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The Correlation of AI Based Automated Hematology Analyzers and Manual Results of Complete Blood Count in Dog and Cat Blood

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Simple Summary

Recently, integrating artificial intelligence (AI) in conjunction with automated blood analyzers into clinical laboratory testing will enhance accuracy, speed, and reliability. This study assessed the performance of the Awalife AI-100Vet multi-functional morphological analyzer when compared to manual CBC testing in dogs and cats. In dogs, the following parameters PCV, hemoglobin, red blood cell count, MCH, white blood cell count, and differential counts (% neutrophils, lymphocytes, monocytes, eosinophils, and reticulocytes) showed significant correlation. In cats, PCV, hemoglobin, WBC count, and percentages of neutrophil, lymphocyte, and eosinophil were also significantly correlated. AUC values of 0.72 in dogs and 0.92 in cats were determined, indicating good diagnostic performance and good agreement with manual methods. Conversely, there are still some discrepancies that require further algorithm refinement to improve recognition of abnormal cell morphology, ensure consistency across sample variations, and enhance accuracy in specific pathological conditions.

Abstract

The complete blood count (CBC) is a diagnostic test to analyze abnormalities of blood cells. Currently, automated hematology analyzers and artificial intelligence technology are being used with automated blood analyzers to ensure accuracy and reliability. This study aimed to evaluate the performance of artificial intelligence (AI) based automated blood cell analyzer, Awalife AI-100Vet Multifunctional Morphological Analyzer, in dog and cat blood samples by comparison with the CBC manual method. In dogs, PCV, hemoglobin, RBC, MCH, WBC, % Neutrophil, %Lymphocyte, %Monocyte, %Eosinophil and %Reticulocyte were all significantly correlated. While in cats, PCV, Hemoglobin RBC, WBC, % Neutrophil, % Lymphocyte, and % Eosinophil were all also significantly correlated. AUC values obtained by the Awalife AI-100Vet analyzer for Hematology testing in dogs and cats were 0.72 and 0.92 respectively. These findings suggest that the Awalife AI-100Vet analyzer demonstrated good accuracy using dog blood for hematology testing as well as excellent accuracy when using cat blood. The AI-based automated blood analyzer has the potential to analyze hematological data and is close to the reference method. However, there are still differences in some parameters. Further optimization of the AI algorithm, which will involve increasing the accuracy of identifying unusual cell shapes, improving stability against various samples, such as stains, and achieving good results when working with unique pathologies, should be carried out.

Keywords: AI based automated; hematology; complete blood count; CBC; dog; cat

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1. Introduction

A complete blood count (CBC), also known as peripheral blood smear, provides a baseline of laboratory tests that are used to evaluate an animal's health. In veterinary medicine, a CBC is used to assist in diagnosing diseases, monitoring the effectiveness of treatments and determining the overall health of a companion animal [1]. In evaluating a CBC, an assessment of red blood cell morphology can be helpful in determining the presence of hematological and/or metabolic abnormalities, the presence of oxidative damage, or to assist in pinpointing the pathogenesis of disease. Four morphologic features of red blood cells (RBCs) were assessed: color, size, shape, and presence of inclusions. In patients with iron deficiency anemia, hypochromasia may be present due to the role of iron in the synthesis of heme. Therefore, RBCs from patients with iron deficiency anemia will have low hemoglobin content [2]. Anemia in animals is classified as a decrease in either the hemoglobin concentration or the number of red blood cells, both of which create a decrease in oxygen-carrying capacity of the blood. Anemia can also be indicated by a decrease in the packed cell volume (PCV), which is referred to as the percentage of RBCs present in the blood. For example, a PCV of less than 35% in dogs is generally accepted as anemic [3], while cats with a PCV of less than 25% are generally accepted as anemic [4]. Anemia is not a disease in its own right but a consequence of some conditions, where the production and/or destruction of erythrocytes is affected. In a CBC, there is a white blood cell count that calculates the total count of white blood cells. These play a crucial role in providing protection against diseases and are an important part of the immune system. CBC with differential is done to determine the types of white blood cells that may indicate certain illnesses, such as infections and allergies [2]. For many years, the conventional method for analyzing animal blood cells was the manual microscopic evaluation of the blood smear. This is due to the fact that dog and cat blood cells have specific morphological properties different from those of humans [5].

As the use of artificial intelligence (AI) within blood count analyzers continues to expand, several functions involving AI, including image analysis, blood cell identification and classification, abnormality detection through morphological changes, and data interpretation, are now available to support complete blood count (CBC) testing [6–8]. This new technology provides an opportunity to fully automate many of the labor-intensive tasks associated with manual CBC testing, thereby enhancing efficiency and accuracy. In addition, both machine learning (ML) and deep learning (DL) techniques can be used to develop various diagnostic tools, including laboratory imaging systems and diagnostic text systems, which improve the workflow of the laboratory [9]. With such systems, laboratory technicians can move from repeated performing microscopic analysis to performing greater levels of interpretation and management of critical pathological results.

AI-based platforms use algorithms developed for the recognition and classification of blood cells through morphological features and, in addition, through taxonomically defined characteristics specific to individual species. With the possibility of automating microscopic examination of blood cells, this will expedite blood cell analysis while also reducing the need for manual examination [10]. Although AI has been in widespread use within the field of human medicine, using AI in veterinary medicine brings some unique challenges. Challenges related to variability of parameters such as platelet counts and leukocyte differentiation, as well as the expertise of those working with abnormal cell populations, have impacted the veterinary community's ability to make clinical decisions about the use of AI systems to assist in veterinary diagnostics [11,12]. Technological development allowed the integration of the capabilities of artificial intelligence into automated blood analysis systems. Technological development has made it possible to integrate the capabilities of artificial intelligence into automated blood systems. Artificial intelligence is capable of recognizing patterns, classifying cells, and detecting abnormalities with the help of technologies of impedance counting, flow cytometry, optical scatter analysis, and algorithms of machine learning [13]. The application of machine learning and deep learning technologies is considered a breakthrough for improving CBC analysis in veterinary medicine. Artificial intelligence, especially machine learning and deep learning algorithms, will be able to contribute to increasing the accuracy and efficiency of CBC analysis in

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veterinary medicine [14,15]. The use of deep learning for classifying white blood cells in peripheral blood smear images.

The natural differences between the dog and cat blood cells add even greater complexity to the task of automated analysis due to peculiarities of cell morphology that can cause problems for artificial intelligence-based recognition [16]. Specifically, feline erythrocytes are much more sensitive to alteration due to storage and artificial modifications than dog cells and thus could affect analyzer functionality in different ways [17]. Studies show that some differences exist in parameters that are important to examine with automated analyzers, especially with regard to samples obtained from sick animals [12,18]. Using animal blood for analysis presents several challenges, which can pose major issues when trying to use an animal blood sample to obtain accurate CBC test results. The most commonly experienced limitation when analyzing animal blood is the speed at which the blood clots after being drawn, as well as the difficulty in obtaining sufficient blood volume (from the patient) to run all of the CBC parameters. Determining whether results obtained from CBC tests are accurate also requires veterinarians or animal scientists to have expertise regarding the interpretation of laboratory results obtained from animal blood samples. Due to these factors, it is important to follow proper procedures during the collection of blood samples and to have expertise available in order to properly verify laboratory results from the analysis of animal blood samples.

This study aims to evaluate the correlation between automated hematology analyzers using AI technology to the standard techniques used to do a CBC in both canines and felines across different presentations and conditions. Enhanced AIs will be reviewed regarding how much improvement can be achieved in the degree of accuracy as well as in reducing the amount of time necessary for the extensive manual interpretation of CBC studies completed in the field of veterinary clinical pathology. Enhanced AI systems can achieve their maximum effectiveness by providing accurate test results and eliminating the lengthy manual interpretative process associated with CBCs in the field of veterinary clinical pathology. Additionally, this study may be useful in the development of evidence-based guidelines on how to implement and interpret AI-enhanced hematology tests in the field of veterinary medicine for companion animals.

2. Materials and Methods

2.1. Ethical Approval of the Study

This prospective method comparison study was conducted at Faculty of Veterinary Technology, Kasetsart University, between March, 2024 and October, 2025. The procedures conducted in this study were approved by the Institutional Animal Care and Use Committee Kasetsart University (protocol number ACKU67-VTN-010 for dog blood and ACKU68-VTN-002 for cat blood) and was carried out in accordance with the principles of the 3Rs concept.

2.2. Study Design and Sample Collection

In the dog groups, based on the study by Thongsahuan et al. [19], the mean (SD) of red blood cell (RBC) count in *B. canis*-infected dogs was 4.49 (1.45) × 10^6/µL, whereas in healthy dogs it was 6.17 (1.00) × 10^6/µL. These values were used to calculate the required sample size using the following formula [20], where $\alpha = 0.05$, $\beta = 0.20$, σ represents the standard deviation, and μ represents the mean.

$$n_2 = \frac{\left(z_{\frac{\alpha}{2}} + z_{1-\beta}\right)^2 \left[\sigma_1^2 + \frac{\sigma_2^2}{r}\right]}{\Delta^2}$$

$$r = \frac{n_2}{n_1}, \Delta = \mu_1 - \mu_2$$

The calculated sample size was nine per group. After increasing the number by 20% to account for potential sample loss, the final sample size per group was 11. Therefore, the number of dogs used to perform the Known-groups validity test was at least 11 dogs per group.

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Based on the above-mentioned study, the highest and lowest prevalence values of anemia in dogs infected with various blood parasites were entered into the following sample size calculation formula [21], where α = 0.05, p represents the estimated proportion of the event (p = 0.81), and d represents the maximum tolerated error (d = 0.81 − 0.63).

$$n = \frac{z^2_{1-\frac{\alpha}{2}} p(1-p)}{d^2}$$

The calculated sample size was 19. After increasing this number by 20% to account for potential sample loss, the final sample size was 23. Therefore, at least 23 dogs were required to perform the diagnostic accuracy test.

In the cat groups, based on the study by Lophaisankit, et al. [22], the mean (SD) of RBC in anemic adult cats was 3.89 (1.84) × 10^6/μL, whereas in normal adult cats, it was 8.67 (0.60) × 10^6/μL. These values were used to calculate the required sample size using the formula by Bernard (2000) [20], same as the dog group, where α = 0.05 and β = 0.20. The calculated sample size was two per group. After increasing the number by 20% to account for potential sample loss, the final sample size per group was three. Therefore, at least three cats per group were used to perform the known-groups validity test. According to the studies by Inpankaew, et al. [23] and Lorsirigool, et al. [24], the estimated prevalence of anemia in cats was approximately 8.0% and 24.24%, respectively. As with the dog groups, these prevalences were entered into the sample size calculation formula [21], where α = 0.05, p = 0.08, and d = 0.24 – 0.08. The calculated sample size was 12. Therefore, at least 12 cats were required to perform the diagnostic accuracy test.

In the correlation analysis section of both groups, the sample size was calculated using the following formula [25], where α = 0.05, β = 0.20, r represents the expected correlation coefficient (r = 0.9), and C = 0.5 × ln[(1 + r) / (1 − r)] = 1.4722.

$$n = \left[\frac{Z_\alpha + Z_\beta}{C}\right]^2 + 3$$

The calculated sample size was 7. After increasing this number by 20% to account for potential sample loss, the final sample size was 9. Therefore, at least 9 dogs and cats were required to perform the convergent validity test.

As described previously in the sample size calculations, blood was taken from 38 animals (27 dogs; 11 cats) from an original total of 80 animals (dogs and cats) presented to the Veterinary Hospital for routine health examinations or various clinical issues. Upon being asked, owners consented, in writing, to the procedure.

For each animal, a 2.0 mL blood sample was collected from cephalic venipuncture into K_2EDTA anticoagulant tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA). All samples were processed within 6 hours of collection and analyzed in parallel using both the Awalife AI100 Vet automated hematology analyzer and reference manual methods.

2.3. Reference Manual Technique

The complete blood count (CBC) remains one of the most frequently ordered laboratory tests in clinical practice, providing critical information about both the quantity and size of circulating blood cells [26]. A standard CBC comprises several measured parameters, including red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Hct), along with calculated red cell indices, platelet count, and white blood cell count (WBC). The standard CBC was done as follows [27]. Hemoglobin Determination

Hemoglobin concentration was measured using the cyanmethemoglobin method. Twenty microliters of whole blood were added to 5 mL of Drabkin's reagent, mixed, and incubated at room temperature for 10 minutes. Absorbance was measured at 540 nm using a spectrophotometer (Shimazu UV-VIS 1900i, Shimazu, Japan). Hemoglobin concentration was calculated using calibration curves.

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2.4. Packed Cell Volume (PCV)

PCV was determined by using the microhematocrit method. Heparinized capillary tubes were filled with blood, sealed with clay, and centrifuged at 10,000 g for 5 minutes. The packed cell volume was measured using a hematocrit reader. Manual Cell Counts

RBC, WBC, and platelet counts were performed using the manual hemocytometer method with appropriate diluting fluids. For WBC counts, blood was diluted 1:20 with Türk's solution (2% acetic acid with gentian violet). For RBC counts, blood was diluted 1:200 with Hayem's solution. All counts were performed in duplicate and averaged.

2.5. Differential White Blood Cell Count

Blood smears were prepared using the wedge technique, air-dried, and stained with Wright-Giemsa stain. A 200-cell differential count was performed under oil immersion (1000× magnification), classifying cells as neutrophils (segmented and band), lymphocytes, monocytes, eosinophils, or basophils. Results were expressed as both percentages and absolute counts. Reticulocyte Count

For reticulocyte enumeration, blood was mixed with new methylene blue stain (1:1 ratio) and incubated for 15 minutes. Smears were prepared, and reticulocytes were counted as a percentage of 1,000 RBCs under oil immersion. Absolute reticulocyte counts were subsequently calculated based on the percentage and the total RBC count.

2.6. Awalife AI-100Vet Multifunctional Morphological Analyzer

The Awalife AI-100Vet analyzer represents the next-generation fully automated diagnostic platform created exclusively for use in veterinary practices. This state-of-the-art setup allows for the complete evaluation of all blood, urine, and fecal sediment samples from all veterinary species, including rodents and reptiles. As such, the Awalife AI-100Vet analyzer will become an integral part of your modern veterinary laboratory. To evaluate a sample of blood, 10 μL of EDTA-anticoagulated whole blood is mixed with the supplied reagent in a test tube, and the patient demographics and species are entered into the Awalife AI-100Vet analyzer. After mixing, 150 μL of the prepared sample is placed on the respective test chip and then placed in the Awalife AI-100Vet analyzer. The results of this analysis will be available in 6 minutes. To obtain the highest degree of analytical accuracy, it is best to analyze all samples within 6 hours post-collection. Statistical analysis

The hematological parameters of dogs and cats, measured using both the manual technique and the AI-100Vet, were presented as mean (standard deviation) for normally distributed data or median (interquartile range) for non-normally distributed data. Normal distribution was assessed using a histogram and the Shapiro-Wilk test. The diagnostic accuracy of the AI-100Vet was analyzed, and the positive predictive value (PPV) and negative predictive value (NPV) were estimated at different prevalence levels using Bayes' theorem. These prevalence levels were based on the minimum and maximum values reported in previous research articles. Known-groups validity was assessed by comparing hematological results between normal and anemic dogs, as determined by the Awalife AI-100Vet analyzer, using either the Student's t-test or the Wilcoxon rank-sum test.

Convergent validity was assessed through correlation analysis between hematological parameters obtained by the manual method and those measured using the Awalife AI-100Vet analyzer, employing either the Pearson or Spearman correlation test. Statistical significance was considered at $P < 0.05$. All data analyses were performed using STATA statistical software, version 17.0 (Stata Corp, Texas, USA). Results

There have been substantial improvements in hematological evaluation for pets through the emergence of an AI-based automated blood analyzer. The latter has become predominantly used to evaluate blood parameters among the companion animals (e.g., dogs and cats), whereas the former is still used as well. There are both pros and cons of applying each method for the examination of CBC parameters in dogs and cats. Thus, the relationship between AI-assisted automated hematology evaluation by means of the Awalife AI-100Vet analyzer and conventional manual methods was studied among the samples from dogs and cats. (B)

The samples were obtained from 38 animals: 27 dogs (15 normal and 12 anemic dogs) and 11 cats (5 normal and 6 anemic cats). Hematological data obtained from normal and anemic dogs, as well as normal and anemic cats, using the reference manual technique revealed a wide range of hematological profiles (Table 1).

Table 1. The hematological parameters of dogs and cats by reference manual technique.

Parameter	**Dog**		**Cat**	
	Normal (n=15)	**Anemia (n=12)**	**Normal (n=5)**	**Anemia (n=6)**
PCV (%), mean (SD)	41.17 (2.68)	30.17 (5.24)	41.14 (5.94)	21.5 (5.17)
Hemoglobin (g/dL), mean (SD)	16.42 (1.60)	11.09 (1.64)	13.07 (1.69)	6.45 (2.16)
RBC (10^6/ µL), mean (SD)	6.76 (0.84)	4.63 (0.82)	9.90 (0.69)	4.54 (2.00)
Plasma protein (g/dL), mean (SD)	7.74 (0.47)	7.63 (1.20)	8.68 (1.77)	9.30 (2.25)
MCV (fL), mean (SD)	61.50 (5.68)	61.14 (11.67)	41.81 (7.02)	56.03 (27.49)
MCH (pg), mean (SD)	24.57 (3.21)	22.83 (5.07)	13.28 (2.03)	19.26 (16.43)
MCHC (g/dL), mean (SD)	39.90 (4.03)	37.47 (6.19)	31.97 (3.29)	32.09 (14.10)
WBC (10^3/ µL), mean (SD)	10.87 (4.96)	11.34 (6.34)	12.66 (8.37)	15.59 (12.09)
Neutrophil (%), mean (SD)	71.47 (12.05)	77.08 (10.26)	58.00 (8.45)	50.00 (17.85)
Lymphocyte (%), median (IQR)	14 (14)	13.5 (9)	34 (3)	46 (24)
Monocyte (%), median (IQR)	6 (5)	3 (4)	0 (2)	3.5 (8)
Eosinophil (%), median (IQR)	5 (6)	3.5 (5)	4 (2)	2 (6)
Basophil (%), median (IQR)	0	0	0	0
Reticulocyte (%), median (IQR)	0.3 (0.4)	0.2 (0.35)	0.03 (0.28)	0.3 (0.3)

The Awalife AI-100vet system offers standardized, high-resolution digital images with clearer visualization of erythrocyte morphology, leukocyte nuclear and cytoplasmic details, and platelet distribution. This results in a more consistent and reproducible morphological assessment with less observer variability than traditional microscopy (100x), which depends on manual observation with greater subjectivity (Figure 1).

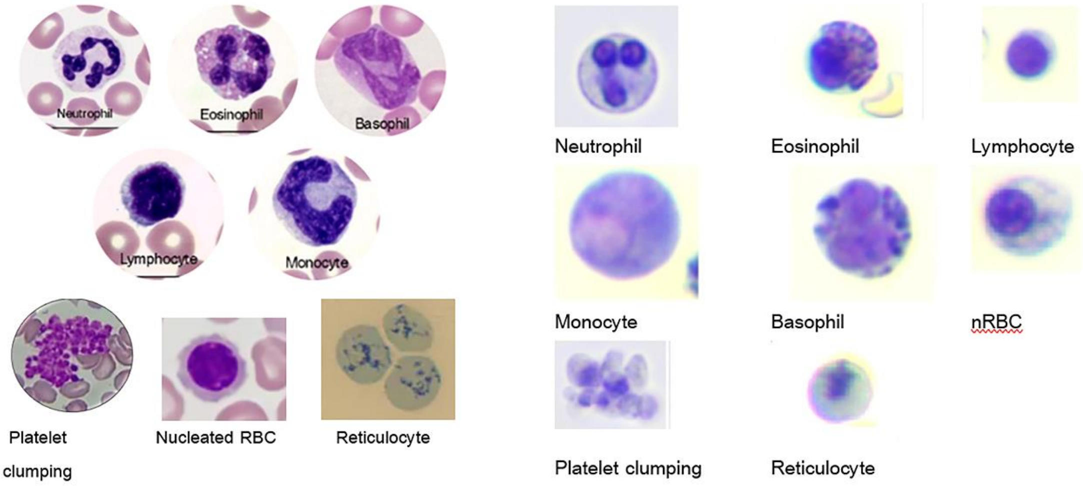

Figure 1. The image presents a comparative visual analysis of blood smear morphology as seen through traditional microscopy, 100X (A) versus the Awalife AI-100vet system, 40X (B) .

The diagnostic accuracy of Awalife AI-100Vet analyzer for the detection of anemia in dogs and cats is shown in Figure 1 and Table 2. The diagnostic accuracy in 27 dogs was 44% of prevalence, an area under the receiver operating characteristic curve (AUC ROC) of 0.72, 50% of sensitivity, 93.3% of specificity, 85.7% of PPV, 70% of NPV, and 7.5 of positive likelihood ratio (LR+). While the diagnostic accuracy in 11 cats was 55% of prevalence, the AUC ROC of 0.92, 83.3% of sensitivity, 100% of specificity, 100% of PPV, 83.3% of NPV, and no answer for LR+.

Table 2. Diagnostic accuracy of the Awalife AI-100Vet Multifunctional Morphological Analyzer for the detection of anemia in dogs and cats.

Animal	Prevalence	AUC ROC	Sensitivity	Specificity	PPV	NPV	LR+
Dog	44%	0.72 (0.55-0.88)	50 (21.1-78.9)	93.3 (68.1-99.8)	85.7 (42.1-99.6)	70 (45.7-88.1)	7.5 (1.0-54.1)
Cat	55%	0.92 (0.75-1.00)	83.3 (35.9-99.6)	100 (47.8-100)	100 (47.8-100)	83.3 (35.9-99.6)	N/A

PPV and NPV of the Awalife AI-100Vet analyzer for the detection of anemia in dogs at different prevalence rates using Bayes' theorem were shown in Table 3. While the PPV and NPV of the Awalife AI-100Vet Multifunctional Morphological Analyzer for the detection of anemia in cats at different prevalence rates using Bayes' theorem are shown in Table 4.

Table 3. Positive predictive value (PPV) and negative predictive value (NPV) of the Awalife AI-100Vet Multifunctional Morphological Analyzer for the detection of anemia in dogs at different prevalence rates using Bayes' theorem.

Animal	Prevalence 30%		Prevalence 55%		Prevalence 80%	
	PPV	NPV	PPV	NPV	PPV	NPV
Dog	76.3	81.3	90.2	60.4	96.8	31.8

Inferring prevalence rates allowed for the analysis of PPV and NPV. According to Maneerat and Somrup [28], who discovered a 34% prevalence of anemia in dogs infected with hemoparasites, the minimum inferred prevalence rate was 30%. According to Abakpa, S. et al. [29], who discovered a 77.7% prevalence of anemia in dogs infected with hemoparasites, the maximum inferred prevalence rate was 80%. When the prevalence of anemia dropped to 30%, NPV increased to 81.3%, while PPV increased to 96.8% when the prevalence of anemia increased to 80%.

Table 4. Positive predictive value (PPV) and negative predictive value (NPV) of the Awalife AI-100Vet Multifunctional Morphological Analyzer for the detection of anemia in cats at different prevalence rates using Bayes' theorem.

Animal	Prevalence 30%		Prevalence 45%		Prevalence 60%	
	PPV	NPV	PPV	NPV	PPV	NPV
Cat	100	93.3	100	88	100	80

The analysis of PPV and NPV was carried out using the prevalence rates. The minimum inferred prevalence rate was 30%, according to Korman, R. M., et al. [30]. In their study, the researchers stated that the prevalence of anemia in cats was 35.6%. On the other hand, the maximum inferred prevalence rate was 60%, according to Do, T. et al. [31]. Their study indicated that the prevalence of anemia in cats was 63.7%. It was seen that when the prevalence of anemia was 60%, the PPV went up to 100%, and when the prevalence of anemia was 30%, the NPV went up to 93.3%.

The AUC values of the Awalife AI-100Vet analyzer for hematology tests in dogs and cats were 0.72 and 0.92, respectively (Table 2 and Figure 2). These results indicate that the Awalife AI-100Vet analyzer demonstrated satisfactory overall accuracy for hematology tests using dog's blood samples and excellent accuracy for tests using cat's blood samples.

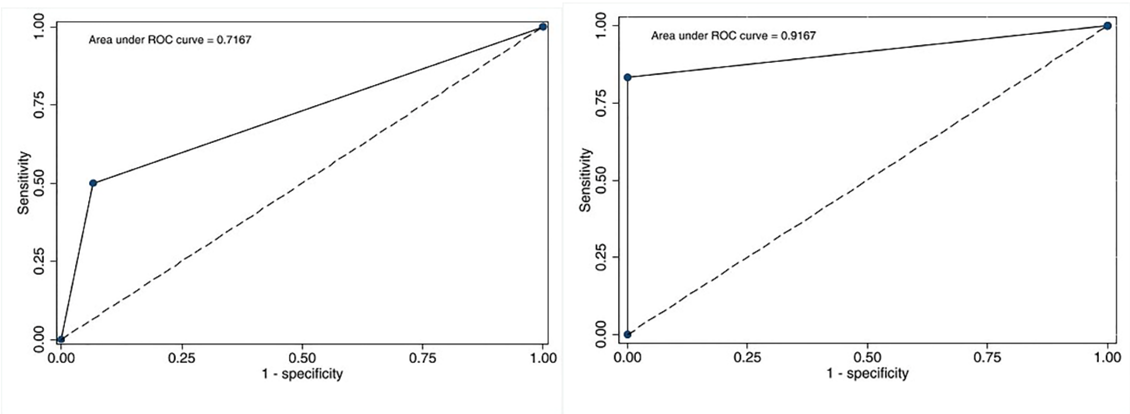

Figure 2. Area under the curve of the receiver-operated characteristics (AUC-ROC) curve for the Awalife AI-100Vet Multifunctional Morphological Analyzer and manual technique for diagnosis of anemia in dogs (left) and cats (right).

The comparison of hematological parameters between normal and anemia groups in dogs and cats, assessed by Awalife AI-100Vet analyzer (Known-groups validity test), is shown in Table 5. In both animals, PCV, hemoglobin, and RBC were statistically significant ($p<0.05$).

Table 5. Comparison of the statistical differences in hematological parameters between normal and anemia groups in dogs and cats by Awalife AI-100Vet Multifunctional Morphological Analyzer (Known-groups validity test).

Parameter	Dog (n=27)			Cat (n=11)		
	Normal (n=20)	Anemia (n=7)	p-value	Normal (n=6)	Anemia (n=5)	p-value
PCV (%), mean (SD)	38.32 (5.55)	30.43 (7.07)	0.006	38.78 (7.84)	20.4 (4.93)	0.001

Hemoglobin (g/dL), mean (SD)	15.06 (2.57)	11.16 (2.91)	0.003	12.26 (2.50)	6.10 (2.22)	0.002
RBC (10^6/ µL), mean (SD)	6.24 (1.17)	4.57 (1.10)	0.003	9.17 (1.89)	4.35 (2.17)	0.003
MCV (fL), mean (SD) in dog, median (IQR) in cat	62.38 (9.03)	58.35 (7.26)	0.298	44.64 (9.12)	46.29 (18.10)	0.584
MCH (pg), mean (SD) in dog, median (IQR) in cat	24.44 (3.52)	21.97 (5.48)	0.181	14.01 (1.84)	13.49 (14.36)	1.000
MCHC (g/dL), mean (SD) in dog, median (IQR) in cat	39.26 (3.62)	37.56 (8.40)	0.620	32.44 (2.73)	35.54 (24.94)	0.584
Reticulocyte (%), median (IQR)	0.20 (0.34)	0.50 (0.60)	0.240	0.02 (0.30)	0.40 (0.30)	0.099

As seen in Table 6, the correlation between hematological parameters in dogs and cats using the reference manual technique and Awalife AI-100Vet analyzer (Convergent validity test) was as follows. PCV, hemoglobin, RBC, MCH, WBC, % Neutrophil, % Lymphocyte, % Monocyte, % Eosinophil, and % Reticulocyte were found to be statistically significant in dogs. Conversely, the following were statistically significant in cats: PCV, hemoglobin, RBC, WBC, % Neutrophil, % Lymphocyte, and % Eosinophil. Scatter plots in dogs (Figure 5) and cats (Figure 6) were used to demonstrate the relationship between hematological parameters determined manually and using the Awalife AI-100Vet analyzer.

Table 6. Correlation between manual and Awalife AI-100Vet hematological assessments in dogs and cats.

Parameter	Dog (n=27)				Cat (n=11)			
	Manual	AI-100Vet	R	p-value	Manual	AI-100Vet	R	p-value
PCV (%), mean (SD)	36.28 (6.82)	41.39 (10.25)	0.61	0.001	30.43 (11.52)	31.66 (14.33)	0.85	0.001
Hemoglobin (g/dL), mean (SD)	14.05 (3.13)	14.68 (3.78)	0.70	<0.001	9.46 (3.93)	11.25 (4.85)	0.76	0.007
RBC (10^6/ µL), mean (SD)	5.81 (1.35)	6.29 (1.66)	0.56	0.002	6.97 (3.16)	6.74 (3.07)	0.87	<0.001
MCV (fL), mean (SD)	61.34 (8.66)	66.15 (4.16)	0.28	0.157	49.57 (21.27)	47.12 (1.81)	0.28	0.409
MCH (pg), median (IQR)	24.42 (4.31)	23.60 (2.48)	0.42	0.030	13.49 (5.15)	16.68 (1.56)	0.24	0.484
MCHC (g/dL), mean (SD)	38.82 (5.14)	35.44 (2.20)	0.06	0.779	32.04 (10.18)	35.98 (1.63)	0.19	0.575
WBC (10^3/ µL), median (IQR)	10.12 (6.0)	12.79 (9.03)	0.56	0.002	10.20 (19.6)	8.49 (4.94)	0.93	<0.001
Neutrophil (%), mean (SD)	73.96 (11.44)	71.15 (11.03)	0.67	<0.001	53.64 (14.33)	54.96 (14.97)	0.93	<0.001

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Lymphocyte (%), mean (SD)	15.74 (9.28)	13.71 (7.33)	0.57	0.002	39.00 (15.69)	31.70 (15.23)	0.84	0.001
Monocyte (%), median (IQR)	4.00 (4.00)	5.17 (7.10)	0.65	<0.001	2.00 (5.00)	3.83 (6.06)	0.57	0.068
Eosinophil (%), median (IQR)	4.00 (7.00)	6.81 (7.49)	0.53	0.005	3.00 (4.00)	5.90 (6.63)	0.81	0.002
Basophil (%), median (IQR)	0	0	-0.05	0.784	0	0	N/A	N/A
Reticulocyte (%), median (IQR)	0.2 (0.48)	0.04 (0.05)	0.51	0.007	0.2 (0.48)	0.01 (0.1)	0.41	0.208

3. Discussion

CBC is still one of the most basic tests used in veterinary practice to determine the health status of an animal, the nature of the disease, its course, and the treatment response. Manual assessment through microscopy was traditionally thought to be the most reliable way to analyze hematological data in veterinary species, especially dogs and cats, due to the distinctive features of their blood cells, which differ significantly from humans [32,33]. Anemia is a disease caused by the reduction in hemoglobin content in the blood, often accompanied by erythrocyte deficiency and/or decrease in hematocrit [34]. The biochemical abnormalities in cases of hemolytic anemia have significant value as diagnostic and prognostic markers in companion animals [35].

The AI could help laboratory technologists in the morphological differentiation of leukocytes. Specifically, it could aid in enhancing the sensitivity of abnormal leukocyte differentiation and decrease the chance of false-negative detection of abnormal white blood cells (WBCs) [36]. By comparing the images of blood smears generated through conventional microscopy with those generated using the Awalife AI-100vet software, emphasizing crucial hematologic parameters comprising various types of white blood cells (neutrophils, eosinophils, basophils, lymphocytes, monocytes), different forms of red blood cells (nucleated RBCs and reticulocytes), and platelet agglomeration, one can conclude that the Awalife AI-100vet software creates images with greater reproducibility and fewer subjective differences compared to conventional microscopy. It also provides comparable or even superior diagnostic clarity. This finding suggests that there might be a potential advantage of applying AI-assisted hematology screening in routine veterinary clinical settings, particularly in those requiring high throughput or resource limitations. Additionally, the AI seems to highlight diagnostic markers, such as granular patterns and nuclear segmentation.

The hematological parameters of 15 normal and 12 anemic dogs, as well as 5 normal and 6 anemic cats, were obtained using the Reference Manual method and revealed diverse hematological profiles (Table 1). Anemia in dogs was usually defined with the help of a hemoglobin concentration lower than 12.5 g/dL. Another frequently used diagnostic criterion is PCV lower than 35% [37]. Anemia in cats is usually characterized by a hemoglobin level that is lower than 9.0 g/dL [38]. However, the diagnosis of anemia in cats may also use other parameters such as hematocrit and RBC. Hemoglobin varies in different species and is usually used to diagnose and classify anemia [2]. The reference standard seems to be the manual method (probably complete blood count with manual differential). The Awalife AI-100Vet Multifunctional Morphological Analyzer showed very different diagnostic results in dogs and cats.

The data show that the test is fairly accurate in dogs (AUC=0.72), very specific (93.3%), and only fairly sensitive (50%). This means that it doesn't often give false positives, but it misses about half of the really anemic dogs. The Awalife AI-100Vet Multifunctional Morphological Analyzer should not be the only test used to find out if a dog has anemia because it is only moderately sensitive (50%). It is still important to check manually by looking at a blood smear, especially for negative results. But the moderate NPV in dogs (70%) means that negative results should be taken with a grain of salt when treating dogs.

From the analysis carried out, the data reveal high performance among cats suffering from anemia. The sensitivity level is relatively high, and it has 100% specificity. Therefore, it seems like the method can be used to diagnose cat anemia effectively; however, the small number of subjects used should be considered. In the case of cats, it is noted that the test exhibits high accuracy (AUC=0.92) with high specificity (100%) and sensitivity of 83.3%. Clinically speaking, the relatively high PPVs (85.7% in dogs and 100% in cats) confirm the validity of the test.

Predictive Values of Awalife AI-100Vet analyzer for detecting anemia with varying levels of prevalence were evaluated (Table 3). This evaluation investigates the diagnostic usefulness of Awalife AI-100Vet Multifunctional Morphological Analyzer under various prevalence conditions for the disease in question based on Bayesian approach.

For veterinary clinics with populations having prevalence rates that differ from those of the study (44% for dogs and 55% for cats), the Bayes-adjusted PPV and NPV from Tables 3 and 4 represent more precise indicators of the test accuracy. The PPV and NPV are significantly affected by the prevalence of the condition under consideration, whereas the sensitivity and specificity of the test do not depend on the population's prevalence. The dependence between the above parameters is described by Bayes' rule, allowing us to determine how the PPV and NPV change depending on the prevalence. For dogs, the Awalife AI-100Vet analyzer showed good diagnostic accuracy, with an AUC score of 0.72 for dog samples, which falls into the "fair" category.

However, as shown by Tables 2 and 3 below, these percentages have different PPV and NPV for varying prevalences in the population under study. In terms of the 44% prevalence, according to Tables 2 and 3, PPV = 85.7% (high PPV) and NPV = 70% (average NPV).

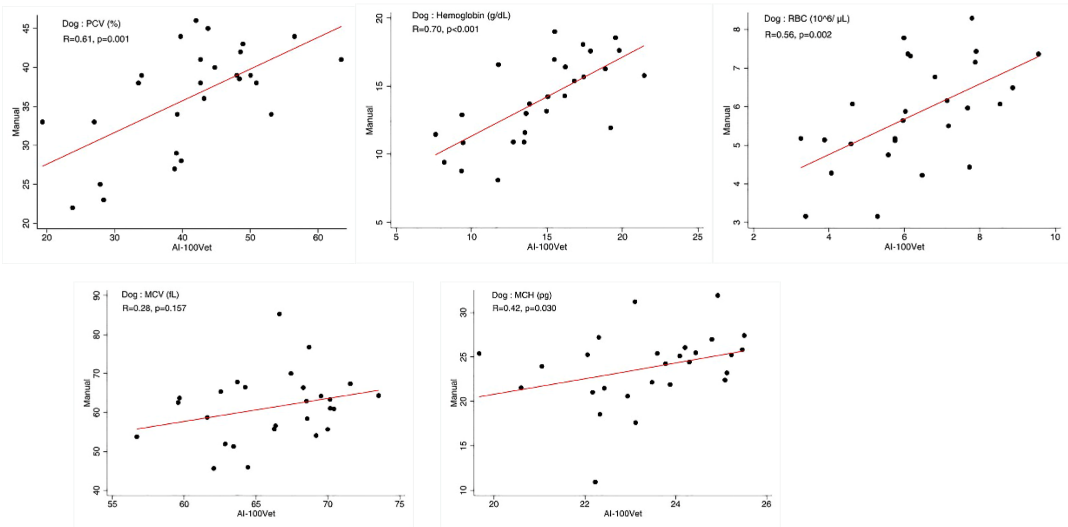
The positive likelihood ratio (LR+) of 7.5 means that the positive result is seven and a half times more likely in the case of an anemic dog compared to a non-anemic dog. For adjusting prevalence using Bayes' theorem, the PPV would reduce, and the NPV would rise in low-prevalence cases and vice versa in high-prevalence cases.

For cats, the analyzer proved highly accurate with an AUC value of 0.92 in cat samples, falling into the "excellent" category for clinical diagnosis, as presented in Tables 2 and 4, having a sensitivity of 83.3% and specificity of 100%. With respect to the prevalence rate of 55%, the analysis revealed that the PPV value was 100% giving the clinicians maximum assurance regarding the validity of any positive result irrespective of the disease prevalence. The NPV of 83.3% implies moderate but strong assurance of the accuracy of negative results. With the perfect specificity among cats, it implies that there were no false positives with the PPV being 100% irrespective of the disease prevalence (see Table 4). Applying Bayesian analysis to prevalence values in Table 4, the NPV value would be high in low prevalence groups while being lower in high prevalence groups as compared to dogs because of its high sensitivity.

Based on the difference in performance between species, cats demonstrated considerably superior performance to that of dogs, with an AUC of 0.92 in cats and 0.72 for dogs, indicating that other factors likely contribute to animal species. When used as a screening test in areas where the prevalence of disease is low, it has been shown to provide low positive predictive value but high negative predictive value for dogs, which suggests that the screening for anemia would be more capable of ruling out anemia as opposed to confirming it. Conversely, when used in high-prevalence areas at referral clinics, the positive predictive value would be high, and the negative predictive value would be low, which would provide a significant degree of confidence in the positives.

The comparison of hematological parameters between normal and anemia groups in dogs and cats by Awalife AI-100Vet Multifunctional Morphological Analyzer (Known-groups validity test) is shown in Table 5. All three parameters, PCV, Hemoglobin, and RBC count, achieved statistically significantly different results between normal and anemic groups ($p<0.05$). This confirms that the analyzer's detection of these core hematologic changes associated with anemia is valid. These are some of the most basic and important parameters used for diagnosing anemia and their substantial decline in the anemic group corresponds with what would be expected to occur given the pathophysiology of anemia. The same trend was noted in the cat group, although the statistical values were similar with a significant decline in PCV, hemoglobin, and RBC ($p<0.05$); thus confirming that these data also support the validity of the analyzer for use in cat samples. However, other red cell indices such as MCV, MCH, and MCHC did not have significantly different results from either species. This is likely due to the variability of presentation of the different types of anemia (normocytic, macrocytic, microcytic) found in veterinary medicine; therefore, these indices may not have much diagnostic value when used alone. Reticulocyte counts for both species did not have any significant differences as well; this could be due to the small sample size tested or because those animals included a small number of cases of non-regenerative anemia. Overall, the analyzer was able to demonstrate very high discrimination ability for the three core hematological parameters; specifically, PCV, hemoglobin, and RBC; thus demonstrating how clinically relevant the analyzer is for diagnosing and monitoring anemia in both dogs and cats.

The evidence of convergent validities test between hematological parameters of dogs and cats by reference manual technique with Awalife AI-100Vet Multifunctional Morphological Analyzer is shown in Table 6. The scatter plots for the relationships between hematological parameters of dogs and cats using the manual reference technique and Awalife AI-100Vet multi-functional morphology analyzer were plotted as separate figures (Figure 2, dogs; Figure 3, cats). These results are consistent with other studies analyzing automated hematology analyzers. As indicated by Bujang and Adnan [39], small sample sizes may give rise to inaccurate estimates of diagnostic accuracy. The prevalent forms of anemia among the sampled populations may differ. For example, dogs are known to be affected by immune-mediated hemolytic anemia with characteristic morphologic features that can confound automated systems [40], while cats tend to develop more uncomplicated anemias secondary to chronic kidney disease [41]. These integrated approaches aim to improve the diagnostic value of CBC by placing hematologic abnormalities in a wider clinical context.



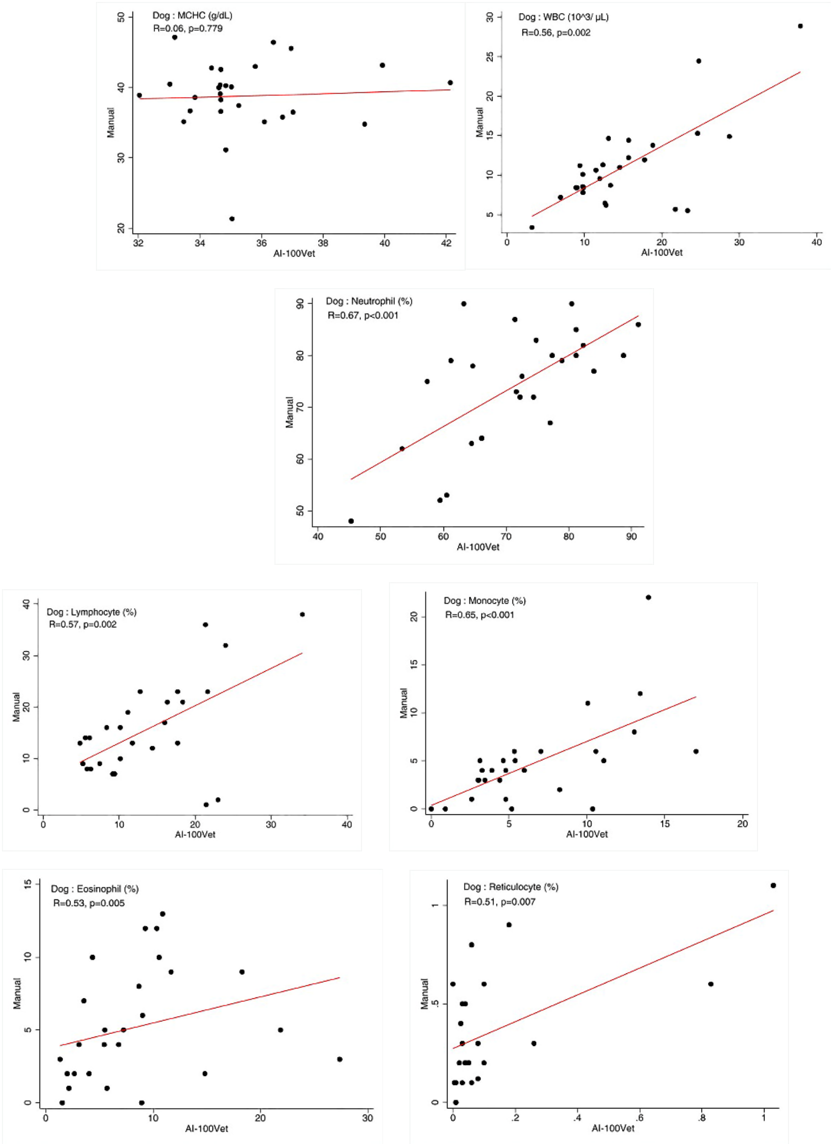
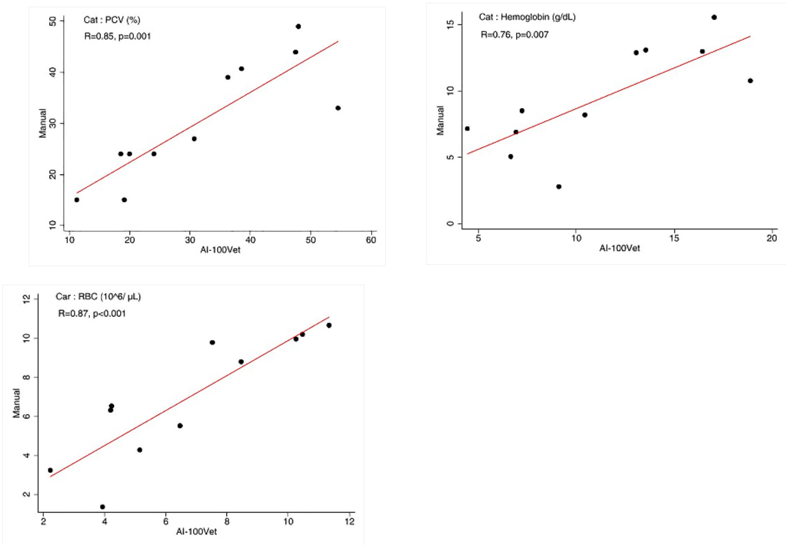


Figure 2. Scatter plot of the Awalife AI-100Vet Multifunctional Morphological Analyzer and reference manual technique for estimated hematological parameters of the dog.



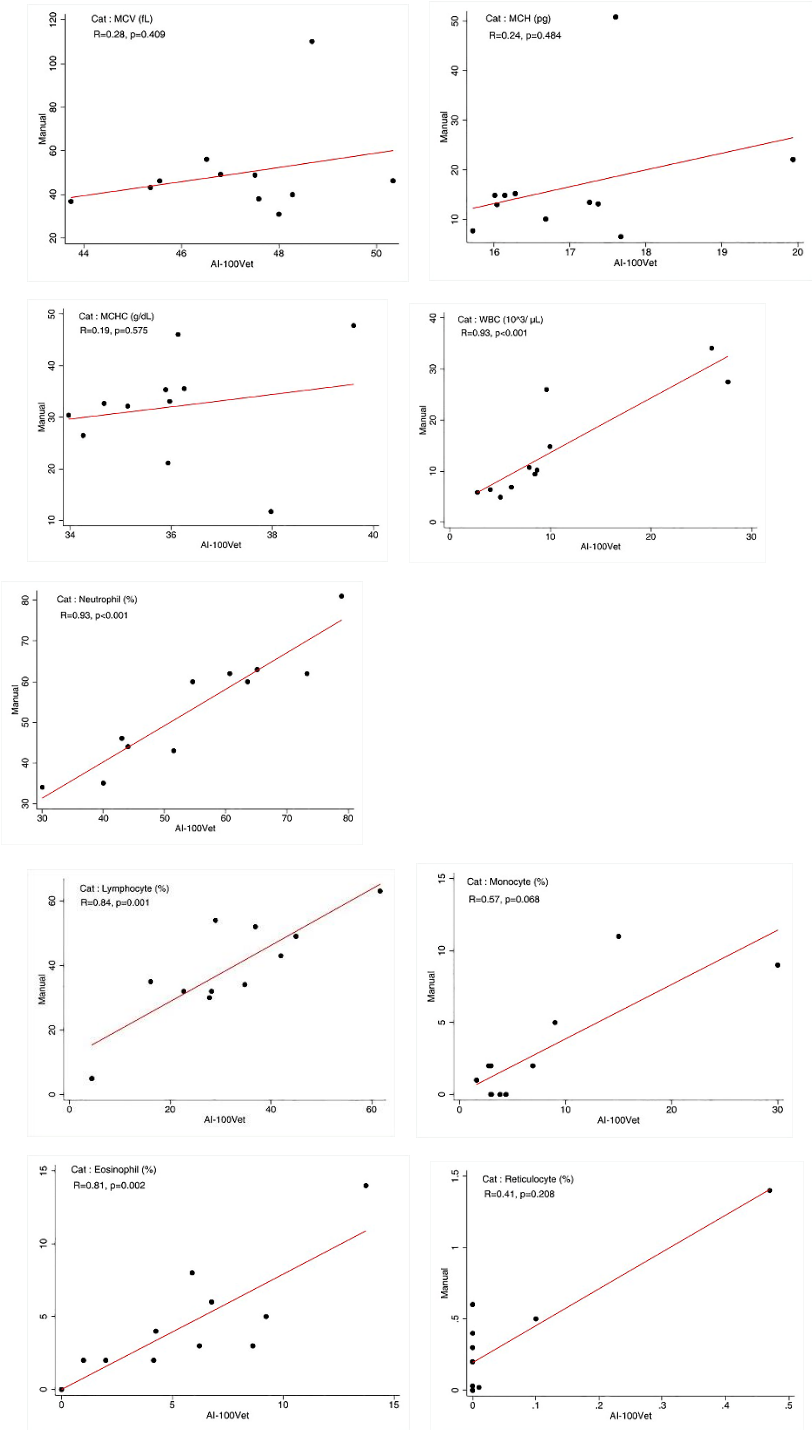


Figure 3. Scatter plot of the Awalife AI-100Vet Multifunctional Morphological Analyzer and reference manual technique for estimated hematological parameters of the cat.

As mentioned in Xiao et al. [42], there are certain shortcomings of AI systems applied to veterinary hematology. They stressed the fact that the overwhelming majority of Artificial Intelligence algorithms require diverse datasets of considerable sizes that can be not available for very rare Hematologic disorders or specific animals. The existing differences are likely to be eliminated with the advancements in animal-specific AI systems and increased cell-resolution capabilities of these systems. Provided that they are used together with internal quality controls, which should include criteria for self-assessment of results. Finally, one may also note that AI-powered automated blood analyzers raise the issue of interpretability and verification of their use in clinical settings. The present research examined the relationship between automated AI analyzers

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and traditional techniques used in complete blood counts for dogs and cats. Our findings reveal important considerations regarding the accuracy, reliability, and limitations of these technologies in veterinary clinical pathology.

This study was limited in sample size because only a small portion of the study population has been diagnosed with anemia; therefore, the authors believe that increasing the number of sampling points or extending the duration of data collection will assist in increasing the available sample size for their research. In addition, the authors believe that obtaining more detail about the types and underlying reasons for the development of anemia (i.e., due to inadequate nutrition, chronic illness, or hemolytic conditions) will provide the authors with more information from which to conduct a more accurate analysis. These additional data would also allow the authors to conduct further investigation into the relationship between the performance of the device and the characteristics of anemia.

Recent research has also shown that by using larger more diverse samples, the diagnostic accuracy of study results is strengthened and the variability in the study results for different kinds of anemia can be better understood. As a result, this will enhance the clinical significance of the study results.

4. Conclusion

According to the findings from this research study, the automated blood cell analyzer (Awalife AI-100Vet), which utilizes artificial intelligence (AI), has shown quite a lot of promise on accurate hematologic parameter assessments in dogs and cats, particularly for cats (felines) where results were found to be most accurate. The analyzer's results are comparable to the reference standard methodology used in manual counting; however, there remains some difference between the two techniques, indicating that the AI algorithms may require additional work to refine them. The AI-100Vet is considered to be a viable and efficient substitute for blood cell counting; however, further continued refinement and/or optimization of the AI-100Vet will lead to greater accuracy and provide an opportunity for universal clinical applicability.

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Institutional Review Board Statement: This prospective method comparison study was conducted at Faculty of Veterinary Technology, Kasetsart University, between March, 2024 and October, 2025. The procedures conducted in this study were approved by the Institutional Animal Care and Use Committee Kasetsart University (protocol number ACKU67-VTN-010 for dog blood and ACKU68-VTN-002 for cat blood) and was carried out in accordance with the principles of the 3Rs concept.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

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