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Preparation of blood samples for quantification of Vitamin B1 (thiamine-di-phosphate) and Vitamin B6 (pyrodoxal-5-phosphate) using the epMotion® 5075

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Abstract

In collaboration with the Isala Hospital in Zwolle, NL, a method for preparation of blood samples for the purpose of quantification of vitamin B1 and vitamin B6 was successfully established for the laboratory.

Vitamin B1 is a vitamin of the B complex, its di-phosphate playing a role in various processes throughout the body, such as during carbohydrate metabolism as well as for specific functions of the nervous system. Vitamin B1 deficiency may indicate diseases such as Beriberi, chronic alcoholism, Wernicke-encephalopathy or Strachan-syndrome. In addition to human conditions, vitamin B1 deficiency diseases are also found in the veterinary field. Vitamin B6 is also a vitamin of the B complex, involved in amino acid metabolism (conversion of transaminases). Since almost all food contains vitamin B6, signs of deficiency are typically not common. However, deficiencies, as well as elevated values, are linked to neurological and psychiatric symptoms.

Determination of the concentration of both vitamins in the blood is performed via HPLC. The entire process of sample preparation is carried out on the epMotion 5075 VAC/TMX, or 5075vt, respectively.



Figure 1: epMotion 5075vt

Introduction

Since tests for the two parameters vitamin B1 and vitamin B6 are ordered with increasing frequency, many samples need to be prepared which, in turn, requires substantial hands-on time in many laboratories. For example, patients presenting with neurological symptoms are referred by their general practitioners to specially educated neurologists who will order tests for vitamin B1 and B6 if vitamin deficiency is suspected. Since neurological diseases are on the rise, increasing numbers of diagnostic tests are ordered in clinics, thus placing a high pipetting burden on clinical laboratories. At the Isala Hospital in Zwolle, NL, it was deliberated how best to meet these increasing demands.

Concentrations of vitamin B1 and vitamin B6 are always determined using HPLC. Since the blood samples cannot be used as such for HPLC measurements, appropriate preparation must be carried out first. These steps are easily automated on the *epMotion*, which additionally protects humans from hazardous liquids such as semicarbazide hydrochloride.

The *epMotion* is a very flexible, fully automated system for a broad variety of pipetting. The *epMotion* is not limited to pipetting; temperature steps, mixing steps and purification can also be performed in fully automated fashion.

Material and Methods

- > Eppendorf *epMotion* 5075 VAC/TMX
- > Vac frame
- > Vac frame holder
- > Reservoir Rack
- > Reservoirs 30 mL/Reservoirs 100 mL
- > Racks
- > Racks for single tubes
- > Tipholder for reloadable tips

- > Eppendorf Centrifuge 5810

- > Agilent® HP1260/1200 liquid chromatograph
- > MF membrane filter
- > HPLC Quard columns

- > Perchloric acid 7 %
- > Neutralization reagent

- > Trichloroacetic acid
- > Semicarbazide hydrochloride

Reagent preparation

Prior to filling the 100 mL reservoirs of the *epMotion* and hanging these into the reservoir rack, the neutralization reagent for vitamin B1 quantification is prepared, and the concentrated perchloric acid is diluted to 7 %. The standards and controls are solubilized in the prescribed amounts of distilled water. The racks containing the previously frozen and now thawed EDTA blood samples are placed on the work table.

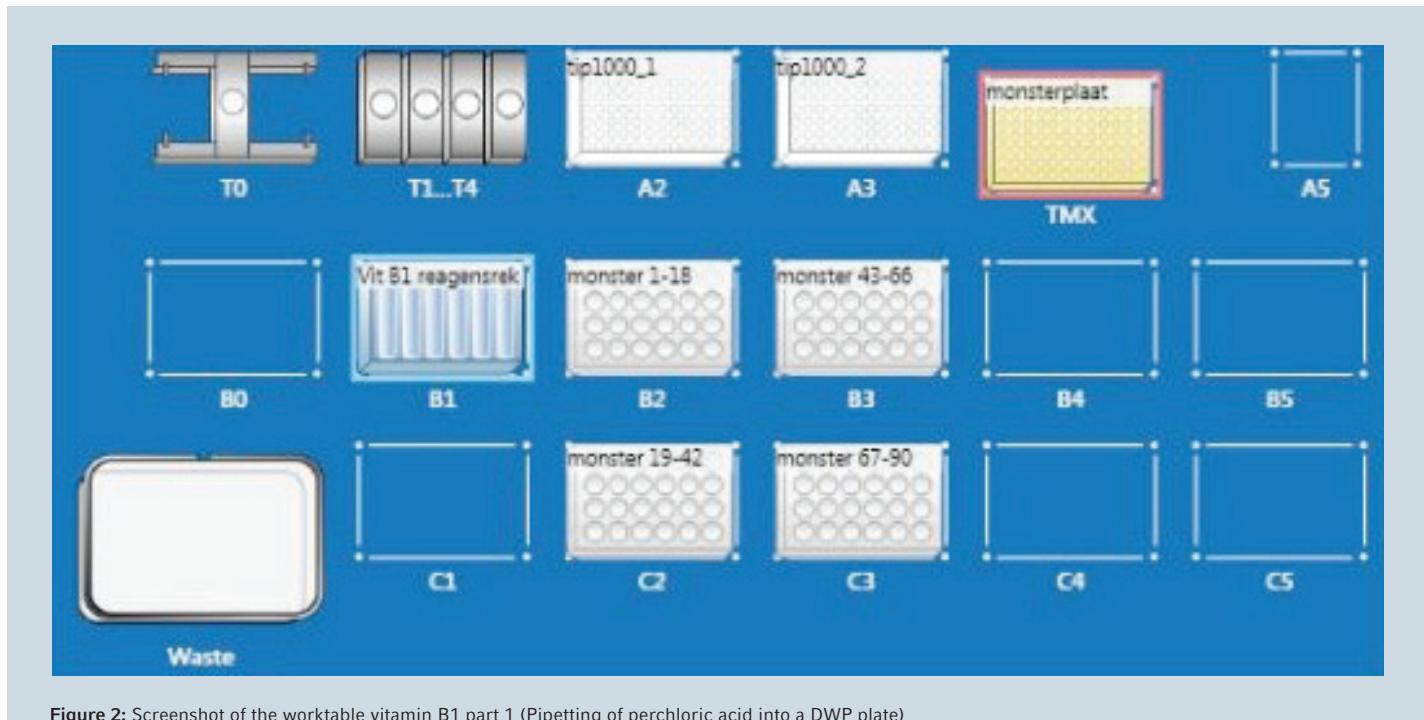


Figure 2: Screenshot of the worktable vitamin B1 part 1 (Pipetting of perchloric acid into a DWP plate)

Position	Labware	Comment
A2	epT.I.P.S.® Motion 1000 μL filter	
A3	epT.I.P.S. Motion 1000 μL filter	
A4	Sample plate	Deepwell plate 96
B1	Reagent reservoirs: Perchloric acid 7 % Neutralization reagent	100 mL reservoir 100 mL reservoir
B2	Racks containing samples, standards and controls	
B3	Racks containing samples, standards and controls	
C2	Racks containing samples, standards and controls	
C3	Racks containing samples, standards and controls	

Table 1: epMotion worktable details for vitamin B1 determination

The same preparation steps are carried out for quantification of vitamin B6, except the trichloroacetic acid is diluted and

the semicarbazide hydrochloride solution is prepared in accordance with the instructions.

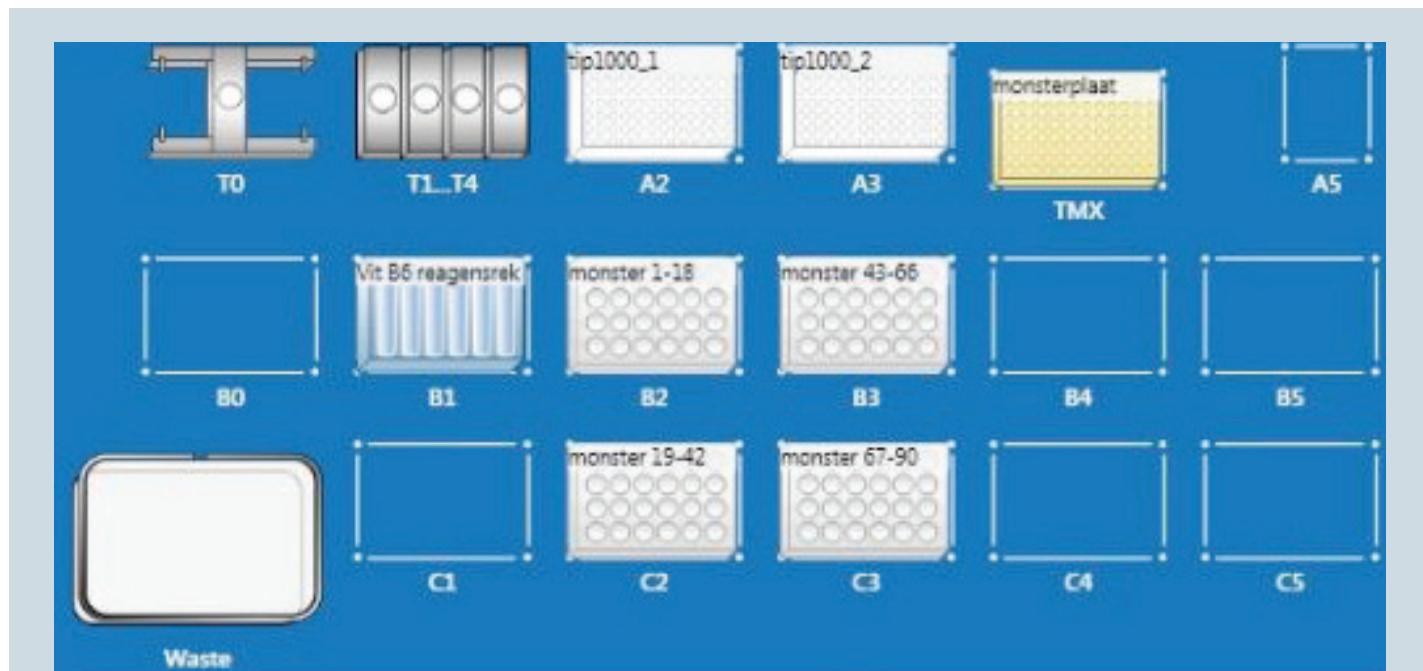


Figure 3: Screenshot of the worktable vitamin B6 part 2 (pipetting into HPLC vials)

Position	Labware	Comment
A2	epT.I.P.S. Motion 1000 μ L filter	
A3	epT.I.P.S. Motion 1000 μ L filter	
A4	Sample plate	Deepwell plate 96
B1	Reagent reservoirs: Trichloroacetic acid Semicarbazide hydrochloride	100 mL reservoir 100 mL reservoir
B2	Racks containing samples, standards and controls	
B3	Racks containing samples, standards and controls	
C2	Racks containing samples, standards and controls	
C3	Racks containing samples, standards and controls	

Table 2: epMotion worktable details for vitamin B6 determination

Procedure

Vitamin B1

The blood samples from EDTA whole blood or heparinized blood are initially frozen for 12 h to ensure that they are hemolysis-free. The samples are distributed to 4 racks on the worktable of the epMotion. 250 μ L of blood per sample are transferred to a DWP plate. The epMotion then dispenses the perchloric acid into the EDTA samples. Following a Thermomixer® step is a centrifugation step (15 minutes at

4500 rpm) in the Eppendorf Centrifuge 5810, outside the epMotion. Subsequently the supernatant is pipetted from the plate into HPLC vials, and neutralization reagent is added. The standard samples and controls are treated in the same manner. The filled HPLC vials are further processed on the Agilent HP 1260/1200.

Vitamin B6

Similar preparation steps are carried out for the quantification of vitamin B6 content in the blood. Samples are also frozen for 12 h. They are then placed on the work-table of the epMotion, and 275 µL of blood are pipetted into a DWP plate, followed by addition of 550 µL trichloroacetic acid. Following a mixing step and a temperature step on the Thermomixer (TMX), the end of this application is marked by a centrifugation step on the Eppendorf Centrifuge 5810 (24 min, 4500 rpm). The supernatants from the plate are then transferred to the individual HPLC vials, and semicarbazide hydrochloride is added. Parallel to vitamin B1 determination, the filled HPLC vials are then further

processed on the Agilent HP 1260/1200. Standards and controls are included.

Vitamin B1 and B6 quantification are performed together, since the combination of both parameters provides a complete diagnostic picture. These applications were tested on an epMotion VAC/TMX; due to spatial constraints the analyses were performed in two separate runs. The newer version of the epMotion, e.g. the epMotion 5075t, would allow both analyses to be performed together, as up to 15 positions are now available on its worktable, depending on the model.

Results and Discussion

Over a period of 22 weeks, one run for vitamin B1 preparation and one run for vitamin B6 preparation were tested on the epMotion. The weekly controls SKML Medium and Control Instruchemie Normal were used for evaluation of accuracy and precision of the pipetting steps on the

epMotion. The results were compared to results obtained with samples prepared manually. The values obtained for samples prepared on the epMotion were superior to those obtained from samples prepared manually with respect to accuracy as well as precision.

	Between-run (n = 22) epMotion			Between-run (n=22) manual		
	\bar{x}_{gem} nmol/L	SD nmol/L	% CV	\bar{x}_{gem} nmol/L	SD nmol/L	% CV
SKML Vit B1/B6 Medium	118	2.32	1.98	118	4.1	3.5
Contr. Instruchemie Normal	119	2.84	2.40	127	4.8	3.8

Table 3: Two different sera were used to quantify the vitamin B1 content: SKL Medium Level and Control Instruchemie Normal. Direct comparison of averages obtained from manual measurement and measurement using the epMotion is not possible as the lot numbers of the sera were different. The sole purpose was comparison of SD and % CV.

	Between-run (n = 22) epMotion			Between-run (n=22) manual		
	\bar{x}_{gem} nmol/L	SD nmol/L	% CV	\bar{x}_{gem} nmol/L	SD nmol/L	% CV
SKML Vit B1/B6 Medium	90	2.11	2.33	93	3.4	3.7
Contr. Instruchemie Normal	93	2.11	2.28	92	3.5	3.8

Table 4: Vitamin B6 was also measured in 2 sera, SKL Medium and Control Instruchemie Normal. Direct comparison of averages obtained from manual measurement and measurement using the epMotion is not possible as the lot numbers of the sera were different. The sole purpose was comparison of SD and % CV.

In addition, comparisons were carried out on EDTA blood samples. To this end, the blood samples were prepared on

the epMotion as well as manually. The HPLC values show very good correlation between the two approaches.

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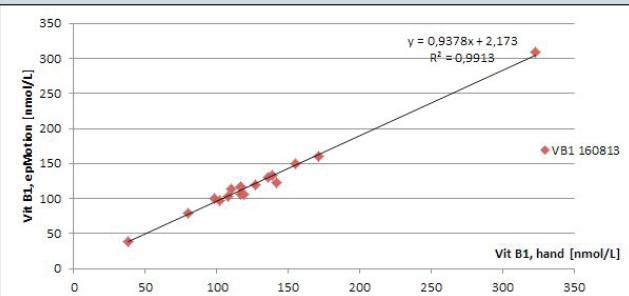


Table 5: Comparison between sample preparation performed manually and on the epMotion for vitamin B1.

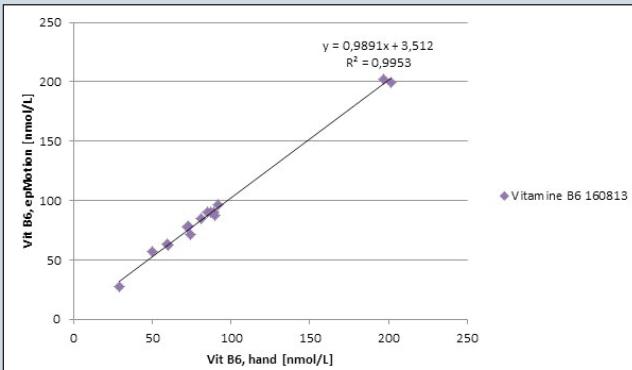


Table 6: Comparison between sample preparation performed manually and on the epMotion for vitamin B6.

Conclusion

Integration of the epMotion into the process of vitamin B1 and B6 quantification successfully reduced a large proportion of the manual labor. The epMotion 5075 enables performance of otherwise elaborate manual steps in a fast and efficient

manner. Accuracy and precision are reliable; thus the epMotion is ideally suited to perform the preparatory steps for HPLC measurement.

References

- [1] Procedure voor het beoordelen van interne controles m.b.v. QC module
- [2] Procedure rondom de organisatie van externe controles voor de Unit Speciale Chemie
- [3] Operating manual for HP 1260 and HP 1200, Agilent
- [4] Operating Manual for epMotion 5075

Ordering information

Description	Order no. international	Order no. North America
epMotion® 5075vt	5075 000.304	5075000304
epMotion® 5075t	5075 000.302	5075000302
Reservoir rack	5075 754.002	960002148
Reservoirs 100 mL	0030 126.513	960051017
Racks	5075 760.002	960002032
epT.I.P.S.® Motion tips 1000 µL, reload, filter	0030 014.510	003014510
Tipholder für epT.I.P.S.	5075 751.399	5075751399
Centrifuge 5810	5810 000.424	5810000424

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