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Clinical evaluation of the ADVIA Centaur XPT chemiluminescent immunoassay for equine insulin measurement

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ABSTRACT

Background: Insulin dysregulation is a key component of equine metabolic syndrome and is commonly assessed using basal insulin concentrations. Different analyzers may yield variable insulin results, limiting comparability.
Aims/Objectives: To assess the analytical performance of the ADVIA Centaur XPT chemiluminescent immunoassay (CLIA) for equine insulin measurement and to establish adapted decision thresholds based on comparison with a previously used CLIA method.
Methods: Precision, linearity, and dilution recovery were assessed for the ADVIA Centaur XPT. A total of 89 equine serum samples submitted for basal insulin testing were measured using the ADVIA Centaur XPT and Immulite 2000 XPi analyzers. Results were compared using Deming regression, Bland-Altman analysis, and weighted kappa statistics to evaluate agreement in classification.
Results: Intra-assay coefficients of variation were 3.56 %, 2.01 %, and 1.92 % for low, medium, and high insulin concentrations, respectively; inter-assay variation was 5.19 %, 5.78 %, and 5.68 %. Deming regression showed a proportional bias, with the ADVIA Centaur XPT consistently measuring lower insulin concentrations compared to the Immulite 2000 XPi. Bland-Altman analysis confirmed this bias across the measurement range. Spearman correlation between the two methods was 0.92 (95 % CI: 0.87–0.94; $P < 0.0001$), indicating a strong association in rank order. Classification agreement using adapted decision limits yielded a weighted kappa of 0.82, indicating strong agreement.
Conclusion: The ADVIA Centaur XPT demonstrated acceptable precision. When insulin concentrations were classified using adapted thresholds, the agreement between the two CLIA analyzers was strong, suggesting that the ADVIA Centaur XPT may be suitable for measurement of equine insulin concentration.

1. Introduction

Equine Metabolic Syndrome (EMS) is a multifactorial disorder increasingly recognized in equine clinical practice due to its significant impact on horse health [1]. EMS is characterized by obesity, regional adiposity, insulin dysregulation (ID), and an increased risk of laminitis, a painful and potentially life-threatening condition [2]. Accurate measurement of insulin concentrations is central to make an accurate diagnosis of EMS, where hyperinsulinemia is a key marker of the disease that is also used to assess prognosis and response to treatment [3].

The diagnosis of EMS relies heavily on the measurement of basal insulin concentrations or dynamic tests [4–6] that assess the insulin response to a glucose challenge. However, performing these dynamic tests under field conditions can be challenging, particularly in settings where resources are limited or where the stress of testing could impact the animal's well-being [7]. Comparative studies [8,9], have explored the relative diagnostic value of these approaches, with basal insulin remaining a practical and widely used method in both clinical and research settings. While basal insulin testing can be more practical, both basal and dynamic tests that rely on insulin measurements, are subject to similar limitations, including inter-laboratory variability, differences in assay methodology, and discrepancies between analytical analyzers [10].

This variability can complicate the diagnosis and management of horses with EMS, as clinicians may receive inconsistent data depending on the laboratory used. This underscores the importance of validating

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the methods used for insulin measurement.

Chemiluminescent immunoassay (CLIA) is a technique that has gained recognition for its high reliability and precision in measuring insulin concentrations, offering several advantages such as high analytical sensitivity, specificity for insulin detection [11–13], and, due to its automation, the ability to process a large number of samples more rapidly compared to RIA (Radioimmunoassay) or ELISA (Enzyme--Linked Immunosorbent Assay). The ADVIA Centaur XPT which utilize CLIA technology, has been commonly used in both human [14] and veterinary medicine [15] showing in equine studies a good Spearman correlation and Deming regression results compared to RIA and ELISA assays [10]. However, despite its use in research, it has not been formally validated for equine use.

In contrast, the Immulite 2000 XPi, has been widely used [10,16,17] for measuring insulin in horses. Although formal validation data are limited [17], its extensive use and the availability of proposed clinical cut-offs [18] have contributed to its adoption in equine clinical practice. However, a recent comparison study [19] reported that approximately 10 % of insulin results obtained with both the Immulite 2000 and 2000 XPi were spuriously high, ranging from two to over tenfold relative to RIA. While RIA has traditionally been favored in research due to their sensitivity and specificity, their limited accessibility, and the need to handle radioactive materials reduce their practicality in routine or large-scale use.

Considering the observed variability, comparing the performance of these machines to measure insulin concentrations in the context of diagnosing EMS is vital to ensure that clinicians have access to accurate and consistent data for the management of this increasingly prevalent condition. It is also important to recognize the limitations in data extrapolation: clinicians should be aware of which analytical system was used, as results from different analyzers may not be directly comparable.

To achieve this, we assessed the analytical and clinical performance (validation) of the ADVIA Centaur XPT machine and compared the insulin concentrations it reported with those obtained using the Immulite 2000 XPi, in order to determine adapted clinical decision thresholds.

2. Materials and methods

2.1. Ethical approval

All samples included in this study were originally submitted for routine diagnostic purposes to a commercial veterinary laboratory in Germany. At the time of submission, owners were informed (via the submission forms and accompanying data protection policy) that their animals' samples and associated anonymized data could be used for research purposes.

In addition, all referring veterinarians were informed about the objectives and scope of this specific study, including the fact that data and samples would be anonymized prior to analysis. Given that the study involved only retrospective analysis of anonymized clinical samples, no formal ethical approval was required.

2.2. Animals and blood samples

A total of 89 equine serum samples, were collected from various locations across Europe to ensure a diverse representation of the equine population. Samples represent a wide range of insulin concentrations and were simultaneously assayed using the Immulite 2000 XPi (Siemens) and ADVIA Centaur XPT (Siemens) systems. According to recent literature [20], a minimum of 40 well-selected patient samples spanning the reportable range is recommended for comparing two analytical methods. The use of 89 samples not only exceeds this minimum requirement but also enhances the robustness and reliability of the comparative analysis between the two systems. The maximum reportable concentration for both assays was 300 μIU/mL, thus, any samples with unquantificable insulin concentrations (> 300 μIU/mL) were excluded from the analysis. Serum samples were received already separated and refrigerated or frozen to ensure stability [21]. All assays were performed at LABOKLIN GmbH & Co. KG in Germany. The study population consisted of 1 stallion (1.1 %), 22 geldings (24.7 %), 35 of unknown sex (39.3 %), 30 mares (33.7 %), and 1 spayed mare (1.1 %).

Different breeds of warmbloods, coldbloods, and ponies were included. The ages of the animals ranged from 4 to 29 years, with a median age of 18 years.

2.3. Evaluation of ADVIA Centaur XPT CLIA (linearity, recovery upon dilution, and precision)

2.3.1. Precision

1. **Intra-assay precision:** Intra-assay precision was evaluated by performing 10 consecutive measurements on three different equine serum samples, each representing a high (mean 149.55 μIU/mL), medium (mean 73.08 μIU/mL), and low (mean 14.49 μIU/mL) insulin concentrations. The coefficient of variation (CV) was calculated for each concentration to determine the repeatability of the machine within a single assay.
2. **Inter-assay precision:** Inter-assay precision was assessed by analyzing three different equine serum samples, again representing high (mean 117.10 μIU/mL), medium (mean 23.83 μIU/mL), and low (mean 2.38 μIU/mL) insulin concentrations, across five different days. The measurements were performed by at least two different operators and with three different batches of reagents. The samples were kept aliquoted and refrigerated throughout the process. This approach allowed for the calculation of the inter-assay CV, providing insight into the machine's reproducibility over time and under varying conditions.

2.3.2. Linearity and recovery

To further validate the ADVIA Centaur XPT's performance, tests for linearity and recovery were conducted. Two equine serum samples with high insulin concentrations (82.87 μIU/mL and 120.44 μIU/mL) were selected for this purpose. Each sample was diluted using Siemens original diluent in a series of steps: 1:1, 1:2, 1:4, and 1:8. The purpose of these dilutions was to assess the linearity of the machine's response across a range of concentrations and to evaluate its accuracy in recovering the expected insulin values after dilution.

For each dilution, the observed insulin concentration was measured and compared to the expected concentration. The recovery upon dilution (RUD) was calculated as a percentage of the expected value. To ensure robustness in the RUD evaluation, the mean of all RUD measurements performed across both samples was calculated.

The coefficient of variation (CV%) was calculated as the ratio of the standard deviation to the mean, multiplied by 100. An intra-assay and inter-assay of ≤10 % were deemed acceptable [22]. Dilutional parallelism was evaluated using linear regression to establish the slope and intercept of the line. Percentage recovery upon dilution was determined by calculating the ratio of measured concentration to expected concentration.

2.4. Comparison between ADVIA Centaur XPT CLIA to Immulite 2000 XPi CLIA

The ADVIA Centaur XPT and Immulite 2000 XPi both utilize chemiluminescent immunoassay (CLIA) technology for insulin measurement but differ in their methods and validation. The ADVIA Centaur XPT uses a direct chemiluminescence method with a two-site sandwich assay, where mouse monoclonal anti-insulin antibodies are labelled with acridinium ester. It has a detection limit of 0.5 μIU/mL and a measurement range of 1.0 to 300 μIU/mL.

In contrast, the Immulite 2000 XPI, which is also based on a two-site sandwich immunoassay, uses an indirect chemiluminescence method

with an enzyme-labelled secondary antibody. It has a detection limit of 1.0 μIU/mL and a measurement range from 2.0 to 300 μIU/mL.

2.5. Statistical analysis

Data analysis was performed using GraphPad Prism 10.3.1.

The Shapiro-Wilk normality test was employed to assess whether the data followed a normal distribution. To compare the results obtained from different assays and to examine the relationships between the two methods, the Wilcoxon matched pairs signed rank test, Spearman correlation, and Deming regression analyses were utilized.

The Deming regression line was used to interpolate the clinical decision limits from the Immulite 2000 XPi to the ADVIA Centaur XPT. Although this approach assumes measurement error in both assays, Deming regression was selected to reflect the absence of a true gold standard and the presence of analytical variability in both analyzers.

Initially, a Bland-Altman analysis using absolute differences was performed to assess overall agreement. However, due to the presence of proportional bias between methods—as revealed by Deming regression—subsequent analyses were based on percentage differences relative to Immulite 2000 XPi. Three separate Bland-Altman analyses were performed: one including all insulin values, another focusing on values below 75 μIU/mL on Immulite 2000 XPi (corresponding to the suspected insulin dysregulation range), and a third for values below 31 μIU/mL on Immulite 2000 XPi (the non-diagnostic range) [18]. Percentage bias and 95 % limits of agreement were calculated and plotted against the average of both methods to account for this proportional relationship.

Cohen's kappa coefficient (κ) was used to assess the degree of agreement between the two assays when classifying samples into the three referred categorical outcomes, based on the established thresholds for the Immulite 2000 XPi and the Deming regression-derived thresholds for the ADVIA Centaur XPT. This statistic provides a more robust measure of agreement than simple percentage agreement by accounting for the possibility of agreement occurring by chance. However, since the insulin concentrations are classified into ordinal categories (non-diagnostic, suspected insulin dysregulation, and compatible with insulin dysregulation), the weighted kappa was used to account for the degree of disagreement between categories. Agreement should be interpreted as follows: <0.20 indicates poor agreement, 0.21–0.40 is considered fair, 0.41–0.60 is moderate, 0.61–0.80 is good, and 0.81–1.0 represents very good strength of agreement [23].

3. Results

3.1. Validation of ADVIA Centaur XPT CLIA (precision, linearity, and recovery upon dilution)

3.1.1. Intra-assay precision

The results (Table 1) showed excellent consistency, with intra-assay CVs of 3.56, 2.01, and 1.92 % for low, medium, and high insulin concentrations, respectively.

3.1.2. Inter-assay precision

The inter-assay (Table 2) CVs were 5.19 %, 5.78 %, and 5.68 % for low, medium, and high insulin concentration, respectively.

Table 1
Number of values, minimum, maximum, mean, standard deviation, and coefficient of variation for each insulin concentration measured with ADVIA Centaur XPT for intra-assay precision.

Number of values	10 (Low)	10 (Medium)	10 (High)
Minimum	13.30	70.53	142.82
Maximum	14.92	75.32	152.80
Mean	14.49	73.02	149.55
Std. Deviation	0.52	1.47	2.88
Coefficient of variation	3.56 %	2.01 %	1.93 %

Table 2
Number of values, minimum, maximum, mean, standard deviation, and coefficient of variation for each insulin concentration measured with ADVIA Centaur XPT for inter-assay precision.

Number of values	5 (Low)	5 (Medium)	5 (High)
Minimum	2.25	21.55	107.45
Maximum	2.55	25.30	122.95
Mean	2.38	23.83	117.10
Std. Deviation	0.12	1.38	6.65
Coefficient of variation	5.20 %	5.79 %	5.68 %

3.1.3. Linearity and recovery upon dilution

The assay demonstrated strong linearity (Fig. 1), with an r^2 value of 0.9916 ($P < 0.0001$), following the equation $Y = 0.9351*X + 2.288$.

Recovery upon dilution (RUD) was evaluated by calculating the mean recovery percentage for all diluted samples. The combined mean recovery was 105.81 % (±15,42), with a standard deviation of 9.69 %.

3.2. Comparison between ADVIA Centaur XPT CLIA to Immulite 2000 XPi CLIA

Insulin concentrations measured by Immulite 2000 XPi in the 89 samples had a median of 11.6 μIU/mL (IQR: 2.34–31.85, range: 2.0–208.0 μIU/mL). The corresponding values measured by the ADVIA Centaur XPT had a median of 6.9 μIU/mL (IQR: 3.25–12.55, range: 1.0–133.3 μIU/mL). The statistical comparison between the ADVIA Centaur XPT and the Immulite 2000 XPi showed a non-normal distribution of the data, as indicated by the Shapiro-Wilk test ($P < 0.0001$). Due to the non-normality, the Wilcoxon matched pairs signed-rank test was applied, confirming significant differences between the assays ($P < 0.0001$).

Following this, a Spearman correlation was performed. The correlation was strong, with a Spearman's correlation coefficient (ρ) of 0.92 (95 % CI: 0.87–0.94; $P < 0.0001$), indicating a high degree of association between the ADVIA Centaur XPT and Immulite 2000 XPi assays [24].

A Deming regression analysis was also conducted (Fig. 2). The Deming regression equation was $Y = 0.4686*X + 0.4906$ ($P < 0.0001$). The r^2 value, which indicates how well the regression line fits the data, is not provided by Deming regression in GraphPad Prism. However, in a standard linear regression, the r^2 value was calculated to be 0.8880.

By applying the Immulite thresholds to the Deming regression equation, the corresponding values for the ADVIA Centaur XPT were calculated. An Immulite 2000 XPi value of 31 μIU/mL corresponded to an estimated 14.98 μIU/mL on the ADVIA Centaur XPT (95 % CI:

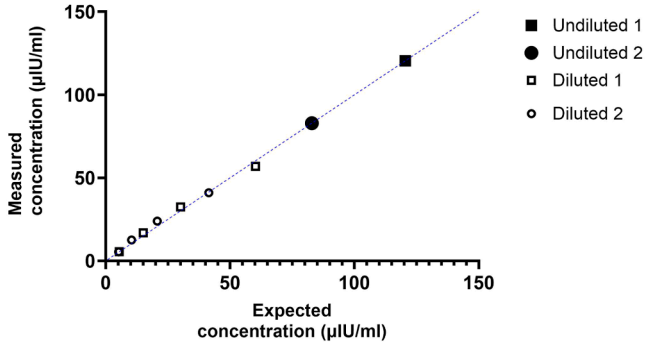

Fig. 1. Evaluation of linearity and recovery upon dilution (RUD) in two different insulin measurements (82.87 and 120.44 μIU/mL) using ADVIA Centaur XPT. Measured vs. expected insulin concentrations are shown. Undiluted samples are represented by filled markers, and diluted samples by open markers. The dashed blue line represents the line of identity ($y = x$).

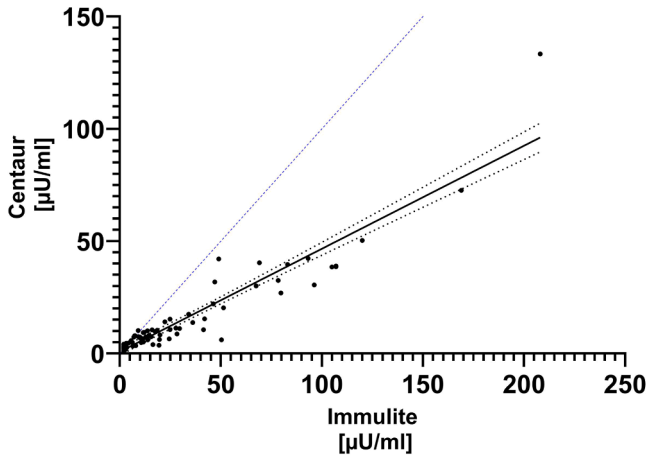

Fig. 2. Deming regression analysis comparing 89 insulin measurements between the ADVIA Centaur XPT and Immulite 2000 XPi systems. The solid black line represents the line of best fit (black dashed lines, 95 % confidence interval), and the blue dashed line identifying perfect agreement ($y = x$).

13.65–16.31 µIU/mL). Similarly, an Immulite 2000 XPi value of 75 µIU/mL corresponded to 35.15 µIU/mL on the ADVIA Centaur XPT (95 % CI: 33.02–37.27 µIU/mL).

Several Bland-Altman plots were generated to further assess the agreement between the ADVIA Centaur XPT and Immulite 2000 XPi CLIA systems (Fig. 3). An initial Bland-Altman analysis using absolute differences was performed for the entire dataset. The bias was −13.86 µIU/mL, with 95 % limits of agreement (LOA) ranging from −56.44 to +28.71 µIU/mL. This indicates that, on average, the ADVIA Centaur XPT measured insulin concentrations 13.86 µIU/mL lower than the Immulite 2000 XPi, with considerable variability across the measurement range.

For the full dataset using percentage differences relative to average values, the mean percentage bias was −39.84 %, with 95 % LOA ranging from −140.7 % to +61.01 %. When restricting the analysis to samples with Immulite concentrations below 75 µIU/mL, the percentage bias decreased slightly to −33.70 %, with 95 % LOA (−135.2 % to +67.79 %). The percentage bias decreased further in the subset of samples with Immulite concentrations below 31 µIU/mL, where the bias was −26.38 % and the limits of agreement ranged from −124.8 % to +72.01 %.

Lastly, the standard kappa result was 0.74 (95 % CI: 0.595–0.884), indicating substantial agreement between the two assays. When accounting for the ordinal nature of the insulin categories, the weighted kappa increased to 0.82, reflecting a very strong level of agreement [25].

4. Discussion

The results of this study provide valuable information, particularly in light of research suggesting the use of conversion factors between different insulin measurement techniques and machines [26]. However, there has been no prior description of the correlation between the Immulite 2000 XPi and the ADVIA Centaur XPT systems, making this study a crucial contribution to understanding how these assays relate to each other.

Although the insulin concentrations measured in this study may initially appear low (median <15 µIU/mL in both analyzers), this distribution is consistent with values typically reported in the general equine population. For example, a normal reference interval of 2.0–21.1 µIU/mL was established in 112 ponies with ad libitum access to hay using the Immulite 2000 chemiluminescent assay [27]. Similarly, another study using the same assay reported peak insulin values below 19.8 µIU/mL in clinically normal ponies fed dry hay, and below 3.9 µIU/mL when fed soaked hay [28]. Additionally, Frank (2011) [29] recommended a cut-off of 20 µIU/mL for basal insulin in healthy horses using the RIA. Taken together, these findings support the notion that

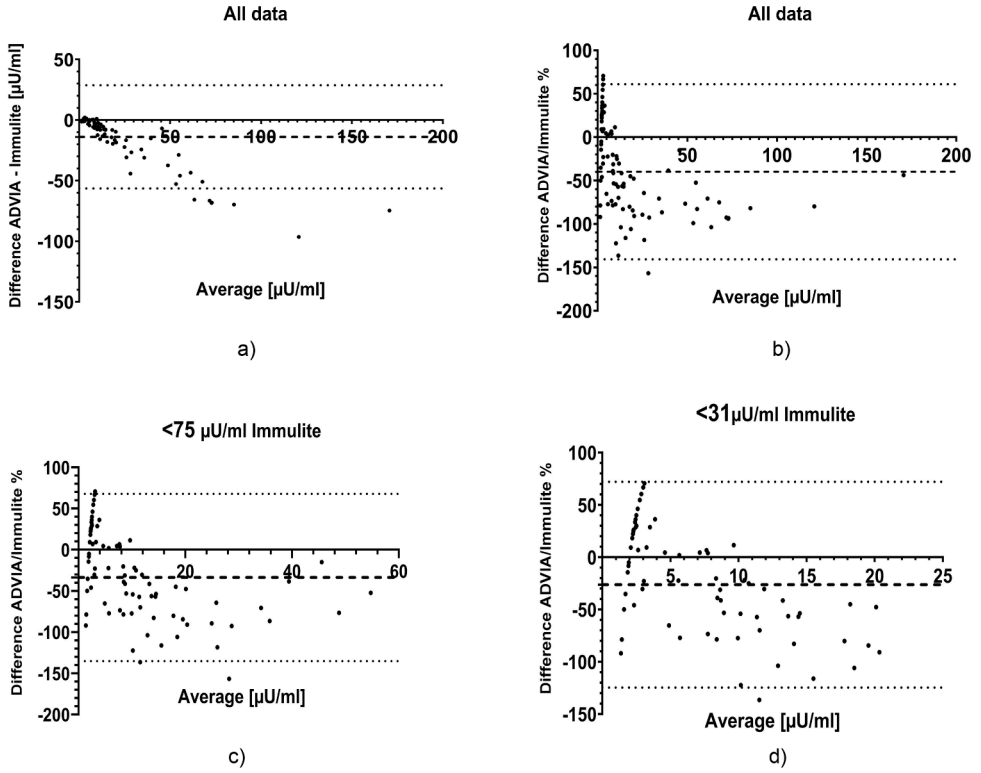

Fig. 3. a) Bland-Altman plot showing the absolute difference between the ADVIA Centaur XPT and Immulite 2000 XPi for all samples ($n = 89$); b) proportional Bland-Altman plot for all samples ($n = 89$); c) proportional Bland-Altman plot for Immulite 2000 XPi insulin concentrations < 75 µIU/mL ($n = 78$); d) proportional Bland-Altman plot for Immulite 2000 XPi insulin concentrations < 31 µIU/mL ($n = 67$). Dashed line = bias; dotted lines = 95 % limits of agreement.

most horses and ponies fall within normal or borderline insulin ranges under standard conditions, reinforcing the relevance of comparing results between the ADVIA Centaur XPT and the Immulite 2000 XPi specifically within this commonly encountered concentration range. Focusing the comparison within this range strengthens the clinical relevance of our findings.

The low intra-assay coefficients of variation for the ADVIA Centaur XPT observed in this study reflect a high level of repeatability within a single run, with minimal variability when the same sample is analysed multiple times under identical conditions. Similarly, the low inter-assay suggests that the ADVIA Centaur XPT provides consistent performance over time. This level of analytical precision supports its suitability for clinical use, where reproducibility is essential.

Recovery upon dilution was consistent and close to the expected values, with a combined mean recovery of 105.81 % (±15.42). While there are no established total allowable error (TAE) limits for insulin measurement in equines, guidelines from the Wadsworth Center of the New York State Department of Health recommend a TAE of ± 25 % for human insulin assays [30], and Westgard defines a TAE for insulin at ± 32.9 % [31]. The ADVIA Centaur XPT's RUD falls well within these recommended thresholds, reinforcing its potential suitability for accurate insulin quantification even after dilution in veterinary contexts.

The results of the Deming regression, supported by a high r^2 value, confirmed a close association between the two analytical systems, indicating a proportional relationship between the two assays but suggesting some systematic differences between the results provided by the two machines with the ADVIA Centaur XPT tending to report lower insulin measurements compared to the Immulite 2000 XPi across the range of values. This indicates that while there is a strong correlation between the two methods, they are not directly interchangeable without further adjustment (adapted cut-off/decision points).

The Bland-Altman analysis also showed this bias and highlighted that the agreement between the assays improves at lower insulin concentrations, with smaller proportional biases, suggesting that the ADVIA Centaur XPT is more directly comparable to the Immulite 2000 XPi in non-diagnostic and borderline cases. However, at higher concentrations, the ADVIA Centaur XPT tends to underestimate insulin concentrations more significantly. Despite this, limits of agreement remained wide across all ranges.

Nevertheless, the weighted kappa suggested that, although there may be differences between the two systems, they are largely in agreement when categorizing insulin concentrations, underscoring the need for method-specific cut-offs intervals and adapted diagnostic thresholds. It should be noted, however, that the low median and interquartile range of insulin concentrations in our dataset may have contributed to an increased agreement in the lower categories and thus influenced the kappa coefficient. While this represents a statistical limitation, the distribution itself, as previously mentioned in relation to the typical insulin values observed in the general equine population, reinforces the clinical relevance of the comparison within this concentration range.

Despite the strengths of this research, some limitations should be noted. The absence of clinical histories for the animals means that the health status of each horse was unknown, and it cannot be determined whether elevated insulin concentrations were associated with clinical disease. Furthermore, the insulin cut-offs proposed are decision limits derived from the assays, but they do not confirm whether an animal is genuinely insulin-dysregulated or not. Unlike reference intervals, which describe the distribution of values statistically derived from a predefined apparently healthy population, clinical decision limits in this case are cut-off values or thresholds designed to categorize patients into one of three groups: a) non-diagnostic, b) suspected insulin dysregulation or c) compatible with insulin dysregulation, regardless of whether the patients are truly healthy or not [32]. Additional diagnostic testing and clinical context are necessary to draw definitive conclusions about the animal's health.

Several factors may account for the differences observed between the ADVIA Centaur XPT and the Immulite 2000 XPi in insulin measurements. Immunoassays are inherently susceptible to analytical interference from structurally related hormones or hormone-binding proteins, which may cross-react with the antibodies used in the assay. Additionally, heterophilic antibodies—formed following exposure to external antigens and capable of binding immunoglobulins from other species—have been identified as a source of interference in a wide range of immunometric assays, including insulin measurement [19]. Differences in the specific antibody reagents and assay design may also play a role. For instance, another paper [10] suggested that alterations in the antigenic epitope recognized by anti-human insulin antibodies used in chemiluminescent immunoassays may reduce binding efficiency, potentially contributing to variability between platforms. These combined factors highlight the complexity of comparing results across different immunoassay systems and support the need for analyzer-specific validation and interpretation. Furthermore, evidence supporting the analytical accuracy of almost all currently available equine insulin assays is lacking (including Immulite 2000 XPi), which complicates the interpretation and generalization of results across studies. The absence of equine-specific standards or access to a reference assay such us LC-MS (Liquid Chromatography-Mass Spectrometry) makes it challenging to assess the accuracy of any equine insulin assay. To our knowledge, insulin LC-MS has been used to measure equine serum insulin concentration in only one study [33].

5. Conclusion

This study demonstrates that the ADVIA Centaur XPT, while not initially validated for use in equine insulin measurement, provides results that can correlate with the Immulite 2000 XPi, a system widely used and validated for this purpose. The precision of the ADVIA Centaur XPT system was excellent, with intra- and inter-assay variability well within acceptable limits. Linearity and recovery upon dilution were also robust, further supporting its potential utility in a clinical setting.

Despite these strengths in the correlation, systematic differences between the two methods were observed. A Deming regression revealed a proportional bias, with the ADVIA Centaur XPT reporting lower insulin concentrations compared to the Immulite 2000 XPi. Bland-Altman analyses further confirmed agreement between the methods, but with proportional bias which demonstrated widening differences between analyzers as insulin concentrations increased.

These biases highlight the importance of adjusting ADVIA Centaur XPT decision limits when used in clinical diagnosis, particularly when determining insulin dysregulation thresholds. Interpolating the Immulite's decision limits using Deming regression allowed for the determination of clinically relevant equivalents for the ADVIA Centaur XPT, providing practical guidelines for practitioners. Cohen's weighted kappa of 0.82 indicated a strong level of agreement between the two systems when classifying insulin dysregulation, provided that decision limits are adapted for each analyzer. This suggests that the ADVIA Centaur XPT can offer comparable classification results to those to the Immulite 2000 XPi when results are interpreted using machine-specific thresholds. This underscores the utility of the ADVIA Centaur XPT for equine insulin measurement in clinical practice. In addition, emphasizes the importance for clinicians to know which system was used to measure insulin concentrations.

In summary, while the ADVIA Centaur XPT shows significant potential as a reliable tool for measuring insulin in equine patients, it is essential for clinicians to account for the observed bias and apply machine-specific decision limits to ensure accurate diagnostic decision-making. Further validation of this analyzer in a healthy equine population would be necessary to establish reliable reference intervals and broaden its clinical application.

Ethics in publishing statement

I testify on behalf of all co-authors that our article submitted followed ethical principles in publishing.

Title: Clinical evaluation of the ADVIA Centaur XPT (Siemens) chemiluminescent immunoassay for equine insulin measurement

All authors agree that:

This research presents an accurate account of the work performed, all data presented are accurate and methodologies detailed enough to permit others to replicate the work.

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All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

CRediT authorship contribution statement

R. Rey-Conejo: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **R.E. Toribio:** Writing – review & editing, Supervision, Conceptualization. **S. Möller:** Writing – review & editing, Resources. **E. Müller:** Writing – review & editing, Resources. **P. Fores-Jackson:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

R.Rey Conejo reports equipment, drugs, or supplies was provided by Laboklin Laboratory for Clinical Diagnostics GmbH & Co. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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