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Homologous Assay Development for Detection of Dexamethasone using HRP as Label Enzyme

Kiran Rangari[1]*, Dinesh Kumar[2], Rekha Meena[3], Divya Verma[4], T.G. Shrivastav[5]

[1]Reader, Department of Reproductive Biomedicine, The National Institute of Health and Family Welfare, New Delhi, India
[2]Technical Assistant, Department of Reproductive Biomedicine, The National Institute of Health and Family Welfare, New Delhi, India; & Assistant Director, FSSAI, New Delhi, India
[3]Research Officer, Department of Reproductive Biomedicine, The National Institute of Health and Family Welfare, New Delhi, India
[4]Ph.D Scholar, The National Institute of Health and Family Welfare, New Delhi, India; & Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi, India
[5]Former Professor, Department of Reproductive Biomedicine, The National Institute of Health and Family Welfare, New Delhi, India

***Address for Correspondence:** Dr Kiran Rangari, Reader, Department of Reproductive Biomedicine, The National Institute of Health and Family Welfare, New Delhi-110067, India
E-mail: kiranrangari@nihfw.org

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ABSTRACT

Background: Dexamethasone is a synthetic corticosteroid similar to cortisol produced naturally by the adrenal glands. As an anti-inflammatory and immunosuppressive agent, it is used in many diseases such as rheumatoid arthritis and allergic anaphylactic shock, and its suppression test to diagnose Cushing's syndrome. Its further use includes its administration before antibiotics in bacterial meningitis, antitumor treatment, for treatment of glucocorticoid resistance, Addison's disease, and congenital adrenal hyperplasia. The drug is abused by using it in animal husbandry as a growth promoter and in horse sports to enhance their performance.

Methods: In this study, the development of homologous ELISA using Dexamethasone-21-hemisuccinate (DEX-21-HS)-Bovine serum albumin antiserum and Dexamethasone-21-hemisuccinate (DEX-21-HS)-Horseradish peroxidase enzyme conjugate has been done. The n-hydroxysuccinimide ester method was used to prepare the immunogen and enzyme conjugate.

Results: The sensitivity 0.25 ng/mL, affinity $2.8x10^{-8}$ L/mol and ED_{50} 4.98 ng/mL of the assay were found. The cross-reactivity of the assay was checked and found with three steroids (Corticosterone- 1.13%, Progesterone- 2.25% and Prednisolone- 6.3%) out of 48 structurally related steroids. Then, analytical variables of the developed assay were studied, such as recovery (98.55% to 105.08%), precision (Inter and Intra- assay coefficient of variation <9.28%), correlation (R^2= 0.98) by utilizing a commercially available Dexamethasone kit for comparison.

Conclusion: This study concluded that low-cost indigenous ELISA for Dexamethasone had been developed, which can give results within 75-80 minutes.

Key-words: Bovine serum albumin, Enzyme conjugate, Dexamethasone, Homologous ELISA, Horseradish peroxidase, Immunogen

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INTRODUCTION

Dexamethasone is a synthetic corticosteroid that resembles the natural hormone cortisol produced by the adrenal glands. It is used as an immunosuppressant and has anti-inflammatory properties. It is used in many diseases, such as rheumatoid arthritis, respiratory diseases, and allergic anaphylactic shock [1–3]. The dexamethasone suppression test is used to diagnose Cushing syndrome [4].

Its other uses include administration before antibiotics in case of bacterial meningitis, to neutralize effects of antitumor treatment, for treatment of glucocorticoid resistance, Addison's disease, and congenital adrenal hyperplasia [5–9].

The drug abuse of Dexamethasone involves its use in animal husbandry as a growth promoter and in horse sports to enhance the performance of horses. Due to its high potency, Dexamethasone is administered in low doses, resulting in low residue levels in biological fluids. Several techniques are present for the determination of 17α-methyltestosterone levels. Its detection was developed by [10] using Radioimmunoassay (RIA), which is a hazardous and lengthy process. LCMS, LCMS/MS, HPLC, and GCMS have been used to detect this drug in different types of samples.

Because of the complex process of these methods mentioned above, they cannot be utilized as a screening system for steroid detection. Therefore, the need was to develop antibody-based, cost-effective, sensitive, specific, and accurate ELISA to detect Dexamethasone levels in food, soil, water, body fluids, and tissue samples.

MATERIALS AND METHODS

The Institutional Animal Ethics Committee (IAEC) at the NIHFW in New Delhi, India, gave ethical consent for this study. Experiments and investigations were conducted at the Institute's Reproductive Biomedicine Laboratory in 2020.

Chemicals and Reagents- Only pure analytical-grade chemicals, salts, and solvents were used in this experiment. *Sigma Chemical, St. Louis, MO, USA* was the source for complete and incomplete Freund's adjuvant, Bovine serum albumin, Dioxan, Dimethyl formamide, 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride (EDAC), N-hydroxysuccinimide (NHS), tetramethylbenzidine/ hydrogen peroxide (TMB/H_2O_2), and thimerosal. Ammonium sulphate, Activated Charcoal, Acetic acid, Dextran, Hydrochloric acid, Sodium Phosphate dibasic, Sodium Chloride, Sodium phosphate monobasic dehydrate, sodium acetate, Sodium azide, Sucrose and Tri-sodium citrate 2-hydrate were obtained from Merck (India) Pvt. Ltd. All Steraloids Inc., Newport, USA supplied the steroids used in the experimentation. HRP was obtained from Bangalore Genei (Bangalore, India). Greiner ELISA plates were used for this study. Syringes were of Dispovan. Variable volumes of micropipettes and multi-stepper pipettes were obtained from Thermo Scientific™ Finnpipette™. Syringe filters were acquired from *Axiva*.

Buffers

a) **Phosphate buffer (10 mM; pH 7.0)**- contains $NaH_2PO_4.2H_2O$: 0.39 g, $Na_2HPO_4.2H_2O$: 0.895 g, 0.1% NaN_3 and 0.9% NaCl for the volume of one Litre.
b) **Antibody dilution buffer (10 mM)**- contains 0.52 g $NaH_2PO_4.2H_2O$, 1.1 g $Na_2HPO_4.2H_2O$, 90 g Sucrose, 100 g ammonium sulphate, 0.5 g sodium azide and 2 g BSA for one Litre volume.
c) **Enzyme conjugate buffer/EC Buffer (10 mM; pH 5.6)**- contains 1N CH_3COOH 1.5 ml/l, CH_3COONa 0.84 g, 0.1% Dextran T-70, 0.3% BSA, and 0.1% Thimerosal for one Litre.
d) **Blocking buffer (10 mM)**- contains 0.2% BSA, 0.9% NaCl, 0.1% Thimerosal, 0.1% Dextran T-70, 0.01% gentamicin sulphate, 0.1% EDTA, and dipotassium salt.

Instruments

- Harrison lyophilizer (India)
- Thermo Scientific™ UV-Visible Double beam Spectrophotometer "Evolution 220" (USA)
- "Tecan Infinite" ELISA plate reader (Tecan Group Ltd., Mannedorf, Switzerland)

Immunogen (Dexamethasone-21-HS-BSA) preparation- BSA was attached with Dexamethasone-21-hemisuccinate (DEX-21-HS) by NHS/carbodiimide method with few modifications as depicted by [11]. 10 mg DEX-21-HS was dissolved in 400 µl DMF and 400 µl of dioxin. The mixture was vortexed and overnight activation was accomplished at 4ºC. Next, a slow, swirling addition of the activated steroid was made to the BSA solution (1 mg BSA in 300 µL distilled water). The reaction mixture was vortexed again and kept at 4°C overnight. Following that, the DEX-21-HS-BSA conjugate got dialyzed three times against deionized water. The residue was removed by centrifugation. The dialysate was collected, lyophilized, and kept at 4°C in 1 mg aliquots.

Immunization of rabbit and collection of antiserum- The DEX-21-HS-BSA antibody was produced in New Zealand

(NZ) white rabbits using the Shrivastav *et al.* [12] approach. A fresh emulsion of 0.5 mL Freund's complete adjuvant and 0.5 mL saline containing 1 mg of immunogen (DEX-21-HS-BSA) was prepared. The rabbit injected 250 µl of emulsion intramuscularly into each of its limbs. On days 7, 14, 21, and 28, repeated injections of emulsions were administered intramuscularly. After the initial five injections, booster doses were administered every 30 days. Beginning with the initial booster dose, blood was routinely drawn from the ninth to the fourteenth day after the booster injection. Blood was centrifuged for 10-15 minutes at 5000 rpm; antiserum recovered was divided into 1 ml aliquots and kept at -30° C.

Normal rabbit serum (NRS) collection- NRS was obtained from non-immunized NZ white rabbits and stored with the process indicated in the previous section.

Enzyme conjugate (DEX-21-HS-HRP) preparation- A modified active ester approach [13] was used to link DEX-21-HS to HRP. 200 µl dioxin and 200 µl DMF were mixed with 5 mg of DEX-21-HS. 100 µl of water containing 10 mg of NHS and 20 mg of EDAC were added to this solution. At 4°C, the reaction mixture was activated for 24 hours. The aqueous HRP (1 mg/3 ml) was mixed with an activated solution of DEX-21-HS and stored at 4°C for one day. The reaction mixture was then run through an equilibrated 10 mM PBS-filled Sephadex G-25 column (gel filtration). To preserve the enzyme activity, 1% (w/v) sucrose, BSA, ammonium sulfate, and the same amount of ethylene glycol were added to the pooled, brown-colored fractions. Aliquots were stored at -30°C.

Checkerboard Assay

Coating of primary antibody- Following the Dutta *et al.* [14] procedure, the ninety-six well microtitre plate was coated with primary antibody immobilization utilizing the immunobridge technique. 250 µl of diluted NRS was added to each well and incubated overnight at 37°C. After incubation, running water rinsed the plate three to four times. 250 µl of diluted goat's anti-rabbit gamma globulin (1:1000) was dispensed into the NRS-coated wells and incubated at 37°C for 2 hours. The plate's contents were then decanted and washed with running water. An ARGG-coated eight-well strip was incubated overnight at 4°C after adding 150 µl of buffer no. 2 to test NSB. In the buffer above, the antiserum was serially diluted as 1:500, 1:1000, 1:2000, and 1:4000. After removing the remaining unabsorbed antibody, the remaining unoccupied sites were blocked with 250 µl of blocking solution and incubated at 37°C for one hour. The plate was dried after decanting the contents and kept at 4°C.

Evaluation for immunoreactivity and determining the optimal loading of primary antibody and HRP conjugate- DEX-21-HS-HRP (100 µl) was diluted 1:500, 1:1000, 1:2000, and 1:4000 in EC buffer and added to wells (1 in 2 dilutions in the vertical pattern). The incubation time was one hour at room temperature. Following incubation, the contents of the wells were decanted and washed twice or three times in running tap water. Each well received 100 µl of TMB/H2O2 and underwent incubation for 15 minutes at room temperature. After stopping the reaction with 100 µl of 0.1 N HCl, the colour intensity at 450 nm was measured using an ELISA plate reader. The antiserum dilution with the least non-specific binding and the highest binding to the corresponding enzyme conjugate dilution was chosen for further studies.

Preparation of calibrators /working standards- Working standards for Dexamethasone (0, 1, 2, 5 10, 25, 50, and 100 ng/ml) were made in buffer or charcoal-treated Serum using Basu et al. procedure [15]. 5% charcoal was added to the Serum and mixed overnight at 4°C for the charcoal treatment. This solution was centrifuged at 3000x g before filtering through a 0.45 µm syringe filter to remove the charcoal.

Assay procedure- Following the Verma [16] procedure,100 µl of standards/samples (0-100 ng/ml) were added to the DEX-21-HS-BSA antiserum-coated microtitre wells. Then 100 µl of DEX-21-HS-HRP was also added to each well. After then, the wells were kept at RT for an hour. The wells were filled, decanted, and flicked many times, while rinsed under running water to eliminate any remaining enzyme conjugate. TMB/H_2O_2 substrate (100 µl) was added to each good incubation at room temperature for 15 minutes. ELISA reader was then used to read the developed yellow colour after adding 100 µl 0.1 N HCl (stop solution) to the wells.

Validation of Dexamethasone immunoassay- Sensitivity, ED_{50}, affinity, specificity (cross-reaction), recovery,

precision, and correlation coefficient were determined as a part of the analytical validation of the assay. The 'IMMUNOCAL' program measured unknown recovery and precision sample concentrations. The correlation coefficient was calculated by comparing the results of the estimation of Dexamethasone in Serum obtained by established ELISA and commercial ELISA.

Sensitivity is the assay's ability to detect the smallest amount of the target analyte under the prescribed standard conditions. The lower detection dose (LDD) is the concentration of analyte (A) that produces a statistically significant change in response than would be expected if analyte (A_0) was not present. After determining A_0 32 times, it is determined as A_0 -2 x S.D [17].

Specificity- The capacity of an assay to identify just the desired analyte in a heterogeneous mixture is known as specificity[18]. Cross-reactivity is the threshold when the assay signal is reduced to 50% of what it would be in the absence of the analyte. It is measured in terms of 'percentage cross-section.

$$\%\text{Cross} - \text{reactivity} = \frac{\text{Concentration of standard at } 50\% \frac{A}{A0}\,(S)}{\text{Concentration of cross} - \text{reactant at } 50\% \frac{A}{A0}\,(C)} X100$$

where, S- Standard and C- cross reactants

Precision is a statistical measure of sample variance in repeated measurements of the same sample. It is also known as reproducibility. Precision measures variability between consecutive analyses of the same sample within and between the assays. The formula used is:

CV % = S. D / mean x 100

Recovery- A recovery study evaluates a test's ability to compute a sample's increasing amount of a standard analyte. As a result, the concentration of the recovered analyte (C) is determined by combining a known analyte concentration (A) with base (B) after the assay. The formula is: **(C-B)/A x 100**

Dexamethasone was added to 10 mL aliquots of seven serum pools at concentrations of 0.75, 1.5, 3.5, 7.5, 15, and 30 ng/mL. The % recovery was calculated as previously described.

Statistical Analysis- Microsoft Excel prepared composite dose-response curves using the Dexamethasone antibody and Dexamethasone enzyme conjugate combination. Standard concentrations were plotted on a log scale of the X-axis, and A/A0 X-100 (absorbance obtained at different standard concentrations divided by absorbance of zero standard concentration) was plotted on Y-axis. Each calculated Value is the mean±S.D. of eight assays. The "IMMUNOCAL" program (in-house developed) was used to deduce the unknown concentrations from Serum. The logit log graph and Scatchard plot were prepared using Microsoft Excel.

RESULTS

Immunoreactivity (Binding)- It was found that 1:500 dilution of Dexamethasone-21-HS-BSA Ab displayed greater binding and least NSB (non-specific binding) with 1:1000 dilution of Dexamethasone-21-HS-HRP enzyme conjugate.

Dose-Response study- Fig. 1 depicts the composite dose-response graph of Dexa-21-HS-BSA and Dexa-21-HS-HRP. The Dexamethasone concentration (ng/mL) is represented as log values on the X-axis, while the A/A0 ratio of standards is plotted on the Y-axis. MS Excel was used to create the graph. The CV% for each standard's A/A0 ratio ranged from 3.23% to 9.77%. The standard curve equations for ELISA relationships were logit-log transformed as y = -1.46x + 1 ng/mL. The affinity constant (1.8 X 10^{-8} L/mol) of the Dexamethasone antibody for Dexamethasone was determined by Scatchard plot (Table 1).

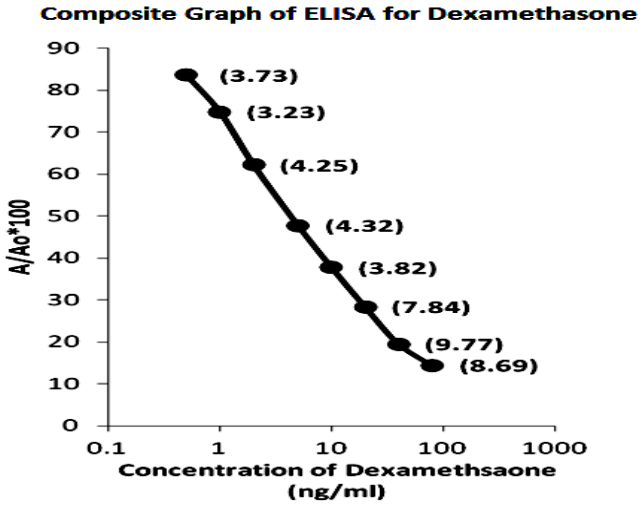

Fig. 1: Composite dose-response curve of homologous ELISA of Dexamethasone using Dexa-21-HS-BSA-Ab and Dexa-21-HS-HRP enzyme conjugates. Each Value is a mean±SD of 8 assays (In duplicate). The coefficient of variation at each concentration is shown in parentheses

Sensitivity- Sensitivity was found to be 0.25 ng/mL (LDD), and ED_{50} calculated was 4.98 ng/mL (Table 1).

Table 1: Analytical Parameters of Homologous ELISA of Dexamethasone

Combination of Assay	Slope and intercept		Sensitivity (ng/mL)	Affinity (L/mol)	ED_{50} (ng/mL)
	m	c			
Dexa-21-HS-BSA Ab with Dexa-21-HS-HRP	-1.46	1.00	0.25	2.8×10^{-8}	4.98

Specificity- Among the 48 steroids checked for cross-reaction, only Corticosterone, Progesterone and Prednisolone showed cross-reactivity of 1.13%, 2.25%, and 6.3%, respectively. The list of all 48 steroids is provided as a supplementary table.

Table 2: Steroids showing percentage cross-reaction with Dexamethasone

Steroid Measured	Cross-reactions (%)
Progesterone	2.25
Corticosterone	1.13
Prednisolone	6.3

Precision and Recovery- The CV% for Inter and Intra assay was <9.28 (Table 3). The Recovery percentage ranged from 98.55% to 105.08% (Table 4).

Table 3: Inter and intra-assay coefficient of variation for the measurement of Dexamethasone in serum pools

Variation	Mean ± S.D.	Coefficient of variation (%)
Intra assay (*n* = 8)	0.57± 0.05	9.28
	1.39 ± 0.11	8.11
	2.11 ± 0.11	8.70
	4.15 ± 0.35	8.53
	7.67 ± 0.60	7.80
	15.38 ± 1.42	9.26
	33.20 ± 2.52	7.58
Inter-assay (*N*= 8)	0.63 ± 0.04	6.39
	1.36 ± 0.11	7.81
	2.10 ± 0.19	8.85
	4.34 ± 0.27	6.18
	8.15 ± 0.73	8.94
	15.76 ± 1.07	6.77
	30.33 ± 2.54	8.37

N= No. of times assays carried out; n =No. of times same sample tested.

Correlation Coefficient- Using our developed ELISA and a commercially available ELISA for Dexamethasone (catalog number E13650603, purchased from Sincere Biotech Co. Ltd., Beijing, China), levels of Dexamethasone in serum samples (n=54) were determined, and the coefficient of correlation was R^2=0.98. Figure 2 shows how the method efficiently fit linear data to 2-D data when X and Y were measured with errors that could account for changes in estimating errors among the test and the standard method. The regression graph was plotted using Graph Pad Prism 6.0 software.

Table 4: Recovery of Dexamethasone from spiked serum pools

Serum pools	Addition of Dexamethasone (ng/ml)	Observed Value (ng/ml)	Expected Value (ng/ml)	Recovery (%)
A (Basal)	–	0.63	-	-
B	0.75	1.36	1.38	98.55
C	1.50	2.10	2.13	98.59
D	3.50	4.34	4.13	105.08
E	7.50	8.15	8.13	100.24
E	15.00	15.76	15.63	100.83
F	30.00	30.33	30.63	99.02

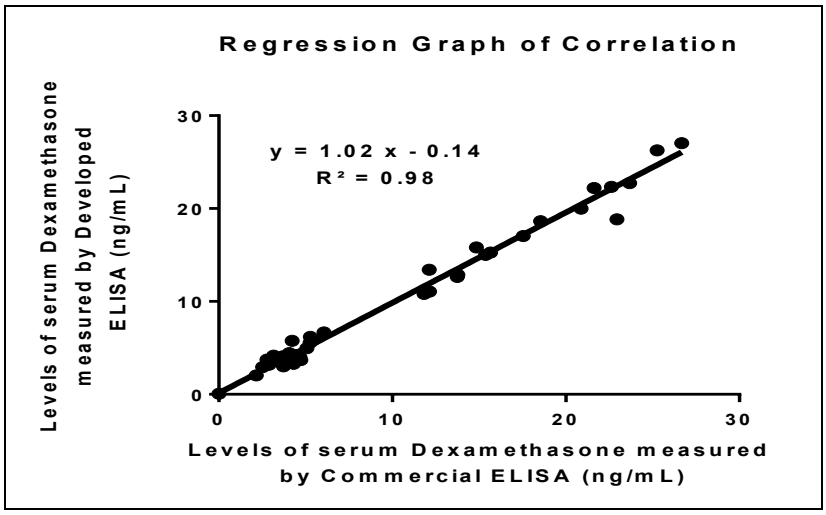

Figure 2: Correlation Coefficient for Dexamethasone

DISCUSSION

Several techniques are available at present for 17α-methyl testosterone detection like, High-Performance Liquid Chromatography, Gas Chromatography, Liquid Chromatography, and Mass Spectrometry; Immunoassays, such as CLIA, RIA, LFIA, and ELISA. The physical methods involve effort, are time-consuming, extra steps, and extensive processing of samples, and require more sample volume (1-2 ml), trained personnel, heavy instrumentation, and machines. RIA needs permission, and health hazards occur due to using radioactive materials. LFIA and CLIA provide only qualitative information. In Competitive ELISA, the competitive reaction is observed among sample antigen and coated antigen with 1° antibody. Less colour means more antigen exists in the sample, so a competitive assay determines even a low amount of antibody [19].

There are several studies on the detection of Dexamethasone by using different methods and techniques, but they show less sensitive results than our ELISA. There are studies on detecting Dexamethasone by ELISA, but they are indirectly competitive and antigen-based; no study of direct competitive ELISA has been reported. The results of this study using Dexa-21-HS-BSA Ab and Dexa-21-HS-HRP EC give very good results. LDD of the present assay was estimated at 0.25 ng/mL and ED_{50} at 4.98 ng/mL, which is very low as compared to earlier Dexamethasone studies of Song which show sensitivity 10 ng/mL by using HPLC followed by immunoaffinity chromatography [20]. Wang *et al.* [21] showed a 0.3 µg/Kg sensitivity in chicken muscle and 0.5 µg/Kg in the chicken liver. Our assay showed good sensitivity, which can correlate well with the small doses of Dexamethasone. The composition and structure of molecules and antigen-antibody bounded affinity interactions are the foundation for developing sensitive and specific ELISA. The sensitivity of an ELISA is governed by the conjugation chemistry involved in the production of immunogen. Li

detected Dexamethasone in milk and pork using lateral flow immunoassays and latex microspheres with a visual detection limit of 0.3 ng/mL in milk and 0.7 µg/Kg in pork. They showed cross-reactions with Prednisone (14%), Hydrocortisone (1.5%), and Betamethasone (24%) [22].

The percentage cross-reaction (specificity) of Dexa-21-HS-BSA antibody was estimated with commercially available structurally similar steroids. Among the 48 steroids checked for cross-reaction, only three (Corticosterone, Progesterone and Prednisolone) showed cross-reactivity of 1.13%, 2.25%, and 6.3%, respectively. The geometry and arrangement of the structurally similar steroid and antibody-binding segments give a pattern of cross-reaction [23].

In the present study, the intra- and inter-assay CV% of each pool is <9.77, and the recovery percentage calculated was from 98.55% to 105.08%. The studies of Wang [21], Vdovenko [24], and Li [22] showed recovery percentages ranged from 83.3-114.2%, 82-142%, and 80-93.3% (milk) and 80-122.8% (pork) respectively. We can say that the homologous assay system based on direct competition has been developed for Dexamethasone using HRP as an enzyme label. It is sensitive, specific and requires only 1 hour 15 minutes for completion.

CONCLUSIONS

A sensitive, specific, reliable, indigenous, and affordable direct competitive ELISA has been developed. Less sample volume and time are required to complete the assay. The ELISA can further determine the Dexamethasone level in food and biological samples (fluids and tissues). The conjugation study of immunogen and enzyme conjugate can be used *in-silico*, such as docking studies. Immunochromatographic paper strips can also be produced for Dexamethasone as a screening method to test its level in biological samples (Urine, blood, Serum, tissues, etc.) and food samples.

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CONTRIBUTION OF AUTHORS

Research Concept- T.G. Shrivastav
Research Design- Kiran Rangari
Supervision- T.G. Shrivastav, Kiran Rangari
Data collection- Dinesh Kumar
Data analysis and interpretation- Dinesh Kumar, Divya Verma
Literature search- Divya Verma, Rekha Meena
Writing article- Kiran Rangari, Rekha Meena, Divya Verma
Critical review- T.G. Shrivastav, Kiran Rangari
Article editing- Kiran Rangari, Divya Verma
Final approval- T.G. Shrivastav, Kiran Rangari

REFERENCES

[1] Abraham SM, Lawrence T, Kleiman A, Warden P, Medghalchi M, et al. Anti-inflammatory Effects of Dexamethasone Are Partly Dependent on Induction of Dual Specificity Phosphatase1. J Exp Med., 2006; 203(8): 1883–89.

[2] Aghai ZH, Kumar S, Farhath S, Kumar MA, Saslow J, et al. Dexamethasone Suppresses Expression of Nuclear Factor-KappaB in the Cells of Tracheobronchial Lavage Fluid in Premature Neonates with Respiratory Distress. Pediatr Res., 2006; 59(6): 811–15.

[3] Shefrin AE, Goldman RD. Use of Dexamethasone and Prednisone in Acute Asthma Exacerbations in Pediatric Patients. Can Fam Physician, 2009; 55(7): 704.

[4] Findling JW, Raff H, Aron DC. The Low-Dose Dexamethasone Suppression Test: A Reevaluation in Patients with Cushing's Syndrome. J Clin Endocrinol Metab., 2004; 89 (3): 1222–26.

[5] Charmandari E, Kino T, Ichijo T, Chrousos GP. Generalized Glucocorticoid Resistance: Clinical Aspects, Molecular Mechanisms, and Implications of a Rare Genetic Disorder. J Clin Endocrinol Metab., 2008; 93(5): 1563–72.

[6] De Gans J, Van de Beek D. Dexamethasone in Adults with Bacterial Meningitis. N Engl J Med., 2002; 347(20): 1549–56.

[7] Harousseau JL, Attal M, Leleu X, Troncy J, Pegourie B, et al. Bortezomib plus Dexamethasone as Induction Treatment Prior to Autologous Stem Cell Transplantation in Patients with Newly Diagnosed Multiple Myeloma: Results of an IFM Phase II Study. haematologica, 2006; 91(11): 1498–505.

[8] Hirvikoski T, Nordenström A, Wedell A, Ritzén M, Lajic S. Prenatal Dexamethasone Treatment of Children at Risk for Congenital Adrenal Hyperplasia:

The Swedish Experience and Standpoint. J Clin Endocrinol Metab., 2012; 97(6): 1881–83.

[9] Zhou J, Ruan L, Li H, Wang Q, Zheng F, et al. Addison's Disease with Pituitary Hyperplasia: A Case Report and Review of the Literature. Endocrine, 2009, 35: 285–89.

[10]Meikle AW, Lagerquist LG, Tyler FHA. Plasma Dexamethasone Radioimmunoassay. Steroids, 1973; 22(2): 193–202.

[11]Shrivastav TG. Preparation of Horseradish Peroxidase Hydrazide and Its Use in Immunoassay. J Immunoassay Immunochem., 2003, 24(3): 301–09. doi: 10.1081/IAS-120022939.

[12]Shrivastav TG, Basu A, Kariya KP. Substitution of Carbonate Buffer by Water for IgG Immobilization in Enzyme-Linked Immunosorbent Assay. J Immunoassay Immunochem., 2003; 24 (2): 191–203. doi: 10.1081/IAS-120020084.

[13]Kumar D, Singh H, Shrivastav T. Homologous ELISA for Detection of Prednisolone in Human Serum. Food Agric Immunol., 2018; 29(1): 369–85.

[14]Dutta P, Shrivastav TG, Thakur SC. Homologous ELISA for Measurement of Medroxyprogesterone Acetate in Serum. Anal Biochem., 2018; 558: 60–68. doi: 10.1016/j.ab.2018.07.010.

[15]Basu A, Shrivastav TG, Maitra SK. A Direct Antigen Heterologous Enzyme Immunoassay for Measuring Progesterone in Serum without Using Displacer. Steroids, 2006; 71(3): 222–30.

[16]Verma D, Shrivastav TG, Thakur SC. Homologous ELISA for Detection of 17α-Methyltestosterone in Serum. Int J Health Sci., 2022; 6(S6), 6457–74.

[17]Rodbard D. Statistical Quality Control and Routine Data Processing for Radioimmunoassays and Immunoradiometric Assays. Clin. Chem. 1974, 20(10): 1255-70.

[18]Abraham GE. Solid-Phase Radioimmunoassay of Estradiol-17β. J Clin Endocrinol Metab., 1969; 29(6): 866–70.

[19]Chaudhari G, Kavil S, Ullal D, Kadam S, Bakre D, et al. An overview on ELISA techniques for analyte detection to new levels: A Review. Editor. Board 2014, 3 (6), 172.

[20]Song Y, Park J, Kim J, Kim C. HPLC Determination of Dexamethasone in Human Plasma. J Liq Chromatogr Relat Technol., 2004; 27(14): 2293–306.

[21]Wang W, He J, Xiao H, Zheng X, Li L. One-Step Indirect Competitive ELISA Development for Dexamethasone Determination in Chicken. J Food Saf Hyg., 2016; 2(3/4): 75–83.

[22]Li X, Chen X, Liu Z, Wang J, Hua Q, et al. Latex Microsphere Immunochromatography for Quantitative Detection of Dexamethasone in Milk and Pork. Food Chem., 2021; 345, 128607.

[23]Nara S, Tripathi V, Chaube SK, Rangari K, Singh H, et al. Influence of Hydrophobic and Hydrophilic Spacer-Containing Enzyme Conjugates on Functional Parameters of Steroid Immunoassay. Anal Biochem., 2008; 373(1): 18–25.

[24]Vdovenko MM, Gribas AV, Vylegzhanina AV, Sakharov IY. Development of a Chemiluminescent Enzyme Immunoassay for the Determination of Dexamethasone in Milk. Anal Methods, 2012; 4(8): 2550–54.