ELISA ASSAY FOR KETAMINE

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Ketamine ((±)-2-(2-chlorophenyl)-2-(methylamino) Cyclohexanone) is a dissociative anesthetic used regularly in animal practice. Ketamine is classified as a Class 2 drug in the RCI Uniform Classification Guidelines for Foreign Substances (2001). The current methods for detection of ketamine employ instrumental methods, but due to complexity and cost, these methods are unsuitable for high volume screening of samples. Therefore, a user-friendly ELISA should prove a valuable screening method for the racing/veterinary industry.

For this assay, ketamine-specific antisera was produced in rabbits by injecting a protein:hapten immunogen. The drug hapten was linked to horseradish peroxidase to form a enzyme:drug conjugate. Antisera coated onto microwells were optimized with the enzyme conjugate using a TMB substrate system. Standard curves were generated by competing the conjugate with known levels of ketamine in buffer. The I-50 (measure of assay sensitivity) was optimized to 10 ng/mL.

Background studies using cleared equine and canine track samples revealed slight interferences with the assay. This interference was alleviated by diluting equine urine samples 1:4 with assay buffer and by diluting equine serum and plasma samples 1:1 with assay buffer.

Standard curves were generated using four common sample matrices (equine plasma, equine serum, equine urine and canine urine). The I-50 is 43 ng/mL in diluted equine plasma and 54 ng/mL in diluted equine serum. The I-50 in diluted equine urine is 95 ng/mL and 22 ng/mL in neat canine urine.

Cross-reactivities for several illegal drugs, therapeutic drugs, masking agents, vitamins, and drug vehicles were determined. Significant cross-reactivity was only seen with the primary ketamine metabolite, norketamine.

Equine administration samples were collected from a Standardbred mare after a single 100 mg dose of ketamine was administered IV. Urine samples were collected up to 24 hours post administration. Ketamine equivalents were detectable in the urine by 1 hour post-administration, peaked at 2 hours, and were still detectable at 8 hours post-administration.

An ELISA assay has been developed for ketamine that should allow for simple screening of race samples. The test is sensitive for the drug and its major metabolite in urine. The test is highly specific and interferences from common matrices can be easily alleviated with a small sample dilution.