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Cortisol

cobas®

Cortisol

REF	Σ	SYSTEM
11875116 122	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro quantitative determination of cortisol in human serum, plasma, urine, and saliva. The determination of cortisol is used for the recognition and treatment of functional disorders of the adrenal gland.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

Cortisol (hydrocortisone) is the most prominent glucocorticosteroid, and it is essential for the maintenance of several body functions. Like other glucocorticosteroids, cortisol is synthesized from the common precursor cholesterol in the zona fasciculata of the cortex of the adrenal gland. For the transport of cortisol in blood, about 90 % of cortisol is bound to corticosteroid binding globulin (CBG) and to albumin. Only a small amount of cortisol circulates unbound in blood and is free to interact with its receptors.[1]

The most important physiological effects of cortisol are the increase of blood glucose levels (enhancement of gluconeogenesis, catabolic action), and its anti-inflammatory and immunosuppressive action.[1]

Synthesis and secretion of cortisol by the adrenal gland are controlled by a negative feedback mechanism within the hypothalamus-pituitary-adrenal cortex-axis. If the cortisol level is low, corticotropin releasing hormone (CRH) is secreted by the hypothalamus, which causes the pituitary to release adrenocorticotropic hormone (ACTH). This stimulates the synthesis and secretion of cortisol by the adrenal gland. Cortisol itself acts in a negative feedback mechanism on the pituitary gland and the hypothalamus. In addition, stress is followed by increased cortisol secretion.[1]

Serum cortisol concentrations normally show a diurnal variation.[1] Maximum concentrations (700 nmol/L or 25.4 µg/dL) are usually reached early in the morning and then concentrations decline throughout the day to an evening level that is about half of the morning concentration. Therefore, for interpretation of results, it is important to know the collection time of the serum sample.

The cortisol status of a patient is used to diagnose the function or malfunction of the adrenal gland, the pituitary, and the hypothalamus.[2,3] Thereby cortisol serum concentrations are used for monitoring of several diseases with an overproduction (e.g. Cushing's syndrome)[4,5] or underproduction (e.g. Addison's disease) of cortisol and for the monitoring of several therapeutic approaches (e.g. dexamethasone suppression therapy in Cushing's syndrome and hormone replacement therapy in Addison's disease).

The determination of cortisol in 24-hour urine is the method of choice for the detection of Cushing's syndrome since cortisol excretion in urine is not subject to the diurnal rhythm of cortisol secretion.[6] This allows a more exact differentiation between healthy individuals and patients with Cushing's syndrome. Cortisol which is excreted into urine without alteration is referred to as urinary free cortisol (UFC). Usually there is a direct proportional relationship between urinary free cortisol and the unbound and hence biologically active cortisol in the blood.[1]

Recent studies have demonstrated that several night-time salivary cortisol measurements are superior to the measurement of urinary free cortisol in establishing the diagnosis of Cushing syndrome.[7,8,9,10]

Determination of night-time salivary cortisol is particularly helpful in children, psychiatric patients, and subjects where a variety of stress factors might affect the adrenal cortex, causing increased adrenal steroid concentrations.[11]

The Elecsys Cortisol assay makes use of a competition test principle using a polyclonal antibody which is specifically directed against cortisol. Endogenous cortisol in the sample which has been liberated from binding protein with danazol competes with exogenous cortisol derivative in the test which has been labeled with ruthenium complex[a)] for the binding sites on the biotinylated antibody.

Urine can be used for analysis after extraction with dichloromethane (to lower interfering substances).

Untreated saliva is used directly after centrifugation.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample is incubated with a cortisol-specific biotinylated antibody and a ruthenium complex labeled cortisol derivative. Depending on the concentration of the analyte in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with sample analyte and in part with ruthenylated hapten.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Reagents - working solutions

The reagent rackpack is labeled as CORT.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-cortisol-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated polyclonal anti-cortisol antibody (ovine) 90 ng/mL; MES[b)] buffer 100 mmol/L, pH 6.0; preservative.

R2 Cortisol-peptide~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Cortisol derivative (synthetic), labeled with ruthenium complex 25 ng/mL; danazol 20 µg/mL; MES buffer 100 mmol/L, pH 6.0; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

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Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	8 weeks

Specimen collection and preparation

Only the specimens listed below were tested.

Serum and plasma:

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-, NH_4^+-heparin, K_2-, K_3-, Na_2-EDTA and sodium citrate plasma. When sodium citrate is used the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

The values obtained for sodium fluoride/potassium oxalate plasma are by 27 % lower than those obtained for serum.

Please note: Due to the circadian rhythm of cortisol levels in serum and plasma, the sample collection time must be noted.

Stable for 5 days at 2-8 °C, 3 months at -20 °C. Freeze only once.[12]

Saliva:

Collect a saliva sample using a Salivette device.

Do not use vials containing citric acid.

Remove the swab from the suspended insert and gently chew for about 2 minutes to saturate the swab with saliva. Replace the swab into the suspended insert and close the tube. Centrifuge the Salivette for 2 minutes at 1000 g to separate off the saliva into the outer tube. Use the clear supernatant for the Elecsys Cortisol assay. Use saliva samples in the same way as serum or plasma specimens.

The centrifuged saliva sample is stable for 5 days at 2-8 °C, 3 months at -20 °C. Freeze only once.[13]

Urine:

Collect 24-hour urine in clean containers without preservatives and measure the volume (L/24 h). If necessary, centrifuge precipitates prior to extraction. Use the extracted urine samples in the analysis in the same way as serum and plasma samples.

Stability of the urine samples: 7 days at 2-8 °C, 3 months at -20 °C. Freeze only once.

Stability of the reconstituted extract: 7 days at 2-8 °C, 4 weeks at -20 °C. Freeze only once.

The sample types listed (serum and plasma) were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 11875124122, Cortisol CalSet, for 4 x 1 mL
- REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

Additionally required for the determination of cortisol in urine:

- Dichloromethane (methylene chloride)
- Suitable glass tubes, pipettes, rotating shaker (e.g. vortex), nitrogen, and extractor hood

Additionally required for the determination of cortisol in saliva:

- Salivette®, sample collection tube (cotton swab tube without preparation), Sarstedt, Nümbrecht, Germany, REF 51.1534

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

Extraction and reconstitution of the urine samples

1. Mix well 600 µL of urine + 3.0 mL of dichloromethane in a glass tube for 7 minutes.
2. Centrifuge for 5 minutes at 2500 g to separate the phases.
3. Remove and discard the aqueous phase and possible residues at the phase interface.
4. Transfer 1.5 mL of the dichloromethane phase into a clean glass tube and, under a hood, reduce until dry by exposing it to a gentle nitrogen flow.
5. Reconstitute the dry residue with 300 µL of Diluent Universal and incubate for 30 minutes at 15-25 °C while occasionally mixing 4 times for 1 minute in a rotating shaker.
6. Analyze the reconstituted sample in the same way as serum and plasma samples.

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the Enzymun-Test Cortisol method. This in turn was standardized via ID-MS.[14]

The Elecsys Cortisol assay showed recovery results from 89-111 % in the IRMM (Institute for Reference Materials and Measurements, Geel,

Belgium)/IFCC-451 Panel (ID/GC/MS),[14,15] which consists of 34 samples in the concentration range of 83-764 nmol/L.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

In addition, other suitable control material can be used.

Urine controls should be extracted and analyzed the same way urine samples are.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in nmol/L, µg/dL or µg/L).

Conversion factors:	nmol/L x 0.03625 = µg/dL
	nmol/L x 0.3625 = µg/L
	µg/dL x 27.586 = nmol/L
	µg/L x 2.7586 = nmol/L

Manual calculation for urinary free cortisol: cortisol excretion over 24-hours (cortisol concentration/24 h):

Multiply the analyzer results by the volume of the 24-hour urine (L/24 h) (when the analyzer result is given in µg/dL, multiply again by 10 = µg/24 h). The average yield of extraction (n = 25) was determined to be 94 %.

Limitations - interference

When performed in serum and plasma, the assay is unaffected by icterus (bilirubin < 1026 µmol/L or < 60 mg/dL), hemolysis (Hb < 1.2 mmol/L or < 1.9 g/dL), lipemia (Intralipid < 2700 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

When performed in urine, the assay is unaffected by 60 mg/dL protein, 750 mmol/L NaCl, 350 mmol/L urea, 5 mmol/L creatinine, 2 mmol/L glucose.

Criterion: Recovery within ± 15 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1100 IU/mL.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

Pregnancy, contraceptives and estrogen therapy give rise to elevated cortisol concentrations.

In samples from patients who have been treated with prednisolone, methylprednisolone or prednisone, falsely elevated concentrations of cortisol may be determined.

During metyrapon tests, 11-deoxycortisol levels are elevated. Falsely elevated cortisol values may be determined due to cross reactions (see section on analytical specificity).

Patients suffering from 21-hydroxylase deficiency exhibit elevated 21-deoxycortisol levels and this can also give rise to elevated cortisol levels.

The time of sample collection must be taken into account when interpreting results due to the cortisol secretion circadian rhythm. Severe stress can also give rise to elevated cortisol levels.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

0.5-1750 nmol/L or 0.018-63.4 µg/dL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.5 nmol/L (< 0.018 µg/dL). Values above the measuring range are reported as > 1750 nmol/L (> 63.4 µg/dL) (or up to 17500 nmol/L or 634 µg/dL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: 0.500 nmol/L (0.018 µg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Serum and plasma samples with cortisol concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:10 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 50 nmol/L or > 1.8 µg/dL.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Urine samples with concentrations above the measuring range can be diluted prior to extraction using urine with a low analyte concentration. This dilution must be taken into account when calculating the cortisol concentration in the urine.

Expected values

Cortisol in serum and plasma

In studies with the Elecsys Cortisol assay, the following values were determined using samples from healthy individuals (5^{th}-95^{th} percentile):

Morning hours 7-10 a.m.: 171-536 nmol/L (6.2-19.4 µg/dL), n = 144

Afternoon hours 4-8 p.m.: 64-327 nmol/L (2.3-11.9 µg/dL), n = 135

Urinary free cortisol

The following values were determined in studies with the Elecsys Cortisol assay covering urine samples from 88 healthy individuals (5^{th}-95^{th} percentile):

100-379 nmol/24 h (36-137 µg/24 h)

Cortisol in saliva

The following values were determined in saliva samples from 154 healthy individuals (95^{th} percentile) using the Elecsys Cortisol assay:

Morning hours 8-10 a.m.: < 19.1 nmol/L (< 0.69 µg/dL)

Afternoon hours 2:30-3:30 p.m.: < 11.9 nmol/L (< 0.43 µg/dL)

Midnight hours: see reference[16]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Cortisol

Cortisol

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in accordance with a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%	nmol/L	µg/dL	%
HS[c)] 1	208	7.53	2.76	0.10	1.3	3.29	0.12	1.6
HS 2	561	20.3	7.40	0.23	1.3	8.36	0.30	1.5
HS 3	1268	46.0	14.0	0.52	1.1	19.9	0.72	1.6
PC U[d)]1	363	13.2	5.08	0.18	1.4	5.67	0.21	1.6
PC U2	865	31.4	8.54	0.31	1.0	12.5	0.45	1.4

c) HS = human serum

d) PC U = PreciControl Universal

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%
HS 1	129	4.69	2.25	0.08	1.7
HS 2	352	12.8	5.19	0.19	1.5
HS 3	717	26.0	12.5	0.45	1.7
PC U1	418	15.1	4.59	0.17	1.1
PC U2	866	31.4	8.90	0.32	1.0

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%
HS 1	124	4.51	2.79	0.10	2.2
HS 2	341	12.4	9.72	0.35	2.8
HS 3	691	25.1	12.4	0.45	1.8
PC U1	410	14.8	6.76	0.25	1.7
PC U2	846	30.7	11.5	0.42	1.4

In order to describe the effect of extraction and reconstitution on precision, the following table may be used in comparison to the respective table above with serum samples.

Precision of the cortisol determination in urine was determined using Elecsys reagents, urine samples, and a urine control by 25-fold extraction in one run (repeatability n = 25) and by measuring 10 urine single extracts in single determinations in 10 runs (intermediate precision n = 10):

Elecsys 2010 and **cobas e** 411 analyzers					
	Repeatability incl. extract.				
Sample	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%
Urine 1	617	22.3	13.3	0.48	2.2
Urine 2	917	33.2	21.2	0.77	2.3
Urine 3	1156	41.9	33.2	1.20	2.9
Urine 4	1683	61.0	39.1	1.42	2.3
Urine Control 1	-	-	-	-	-

Elecsys 2010 and **cobas e** 411 analyzers					
	Intermediate precision incl. extract.				
Sample	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%
Urine 1	639	23.2	15.9	0.58	2.5
Urine 2	922	33.4	29.4	1.07	3.2
Urine 3	1162	42.1	28.7	1.04	2.5
Urine 4	1625	58.9	30.0	1.09	1.8
Urine Control 1	77.8	2.82	3.66	0.13	4.7

Precision of the cortisol determination in saliva was determined using Elecsys reagents, native saliva samples, and spiked saliva samples in one run (repeatability, n = 21) and in a single determination of 10 runs (intermediate precision, n = 10). Different saliva samples were used for the determination of repeatability and intermediate precision:

Elecsys 2010 and **cobas e** 411 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%
Saliva 1	4.68	0.170	0.287	0.010	6.1
Saliva 2	11.5	0.417	0.309	0.011	2.7
Saliva 3	15.1	0.547	0.611	0.022	4.0
Saliva 4	15.9	0.576	0.245	0.009	1.5
Saliva 5	19.8	0.718	0.611	0.022	2.8

Elecsys 2010 and **cobas e** 411 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	nmol/L	µg/dL	nmol/L	µg/dL	%
Saliva 1	2.08	0.075	0.696	0.025	33.4
Saliva 2	8.05	0.292	0.924	0.033	11.5
Saliva 3	13.1	0.475	0.938	0.034	7.1
Saliva 4	34.6	1.25	1.69	0.061	4.9
Saliva 5	42.5	1.54	1.76	0.064	4.1

Method comparison

Serum:

A comparison of the Elecsys Cortisol assay (y) with the Enzymun-Test Cortisol method (x) in 95 clinical serum samples gave the following correlations (nmol/L):

Passing/Bablok[17]	Linear regression
y = 1.11x - 25.3	y = 1.08x - 22.2
τ = 0.885	r = 0.985

The sample concentrations were between approximately 100 and 1240 nmol/L or 3.6 and 45 µg/dL.

Urine:

A comparison of the Elecsys Cortisol assay (y) with a commercially available cortisol test (x) in 127 extracted urine samples gave the following correlations (nmol/L):

Passing/Bablok[17]

Slope: 1.32 (95 % confidence range: 1.26-1.44)

Intercept: 2.32 (95 % confidence range: -7.82-7.18)

τ = 0.787

Linear regression:

Slope: 1.23 (95 % confidence range: 1.20-1.26)

Intercept: 15.3 (95 % confidence range: 9.89-20.70)

r = 0.990

The sample concentrations were between 5.52 and 1402 nmol/L or 0.20 and 50.82 µg/dL for the commercially available cortisol test.

Saliva:

A comparison of the Elecsys Cortisol assay (y) with a commercially available cortisol test developed specifically for the determination of cortisol in saliva (x) in 326 saliva samples gave the following correlations (nmol/L):

Passing/Bablok[17]

Slope: 1.12 (95 % confidence range: 1.03-1.22)

Intercept: 0.52 (95 % confidence range: -0.06-0.83)

τ = 0.531

Linear regression:

Slope: 0.90 (95 % confidence range: 0.87-0.94)

Intercept: 1.71 (95 % confidence range: 1.47-1.96)

r = 0.942

The sample concentrations were between 1.29 and 50.4 nmol/L or 0.05 and 1.83 µg/dL for the commercially available cortisol test.

Analytical specificity

For the antibody derivate used, the following cross-reactivities (%) were found:

a) substance added per 10 µg/mL:

corticosterone	5.8
cortisol-21-sulfate	0.04
cortisone	0.30
11-deoxycorticosterone	0.69
11-deoxycortisol	4.1
dexamethasone	0.08
17-α-hydroxyprogesterone	1.50
prednisone	0.28
progesterone	0.35

b) substance added per 1 µg/mL:

21-deoxycortisol	45.4
6-β-hydroxycortisol	158

c) substance added per 0.1 µg/mL:

allotetrahydrocortisol	165
prednisolone	171
6-α-methylprednisolone	389

Functional sensitivity

< 8.5 nmol/L (< 0.308 µg/dL)

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %. It has been determined using low concentration saliva samples.

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For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

CE

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