Cortisol & Cortisone Dilute & Shoot in Urine

1180 M CODS

Instructions for use, LC-MS/MS assay

Diagnotix BV
De Plassen 4
9902 SE Appingedam
The Netherlands

SRN: NL-MF-000000236

UDI-DI: 08720514312575

+31 (0)596 - 20 10 62
info@diagnotix.com
techsupport@diagnotix.com

Document version: 2.1
Replaces: 2.0
Date of release: 28-02-2025

CE (EU) 2017/746 - IVD Medical Device

Contents

1. General Information

1.1 Information for the Device

1180 M CODS - Cortisol & Cortisone Dilute & Shoot Reagent Set
UDI-DI: 08720514312575

For information on the individual components of this set, refer to chapter 2 of these instructions for use.

1.2 Intended Purpose

1.2.1 Measurand

Cortisol
Hydrocortisone

Cortisone

1.2.2 Function

The function of this device is to aid in the diagnosis of several conditions, refer to paragraph 1.2.3, by the determination of Cortisol and Cortisone levels in 24h human urine, performed by automated quantitative LC-MS assay technology.

1.2.3 Specific Information indented to be provided

Deviant measurand values can be an indication of the following physiological or pathological states and/or conditions:

Cortisol:
- Endogenous Cushing's syndrome
- Non-autonomous hypercortisolism
- Psychiatric disorders
- Morbid obesity
- Diabetes mellitus
- Alcoholism
- Apparent mineralocorticoid excess (AME) syndrome

Cortisone:
- Apparent mineralocorticoid excess (AME) syndrome
- Congenital adrenal hyperplasia
- Adrenal insufficiency

1.2.4 Required Specimen

Human urine

1.2.4.1 Conditions for collection, handling and preparation of specimen

Use at least 1 ml of urine collected over a 24 hour period. Store samples for 14 days at 2 - 8 °C or 2 months at - 20 °C.

1.2.5 Testing Population

Patients known or suspected to be suffering from the conditions specified under paragraph 1.2.3.

1.3 Intended User

Laboratory Professional Use

1.4 Test Principle

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

Prior to the LC-MS/MS analysis a sample dilution is performed to dilute the sample matrix and to spike with the internal standard.

After separation by chromatography on an analytical C-18 column, the measurands 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 (Multiple Reaction Monitoring) mode. 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.

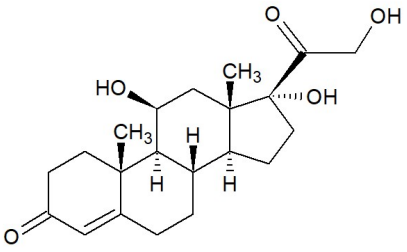
1.5 Clinical Background

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.

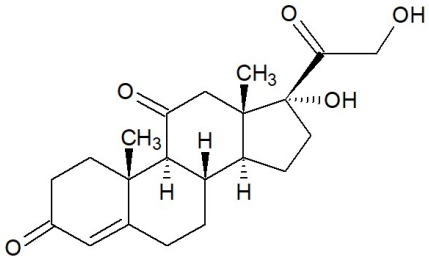
As symptoms are always non-specific, including hypertension, truncal obesity and mood disorders, specific biochemical tests are required for diagnosing CS.

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 urine 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

1.5 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] Antonelli, G. et al. 2014

2. Components and Accessories

2.1 Description of Components

All components are for LC-MS/MS use only, components also may contain other ingredients which might influence the measurement. All declared stabilities are only valid in case of no bacterial contamination.

2.1.1 Calibrators and Controls

1042 CAL M CORT | Cortisol & Cortisone Set
UDI-DI: 8720514310519

A six-point lyophilized calibrator at clinically relevant levels, refer to the value data sheet provided with each set for specific values per production batch.

1041 CON M CORT | Cortisol & Cortisone Control Set
UDI-DI: 8720514310526
1054 M CORT | Cortisol & Cortisone Control I
UDI-DI: 8720514310892
1055 M CORT | Cortisol & Cortisone Control II
UDI-DI: 8720514310908
1056 M CORT | Cortisol & Cortisone Control III
UDI-DI: 8720514310632

Three levels of lyophilized controls at clinically relevant levels for quality control purposes, refer to the value data sheet provided with each set for specific values per production batch.

2.1.1.1 Handling

Reconstitute the calibrators and controls as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Cortisol Dilute & Shoot Calibrator Set and Controls with exactly 1000 µl distilled or deionised water using a volumetric pipette.
3. Re-place 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.

2.1.1.2 Stability and Storage

The stability of the calibrators and controls are:

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

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

2.1.2 Internal Standard

1049 M CODS | Cortisol & Cortisone Internal Standard
UDI-DI: 8720514310595

A lyophilized deuterated version of the measurand, dissolved in an inert substance. Used to identify and correct potential deviating values, due to errors or varying circumstances in sample preparation or within the LC-MS/MS.

Active ingredient(s): Hydrocortisone D4 & Cortisone-D8

2.1.2.1 Handling

Reconstitute the internal standard as follows:

1. Carefully remove the cap and rubber plug avoiding any loss of contents.
2. Reconstitute Cortisol Dilute & Shoot Internal Standard D4 D8 with 6.0 ml 1190 M CODS Diluting Solution.
3. Re-place 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. After reconstitution pour this vial into the Diluting Solution bottle and mix thoroughly.

2.1.2.2 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	2 weeks

2.1.3 Diluting Solution

1190 M CODS | Cortisol & Cortisone Dilute & Shoot Diluting Solution
UDI-DI: 8720514312674

A solution provided to dilute the sample before use on an LC-MS/MS.

2.1.3.1 Handling

The Reagent is liquid and ready for use.

2.1.3.2 Storage and Stability

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC.

2.1.4 Mobile Phases

1191 M CODS | Cortisol & Cortisone Dilute & Shoot Mobile Phase I
UDI-DI: 8720514312681
1192 M CODS | Cortisol & Cortisone Dilute & Shoot Mobile Phase II
UDI-DI: 8720514312698

Two mobile phases are added to tune and carry the sample through the LC-MS. Different ratios of the mobile phases will allow different components to eluate from the column at differing speeds.

2.1.4.1 Handling

The Reagents are liquid and ready for use.

2.1.4.2 Stability and storage

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC.

2.1.5 Autosampler Washing Solution

1196 M CODS | Cortisol & Cortisone Dilute & Shoot Autosampler Washing Solution
UDI-DI: 8720514312735

A solution used to clean the LC-MS/MS system after use, specifically designed to remove residue from testing the measurand.

Active ingredient(s): Methanol 50%-<75%

2.1.5.1 Handling

The Reagent is liquid and ready for use.

2.1.5.2 Stability and storage

Store at 2 - 8 °C. After first opening the Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC.

2.2 List of components provided

1180 KIT M CODS - Complete Kit for Cortisol & Cortisone Dilute & Shoot in urine

Contents (for 300 assays):

Cortisol & Cortisone Calibrator Set (Calibrator 1 – 6)	1042 CAL M CORT	6 x 2 x 1 ml
Cortisol & Cortisone Internal Standard	1049 M CORT	3 x 6 ml
Cortisol & Cortisone Dilute & Shoot Diluting Solution	1190 M CODS	3 x 100 ml
Cortisol & Cortisone Dilute & Shoot Mobile Phase I	1191 M CODS	1 x 500 ml
Cortisol & Cortisone Dilute & Shoot Mobile Phase II	1192 M CODS	1 x 500 ml
Cortisol & Cortisone Dilute & Shoot Autosampler washing solution	1196 M CODS	1 x 1000 ml
Cortisol & Cortisone Dilute & Shoot Manual		

2.3 Separately available materials and components

Cortisol & Cortisone Calibrator Set (Calibrator 1 – 6)	1042 CAL M CORT	6 x 2 x 1ml
Cortisol & Cortisone Dilute & Shoot Internal standard D3	1189 M CODS	1 x 6 ml
Cortisol & Cortisone Dilute & Shoot Diluting Solution	1190 M CODS	1 x 100 ml
Cortisol & Cortisone Dilute & Shoot Mobile Phase I	1191 M CODS	1 x 500 ml
Cortisol & Cortisone Dilute & Shoot Mobile Phase II	1192 M CODS	1 x 500 ml
Cortisol & Cortisone Dilute & Shoot Autosampler washing solution	1196 M CODS	1 x 1000 ml

Analytical column Acquity UPLC HSS T3 Column 1.8 µm, 2.1mm x 100 mm	186003539	1 pc
Cortisol & Cortisone Control I	1054.10 M CORT	10 x 1 ml
Cortisol & Cortisone Control II	1055.10 M CORT	10 x 1 ml
Cortisol & Cortisone Control III	1056.10 M CORT	10 x 1 ml
Cortisol & Cortisone Control Set	1041 CON M CORT	3 x 3 x 1 ml

3. Warnings, precautions, measures and limitations of use

3.1 General

The device and its components must only be used in line with the intended purpose by the intended user as stated in chapter 1. Due to their nature, most reagents of this device contain or are largely composed of hazardous substances. Please refer to the Safety Data Sheets (SDS) for each of the components for specific hazards and measures to be taken.

Used components should be discarded and are not suitable for re-use.

3.1.1 Potentially infectious material

The human urine used for manufacturing calibrators and controls was tested for the following infectious markers and found negative: HBsAg, HIV-1, HIV-2 and HIV p24 Ag antibodies, Anti-HTLV 1 and 2, HCV and HIV genome. Nevertheless, the urine controls should be considered as potentially infectious and treated with appropriate care.

3.2 CMR substances

No CMR substances are used in the manufacturing of this kit or its components.

3.3 Disposal

For the safe disposal of the components of this kit, please refer to the safety data sheet of the component in question.

4. Assay procedure

4.1 Settings and procedure

4.1.1 Required instruments and LC modules

Using this test kit requires a UHPLC system with tandem mass spectrometer (LC-MS/MS) with the following modules:

- Autosampler
- UHPLC gradient pump
- Column heater
- Degasser

4.2 The analytical system

4.2.1 Preparation of the analytical system

- Flush the LC system excluding the column.
- Set the UHPLC pump at a flow rate of 1 ml/min and flush the system for 10 minutes with Mobile Phase 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 0.4 ml/min.
- Set the column heater to 45°C.
- Equilibrate the column for 15 minutes with Mobile Phase I.
- Start the program for the gradient and equilibrate for another 10 minutes.

4.2.2 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-MS/MS Parameters and Condition

4.3.1 LC Parameters

UHPLC pump Flow rate 0.4 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 1000 bar.
1 bar = 14.5 PSI

4.3.2 Autosampler Conditions

Injection volume:	10-20 µL
Sample temperature:	10 °C
Runtime:	5.5 min
Column temperature:	45 °C ± 5 °C alarm
Needle wash:	pre-injection 0 sec.
	Post-injection 12 sec.
Seal Wash:	10:90 ACN:H2O
Wash Solvent:	Autosampler Washing Solution; 30:70 H2O:MeOH

4.3.3 Gradient

Time (min)	**Flow Rate (mL/min)**	**%A**	**%B**	**Curve**
0.00	0.40	70	30	Initial
1.00	0.40	30	70	6
2.60	0.40	30	70	6
2.61	0.40	0	100	6
3.60	0.40	0	100	6
3.61	0.40	70	30	6
5.00	0.40	70	30	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.

4.3.4 MS Conditions (e.g. Waters Xevo TQS)

MS System:	(Waters Xevo TQS-micro)
Ion mode:	Electrospray
Capillary voltage:	1.0 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	600°C
Desolvation gas flow:	750L/hr
Detection mode:	ESI
Dwell time:	0.078
Collision gas:	Argon

Substance	Precursor	Product	Cone	Collision
Cortisol	363.15	97.08	30	25
Cortisol	363.15	120.9	30	25
Cortisol D4	367.15	97.08	30	25
Cortisol D4	367.15	120.9	30	25

Substance	Precursor	Product	Cone	Collision
Cortisone	361.1	121	15	25
Cortisone	361.1	163	15	25
Cortisone D8	369.1	169.1	15	25

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

4.4 Sample Preparation

4.4.1 Reconstitution of the lyophilised Calibrators / Controls.

Refer to paragraph 2.1.1.1 and the product value data sheets.

4.4.2 Sample preparation (sample, calibrator or control)

1. Centrifuge Calibrator, Control, Patient sample (5 min, 10000 x g or more).
2. Pipette 100 µl of the centrifuged supernatant into a vial.
3. Add 900 µl Cortisol Dilute & Shoot Dilution solution with Internal Standard D4/8.
4. Mix well and transfer the samples into vials or 96 well plate which is suitable for the auto sampler in use and Inject 10-20 µl in the LC-MS/MS.

4.4.3 Sample Preparation with pipette robot

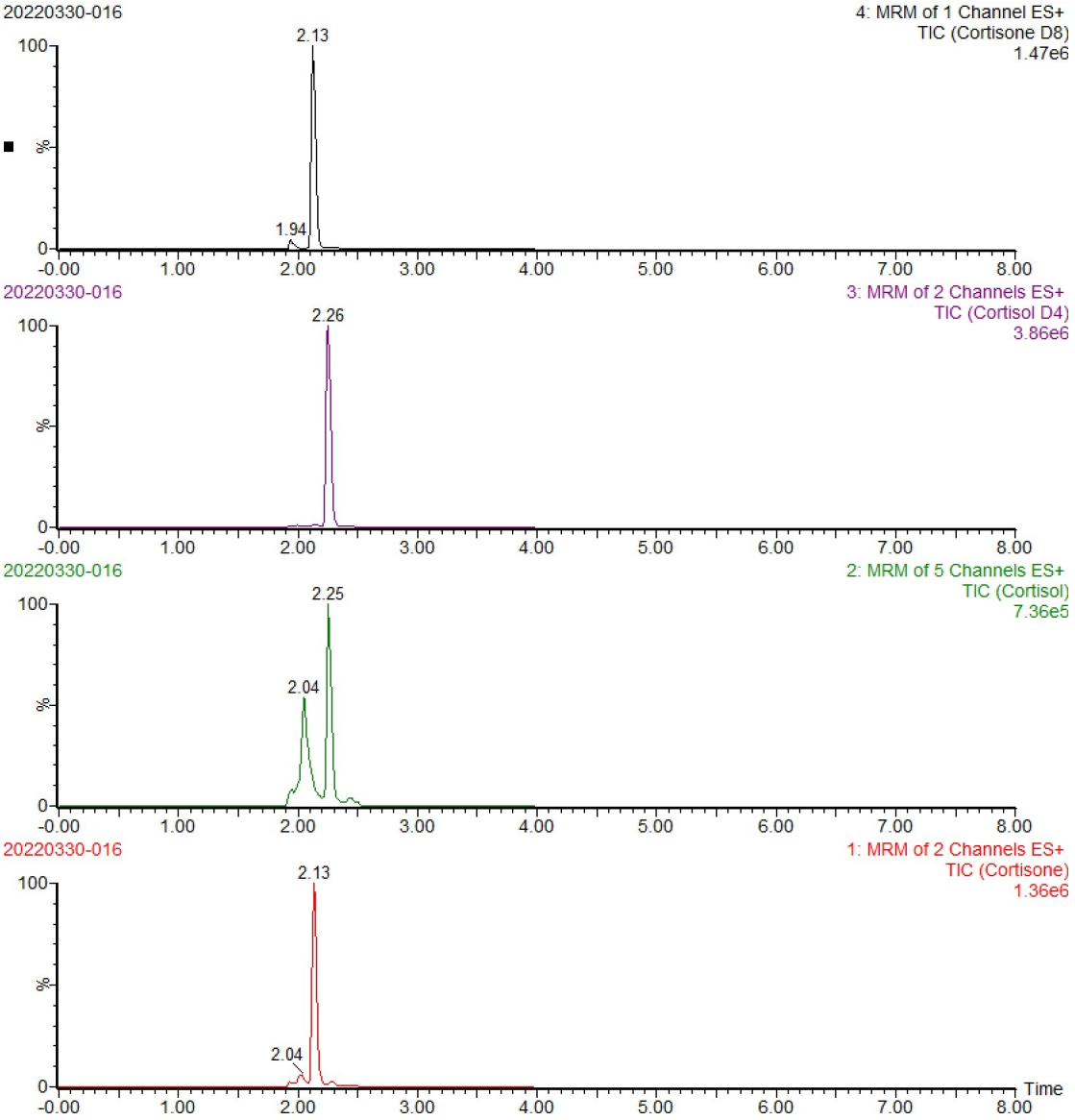
Into a 2 ml 96 well plate:

1. Centrifuge Calibrator, Control, Patient sample (5 min, 10000 x g or more).
2. Pipette 100 µl of the centrifuged supernatant into the plate.
3. Add 900 µl Cortisol Dilute & Shoot Dilution solution with Internal Standard D4/8 to each well.
4. Mix well and transfer the sample into a 96 well plate which is suitable for the auto sampler in use and Inject 10-20 µl in the LC-MS/MS.

4.5 Interpretation of results

4.5.1 Examples of chromatograms

Example chromatogram of a Patient sample, recorded with a Waters LC-MS/MS TQS-micro:



4.5.2 Results from LC-MS and Reference Values

The assay will result in a certain value for the measurand, which will need to be compared to applicable reference values to be interpreted for the specific patient.

For illustrative purposes only, an example of reference values for this device can be used as follows:

Reference ranges for urine (24h)

	nmol/l
Cortisol	5 - 133
Cortisone	74 - 328

The indicated reference ranges are taken from scientific literature[2]. It is recommended that each laboratory establishes its own reference ranges.

The inclusion of this information is required by Annex I, section 20.4.1 (v) of the IVDR. Diagnotix employs no medically trained professionals and can only indicate possible ways of interpreting results based on published literature. Always consult a trained medical professional with expertise in the area of interest for this kit for interpretation of results.

Interpretation of the results of this test also depends in a significant way on the individual characteristics of the patient involved. Diagnotix recommends taking these inputs into consideration as well.

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

5. Summary of Analytical Performance Characteristics

5.1 Repeatability (Simple Precision)

The repeatability, or simple precision, was analyzed by measuring a patient sample, Cortisol Dilute and shoot control I* and Cortisol Dilute and shoot control III* 22 times from one sample within two hours from each other. From these results the Coefficient of Variation (CV) is calculated and compared to the precision verification goal which in turn is calculated using the biological variation.

Sample	**Simple precision Cortisol (CV%)**	**Simple precision Cortisone (CV%)**
1193 M CODS Cortisol Dilute and shoot Control I *	2.0	1.7
1195 M CODS Cortisol Dilute and shoot Control III *	1.0	1.2
Patient sample Urine	1.4	0.9

5.2 Reproducibility (Complex Precision)

The Reproducibility, or Complex Precision, was analyzed by measuring a patient sample, Cortisol Dilute & Shoot control I* and Cortisol Dilute & Shoot control III* in duplicate 22 times. Each time the sample preparation had a variance (different analyst, pipet, day, reagent temperature and/or calibration). This to simulate twenty different days in a laboratory. From these results the Coefficient of Variation (CV) is calculated and compared to the precision verification goal which in turn is calculated using the biological variation.

Sample	**Complex precision Cortisol (CV%)**	**Complex precision Cortisone (CV%)**
1193 M CODS Cortisol Dilute and shoot Control I *	3.0	2.9
1195 M CODS Cortisol Dilute and shoot Control III *	2.5	2.6
Patient sample Urine	3.2	3.4

* At the time of validation, controls were named differently. There is no chemical difference between the controls mentioned here and at section 2.1, 2.2 and 2.3.

5.3 Linearity

The linearity was analyzed by preparing a series of incrementally increasing Cortisol and Cortisone concentrations twice. These samples were measured in duplicate from which the linearity was verified and upper limed of detection was calculated.

Analyte	**Linearity (nmol/l)**
Cortisol	1200
Cortisone	3100

5.4 Limit of Blank

The Limit of Blank (LOB) was analyzed by measuring Cortisol Dilute & Shoot Calibrator 1 (zero) * 20 times and Calibrator 2 (non-zero) 5 times. From the responses the LOB was calculated.

Analyte	**LOB (nmol/l)**
Cortisol	0
Cortisone	0.218

5.5 Carryover

To verify that there is no carryover two samples were prepared. One low (Calibrator 2)* and one high (Calibrator 6)*. The samples were divided into eleven low samples and ten high samples. The samples were measured in a particular order after which the datasets were analyzed.

Analyte	**Passes**
Cortisol	Yes
Cortisone	Yes

* At the time of validation, calibrators were named differently. There is no chemical difference between the calibrators mentioned here and at section 2.1, 2.2 and 2.3.

5.6 Accuracy

The accuracy of the method was determined by participating in the subscription schemes from the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML). This organization gathers results from all contributing laboratories and establishes a consensus or average. This in turn is compared to the results from Diagnotix.

All measured samples met the requirement of +/- 15%.

Analyte	**Accuracy**
Cortisol	Pass
Cortisone	Pass

6. Change log

<table>
<tr><th>Version Change</th><th>Changed section</th><th>What has been changed</th><th>Date</th></tr>
<tr><td>From V2.0 to 2.1</td><td>2.1.2
2.2</td><td>Product code internal standard has been changed.
Product formulation has not been changed, therefore there is no chemical change and the product performance remains the same.
<table>
<tr><th>Previous product code</th><th>New product code</th></tr>
<tr><td>1189 M CODS</td><td>1049 M CORT</td></tr>
</table></td><td>28-FEB-2025</td></tr>
<tr><td>From V1.1 to V2.0</td><td>2.1.1
2.2
2.3
5</td><td>Calibrators and controls product name and product code. At section 2.1.1, 2.2 and 2.3 product name and product code has been changed. At section 5 notes are added when precursor product name and codes been used.
Product formulation has not been changed, therefore there is no chemical change and the product performance remains the same.
<table>
<tr><th>Previous product code</th><th>New product code</th></tr>
<tr><td>1182 CON M CODS</td><td>1041 CON M CORT</td></tr>
<tr><td>1181 CAL M CODS</td><td>1042 CAL M CORT</td></tr>
<tr><td>1193 M CODS</td><td>1054 M CORT</td></tr>
</table></td><td>8-NOV-2024</td></tr>
</table>

		1194 M CODS	1055 M CORT	
		1195 M CODS	1056 M CORT	

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