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Instructions for Use

Steroid Panel LC-MS

Liquid Chromatography-Mass Spectrometry Assay (LC-MS Assay) for the quantitative determination of steroidal hormones in human serum and plasma (EDTA).

REF 30191875

Σ 96

Part A: 2°C 25°C

Part B: -20°C

EU: IVD CE

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REVISION HISTORY OF INSTRUCTIONS FOR USE

Changes from the previous version 2022-05 to actual version 2022-06	
Chapter 16.1.	Additional information
Chapter 21.2.	Additional information

1. INTENDED USE

Liquid Chromatography-Mass Spectrometry Assay (LC-MS Assay) for the quantitative determination of steroidal hormones in human serum and plasma (EDTA).

2. INTENDED PURPOSE

Liquid Chromatography-Mass Spectrometry (LC-MS) assay for the quantitative determination of steroid hormones (11-deoxycorticosterone, 11-deoxycortisol, 17-hydroxypregnenolone, 17-hydroxyprogesterone, 21-deoxycortisol, aldosterone, androstenedione, corticosterone, cortisol, cortisone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, dihydrotestosterone, estradiol, estrone, progesterone, testosterone) and dexamethasone in human serum and EDTA plasma.
This test is intended as an aid to diagnosis, recurring testing or monitoring for patients requiring measurement of the relevant steroid hormone levels. The patient population includes adults and children with suspected or confirmed endocrine disorders or neoplasms. Furthermore, this test is intended for monitoring health status by assessing relevant steroids as physiological markers for fertility.
This product is not intended for the determination of estrone in children.
This product is not appropriate for use in neonatal screening.
The Steroid Panel LC-MS assay is a liquid chromatographic mass spectrometric assay (LC-MS), based on the principle of chromatographic separation and measured on a mass spectrometer. The assay is semiautomated requiring general-purpose laboratory instruments and consumables such as extraction manifolds, evaporators, liquid chromatography mass spectrometry system, vortexer, roller mixer and pipettes to execute the test. Test results may be calculated based on a standard curve and compared to laboratory established reference ranges from healthy individuals (i.e., reference intervals). The test kit is intended for professional laboratory use by trained personnel. The test kit is not for home or layperson use.

3. SUMMARY AND EXPLANATION

Steroid hormones are important signaling molecules that regulate many important physiological and the underlying biochemical processes. They are typically divided into two large groups based on their primary physiological roles: corticosteroids mostly from adrenal cortex and sex steroids mostly from gonads and placenta.[1,2] Corticosteroids are further divided into mineralocorticoids and glucocorticoids. The former is involved in salt and water balance, while the latter is involved in energy metabolism. Sex steroids are further categorized into progestogens, androgens and estrogens for their roles in pregnancy, male and female sexual developments. Measurement of steroids as physiological markers for fertility is helpful for monitoring health status. Steroid hormones exert their functions by binding to their respective receptors and trigger either gene expression events or kinase signaling.[3,4]
The enzymes responsible for the biosynthesis of steroid hormones can be classified into two groups, cytochrome P450 (CYP) and hydroxysteroid dehydrogenases (HSD). Loss-of-function and gain-of-function mutations of many of these genes lead to disruption of hormone homeostasis and cause many different disease conditions.[5] Endocrine disorders like congenital adrenal hyperplasia, aromatase deficiency and apparent mineralocorticoid excess syndrome have strong genetic associations, while certain cases of hyperandrogenism, amenorrhea and adrenal insufficiency are also caused by these enzyme lesions.[5]
Neoplasm is one other common cause of steroid hormone related disorders like Cushing's syndrome, aldosteronism or adrenal incidentaloma, and often associated with altered hormone levels.[6-8] Given steroids' central roles in regulating sexual development, fertility related diagnosis often also includes steroid testing.[9,10] Premature adrenarche can cause hyperandrogenism and polycystic ovarian syndrome later on and affect fertility.[11]
Given the strong association of steroid hormones and endocrine disorders, steroid testing has become an integral part in diagnostic procedures and is recommended by many clinical practice guidelines.[6,8,12-14]
LC-MS based assays have evolved into the gold standard and considered state of the art in steroid hormone testing,[15-18] and their use have also been endorsed by clinical practice guidelines.[6,12]

4. TEST PRINCIPLE

The assay procedure follows the basic principles of a chromatographic separation. Samples are purified through a solid phase extraction step. After reconstitution, samples are subjected to high performance liquid chromatography (HPLC) separation on a reversed phase column. Compounds are sequentially eluted from the chromatographic column with a binary gradient using an aqueous/organic solvent system. The HPLC system is coupled to a triple quadrupole mass spectrometer that is being used for substance specific detection of the analytes. The detection system uses a twofold separation of charged intermediates and fragments of the target analytes. To control for matrix effects, isotope-labelled internal standards are added to calibrators, controls and samples. The responses of the analytes are normalized to the internal standards and this allows for the calculation of a calibration curve that can be used to calculate concentrations of steroids in serum and plasma (EDTA) samples.

The included HPLC method is based on non-polar interactions. After separation, the analytes are ionized via electrospray ionization (ESI) and the appropriate mass-to-charge-ratio is detected via triple quadrupole mass spectrometry. During ionization both negative and positive ionization modes are used. Based on the broad spectrum of analytes, the steroids were split in two LC-MS chromatographic runs. Using one extraction method, all 18 steroids can be analyzed using different LC-MS methods.

Run 1 includes:
11-deoxycorticosterone, 11-deoxycortisol, 17-hydroxyprogesterone, 21-deoxycortisol, aldosterone, androstenedione, corticosterone, cortisol, cortisone, dexamethasone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, dihydrotestosterone, progesterone, testosterone.

Run 2 includes:
17-hydroxypregnenolone, estradiol, estrone.

5. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only by GLP trained professionals.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Broken glass may cause injury. Handle glass vessels with caution.
5. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
6. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
8. Chemicals and prepared, used, unused or expired reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
9. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
10. All serious incidents that have occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
11. Avoid contact with solutions provided. They may cause skin irritations and burns.
12. Mobile Phase A and B do contain ammonium fluoride (< 5 mM); follow safety guidelines for disposal. The solutions must not come into contact with acid.
13. All reagents of this kit containing human serum or plasma have been tested and were found negative for anti-HIV I/II, HBsAg and anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely. For this reason reagents should be treated as potential biohazards in use and for disposal.

6. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at different temperatures:

- **Part A at 2 - 25°C**
- **Part B at -20°C** (orange labeled)

For components included in Part A and Part B see MATERIALS SUPPLIED

Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

The kit is stable up to 6 months after the first opening (not exceeding the expiry date) when stored at indicated storage temperature. The unopened reagents are stable until the expiry date indicated.

After elution, the utilized wells of Extraction Columns and Deep Well Collection Plate cannot be reused. But wells which were not used may be used for extraction of further samples and the Extraction Columns should, therefore, be stored at 18 - 25°C (ambient temperature) protected from dust.

For storage of HPLC column, purge chromatographic column with a mixture of water/methanol (50:50, *v*/*v*, neat, no added additives, for additional information see information enclosed with the chromatographic column).

7. SPECIMEN COLLECTION AND STORAGE

Specimen
Serum and plasma (EDTA)

Specimen collection
The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Sample Collection Device
Gel separation tubes including clot activators can cause inference with steroid hormones due to adsorption.
It is recommended not to use gel separation tubes.

Specimen storage
Samples can be stored at 2 - 8°C for up to 3 days
21-deoxycortisol and dexamethasone are not stable in plasma (EDTA). Please keep the samples frozen at -20°C for analysis of 21-deoxycortisol and dexamethasone. Avoid repeated freeze-thaw cycles. Keep away from heat or direct sunlight.

8. MATERIALS SUPPLIED

Storage	Quantity	Symbol	Component
Part A 2 - 25°C	2 x 25 mL	PHASE A CONC	**Mobile Phase A,** Concentrate (20x) Contains: ammonium fluoride < 5 mM
	2 x 25 mL	PHASE B CONC	**Mobile Phase B,** Concentrate (20x) Contains: ammonium fluoride < 5 mM
	1 x 20 mL	EXTRACT CONC	**Extraction Solution** Concentrate (3.35x) Contains: 2-propanol
	1 x 15 mL	WASH 1 CONC	**Wash Solution 1,** Concentrate (11x) Contains: Methanol
	1 x 10 mL	WASH 2 CONC	**Wash Solution 2,** Concentrate (16x) Contains: Methanol
	1 x 10 mL	ELUTION CONC	**Elution Solution,** Concentrate (12x) Contains: Methanol
	1 x 20 mL	RECONST CONC	**Reconstitution Solution,** Concentrate (2.5x) Contains: Methanol
	1 x 96	SPE COL	**Extraction Columns,** Ready to use.
	1 x 96	COLL PLATE	**Deep Well Collection Plate** Additional Deep Well Collection Plate can be ordered separately from IBL under REF: IB70018
	1 x 96	PLATE SEAL	**Pre-Slit Seal** for Deep Well Collection Plate
	3 x	FOIL	**Adhesive Foil**
Part B -20°C	1 x 6 x 1.3 mL	CAL A-F LYO	**Standard A-F**, lyophilized Concentrations see CALCULATION OF RESULTS
	1 x 1.3 mL	CAL ZERO LYO	**Standard Zero**, lyophilized Concentrations see CALCULATION OF RESULTS
	1 x 5.4 mL	INT STD	**Internal Standard,** Ready to use. Contains: Methanol Concentrations see CALCULATION OF RESULTS
	1 x 2 x 1.3 mL	CONTROL H LYO CONTROL L LYO	**Control High and Control Low**, lyophilized Concentrations / acceptable ranges see QC certificate.

9. MATERIALS REQUIRED BUT NOT SUPPLIED

1. HPLC Column, (can be ordered separately from IBL under: 30215928) flush and equilibrate before use (see information enclosed with the column)
2. Methanol (LC-MS grade) approx. 1.5 L
3. Water (LC-MS grade) approx. 1.5 L
4. Paper towels, pipette tips and timer
5. Waste plate for 96 well extraction columns (not mandatory for automated positive pressure extraction via Resolvex® A200)
6. Tuning Solutions (recommended), tune mass spectrometer before usage according to MS TUNING

10. DEVICES REQUIRED BUT NOT SUPPLIED

1. LC-System comparable to: Shimadzu CBM-20A and LC-20AD including sample cooler and column heater
2. MS-System comparable to: Sciex 5500 Triple Quad ESI (Turbo V Ion Source)
3. Centrifuge (preferably refrigerated); ≥ 2000 x g
4. Sample concentrator by use of nitrogen (suitable for 96-Well-Plates, heatable up to 40°C)
5. Positive pressure manifold for SPE sample preparation (manual positive pressure manifold for 96-Well-Plates comparable to Tecan - Resolvex® M10, 60 - 100 PSI, automated positive pressure manifold for 96-Well-Plates comparable to Tecan – Resolvex® A200)
6. Roller mixer
7. Orbital shaker (600 - 900 rpm)
8. Pipettes (< 3% CV). Volume: 50 µL; 100 – 1000 µL

If you have any questions about the compatibility of the devices, please contact: ProductSupport.IBL@tecan.com

11. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, drying times, temperatures and pre-treatment steps have to be performed strictly according to the instructions. In addition, the chromatographic column (REF: 30215928) has to be used to ensure performance. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18 - 25°C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming using a roller mixer.
3. Avoid contamination of reagents, pipettes and wells. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells.
4. Use a pipetting scheme to verify an appropriate plate layout.

12. MS TUNING

MS Tuning is highly recommended for optimizing the MRM transitions. The parameters for the MRM transitions shown in TEST PROCEDURE are for orientation purposes.
For MS tuning it is highly recommended to tune the instrument using external steroid standard material. It is important to tune the steroids and the internal standards included in this panel strictly according to the mass-to-charge-ratio of transitions listed in TEST PROCEDURE.
For more information please contact: ProductSupport.IBL@tecan.com

13. PRE-TEST SETUP INSTRUCTIONS

The contents of the kit for 96 determinations can be divided into four separate runs, since the standards and controls are suitable for four replicates in the sample preparation (Extraction).

If automation of positive pressure extraction of Tecan Resolvex® A200 would be performed, the volumes are sufficient for two runs with 96 determinations.

13.1. Preparation of lyophilized components

Dissolve component	with	Diluent	Remarks	Storage	Stability
CAL A-F LYO	1.3 mL	Water (LC-MS grade)	Mix 15 minutes on a roller mixer.	≤ - 20°C Aliquots*	4 weeks
CAL ZERO LYO	1.3 mL	Water (LC-MS grade)	Mix 15 minutes on a roller mixer.	≤ - 20°C Aliquots*	4 weeks
CONTROL H LYO	1.3 mL	Water (LC-MS grade)	Mix 15 minutes on a roller mixer.	≤ - 20°C Aliquots*	4 weeks
CONTROL L LYO	1.3 mL	Water (LC-MS grade)	Mix 15 minutes on a roller mixer.	≤ - 20°C Aliquots*	4 weeks

* It is recommended to limit the number of freeze /thaw cycles to a maximum of 3.

13.2. Preparation of concentrated components

Dilute / dissolve	Component	with	Diluent	Relation	Remarks	Storage	Stability
50 mL	PHASE A CONC	950 mL	Water (LC-MS grade)	1:20	Mix vigorously.	18 - 25°C	2 weeks
50 mL	PHASE B CONC	950 mL	Methanol (LC-MS grade)	1:20	Mix vigorously.	18 - 25°C	4 weeks
20 mL	EXTRACT CONC	47 mL	Water (LC-MS grade)	1:3.35	Mix vigorously.	18 - 25°C	8 weeks
15 mL	WASH 1 CONC	150 mL	Water (LC-MS grade)	1:11	Mix vigorously.	18 - 25°C	8 weeks
10 mL	WASH 2 CONC	60 mL	Methanol (LC-MS grade)	1:16	Mix vigorously.	18 - 25°C	8 weeks
		90 mL	Water (LC-MS grade)				
10 mL	ELUTION CONC	110 mL	Methanol (LC-MS grade)	1:12	Mix vigorously.	18 - 25°C	8 weeks
20 mL	RECONST CONC	30 mL	Water (LC-MS grade)	1:2.5	Mix vigorously.	18 - 25°C	8 weeks

13.3. Dilution of Samples

Samples suspected to contain concentrations above the measuring range have to be diluted as follows:

Sample	to be diluted	with	Remarks
Serum and plasma (EDTA)	> highest standard and / or > 3600 ng/mL dehydroepiandrosterone sulfate	Water (LC-MS grade)	prior to extraction step, dilute into the concentration range between Standard D - E

13.4. LC-MS pre-run setup

Equilibrate the analytical system at least 20 minutes before injecting any samples. To equilibrate HPLC column before first use or after storage, displace storage medium with mobile phase mixture corresponding to starting conditions (PHASE A / PHASE B 60:40, *v/v*) for 20 minutes at a flowrate of 0.35 mL/min until column backpressure is stable. For further details of HPLC column usage, please refer to the information enclosed to chromatographic column. For information on MS/MS setup, refer to the documentation and procedures supplied with your LC-MS system. Inject blank samples or Mobile Phase A to check for carry-over of analytes. For storage conditions of HPLC column, see STORAGE AND STABILITY.

13.5. Extraction of Samples, Standards and Controls

If necessary, centrifuge the samples prior to extraction in order to avoid clogging of the columns.

Each sample, Standard and Control has to be extracted. The dried extracts (after evaporation of eluate) may be stored at ≤-20°C up to 5 weeks, except for the analysis of 17-hydroxyprogesterone – in this case, the extraction has to be performed without interruption.

14. Extraction procedure for semi-automated positive pressure manifold

Positive pressure extraction for the Steroid Panel LC-MS is semi-automatable on the Resolvex® A200 and comparable to manual sample extraction using Resolvex® M10.
If an automated positive pressure manifold like Resolvex® A200 is used, the method will guide through the extraction procedure.
For the use of products on automated instruments it is absolutely necessary to perform and maintain a validation according to legal requirements. A successful validation of the process is a prerequisite for diagnostic use. The responsibility for the implementation and documentation of validation in accordance with the country-specific requirements is assumed by the organization or institution.
For more information please contact: ProductSupport.IBL@tecan.com

15. Extraction procedure

Column Conditioning:

1.	Place the Extraction Columns upon waste plate.
2.	Add **1 x 500 µL** of **methanol (LC-MS grade)** to the appropriate columns.
3.	Let the solvent pass through the column by positive pressure until no residue left on columns. Discard eluate.
4.	Add **1 x 500 µL** of **water (LC-MS grade)** to the appropriate columns.
5.	Let the solvent pass through the column by positive pressure until no residue left on columns. Discard eluate.

Sample Application:

<table>
<tr><td>6.</td><td colspan="2">Place the Extraction Columns upon waste plate.</td></tr>
<tr><td>7.</td><td colspan="2">Add 250 µL of diluted Extraction Solution to the appropriate columns.</td></tr>
<tr><td>8.</td><td colspan="2">Add 250 µL of reconstituted standards, reconstituted controls in appropriate wells</td></tr>
<tr><td>9.</td><td>If serum is used:</td><td>If plasma (EDTA) is used:</td></tr>
<tr><td></td><td>Add 250 µL of serum samples to the appropriate wells following pipetting schema.</td><td>a) Dilute plasma samples with same amount of LC-MS grade water (1:1, v/v), mix vigorously
b) Add 500 µL of diluted plasma samples to the appropriate wells following pipetting schema.</td></tr>
<tr><td>10.</td><td colspan="2">Add 50 µL of Internal Standard to the appropriate columns.</td></tr>
<tr><td>11.</td><td colspan="2">Mix 1 minute on an orbital shaker (600 - 900 rpm)</td></tr>
<tr><td>12.</td><td colspan="2">Let pass through the column by positive pressure until no residue left on columns. Discard eluate.</td></tr>
</table>

Washing:

13.	Add **1 x 900 µL** of diluted **Wash Solution 1** to the appropriate columns.
14.	Let the solvent pass through the column by positive pressure until no residue left on columns. Discard eluate.
15.	Add **1 x 900 µL** of diluted **Wash Solution 2** to the appropriate columns.
16.	Let the solvent pass through the column by positive pressure until no residue left on columns. Discard eluate.

Elution of Extract:

17.	Place the Extraction Columns upon the **Deep Well Collection Plate**.
18.	Add **250 µL** of diluted **Elution Solution** to the respective columns.
19.	Let the solvent pass through the column by positive pressure until no residue left on columns. **Keep the eluate in place.**
20.	Add another **250 µL** of diluted **Elution Solution** to the respective columns.
21.	Let the solvent pass through the column by positive pressure until no residue left on columns. **Keep the eluate in place.**
22.	Remove columns from the tubes. Avoid drops to be left at the columns.

Evaporation and Reconstitution of Extract:

23.	Evaporate the eluate to dryness using a stream of nitrogen on a block heater heated to 40°C (approx. 1 hour). *Note: The dried extracts may be stored at ≤-20°C up to 5 weeks, except for the analysis of 17-hydroxyprogesterone – in this case, the extraction have to be performed without interruption.*
24.	Reconstitute samples with **100 µL** of diluted **Reconstitution Solution**
25.	Firmly place a **plate seal** on the collection plate.
26.	Mix **15 minutes** on an orbital shaker (i. e. 600 - 900 rpm).
27.	Place the **Deep Well Collection Plate** in HPLC autosampler.

16. TEST PROCEDURE

The LC-MS measurement is performed in two different runs to cover all analytes – Only a different LC-MS method is required. Prepared samples are enough for duplicate injections for both runs if desired.

The two runs target different analytes, which are listed below:

Run 1	11-deoxycorticosterone, 11-deoxycortisol, 17-hydroxyprogesterone, 21-deoxycortisol, aldosterone, androstenedione, corticosterone, cortisol, cortisone, dexamethasone, dehydroepiandrosterone, dehydroepiandrosterone sulfate, dihydrotestosterone, progesterone, testosterone
Run 2	17-hydroxypregnenolone, estradiol, estrone

16.1. General setting of the LC system

The liquid chromatography system must be equipped with the chromatographic column provided with the kit and must be equilibrated with chromatography phases A and B according to chapter LC-MS pre-run setup.

The LC parameters listed below must be set for both analytical runs

LC Parameters	Setting for Run 1	Setting for Run 2
Flow	0.35 mL/min	0.35 mL/min
Total run time	10 minutes	6 minutes
Column oven temperature	40°C	40°C
Autosampler temperature	10°C	10°C

Additionally, it is recommended to measure blanks (e. g. solvent blanks) between every 10 - 20 samples as well as own quality control samples (for further information regarding Quality Control see QUALITY CONTROL). The internal standard can be used as non-extracted reference in order to check the performance of the method (max. 100 µL).

For Run 1 and Run 2 solvent gradients are summarized in figure 1 and 2, respectively.

Time [min]	Module	Event	Parameter
0.00	Pumps	Pump B Conc.	45
0.80	Pumps	Pump B Conc.	45
1.50	Pumps	Pump B Conc.	55
6.00	Pumps	Pump B Conc.	65
6.70	Pumps	Pump B Conc.	95
8.90	Pumps	Pump B Conc.	95
9.00	Pumps	Pump B Conc.	45
10.00	Controller	Stop	

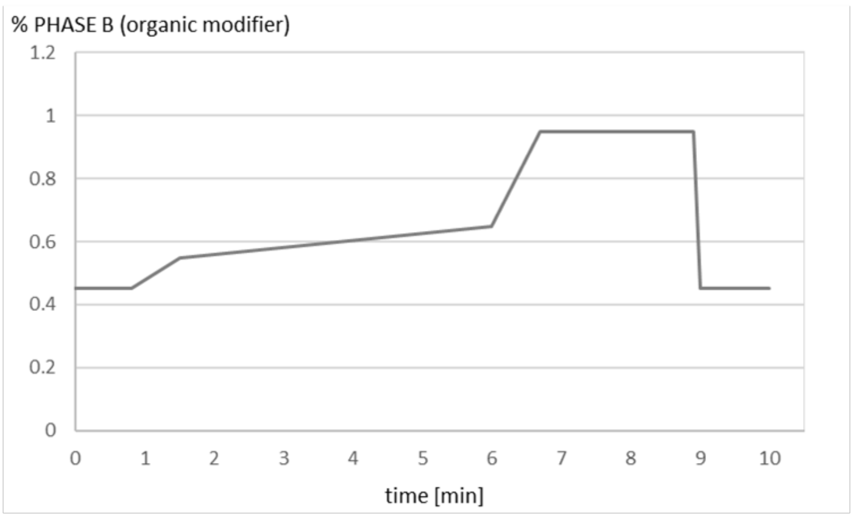

Figure 1: Liquid Chromatography Gradient of Run 1.

Time [min]	Module	Event	Parameter
0.00	Pumps	Pump B Conc.	45
0.55	Pumps	Pump B Conc.	45
1.00	Pumps	Pump B Conc.	70
5.00	Pumps	Pump B Conc.	95
5.50	Pumps	Pump B Conc.	95
5.51	Pumps	Pump B Conc.	45
6.00	Controller	Stop	

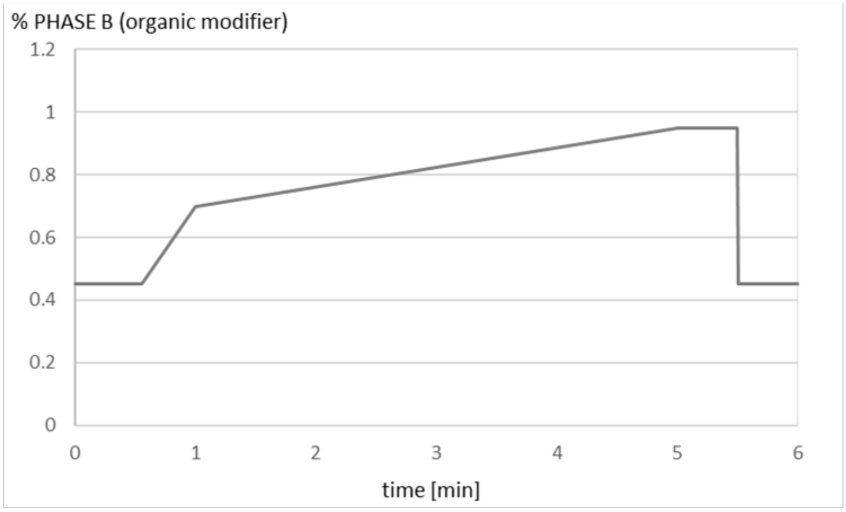

Figure 2: Liquid Chromatography Gradient of Run 2.

16.2. General Settings of MS system

The mentioned mass spectrometry parameters have to be set to the following values

MS Parameters	ESI Positive	ESI Negative
Curtain Gas (CUR)	35	35
Collison Gas (CAD)	8	8
Ion Spray Voltage (IS)	4500 V	-4500 V
Temperature (TEM)	600°C	600°C
Ion Source Gas 1 (GS1)	40	40
Ion Source Gas 2 (GS2)	60	60

16.3. Example Chromatogram

Figure 3 to 6 shows exemplary chromatograms for Run 1 and 2, respectively.

1. aldosterone	9. androstenedione
2. cortisone	10. 11-deoxycorticosterone
3. dehydroepiandrosterone sulfate	11. testosterone
4. cortisol	12. dehydroepiandrosterone
5. 21-deoxycortisol	13. 17-hydroxyprogesterone
6. corticosterone	14. dihydrotestosterone
7. dexamethasone	15. progesterone
8. 11-deoxycortisol	

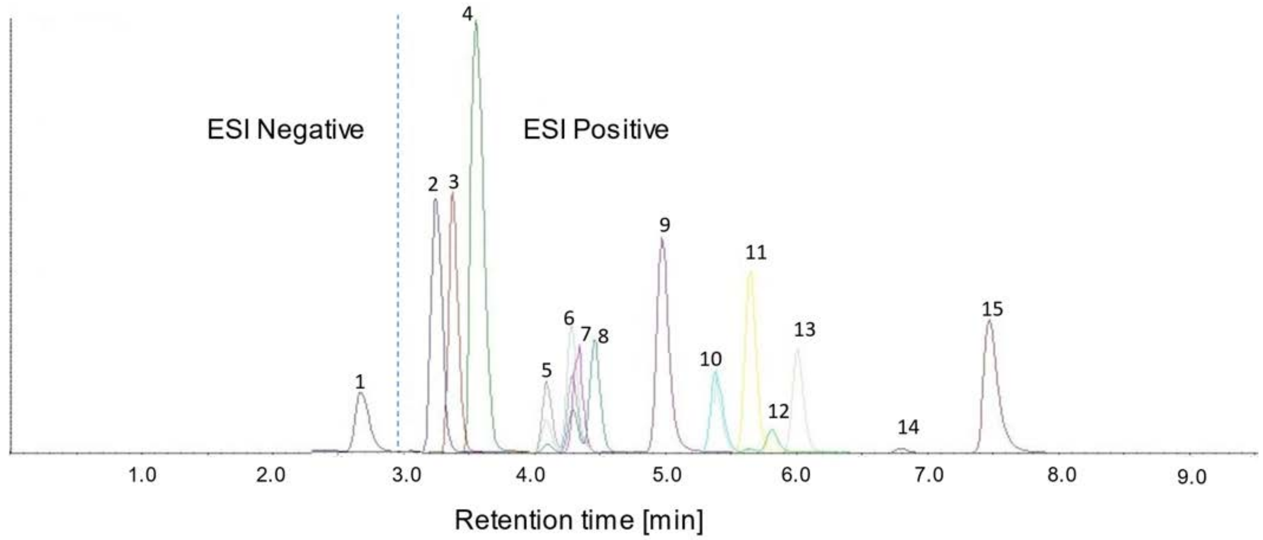

Figure 3: Exemplary chromatogram of Standard F - Run 1. The dotted line represents the switching between ESI negative and ESI positive mode. An injection volume of 20 µL was used

1. estrone
2. estradiol
3. 17-hydroxypregnenolone

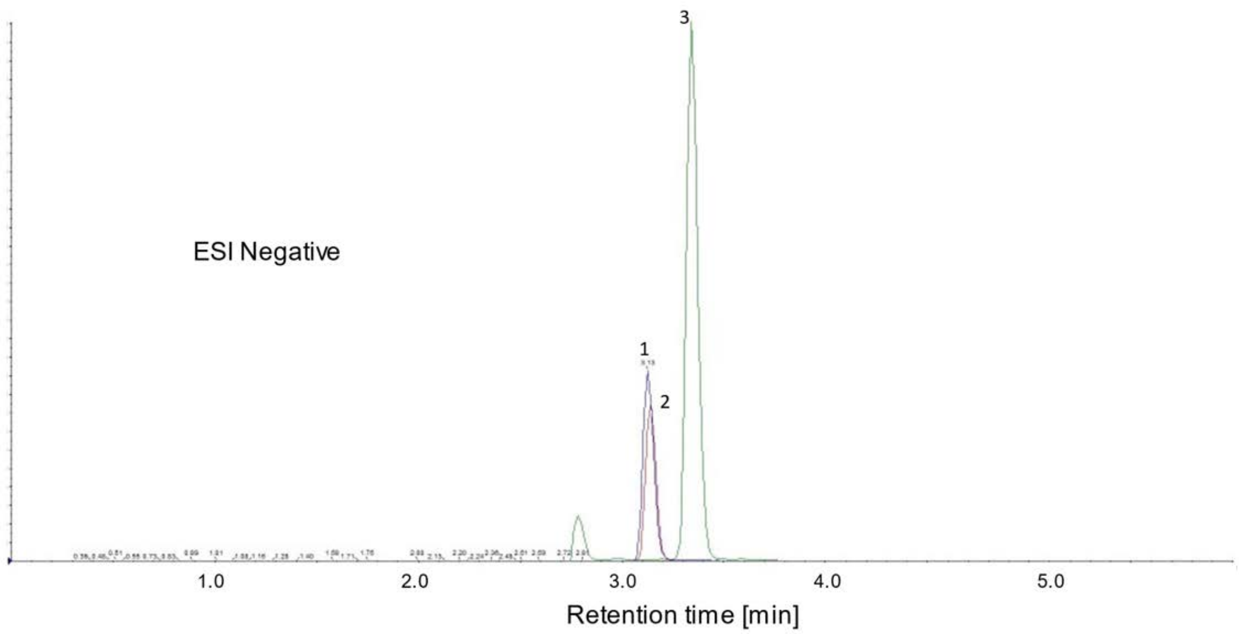

Figure 4: Exemplary chromatogram of Standard F - Run 2. An injection volume of 20 µL was used.

1. aldosterone-D4	9. androstenedione-13C3
2. cortisone-13C3	10. 11-deoxycorticosterone-13C3
3. dehydroepiandrosterone sulfate-D5	11. testosterone-13C3
4. cortisol-D4	12. dehydroepiandrosterone-D5
5. 21-deoxycortisol-D8	13. 17-hydroxyprogesterone-D8
6. corticosterone-13C3	14. dihydrotestosterone-D3
7. dexamethasone-D5	15. progesterone-D9
8. 11-deoxycortisol-D5	

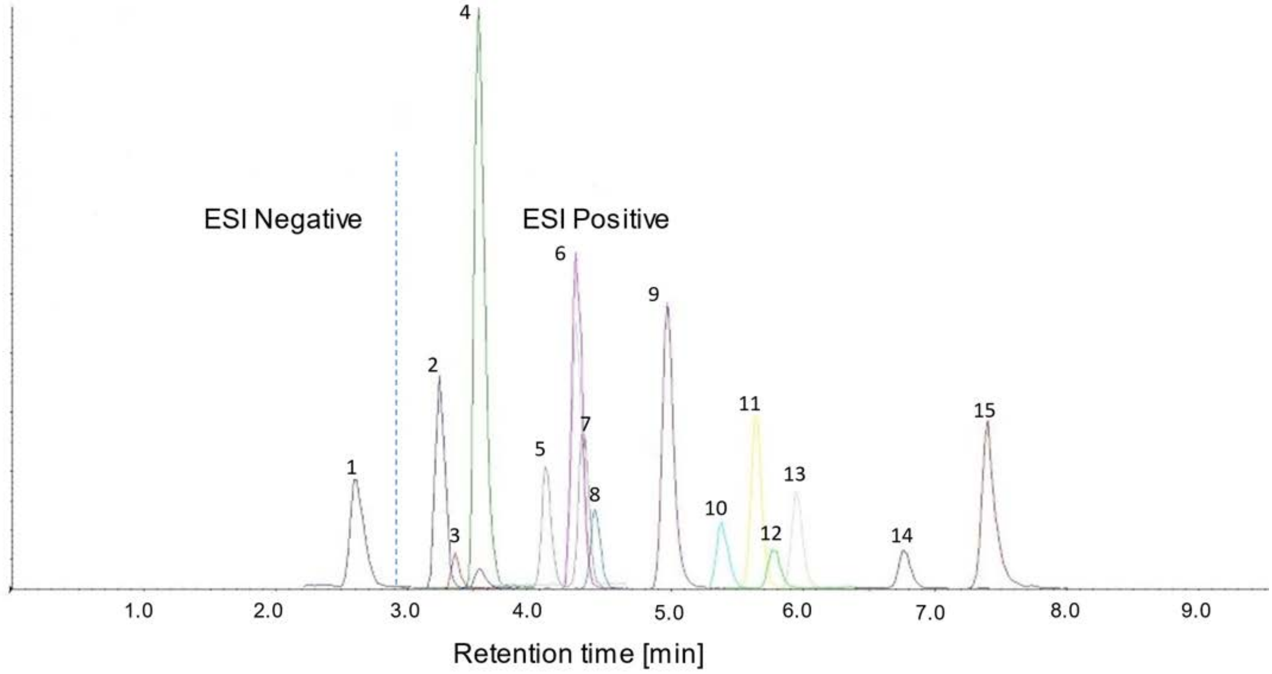

Figure 5: Exemplary chromatogram of internal standard - Run 1. An injection volume of 20 µL was used.

1. estrone-13C3
2. estradiol-13C3
3. 17-hydroxypregnenolone-13C3-D2

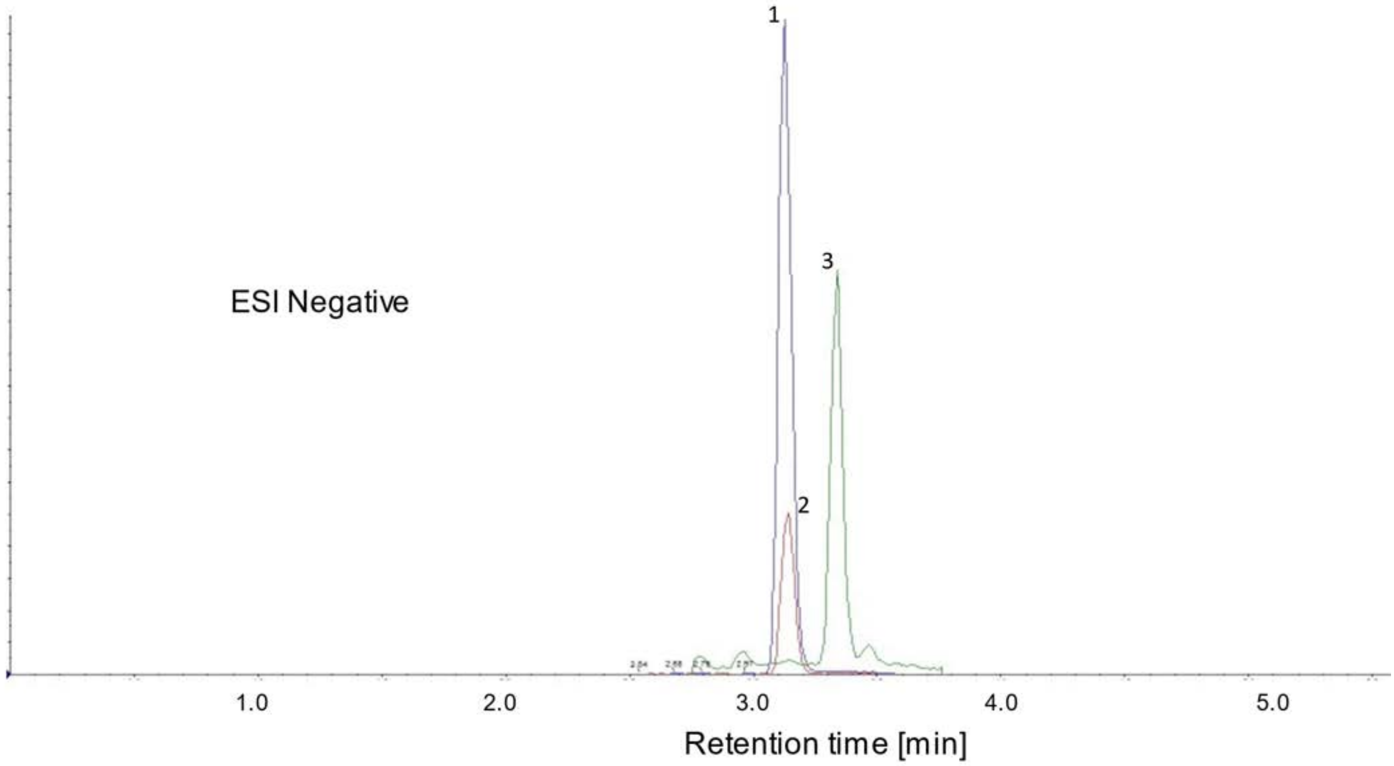

Figure 6: Exemplary chromatogram of internal standard - Run 2. An injection volume of 20 µL was used.

16.4. MRM-Transitions

The following tables list the respective transitions for all analytes and the respective isotopically labelled internal standard (IS). The addition “a” refers to the Quantifier and “b” to the Qualifier.

The mentioned potentials (Declustering Potential DP, Entrance Potential EP, Collision Energy CE, Collision Cell Exit Potential CXP), retentions times and transition values serve for orientation only, an individual tuning is highly recommended (see MS TUNING).

16.4.1. Transitions and respective parameters of Run 1 (ESI negative/MRM -)

Q1	Q3	Compound	RT [min]	DP	EP	CE	CXP
359.152	188.986	aldosterone_a	2.80	-115	-10	-22	-21
359.152	331.132	aldosterone_b	2.80	-115	-10	-24	-19
363.113	190.012	aldosterone-D4_a	2.80	-130	-10	-26	-17
363.113	335.17	aldosterone-D4_b	2.80	-130	-10	-22	-19

16.4.2. Transitions and respective parameters of Run 1 (ESI positive/MRM +)

Q1	Q3	Compound	RT [min]	DP	EP	CE	CXP
331.292	97.05	11-deoxycorticosterone_a	5.40	71	10	27	16
331.292	109.05	11-deoxycorticosterone_b	5.40	71	10	31	12
334.23	100.061	11-deoxycorticosterone-13C3_a	5.40	181	10	29	12
334.23	112.076	11-deoxycorticosterone-13C3_b	5.40	181	10	33	14
347.27	97.07	11-deoxycortisol_a	4.40	131	10	29	10
347.27	109.07	11-deoxycortisol_b	4.40	131	10	31	6
352.2	100.09	11-deoxycortisol-D5_a	4.40	171	10	35	14
352.2	113.01	11-deoxycortisol-D5_b	4.40	171	10	35	12
331.279	97.06	17-hydroxyprogesterone_a	5.87	1	10	27	12
331.279	109.03	17-hydroxyprogesterone_b	5.87	1	10	35	20
339.3	100	17-hydroxyprogesterone-D8_a	5.87	1	10	31	18
339.3	113.1	17-hydroxyprogesterone-D8_b	5.87	1	10	37	4
347.29	311.235	21-deoxycortisol_a	3.96	1	10	21	10
347.29	121.076	21-deoxycortisol_b	3.96	1	10	31	8
355.26	319.26	21-deoxycortisol-D8_a	3.96	6	10	23	28
355.26	125.09	21-deoxycortisol-D8_b	3.96	6	10	31	14
287.195	97.047	androstenedione_a	5.00	106	10	29	14
287.195	109.058	androstenedione_b	5.00	106	10	31	6
290.219	100.061	androstenedione-13C3_a	5.00	131	10	29	12
290.219	112.066	androstenedione-13C3_b	5.00	131	10	29	12
347.272	121.063	corticosterone_a	4.14	81	10	29	16
347.272	91.051	corticosterone_b	4.14	81	10	73	14
350.202	332.218	corticosterone-13C3_a	4.14	76	10	21	16
350.202	124.059	corticosterone-13C3_b	4.14	76	10	31	10
363.22	121.08	cortisol_a	3.45	41	10	29	14
363.2	91.09	cortisol_b	3.45	41	10	83	42
367.23	121.08	cortisol-D4_a	3.45	36	10	29	6
367.23	77.05	cortisol-D4_b	3.45	36	10	73	18
361.23	163.12	cortisone_a	3.25	126	10	31	16
361.23	91.07	cortisone_b	3.25	126	10	81	14
364.075	166.11	cortisone-13C3_a	3.25	121	10	31	14
364.075	105.08	cortisone-13C3_b	3.25	121	10	49	12
393.178	373.186	dexamethasone_a	4.15	36	10	11	34
393.178	355.187	dexamethasone_b	4.15	36	10	15	22
398.08	378.211	dexamethasone_D5_a	4.15	31	10	13	26
398.08	360.184	dexamethasone_D5_b	4.15	31	10	17	16
289.23	271.2	dehydroepiandrosterone_a	5.90	31	10	11	32

Q1	Q3	Compound	RT [min]	DP	EP	CE	CXP
289.23	253.2	dehydroepiandrosterone_b	5.90	31	10	15	22
294.117	258.209	dehydroepiandrosterone-D5_a	5.90	91	10	15	22
294.117	218.233	dehydroepiandrosterone-D5_b	5.90	91	10	25	20
271.213	253.188	dehydroepiandrosterone-sulfate_a	3.43	136	10	19	22
271.213	91.054	dehydroepiandrosterone-sulfate_a	3.43	136	10	63	40
276.238	258.113	dehydroepiandrosterone-sulfate_a	3.43	211	10	19	34
276.238	97.043	dehydroepiandrosterone-sulfate_a	3.43	211	10	29	10
291.102	255.122	dihydrotestosterone_a	6.90	111	10	23	38
291.102	159.146	dihydrotestosterone_b	6.90	111	10	29	16
294.254	258.22	dihydrotestosterone-D3_a	6.90	151	10	21	30
294.254	91.051	dihydrotestosterone-D3_b	6.90	151	10	65	14
315.29	97.07	progesterone_a	7.50	126	10	27	18
315.29	109.07	progesterone_b	7.50	126	10	31	16
324.38	100.08	progesterone-D9_a	7.50	121	10	27	12
324.38	113.08	progesterone-D9_b	7.50	121	10	33	14
289.241	97.043	testosterone_a	5.70	136	10	29	16
289.241	109.059	testosterone_b	5.70	136	10	31	52
292.229	100.061	testosterone-13C_a	5.70	106	10	27	18
292.229	112.087	testosterone-13C_b	5.70	106	10	29	18

16.4.3. Transitions and respective parameters of Run 2 (ESI negative/MRM -)

Q1	Q3	Compound	RT [min]	DP	EP	CE	CXP
331.1	287.3	17-hydroxypregnenolone_a	3.26	-70	-10	-30	-17
331.1	303.3	17-hydroxypregnenolone_b	3.26	-70	-10	-30	-17
335.15	289.114	17-hydroxypregnenolone-13C2-D2_a	3.26	-110	-10	-26	-17
335.15	316.116	17-hydroxypregnenolone-13C2-D2_b*	3.26	-110	-10	-26	-27
271.19	144.99	estradiol_a	3.06	-40	-10	-50	-17
271.19	182.99	estradiol_b	3.06	-40	-10	-54	-17
274.074	148.003	estradiol-13C3_a	3.06	-35	-10	-50	-17
274.074	185.991	estradiol-13C3_b	3.06	-35	-10	-52	-21
269.218	144.901	estrone_a	3.04	-20	-10	-46	-17
269.218	142.966	estrone_b	3.04	-20	-10	-70	-17
272.185	147.975	estrone-13C3_a	3.04	-155	-10	-48	-13
272.185	145.982	estrone-13C3_b	3.04	-155	-10	-66	-9

*For the quantitative evaluation of 17-hydroxypregnenolone, the use of 17-hydroxypregnenolone-13C2-D2_b is recommended.

17. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or comparable standards/laws. User and/or laboratory must have a validated system to get diagnosis according to GLP. All kit controls must be found within the acceptable ranges as stated in the QC certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.
It is recommended to participate at appropriate quality assessment trials.

18. CALCULATION OF RESULTS

For every batch of measurements, a calibration data set has to be acquired with the calibrator set of this kit. In the following the concentrations of standards and internal standards are listed.

To account for possible matrix effects, internal standard mix is added to every calibrator, control and sample before extraction. To generate calibration curves, the ratio of peak areas of analyte and corresponding internal standard are plotted on the y-axis and the corresponding concentrations of the calibrators on the x-axis. To check for correct conditions during the quantification experiment and calculation method please run the two controls (high and low) in the beginning of the batch.

For all analytes, it is recommended to calculate the results with quadratic regression.

In case of diluted samples the values have to be multiplied with the corresponding dilution factor. Samples showing concentrations above the measuring range have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and re-assayed.

Concentration Standards and Controls [ng/mL]

Analyte	0	A	B	C	D	E	F	Control Low	Control High	Internal Standard
Run 1										
11-deoxycorticosterone	0.00	0.04	0.11	0.28	0.74	1.94	5.13	0.19	3.54	1.00
11-deoxycortisol	0.00	0.10	0.26	0.70	1.84	4.86	12.8	0.48	8.84	2.00
17-hydroxyprogesterone	0.00	0.10	0.26	0.70	1.84	4.86	12.8	0.48	8.84	2.00
21-deoxycortisol	0.00	0.10	0.26	0.70	1.84	4.86	12.8	0.48	8.84	3.00
aldosterone	0.00	0.10	0.22	0.46	0.99	2.14	4.59	0.34	3.37	3.00
androstenedione	0.00	0.10	0.26	0.70	1.84	4.86	12.2	0.48	8.84	2.00
corticosterone	0.00	0.30	0.79	2.09	5.52	14.6	38.5	1.44	26.5	5.00
cortisol	0.00	2.00	5.28	13.9	36.8	97.2	256	9.61	177	25.0
cortisone	0.00	0.50	1.32	3.48	9.20	24.3	64.1	2.40	44.2	5.00
dexamethasone	0.00	0.50	1.32	3.48	9.20	24.3	64.1	2.40	44.2	20.0
dehydroepiandrosterone	0.00	1.00	2.15	4.62	9.94	21.4	45.9	3.39	33.7	50.0
dehydroepiandrosterone sulfate	0.00	50.0	132	348	920	2429	6412	240	4420	500
dihydrotestosterone	0.00	0.15	0.24	0.38	0.61	0.98	1.57	0.31	1.28	4.00
progesterone	0.00	0.10	0.26	0.70	1.84	4.86	12.8	0.48	8.84	2.00
testosterone	0.00	0.04	0.13	0.41	1.31	4.19	13.4	0.27	8.81	1.00
Run 2										
17-hydroxypregnenolone	0.00	0.30	0.79	2.09	5.52	14.57	38.47	1.44	26.52	4.00
estradiol	0.00	0.03	0.08	0.21	0.55	1.46	3.85	0.14	2.65	1.00
estrone	0.00	0.01	0.03	0.07	0.18	0.49	1.28	0.05	0.88	1.00

19. EXPECTED VALUES

Reference ranges of apparently healthy population of the 17 analytes, measurable with the Steroid Panel LC-MS, were determined by peer-reviewed scientific literature and confirmed by in-house measurement of 147 serum samples from male and female apparently healthy individuals. Since dexamethasone is a synthetic glucocorticoid medication, no endogenous reference values are evaluable.
The reference intervals of apparently healthy population as derived from peer-reviewed literature is shown in the tables.
For better comparability the reference ranges were converted to ng/mL.

Reference interval: **11-deoxycorticosterone**
Following reference values are published for apparently healthy subjects:

Population		**Analyte**			**Reference**
Gender	**Age**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female*	19 - 77 years	plasma		0.155 ng/mL	Eisenhofer et al [21]
male	18 - 81 years	plasma		0.178 ng/mL	Eisenhofer et al [21]
overall	< 10 days (preterm)	serum	0.01 ng/mL	0.159 ng/mL	Fiet et al [24]
overall	< 10 days (full-term)	serum	0.012 ng/mL	0.095 ng/mL	Fiet et al [24]
overall	10 days - 18 years	serum	0.01 ng/mL	0.27 ng/mL	Fiet et al [24]
female	0 - 1 years	serum	0.05 ng/mL	0.47 ng/mL	Kulle et al [27]
female	1 - 7 years	serum	0.06 ng/mL	0.56 ng/mL	Kulle et al [27]
female	7 - 13 years	serum	0.06 ng/mL	0.51 ng/mL	Kulle et al [27]
female	13 - 16 years	serum	0.06 ng/mL	0.32 ng/mL	Kulle et al [27]
female	>16 years	serum	0.06 ng/mL	0.54 ng/mL	Kulle et al [27]
male	0 - 1 years	serum	0.06 ng/mL	0.47 ng/mL	Kulle et al [27]
male	1 - 7 years	serum	0.06 ng/mL	0.35 ng/mL	Kulle et al [27]
male	7 - 13 years	serum	0.06 ng/mL	0.60 ng/mL	Kulle et al [27]
male	13 - 16 years	serum	0.06 ng/mL	0.60 ng/mL	Kulle et al [27]
male	>16 years	serum	0.06 ng/mL	0.75 ng/mL	Kulle et al [27]

*(no oral contraceptive)

Reference interval **11-deoxycortisol**
Following reference values are published for apparently healthy subjects:

Population		**Analyte**			**Reference**
Gender	**Age**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female (n =120)	Adults	serum [A]	0.13 ng/mL	2.65 ng/mL	Parikh et al [31]
female (n =120)	Adults	serum [P]	0.01 ng/mL	1.10 ng/mL	Parikh et al [31]
male (n = 62)	Adults	serum [A]	0.11 ng/mL	2.32 ng/mL	Parikh et al [31]
male (n = 62)	Adults	serum [P]	0 ng/mL	0.80 ng/mL	Parikh et al [31]
female	0 - 1 years	serum	0 ng/mL	1.83 ng/mL	Kyriakopoulou et al [22]
female	1 - 2 years	serum	0.03 ng/mL	0.30 ng/mL	Kyriakopoulou et al [22]
female	2 - 7 years	serum	0.02 ng/mL	0.37 ng/mL	Kyriakopoulou et al [22]
female	7 - 12 years	serum	0.03 ng/mL	0.79 ng/mL	Kyriakopoulou et al [22]
female	12 - 19 years	serum	0 ng/mL	0.79 ng/mL	Kyriakopoulou et al [22]
male	0 - 1 years	serum	0 ng/mL	1.83 ng/mL	Kyriakopoulou et al [22]
male	1 - 2 years	serum	0.03 ng/mL	0.30 ng/mL	Kyriakopoulou et al [22]
male	2 - 7 years	serum	0.02 ng/mL	0.37 ng/mL	Kyriakopoulou et al [22]
male	7 - 12 years	serum	0.03 ng/mL	0.79 ng/mL	Kyriakopoulou et al [22]
male	12 - 19 years	serum	0 ng/mL	0.79 ng/mL	Kyriakopoulou et al [22]

[A] (AM)
[P] (AM)

Reference interval **17-hydroxyprogesterone**
Following reference values are published for apparently healthy subjects:

Population				Analyte			Reference
Gender	**n**	**Age**	**Phase**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female		Adults	luteal	plasma	0.12 ng/mL	2.74 ng/mL	Eisenhofer et al [21]
female		Adults	follicular	plasma	0.12 ng/mL	1.65 ng/mL	Eisenhofer et al [21]
female		Adults	oral contraceptives	plasma	0.04 ng/mL	0.66 ng/mL	Eisenhofer et al [21]
female		Adults	post-menopausal	plasma	0.06 ng/mL	1.03 ng/mL	Eisenhofer et al [21]
male		Adults		plasma	0.29 ng/mL	2.06 ng/mL	Eisenhofer et al [21]
female		Adults	1. trimester	serum	0.48 ng/mL	3.77 ng/mL	Soldin et al [23]
female		Adults	2. trimester	serum	1.13 ng/mL	4.67 ng/mL	Soldin et al [23]
female		Adults	3. trimester	serum	1.53 ng/mL	7 ng/mL	Soldin et al [23]
female		Adults	postpartum	serum	< 0.1 ng/mL	4.95 ng/mL	Soldin et al [23]
female		Adults	60 min after ACTH stimulation	serum	0.46 ng/mL	4.13 ng/mL	Ueland et al [30]
female		Adults	contraceptives	serum	0.36 ng/mL	4.36 ng/mL	Ueland et al [30]
male		Adults	60 min after ACTH stimulation	serum	0.69 ng/mL	2.97 ng/mL	Ueland et al [30]
overall		< 10 days	preterm	serum	1.27 ng/mL	3.8 ng/mL	Fiet et al [24]
overall		< 10 days	full-term	serum	0.14 ng/mL	2.83 ng/mL	Fiet et al [24]
overall		10 days - 18 years		serum	0.054 ng/mL	2.75 ng/mL	Fiet et al [24]
female	156	<1 year		serum		0.76 ng/mL	Bae et al [25]
female	47	1 - 5 years		serum		0.64 ng/mL	Bae et al [25]
female	271	5 - 10 years		serum		0.59 ng/mL	Bae et al [25]
female	282	10 - 15 years		serum		2.36 ng/mL	Bae et al [25]
female	118	15 - 20 years		serum		2.10 ng/mL	Bae et al [25]
female	187	20 - 40 years		serum		2.65 ng/mL	Bae et al [25]
female	120	40 - 60 years		serum		1.76 ng/mL	Bae et al [25]
female	112	60 - 80 years		serum		0.66 ng/mL	Bae et al [25]
male	124	<1year		serum		0.80 ng/mL	Bae et al [25]
male	206	1 - 5 years		serum		0.85 ng/mL	Bae et al [25]
male	286	5 - 10 years		serum		0.69 ng/mL	Bae et al [25]
male	341	10 - 15 years		serum		1.28 ng/mL	Bae et al [25]
male	155	15 - 20 years		serum	0.42 ng/mL	2.26 ng/mL	Bae et al [25]
male	219	20 - 40 years		serum	0.28 ng/mL	2.08 ng/mL	Bae et al [25]
male	142	40 - 60 years		serum	0.24 ng/mL	1.71 ng/mL	Bae et al [25]
male	103	60 - 80 years		serum	0.21 ng/mL	1.46 ng/mL	Bae et al [25]

Reference interval **21-deoxycortisol**
Following reference values are published for apparently healthy subjects:

Population		Analyte			Reference
Gender	Age	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female	19 - 77 years	plasma		0.077 ng/mL	Eisenhofer et al [21]
male	18 - 81 years	plasma		0.159 ng/mL	Eisenhofer et al [21]
overall	< 10 days (preterm)	serum	0.027 ng/mL	0.209 ng/mL	Fiet et al [24]
overall	< 10 days (full-term)	serum	0.027 ng/mL	0.113 ng/mL	Fiet et al [24]
overall	10 days - 18 years	serum	0.027 ng/mL	0.103 ng/mL	Fiet et al [24]

Reference interval **aldosterone**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	n	Age	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female		Adults	Heparin plasma [A]	0.0036 ng/mL	0.16 ng/mL	Einsenhofer et al [21]
male		Adults	Heparin plasma [A]	0.0072 ng/mL	0.24 ng/mL	Einsenhofer et al [21]
female	156	<1 year	serum		1.11 ng/mL	Bae et al [25]
female	47	1 - 5 years	serum		0.63 ng/mL	Bae et al [25]
female	269	5 - 10 years	serum		0.30 ng/mL	Bae et al [25]
female	280	10 - 15 years	serum		0.30 ng/mL	Bae et al [25]
female	112	15 - 20 years	serum		0.39 ng/mL	Bae et al [25]
female	188	20 - 40 years	serum		0.36 ng/mL	Bae et al [25]
female	120	40 - 60 years	serum		0.22 ng/mL	Bae et al [25]
female	112	60 - 80 years	serum		0.12 ng/mL	Bae et al [25]
male	124	<1 year	serum		1.03 ng/mL	Bae et al [25]
male	206	1 - 5 years	serum		0.37 ng/mL	Bae et al [25]
male	186	5 - 10 years	serum		0.31 ng/mL	Bae et al [25]
male	340	10 - 15 years	serum		0.24 ng/mL	Bae et al [25]
male	153	15 - 20 years	serum		0.24 ng/mL	Bae et al [25]
male	220	20 - 40 years	serum		0.17 ng/mL	Bae et al [25]
male	142	40 - 60 years	serum		0.14 ng/mL	Bae et al [25]
male	103	60 - 80 years	serum		0.16 ng/mL	Bae et al [25]

[A] (AM)

Reference interval **androstenedione**
Following reference values are published for apparently healthy subjects:

Population				Analyte		Reference
Gender	n	Age	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female		0 - 15 days	serum	0 ng/mL	0.73 ng/mL	Kyriakopoulou et al [22]
female		15 - 365 days	serum	0.026 ng/mL	1.63 ng/mL	Kyriakopoulou et al [22]
female		1 - 6 years	serum	0.027 ng/mL	0.16 ng/mL	Kyriakopoulou et al [22]
female		6 - 10 years	serum	0.058 ng/mL	0.26 ng/mL	Kyriakopoulou et al [22]
female		10 - 12 years	serum	0 ng/mL	0.73 ng/mL	Kyriakopoulou et al [22]
female		12 - 15 years	serum	0.21 ng/mL	1.72 ng/mL	Kyriakopoulou et al [22]
female		15 - 19 years	serum	0.14 ng/mL	1.85 ng/mL	Kyriakopoulou et al [22]
male		0 - 15 days	serum	0 ng/mL	0.73 ng/mL	Kyriakopoulou et al [22]
male		15 - 365 days	serum	0.026 ng/mL	1.63 ng/mL	Kyriakopoulou et al [22]
male		1-6 years	serum	0.027 ng/mL	0.16 ng/mL	Kyriakopoulou et al [22]
male		6-10 years	serum	0.058 ng/mL	0.26 ng/mL	Kyriakopoulou et al [22]
male		10-12 years	serum	0 ng/mL	0.73 ng/mL	Kyriakopoulou et al [22]
male		12-15 years	serum	0.15 ng/mL	0.58 ng/mL	Kyriakopoulou et al [22]
male		15-19 years	serum	0.25 ng/mL	1.02 ng/mL	Kyriakopoulou et al [22]
female	156	<1 year	serum		0.200 ng/mL	Bae et al [25]
female	47	1 - 5 years	serum		0.152 ng/mL	Bae et al [25]
female	269	5 - 10 years	serum		0.478 ng/mL	Bae et al [25]
female	280	10 - 15 years	serum	0.132 ng/mL	1.962 ng/mL	Bae et al [25]
female	112	15 - 20 years	serum	0.361 ng/mL	2.240 ng/mL	Bae et al [25]
female	188	20 - 40 years	serum	0.378 ng/mL	2.111 ng/mL	Bae et al [25]
female	120	40 - 60 years	serum	0.266 ng/mL	1.635 ng/mL	Bae et al [25]
female	112	60 - 80 years	serum	0.163 ng/mL	1.037 ng/mL	Bae et al [25]
male	124	<1 year	serum		0.203 ng/mL	Bae et al [25]
male	206	1 - 5 years	serum		0.172 ng/mL	Bae et al [25]
male	286	5 - 10 years	serum		0.395 ng/mL	Bae et al [25]
male	340	10 - 15 years	serum	0.080 ng/mL	1.048 ng/mL	Bae et al [25]
male	153	15 - 20 years	serum	0.401 ng/mL	1.641 ng/mL	Bae et al [25]
male	220	20 - 40 years	serum	0.404 ng/mL	1.776 ng/mL	Bae et al [25]
male	142	40 - 60 years	serum	0.358 ng/mL	1.887 ng/mL	Bae et al [25]
male	103	60 - 80 years	serum	0.263 ng/mL	1.366 ng/mL	Bae et al [25]

Reference interval **corticosterone**
Following reference values are published for apparently healthy subjects:

Population		Matrix	Analyte		Reference
Gender	Age		lower limit (2.5%)	upper limit (97.5%)	
female	19 - 77 years	plasma	0.616 ng/mL	23.158 ng/mL	Eisenhofer et al [21]
male	18 - 8 1 years	plasma	0.601 ng/mL	14.765 ng/mL	Eisenhofer et al [21]
female	1 - 12 months	serum	0.102 ng/mL	5.613 ng/mL	Kyriakopoulou et al [22]
female	1 - 4 years	serum	0.226 ng/mL	1.356 ng/mL	Kyriakopoulou et al [22]
female	4 - 6 years	serum	0.346 ng/mL	1.498 ng/mL	Kyriakopoulou et al [22]
female	6 - 15 years	serum	0.160 ng/mL	3.349 ng/mL	Kyriakopoulou et al [22]
female	15 - 19 years	serum	0.310 ng/mL	5.554 ng/mL	Kyriakopoulou et al [22]
male	<1 month	serum	0.051 ng/mL	7.289 ng/mL	Kyriakopoulou et al [22]
male	1 - 12 months	serum	0.102 ng/mL	5.613 ng/mL	Kyriakopoulou et al [22]
male	1 - 4 years	serum	0.226 ng/mL	1.356 ng/mL	Kyriakopoulou et al [22]
male	4 - 6 years	serum	0.346 ng/mL	1.498 ng/mL	Kyriakopoulou et al [22]
male	6 - 15 years	serum	0.160 ng/mL	3.349 ng/mL	Kyriakopoulou et al [22]
male	15 - 19 years	serum	0.310 ng/mL	5.554 ng/mL	Kyriakopoulou et al [22]

Reference interval **cortisol**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	n	Age	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female	293	Adults	Heparin plasma [A]	43.9 ng/mL	254.1 ng/mL	Einsenhofer et al [21]
male	232	Adults	Heparin plasma [A]	48.6 ng/mL	241.0 ng/mL	Einsenhofer et al [21]
female	156	<1 year	serum	21.7 ng/mL	159.6 ng/mL	Bae et al [25]
female	47	1 - 5 years	serum	28.4 ng/mL	240.3 ng/mL	Bae et al [25]
female	269	5 - 10 years	serum	32.5 ng/mL	155.3 ng/mL	Bae et al [25]
female	280	10 - 15 years	serum	34.7 ng/mL	182.1 ng/mL	Bae et al [25]
female	112	15 - 20 years	serum	49.4 ng/mL	462.5 ng/mL	Bae et al [25]
female	188	20 - 40 years	serum	47.6 ng/mL	428.7 ng/mL	Bae et al [25]
female	120	40 - 60 years	serum	59.5 ng/mL	314.7 ng/mL	Bae et al [25]
female	112	60 - 80 years	serum	55.5 ng/mL	257.5 ng/mL	Bae et al [25]
male	124	<1 year	serum	24.2 ng/mL	198.6 ng/mL	Bae et al [25]
male	206	1 - 5 years	serum	34.4 ng/mL	189.8 ng/mL	Bae et al [25]
male	286	5 - 10 years	serum	36.3 ng/mL	153.2 ng/mL	Bae et al [25]
male	340	10 - 15 years	serum	30.3 ng/mL	164.4 ng/mL	Bae et al [25]
male	153	15 - 20 years	serum	41.4 ng/mL	214.9 ng/mL	Bae et al [25]
male	220	20 - 40 years	serum	47.2 ng/mL	229.1 ng/mL	Bae et al [25]
male	142	40 - 60 years	serum	62.4 ng/mL	245.4 ng/mL	Bae et al [25]
male	103	60 - 80 years	serum	66.4 ng/mL	233.8 ng/mL	Bae et al [25]

[A] (AM)

Reference interval **cortisone**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	N	Age	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female	120	Adults	serum [A]	12 ng/mL	39.4 ng/mL	Parikh et al [31]
female	120	Adults	serum [P]	5 ng/mL	26.5 ng/mL	Parikh et al [31]
overall	182	Adults	serum [A]	14.6 ng/mL	34.6 ng/mL	Parikh et al [31]
overall	182	Adults	serum [P]	4.3 ng/mL	31.8 ng/mL	Parikh et al [31]
female	155	<1 year	serum	10.4 ng/mL	42.0 ng/mL	Bae et al [25]
female	47	1 - 5 years	serum	7.6 ng/mL	39.9 ng/mL	Bae et al [25]
female	270	5 - 10 years	serum	7.9 ng/mL	31.9 ng/mL	Bae et al [25]
female	281	10 - 15 years	serum	7.6 ng/mL	36.4 ng/mL	Bae et al [25]
female	116	15 - 20 years	serum	10.4 ng/mL	36.4 ng/mL	Bae et al [25]
female	182	20 - 40 years	serum	14.0 ng/mL	38.2 ng/mL	Bae et al [25]
female	101	40 - 60 years	serum	9.2 ng/mL	38.3 ng/mL	Bae et al [25]
female	104	60 - 80 years	serum	9.0 ng/mL	28.3 ng/mL	Bae et al [25]
male	124	<1 year	serum	9.3 ng/mL	42.2 ng/mL	Bae et al [25]
male	206	1 - 5 years	serum	9.2 ng/mL	37.2 ng/mL	Bae et al [25]
male	286	5 - 10 years	serum	7.9 ng/mL	30.2 ng/mL	Bae et al [25]
male	341	10 - 15 years	serum	7.2 ng/mL	38.8 ng/mL	Bae et al [25]
male	155	15 - 20 years	serum	13.6 ng/mL	44.6 ng/mL	Bae et al [25]
male	214	20 - 40 years	serum	15.5 ng/mL	34.0 ng/mL	Bae et al [25]
male	114	40 - 60 years	serum	10.1 ng/mL	31.5 ng/mL	Bae et al [25]
male	92	60 - 80 years	serum	10.0 ng/mL	29.2 ng/mL	Bae et al [25]

[A] (AM)
[P] (PM)

Reference interval **dehydroepiandrosterone**
Following reference values are published for apparently healthy subjects:

Population		Analyte			Reference
Gender	**Age**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female	0.5 - 2 years	serum		1.99 ng/mL	Kushnir et al [26]
female	2 - 4 years	serum		0.85 ng/mL	Kushnir et al [26]
female	4 - 6 years	serum		1.03 ng/mL	Kushnir et al [26]
female	6 - 7 years	serum		1.79 ng/mL	Kushnir et al [26]
female	7 - 10 years	serum	0.14 ng/mL	2.35 ng/mL	Kushnir et al [26]
female	10 - 12 years	serum	0.43 ng/mL	3.78 ng/mL	Kushnir et al [26]
female	12 - 14 years	serum	0.89 ng/mL	6.21 ng/mL	Kushnir et al [26]
female	14 - 16 years	serum	1.22 ng/mL	7.01 ng/mL	Kushnir et al [26]
female	16 - 18 years	serum	1.42 ng/mL	0.9 ng/mL	Kushnir et al [26]
female	Tanner Stage 1	serum	0.14 ng/mL	2.76 ng/mL	Kushnir et al [26]
female	Tanner Stage 2	serum	0.83 ng/mL	4.87 ng/mL	Kushnir et al [26]
female	Tanner Stage 3	serum	1.08 ng/mL	7.56 ng/mL	Kushnir et al [26]
female	Tanner Stage 4-5	serum	1.24 ng/mL	7.88 ng/mL	Kushnir et al [26]
male	0.5 - 2 years	serum		2.5 ng/mL	Kushnir et al [26]
male	2 - 4 years	serum		0.63 ng/mL	Kushnir et al [26]
male	4 - 6 years	serum		0.95 ng/mL	Kushnir et al [26]
male	6 - 7 years	serum	0.06 ng/mL	1.93 ng/mL	Kushnir et al [26]
male	7 - 10 years	serum	0.1 ng/mL	2.08 ng/mL	Kushnir et al [26]
male	10 - 12 years	serum	0.32 ng/mL	3.08 ng/mL	Kushnir et al [26]
male	12 - 14 years	serum	0.57 ng/mL	4.1 ng/mL	Kushnir et al [26]
male	14 - 16 years	serum	0.93 ng/mL	6.04 ng/mL	Kushnir et al [26]
male	16 - 18 years	serum	1.17 ng/mL	6.52 ng/mL	Kushnir et al [26]
male	Tanner Stage 1	serum	0.11 ng/mL	2.37 ng/mL	Kushnir et al [26]
male	Tanner Stage 2	serum	0.37 ng/mL	3.66 ng/mL	Kushnir et al [26]
male	Tanner Stage 3	serum	0.75 ng/mL	5.24 ng/mL	Kushnir et al [26]
male	Tanner Stage 4 - 5	serum	1.22 ng/mL	6.7 ng/mL	Kushnir et al [26]
overall	19 - 30 years	serum		13 ng/mL	Mayo Clinic [28]
overall	31 - 40 years	serum		8 ng/mL	Mayo Clinic [28]
overall	41 - 50 years	serum		8 ng/mL	Mayo Clinic [28]
overall	51 - 60 years	serum		6 ng/mL	Mayo Clinic [28]
overall	>61 years	serum		5 ng/mL	Mayo Clinic [28]
overall	1 - 5 years	serum		0.05 ng/mL	Gardner et al [29]
overall	6 - 7 years	serum		0.63 ng/mL	Gardner et al [29]
overall	8 - 10 years	serum		1.11 ng/mL	Gardner et al [29]
overall	11 - 12 years	serum		1.86 ng/mL	Gardner et al [29]
overall	13 - 14 years	serum		2.02 ng/mL	Gardner et al [29]
overall	15 - 16 years	serum	0.39 ng/mL	4.81 ng/mL	Gardner et al [29]
overall	17 - 19 years	serum	0 ng/mL	4.91 ng/mL	Gardner et al [29]
overall	20 - 50 years	serum	0.31 ng/mL	7.01 ng/mL	Gardner et al [29]

Reference interval **dehydroepiandrosterone sulfate**
Following reference values are published for apparently healthy subjects:

Population			**Analyte**			**Reference**
Gender	**n**	**Age**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female		18 - 81 years	Heparin plasma [A]	313 ng/mL	2740 ng/mL	Eisenhofer et al [21]
female		Postmenopausal	Heparin plasma [A]	135 ng/mL	4582 ng/mL	Eisenhofer et al [21]
male		18-81 years	Heparin plasma [A]	338 ng/mL	3693 ng/mL	Eisenhofer et al [21]
female	156	<1 year	serum		210 ng/mL	Bae et al [25]
female	47	1 - 5 years	serum		258 ng/mL	Bae et al [25]
female	269	5 - 10 years	serum		1043 ng/mL	Bae et al [25]
female	280	10 - 15 years	serum	173 ng/mL	2134 ng/mL	Bae et al [25]
female	112	15 - 20 years	serum	435 ng/mL	4054 ng/mL	Bae et al [25]
female	188	20 - 40 years	serum	439 ng/mL	2963 ng/mL	Bae et al [25]
female	120	40 - 60 years	serum	162 ng/mL	2384 ng/mL	Bae et al [25]
female	112	60 - 80 years	serum	41 ng/mL	1776 ng/mL	Bae et al [25]
male	124	<1 year	serum		195 ng/mL	Bae et al [25]
male	206	1 - 5 years	serum		166 ng/mL	Bae et al [25]
male	286	5 - 10 years	serum		995 ng/mL	Bae et al [25]
male	340	10 - 15 years	serum	184 ng/mL	2521 ng/mL	Bae et al [25]
male	153	15 - 20 years	serum	556 ng/mL	4415 ng/mL	Bae et al [25]
male	220	20 - 40 years	serum	660 ng/mL	4706 ng/mL	Bae et al [25]
male	142	40 - 60 years	serum	243 ng/mL	3737 ng/mL	Bae et al [25]
male	103	60 - 80 years	serum	199 ng/mL	3191 ng/mL	Bae et al [25]

[A] (AM)

Reference interval **dihydrotestosterone**
Following reference values are published for apparently healthy subjects:

Population		**Analyte**			**Reference**
Gender	**Age**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female	20 - 79 years	plasma		0.212 ng/mL	van der Veen et al [32]
female *	20 - 39 years	plasma		0.099 ng/mL	van der Veen et al [32]
male	20 - 79 years	plasma	0.26 ng/mL	0.99 ng/mL	van der Veen et al [32]
female	0 - 7 days	plasma		0.03 ng/mL	Kulle et al [27]
female	0.5 - 3 months	plasma		0.03 ng/mL	Kulle et al [27]
female	3 - 6 months	plasma		0.03 ng/mL	Kulle et al [27]
female	6 - 12 months	plasma		0.03 ng/mL	Kulle et al [27]
female	1 - 4 years	plasma		0.12 ng/mL	Kulle et al [27]
female	4 - 7 years	plasma		0.12 ng/mL	Kulle et al [27]
female	7 - 10 years	plasma		0.15 ng/mL	Kulle et al [27]
female	10 - 13 years	plasma		0.23 ng/mL	Kulle et al [27]
female	13 - 16 years	plasma		0.29 ng/mL	Kulle et al [27]
female	16 - 19 years	plasma		0.29 ng/mL	Kulle et al [27]
male	0 - 7 days	plasma		0.20 ng/mL	Kulle et al [27]
male	0.5 - 3 months	plasma		0.75 ng/mL	Kulle et al [27]
male	3 - 6 months	plasma		0.23 ng/mL	Kulle et al [27]
male	6 - 12 months	plasma		0.12 ng/mL	Kulle et al [27]
male	1 - 4 years	plasma		0.38 ng/mL	Kulle et al [27]
male	4 - 7 years	plasma		0.23 ng/mL	Kulle et al [27]
male	7 - 10 years	plasma		0.17 ng/mL	Kulle et al [27]
male	10 - 13 years	plasma		0.55 ng/mL	Kulle et al [27]
male	13 - 16 years	plasma		0.93 ng/mL	Kulle et al [27]
male	16 - 19 years	plasma		0.55 ng/mL	Kulle et al [27]

* Oral contraceptives

Reference interval **progesterone**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	Age [years]	Menstrual cycle	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female	24 - 44	Follicular, 1 - 7 days	serum	0.072 ng/mL	2.44 ng/mL	Frederiksen et al [19]
female	24 - 44	Follicular, 8 - 14 days	serum	0.075 ng/mL	16.30 ng/mL	Frederiksen et al [19]
female	24 - 44	Luteal, 15+	serum	0.088 ng/mL	26.09 ng/mL	Frederiksen et al [19]
female	24 - 44	Luteal, 15+ (confirmed ovulation)	serum	3.391 ng/mL	26.12 ng/mL	Frederiksen et al [19]
female	18 - 81	Follicular	Heparin plasma [A]	0.019 ng/mL	5.85 ng/mL	Eisenhofer et al [21]
female	18 - 81	Luteal	Heparin plasma [A]	0.022 ng/mL	26.06 ng/mL	Eisenhofer et al [21]
female	18 - 81	Oral contraceptives	Heparin plasma [A]	0.003 ng/mL	0.31 ng/mL	Eisenhofer et al [21]
female	18 - 81	Post-menopausal	Heparin plasma [A]	0.009 ng/mL	3.59 ng/mL	Eisenhofer et al [21]
male	18 - 81		Heparin plasma [A]	0.013 ng/mL	21.98 ng/mL	Eisenhofer et al [21]
female	0 - 12		serum	0 ng/mL	0.22 ng/mL	Kyriakopoulou et al [22]
female	12 - 19		serum	0.11 ng/mL	2.19 ng/mL	Kyriakopoulou et al [22]
male	0 - 12		serum	0 ng/mL	0.25 ng/mL	Kyriakopoulou et al [22]
male	12 - 19		serum	0.03 ng/mL	0.3 ng/mL	Kyriakopoulou et al [22]

[A] (AM)

Reference interval **testosterone**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	n	Age	Matrix	lower limit (2.5%)	upper limit (97.5%)	
female		0 - 15 days	serum	0.017 ng/mL	0.132 ng/mL	Kyriakopoulou et al [22]
female		15 - 365 days	serum	0.006 ng/mL	0.063 ng/mL	Kyriakopoulou et al [22]
female		1 - 13 years	serum	0.017 ng/mL	0.323 ng/mL	Kyriakopoulou et al [22]
female		13 - 16 years	serum	0.144 ng/mL	0.392 ng/mL	Kyriakopoulou et al [22]
female		16 - 19 years	serum	0.032 ng/mL	0.666 ng/mL	Kyriakopoulou et al [22]
male		0 - 15 days	serum	0.072 ng/mL	1.830 ng/mL	Kyriakopoulou et al [22]
male		15 - 365 days	serum	0.014 ng/mL	2.570 ng/mL	Kyriakopoulou et al [22]
male		1 - 13 years	serum	0.017 ng/mL	0.323 ng/mL	Kyriakopoulou et al [22]
male		13 - 16 years	serum	0.280 ng/mL	4.530 ng/mL	Kyriakopoulou et al [22]
male		16 - 19 years	serum	0.277 ng/mL	6.740 ng/mL	Kyriakopoulou et al [22]
female	156	<1 years	serum		0.943 ng/mL	Bae et al [25]
female	47	1 - 10 years	serum		0.141 ng/mL	Bae et al [25]
female	280	10 - 15 years	serum	0.040 ng/mL	0.502 ng/mL	Bae et al [25]
female	112	15 - 20 years	serum	0.112 ng/mL	0.620 ng/mL	Bae et al [25]
female	188	20 - 40 years	serum	0.095 ng/mL	0.591 ng/mL	Bae et al [25]
female	120	40 - 60 years	serum	0.078 ng/mL	0.461 ng/mL	Bae et al [25]
female	112	60 - 80 years	serum	0.075 ng/mL	0.459 ng/mL	Bae et al [25]
male	124	<1 years	serum		1.156 ng/mL	Bae et al [25]
male	206	1 - 10 years	serum		0.092 ng/mL	Bae et al [25]
male	340	10 - 15 years	serum	0.032 ng/mL	7.435 ng/mL	Bae et al [25]
male	153	15 - 20 years	serum	2.391 ng/mL	8.860 ng/mL	Bae et al [25]
male	220	20 - 40 years	serum	2.301 ng/mL	8.404 ng/mL	Bae et al [25]
male	142	40 - 60 years	serum	1.800 ng/mL	7.709 ng/mL	Bae et al [25]
male	103	60 - 80 years	serum	1.834 ng/mL	7.585 ng/mL	Bae et al [25]

Reference interval **17-hydroxypregnenolone**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	**n**	**Age**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female	126	7 - 9 years	serum, plasma		2.12 ng/mL	Kushnir et al [26]
female	83	10 - 11 years	serum, plasma		3.2 ng/mL	Kushnir et al [26]
female	84	12–13 years	serum, plasma		3.62 ng/mL	Kushnir et al [26]
female	83	14 – 15 years	serum, plasma	0.28 ng/mL	4.22 ng/mL	Kushnir et al [26]
female	83	16–17 years	serum, plasma	0.25 ng/mL	4.19 ng/mL	Kushnir et al [26]
female	56	18–51 years	serum, plasma, Premenopausal		2.08 ng/mL	Kushnir et al [26]
male	126	7–9 years	serum, plasma		1.87 ng/mL	Kushnir et al [26]
male	83	10–11 years	serum, plasma		3.87 ng/mL	Kushnir et al [26]
male	83	12–13 years	serum, plasma		3.66 ng/mL	Kushnir et al [26]
male	84	14–15 years	serum, plasma	0.32 ng/mL	4.28 ng/mL	Kushnir et al [26]
male	84	16–17 years	serum, plasma	0.31 ng/mL	4.78 ng/mL	Kushnir et al [26]
male	56	18–52 years	serum, plasma	0.36 ng/mL	4.09 ng/mL	Kushnir et al [26]
overall		< 10 days (preterm)	serum	3.77 ng/mL	35.62 ng/mL	Fiet et al [24]
overall		< 10 days (full-term)	serum	0.43 ng/mL	41.0 ng/mL	Fiet et al [24]
overall		10 days - 18 years	serum	0.41 ng/mL	4.80 ng/mL	Fiet et al [24]

Reference interval **estradiol**
Following reference values are published for apparently healthy subjects:

Population				Analyte			Reference
Gender	**n**	**Age**	**Menstrual cycle**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female		24 - 44 years	Follicular, 1 - 7 days	serum	0.022 ng/mL	0.075 ng/mL	Frederiksen et al [19]
female		24 - 44 years	Follicular, 8-14 days	serum	0.031 ng/mL	0.277 ng/mL	Frederiksen et al [19]
female		24 - 44 years	Luteal, 15+	serum	0.020 ng/mL	0.254 ng/mL	Frederiksen et al [19]
female		24 - 44 years	Luteal, 15+ (confirmed ovulation)*	serum	0.041 ng/mL	0.291 ng/mL	Frederiksen et al [19]
female		7 - 10 years		serum		0.035 ng/mL	Kushnir et al [20]
female		10 - 13 years		serum		0.087 ng/mL	Kushnir et al [20]
female		13 - 16 years		serum	0.009 ng/mL	0.248 ng/mL	Kushnir et al [20]
female		16 - 18 years		serum	0.002 ng/mL	0.266 ng/mL	Kushnir et al [20]
male		7 - 10 years		serum		0.006 ng/mL	Kushnir et al [20]
male		10 - 13 years		serum		0.010 ng/mL	Kushnir et al [20]
male		13 - 16 years		serum	0.001 ng/mL	0.036 ng/mL	Kushnir et al [20]
male		16 - 18 years		serum	0.003 ng/mL	0.034 ng/mL	Kushnir et al [20]
female	156	< 1 year		serum		0.022 ng/mL	Bae et al [24]
female	47	1 - 5 years		serum		0.017 ng/mL	Bae et al [24]
female	557	5 - 10 years		serum		0.012 ng/mL	Bae et al [24]
female	629	10 - 15 years		serum		0.228 ng/mL	Bae et al [24]
female	321	15 - 20 years		serum		0.255 ng/mL	Bae et al [24]
female	236	20 - 40 years		serum		0.302 ng/mL	Bae et al [24]
female	119	40 - 60 years		serum		0.255 ng/mL	Bae et al [24]
female	112	60 - 80 years		serum		0.038 ng/mL	Bae et al [24]
male	124	<1 year		serum		0.020 ng/mL	Bae et al [24]
male	206	1 - 5 years		serum		0.019 ng/mL	Bae et al [24]
male	946	5 - 10 years		serum		0.010 ng/mL	Bae et al [24]
male	731	10 - 15 years		serum		0.028 ng/mL	Bae et al [24]
male	335	15 - 20 years		serum		0.046 ng/mL	Bae et al [24]
male	223	20 - 40 years		serum		0.043 ng/mL	Bae et al [24]
male	143	40 - 60 years		serum		0.036 ng/mL	Bae et al [24]
male	103	60 - 80 years		serum		0.045 ng/mL	Bae et al [24]
female		<18 years		plasma		0.040 ng/mL	Denver et al [34]
female		Adults	Premenopausal	plasma	0.015	0.350	Denver et al [34]
female		Adults	Pregnancy	plasma	0.188	7.192	Denver et al [34]
female		Adults	Postmenopausal	plasma		0.010	Denver et al [34]
male		<18 years		plasma		0.038	Denver et al [34]
male		Adults		plasma	0.010	0.040	Denver et al [34]
female		Adults	Menstrual cycle 2 - 4 days	plasma	0.040	0.300	Bergemann et al [35]
female		Adults	Menstrual cycle 10 - 12 days	plasma	0.150	0.350	Bergemann et al [35]
female		Adults	Menstrual cycle 20 - 22 days	plasma	0.030	0.200	Bergemann et al [35]

* Ovulation confirmed by progesterone > 10 nmol/L, also applied to other tables.

Reference interval **estrone**
Following reference values are published for apparently healthy subjects:

Population			Analyte			Reference
Gender	**Age [years]**	**Menstrual cycle**	**Matrix**	**lower limit (2.5%)**	**upper limit (97.5%)**	
female	24 - 44	Follicular, 1-7 days	serum	0.018 ng/mL	0.142 ng/mL	Frederiksen et al [19]
female	24 - 44	Follicular, 8-14 days	serum	0.036 ng/mL	0.472 ng/mL	Frederiksen et al [19]
female	24 - 44	Luteal, 15+	serum	0.024 ng/mL	0.342 ng/mL	Frederiksen et al [19]
female	24 - 44	Luteal, 15+ (confirmed ovulation)	serum	0.060 ng/mL	0.416 ng/mL	Frederiksen et al [19]
female	0.3 - 7		serum	0.0011 ng/mL	0.005 ng/mL	Frederiksen et al [19]
male	0.3 - 7		serum	0.0006 ng/mL	0.004 ng/mL	Frederiksen et al [19]
female	7 - 10		serum		0.025 ng/mL	Kushnir et al [20]
female	10 - 13		serum		0.042 ng/mL	Kushnir et al [20]
female	13 - 16		serum	0.008 ng/mL	0.105 ng/mL	Kushnir et al [20]
female	16 - 18		serum	0.004 ng/mL	0.133 ng/mL	Kushnir et al [20]
male	7 - 10		serum		0.006 ng/mL	Kushnir et al [20]
male	10 - 13		serum		0.010 ng/mL	Kushnir et al [20]
male	13 - 16		serum	0.001 ng/mL	0.030 ng/mL	Kushnir et al [20]
male	16 - 18		serum	0.001 ng/mL	0.032 ng/mL	Kushnir et al [20]
female	Adults	Postmenopausal	serum	0.007 ng/mL	0.040 ng/mL	Mayo clinic [33]
male	Adults		serum	0.010 ng/mL	0.060 ng/mL	Mayo clinic [33]

In cases where zero is given in the age group, this is not intended for use in neonatal screening. It is part of the age group including zero taken directly from literature. This applies to all tables containing reference intervals in this report.

Warning: Very low values in apparently healthy or affected population may result in concentration below limit of quantitation.

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests. It is recommended that each laboratory establishes its own range of normal values.

20. LIMITATIONS OF THE PROCEDURE

Specimen collection and storage have a significant effect on the test results. See SPECIMEN COLLECTION AND STORAGE for details. For interferences, see PERFORMANCE.

21. PERFORMANCE

21.1. LoQ and Measuring Range

The LoQ study was conducted during three independent runs. The runs were performed with at least three different low concentrated human serum samples. Each sample was tested in 5-fold determination (biological replicate) using two reagent lots. The data was evaluated on basis of CLSI guideline EP17-A2.

The measuring range is defined between the limit of quantitation (LoQ) and the highest measured concentration showing linearity (upper limit of measuring interval – ULMI).

Analyte	LoQ [ng/mL]	ULMI [ng/mL]
11-deoxycorticosterone	0.022	5.13
11-deoxycortisol	0.168	12.8
17-hydroxyprogesterone	0.092	12.8
21-deoxycortisol	0.098	12.8
aldosterone	0.070	4.59
androstenedione	0.057	12.8
corticosterone	0.265	38.5
cortisol	1.516	256
cortisone	0.748	64.1
dexamethasone	0.620	64.1
dehydroepiandrosterone	0.621	45.9
dehydroepiandrosterone sulfate	61.2	3600
dihydrotestosterone	0.084	1.57
progesterone	0.080	12.8
testosterone	0.040	13.4
17-hydroxypregnenolone	0.166	38.5
estradiol	0.017	3.85
estrone	0.006	1.28

21.2. Interference

The mentioned substances and their influence on the quantitation of analytes are analyzed via spiking.
Highlighted substances (☒) will influence the results of respective analyte >20%.
No interference is shown until the mentioned concentration for the checked (✓) substances.
Additionally, interferences with oxazepam and normethandrone cannot be excluded.

✓ **NO interference** ☒ **interference**	Hemoglobin 12 mg/mL	Bilirubin 1 mg/mL	Glyceryl trioleate 10 mg/mL	ProClin300 1.0%	Fenofibrate 0.1%	18-hydroxycorticosterone 10 ng/mL	Penicillin 1 mg/mL	Methicillin 0.1 mg/mL	11β-hydroxyprogesterone 200 ng/mL	Ethylenediaminetetraacetic acid (EDTA) 1.66 mg/mL	Betamethasone 50 ng/mL	Epitestosterone 40 ng/mL	Sodium azide 0.1%
11-deoxycorticosterone	✓	✓	✓	✓	✓	✓	✓	✓	☒	✓	✓	✓	✓
11-deoxycortisol	✓	✓	✓	✓	✓	✓	✓	✓	☒	✓	✓	✓	✓
17-hydroxyprogesterone	☒	☒	✓	☒	☒	☒	✓	✓	☒	☒	☒	☒	✓
21-deoxycortisol	✓	✓	✓	☒	✓	☒	✓	✓	☒	✓	✓	☒	☒
aldosterone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
androstenedione	✓	☒	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
corticosterone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
cortisol	✓	✓	✓	✓	✓	✓	☒	✓	✓	✓	✓	✓	✓
cortisone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
dexamethasone	✓	☒	✓	✓	✓	✓	✓	✓	✓	✓	☒	✓	✓
dehydroepiandrosterone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
dehydroepiandrosterone sulfate	✓	✓	✓	✓	☒	✓	☒	✓	✓	✓	✓	✓	✓
dihydrotestosterone	✓	✓	✓	✓	✓	✓	✓	✓	✓	☒	✓	☒	✓
progesterone	✓	✓	✓	☒	✓	☒	✓	✓	✓	✓	✓	✓	✓
testosterone	☒	✓	☒	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
17-hydroxypregnenolone	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
estradiol	☒	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
estrone	☒	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

21.3. Metrological Traceability

The concentrations assigned to kit standards are metrological traceable to SI unit ng/mL through an unbroken chain of calibration from Certified Reference Material to the kit Standards according to ISO 17511:2020.
The combined uncertainty of measurement for each analyte is assessed as the sum of all single uncertainties of methods used in the calibration hierarchy.

Analyte	Combined uncertainty of measurement [%]
11-deoxycorticosterone	6.7
11-deoxycortisol	7.4
17-hydroxyprogesterone	15.1
21-deoxycortisol	12.2
aldosterone	9.1
androstenedione	6.3
corticosterone	6.7
cortisol	11.9
cortisone	7.6
dexamethasone	8.0
dehydroepiandrosterone	16.0
dehydroepiandrosterone sulfate	15.9
dihydrotestosterone	8.0
progesterone	11.2
testosterone	7.8
17-hydroxypregnenolone	7.0
estradiol	7.5
estrone	4.9

21.4. Recovery

The recovery study was conducted by spiking different amounts of analyte into 3 serum samples and the recovery of predicted value was calculated according to CLSI guideline C62-A and C50. The mean recovery of every analyte is shown in the following table.

Analyte	Recovery [%]	Range [%]
11-deoxycorticosterone	115.1	114.4 - 115.9
11-deoxycortisol	107.9	103.1 - 112.7
17-hydroxyprogesterone	117.5	103.6 - 135.5
21-deoxycortisol	100.2	88.7 - 106.8
aldosterone	96.5	93.1 - 100.7
androstenedione	121.8	117.3 - 129.1
corticosterone	101.5	99.3 - 103.4
cortisol	102.9	98.9 - 106.0
cortisone	98.4	92.3 - 104.1
dexamethasone	102.0	98.3 - 107.7
dehydroepiandrosterone	104.8	100.6 - 112.4
dehydroepiandrosterone sulfate	107.6	101.5 - 115.7
dihydrotestosterone	96.9	92.4 - 101.8
progesterone	102.3	101.6 - 102.9
testosterone	94.9	90.7 - 102.9
17-hydroxypregnenolone	104.3	100.6 - 109.2
estradiol	116.1	102.3 - 209.3
estrone	104.9	102.3 - 109.3

21.5. Linearity

Linearity was evaluated based on CLSI guideline EP06-A. Four different samples – including human plasma (EDTA) and serum – were diluted serially and the recovery in dilution was analyzed based on linear regression. Linearity is ensured until the upper limit of measuring interval (ULMI).

Analyte	**Recovery [%]**	**ULMI [ng/mL]**
11-deoxycorticosterone	91.9	5.13
11-deoxycortisol	99.4	12.8
17-hydroxyprogesterone	92.0	12.8
21-deoxycortisol	91.6	12.8
aldosterone	108.1	4.59
androstenedione	91.5	12.8
corticosterone	100.6	38.5
cortisol	108.4	256
cortisone	99.0	64.1
dexamethasone	90.7	64.1
dehydroepiandrosterone	106.2	45.9
dehydroepiandrosterone sulfate	102.6	3600
dihydrotestosterone	96.1	1.57
progesterone	91.3	12.8
testosterone	95.2	13.4
17-hydroxypregnenolone	98.4	38.5
estradiol	89.0	3.85
estrone	98.0	1.28

21.6. Precision

21.6.1. Intra and inter assay precision

Intra and inter assay precision were evaluated on basis of CLSI guideline EP05-A3 using 20 different days, 2 runs per day and 2 replicates per day (20x2x2 experiment). In each run, 5 different serum samples of different concentration levels were analyzed.

Intra-Assay:

Analyte	**Mean CV [%]**	**Range CV [%]**
11-deoxycorticosterone	4.4	3.8 – 5.3
11-deoxycortisol	4.6	3.3 – 5.4
17-hydroxyprogesterone	10.7	5.5 – 14.5
21-deoxycortisol	8.3	3.9 – 11.8
aldosterone	6.3	5.3 – 7.9
androstenedione	4.0	3.8 – 4.4
corticosterone	4.9	3.4 – 5.6
cortisol	5.1	4.4. – 6.1
cortisone	5.3	5.0 – 5.9
dexamethasone	7.4	5.8 – 8.6
dehydroepiandrosterone	6.3	4.4 – 8.4
dehydroepiandrosterone sulfate	8.3	7.0 – 9.5
dihydrotestosterone	4.9	4.0 – 5.6
progesterone	5.8	3.6 – 7.8
testosterone	4.7	4.0 – 6.3
17-hydroxypregnenolone	4.3	3.5 – 5.2
estradiol	3.6	2.6 – 4.5
estrone	3.6	2.9 – 4.1

Inter-Assay:

Analyte	Mean CV [%]	Range CV [%]
11-deoxycorticosterone	6.9	6.2 – 8.0
11-deoxycortisol	8.2	5.8 – 9.7
17-hydroxyprogesterone	14.2	10.7 – 18.0
21-deoxycortisol	13.2	10.9 – 16.3
aldosterone	9.5	8.4 – 10.6
androstenedione	6.2	5.0 – 7.2
corticosterone	8.6	7.0 – 10.6
cortisol	10.3	7.8 – 12.5
cortisone	7.2	6.8 – 7.8
dexamethasone	12.3	10.9 – 13.5
dehydroepiandrosterone	12.1	10.0 – 16.3
dehydroepiandrosterone sulfate	14.4	12.8 – 17.0
dihydrotestosterone	8.8	7.3 – 11.1
progesterone	10.9	9.2 – 12.9
testosterone	7.0	5.6 – 9.1
17-hydroxypregnenolone	7.7	5.5 – 9.4
estradiol	6.4	5.0 – 9.2
estrone	5.2	4.6 – 6.1

21.6.2. Between lot precision

The between lot precision, including the between user precision, was conducted with 5 samples during 3 days of testing using 3 reagent lots and 3 different investigators. Each sample was tested in five replicates per run. The mean CV of the 5 samples was analyzed according to CLSI guideline EP05 for each analyte.

Analyte	Mean CV [%]	Range CV [%]
11-deoxycorticosterone	5.2	3.4 – 6.6
11-deoxycortisol	6.0	2.9 – 8.1
17-hydroxyprogesterone	14.5	6.3 – 29.6
21-deoxycortisol	11.4	6.5 – 14.5
aldosterone	8.6	5.5 – 10.8
androstenedione	4.6	2.6 – 8.6
corticosterone	5.2	3.0 – 7.7
cortisol	11.1	6.2 – 16.9
cortisone	6.3	3.4 – 9.7
dexamethasone	6.8	5.4 – 8.7
dehydroepiandrosterone	15.7	8.2 – 35.6
dehydroepiandrosterone sulfate	15.3	12.1 – 17.5
dihydrotestosterone	7.4	5.8 – 8.8
progesterone	10.3	6.5 – 19.2
testosterone	7.2	4.7 – 9.8
17-hydroxypregnenolone	5.5	4.4 – 7.5
estradiol	6.2	5.5 – 7.9
estrone	2.3	1.6 – 2.9

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23. TROUBLE SHOOTING

The following list will give an overview of challenges and possible cases as well as possible solution approaches. It offers only an overview and does not represent a complete trouble shooting list. If technical problems persist, please consult the instrument manufacturer or ProductSupport.IBL@tecan.com.

Challenge	Possible Cause	Corrective Action
Loss of sensitivity	Instrument or ion source requires tuning and optimizing.	Repeat MS tuning
	Instrument or ion source parameters are wrong	Check instrument and ion source parameter according to CALCULATION OF RESULTS
	Dirty ESI probe	Clean ESI probe
	Dirty curtain or orifice plate	Clean affected plate
	Dirty triple quadrupole unit (e. g. QJet or Q0)	Clean respective parts of the mass spectrometer
	Leaks	Inspect the syringe, the sample line and all other fittings and tubing. Make sure fittings and tubing are of correct type and size.
	The sample has degraded or has a too low concentration	Check storage time, temperature and expiration dates of used solvents and standards (see MATERIALS REQUIRED BUT NOT SUPPLIED and PRE-TEST SETUP INSTRUCTIONS). If possible, use a fresh sample.
	The ion source and/or the probe is not installed properly, or it is faulty.	Check installation of ion source and probe. Check the condition and position of the probe. If necessary, replace probe.
	Mobile phase was reconstituted wrongly	Check if mobile was correctly reconstituted according to PRE-TEST SETUP INSTRUCTIONS.
Arcing or sparks occur	Position of corona discharge needle is faulty	Adjust corona needle towards the curtain plain, and away from the stream of heater gas.
Ghost peaks	Peaks from previous run occur	Flush column, measure blanks as recommended between 10 - 25 patient samples, check if the LC gradient was performed according to TEST PROCEDURE.
	LC-MS method is wrong	Check LC-MS method according to TEST PROCEDURE.
	Contamination of LC- and/or MS-system	Flush tubings, chromatographic column and autosampler needle, check by using double blanks
	Contamination due sample preparation	Check if sample preparation was performed correctly according to PRE-TEST SETUP INSTRUCTIONS. Check if preparation solvents were correctly reconstituted with LC-MS-grade solvents according to PRE-TEST SETUP INSTRUCTIONS.
	Contamination of LC solvents	Check if preparation solvents were correctly reconstituted with LC-MS grade solvents according to PRE-TEST SETUP INSTRUCTIONS. Check storage temperature, storage time and expiration date of mobile phases. Do not store LC solvents in direct sunlight, it is recommended to use brown glass bottles. Use solvent filters in LC solvents.
	Air in the LC-system	Check for leaks. Use degassed solvents, check functionality of degasser. Purge LC-system.
Noisy Baseline	Contaminant buildup	Flush column. Check if solvents are LC-MS grade. Check if mobile phase was correctly reconstituted according to PRE-TEST SETUP INSTRUCTIONS, check storage time, temperature and experience dates of mobile phases and chromatographic column.
	Electrical inferences	Check for inferences, contact MS instrument service if necessary.
	Unsuitable sample volume	Use 20 µL Injection Volume.
Drifting	Contaminant buildup	See noisy baseline

Challenge	Possible Cause	Corrective Action
Baseline	Random temperature changes in e. g. column heater	Check function and conditions of column heater.
	Room temperature changes	Check room temperature and ensure a constant temperature.
Tailing of peaks	Extra dead volume	Reduce length of tubing. Check fittings. Ensure injector seal is tight.
	Old chromatographic column	Check the number of runs that have been run on the chromatographic column, change the column as recommended. Use advised storage solvents (see PRE-TEST SETUP INSTRUCTIONS). Flush column according to information enclosed to the chromatographic column. Replace column if necessary.
	Constitution of the mobile phases	See ghost peaks.
	Physical damage of the chromatographic column	Replace column.
	Interference	Flush column according to information enclosed to the chromatographic column. Check possible interferences.
Fronting of peaks	Unswept dead volume	See tailing of peaks.
	Column overloading	Use 20 µL Injection Volume.
	Interference	Flush column according to information enclosed to the chromatographic column. Check possible interferences.
	Physical damage of the chromatographic column	Replace column.
Broad peaks	Column overloading	Use 20 µL Injection Volume.
	Contamination of LC solvents	See ghost peaks
Split peaks/ double peaks	Interference	Flush column according to information enclosed to the chromatographic column. Check possible interferences.
	Column void or channeling	Replace column
	Column overloading	Use 20 µL Injection Volume.
	Wrong MS settings (e. g. dwell time)	Check MS conditions according to TEST PROCEDURE

The listed troubleshooting represents only selected error causes and their solution approaches. It does not replace maintenance and/or regular performance check-ups. The information is based on the Sciex System User Guide and Turbo V Ion Source Operation Guide (API Sciex, 5500 Series of Instruments, System User Guide, RUO-IDV-05-6768-A, December 2017 and API Sciex, Turbo V Ion Source, Operation Guide, RUO-IDV-05-6147-C, January 2022).

Symbols / Symbole / Symboles / Símbolos / Simboli / Símbolos / Σύμβολα

REF	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.–Cat.: / N.º Cat.: / Αριθμός-Κατ.:
LOT	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
Σ	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
CONC	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
LYO	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλιασμένο
IVD	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
BIO	Contains biological material of human origin / Enthält biologisches Material menschlichen Ursprungs / Contient une substance biologique d'origine humaine / Contiene material biológico de origen humano / Contiene materiale biologico di origine umana / Contém material biológico de origem humana / Περιέχει βιολογικό υλικό ανθρώπινης προέλευσης
BIO	Contains biological material of animal origin / Enthält biologisches Material tierischen Ursprungs / Contient une substance biologique d'origine animale / Contiene material biológico de origen animal / Contiene materiale biologico di origine animale / Contém material biológico de origem animal / Περιέχει βιολογικό υλικό ζωικής προέλευσης
UDI	Unique Device Identification / Eindeutige Gerätekennung / Identifiant de dispositif unique / Identificación única de producto / Identificatore univoco del dispositivo / Identificador de dispositivo único / Μοναδικός αναγνωριστικός κωδικός προϊόντος
i	Read instructions before use / Arbeitsanleitung lesen / Lire la fiche technique avant emploi / Lea las instrucciones antes de usar / Leggere le istruzioni prima dell'uso / Ler as instruções antes de usar / Διαβάστε τις οδηγίες πριν την χρήση
	Keep away from heat or direct sun light / Vor Hitze und direkter Sonneneinstrahlung schützen / Garder à l'abri de la chaleur et de toute exposition lumineuse / Manténgase alejado del calor o la luz solar directa / Non esporre ai raggi solari / Manter longe do calor ou luz solar directa / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Armazenar em: / Αποθήκευση στους:
2-8°C	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazenar a: 2 - 8°C / Conservare a: 2-8°C / Armazenar em: 2-8°C / Αποθήκευση στους: 2-8°C
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbricante: / Παραγωγός:
	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
!	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

LIMITATION OF LIABILITY: IN ALL CIRCUMSTANCES THE EXTENT OF MANUFACTURER'S LIABILITY IS LIMITED TO THE PURCHASE PRICE OF THE KIT(S) IN QUESTION. IN NO EVENT SHALL MANUFACTURER BE LIABLE FOR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING DAMAGES FOR LOST PROFITS, LOST SALES, INJURY TO PERSON OR PROPERTY OR ANY OTHER INCIDENTAL OR CONSEQUENTIAL LOSS.

The labelling of hazardous substances is according to European directive.
For further country-specific classifications, please refer to the corresponding safety data sheet.

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