CORTISOL-RIA-CT

KIRI28000

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve - Belgium

History

Resume of change :

Previous Version : 151021/1	**Current Version :** 191016/1
I. INTENDED USE Radioimmunoassay for the determination of human Cortisol in serum, plasma or urine without extraction. **For research use only, not for use in diagnostic procedures.**	***I. INTENDED USE*** Radioimmunoassay for the determination of human Cortisol in serum, plasma, urine or saliva without extraction. **For research use only, not for use in diagnostic procedures.** For professional use only. **The whole procedure has been adapted to the determination of salivary cortisol**
CONTROL | **N** Controls – 1 and 2 in human serum with azide (<0.1%)	**CONTROL** | **N** Controls – 1 and 2 in human plasma with azide (<0.1%)
IX. SPECIMEN COLLECTION AND PREPARATION - Saliva samples should be collected using adequate sampling devices e.g. Salivette (Starstedt cat n° 51.1534).	***IX. SPECIMEN COLLECTION AND PREPARATION*** - Saliva samples should be collected using adequate sampling devices e.g. Salivette (Sarstedt cat n° 51.1534.500).
X. PROCEDURE FOR SERUM, PLASMA AND URINE SAMPLES **A. Handling notes** No indication	***X. PROCEDURE FOR SERUM, PLASMA AND URINE SAMPLES*** **A. Handling notes** Each tube can only be used once.

B. Specificity

Compound	Cross-Reactivity (%)
Cortisol	100.00
17-OH-Progesterone	5.60
DOC	0.74
Corticosterone	0.51
11-DOC	0.27
Prednisone	0.25
Estradiol	0.17
Prednisolone	0.10
Testosterone	0.08
Progesterone	0.00

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Cortisol		cpm	B/Bo (%)
Total count		63729	
Calibrator	0 µg/L	35476	100.0
	10 µg/L	31047	87.5
	30 µg/L	25851	72.9
	100 µg/L	17182	48.4
	300 µg/L	9399	26.5
	1000 µg/L	4050	11.4

XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Cortisol levels vary diurnally and as a function of suppression manoeuvres. Morning samples results are generally 2 to 3 times higher than these obtained with afternoon samples. Morning samples generally read less than 5 µg/L following metyrapone testing or dexamethasone suppression.

Identification	Range (*) (µg/L)
Serum samples	
Morning	50 – 250 µg/L
Afternoon	25 -125 µg/L
24 h urine samples	7 – 96 µg/L

(*) The range is based on 2.5 % and 97.5 % percentiles

LOT : 151021/1

No history

B. Specificity

Compound	Cross-Reactivity (%)
Cortisol	100.00
17-OH-Progesterone	0.8
21-DOC	1.7
Corticosterone	1.4
11-DOC	89.5
Prednisone	0.7
Estradiol	0.04
Prednisolone	10.9
Testosterone	0.3
Progesterone	0.06

XIII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Serum/plasma/urine Cortisol		cpm	B/Bo (%)
Total count		60203	
Calibrator	0 µg/L	43148	100.0
	17 µg/L	40143	93.0
	34 µg/L	35177	81.5
	100 µg/L	26815	62.2
	274 µg/L	15359	35.6
	450 µg/L	11342	26.3

XVII. REFERENCE INTERVALS

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Cortisol levels vary diurnally and as a function of suppression manoeuvres. Morning samples results are generally 2 to 3 times higher than these obtained with afternoon samples. Morning samples generally read less than 5 µg/L following metyrapone testing or dexamethasone suppression.

Identification	Range (*) (µg/L)
Serum samples	
Morning collection	50 – 270 µg/L
Afternoon collection	25 – 119 µg/L
24h Urine samples	6 -75 µg/24H
Saliva samples (morning collection)	1.2 – 7.5 µg/L

(*) The range is based on 2.5 % and 97.5 % percentiles

Version : 191016/1

History added

en

Read entire protocol before use.

CORTISOL-RIA-CT

I. INTENDED USE

Radioimmunoassay for the determination of human Cortisol in serum, plasma, urine or saliva without extraction. **For research use only, not for use in diagnostic procedures.**
For professional use only.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource CORTISOL-RIA-CT Kit

B. Catalog number : KIRI28000 : 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

For technical assistance or ordering information in the United States contact :
Immuno-Biological Laboratories, Inc. (IBL-America)
Tel : 1-888-523-1246 Fax : 1-763-780-2988 Email : info@ibl-america.com

III. BACKGROUND

A. Biological activity

Cortisol is the major glucocorticoid produced and secreted by the adrenal gland. In response to different stimuli (diurnal rhythm, stress, low blood sugar concentration), the cerebral cortex stimulates the hypothalamus to release the CRF (corticotrophin releasing factor). CRF causes the release from the pituitary gland of ACTH (adrenocorticotropic hormone). Glucocorticoids are then synthesized in response to ACTH.
Since Cortisol levels depend upon the interaction of the hypothalamus, pituitary and adrenal glands, determination of urine cortisol levels can also aid in the study of the diseases states of these glands.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I labelled steroid competes with the steroid to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. Neither extraction nor chromatography are required because of the high specificity of the coated antibodies. After 45 minutes incubation at 37°C (180 minutes for salivary procedure), an aspiration step terminates the competition reaction. The tubes are then washed with 3 ml of wash solution and aspirated again. A calibration curve is plotted and the cortisol concentrations of the samples are determined by dose interpolation from the calibration curve.
The method is calibrated against LC-MS reference method.

V. REAGENTS PROVIDED

Reagents	96 Tests Kit	Colour Code	Reconstitution
Tubes coated with anti-cortisol (monoclonal)	2 x 48	yellow	**Ready** for use
Ag | **^{125}I** TRACER: ^{125}Iodine labelled cortisol in phosphate-citrate buffer with proteins, ANS, and azide (<0.1%)	1 vial 52 ml 116 kBq	red	**Ready** for use
CAL | **0** Zero Calibrator in human serum and azide (0.1%)	1 vial 1 ml	yellow	**Ready** for use
CAL | **N** Calibrators - N = 1 to 5 **(see exact values on vial labels)** in human serum and azide (0.1%)	5 vials 0.5 ml	yellow	**Ready** for use
WASH | **SOLN** | **CONC** Wash solution (TRIS-HCl)	1 vial 10 ml	brown	**Dilute** 70 x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls – 1 and 2 in human plasma with azide (<0.1%)	2 vials lyophilised	silver	**Add** 0.5 ml distilled water

Note : Use the zero calibrator for sera dilutions. If needed, urines can be diluted in an ordinary phosphate saline buffer (PBS). Saliva can be diluted in physiological serum (see XI)

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Pipettes for delivery of: 25 µl and 500 µl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Water bath at 37°C
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).
9. Physiological serum (NaCl 9 g/L solution), for salivary procedure

VII. REAGENT PREPARATION

A. **Controls**: Reconstitute the controls with 0.5 ml distilled water.
B. **Working Wash solution**: Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.
C. **Calibrators and controls preparation for salivary procedure: see XI**

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, controls are stable for 7 days at 2-8°C. For longer storage periods, aliquots should be made and kept at –20°C for maximum 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Keep serum, plasma or urine at 2-8°C for 1-2 days. Keep frozen for longer periods. Highly lipemic or haemolyzed samples must be discarded. Presence of fibrin filaments in the plasma can interfere with the assay. Use clear samples only.
- Collect urine during 24 hours without preservative. Record the total volume.
- Avoid subsequent freeze-thaw cycles.
- Saliva samples should be collected using adequate sampling devices e.g. Salivette (Sarstedt cat n° 51.1534.500). Eating, drinking or brushing teeth have to be avoided 30 minutes before sampling. In case of mouth lesions, inflammation or diseases the sampling must be also avoided (blood contamination). Saliva samples can be stored at 2-8°C for one week or frozen for longer periods. Samples have to be frozen, thawed and centrifuged at least once (in order to separate the mucins).

X. PROCEDURE FOR SERUM, PLASMA AND URINE SAMPLES

A. Handling notes

Do not use the kit or components beyond expiry date.
Do not mix materials from different kit lots.
Bring all the reagents to room temperature prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling.
Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.
Respect the incubation times.
Prepare a calibration curve for each run, do not use data from previous runs.
Each tube can only be used once.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, controls and samples and dispense 25 µl of each into the respective tubes.
3. Dispense 500 µl of ^{125}Iodine labelled Cortisol into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for 45 minutes at 37° +/- 2°C, in a water bath.
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

XI. PROCEDURE FOR SALIVARY CORTISOL MEASUREMENT

A. Calibrators and controls

1. Using physiological serum (NaCl 9g/L in distilled water), dilute the calibrators in glass or plastic tubes: **Calibrator 1: 1/20 and 1/10.**
   Calibrators 2 to 5: 1/10. The needed volume to perform the ria in duplicate is 400 µl; therefore, we recommend the following dilutions:
   1/20 = 25 µl calibrator 1 + 475 µl physiological serum
   1/10 = 50 µl calibrator 1 to 5 + 450 µl physiological serum
2. **Dilute the controls 1/10 in physiological serum**
3. **Do not use the Calibrator 0: it is replaced, in the test, by physiological serum.**

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, controls and samples and dispense **200 µl** of each into the respective tubes.

3. Dispense 500 µl of ^{125}Iodine labelled Cortisol into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for **180 minutes at 37° +/- 2°C**, in a water bath.
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

XII. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B0\ (\%) = \frac{\text{Counts (Calibrato r or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B0(%)) values for each calibrator point as a function of the cortisol concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B0 (%)) values, determine the cortisol concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled cortisol (B0/T) must be checked.
7. **Remark: for salivary measurement, it is mandatory to modify the concentrations of the calibrators, due to the preliminary dilutions (see *XI),* before calculating the calibration curve.**

XIII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

Serum/plasma/urine Cortisol		cpm	B/Bo (%)
Total count		60203	
Calibrator	0 µg/L	43148	100.0
	17 µg/L	40143	93.0
	34 µg/L	35177	81.5
	100 µg/L	26815	62.2
	274 µg/L	15359	35.6
	450 µg/L	11342	26.3

Salivary Cortisol	cpm	B/Bo (%)
Total count	32041	
0.0 µg/L	20134	100.0
0.9 µg/L	18696	92.9
1.7 µg/L	18009	89.4
3.4 µg/L	16161	80.3
10.0 µg/L	12602	62.6
27.4 µg/L	7789	38.7
45.0 µg/L	5849	29.1

XIV. SERUM, PLASMA, URINE: PERFORMANCES AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was **0.9 µg/L**.

B. Specificity

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
Cortisol	100.00
17-OH-Progesterone	0.8
21-DOC	1.7
Corticosterone	1.4
11-DOC	89.5
Prednisone	0.7
Estradiol	0.04
Prednisolone	10.9
Testosterone	0.3
Progesterone	0.06

Note: this table shows the cross-reactivity for the anti cortisol

C. Precision

INTRA-ASSAY PRECISION / INTER-ASSAY PRECISION

Serum	N	Mean (µg/L)	CV (%)	Serum	N	Mean (µg/L)	CV (%)
A	5	1.10	6.2	A	8	1.95	8.7
B	5	3.70	5.2	B	8	5.48	11.5
C	5	28.30	7.7	C	8	36.85	15.1

D. Accuracy

DILUTION TEST

Dilution (serum)	Theoretical Concent. (µg/L)	Measured Concent. (µg/L)
1/1	-	630.3
1/2	315.2	298.5
1/4	157.6	159.3
1/8	78.8	81.3
1/16	39.4	39.1
1/32	19.7	21.7
1/64	9.9	11.1

Samples were diluted with zero calibrator.

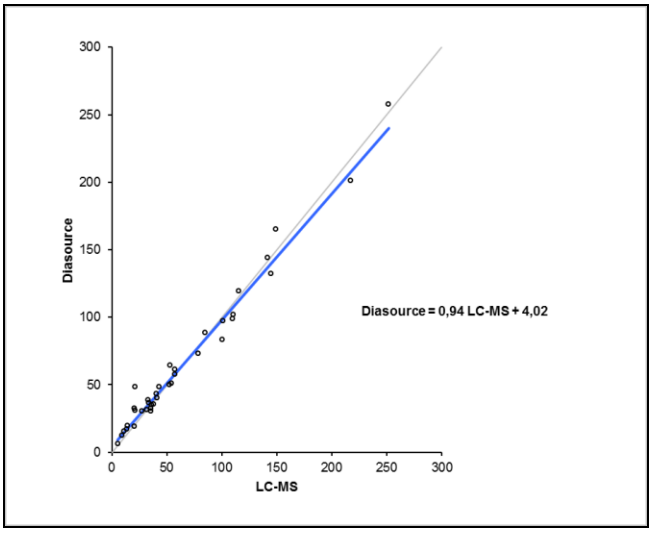
RECOVERY TEST

Sample (µg/L)	Added Cortisol (µg/L)	Recovered Cortisol (µg/L)	Recovered (%)
3.0	30.0	17.7	93.2
3.0	100.0	48.7	105.7
3.0	300.0	149.2	101.5
3.0	1000.0	509.2	98.5

Conversion factor :

From µg/L to nmol/L : x 2.758
From nmol/L to µg/L : x 0.363

E. Calibration against LC-MS



XV. SALIVARY PROCEDURE PERFORMANCES

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was **0.53 µg/L**.

B. Precision

INTRA-ASSAY PRECISION INTER-ASSAYS PRECISION

Sample	N	Mean (µg/L)	CV (%)	Sample	N	Mean (µg/L)	CV (%)
A	12	7.9	10.4	A	8	15.8	4.7
B	12	18.7	10.4	B	8	13.2	4.7

C. Accuracy

DILUTION TEST

Dilution	Theoretical conc. (µg/L)	Measured conc. (µg/L)
1/1	**	11.46
1/2	5.73	5.31
1/4	2.87	3.00
1/8	1.43	1.30
1/16	0.72	0.68
1/32	0.36	0.35

RECOVERY TEST

Sample (µg/L)	Added Cortisol (µg/L)	Recovered Cortisol (µg/L)	Recovered (%)
2.29	0.60	0.52	97.2
2.29	1.20	1.32	103.4
2.29	3.81	3.66	97.5
2.29	10.90	11.82	107.0
2.29	18.00	16.33	91.8

XVI. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

XVII. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Cortisol levels vary diurnally and as a function of suppression manoeuvres. Morning samples results are generally 2 to 3 times higher than these obtained with afternoon samples. Morning samples generally read less than 5 µg/L following metyrapone testing or dexamethasone suppression.

Identification	Range (*) (µg/L)
Serum samples	
Morning collection	50 – 270 µg/L
Afternoon collection	25 – 119 µg/L
24h Urine samples	6 -75 µg/24H
Saliva samples (morning collection)	1.2 – 7.5 µg/L

(*) The range is based on 2.5 % and 97.5 % percentiles

XVIII. PRECAUTIONS AND WARNINGS

Safety

For research use only, not for use in diagnostic procedures.
This kit contains ^{125}I (half-life: 60 days), emitting ionizing X (28 keV) and γ (35.5 keV) radiations.
This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.
All radioactive handling should be executed in a designated area. away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.
Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.
The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.
Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

XIX. BIBLIOGRAPHY

1. Abraham, GE, Ed. (1981)
Radioassay Systems in Clinical Endocrinology.
Marcel Dekker Inc, New York

2. Rittler M and Geisler D (1983)
Radioimmunoassay of urinary cortisol: comparison of the procedures with and without previous cortisol extraction.
Meth. Find. Exptl. Clin. Pharmacol. 5(6), 403-406.

XX. SUMMARY OF PROTOCOLS

SERUM, PLASMA AND/OR URINE SAMPLES	**TOTAL COUNTS µl**	**CALIBRATORS µl**	**SAMPLE(S) CONTROLS µl**
Calibrators (0 to 5) Samples, Controls Tracer	- - 500	25 - 500	- 25 500
Incubation	45 minutes at 37 +/- 2°C, in a water bath		
/Separation Working Wash solution Separation	-	Aspirate (or decant) 3.0 ml Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

SALIVA SAMPLES	**TOTAL COUNTS µl**	**CALIBRATORS µl**	**SAMPLE (S) CONTROLS µl**
Calibrators (0 to 6)* Samples, Controls Tracer	- - 500	200 - 500	- 200 500
Incubation	180 minutes at 37 +/- 2°C, in a water bath		
/Separation Working Wash solution Separation	-	Aspirate (or decant) 3.0 ml Aspirate (or decant)	
Counting	Count tubes for 60 seconds		

* See XI for calibrators' preparation

DIAsource Catalogue Nr : KIRI28000	Revision nr : 191016/1

Revision date : 2019-10-16

Distributed by:

Immuno-Biological Laboratories, Inc. (IBL-America)
8201 Central Ave. NE, Suite P
Minneapolis, MN 55432, USA
Phone: (888) 523-1246
Fax.: (763) 780-2988
Web: www.ibl-america.com
Email: info@ibl-america.com