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ORIGINAL RESEARCH

Analytic and quality control validation and assessment of field performance of a point-of-care chemistry analyzer for use in the White rhinoceros

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Background: A chemistry point-of-care analyzer would be useful for evaluating injured wildlife, particularly White rhinoceros (*Ceratotherium simum*) that survive poaching attempts. The IDEXX VetTest could be suitable, but species-specific validation, development of a statistical quality control (QC) strategy, and evaluation under field conditions are necessary.

Objectives: The objectives were to (1) validate the VetTest for the White rhinoceros, (2) perform QC validation on the VetTest and generate a statistical QC strategy, and (3) apply this QC strategy to monitor performance under typical field conditions.

Methods: Differences between White rhinoceros heparin plasma and serum, short-term imprecision, and reportable range using rhinoceros plasma and long-term imprecision using commercial quality control material (QCM) were assessed against prescribed total allowable error (TE_a) for up to 15 analytes. Quality control validation was performed using data from the long-term imprecision study and TE_a. A QC strategy using QCM was developed and used to monitor performance under field conditions.

Results: Imprecision was acceptable for all analytes except for ALP, ALT, and AST at low activities. The reportable range for AST and LDH differed from the manufacturer's specifications. Eleven analytes were suitable for statistical QC using the 1_{3s} rule, 3 using the 2_s rule; ALP was not suitable. In the field, observed error was < TE_a for all 15 analytes and the sigma metric was > 3.0 for 12 analytes.

Conclusions: The VetTest is suitable for use in the White rhinoceros. Statistical QC is possible for most analytes and useful for evaluation of field performance.

Introduction

From 2014 to 2015, 2390 rhinoceros (mainly White rhinoceros, *Ceratotherium simum*) were killed through poaching in South Africa.[1] Some animals survive poaching attempts and require veterinary care for their injuries.[2] Clinical pathology plays an important role in the initial evaluation and ongoing monitoring of these animals; however, the delay between blood sampling and subsequent analysis at a reference laboratory can be up to 24 hours.[3] These compromised animals generally need to be chemically immobilized each time a veterinary procedure (including blood sampling) is performed, which is associated with the risk of respiratory depression, hypertension, and renarcotization.[4] A patient-side point-of-care analyzer (POCA) would provide immediate clinical information, limit immobilization events, and decrease the risk of preanalytic errors associated with sample transport. Most POCAs used in veterinary practice are only validated for use in domesticated animals in a stable environment such as a veterinary practice laboratory. Before a POCA can be used for the White rhinoceros, the analyzer must be validated for the species in question.[5] Furthermore, recent guidelines published by the American Society for Veterinary Clinical Pathology (ASVCP) have emphasized the need for a quality control (QC) strategy for POCAs.[6] Monitoring analyzer performance becomes particularly important when considering that a POCA used out in the field for wildlife is subject to a set of challenges not encountered in a stable practice or laboratory environment. These include factors like varying weather conditions, uneven roads, inconsistent power supply, and dust. Although POCAs are commonly used for field work on nondomesticated animals, validation studies have not evaluated stability of performance in the field.[7] The aims of this study were to (1) perform analytic validation of a POCA for use in the White rhinoceros, (2) to perform QC validation and formulate a statistical QC strategy for the POCA, and (3) to assess the performance of the POCA under anticipated field conditions.

Materials and Methods

Analyzer

The POCA evaluated in this study was the IDEXX VetTest chemistry analyzer (IDEXX Laboratories, Inc., Westbrook, ME, USA). This analytic system uses reflectance photometry with dry-slide technology.[8] Spectral analysis uses 6 lamps, each with a different wavelength, and reactions take place at 37°C. The ambient operating temperature range for the analyzer is 19–27°C and the humidity range 30–50%. Slides must be stored at −18°C and can be used directly from the freezer.[9] A maximum of 12 slides can be used per run. The VetTest used in this study was placed in the clinical pathology laboratory of the Onderstepoort Veterinary Academic Hospital under recommended operating conditions for the purpose of analyzer and QC validation. A full maintenance (including analysis of the manufacturer's quality control material [QCM]) was performed on the analyzer before the study began. All analyses aside from those in the field study were carried out by one investigator (E.H.H.) after training by an IDEXX technician. A second investigator (J.P.P.) assisted in the field study.

Performance goals

The ASVCP-prescribed total allowable error (TE_a) goals were used for both the method validation and the QC validation.[10] For imprecision studies, ASVCP guidelines state that the imprecision, represented by the CV, should be < TE_a.[11] However, the total observed error (TE_{obs}) should also be < TE_a.[11] As TE_{obs} is calculated by multiplying the CV by a factor of 2, the requirement for the imprecision studies here was CV < 0.5 TE_a, in order to fulfill the criteria of $TE_{obs} < TE_a$.[10]

Method validation

Analytes and samples

Fifteen analytes were evaluated: albumin (ALB), ALP, ALT, AST, total calcium (CA), CK, creatinine (CREA), GGT, glucose (GLU), lactate (LAC), LDH, magnesium (MG), inorganic phosphate (PHOS), total proteins (TP), and urea. The analytic methods are shown in Table S1.[8,9] Two types of sample material were used: an assayed human QCM (Bio-Rad Liquid Assayed Multiqual Level 1 and 2, Lot 45701/45702; Bio-Rad Laboratories Inc., Hercules, CA, USA) and samples from White rhinoceros. The rhinoceros samples had been collected previously for other studies and included blood from both healthy animals, immobilized for the purposes of translocation or preventive dehorning, and from clinically ill animals. Blood was collected from the auricular vein directly into serum and heparin tubes (BD Vacutainer; Becton and Dickinson, Plymouth, UK), stored in a cooler box with ice packs and centrifuged within 24 h; serum and heparin plasma were aliquoted and stored at −80°C. Samples were up to 3 years old. Results from previous analyses of these samples on the laboratory's wet chemistry analyzer (Cobas Integra 400 Plus; Roche Diagnostics Ltd., Rotkreuz, Switzerland) were used to guide sample selection for the various experiments. Samples were excluded if gross hemolysis, lipemia, or icterus were present. Approval to use the samples was granted by the University of Pretoria Animal Ethics Committee (V042-15).

Comparison of serum vs heparin plasma

Twenty paired serum and heparin plasma samples were used. Paired samples were thawed simultaneously; a panel of all analytes aside from LAC was measured on serum first followed by plasma for 10 paired samples, and vice versa for the next 10 samples.

Short-term imprecision

A high and a low pool were created for each analyte using White rhinoceros plasma. Pools were kept at room temperature after being made up and were used within 12 h. Twenty measurements were performed for each analyte on each pool in 2 runs consisting of 10 analyses each, with the second run immediately following the first.

Long-term imprecision

The long-term imprecision study was carried out by running a panel containing all analytes on 2 levels of QCM once daily. Twenty such measurements were performed over a period of 31 days. Slides were not inserted in a particular order and a batch of 12 slides followed by a batch of 3 were used to complete the panel of 15 analytes. No results were obtained on 2 occasions for some analytes due to a slide spotting error and the missing data were obtained by running an extra panel for these analytes on day 31. The same QCM lot was used for all runs and the material was handled according to the manufacturer's recommendations.

Reportable range

Evaluation of linearity and reportable range was carried out using rhinoceros plasma for AST, CK, LAC, LDH, and TP. For each analyte, samples with a known high concentration were analyzed once in order to ensure that the analyte was within the reportable range. If this was successful, a further analysis in duplicate was immediately carried out to determine the mean analyte concentration; this sample was designated as level 5. If the result was outside of the measurement range, distilled water was used to dilute the sample in a ratio of 1:2 and remeasured. Dilution and analysis were continued until a result was achieved, at which point a further duplicate analysis was carried out and the sample was designated as level 5. A dilution series was prepared using distilled water (level 1 blank) and level 5 in ratios of 3:1 (level 2), 1:1 (level 3), and 1:3 (level 4). Levels 1–4 were then analyzed in triplicate.

Statistical analysis and calculations

For the serum and plasma comparison, data were first tested for normality using the Kolmogorov–Smirnov test. For nonparametric data (ALP, ALT, CA, and CK), the median and interquartile range were calculated, and the difference between serum and plasma was assessed using the Wilcoxon matched-pair signed rank test. For data with a normal distribution, the mean and 95% CI were calculated and the paired t-test was used to assess differences between serum and plasma. The difference between the mean or median was calculated as a percentage of the value for serum for each analyte and compared to TE_a. The Spearman's correlation coefficient rho (r) was also calculated in order to assess the association between serum and plasma results. Level of significance was set at $P < .05$.

For both imprecision studies, the CV for each analyte, expressed in percentage, was calculated by dividing the SD by the mean multiplied by 100 for each pool or level.

For the reportable range study, means were calculated from the triplicate measurements and plotted against target values of the dilution series. The resulting graph was inspected visually for linearity over the range of values, and the slope and intercept were calculated using ordinary least squares regression analysis.

The programs and statistical tools used were Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

QC validation

Quality control validation was performed using the CVs obtained from the long-term imprecision study. As the supplier of the QCM did not supply target values for this analyzer, a useful estimate of bias for the purpose of QC validation could not be calculated and was set at zero for these calculations. The TE_{obs} for each analyte for each level of QCM was calculated as[10]

$$TE_{obs}\,(\%) = 2CV$$

The sigma metric (σ) was calculated as[12]

$$\sigma = TE_a\,(\%)/CV\,(\%)$$

The selection of appropriate control rules was performed in 2 steps. First, the TE_a and CV for each analyte were matched to 2 simple control rules using a table from a recent publication which was formulated in order to assist in selection of appropriate control rules for in-clinic analyzers.[13] This table provides for the use of either a 1_{3s} rule with $n = 1$, probability of error detection (P_{ed}) of ≥ 85% and probability of false rejection (P_{fr}) of 0%, or a 1_{3s} rule with $n = 2$, P_{ed} of > 90% and $P_{fr} = 0\%$. The 1_{3s} $n = 1$ rule was used preferentially. When reviewing the suitability of the 1_{3s} $n = 1$ rule, the CV corresponding with that considered to be the more clinically relevant QCM level was used. Second, for analytes which could not be monitored by one of these rules, a sigma-metric QC design tool was used to identify candidate rules.[12] Final rule selection was based on the criteria of $n \leq 2$, and that a simple rule was preferred over a multirule.

QC strategy

The 2_s or 3_s control rule limits were calculated from the SDs of the original set of QCM measurements. The mean values from the original 20 measurements served as the target values. A protocol for future statistical QC was developed based on the selected control rules and limits for the chosen levels. Levey–Jennings charts were created for each analyte.

Field performance

In order to simulate anticipated field conditions, the analyzer was placed in the closed back of a 4-wheel drive vehicle which was driven around on dirt roads and uneven jeep tracks for 4 days in summer (November 2015). The vehicle was stationary during analyses, usually in the shade of a tree. Vehicle air-conditioning was left running at all times. Electricity was supplied via a 350W uninterruptible power supply unit (WAECO Sinepower MSI 412; Dometic WAECO International GmbH, Emsdetten, Germany) from the vehicle's 12V battery. The analyzer was placed inside a hard molded plastic airtight box with custom foam padding (Pelican Products Inc., Torrance, CA, USA). The analyzer was kept in this box during transport and taken out during measurement to facilitate operation of the ventilator fan. Slides were kept in a polystyrene cooler box with ice packs. Aliquots of QCM were placed frozen into this box at the start of the trip and thawed as needed. Ten sets of QCM measurements were carried out according to the QC strategy over 4 days and results of the QC analyses were recorded on the bespoke Levey–Jennings charts. The following were additionally recorded for each analysis: cooler box temperature, ambient temperature in the back of the vehicle, ambient outside temperature, and any analyzer warnings. Temperatures were measured using digital thermometers with or without a probe. The analyzer was checked at the end of each day for dust inside the rotor cover and dusted if necessary. The mean and SD of the QC results from the field performance (fp) study was used to calculate the CV_{fp}, $bias_{fp}$, TE_{fp}, and σ_{fp} as follows[10]

$$\mathrm{Bias}_{fp}\,(\%) = (\mathrm{target} - \mathrm{mean}_{fp})/\mathrm{target} \times 100$$

$$\mathrm{TE}_{fp}\,(\%) = 2\mathrm{CV}_{fp} + \mathrm{bias}_{fp}$$

$$\sigma_{fp} = (\mathrm{TE}_a - \mathrm{bias}_{fp})/\mathrm{CV}_{fp}$$

Results

Method validation

Heparin plasma vs serum

Results are presented in Table 1. Magnesium was significantly higher and PHOS lower in plasma compared to serum. The percentage difference between the medians or means obtained was within the TE_a for all analytes. There was only moderate correlation for ALT, AST, LDH, and TP.[14]

Short-term imprecision

The CVs varied between low and high species-specific pools, but were < 7% for all analytes except the low pools for ALT and AST (Table 2). The CV for the low pool of ALT and AST was > 0.5 TE_a; all other CVs met the performance goals.

Long-term imprecision

Slide spotting failures occurred on 2 occasions, on day 3 with level 1 (ALB, AST, and GLUC) and day 18 with

Table 1. Comparison of biochemistry analytes of White rhinoceros measured in serum and heparin plasma using the IDEXX VetTest chemistry analyzer.

Analyte	Serum	Heparin Plasma	% Difference	*P*-Value	*r*
Albumin (g/L)	30.2 (26.4–34.0)	31.2 (28.4–34.0)	3.3	.413	.78*
ALP (U/L)	107 (81–133)	82 (61–103)	−23.5	.210	.76*
ALT (U/L)	30 (24–36)	24 (17–31)	−20.0	.397	.43
AST (U/L)	63 (42–83)	64 (44–83)	2.1	.886	.58*
Calcium (mmol/L)	3.09 (2.97–3.21)	3.03 (2.94–3.09)	−1.94	.360	.67*
CK (U/L)	249 (177–331)	225 (146–304)	−9.64	.116	.97*
Creatinine (μmol/L)	125 (99–152)	122 (99–146)	−2.6	.406	.86*
GGT (U/L)	23 (18–28)	23 (19–28)	−0.4	.944	.76*
Glucose (mmol/L)	6.5 (5.2–7.8)	7.0 (5.8–8.2)	6.4	.518	.81*
Lactate dehydrogenase (U/L)	1291 (1112–1469)	1374 (1253–1495)	6.5	.264	.50*
MG (mmol/L)	1.18 (1.10–1.27)	1.22 (1.15–1.29)	3.1	.043	.82*
Phosphate (mmol/L)	1.60 (1.39–1.82)	1.53 (1.33–1.73)	−4.7	.025	.96*
Total proteins (g/L)	87 (79–94)	87 (83–92)	1.0	.767	.60*
Urea (mmol/L)	6.3 (5.0–7.5)	6.3 (5.0–7.5)	−0.3	.781	.98*

Values are presented as mean (95% CI) or median (interquartile range) (ALP, ALT, CA, and CK). Percentage difference is the difference between the mean or median plasma values compared to the serum values. *P* values were obtained using the paired *t*-test or Wilcoxon matched-pair signed rank test. *r* represents Spearman's correlation coefficient.

*$P < .05$ for *r*.

Table 2. Short-term and long-term imprecision studies determined on White rhinoceros heparin plasma pools and commercial human quality control materials, respectively.

Analyte (Unit)	Short-term Imprecision Mean	Short-term Imprecision CV (%)	Long-term Imprecision Mean	Long-term Imprecision CV (%)
Albumin (g/L)	24	0.9	30	2.5
	34	1.5	37	3.7
ALP (U/L)	92	6.7	108	14.1
	279	4.3	228	9.3
ALT (U/L)	22	34.7	94	8.6
	236	2.2	179	3.9
AST (U/L)	41	23.2	107	12.8
	837	3.1	278	3.4
Calcium (mmol/L)	3.01	1.8	2.77	1.3
	3.38	1.0	3.35	1.1
CK (U/L)	108	3.5	208	5.0
	990	4.1	395	5.1
Creatinine (μmol/L)	92	2.9	198	3.8
	319	1.0	709	1.7
GGT (U/L)	24	4.4	112	1.2
	65	1.4	138	1.2
Glucose (mmol/L)	4.55	2.0	6.80	1.5
	12.39	1.0	20.04	1.4
Lactate (mmol/L)	6.03	1.8	3.19	2.7
	11.82	0.9	5.55	1.6
Lactate dehydrogenase (U/L)	1269	3.7	425	7.2
	1750	6.7	1094	4.2
Magnesium (mmol/L)	1.14	1.6	1.10	1.7
	1.32	2.2	1.55	2.0
Phosphate (mmol/L)	0.92	1.8	1.56	5.0
	2.22	2.2	2.52	1.3
Total proteins (g/L)	60	1.2	57	2.0
	110	1.2	69	2.2
Urea (mmol/L)	4.7	2.4	13.8	3.2
	20.7	1.7	22.3	2.6

level 2 (ALB, ALP, and ALT). Each time these were the 3 slides in the second batch and the failure was due to inadequate sample material in the cup. Imprecision was < 10% for all analytes except for ALP and AST level 1, where imprecision was > 0.5 TE_a (12.5%) (Table 2).

Reportable range

All 5 analytes showed a linear range under dilution, with linear correlation coefficients of 0.98 for AST and ≥ 0.99 for CK, LAC, LDH, and TP. The analytic range, slope, and intercept of the regression lines are shown in Table 3. Level 1 and level 5 values were close to the manufacturer's reportable range for CK, LAC, and TP.[9] The highest measurable activity was 885 U/L for AST (reported range 0–1083 U/L). The measured analytic range for LDH was 117–1781 U/L, in contrast to the manufacturer's reported range of 50–2800 U/L.[9]

Table 3. Results of the linearity study for 5 analytes in White rhinoceros plasma obtained by regression analysis.

Analyte (Unit)	Analytical Range	*r*	Intercept	Slope
AST (U/L)	0–885	.98	−21 (−178–136)	0.96 (0.67–1.25)
CK (U/L)	0–1522	.99	90 (−153–334)	1.00 (0.74–1.24)
Lactate (mmol/L)	0–10.53	> .99	0.45 (−0.75–1.65)	0.99 (0.80–1.17)
LDH (U/L)	117–1781	> .99	65 (−78–207)	0.99 (0.86–1.11)
Total protein (g/L)	0–109	> .99	4 (−6–15)	0.98 (0.81–1.14)

r represents the linear correlation coefficient. Results for the intercept and slope of the regression line are presented with 95% CI in parentheses.

QC validation

Table 4 contains the TE_{obs} and σ values as well the selected QC rules with corresponding P_{ed} and P_{fr}. The TE_{obs} was < TE_a for all controls except ALP level 1 and AST level 1. A σ value of ≥ 6.0 was obtained for both QC levels for 5 analytes and for one QC level for 6 analytes. Alkaline phosphatase had σ < 3.0 for both QCM levels, and ALT, AST, and LDH had σ < 3.0 for level 1. Six analytes were suitable for statistical QC using the 1_{3s} $n = 1$ rule at the clinically relevant QCM level. A further 5 analytes were suitable for statistical QC using the 1_{3s} $n = 2$ rule. Statistical QC could be applied to LDH, TP, and urea using the 1_{2s} $n = 2$ rule with a P_{ed} of > 85%; however, the 1_{2s} rule is associated with a P_{fd} of 9% for each measurement. Alkaline phosphatase was not suitable for statistical QC using a TE_a of 25%; a 1_{2s} $n = 2$ rule gave a P_{ed} of 30%.

QC strategy

The target values and rule limits for each analyte are shown in Table 5. Analytes monitored with QCM level 1 were ALB, ALP, ALT, AST, CK, CREA, GGT, GLU, LDH, MG, TP, and urea. Analytes monitored with QCM level 2 included ALB, ALP, ALT, AST, CA, CK, CREA, LAC, LDH, PHOS, TP, and urea.

Field performance study

Outdoor temperatures ranged from 24.4 to 35.0°C. Temperatures in the back of the vehicle ranged from 24.4 to 30.0°C and exceeded 27.0°C on 3 occasions. The temperature inside the cooler box ranged from −4.7 to 4.0°C, with the temperatures increasing over the course of each day. The analyzer gave temperature warnings at the end of analysis when the ambient

Table 4. Total observed error, sigma metric, and selected quality control rules for 15 analytes measured using the IDEXX VetTest chemistry analyzer using 2 levels of quality control material (QCM). The more clinically relevant QCM level is bolded. The probability of error detection and false rejection for each rule are shown.

Analyte	QCM Level	TE_{obs} (%)	Sigma Metric	Suitable for 1_{3s} $n = 1$ $P_{ed} > 85\%$	Suitable for 1_{3s} $n = 2$ $P_{ed} > 90\%$	Rule Selected	P_{ed}	P_{fr}
Albumin	**1**	4.9	6.0	No	Yes	1_{3s} $n = 2$	> 90%	0%
	2	7.4	4.1	No				
ALP	1	28.2	2.8	No				
	2	18.6	2.7	No	No	1_{2s} $n = 2$	30%	9%
ALT	1	17.2	2.9	No				
	2	7.8	6.4	No	Yes	1_{3s} $n = 2$	> 90%	0%
AST	1	25.7	2.0	No				
	2	6.8	7.4	No	Yes	1_{3s} $n = 2$	> 90%	0%
Calcium	1	2.6	7.7	No				
	2	2.2	9.7	Yes	Yes	1_{3s} $n = 1$	> 85%	0%
CK	1	10.0	6.0	No				
	2	10.2	5.9	No	Yes	1_{3s} $n = 2$	> 90%	0%
Creatinine	**1**	7.7	5.3	No	Yes	1_{3s} $n = 2$	> 90%	0%
	2	3.3	11.8	Yes				
GGT	**1**	2.5	16.7	Yes	Yes	1_{3s} $n = 1$	> 85%	0%
	2	2.4	16.7	Yes				
Glucose	**1**	3.8	13.4	Yes	Yes	1_{3s} $n = 1$	> 85%	0%
	2	2.8	14.3	Yes				
Lactate	1	5.5	14.8	Yes				
	2	3.1	25.0	Yes	Yes	1_{3s} $n = 1$	> 85%	0%
LDH	1	14.3	2.7	No				
	2	8.5	4.8	No	No	1_{2s} $n = 2$	> 90%	9%
Magnesium	**1**	3.4	11.8	Yes	Yes	1_{3s} $n = 1$	> 85%	0%
	2	3.9	10.0	Yes				
Phosphate	1	10.0	3.0	No				
	2	2.5	11.5	Yes	Yes	1_{3s} $n = 1$	> 85%	0%
Total protein	1	4.1	5.0	No				
	2	4.5	4.5	No	No	1_{2s} $n = 2$	> 90%	9%
Urea	**1**	6.4	3.8	No				
	2	5.3	4.6	No	No	1_{2s} $n = 2$	85%	9%

TE_{obs} indicates total observed error; σ, sigma metric; P_{ed}, probability of error detection; P_{fr}, probability of false rejection.

temperature was > 27°C, but still delivered results. There was no visible dust seen inside the rotor cover. Results were outside of control limits for CREA and LDH once, and for AST, GGT, and urea twice. All these QC failures, except for one GGT measurement, were associated with a high temperature warning. There were multiple failures for TP (7 times for level 1, 9 times for level 2) with results above the upper limit as shown in the Levey–Jennings charts in Figure 1. The TE_{fp} was < TE_a for all analytes. The σ was > 3.0 for all analytes except for ALP and urea level 1 and TP both levels (Table S2).

Discussion

Overall, the POCA fulfilled most of the method validation requirements and can be used for the White rhinoceros. Application of ASVCP guidelines for quality control in POCAs based on QC validation was successfully applied. The resulting quality control strategy was used to assess performance of the analyzer in the field, with acceptable results.

The user manual for the VetTest states that serum and lithium heparin plasma may be used interchangeably for analytes examined in this study except LAC, but no further information is given.[9] Lactate can be measured from heparin plasma if centrifuged and separated from red blood cells within 5 minutes of collection.[9] This study revealed significant differences for MG and PHOS concentrations between heparin plasma and serum for the White rhinoceros as well as only moderate correlations for 4 other analytes (ALT, AST, LDH, and TP). The differences found here could be method or species-related. A study comparing results for White rhinoceros heparin plasma and serum using another POCA found differences for ALP, AST, GGT, TP, BUN, CK, and ALB, which differs from the

Table 5. Quality control strategy for the IDEXX VetTest chemistry analyzer using either one or 2 levels of quality control material (QCM), based on results of quality control validation.

Analyte (Unit)	QCM Level	Target	Limits
Albumin (g/L)	1	30	28–32
	2	37	33–42
ALP (U/L)	1	108	77–138
	2	228	186–271
ALT (U/L)	1	94	70–118
	2	179	158–200
AST (U/L)	1	107	66–148
	2	278	250–306
Calcium (mmol/L)	2	3.35	3.24–3.47
CK (U/L)	1	208	176–239
	2	395	335–456
Creatinine (μmol/L)	1	198	175–221
	2	709	674–745
GGT (U/L)	1	112	108–116
Glucose (mmol/L)	1	6.80	6.49–7.10
Lactate (mmol/L)	2	5.55	5.3–5.8
LDH (U/L)	1	425	364–486
	2	1094	1001–1186
Magnesium (mmol/L)	1	1.10	1.04–1.15
Phosphate (mmol/L)	2	2.52	2.42–2.62
Total protein (g/L)	1	57	55–59
	2	69	66–72
Urea (mmol/L)	1	13.8	12.9–14.7
	2	22.3	21.1–23.5

QCM indicates quality control material.

findings here.[15] Differences in MG and PHOS in heparin plasma vs serum have not been reported in dogs, cattle, horses, or sheep.[16–19] The reason for the findings here are unclear; however, care should be taken in using plasma and serum interchangeably in this and other species. Using plasma rather than serum delivers faster results as the sample does not need to be left to clot before centrifugation. This is potentially critical when attending to an injured or immobilized White rhinoceros. Based on the findings presented here, all further experiments were conducted on heparin plasma and not serum.

White rhinoceros plasma was used for the short-term imprecision study in order to assess species-specific imprecision. A commercial liquid QCM was used in the long-term imprecision study and for QC validation, as in other studies, as this type of material is practical and commonly used for internal QC.[13,20–22] Short-term imprecision was acceptable for most analytes, similar to results from other studies using equine serum and canine and pigeon plasma.[8,23,24] The imprecision for AST of 23% and ALT of 35% at low activity levels was > 0.5 TE_a (12.5%); however, this may be of little or no clinical significance at low levels of results. Previous studies reported an imprecision of 2–8% for AST at similar or higher activity levels but were performed on material from other species.[8,23,24] None of these studies calculated the short-term imprecision of ALT on patient samples; however, a recent study found a CV of 7% for ALT, using a commercial QCM, at an activity of 37 IU/L.[20] These rhinoceros-specific measures of imprecision reported in our study can be used to aid in interpretation of patient data in the future.

Long-term imprecision fell within performance goals for all analytes except for ALP and AST activity at the lower level. A long-term imprecision of 2–4% for ALP and 2–7% for AST has been reported for similar or slightly higher enzyme activities in some studies examining imprecision in this analyzer.[8,20,24] The results for long-term imprecision in this and the afore-mentioned studies are in contrast to data obtained from this analyzer in use in veterinary practices, where imprecision was often much higher than obtained here.[13] It is likely that the high imprecision observed inpractice is due to the low enzyme activities in these samples and is usually not clinically relevant.[10]

The activities of AST, CK, LAC, LDH, and TP were chosen for the reportable range study as high levels had been noted while making up the pools for the imprecision study, and linearity up to the upper reported analytic limit is potentially of clinical importance. It was however not possible to obtain results for AST and LDH near the upper reported limits. No published study on reportable range for this analyzer on any material was found, and it is impossible to conclude whether the reported ranges for these analytes are inaccurate or whether there are interfering substances present in White rhinoceros plasma leading to these findings. As many injured White rhinoceros suffer from a myopathy with very high reported activities of muscle enzymes, it may be prudent for clinicians to perform a 1:4 or 1:9 dilution before running AST, CK, and LDH.[3]

Bias estimates for a POCA can firstly provide information for the assessment and monitoring of analytic performance and secondly be used to determine whether RI derived for another method are valid.[5] Initial species-specific analyzer performance was assessed here based on linearity and precision studies using White rhinoceros plasma. Bias is ideally calculated during instrument performance studies from a method comparison experiment, where the field method is compared to a gold standard method.[5,25] This was not possible here due to the lack of a gold standard. A wet chemistry analyzer (Cobas Integra 400 Plus) is used in the authors' laboratory for routine rhinoceros samples, but this analyzer has not been validated for this species and is another field method. Results of a method

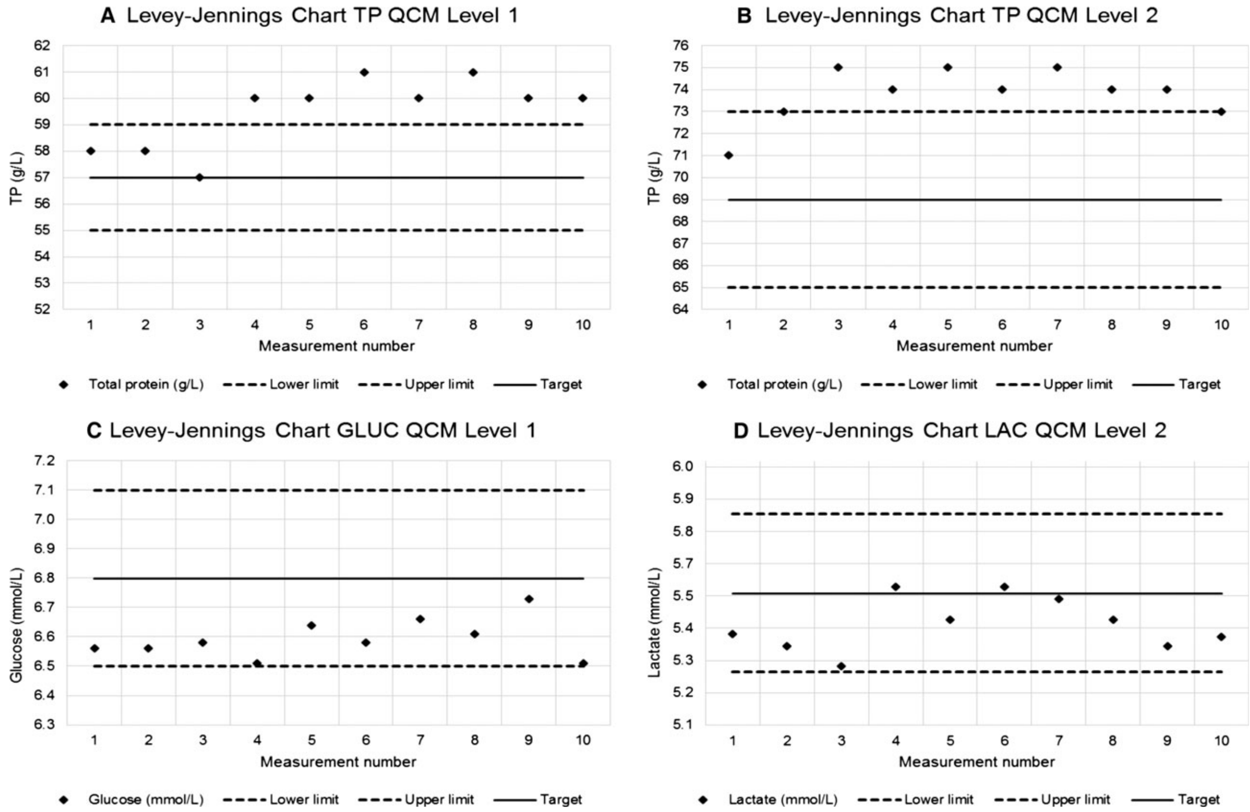

Figure 1. Levey–Jennings charts showing the results for total proteins (TP) for 2 levels (**A** and **B**); and glucose (GLUC) (**C**) and lactate (LAC) (**D**) for one level of quality control material (QCM) measured 10 times on the IDEXX VetTest chemistry analyzer over 4 days under field conditions. The solid black line represents the target and the dotted lines represent the predetermined control limits. The TP was out of control limits 7/10 times for level 1 and 9/10 times for level 2. All results for GLUC and LAC are within limits. All 3 analytes were measured by the 562 nm lamp.

comparison between the VetTest and Cobas Integra for 10 of the analytes investigated here are detailed in a separate publication.[26] Using bias obtained from comparison with a field method for quantification of TE_a and QC validation can overestimate the error assigned to the comparative method and was not used in the calculations here.[21] Bias can also be quantified using mean values provided for an assayed QCM as “true” values; however, these were not available for this method and using the targets supplied for other methods is of questionable value.[5] Method-specific target values were calculated from 20 measurements of the QCM and bias was subsequently measured for the purposes of analytic performance monitoring during the field performance study and incorporated into TE_{obs} calculations for that part of the study.

Designing a QC plan based on the use of validated control rules is regarded as the gold standard for interpreting QC data, even for veterinary POCAs.[6,27] Furthermore, daily monitoring of POCA instrument performance is recommended by the ASVCP.[6] The routine QC procedure prescribed for the VetTest by the manufacturer is an analysis once a month using QC material supplied by the manufacturer.[9] A set of slides for 6 analytes, each testing one of the 6 lamps is also supplied. Results of the QC analysis are presented against a “reference range” which appears on the analyzer printout. Information concerning the derivation of this range, including the number of SDs it represents (2 or 3) is not available and no target mean values are provided. This strategy is not in line with current best practice guidelines and an alternative QC plan, following these guidelines, was therefore designed.[6] Where possible, the 1_{3s} rule was selected as this rule is considered to be most suitable for POCAs.[6,13] The 1_{3s} rule was suitable for use in 73% (11/15) of analytes. It has been suggested that POCAs should have > 75% of analytes controllable by the 1_{3s} rule, with the probability for false rejection (P_{fr}) $\leq$ 5% and the probability of error detection (P_{ed}) $\geq$ 85%, in order to be fit for statistical quality control in a clinic environment.[6] The use of other control rules requires the application of QC validation procedures, and was performed here. It was not possible to use statistical QC for ALP based on the data in this study, as a P_{ed} 30% is unacceptably low. The utility of running ALP on this analyzer is questionable if using a TE_a of 25%. Increasing the TE_a is a possibility, and a new TE_a could be calculated based on RI and clinical decision limits.[28] These data are however not available yet for White rhinoceros.

The 1_{2s} rule provided an adequate $P_{ed} > 85\%$ for 3 analytes for which the 1_{3s} rule was not suitable. Although the 1_{2s} rule is associated with a high P_{fr}, it is simpler for clinicians to apply than a multirule. Another option is to use a less stringent rule with more levels of QCM, but the cost of these additional QCM levels needs to be weighed up against the cost of repeating the analysis using the QCM levels already in use. Changes of QCM lots and recalibration through software updates could affect the control limits derived for this study and new data may need to be generated in the event of a new lot or software update.[6]

Published information regarding evaluation of POCA performance in the field is available but scarce and focuses on method comparison between the POCA and a reference laboratory analyzer.[7,29–31] The evaluation of bias, however, does not assess stability of the system over time. A human study evaluating a clinical chemistry analyzer in a military field laboratory followed a protocol advocated by the U.S National Committee for Laboratory Standards, in which precision, linearity, and accuracy were monitored.[29] This protocol was carried out in a premobilization, mobilization, and postmobilization phase in that study.[29] Monitoring of both accuracy and precision over time is more likely to reflect performance. The use of statistical QC facilitates measurement of both bias and imprecision against preset goals and was thus the objective evaluation tool used in this study. The analyzer generally performed well under field conditions, except when vehicle ambient temperature exceeded 27°C. This is in line with the manufacturer's operating specifications and indicates the importance of measuring ambient temperature in the field, and keeping the operating environment as cool as possible. The cause for the TP QC failures was not clear and the positive bias present in the TP results represents a systematic error. The TP is measured with the green 562 nm lamp, along with LAC and GLUC, which had good QC results with $\sigma > 6.0$ for both. The TP slides were kept under the same conditions as the other slides. The manufacturer states that all slides can be recycled from cold storage to room temperature up to 5 times—some but not all of these slides would have undergone a temperature increase to a maximum of 4°C only once during the course of the experiment, therefore inaccuracies due to temperature changes seem unlikely.[9] The same lot of TP slides was used for the long-term imprecision and field performance and lot-to-lot variation can be ruled out. In a clinical scenario, the next step in troubleshooting would be to contact the manufacturer for further technical assistance, before running further patient samples. The formulation of bespoke Levey–Jennings charts and concurrent recording of environmental conditions assisted with troubleshooting of QC failures. The Levey–Jennings charts in particular provide a user-friendly method of recording and assessment for operators not familiar with the concepts of QC.

The VetTest proved suitable for use in the White rhinoceros with heparin plasma samples, although the upper reportable limits for AST and LDH were much lower than those provided by the manufacturer for other species. Method comparison data and RI for this POCA are presented in a separate study.[26]

This study provides an example of how QC validation and statistical QC can be applied to a POCA in line with ASVCP guidelines.[6] Other aspects of quality assurance should however not be ignored. Operator training, formulation of standard operating procedures, and comparability testing, for example, are all important elements of a total quality management strategy, and should be considered for this analyzer.

Providing clinical pathology data for wildlife means that patient-side analyzers may have to function in varying and challenging environmental conditions. The evaluation of performance using statistical QC shown here provides an example of how the stability of an analytic system can be evaluated under field conditions. Performing and evaluating QC each time the analyzer is used in the field will be vital to ensure the quality of patient results.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Analytes and analytical methods chosen for investigation in White rhinoceros on the IDEXX VetTest dry chemistry analyzer.

Table S2. Results of a field performance study on the IDEXX VetTest where 10 measurements of quality control material were made over 4 days and evaluated against control rules formulated during a quality validation process.