Research Article

Comparative Study of Biochemical Accuracy, Reliability, Relevancy between Traditional and Automated Methods for Blood Analyses in Broiler Chickens

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Abstract | This study aimed to evaluate the accuracy, reliability, and agreement between conventional manual techniques and modern automated analyzers in measuring hematological and biochemical parameters in broiler chickens. Blood samples were collected from fifty female broiler chickens at 35 days of age with an average body weight of 2300 ± 40 g. Samples were obtained from the brachial vein, where whole blood was used for hematological analysis and serum was used for biochemical measurements. Hematological parameters included red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count, and the heterophil to lymphocyte ratio (H/L ratio). Serum biochemical parameters included glucose, total protein, albumin, cholesterol, triglycerides, and liver enzymes (AST and ALT). Analyses were performed using both conventional laboratory methods and automated analyzers. The results were evaluated based on mean values, standard error of the mean (SEM), coefficient of variation (CV %), and analysis time.The results showed no significant differences between the conventional and automated methods for all evaluated hematological and biochemical parameters, with values remaining within the established physiological reference ranges for broiler chickens. However, the automated method demonstrated superior analytical performance, as indicated by consistently lower CV% values compared with the conventional method, reflecting higher measurement precision and stability. Additionally, automated analyzers required substantially less analysis time per sample than the conventional procedures, highlighting their greater efficiency in laboratory workflow.

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Introduction

Poultry are among the primary animal sources of dietary protein, contributing significantly to human nutrition by providing essential amino acids, vitamins, and minerals. They play a central role in global food security and improving human health, particularly in developing countries. Poultry meat and eggs are preferred food sources due to their high quality, digestibility, and relatively low cost compared to other animal proteins such as beef or lamb (Al-Salhi and Al-Shatty, 2023; Al-Salhi, 2026a). Moreover, the poultry industry provides substantial economic benefits and employment opportunities, especially for small-scale and rural farmers, making the improvement of poultry health and product quality a key goal in both research and practical applications (Aljebory and Naji, 2021; Khalaf *et al*., 2025).

The health and productivity of poultry are influenced by a wide range of factors, including infectious and non-infectious diseases, environmental conditions, and nutrition. In this context, laboratory tests, particularly complete blood counts (CBC) and biochemical analyses, are essential tools for evaluating birds' health status, diagnosing diseases, and monitoring treatment responses Hematological and biochemical parameters reflect key physiological functions, such as immune response, metabolic activity, and stress tolerance, making them critical indicators of avian health (Naser *et al*., 2025; Al-Salhi, 2026).

Despite the importance of hematological and biochemical analyses in poultry research, the literature still shows a noticeable gap in studies comparing conventional manual methods with modern automated techniques. In the field of biochemical analysis, several recent studies have evaluated the reliability of portable analyzers, such as the i-STAT and VetScan VS2, in comparison with conventional laboratory methods for assessing key biochemical indicators in poultry. Sauer *et al*. (2020) reported that both devices showed good agreement with laboratory results for glucose, total protein, albumin, cholesterol, and triglycerides in laying hens, although minor differences should be taken into account when interpreting the results. Similarly, Ruiz-Jimenez *et al*. (2021) emphasized that portable analyzers provide rapid and practical results that are highly suitable for field conditions; however, they cannot fully replace conventional laboratory analyzers for all biochemical parameters due to certain measurement variability. More recently, Ruiz-Jimenez *et al*. (2022) demonstrated that the VetScan VS2 analyzer exhibited high agreement with conventional laboratory measurements for glucose, total protein, albumin, and cholesterol, while also highlighting the need to establish device- and age-specific reference intervals to ensure accurate interpretation of the results.

In the context of hematological analysis, a recent study by Legroux *et al*. (2025) indicated that automated analyzers, such as Sysmex XT-2000iV and Sysmex XN-1000V, can provide accurate measurements for some variables, including hematocrit. Nevertheless, these automated systems cannot entirely replace conventional manual methods across all hematological parameters, particularly for white blood cell counts and differential counts, which may exhibit consistent bias or measurement errors.

Based on these research gaps, the present study aims to evaluate the accuracy, reliability, and agreement between conventional manual methods and modern automated analyzers for hematological and biochemical parameters in broiler chickens. This includes analyzing blood samples using both methods, comparing numeric results with reference values, and determining whether significant differences exist between the two approaches in terms of result accuracy, reproducibility, and agreement. The study also seeks to provide clear recommendations for the most reliable analytical methods for research and practical applications, ultimately improving health monitoring and management in poultry production.

Materials and Methods

Blood sampling

Blood samples were collected from fifty female broiler chickens at 35 days of age, with an average body weight of 2300 ± 40 g (Mean ± SEM). Blood was obtained from the wing vein (brachial vein) using sterile disposable syringes. A total of 6 mL of blood was collected from each bird: 3 mL from the right wing for hematological (cellular) analysis and immediately transferred into tubes containing EDTA as an anticoagulant, and 3 mL from the left wing for biochemical and chemical parameter analysis, placed into plain tubes without anticoagulant for serum separation. Serum samples were obtained by centrifugation at 3000 rpm for 10 minutes. All analyses were performed immediately after sample collection by the specialized research team to ensure the stability of blood components and minimize potential alterations due to storage or waiting time.

As shown in Figure 1, the workflow diagram illustrates the process of taking blood samples from broiler chickens and the subsequent laboratory analyses, including serum biochemical analysis and hematological analysis.

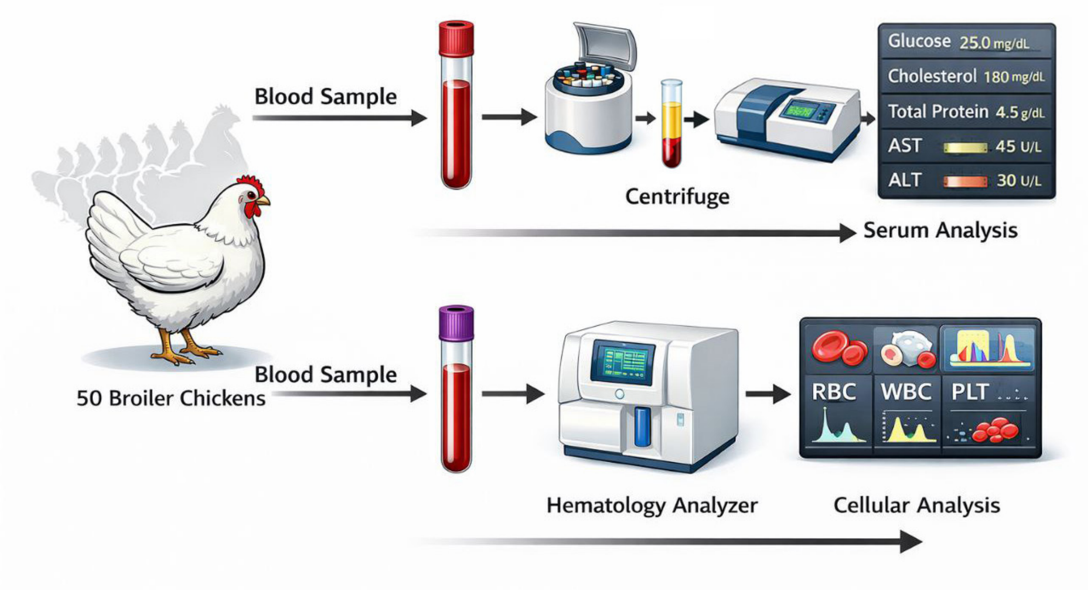

Figure 1: *Simplified schematic diagram illustrating blood sampling and subsequent hematological and biochemical analyses in broiler chickens.*

Measurement of parameters using conventional and modern methods

Hematological parameters included red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), packed cell volume (PCV), platelets, and the heterophil/lymphocyte ratio (H/L ratio). Biochemical parameters included glucose, total protein, albumin, cholesterol, triglycerides, and liver enzymes (AST and ALT).

Conventional analyses were performed on the right wing samples for hematology and left wing serum samples for biochemistry. RBC, WBC, platelets, and H/L ratio were measured using a Hemocytometer with Turk's solution, Hb using the Cyanmethemoglobin method, and PCV using the Microhematocrit method. Biochemical parameters were measured using a Spectrophotometer, a widely used conventional method in poultry research, serving as a reference for comparison with automated analyses (Tietz, 1995; Al-Salhi, 2025).

Modern automated analyses were conducted using Automated Hematology Analyzer for hematological parameters and Automated Biochemistry Analyzer for biochemical parameters. Hematological parameters were analyzed using an automated hematology analyzer (Mindray BC-2800, Mindray, Shenzhen, China), while serum biochemical parameters were measured using an automated biochemistry analyzer (Mindray BS-120, Mindray, Shenzhen, China). All devices were calibrated beforehand using standard control materials to ensure analytical accuracy, reliability, and measurement precision. The automated measurements were then compared with conventional analyses to evaluate the accuracy, agreement, and precision of both analytical approaches.

Reference ranges compiled from established avian hematology sources and commonly reported physiological values for healthy broiler chickens are presented in Tables 1 and 2.

Table 1: *Reference ranges of hematological parameters in broiler chickens*

Parameter	Reference range	Unit	Scientific references
RBC (Red blood cells)	2.5–3.5	$\times 10^6$ cells/µL	Feldman *et al.*, 2000; Jain, 1993
WBC (White blood cells)	12–30	$\times 10^3$ cells/µL	Bounous and Stedman, 2000
Hemoglobin (Hb)	7–13	g/dL	Jain, 1993; Banerjee, 2008
Packed Cell Volume (PCV)	22–35	%	Campbell, 2013
Platelets (Thrombocytes)	30–100	$\times 10^3$ cells/µL	Campbell, 2013
Heterophils	20–40	%	Maxwell, 1993
Lymphocytes	45–70	%	Maxwell, 1993
Heterophil/Lymphocyte Ratio (H/L ratio)	0.2–0.5	Ratio	Maxwell, 1993

Table 2: *Reference ranges of serum biochemical parameters in broiler chickens*

Parameter	Reference range	Unit	Scientific references
Glucose	200–350	mg/dL	Tietz, 1995; Kaneko *et al.*, 2008
Total Protein	3.0– 6	g/dL	Kaneko *et al.*, 2008
Albumin	1.2–2.5	g/dL	Tietz, 1995
Cholesterol	100–200	mg/dL	Lumeij, 2008
Triglycerides	30–150	mg/dL	Kaneko *et al.*, 2008
AST (Aspartate Aminotransferase)	150–350	U/L	Lumeij, 2008
ALT (Alanine aminotransferase)	2–10	U/L	Tietz, 1995

To further compare the efficiency and reliability of both analytical approaches, additional assessments were conducted, including analysis time measurement, repeatability testing, and evaluation of analytical precision.

Analysis time measurement

The analysis time for each sample was recorded using both manual and automated methods, from the start of the procedure until the final result was obtained for each parameter. All measurements were performed by the specialized research team to ensure accuracy and operational efficiency. The mean analysis time for each method was calculated to evaluate processing speed and overall workflow efficiency.

Repeatability and reliability assessment

To evaluate reliability, each sample was analyzed three consecutive times using each method, with each measurement considered an independent reading. The mean value for each sample was used in the final analysis, allowing comparison of repeatability and consistency between the two methods.

Analytical precision and measurement stability

The coefficient of variation (CV %) was calculated for each parameter based on repeated measurements of each sample, serving as a measure of analytical precision and measurement stability. Lower CV% values indicate higher consistency and reliability, enabling an objective comparison of the performance of manual and automated methods (Sokal and Rohlf, 2012).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Differences between conventional (manual) and automated methods for each hematological and biochemical parameter were evaluated using paired t-tests. Analytical precision and measurement stability were assessed by calculating the coefficient of variation (CV %) based on repeated measurements. Analysis time between methods was also compared to evaluate processing efficiency. Statistical significance was considered at $P \leq 0.05$. All statistical analyses were performed using SPSS software version 25 (IBM SPSS Statistics, Chicago, IL, USA).

Results and Discussion

Hematological analysis and method performance

As shown in Table 3 and Table 4, no significant differences were observed between the conventional and automated methods for all evaluated hematological parameters, including red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), packed cell volume (PCV), platelet count, and the heterophil to lymphocyte ratio (H/L ratio). The mean values obtained using both analytical approaches were highly comparable, and all measured parameters fell within the established physiological reference ranges for broiler chickens. These findings indicate a high level of consistency between the two analytical methods in determining hematological characteristics.

In contrast, Table 4 demonstrates significant differences in analytical performance between the two methods. The automated method consistently showed lower coefficient of variation (CV %) values across all evaluated parameters compared with the conventional method, indicating greater analytical precision and measurement consistency. Furthermore, the automated analysis required substantially less processing time per sample than the conventional procedure, highlighting its superior efficiency in laboratory workflow.

Table 3: *Comparison of hematological parameters between conventional and automated methods in broiler chickens*

Parameter	Conventional method (Mean ± SEM)	Automated method (Mean ± SEM)	Reference range	Sig.
RBC (×10^6 cells/µL)	2.90 ± 0.09	2.95 ± 0.05	2.5 – 3.5	NS
WBC (×10^3 cells/µL)	20.2 ± 0.82	21.1 ± 0.56	12 – 30	NS
Hemoglobin (g/dL)	10.3 ± 0.28	10.6 ± 0.19	7 – 13	NS
PCV (%)	30.8 ± 0.74	31.2 ± 0.51	22 – 35	NS
Platelets (×10^3 cells/µL)	65.7 ± 2.91	67.9 ± 1.83	30 – 100	NS
H/L Ratio	0.36 ± 0.02	0.34 ± 0.01	0.2 – 0.5	NS

NS: No significant difference between conventional and automated methods (P > 0.05).
Data are expressed as Mean ± SEM (n = 50).
Each sample was measured three times and the average value was used.

Table 4: *Analytical performance comparison between conventional and automated methods for hematological analysis*

Parameter	CV% conventional	CV% automated	Analysis time conventional (min/sample)	Analysis time automated (min/sample)	Sig.
RBC (×10^6 cells/µL)	7.2 ^{a}	3.4 ^{b}	23.2 ± 0.8 ^{a}	3.9 ± 0.3 ^{b}	*
WBC (×10^3 cells/µL)	8.1 ^{a}	3.9 ^{b}	20.1 ± 0.8 ^{a}	3.8 ± 0.2 ^{b}	*
Hemoglobin (g/dL)	6.5 ^{a}	2.8 ^{b}	18.4 ± 0.6 ^{a}	3.5 ± 0.3 ^{b}	*
PCV (%)	6.9 ^{a}	3.1 ^{b}	16.9 ± 0.5 ^{a}	3.2 ± 0.1 ^{b}	*
Platelets (×10^3 cells/µL)	9.4 ^{a}	4.6 ^{b}	22.7 ± 0.9 ^{a}	4.1 ± 0.2 ^{b}	*
H/L Ratio	8.7 ^{a}	4.1 ^{b}	20.4 ± 0.9 ^{a}	4.8 ± 0.5 ^{b}	*

NS: No significant difference between conventional and automated methods (P > 0.05).
Data are expressed as Mean ± SEM (n = 50).
Each sample was measured three times and the average value was used.
**Different superscript letters (a, b) within the same row indicate significant differences between methods (P ≤ 0.05).*
CV%: Coefficient of variation calculated from repeated measurements.
Data are expressed as Mean ± SEM (n = 50).

The absence of significant differences between the two analytical approaches in the mean values of the hematological parameters suggests a strong level of agreement between the conventional manual techniques and the automated analyzers in evaluating blood characteristics of broiler chickens. This consistency supports the reliability of automated systems for routine hematological assessment while maintaining results comparable to those obtained using established manual methodologies.

Moreover, the lower CV% values observed in the automated method indicate a higher level of analytical precision and measurement stability compared with the conventional approach. This improvement can largely be attributed to the standardized operational procedures and electronic measurement systems incorporated in automated analyzers, which minimize operator-dependent variability during sample processing. Additionally, the markedly reduced analysis time associated with automated techniques reflects their operational efficiency, making them particularly suitable for laboratories handling large numbers of samples in both research and diagnostic settings.

The findings of the present study are in agreement with those reported by Legroux *et al*. (2025), who indicated that automated hematology analyzers provide accurate measurements for most blood parameters, although limited discrepancies may occur in certain variables, such as white blood cell counts. The results also align with those of Ruiz-Jimenez *et al*. (2022), who observed moderate to high agreement between portable analyzers and conventional laboratory methods in assessing blood parameters in poultry. In comparison, our study demonstrated complete concordance between conventional and automated methods across all hematological indicators, with notable improvements in precision, measurement stability, and analysis time using automated techniques, further supporting the reliability of these systems for routine use in research and diagnostic laboratories.

Serum biochemical analysis and method performance

As presented in Table 5 and Table 6, no significant differences were detected between the conventional and automated methods for all evaluated serum biochemical parameters, including glucose, total protein, albumin, cholesterol, triglycerides, and the liver enzymes AST and ALT. The mean values obtained from both analytical approaches were closely comparable, and all measured parameters were within the established physiological reference ranges for broiler chickens. These findings indicate a strong level of agreement between the two analytical methods in assessing serum biochemical characteristics.

However, Table 6 reveals significant differences in analytical performance between the two methods. The automated method consistently produced lower coefficient of variation (CV %) values compared with the conventional method across all measured parameters, indicating improved analytical precision and measurement stability. In addition, the automated analysis required considerably less processing time per sample than the conventional procedure, reflecting greater efficiency in laboratory operations.

The absence of significant differences between the two analytical approaches in the mean values of serum biochemical parameters suggests a high level of agreement between conventional laboratory techniques and modern automated analyzers in evaluating blood serum components in broiler chickens. This consistency supports the applicability of automated systems for routine biochemical analysis while maintaining results comparable to those obtained through traditional laboratory procedures.

Furthermore, the lower CV% values observed in the automated method indicate a higher degree of analytical precision and measurement stability

Table 5: *Comparison of serum biochemical parameters between conventional and automated methods in broiler .*

Parameter	Conventional method (Mean ± SEM)	Automated method (Mean ± SEM)	Reference range	Sig.
Glucose (mg/dL)	268.4 ± 6.7	271.2 ± 4.5	200 – 350	NS
Total Protein (g/dL)	4.32 ± 0.12	4.41 ± 0.09	3.0 – 6.0	NS
Albumin (g/dL)	1.78 ± 0.05	1.82 ± 0.04	1.2 – 2.5	NS
Cholesterol (mg/dL)	148.6 ± 4.9	151.3 ± 3.7	100 – 200	NS
Triglycerides (mg/dL)	86.7 ± 3.6	89.5 ± 2.8	30 – 150	NS
AST (U/L)	241.3 ± 8.1	247.6 ± 6.4	150 – 350	NS
ALT (U/L)	6.4 ± 0.3	6.6 ± 0.2	2 – 10	NS

NS: No significant difference between conventional and automated methods ($P > 0.05$).
Data are expressed as Mean ± SEM ($n = 50$).
Each sample was measured three times and the average value was used.

Table 6: *Analytical performance comparison between conventional and automated methods for serum biochemical analysis*

Parameter	CV% conventional	CV% automated	Analysis time conventional (min/sample)	Analysis time automated (min/sample)	Sig.
Glucose (mg/dL)	6.8 ^{a}	3.2 ^{b}	18.7 ± 0.6 ^{a}	3.4 ± 0.2 ^{b}	*
Total Protein (g/dL)	7.5 ^{a}	3.6 ^{b}	17.9 ± 0.5 ^{a}	3.1 ± 0.1 ^{b}	*
Albumin (g/dL)	6.9 ^{a}	3.1 ^{b}	16.8 ± 0.6 ^{a}	3.4 ± 0.3 ^{b}	*
Cholesterol (mg/dL)	7.8 ^{a}	3.9 ^{b}	20.2 ± 0.8 ^{a}	3.5 ± 0.4 ^{b}	*
Triglycerides (mg/dL)	8.4 ^{a}	4.1 ^{b}	19.4 ± 0.7 ^{a}	3.6 ± 0.2 ^{b}	*
AST (U/L)	7.3 ^{a}	3.5 ^{b}	22.3 ± 0.4 ^{a}	3.9 ± 0.7 ^{b}	*
ALT (U/L)	6.6 ^{a}	3.0 ^{b}	20.1 ± 0.9 ^{a}	3.8 ± 0.5 ^{b}	*

**Different superscript letters (a, b) within the same row indicate significant differences between methods ($P \leq 0.05$).*
CV%: Coefficient of variation calculated from repeated measurements.
Data are expressed as Mean ± SEM ($n = 50$).

compared with the conventional approach. This improvement can be attributed to the standardized analytical processes and electronic detection systems integrated into automated analyzers, which reduce operator-dependent variability during laboratory procedures. Additionally, the markedly reduced analysis time highlights the operational efficiency of automated systems, making them particularly advantageous for research and diagnostic laboratories that require rapid processing of large numbers of samples.

The findings of the present study are consistent with those reported by Ruiz-Jimenez *et al.* (2021), who observed that portable analyzers such as i-STAT and VetScan VS2 showed moderate to high correlation coefficients for most biochemical parameters in chickens, with generally good agreement with conventional laboratory methods despite minor differences in some analytes. The results also align with Sauer *et al.* (2020), who compared i-STAT1 and VetScan VS2 for several blood chemistry and electrolyte indicators in laying hens and found substantial concordance across many parameters, although slight variations were noted that should be considered when interpreting results. Furthermore, Ruiz-Jimenez *et al.* (2022) reported that portable analyzers can provide reliable biochemical data in poultry and establish age-specific reference intervals for certain analytes, supporting their use in both research and practical applications. Collectively, these studies reinforce that automated biochemical analyses deliver accurate and reliable measurements comparable to conventional methods, while also enhancing operational efficiency and reducing analysis time.

Despite the promising findings, some limitations of the present study should be acknowledged. The experiment was conducted using only female broiler chickens at a single age (35 days), which may limit the generalizability of the results. Hematological and biochemical parameters may vary depending on sex, age, breed, and physiological status. Therefore, future studies including different sexes, age groups, and broiler strains would provide a more comprehensive evaluation of the agreement between conventional and automated analytical methods in poultry.

Conclusions and Recommendations

Based on the results of the present study, no significant differences were observed between conventional manual methods and automated analyzers in the measurement of hematological and serum biochemical parameters in broiler chickens. Both methods produced comparable values within the physiological reference ranges, indicating good agreement in the assessment of these blood indicators. However, automated analyzers demonstrated superior analytical performance, as reflected by lower coefficients of variation and substantially shorter analysis time per sample. These findings suggest that automated analytical systems provide reliable and precise measurements of hematological and biochemical parameters in broiler chickens while significantly improving laboratory efficiency. Therefore, automated analyzers represent a practical and efficient alternative to conventional manual methods for routine laboratory analyses in both research and diagnostic settings.

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Novelty Statement

The novelty lies in the integrated comparison of manual and automated methods for broiler blood analysis, assessing accuracy, precision, and processing time in a single framework. The study demonstrates superior precision and efficiency of automated systems without significant differences in measured values.

Author's Contribution

Ahmed A. Al-Salhi: Manuscript writing, data analysis, and overall supervision
Amena L. Al-Shareefi: Study design and scientific review
Nawal J. Shanshool: Sample collection and laboratory analyses
Saif Sh. Kamel: Statistical analysis and final revision.

Generative AI and AI-assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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