Metanephrines in (EDTA) plasma

1060 M MET

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

diagnotix BV
De Plassen 4
9902 SE Appingedam
The Netherlands

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

Document version: 3.0
Replaces: 2.10
Date of release: 03-02-2021

CE 98/79/EC - IVD Medical Devices

Contents

Contents **2**

1. Introduction **4**

1.1 Intended Use *4*

1.2 Intended User *4*

1.3 Notice Regarding Serious Incidents *4*

1.4 IVD symbols *4*

1.2 Clinical background *5*

1.3 Description of the analytical procedure *6*

2. Components of the Metanephrines Kit **7**

2.1 Ordering information *7*

2.2 Safety information *8*

2.3 Storage conditions and lifetime of kit components *8*

2.3.1 Calibrators and controls 8

2.3.1.1 Handling 8

2.3.1.2 Stability and storage 9

2.3.2 Internal standard 9

2.3.2.1 Handling 9

2.3.2.2 Stability and storage 9

2.3.3 Mobile Phases 9

2.3.3.1 Handling 9

2.3.3.2 Stability and storage 9

2.3.4 Washing Solutions 9

2.3.4.1 Handling 10

2.3.4.2 Stability and storage 10

2.3.5 Elution solution 10

2.3.5.1 Handling 10

2.3.5.2 Stability and storage 10

2.3.6 Buffer 10

2.3.6.1 Handling 10

2.3.6.2 Stability and storage 10

2.3.7 Autosampler washing solution 10

2.3.7.1 Handling 10

2.3.7.2 Stability and storage 10

3. Required instruments **11**

3.1 Required LC Modules *11*

4. The analytical system **11**

4.1 Preparation of the analytical system *11*

4.2 Starting the analytical system *11*

4.3 LC-MS/MS Parameters and Conditions 11
4.3.1 LC Parameters 11
4.3.2 Autosampler Conditions 12
4.3.3 Gradient 12
4.3.4 MS Conditions (e.g. Waters Xevo TQS) 12

5. Sample 13

5.1 Sample material 13

5.2 Sample preparation 13
5.2.1 Reconstitution of the lyophilised Calibrators / Controls. 13

5.3 Examples of chromatograms 14

6. Reference Ranges 15

7. References 16

1. Introduction

1.1 Intended Use

This LC-MS/MS kit is intended for the determination of free Metanephrines, Normetanephrines and 3-methoxytyramine (3-MT) in (EDTA) plasma. The components in this 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

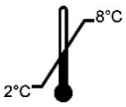
Order Number

Lot Number

For in vitro diagnostic use


See instructions for use


Manufacturer



Temperature limits

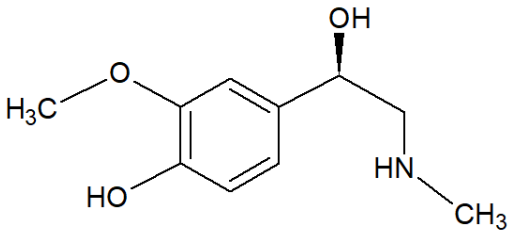
Contains sufficient for < n > tests


Expiry date

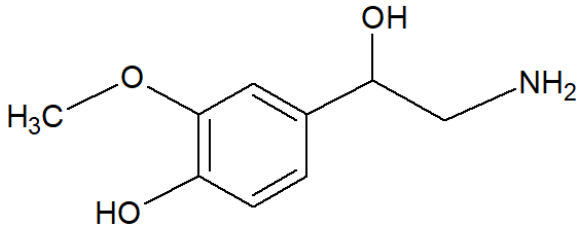
1.2 Clinical background

The Free Metanephrines test in (EDTA) plasma measures three metabolites of the catecholamines epinephrine, norepinephrine and dopamine. These catecholamines are made in the adrenal gland to regulate the heartbeat, blood pressure and glucose concentrations. They can also be released in excess by rare tumors, such as pheochromocytomas and paragangliomas, on adrenal and extra-adrenal chromaffin tissue. The majority of these tumors release epinephrine and norepinephrine. Some rare tumors however excrete mostly, or sometimes solely, dopamine.

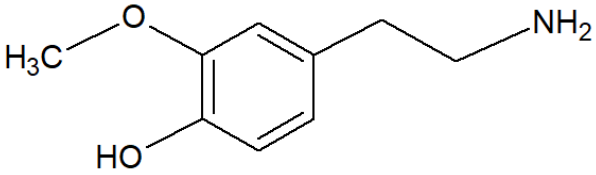
This is why all three the catecholamines are important to be determined when one of these tumors is suspected. There are however some difficulties with determining catecholamines, some tumors, for example, are biochemically silent or only periodically secrete these catecholamines making false negatives a possibility. False positives may also arise because of panic disorders or congestive heart failure. Their metabolites, metanephrine, normetanephrine and 3-methoxytyramine (3-MT) have superior diagnostic sensitivity and specificity compared to urinary and plasma catecholamines and so are recommended for the diagnosis of pheochromocytomas and paragangliomas.[1]



Metanephrine



Normetanephrine



3-methoxytyramine

[1] Weismann, D. et al (2015)

1.3 Description of the analytical procedure

Metanephrine, normetanephrine and 3-MT are determined from human (EDTA) plasma by UHPLC with positive ion electrospray LC-MS/MS.

Prior to the LC-MS/MS analysis a purification of the sample spiked with internal standard is performed with SPE-columns. Solid-phase extraction (SPE) is a method of sample preparation that concentrates and purifies analytes from solution by adsorption onto a disposable solid-phase cartridge, followed by elution of the analyte with a solvent appropriate for instrumental analysis.

After sample preparation, the sample is injected into a LC- MS/MS system and metanephrines, normetanephrines and 3-MT are ionized by electrospray ionization (ESI) and detected by MS/MS.

Measurement of the analytes is carried out in MRM (Multiple Reaction Monitoring) mode. In this mode only selected ions, known as 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.

The analytical method enables a quantitation in biological matrices by use of isotope-labelled internal standards.

Two MRM-transitions (quantifier, qualifier) are used for each analyte.

2. Components of the Metanephrines Kit

2.1 Ordering information

1060 KIT M MET - Complete Kit for Metanephrines in (EDTA) plasma

Contents (for 200 assays):

Metanephrines Calibrator Set (Calibrator 1 – 6)	1061 CAL M MET	6 x 2 x 2 ml
Metanephrines Internal Standard D3 D4	1069 M MET	8 x 6 ml
Metanephrines Mobile Phase I	1070 M MET	1 x 250 ml
Metanephrines Mobile Phase II	1071 M MET	2 x 500 ml
Metanephrines Washing Solution 1	1072 M MET	2 x 220 ml
Metanephrines Washing Solution 2	1073 M MET	2 x 220 ml
Metanephrines Washing Solution 3	1074 M MET	2 x 100ml
Metanephrines Elution Solution	1078 M MET	2 x 30 ml
Metanephrines Buffer	1079 M MET	2 x 60 ml
Metanephrines Autosampler Washing Solution	10601 M MET	1 x 1000 ml
Metanephrines Manual		

Separately available components:

Metanephrines Calibrator Set (Calibrator 1 – 6)	1061 CAL M MET	6 x 2 x 2 ml
Metanephrines Controls Set (control I – III)	1062 CON M MET	3 x 3 x 2 ml
Metanephrines Internal Standard D3 D4	1069 M MET	6 ml
Metanephrines Mobile Phase I	1070 M MET	250 ml
Metanephrines Mobile Phase II	1071 M MET	500 ml
Metanephrines Washing Solution 1	1072 M MET	220 ml
Metanephrines Washing Solution 2	1073 M MET	220 ml
Metanephrines Washing Solution 3	1074 M MET	100 ml
Metanephrines Elution Solution	1078 M MET	30 ml
Metanephrines Buffer	1079 M MET	60 ml

Metanephrines Autosampler Washing Solution	10601 M MET	1000 ml

Column Acquity UPLC BEH Amide 1.7µm 2.1 x 100mm	186004801	1 pcs
Metanephrines Control I	1075 M MET	10 x 2 ml
Metanephrines Control II	1076 M MET	10 x 2 ml
Metanephrines Control III	1077 M MET	10 x 2 ml
SPE Columns	186006342	2 x 100

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 plasma 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

1061 CAL M META | Metanephrines Calibrator Set
1062 CON M META | Metanephrines Control Set
1075 M META | Metanephrines Control I
1076 M META | Metanephrines Control II
1077 M META | Metanephrines 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 Metanephrines Calibrator Set and Controls with exactly 2,0 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 been dissolved, do not shake. Avoid foaming.
5. Let stand for another 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	2 weeks.
After reconstitution:	- 20 °C	1 month.

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

2.3.2 Internal standard

1069 M MET | Metanephrines Internal Standard D3 D4

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 four Metanephrines Internal Standard each with exactly 6 ml buffer (1079 M MET) using a volumetric pipette.
3. Swirl the vial carefully and mix thoroughly making sure that all traces of dry material have dissolved, do not shake. Avoid foaming.
4. Let stand for 15 minutes at room temperature.
5. Swirl the vial carefully, do not shake. Avoid foaming.
6. After reconstitution pour both vials in the buffer bottle used for the reconstitution.

2.3.2.2 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.

2.3.3 Mobile Phases

1070 M MET | Metanephrines Mobile Phase I
1071 M MET | Metanephrines Mobile Phase II

2.3.3.1 Handling

The Reagents are liquid and ready for use.

2.3.3.2 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 4 weeks on the UHPLC.

2.3.4 Washing Solutions

1072 M MET | Metanephrines Washing Solution 1
1073 M MET | Metanephrines Washing Solution 2
1074 M MET | Metanephrines Washing Solution 3

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 Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C.

2.3.5 Elution solution

1078 M MET | Metanephrines Elution 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 6 weeks if closed and stored at 2 - 8 °C.

2.3.6 Buffer

1079 M MET | Metanephrines Buffer

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 6 weeks if closed and stored at 2 - 8 °C.

2.3.7 Autosampler washing solution

10601 M MET | Methanephrines 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 6 weeks if closed and stored at 2 - 8 °C or 4 weeks on the UHPLC.

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

- Prime the LC system with Mobile Phases I and II excluding the column.
  Prime the autosampler with the Metanephrines Autosampler washing solution.
- 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 30 °C.
- Equilibrate the column for 15 minutes with Mobile Phase II.

Start the program for the gradient and equilibrate for another 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-MS/MS Parameters and Conditions

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 30 °C for the complete UHPLC system the back pressure should not exceed 800 bar. 1 bar = 14.5 PSI

4.3.2 Autosampler Conditions

Needle volume:	30 µL
Injection volume:	5-20 µL
Sample syringe volume:	250 µL
Sample temperature:	10 °C
Runtime:	4.0 min
Column temperature:	30 °C ± 2 °C alarm
Needle wash:	15 seconds
Seal Wash:	90:10 H2O:ACN
Wash Solvent:	Metanephrines Autosampler washing solution

Or: the injection needle has to be flushed after sampling (minimising sample carryover). For this purpose, please use the settings recommended by the manufacturer of the auto sampler in use.

4.3.3 Gradient

Time (min)	**Flow Rate (mL/min)**	**%A**	**%B**	**Curve**
0.00	0.40	0	100	Initial
0.75	0.40	0	100	6
1.50	0.40	0	100	6
3.00	0.40	20	80	1
4.00	0.40	0	100	1

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)

The mass transitions are an indication, the optima can differ vary slightly between different LC-MS/MS systems.

Analyte/IS	Quantifier MRM		Qualifier MRM	
	precursor	product	precursor	product
3-MT	151.2	91	151.2	119
Metanephrine	180	148	180	165
Normetanephrine	165.9	134.0	165.9	106.0
d4-3-MT	154.9	95	154.9	123
d3-Metanephrine	183	151	183	168
d3-Normetanephrine	168.9	137	168.9	109

Analyte	Rt(min)	Internal standard (IS)	Rt(min)
3-MT	1.69	d4-3-MT	1.69
Metanephrine	1.86	d3-Metanephrine	1.86
Normetanephrine	2.48	d3-Metanephrine	2.48

5. Sample

5.1 Sample material

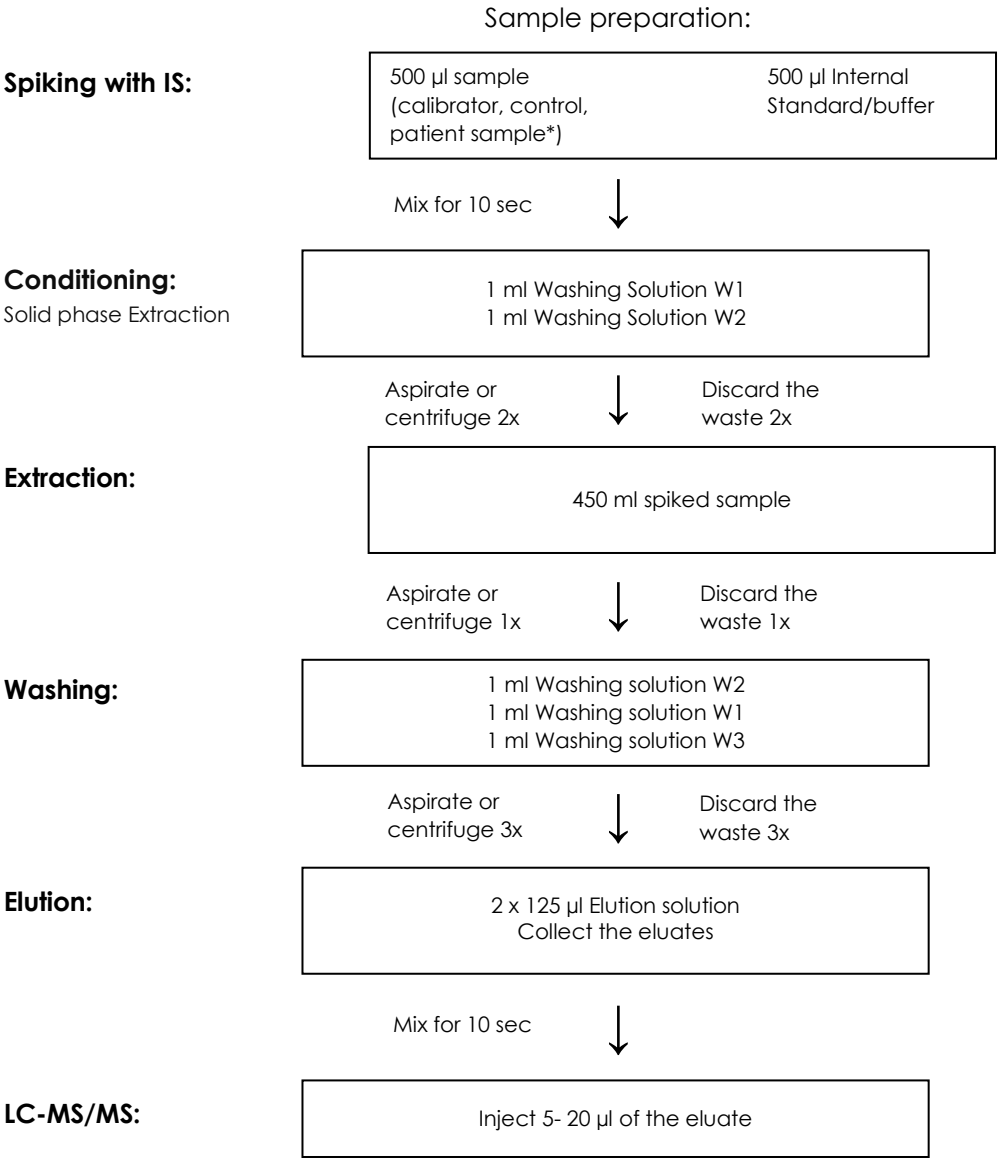
Use blood plasma (EDTA-tubes) after sample collection directly centrifuge and separate the plasma and blood cells.

Plasma samples can be stored: 3 months (- 20 °C)
Avoid freeze-thaw cycles.

5.2 Sample preparation

5.2.1 Reconstitution of the lyophilised Calibrators / Controls.

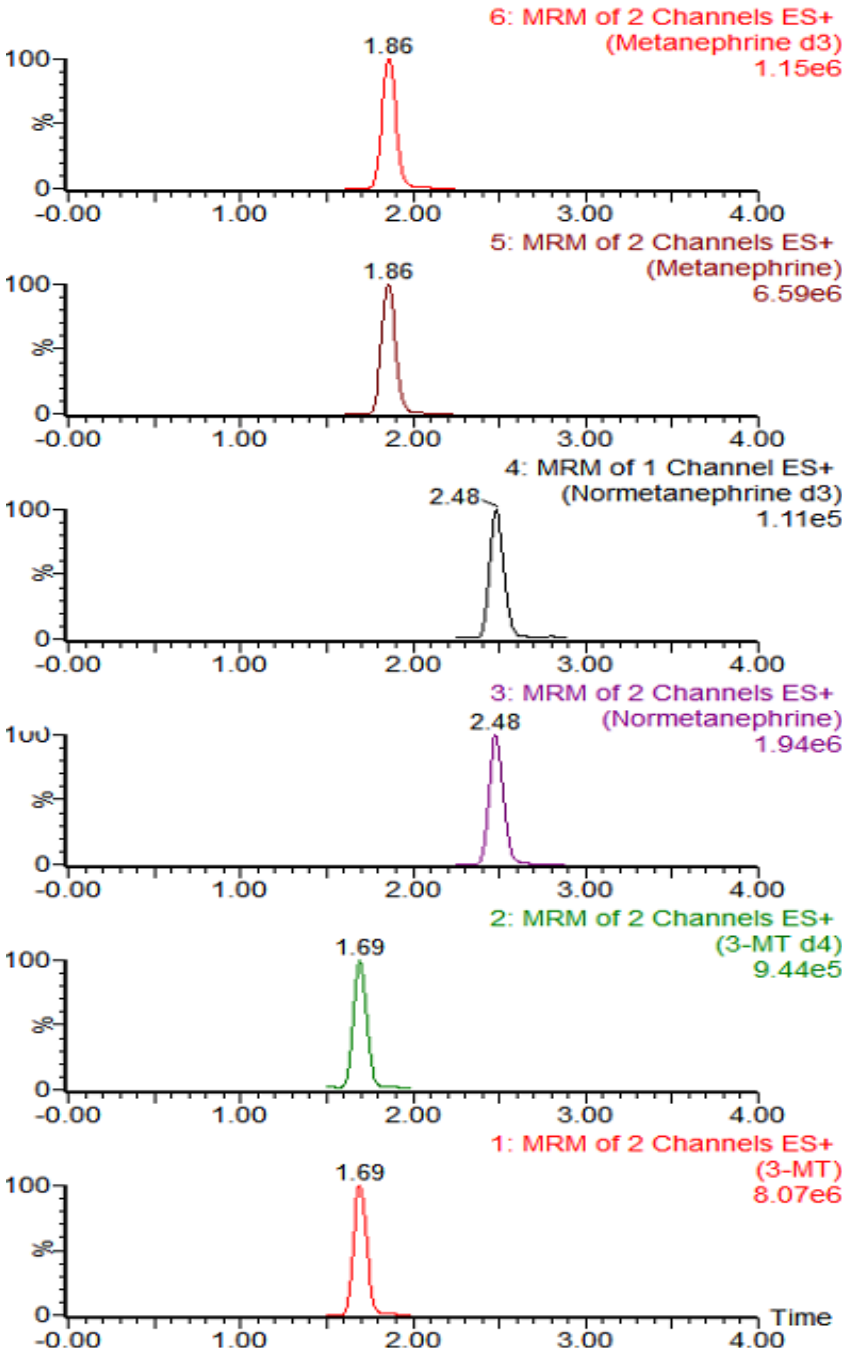
See 2.3.1.1 and the product data sheets



*** Note that patient samples need to be centrifuged (10 minutes at 3000 g) prior to spiking with internal standard.**

5.3 Examples of chromatograms

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



6. Reference Ranges

	nmol/l
metanephrine	0.07 - 0.33
normetanephrine	0.23 - 1.07
3-MT	< 0.17

The indicated reference ranges are taken from scientific literature and were determined from healthy individuals. The blood was drawn in sitting position.[2]

It is recommended that each laboratory establishes its own reference ranges.

[2] De Jong, W.H.A., et al

7. References

1. Weismann D, et al. (2015). Measurements of plasma metanephrines by immunoassay vs liquid chromatography with tandem mass spectrometry for diagnosis of pheochromocytoma. *European Journal of Endocrinology, 172(3)*, 251-260.

2. De Jong, W.H.A., et al (2007). Plasma Free Metanephrine Measurement Using Automated Online Solid-Phase Extraction HPLC–Tandem Mass Spectrometry. *Clinical Chemistry, 53(9)*, 1684-1693.