ImmuChem™ Coated Tube

Cortisol

^{125}I RIA Kit

For In Vitro Diagnostic Use

MP Biomedicals, LLC
Diagnostics Division
13 Mountain View Avenue
Orangeburg, NY 10962

CE

07815211.7
Q03-177, Q05-032 (3/05)

LICENSING REQUIREMENTS

MP Biomedical, LLC is permitted to transfer radioactive materials only after the receipt of a copy of the customer's Radioisotope License. In emergency situations, the purchaser may furnish oral certification of the above information provided a copy of the license is received by MP Biomedicals within ten (10) days.

ORDERING INFORMATION

ORDER TODAY by CALLING MP Biomedicals Customer Service Department in Irvine, California.

(800) 888-7008
or
Fax (949) 260-1079

Price quotations for standing orders and quantity purchases will be supplied upon request. Since we are unable to restock this product, all sales are final. All shipments are F.O.B. Orangeburg, NY.

ImmuChem™ Cortisol CT

CATALOG No. 07-221102 (100 Tubes)
CATALOG No. 07-221105 (500 Tubes)
CATALOG No. 07-221106 (1000 Tubes)

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I. INTRODUCTION

A. INTENDED USE

The ImmuChem™ Cortisol ^{125}I kit is designed to measure this steroid in unextracted serum.

B. CLINICAL PHYSIOLOGY

Cortisol is the principle glucocorticoid secreted by the adrenal cortex. Adrenal secretion of cortisol is modulated by a complex negative feedback mechanism involving the central nervous system, hypothalamus, pituitary, and adrenals. ACTH released from the pituitary augments adrenal secretion of cortisol while falling levels of cortisol are associated with rising levels of ACTH. Normally there is a diurnal variation of cortisol with the highest values measurable in the morning blood samples and the lowest values obtained in the late afternoon.

C. CLINICAL APPLICATIONS

1. BASELINE MEASUREMENTS

   In the diagnosis of Cushing's syndrome, early morning and later afternoon measurements may be utilized to assess the normal cortisol diurnal variation. In Cushing's syndrome, this diurnal variation is frequently lost, with the afternoon values usually elevated above the normal range. A negative test is strong evidence against the diagnosis of Cushing's syndrome. Because there are many false positive tests, the use of a baseline serum cortisol measurement is of limited diagnostic power.[1]

2. THE OVERNIGHT DEXAMETHASONE SUPPRESSION TEST

   This is a screening test for Cushing's syndrome. Pituitary ACTH secretion peaks between 4 and 6 a.m. in individuals on a normal sleep-wake cycle. This in turn causes an increase in the cortisol secretion approximately thirty (30) minutes after the ACTH peak. The administration of dexamethasone at 11 p.m. will inhibit the release of the major portion of the morning secretion of ACTH and thereby suppress the morning cortisol secretion. This is one of the more powerful screening tests for Cushing's syndrome. Most authorities agree that a negative test excludes this diagnosis.[1-3]

   With the administration of dexamethasone, serum cortisol normally suppresses to less than 2.5 µg/dL. False negative suppression in patients with Cushing's syndrome are rare, but false positives are common in patients who are depressed, have slept poorly, are under severe emotional stress, or are obese. When this test is negative (less than 2.5 µg/dL), the diagnosis of Cushing's syndrome can be excluded.[1-3]

3. THE IM ACTH STIMULATION TEST

   This test is used for the diagnosis of adrenal insufficiency. In this test, a fragment consisting of the 1-24 amino acid sequence of ACTH called Cosyntropin (Cortrosyn) is used as opposed to the entire 1-39 amino acid ACTH molecule. Cosyntropin is of uniform potency by weight, acts rapidly and does not produce allergic responses.[4,5]

   In normal patients, there is an increment in plasma cortisol concentrations exceeding 7 µg/dL over the baseline concentration. An abnormal result from this test should be followed by an IV stimulation test.

II. PRINCIPLE OF THE TEST

Radioimmunoassays (RIA) depend on the ability of an antibody to bind its antigen. To quantitate the antigen, the radioactive and nonradioactive forms of the antigen compete for binding sites on its specific antibody. As more nonradioactive antigen is added, less radioactive antigen remains bound until equilibrium between the free and antibody- bound antigen occurs.

In the ImmuChem™ CORTISOL assay, the antibody is covalently bound to the inner surface of a polypropylene tube. Thus, antibody-bound antigen is also bound to the tube wall. No second antibody, charcoal, or other agent is needed and no centrifugation is required. At the conclusion of the assay, free antigen is aspirated or decanted, leaving only antibody-bound antigen. The coated tube is then counted in a gamma counter to determine the amount of antibody-bound cortisol ^{125}I. Levels of cortisol in the sample are determined graphically from a standard curve constructed with results obtained from the cortisol standards.

III. REAGENTS PROVIDED AND LABEL COLOR CODE

COMPONENT	LABEL COLOR BAR	VOLUME OR QUANTITY	
		100T KIT	500T KIT
Anti-Cortisol Tubes Cat. No. 07-221110	Yellow	4 x 25 Tubes	20 x 25 Tubes
Cortisol Standards (6) Cat. No. 07-221130	Green	0.5 mL each*	3 x 0.5 mL each*
Cortisol-^{125}I Cat. No. 07-221121	Blue	2 x 52 mL	10 x 52 mL

*0 µg/dL standard contains 1 mL.

IV. REAGENT DESCRIPTION (FOR IN-VITRO DIAGNOSTIC USE)

A. ANTI-CORTISOL COATED TUBES ANTI TUBES

Cortisol-3-O-Carboxymethyloxime-BSA was used as the antigen to generate the antiserum in rabbits. This antiserum is covalently bound to the inner surface of a polypropylene tube and is titered to provide 40 to 60% total binding in the absence of non radioactive Cortisol.

STORAGE: *2 to 8°C. Reseal the plastic bag after use.*
STABILITY: *Refer to the expiration date on the bag.*

B. CORTISOL STANDARDS* STD 1-6

Six standards are provided at the following concentrations: 0, 1.0, 3.0, 10, 30, and 100 µg/dL. These standards have been prepared in a human serum matrix and contain sodium azide and gentamicin sulfate.

STORAGE: *The standards are stable for 1 month at 2 to 8°C. For longer storage, freeze below -15°C. Standards should not be subjected to more than 4 freeze-thaw cycles.*
STABILITY: *Refer to the expiration date on the vial.*

*NOTE: These standards are of human serum origin and although this blood has been tested and found negative for HIV-1/2 antibody, HCV antibody and HbsAg, it should be handled with the same safety precautions afforded any human sample.

C. CORTISOL-^{125}I ^{125}I TRACER

This radioactive material contains less than 3 microcuries per vial on the date of calibration. 1.0 mL of this material will provide approximately 45,000 cpm on calibration day, using a counter with 75% efficiency.

STORAGE: *2 to 8°C.*
STABILITY: *Refer to the expiration date on the vial.*

V. LIMITATIONS, PRECAUTIONS AND GENERAL COMMENTS

NOTE: These reagents contain sodium azide which has a tendency to build up in lead or copper plumbing forming potentially explosive metal azides. Always flush large quantities of water through the plumbing after the disposal of these reagents. Radioactive waste should be disposed according to the U.S. NRC guidelines.

A. Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.

B. A standard curve must be established with every assay.

C. The reagents provided in this kit have been designed and optimized for the measurement of cortisol from human serum or plasma. Anyone performing animal research work must establish their own physiological ranges. Should assistance be required, please contact the MP Biomedicals Technical Services department.

D. The reagents supplied in this kit are for IN-VITRO DIAGNOSTIC USE ONLY.

E. The use of grossly hemolized or lipemic samples should be avoided.

F. A diurnal variation does occur in cortisol secretion, therefore it is important to be aware of the time of day that the sample was drawn.

G. The kit reagents and materials are intended for use as an integral unit. Do not mix various lots of any component reagent within an individual run.

H. RADIOACTIVE MATERIALS

Please observe the following precautions when handling this radioactive material:

1. This radioactive material may be received, acquired, possessed and used only by those licensed to do so. Its use is solely for in-vitro clinical or laboratory tests not involving the internal or external administration of the materials, i.e. radioactivity, to humans or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of, and with a general license from, the U.S. NRC or the state with which the U.S. NRC has entered into agreement for the exercise of regulatory authority.

2. Immediately upon the receipt of this kit, check for breakage and verify the contents as per the packing list. Should there be any breakage or questions regarding this kit's contents, immediately notify your distributor by telephone.

3. Kit reagents should be stored and used only at clean, designated work stations of the laboratory. Although the exposure to radiation from the small amount of isotope supplied is negligible, it is a good practice to designate a storage area at least 10 feet from any work station. Furthermore, persons under the age of 18 should not be permitted to handle radioactive material or enter an area where it is either stored or used.

4. Should there be spillage of any of the radioactive material, the following clean-up procedure is recommended: while wearing disposable gloves, blot the spillage with an absorbent material. Dispose of this material as radioactive waste. Remove the gloves, tear to prevent further usage and discard as regular waste. Finally, wash your hands thoroughly.

5. The pipetting of radioactive material by mouth should be avoided. Smoking, eating or drinking while performing tests involving radioactive material should not be permitted. Lastly, persons handling radioactive material should wash their hands immediately after handling and prior to leaving the laboratory area.

VI. SPECIMEN COLLECTION AND HANDLING

Plasma: Draw blood into a green capped (heparin) Vacutainer™ tube. Separate the plasma by centrifugation and store the plasma in a refrigerator (up to 1 week) or store frozen.

Serum: Draw blood into a red capped Vacutainer™ tube. Allow the blood to clot for at least thirty minutes at room temperature. Separate the serum and store under the same conditions as the plasma.

VII. EQUIPMENT AND REAGENTS REQUIRED

* 1. Pipettors and/or pipettes that can accurately and precisely deliver the required volumes (25 and 1000 microliters).
2. A test tube rack.
3. A laboratory vortex mixer.
4. A water bath, 37°C ± 1.
5. An aspirator with a trap.
6. Gamma counter.

*Available from MP Biomedicals

VIII. ASSAY PROCEDURE

A. ASSAY PREPARATIONS

1. Bring all standards, samples, controls, coated tubes, and CORTISOL-^{125}I to room temperature prior to use.
2. Place the required number of anti-CORTISOL tubes in a test tube rack. Reseal the unused tubes in the plastic bag along with the desiccant and refrigerate.
3. Add all solutions in the quantities specified directly from the reagent vials.

B. ASSAY STEPS

1. Pipette 25 µL of each standard, control and patient sample into its respective coated tube.
2. Add 1.0 mL of the CORTISOL-^{125}I to all tubes and vortex all the tubes.
3. Incubate for FORTY-FIVE (45) minutes at 37 ± 1°C.
4. Aspirate or decant the contents of the tubes. (If decanting, touch the rim of the tubes on absorbent paper before turning upright.)
5. Count the tubes in a gamma counter calibrated for ^{125}I.

C. QUALITY CONTROL

Serum pools or commercially available controls containing a low, normal, and high concentration of Cortisol should routinely be assayed as unknowns. The concentrations of these controls should be plotted on a Levy-Jennings type system in order to monitor the performance and reliability of the assay. For further information, see:

DAVID RODBARD: "Statistical Quality Control and Routine Data Processing for Radioimmunoassays and Immunoradiometric Assays." CLIN CHEM 20/10, 1255-1270 (1974).

IX. PROTOCOL

Tube No.	Description	Standard or Unknown	CORTISOL ^{125}I		
1,2	0 µg/dL	25 µL	1000 µL	VORTEX, INCUBATE 37°C FOR 45 MINUTES	ASPIRATE, OR DECANT / BLOT, COUNT
3,4	1.0 µg/dL				
5,6	3.0 µg/dL				
7,8	10 µg/dL				
9,10	30 µg/dL				
11,12	100 µg/dL				
13,14	Control 1				
15,16	Control 2				
17,18	Control 3				
19,20	Sample				
21,22	etc.	▼	▼		

X. CALCULATIONS

A. Take the average counts of all duplicate tubes. Divide all averaged counts by the averaged counts of the zero standard and multiply by 100. This yields %B/Bo.

B. Formula:

$$\%B/Bo = \frac{\overline{CPM}\ (sample)}{\overline{CPM}\ (zero\ standard)} \times 100$$

C. Plot percent bound versus the concentration of the cortisol standards (1 to 100 µg/dL). This yields the standard curve. This curve may be plotted on linear, semi-log, or log-logit graph paper. Patient samples may then be read directly from this plot.

SAMPLE CALCULATIONS

$$\%B/Bo = \frac{\overline{CPM}\text{ (sample)}}{\overline{CPM}\text{ (zero standard)}} \times 100$$

$$= \frac{14056}{17355} \times 100$$

$$= 81\%$$

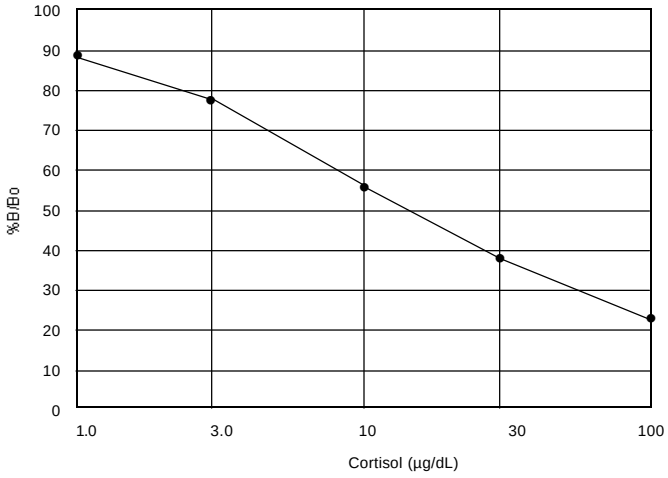
This calculation is for example only. The user must construct a standard curve each time the assay is run.

XI. SAMPLE ASSAY

STANDARD	CPM	AVG CPM	%B/Bo	Cortisol (µg/dL)
0 µg/dL	26,016			
	25,272			
	24,487	25,258		
1 µg/dL	22,437			
	22,955			
	22,316	22,569	89	
3 µg/dL	20,035			
	19,395			
	19,409	19,613	78	
10 µg/dL	14,484			
	14,047			
	14,280	14,270	56	
30 µg/dL	9,497			
	9,697			
	9,602	9,599	38	
100 µg/dL	5,568			
	5,897			
	5,455	5,640	22	
Control I	17,570			
	18,143			
	18,178	17,964	71	4.8
Control II	12,094			
	12,263			
	11,958	12,105	48	18.4
Control III	7,761			
	7,948			
	7,746	7,818	31	48.7

XII. SAMPLE STANDARD CURVE

NOTE: This curve serves only as an example. Cortisol values must not be derived from it.



XIII. EXPECTED PHYSIOLOGICAL RANGES

The following data was obtained from one demographic patient sampling.

MORNING SAMPLES (8:30 A.M.) 7 to 24 µg/dL

AFTERNOON SAMPLES (4:30 P.M.) 3 to 11 µg/dL

As with any diagnostic test, differences in physiological ranges may be encountered from laboratory to laboratory due to patient demographics, laboratory techniques, and population sampling. These ranges should only be used as a guideline. We recommend each laboratory establish its own ranges using a statistically significant number of characterized patient specimens in each diagnostic catagory.

XIV. PERFORMANCE CHARACTERISTICS

A. PARALLELISM (linearity of dilutions)

Sample	Neat	1:2	1:4	1:8	1:16
1	31.7	13.8x2=27.6	6.99x4=28.0	3.49x8=27.9	1.80x16=28.8
2	91.7	49.4x2=98.8	23.1x4=92.4	12.0x8=96.0	5.28x16=84.5

All values are in µg/dL.

B. RECOVERY

Sample	Cortisol Added (µg/dL)	Expected Result (µg/dL)	Observed Result (µg/dL)	% Recovered
6.3	2	8.3	8.1	98
	5	11.3	10.8	96
	10	16.3	14.9	91
	20	26.3	25.6	97
	40	46.3	49.7	107
4.5	2	6.5	7.0	108
	5	9.5	11.1	117
	10	14.5	14.6	101
	20	24.5	24.1	98
	40	44.5	49.4	111

C. PATIENT SAMPLE CORRELATION

Ninety samples were analyzed in duplicate using the ImmuChem™ CORTISOL-^{125}I RIA Kit and a commercially available RIA kit. The results are given below (ImmuChem™ = y-axis):

sampling number (n) = 90

y-intercept = 0.8

slope = 1.12

correlation coefficient = 0.972

D. INCUBATION TIME STUDY

Four assays were set up simultaneously and incubated for varying periods at 37°C. The results are shown below.

DESCRIPTION	30 MIN	45 MIN	60 MIN	90 MIN
Bo/T	40.6	49.0	52.8	57.4
1.0 µg/dL	88	85	84	85
3.0 µg/dL	75	72	72	76
10 µg/dL	57	53	53	56
30 µg/dL	39	38	35	36
100 µg/dL	24	22	22	20
CONTROL 1 (VALUE)	71 (4.3)	66 (4.9)	67 (4.3)	68 (4.8)
CONTROL 2 (VALUE)	48 (18)	44 (19)	45 (18)	44 (19)
CONTROL 3 (VALUE)	33 (47)	30 (51)	29 (52)	28 (56)

All values are in µg/dL.

E. INTERFERING SUBSTANCES

Substance	Serum CORTISOL Value (µg/dL)	Amount Added	Observed CORTISOL Value (µg/dL)
Triglycerides	18.9	222 mg/dL	18.3
Hemoglobin	18.9	100 mg/dL	19.6
Bilirubin	18.9	4.9 mg/dL	20.6
Gentamicin	18.9	0.25 mg/dL	18.0

F. INTRA-ASSAY VARIATION

Three samples with high, medium and low concentrations of cortisol were assayed in 5 sets of 10 replicates. The standard deviations and coefficients of variations were determined.

	Control A	Control B	Control C
n	50	50	50
Mean	4.7	18.1	47.4
S.D.	0.42	0.96	2.9
C.V.	8.9%	5.3%	6.1%

G. INTER-ASSAY VARIATION

	Control A	Control B	Control C
n	55	56	56
Mean	4.96	18.7	50.0
S.D.	0.46	1.4	3.8
C.V.	9.3%	7.5%	7.6%

H. MINIMUM DETECTABLE DOSE

Ten duplicates of the zero standard were set up in an assay to determine the minimum quantity of cortisol detectable by this assay. By subtracting two standard deviations from the mean of the zero tubes, we find the minimum detectable dose of cortisol in this system is approximately 0.17 µg/dL.

XV. PROCEDURE FOR URINARY CORTISOL DETERMINATION

A. SPECIMEN COLLECTION

1. Collect the 24 hour urine specimen in a large plastic bottle (about 3000 mL capacity) containing 10 grams of boric acid.
2. Keep the container in a cool and dark place during the collection period.
3. Record the total volume of urine.
4. Store at 2-8°C for up to 7 days. For longer storage, store frozen at -15°C.

B. EXTRACTION

1. Chill an adequate volume of methylene chloride in an ice bath.
2. Add 0.5 mL of a well mixed 24 hour urine sample to an appropriately labeled glass tube (10x75 or 12x75 mm size).

   If the urine sample appears cloudy, centrifuge 0.7 mL for 5 minutes at 1500 g. Transfer 0.5 mL of the supernatant to an appropriately labeled tube.
3. Add 1 mL of chilled methylene chloride. Cap and vortex thoroughly. Centrifuge for 5 minutes at 1500 g to separate the phases.
4. Transfer 50 µL of the lower phase into an appropriately labeled anti-Cortisol tube. Run all tubes in duplicate.
5. Evaporate to complete dryness under a hood with mild warming (not to exceed 37°C).
6. Follow the assay procedure. No more than 30 minutes should elapse between the transfer of the extract into the coated tube and the addition of the tracer.
7. A blank should be run by extracting 0.5 mL of deionized water. The blank should read nondetectable when run in the assay.

COMMENTS FOR EXTRACTION PROCEDURE

- To prevent dripping, rinse the 1 mL and the 50 µL pipets several times in cold methylene chloride before transferring it into the tubes.
- Do not wipe the tip when pipetting the 50 µL sample into the coated tube.

C. ASSAY PROCEDURE

1. Pipet 25 µL of each of the serum standards into the coated tubes.
2. Pipet 25 µL of the controls into the appropriate tubes.
3. Pipet 25 µL of the 0 µg/dL standard into all the dried urinary sample tubes.
4. Add 1.0 mL of the Cortisol tracer (^{125}I) to all the tubes and vortex briefly.
5. Incubate all assay tubes at 37°C for 45 minutes.
6. Aspirate or decant the tubes in the same order pipetted.
7. Count the tubes for one minute in a gamma counter.
8. Determine the concentration of cortisol in the extracted sample by reading it against the standard curve generated by the cortisol serum standards.
9. Calculate the urinary cortisol as follows:

   µg Cortisol/24 hours = µg/dL cortisol x 0.01 dL/mL x mL urine per 24 hours.

D. REFERENCE RANGE

10-100 micrograms per 24 hours.

XVI. SPECIFICITY OF THE ANTISERUM

The following compounds have been checked for cross-reactivity. The percentages indicate the cross-reactivity at 50% displacement on the CORTISOL standard curve.

COMPOUND	% CROSS REACTION
Cortisol	100.00
Prednisolone	45.6
11-Desoxycortisol	12.3
Corticosterone	5.5
Prednisone	2.7
Cortisone	2.1
17α-Hydroxyprogesterone	1.0
Progesterone	0.25
Dexamethasone	<0.10
Dihydrotestosterone	<0.10
Testosterone	<0.10

XVII. REFERENCES

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3. Ashcraft, M.W., A.J. van Herle, S.L. Vener, D.L. Geffner: Serum cortisol levels in Cushing's syndrome after low- and high-dose dexamethasone suppression. Ann. Intern. Med. 97:**21**, 1982.
4. Oelkers, W., S. Diederich, V. Bahr: Diagnosis and therapy surveillance in Addison's disease: rapid adrenocorticotropin (ACTH) test and measurement of plasma ACTH, renin activity, and aldosterone. J. Clin. Endocrinol. Metab. 75:**259**, 1992.
5. Baxter, J.D. and J.B. Tyrrell: The Adrenal Cortex. In, Felig, P., Baxter, J.D., Broadus A.E., Frohman, L.A. (eds): Endocrinology and Metabolism, 1987, McGraw-Hill, p. **578**.