BECKMAN COULTER

CE 2797

Cortisol RIA KIT

Instruction for use in local language is available at beckmancoulter.com/techdocs.

REVISION HISTORY

Previous version: IFU-IM1841-06	**Current version:** IFU-IM1841-07
MATERIALS PROVIDED **Calibrators: five 0.5 mL vials and one 5 mL vial of «zero» calibrator** (ready-to-use) The calibrator vials contain from 0 to approximately 2,000 nM of cortisol in buffer with bovine serum albumin and sodium azide (<0.1%). The exact concentration is indicated on each vial label. The calibrators are traceable to a certified reference material (Cerilliant). **Control sample: one 0.5 mL vial** (ready-to-use) The vial contains cortisol in a buffer with bovine serum albumin and sodium azide (<0.1%). The concentration range is indicated on a supplement. The control sample is traceable to a certified reference material (Cerilliant).	**Calibrators: five 0.5 mL vials and one 5 mL vial of «zero» calibrator** (ready-to-use) The calibrator vials contain from 0 to approximately 2,000 nM of cortisol in buffer with bovine serum albumin and sodium azide (<0.1%). The calibrators are traceable to a certified reference material (Cerilliant). The exact concentration is indicated on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs). **Control sample: one 0.5 mL vial** (ready-to-use) The vial contains cortisol in a buffer with bovine serum albumin and sodium azide (<0.1%). The control sample is traceable to a certified reference material (Cerilliant). The concentration range is indicated on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs).
Standard curve *(Example of standard curve, do not use for calculation. Use the concentration of calibrators indicated on each vial label. The concentrations are lot specific, check carefully.)*	Example of standard curve is given on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs). The measured data are indicative only, do not use them for calculation of your results.

REF IM1841

FOR PROFESSIONAL USE ONLY

INTENDED PURPOSE

Cortisol RIA KIT is an in vitro diagnostic manual medical device intended to be used by healthcare professionals for the quantitative measurement of cortisol in human serum, plasma and urine. Measurement of cortisol is intended to be used as an aid in diagnosis of adrenal related disorders, such as Cushing's syndrome and Addison's Disease in general population [1, 2, 3, 4, 5, 6].

PRINCIPLE

The radioimmunoassay of cortisol is a competition assay. Samples and calibrators are incubated with ^{125}I-labeled cortisol, as a tracer, in monoclonal antibody-coated tubes. After incubation, the contents of the tubes are aspirated so as to remove unbound ^{125}I-labeled tracer. The bound radioactivity is then determined in a gamma counter. The cortisol concentrations in the samples are obtained by interpolation from the standard curve. The concentration of cortisol in the samples is indirectly proportional to the radioactivity.

WARNING AND PRECAUTIONS

General remarks:

- The vials with calibrators and controls should be opened as shortly as possible to avoid excessive evaporation.
- Do not mix the reagents from kits of different lots.
- A standard curve must be established with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

Protection against ionizing radiation

The purchase, possession, utilization, and transfer of radioactive material is subject to the regulations of the country of use. Adherence to the basic rules of radiation safety should provide adequate protection.

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipetting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.

- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.

Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can react with lead, copper or brass to form explosive metal azides. Sodium azide disposal must be in accordance with appropriate local regulations.

Material of human origin

All patient specimens should be handled as potentially infectious and waste should be discarded according to the country rules.

Dichloromethane

Dichloromethane is a highly volatile and inflammable solvent. Extraction and evaporation must be done in a ventilated hood. Keep away from any open flame. Do not pipet reagents by mouth.

The summary of safety and performance for this in vitro diagnostic medical device is available to the public in the European database on medical device (EUDAMED) when this database is available, and the information has been uploaded by the Notified Body. The web address of the EUDAMED public web site is: https://ec.europa.eu/tools/eudamed.

To search the information about this product in EUDAMED, use BUDI-DI: 150995905IM184164.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS Safety Data Sheet is available at beckmancoulter.com/techdocs

SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

Serum and plasma samples

- Serum or EDTA plasma are the recommended sample types.
- Allow serum samples to clot completely before centrifugation.
- Serum and plasma samples may be stored at 2-8°C, if the assay is to be performed within 24 hours. For longer storage keep frozen (at < -18°C, 1 year maximum), after aliquoting so as to avoid repeated freezing and thawing. Thawing of sample should be performed at room temperature.
- If samples have concentrations greater than the highest calibrator, they must be diluted into the zero calibrator.

Urine samples

- Collect 24-hour urine in flask.
- Determine volume.
- If necessary, store aliquoted at < -18°C, 1 year maximum. Thawing of sample should be performed at room temperature.
- If samples have concentrations greater than the highest calibrator, they must be diluted in distilled water.

MATERIALS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2-8°C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take them into account.

Tubes: 2 x 50 (ready-to-use)

^{125}I-Tracer: one 55 mL vial (ready-to-use)

The vial contains 185 kBq, at the date of manufacture, of ^{125}I-labeled cortisol in buffer containing proteins, sodium azide (<0.1%) and a dye.

Calibrators: five 0.5 mL vials and one 5 mL vial of «zero» calibrator (ready-to-use)

The calibrator vials contain from 0 to approximately 2,000 nM of cortisol in buffer with bovine serum albumin and sodium azide (<0.1%). The calibrators are traceable to a certified reference material (Cerilliant).

The exact concentration is indicated on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs).

The zero calibrator may be ordered separately, too (REF. IM1959 - 10 mL, REF. IM3444 - 250 mL).

Note: Occasional presence of turbidity in the zero calibrator does not affect assay performance.

Control sample: one 0.5 mL vial (ready-to-use)

The vial contains cortisol in a buffer with bovine serum albumin and sodium azide (<0.1%). The control sample is traceable to a certified reference material (Cerilliant).

The concentration range is indicated on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs).

MATERIALS REQUIRED, BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- Precision micropipette (50 µL).
- Semi-automatic pipette (500 µL).
- Vortex type mixer.
- Horizontal or orbital shaker.
- Aspiration system.
- Gamma counter set for ^{125}I.

For the assay of urine extract cortisol (optional)

- Analytical grade dichloromethane (methylene chloride), not ethanol-stabilized.
- Glass tubes or vials fitted with teflon-lined screw caps.
- 2 and 5 mL glass pipets.
- Evaporator.

PROCEDURE

Extraction of urine (optional, see Assay procedure for direct assay of urine cortisol).

Note: The extraction must be done in clean glass vials or tubes, pre-rinsed with dichloromethane and fitted with teflon or glass stoppers.

Bring samples to room temperature and mix well before starting extraction.

Samples only are extracted before assay; do not extract calibrators.

- Cool a sufficient volume of dichloromethane in ice-water bath.
- Number tubes or flasks.
- Add 500 µL of urine sample to numbered tubes kept on ice.
- Add 5 mL of dichloromethane using a glass pipet, stopper carefully and vortex for one minute.
- Let the two phases separate at room temperature.
- Take off upper, aqueous phase by aspiration.
- Take 2 mL of organic phase using glass pipet or syringe.
- Evaporate organic phase completely in evaporator. Do not heat.
- Take up organic extract in 200 µL of zero calibrator. Wait 15 minutes and then vortex vigorously.

Preparation of reagents

Let all the reagents come to room temperature.

Assay procedure for serum, plasma and urine extracts:

Step 1 Additions*	Step 2 Incubation	Step 3 Counting
To coated tubes, add successively: 50 µL of calibrator, control, serum sample, plasma sample or urine extract and 500 µL of tracer. Vortex gently 1-2 seconds.	Incubate 1 hour at 18-25°C with shaking (≥ 400 rpm).	Aspirate carefully the content of tubes (except the 2 tubes «total cpm»). Count bound cpm (B) and total cpm (T) for 1 min.

* Add 500 µL of tracer to 2 additional tubes to obtain total cpm.

Assay procedure for direct assay of urine cortisol:

Step 1* Additions	Step 2 Incubation	Step 3 Counting
To coated tubes, add successively: 50 µL of calibrator or control or 50 µL of urine sample followed by 50 µL of zero calibrator** and 500 µL of tracer. Vortex gently 1-2 seconds.	Incubate 1 hour at 18-25°C with shaking (≥ 400 rpm).	Aspirate carefully the content of tubes (except the 2 tubes «total cpm»). Count bound cpm (B) and total cpm (T) for 1 min.

* Add 500 µL of tracer to 2 additional tubes to obtain total cpm.

** Additional 50 µL of zero calibrator is added to tubes with urine samples only.

RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of analyte concentrations in samples measured at the same time as the calibrators.

Standard curve

Example of standard curve is given on the Certificate of Analysis provided with the kit and on the Beckman Coulter website (beckmancoulter.com/techdocs). The measured data are indicative only, do not use them for calculation of your results.

The results in the quality control department were calculated using *spline* curve fit with logit of B/T or B/B_0 on the vertical axis and log of analyte concentration of the calibrators on the horizontal axis.

Other calculation methods may give slightly different results.

Samples

For each sample, locate ratio B/T or B/B_0 on the vertical axis and read off the corresponding analyte concentration on the horizontal axis.

To convert concentrations from nmol/L to ng/mL, multiply results by 0.362.

Serum, plasma or urine samples assayed after extraction

The concentration is in nanomoles per liter (nM). The calculation of the daily urinary cortisol excretion is given in the following paragraph.

Urine samples assayed by direct procedure

The value must be multiplied by **1.09**, the dilution factor due to the addition of 50 µL of zero calibrator.

It is suggested that in cases where there is disagreement with clinical data or other measurements, to check the value obtained in the direct assay by an indirect assay after dichloromethane extraction.

The daily excretion of urine cortisol (Q) (nmol/24hours) is:

$$Q = U \times V$$

where

U = urine cortisol value (nM)

V = volume of urine in liters/24 hours

EXPECTED VALUES

We recommend each laboratory to establish its own reference values. The following values obtained from healthy subjects are indicative only.

Sera:

Morning: 263 to 724 nM (n = 69) Evening: 49 to 430 nM (n = 84)

Urines (n = 66)

Assay	Mean (nmol/24h)	Standard deviation (nmol/24h)	Extremes values for 95% of population (nmol/24h)
Direct	114	55	38 - 208
After extraction	103	45	30 - 197

Detail information about expected values for children (sorted according to age and sex) can be found in the data sheet "APPENDIX".

QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly in the same way as the assay samples, and it is recommended that their results be analyzed using appropriate statistical methods.

Failure to obtain the appropriate values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: imunochem@beckman.com

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of EU Member State in which the user and/or patient is located.

PERFORMANCE CHARACTERISTICS

(for more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Sensitivity

Limit of Detection (LoD): 8.60 nM

The LoD of the assay is 8.60 nM, determined consistent with guidelines in CLSI document EP17-A2 [7] based on the proportions of false positives (α) less than 5% and false negatives (β) less than 5%; using determinations, with 128 blank and 120 low level samples; and Limit of Blank (LoB) of 4.44 nM.

Specificity

The antibody used in the immunoassay is highly specific for cortisol. Extremely low cross reactivities were obtained against other naturally occurring steroids (Aldosterone, Corticosterone, Cortisone, 11-Desoxycortisol, Progesterone, etc.) or therapeutic drugs that may be present in patient samples (Prednisolone, Prednisone, Spironolactone, etc.).

Precision

Repeatability and within-laboratory precision

The precision of the assay was determined consistent with guidelines in CLSI document EP05-A3 [8]. For repeatability the coefficients of variation were found below or equal to 7.49 % for serum samples. For within-laboratory precision the coefficients of variation were found below or equal to 13.7 % for serum samples.

Accuracy

Linearity

The assay demonstrated to be linear from 12.06 to 2,520 nM using serum samples (determined consistent with guidelines in CLSI document EP06-A [9]).

Dilution test

High-concentration serum samples were serially diluted with the zero calibrator. The recovery percentages obtained were between 85.7% and 115%.

Recovery test

Low-concentration serum samples were spiked with known quantities of cortisol. The recovery percentages obtained were between 95.2% and 113%.

Measurement range (from LoD to the highest calibrator): 8.60 to approximately 2,000 nM.

LIMITATIONS

Failure to follow these instructions for use (IFU) may significantly affect results.

Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information.

Do not use hemolyzed, lipemic or icteric samples. For more details, see Appendix, § Interference.

Treatment with high doses of corticoids (e.g. prednisolone and prednisone) may lead to an artefactual increase in serum and urine cortisol, due to interference by the substance administered or certain of its metabolites. It is therefore inadvisable to perform direct cortisol assays on patients receiving systemic corticotherapy.

In immunoassays, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Immunoassays may be also affected by presence of anti-avidin or anti-streptavidin antibodies, as well as by the presence of autoantibodies directed against the determined analyte. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies [10, 11, 12].

APPENDIX

PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

Interference

Serum samples containing cortisol concentrations (low and high) were spiked with multiple concentrations of the substances listed below and assayed using Cortisol RIA KIT. Values were calculated as described in CLSI EP07, 3rd ed. [13]. Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). No interference (defined as a shift in dose > 15 %) was found for addition of interferent up to concentration stated in the table below.

Interferent	Test concentration
Acetylsalicylic acid	37.65 µg/mL
Ascorbic acid	50.06 µg/mL
Biotin	1,568 ng/mL
Conjugated bilirubin	514.1 µg/mL
Hemoglobin	10,276 µg/mL
Heparin	7,971 ng/mL
Cholesterol	7.28 mg/mL
Ibuprofen	233.4 µg/mL
Prednisone	125.1 ng/mL
Prednisolone	106.7 ng/mL
Rheumatoid factor	29.82 IU/mL
Triglycerides	8.56 mg/mL
Unconjugated bilirubin	380.9 µg/mL

In spite of hemoglobin, bilirubin (conjugated, unconjugated), triglycerides, prednisone and prednisolone interference data in the table, we advise to avoid using hemolyzed, lipemic or icteric samples or samples of those patients who are receiving systemic corticotherapy.

Specificity

The percent cross-reactivity is expressed as the ratio of the cortisol concentration to the concentration of the reacting compound at 50% binding of the zero calibrator.

Data on cross-reactivity with several endogenous and pharmaceutically employed steroids are presented in the following table:

Steroid	% Cross- reactivity	Steroid	% Cross- reactivity
Cortisol	100	Tetrahydro-11-desoxycortisol	<0.1
11-Desoxycortisol	18	Dexamethasone	<0.1
Corticosterone	8.4	Substance E	<0.1
21-Desoxycortisol	7.5	α-Cortolone	<0.1
Desoxycorticosterone	7.3	Hydrocortisone-21-sulfate	<0.1
17α-Hydroxyprogesterone	3.5	Etiocholanolone	<0.1
Dihydrocortisol	2.4	11β-Hydroxyetiocholanolone	<0.1
5α-Dihydrocortisone	2.3	Prégnenolone sulfate	<0.1
5β-Dihydrocortisone	<0.1	Estradiol	<0.1
Progesterone	1.8	Estriol	<0.1
Cortisone	1.5	Estrone	<0.1
Pregnenolone	1.1	DHEA-sulfate	<0.1
Allotetrahydrocortisone	0.8	Androstenedione	<0.1
21-Desoxycortisone	0.13	Spironolactone	<0.1
6α-Methylprednisolone	0.27	18-Hydroxycorticosterone	<0.1
6β-Hydroxycortisol	<0.1	Aldosterone	<0.1
β-Cortolone	<0.1	Danazol	<0.1
20α-Dihydrocortisol	<0.1	5α-Dihydrotestosterone	<0.1
Tetrahydrocortisone	<0.1	19-Norethisterone	<0.1
β-Cortol	<0.1	Testosterone	<0.1
Tetrahydrocortisol	<0.1	17-hydroxypregnanolone	ND
Pregnanetriolone	ND	Pregnanetriol	ND
18-Hydroxycortisol	ND		

· ND = Non-Detectable

Precision

Repeatability and within-laboratory precision

Samples were assayed for 20 days, 2 runs per day, in triplicates per run. Assays were performed by two lab technicians, by two reagent lots. There were 120 individual measurements per sample with no invalid results.

Serum	Mean (nM)	Repeatability		Within laboratory precision	
		SD (nM)	C.V. (%)	SD (nM)	C.V. (%)
S1	1,769	113.2	6.40	133.3	7.54
S2	831.7	49.50	5.95	67.13	8.07
S3	415.9	20.90	5.03	28.66	6.89
S4	264.2	15.10	5.72	20.66	7.82
S5	139.4	9.28	6.66	13.32	9.56
S6	34.04	2.55	7.49	4.68	13.74

EDTA plasma	Mean (nM)	Repeatability		Within laboratory precision	
		SD (nM)	C.V. (%)	SD (nM)	C.V. (%)
P1	1,585	81.46	5.14	169.1	10.67
P2	776.0	38.52	4.96	87.91	11.33
P3	415.0	20.63	4.97	31.87	7.67
P4	34.14	2.78	8.15	4.17	12.22
P5	278.0	14.59	5.25	23.47	8.44
P6	118.0	6.20	5.26	12.05	10.21

Urine	Mean (nM)	Repeatability		Within laboratory precision	
		SD (nM)	C.V. (%)	SD (nM)	C.V. (%)
U1	1,283	87.65	6.83	152.0	11.85
U2	932.3	51.23	5.49	82.61	8.86
U3	367.1	23.23	6.33	31.47	8.57
U4	177.6	8.62	4.85	16.09	9.06
U5	74.18	4.48	6.04	8.05	10.85
U6	232.7	10.65	4.58	23.02	9.89

Urine extract	Mean (nM)	Repeatability		Within laboratory precision	
		SD (nM)	C.V. (%)	SD (nM)	C.V. (%)
UE1	794.1	39.47	4.97	156.0	19.64
UE2	518.0	25.75	4.97	122.0	23.55
UE3	186.4	11.03	5.92	39.19	21.02
UE4	72.44	4.25	5.86	15.60	21.54
UE5	31.22	3.25	10.42	5.99	19.18
UE6	91.80	5.57	6.07	19.43	21.16

Accuracy

Linearity

The assay demonstrated to be linear from 8.60 to 2,864 nM using EDTA plasma samples (determined consistent with guidelines in CLSI document EP06-A [9]).

The assay demonstrated to be linear from 17.86 to 2,698 nM using urine samples (determined consistent with guidelines in CLSI document EP06-A [9]).

The assay demonstrated to be linear from 15.84 to 3,391 nM using urine extracts (determined consistent with guidelines in CLSI document EP06-A [9]).

Dilution test

Samples were diluted in the zero calibrator and assayed according to the procedure of the kit.

Serum	Dilution factor	Measured (nM)	Expected (nM)	Ratio (%) Measured/ Expected
S1	-	308.6	-	-
	1:2	157.8	154.3	102.3
	1:4	69.2	77.1	89.7
	1:8	38.9	38.6	100.8
	1:16	18.3	19.3	95.1
	1:32	8.5	9.6	88.2
S2	-	436.7	-	-
	1:2	228.7	218.4	104.7
	1:4	113.5	109.4	103.9
	1:8	63.0	54.6	115.4
	1:16	27.7	27.3	101.5
	1:32	13.1	13.6	96.1
S3	-	520.6	-	-
	1:2	278.0	260.3	106.7
	1:4	144.9	130.1	111.3
	1:8	74.7	65.1	114.8
	1:16	35.9	32.5	110.2
	1:32	17.1	16.3	105.1
S4	-	1,138	-	-
	1:2	560.2	569.0	98.5
	1:4	278.4	284.5	97.8
	1:8	139.9	142.2	98.4
	1:16	61.0	71.1	85.7
	1:32	32.9	35.6	92.6
S5	-	1,432	-	-
	1:2	811.6	716.0	113.3
	1:4	349.7	358.2	97.7
	1:8	187.8	179.3	104.9
	1:16	95.6	89.5	106.7
	1:32	48.8	44.8	108.9

EDTA plasma	Dilution factor	Measured (nM)	Expected (nM)	Ratio (%) Measured/ Expected
P1	-	410.4	-	-
	1:2	202.0	205.2	98.47
	1:4	86.40	102.6	84.22
	1:8	43.72	51.29	85.23
	1:16	22.71	25.65	88.55
	1:32	13.68	12.82	106.7
P2	-	534.3	-	-
	1:2	254.5	267.1	95.27
	1:4	121.6	133.6	91.07
	1:8	59.93	66.78	89.74
	1:16	27.03	33.39	80.95
	1:32	13.39	16.70	80.20
P3	-	377.7	-	-
	1:2	171.7	188.9	90.93
	1:4	75.66	94.43	80.12
	1:8	38.07	47.22	80.63
	1:16	20.89	23.61	88.49
P4	-	327.0	-	-
	1:2	165.1	163.5	101.0
	1:4	82.97	81.76	101.5
	1:8	36.62	40.88	89.58
	1:16	18.89	20.44	92.42
P5	-	754.4	-	-
	1:2	358.2	377.2	94.96
	1:4	182.2	188.6	96.62
	1:8	77.42	94.30	82.10
	1:16	37.86	47.15	80.29
	1:32	20.16	23.58	85.51

Samples were diluted in distilled water and assayed according to the assay procedure for direct assay of urine.

Urine (direct)	Dilution factor	Measured (nM)	Expected (nM)	Ratio (%) Measured/ Expected
U1	-	342.8	-	-
	1:2	203.0	171.4	118.4
	1:4	81.04	85.70	94.56
	1:8	34.33	42.85	80.11
U2	-	524.5	-	-
	1:2	271.5	262.3	103.5
	1:4	141.8	131.1	108.1
	1:8	53.96	65.57	82.30
	1:16	29.33	32.78	89.47
	1:32	13.89	16.39	84.74
U3	-	578.5	-	-
	1:2	295.8	289.2	102.3
	1:4	138.3	144.6	95.63
	1:8	66.08	72.31	91.39
	1:16	28.96	36.15	80.10
U4	-	513.5	-	-
	1:2	261.1	256.7	101.7
	1:4	129.5	128.4	100.9
	1:8	62.16	64.18	96.85
	1:16	27.42	32.09	85.44
U5	-	298.1	-	-
	1:2	156.9	149.1	105.3
	1:4	77.78	74.53	104.4
	1:8	31.83	37.26	85.42

Samples were extracted, diluted in zero calibrator and assayed according to the assay procedure of the kit.

Urine (extract)	Dilution factor	Measured (nM)	Expected (nM)	Ratio (%) Measured/ Expected
UE1	-	272.6	-	-
	1:2	139.7	136.3	102.5
	1:4	56.90	68.15	83.49
	1:8	30.75	34.08	90.24
	1:16	13.86	17.04	81.35
UE2	-	489.8	-	-
	1:2	234.9	244.9	95.90
	1:4	121.4	122.5	99.15
	1:8	59.47	61.23	97.13
	1:16	33.11	30.61	108.2
	1:32	14.59	15.31	95.32
UE3	-	529.1	-	-
	1:2	295.0	264.5	111.5
	1:4	139.6	132.3	105.5
	1:8	64.36	66.13	97.32
	1:16	31.05	33.07	93.90
	1:32	16.72	16.53	101.1
UE4	-	444.1	-	-
	1:2	220.5	222.1	99.28
	1:4	104.8	111.0	94.38
	1:8	49.74	55.52	89.60
	1:16	24.71	27.76	89.02
	1:32	11.97	13.88	86.25
UE5	-	254.8	-	-
	1:2	114.3	127.4	89.74
	1:4	54.86	63.69	86.13
	1:8	26.06	31.85	81.83
	1:16	13.89	15.92	87.23

Recovery test

Samples were spiked with known quantities of cortisol and assayed according to the assay procedure of the kit.

Serum	Endogen. conc.	Added conc.	Expected conc.	Measured conc.	Ratio (%) Measured/ Expected
	(nM)				
S1	24.8	15	39.8	39.9	100.3
	24.8	50	74.8	84.6	113.1
	24.8	150	174.9	185.8	106.2
	24.8	500	524.9	552.0	105.2
	24.8	1,501	1,526	1,655	108.4
S2	70.4	15	85.4	87.0	101.9
	70.4	50	120.4	130.0	107.9
	70.4	150	220.4	231.2	104.8
	70.4	500	570.4	611.5	107.1
	70.4	1,501	1,572	1,675	106.5
S3	142.5	15	157.5	149.9	95.2
	142.5	50	192.5	187.4	97.3
	142.5	150	292.6	295.4	100.9
	142.5	500	642.6	723.9	112.6
	142.5	1,501	1,644	1,722	104.7
S4	261.3	15	276.3	275.9	99.8
	261.3	50	311.4	337.8	108.5
	261.3	150	411.4	427.4	103.8
	261.3	500	761.5	806.0	105.8
	261.3	1,501	1,763	1,862	105.6
S5	353.0	15	368.0	367.4	99.8
	353.0	50	403.0	405.8	100.7
	353.0	150	503.0	513.1	102.0
	353.0	500	853.0	891.1	104.4
	353.0	1,501	1,854	1,911	103.1

EDTA plasma	Endogen. conc.	Added conc.	Expected conc.	Measured conc.	Ratio (%) Measured/ Expected
	(nM)				
P1	22.06	9.59	31.65	37.86	119.6
	20.99	19.55	40.54	35.40	87.33
	22.35	23.55	45.89	44.29	96.51
P2	27.76	14.69	42.45	47.74	112.5
	28.29	36.96	65.25	77.97	119.5
	27.09	66.36	93.45	104.0	111.3
P3	39.76	19.55	59.31	62.09	104.7
	41.26	41.32	82.58	91.74	111.1
	41.53	101.27	142.8	163.5	114.5
P4	46.97	23.55	70.52	72.38	102.6
	45.22	49.88	95.10	91.04	95.73
	46.08	101.3	147.3	152.5	103.5
P5	50.16	28.08	78.24	72.88	93.15
	48.01	58.22	106.23	108.8	102.4
	48.91	125.0	173.9	180.89	104.0

Urine (direct)	Endogen. conc.	Added conc.	Expected conc.	Measured conc.	Ratio (%) Measured/ Expected
	(nM)				
U1	55.66	23.55	79.21	84.11	106.2
	53.93	45.63	99.55	95.74	96.17
	53.93	125.0	178.9	182.9	102.2
U2	26.97	13.44	40.41	43.12	106.7
	27.84	23.55	51.39	47.21	91.87
	26.97	45.63	72.60	62.25	85.75
U3	19.15	13.44	32.59	26.92	82.60
	19.77	23.55	43.32	37.05	85.53
	19.15	45.63	64.78	60.53	93.44
U4	68.95	32.55	101.50	92.97	91.60
	65.61	66.36	131.97	138.64	105.1
	67.24	136.6	203.9	208.4	102.2
U5	35.38	23.55	58.93	51.29	87.04
	34.71	36.96	71.67	63.55	88.67
	33.24	66.36	99.60	94.22	94.60

Urine (extract)	Endogen. conc.	Added conc.	Expected conc.	Measured conc.	Ratio (%) Measured/ Expected
	(nM)				
UE1	27.98	23.55	51.53	59.15	114.8
	27.10	45.63	72.73	61.66	84.78
	27.10	125.0	152.1	146.6	96.40
UE2	10.35	13.44	23.79	21.53	90.51
	10.68	23.55	34.23	33.75	98.59
	10.35	45.63	55.98	51.19	91.45
UE3	4.55	13.44	17.98	14.93	83.02
	4.69	23.55	28.24	28.15	99.67
	4.55	45.63	50.17	56.16	111.9
UE4	43.38	32.55	75.92	90.25	118.9
	41.27	66.36	107.6	126.7	117.7
	42.30	136.6	178.9	200.2	111.9
UE5	13.81	23.55	37.36	35.40	94.76
	13.55	36.96	50.51	52.08	103.1
	12.97	66.36	79.34	79.28	99.93

Expected values for children

Results are sorted according to the age and sex.

Cortisol (nM)						
Boys	**N**	**Median**	**Min.**	**Max.**	**2.5th percentile**	**97.5th percentile**
<1 month	26	150.3	59.60	472.5	62.60	416.4
1-2 months	28	161.6	33.40	653.0	43.32	641.9
3-5 months	14	353.9	120.5	516.5	130.5	500.3
6-11 months	46	218.2	80.30	686.2	117.4	570.3
1-8 years	36	377.9	153.9	682.1	167.6	676.7
9-14 years	31	274.3	126.5	484.1	180.4	476.4

Cortisol (nM)						
Girls	**N**	**Median**	**Min.**	**Max.**	**2.5th percentile**	**97.5th percentile**
< 1 month	16	89.50	35.80	450.3	35.88	414.1
1 - 2 months	16	123.0	26.30	605.8	27.39	579.2
3 - 5 months	27	196.6	42.10	621.1	52.05	614.9
6 - 11 months	31	254.8	105.6	766.2	116.6	658.4
1 - 8 years	31	324.1	140.0	645.2	154.6	537.1
9 - 14 years	34	252.1	112.5	632.0	115.1	610.1

^{125}I Characteristics

$T_{1/2}$ (^{125}I) = 1443 h = 60.14 d

^{125}I	E (MeV)	%
γ	0.035	6.5
K_α X-ray	0.027	112.5
K_β X-ray	0.031	25.4

Correlations

Serum and EDTA plasma values for 20 samples (serum values ranging from 234.5 to 667.6 nM) were compared using the IM1841 Cortisol RIA KIT. Results are as follows:

[EDTA-plasma] = 0.9874[serum] + 8.7389, R = 0.9771

Direct assay of urinary Cortisol and after extraction for 108 urine samples (values of direct urinary cortisol ranging from 9.80 to 2,180 nM) were compared using the IM1841 Cortisol RIA KIT.

Results are as follows: [Direct] = 1.05 [Extraction] + 5.40, R = 1.00

Symbols Key

REF	Product Reference / Référence du produit / Produktreferenz / Riferimento prodotto / Número de referencia del producto / Referência do produto / Produktreferens / Κωδικός αναφοράς προϊόντος / 产品参考 / Gaminio nuoroda / Termékszám / Dane referencyjne produktu / Reference k produktu / Referenčné označenie výrobku / 제품 참조 자료 / Ürün Referansı / Ссылка на продукт / Референца за производ / 產品參考
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