Vitamin B6 enzymatic

Procedure

KK-VB6

A Commitment to Diagnostics

Intended Use

The Vitamin B6 enzymatic assay (KK-VB6) is intended for the quantitative determination of Pyridoxal 5'-Phosphate (PLP, vitamin B6) in EDTA plasma. The BÜHLMANN Vitamin B6 enzymatic assay allows for detection of potential vitamin B6 deficiency or overdose.

Principle of the Assay

L-Tyrosine is decarboxylated by a vitamin B6 (PLP)-dependent enzyme, tyrosine-apo-decarboxylase to tyramine. The activity of the apo-enzyme is directly proportional to the amount of PLP present in the reaction mixture.

Tyramine is then oxidized to p-hydroxybenzyl aldehyde and hydrogen peroxide (H_2O_2) by the action of tyramine oxidase.

The H_2O_2 reacts with 4-aminoantipyrine and TOOS in the presence of horseradish peroxidase to obtain a quinoneimine (purple dye) the absorbance of which is measured at 546 nm (520-595 nm).

Manual procedure

Reagents have to be adjusted to 18 - 28 °C .

Dilute EDTA plasma and Controls 1:40 in Dilution Buffer

Pipett 50 µl Substrate R1 into each well

Pipett 50 µl Calibrators 0, 20, 200 nmol/L
into the respective wells

Pipett 50 µl Control low and normal (diluted)
into the respective wells

Pipett 50 µl diluted sample into the subsequent wells.

Pipett 50 µl Apo-Enzyme R2 into each well.

shake and incubate for 30 + 5 min at 37°C in a plate incubator

Pipett 100 µl Enzyme R3 into each well

shake and incubate for 15 + 3 min at 37°C in a plate incubator

Read OD at 546 nm (alternatively at 520-595 nm)

Use endpoint mode with two calibrators (20 and 200 nmol/L). Calibrator 0 is used as Blank. Have a standard curve created by using linear curve-fitting.

Special Equipment

Manual procedure:
Microtiterplate reader with a filter at 546 nm, (520-595 nm) incubation chamber at 37°C and software suitable for endpoint measurements.

Microtiterplates, e.g. NUNC Maxisorb F8

Pre-Analytics

Samples required:	~500 µl EDTA plasma dilute 1:40 in dilution buffer **Lipemic plasma:** Samples should be taken from fasting individuals due to interferences with the photometric determination. **Hemolytic plasma:** Slightly hemolytic samples can be used.
Sample collection:	Draw blood into EDTA venipuncture tubes
Sample storage:	at 2-8°C up to 12 h protected from light. at -20°C for at least 3 months

Kit components

	KK-VB6
Tests	100
Dilution Buffer	1 x 60 ml
Enzyme Buffer	1 x 13 ml
Substrate R1	1 x lyophilized
Apo-Enzyme R2	1 x lyophilized
Enzyme R3	2 x lyophilized
Calibrator Set	1 x 3 lyophilized
Control Set normal / high	1 x 2 lyophilized

Reconstituted reagents are stable for 2 months at 2-8 °C except of Apo-Enzyme, Calibrator, and Controls (undiluted) which are stable for 2 months at ≤-20 °C; store in aliquots, if reagent is needed for more than 3 runs. Controls have to be diluted 1:40 prior to usage.

BÜHLMANN

Vitamin B6 enzymatic

Characteristics KK-VB6

A Commitment to Diagnostics

Assay Performance Data

Data have been established with the manual procedure on microtiterplates.

Dilution linearity 9-250 nmol/L

Spiking recovery 81-105 %

3 samples were spiked with increasing amounts of PLP and analysed in 3 runs.

Sensitivity

LoB: < 7 nmol/L

LoD: < 7 nmol/L

LoQ: < 10 nmol/L

Repeatability <10 %

Total precision <15 %

Diluted EDTA samples n=5 were tested over a period of 20 work days in 2 runs per day.

Precision Profile

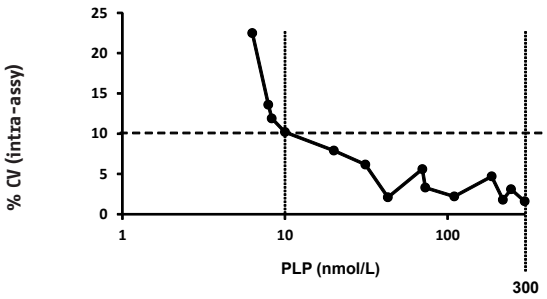

Figure 1: Precision Profile

Specificity of the Enzyme	Max. conc. tested nmol/L	Reactivity %
Component		
Pyridoxal (PL)	10'000	≤0.1 %
Pyridoxin (PN)	10'000	≤0.1 %
Pyridoxamine (PM)	10'000	≤0.1 %
4-pyridoxic acid (PA)	10'000	≤0.1 %
Pyridoxamine 5'-phosphate (PMP)	1200 10'000	≤0.2 % ≤0.8 %

Method comparison HPLC vs enzymatic

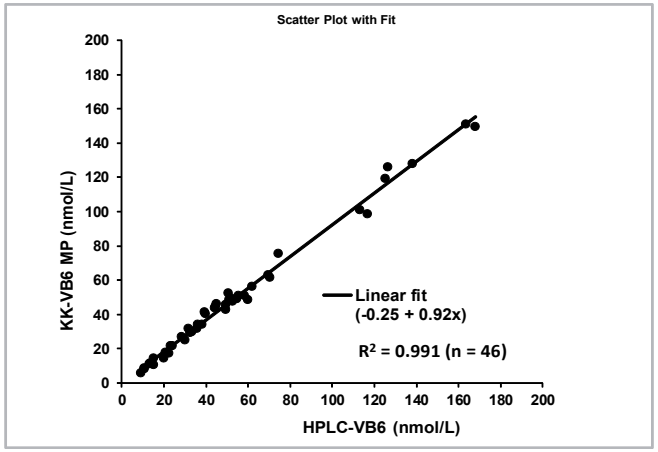

Figure 2: Method comparison with EDTA plasma samples

Normal Values

Apparently healthy adults	
n	60
Median (nmol/L)	23
95th Percentile (nmol/L)	172.5
2.5th Percentile (nmol/L)	57.5

Interfering Substances

No interference is detected with the following substances up to the listed concentrations:

Lipemic samples: triglycerides: Intralipid® 200 mg/dL; equivalent to 5.6 mmol/L triglycerides

Hemolytic samples: haemoglobin: 3.2 mmol/L; 500 mg/dL

Icteric samples: conjugated bilirubin: 360 µmol/L; 30 mg/dL, unconjugated bilirubin: 214 µmol/L; 12.5 mg/dL

Other substances and/or factors have not been investigated in this study. Interferences cannot be excluded.

Ordering code:
KK-VB6 100 tests

CE-marked product

BÜHLMANN Laboratories AG
Baselstrasse 55
CH–4124 Schönenbuch/Basel
Switzerland

Phone +41 61 487 12 12
Fax orders +41 61 487 12 99
info@buhlmannlabs.ch
www.buhlmannlabs.ch

TF055ML-03 E