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Two Point-of-Care Cardiac Troponin I Immunoassays Have Acceptable Analytical Performance for the Detection of Measurands of Cardiac Troponin I Cardiac Muscle Homogenates From Southern-Central Black Rhinoceros (*Diceros bicornis minor*) and Southern White Rhinoceros (*Ceratotherium simum simum*)

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ABSTRACT

Background: Skeletal and possible cardiac muscle damage has been reported in chemically immobilized and transported African rhinoceros during conservation-related activities. The extent of cardiac muscle injury in these rhinoceros is unknown due to a lack of validated cardiac troponin I (cTnI) assays. However, recently, five human cTnI assays were deemed suitable for analytical validation in African rhinoceros based on cTnI sequencing results.

Objectives: The first objective was to validate two cTnI immunoassay point-of-care analyzers (POCAs) in African rhinoceros and, secondly, to perform quality control (QC) validation for the POCAs.

Methods: Analytical validation of the Stratus CS Acute Care Troponin I cTnI immunoassay and Atellica VTLi high sensitivity cTnI (hs-cTnI) assay was performed using rhinoceros serum samples and species-specific cardiac muscle homogenate. Experiments included precision studies, reportable range, hemoglobin interference studies, recovery studies, and detection limit studies, with results assessed against prescribed total allowable error (TE_a) performance goals. Commercial quality control material (QCM) data were used to calculate bias and imprecision for QC validation.

Results: Imprecision was acceptable (1.9%–10.3%) and met low cTnI concentration performance goals. Reportable ranges were similar to the manufacturer's specifications. High hemoglobin concentrations in white rhinoceros resulted in a positive bias in the Stratus CS. A simple 1_{3s} QC rule using two levels of QCM and a TE_a of 70% could be used in both analyzers, except at very low cTnI concentrations in the Atellica VTLi.

Conclusions: Both cTnI POCAs are suitable for use in African rhinoceros, and analytical performance goals for low cTnI concentrations in hs-cTnI assays were met.

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1 | Introduction

The two extant species of rhinoceros native to Africa, the black rhinoceros (*Diceros bicornis*) and the white rhinoceros (*Ceratotherium simum*), are endangered, with most animals living in fairly small, isolated populations in conservancies, private game reserves, and intensive protection zones in sub-Saharan Africa [1, 2]. A small number are living under managed care in global zoological institutions [3]. National and international rhinoceros conservation strategies include humane horn trimming (dehorning) and translocation of animals to safe areas. Both dehorning and translocation involve capture using chemical immobilization; translocated animals must also undergo temporary captivity, transport, and release into a novel environment [4]. Both chemical immobilization, which involves the administration of potent opioids like etorphine or thiafentanil, and transport result in pathophysiological changes that impact the cardiovascular and musculoskeletal systems. These include severe hypertension, hypoxemia, tachycardia, electrolyte imbalances, and increased activities of serum aspartate aminotransferase and creatine kinase, indicating muscle damage [4, 5]. Although not reported in rhinoceros, capture myopathy, a syndrome that involves both skeletal and heart muscle, is well documented during capture and translocation procedures in various wild-life species [6]. Cardiac troponins are the biomarkers of choice for the detection of reversible and irreversible cardiomyocyte injury in human and veterinary medicine [7–9]. Investigation of the extent of cardiac muscle injury during chemical immobilization and transport and the role of cardiomyocyte injury in capture myopathy in rhinoceros has been hampered by the lack of validated cardiac troponin I (cTnI) immunoassays.

We recently sequenced the cTnI gene of both southern-central black and southern white rhinoceros and evaluated the homology, compared to humans, of epitopes on the predicted cTnI protein that are targeted by the detection and capture antibodies in six automated human cTnI assays [10]. Of the five assays that were deemed suitable for further evaluation, we selected two point-of-care (POC) methods for further investigation based on accessibility, potential for use in field conditions, and conserved targeted epitopes. The Stratus CS Acute Care Troponin I conventional cTnI immunoassay (Siemens Healthineers, Erlangen, Germany) has been used in dogs, horses, sheep, pigs, rabbits, rats, and mice [11, 12]. The Atellica VTLi high sensitivity cTnI assay (Siemens Healthineers, Erlangen, Germany) has recently been introduced into the human medical environment and has not yet been validated for use in any veterinary species, to our knowledge.

Both assays require analytical validation before use in rhinoceros [13]. Additionally, the implementation of quality assurance and quality control (QC) strategies is important for POC analyzers (POCA), which are invaluable in wildlife and often used in field settings [14, 15]. Veterinary analytical method validation guidelines and analytical error goals are provided by the American Society of Veterinary Clinical Pathology (ASVCP) [16, 17]. The International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers (IFCC TF-CB) additionally mandates analytical performance specifications for high sensitivity cTnI immunoassays [18].

This study aimed to first perform analytical validation of two cTnI immunoassay POCA for use in African rhinoceros and, secondly, to perform QC validation for the POCA.

2 | Materials and Methods

2.1 | Analyzers and Assay Methods

The Stratus CS Acute Care Troponin I method is a two-site sandwich assay with one detection and one capture antibody based on solid phase radial partition immunoassay technology. Cardiac troponin concentration is reported in ng/mL ($\mu\text{g/L}$), to two significant figures as per IFCC TF-CB guidelines [18]. The manufacturer's reportable range is 0.03–50 ng/mL. The Atellica VTLi uses a high sensitivity (hs) cTnI test, which is also a two-site sandwich immunoassay with one detection and three capture antibodies; a key component of this assay is super-magnetic nanoparticles that covalently bind to the capture antibodies [19]. Cardiac troponin concentration is reported in ng/L and as whole numbers without decimal points, as per IFCC TF-CB guidelines [18]. The manufacturer's reportable range is 2 (plasma) or 4 (whole blood) to 50 ng/L. Both immunoassays use monoclonal antibodies derived from mice. Single-use reagent cartridges, stored at 2°C–8°C, are used in both analyzers. Both analyzers used in this study were placed in a campus clinical pathology laboratory under recommended operating conditions for analyzer and QC validation. Full maintenance, including analysis of manufacturer-recommended quality control material (QCM) (including calibration cartridges in the case of the Stratus CS analyzer), was performed on both analyzers throughout the study. Analyzer maintenance was performed as advised by the respective manufacturers, and QCM was analyzed at the start of an analytical run on the days that samples were analyzed. One level of QCM was used in the Stratus (MAS CardioImmune XL, Liquid assayed cardiac marker control Level 2, Lot CXL25042; Thermo Scientific, Fremont, CA, USA) and three levels of QCM were used in the Atellica VTLi (Pathonorm, Cardiac Acute Lig L-1, L-2, and L3, Lot 2208906, 2203830, 2208908, respectively, SERO AS, Stasjonsveien, Billingstad, Norway). The QC results performed on the Stratus CS should be reported to three significant figures based on IFCC TF-CB guidelines; however, the analyzer only reported two decimal places [18]. In the Atellica VTLi, the QC results were reported with one decimal point as advised by the IFCC TF-CB [18]. All analyses were carried out by one investigator (Y.R.).

2.2 | Performance Goals

The ASVCP recommended total allowable error (TE_a) goal for troponins is 70% [17]. However, this “consensus” goal was based on a single expert opinion and we decided to also compare the analytical performance against a stricter requirement of 30% (or 0.9 ng/mL), as recommended by the American Association of Bioanalysts (AAB) [20, 21]. Based on Westgard and ASVCP guidelines, the requirements for short-term and long-term replication studies are; standard deviation (SD) $<0.25 TE_a$ and SD $<0.33 TE_a$, expressed in the units of the test, respectively [16, 22]. The IFCC TF-CB performance specification of total analytical error of $<3.5 \text{ ng/L}$ for the Atellica hs-cTnI immunoassay

at ≤ 10 ng/L was also applied in the precision experiments [23]. The acceptable performance for the interference and recovery studies was that the estimated proportional systematic error (SE) was < 0.5 TE_a, and SE $<$ TE_a, respectively [16].

2.3 | Method Validation

2.3.1 | Samples

Sample materials used were serum from 6 southern white (*C. simum simum*) and 3 southern-central black (*D. bicornis minor*) rhinoceros, cardiac muscle homogenate from 2 white and 1 black rhinoceros, and red cell pellets from 5 white rhinoceros orphans. For the preparation of the muscle homogenate, myocardium from the left ventricle was obtained from 2 white rhinoceros and 1 black rhinoceros immediately after death or euthanasia due to causes unrelated to the study. Multiple cardiac muscle blocks, ≤ 5 mm in any dimension, were prepared, blotted on tissue paper to remove excess blood, trimmed of obvious connective tissue and fat, and stored at -80°C in a preservative-free tube. The storage duration from collection to muscle homogenate preparation ranged from 8–22 months and 1–5 months for white and black rhinoceros samples, respectively. The muscle homogenate was prepared as previously described, following the manufacturer's instructions (Bio-Plex Cell Lysis Kit; Bio-Rad) [24]. Briefly, the frozen muscle was transferred to wet ice and cut into $1\text{ mm} \times 1\text{ mm}$ pieces (100–300 mg, in total), placed in tubes containing steel beads (Bead types type F; Macherey-Nagel) and lysing solution, and mechanically homogenized (Precellys 24 homogenizer; Bertin Technologies). The tissue homogenate was centrifuged, and the resulting supernatants were stored at -80°C for 3–6 months.

The rhinoceros serum samples had been previously collected for other studies and included healthy animals immobilized for translocation or dehorning. Blood was collected from the auricular vein directly into serum tubes (BD Vacutainer; Becton and Dickinson, Plymouth, UK), stored in a cooler box with ice packs, and centrifuged within 24 h. The serum was aliquoted and stored at -80°C . The storage duration from collection to serum pool preparation ranged from 8–22 months and 1–5 months for white and black rhinoceros samples, respectively. Samples were excluded if gross hemolysis, lipemia, or icterus was present. Small amounts of cardiac muscle lysate supernatant were added to aliquots of stored serum to achieve the desired concentrations of cardiac muscle homogenate. Serum from 2 to 3 animals was used to create pools for the different experiments.

Approval for the use of the samples was granted by the University of Pretoria's Ethics Committees (REC205-21).

2.3.2 | Short-Term Imprecision

A high and low sample pool was created for each analyzer and species, using the species-specific cardiac muscle homogenates and serum. Pools were kept at 4°C after being prepared and were used within 8 h. Twenty measurements were performed on each pool. Both pools were measured in the same analytical run, except in the case of the Atellica VTLI, white rhinoceros, where

the pools were measured in two separate analytical runs due to the analyzer's results storage capacity being reached, necessitating the download of results before analysis could proceed.

2.3.3 | Long-Term Imprecision

A high and low sample pool was created and aliquoted for each analyzer and species. The aliquots were stored at -20°C . Four replicate measurements on each pool were performed daily for 5 days. Both low- and high-concentration pooled aliquots were measured in the same analytical run.

2.3.4 | Reportable Range and Linearity

A high and low sample pool was created for each species and analyzer, using each analyzer's manufacturer's reportable range as a guideline. The high and low pools were designated as level 5 and level 1, respectively. A dilution series was prepared using the low pool (level 1) and the high pool (level 5) in ratios of 3:1 (level 2), 1:1 (level 3) and 1:3 (level 4). Levels 1–5 were measured in duplicate on the Atellica VTLi and in triplicate on the Stratus CS in the same analytical run.

2.3.5 | Hemoglobin Interference

White rhinoceros hemolysate was prepared using EDTA-anticoagulated whole blood samples leftover from research samples unrelated to this project, obtained from conscious, healthy white rhinoceros orphans. The whole blood samples were centrifuged at $1520g$ for 10 min, and the plasma was discarded, and the red cell pellets were frozen at -20°C . For this experiment, the pellets were thawed to room temperature and mechanically lysed by repeated passage through a small gauge needle attached to a 20 mL plastic syringe. Thereafter, the samples were centrifuged at $1520g$ for 8 min, and the supernatant was harvested. The hemolysate was added to pooled serum samples of each rhinoceros species. The pooled serum used was free of gross hemolysis, and hemolysate was added at three different concentrations, namely 1.0, 2.0, and 3.0 g/L of hemoglobin, as determined spectrophotometrically (ADVIA 2120i, Siemens, Germany) (Figure 1). Distilled water was added to the control serum specimens at equivalent volumes. The pairs of samples were analyzed in duplicate. The same samples were measured on both analyzers in the same analytical run for each analyzer.

2.3.6 | Recovery

Species-specific cardiac muscle homogenate was diluted with pooled serum samples from the same species to create a standard spiking solution for each analyzer, with relatively high cTnI concentrations to allow the adding of small amounts to minimize specimen dilution (dilution should be $< 10\%$) [16]. Serum samples were spiked to four different cardiac muscle homogenate concentrations for each species per analyzer. Distilled water was added to the control serum specimens at equivalent volumes. Duplicate measurements of both spiked and control specimens were performed during one analytical run.

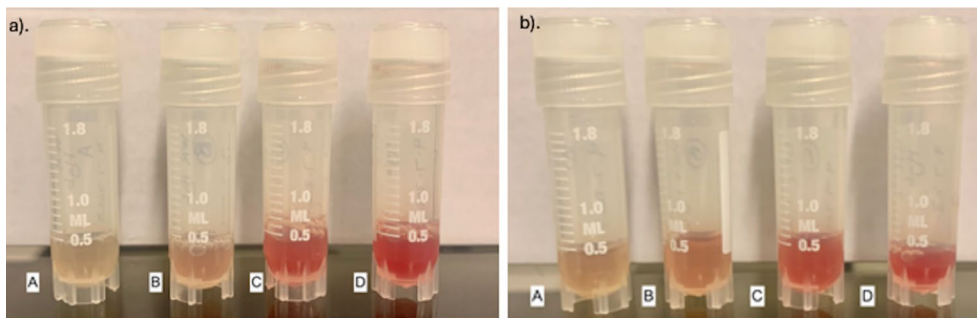


FIGURE 1 | Hemoglobin interference study, different quantities of hemoglobin added to (a) white rhinoceros and (b) black rhinoceros serum samples to reach increased concentrations that are anticipated to occur in patient samples. (A) 0 g/L hemoglobin; (B) 1 g/L hemoglobin; (C) 2 g/L hemoglobin and (D) 3 g/L hemoglobin.

2.3.7 | Detection Limit Study

The limit of blank (LoB) and limit of detection (LoD) were determined. In the LoB experiment, 20 replicates were performed on distilled water in one analytical run on both analyzers. In the LoD experiment, species-specific pool serum samples of low concentration (close to the manufacturer-reported LoD of each analyzer) were aliquoted and stored at -20°C . Four replicate measurements of each aliquot were performed daily for 5 days.

2.3.8 | Statistical Analysis and Calculations

The statistical analysis and calculations were performed as previously described and are briefly outlined below [16, 22].

For both imprecision studies, SD, mean, and coefficient of variation (CV) were calculated. The CV was expressed in percentage and calculated by dividing the SD by the mean multiplied by 100 for each pool [16].

For the reportable range study, means were calculated from the duplicate or triplicate measurements and plotted against the target values of the dilution series. The resultant graphs were inspected visually for linearity over the range of values, and the slope and intercept were calculated using linear regression analysis.

Calculation of the SE due to hemoglobin interference was performed by determining the mean for the duplicates of the hemoglobin-containing sample and the control and determining the difference between the two. Lastly, the mean difference or bias for all specimens at a given concentration of interferent was calculated.

In the recovery studies, the concentration of the measurand added was calculated as follows:

$$\text{Concentration of standard} \times \left(\frac{\text{mL standard added}}{(\text{mL of standard added} + \text{mL of sample added})} \right)$$

The mean of the replicate measurements of all samples was then calculated, followed by the calculation of the difference between the spiked sample and the control. The recovery was

then calculated by dividing the difference by the amount added. This was followed by calculating the mean of the recoveries of all the pairs tested and finally calculating the proportional SE as $100\% - \text{recovery}\%$.

In the detection limit studies, the LoB and LoD were estimated by the mean value of the blank + $(1.65 \times \text{SD})$ of the blank and the low concentration sample, respectively. The limit of quantification (LoQ) was estimated as the mean of the blank + $(2 \times \text{SD})$ of the low concentration sample [16].

The programs and statistical tools used were Microsoft Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) and MedCalc version 22.021 (MedCalc Software Ltd., Ostend, Belgium).

2.4 | Quality Control Validation

Quality control validation, specifically setting control limits, was performed using the observed analytical performance of both analyzers and a TE_a of 30% or 70%. One to three levels of QCM were analyzed at the start of each analytical run on both analyzers during the method validation experiments. This data were used to calculate the CV (formula described earlier). The target mean values provided by the QCM suppliers of the material used in the Stratus CS and Atellica VTLi were method and analyzer specific. However, as bias was present with initial QCM measurements and because calibration cannot be performed by an operator for the Atellica VTLi to correct the bias, target mean values for QCM for both analyzers for this study were calculated from the initial 5–10 measurements per QCM level. The bias was subsequently calculated as [16]

$$\text{Bias} (\%) = \frac{(\text{mean}_{\text{measured}} - \text{mean}_{\text{target}})}{\text{mean}_{\text{target}}} \times 100$$

The observed TE (TE_{obs}) was expressed in percentage and calculated as [16]

$$\text{TE}_{\text{obs}} (\%) = (2 \times \text{CV}) + |\text{bias}|$$

Analyzer performance was also evaluated by calculating the sigma metric (σ).

$$\sigma = \frac{TE_a(\%)}{CV(\%)}$$

The selection of appropriate control rules was performed by visual analysis of the normalized Westgard Operating Specifications Charts (OPS Specs Charts) after using the Normalized OPS Specs Calculator to determine the various operating points (www.westgard.com) [25, 26]. A sigma-metric QC design tool was also used to identify candidate rules if none were found using the OPS Specs Charts [27]. The final rule selection was based on the criteria of $n \leq 2$, with a probability of error detection (P_{ed}) of $> 85\%$ and a probability of false rejection (P_{fr}) of $< 5\%$, and a simple rule was preferred over a multirule. The CVs from both analyzers were entered into a “reverse approach” calculation using the sigma-metric QC design tool to assess which amount of total error could be controlled with a simple QC rule.

3 | Results

3.1 | Method Validation

3.1.1 | Short-Term Imprecision

In the Stratus CS, the CV ranged from 2.3%–3.8% and 2.5%–8.0% in the homogenate spiked serum pools of the black and white rhinoceros, respectively. The CV ranged from 5.3%–5.5% and 8.1%–10.3% in the black and white rhinoceros, respectively, in the Atellica VTLi. In the Stratus CS, all the SDs met both performance goals in the two rhinoceros species, with the SDs measured in the low pools equal to the AAB performance goal (30% TE_a). In the Atellica VTLi, when using AAB cTnI TE_a (30%), the white rhinoceros high pool SD failed. The low pool SDs of the black and white rhinoceros were equal to both the AAB and IFCC TF-CB performance goals (30% and 3.5 ng/L TE_a , respectively). The results are summarized in Tables 1 and 2.

3.1.2 | Long-Term Imprecision

In the Stratus CS the CV ranged from 2.8%–8.3% and 1.9%–6.3% in the black and white rhinoceros, respectively. The CV ranged from 2.8%–8.0% and 7.4%–8.0% in the black and white rhinoceros, respectively, in the Atellica VTLi. All SDs met both performance goals, with the SD measured on the Stratus CS in the white rhinoceros low pool being equal to the AAB performance goal (30% TE_a). The results are summarized in Tables 1 and 2.

3.1.3 | Reportable Range

The cardiac muscle homogenate showed a linear range under dilution for both species in both analyzers. The analytic range, slope, and intercept of the regression lines are shown in Table 3. Levels 1 and 5 values were close to the manufacturer's reportable range for both species in both analyzers.

TABLE 1 | Precision study results for the Siemens Stratus CS in African rhinoceros.

Black rhinoceros	Pool mean (ng/mL)	SD (ng/mL)	CV (%)	Acceptable SD at 70% TE_a	Acceptable SD at 30% TE_a	White rhinoceros	Pool mean (ng/mL)	SD (ng/mL)	CV (%)	Acceptable SD at 70% TE_a	Acceptable SD at 30% TE_a
Short-term imprecision											
Low pool	0.13	0.01	3.8	0.02	0.01	Low pool	0.14	0.01	8.0	0.03	0.01
High pool	11.39	0.26	2.3	1.99	0.86	High pool	8.60	0.22	2.5	1.51	0.65
Long-term imprecision											
Low pool	0.16	0.01	8.3	0.04	0.02	Low pool	0.11	0.01	6.3	0.03	0.01
High pool	10.91	0.31	2.8	2.52	1.08	High pool	7.91	0.15	1.9	1.83	0.78

Abbreviations: CV, coefficient of variation; SD, standard deviation; TE_a , total allowable error. TE_a in ng/mL calculated as 70% or 30% of pool mean and acceptable. SD: $< 0.25 TE_a$ for short-term imprecision; < 0.33 for long-term imprecision.

TABLE 2 | Precision study results for the Siemens Atellica VTli in African rhinoceros.

Black rhinoceros	Pool mean (ng/L)	SD (ng/L)	CV (%)	Acceptable		White rhinoceros	Pool mean (ng/L)	SD (ng/L)	CV (%)	Acceptable		Acceptable	
				SD (ng/L) at 70% TE ^a	SD (ng/L) at 30% TE ^a					SD (ng/L) at 70% TE ^a	SD (ng/L) at 30% TE ^a		
Short-term imprecision													
Low pool	11	1	5.3	2	1	1	12	1	10.3	2	1	1	1
High pool	1062	59	5.5	186	80	80	988	80	8.1	173	74	74	74
Long-term imprecision													
Low pool	11	1	8.0	3	1	1	12	1	8.0	3	1	1	1
High pool	1237	35	2.8	286	122	122	1186	88	7.4	274	117	117	117

Note: Bold text—performance goal exceeded.

Abbreviations: cTnI, cardiac troponin I; CV, coefficient of variation; IFCC TF-CB, International Federation of Clinical Chemistry and Laboratory Medicine Task Force on Clinical Applications of Bio-Markers; SD, standard deviation; TE_a, total allowable error.

^aTE_a in ng/L calculated as 70% or 30% of pool mean and acceptable SD: <0.25 TE_a for short-term imprecision; <0.33 for long-term imprecision.

^bTE_a of 3.5 ng/L at low cTnI concentrations (≤10 ng/L) as defined by IFCC TF-CB.

3.1.4 | Hemoglobin Interference

Both performance goals were met in all the hemoglobin interference experiments, except white rhinoceros samples analyzed on the Stratus CS. The average observed interference was 0.02 ng/mL in white rhinoceros, and it was equal to the acceptable allowed SE using the 70% TE_a performance goal but exceeded the 30% TE_a performance goal. In black rhinoceros, the average observed interference on the Stratus CS was also 0.02 ng/mL. The interference was associated with the samples that had increased concentrations of hemoglobin (> 2 g/L). The average observed interference noted in the Atellica VTli for black and white rhinoceros, respectively, was −2 and 1 ng/L. In white rhinoceros samples, the observed interference in the Atellica VTli was equal to the acceptable allowed SE using the 30% TE_a performance goal. Results are summarized in Table 4.

3.1.5 | Recovery

In the Stratus CS, the average recovery in black and white rhinoceros samples was 110% and 102%, respectively. The proportional SE was −10% and −2% in the black and white rhinoceros, respectively. Both the performance goals (TE_a of 30% or 70%) were met. In the Atellica VTli, the average recovery in black and white rhinoceros samples was 85% and 58%, respectively. The proportional SE was 15% and 42% in the black and white rhinoceros, respectively. When using the ASVCP cTnI TE_a, the performance goals were met; however, only the black rhinoceros samples met the performance goal when assessed against the AAB cTnI TE_a.

3.1.6 | Detection Limit Study

For both black and white rhinoceros, the LoB and LoD on the Stratus CS were <0.03 and 0.04 ng/mL, respectively. The manufacturer reported analytical sensitivity is <0.03 ng/mL. The LOQ for both species on the Stratus CS was 0.05 ng/mL.

The LoB for both rhinoceros species was 0.06 ng/L on the Atellica VTli, which is lower than reported by the manufacturer (0.55 ng/L). The LoD on this analyzer was 0.88 ng/L for black rhinoceros and 0.81 ng/L for white rhinoceros. This is lower than the manufacturer-reported LoD (1.2–1.6 ng/L). The LoQ was 1.05 and 0.97 ng/L in the black and white rhinoceros, respectively.

3.2 | QC Validation

The results are summarized in Table 5, with suitable candidate QC rules as advised for POCAs highlighted in blue [14, 15]. For the Stratus CS, the target mean calculated using 10 measurements was the same as the method-specific target mean provided by the manufacturer, and therefore the manufacturer target mean was used for bias calculations. For the Stratus CS, 42 QCM data points were available in a 6-month period for level 2 of the commercial QCM. The assay was suitable for statistical QC using the 1_{2,5s} *n* = 2 rule at the 30% TE_a performance goal; however, it was associated with a *P*_{ed} of 80% and a *P*_{fr} of 3%; no

TABLE 3 | Results of the linearity study of cardiac troponin I in African rhinoceros serum obtained by regression analysis.

Analyzer	Rhinoceros species	Analytical range	<i>r</i>	Intercept	Slope
Stratus CS (ng/mL)	Black rhinoceros	0.03–40.13	0.99	2.62	0.98
	White rhinoceros	0.05–38.39	1.00	1.10	0.96
Atellica VTLi (ng/L)	Black rhinoceros	3–1250	0.99	−6.45	0.97
	White rhinoceros	3–1250	1.00	−33.18	1.01

TABLE 4 | Hemoglobin interference results for the Siemens Stratus CS and Atellica VTLi in African rhinoceros.

	Mean of paired sample measurements	Average interference/SE	Acceptable SE at 70% TE _a ^a	Acceptable SE at 30% TE _a ^a
Siemens Stratus CS (ng/mL)				
Black rhinoceros	0.25	0.02	0.09	0.04
White rhinoceros	0.05	0.02	0.02	0.01
Siemens Atellica VTLi (ng/L)				
Black rhinoceros	36	−2	13	5
White rhinoceros	6	1	2	1

Note: Bold text—performance goal exceeded.

Abbreviations: SE, systematic error; TE_a, total allowable error.

^aTE_a in ng/mL (Stratus CS) or ng/L (Atellica VTLi) calculated as 70% or 30% of measurements mean and acceptable SE: <0.5 TE_a.

TABLE 5 | Results of the quality control (QC) validation from observed analytical performance using commercial QC material.

	Stratus CS: MAS cardioImmune XL, level 2 (ng/mL)	Atellica VTLi		
		Pathonorm cardiac acute lig L-1 (ng/L)	Pathonorm cardiac acute lig L-2 (ng/L)	Pathonorm cardiac acute lig L-3 (ng/L)
Target mean	0.62 ^a	16	33	277
Observed mean	0.59	16	32	278
SD	0.04	3	2	25
CV (%)	7.2	17.7	7.1	9.0
Bias (%)	−5.5	3.9	−1.1	0.5
TE _{obs} (%)	19.8	39.3	15.4	18.4
Sigma metric (σ) at 30% TE _a	4.2	1.7	4.2	3.3
Sigma metric (σ) at 70% TE _a	9.8	3.9	9.8	7.8
QC rule candidates at 30% TE _a , <i>n</i> = 2	1 _{2.5s} P _{ed} : 80% P _f : 3%	1 _{2s} P _{ed} : 5% P _f : 3%	1 _{2s} P _{ed} : 90% P _f : 9% 1 _{2.5s} P _{ed} : 80% P _f : 3%	1 _{2.5s} P _{ed} : 40% P _f : 3%
QC rule candidates at 70% TE _a , <i>n</i> = 2	1 _{3s} P _{ed} : > 90% P _f : 0% 1 _{3.5s} P _{ed} : 90% P _f : 0% 1 _{2.5s} P _{ed} : 90% P _f : 3%	1 _{2.5s} P _{ed} : 75% P _f : 3%	1 _{3s} P _{ed} : > 90% P _f : 0% 1 _{3.5s} P _{ed} : 90% P _f : 0% 1 _{2.5s} P _{ed} : 90% P _f : 3%	1 _{3s} P _{ed} : > 90% P _f : 0% 1 _{3.5s} P _{ed} : 90% P _f : 0% 1 _{2.5s} P _{ed} : 90% P _f : 3%

Abbreviations: σ, sigma metric; CV, coefficient of variation; *n*, number of quality control measurements performed; P_{ed}, probability of error detection; P_f, probability of false rejection; QC, quality control; QCM, quality control material; SD, standard deviation; TE_a, total allowable error; TE_{obs}, total observed error.

^aUsed the QCM manufacturer-provided target mean. The remaining target means were calculated from analyzer-specific measurements. The rules in blue met the simple rule requirements, with a probability of error detection > 85% and a probability of false rejection < 5% using two levels of quality control material.

rules were available for a higher P_{ed} . When using the 70% TE_a performance goal, 3 suitable QC rule candidates were available with a P_{ed} of $\geq 90\%$ and a P_{fr} of 0%–3% using 2 levels of QCM.

For the Atellica VTLi, 18, 14, and 23 QCM data points were available for levels 1–3 of the commercial QCM, respectively, in an 8-month period. Target means were calculated using 8, 5, and 10 measurement data points, respectively, for QCM levels 1, 2, and 3. These values differed from those provided by the manufacturer, and the calculated measurement means were used for bias calculations. The remaining data points for each QCM level were used for QC performance calculations. When using the 30% TE_a performance goal, candidate QC rules with a P_{ed} of $\geq 90\%$ and a P_{fr} of 0%–3% using 2 levels of QCM were not available for any of the QCM levels. This was also true when using the 70% TE_a performance goal for QCM level 1, but 3 suitable QC rule candidates were available with a P_{ed} of $\geq 90\%$ and a P_{fr} of 0%–3% using 2 levels of QCM for QCM levels 2 and 3. The total error that could be controlled, using a simple QC rule ($1_{2.5s}$, P_{ed} of 90% and P_{fr} of 3%, $n=2$), when entering the CVs from both analyzers (excluding the Atellica VTLi, level 1) was 33%–42%.

4 | Discussion

Both POCAs fulfilled most of the method validation requirements and can be used to establish reference intervals for black and white rhinoceros to assist in the clinical interpretation of results. Furthermore, candidate QC rules suitable to control the level of error, as suggested by the ASVCP, were identified based on observed analytical performance. These rules can be used to assess the performance of the analyzers once validated with prospective QC data points, which is the penultimate step before implementation [28].

Deciding on whether an instrument or method is suitable for its intended use in veterinary testing is based on predetermined quality goals, namely, the TE_a and the strictest requirement should be used [16]. The consensus ASVCP TE_a for cardiac troponin is 70%, which is based on clinical interpretation of results [17, 20]. A consensus TE_a can also be based on biological variation, which is the most stringent quality goal or state-of-the-art performance where analytical performance is used to determine the lowest TE_a that can be controlled [29]. A more stringent TE_a of 30%, recommended by the AAB, was also used in this study, as the ASVCP guideline was derived from a single expert opinion based on cardiac troponin results in dogs and cats and because of the lack of published data on the clinical and biological variation of cardiac troponin results in African rhinoceros. The AAB recommendation is based on the “final rule” mandated by the Centers for Medicare & Medicaid Services according to the Clinical Laboratory Improvement Amendments of Proficiency Testing Regulations Related to Analytes and Acceptable Performance, published in the USA Federal Register in July 2022 and revised on the 8 July 2024, which will be implemented in January 2025 [21]. Furthermore, additional analytical performance goals are required by the IFCC TF-CB for hs-cTnI assays as cardiac troponin testing is the standard of practice for the diagnosis of acute myocardial infarction, early rule-out, risk stratification, and outcome assessment in patients with acute coronary syndrome [30].

Among other things, these recommendations focus on QC utilization, validation of the lower reportable analytical limits, correct reporting of units in measurable concentration for patients and QCM, and imprecision goals at the 99th percentile upper reference limits (99th URLs) [18]. All the method validation experiments performed for both assay methods met the ASVCP TE_a performance goal. The AAB TE_a performance goal was not met in the white rhinoceros short-term replication experiment on the Atellica VTLi, but the imprecision at concentrations near 10 ng/L was acceptable for the Atellica VTLi based on the IFCC TF-CB hs-cTnI assay guidelines. The AAB TE_a performance goal was not met for the white rhinoceros hemoglobin interference experiment on the Stratus CS, indicating that hemolyzed samples from white rhinoceros are not suitable for cTnI measurement with the Stratus CS. The error present with the white rhinoceros recovery experiment on the Atellica VTLi also exceeded the AAB TE_a . However, based on all results, both analyzers are suitable for the preliminary measurement of cTnI in both rhinoceros species despite not fulfilling all criteria for the 30% performance goal.

Although the Stratus CS has not been validated for use in veterinary species, it has been used to generate feline and canine cTnI reference intervals and assess cardiac muscle reactivity in pigs, sheep, rabbits, rats, and mice [11, 12]. There are, therefore, no veterinary-specific repeatability results available for comparison, but the reported within-run CV in human validation studies using commercial QCM is low ($< 3\%$) [31]. In our study, only the imprecision for the white rhinoceros low pool was higher than reported for low pool and QCM concentrations by the manufacturer (2.7%–4.3%; only repeatability data available). The Atellica VTLi has not been used or validated for use in veterinary medicine, and repeatability results can only be compared to results obtained from human validation studies and the manufacturer's findings detailed on the product datasheet. The reported CV% ranged from 4.1%–8.0% and 3.4%–9.3% in a recent validation study and the manufacturer results, respectively [19]. This is comparable to the CV obtained in the white rhinoceros high pool, which failed the 30% TE_a performance goal. The experiment was performed by one operator under standardized conditions and procedures, and therefore, the CV obtained at a high cardiac muscle homogenate concentration in white rhinoceros serum most likely reflects the analyzer's inherent analytical performance capability. However, the CV was $< 10\%$, fulfilling the recommended high precision for a hs-cTnI method [32, 33].

In human medicine, very low cTnI concentrations, only measurable by hs-cTnI assays, are used for medical decision-making specifically in the diagnosis of acute myocardial infarction [23]. This necessitates the evaluation of analytical performance for hs-cTnI assays at low cTnI concentrations (≤ 10 ng/L), as significant bias has previously been identified at low troponin concentrations in hs-cTn assays [23]. Therefore, the IFCC TF-CB recommends that long-term analytical performance at low cTnI concentrations be monitored using a performance goal of SD 3.5 ng/L [23]. Furthermore, the 99th URLs are used as diagnostic cut-off values in the diagnosis of acute coronary syndrome in humans [34] and it is recommended that hs-cTnI assays should have a CV of $\leq 10\%$ at the 99th URLs, which allows confident reporting of hs-cTnI values and serial changes in cardiac troponin

results over time [30]. According to the Atellica VTLi manufacturer information and a recent study [19], this could be achieved for the assay based on their validations, but 99th URLs have not yet been determined for rhinoceros, so this performance goal was not considered in our study.

According to the manufacturer and confirmed in human validation studies, hemoglobin concentrations of up to 10 g/L do not result in a significant level of interference in the Stratus CS [31]. No significant interference was found in the black rhinoceros samples up to the evaluated concentration of 3 g/L hemoglobin, but this was not true for the white rhinoceros samples containing ≥ 2 g/L hemoglobin. A possible explanation for this discrepancy between the two rhinoceros species is that the troponin concentration in the pooled serum of white rhinoceros in this experiment was very low and near the detection limit of 0.04 ng/mL (0.05 ng/mL vs. 0.25 ng/mL in pooled serum from black rhinoceros, Table 4) and the SE may not be evident at higher cTnI concentration in the Stratus CS. However, the hemolysate was prepared from white rhinoceros red blood cell pellets, and a possible species-specific matrix effect resulting in interference cannot be excluded. Similar to human validation studies, hemoglobin did not significantly affect the Atellica VTLi results in rhinoceros, supporting manufacturer claims [19].

The proportional error from the recovery experiment in white rhinoceros samples analyzed on the Atellica VTLi exceeded the 30% total error goal but performed within acceptable limits when compared to the ASVCP guidelines. Conversely, the Atellica VTLi recovery in the black rhinoceros samples met both performance goals. The same reagent cartridge lot was used in the black and white rhinoceros experiments. Therefore, it is plausible that a species-specific matrix effect was the cause of the bias present in white rhinoceros. As this analyzer has not been used in publications concerning other animal species, it is difficult to determine the source of bias. Although not investigated in this study due to costs and time constraints, assessing whether similar results are noted in lithium-heparinized anticoagulated plasma or whole blood samples, the recommended sample type specimens in the Atellica VTLi, will be useful. Evidence suggests that heparin binding to troponins results in lower cTnI concentration in heparin-anticoagulated plasma than in serum [35].

When validating an immunoassay, detection limit experiments are recommended especially when a low value may be of clinical significance [16]. With the advent of hs-cTn assays and the use of the 99th URLs for clinical decision-making, clinicians are incorporating both detectable and nondetectable hs-cTn concentrations into their clinical decision-making process [18]. Emerging clinical evidence suggests that “undetectable levels,” concentrations less than either the LoB or LoD, can be used to safely rule out acute myocardial infarction using a single hs-cTn value when assessed against the 99th percentile upper reference limit determined for the assay [30]. It is recommended that the LoD should be defined as the lower analytical reportable limit and be communicated to clinicians, especially if the LoQ is being used for the lower reporting limit [18]. To ensure that detectable hs-cTn concentrations can be consistently quantified over time due to reported drift over time at the 99th percentile medical decision limit of hs-cTn assays, it is advised that clinical

laboratories should validate LoB, LoD (outside of the United States of America [US]) and LoQ (as per US Food and Drug Administration [FDA] regulations) at a minimum on an annual basis or more frequently as deemed necessary [18]. Even though high values of cTnI rather than low concentrations are considered significant in veterinary patients, detection limit studies also guide decisions on whether an assay will be useful in a particular species, that is the concentration of the analyte of interest quantifiable in health by the assay. Serum cTnI in healthy dogs and cats as measured on the Stratus CS has been reported to often be below the detection limit of the assay (0.03 ng/mL) [12]. Extrapolating from dogs and cats and considering the LoD of 0.04 ng/mL determined in this study for the assay, likely, the serum cTnI concentrations in healthy rhinoceros will also be below the analytical detection limit of this assay. The hs-cTnI assay used by the Atellica VTLi is likely to be more useful for serum cTnI quantification, especially when establishing reference intervals in healthy animals or for monitoring changes at low concentration levels.

The ASVCP quality assurance guidelines recommend weekly measurement of QCM for unit devices in POCA [14]. Quality control limits established during validation instead of manufacturer target ranges should be used in assessing method performance [28]. A simple QC rule, such as $1_{2.5s}$ or 1_{3s} with a P_{ed} of $\geq 85\%$ and P_{fr} of $\leq 5\%$, using ≤ 2 levels of QCM is preferred in veterinary medicine POCA [14]. This contrasts with the IFCC TF-CB's recommendation of daily measurement of 3 QCM concentrations for hs-cTn assays and 2 for contemporary cTn assays, which is impractical for POCA in veterinary practices [18]. Using ASVCP cTnI TE_a , appropriate QC rules could be achieved for both analyzers, except for the Atellica VTLi when using the low QCM level, where the P_{ed} was below the 85% requirement. In the Atellica VTLi, this was made achievable in part by decreasing the bias associated with using the provided method-specific QCM manufacturer target means and instead using target means determined from initial QCM measurements in our laboratory. This was done since calibration, which is used to reduce bias in many reference laboratory instruments, could not be conducted on the Atellica VTLi [13, 16, 17]. A master calibration curve is created for each lot of cartridges and included in the cartridge's radio frequency identification tag, defining the correlation between signal change differences and analyte concentration [36]. Calibration could be done on the Stratus CS during each reagent lot change, and the computed measured target mean was comparable to the target mean provided by QCM manufacturers. Because the Atellica VTLi analyzer's target means were calculated internally, the evaluation of QCM results was initially focused on precision. It was also limited by the low number of QCM data points available, which were further reduced due to the calculation of method-specific target means. To calculate bias and CV, a minimum of 20 QCM data points are required [16].

When using a total error goal of 30%, neither analyzer qualified for the use of a simple candidate QC rule with the desired P_{ed} and P_{fr} . This was primarily due to the high CVs obtained. The CVs for the lower concentration QCM target means were comparable to the CVs observed in the low pool precision studies, except for the Atellica VTLi's low-level QCM, which exceeded the highest low pool CV obtained (10.3% in the low pool vs. 17.7%

for level 1 QCM). This is also the only QCM level with a $\sigma < 3.0$, indicating inadequate analytical performance and the need for additional statistical and non-statistical QC [28]. Currently, there are no published guidelines for the concentration of QCM that should be used to monitor the performance of cTnI assays, as well as the permissible imprecision and bias at concentrations equal to or less than the 99th percentile [18]. However, monitoring analytical performance at low concentrations is critical, as reported quantitative shifts may result in analytical variations within the low concentration ranges that exceed the stated degree of concentration change used in clinical research for serial cTnI measurements [18]. High-sensitivity-cTnI POCAs should adhere to the proposed performance specification of $< 35\%$ of the TE at low hs-cTnI [18]. To achieve this, the 'reverse approach' [14] can be used to determine the minimum total error that can be managed with a simple QC rule [28]. However, manual OPSpec Charts are not sufficient for this purpose, and a statistical computerized software program like EZ Rules 3 (EZ RULES 3, Westgard QC, Madison, WI, USA) is necessary [28]. When the CVs from both analyzers were entered into a 'reverse approach' calculation using the Westgard sigma-metric design tool, the total error that could be controlled with a simple QC rule was 33%–42%. This computed minimum total error that can be controlled may subsequently be used as a consensus TE_a goal for cTnI in veterinary laboratory medicine. The next step is to select a final simple rule, calculate control limits at the specified QCM levels, and use statistical QC to monitor analytical performance on both analyzers over time.

This study has certain limitations that should be considered. Firstly, the validation of the assays was conducted using biobank-stored species-specific serum pools; however, they have not been validated for the use of serum or muscle homogenates. The Stratus CS assay requires lithium or sodium heparin anticoagulated whole blood, while the Atellica VTLi assay has been validated for use with lithium-heparinized whole blood or plasma [19, 37]. Ideally, the effect of serum should have been compared to lithium-heparinized plasma samples using an interference study, but only serum and not plasma samples were available. The contribution of a matrix effect due to the use of serum or species-specific differences could be a reason for performance goals not being met in the recovery experiment for the white rhinoceros.

Troponin I exists in various subforms, and it is found as slow-twitch and fast-twitch skeletal (skTn) or cardiac (cTn) isoforms. The sequence homology between skTnI and cTnI is approximately 40% [38]. The analytical specificity, i.e., cross-reactivity of the immunoassay with skTnI, was not evaluated in this study. Nevertheless, a veterinary study found cross-reactivity with skTnI when assessing cardiac muscle reactivity to Stratus CS antibodies [11]. However, this assay was highly selective for myocardium, with a reactivity level over 1000 times greater than that of skeletal muscle [11]. Furthermore, it is unlikely that significant cross-reactivity will occur, given the low reported degrees of cross-reactivity for skTnI in the Atellica VTLi and Stratus CS cTnI assays, which are less than 0.01% and 0.04%, respectively. Although cross-reactivity is expected to be minimal in these assays, it is essential to consider it when interpreting serum cTnI levels in rhinoceros that have experienced substantial skeletal muscle injury.

Extracted species-specific cardiac homogenates were used to spike the pooled serum samples in various method validation experiments, because isolated and purified rhinoceros cTnI standard solutions are not commercially available. It is therefore presumed and not proven that mainly cTnI molecules cross-reacted with the evaluated immunoassay antibodies. However, in previous studies, cTnI immunoassay has been successfully validated in various marine mammals using species-specific cardiac muscle homogenate-spiked serum samples [24].

This study did not establish reference intervals, and although the assays were determined to be suitable for further investigation into the measurement of serum cTnI in African rhinoceros, further research into reference limits and serum cTnI concentrations in healthy rhinoceros is necessary to interpret clinical data comprehensively.

5 | Conclusion

The two evaluated POC cTnI immunoassays demonstrated acceptable analytical performance for the detection of measurands of cTnI in cardiac muscle homogenate from African rhinoceros. The Atellica VTLi has potential for use in the field due to its small size and low detection limits. Ideally, field performance evaluation should be performed to assess the effect of ambient outside temperatures and ease of use. Reference intervals should be established for both species to assist in the clinical interpretation of results obtained using these assays. Although a simple QC rule has been validated for both analyzers based on ASVCP guidelines on total error, further investigation into the lowest total error that can be controlled, based on the analytical performance of each analyzer, is needed.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

1. A. Chanyandura, V. K. Muposhi, E. Gandiwa, and N. Muboko, "An Analysis of Threats, Strategies, and Opportunities for African Rhinoceros Conservation," *Ecology and Evolution* 11 (2021): 5892–5910.
2. R. Emslie, "*Ceratotherium simum* ssp. *simum*. The IUCN Red List of Threatened Species 2020: E.T39317A45814320," 2020, <https://doi.org/10.2305/IUCN.UK.2020-1.RLTS.T39317A45814320.en>.
3. S. M. Ferreira, S. Ellis, and G. Burgess, "African and Asian Rhinoceroses—Status, Conservation and Trade. A Report From the IUCN Species Survival Commission (IUCN SSC) African and Asian Rhino Specialist Groups and TRAFFIC to the CITES Secretariat Pursuant to Resolution Conf. 9.14 (Rev. CoP17)," 2022.
4. F. Pohlin, M. Hofmeyr, E. H. Hooijberg, et al., "Challenges to Animal Welfare Associated With Capture and Long Road Transport in Boma-Adapted Black (*Diceros bicornis*) and Semi-Captive White

- (*Ceratotherium simum*) Rhinoceroses,” *Journal of Wildlife Diseases* 56 (2020): 294–305.
5. P. Buss, M. Miller, A. Fuller, et al., “Cardiovascular Effects of Etorphine, Azaperone, and Butorphanol Combinations in Chemically Immobilized Captive White Rhinoceros (*Ceratotherium simum*),” *Journal of Zoo and Wildlife Medicine* 47 (2016): 834–843.
 6. D. Breed, L. Meyer, J. Steyl, et al., “Conserving Wildlife in a Changing World: Understanding Capture Myopathy—A Malignant Outcome of Stress During Capture and Translocation,” *Conservation Physiology* 7 (2019): coz027.
 7. S. M. Wells and M. Sleeper, “Cardiac Troponins,” *Journal of Veterinary Emergency and Critical Care* 18 (2008): 235–245.
 8. M. B. Ashraf, M. A. Akter, M. Saha, et al., “Clinicopathological Evaluation on Capture Myopathy Due to Chemical Immobilization in Spotted Deer,” *Turkish Journal of Veterinary Research* 3 (2019): 73–79.
 9. N. Cámara, E. Sierra, A. Fernández, et al., “Capture Myopathy and Stress Cardiomyopathy in a Live-Stranded Risso’s Dolphin (*Grampus griseus*) in Rehabilitation,” *Animals* 10 (2020): 220.
 10. Y. Rautenbach, S. D. C. Parsons, A. K. Loots, et al., “Genetic Characterization of Diagnostic Epitopes of Cardiac Troponin I in African Rhinoceros,” *Journal of Veterinary Diagnostic Investigation* 37, no. 2 (2025): 263–271.
 11. P. J. O’Brien, Y. Landt, and J. H. Ladenson, “Differential Reactivity of Cardiac and Skeletal Muscle From Various Species in a Cardiac Troponin I Immunoassay,” *Clinical Chemistry* 43 (1997): 2333–2338.
 12. M. M. Sleeper, C. A. Clifford, and L. L. Laster, “Cardiac Troponin I in the Normal Dog and Cat,” *Journal of Veterinary Internal Medicine* 15 (2001): 501–503.
 13. B. Flatland, K. R. Friedrichs, and S. Klenner, “Differentiating Between Analytical and Diagnostic Performance Evaluation With a Focus on the Method Comparison Study and Identification of Bias,” *Veterinary Clinical Pathology* 43 (2014): 475–486.
 14. B. Flatland, K. P. Freeman, L. M. Vap, K. E. Harr, and ASVCP, “ASVCP Guidelines: Quality Assurance for Point-of-Care Testing in Veterinary Medicine,” *Veterinary Clinical Pathology* 42 (2013): 405–423.
 15. E. H. Hooijberg, G. Steenkamp, J. P. du Preez, and A. Goddard, “Analytic and Quality Control Validation and Assessment of Field Performance of a Point-of-Care Chemistry Analyzer for Use in the White Rhinoceros,” *Veterinary Clinical Pathology* 46 (2017): 100–110.
 16. J. E. Arnold, M. S. Camus, K. P. Freeman, et al., “ASVCP Guidelines: Principles of Quality Assurance and Standards for Veterinary Clinical Pathology (Version 3.0),” *Veterinary Clinical Pathology* 48, no. 4 (2019): 542–618, <https://doi.org/10.1111/vcp.12810>.
 17. K. E. Harr, B. Flatland, M. Nabity, K. P. Freeman, and ASVCP, “ASVCP Guidelines: Allowable Total Error Guidelines for Biochemistry,” *Veterinary Clinical Pathology* 42 (2013): 424–436, <https://doi.org/10.1111/vcp.12101>.
 18. A. H. B. Wu, R. H. Christenson, D. N. Greene, et al., “Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion From the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine,” *Clinical Chemistry* 64 (2018): 645–655.
 19. R. H. Christenson, L. D. S. Frenk, H. J. de Graaf, et al., “Point-of-Care: Roadmap for Analytical Characterization and Validation of a High-Sensitivity Cardiac Troponin I Assay in Plasma and Whole Blood Matrices,” *Journal of Applied Laboratory Medicine* 7 (2022): 971–988.
 20. F. Porciello, M. Rishniw, W. Herndon, F. Birettoni, M. T. Antognoni, and K. W. Simpson, “Cardiac Troponin I Is Elevated in Dogs and Cats With Azotaemia Renal Failure and in Dogs With Non-Cardiac Systemic Disease,” *Australian Veterinary Journal* 86 (2008): 390–394.
 21. “Clinical Laboratory Improvement Amendments of 1988 (CLIA) Proficiency Testing Regulations Related to Analytes and Acceptable Performance,” accessed 11 July 2024, <https://www.federalregister.gov/documents/2022/07/11/2022-14513/clinical-laboratory-improvement-amendments-of-1988-clia-proficiency-testing-regulations-related-to>.
 22. J. O. Westgard, N. B. Person, and S. A. Westgard, *Basic Method Validation*, 4th ed. (Westgard QC Inc., 2020).
 23. P. A. Kavsak, A. S. Jaffe, D. N. Greene, R. H. Christenson, F. S. Apple, and A. H. B. Wu, “Total Analytic Error for Low Cardiac Troponin Concentrations (≤ 10 Ng/L) by Use of a High-Sensitivity Cardiac Troponin Assay,” *Clinical Chemistry* 63 (2017): 1043–1045.
 24. M. J. Joblon, J. E. Flower, L. A. Thompson, et al., “Investigation of the Use of Serum Biomarkers for the Detection of Cardiac Disease in Marine Mammals,” *Journal of Zoo and Wildlife Medicine* 53, no. 2 (2022): 373–382, <https://doi.org/10.1638/2021-0152>.
 25. J. O. Westgard, “Normalized OPSpecs Calculator,” 2024, <https://www.westgard.com/normalized-opspecs-calculator.html>.
 26. J. O. Westgard, “Internal Quality Control: Planning and Implementation Strategies,” *Annals of Clinical Biochemistry* 40 (2003): 593–611.
 27. J. O. Westgard, “The Sigma-Metrics QC-Selection Tool,” in *Basic QC Practices Training in Statistical Quality Control for Medical Laboratories* (Westgard QC, 2010), 231–250.
 28. K. Freeman, S. Klenner-Gastreich, and J. Korchia, “The Importance of Quality Control Validation and Relationships With Total Error Quality Goals and Bias in the Interpretation of Laboratory Results,” *Veterinary Clinical Pathology* 53 (2024): 65–74.
 29. S. Sandberg, C. G. Fraser, A. R. Horvath, et al., “Defining Analytical Performance Specifications: Consensus Statement From the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine,” *Clinical Chemistry and Laboratory Medicine* 53 (2015): 833–835.
 30. F. S. Apple, Y. Sandoval, A. S. Jaffe, J. Ordonez-Llanos, and IFCC Task Force on Clinical Applications of Cardiac Bio-Markers, “Cardiac Troponin Assays: Guide to Understanding Analytical Characteristics and Their Impact on Clinical Care,” *Clinical Chemistry* 63 (2017): 73–81.
 31. B. Bénéteau-Burnat, B. Baudin, and M. Vaubourdolle, “Evaluation of Stratus CS Stat Fluorimetric Analyser for Measurement of Cardiac Markers Troponin I (cTnI), creatine Kinase MB (CK-MB), and Myoglobin,” *Journal of Clinical Laboratory Analysis* 15 (2001): 314–318.
 32. D. R. Lazar, F. L. Lazar, C. Homorodean, et al., “High-Sensitivity Troponin: A Review on Characteristics, Assessment, and Clinical Implications,” *Disease Markers* 2022 (2022): 9713326, <https://doi.org/10.1155/2022/9713326>.
 33. F. S. Apple, P. O. Collinson, and Biomarkers ITFoCAoC, “Analytical Characteristics of High-Sensitivity Cardiac Troponin Assays,” *Clinical Chemistry* 58, no. 1 (2012): 54–61, <https://doi.org/10.1373/clinchem.2011.165795>.
 34. A. Clerico, M. Zaninotto, A. Ripoli, et al., “The 99th Percentile of Reference Population for cTnI and cTnT Assay: Methodology, Pathophysiology and Clinical Implications,” *Clinical Chemistry and Laboratory Medicine* 55, no. 11 (2017): 1634–1651, <https://doi.org/10.1515/cclm-2016-0933>.
 35. H. Stiegler, Y. Fischer, J. F. Vazquez-Jimenez, et al., “Lower Cardiac Troponin T and I Results in Heparin-Plasma Than in Serum,” *Clinical Chemistry* 46 (2000): 1338–1344.
 36. W. F. Peacock, J. L. Januzzi, F. de Theije, et al., “Methods of the Pivotal Trial of the Atellica VTLi Point of Care Emergency Department

High Sensitivity Troponin Evaluations,” *Clinical Biochemistry* 121–122 (2023): 110679.

37. J.-P. Chapelle, M.-C. Aldenhoff, L. Pierard, and J. Gielen, “Comparison of Cardiac Troponin I Measurements on Whole Blood and Plasma on the Stratus CS Analyzer and Comparison With AxSYM,” *Clinical Chemistry* 46 (2000): 1864–1866.

38. H. Hyytiä, T. Heikkilä, P. Hedberg, T. Puolakanaho, and K. Pettersson, “Skeletal Troponin I Cross-Reactivity in Different Cardiac Troponin I Assay Versions,” *Clinical Biochemistry* 48 (2015): 313–317.