Cortisol & Cortisone in urine/saliva

1040 M CORT

Instruction manual for LC-MS/MS assay
for in vitro diagnostic use

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1. Introduction

1.1 Intended Use

The LC-MS/MS kit is intended for the determination of Cortisol and Cortisone in urine and saliva

The components in de kit must be used as stated in the user manual.

1.2 Intended User

This kit is designed for (healthcare) laboratory professional use. Diagnotix recommends that users adhere to ISO 15189 Medical Laboratories.

1.3 Notice Regarding Serious Incidents

Following (EU) 2017/746 Annex I, Chapter III, 20.4.1 af), any serious incident that has occurred in relation to this 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.

1.4 IVD symbols

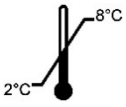
Order Number

Lot Number

For in vitro diagnostic use

See instructions for use

Manufacturer



Temperature limits

Contains sufficient for 200 tests

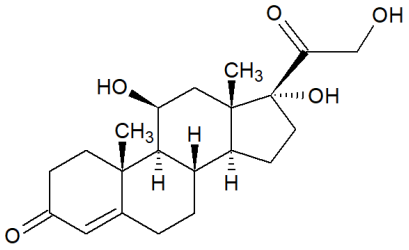
Expiry date

1.2 Clinical background

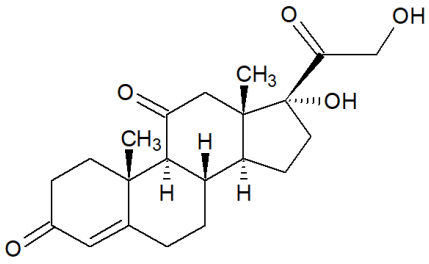
Urinary free cortisol (UFC) analysis represents the first biochemical laboratory approach for the screening of endogenous Cushing's syndrome (CS). Endogenous CS is caused by prolonged exposure to elevated levels of endogenous cortisol that may occur from excess production by one or both adrenal glands, or from overproduction of the adrenocorticotropic hormone (ACTH), which normally regulates cortisol production. As symptoms are always non-specific, including hypertension, truncal obesity and mood disorders, specific biochemical tests are required for diagnosing CS.

One of the first-line tests for diagnosis, the measurement of 24-h UFC, can also be useful in other clinical conditions characterized by a high serum cortisol level, such as in non-autonomous hypercortisolism (pseudo-CS), psychiatric disorders, morbid obesity, poorly controlled diabetes mellitus and alcoholism. Altered cortisol metabolism is also responsible for a condition called apparent mineralocorticoid excess (AME) syndrome. Type 2 11β-hydroxysteroid dehydrogenase (11β-HSD) regulates the cortisol level by oxidizing it to its inactive form, cortisone.

While cortisol is mainly essentially secreted by the adrenal gland, cortisone is mainly produced by 11β-HSD type 2 in multiple human tissues, which interconverts bioactive cortisol to hormonally inactive cortisone to prevent activation of the mineralocorticoid receptor by cortisol. Hence the simultaneous determination of cortisol and cortisone can help in the diagnosis of AME syndrome, but also in congenital adrenal hyperplasia and adrenal insufficiency.[1]



Cortisol



Cortisone

[1] Antonelli, G. et al. 2014

1.3 Description of the analytical procedure

Cortisol and Cortisone are determined from human urine or saliva by UHPLC with positive ion electrospray LC-MS/MS.

Prior to the LC-MS/MS analysis a sample clean-up is performed to remove the sample matrix and to spike with the internal standard.

After separation by chromatography on an analytical C-18 column, Cortisol and Cortisone are ionized by electrospray ionization (ESI) and detected by LC-MS/MS.

Electrospray ionization is a soft ionization technique where a strong electric field is applied to the liquid passing through the ESI-capillary of the MS-source. The ions are mostly performed in solution before desorption and then transferred into the ion path of the tandem mass spectrometer which consists of three quadrupoles (two mass selectors connected by a collision cell).

Measurement of the analytes is carried out in MRM mode. (MRM: Multiple Reaction Monitoring). In this mode only selected ions (precursor ions) with a defined mass/charge (M/z) ratio are isolated in the first quadrupole and subsequently transferred into the collision cell, where they are fragmented by impact with an inert gas (argon or nitrogen) at defined voltage settings. Among the fragments generated (known as product ions) only those with a defined M/z ratio can pass the third quadrupole for final detection. In this way the MRM mode ensures a selective identification and quantification of the target analytes.

2. Components of the Cortisol & Cortisone Kit

2.1 Ordering information

1040 KIT M CORT | Complete Kit for Cortisol and Cortisone in urine and saliva

Contents (for 200 assays)**:**

Cortisol & Cortisone Calibrator Set (Calibrator 1 – 6)	1042 CAL M CORT	6 x 2 x 2 ml
Cortisol & Cortisone Internal Standard D4 D8	1049 M CORT	2 x 6 ml
Cortisol & Cortisone Washing Solution	1053 M CORT	2 x 100 ml
Cortisol & Cortisone Mobile Phase I	1051 M CORT	1 x 500 ml
Cortisol & Cortisone Mobile Phase II	1052 M CORT	1 x 500 ml
Cortisol & Cortisone Elution Solution	1050 M CORT	2 x 100 ml
Cortisol & Cortisone Reconstitution Solution	1057 M CORT	1 x 100 ml
Cortisol & Cortisone Conditioning Solution	1058 M CORT	2 x 100 ml
Cortisol & Cortisone Autosampler Washing Solution	10401 M CORT	1 x 1000 ml
Cortisol & Cortisone Manual		

Separately available components:

Cortisol & Cortisone Calibrator Set (Calibrator 1 – 6)	1042 CAL M CORT	6 x 2 x 2 ml
Cortisol & Cortisone Internal Standard D4 D8	1049 M CORT	6 ml
Cortisol & Cortisone Elution Solution	1050 M CORT	100 ml
Cortisol & Cortisone Mobile Phase I	1051 M CORT	500 ml
Cortisol & Cortisone Mobile Phase II	1052 M CORT	500 ml
Cortisol & Cortisone Washing Solution	1053 M CORT	100 ml
Cortisol & Cortisone Reconstitution Solution	1057 M CORT	100 ml
Cortisol & Cortisone Conditioning Solution	1058 M CORT	100 ml
Cortisol & Cortisone Autosampler Washing Solution	10401 M CORT	1000 ml

Analytical Column Acquity UPLC BEH C18 Column 1.7 µm, 2.1 mm x 50 mm	186002350	1 pc
Cortisol & Cortisone Control I	1054 M CORT	10 x 2 ml
Cortisol & Cortisone Control II	1055 M CORT	10 x 2 ml
Cortisol & Cortisone Control III	1056 M CORT	10 x 2 ml
Cortisol & Cortisone Control Set	1041 CON M CORT	3 x 3 x 2 ml
SPE Tubes HLB 30 mg/1 ml, 100/pk	WAT094225	100 pcs
SPE 96-Well Plate 30 mg, 1/pk	WAT058951	1 pc

2.2 Safety information

Several components are chemical preparations and may contain hazardous substances. For safety information, please consult the Material Safety Data Sheet (MSDS) of each component.

The raw material donor blood was tested for HBsAg, anti-HIV 1/2 and anti-HCV. However, because no test method can offer complete assurance that products derived from human sources will not transmit infectious agents, it is recommended that this product be handled with the same precautions as patient samples.

2.3 Storage conditions and lifetime of kit components

Please unpack the kit components from the transport packaging *immediately upon receipt* and follow the instructions for storage conditions indicated on the product labels.

2.3.1 Calibrators and controls

1041 CON M CORT | Cortisol & Cortisone Control Set
1042 CAL M CORT | Cortisol & Cortisone Calibrator Set
1054 M CORT | Cortisol & Cortisone Control I
1055 M CORT | Cortisol & Cortisone Control II
1056 M CORT | Cortisol & Cortisone Control III

2.3.1.1 Handling

Reconstitute the calibrators and controls as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Cortisol & Cortisone Calibrator Set and Controls with exactly 2 ml distilled or deionised water using a volumetric pipette.
3. Replace the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Swirl the vial carefully, do not shake. Avoid foaming.
7. Use the preparation as a patient sample.

2.3.1.2 Stability and storage

The stability of the calibrators and controls are:

Before reconstitution:	2 - 8 °C	Until expiry date printed on the product label
After reconstitution:	2 - 8 °C	1 week
After reconstitution:	- 20 °C	1 month

The declared stated stabilities are only valid in case of no bacterial contamination.

2.3.2 Internal Standard D4 D8

1049 M CORT | Cortisol & Cortisone Internal Standard D4 D8

2.3.2.1 Handling

Reconstitute the internal standard as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute the Cortisol & Cortisone Internal Standard D4 D8 with exactly 6.0 ml distilled or deionised water using a volumetric pipette.
3. Replace the plug and let stand during 15 minutes.
4. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
5. Let stand for 15 minutes at room temperature.
6. Swirl the vial carefully, do not shake. Avoid foaming.

2.3.2.2 Stability and storage

The stability of the internal standard is:

Before reconstitution:	2 - 8 °C	Until expiry date printed on the product label
After reconstitution:	2 - 8 °C	1 week
After reconstitution:	- 20 °C	1 month

The declared stated stabilities are only valid in case of no bacterial contamination.

2.3.3 Elution Solution

1050 M CORT | Cortisol & Cortisone Elution Solution

2.3.3.1 Handling

The Reagent is liquid and ready for use.

2.3.3.2 Stability and storage

The stability of the washing solution is:

Store at 2 - 8 °C	After first opening the Reagent can be used for 8 weeks if closed and stored at 2 - 8 °C.

2.3.4 Mobile Phases

1051 M CORT | Cortisol & Cortisone Mobile Phase I
1052 M CORT | Cortisol & Cortisone Mobile Phase II

2.3.4.1 Handling

The Reagents are liquid and ready for use.

2.3.4.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagents can be used for 8 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC.

2.3.5 Washing Solution

1053 M CORT | Cortisol & Cortisone Washing Solution

2.3.5.1 Handling

The Reagent is liquid and ready for use.

2.3.5.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 8 weeks if closed and stored at 2 - 8 °C.

2.3.6 Reconstitution Solution

1057 M CORT | Cortisol & Cortisone Reconstitution Solution

2.3.6.1 Handling

The Reagent is liquid and ready for use.

2.3.6.2 Stability and storage

Store at 2 - 8 °C.	After first opening the Reagent can be used for 8 weeks if closed and stored at 2 - 8 °C.

2.3.7 Autosampler Washing Solution

10401 M CORT | Cortisol & Cortisone Autosampler Washing Solution

2.3.7.1 Handling

The Reagent is liquid and ready for use.

2.3.7.2 Stability and storage

Store at 2 - 8 °C.	After first opening the Reagent can be used for 8 weeks if closed and stored at 2 - 8 °C.

2.3.8 Conditioning Solution

1058 M CORT | Cortisol & Cortisone Conditioning Solution

2.3.8.1Handling

The Reagent is liquid and ready for use.

2.3.8.2 Stability and storage

Store at 2 - 8 °C	After first opening the Reagent can be used for 8 weeks if closed and stored at 2 - 8 °C.

3. Required instruments

Using this test kit requires a UHPLC system with tandem mass spectrometer (LC-MS/MS).

3.1 Required LC Modules

- Auto sampler
- UHPLC gradient pump
- Column heater
- Degasser

4. The analytical system

4.1 Preparation of the analytical system

- Flush the LC system excluding the column.
- Set the UHPLC pump at a flow rate of 2-4 ml/min and flush the system for 3 minutes with Mobile Phases I and II (50 : 50).
- Connect the column with the column heater . (see arrow marking on the column)

After flushing the system, the equilibration is performed as follows:

- Set the UHPLC pump to a flow rate of 0.25 ml/min.
- Set the column heater to 45 °C.
- Start the program for the gradient and equilibrate for 10 minutes.

4.2 Starting the analytical system

- Equilibrate the system.
- Check the temperature of the column.
- Initialize the injector.
- Start the programme on the LC-MS/MS system.

4.3 LC-MSMS Parameters and Conditions

4.3.1 LC Parameters

UHPLC pump	Flow rate 0.25 ml/min
Mobile Phases I and II	Close the bottles to avoid alteration of RT's through evaporation of the mobile phases
Column	The column is installed in the column heater 45 °C For the complete UHPLC system the backpressure should not exceed 1200 bar. 1 bar = 14.5 PSI

4.3.2 Autosampler Conditions (for example Waters Acquity I class FTN)

Injection volume:	5 - 20 µL
Sample temperature:	10 °C
Runtime: 4.5	min
Column temperature:	45 °C
Needle wash:	wash twice for 6 seconds
Seal Wash:	90:10 H2O:ACN
Wash Solvent:	Autosampler Washing Solution

4.3.3 Gradient (for example Waters Acquity I class Binary Solvent Manager)

Time (min)	**Flow Rate* (mL/min)**	**%A**	**%B**	**Curve**
0.00	0.25 - 0.3	85	15	Initial
1.40	0.25 - 0.3	60	40	6
2.30	0.25 - 0.3	60	40	6
2.40	0.25 - 0.3	0	100	6
2.60	0.25 - 0.3	0	100	6
2.70	0.25 - 0.3	85	15	6
5.00	0.25 - 0.3	85	15	6

Please note that the gradient is dependent on the analyser used. End users will need to define the optimal gradient for the analyser in use.

* Flow Rate depends on the pressure of the pumps used.

4.3.4 MS Conditions (for example Waters Xevo TQS)

MS Systeem:	(Waters Xevo TQS)
Ion mode:	Positive electrospray
capillary voltage:	1.0 kV
Source temperature	150 °C
Desolvation temperature	600 °C
Desolvation gas flow	1000 L/hr
Cone gas flow	150 L/hr
Detection mode	MRM
Dwell time	Auto
Collision Gas	Argon

Precursor	Product	Dwell (sec)	Cone (V)	Collision (eV)	Delay(s)	Compound
363.10	97.00	0.037	15.00	25.00	auto	Cortisol
363.10	121.00	0.037	15.00	25.00	auto	Cortisol
367.00	97.00	0.037	15.00	25.00	auto	Cortisol d4
367.00	121.00	0.037	15.00	25.00	auto	Cortisol d4

Precursor	Product	Dwell (sec)	Cone (V)	Collision (eV)	Delay(s)	Compound
361.10	121.00	0.037	15.00	25.00	auto	Cortisone
361.10	163.00	0.037	15.00	25.00	auto	Cortisone
369.10	169.10	0.041	15.00	25.00	auto	Cortisone d8

These conditions are an indication, the optima can differ slightly between different LC-MS/MS systems.

5. Sample

5.1 Sample material

5.1.1 Urine

Use at least 1 ml of urine collected over a 24 hour period.
Samples can be stored: 14 days at 2 - 8 °C
2 months at - 20 °C

Note that patient samples need to be centrifuged (5 minutes at 3000 g for urine samples and 5 minutes at 900g for saliva samples) prior to spiking with internal standard.

5.1.2 Saliva

Use at least 500 µl of saliva.
Samples can be stored: 14 days at 2 - 8 °C
2 months at - 20 °C

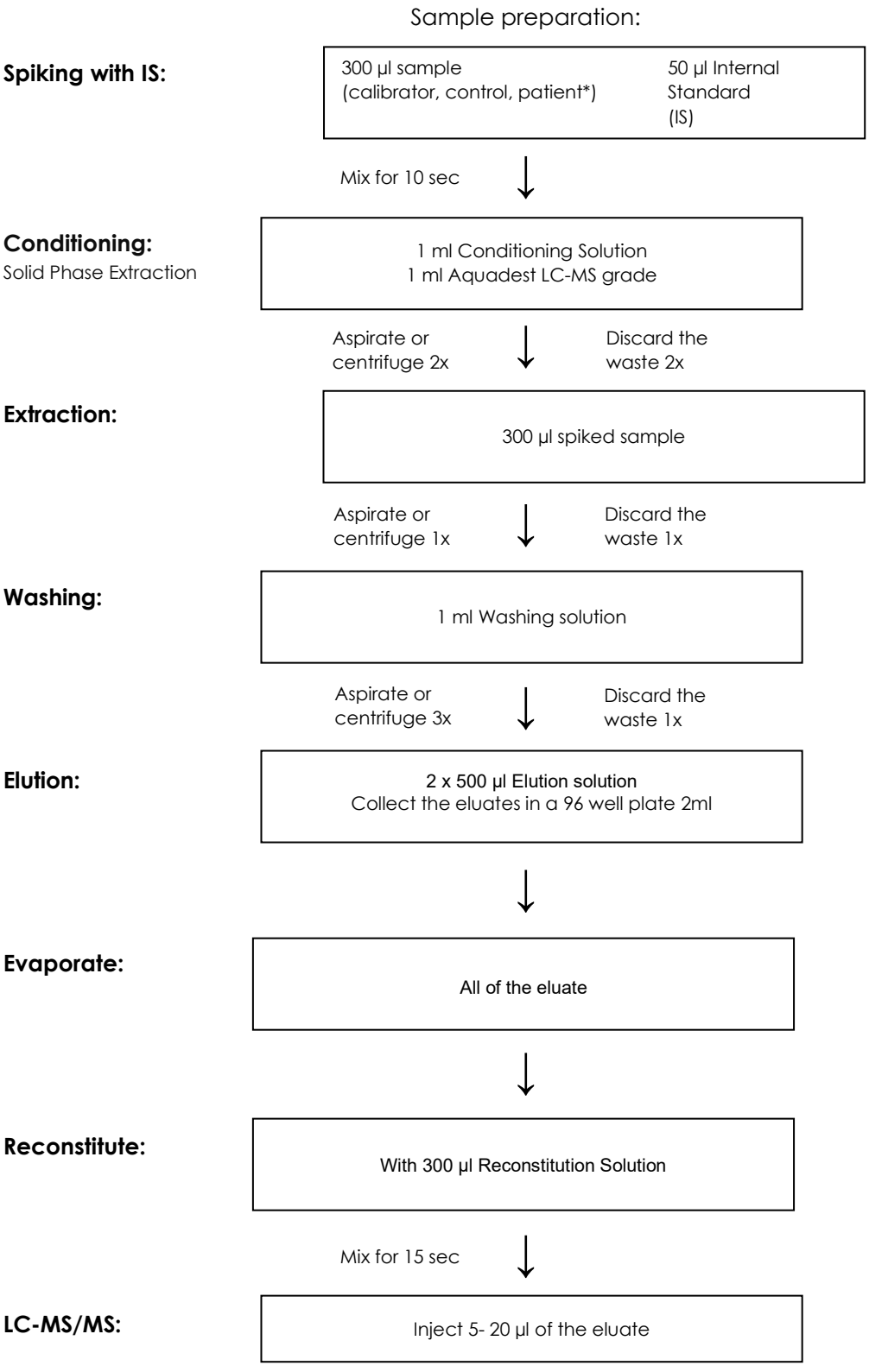
Note that patient samples need to be centrifuged (5 minutes at 3000 g for urine samples and 5 minutes at 900g for saliva samples) prior to spiking with internal standard.

5.2 Sample preparation

5.2.1 Reconstitution of the lyophilised Calibrators / Controls.

See 2.3.1.1 and the product data sheets.

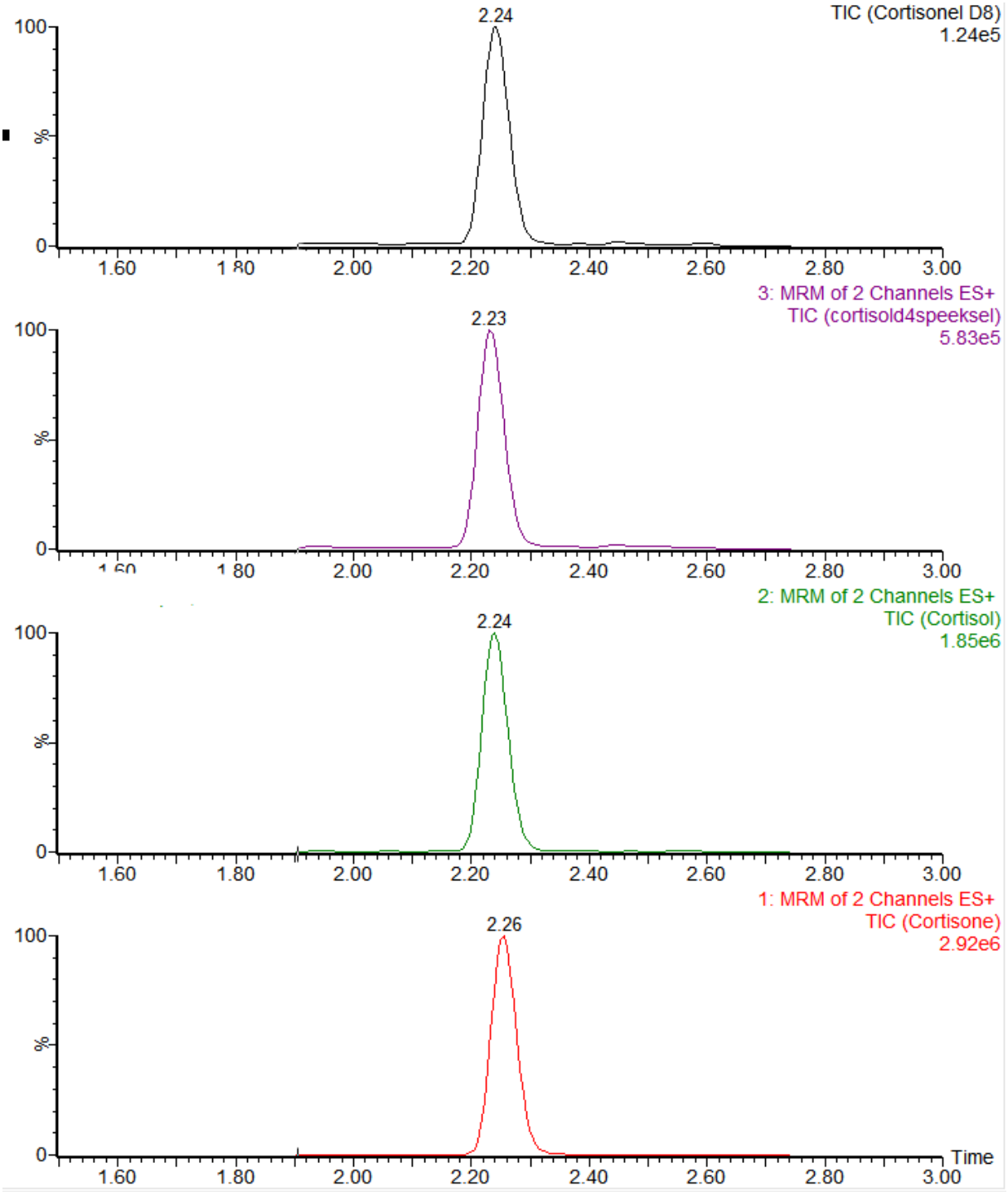
5.2.2 Sample preparation (urine, saliva, calibrator or control)



*** Note that patient samples need to be centrifuged (5 minutes at 3000 g for urine samples and 5 minutes at 900g for saliva samples) prior to spiking with internal standard.**

5.3 Examples of chromatograms

Example chromatogram of a Patient urine sample, recorded with the Waters UHPLC I-Class and the LC-MS/MS system Xevo TQS.



6. Test data (Validation report)

6.1 Linearity

	nmol/l
Cortisol	3787.17
Cortisone	3727.35

6.2 Limit of detection

	nmol/l
Cortisol	0.2
Cortisone	0.2

Note:

The limit of quantification (LOQ) is not given for the reason that the LOQ depends on the LC-MS/MS used for the measurement. We recommend that each laboratory determines the LOQ on the LC-MS/MS that will be used for this method.

6.3 Repeatability

Cortisol

Item	**Measured value (nmol/l)**	**Standard Deviation (nmol/l)**	**CV (%)**	**N**
Calibrator 3	10.0	0.37	3.70	20
Calibrator 5	190.1	9.15	4.82	20
Patient material (urine)	33.7	0.98	2.91	20

Cortisone

Item	**Measured value (nmol/l)**	**Standard Deviation (nmol/l)**	**CV (%)**	**N**
Calibrator 3	10.1	0.34	3.36	20
Calibrator 5	187.3	10.27	5.49	20
Patient material (urine)	122.9	3.27	2.66	20

6.4 Reference Ranges

Reference ranges for urine (24h)

	nmol/l
Cortisol	5 - 133
Cortisone	74 - 328

Reference ranges for saliva

	nmol/l
Cortisol	
8.00 hrs	1.6 - 19.3
16.00 hrs	0.5 - 5.0
23.00 hrs	0.2 - 3.4
Cortisone	
8.00 hrs	N.A.
16.00 hrs	N.A.
23.00 hrs	N.A.

The indicated reference ranges are taken from scientific literature[2].

It is recommended that each laboratory establishes its own normal range.

[2] UMCG own research 2007, https://www.umcg.nl/NL/UMCG/Afdelingen/Laboratoriumgeneeskunde/Professionals/bepalingenwijzer/Paginas/bepalingenwijzer.aspx

7. References

1. Antonelli, G. et al. (2014) Cortisol and cortisone ratio in urine: LC-MS/MS method validation and preliminary clinical application. *Clinical Chemistry and Laboratory Medicine*, *52(2)*, 213-220.

2. UMCG own research 2007, https://www.umcg.nl/NL/UMCG/Afdelingen/Laboratoriumgeneeskunde/Professionals/bepalingenwijzer/Paginas/bepalingenwijzer.aspx