Technical Specifications
PreTRM® Test for Risk Management

PreTRM®
Test for Risk Management

Intended Use/Indications for Use

The PreTRM Test for Risk Management predicts the risk of spontaneous preterm birth (before 37 weeks) in asymptomatic women (no signs or symptoms of preterm labor with intact membranes) ≥18 years old with a singleton pregnancy. The PreTRM Test is performed via a single blood draw between 18wk - 20wk/6d (126-146 days) gestation. It is not intended for use in women who have a multiple pregnancy, have a known or suspected fetal anomaly, or are on any form of progesterone therapy after the first trimester.

The PreTRM test is a laboratory-developed test (LDT) and is performed exclusively by Sera Prognostics Clinical Laboratory, Salt Lake City, Utah.

Analytical Test Method

SAMPLE WORKFLOW

Thawed serum samples are diluted in buffer, filtered to remove particulates, then depleted of high abundance proteins using an automated antibody-based affinity removal system. Depleted samples are then digested with a protease to generate peptides that serve as surrogate analytes for the proteins of interest. Samples are fortified with stable isotope internal standards. The abundances of diagnostic and quality control proteins from fully processed samples are detected by liquid chromatography-mass spectrometry. There are two proteins used to determine the individualized risk of spontaneous preterm birth, insulin-like growth factor-binding protein 4 (IBP4) and sex hormone binding globulin (SHBG).

DATA ANALYSIS

A proteomic score is calculated using the relative abundances of the two signature analytes, IBP4 and SHBG. The individual risk of spontaneous preterm birth before 37 weeks is reported as a Bayesian posterior probability based on the patient's proteomic score. A proprietary algorithm utilizes the internal standard-normalized relative abundances of the diagnostic analytes and the patient's prepregnancy body mass index (BMI) to generate a qualitative risk prediction

QUALITY CONTROL MAKEUP

QC1 - Pooled serum from multiple female donors, not pregnant
QC2 - Pooled serum from multiple female donors, pregnant

Performance Characteristics

LIMITS OF QUANTITATION

The lower and upper limits of quantitation were determined by calculating the %CV of diagnostic protein abundances after replicate analysis of samples across a concentration range that encompasses intended use samples, then comparing these %CVs to an acceptable upper threshold of ≤20%. The upper and lower LOQ for IBP4 are 0.040and 210, respectively. The upper and lower LOQ for the SHBG are 0.011 and 291, respectively.

PROCESS CONTROLS

- Depletion Efficiency
- Digestion Performance
- Proteomic Score-Based Batch Quality Control
- Matrix Verification
- Abundance Measurement Within Validated Measurement Range

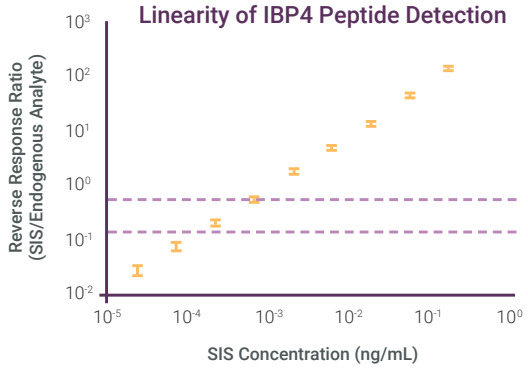
Average IBP4 Reverse Response Ratio	Reverse Response Ratio %CV			Average SHBG Reverse Response Ratio	Reverse Response Ratio %CV		
	Instrument 1	Instrument 2	Instrument 3		Instrument 1	Instrument 2	Instrument 3
0.019	13.21	22.81	25.20	0.011	4.18	3.51	7.88
0.040	10.64	8.06	18.99	0.031	3.58	3.82	6.05
0.099	7.67	10.61	17.58	0.093	3.90	4.17	5.67
0.261	5.94	7.75	11.10	0.290	2.40	3.57	9.23
0.768	4.85	4.59	9.15	0.889	3.44	4.40	5.05
2.349	4.90	6.56	8.72	2.769	4.40	3.54	5.34
6.910	4.39	6.61	10.39	8.660	3.37	1.86	3.70
20.161	3.65	7.70	8.10	27.147	4.57	3.56	3.89
64.870	3.88	5.29	7.78	85.917	2.29	2.93	5.24
209.911	5.21	6.22	8.52	291.267	1.86	2.71	4.43

Assay Reportable Range

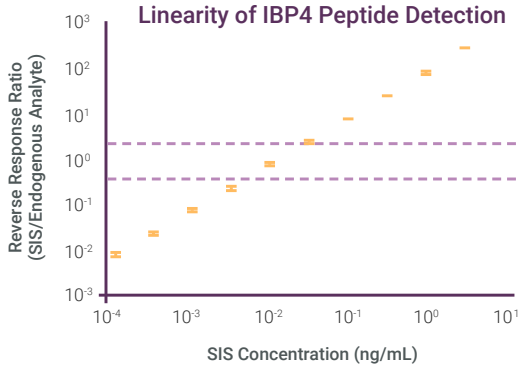
The lowest and highest reportable individualized risk of spontaneous preterm birth are ≤7.3% and ≥60%, respectively. The lower bound is estimated from the United States population baseline rate of spontaneous preterm birth in 2014, contemporaneous with the clinical studies. The upper bound is truncated to the highest score observed in the clinical validation studies.

Linearity

Reverse calibration curves were generated by dividing the internal standard responses by the responses from a constant signal from endogenous diagnostic analytes. Both diagnostic analytes exhibited a linear response, on multiple detection systems, across a range of diagnostic analyte abundances determined by assaying a large number of samples from an intended use population (indicated by the dashed horizontal lines in the plots below).



R square	LCMS01	LCMS02	LCMS03
	0.9961	0.9951	0.9913



R square	LCMS01	LCMS02	LCMS03
	0.9983	0.9977	0.9959

Analytical Specificity/Interference

At each peptide's determined retention time, the mass spectrometer was programmed to monitor two parent-product ion m/z transitions for each peptide and the supporting heavy-labeled analogue. The signal ratio of the two transitions (i.e., transition ratio) was calculated. Retention time, m/z of parent and product ions, and matching transition ratios between the endogenous peptide analyte and the exogenous heavy-labeled analogue measured in 413 samples over 43 days confirmed each signal was from the expected endogenous analyte.

A commercial endogenous interferent panel, at concentrations exceeding those found in clinical specimens, was tested on samples from clinical studies. No significant effect on the proteomic score from these interferents was observed.

Precision

Six samples, each with four replicates per batch, were analyzed in twenty-one batches for intra-batch and inter-batch precision. Modeling of diagnostic analyte precision and what impact on proteomic score would cause a clinically meaningful change in the calculated individualized risk of spontaneous preterm birth, resulted in the a priori establishment of ≤20% CV for acceptable diagnostic analyte normalized response.

Inter-batch Precision (Reproducibility)

Reproducibility						
Sample	Sample Characteristics	N	SHBG %CV	IBP4 %CV	Mean SHBG	Mean IBP4
15-6018	Low IBP4	84	9.67	11.41	1.103	0.192
15-6036	High IBP4, High SHBG	83	9.73	9.49	1.766	0.323
15-6054	Midrange Proteomic Score	84	11.76	10.05	0.861	0.264
15-6092	Midrange Proteomic Score	83	11.89	9.52	0.882	0.285
15-6138	Midrange Proteomic Score	83	9.00	8.87	0.909	0.310
Precision QC 1	Low SHBG	84	12.71	10.22	0.188	0.253

Acceptance criteria were <20% CV across all 21 batches.

Intra-batch Precision (Repeatability)

	Precision 01		Precision 02		Precision 03		Precision 04		Precision 05		Precision 06		Precision 07	
Sample	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4
15-6018	4.63	7.89	5.10	5.12	6.62	11.97	6.41	1.90	10.40	7.76	5.20	13.01	3.02	6.43
15-6036	7.26	2.72	1.50	5.22	4.42	9.71	2.56	4.37	9.92	7.04	4.08	7.77	6.77	12.98
15-6054	1.97	5.47	9.20	7.96	8.38	6.79	4.65	9.38	8.54	4.84	4.95	9.84	2.29	9.98
15-6092	5.98	5.73	16.62	7.87	2.16	4.38	3.70	10.94	21.42	10.87	4.25	7.80	4.38	6.69
15-6138	3.83	9.82	8.79	7.96	8.45	6.71	4.96	4.03	9.85	7.59	5.27	3.61	3.19	6.79
Precision QC 1	5.90	4.02	12.52	10.89	7.51	7.64	19.29	19.76	22.16	13.28	9.75	5.79	5.35	2.80

	Precision 08		Precision 09		Precision 10		Precision 11		Precision 12		Precision 13		Precision 14	
Sample	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4
15-6018	7.04	7.07	8.91	11.09	8.46	5.62	3.35	8.67	9.54	15.52	7.43	8.67	7.71	5.66
15-6036	7.07	4.77	5.69	7.21	6.00	6.29	8.60	6.96	8.85	10.91	4.44	7.06	5.59	8.26
15-6054	8.01	13.61	4.93	8.19	1.63	2.25	6.31	3.47	16.82	14.34	14.37	3.19	7.26	5.30
15-6092	4.07	7.18	6.86	8.35	2.63	11.64	4.82	10.78	7.54	6.27	19.52	9.16	7.61	4.97
15-6138	6.60	10.46	7.20	5.18	2.80	1.77	5.42	5.82	9.90	2.35	3.90	3.37	1.44	3.77
Precision QC 1	10.67	11.29	7.63	9.88	3.71	11.43	2.76	3.77	12.12	5.34	16.91	9.81	7.69	9.09

	Precision 15		Precision 16		Precision 17		Precision 18		Precision 19		Precision 20		Precision 21	
Sample	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4	SHBG	IBP4
15-6018	4.55	8.90	10.53	7.64	1.16	3.42	4.66	10.60	6.32	7.63	11.15	12.88	7.51	4.87
15-6036	4.17	2.15	7.81	12.31	9.18	13.38	10.59	8.18	10.53	4.64	6.70	7.94	4.19	6.84
15-6054	8.66	6.92	3.32	9.85	21.64	11.35	14.90	5.29	3.64	6.81	5.57	4.53	1.89	8.57
15-6092	6.16	6.81	3.56	6.62	4.67	2.41	17.27	5.73	5.00	5.02	5.56	7.86	8.33	9.35
15-6138	10.96	15.13	2.37	2.67	0.98	11.12	14.30	6.38	3.07	10.55	11.96	9.81	5.71	3.53
Precision QC 1	4.86	5.10	2.07	6.93	5.88	7.95	10.00	9.92	3.49	8.59	4.70	10.46	3.36	9.06

Acceptance criteria were that both diagnostic analytes within batch have <20% CVs in 20 of 21 batches (95% agreement). (Two data points were dropped for sampling handling error; one was dropped for a trypsin digestion error.)

REFERENCES: 1. Martin JA, Hamilton B, et al. Births: Final Data for 2012. National Vital Statistics Report. Centers for Disease Control and Prevention. 2013;62:9. **2.** Saade GR, Boggess KA, Sullivan SL et al. Development and validation of a spontaneou: s preterm delivery predictor in asymptomatic women. An J Obstet Gynecol May 2016;214(5):633.e1-24. **3.** Bradford C, Severinsen R, Pugmire T, Rasmussen M, Stoddard K, Uemura Y, Wheelwright S, Mentinova M, Chelsky D, Hunsucker SW, Kearney P, Hickok D, Fleischer TC, Ichetovkin I, Boniface JJ, Critchfield GC, Peltier JM: Analytical validation of protein biomarkers for risk of spontaneous preterm birth. Clin Mass Spectrom 2017, 3:25-38.A commercial endogenous interferent panel, at concentrations exceeding those found in clinical specimens, was tested on samples from clinical studies. No significant effect on the proteomic score from these interferents was observed.

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The Pregnancy Company®