Validation of Immulite® 2000XPI chemiluminescent immunoassay for sheep serum progesterone measurement

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Introduction

Monitoring circulating progesterone concentration (P4) is an important component of basic and applied reproduction research and clinical settings. Radioimmunoassay (RIA) is considered the gold standard method for measuring P4 in sheep blood, but it generates radioactive waste, has low throughput and requires licensed facilities. Immulite® 2000XPI (Siemens) is a fully automated immunoassay system marketed for human use to measure concentrations of different analytes including P4. Our objectives were therefore to: 1) validate sheep serum P4 measurement with the Immulite 2000XPI; and 2) characterize the analytical performance of the Immulite 2000XPI P4 immunoassay (IPI) across the reportable concentration range.

Materials and methods

The immunoassay validation protocols included characterization of the method linearity, and within-run and between-run precision through calculation of the coefficient of variation (CV). The method accuracy was assessed through the calculation of the recovery percentage and spiking-recovery (SR) bias, and the observed total error (Teo(R) = bias + 2CV) across the reportable range (0.2-40 ng/mL). A method comparison study was also performed using 141 serum samples from 30 Florida Native ewes. Passing-Bablok regression and Bland-Altman plots were used to determine the accuracy of the IPI against the reference method (RIA).

Results

The IPI was linearly related to the true value (R2 = 0.993) across the reportable range. The within-run precision CV for P4 of 0.5, 2, 5 and 10 ng/mL measured by IPI were 10.6%, 3.6%, 3.3% and 3.4%, respectively. The between-run precision for P4 of 0.5, 2, 5 and 10 ng/mL were 7.6%, 1.6%, 2.7% and 2.0%. The SR bias was 11.8%, 11.6%, -8.8% and 10.9% for P4 of 0.5, 2, 5 and 10 ng/mL, respectively. At 0.5 and 10 ng/mL, TEo was ~30% and ~20%, respectively (TEo remained similar regardless of the considered bias). The method-comparison study showed that the IPI measured P4 1.9 ng/mL (118.1%) higher than RIA. The results of this study recommend use of the following equation to correct the readings of IPI: serum P4 = 0.81 x (P4)IPI + 0.33.

Significance

Immulite 2000XPI P4 immunoassay provides a precise, accurate, and reliable safe method for measuring P4 in the serum of sheep. These analytical performance parameters should be considered in the interpretation of results and for future expert consensus discussions to determine recommendations for allowable total error (TEa).

