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Measurement of sVEGF R1 and PlGF in serum: comparing prototype assays from Beckman Coulter, Inc. to R&D Systems microplate assays

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Abstract

Objective: To compare the performance of prototype Access® sVEGF R1 and PlGF automated immunoassays from Beckman Coulter to the Quantikine® microplate ELISA assays by R&D Systems.

Methods: Samples obtained from pregnant women, non-pregnant women, and men were assayed according to manufacturers' instructions.

Results: Compared to the Quantikine assays, the Access assays demonstrated improved precision, increased sensitivity, broader dynamic ranges, and reduced analysis time. The Access assays were found to be specific for free sVEGF R1 and free PlGF.

Conclusion: There was good correlation between the Access and Quantikine assays. Superior performance by Access assays may have important prenatal diagnostic implications.

Keywords

preeclampsia; angiogenesis; placental growth factor; soluble vascular endothelial growth factor; sFlt-1

Introduction

There is growing evidence that supports the role of angiogenic and anti-angiogenic proteins in the pathophysiology of preeclampsia. A strong correlation has been found repeatedly between decreased concentrations of placental growth factor (PlGF) and increased

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concentrations of soluble vascular endothelial growth factor receptor 1 (sVEGF R1; also referred to as soluble fms-like tyrosine kinase-1 or sFlt-1) in pregnant women with preeclampsia as well as in women who are asymptomatic but develop the condition later in pregnancy (1–4). A number of studies have reported disease-related alterations in the concentrations of these biomarkers at various gestational intervals including at the time of delivery (3), at the time of preeclampsia diagnosis (4), several weeks prior to diagnosis (5, 6, 7), during the second trimester (8, 9) and as early as the first trimester (8, 10). Severity of disease has been associated with very low concentrations of PlGF (11, 12, 13) as well as with changes in both proteins between the first and second trimesters (14). More recently, investigators have been exploring the utility of these markers in the differential diagnosis of preeclampsia in complicated pregnancies (4, 15).

Vascular endothelial growth factor (VEGF), PlGF and sVEGF R1 are produced by trophoblasts, play key roles in regulating angiogenesis and are critical for successful placentation (16, 17, 18, 19). Both VEGF and PlGF promote angiogenesis by interacting with members of the VEGF receptor family found primarily on endothelial cells. These growth factors drive differentiation of, and are required to maintain, normal endothelial function. sVEGF R1, a soluble isoform of the transmembrane receptor for VEGF and PlGF, counteracts these angiogenic effects by binding circulating VEGF and PlGF and preventing activation of the membrane-bound receptor (18). An increase in the production of sVEGF R1 may trigger the maternal endothelial dysfunction that results in the clinical findings of hypertension, proteinuria, and edema (20).

Most of the studies exploring the usefulness of PlGF and sVEGF R1 as diagnostic markers for preeclampsia have measured these proteins using the Quantikine ELISA kits from R&D Systems (Minneapolis, MN). These assays are available for Research Use Only. Manual microplate assays such as these are considered to be labor intensive. We used advanced analytic techniques to create rapid, precise and reproducible automated assays adaptable for routine use in hospital and reference laboratories. Thus, the objective of this study was to evaluate the performance and analytical characteristics of prototype automated Access immunoassays for PlGF and sVEGF R1 in development. Not available for diagnostic procedures in comparison with the Quantikine microplate kits.

Methods

Study subjects

All subjects provided written informed consent prior to the collection of blood specimens. The utilization of samples for research purposes was approved by the Institutional Review Boards of Abbott Northwestern Hospital, Minneapolis, MN; The Toledo Hospital, Toledo, OH; Wayne State University, Detroit, MI; and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, NIH, DHHS.

Methods comparison testing

Serum specimens were obtained from pregnant women (n=182) enrolled in a prospective clinical trial evaluating the potential clinical utility of the Access assays. Second and third

trimester specimens from both normotensive and preeclamptic subjects were included to cover the complete measuring range of each assay.

The Access PlGF and sVEGF R1 assays are one-step immunoenzymatic (“sandwich”) assays. A sample is added to a reaction vessel along with paramagnetic particles coated with mouse monoclonal anti-human PlGF or mouse monoclonal anti-human sVEGF R1, blocking agent and a second monoclonal antibody conjugated to alkaline phosphatase. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of PlGF or sVEGF R1 in the sample, with the amount of analyte in the sample determined from a stored, multi-point calibration curve. Reagents for both Access assays were provided in ready-to-use reagent cartridges and the assays were run on Access 2 automated immunoassay analyzers. Manual two-step microplate ELISAs for PlGF and sVEGF R1, (Quantikine kits, R&D Systems, Minneapolis, MN) were run according to the manufacturer’s instructions. All specimens analyzed using the sVEGF R1 Quantikine assay were pre-diluted 1:20 prior to analysis. All other assays were run using neat samples.

For low analyte concentration reproducibility analyses, EDTA plasma was obtained from normal pregnant subjects (n=12) between 8.0 and 11.7 weeks of gestation and from men (n=10) and non-pregnant women (n=10). Blinded samples were run in duplicate on the PlGF and sVEGF R1 Access assays. Replicate coefficients of variation (CVs) were calculated for each sample (Table 1).

To identify the impact of ligand binding in the Access assays, purified PlGF and sVEGF R1 proteins were commercially obtained from R&D Systems. Ligand was titrated into a fixed concentration of each analyte and allowed to equilibrate for one hour at room temperature prior to analysis. Increasing molar concentrations of PlGF were spiked into a baseline sample containing 20,000 pg/mL sVEGF R1 and then assayed for sVEGF R1. Likewise, increasing molar concentrations of sVEGF R1 were spiked into a baseline sample containing 2,000 pg/mL PlGF and then assayed for PlGF.

The normality of the data was tested using the Shapiro-Wilk test. Because sVEGF R1 and PlGF concentrations were not normally distributed, non-parametric tests were used for analyses. Passing-Bablok regression was used to measure overall agreement between the Access and Quantikine methods. Pearson r statistic was calculated as a measurement of correlation. Bland-Altman analysis was used to detect bias between methods as a function of analyte concentration. All statistical calculations were performed using the Analyze-it® software add-on version 1.63 (Analyze-it Software Ltd., Leeds, UK) for Microsoft Excel.

Results

Comparison between the Access and Quantikine PlGF assays (n=182) demonstrated good agreement between methods. Passing Bablok regression analysis (Figure 1A) demonstrated a slope of 1.48 (95% CI of 1.36 to 1.61) and an intercept of −71.2 (−103.0 to −33.2 = 95%

CI). The Pearson r statistic was 0.81. A Bland-Altman plot (Figure 1B) showed a relative even spread of biases for individual samples with no strong trend for the sample set as a whole.

Method comparison between the Access and Quantikine sVEGF R1 assays (n=182) demonstrated a lesser agreement. Although both assays measure sVEGF R1, correlation differences are expected as the two methods measure different forms of the analyte [free (Access) vs. total (Quantikine)]. A Bland-Altman plot (Figure 2A) showed a dose-dependent bias for individual samples. Relative reported doses for the Access vs. the Quantikine methods become smaller as the analyte concentration in the samples decreases. This is to be expected if at low sVEGF R1 concentrations a decreasing proportion of the sVEGF R1 exists in the free form. Passing Bablok regression analysis (Figure 2b) demonstrated a slope of 2.63 (2.24 to 3.06 = 95% CI) and an intercept of −2194 (−2981 to −1630 = 95% CI). The Pearson r statistic was 0.77.

The Access assays demonstrated interference upon titration of purified sVEGF R1 into the PlGF assay and separately, PlGF into the sVEGF R1 receptor assay. As ligand:receptor molar ratios approached 1:1, significant signal reductions were observed for both assays. Further testing indicated that this was not due to cross-reactivity (data not shown) but rather to a strong preference in the assays for free analytes. Figures 3a and 3b detail the interactions observed.

The limits of reproducible detection for the Access sVEGF R1 and PlGF assays were challenged using plasma samples obtained from ten men, ten non-pregnant women and twelve pregnant women in their first trimester. All samples demonstrated low coefficients of variation which were within the precision claimed for the assays. Unlike the Quantikine microplate kit, all samples, including the male and non-pregnant female samples were within the detection limit of the Access assays. Both Access assays demonstrated significant dose separation between non-pregnant and first trimester pregnancy samples (Table 1).

The assay time, sample volume and other assay characteristics of each method are compared in Table 2.

Discussion

The Quantikine microplate ELISAs for sVEGF R1 and PlGF have been used almost exclusively by investigators to evaluate the concentrations of these molecules in preeclampsia and related complications of pregnancy. Because of this extensive research history, an understanding of the relationships between the Access automated assays for PlGF and sVEGF R1 and their Quantikine predecessor microplate assays was desired. The results reported herein show that the Access prototype assays offer significant improvements in analytical performance over the Quantikine microplate assays.

Early development of the Access prototypes included screening and identification of antibody pairs which allow detection and quantitation of free or total analyte. Data from early feasibility studies indicated that assays for free analyte provided greater separation between patients with preeclampsia and those with normal pregnancies than assays of total

analyte. Measurement of free analyte is likely to reflect the amount of biologically active molecules available. Indeed, Christinger et al determined the binding affinity (IC50) between PlGF and sVEGF R1 to be approximately 5×10^{-10}, which effectively creates a non-dissociating complex (21). That is, once PlGF binds to sVEGF R1 in circulation, the pro-angiogenic effects of PlGF are effectively blocked. Our early findings suggest that measurement of bioavailable molecules may be more useful in the identification of pregnant women at risk for preeclampsia.

Concerns about the limitations of the microplate assays have been raised by other investigators, specifically imprecision and relatively poor low-end sensitivity of the PlGF assay (8). Precision of both Access automated assays was found to be better than their microplate counterparts (Table 2). Other investigators have reported within run and between run %CVs of 3.9–9.8 and 1.4–4.7, respectively for the Quantikine sVEGF R1 assay (7, 21, 22) and within run and between run %CVs of 4.8–13.1 and 4.1–6.0, respectively, for the Quantikine PlGF assay (7, 22). The manufacturer's directions for use note within run and between run %CVs of 2.6–3.8 and 7.0–8.1 for sVEGF R1 and 3.6–7.0 and 10.9–11.8 for PlGF. Poor low-end sensitivity of the microplate PlGF assay has limited studies aimed at understanding the role of PlGF in early placental development since the concentrations detected in first trimester blood specimens are often too low to be reliably measured. Romero et al reported that, in general, PlGF was not measurable (using the Quantikine microplate method) in most patients in early pregnancy (7). Indeed, 69% of patients destined to develop preterm and term preeclampsia, as well as 84% of patients who delivered a small-for-gestational-age (SGA) neonate had undetectable plasma concentrations of PlGF in early pregnancy (first and early second trimester), whereas only 33% of controls had undetectable plasma concentrations of PlGF ($p<0.0001$). Plasma PlGF concentration became detectable on average at 10.7 weeks, 9.4 weeks and 9.7 weeks of gestation in patients with term preeclampsia, preterm preeclampsia and SGA neonates, respectively. Among normal pregnancies, the plasma concentration of PlGF became detectable on average at 8.5 weeks of gestation. The sensitivity of the Quantikine PlGF assay is 9.52 pg/mL (8) while the Access PlGF assay is <1.0 pg/mL. The ability to measure low concentrations of PlGF in the first trimester could aid in defining the relationship between PlGF and placental development and may offer insight into the genesis of preeclampsia.

Our data suggest that the method used for sVEGF R1 and PlGF quantitation will need to be clearly identified in future reports. Assays may not sufficiently agree to achieve interchangeable or biologically equivalent results. The measuring range and analytical sensitivity between platforms differ, which also contributes to discrepant interpretation. In particular, the sensitivity of the PlGF assay used may not allow for quantitation in specimens from males, non-pregnant females, and very early gestational ages. In turn, the Quantikine sVEGF R1 assay requires significant dilution of specimens from women in their third trimester or from women with preeclampsia to achieve reportable results. Because the automated Access assay for sVEGF R1 measures unbound protein whereas the microplate assay measures total sVEGF R1, analyte concentration bias between methods is expected which may affect clinical interpretation of the results. Indeed, studies using the Access free sVEGF R1 assay demonstrated improved disease state discrimination compared to published reports which used the microplate method (manuscript submitted). Additional clinical

studies are underway to determine reference ranges as well as the optimal utilization of the automated assays in clinical situations.

Access assay characteristics offer benefits over those of the corresponding Quantikine assays. The lower specimen volumes used by the Access assays offer advantages to researchers involved in clinical studies who must carefully select laboratory tests when they are limited by specimen volume. In addition, the enhanced precision eliminates the need to run duplicate measurements as recommended for the microplate assays. Another benefit of automation is speed of analysis and rapid turn around time. Microplate assays, in general, are labor intensive and not well suited for routine and rapid analysis in a clinical laboratory setting. With reduced assay time and large dynamic measuring ranges for the Access automated assays, analyzed samples do not need repeat testing.

Analytical improvements should have positive clinical implications. Beckman Coulter, Inc. has designed automated immunoassays for PlGF and sVEGF R1 with the requirements of clinicians, patients, and busy clinical laboratories in mind. The impact of improved performance (speed, reproducibility, ease of use, calibration stability, reagent lot-to-lot stability) will extend beyond the laboratory when these automated assays are adopted as diagnostic tools.

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References

1. Bersinger NA, Odegard RA. Second- and third-trimester serum levels of placental proteins in preeclampsia and small-for-gestational age pregnancies. Acta Obstet Gynecol Scand 2004; 83:37–45. [PubMed: 14678084]
2. Unal ER, Robinson CJ, Johnson DD, Chang EY. Second-trimester angiogenic factors as biomarkers for future-onset preeclampsia. Am J Obstet.Gynecol 2007; 197:211–14. [PubMed: 17689655]
3. Thadhani R, Mutter WP, Wolf M, Levine RJ, Taylor RN, Sukhatme VP, Ecker J, Karumanchi SA. First trimester placental growth factor and soluble fms-like tyrosine kinase 1 and risk for preeclampsia. J Clin Endocrin Metabol 2004; 89:770–775.
4. Robinson CJ, Johnson DD, Chang EY, Armstrong DM, Wang W. Evaluation of placenta growth factor and soluble fms-like tyrosine kinase 1 receptor levels in mild and severe preeclampsia. Am.J.Obstet.Gynecol 2006; 195:255–59. [PubMed: 16813756]
5. Chaiworapongsa T, Romero R, Espinoza J, Bujold E, Mee KY, Goncalves LF et al. Evidence supporting a role for blockade of the vascular endothelial growth factor system in the pathophysiology of preeclampsia. Young Investigator Award. Am.J Obstet Gynecol 2004; 190:1541–47. [PubMed: 15284729]
6. Ohkuchi A, Hirashima C, Matsubara S, Suzuki H, Takahashi K, Arai F et al. Alterations in placental growth factor levels before and after the onset of preeclampsia are more pronounced in women with early onset severe preeclampsia. Hypertens.Res 2007; 30:151–59. [PubMed: 17460385]
7. Teixeira PG, Cabral AC, Andrade SP, Reis ZS, da Cruz LP, Pereira JB et al. Placental growth factor (PlGF) is a surrogate marker in preeclamptic hypertension. Hypertens. Pregnancy 2008; 27:65–73. [PubMed: 18293205]

8. McKeeman GC, Ardill JES, Caldwell CM, Hunter AJ, McClure N. Soluble vascular endothelial growth factor receptor-1 (VEGFR-1) is increased throughout gestation in patients who have preeclampsia develop. Am J Obstet Gynecol 2004; 191:1240–1246. [PubMed: 15507947]

9. Tsatsaris V, Goffin F, Munaut C, Brichant JF, Pignon MR, Noel A et al. Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. J. Clin. Endocrinol. Metab 2003; 88:5555–63. [PubMed: 14602804]

10. Masuyama H, Suwaki N, Nakatsukasa H, Masumoto A, Tateishi Y, Hiramatrsu Y. Circulating angiogenic factors in preeclampsia, gestational proteinuria, and superimposed preeclampsia on chronic glomerulonephritis. Am J Obstet Gynecol 2006; 194:551–556. [PubMed: 16458660]

11. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk for preeclampsia. N Engl J Med 2004; 350:672–683. [PubMed: 14764923]

12. Krauss T, Pauer HU, Augustin HG. Prospective analysis of placental growth factor (PlGF) concentrations in the plasma of women with normal pregnancy and pregnancies complicated by preeclampsia. Hypertension in Pregnancy 2004; 23:101–111. [PubMed: 15117604]

13. Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Reez O, Mazaki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine R, Karumanchi SA. A longitundinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Maternal-Fetal Neonatal Med 2008; 21:9–23.

14. Eskild A, Nilsen TI, Jeansson S, Jenum PA, Staff AC. Changes in circulating level of angiogenic factors from the first to second trimester as predictors of preeclampsia. Am J Obstet.Gynecol 2007; 196:239–6. [PubMed: 17346536]

15. Salahuddin S, Lee Y, Vadnais M, Sachs BP, Karumanchi SA, Lim K. Diagnostic utility of soluble fms-like tyrosine kinase 1 and soluble endoglin in hypertensive diseases of pregnancy. Am J Obstet Gynecol 2007; 197;28e1–28.e1–6.

16. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science 2005; 308: 1502–1504.

17. Maynard SE, Moore Simas TA, Solitro MJ, Rajan A, Crawford S, Soderland P, Meyer B. Circulating angiogenic factors in singleton vs. multiple-gestation pregnancies. Am J Obstet Gynecol 2008; 200e1–200e7

18. Carmeliet P Mechanisms of angiogenesis and arteriogenesis. Nat Med 2000; 6 (4): 389–395. [PubMed: 10742145]

19. Taylor RN, Grimwood J, Taylor RS, McMaster MT, Fisher SJ, North RA. Longitudinal serum concentrations of placental growth factor: evidence for abnormal placental angiogenesis in pathologic pregnancies. Am.J.Obstet.Gynecol 2003; 188:177–82. [PubMed: 12548214]

20. Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J.Clin.Invest 2003; 111:649–58. [PubMed: 12618519]

21. Christinger HW, Fuh G, de Vos AM, and Wiesmann C. The crystal structure of placental growth factor in complex with domain 2 of vascular endothelial growth factor receptor-1. Biol. Chem 2004; 279 (11), 10382–10388.

22. Lim JH, Kim SY, Park SY, Yang JH, Kim MY, and Hyun Mee Ryu MH. Effective prediction of preeclampsia by a combined ratio of angiogenesis-related factors. Obstet Gynecol 2008; 111:1403–9. [PubMed: 18515525]

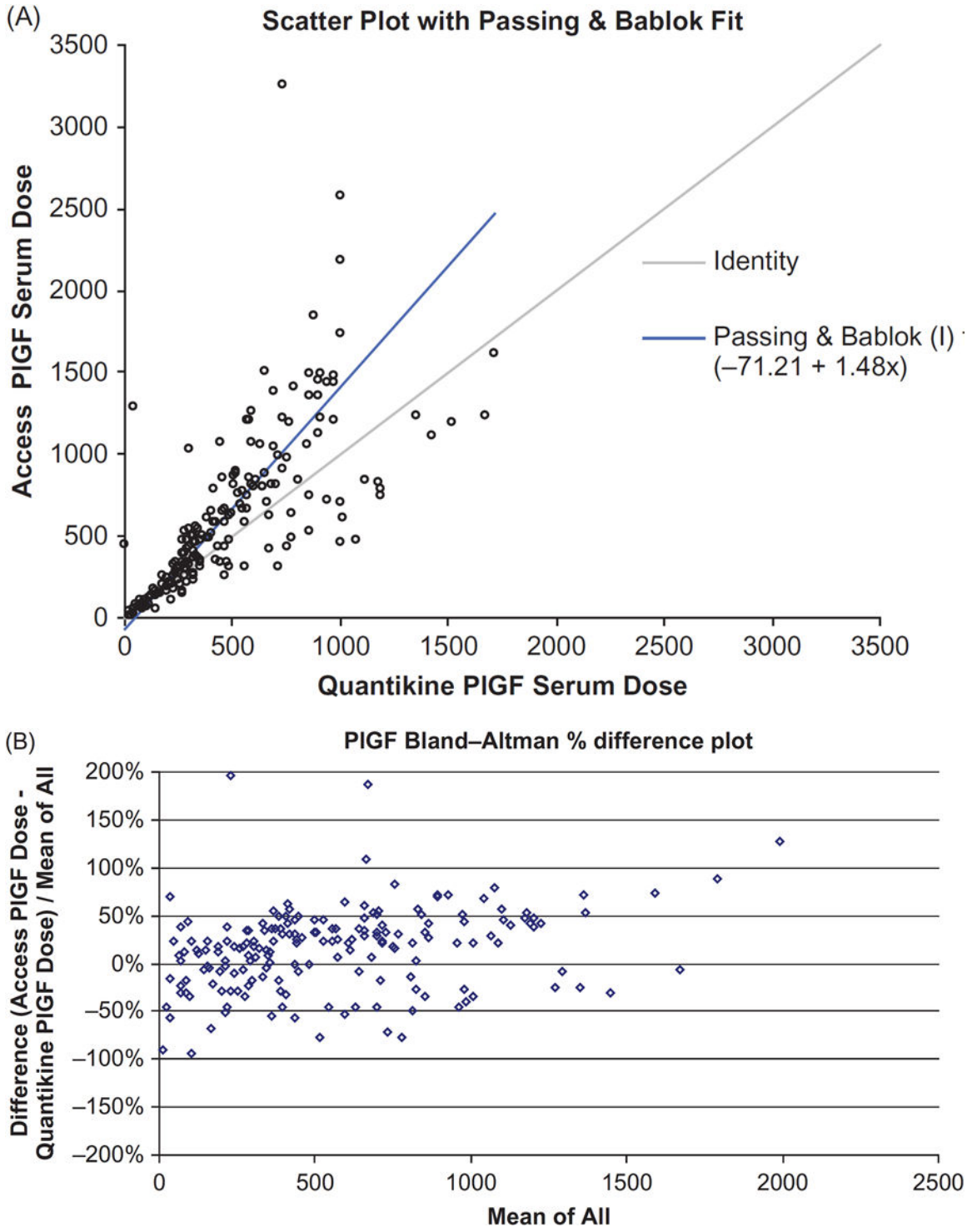

Figure 1. Agreement between Quantikine microplate PlGF assay and Access automated PlGF assay.

(A) Passing-Bablok regression analysis. **(B)** Bland–Altman plot.

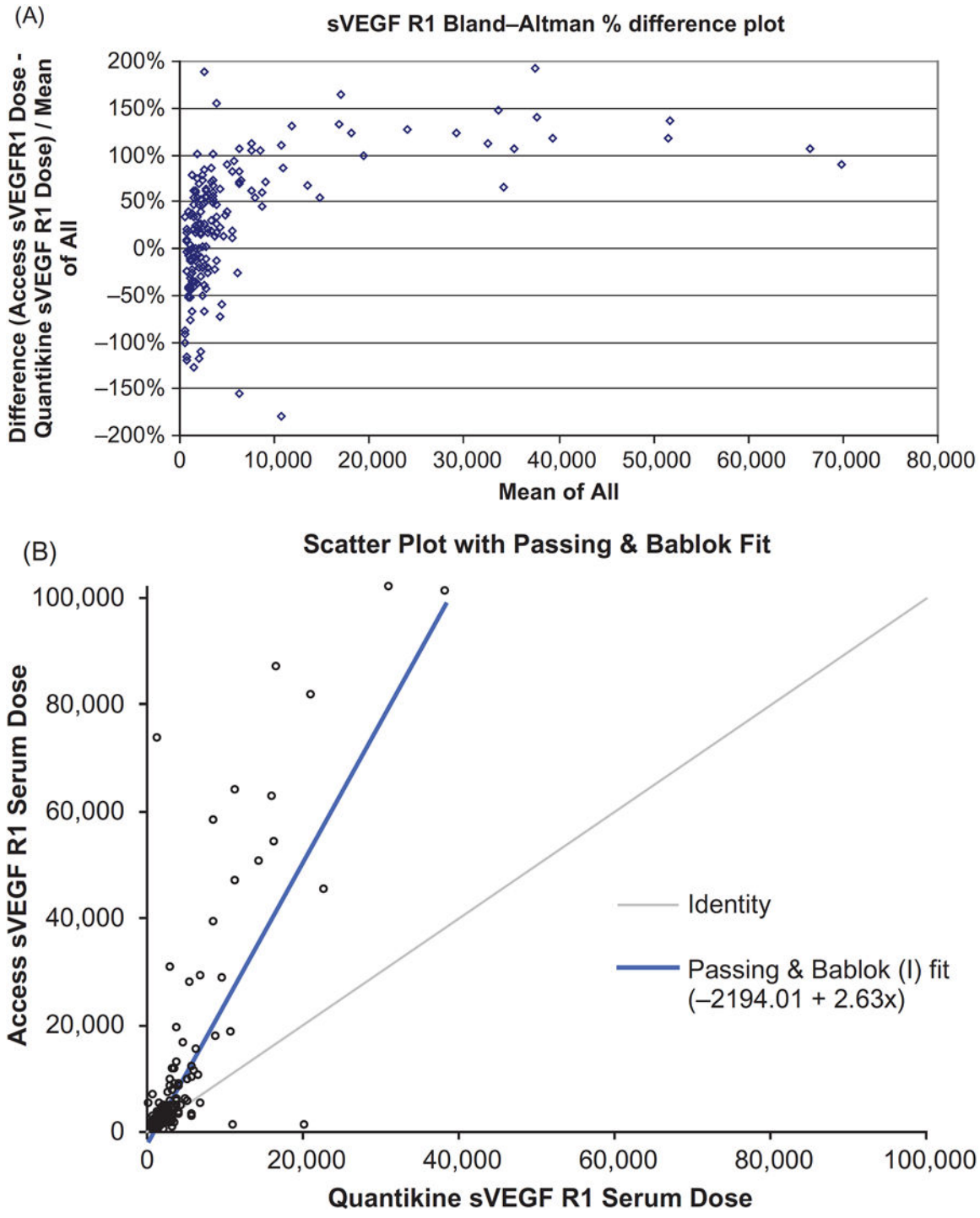

Figure 2. Agreement between Quantikine microplate sVEGF R1 assay and Access automated sVEGF R1 assay.

(A) Bland–Altman plot and **(B)** Passing-Bablok regression analysis.

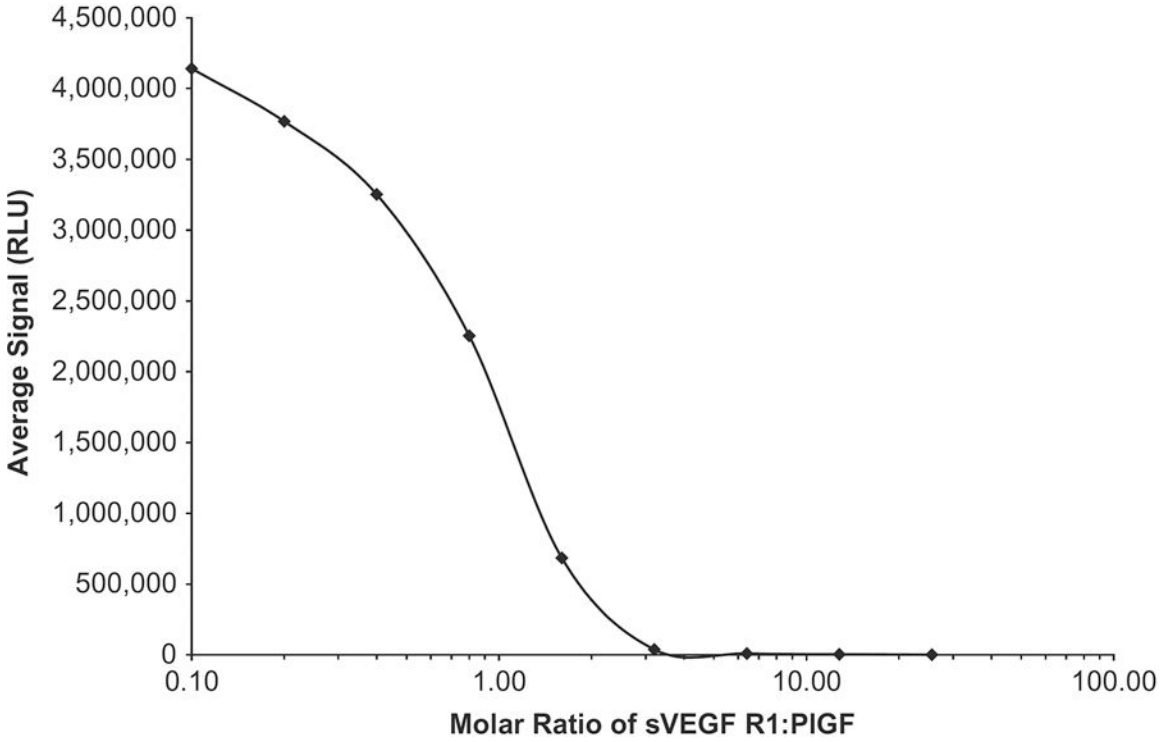

Figure 3A. sVEGF R1 Titration into Access PlGF Assay.

The Access PlGF assay signal is reduced by the presence of molar excesses of purified sVEGF R1. The Quantikine PlGF assay insert indicates a similar interference. This is consistent with a binding model in which both the Access and Quantikine assays preferentially detect the free (non-receptor bound) form of PlGF.

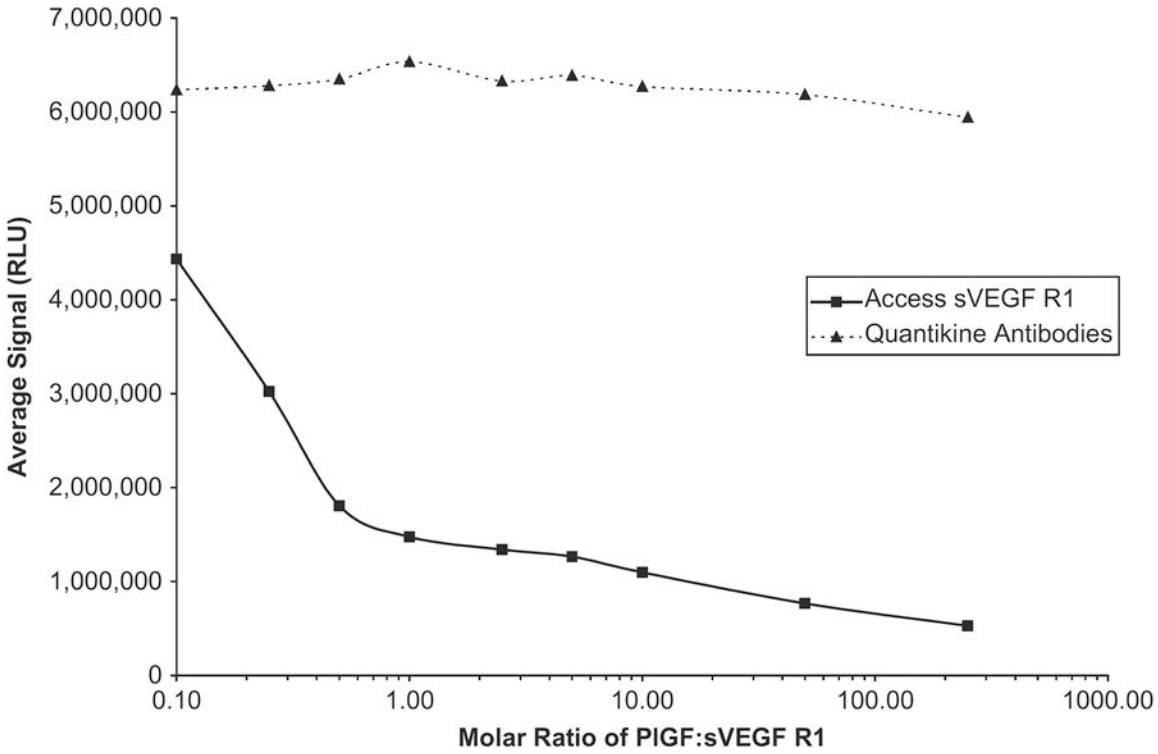

Figure 3B. PlGF titration into Access sVEGF R1 assay.

The Access sVEGF R1 assay signal is reduced by the presence of molar excesses of purified PlGF (solid line), while the antibody pair used in the Quantikine sVEGF R1 assay is not affected by PlGF concentration (dashed line). This indicates that the Quantikine assay measures total sVEGF R1 while Access preferentially measures the free, non-ligand containing form of the receptor.

Table 1.

Median concentrations of sVEGF and PlGF measured by the Access system in human samples

	sVEGF R1		PlGF	
	Median Dose (pg/mL)	**Median CV (%)**	**Median Dose (pg/mL)**	**Median CV (%)**
Male	54.74	2.30	2.87	2.81
Female non-pregnant	63.00	1.78	2.83	2.05
Female pregnant	2881.46	0.97	18.74	2.49

Table 2:

Comparison of Assay Characteristics

Characteristic	R&D Systems PlGF	Access PlGF		R&D Systems sVEGF R1	Access sVEGF R1
Assay Time	~ 4.5 hours	< 1 hour		~ 4.5 hours	< 1.5 hour
Sample Volume	100 μL	83 μL		100 μL	25 μL
Dynamic Range	15.6 – 1,000 pg/mL	1 – 5,000 pg/mL		31.2 – 2,000 pg/mL	15 – 100,000 pg/mL
With-in Run CV	3.6 – 7.0%	2.00%		2.6 – 3.8%	2.10%
Between Run CV	10.9 – 11.8%	2.49%		7.0 – 8.1%	2.80%