

EFFECT OF AFFINITY PURIFICATION ON THE SENSITIVITY AND PRECISION
OF A SIMPLIFIED RAPID ENZYME LINKED IMMUNOSORBENT ASSAY
(ELISA) FOR DEXAMETHASONE

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Two colorimetric ELISA methods (3 and 2 step) have been investigated for dexamethasone which required 2.15 h or 1.05 h total assay time. The first was a conventional 3 step indirect competitive ELISA using alkaline phosphatase labelled second antibody on 10 µgml⁻¹ coating concentration of the drug conjugate.

A new two step method to be reported was a premix of first and second antibody (single reagent) at 1:1000, 1:125 and 1:1000, 1:250 dilution, respectively in equal volumes. The limit of detection (LOD) with respect to 50% reduction in back ground signal for 3 step unpurified and purified antiserum were found to be 1.3 x 10⁻⁹ and 5.0 x 10⁻¹¹ mol l⁻¹ and for 2 step unpurified antiserum at 1:1000, 1:125 and 1:1000, 1:250 Ab₁ and Ab₂ concentrations were found to be 1.5 x 10⁻⁸ and 1.1 x 10⁻⁸ mol l⁻¹ respectively. The two step ELISA is more quicker than the 3 step ELISA which could be completed in 65 min. compared with 135 min. respectively.

Keywords: Enzyme Linked Immunosorbent Assay (ELISA).

Introduction

Dexamethasone is a synthetic glucocorticoid which is used widely in veterinary medicine and is given orally, parenterally and local injection, by inhalation and as applied topically in the management of various disorders in which corticosteroids are indicated. Its lack of mineralocorticoid properties makes dexamethasone particularly suitable for treating cerebral oedema and congenital adrenal hyperplasia (1, 2).

It is a potent anti-inflammatory agent and has become a popular alternative to proper rest to keep horses in training. Clinical signs of inflammation including

heat, swelling and pain are markedly reduced with its use even though complete healing has not occurred consequently, racehorses kept in training without sufficient rest or treated with the drug for prolonged periods suffer in human and adverse reactions, reaction.

Its use may provide an unfair advantage in performance for racing horses and methods of screening for its presence in equine urine and serum must be available. For this reason, a method for detecting the use of dexamethasone and related steroids in horses is necessary.

Precision: Within assay precision was determined for unpurified and affinity purified antiserum (n=4) by three step ELISA by using dexamethasone samples of 1.0×10^{-9} and 1.0×10^{-8} mol l⁻¹ and for two step ELISA within (n=12) and between assay (n=6) precisions were determined for unpurified antiserum. by using the 1.0×10^{-9} , 1.0×10^{-8} and 1.0×10^{-7} mol l⁻¹ standards of dexamethasone.

Sensitivity: The ELISA was performed with dexamethasone standards and 24 sample blanks (assay buffer). The mean standard deviations of the blanks were used to estimate the limit of detection of dexamethasone from the standard curve at 95% confidence level (i.e the concentration of dexamethasone at the absorbance at zero mean minus 2x s.d. of zero) and the concentrations of dexamethasone corresponding to 50% signal reduction were also determined for each assay.

Results

Standard curves: The standard curves and percentage signal curves for three step and two step ELISA for unpurified and purified antiserum are shown in Figs 1-8. The steepest part of standard curve for 3 step ELISA was between 1×10^{-9} mol l⁻¹

and 1×10^{-8} mol l⁻¹ and for two step ELISA 1×10^{-9} mol l⁻¹ and 1×10^{-7} mol l⁻¹ dexamethasone respectively.

Precision: The relative standard deviation (*s_r*) for unpurified and purified antiserum for three step ELISA were 3.5%, 2.10% (n=4) and 13.92%, 10.45% (n=6) for the 1×10^{-9} mol l⁻¹, 1×10^{-8} mol l⁻¹ dexamethasone standards respectively. The *Sr* for two step ELISA for unpurified antiserum were 2.81, 4.97, 7.22% (n=4) for 1:1000, 1:125 Ab₁ and Ab₂ dilution and for 1:1000, 1:250 were 3.80, 3.95 and 7.19% (n=12) respectively for 1×10^{-9} mol l⁻¹, 1×10^{-8} mol l⁻¹ and for 1×10^{-7} mol l⁻¹ within assay precision and 2.68, 2.88, 6.20% (n=6) were also calculated for between assay precision for the same standards in the two step ELISA (Table 1).

Sensitivity: The limit of detection (LOD) at 95% confidence for 3 step unpurified and purified antiserum and for 2 step unpurified antiserum are shown in tables 1 and 2. The concentrations of dexamethasone which produced 50% signal reduction are shown in table 2.

Table 1. Precision and limit of detection (LOD) at 95% confidence.

Assay format	Within Assay <i>Sr</i> (%)			LOD (95%) (mol l ⁻¹)	Between Assay <i>Sr</i> .(%)		
	1.0×10^{-9} (mol l ⁻¹)	1.0×10^{-8} (mol l ⁻¹)	n		1.0×10^{-9} (mol l ⁻¹)	1.0×10^{-8} (mol l ⁻¹)	n
3 step (unpurified 1:1000, 1:500)	3.5	2.10	4	2.0×10^{-11}	4.8	8.8	4
3 step (purified 1:1000, 1:500)	13.92	10.45	6	1.0×10^{-11}	2.7	9.4	3
2 step (Ab ₁ , Ab ₂ = 1:1000, 1:250 unpurified)	2.81	4.97	4	4.0×10^{-11}	-	-	-
2 step (Ab ₁ , Ab ₂ = 1:1000, 1:250 unpurified)	3.80	3.95	12	5.0×10^{-11}	2.68	2.88	6

Table 2. Limit of detection (LOD) and concentrations of drug producing 50% signal reduction

Method	LOD (mol l ⁻¹)	50% signal Conc (mol l ⁻¹)
3 Step (unpurified)	1.5 x 10 ⁻¹¹	1.3 x 10 ⁻⁹
3 Step (purified)	8.0 x 10 ⁻¹²	5.0 x 10 ⁻¹¹
2 Step (unpurified) (1:1000, 1:125 Ab ₁ & Ab ₂)	3.0 x 10 ⁻¹¹	1.5 x 10 ⁻⁸
2 Step (unpurified) (1:1000, 1:250 Ab ₁ & Ab ₂)	4.0 x 10 ⁻¹¹	1.1 x 10 ⁻⁸

Discussion

During the investigations, the three step and two step assays were performed by using unpurified and affinity purified antiserum at different antibody dilutions (Table 1) on pre-coated plates with 10 µg ml⁻¹ of the drug conjugate. The results for the three and two step assay formats have been shown in table 1 and figures 1-4. The results for the three step assay indicated that the affinity purified antibodies showed lower background signal than the unpurified antibodies (Figs 1, 2) and was more sensitive than the unpurified antiserum, due to the fact that the affinity purification also removed the PTG-binding antibodies. The steepest part for unpurified and affinity purified antiserum (1:1000, 1:500 Ab₁: Ab₂ dilutions) were found between the concentration ranges of 1.0 x 10⁻⁸ - 1.0 x 10⁻⁹ and 1.0 x 10⁻¹⁰ - 1.0 x 10⁻¹¹ mol l⁻¹ of dexamethasone respectively. The concentrations of dexamethasone which produced 50% reduction in signal have also been shown in table 2 and figures 5, 6 and were 1.3 x 10⁻⁹ and 5.0 x 10⁻¹¹ mol l⁻¹ respectively. These results also indicate that the three step assay format with affinity purified antibodies showed the better sensitivity.

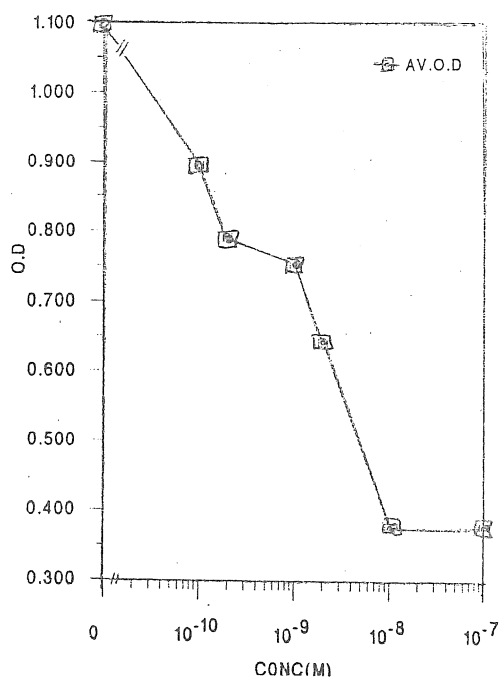


Fig. 1. Standard curve of dexamethasone for three step ELISA for unpurified antiserum (1:1000, 1:500 Ab₁ & Ab₂ dilutions)

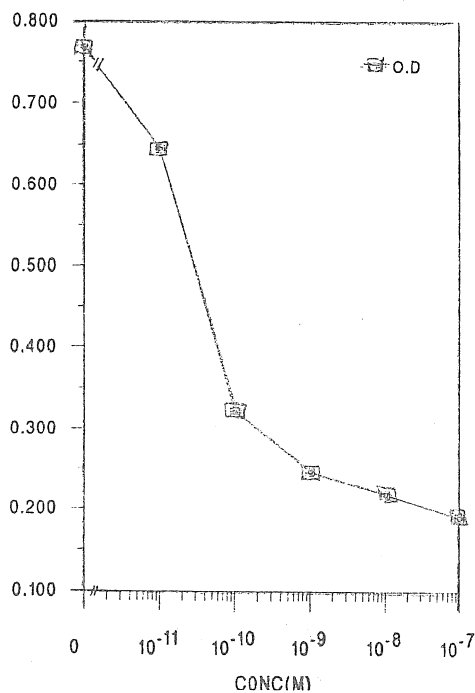


Fig. 2. Standard curve of dexamethasone for three step ELISA for affinity purified antiserum (1:1000, 1:500 Ab₁ & Ab₂ dilutions)

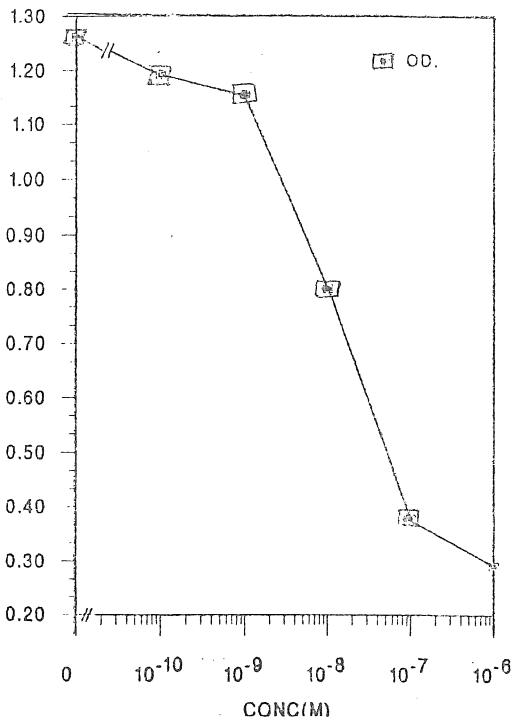


Fig. 3. Standard curve of dexamethasone for two step ELISA for unpurified antiserum (1:1000, 1:125 Ab₁ & Ab₂ dilutions)

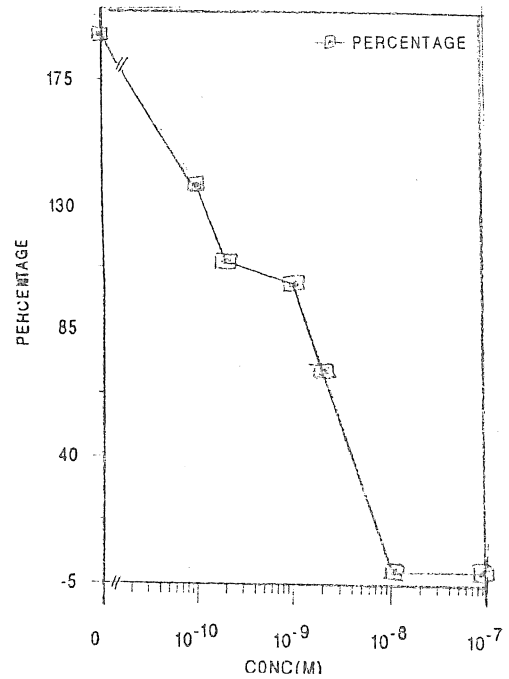


Fig. 5. Graph between conc. and percentage signal for three step ELISA for unpurified antiserum (1:1000, 1:500 Ab₁ & Ab₂ dilutions)

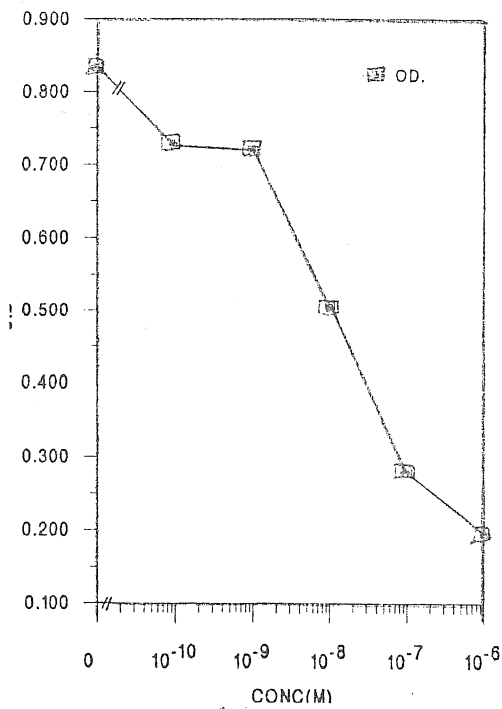


Fig. 4. Standard curve of dexamethasone for two step ELISA for unpurified antiserum (1:1000, 1:125 Ab₁ & Ab₂ dilutions)

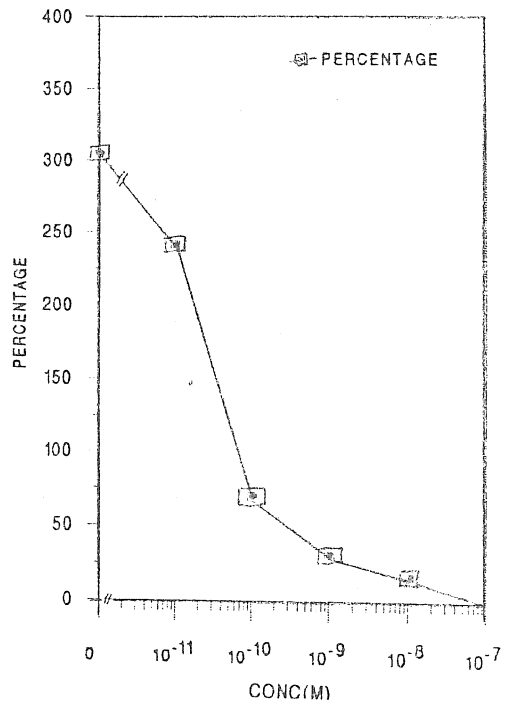


Fig. 6. Graph between conc and percentage signal for three step ELISA for affinity purified antiserum (1:1000, 1:500 Ab₁ & Ab₂ dilutions)

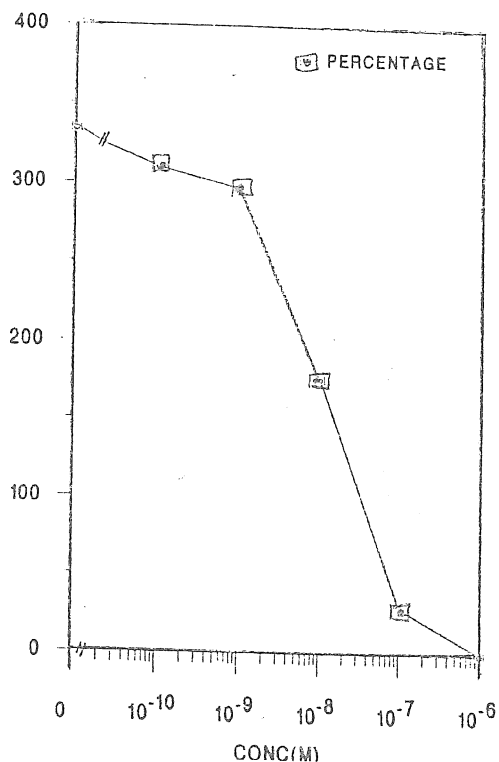


Fig. 7. Graph between conc. and percentage signal for two step ELISA for unpurified antiserum (1:1000, 1:125 Ab₁ & Ab₂ dilutions)

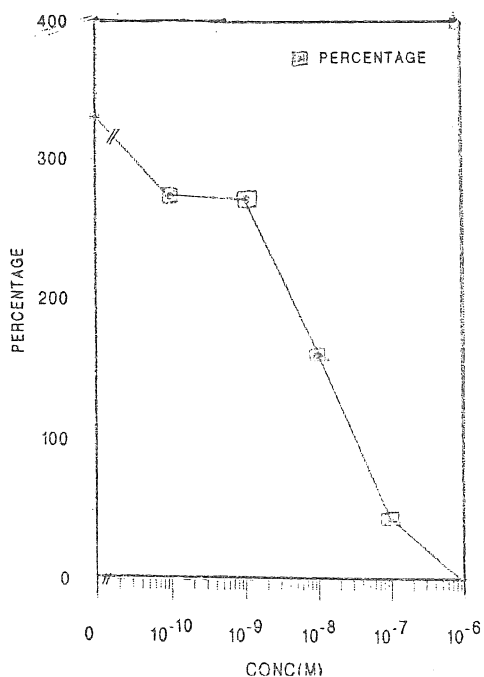


Fig. 8. Graph between conc. and percentage signal for two step ELISA for unpurified antiserum (1:1000, 1:250 Ab₁ & Ab₂ dilutions)

The results for two step ELISA for unpurified antiserum have also been shown in table 1 and Figs 3,4. In this study, the two different antibody concentrations, i.e. 1:1000, 1:125 and 1:1000, 1:250 Ab₁ : Ab₂ were used as a premix in equal volumes (single reagent in order to study the sensitivity and precision data). The results indicate that both antibody dilutions showed the same trend in linearity between the concentration ranges of dexamethasone 1.0×10^{-7} to 1.0×10^{-9} mol l⁻¹ (Figs 2, 3). The concentrations of dexamethasone which produced 50% reduction in signal have also been shown in table 2 and figures 7, 8 and were 1.5×10^{-8} and 1.1×10^{-7} mol l⁻¹ respectively. The two step ELISA for affinity purified antiserum with the premix of single reagent, 1:100, 1:250 dilution of 1st and 2nd antibody in equal volumes, showed linearity between 1.0×10^{-6} and 1.0×10^{-8} mol l⁻¹ and it also showed lower blank values than the unpurified antiserum (O.D. = 0.50 and 0.840 respectively).

The total assay time to complete the three step and two step ELISAs were 125 and 65 minutes respectively. The two step ELISA was quicker than the three step ELISA and it was also quicker than the reported ELISA (9) which require 2.5 h.

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