤0.05) higher (17.09±0.592×109/L) than WBC-1 and WBC-2, and lower than WBC-B, though within the normal physiological range. Similar results were attained for gender-based and age-based groups. The WBC count in another study (Abdel-Lattif & Al-Muhja, 2021; Zamfirescu et al., 2009) showed the mean (±SE) values of 18.60±0.69 and 15.41±1.21 which are nearly equal to the value obtained through WBC-R of present study. While, the lower values have also been reported for sheep WBCs being 9.13±0.59 and 7.3±1.8 (Ahmadi-hamedani et al., 2016; Frye et al., 2022). Such huge variation in WBC count for sheep inevitably depicts that starting from blood collection, transport and analysis till the use of various techniques (manual and/or automated) affect the count and need to be kept in strict consideration.

Table 2. Gender-Wise WBC Count as Deduced through Manual Methods (WBC-1 and WBC-2) and Hematology Analyzers (WBC-R and WBC-B) for Apparently Healthy Sipli Sheep (n = 60)

Methods	Mean ± SE	Median (IQR)	(Min.-Max.)	Range	RI (25^{th}-90^{th})	95% CI
Males (n = 17)						
WBC-1	3.23±0.185[a]	3.00 (0.85)	(2.10-5.20)	3.10	2.80-4.64	2.84-3.62
WBC-2	3.76±0.252[a]	3.70 (1.70)	(2.30-6.00)	3.70	2.80-5.44	3.23-4.29
WBC-R	18.50±1.358[b]	17.18 (8.78)	(9.70-29.37)	19.67	14.82-27.25	15.62-21.38
WBC-B	48.83±3.229[c]	50.76 (19.21)	(27.97-72.92)	44.95	36.71-70.60	41.99-55.68
Females (n = 43)						
WBC-1	3.52±0.167[a]	3.20 (1.00)	(2.10-7.00)	4.90	2.80-5.42	3.18-3.86
WBC-2	4.01±0.126[a]	3.70 (1.20)	(2.60-5.80)	3.20	3.50-5.20	3.75-4.26
WBC-R	16.54±0.618[b]	16.33 (5.37)	(9.45-25.83)	16.38	13.50-22.10	15.29-17.79
WBC-B	47.77±1.372[c]	48.37 (12.44)	(28.67-67.48)	38.81	41.08-59.41	45.00-50.54

SE=Standard Error; IQR=Interquartile Range; RI=Reference Interval; CI=Confidence Interval; [a, b, c] Superscripts indicate the significance at (P≤0.05) for different methods of WBC counting within gender-based groups.

Table 3. Age-Wise WBC Count as Deduced through Manual Methods (WBC-1 and WBC-2) and Hematology Analyzers (WBC-R and WBC-B) for Apparently Healthy Sipli Sheep (n = 60)

Methods	Mean ± SE	Median (IQR)	(Min.-Max.)	Range	RI (25^{th} 90^{th})	95%CI
G1 = up till one year						
WBC-1	3.23±0.278 [a]	3.00 (0.50)	(2.30-5.50)	3.20	2.82-5.32	2.60-3.86
WBC-2	3.89±0.230[a]	3.55 (1.30)	(2.90-4.80)	1.90	3.40-4.79	3.36-4.41
WBC-R	14.43±0.926[b]	15.02 (4.87)	(9.45-18.87)	9.42	11.89-18.72	12.33-16.52
WBC-B	48.81±2.837[c]	48.56 (6.26)	(32.41-67.48)	35.07	45.46-66.23	42.39-55.22
G2 = from one to two years						
WBC-1	3.45±0.191[a]	2.90 (1.00)	(2.10-7.00)	4.90	2.80-5.24	3.06-3.84
WBC-2	3.77±0.149[a]	3.60 (1.20)	(2.30-6.00)	3.70	3.10-5.02	3.46-4.07
WBC-R	17.98±0.821[b]	17.18 (5.90)	(9.46-29.37)	19.91	15.02-25.55	16.31-19.64
WBC-B	51.12±1.520[c]	51.40 (13.45)	(35.72-72.92)	37.20	43.31-63.25	48.03-54.21
G3 = above two years						
WBC-1	3.55±0.213[a]	3.40 (0.90)	(2.60-5.80)	3.20	3.00-5.02	3.09-4.01
WBC-2	4.38±0.232[a]	4.50 (1.50)	(2.70-5.80)	3.10	3.70-5.62	3.88-4.87
WBC-R	16.81±1.115[b]	15.40 (7.18)	(9.70-24.81)	15.11	13.56-23.26	14.42-19.20
WBC-B	40.47±2.745[c]	38.12 (12.70)	(27.97-70.03)	42.06	33.12-57.88	34.58-46.36

SE=Standard Error; IQR=Interquartile Range; RI=Reference Interval; CI=Confidence Interval; G1= up till 1 year; G2= from 1 to 2 years, G3= above 2 years.
[a, b, c] Superscripts indicate the significance at (P≤0.05) for different methods of WBC counting within age-based groups.

In contrast, the results obtained through manual methods (WBC-1 and WBC-2) in the present study showed the mean (± SE) values were lower than normal physiological range for sheep (3.44-3.94×109/L). Similarly, values attained through other hematology analyzer (WBC-B) showed the values were higher than normal physiological range for sheep (48.07×109/L). The lower values than normal physiological range obtained through manual methods could reasonably be clarified through the subjectivity of the hemocytometry method. Moreover, the higher values than normal physiological range obtained through hematology analyzer (WBC-B) may be because of the built-in capabilities of equipment (Clark et al., 2019; Hippel, 2007).

As the WBC count attained through multispecies automated hematology analyzer (WBC-R) was within normal physiological range for sheep in the present study, hence logilinear regression was implied between the data of WBC-R and the data obtained through other three methods i.e. WBC-1, WBC-2 and WBC-B (Figure 2).

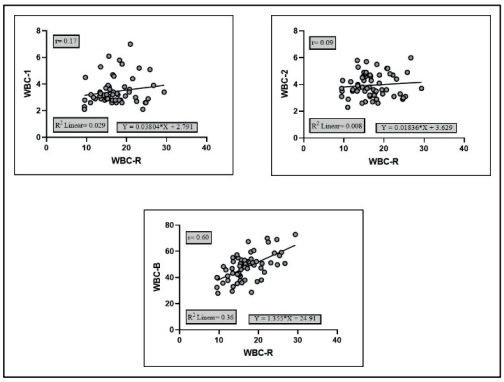

Figure 2. Scatterplots illustrating log-linear regression relationships between WBC counts obtained via the Rayto hematology analyzer (WBC-R) and three comparison methods in apparently healthy Sipli sheep (n = 60): a) manual method WBC-1, b) manual method WBC-2, and c) automated analyzer WBC-B. Each plot demonstrates the strength and pattern of association between WBC-R and the respective methods

A weak relationship was noticed between the WBC count attained through hematology analyzer (WBC-R) versus manual methods (WBC-1 and WBC-2) as indicated by weak r-values and r-square values. Moderately high level of relation was noticed only between WBC-R and WBC-B (r-value = 0.60; r-square value = 0.36; 36% probability). The results are in strong concordance to previous research works which have endorsed that manual quantification of cells (through hemocytometry) gives erroneous results and is prone to subjectivity whereas automated analyzers are precise and provide accuracy (Daves et al., 2024; Ma et al., 2023; Michael et al., 2022; Vis & Huisman, 2016). However, a weak correlation (36% probability) between the two automated multispecies hematology analyzers of the present study is not in line with previous studies which have reported a strong correlation between different hematology analyzers (Grebert et al., 2021; Vis & Huisman, 2016).

Automated multispecies hematology analyzers are widely regarded as the gold standard for assessing blood characteristics. In this study, the level of agreement between two such analyzers (WBC-R and WBC-B) was evaluated using B & A analysis, Cronbach's alpha, and ICC. The B & A plot (Figure 3) revealed a weak agreement between the analyzers, with a mean bias of -30.97 (upper limit: -14.56, lower limit: -46.77) and a standard deviation of bias of 8.37.

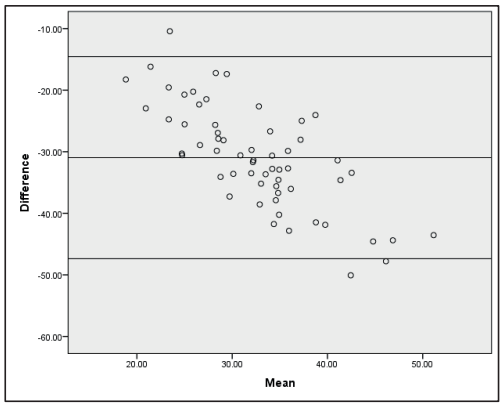

Figure 3. Bland-Altman scatterplot illustrating the agreement between WBC counts obtained from two automated hematology analyzers - WBC-R and WBC-B - in Sipli sheep (n = 60). The central horizontal line represents the mean difference (bias) between the two methods, calculated as –30.97. The upper and lower limits of agreement are depicted as –14.56 and –46.77, respectively, based on the standard deviation (SD) of the bias, which was 8.37.

Cronbach's alpha and ICC results (Table 4) further supported this finding, with the ICC value of 0.619 indicating moderate reliability

under a two-way random effects model for consistency. The LCCC reflected low accuracy (0.086), while precision was moderate (0.603). Additionally, the mean bias error (MBE) was calculated at 30.268, with WBC-B consistently reporting higher WBC counts compared to WBC-R. These findings contrast with prior studies that reported stronger agreement among different multispecies hematology analyzers, underscoring the variability in analyzer performance and the need for validation before clinical use (Brouwer et al., 2017; Cook et al., 2016; Michael et al., 2022; Rishniw & Pion, 2016).

Table 4. Cronbach's Alpha, Intraclass Correlation, Lin's Concordance Correlation Coefficient and Mean Bias Error (MBE) between WBC-R and WBC-B in Apparently Healthy Sipli Sheep (n = 60)

WBC-R vs WBC-B						
Intraclass correlation		95% CI	Cronbach's Alpha	Accuracy	Precision	MBE
Single measure	0.449	0.221-0.629	0.619	0.086	0.603	30.268
Average measure	0.619	0.363-0.773				

CI = Confidence Interval

CONCLUSIONS

The present study concludes that hemocytometer-based manual techniques for counting WBCs in sheep are likely inaccurate, as they tend to yield underestimated values.

Among the 3-part multispecies hematology analyzers evaluated in this study (Rayto RT-7600Vet and Biobase BK-5000Vet, China), the Rayto RT-7600Vet produced WBC counts closest to the physiological range for sheep. However, when using 3-part analyzers with predefined settings for sheep, caution is required, as their accuracy may vary.

To ensure reliable hematological assessment in sheep, it is essential to establish species-specific reference intervals (RIs) for each analyzer.

These findings provide valuable guidelines for research laboratories and clinical settings, particularly those with limited resources, emphasizing the cautious interpretation of results.

It is recommended that laboratories establish analyzer-specific reference intervals (RIs) and coefficient of variation (CV %) values for all hematological parameters.

Regular validation protocols should be implemented for both 3-part and 5-part hematology analyzers to ensure accuracy and reliability.

Additionally, RIs should be derived from larger populations of healthy animals to improve the precision and applicability of these techniques in clinical and research settings.

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