Validation of a commercially available radioimmunoassay and species-specific ELISAs to measure high concentrations of insulin in equine serum

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Reasons for performing study: Insulin in equine serum is commonly measured using a radioimmunoassay (RIA) validated using samples with low serum insulin concentrations. Recently problems have been reported, particularly when measuring high insulin concentrations. An equine enzyme-linked immunosorbent assay (ELISA) is now available which reportedly agrees well with RIA values from samples with low insulin concentrations.

Objectives: To validate the RIA and equine ELISAs for use with samples containing high insulin concentrations and to compare the different assay techniques.

Methods: Serum insulin concentrations were measured in equine samples using a commercially available RIA kit and a commercially available ELISA kit and values compared. The intra and inter-assay repeatability and dilutional parallelism for each assay was determined. The effect of standard and sample diluent and freeze-thawing, and cross-reaction with human C-peptide were determined.

Results: The mean ± SD intra-assay CV was 6.5 ± 5.1% and 10.6 ± 11.0%, and inter-assay CV was 7.4 ± 3.4% and 9.0 ± 4.6% for the RIA and ELISA respectively. Dilutional parallelism for the samples was observed using insulin-depleted saline (IDS), but not PBS, distilled water or zero standard for the RIA and using zero calibrator, but not IDS for the ELISA. The median (range) difference in insulin concentration after freeze-thawing was 0.94 μU/ml (-128.4 to 83.2 μU/ml) and 15.6 μU/ml (-343.1 to 514.4 μU/ml) for the RIA and ELISA respectively. There was no cross-reaction with human C-peptide. The mean ± SD bias between the ELISA and RIA was -18.5±25.5 μU/ml and -185.3±98.7 μU/ml for samples with measured insulin concentrations < or > 175 μU/ml, respectively.

Conclusions: Equine samples should be diluted with IDS and zero calibrator in the RIA and ELISA respectively. Freeze thawing has a variable effect. Samples with measured insulin concentrations <175 μU/ml in the ELISA agreed reasonably well with the RIA, but the bias increased as the measured insulin concentration increased.