

Appendix 1. List of Biomarker Abbreviations and Units

Biomarker	Biomarker full name	Units
ADAM 9	Disintegrin and metalloproteinase domain-containing protein 9	pg/ml
Angiogenin	Angiogenin	µg/ml
ANP	Natriuretic peptide A	ng/ml
Arginase-1	Arginase 1	ng/ml
Arginase-2	Arginase 2	ng/ml
BNP	Brain natriuretic peptide	ng/ml
Caspase	Caspase	ng/ml
CCL23	MIP3, C motif chemokine 23	ng/ml
C-Met-109a	Tyrosine kinase	ng/ml
C-Met-111a	Tyrosine kinase	ng/ml
CPA-4	Carboxypeptidase A4	ng/ml
CRP	C reactive protein	µg/ml
CXCL10	CXC motif chemokine 10	ng/ml
Cystatin	Cystatin	ng/ml
Elafin-131	Elafin	ng/ml
Elafin-132	Elafin	ng/ml
Endoglin	Endoglin	ng/ml
Endothelin	Endothelin	pg/ml
Ephrin	Ephrin	pg/ml
ESAM-1	Endothelial Cell-selective adhesion molecule	ng/ml
FAS	Tumour necrosis factor receptor superfamily member 6	ng/ml
FasL	Tumour necrosis factor ligand superfamily member 6	ng/ml
FIH	Hypoxia inducible factor 1-alpha inhibitor	ng/ml
HbF	Haemoglobin Fetal	ng/ml
ICAM-1	Intercellular adhesion molecule 1	ng/ml
IL-1ra	Interleukin 1 receptor antagonist	pg/ml
Kunitz-2 (HAI-2) 34a	Kunitz-type protease inhibitor 2	ng/ml
Kunitz-2 (HAI-2) 35b	Kunitz-type protease inhibitor 2	ng/ml
Kunitz-2 (HAI-2) 40b	Kunitz-type protease inhibitor 2	ng/ml

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Leptin	Leptin	ng/ml
Leptin receptor	Leptin receptor	ng/ml
MIF-49a	Macrophage migration inhibitory factor	ng/ml
MIF-49b	Macrophage migration inhibitory factor	ng/ml
MMP-9	Matrix metalloproteinase-9	ng/ml
Nephrin-100a	Nephrin	ng/ml
Nephrin-101a	Nephrin	ng/ml
NGAL	Neutrophil gelatinase-associated lipocalin	ng/ml
PAI-1	Plasminogen activator inhibitor 1	ng/ml
PAI-2	Plasminogen activator inhibitor 2	ng/ml
PAPP-A	Pregnancy specific plasma protein A	ng/ml
PCT-95a	Procalcitonin	pg/ml
PCT-99b	Procalcitonin	pg/ml
Pentraxin-3-64a	Pentraxin-related protein PTX3	ng/ml
Pentraxin-3-67a	Pentraxin-related protein PTX3	ng/ml
Periostin	Periostin	ng/ml
PIGF	Placental Growth Factor	pg/ml
Podocalyxin	Podocalyxin	ng/ml
sFlt-1	Soluble fms-like tyrosine kinase-1	ng/ml
ST2-116b	Interleukin-1 receptor-like 1	ng/ml
ST2-75b	Interleukin-1 receptor-like 1	ng/ml
TGFβ-1	Transforming growth factor beta-1	ng/ml
TIMP-1	Metelloproteinase Inhibitor 1	ng/ml
TNFR1A	Tumour necrosis factor receptor superfamily member 1A	ng/ml
VEGF-C	Vascular endothelial growth factor C	ng/ml
Visfatin	Visfatin	ng/ml
WAP4C-HE4-85b	WAP four disulfide core domain protein 2	ng/ml
WAP4C-HE4-91a	WAP four disulfide core domain protein 2	ng/ml

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Appendix 2.

The additional 57 biomarker assays were analysed in a central laboratory facility (Alere, San Diego, CA) at room temperature, following development of the assays to determine optimal formatting (i.e. sandwich format, competitive, diluted format, single analyte) with customisation of the Luminex assays to perform with each other on a single format, usually as a non-diluted neat assay optimized for each analyte. A list of biomarker assay information (low and high cut-offs, assay coefficient variable and assay format) is given in table S2.

The Luminex sandwich assays used a mouse-derived recombinant Fab conjugated to a magnetic bead as the capture and a biotin-conjugated recombinant Fab as the assay detection, optimised for use in Luminex xMap technology (Alere, San Diego). Recombinant Fab conjugated magnetic beads were added to the plate and washed. Sample was then incubated with the beads, followed by incubation with the detection antibody. The plate was washed, incubated with streptavidin-labeled phycoerythrin, washed and then read using a Luminex 200 reader. The Luminex competitive assay format used a recombinant Fab conjugated to the bead and a biotin-conjugated antigen as the assay detection.

Recombinant Fab conjugated magnetic beads were added to the plate and washed. The sample and detection reagent were premixed, added simultaneously and then incubated. The final steps were as described for the Luminex sandwich assay.

The micro-titer ELISA assays used a streptavidin coated plate and biotin or fluorescein conjugated recombinant Fabs. The ELISA sandwich assay used a biotin-conjugated recombinant Fab as the capture and a fluorescein-conjugated recombinant Fab as the detection antibody. Capture antibody was coated on the plate, incubated, washed and sample added. After sample incubation, the plate was washed and then incubated with detection antibody. Following washing, the plate was incubated with anti-fluorescein antibody conjugated to alkaline phosphatase, washed, fluorescent substrate added and then read using a Tecan infinite F200 reader.

The ELISA competitive assay used a biotin-conjugated antigen as the capture and a fluorescein-conjugated recombinant Fab as the detection antibody. The plate was coated with capture antigen, incubated and washed. Addition of sample was immediately followed

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by addition of the detection antibody and incubated. The final steps were the same as the ELISA sandwich.

Each assay used an eight-point dose curve prepared gravimetrically in EDTA plasma or buffer. Immunoassays utilizing human plasma were performed in 384-well microtitre plates using Perkin-Elmer Minitrak robotic liquid handling system for all liquid handling steps. Test samples were added to the 384-well plate, containing wells for a calibration curve consisting of multiple analyte concentrations and control samples. Calibration curves were prepared gravimetrically in plasma from healthy donors. For sandwich assays, one concentration in each set of calibrators included neutralizing antibody for correction of endogenous antigen present in the plasma pool.

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Appendix 3. Biomarker Assay Information

Biomarker	Low Cutoff	High Cutoff	Assay Coefficient Variable	Assay format
PIGF	12	3000	13	Sandwich, Luminex
ADAM-9	38.07	7913.74	11	Sandwich, Luminex
Angiogenin	0.14	61.24	7	Competitive, Luminex
ANP	0.048	71.93	13	Sandwich, Luminex
Arginase-1-10a	0.035	30.50	9	Sandwich, Luminex
Arginase-2-11a	1.318	378.14	15	Sandwich, Luminex
BNP	0.007	5.83	18	Sandwich, Luminex
Caspase	0.292	114.29	13	Sandwich, Luminex
CCL23	0.009	3.52	7	Sandwich, Luminex
C-Met-109a	7.999	453.54	11	Sandwich, Luminex
C-Met-111a	77.147	1035.48	7	Sandwich, Luminex
CPA-4	0.119	19.14	5	Sandwich, Luminex
CRP	0.07871	141.96	5	Competitive, Luminex
CXCL10	0.006	5.81	10	Sandwich, Luminex
Cystatin	165.009	9072.87	21	Competitive, Microtitre
Elafin-131	28.670	42668.61	10	Competitive, Luminex
Elafin-132	21.159	42668.61	5	Competitive, Luminex
Endoglin	1.981	654.84	18	Sandwich, Microtitre
Endothelin-1	0.704	901.9	13	Sandwich, Luminex
Ephrin	43.71	4009.97	20	Sandwich, Luminex
ESAM-1	1.073	32.77	9	Sandwich, Luminex
FAS	0.115	152.86	22	Sandwich, Luminex
FasL	0.156	30.20	11	Sandwich, Luminex
FIH	0.003	18.50	6	Sandwich, Luminex
HbF	0.848	386.32	18	Sandwich, Microtitre
ICAM-1	106.275	30231.73	6	Competitive, Luminex
IL-1ra	0.477	1434.20	9	Sandwich, Luminex
Kunitz-2-34a	0.016	10.17	19	Sandwich, Luminex

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Kunitz-2-35b	0.140	57.15	13	Sandwich, Luminex
Kunitz-2-40b	0.159	57.38	7	Sandwich, Luminex
Leptin	5.509	148.56	11	Sandwich, Luminex
Leptin receptor	2.244	1079.61	9	Sandwich, Luminex
MIF-49a	3.912	25.99	10	Sandwich, Luminex
MIF-49b	0.414	70.85	9	Sandwich, Luminex
MMP-9	4.542	202.82	5	Sandwich, Luminex
Nephrin-100a	0.517	19.98	25	Sandwich, Luminex
Nephrin-101a	0.094	19.74	22	Sandwich, Luminex
NGAL-MT	0.625	2924.00	22	Sandwich, Microtitre
PAI-1-52b	0.194	103.48	12	Sandwich, Luminex
PAI-2	0.047	77.90	5	Sandwich, Luminex
PAPP-A	0.189	812.10	7	Sandwich, Luminex
PCT-95a	12.22	9165.34	14	Sandwich, Luminex
PCT-99b	9.55	3982.50	14	Sandwich, Luminex
Pentraxin-3-64a	0.221	91.01	10	Sandwich, Luminex
Pentraxin-3-67a	0.940	59.41	16	Sandwich, Luminex
Periostin	0.538	107.38	7	Sandwich, Luminex
Podocalyxin	0.075	20.79	15	Sandwich, Luminex
sFlt-1	0.006	27.86	10	Sandwich, Luminex
ST2-116b	0.038	21.25	14	Sandwich, Luminex
ST2-75b	0.075	44.63	6	Sandwich, Luminex
TGFβ-1	0.040	63.98	9	Sandwich, Luminex
TIMP-1	9.127	1917.45	5	Competitive, Luminex
TNFR-1A	0.230	31.02	18	Sandwich, Luminex
VEGF-C	0.527	74.07	5	Sandwich, Luminex
Visfatin	2.535	1738.71	13	Sandwich, Luminex
WAP4C-HE4-85b	0.129	89.71	11	Sandwich, Luminex
WAP4C-HE4-91a	1.516	54.27	6	Sandwich, Luminex

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Appendix 4. Results of Factor Analysis: Loadings of Biomarkers on Five Largest Factors (Eigenvalues > 2) After Varimax Rotation Showing Loadings > 0.6 Only and Uniqueness > 0.6

Biomarker	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Uniqueness
ADAM-9		0.89				0.17
ANP	0.72					0.32
Arginase-2			0.82			0.20
BNP			0.63			0.35
Caspase					0.70	0.25
CCL23	0.62					0.46
Cystatin C						0.77
Elafin						0.85
Endoglin				0.81		0.29
ESAM-1	0.76					0.36
FAS	0.67					0.40
FasL		0.85				0.24
FIH					0.73	0.40
Kunitz-2	0.73					0.36
Leptin						0.67
MIF-49b					0.64	0.44
Nephrin			0.87			0.15
NGAL						0.63
PAI-1		0.67				0.33
PAPP-A						0.70
Pentraxin			0.65			0.31
Pentraxin		0.73				0.31
PIGF				0.83		0.31
Podocalyxin			0.78			0.19
Procalcitonin			0.80			0.26
sFlt-1				0.86		0.15

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TGFβ-1	0.75					0.37
TIMP-1					0.70	0.41
TNFR-1A	0.82					0.31
WAP4C-HE4-85b	0.70					0.25
WAP4C-HE4-91a	0.77					0.33

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Appendix 5. Odds Ratios for Preeclampsia Requiring Delivery Within 14 Days of Sampling Derived From Multiple Logistic Regression Analysis of the Five Factors

Factor	Biomarkers contained in factor	Odds Ratio (95% confidence intervals)
1	ANP, CCL23, ESAM-1, FAS, TGF β -1, TNFR-1A, WAP4C-HE4-85b, WAP4C-HE4-91a	1.30 (0.91 to 1.86)
2	ADAM-9, Ephrin, FasL, Kunitz 35b , Kunitz 40b, Nephrin, PAI-1, Pentraxin	0.63 (0.36 to 1.10)
3	Arginase-2, BNP, Nephrin, PCT-95a, Pentraxin, Podocalyxin	1.61 (1.07 to 2.42)
4	PIGF, Endoglin, sFlt-1	12.6 (6.44 to 24.74)
5	Caspase, FIH, MIF, TIMP-1	1.32 (0.91 to 1.92)

Odds ratios are for a change of one standard deviation in the factor score.

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Appendix 6. Individual Biomarker ROC Areas (95% Confidence Intervals) for Pre-eclampsia Requiring Delivery Within 14 Days of Sampling in Women Presenting Between 20⁺⁰ and 34⁺⁶ Weeks of Gestation, Listed in descending ROC Area

Biomarkers	ROC areas (95% confidence intervals)
ADAM-9	0.51 (0.46 to 0.55)
Angiogenin	0.55 (0.47 to 0.63)
ANP	0.70 (0.63 to 0.77)
[Arginase-1]	0.54 (0.46 to 0.62)
Arginase-2	0.56 (0.48 to 0.64)
BNP	0.75 (0.69 to 0.82)
Caspase	0.56 (0.48 to 0.64)
[CCL23]	0.54 (0.47 to 0.61)
[C-Met-109a]	0.56 (0.49 to 0.64)
[C-Met-111a]	0.55 (0.48 to 0.63)
[CPA-4]	0.63 (0.56 to 0.70)
CRP	0.52 (0.45 to 0.60)
CXCL10	0.58 (0.49 to 0.66)
Cystatin	0.68 (0.61 to 0.75)
Elafin 131	0.50 (0.41 to 0.58)
Elafin 132	0.50 (0.42 to 0.58)
Endoglin	0.83 (0.79 to 0.88)
[Endothelin]	0.58 (0.51 to 0.66)
[Ephrin]	0.51 (0.48 to 0.53)
[ESAM-1]	0.51 (0.43 to 0.58)
[FAS]	0.50 (0.43 to 0.58)
[FasL]	0.56 (0.50 to 0.61)
FIH	0.55 (0.47 to 0.63)
[HbF]	0.52 (0.45 to 0.60)
ICAM-1	0.55 (0.47 to 0.63)
[IL1-RA]	0.58 (0.50 to 0.65)
Kunitz-2-34a	0.62 (0.55 to 0.69)

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[Kunitz-2-35b]	0.52(0.44 to 0.60)
[Kunitz-2-40b]	0.53 (0.46 to 0.60)
[Leptin]	0.59 (0.52 to 0.66)
[Leptin receptor]	0.48 (0.41 to 0.55)
[MIF-49a]	0.51 (0.43 to 0.59)
[MIF-49b]	0.60 (0.52 to 0.67)
[MMP-9]	0.54 (0.47 to 0.62)
Nephrin-100a	0.52 (0.46 to 0.57)
Nephrin-101a	0.53 (0.45 to 0.61)
NGAL	0.67 (0.61 to 0.74)
PAI-1	0.62 (0.55 to 0.70)
[PAI-2]	0.61 (0.53 to 0.68)
[PAPP A]	0.65 (0.57 to 0.72)
PCT 95a	0.65 (0.58 to 0.72)
PCT-99b	0.62 (0.55 to 0.70)
Pentraxin 3 64a	0.65 (0.58 to 0.71)
Pentraxin 3 67a	0.60 (0.54 to 0.66)
Periostin	0.64 (0.56 to 0.71)
[PIGF]	0.87 (0.83 to 0.92)
Podocalyxin	0.52 (0.45 to 0.60)
sFlt-1	0.83 (0.78 to 0.88)
ST2-116b	0.76 (0.70 to 0.82)
ST2-75b	0.71 (0.64 to 0.78)
[TGFβ-1]	0.55 (0.47 to 0.62)
TIMP-1	0.59 (0.52 to 0.67)
[TNFR-1A]	0.59 (0.51 to 0.67)
VEGF-C	0.65 (0.58 to 0.72)
Visfatin	0.65 (0.58 to 0.73)
WAP4C-HE4-85b	0.67 (0.61 to 0.74)
WAP4C-HE4-91a	0.65 (0.59 to 0.72)

[] indicates low concentrations of biomarker correlated to disease.

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Appendix 7. Individual Median Biomarker Concentrations (Quartiles) in Women Presenting Between 20⁺⁰ and 34⁺⁶ Weeks of Gestation, Listed in Alphabetical Order

Biomarker	Women with preeclampsia requiring delivery within 14 days n=76	All other participants n=210	All women n=286
ADAM-9 (pg/ml)	88.2% below limit of detection	86.7% below limit of detection	87.1% below limit of detection
Angiogenin (µg/ml)	11.0 (7.04 to 18.9)	10.6 (6.92 to 16.3)	10.7 (6.92 to 16.6)
ANP (ng/ml)	2.33 (0.87 to 6.86)	0.81 (0.39 to 1.82)	1.03 (0.45 to 2.62)
Arginase-1 (ng/ml)	0.75 (0.44 to 1.29)	0.68 (0.42 to 1.06)	0.69 (0.43 to 1.10)
Arginase-2 (ng/ml)	13.1 (7.86 to 18.9)	11.1 (7.25 to 16.0)	11.4 (7.44 to 17.3)
BNP (pg/ml)	0.17 (0.12 to 0.30)	0.10 (0.06 to 0.14)	0.11 (0.07 to 0.18)
Caspase (ng/ml)	4.42 (1.79 to 9.72)	3.25 (1.52 to 7.02)	3.45 (1.56 to 7.54)
CCL23 (ng/ml)	0.24 (0.19 to 0.33)	0.27 (0.19 to 0.35)	0.26 (0.19 to 0.34)
C-Met-109a (ng/ml)	125 (96.2 to 147)	135 (100 to 171)	132 (99.9 to 164)
C-Met-111a (ng/ml)	375 (310 to 446)	394 (328 to 483)	387 (322 to 467)
CPA-4 (ng/ml)	2.37 (1.84 to 2.90)	2.69 (2.14 to 3.45)	2.59 (2.08 to 3.35)
CRP (µg/ml)	15.7 (5.34 to 31.0)	13.7 (7.00 to 25.9)	14.0 (6.70 to 27.9)
CXCL10 (ng/ml)	0.25 (0.16 to 0.35)	0.22 (0.16 to 0.30)	0.23 (0.16 to 0.31)
Cystatin (ng/ml)	3872 (2736 to 6319)	2746 (1955 to 3922)	2939 (2101 to 4597)
Elafin-131 (ng/ml)	123 (86.5 to 189)	131 (95.6 to 171)	128 (91.8 to 175)
Elafin-132 (ng/ml)	59.9 (38.8 to 155)	64.2 (42.5 to 103)	63.3 (41.9 to 111)
Endoglin (ng/ml)	180 (126 to 280)	38.5 (17.5 to 106)	68.0 (24.7 to 160)
Endothelin (ng/ml)	94.4% below limit of detection	92.9% below limit of detection	93.0% below limit of detection
Ephrin (pg/ml)	97.4% below limit of detection	96.2% below limit of detection	96.5% below limit of detection
ESAM-1 (ng/ml)	5.07 (4.54 to 6.74)	5.21 (4.40 to 6.29)	5.18 (4.41 to 6.32)
FAS (ng/ml)	2.78 (2.14 to 3.51)	2.73 (2.05 to 3.74)	2.75 (2.08 to 3.65)
FasL (ng/ml)	0.13 (0.13 to 0.13)	0.13 (0.13 to 0.17)	0.13 (0.13 to 0.15)

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FIH (ng/ml)	0.21 (0.07 to 0.51)	0.14 (0.07 to 0.35)	0.15 (0.07 to 0.38)
HbF (ng/ml)	43.7 (22.8 to 83.3)	50.6 (25.2 to 81.2)	48.7 (24.2 to 81.2)
ICAM-1 (ng/ml)	698 (518 to 991)	622 (491 to 845)	635 (497 to 883)
IL-1ra (pg/ml)	98.6% below limit of detection	100% below limit of detection	99.7% below limit of detection
Kunitz 2-34a (ng/ml)	0.48 (0.35 to 0.61)	0.40 (0.28 to 0.52)	0.42 (0.29 to 0.56)
Kunitz 2-35b (ng/ml)	0.26 (0.13 to 0.43)	0.28 (0.15 to 0.44)	0.28 (0.14 to 0.43)
Kunitz 2-40b (ng/ml)	0.18 (0.14 to 0.35)	0.20 (0.14 to 0.44)	0.20 (0.14 to 0.42)
Leptin (ng/ml)	17.2 (13.7 to 22.8)	17.1 (10.8 to 24.9)	17.1 (11.9 to 24.8)
Leptin receptor (ng/ml)	133 (112 to 157)	155 (112 to 195)	148 (112 to 189)
MIF-49a (ng/ml)	10.7 (9.15 to 13.4)	10.9 (9.50 to 12.7)	10.9 (9.33 to 13.0)
MIF-49b (ng/ml)	9.85 (6.12 to 18.7)	7.89 (5.56 to 11.6)	8.47 (5.62 to 13.2)
MMP-9 (ng/ml)	38.8 (30.1 to 54.6)	42.0 (31.0 to 59.1)	41.1 (30.7 to 57.5)
Nephrin-100a (ng/ml)	77.6% below limit of detection	81.4% below limit of detection	80.4% below limit of detection
Nephrin-101a (ng/ml)	0.38 (0.19 to 0.60)	0.33 (0.18 to 0.56)	0.34 (0.19 to 0.59)
NGAL (ng/ml)	54.1 (39.4 to 75.8)	38.8 (24.7 to 56.5)	41.7 (27.4 to 63.7)
PAI-1 (ng/ml)	0.65 (0.37 to 0.97)	0.43 (0.22 to 0.72)	0.46 (0.25 to 0.78)
PAI-2 (ng/ml)	9.95 (7.64 to 12.8)	11.5 (9.03 to 13.8)	11.3 (8.72 to 13.7)
PAPP-A (ng/ml)	62.4 (25.9 to 145)	117 (57.0 to 187)	98.6 (46.9 to 172)
PCT-95a (pg/ml)	0.07 (0.04 to 0.13)	0.05 (0.03 to 0.08)	0.05 (0.03 to 0.09)
PCT-99b (pg/ml)	82.6% below limit of detection	69.5% below limit of detection	73.7% below limit of detection
Pentraxin 3- 64a (ng/ml)	59.2% below limit of detection	79.5% below limit of detection	74.1% below limit of detection
Pentraxin 3-67a (ng/ml)	3.39 (2.16 to 5.06)	2.05 (0.95 to 3.98)	2.43 (1.27 to 4.45)
Periostin (ng/ml)	10.0 (7.46 to 12.4)	8.36 (6.20 to 10.4)	8.82 (6.44 to 11.1)
PIGF (pg/ml)	8.60 (3.07 to 21.8)	138 (28.6 to 381)	54.8 (13.2 to 282)
Podocalyxin (ng/ml)	0.13 (0.07 to 0.27)	0.12 (0.07 to 0.23)	0.13 (0.07 to 0.23)
sFlt-1 (ng/ml)	5.06 (2.98 to 7.98)	1.10 (0.60 to 2.24)	1.60 (0.68 to 4.53)
ST2-116b (ng/ml)	2.02 (1.12 to 3.59)	0.78 (0.51 to 1.58)	1.02 (0.60 to 1.97)
ST2-75b (ng/ml)	8.54 (5.77 to 14.71)	5.76 (3.56 to 8.39)	6.27 (3.85 to 9.69)

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TGFβ-1 (ng/ml)	1.91 (1.46 to 2.31)	1.95 (1.52 to 2.42)	1.93 (1.51 to 2.39)
TIMP-1 (ng/ml)	132 (97.5 to 190)	112 (89.6 to 156)	119 (91.0 to 164)
TNFR1A (ng/ml)	9.22 (6.11 to 13.4)	7.45 (5.58 to 10.6)	7.64 (5.73 to 11.5)
VEGF-C (ng/ml)	16.3 (14.2 to 18.8)	14.2 (12.4 to 16.7)	14.9 (12.8 to 17.3)
Visfatin (ng/ml)	2.94 (1.83 to 4.15)	2.03 (1.28 to 2.67)	2.22 (1.35 to 3.20)
WAP4C-HE4-85b (ng/ml)	1.90 (1.44 to 3.06)	1.37 (0.94 to 2.05)	1.50 (1.04 to 2.30)
WAP4C-HE4-91a (ng/ml)	14.8 (13.0 to 17.9)	13.3 (11.2 to 15.7)	13.8 (11.9 to 16.2)

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Appendix 8. Characteristics of Participants at Booking and Enrolment in Women Presenting Between 35⁺⁰ and 36⁺⁶ Weeks of Gestation (According to Diagnosis of Preeclampsia)

Characteristics	Women with preeclampsia requiring delivery within 14 days n=67	All other participants n=70	p value	All women n=137
At booking:				
Age (years)	32.3	32.5	0.87	32.4
BMI (kg/m ²)	28.39	28.64	0.66	28.63
White ethnicity	44 (66)	44 (63)	0.73	88 (64)
Singleton pregnancy	55 (82)	68 (97)	0.005	123 (90)
Highest first trimester systolic BP (mmHg)	115 (110 to 123)	120 (110 to 130)	0.14	118 (110 to 127)
Highest first trimester diastolic BP (mmHg)	70 (65 to 80)	71 (64 to 80)	0.70	70 (65 to 80)
Smoker at booking	10 (15)	11 (15)	0.97	21 (15)
Quit smoking during pregnancy	6 (9)	7 (10)	0.79	13 (10)
Previous medical history:				
Preeclampsia requiring delivery <34/40	1 (2)	5 (7)	0.33	6 (4)
Chronic hypertension	2 (3)	8 (11)	0.08	10 (7)
Known SLE or APS	0	0	-	0
Pre-existing diabetes mellitus	3 (5)	1 (1)	0.32	4 (3)
Renal disease	2 (3)	2 (3)	0.97	4 (3)
At enrolment:				

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Gestational age at sampling (weeks)	35.9	36.0	0.54	35.9
New onset hypertension	52 (78)	40 (57)	0.01	21 (15)
Worsening of hypertension	10 (15)	11 (16)	0.90	21 (15)
New onset of dipstick proteinuria (1+ or greater)	49 (73)	36 (51)	0.01	85 (62)
Highest systolic BP (mmHg)	150 (142 to 160)	140 (130 to 148)	<0.001	144 (132 to 153)
Highest diastolic BP (mmHg)	96 (90 to 100)	90 (82 to 96)	0.001	94 (86 to 99)
Alanine transaminase (U/L)	17 (13 to 25)	12 (10 to 19)	0.007	15 (11 to 21)
Creatinine (mg/dl)	60 (50 to 69)	53 (46 to 62)	0.03	55 (48 to 66)
Uric acid (mg/dl)	342 (287 to 380)	270 (210 to 320)	0.001	314 (238 to 360)
Platelet count (x10 ⁹ /l)	193 (158 to 252)	235 (190 to 279)	0.002	213 (176 to 263)

Values given are median (quartiles) or n (%) as appropriate.

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Appendix 9. Characteristics of Delivery and Maternal and Neonatal Outcome in Women Presenting Between 35⁺⁰ and 36⁺⁶ Weeks of Gestation

Characteristics	Women with preeclampsia requiring delivery within 14 days n=67	All other participants n=70	p value	All women n=137
Onset of labour				
Spontaneous	6 (9)	19 (27)	0.01	25 (18)
Induced	43 (64)	31 (44)	0.02	74 (54)
Pre-labour caesarean section	18 (27)	20 (29)	0.82	38 (28)
Mode of delivery				
Spontaneous vaginal delivery	19 (28)	34 (49)	0.02	53 (39)
Assisted vaginal delivery	10 (15)	4 (6)	0.09	14 (10)
Caesarean section	37 (55)	32 (46)	0.23	69 (50)
Adverse maternal outcome*	30 (45)	14 (20)	0.003	44 (32)
Gestation at delivery (weeks)	36.7	38.5	<0.001	37.4
Neonatal outcomes				
Intrapartum /Fetal death	0	0		0
Neonatal death	0	0		0
Birth weight (g)	2490 (2250 to 2970)	3330 (2835 to 3535)	<0.001	2970 (2430 to 3360)
Small for gestational age (<10 th birth weight centile)	25 (46)	14 (21)	0.005	39 (32)
Small for gestational age (<3 rd birth weight centile)	14 (26)	11 (16)	0.21	25 (20)
Small for gestational age (<1 st birth weight centile)	6 (11)	5 (7)	0.50	11 (9)
Adverse perinatal outcome†	3 (6)	6 (9)	0.48	9 (7)

Values given are median (quartiles) or n (%) as appropriate.

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*Adverse maternal outcome defined as presence of any of the following complications: maternal death, eclampsia, stroke, cortical blindness or retinal detachment, hypertensive encephalopathy, systolic blood pressure ≥ 160 mmHg, myocardial infarction, Intubation (other than for caesarean section), pulmonary oedema, platelets $< 50 \times 10^9/L$ ~~transfusion~~, disseminated intravascular coagulation, thrombotic thrombocytopenic purpura/ haemolytic uraemic syndrome, hepatic dysfunction (alanine transaminase ≥ 70 IU/L), hepatic haematoma or rupture, acute fatty liver of pregnancy, creatinine $> 150 \mu\text{mol/L}$, renal dialysis, placental abruption, major postpartum haemorrhage, major infection.

†Adverse perinatal outcome defined as presence of any of the following complications: antepartum/ intrapartum fetal or neonatal death, neonatal unit admission for > 48 hrs at term, intraventricular haemorrhage, periventricular leucomalacia, seizure, retinopathy of prematurity, respiratory distress syndrome, bronchopulmonary dysplasia or necrotising enterocolitis.

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Appendix 10. Individual Biomarker ROC Areas (95% Confidence Intervals) for Pre-eclampsia Requiring Delivery Within 14 Days of Sampling in Women Presenting Between 35⁺⁰ and 36⁺⁶ Weeks of Gestation, Listed in Descending ROC area

Biomarkers	ROC areas (95% confidence intervals)
ADAM-9	0.57 (0.51 to 0.64)
Angiogenin	0.50 (0.40 to 0.59)
ANP	0.69 (0.60 to 0.78)
Arginase-1	0.58 (0.48 to 0.68)
[Arginase-2]	0.54 (0.45 to 0.64)
BNP	0.70 (0.61 to 0.78)
Caspase	0.50 (0.41 to 0.60)
[C-Met-109a]	0.57 (0.48 to 0.67)
[C-Met-111a]	0.60 (0.50 to 0.70)
[CCL23]	0.60 (0.50 to 0.69)
[CPA-4]	0.55 (0.45 to 0.64)
CRP	0.47 (0.37 to 0.57)
CXCL10	0.62 (0.52 to 0.71)
Cystatin	0.65 (0.56 to 0.74)
Elafin 131	0.44 (0.34 to 0.53)
Elafin 132	0.59 (0.49 to 0.69)
Endoglin	0.72 (0.64 to 0.81)
[Endothelin]	0.71 (0.62 to 0.80)
[Ephrin]	0.50 (0.48 to 0.52)
[ESAM-1]	0.57 (0.48 to 0.67)
[FAS]	0.58 (0.48 to 0.67)
[FasL]	0.52 (0.44 to 0.60)
FIH	0.45 (0.35 to 0.55)
[HbF]	0.50 (0.40 to 0.60)
ICAM-1	0.48 (0.38 to 0.58)
[IL1-RA]	0.56 (0.47 to 0.66)

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Kunitz-2-34a	0.64 (0.54 to 0.73)
[Kunitz-2-35b]	0.53 (0.44 to 0.63)
[Kunitz-2-40b]	0.60 (0.50 to 0.69)
[Leptin]	0.57 (0.48 to 0.67)
[Leptin receptor]	0.48 (0.38 to 0.58)
[MIF-49a]	0.59 (0.49 to 0.68)
[MIF-49b]	0.54 (0.45 to 0.64)
[MMP-9]	0.51 (0.42 to 0.61)
Nephrin-100a	0.55 (0.49 to 0.61)
Nephrin-101a	0.55 (0.45 to 0.64)
NGAL	0.67 (0.58 to 0.76)
PAI-1	0.58 (0.48 to 0.68)
[PAI-2]	0.51 (0.41 to 0.61)
[PAPP A]	0.64 (0.55 to 0.74)
PCT 95a	0.61 (0.52 to 0.71)
PCT-99b	0.68 (0.59 to 0.77)
Pentraxin 3 64a	0.61 (0.53 to 0.69)
Pentraxin 3 67a	0.64 (0.55 to 0.73)
Periostin	0.61 (0.52 to 0.71)
[PIGF]	0.75 (0.67 to 0.83)
Podocalyxin	0.56 (0.46 to 0.65)
sFlt-1	0.75 (0.67 to 0.83)
ST2-116b	0.71 (0.62 to 0.80)
ST2-75b	0.66 (0.57 to 0.75)
[TGFβ-1]	0.61 (0.52 to 0.71)
TIMP-1	0.56 (0.46 to 0.66)
[TNFR-1A]	0.70 (0.61 to 0.78)
VEGF-C	0.64 (0.55 to 0.74)
Visfatin	0.67 (0.57 to 0.76)
WAP4C-HE4-85b	0.67 (0.58 to 0.76)
WAP4C-HE4-91a	0.65 (0.55 to 0.74)

[] indicates low concentrations of biomarker correlated to disease.

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Appendix 11. Individual Median Biomarker Concentrations (Quartiles) in Women Presenting Between 35⁺⁰ and 36⁺⁶ Weeks of Gestation

Biomarker	Preeclampsia requiring delivery within 14 days n=67	All other participants n=70	All women n=137
ADAM-9 (pg/ml)	74.6% below limit of detection	88.6% below limit of detection	81.8% below limit of detection
Angiogenin (µg/ml)	11.0 (7.70 to 15.8)	10.6 (8.23 to 19.5)	10.9 (8.23 to 17.4)
ANP (ng/ml)	2.55 (0.97 to 4.89)	0.92 (0.43 to 2.06)	1.54 (0.62 to 3.55)
Arginase-1 (ng/ml)	0.91 (0.50 to 1.67)	0.64 (0.40 to 1.22)	0.68 (0.43 to 1.38)
Arginase-2 (ng/ml)	10.2 (6.92 to 17.0)	9.70 (5.40 to 15.8)	10.1 (6.17 to 15.8)
BNP (pg/ml)	0.15 (0.08 to 0.24)	0.09 (0.05 to 0.15)	0.11 (0.06 to 0.19)
Caspase (ng/ml)	3.39 (1.54 to 7.06)	3.71 (1.64 to 7.11)	3.61 (1.64 to 7.06)
CCL2314b (ng/ml)	0.25 (0.19 to 0.34)	0.22 (0.16 to 0.28)	0.23 (0.17 to 0.29)
C-Met-109a (ng/ml)	152 (106 to 182)	133 (98.1 to 175)	143 (102 to 180)
C-Met-111a (ng/ml)	432 (351 to 551)	389 (334 to 445)	411 (341 to 495)
CPA-4 (ng/ml)	2.90 (2.23 to 3.73)	2.67 (2.16 to 3.42)	2.84 (2.21 to 3.53)
CRP (µg/ml)	9.76 (6.22 to 20.9)	11.6 (5.13 to 25.6)	10.9 (5.89 to 21.7)
CXCL10 (ng/ml)	0.30 (0.20 to 0.46)	0.23 (0.16 to 0.38)	0.28 (0.12 to 0.41)
Cystatin (ng/ml)	3740 (2880 to 7400)	2879 (2127 to 4705)	3274 (2461 to 5232)
Elafin-131 (ng/ml)	115 (90.8 to 168)	145 (95.8 to 188)	133 (93.0 to 176)
Elafin-132 (ng/ml)	68.2 (47.6 to 110)	52.1 (37.0 to 89.5)	59.8 (40.0 to 96.8)
Endoglin (ng/ml)	143 (87.2 to 209)	77.0 (44.1 to 124)	104 (60.1 to 177)
Endothelin (ng/ml)	2.28 (1.47 to 3.76)	1.52 (0.82 to 2.36)	1.97 (1.25 to 2.91)
Ephrin (pg/ml)	98.5% below limit of detection	98.6% below limit of detection	98.5% below limit of detection
ESAM (ng/ml)	5.99 (4.57 to 7.35)	5.62 (4.25 to 6.10)	5.73 (4.33 to 6.75)
FAS (ng/ml)	2.64 (2.05 to 3.85)	2.46 (1.73 to 3.25)	2.57 (1.91 to 3.48)
FasL (ng/ml)	0.13 (0.06 to 0.35)	0.19 (0.95 to 0.43)	0.18 (0.07 to 0.36)
FIH (ng/ml)	0.17 (0.06 to 0.35)	0.19 (0.10 to 0.43)	0.18 (0.07 to 0.37)

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HbF (ng/ml)	42.2 (18.6 to 78.5)	39.9 (19.7 to 80.1)	41.2 (19.6 to 78.5)
ICAM-1 (ng/ml)	614 (492 to 845)	630 (535 to 887)	618 (518 to 848)
IL-1ra (pg/ml)	0.24 (0.17 to 0.35)	0.02 (0.01 to 0.03)	0.23 (0.02 to 0.03)
Kunitz 2-34a (ng/ml)	0.53 (0.36 to 0.82)	0.43 (0.30 to 0.54)	0.46 (0.35 to 0.64)
Kunitz 2-35b (ng/ml)	0.34 (0.23 to 0.60)	0.32 (0.20 to 0.50)	0.33 (0.23 to 0.55)
Kunitz 2-40b (ng/ml)	0.39 (0.14 to 0.63)	0.25 (0.14 to 0.47)	0.33 (0.14 to 0.55)
Leptin (ng/ml)	19.5 (12.7 to 27.0)	15.7 (12.9 to 21.5)	17.3 (12.9 to 25.0)
Leptin receptor (ng/ml)	148 (108 to 188)	149 (109 to 198)	148 (109 to 191)
MIF-49a (ng/ml)	11.4 (9.58 to 14.5)	10.5 (9.00 to 11.9)	11.0 (9.36 to 13.4)
MIF-49b (ng/ml)	9.82 (7.13 to 15.0)	8.80 (6.37 to 14.4)	9.35 (7.06 to 14.4)
MMP-9 (ng/ml)	41.6 (27.9 to 54.6)	38.8 (26.2 to 60.2)	39.9 (27.7 to 57.3)
Nephrin-100a (ng/ml)	79.1% below limit of detection	88.6% below limit of detection	83.9% below limit of detection
Nephrin-101a (ng/ml)	0.32 (0.14 to 0.53)	0.29 (0.10 to 0.48)	0.31 (0.11 to 0.51)
NGAL (ng/ml)	51.8 (37.2 to 62.5)	35.2 (24.8 to 53.2)	45.3 (30.2 to 59.7)
PAI-1 - 52b (ng/ml)	0.65 (0.39 to 1.02)	0.56 (0.32 to 0.79)	0.58 (0.36 to 0.91)
PAI-2-55a (ng/ml)	11.5 (8.2 to 13.7)	10.7 (8.86 to 14.7)	11.3 (8.74 to 14.4)
PAPP-A (ng/ml)	24.2 (13.6 to 52.2)	58.2 (19.5 to 118)	32.4 (16.0 to 87.8)
PCT-95a (pg/ml)	0.06 (0.03 to 0.09)	0.05 (0.03 to 0.08)	0.05 (0.03 to 0.09)
PCT-99b (pg/ml)	0.22 (0.01 to 0.05)	0.01 (0.01 to 0.03)	0.02 (0.01 to 0.04)
Pentraxin 3- 64a (ng/ml)	50.7% below limit of detection	71.4% below limit of detection	61.3% below limit of detection
Pentraxin 3-67a (ng/ml)	3.13 (2.00 to 4.78)	1.85 (0.90 to 3.61)	2.54 (1.18 to 4.13)
Periostin (ng/ml)	9.41 (7.26 to 10.7)	7.84 (6.55 to 9.65)	8.30 (6.99 to 10.5)
PIGF (pg/ml)	18.8 (12.6 to 42.7)	66.2 (27.4 to 201)	31.9 (14.6 to 98.9)
Podocalyxin (ng/ml)	0.13 (0.07 to 0.22)	0.10 (0.70 to 0.18)	0.10 (0.07 to 0.21)
sFlt-1 (ng/ml)	2.68 (1.41 to 4.15)	1.40 (0.97 to 2.10)	1.82 (1.06 to 2.90)
ST2-116b (ng/ml)	10.9 (6.72 to 16.3)	7.27 (4.96 to 11.2)	8.29 (6.05 to 13.6)
ST2-75b (ng/ml)	2.15 (1.64 to 2.43)	1.78 (1.30 to 2.24)	1.95 (1.46 to 2.41)
TGFβ-1 (ng/ml)	123 (92.8 to 167)	106 (80.2 to 166)	117 (87.3 to 166)
TIMP (ng/ml)	10.1 (7.63 to 14.9)	7.50 (6.05 to 9.85)	8.18 (6.53 to 12.2)
TNFR1A (ng/ml)	16.2 (14.2 to 18.7)	14.6 (12.4 to 16.5)	15.3 (13.2 to 17.8)

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VEGF-C (ng/ml)	5.35 (2.98 to 7.92)	2.22 (1.11 to 3.99)	3.56 (1.68 to 6.22)
Visfatin (ng/ml)	2.38 (1.77 to 3.94)	1.84 (1.29 to 2.83)	2.11 (1.56 to 3.31)
WAP4C-HE4-85b (ng/ml)	1.90 (1.32 to 2.61)	1.30 (0.94 to 2.14)	1.55 (1.12 to 2.42)
WAP4C-HE4-91a (ng/ml)	16.7 (14.2 to 18.0)	14.3 (12.3 to 16.1)	15.0 (12.8 to 17.5)

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