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Technical note

# Stability of placental growth factor, soluble fms-like tyrosine kinase 1, and soluble fms-like tyrosine kinase 1 e15a in human serum and plasma

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ABSTRACT

Placental growth factor (PlGF), total soluble fms-like tyrosine-kinase 1 (sFlt-1) and its placental-specific variant, sFlt-1 e15a, show promise as biomarkers for the prediction and diagnosis of preeclampsia. This study describes the degradation of PlGF, sFlt-1 and sFlt-1 e15a within maternal serum and plasma to assist clinical implementation. Whole blood was refrigerated at 4 °C for up to 48 h prior to centrifugation for isolation of plasma and serum. PlGF and sFlt-1 were quantified using the B.R.A.H.M.S Kryptor Compact PLUS; sFlt-1 e15a via a custom ELISA. All three analytes are stable for at least 48 h at 4 °C. Serum and plasma performed comparably.

## 1. Introduction

Preeclampsia is a heterogenous condition that affects 2–8% of pregnancies, and often poses many challenges with regards to accurate prediction and diagnosis. As a result, it is a significant contributor to maternal and perinatal morbidity and mortality [1–3]. Fortunately, over recent decades, studies have demonstrated the presence of biomarkers in maternal blood samples, which show promise in enhancing the prediction and diagnosis of this disease [3,4]. Recent candidates of interest include angiogenic factor, placental growth factor (PlGF), and anti-angiogenic factor, soluble fms-like tyrosine kinase-1 (sFlt-1) [5,6]. These factors are key to the pathophysiology of preeclampsia; elevated circulating sFlt-1 released from the preeclamptic placenta results in an imbalance of angiogenic to anti-angiogenic factors, which leads to widespread endothelial dysfunction and the clinical features of preeclampsia [7]. PLGF is a subtype of vascular endothelial growth factor that is abundantly produced by the placenta and promotes angiogenic activity. sFlt-1 is an anti-angiogenic factor, that binds to and antagonizes the actions of PLGF. There are four known splice variants of sFLT-1, of which, sFlt-1 i13 and sFlt-1 e15a are expressed in pregnant women [6,8]. The latter is a placental-specific variant of sFlt-1 that offers promise for improving preeclampsia prediction, because it removes the potentially distorting effect of sFlt-1 i13, which is the main variant produced by the endothelium [6]. Studies have demonstrated that pregnancies complicated by preeclampsia have higher concentrations of sFlt-1 and sFlt-1 e15a, and lower concentrations of PlGF, in comparison to normal pregnancies. These biochemical changes appear to precede the clinical features of disease. Therefore, measuring their levels could improve the prediction and diagnosis of preeclampsia.

Accurate assessment of the levels of sFlt-1, sFlt-1 e15a and PLGF requires an understanding of their stability *ex vivo* during the collection and analysis process. Knowledge about the natural degradation course of these analytes under routine collection and storage procedures is limited, and non-existent in the case of sFlt-1 e15a. Furthermore, the stability of sFlt-1 is not documented in whole blood and there is no information regarding how plasma compares to serum for each analyte. This knowledge is necessary to better inform the translation of these biomarkers into clinical practice. Therefore, we aimed to assess the stability of these biomarkers over time in whole blood before assessing analyte levels in both serum and plasma.

## 2. Methods

### 2.1. Study participants

We conducted a prospective cohort study at Monash Health, Melbourne, Australia, and recruited five pregnant women greater than 37 weeks' gestation who were admitted for induction of labor. Participants that were unable to consent were excluded from this study. Ethics approval was obtained from the Monash Health Human Research

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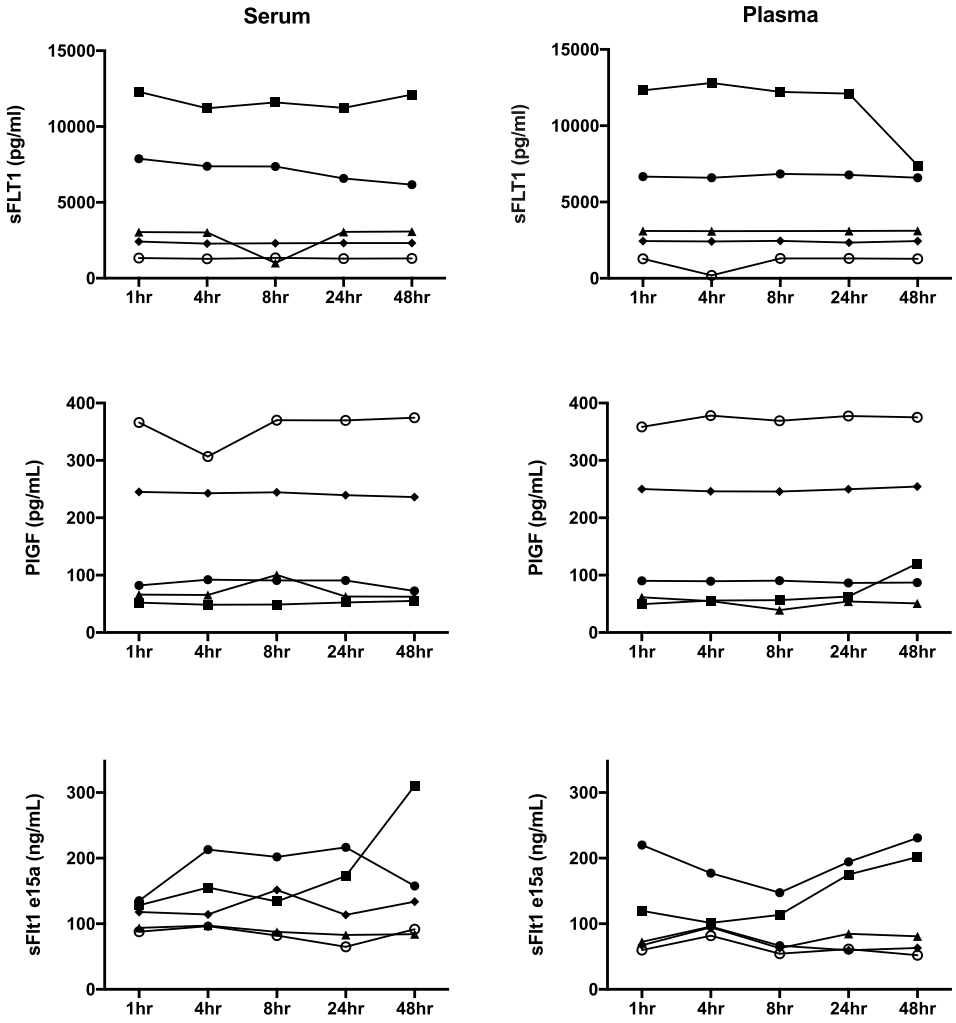


**Fig. 1.** Serum and plasma concentrations of sFlt-1, PlGF and sFlt-1 e15a remain stable in maternal whole blood when stored at 4 °C for up to 48 h. Data is presented for each individual participant, as represented by the different marker symbols, to highlight the analyte stability across time.

Ethics Committee (HREC: 9878).

### 2.2. Sample collection and processing

Eighty milliliters of whole blood were collected prior to commencement of the induction of labor. Each participant's blood was equally divided between five serum-separating (SST) tubes and five EDTA (Ethylenediaminetetraacetic acid) plasma pathology tubes (Thermo Fisher Scientific, Waltham, MA, US). These tubes were refrigerated at 4 °C for varying time intervals after which they were centrifuged at 1200 g for 20 min. The time intervals from refrigeration to processing were one, four, eight, 24- and 48-hours post-collection respectively. Serum and plasma samples were then aliquoted and stored at −80 °C until analysis.

### 2.3. PlGF, total sFlt-1 and sFlt-1 e15a assays

sFlt-1 and PLGF levels were measured using the B.R.A.H.M.S Kryptor Compact PLUS (Thermo Fisher Scientific) and in accordance with the manufacturer's specifications. The B.R.A.H.M.S Kryptor is a fully-automated immunoassay platform, which has been well validated for the measurement of these biomarkers in pregnancy [9]. sFlt-1 e15a was measured using a non-commercially available enzyme-linked immunosorbent assay (ELISA). This ELISA was developed in-house, and its methodology has been previously published in the literature [10]. Findings were analyzed on SoftMax Pro 6.4 (Molecular Devices, San Jose, CA, US). The inter-assay coefficient of variation was 11%.

### 2.4. Statistical analysis

Statistical analysis was performed using repeated measures Friedman's two-way ANOVA by ranks to compare the level of each analyte between different time points in both serum and plasma respectively. Analysis of each analyte in serum compared to plasma at each matched time point was performed using Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$. Analysis was undertaken using IBM SPSS 25.0 (IBM Corp, Armonk, NY, US).

## 3. Results

Samples were collected from five women, aged 27–36 years. All women were greater than 37 weeks of gestation (38–40.1 weeks), four women (80%) were multiparous, and the indications for induction of labor included pre-existing diabetes, gestational diabetes, and reduced fetal movements. The degradation courses of PlGF, sFlt-1 and sFlt-1 e15a per participant are shown in Fig. 1. PlGF, sFlt-1 and sFlt-1 e15a are stable in serum at 4 °C for at least 48 h, with no statistically significant change in concentrations over this time ($p = 0.837$, $p = 0.134$, and $p = 0.551$ respectively). These findings are also replicated in plasma samples, where there was no significant change in the concentrations of PlGF, sFlt-1 and sFlt-1 e15a over 48 h ($p = 0.891$, $p = 0.651$, and $p = 0.428$ respectively).

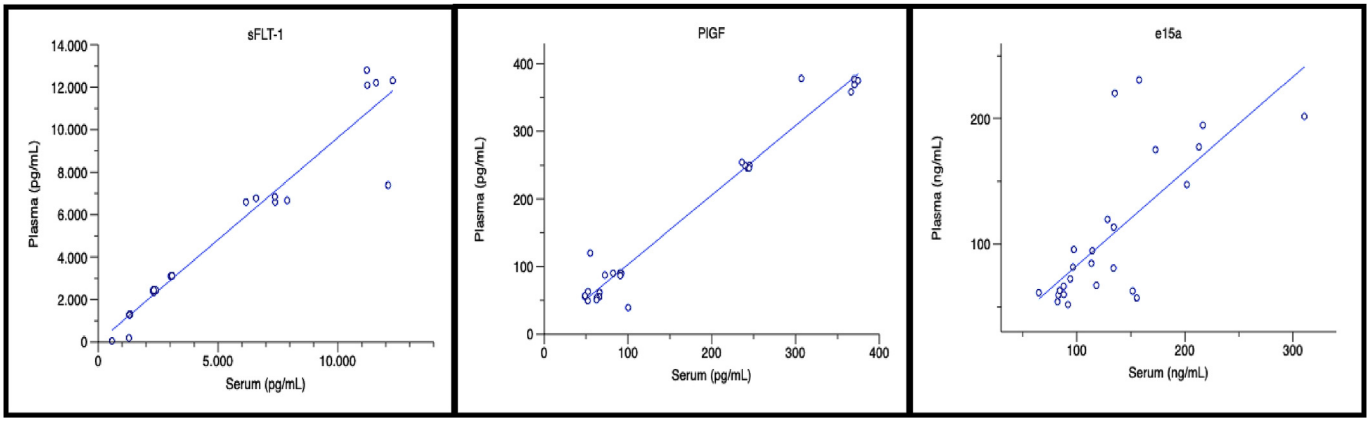


**Fig. 2.** Correlation between serum and plasma performance for sFlt-1, PlGF and sFlt-1 e15a up to 48 h.

Furthermore, there is no significant difference between serum and plasma levels of PlGF and sFlt-1 at any given time point. However, there is a significantly higher sFlt-1 e15a in serum compared to plasma, mainly due to differences at four and eight hours. Nevertheless, there remains a strong correlation between serum and plasma levels of sFlt-1 e15a (Spearman's rho 0.738, $p < 0.001$) (Fig. 2). Similarly, there is also a strong correlation between serum and plasma for both PlGF and sFlt-1 levels, indicating both plasma and serum may be used to measure these analytes (Spearman's rho 0.817 and 0.972 respectively; $p < 0.001$ for both) (Fig. 2).

## 4. Discussion

The stability of PlGF in whole blood and serum at various temperatures has previously been described [11,12]. There is no significant change in PlGF levels over a few hours at room temperature, and at least for 48 h if refrigerated [11,12]. This suggests that collection and storage processes preserve PlGF accurately. Similarly, sFlt-1 appears stable when stored frozen at −80 °C for up to three years [11]. However, the stability of sFlt-1 from collection until freezing has not been investigated to our knowledge. This study is the first to examine the degradation of sFlt-1 e15a.

These results suggest that PlGF, sFlt-1 and sFlt-1 e15a do not undergo significant degradation for at least 48 h from collection until centrifugation when stored at 4 °C. For both serum and plasma, the levels of PlGF, sFlt-1 and sFlt-1 e15a are relatively static. The upward trend that occurs for plasma PlGF, and for both plasma and serum sFlt-1 e15a, appears to be consistent with the available literature [12]. We postulate that this occurs due to a theoretical dissociation of bound PlGF from sFlt-1, and by extension sFlt-1 e15a with time. As a result, there is an increased concentration of unbound measurable forms of PLGF and sFLT-1 e15a in the circulation. The knowledge that PlGF, sFlt-1 and sFlt-1 e15a are stable in whole blood at 4 °C for up to two days from collection supports efforts at practical implementation in the clinical or research setting. Serum and plasma perform almost interchangeably for all three biomarkers.

## Conflicts of interest

This study received support from Thermo Fisher Scientific through provision of complimentary reagents and equipment. M. Reddy receives support from the National Health and Medical Research Council (GNT1151281), while both M. Reddy and K.R. Palmer receive funding from the Royal Australian and New Zealand College of Obstetricians and Gynaecologists. Remaining authors declare no existing conflict of interest.

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