Vitamin B1 & B6 in whole blood

1020 M VB1B6

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

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CE 98/79/EC - IVD Medical Devices

Contents

1. Introduction

1.1 Intended Use

This LC-MS/MS kit is intended for the determination of Vitamin B1 & B6 in whole blood.

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

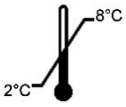
Order Number

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

Water-soluble B Vitamins are important cofactors in cell metabolism. Two water-soluble vitamins with clinical relevance are Vitamins B1 and B6. Thiamine Diphosphate (TDP) is the biologically active form of Vitamin B1 and is required for various metabolic functions. Prolonged deficiency can cause Beriberi, a debilitating neurological disease.[1] Pyridoxal 5-phosphate (PLP) is the biologically active form of Vitamin B6 and is a coenzyme for a number of transamination reactions. It plays critical roles in chronic disease and pro-inflammatory response.[2] Additionally, both Vitamin B6 and B1 have also been linked to increased survival rates in the elderly.[3]

Thiamine deficiency may give rise to manifestations in the cardiovascular and nervous systems. There are two forms; dry Beriberi, which consists of sensorimotor neuropathy and Wernicke-Korsakoff syndrome, and wet Beriberi, which consists of edema and cognitive heart failure, but little CNS manifestations. The clinical picture of Wernicke's encephalopathy, with or without Korsakoff syndrome, is frequently encountered in alcoholics, with predominant oculomotor and cerebellar symptomatology, although it can also be seen in other conditions, such as hyperemesis, dialyses, and post-gastrointestinal surgery.

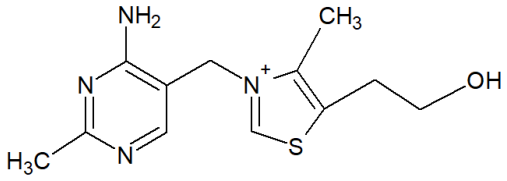
Pyridoxine deficiency on a nutritional basis has been recognized as a rare cause of serve and even fatal convulsions in neonates and infants. Pyridoxine dependency develops during fetal life as a genetic disorder and causes both intrauterine and postnatal seizures.

Neurologic disorders reflecting both pyridoxine deficiency and pyridoxine toxicity have been recognized. Both overdose and deficiency may cause peripheral neuropathy. Pyridoxine deficiency causes injury of motor and sensory axons, whereas an overdose of pyridoxine causes a pure sensory neuropathy or neuronopathy with sensory ataxia.

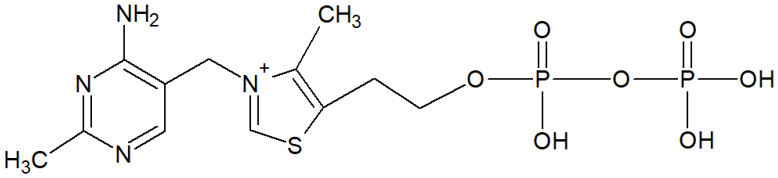
Mass spectrometry based methods have been tested for the determination of Vitamin B1 & B6. Furthermore chromatographic separation for both of the vitamins is critical and not easy. This method can be used for the routine analysis of Vitamin B1 & B6 in whole blood. Sample preparation is simple and rapid and analogous for the different biological matrices. A six-point lyophilized whole blood calibrator at clinically relevant levels has been added to the kit. Lyophilized blood controls are also available for quality assurance.

Two isotope-labelled internal standard Pyridoxal-5'-phosphate (methyl D3) and Vitamin B1 pyrophosphate (methyl D3) are added to compensate for matrix effects and measurement variations. Samples are analyzed using positive ion electrospray in MRM mode for maximum sensitivity and selectivity.

[1] Stanley, N. N. "Cardiac Beriberi: Two Modes of Presentation." BMJ: 567-569.
[2] Huang, et al. "Vitamin B6 Supplementation Improves Pro-inflammatory Responses in Patients with Rheumatoid Arthritis." European Journal of Clinical Nutrition (2010): 1007-013
[3] Huang, et al. "Prediction of All-cause Mortality by B Group Vitamin Status in the Elderly." Clinical Nutrition (2011): 191-98.

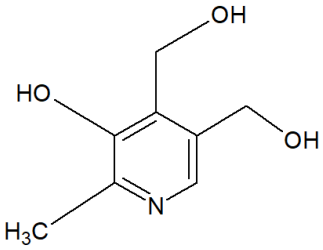


Thiamine

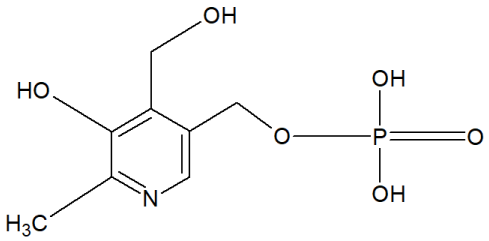


Thimaine pyrophosphate (TPP)
(Thiamine diphosphate)

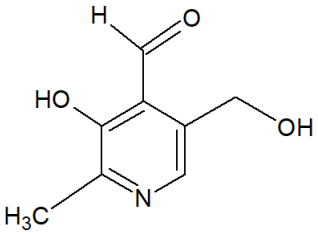
Vitamin B1 (above)



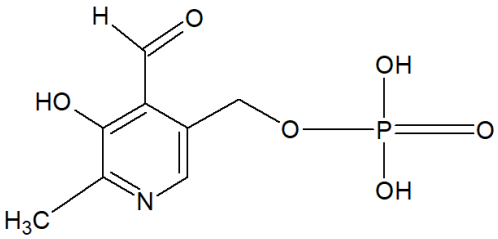
Pyridoxine



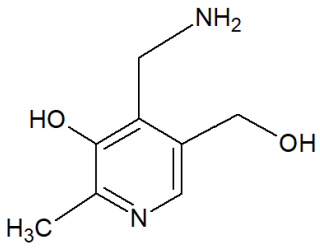
Pyridoxine-5'-phosphate



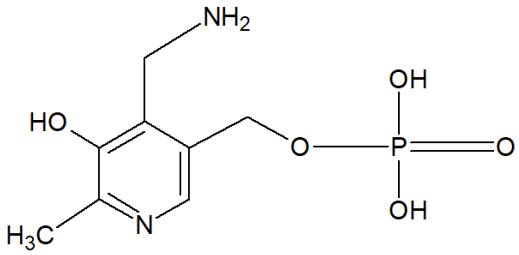
Pyridoxal



Pyridoxal-5'-phosphate (PLP) 



Pyrodoxamine



Pyridoxamine-5'-phosphate

Vitamin B6 group (above)

1.3 Description of the analytical procedure

Vitamin B1 & B6 are determined from human whole blood 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, Vitamin B1 and Vitamin B6 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.

2. Components of the Vitamin B1 & B6 Kit

2.1 Ordering information

1020 KIT M VB1B6 - Complete Kit for Vitamin B1& B6 in whole blood

Contents (for 300 assays):

Vitamin B1 & B6 Calibrator Set (Calibrator 1 – 6)	1022 CAL M VB1B6	6 x 2 x 500 µl
Vitamin B1 & B6 Internal Standard D3	1029 M VB1B6	3 x 6 ml
Vitamin B1 & B6 Deproteinization Solution	1030 M VB1B6	3 x 46 ml
Vitamin B1 & B6 Mobile Phase I	1031 M VB1B6	1 x 500 ml
Vitamin B1 & B6 Mobile Phase II	1032 M VB1B6	1 x 250 ml
Vitamin B1 & B6 Autosampler washing solution	10201 M VB1B6	1 x 1000 ml
Vitamin B1 & B6 Manual		

Separately available components:

Vitamin B1 & B6 Calibrator Set (Calibrator 1 – 6)	1022 CAL M VB1B6	6 x 2 x 500 µl
Vitamin B1 & B6 Internal standard D3	1029 M VB1B6	1 x 6 ml
Vitamin B1 & B6 Deproteinization Solution	1030 M VB1B6	1 x 46 ml
Vitamin B1 & B6 Mobile Phase I	1031 M VB1B6	1 x 500 ml
Vitamin B1 & B6 Mobile Phase II	1032 M VB1B6	1 x 250 ml
Vitamin B1 & B6 Autosampler washing solution	10201 M VB1B6	1 x 1000 ml

Analytical column Xbridge BEH C18 XP Column 2.5 µm, 3mm x 75 mm	186006034	1 pc
Vitamin B1 & B6 Control I	1033 M VB1B6	10 x 500 µl
Vitamin B1 & B6 Control II	1034 M VB1B6	10 x 500 µl
Vitamin B1 & B6 Control III	1035 M VB1B6	10 x 500 µl
Vitamin B1 & B6 Control Set	1021 CON M VB1B6	3 x 3 x 500 µl

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

1022 CAL M VB1B6 | Vitamin B1 & B6 Calibrator Set
1021 CON M VB1B6 | Vitamin B1 & B6 Control Set
1033 M VB1B6 | Vitamin B1 & B6 Control I
1034 M VB1B6 | Vitamin B1 & B6 Control II
1035 M VB1B6 | Vitamin B1 & B6 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 Vitamin B1 & B6 Calibrator Set and Controls with exactly 500 µl 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	48 hours
After reconstitution:	- 20 °C	2 weeks

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

2.3.2 Internal standard

1029 M VB1B6 | Vitamin B1 & B6 Internal Standard D3

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 Vitamin B1 & B6 Internal Standard D3 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	48 hours
After reconstitution:	- 20 °C	1 week

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

2.3.3 Deproteinization Solution

1030 M VB1B6 | Vitamin B1 & B6 Deproteinization 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 Deproteinization Solution is:

Store at 2 - 8 °C	After first opening the Reagent can be used for 3 weeks if closed and stored at 2 - 8 °C

2.3.4 Mobile Phases

1031 M VB1B6 | Vitamin B1 & B6 Mobile Phase I
1032 M VB1B6 | Vitamin B1 & B6 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 Reagent can be used for 6 weeks if closed and stored at 2 - 8 °C or 2 weeks on the UHPLC

2.3.5 Autosampler washing solution

10201 M VB1B6 | Vitamin B1 & B6 Autosampler 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 6 weeks if closed and stored at 2 - 8 °C or 2 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

- 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 0.6 ml/min.
- Set the column heater to 30°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 Starting the analytical system

- Equilibrate the system.
- Check the temperature of the column.
- Initialize the injector.
- Start the programme on the LCMSMS system.

4.3 LC-MS/MS Parameters and Conditions

4.3.1 LC Parameters

UHPLC pump	Flow rate 0.6 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 backpressure should not exceed 800 bar. 1 bar = 14.5 PSI

4.3.2 Autosampler Conditions

Injection volume:	10-20 µL
Sample temperature:	10 °C
Runtime:	2.5 min
Column temperature:	30 °C ± 2 °C alarm
Needle wash:	wash twice for 6 seconds
Seal Wash:	10:90 ACN:H2O
Wash Solvent:	Autosampler Washing Solution; 90:10 H2O:MeOH

4.3.3 Gradient

Time (min)	**Flow Rate (mL/min)**	**%A**	**%B**	**Curve**
0.00	0.60	95	5	Initial
0.60	0.60	70	30	6
1.20	0.80	3	97	11
1.70	0.80	95	5	11
1.90	0.60	95	5	11

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)
Ion mode:	Electrospray
Capillary voltage:	1.0 kV
Polarity:	positive
Source temperature:	150 °C
Desolvation temperature:	600°C
Desolvation gas flow:	1000L/hr
Detection mode:	MRM
Dwell time:	0.019 sec
Collision gas:	Argon / Nitrogen

Substance	**Precursor**	**Product**
Vitamin B6	248.00	94.00
Vitamin B6	248.00	150.00
Vitamin B6 D3	251.00	153.00

Substance	**Precursor**	**Product**
Vitamin B1	425.20	122.45
Vitamin B1	425.02	304.00
Vitamin B1 D3	428.20	125.45

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

5. Sample

5.1 Sample material

Use whole blood (EDTA- and Heparin-tubes)
Samples can be stored: 1 month (- 20°C)

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 (whole blood, calibrator or control)

1. 50 µl Vitamin B1 & B6 Internal Standard D3.
2. Add 50 µl sample (Calibrator, Control, Patient sample).
3. Mix immediately and add 400 µl Vitamin B1 & B6 Deproteinization Solution in the tubes using a vortex mixer for 30 seconds.
4. After mixing on a vortex set the tube immediately in a shaker for another 30 minutes.
5. Make sure that all the tubes have been shaken for at least 30 minutes.
6. Centrifuge (5 min, 10000 x g or more).
7. Use 200 µl centrifuged supernatant to a vial or 96 well plate, which is suitable for the auto sampler in use and Inject 10-20 µl in the LC-MS/MS.

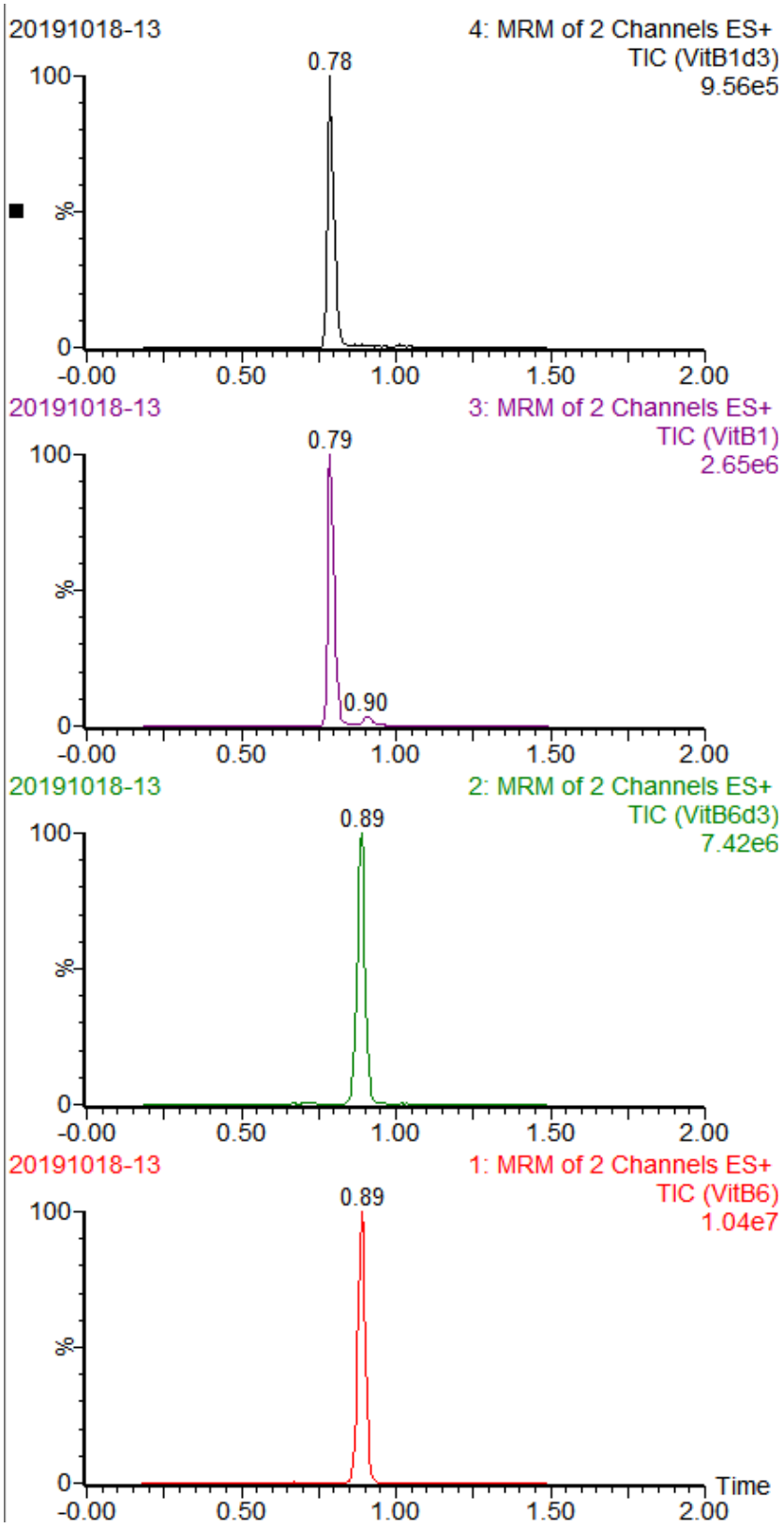
5.2.3 Sample Preparation with pipette robot

Into a 2 ml 96 well plate:

1. 50 µl Vitamin B1 & B6 Internal Standard D3.
2. Whilst mixing the plate, add 50 µl sample (Calibrator, Control, Patient sample), and leave mixing for 15 minutes.
3. Whilst continuing to mix the plate, add 400 µl Vitamin B1 & B6 Deproteinization Solution and leave mixing for at least 30 minutes.
4. Once mixing is complete, centrifuge (5 min , 10000x g or more).
5. Transfer the samples into a 1 ml 96 well collection plate for injection on the UHPLC/MS/MS system (Needle placement 2 mm) or inject directly off the pellet (Needle placement 10 mm) 10-20 µl in the LC-MS/MS.

5.3 Examples of chromatograms

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



6. Test data (Validation report)

6.1 Linearity

	nmol/l
Vitamin B1	8000
Vitamin B6	3000

6.2 Limit of quantification

	nmol/l
Vitamin B1	9.4
Vitamin B6	8.0

6.3 Repeatability

Vitamin B1

Item	**Measured value (nmol/l)**	**Standard Deviation (nmol/l)**	**CV (%)**	**N**
Level I	41.1	1.0	2.4	20
Level III	212.1	4.0	1.9	20
Patient material	147.5	2.9	1.9	20

Vitamin B6

Item	**Measured value (nmol/l)**	**Standard Deviation (nmol/l)**	**CV (%)**	**N**
Level I	22.5	0.5	2.4	20
Level III	122.7	2.5	2.1	20
Patient material	90.9	2.0	2.2	20

6.4 Reference Ranges

	nmol/l
Vitamin B1	60 – 120 [4]
Vitamin B6	35 – 110 [5]

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

[4] Hooijkaas, et al. "Handboek medische laboratoriumdiagnostiek" (2013): 820 - 821
[5] Hooijkaas, et al. "Handboek medische laboratoriumdiagnostiek" (2013): 825

7. References

1. Stanley, N. N. "Cardiac Beriberi: Two Modes of Presentation." BMJ: 567-569.
2. Huang, et al. "Vitamin B6 Supplementation Improves Pro-inflammatory Responses in Patients with Rheumatoid Arthritis." European Journal of Clinical Nutrition (2010): 1007-013.
3. Huang, et al. "Prediction of All-cause Mortality by B Group Vitamin Status in the Elderly." Clinical Nutrition (2011): 191-98.
4. Hooijkaas, et al. "Handboek medische laboratoriumdiagnostiek" (2013): 820 - 821.
5. Hooijkaas, et al. "Handboek medische laboratoriumdiagnostiek" (2013): 825.