Assessment of vitamin K status by fully automated IDS-iSYS Ina*K*tif MGP

Dagmar Kasper[1], Mhammed Bougoussa[1], Cees Vermeer[2]
[1]IDS Plc, Boldon, UK
[2]VitaK, Maastricht University, The Netherlands

Introduction

Matrix Gla-protein (MGP) is a vitamin K-dependent protein acting as a potent inhibitor of soft tissue calcification, mainly in the vessel wall and cartilage [1]. During its synthesis MGP undergoes extensive posttranslational modification, i.e. serine phosphorylation and glutamate carboxylation (to form Gla). Vascular vitamin K insufficiency is associated with the production of desphospho-uncarboxylated MGP (dp-ucMGP), which has no calcification inhibitory activity and which is set free in the circulation, where it can be quantified by assays based on the sandwich ELISA principle. Most data presently available in the literature are based on a hand-made microtiter plate assay designed at VitaK laboratories [2]. Here we present a fully automated version of the same assay, which is produced by IDS: the Ina*K*tif MGP assay.

Materials & Methods

Antibodies used: Monoclonal antibodies against the phosphoserine domain (aa 3-15) and the Gla-domain (aa 35-49) in human MGP.
Assay principle: The IDS Automated Analyser IDS-iSYS Ina*K*tif MGP assay uses both highly specific monoclonal antibodies. One biotinylated antibody is coupled to magnetic particles (MP). The second antibody is coupled to an acridinium ester derivative. 50 µl of sample is incubated with 20 µL of MP and the second antibody. After 60 min of incubation, followed by a washing step, triggers are added whereby the luminescence measured is directly proportional to the Ina*K*tif MGP concentration present in the sample. The time to first result is 63 minutes. The throughput of the assay is 89 tests/hour.
Assay Diagram:

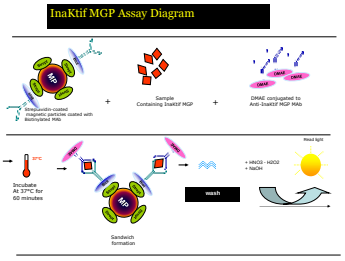


Figure 1: Schematic diagram of the IDS-iSYS Ina*K*tif MGP assay. The signal produced is proportional to the Ina*K*tif MGP concentration in the sample

Assay calibration. The IDS Ina*K*tif MGP assay is calibrated against an in house standard prepared in horse serum using 2-point calibrations for master curve repositioning. The analytical range is 200-10000 pM.
Sample preparation: Blood (taken by venipuncture) should be collected in EDTA; plasma should be prepared and kept frozen at -80°C until testing

Results

Sensitivity: Sensitivities LOB, LOD and LOQ were evaluated in accordance with **CLSI EP17-A** Guideline. LOB and LOD were determined respectively at 47 and 119 pM.
Precision: Precision was evaluated according to **CLSI EP-5A** Guideline. Five control plasma samples were assayed in duplicate twice a day for a minimum of 20 days.

Control	N	Mean	Within - Run		Total	
		pM	*SD*	*CV%*	*SD*	*CV%*
1	80	555	35	6.2	80	14.4
2	80	1033	49	4.7	108	10.5
3	80	2493	204	4.2	198	7.9
4	80	4386	114	2.6	296	6.7
5	80	7741	221	2.9	468	6.0

Table 1: IDS-iSYS InaKtif MGP assay precision

Linearity: The linearity was realized according to the EP-6A CLSI recommendation. The IDS-iSYS Ina*K*tif MGP assay shows a good linearity over the dynamic range (figure 2).

	Observed	Expected	O/E (%)
X1	0		
0,875X1+0,125X2	807	937	86
0,750X1+0,250X2	1951	1873	104
0,625X1+0,375X2	3174	2810	113
0,500X1+0,500X2	4121	3746	110
0,375X1+0,625X2	4875	4683	104
0,250X1+0,750X2	5469	5620	97
0,125X1+0,875X2	7024	6556	107
X2	7670	7493	102
		Average	**100%**

Table 2: IDS-iSYS Ina*K*tif MGP linearity over the dynamic range

Recovery: 3 serum samples were spiked with 2 different amounts of known MGP concentration. The recovery was determined by comparing recovered concentrations to the added concentrations. Data are shown in table 3.

Ina*K*tif MGP conc. (pM)	MGP added (pM)	Measured values (pM)	Recovered (pM)	Recovery (%)
89	1383	1206	1117	86%
89	2677	2406	2317	90%
267	1561	1431	1164	90%
267	2855	2613	2346	91%
1269	2563	2672	1403	108%
1269	3857	4041	2772	107%
			Average	**95%**

Table 3: Recovery results with IDS-iSYS Ina*K*tif MGP assay

Correlation between Ina*K*tif MGP assay and hand-made ELISA: The IDS-iSYS Ina*K*tif MGP assay was compared with the VitaK homemade ELISA (figure 2). Linear regression was used for comparative data:
IDS-iSYS Ina*K*tif MGP = 1.056 (x) + 54.34 with a coefficient of correlation R= 0.98.

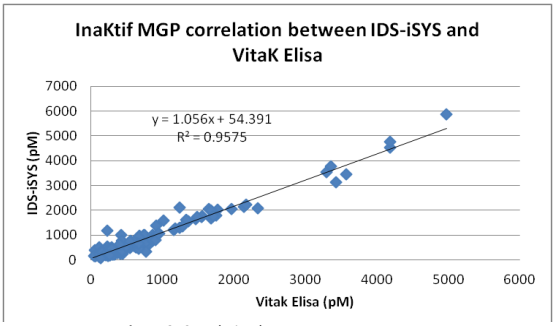
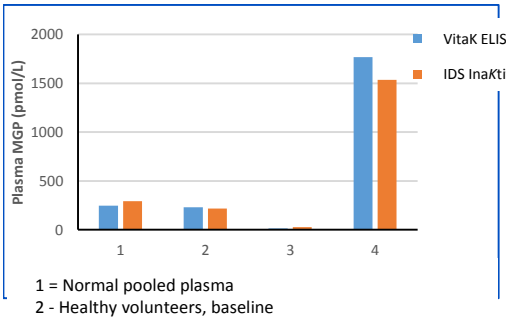


Figure 2: Correlation between two assays

Vitamin K status The microtiter plate assay and the Ina*K*tif MGP assay were measured in volunteers before and after vitamin K supplementation, and in patients on vitamin K antagonist treatment (figure 3).



1 = Normal pooled plasma
2 - Healthy volunteers, baseline
3 = Healthy volunteers, 1 month of high vit. K treatment
4 = Patients on long-term oral anticoagulant treatment

Conclusions

- The automated dp-ucMGP assay closely resembles the microtiter plate version. Therefore, data obtained with both assays are comparable.
- The Ina*K*tif MGP assay provides a quantitative measure for the vitamin K status of the vessel wall.
- Poor vascular vitamin K status was demonstrated to be a major risk factor for cardiovascular morbidity and mortality, both in patients and in the healthy population
- Studies in which cardiovascular end points are monitored should be stratified for dp-ucMGP.
- Poor vitamin K status is a modifiable risk factor for arterial calcification, and the Ina*K*tif MGP assay is the most direct way to identify patients at risk and to monitor the effect of vitamin K supplementation.

References

1. Cranenburg, E.C.M., et al. Vitamin K, the coagulation vitamin that became omnipotent. *Thromb. Haemostas. 98 (2007) 120-125.*
2. Cranenburg E.C.M., et al. Characterization and potential diagnostic value of circulating matrix Gla protein (MGP) species. *Thromb. Haemostas. 104 (2010) 811-822.*