Simoa® Planar Array Human Angiogenesis Panel 1

SP-X™ Data Sheet Item 85-0004

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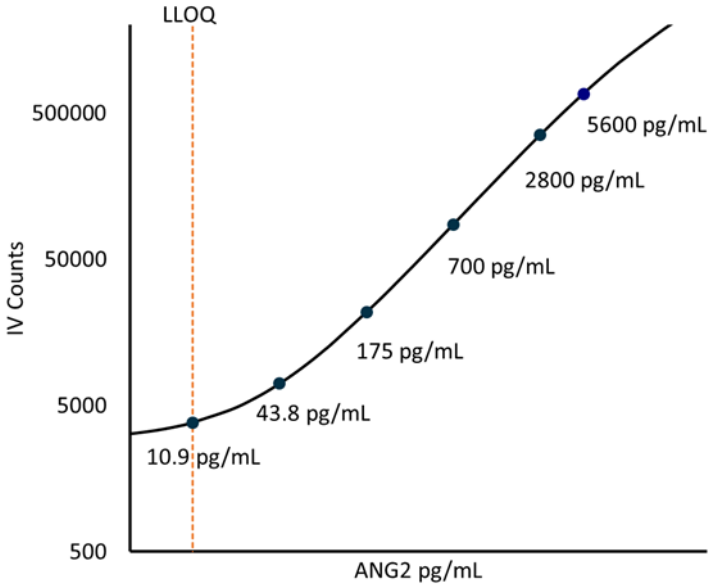
The Simoa® Planar Array Human Angiogenesis Panel 1 is a multiplex immunoassay designed for the Quanterix SP-X™ Imaging and Analysis system which simultaneously measures eight important angiogenic factors in blood. The eight targets are Angiopoietin-2 (Ang-2), Heparin-binding EGF-like growth factor (HB-EGF), Vascular endothelial growth factor C (VEGFC), Placental growth factor (PlGF), Basic fibroblast growth factor (FGFb), Hepatocyte growth factor (HGF), Platelet-derived growth factor BB (PDGFBB), and Vascular endothelial growth factor (VEGF).

Description – Angiopoietin 2 (Ang2)

There are three genuine angiopoietins (Ang1, -2 and -4) in human, which modulate vascularization via binding to the endothelial receptor Tie2, complementing the VEGF system in later stages of vascular development. They form homomeric multimers of 3 to 6. Some evidence has suggested Ang2 is an antagonist of Tie-2, yet it has also been found to complement Ang1 activation of Tie2, making the interpretation of its role complex. It modulates endothelial permeability and barrier function and Tie2 signaling aids in maintaining a quiescent, well-functioning vasculature. Secreted by endothelium, plasma levels of Ang-2 are elevated in inflammatory and angiogenic diseases including sepsis, malaria, and cancer. Ang-2 expression has been documented in a range of human cancers, including glioblastoma, melanoma, prostate adenocarcinoma, and renal cell carcinoma.

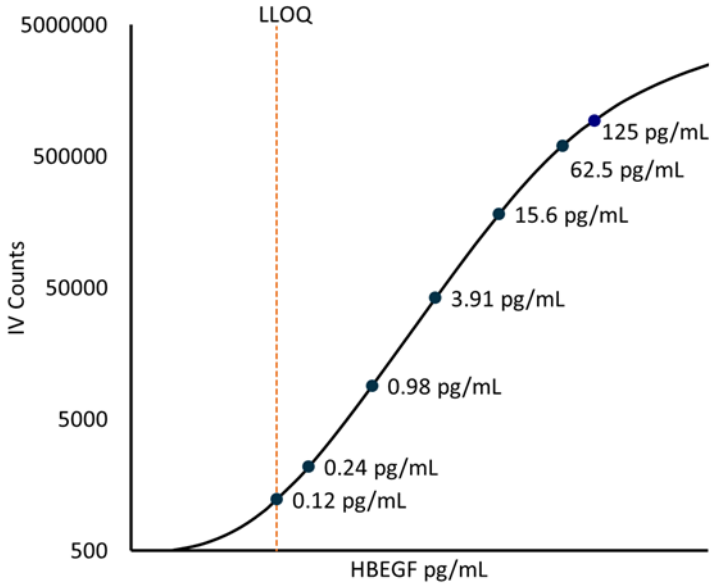
Ang 2: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – Heparin binding EGF like growth factor (HBEGF)

HBEGF is related to EGF (epidermal growth factor) and acts as a potent mitogen and chemotactic factor for smooth muscle cells and fibroblasts. The soluble, mature form is cleaved from a transmembrane version and activates EGF receptor subtypes HER1 and HER4. HBEGF acts in normal physiological processes like blastocyte implantation and wound healing, in which it is down-regulated by miR-132 during the transition from inflammation to proliferation; and the pathological processes of tumor growth, hyperplasia, atherosclerosis and diphtheria in which it functions as the receptor for the toxin. In mice, *in vivo* administration of HBEGF has beneficial effects in models of pre-term brain injury and diabetes, and chronic tympanic membrane perforations. It signals through autocrine, paracrine and juxtacrine activity when packaged in exosomes.

HBEGF: Calibrator concentrations and Lower Limit of Quantification depicted.



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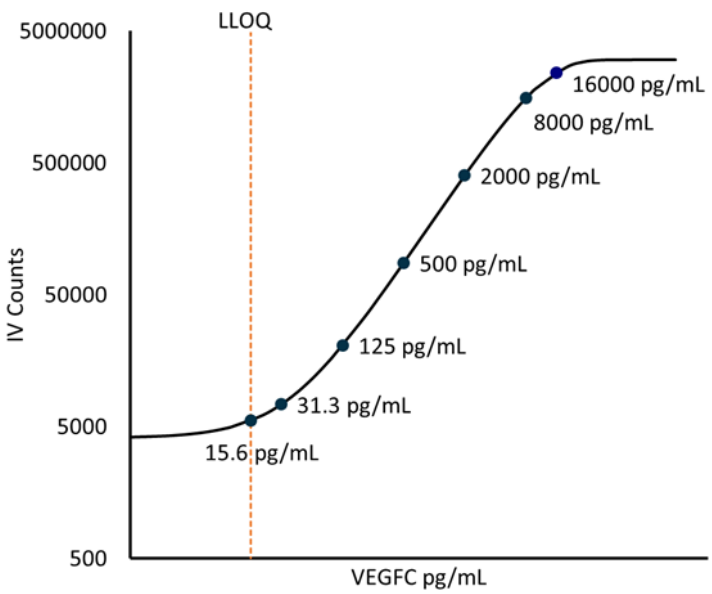
Description – Vascular endothelial growth factor C (VEGFC)

Vascular endothelial growth factor C (VEGF-C) is a member of the platelet-derived growth factor / vascular endothelial growth factor (PDGF/VEGF) family. It promotes lymphogenesis and angiogenesis through its receptors, VEGFR-3 and VEGFR-2, and plays an important role in both neuronal development and blood pressure regulation. Lack of VEGF-C results in lymphedema, whilst increased expression of VEGF-C is implicated in tumor angiogenesis and metastasis.

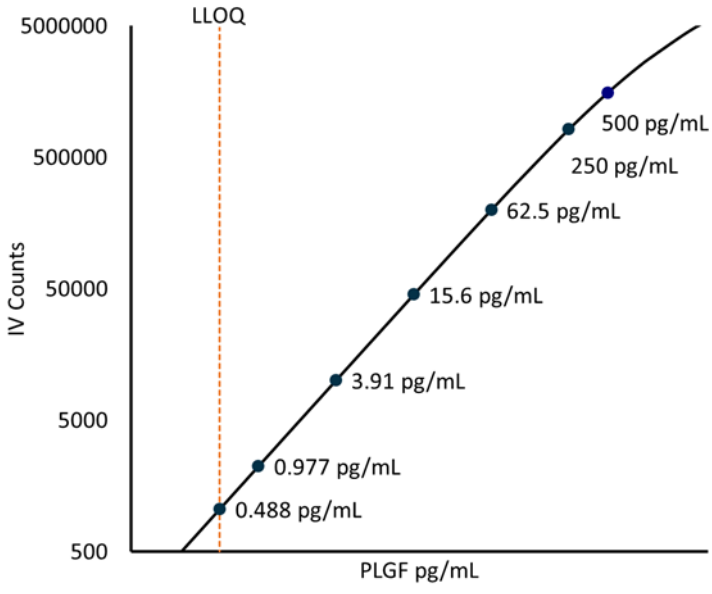
Description – Placental growth factor (PlGF)

PlGF, a member of the VEGF family of cytokines, plays important roles in the development and growth of the vascular or lymphatic endothelia. PlGF, secreted as a homodimer but may also form PlGF/VEGF heterodimers, synergistically enhances VEGF-induced angiogenesis and vascular permeability. PlGF is expressed mainly in placental trophoblast during pregnancy, and may also be expressed in vascular cells, fibroblasts, leukocytes, hepatocytes, bone marrow-derived cells, neurons, epithelial cells and tumor cells. Serum levels of PlGF peak during pregnancy at weeks 26 – 28, and low serum PlGF is associated with the development of preeclampsia later in pregnancy. PlGF is a potential prognostic marker for tumor progression in several types of cancer, including renal, colorectal, gastric, breast, and lung. PlGF also plays a role in inflammatory conditions such as atherosclerosis and rheumatoid arthritis. PlGF is elevated in individuals with Sickle Cell Disease (SCD) and thought to play a role in the pathophysiology of pulmonary hypertension in patients with SCD.

VEGFC Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



PLGF Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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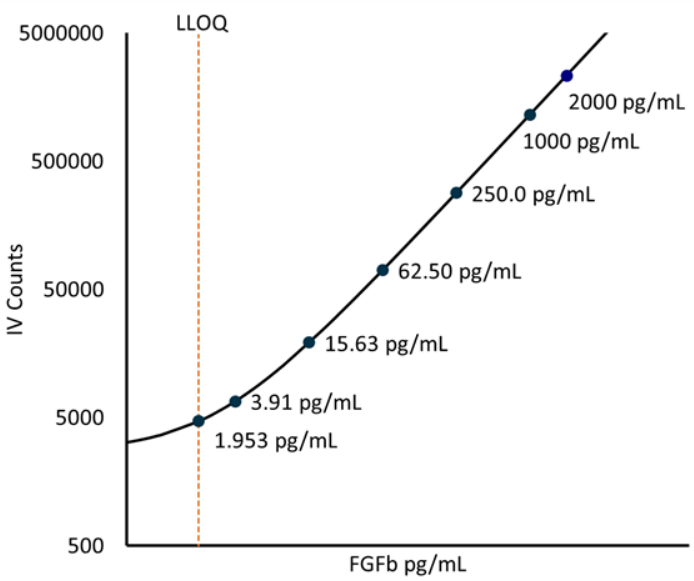
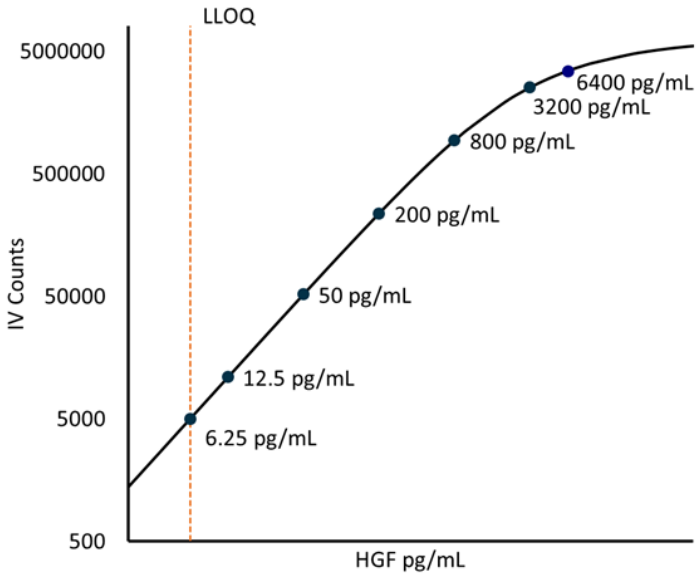
Description – Basic fibroblast growth factor (FGFb)
FGFb (Basic fibroblast growth factor, also known as FGF2 and FGF-β) is a growth factor and signaling protein encoded by the FGF2 gene. FGFb binds to and exerts its effects via the fibroblast growth factor receptor (FGFR). It possesses broad mitogenic and cell survival activities, and is involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion.

Description – Hepatocyte growth factor (HGF)
Hepatocyte growth factor (HGF) is a pleiotropic growth factor secreted by mesenchymal stromal cells, which targets and acts upon epithelial cells, endothelial cells, haemopoietic progenitor cells and T cells. HGF regulates cell growth, cell motility, and morphogenesis by activating a tyrosine kinase signaling cascade after binding to its receptor, tyrosine-protein kinase Met (c-MET). HGF is involved in the regulation of growth, motility, and morphogenesis of various cell types, and plays an important role in wound-healing, tissue or organ regeneration, angiogenesis, and carcinogenesis.

FGFb Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



HGF Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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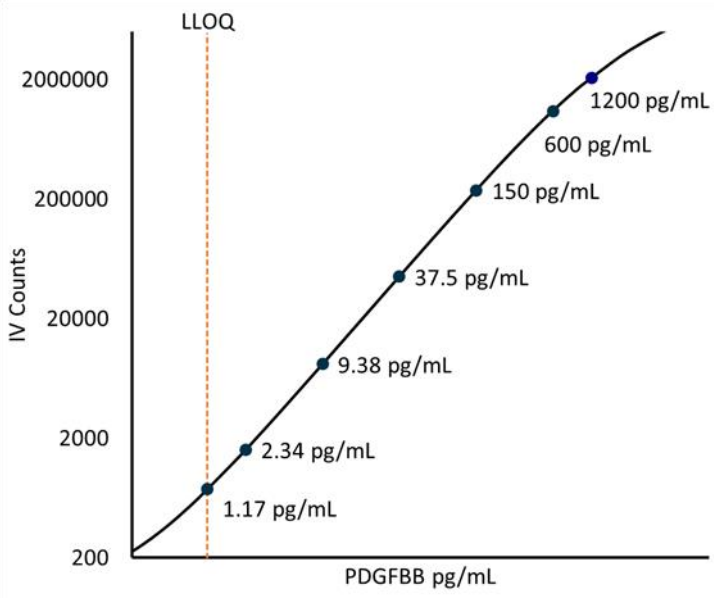
Description – Platelet-derived growth factor BB (PDGFBB)

The PDGF family is related to VEGF and composed of members PDFG-AA, -AB, -BB (disulfide-linked dimers of polypeptide chains A or B), -C and -D. PDGFs act as serum growth factor for fibroblasts, smooth muscle cells (SMCs), and glia cells, and have crucial roles during development but no known normal physiological function in the adult. PDGF-BB binding promotes homo- and hetero-dimerization of the tyrosine kinase receptor domains PDGFR-α and PDGFR-β, although *in vivo* there is little evidence for PDGF-AB or PDGFR- α β dimerization. PDGF-B is mainly expressed in vascular endothelial cells, megakaryocytes, and neurons. PDGF activity has been implicated in a variety of cancers, and numerous studies have demonstrated that PDGF-B/PDGFR-β autocrine signaling promotes self-sufficiency in cellular growth signals. They have also been associated with pulmonary hypertension, retinal vascular disease and fibrotic diseases. PDGF-BB has been clinically used to improve wound healing through topical treatment.

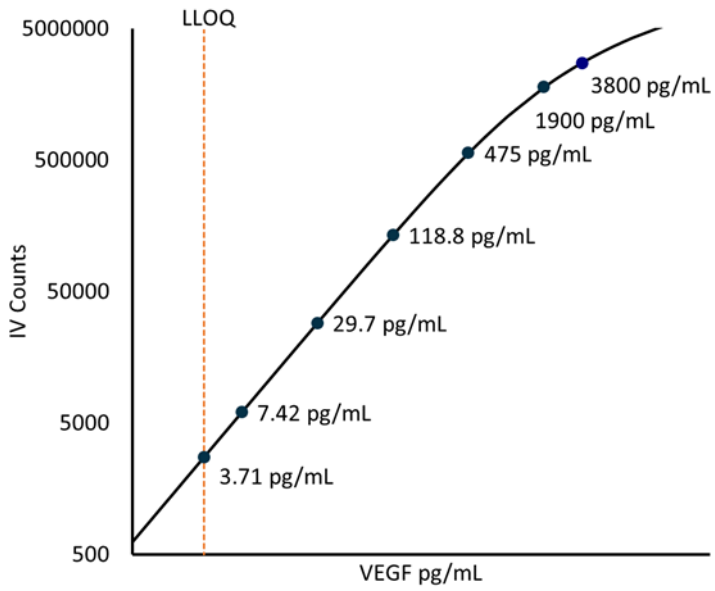
PDGFBB Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



Description – Vascular endothelial growth factor (VEGF)

Vascular endothelial growth factor (VEGF) is a 27KDa signaling protein produced by cells that stimulates vasculogenesis and angiogenesis. The VEGF family includes VEGF-A (VEGF), VEGF-B, PlGF, VEGF-C and VEGF-D. VEGF is a heparin binding protein and exists as a disulfide linked homo-dimer. It has at least 6 isoforms produced by alternative splicing. The Simoa Human VEGF assay detects the 165 amino acid form of the factor (VEGF-165), which is the most common form in tissues. VEGF induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis, and induces permeabilization of blood vessels. Though VEGF acts primarily on endothelial cells, it also binds to receptors on HSCs, monocytes, osteoblasts and neuronal cells. VEGF is also involved in vasodilation through induction of endothelial nitric oxide synthase and the subsequent increase in nitric oxide production. Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus. When VEGF is over-expressed, it can contribute to disease; cancers that can express VEGF are able to grow and metastasize.

VEGF Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



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Minimum Required Dilution (MRD) and Tests per kit

Diluted Sample volume (1:4 Dilution) *	50 µL per measurement
Tests per kit	96

***See Kit Instruction for details**

Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve across 3 reagent lots (13 runs total). Analytical Lower Limit of Quantification (LLOQ) is the lowest calibration standard with back-calculated concentration pooled CV <20% and relative error <25%.

	Analytical LLOQ	Functional LLOQ (x MRD)
ANG2	**10.9 pg/mL** pooled CV 13% mean recovery 112%	**43.8 pg/mL**
HBEGF	**0.122 pg/mL** pooled CV 6% mean recovery 95%	**0.488 pg/mL**
VEGFC	**15.6 pg/mL** pooled CV 13% mean recovery 112%	**62.5 pg/mL**
PLGF	**0.488 pg/mL** pooled CV 8% mean recovery 87%	**1.95 pg/mL**
FGFb	**1.95 pg/mL** pooled CV 18% mean recovery 86%	**7.81 pg/mL**
HGF	**6.25 pg/mL** pooled CV 3% mean recovery 95%	**25.0 pg/mL**
PDGFBB	**1.17 pg/mL** pooled CV 10% mean recovery 85%	**4.69 pg/mL**
VEGF	**3.71 pg/mL** pooled CV 10% mean recovery 90%	**14.8 pg/mL**

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve across 3 reagent lots (13 runs total).

	LOD
ANG2	**10.5 pg/mL** range 7.0 – 4.1
HBEGF	**0.046 pg/mL** range 0.025 – 0.081
VEGFC	**5.67 pg/mL** range 3.29 – 8.26
PLGF	**0.13 pg/mL** range 0.08 – 0.20
FGFb	**1.37 pg/mL** range 0.68 – 1.81
HGF	**0.74 pg/mL** range 0.25 – 0.94
PDGFBB	**0.46 pg/mL** range 0.10 – 0.89
VEGF	**0.88 pg/mL** range 0.51 – 1.15

Assay Ranges: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD.

	Assay Range
ANG2	0 – 22400 pg/mL
HBEGF	0 – 500 pg/mL
VEGFC	0 – 64000 pg/mL
PLGF	0 – 2000 pg/mL
FGFb	0 – 8000 pg/mL
HGF	0 – 25600 pg/mL
PDGFBB	0 – 4800 pg/mL
VEGF	0 – 15200 pg/mL

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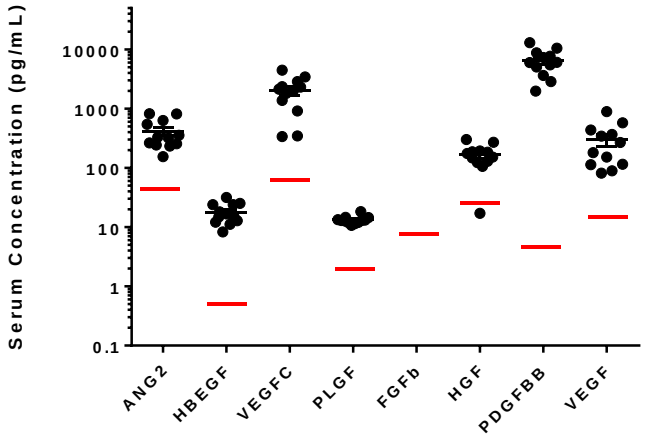
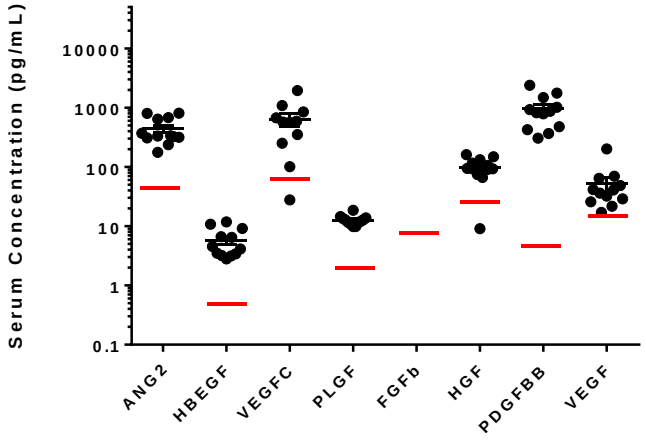
Endogenous Serum and Plasma Readings: Healthy donor matched EDTA plasma (n=12) and serum (n=12) samples were measured. Bars depict median with interquartile range. Red lines represent functional LLOQ.





	Sample Type	Median Conc pg/mL	% Above LOD	% Above LLOQ
ANG2	Serum	326.5	100%	100%
	EDTA	334.5	100%	100%
HBEGF	Serum	15.8	100%	100%
	EDTA	4.3	100%	100%
VEGFC	Serum	2074.0	100%	100%
	EDTA	573.5	92%	83%
PLGF	Serum	12.9	100%	100%
	EDTA	12.5	100%	100%
FGFb	Serum	ND	0%	0%
	EDTA	ND	0%	0%
HGF	Serum	163.5	100%	92%
	EDTA	94.0	100%	92%
PDGFBB	Serum	6039.5	100%	100%
	EDTA	847.9	100%	100%
VEGF	Serum	225.1	100%	100%
	EDTA	38.5	100%	100%

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Precision: Measurements of 3 serum or plasma-based panels and 2 calibrator-based controls. Triplicate measurements were made across 3 reagent lots (13 runs total).

Mean (pg/mL)	ANG2	HBEGF	VEGFC	PLGF	FGFb	HGF	PDGFBB	VEGF
Control 1	2783	59.7	7390	210	910	3110	617	1692
Control 2	666	16.0	1778	56.3	257	779	170	449
Control 3	ND	1.22	127	4.23	17.5	63.4	10.4	32.2
Panel 1	135	10.6	1678	12.8	1633	83.4	1352	88.6
Panel 2	244	3.83	391	18.2	558	183	545	37.8

Inter-run CV	ANG2	HBEGF	VEGFC	PLGF	FGFb	HGF	PDGFBB	VEGF
Control 1	3.3%	6.9%	6.0%	5.5%	11.1%	3.6%	6.4%	5.9%
Control 2	9.3%	11.7%	8.7%	8.2%	13.0%	8.5%	11.1%	9.7%
Control 3	ND	18.5%	17.9%	18.2%	19.5%	14.4%	15.8%	15.0%
Panel 1	16.6%	14.5%	11.6%	16.2%	8.5%	12.5%	5.1%	15.6%
Panel 2	14.1%	19.2%	17.4%	14.3%	16.4%	13.1%	7.1%	17.1%

Intra-run CV	ANG2	HBEGF	VEGFC	PLGF	FGFb	HGF	PDGFBB	VEGF
Control 1	2.0%	2.3%	6.5%	3.2%	3.9%	2.3%	2.1%	1.6%
Control 2	2.5%	2.2%	2.2%	3.9%	6.9%	4.5%	4.5%	1.2%
Control 3	ND	4.2%	16.9%	5.2%	9.2%	2.4%	6.3%	2.0%
Panel 1	6.3%	1.9%	6.0%	2.1%	4.3%	4.1%	2.1%	1.9%
Panel 2	4.0%	3.0%	9.9%	1.9%	4.2%	2.2%	2.5%	2.7%

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Spike and Recovery: 5 EDTA plasma samples and 6 serum samples were spiked at high and low concentrations within the range of the assay.

ANG2	**Mean 70%** range 40%–92%
HBEGF	**Mean 103%** range 96%–112%
VEGFC	**Mean 89%** range 36%–120%
PLGF	**Mean 79%** range 67%–95%
FGFb	**Mean 64%** range 22%–94%
HGF	**Mean 79%** range 64%–100%
PDGFBB	**Mean 82%** range 51%–103%
VEGF	**Mean 86%** range 70%–101%

Dilution Linearity: 4 spiked serum and plasma samples were diluted 4x according to the MRD, and then serially diluted 2x with Sample Diluent six times, for final dilution of 1:128.

ANG2	**Mean 124%** range 96%–174%
HBEGF	**Mean 99%** range 77%–112%
VEGFC	**Mean 127%** range 90%–235%
PLGF	**Mean 126%** range 101%–144%
FGFb	**Mean 131%** range 100%–198%
HGF	**Mean 133%** range 109%–169%
PDGFBB	**Mean 171%** range 118%–312%
VEGF	**Mean 118%** range 102%–145%

Parallelism: 2 serum and plasma samples (not spiked) were diluted 2X serially from 4x (MRD) to 128x with Sample Diluent.

ANG2	**Mean 111%** range 75%–152%
HBEGF	**Mean 95%** range 65%–127%
VEGFC	**Mean 172%** range 100%–244%
PLGF	**Mean 121%** range 84%–156%
FGFb	**Not Detectable**
HGF	**Mean 125%** range 104%–145%
PDGFBB	**Mean 225%** range 133%–386%
VEGF	**Mean 123%** range 98%–147%

Single-plex Correlation: Sample concentrations derived from single-plex standard curves were compared to the same samples calculated from the 8-plex standard curve. The average correlation between multi-plex and single-plex assays over the entire dynamic range is shown in the table below.

	Correlation
ANG2	82%
HBEGF	99%
VEGFC	114%
PLGF	117%
FGFb	90%
HGF	83%
PDGFBB	104%
VEGF	88%

Cross-reactivity: During assay validation, cross-reactivity was assessed by testing single antigen at the concentration of the third highest calibrator in the presence of all detection antibodies, and single detection antibodies in the presence of all antigens in assay buffer. In addition, cross-reactivity of single detection antibodies was assessed in sample matrix. All plexes showed less than 1.2% cross-reactivity.

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