



# +RECIPE

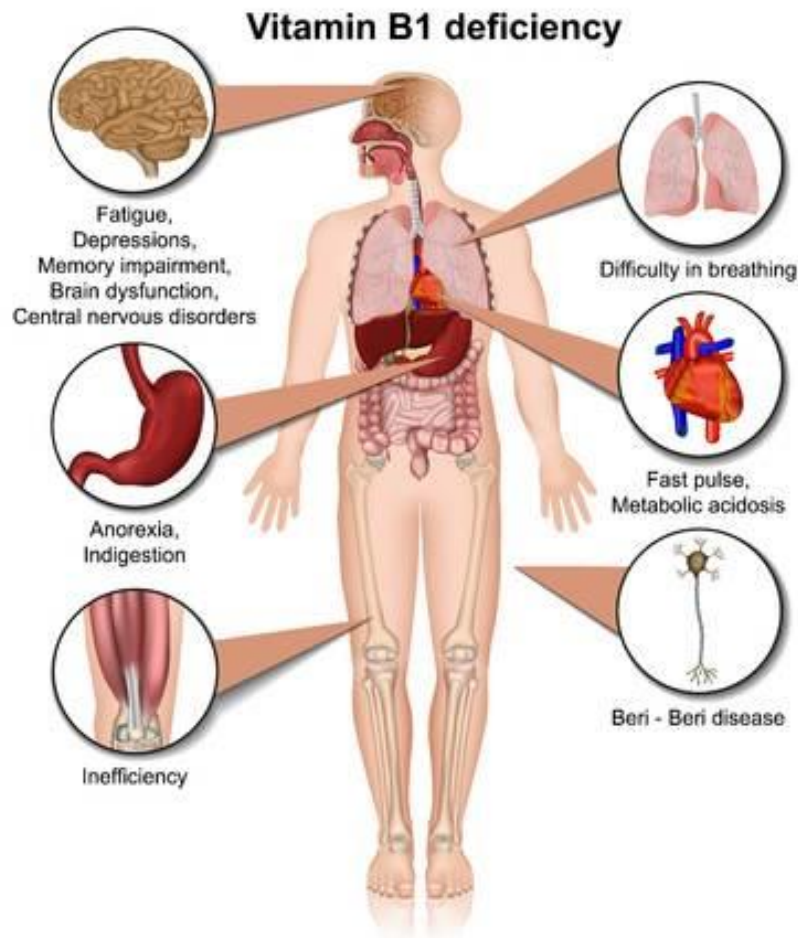
## Simultaneous Determination of Vitamins B1/B2/B6 in Whole Blood by LC MS/MS



Klaus Martin Knirsch

VitaTox Conference 9.September 2020

# Clinical Background Vitamin B1



- Vitamin B1 (Thiamine) is an essential vitamin required for carbohydrate metabolism, brain function, and peripheral nerve myelination.
- Thiamine is obtained from the diet. Body stores are limited and deficiencies can develop quickly.
- Approximately 80% of all chronic alcoholics are thiamine deficient due to poor nutrition.
- Deficiency also can occur in individuals who are elderly, have chronic gastrointestinal problems, have marked anorexia, are on cancer treatment, or are receiving diuretic therapy.
- Severe deficiency causes congestive heart failure (wet beriberi), peripheral neuropathy (dry beriberi), Wernicke encephalopathy (a medical emergency that can progress to coma and death), and Korsakoff syndrome
- Thiamine deficiency is a treatable. It appears that no conditions are directly attributable to thiamine excess
- Whole blood thiamine testing (with thiamine pyrophosphate (TPP) as the active form) is superior to currently available alternative tests for assessing thiamine status. Serum or plasma thiamine testing suffers from poor sensitivity and specificity, and less than 10% of blood thiamine is contained in plasma

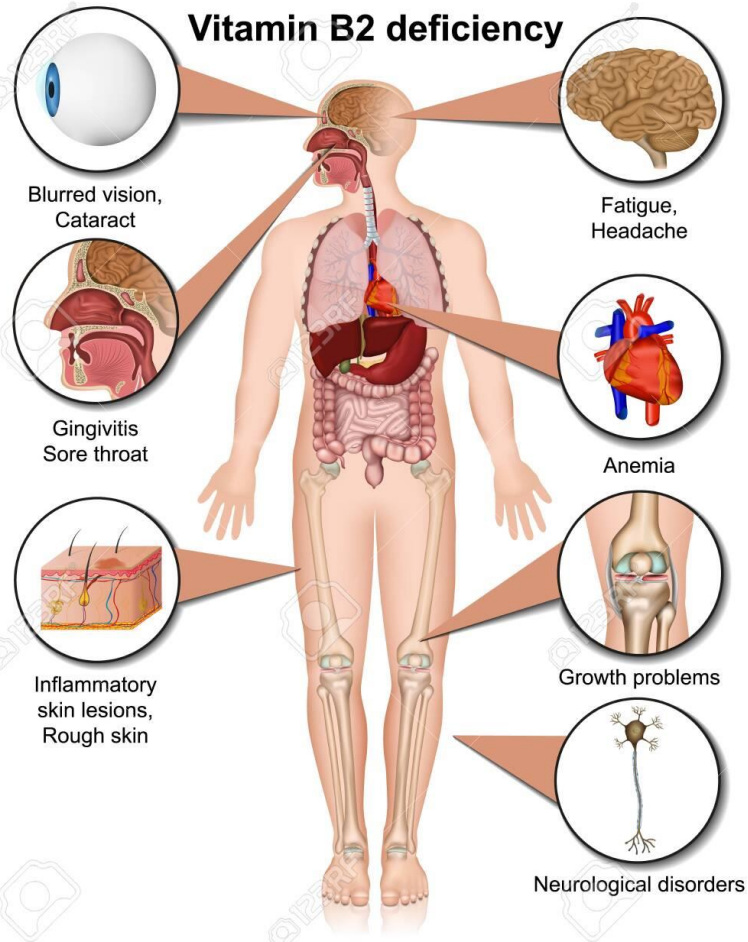
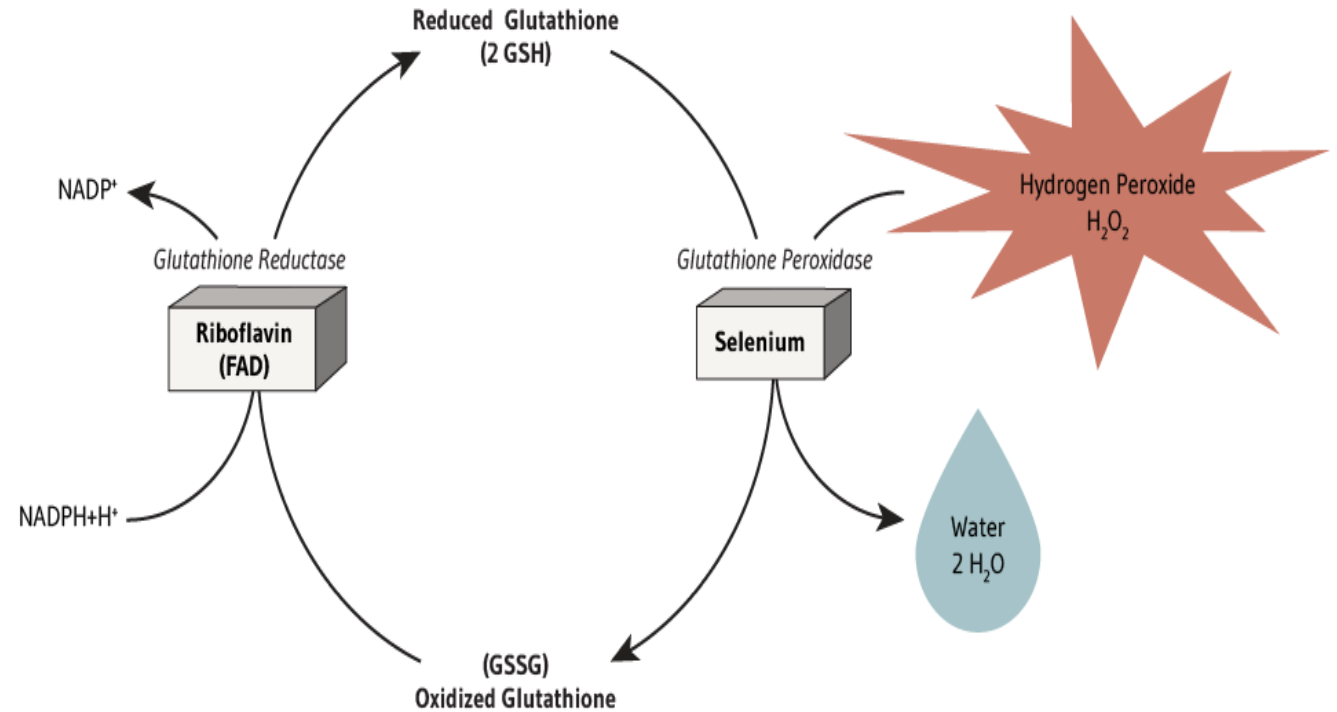


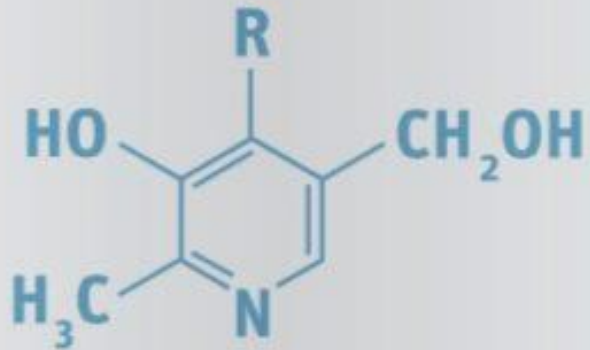
Figure 1. Glutathione Oxidation Reduction (Redox) Cycle



One molecule of hydrogen peroxide is reduced to two molecules of water, while two molecules of glutathione (GSH) are oxidized in a reaction catalyzed by the selenoenzyme, glutathione peroxidase. Oxidized glutathione (GSSG) may be reduced by the flavin adenine dinucleotide (FAD) dependent enzyme, glutathione reductase.

# Clinical Background Vitamin B6

+RECIPE



R = CH<sub>2</sub> OH = Pyridoxine

R = CHO = Pyridoxal

R = CH<sub>2</sub> NH<sub>2</sub> = Pyridoxamine

- There are three different natural forms of vitamin B6, namely pyridoxine, pyridoxamine, and pyridoxal (PL)
- For human metabolism, the active derivative of the vitamin, pyridoxal 5`-phosphate (PLP), is of major importance as the metabolically active coenzyme form
- Vitamin B6 in coenzyme forms performs a wide variety of functions in the body
  - PLP is involved in amino acid metabolism, carbohydrates, and lipids
  - Vitamin B6 also plays a role in cognitive development through the biosynthesis of neurotransmitters and in maintaining normal levels of homocysteine
  - Vitamin B6 is involved in gluconeogenesis and glycogenolysis, immune function and hemoglobin formation
- Vitamin B6 deficiency as well as an excess of this vitamin can cause neurological symptoms
- In healthy people, either plasma or red cell concentrations of PLP can be used as markers of vitamin B6 status, because they are highly correlated. However, in critically ill patients a shift of PLP from plasma to red cells has been observed, implying that whole blood may be a more reliable matrix
- Because Vitamin B1 has to be analysed in Whole Blood the clinically useful combined analysis with Vitamin B6 has to be performed in Whole blood as well

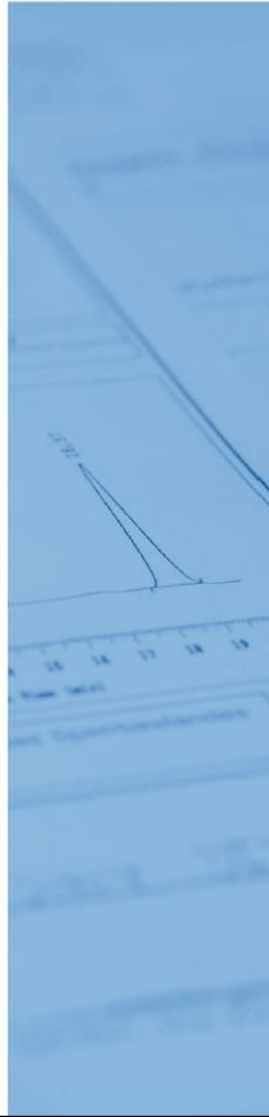
Instruction Manual

ClinMass® LC-MS/MS Complete Kit

**Vitamins B1, B2, B6  
in Whole Blood**

**REF** MS13000  
**IVD** For in vitro diagnostic use

**CE** IVDD, 98/79/EC



LC Parameters:

1 Binary Pump

Injection Volumen: 5-50 µL

Injection Interval: 4 Min (B1/B2/B6); 2.5 Min (B1/B6)

Sample Prep.

**Option 1: Sample prep vitamins B<sub>1</sub>, B<sub>2</sub> and B<sub>6</sub>:**

Precipitation/  
Dotation with IS:

100 µl Precipitant P	50 µl sample (calibrator, control, patient)	20 µl IS (solved in water)
----------------------	---	-------------------------------

mix immediately for 45 sec ↓ (vortex mixer)

centrifuge ↓ (10 min, ≥10000 x g)

LC-MS/MS Analysis:

Inject 5–50 µl of the supernatant
-----------------------------------

**Option 2: Sample prep vitamins B<sub>1</sub> and B<sub>6</sub>:**

Precipitation/  
Dotation with IS:

100 µl IS (solved in Precipitant P)	50 µl sample (calibrator, control, patient)
-------------------------------------	--

mix immediately for 45 sec ↓ (vortex mixer)

centrifuge ↓ (10 min, ≥10000 x g)

LC-MS/MS Analysis:

Inject 5–50 µl of the supernatant
-----------------------------------

# Chromatogramm

## Instruments verified:



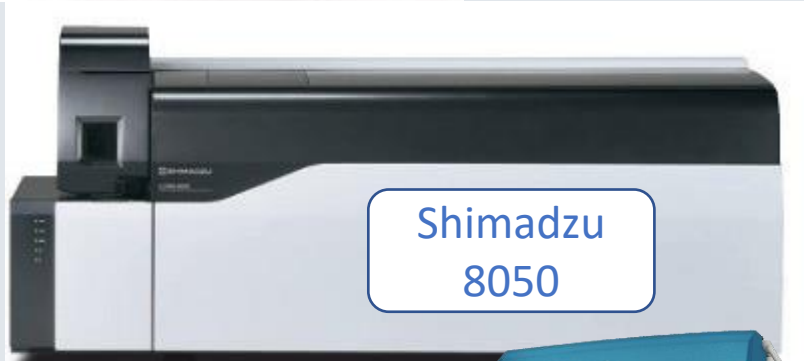
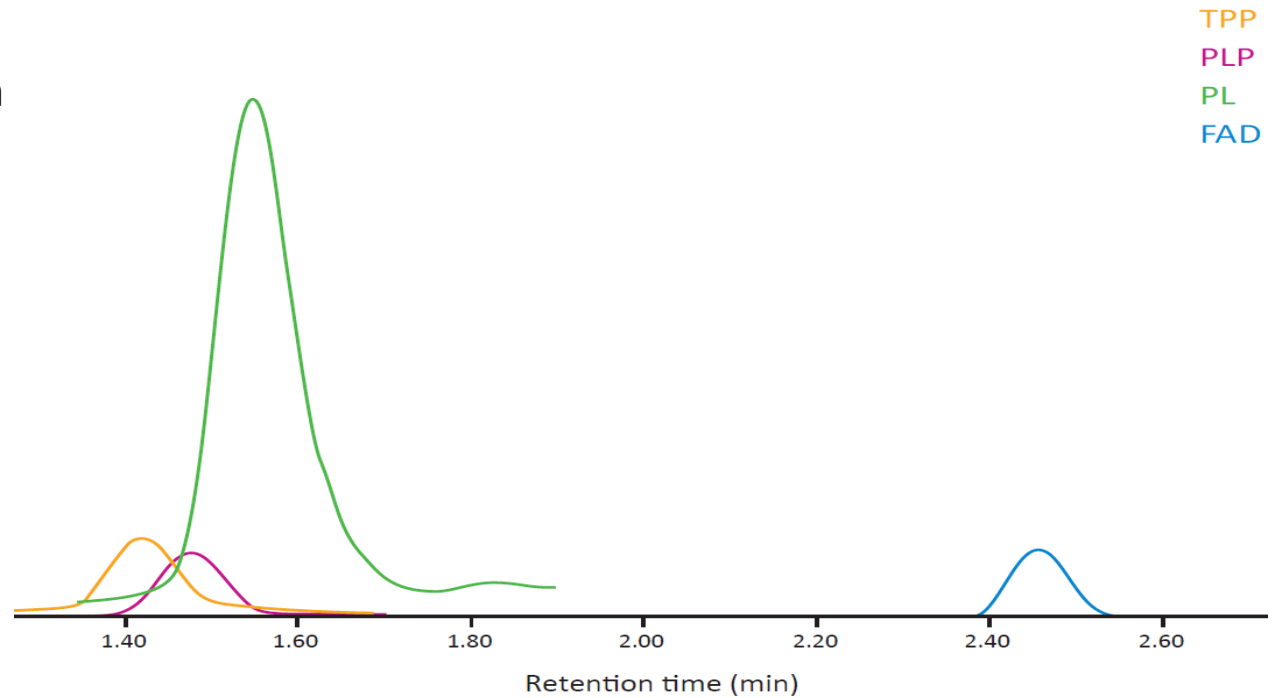
Waters Xevo TQ-S

Analytes and IS:

4 isotope labeled internal standards

Analyte	RT [min]	Internal Standard IS	RT [min]
Thiaminepyrophosphate (TPP)	1.43	d <sub>3</sub> -Thiaminepyrophosphate	1.43
Flavinadeninedinucleotide (FAD)	2.46	<sup>13</sup> C <sub>5</sub> -Flavinadeninedinucleotide	2.46
Pyridoxal (PL)	1.55	d <sub>3</sub> -Pyridoxal	1.54
Pyridoxalphosphate (PLP)	1.47	d <sub>3</sub> -Pyridoxalphosphate	1.47

Chromatogramm



Shimadzu 8050



Sciex API 4500+ 5500

Table 5. Mass transitions of the analytes and internal standard compounds

Analyte / IS	Quantifier MRM		Qualifier MRM	
	Precursor [m/z]	Product [m/z]	Precursor [m/z]	Product [m/z]
Thiaminepyrophosphate (TPP)	425.0	122.1	425.0	304.0
Flavinadeninedinucleotide (FAD)	786.2	348.1	786.2	439.1
Pyridoxal (PL)	168.1	150.1	168.1	94.1
Pyridoxalphosphate (PLP)	248.0	150.1	248.0	94.1
d <sub>3</sub> -Thiaminepyrophosphate	428.0	125.1		
<sup>13</sup> C <sub>5</sub> -Flavinadeninedinucleotide	791.2	353.1		
d <sub>3</sub> -Pyridoxal	171.1	153.1		
d <sub>3</sub> -Pyridoxalphosphate	251.0	153.1		

Table 6. Assignment of the analytes to the isotope-labelled substances in the IS

Analyte	RT [min]	Internal Standard IS	RT [min]
Thiaminepyrophosphate (TPP)	1.43	d <sub>3</sub> -Thiaminepyrophosphate	1.43
Flavinadeninedinucleotide (FAD)	2.46	<sup>13</sup> C <sub>5</sub> -Flavinadeninedinucleotide	2.46
Pyridoxal (PL)	1.55	d <sub>3</sub> -Pyridoxal	1.54
Pyridoxalphosphate (PLP)	1.47	d <sub>3</sub> -Pyridoxalphosphate	1.47

- Linearity: TPP: 5.74 – 300 µg/l  
FAD: 8.11 – 1200 µg/l  
PLP: 4.56 - 200 µg/l
- Precision (Intraassay): 5.8% (FAD) to 8.1% (PLP)  
(Interassay): 6.7% (FAD)to 14.0% (PLP)
- Reference ranges:

Analyte	Reference ranges	
	[µg/l]	[nmol/l]
Thiaminepyrophosphate (TPP) [8]	28–85	66.5–200
Flavinadeninedinucleotide (FAD) [9]	188.5–306.4*	240–390*
Pyridoxalphosphate (PLP) [10]	8.7–27.2	35–110

\*2.5–97.5 % Percentiles



➤ ClinCal® Calibrator: Whole Blood Calibrators Set (lyophilized):

- Level 0-3



➤ ClinChek® Controls: Whole Blood Controls (lyophilized):

- Level I & II



➤ Stability:

1 d at 15-30 °C  
7 d at 2- 8 °C  
90d at -18 °C

➤ Available for all LDT's based on LC MS/MS including Opti-Mix and internal standards



Thank you for attention!

