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# Summary of Bionote Study: Assessment of Vcheck® analyzer for rapid progesterone concentration measurement including recommendations for achieving the optimal breeding time

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## Background

Serum progesterone concentration plays critical role in determining the optimal breeding time in bitches and diagnosing reproductive-related issues. This study aimed to conduct a comparative analysis of serum progesterone results obtained from commercial point-of-care immunological analyzers, namely, Vcheck®, with those obtained using chemiluminescent microparticle immunoassay (CMIA). Our overarching goal was to evaluate these analyzers' accuracy and establish standardized guidelines for optimal breeding timing.

## Introduction

The optimal breeding time in bitches requires an assessment of serum progesterone concentrations [1]. However, this evaluation serves multiple essential purposes, including identifying reproductive irregularities, such as hypoluteoidism [2], and confirming luteolysis before parturition [3–5]. Notably, a characteristic increase in serum progesterone concentrations during the estrus period often exceeding 1 ng/mL, indicates significant hormonal changes. In bitches, ovulation typically occurs 36–50 h after the luteinizing hormone (LH) peak [6], which correlates with serum progesterone concentrations of approximately 2.02 0.18 ng/mL [7]. These concentrations then escalate to a range of 4.00–10.00 ng/mL on the day of ovulation [8], indicating a significant hormonal shift and the onset of ovulation. Intriguingly, despite this range, Seefeldt ***et al***. [9], Marseloo ***et al***. [10], and Mir ***et al***. [11] suggested a serum progesterone concentration of 5.00–8.00 ng/mL, introducing contrasting perspectives on determining the ovulation.

Various techniques, such as radioimmunoassay (RIA) [12, 13], liquid chromatography-tandem mass spectrometry (LC-MS) [14, 15], and chemiluminescence immunoassay (CLIA) [14, 16], are used to measure serum progesterone concentration in veterinary medicine. An accurate and reliable alternative is the CLIA method, adept at assaying serial blood samples with guaranteed safety, speed, accuracy and repeatability [17]. CLIA addresses the drawbacks associated with RIA and enzyme immunosorbent assay and serves as a robust solution for precise serum progesterone monitoring, aiding in accurate ovulation prediction and confirmation. Moreover, recent advances, such as point-of-care analyzers, have transformed the measurement of progesterone. These advancements, including rapid fluorescence immunochromatography assay, surface plasmon field-enhanced fluorescence spectroscopy, lateral flow immunochromatography and competitive enzyme-linked fluorescence assay [18–21], enhance serum progesterone monitoring, ultimately improving veterinary practice in managing reproductive processes.

However, differences in serum progesterone levels are due to the use of different laboratory techniques and variations between bitches. As a result, the precise identification of the ideal mating period necessitates the collection of multiple consecutive blood samples during both the proestrus and estrus phases, which can then be compared with established gold standards or reference laboratory procedures. This investigation involves a comparative analysis of serum progesterone findings derived from a commercial point-of-care analyzer, namely Vcheck®, in contrast to those acquired through chemiluminescent microparticle immunoassay (CMIA) using the same serum samples.

## Materials and Methods

Ninety-four serum samples from bitches were analyzed using the Vcheck® analyzer and compared with CMIA. Thorough documentation included the mean, standard deviation, 95% confidence interval (CI), and minimum and maximum values of serum progesterone concentrations. Furthermore, Pearson's correlation coefficient, Lin's concordance correlation coefficient, and the bias correction factor were meticulously recorded.

## Results

The mean progesterone concentration measured using the Vcheck® analyzer was significantly lower than that measured using CMIA, with a mean difference of 1.26 ng/mL of serum. The Bias correction factor was 0.935, which was nearly 1.00, indicating that the line of best-fit was on the perfect line of agreement, providing insight into the measurement accuracy. Pearson's correlation coefficient, a measure of precision, was also close to 1 (0.939), confirming the reliability of the data. Furthermore, Lin's concordance correlation coefficient was 0.877, indicating a fair overall agreement between the Vcheck® and CMIA methods. These results support the validity of the Vcheck® analyzer's results. The present study was developed by aligning with established CMIA guidelines and adapting them using the range and 95% CI derived from each set of results, ensuring a standardized and rigorous approach.

Table 1: Provides a comprehensive overview of the means, SD, 95% CI, and range of serum progesterone concentrations. These values were determined through measurements taken during various phases, including early proestrus, LH peak, pre-ovulation, ovulation, post-ovulation and all bitch periods. This detailed presentation enables us to understand the distribution of progesterone levels in different phases.

Mean, SD, 95% CI, Min, and Max value for serum progesterone concentration with quantification using the CMIA and Vcheck® for estimates during the early proestrus, LH peak, pre-ovulation, ovulation, post-ovulation and all periods of the bitch.

| Period | CMIA | | | Vcheck® | | |
|---|---|---|---|---|---|---|
| | Mean ± SD | 95% CI | Min-Max | Mean ± SD | 95% CI | Min-Max |
| Proestrus | 1.24 ± 0.37 | 1.11 - 1.38 | 1.00 - 1.98 | 1.36 ± 0.54 | 1.16 - 1.56 | 1.00 - 2.81 |
| LH peak | 2.65 ± 0.36[a] | 2.35 - 2.95 | 2.17 -2.98 | 1.88 ± 0.55[b] | 1.42 - 2.33 | 1.00 - 2.73 |
| Pre-ovulaiton | 3.71 ± 0.52 | 3.31 - 4.10 | 3.00 - 4.44 | 3.35 ± 1.53 | 2.17 - 4.53 | 1.77 - 7.01 |
| Ovulation | 6.79 ± 1.32[a] | 6.31 - 7.27 | 5.09 - 9.54 | 5.36 ± 2.48[b] | 4.47 - 6.25 | 1.00 - 10.60 |
| Post-ovulation | 16.47 ± 7.18[a] | 12.32 - 20.61 | 10.11 - 30.00 | 11.7 ± 5.90[b] | 10.26 - 14.85 | 5.77 - 21.27 |
| All period | 5.75 ± 5.79[a] | 4.57 - 6.94 | 1.00 - 30.00 | 4.50 ± 4.39[b] | 3.60 - 5.40 | 1.00 - 21.27 |

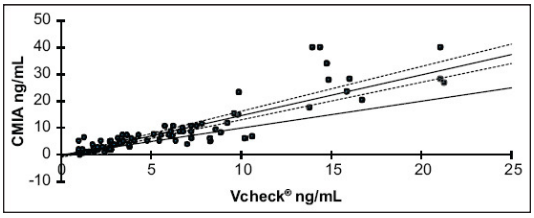
[a, b]values within the same row with different superscripts mean statistically significant difference ($p < 0.05$). SD=Standard deviation, CI=Confidence interval, Min=minimum, Max=maximum, CMIA=Chemiluminescence microparticle immunoassay

We found an intriguing finding when we compared these values: The average values of all samples obtained using Vcheck® were significantly lower than those obtained using CMIA, with an average difference of 1.26 ng/mL (Table-2 and Figure-1). This difference is not only statistically significant but also significantly impacts the accuracy and precision of the measurements.

Table 2: Measure of agreement: Concordance correlation coefficient, Pearsons' correlation coefficient, ant bias correction factor.

| 95% limits of agreement (Bland and Altman) | | | Lin's concordance correlation coefficient | 95% confidence interval | | Pearsons' correlation coefficient | Bias correction factor |
|---|---|---|---|---|---|---|---|
| Average difference | Lower | Upper | | Lower | Upper | | |
| 1.26 | -3.16 | 5.68 | 0.877 | 0.834 | 0.910 | 0.939 | 0.935 |

Figure 1: Passing-Bablok regression plot depicting serum progesterone measurements by Vcheck® against the chemiluminescence microparticle immunoassay serum progesterone measurements. The thin line is the identity line. The thick line represents the regression line and the dotted lines represent its 95% confidence interval.




## Conclusion

The Vcheck® analyzer provides a rapid assessment of serum progesterone concentration in bitches, with results comparable to those measured using the CMIA technique. However, when considering the use of the Vcheck® analyzer, it is recommended that the results should be interpreted carefully and the interpretation guidelines should be followed. In conclusion, Vcheck® provides a reliable and convenient method for veterinarian practitioners to measure canine progesterone levels in a clinical/hospital setting.

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