Validation of the MiniReader – Progesterone ELISA test

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The MiniReader measures the progesterone level in serum samples of dogs and cattle based on an ELISA reaction.

ELISA (Enzyme Linked Immuno-Sorbent Assay) is an analytical assay to detect the presence of a specific substance in a liquid sample. This is performed by antigen–antibody binding, where the antibody is linked to an enzyme and added to the sample with the antigen. The reaction will produce a signal, most commonly a colour change, indicating the quantity of antigen (in this case Progesterone) in the sample. This change in colour is detected by the MiniReader through photometry. The result is displayed in ng/ml based on a specific calibration curve derived using three standards.

The test kit used with the MiniReader includes reagents for the ELISA reaction, sample wells and three standards. The standards contain a defined concentration of synthetic progesterone and are prepared using the identical procedure as that for the samples. The standard values allow an adjustment of the internal calibration curve of the MiniReader to eliminate factors, other than progesterone concentration, which could influence the result.

For the validation procedure of the MiniReader, Minitube employed a variety of tests including a comparison to a reference method, an evaluation of the measurement range, and cross reactivity analyses. Precision, recovery and linearity of the results were also examined as described below.

Comparison of MiniReader to a reference method

Serum samples (67) from female dogs were sent to a standard laboratory as reference . The same samples were analysed with the MiniReader test kit and measured with the MiniReader. A high conformity over the entire range could be confirmed (Figure 1).
In Figure 2, the Concordance-Correlation-Coefficient (CCC) as indicator of the agreement between measurement methods is 0.922. The closer this value to 1 is, the better the two methods agree. A CCC of 0.922 describes a nearly complete agreement.

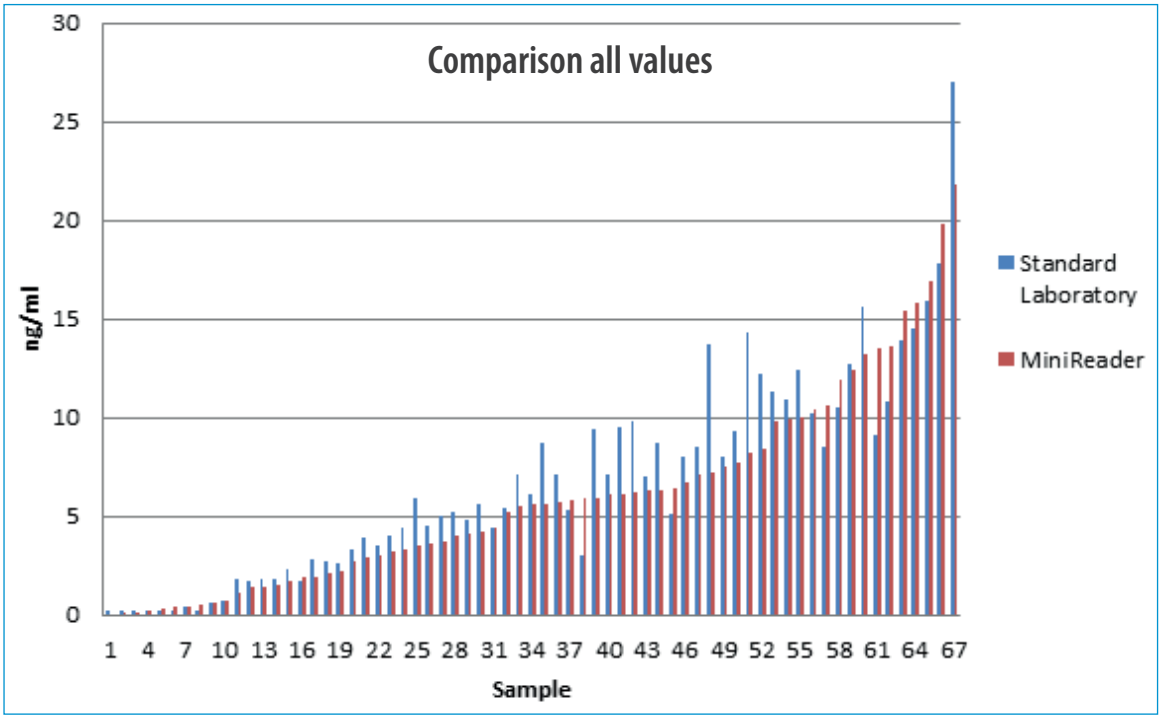

Fig. 1: Comparison of Standard Laboratory vs. MiniReader values for 67 serum samples.

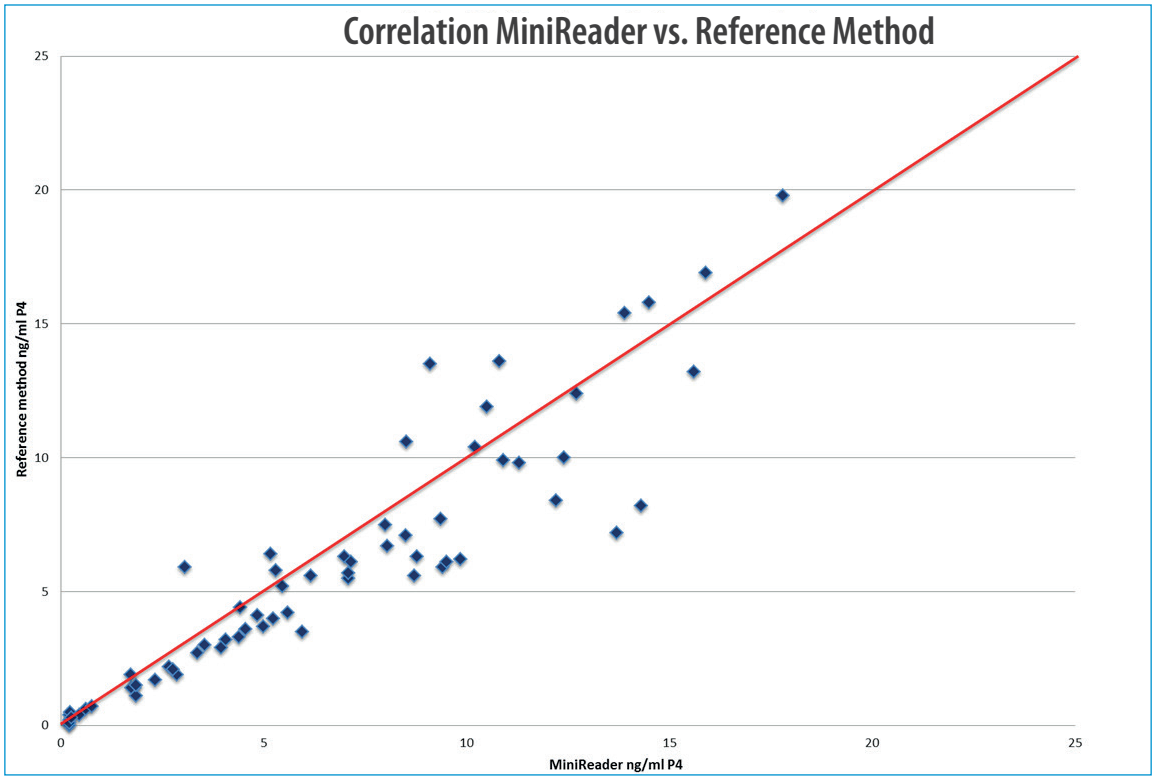

Fig. 2: Concordance of measurement methods: All measurements are distributed close to the line of perfect concordance (red line).

Measurement range

Samples with known progesterone concentration were measured with the MiniReader and the optical density was reported. The measurement range of the assay is shown to be between 0 and 30 ng/ml (Figure 3). The analytical sensitivity was determined to be 0.3 ng/ml. The analytical sensitivity describes the performance of a diagnostic test. It is the lowest detectable progesterone concentration, varying significantly from zero value. A sensitivity of 0.3 ng/ml is adequate for this test kit since the relevant levels are much higher.

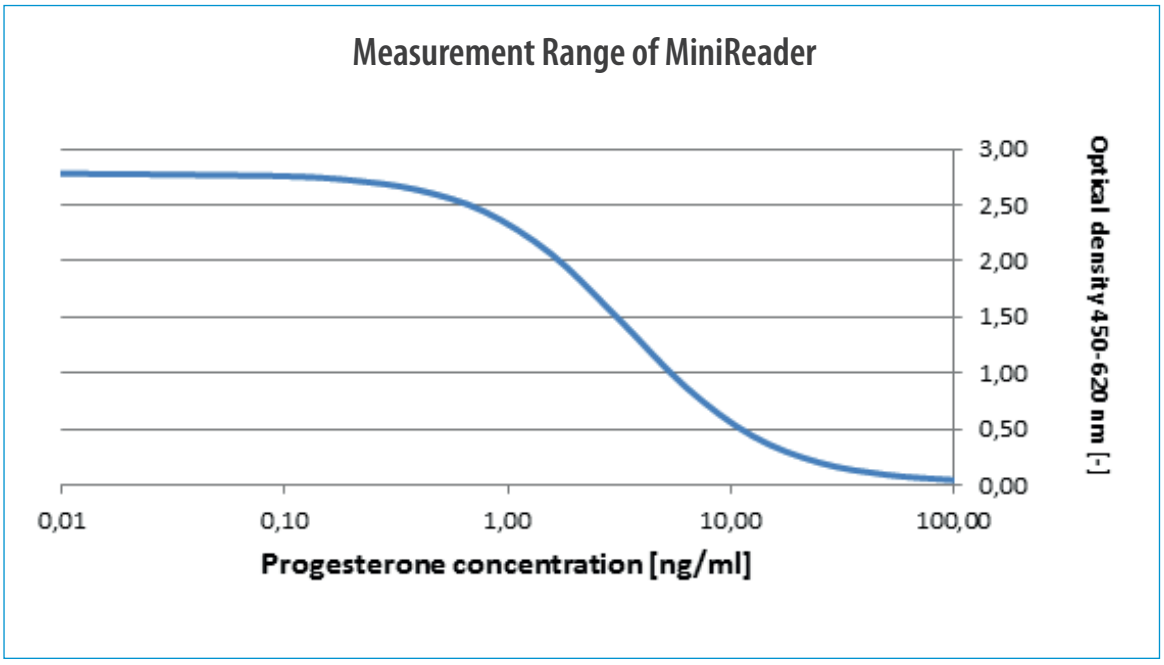

Fig. 3: Standard curve indicating the measurement range of the MiniReader

Cross reactivity (specificity of antibodies)

It is important to exclude the possibility that other substances commonly present in a given serum sample will react like Progesterone in the assay. Therefore, the substances shown in table 1 were tested for possible cross reactivity in the MiniReader assay. The result was <0.1 % for all hormones tested.

Substance	Cross reactivity
Progesterone	100 %
17 H-Progesterone	< 0.1 %
Pregnenolone	< 0.1 %
Testosterone	< 0.1 %
ß-Estradiol I	< 0.1 %
Cortisol	< 0.1 %

Table 1: Cross reactivity of other substances commonly found in serum samples

Precision

An intra-assay and an inter-assay were performed to determine the precision of the assays. For this purpose, the deviations from the measuring results for 3 serum samples were determined within a series of measurements (Intra-assay) and from test series to test series (Inter-assay). Table 2 shows the results of the intra-assay and table 3 shows the result of the inter-assay.

	Sample 1	Sample 2	Sample 3
n	10	10	10
Mean value [ng/ml]	0.99	2.90	13.54
Standard deviation [ng/ml]	0.15	0.34	1.96
Coefficient of variation	15 %	12 %	14 %

Table 2: Intra–assay of three serum samples

	Sample 1	Sample 2	Sample 3
n	5	5	5
Mean value [ng/ml]	1.12	3.0	12.60
Standard deviation [ng/ml]	0.08	0.27	1.21
Coefficient of variation	7 %	9 %	10 %

Table 3: Inter-assay of three serum samples

In order to assess the intra-assay each sample was measured 10 times. The average value for sample 1 was 1.0 ng/ml, for sample 2 it was 2.9 ng/ml, and for sample 3 it was 13.5 ng/ml. The standard deviation was 0.1, 0.3 and 2.0 ng/ml, respectively. The coefficient of variation of sample 1 was 15 %, for sample 2 it was 12 % and for sample 3 it was 14 %.

For evaluation of the inter-assay, 5 measurements were conducted with each sample. The average values were 1.1, 3.0 and 12.6 ng/ml for sample 1, 2 and 3, respectively. Standard deviations were determined as follows: 0.1 ng/ml for sample 1, 0.3 ng/ml for sample 2 and 1.2 ng/ml for sample 3. The coefficients of variation of the three serum samples were 7 % for the serum sample with the lowest progesterone level, 9 % for the medium level, and 10 % for the serum sample with the highest content of progesterone.

The coefficient of variation is defined as the ratio of standard deviation to mean value and should be <20 %.

Recovery

The recovery was determined with solutions of defined progesterone concentration being added to a serum sample. A sample was mixed 1:1 with different standard solutions. The expected progesterone concentration was calculated and the sample was measured with the MiniReader.

The expected concentration results from the zero value of the sample plus the progesterone level of the standard.
The recovery in % was calculated as the ratio of measured progesterone concentration to expected progesterone concentration.

P4-concentration of the standard [ng/ml]	Expected P4-concentration [ng/ml]	Measured P4-concentration [ng/ml]	Recovery
18.0	10.6	9.2	87 %
7.0	5.1	4.8	94 %
3.0	3.1	3.1	100 %
0.5	1.9	1.7	89 %

Table 4: Determination of recovery after addition of defined progesterone solutions

A recovery of 87 % to 100 % was found.

Linearity

To determine linearity, a serum sample was diluted linearly in a sample buffer. The progesterone concentrations were calculated and then measured with the MiniReader. The recovery in % was calculated as the ratio of measured progesterone concentration to expected progesterone concentration.

Dilution	Expected P4-concentration [ng/ml]	Measured P4-concentration [ng/ml]	Recovery
1:2	8.8	8.2	93 %
1:4	4.4	4.9	111 %
1:8	2.2	2.5	114 %
1:16	1.1	1.2	109 %
1:32	0.6	0.5	91 %

Table 5: Determination of linearity of a serum sample

Summary

The MiniReader and MiniReader test kits are a thoroughly tested and very reliable method for measuring the progesterone level in canine serum samples. The results shown in ng/ml on the display allow an immediate assessment of the Progesterone level. The MiniReader is a valuable diagnostic tool for deciding further treatment of the animal.