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Analytical Services

PLASMA CORTISOL

GENERAL INFORMATION

Introduction: Plasma cortisol concentrations are determined using a double antibody radioimmunoassay. This in-house assay uses a commercial radioactive cortisol tracer (Bio-Mediq DPC Pty Ltd, Auckland).

Intended Use: Elevated cortisol concentrations are an indicator of physiological stress. The adrenal gland secretes glucocorticoids in response to the hypothalamic-pituitary-adrenal gland (HPA) axis activation. The window for collection from cows is from 30 mins to 4.5 hours after ACTH administration, and 30 to 90 mins after CRH administration (Verkerk et. al. 1994; Morrow et. al. 2002). Actual sample collection timing depends on hormone dosage, type of stress and experimental aim.

Normal Range Bovine: 1–200 ng/ml cortisol
Limitations of the Procedure: No single assay should be used as the only basis for arriving at a diagnostic conclusion. Faecal corticosterone and/or ACTH may be measured along with Plasma cortisol.

SAMPLE COLLECTION, PREPARATION AND STORAGE

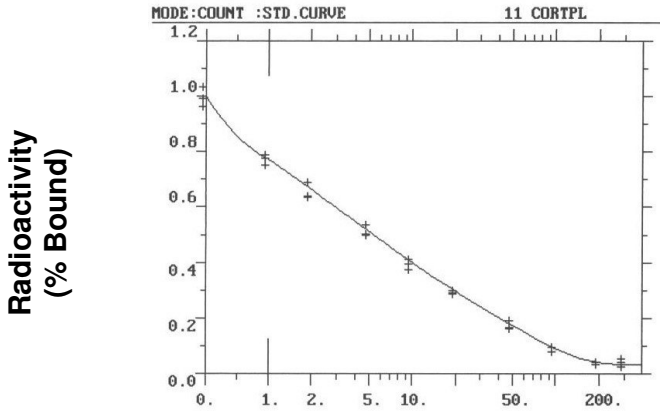
Type: Plasma
Collection: Blood is collected in heparinised green-top blood tubes and 1-2 ml plasma transferred to sample tube.
Storage: Samples should be frozen at –20°C or lower.

ASSAY

Method (Fisher et. al. 2002):

1. Standards, controls and samples are added to the tubes along with primary antibody and radioactive cortisol tracer to allow competitive binding.
2. Second antibody (pre-precipitated) is added to complex with and separate the bound cortisol antigen from the free antigen.
3. After centrifuging the pellet complexes, the supernatant is removed, leaving amounts of bound tracer antigen and sample antigen attached to the primary antibody.
4. Counts per minute in the resulting pellet are determined using a gamma counter. This is a competitive assay, i.e. the higher the concentration of cortisol in the sample, the lower the amount of radioactive cortisol detected.

Sensitivity: 1 ng/ml
Range of Assay: 1 –300 ng/ml
Typical Standard Curve:



Quality Assurance:
Three controls are repeated at every 100 samples throughout the assay to monitor within assay and between assay variation.

	High 80 ng/ml	Medium **45 ng/ml**	**Low 5 ng/ml**
Within Assay CV	12.0%	12.1%	13.6%
Between Assay CV	6.4%	6.7%	7.7%

REFERENCES

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