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Validation of a New and Improved Progesterone Assay on the IMMULITE Immunoassay System

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Abstract

Background: Progesterone is a steroid hormone essential in the preparation for and maintenance of pregnancy. During pregnancy the placenta is the major source of the hormone. In non-pregnant females it is secreted mainly by the corpus luteum, and small quantities are secreted by the adrenal cortex in males and females. Progesterone levels are low during the follicular phase and increase sharply during the luteal phase of the menstrual cycle in females. Measurement of serum progesterone may provide valuable information on the adequacy of the luteal phase of the menstrual cycle, on the effectiveness of ovulation induction, and in monitoring patients at risk for abortion during the early weeks of pregnancy.

Methods: The new and improved progesterone assay (LKPW), already available on the IMMULITE® 2000 and 2000 XPi Immunoassay Systems (L2KPW), is now also available on the IMMULITE® and IMMULITE® 1000 Immunoassay Systems. The assay time has been shortened to 30 minutes from 60 minutes, sample volume has been reduced from 50 µL to 25 µL, and the low-end accuracy has been improved.

The test design is based on a competitive immunoassay format. Progesterone in patient sample competes with progesterone conjugated to bovine calf intestine alkaline phosphatase for binding to the progesterone antibodies coated onto the solid phase (bead). Unbound enzyme conjugate is then removed by a centrifugal wash. Finally, a chemiluminescent substrate is added to the bead and generates a signal which is inversely related to the amount of progesterone present in the patient sample. The assay range is 0.20–40 ng/mL. Two method comparisons were performed against the predicate device, the IMMULITE 2000/2000 XPi Progesterone Assay (L2KPW), using remnant samples from non-pregnant and pregnant females.

Results: For the method comparison performed at one site with three lots of the new IMMULITE Progesterone Assay (LKPW) on 168–172 samples, linear regression analysis yielded slopes of 1.08, 0.99, and 1.02, and correlation coefficients of 0.988, 0.991, and 0.986, respectively. For the method comparison performed at a second site using one IMMULITE kit lot and 400 remnant samples, the slope was 0.99 and the correlation coefficient 0.99. The three lots performed equivalently to the current IMMULITE 2000/2000 XPi Progesterone Assay.

Conclusion: The new progesterone assay for the IMMULITE and IMMULITE 1000 systems is comparable to the assay currently available on the IMMULITE 2000 and 2000 XPi systems, with a faster turnaround time and overall improved performance over the former assay.

[a]This assay has not been FDA cleared for veterinary applications.

Background

Progesterone levels vary throughout the menstrual cycle and multiple measurements may be used to help identify and manage some causes of infertility. Additionally, the hormone can be measured to determine if a woman has ovulated, when ovulation occurred, and monitor the success of induced ovulation. During early pregnancy, progesterone may be ordered, along with other tests, to help diagnose an ectopic or failing pregnancy as decreased levels are observed in such cases. The effectiveness of progesterone injections to help support early pregnancy may also be monitored. The test may also be used as an aid to diagnosis of abnormal uterine bleeding in non-pregnant women.[1]

This new assay was developed to match the performance of the progesterone assay that is currently available for the IMMULITE 2000 and 2000 XPi systems (L2KPW) for human and veterinary applications[a], including breeding management, as indicated in the literature.[2,3]

Materials and Methods

The assay design is based on a one-cycle, competitive immunoassay format. The serum progesterone sample of a subject is incubated with a bead coated with progesterone antiserum, and a reagent containing progesterone conjugated to bovine calf intestine alkaline phosphatase. Progesterone from the patient serum competes with progesterone alkaline phosphatase (Progesterone-AP) conjugate for binding to the antibodies on the solid phase. Unbound enzyme conjugate is then removed by a centrifugal wash. Chemiluminescent substrate is added to the beads in the final step, and a signal is generated, as shown in Figure 1.

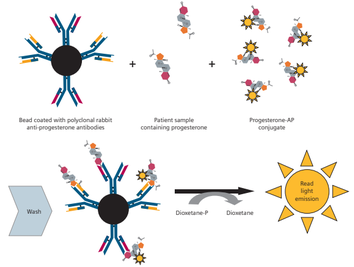


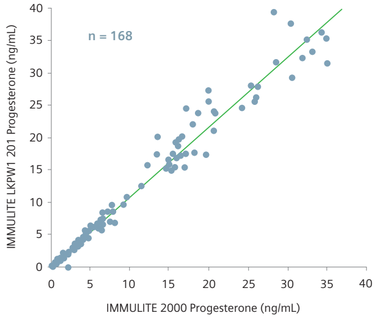
Figure 1. Schematic diagram of the IMMULITE Progesterone assay format.

This method replaced the former two-cycle Progesterone assay (LKPG) on the IMMULITE family of systems. Counts Per Second (CPS), detected by the system, are inversely proportional to the amount of progesterone present in the patient sample. The detectable range of the assay is from 0.2 to 40 ng/mL.

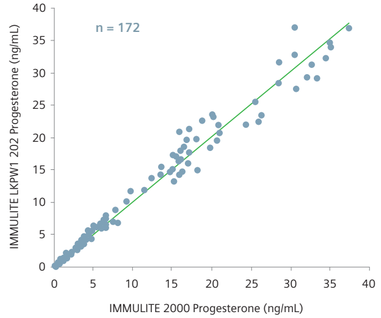
Three lots, LKPW 201, LKPW 202, and LKPW 203 were tested and compared to the predicate IMMULITE 2000/2000 XPi Progesterone assay (commercially available) in method comparison studies at two testing sites (only LKPW 203 was tested against the predicate at one of the sites). Remnant serum samples from pregnant and non-pregnant females collected in both red top and SST (serum separator tubes) blood collection tubes were tested. The results were analyzed using Deming regression or Ordinary Least Squares (OLS) analyses, and estimates for the slope and intercept included 95% confidence intervals.

Results

The method comparison study performed at the first testing site (Tarrytown, NY) included all three lots (LKPW201, LKPW 202, and LKPW 203), and 168-172 individual subject serum samples were run. OLS regression analyses yielded slopes of 1.08, 0.99, and 1.02, with correlation coefficients of 0.988, 0.991, and 0.986, respectively, as shown in Figures 2a,b and c.



a.



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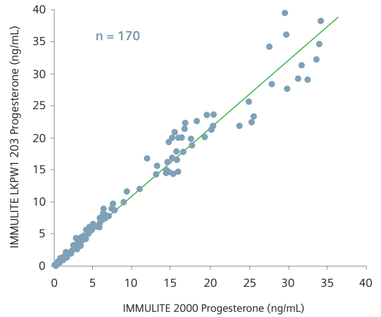

c.

Figure 2. Method comparison of three IMMULITE Progesterone Assay kits vs. the IMMULITE 2000/2000 XPi Progesterone Assay at site 1 (Tarrytown, NY).

At the second testing site (CRED; Sherbrooke, Canada), only one lot (LKPW203) was included in the study, and 400 individual subject serum samples were run. The Deming regression analysis yielded a slope of 0.99, with a correlation coefficient of 0.99, as shown in Figure 3. The samples included were both diluted and neat from pregnant and non-pregnant females.

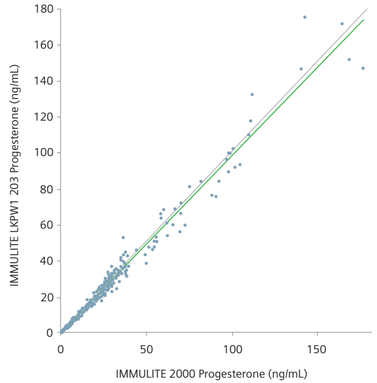

Figure 3. Method comparison of the IMMULITE Progesterone Assay (kit lot 203) vs. the IMMULITE 2000/2000 XPi Progesterone Assay (n=400) at site 2 (CRED; Sherbrooke, Canada)

Conclusions

The results presented in Figures 2 and 3 support the conclusion that the new Progesterone Assay for the IMMULITE and IMMULITE 1000, which has a faster turnaround time and overall improved performance over the former assay, is comparable to the IMMULITE 2000/2000 XPi Progesterone Assay.

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Acknowledgements

The authors would like to recognize the excellent technical contributions for this project from Joe Ma, Lora Lee Barreto, and Henry Wang from Siemens Healthcare Diagnostics Inc. in Tarrytown, New York.

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