Comparison of portable and conventional laboratory analyzers for biochemical tests in chickens

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ABSTRACT Antemortem blood biochemical and blood gas analyses are routinely used in health screening and diagnosis of disease in domestic veterinary species. These testing modalities are not routinely performed in poultry, in part, due to the distance from the diagnostic laboratory. Portable blood analyzers such as the i-STAT and VetScan (**VS2**) can be used to obtain results on the farm without delay, potentially offering a more practical option for poultry practitioners. We investigated the time effect on blood chemistry values and compared the results obtained using the i-STAT and VS2 with those obtained using conventional laboratory analyzers (GEM Premier 3000 and Cobas c501, respectively). We tested blood from 60 healthy chickens. Each sample was tested in triplicate using each of the portable analyzers and once using conventional analyzers. All samples were analyzed within 60 minutes of collection. The concentrations of some analytes were outside the limit of detection of the portable analyzers (i.e., bile acids). Although statistically significant differences were found for some biochemical analytes over time, the actual mean or median differences were too small to be considered of clinical importance. As observed in mammals, significant time-dependent changes in blood gas analytes were observed in whole blood samples exposed to ambient air. Correlation coefficients between portable and conventional analyzers were moderate to high for most of the analytes. For the most part, there was an agreement between the portable and conventional analyzers. We identified constant and proportional biases in the measurement of multiple analytes by both the i-STAT and VS2. Future studies are warranted to establish analyzer-specific reference intervals for poultry.

Key words: analyzer, biochemical analysis, broiler, conventional, portable

INTRODUCTION

Antemortem blood tests, including the complete blood count and biochemical analysis, are routinely used to evaluate health status, diagnose disease, guide medical decisions, and assess the progression of disease in a variety of veterinary species (Harr, 2006). The lack of quality of biochemical data in poultry may impair our ability to properly diagnose diseases and monitor flock health status (Martin et al., 2010; Ammersbach et al., 2015). Although other routine poultry diagnostic methods including necropsy, serology, and microbiologic testing are excellent for diagnosis of infectious diseases (Majo and Dolz, 2019; Collett and Smith, 2020), these testing modalities neither are sufficient to diagnose metabolic conditions (Crespo, 2020) nor can be used as part of flock health management to determine nutritional deficiencies before the development of clinical signs. Therefore, the antemortem biochemical analysis may be an important diagnostic modality in poultry medicine that deserves further investigation.

A delay between sample collection and testing can cause significant changes in the concentration of some blood analytes owing to factors including hemolysis, continued cellular metabolism, evaporation, and altered enzyme activities (Scanes, 2015). In human and small animal medicine, this may not represent a significant challenge, given that in most cases, clinics and hospitals have an on-site laboratory and blood samples can be tested with minimal delay. In production animals, prompt testing may be hindered by the physical distance and time needed to reach a laboratory. In addition, avian red blood cells are nucleated, and their metabolism could be faster than their mammalian counterparts (Scanes, 2015), potentially accelerating storage-related changes to analyte concentrations.

Several studies have reported reference intervals for hematologic, biochemistry, and blood gas analytes in production poultry (Beljan et al., 1971; Ross et al., 1978; Hopkinson et al., 1990; Martin et al., 2010; Schaal et al., 2016; Board et al., 2019; Sauer et al., 2019). However, owing to lack of prompt access to laboratory services, requirements for sample preservation and handling, and cost, these tests are rarely used in routine poultry medicine (Martin et al., 2010). Portable analyzers, such as the i-STAT (Abbott Laboratories, Chicago, IL) or VetScan VS2 (**VS2**; Abaxis, Inc., Union City, CA), can be used to measure biochemical analytes directly on the farm, thus mitigating sample handling and transportation challenges. These analyzers have been used in clinical settings for other avian species, including Strigiformes and Psittacines (Johnston et al., 2007; Greenacre et al., 2008; Ammersbach et al., 2015).

Before the i-STAT and VS2 portable analyzers can be used in commercial poultry with confidence, it is important to determine whether the results obtained using these instruments are comparable with those obtained using conventional laboratory analyzers. Hence, the aims of this study were to 1) assess the comparability of the i-STAT using the CG8+ cartridge and VS2 using the Avian/Reptile rotor with their respective conventional laboratory analyzers: the GEM 3000 and Cobas c501, and 2) evaluate the effects that time has on blood analytes.

MATERIALS AND METHODS

Animal and Housing

Five thousand 1-day-old Ross 708 chickens provided by a commercial broiler integrator were housed at the poultry barn of the Teaching Animal Unit at the College of Veterinary Medicine, North Carolina State University (**CVM-NCSU**). The chickens were vaccinated *in ovo* for Marek's Disease and Infectious Bursal Disease. They were vaccinated for Coccidiosis and Infectious Bronchitis Virus after hatching and before delivery. All birds were managed under the same environmental conditions. A commercial feed was provided by the integrator. All animal handling and blood collection protocols were reviewed and approved by the NCSU Institutional Animal Care and Use Committee (IACUC protocol number: 19-001).

Sample Collection and Handling

Sixty chickens were sampled in 3 groups of 20 chickens on 3 different days. At each sampling time, birds were marked with food coloring dye to ensure that no bird was tested more than once. Venipuncture was performed via the jugular vein in nonanesthetized chickens using disposable 1-mL or 3-mL syringes with 21-gauge heparin flashed needles. As recommended by Owen, 2011, to prevent hemolysis, the needle was removed before the immediate transfer of 1 to 2 mL of blood into a lithium heparin collection tube (BD Biosciences, Franklin Lakes, NJ). The blood was gently mixed and stored at 4°C until analysis.

Blood Chemistry and Gas Analysis

The following analytes were measured in whole blood using CG8+ cartridges on the i-STAT: sodium (**Na$^+$**; mmol/L), potassium (**K$^+$**; mmol/L), ionized calcium (**iCa**; mmol/L), glucose (**Glu**; mg/dL), hematocrit (**Hct**), pH, partial pressure of oxygen (**pO$_2$**; mm Hg), partial pressure of carbon dioxide (**pCO$_2$**; mm Hg), bicarbonate (**HCO$_3$**; mmol/L), total carbon dioxide (**tCO$_2$**; mmol/L), base excess (**BE**; mmol/L), and oxygen saturation (**sO$_2$**; %). The samples were measured according to the manufacturer's instructions, and instrumentation was kept within the recommended operating temperature parameters. The results obtained using the i-STAT were compared with those obtained using the GEM Premier 3000 blood gas system (Instrumentation Laboratories, Bedford, MA), which was calibrated and maintained by the CVM-NCSU Clinical Pathology laboratory.

The following analytes were measured in whole blood using avian/reptilian specific reagent rotors on the VS2: aspartate aminotransferase (**AST**; U/L), bile acids (μmol/L), creatine kinase (**CK**; U/L), uric acid (**UA**; mg/dL), Glu (mg/dL), total calcium (**Ca**; mg/dL), phosphorus (**P**; mg/dL), total protein (**TP**; g/dL), albumin (**Alb**; g/dL), K$^+$ (mmol/L), and Na$^+$ (mmol/L). The samples were measured according to the manufacturer's instructions, and instrumentation was kept within the recommended operating temperature parameters. The results obtained using the VS2 were compared with those obtained using the Roche Cobas c501 chemistry analyzer (Roche Diagnostics, Basel, Switzerland), which is calibrated and maintained by the CVM-NCSU Clinical Pathology laboratory.

Figure 1 is a schematic of the experimental workflow. In brief, each blood sample was analyzed in 3 serial replicates in each instrument, approximately 20 minutes apart. Blood samples were kept at 4°C between testing times, and all analyses were completed within 60 minutes. The first 2 replicates (time 1 [T1] and time 2 [T2]) were completed at the Teaching Animal Unit poultry barn. The third replicate (T3) was completed in the Clinical Pathology laboratory at CVM-NCSU to allow simultaneous measurement by the portable analyzers and the GEM Premier 3000 and Cobas c501. Analysis using the Cobas c501 required an additional step to separate the plasma from the sample by centrifugation at 800 × g for 10 minutes. The values obtained using each portable analyzer at T3 were compared with results obtained simultaneously using either the GEM Premier 3000 or Cobas c501.

Statistical Analysis

Statistical analyses and figures were obtained using MedCalc software (Medcalc version 19.1.5; Ostend, Belgium; https://www.medcalc.org; 2020). Descriptive
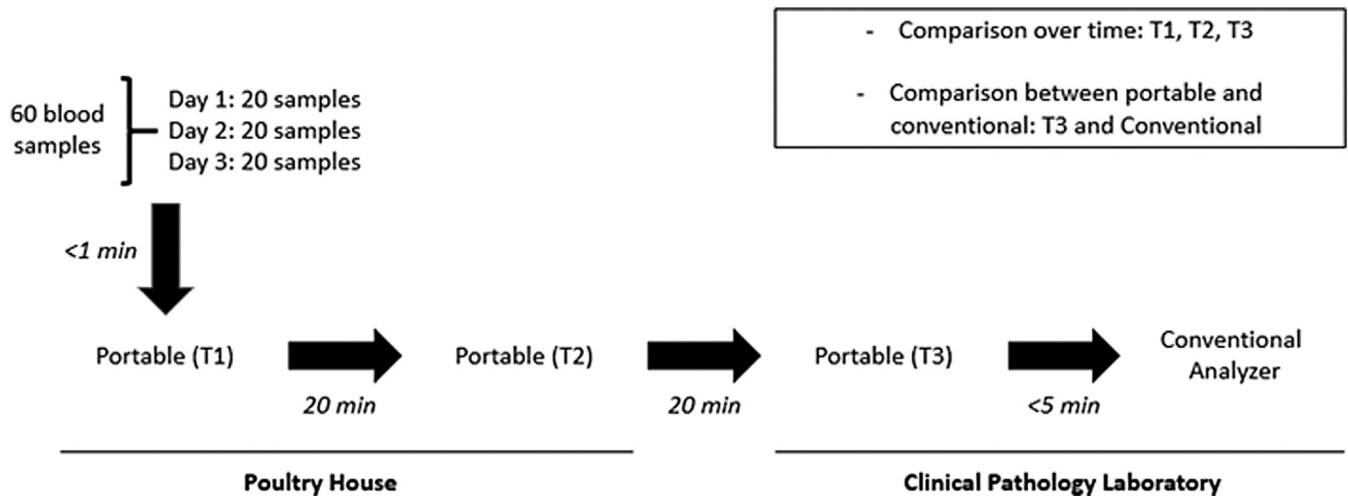
Figure 1. Experimental design and flow of the blood samples.

statistics were estimated for each analyte using the portable analyzers at each time of measurement and the conventional instruments. The normality of the data was determined using the D'Agostino-Pearson normality test (D'agostino, Belanger and D'agostino, 1990). If data were not normally distributed, nonparametric tests were considered.

Comparison of Analytes Measured Over Time Using the Portable Analyzers

Repeated measures ANOVA or Friedman's tests were considered for comparisons of analytes through time for the i-STAT and VS2 analyzers. The α-value was set at ≤0.05. Mountain plots were used for visualization of possible differences between times for the same analyte. (Krouwer and Monti, 1995). These plots compare the median of tests for T2 and T3 against T1 (given that T1 was in the immediacy of blood collection). In this plot, the closer the median difference value is to 0 (median of T2 and T3 values to T1), the closer the test results are to T1.

Comparison of Portable and Conventional Analyzers

Pearson correlation coefficients were obtained to measure the linear association of portable and conventional analyzers. Bland–Altman plots were used for visualization and quantification of the agreement of the results obtained by the i-STAT and VS2 analyzers at T3 with the results obtained using the respective conventional analyzers. Passing–Bablok regression analysis was used to estimate constant and proportional bias between analytical methods. Constant bias indicates that the test analyzer consistently measures an analyte concentration to be higher or lower in comparison with the reference analyzer. Proportional bias indicates that the differences in measurements of the test and reference analyzer are proportional to the level of measurement. This is a nonparametric regression, and nonlinear

samples are not suitable for Passing–Bablok analysis (Altman and Bland, 1983; Martin Bland and Altman, 1986; Jensen and Kjelgaard-Hansen, 2006).

RESULTS

Descriptive statistics of all the analytes are summarized in Tables 1 and 2. Data results outside the detection range of the instruments were excluded. More than 90% of the bile acid measurements were lower than the detection limits of the VS2; thus, this analyte was excluded from further analysis. For CK, only 41 samples (68%) were included. The other 19 samples had a CK value higher than the limit of detection for the VS2 and were excluded.

All the blood gas analytes (i.e., HCO_3, pCO_2, pO_2, sO_2, tCO_2, BE, and pH) measured using the i-STAT analyzer showed statistically significant differences between measurements taken at T1, T2, and T3 (Table 3). Ionized calcium and K^+ showed statistically significant differences between times as well. Glucose, Hct, and Na^+ did not show a statistically significant difference between measurement times (Table 3). The mountain plot for pO_2 (Figure 2) is a representative plot illustrating the time-dependent differences in blood gas concentrations.

On the VS2, statistically significant differences in replicate measurements were observed for AST, CK, UA, Ca, P, K^+, and Na^+ (Table 4). Notably, the numeric differences were small for each of these analytes. Glucose, TP, and ALB did not show any statistically significant differences between time points (Table 4). The mountain plot for TP (Figure 3) is a representative example illustrating an analyte with no statistically significant time-dependent differences in concentration.

The i-STAT showed a constant negative bias for Hct and a proportional bias for pCO_2. Base excess, pH, and sO_2 had both constant and proportional bias. The Bland–Altman plots showed a high agreement (>95%) between the i-STAT and GEM 3000 for iCa, K^+, Na^+,

Table 1. Descriptive statistics for the biochemical analytes measured using the i-STAT with CG8+ cartridges and the GEM 3000 analyzers.

Analyte	i-STAT (T1)			i-STAT (T2)			i-STAT (T3)			GEM 3000		
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD
Glu	228	227.86	22.14	228	228.29	22.13	229.5	228.57	21.74	227	226.22	22.1
Hct	19	20.54	5.25	19	20.84	5.17	19	20.65	5.25	25	27.2	5.98
iCa	1.16	1.17	0.15	1.17	1.16	0.15	1.16	1.15	0.14	1.28	1.27	0.13
K^+	5	5.13	0.69	5	5.1	0.68	5.1	5.08	0.62	5	5.2	0.7
Na^+	142	142.68	8.35	142	142.89	8.33	142	143.05	8.23	143	145.63	7.46
BE	-0.5	0.63	5.22	0	1.1	5.66	1	1.58	5.96	1.5	2.14	4.28
HCO_3	24.15	24.52	4.01	23.6	24.18	4.16	23.1	23.9	4.25	24.1	24.78	4.32
pCO_2	34.65	34.96	8.2	32.1	31.7	8.44	29.9	28.28	8.62	31	30.33	9.52
pH	7.44	7.46	0.12	7.5	7.5	0.14	7.52	7.55	0.16	7.54	7.54	0.12
pO_2	52	59.9	20.88	56	67.53	28.96	93	99.55	41.7	127	123.86	45.5
sO_2	88	88.39	6.89	91	91.18	5.84	98	95.81	4.94	99	97.58	3.45
tCO_2	25.5	25.65	4.11	25	25.19	4.22	24	24.9	4.69	25.15	25.71	4.43

Abbreviations: BE, base excess; Glu, glucose; Hct, hematocrit; iCa, ionized calcium; K^+, potassium; Na^+, sodium; pO_2, partial pressure of oxygen; sO_2, oxygen saturation; tCO_2, total carbon dioxide.

HCO_3, pCO_2, pH, and tCO_2. Agreement was moderate (50–95%) for Glu, Hct, BE, pO_2, and sO_2 (Table 5, Figure 4). None of the analytes showed less than 50% of agreement.

The statistical results for the comparison between VS2 and Cobas c501 are summarized in Table 6. The VS2 had a constant positive bias for Ca and TP and a negative constant bias for Na^+. The VS2 had a negative proportional bias for Glu. The VS2 had combined constant and proportional biases for AST, UA, and K^+. The Bland–Altman plots showed a high agreement (>95%) between the VS2 and the Cobas c501 for Ca, TP, Alb, K^+, and Na^+ (Table 6 and Figure 5). Moderate agreement (50–94%) was observed for AST, CK, UA, Glu, and P. None of the analytes had a poor agreement (<50%).

DISCUSSION

Because the i-STAT and the VS2 cartridges measured different analytes, we keep the discussion for these analyzers separate. We first discuss the finding for the i-STAT over time, followed by the VS2. Then, we discuss differences between the portable and the conventional analyzers.

Significant time-dependent differences in blood gas concentrations (e.g., pO_2, sO_2, pCO_2, tCO_2) were observed as measured using the i-STAT. The blood collection container was opened 3 different times for analysis, exposing the sample to air. Ambient air oxygen and carbon dioxide concentrations are higher and lower than in blood, respectively. In mammalian species, blood gases diffuse readily, and it has been shown that the presence of even 1% of air bubbles in a blood sample can result in significant modifications in the blood gases concentration (e.g., pO_2, sO_2, pCO_2, or tCO_2) (Lu et al., 2003). In addition, there is evidence that modifications in blood pH are inversely related to changes in pCO_2 concentrations (Scanes, 2015). In our studies, we demonstrate that similar rapid changes in blood gas concentrations are observed in avian blood exposed to ambient air. The decreases in iCa and K^+ concentration over time are most likely due to the increased pH. Increased pH, or alkalosis, promotes calcium binding to blood proteins, thus decreasing measured iCa (Wang et al., 2002). Alkalosis also induces transcellular shifting of K^+. Extracellular K^+ is driven into the cells in exchange for intracellular hydrogen, thus reducing measured serum/plasma K^+ (Aronson and Giebisch, 2011). Glucose content was relatively stable across time points, which was expected given the short

Table 2. Descriptive statistics for the biochemical analytes measured using the Vetscan VS2 (VS2) with Avian/Exotic rotors and the Cobas c501 analyzers.

Analyte	VS2 (T1)			VS2 (T2)			VS2 (T3)			Cobas c501		
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD
AST	173	236.8	157.5	173	240.6	158	178	236.7	150.8	172.0	239.6	168.6
CK	874	1,123.2	641.5	887	1,130.5	651.1	884	1,111.3	639.6	905.0	1,190.6	693.1
UA	4.9	5.1	2.0	4.9	5.2	2.0	4.6	5.2	2.1	5.6	5.9	1.9
Glu	235	235.3	16.4	236.0	235.5	15.8	233.5	235.3	16.2	250.5	252.2	17.8
Ca	11.3	11.4	0.7	11.5	11.5	0.7	11.4	11.4	0.6	10.9	10.9	0.9
P	6.5	6.6	0.8	6.8	6.8	0.8	6.8	7.0	0.9	6.9	7.0	0.8
TP	2.9	2.9	0.6	2.8	2.9	0.6	2.8	2.9	0.5	2.6	2.7	0.6
Alb	2.2	2.2	0.3	2.2	2.2	0.3	2.2	2.2	0.3	1.0	1.1	0.3
K^+	5.9	6.6	1.7	5.9	6.8	1.8	5.8	6.7	1.9	5.0	5.1	0.5
Na^+	150	151.3	4.0	150	150.7	4.1	149	150.0	3.7	150.5	151.3	3.6

Abbreviations: Alb, albumin; AST, aspartate aminotransferase; CK, creatine kinase; Glu, glucose; K^+, potassium; Na^+, sodium; TP, total protein; UA, uric acid.

Table 3. Friedman test results for the difference in the results over time for the i-STAT analyzer.

Analytes	P-value	Difference	Clinical relevance of the difference[1]
Glu	0.42350	—	—
Hct	0.24938	—	—
iCa	0.01813	T1 vs T3	Negligible
K^+	0.00016	T3 vs T1 and T2	Negligible
Na^+	0.32261	—	—
BE	0.00055	T3 vs T1 and T2	Negligible
HCO_3	<0.00001	T1 vs T2 and T3	Negligible
pCO_2	<0.00001	T1 vs T2 and T3, T2 vs T3	Negligible
pH	<0.00001	T1 vs T2 and T3	Negligible
pO_2	<0.00001	T1 vs T2 and T3	Effect of opening tubes
sO_2	<0.00001	T1 vs T2 and T3	Effect of opening tubes
tCO_2	<0.00001	T1 vs T2 and T3	Negligible

Abbreviations: BE, base excess; Glu, glucose; Hct, hematocrit; iCa, ionized calcium; K^+, potassium; Na^+, sodium; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; sO_2, oxygen saturation; tCO_2, total carbon dioxide.
[1]Compared with means or medians from Table 1.

duration of the experiment and that the samples were stored in ice, thus decreasing cellular metabolism (Lumeij, 1987).

For analytes measured using the VS2, we observed statistically significant differences in concentrations of AST, CK, UA, Ca, P, K^+, and Na^+ over time. When interpreting these findings, it is critical to consider the degree of change in each analyte because a statistically significant change may not correspond to a clinically relevant change (Ranganathan et al., 2015). Over the short time course of these experiments, we interpreted the time-dependent changes in all analytes measured using the VS2 to likely be negligible and unlikely to change clinical interpretation. Still, it is important to note that there are no studies of stability on avian or reptilian blood; thus, further studies on this subject are advised.

In our study, the i-STAT analyzer with the CG8+ cartridge showed moderate to high agreement for measurements in comparison with the GEM 3000 across the entire range of values measured. Steinmetz et al., 2007 evaluated the performance of the i-STAT CG7+ cartridge, which measures the same analytes as the CG8+ except for Glu, to the Siemens Rapidlab 800 analyzer in anesthetized chickens and found moderate to high correlation between all the analytes, except for K^+ and BE. The agreement between the portable and the conventional analyzer used in this study was not reported.

The agreement between VS2 and Cobas c501 was high for Ca, TP, Alb, K^+, and Na^+ and moderate (90–95%) for AST, CK, UA, Glu, and P. In contrast, other studies on Psittacines (Greenacre et al., 2008) and Strigiformes (Ammersbach et al., 2015) found high to moderate agreement in fewer analytes. Nevertheless, the results from both studies coincided with ours for AST, CK, Glu, and TP.

It is important to consider the method that each of the instruments uses to report the concentration of the analytes because differences could contribute to the degree of agreement (Jensen and Kjelgaard-Hansen, 2006). All 4 analyzers used in this study use ion-selective electrode
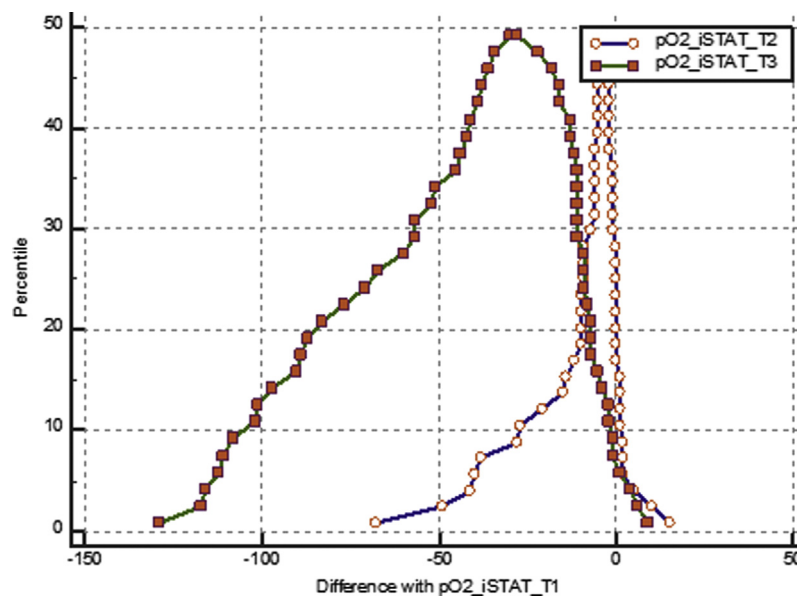

Figure 2. Mountain plot showing an increase in the partial pressure of oxygen (pO_2) in whole blood at T2 and T3 compared with T1, as measured using the i-STAT.

Table 4. Friedman test results for the difference in the results over time for the Vetscan VS2 analyzer.

Analytes	P-value	Difference	Clinical relevance of the difference[1]
AST	<0.00001	T2 vs T1 and T3	Negligible
CK	0.00270	T1 vs T2 and T3	Negligible
UA	0.00008	T2 vs T1 and T3	Negligible
Glu	0.16396	—	—
Ca	<0.00001	T2 vs T1 and T3	Negligible
P	0.03695	T2 vs T3	Negligible
TP	0.09216	—	—
Alb	0.51123	—	—
K^+	0.03695	T2 vs T3	Negligible
Na^+	0.03695	T2 vs T3	Negligible

Abbreviations: Alb, albumin; AST, aspartate aminotransferase; CK, creatine kinase; Glu, glucose; K^+, potassium; Na^+, sodium; TP, total protein; UA, uric acid.
[1]Compared to means or medians from Table 2.

potentiometry to measure the concentration of Na^+, K^+, and pH. Still, the exact composition of the electrodes may differ, contributing to minor differences in the measurement of these analytes. The blood gas analytes, HCO_3 and BE, are calculated values derived from the directly measured pH and pCO_2. The GEM 3000 and i-STAT use the same calculations for HCO_3 and BE: $HCO3 = 10^{(pH+log\ (pCo2)-7.608}$ and $BE = HCO3-24.8+16.2*(pH-7.4)$ (Instrumentation Laboratory, 2002; Abaxis Inc., 2007; Abbott, 2013; Roche Diagnostics, 2015). Therefore, differences in the calculation do not explain the differences in the reported values for these analytes. Temperature is another important factor that could affect the results, particularly for pH. Because pH is temperature dependent, it is typically measured at 37°C, and a correction is needed whenever the temperature of the sample varies (CLSI, 2009). In this study, the blood was at chickens' temperature only for the T1 analysis, and it was kept at 4°C afterward. The GEM 3000 preheats the samples at 37°C before analysis (Instrumentation Laboratory, 2002), but the i-STAT does not (Abbott, 2013); thus, it is possible that

the reported i-STAT pH measurements at T2 and T3 are higher than if they had been measured in blood at 37°C. The effect of temperature on the measurement of pH in avian blood is beyond the scope of the present study, but would warrant further investigation and consideration, particularly for studies using blood pH as a critical indicator of health or metabolic status.

Passing–Bablok regression analysis (Jensen and Kjelgaard-Hansen, 2006) was used to determine constant and proportional bias of each analyte. The analysis showed either constant and/or proportional bias for some analytes (Tables 5 and 6). It is important to take biases into account because they can lead to erroneous interpretations of the results (Bilic-Zulle, 2011). With a constant bias, the new instrument values stay higher or lower than the reference instrument values by a certain amount as the level of the analyte is increased. If the exact amount of modification is calculated, then this error can be fixed by just adjusting the results given by the new instrument. On the other hand, in a proportional bias, the new analyzer values are a fixed percentage of the reference analyzer values at all
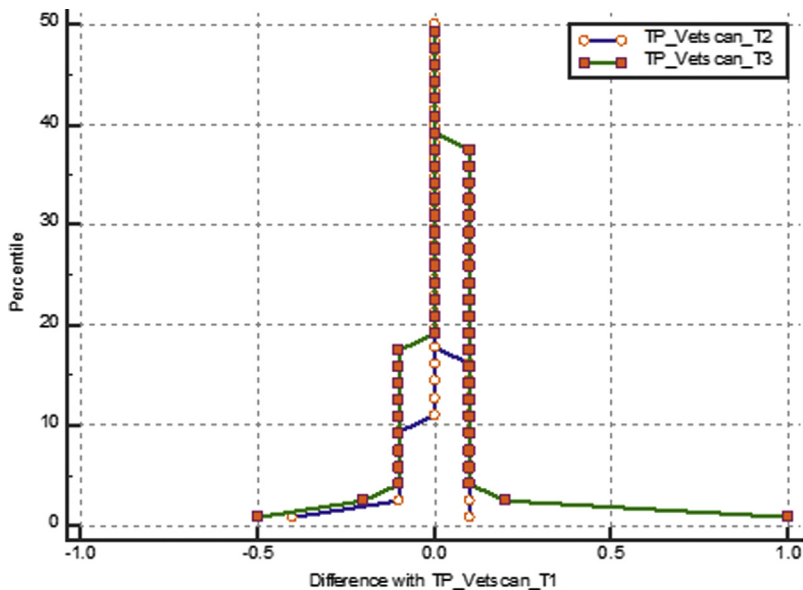

Figure 3. Mountain plot showing no significant differences in total protein (TP) in whole blood at T2 or T3 compared with T1, as measured using the Vetscan VS2.

Table 5. Correlation, Passing–Bablok regression with constant and proportional bias, and Bland–Altman results for agreement between the i-STAT and the GEM 3000 analyzers.

| Analytes | Correlation[1] | Passing–Bablok linear regression analysis | | | | | | Bland–Altman plot | |
		y-intercept	95% CI	Constant bias[2]	Slope	95% CI	Proportional bias[3]	WL[4]	%
Glu	0.81	21.53	−5.16 to 48.06	No	1.17	1 to 1.33	No	50/54	92.59
Hct	0.93	−3.22	−6 to −1.68	Yes	0.89	0.383 to 1.00	No	40/44	93.02
iCa	0.83	−0.27	−0.56 to 0.046	No	1.11	0.94 to 1.35	No	43/42	97.72
K^+	0.94	0.32	0 to 0.76	No	0.94	0.86 to 1.00	No	55/57	96.49
Na^+	0.69	−6.89	−37.50 to 1.00	No	1.04	1 to 1.25	No	55/57	96.49
BE	0.91	−1.54	−1.59 to −1.55	Yes	1.15	1.02 to 1.28	Yes	53/56	94.64
HCO_3	0.90	1.08	−1.64 to 3.75	No	0.91	0.79 to 1.02	No	55/56	98.21
pCO_2	0.91	1.90	−0.70 to 4.59	No	0.90	0.81 to 0.98	Yes	55/57	96.49
pH	0.95	−1.08	−1.69 to −0.44	Yes	1.14	1.06 to 1.22	Yes	55/57	96.49
pO_2	0.76	−6.77	−22 to 4.75	No	0.91	0.81 to 1.06	No	54/57	94.74
sO_2	0.80	−66.66	−100 to −37.69	Yes	1.67	1.38 to 2.00	Yes	54/56	94.73
tCO_2	0.90	1.05	−2.53 to 3.85	No	0.91	0.80 to 1.05	No	54/56	96.43

Abbreviations: BE, base excess; Glu, glucose; Hct, hematocrit; iCa, ionized calcium; K^+, potassium; Na^+, sodium; pCO_2, partial pressure of carbon dioxide; pO_2, partial pressure of oxygen; sO_2, oxygen saturation; tCO_2, total carbon dioxide.
[1]Pearson correlation coefficient.
[2]Constant bias: the confidence interval for the y-intercept must include 0; otherwise, there is evidence of bias.
[3]Proportional bias: the confidence interval for the slope must include 1; otherwise, there is evidence of bias.
[4]WL indicates values that were within the limits of agreement based on the Bland–Altman plot. Denominators per analyte vary.

concentrations tested. We demonstrated constant and proportional biases for BE, pH, and sO_2 when using the CG8+ cartridge in the i-STAT analyzer, and for AST, UA, and K^+ using the avian and reptilian rotor in the VS2 analyzer. The presence of both constant and proportional biases complicates the interpretation of these analytes when using published reference intervals. Together, these results underscore the need to establish reference intervals for each analytic device.

Intriguingly, 19 of the 60 samples were above the limits of detection for CK, an enzyme found primarily in myocytes and commonly used as an indicator of muscular disease in poultry and many other species (Lumeij, 1997).

Increased CK activity has been associated with muscle damage owing to either a normal physiological response to exercise (Mougios, 2007; Baird et al., 2012; Kindermann, 2016) or pathologic conditions such as rhabdomyolysis, infections, metabolic disorders, prolonged inactivity, or temperature-induced states as malignant hyperthermia (Cervellin et al., 2010; Torres et al., 2015). Increases in this enzyme content have also been noted in poultry when the birds are subjected to acute heat stress (Mitchell and Sandercock, 1995; Sandercock et al., 2006) and ionophore toxicity (Dowling, 1992). Further studies will need to be conducted to determine if the marked CK elevations observed in
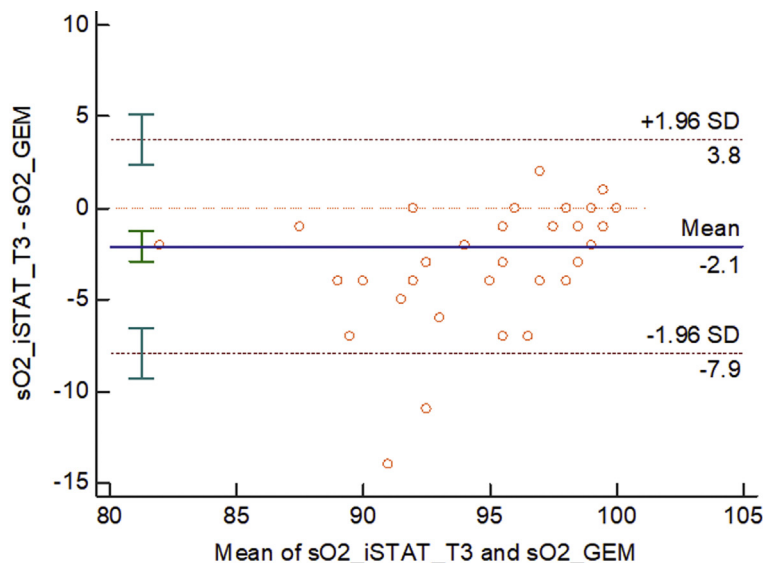
The mean line indicates the difference between both tests expected to be = 0; dotted lines are the upper and lower 95% confidence interval for the mean; and the 95% confidence of limits of agreement represented by the vertical bars. The closer the data points are to the mean line or are within the 95% confidence interval for the mean represent the agreement between tests.

Figure 4. Bland–Altman plot comparing oxygen saturation (sO_2) results measured using the i-STAT and GEM 3000; difference between both tests plotted against the mean difference between the tests.

Table 6. Correlation, Passing–Bablok regression with constant and proportional bias, and Bland–Altman results for agreement between the VS2 and the Cobas c501 analyzers.

| Analytes | Correlation[1] | Passing–Bablok linear regression analysis | | | | | | Bland–Altman plot | |
		y-intercept	95% CI	Constant bias[2]	Slope	95% CI	Proportional bias[3]	WL[4]	%
AST	0.98	19.60	15.42 to 22.17	Yes	0.90	0.89 to 0.93	Yes	51/54	94.44
CK	0.90	−65.69	−543. To 42.57	No	1.05	0.93 to 1.60	No	18/19	94.74
UA	0.88	−1.38	−1.83 to −0.91	Yes	1.11	1.03 to 1.18	Yes	55/59	93.22
Glu	0.87	21.53	−5.16 to 48.06	No	0.24	0.74 to 0.95	Yes	56/60	93.33
Ca	0.84	1.27	0.50 to 2.66	Yes	0.93	0.80 to 1.00	No	58/60	96.67
P	0.55	−0.10	−1.69 to 0.77	No	1.00	0.88 to 1.23	No	51/58	87.93
TP	0.97	0.20	0.20 to 0.20	Yes	1.00	1.00 to 1.00	No	57/60	95.00
Alb	0.37	0.60	0.00 to 1.15	No	1.5	1.00 to 2.00	No	59/60	98.33
K^+	0.50	−12.86	−19.71 to −8.13	Yes	3.85	2.91 to 5.23	Yes	55/57	96.50
Na^+	0.77	−12.73	−51.50 to −1.00	Yes	1.08	1.00 to 1.33	No	57/60	95.00

Abbreviations: Alb, albumin; AST, aspartate aminotransferase; CK, creatine kinase; Glu, glucose; K^+, potassium; Na^+, sodium; TP, total protein; UA, uric acid.

[1]Pearson correlation coefficient.

[2]Constant bias: the confidence interval for the y-intercept must include 0; otherwise, there is evidence of bias.

[3]Proportional bias: the confidence interval for the slope must include 1; otherwise, there is evidence of bias.

[4]WL indicates the number of values that were within the limits of agreement based on the Bland–Altman plot. Denominators per analyte vary.

this study were due to the rapid but physiological growth of the modern chicken's muscles or if there is any chronic muscular damage or environmental condition involved.

Although clinically relevant differences in concentration of analytes are negligible within 1 hour of collection, time-dependent changes in multiple analytes were still observed, indicating the importance of testing blood samples as soon as possible after collection. The changes in blood gas concentrations reported here demonstrate the need to minimize exposure to air. Future studies to determine the effect of time on blood gas analytes in whole blood collected anaerobically may be warranted.
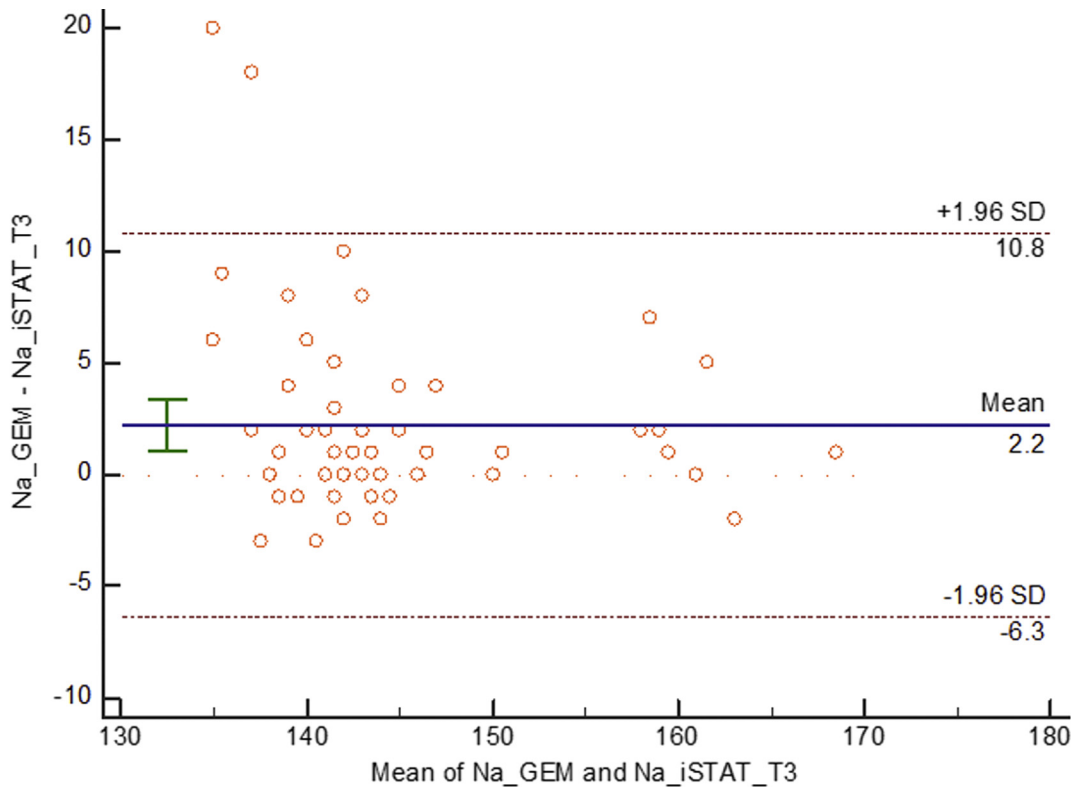
The mean line indicates the difference between both tests expected to be = 0; dotted lines are the upper and lower 95% confidence interval for the mean; and the 95% confidence of limits of agreement represented by the vertical bars. The closer the data points are to the mean line or are within the 95% confidence interval for the mean represent the agreement between tests.

Figure 5. Bland–Altman plot comparing sodium (Na^+) results measured using the Vetscan VS2 and Cobas c501; difference between both tests plotted against the mean difference between the tests.

Although the overall agreement was moderate to high for most analytes, constant, proportional, and mixed constant and proportional biases were observed for both analyzers. Thus, interpretation of results reported by the i-STAT or VS2 using published poultry reference intervals from conventional analyzers may lead to an erroneous diagnosis. Analyzer-specific reference intervals must be established for these analyzers to have confidence in the results.

DISCLOSURES

The authors declare that there are no conflicts of interest.

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