Sample stability of PlGF

Introduction

Pre-eclampsia is a pregnancy disorder affecting 2-8% of pregnant women. Recently biochemical markers, including placenta growth factor (PIGF) and pregnancy-associated plasma protein (PAPP-A), have given promising results in pre-eclampsia screening. While PAPP-A is an established marker employed in Down screening, PIGF is a new analyte and, as yet, there is no published data on its stability in whole blood and serum. Sample stability is a critical issue and reliable sample analysis relies on the fact that sample stability is known and samples have been stored in an appropriate way.

The stability of PIGF has now been studied in whole blood and serum. Samples from pregnant women were kept at different temperatures and PIGF concentrations were measured using specific immunoassays at different time points.
Materials and methods
Serum samples were obtained during the 1st trimester of pregnancy. Fresh serum samples were pooled, using 2-5 samples per pool. The serum pools were aliquoted and stored at different temperatures for various times. Three sample pools per each combination of temperature and time were stored. Exact storage temperatures were monitored by data loggers. Furthermore the effect of freezing and thawing was tested using up to 6 freeze/thaw cycles.

Whole blood samples were obtained from pregnant women during the 2nd and 3rd trimester of pregnancy. Samples were stored at three different temperatures (+4°C, room temperature (RT) and +30°C), three samples per temperature, prior to separation of serum by centrifugation. Storage times were 5 hours, 1 day, and 3 days at all tested temperatures. A whole blood sample stored for 30 minutes at RT before centrifugation served as a reference. After separation of serum, the samples were aliquoted and stored at -20°C until analysis.

The concentration of PlGF in samples was tested using the DELFIA Xpress PlGF kit (PerkinElmer, 6007-0010)* with the DELFIA® Xpress clinical random access platform. The kit uses the two-site immunofluorometric assay principle. Samples were run in three replicates.

The results obtained were analyzed using fitted linear regression. A t-statistic test was used to check whether the slope of the generated regression line was significantly different from zero (α = 0.05). If the slope did not deviate significantly from zero then the change in analyte concentration was considered insignificant. If the slope was significant, storage times when the regression lines exceeded 5% and 10% change in analyte concentration were calculated.

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The stability of PlGF in whole blood was studied by storing whole blood samples at different temperatures (+4°C, RT, and +0°C) for 5 hours, 1 day, and days before centrifugation. Results of the study are presented in Table 2.

Results
Stability in serum
PlGF assay data points were compared to the 0-point result. As an example Figure 1 shows the results for RT storage.

Table 1: Maternal serum samples were stored at different temperatures, and storage times when the change in PlGF concentration exceeded 5% and 10% are given.

Table 2. The stability of PlGF in whole blood at different temperatures was tested, and storage times when change in concentration exceeded 5% and 10% are given.

Conclusions
Long term storage of serum samples for PlGF analysis is preferably at -20°C because the analyte was stable in serum at -20°C at least for 6 months according to an ongoing study. PlGF was stable in serum at +4°C at least for the maximum tested storage time of 30 days. At RT and +30°C serum stability is clearly lower. Setting a criterion for a maximum concentration change to 5%, PlGF was stable for 2.5 days and 1 day at RT and +30°C, respectively. A plausible explanation for the observed increase in concentration in serum samples during storage is the fact that PlGF partially exists in complex with soluble fms-like tyrosine kinase-1 (sFlt1) and some of the PlGF is released from the complex. The DELFIA Xpress assay measures free PlGF. The effect of freezing and thawing on the analyte levels in serum samples was less than 5% when tested for up to 6 freeze/thaw cycles.

In whole blood, PlGF was stable only at +4°C for the maximum tested storage time of 3 days. At higher temperatures it was stable in whole blood for 1 day. However, if the more relaxed criterion for the maximum concentration change of 10% is used then PlGF in whole blood was stable at RT for 2 days.