TECHNICAL SPECIFICATIONS

# 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.[1,2] The PreTRM Test is performed via a single blood draw between 18wk - 20wk/6d (126-146 days) gestation.[3]

The PreTRM test is a laboratory-developed test (LDT) and is performed exclusively by Sera Prognostics Clinical Laboratory, Salt Lake City, Utah.[3]

## Analytical Test Method

### SAMPLE WORKFLOW

For depletion methodologies,* samples are diluted in buffer, filtered to remove particulates, then depleted of high abundance proteins using an automated antibody-based affinity method. For antibody capture methodologies, samples are enriched for the targeted proteins using affinity capture after having been reconstituted from the sample collection device using a buffer. After these initial steps which differ depending on the test systems used, the rest of the methodology is the same. All 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 (SIS). 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).

*samples originating from NY state will be tested using depletion methodologies

### 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 uses the internal standard-normalized relative abundances of the diagnostic analytes and the patient's pre-pregnancy body mass index (BMI) to generate a qualitative risk prediction.

## Process Controls

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

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# Performance Characteristics

## LIMITS OF QUANTITATION

The lower and upper limits of quantitation were determined from linearity experiments, in which recombinant IBP4 and SHBG proteins were spiked into blank serum matrix at known nominal concentrations. The upper limit of quantitation was the highest nominal concentration of each analyte that yielded an acceptable correlation (R2) and had a calculated concentration within ±20% of the nominal concentration and had a CV ≤ 20%. The lower limit of quantitation met the same criteria but also had a signal-to-background > 5.

**Table 1.** Linearity test accuracy results for SHBG

Values in green were deemed to meet all linearity acceptance criteria for SHBG.

| Sample | Nominal Concentration (ug/mL) | LCMS03 (% bias) | LCMS04 (% bias) | LCMS05 (% bias) | LCMS06 (% bias) |
|---|---|---|---|---|---|
| Cal1 | 0.97 | -2.0 | -5.0 | -19.0 | -19.0 |
| Cal2 | 1.38 | -17.0 | -17.0 | -19.0 | -20.0 |
| Cal3 | 1.98 | - | -19.0 | -9.0 | 1.0 |
| Cal4 | 2.82 | -20.0 | - | -16.0 | 5.0 |
| Cal5 | 4.04 | -16.0 | -17.0 | -12.0 | 19.0 |
| Cal6 | 8.24 | -9.0 | -19.0 | -8.0 | 6.0 |
| Cal7 | 16.81 | -9.0 | -4.0 | -9.0 | -13.0 |
| Cal8 | 24.01 | -1.0 | -1.0 | -9.0 | -5.0 |
| Cal9 | 34.30 | 0.0 | -9.0 | -6.0 | -6.0 |
| Cal10 | 49.00 | -1.0 | 2.0 | 3.0 | 4.0 |

After applying a linear fit across a range of nominal concentrations that yield the best calculated accuracy of the SHBG concentration, a range of nominal concentrations was found that: yielded a good correlation for all instruments; covered the apparent concentration values for QC1 and QC2 (4.2 to 45.4 ug/mL); covered the range of response ratios expected for the intended use patient population; and yielded bias values within ±20%. The difference between the observed and expected response is divided by the expected response to measure bias as a ratio. There was no systematic bias in the accuracy values and accordingly, the assay was deemed to yield acceptable results across all instruments.

# Performance Characteristics

**Figure 1.** Example correlation plots for IBP4 and SHBG

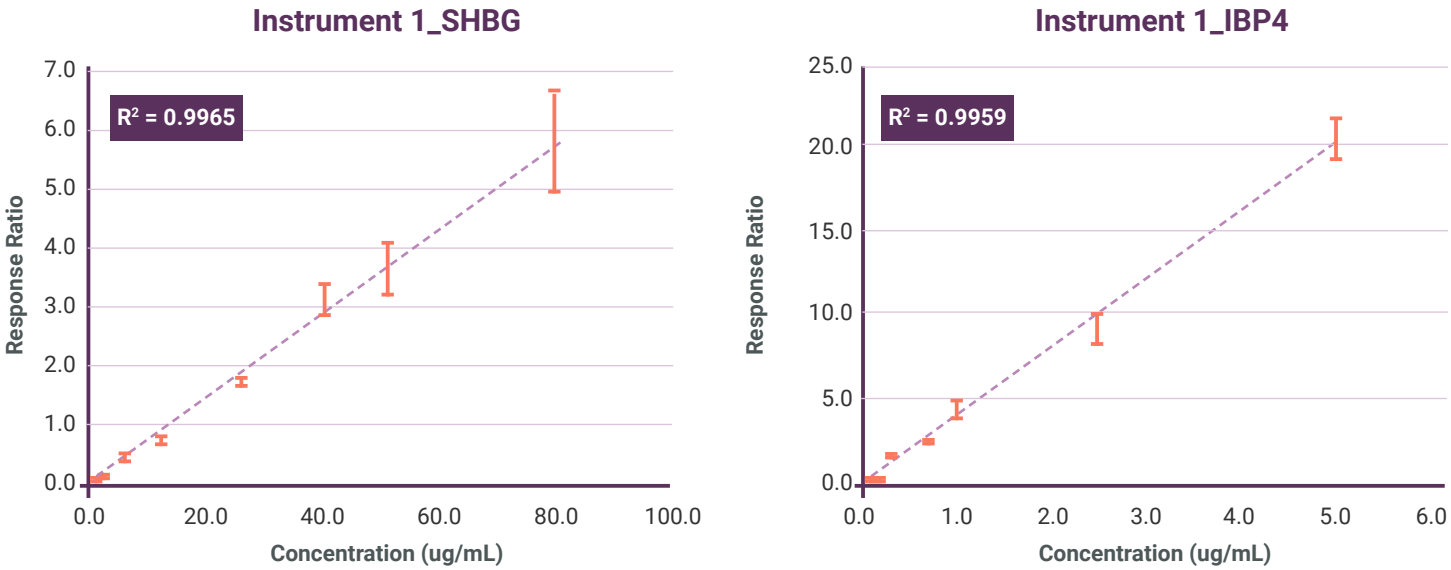


**Table 2.** Linearity test accuracy results for IBP4

Values in green were deemed to meet all linearity acceptance criteria for IBP4.

| Sample | Concentration (ug/mL) | Instrument 1 (% bias) | Instrument 2 (% bias) | Instrument 3 (% bias) | Instrument 4 (% bias) |
|---|---|---|---|---|---|
| Cal1-01 | 0.05 | - | 2.0 | 9.0 | -7.0 |
| Cal2-01 | 0.07 | 11.0 | 2.0 | -2.0 | -20.0 |
| Cal3-01 | 0.10 | -6.0 | -7.0 | 14.0 | -11.0 |
| Cal4-01 | 0.14 | -4.0 | -12.0 | 2.0 | 13.0 |
| Cal5-01 | 0.20 | -1.0 | -8.0 | 5.0 | -14.0 |
| Cal6-01 | 0.41 | 7.0 | -4.0 | -8.0 | -12.0 |
| Cal7-01 | 0.84 | 2.0 | 2.0 | -1.0 | -5.0 |
| Cal8-01 | 1.20 | 4.0 | 5.0 | 1.0 | 0.0 |
| Cal9-01 | 1.72 | 1.0 | -9.0 | -3.0 | 2.0 |
| Cal10-01 | 2.45 | -1.0 | 2.0 | 6.0 | 5.0 |

After applying a linear fit across a range of nominal concentrations that yield the best calculated accuracy of the IBP4 concentration, a range of nominal concentrations was found that: yielded a good correlation for all instruments; covered the apparent concentration values for QC1 and QC2 (0.16 to 0.67 ug/mL); covered the range of response ratios expected for the intended use patient population; and yielded bias values ≤ 20%. There was no systematic bias in the accuracy values. Accordingly, the assay was deemed to yield an acceptable linearity for IBP4 across all instruments.

# Performance Characteristics

**Table 3.** Signal to background at lower limit of quantitation

| Protein | Sample | Instrument 1 | | | Instrument 2 | | | Instrument 3 | | | Instrument 4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Signal | Background | Ratio | Signal | Background | Ratio | Signal | Background | Ratio | Signal | Background | Ratio |
| SHBG | Cal4 | 0.1318 | 0.0007 | **188.3** | 0.1230 | 0.0020 | **61.5** | 0.1230 | 0.0137 | **9.0** | 0.1050 | 0.0110 | **9.5** |
| IBP4 | Cal2 | 0.5118 | 0.0125 | **40.9** | 0.2510 | 0.0140 | **17.9** | 0.3815 | 0.0161 | **23.7** | 0.2720 | 0.0140 | **19.4** |

**Table 4.** Imprecision at lower limit of quantitation

| Protein | Sample | Concentration (ug/mL) | Instrument 1 (% CV) | Instrument 2 (% CV) | Instrument 3 (% CV) | Instrument 4 (% CV) |
|---|---|---|---|---|---|---|
| SHBG | Cal4 | 2.5 | 9.0 | 14.0 | 19.0 | 12.0 |
| IBP4 | Cal2 | 0.1 | 16.0 | 19.0 | 17.0 | 17.0 |

**Table 5.** Linearity test linear fit results for SHBG

All four instruments yielded linear correlation coefficients that met acceptance criteria for SHBG linearity.

| Instrument | Correlation Coefficient ($R^2$) | Pass/Fail ($R^2 > 0.99$) |
|---|---|---|
| LCMS03 | 0.9952 | PASS |
| LCMS04 | 0.9965 | PASS |
| LCMS05 | 0.999 | PASS |
| LCMS06 | 0.9996 | PASS |

**Table 6.** Linearity test linear fit results for IBP4

All four instruments yielded linear correlation coefficients that met acceptance criteria for IBP4 linearity.

| Instrument | Correlation Coefficient ($R^2$) | Pass/Fail ($R^2 > 0.99$) |
|---|---|---|
| LCMS03 | 0.999 | PASS |
| LCMS04 | 0.9959 | PASS |
| LCMS05 | 0.9998 | PASS |
| LCMS06 | 0.9996 | PASS |

## Assay Reportable Range

The lowest and highest reportable individualized risks 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 20141, contemporaneous with the clinical studies. The upper bound is truncated to the highest score observed in clinical validation studies.

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# Performance Characteristics

## Analytical Specificity/Interference

Interference and specificity were evaluated by spiking a pooled serum sample derived from our intended use population with two levels of Triglyceride Rich Lipoproteins, conjugated and unconjugated bilirubin, and hemolysate at levels considered clinically high and pathological. No significant shift in the results ($p \geq 0.05$) were detected at any level of interference. Additionally, no level of interference shifted the retention time in a way that prevented specific identification of the target analyte or changed its qualitative to quantitative transition ratio significantly.

## Precision – Intrabatch (Repeatability)

Intrabatch precision was evaluated by testing samples (N = 21 for each sample type) that represented high, low and threshold test results from our intended use population. These samples were analyzed repeatedly within a day to demonstrate that the assay could yield CVs ≤ 20%. Samples were tested for both response ratios and proteomic score, but the score result is used in clinical decision making.

**Table 7.** Within-batch imprecision (repeatability) of analytes by batch

| SAMPLE | Analyte/Score | N | % CV | Pass/Fail |
|---|---|---|---|---|
| Low | IBP4 | 20* | 20.0 | Pass |
| | SHBG | 20* | 15.4 | Pass |
| | Score | 20* | 4.9 | Pass |
| High | IBP4 | 21 | 6.0 | Pass |
| | SHBG | 21 | 5.0 | Pass |
| | Score | 21 | 3.7 | Pass |
| Threshold | IBP4 | 21 | 16.7 | Pass |
| | SHBG | 21 | 12.2 | Pass |
| | Score | 21 | 9.6 | Pass |

* One replicate dropped owing to liquid handling issue

The method demonstrated acceptable repeatability.

## Precision – Interbatch (Reproducibility)

Interbatch precision was evaluated by testing samples (N = 161 for each sample type) that represented high, low and threshold test results from the intended use population. These samples were analyzed repeatedly across 21 batches spread over more than 20 days across all test systems and personnel, to demonstrate that the assay could yield overall CVs of ≤ 20% across that time frame.

**Table 8.** Interbatch imprecision (reproducibility) of analytes

| SAMPLE | Analyte/Score | N | % CV | Pass/Fail |
|---|---|---|---|---|
| Low | IBP4 | 161 | 15.4 | Pass |
| | SHBG | 161 | 15.3 | Pass |
| | Score | 161 | 7.4 | Pass |
| High | IBP4 | 160* | 16.9 | Pass |
| | SHBG | 160* | 16.8 | Pass |
| | Score | 160* | 19.9 | Pass |
| Threshold | IBP4 | 160* | 19.5 | Pass |
| | SHBG | 160* | 16.9 | Pass |
| | Score | 160* | 10.6 | Pass |

* One replicate dropped owing to liquid handling issue

The method demonstrated acceptable reproducibility.

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# Performance Characteristics

## Frozen Serum Samples

### LIMITS OF QUANTITATION

The lower and upper limits of quantitation (LOQ) were determined by calculating the CVs of diagnostic protein abundances after replicate analysis of samples across a concentration range that encompasses intended use samples, then comparing the CVs to an acceptable upper threshold of ≤ 20%.

**Table 9.** Reverse Response Ratio % CVs Across Concentrations

| 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 20141, contemporaneous with the clinical studies. The upper bound is truncated to the highest score observed in clinical validation studies.

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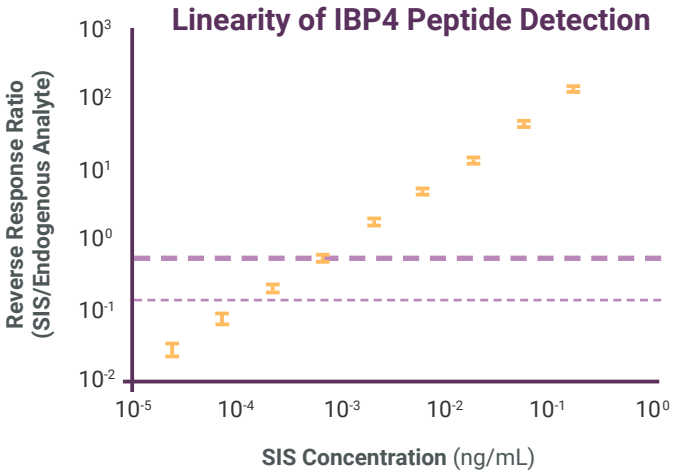
# Performance Characteristics

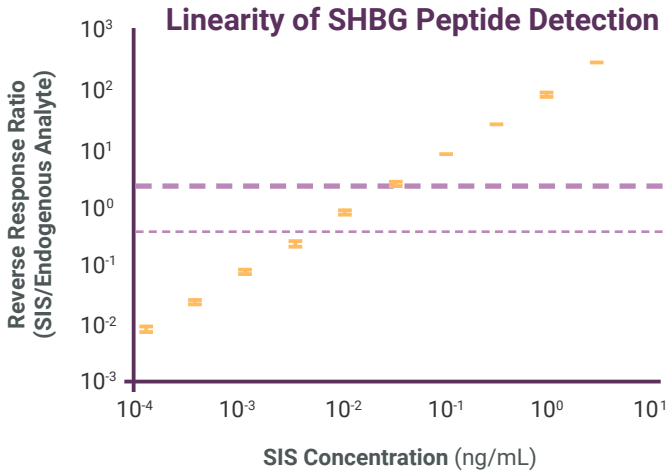
## Linearity

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).

**Figure 2.** Reverse Calibration Curves




| $R^2$ square | LCMS01 | LCMS02 | LCMS03 |
|---|---|---|---|
| | 0.9961 | 0.9951 | 0.9913 |




| $R^2$ 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 mass-to-charge ratio (m/z) transitions for each peptide and the supporting heavy-labeled analogue. The signal ratio of the two transitions (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 that each signal was from the expected endogenous analyte.

A commercially available 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.

**FROZEN SERUM SAMPLES**
using Depletion Methodologies with LCMS Analysis

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# Performance Characteristics

## Table 10. Inter-batch Precision (Reproducibility)

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

| 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 |

## Table 11. 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: Both diagnostic analytes within batch must have CVs ≤ 20% in 20 of 21 batches (95% agreement). (Two data points were dropped for sampling handling error; one was dropped for a trypsin digestion error.)

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# Performance Characteristics

## LIMITS OF QUANTITATION

The lower and upper limits of quantitation were determined from linearity experiments, in which recombinant IBP4 and SHBG proteins were spiked into blank serum matrix at known nominal concentrations. The upper limit of quantitation was the highest nominal concentration of each analyte that yielded an acceptable correlation (R2) and had a calculated concentration within 20% of the nominal concentration and had a CV ≤20%. The lower limit of quantitation met the same criteria but also had a signal-to-background > 5.

**Table 1.** Linearity test accuracy results for SHBG

Values in green were deemed to meet all linearity acceptance criteria for SHBG. LCMS06 did not reach acceptable linearity criteria up to the nominal concentration of 50 ug/mL needed to cover QC ranges and expected patient results.

**Linearity Test Calculated vs. Nominal Concentration Results for SHBG**

| Sample | Nominal Concentration (ug/mL) | Lowest Observed RR *LLOQ | Highest Observed RR **ULOQ | INST 1 Day1 (% bias) | INST 2 Day1 (% bias) | INST 1 Day2 (% bias) | INST 2 Day2 (% bias) | INST 1 Day3 (% bias) | INST 2 Day3 (% bias) |
|---|---|---|---|---|---|---|---|---|---|
| Cal2 | 1.38 | 0.08* | 0.12 | 17.0 | 7.0 | -6.0 | 12.0 | 19.1 | 18.0 |
| Cal3 | 1.98 | 0.12 | 0.16 | 18.0 | 11.0 | 12.0 | - | 17.4 | 12.9 |
| Cal4 | 2.82 | 0.18 | 0.28 | 2.0 | 0.0 | -4.0 | 5.0 | -4.4 | -1.9 |
| Cal5 | 4.04 | 0.30 | 0.37 | 7.0 | 4.0 | -1.0 | 5.0 | -6.7 | -4.6 |
| Cal6 | 5.76 | 0.44 | 0.66 | -3.0 | -4.0 | -7.0 | -5.0 | -1.9 | -2.2 |
| Cal7 | 8.24 | 0.65 | 0.83 | 3.0 | 3.0 | 2.0 | 4.0 | -2.9 | -1.8 |
| Cal8 | 11.76 | 0.84 | 1.29 | 0.0 | 1.0 | 3.0 | 4.0 | -2.3 | -2.4 |
| Cal9 | 16.81 | 1.29 | 1.60 | 8.0 | 8.0 | -3.0 | -1.0 | 0.1 | 1.2 |
| Cal10 | 24.01 | 1.67 | 2.66 | 3.0 | 3.0 | 10.0 | 9.0 | 0.3 | -4.1 |
| Cal11 | 34.30 | 3.10 | 4.09 | -20.0 | -18.0 | -15.0 | -16.0 | 2.4 | 5.4 |
| Cal12 | 49.00 | 3.70 | 4.91** | 4.0 | 4.0 | 10.0 | 8.0 | -1.0 | -1.6 |

After applying a linear fit across a range of nominal concentrations that yield the best calculated accuracy of the SHBG concentration, a range of nominal concentrations was found that: yielded a good correlation for all instruments used in validation; covered the apparent concentration values for QC1 and QC2 (4.2 to 45.4 ug/mL); covered the range of response ratios expected for the intended use patient population; and yielded bias values within ±20. The difference between the observed and expected response is divided by the expected response to measure bias as a ratio. There was no systematic bias in the accuracy values and accordingly, the assay was deemed to yield acceptable results across all instruments.

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**Figure 1.** Example correlation plots for IBP4 and SHBG

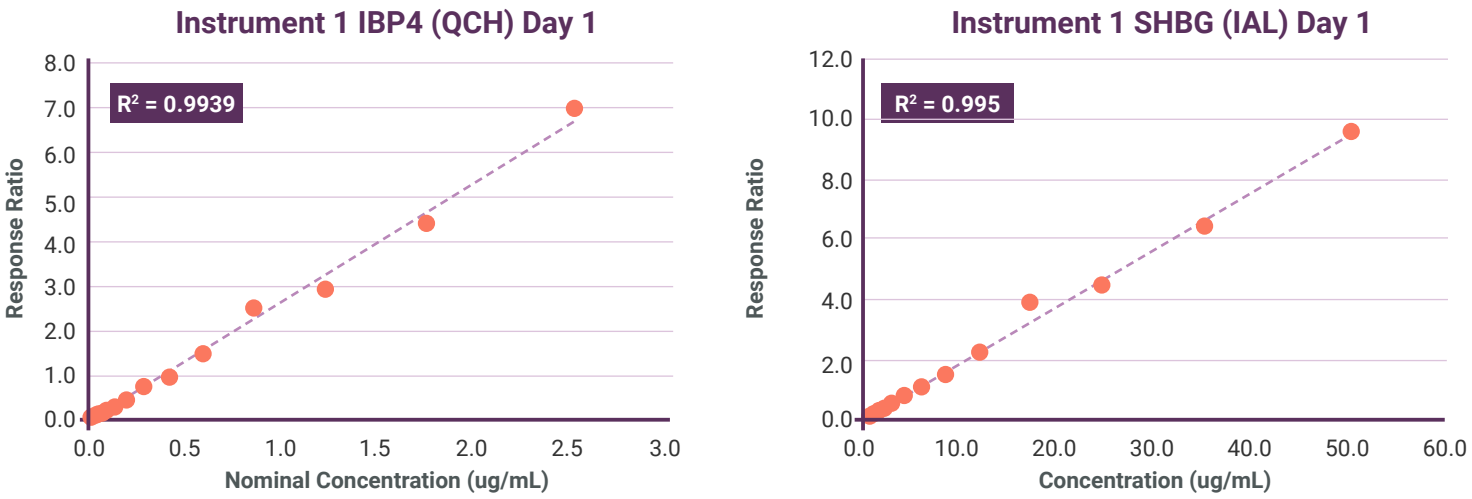


**Table 2.** Linearity test accuracy results for IBP4

Values in green were deemed to meet all linearity acceptance criteria for IBP4.

**Linearity Test Calculated vs. Nominal Concentration Results for IBP4**

| Sample | Nominal Concentration (ug/mL) | Lowest Observed RR *LLOQ | Highest Observed RR **ULOQ | INST 1 Day1 (% bias) | INST 2 Day1 (% bias) | INST 1 Day2 (% bias) | INST 2 Day2 (% bias) | INST 1 Day3 (% bias) | INST 2 Day3 (% bias) |
|---|---|---|---|---|---|---|---|---|---|
| Cal2 | 0.07 | 0.05* | 0.15 | 11.4 | 2.8 | 18.4 | 16.5 | 16.5 | 12.2 |
| Cal3 | 0.10 | 0.08 | 0.19 | 15.4 | 11.0 | 19.5 | - | 18.8 | 6.3 |
| Cal4 | 0.14 | 0.11 | 0.27 | 1.1 | -1.3 | 7.7 | 9.0 | -8.0 | -0.8 |
| Cal5 | 0.20 | 0.20 | 0.34 | 3.0 | 5.5 | 3.6 | 6.6 | -9.9 | -2.1 |
| Cal6 | 0.29 | 0.29 | 0.48 | -3.1 | -2.7 | -5.8 | -5.0 | -6.0 | -0.5 |
| Cal7 | 0.41 | 0.43 | 0.62 | 5.0 | 3.7 | 0.3 | 2.4 | 3.5 | -2.3 |
| Cal8 | 0.59 | 0.53 | 0.93 | 0.7 | 1.6 | 2.5 | 3.3 | -4.4 | 0.4 |
| Cal9 | 0.84 | 0.86 | 1.23 | 7.1 | 6.9 | -2.8 | -2.6 | 2.3 | -1.1 |
| Cal10 | 1.20 | 1.08 | 1.80 | 2.4 | 3.7 | 8.8 | 7.4 | 0.2 | -3.6 |
| Cal11 | 1.72 | 1.86 | 2.80 | -16.9 | -18.1 | - | - | 1.6 | 5.1 |
| Cal12 | 2.45 | 2.38 | 3.39** | 5.5 | 7.2 | 6.7 | 7.8 | -0.8 | -1.5 |

After applying a linear fit across a range of nominal concentrations that yield the best calculated accuracy of the IBP4 concentration, a range of nominal concentrations was found that: yielded a good correlation for all instruments; covered the apparent concentration values for QC1 and QC2 (0.16 to 0.67 ug/mL); covered the range of response ratios expected for the intended use patient population; and yielded bias values within ±20%. The difference between the observed and expected response is divided by the expected response to measure bias as a ratio. There was no systematic bias in the accuracy values and accordingly, the assay was deemed to yield acceptable results across all instruments.

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# Performance Characteristics

**Table 3.** Signal to background at lower limit of quantitation

| Sample | Instrument 1 | | | Instrument 2 | | |
|---|---|---|---|---|---|---|
| | Average Signal (RR) | Background (UTAK Blank Serum) | Ratio | Average Signal (RR) | Background (UTAK Blank Serum) | Ratio |
| Cal2 | 0.0934 | 0.001 | **93.4** | 0.107 | 0.001 | **107.1** |
| Cal2 | 0.1306 | 0.001 | **130.6** | 0.1253 | 0.001 | **125.3** |

The ratio obtained by dividing the signal obtained at the LLOQ by the background signal was found to be ≥5 times the analyte response at the zero calibrator. Accordingly, the assay was deemed to yield acceptable results across all instruments.

**Table 4.** Imprecision at lower limit of quantitation

| Protein | Sample | Concentration (ug/mL) | INST 1 Day 1 (% CV) | INST 2 Day 1 (% CV) | INST 1 Day 2 (% CV) | INST 2 Day 2 (% CV) | INST 1 Day 3 (% CV) | INST 2 Day 3 (% CV) |
|---|---|---|---|---|---|---|---|---|
| IBP4 | Cal2 | 0.07 | 11% | 3% | 18% | 17% | 17% | 12% |
| SHBG | Cal2 | 1.38 | 17% | 7% | 6% | 12% | 19% | 18% |

**Table 5.** Linearity test linear fit results for SHBG

Both instruments yielded linear correlation coefficients that met acceptance criteria for SHBG linearity.

| Instrument | Correlation Coefficient ($R^2$) | Pass/Fail ($R^2 > 0.99$) |
|---|---|---|
| LCMS03 | 0.9947 | PASS |
| LCMS04 | 0.9942 | PASS |

**Table 6.** Linearity test linear fit results for IBP4

Both instruments yielded linear correlation coefficients that met acceptance criteria for IBP4 linearity.

| Instrument | Correlation Coefficient ($R^2$) | Pass/Fail ($R^2 > 0.99$) |
|---|---|---|
| LCMS03 | 0.9981 | PASS |
| LCMS04 | 0.9982 | PASS |

## Assay Reportable Range

The lowest and highest reportable individualized risks 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 20141, contemporaneous with the clinical studies. The upper bound is truncated to the highest score observed in clinical validation studies.

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# Performance Characteristics

## Analytical Specificity/Interference

Interference and specificity were evaluated by spiking a pooled serum sample derived from our intended use population ith two levels of Triglyceride Rich Lipoproteins, conjugated and unconjugated bilirubin, and hemolysate at levels considered clinically high and pathological. No significant shift in the results ($p \geq 0.05$) was detected at any level of interference. Additionally, no level of interference shifted the retention time in a way that prevented specific identification of the target analyte or changed its qualitative to quantitative transition ratio significantly.

## Precision – Intrabatch (Repeatability)

Intrabatch precision was evaluated by testing samples (N = 28 for each sample type) that represented high, low and threshold test results from our intended use population. These samples were analyzed repeatedly within a day to demonstrate that the assay could yield CVs ≤ 20%. Samples were tested for both response ratios and proteomic score, but the score result is used in clinical decision making.

**Table 7.** Within-batch imprecision (repeatability) of analytes by batch

| SAMPLE | Analyte/Score | N | % CV | Pass/Fail |
|---|---|---|---|---|
| High | IBP4 | 28 | 1.1 | Pass |
| | SHBG | 28 | 1.5 | Pass |
| | Score | 28 | 1.5 | Pass |
| Low | IBP4 | 28 | 2.4 | Pass |
| | SHBG | 28 | 1.1 | Pass |
| | Score | 28 | 1.1 | Pass |
| Threshold | IBP4 | 28 | 2.5 | Pass |
| | SHBG | 28 | 1.2 | Pass |
| | Score | 28 | 1.5 | Pass |

The method demonstrated acceptable repeatability.

## Precision – Interbatch (Reproducibility)

Interbatch precision was evaluated by testing samples (N = 294 for each sample type) that represented high, low and threshold test results from the intended use population. These samples were analyzed repeatedly across 21 batches spread over more than 20 days across all test systems and personnel, to demonstrate that the assay could yield overall CVs of ≤ 20% across that time frame.

**Table 8.** Interbatch imprecision (reproducibility) of analytes

| SAMPLE | Analyte/Score | N | % CV | Pass/Fail |
|---|---|---|---|---|
| High | IBP4 | 294 | 9.2 | Pass |
| | SHBG | 294 | 6.5 | Pass |
| | Score | 294 | 7.7 | Pass |
| Low | IBP4 | 294 | 8.4 | Pass |
| | SHBG | 294 | 6.4 | Pass |
| | Score | 294 | 3.3 | Pass |
| Threshold | IBP4 | 294 | 13.5 | Pass |
| | SHBG | 294 | 4.5 | Pass |
| | Score | 294 | 3.3 | Pass |

The method demonstrated acceptable reproducibility.

# Performance Characteristics

## LIMITS OF QUANTITATION

The lower and upper limits of quantitation were determined from linearity experiments, in which recombinant IBP4 and SHBG proteins were spiked into blank whole blood matrix at known nominal concentrations. The upper limit of quantitation was the highest nominal concentration of each analyte that yielded an acceptable correlation (R2) and had a calculated concentration within ±20% of the nominal concentration and had a CV ≤20%. The lower limit of quantitation met the same criteria but also had a signal-to-background > 5.

**Table 1.** Linearity test accuracy results for SHBG

Values in green were deemed to meet all linearity acceptance criteria for SHBG.

| Sample | Nominal Concentration (ug/mL) | Lowest Observed RR *LLOQ | Highest Observed RR **ULOQ | INST 1 Day1 (% bias) | INST 2 Day1 (% bias) | INST 1 Day2 (% bias) | INST 2 Day2 (% bias) | INST 1 Day3 (% bias) | INST 2 Day3 (% bias) |
|---|---|---|---|---|---|---|---|---|---|
| Cal2 | 0.69 | 0.086* | 0.137 | -19.4 | -18.5 | -17.1 | -13.4 | -4.7 | -19.8 |
| Cal3 | 0.99 | 0.122 | 0.188 | -10.9 | -11.4 | -10.5 | -15.7 | 4.9 | -9.0 |
| Cal4 | 1.41 | 0.164 | 0.256 | -8.1 | -9.1 | 3.8 | 7.0 | 2.6 | -4.6 |
| Cal5 | 2.02 | 0.237 | 0.384 | -3.4 | -6.6 | -6.9 | -6.2 | 6.7 | 0.9 |
| Cal6 | 2.88 | 0.319 | 0.574 | -1.6 | -3.0 | -9.0 | -8.8 | 6.3 | 3.2 |
| Cal7 | 4.12 | 0.542 | 0.772 | -0.3 | -4.1 | -0.2 | 0.1 | -4.0 | -5.4 |
| Cal8 | 5.88 | 0.762 | 1.082 | 0.4 | 1.0 | -0.8 | 0.2 | -2.1 | -2.8 |
| Cal9 | 8.40 | 1.095 | 1.565 | -0.7 | -0.2 | 3.1 | 4.0 | -2.3 | -0.3 |
| Cal10 | 12.01 | 1.321 | 2.277 | 1.9 | 2.2 | 1.1 | 0.6 | 0.3 | 2.7 |
| Cal11 | 17.15 | 2.047 | 3.355 | 4.7 | 6.2 | 2.9 | 2.0 | 5.0 | 6.4 |
| Cal12 | 24.50 | 3.200 | 4.700 | -1.7 | -0.8 | 0.6 | 0.0 | -3.3 | -3.2 |
| Cal13 | 35.00 | 4.592 | 6.405** | 0.1 | -1.0 | -1.2 | -0.7 | -0.9 | -0.2 |

After applying a linear fit across a range of nominal concentrations that yield the best calculated accuracy of the SHBG concentration, a range of nominal concentrations was found that: yielded a good correlation for all instruments used in validation; covered the apparent concentration values for QC1 and QC2 (0.05 to 20.0 ug/mL); covered the range of response ratios expected for the intended use patient population; and yielded bias values within ±20%. There was no systematic bias in the accuracy values and accordingly, the assay was deemed to yield acceptable results across all instruments.

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# Performance Characteristics

**Figure 1.** Example correlation plots for IBP4 and SHBG

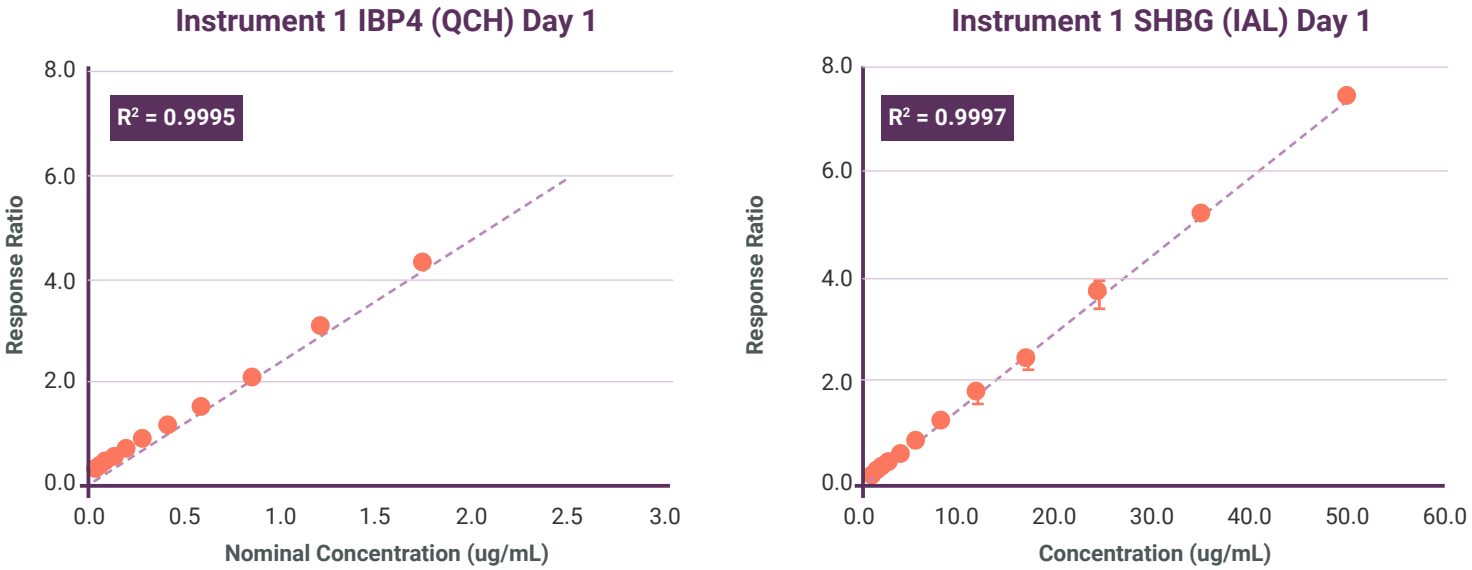


**Table 2.** Linearity test accuracy results for IBP4

Values in green were deemed to meet all linearity acceptance criteria for IBP4.

| Sample | Concentration (ug/mL) | Lowest Observed RR *LLOQ | Highest Observed RR **ULOQ | INST 1 Day 1 (% bias) | INST 2 Day 1 (% bias) | INST 1 Day 2 (% bias) | INST 2 Day 2 (% bias) | INST 1 Day 3 (% bias) | INST 2 Day 3 (% bias) |
|---|---|---|---|---|---|---|---|---|---|
| Cal1 | 0.02 | 0.200* | 0.308 | -16.4 | -15.6 | -14.2 | -19.8 | -3.4 | -11.0 |
| Cal2 | 0.03 | 0.224 | 0.323 | -12.2 | -17.3 | -2.5 | -19.3 | -19.7 | -15.2 |
| Cal3 | 0.05 | 0.246 | 0.351 | 0.6 | -11.7 | -7.7 | -5.9 | 11.6 | -5.5 |
| Cal4 | 0.07 | 0.289 | 0.447 | -5.7 | -10.6 | 16.3 | 6.7 | 3.0 | -13.8 |
| Cal5 | 0.10 | 0.361 | 0.518 | -2.3 | -4.4 | 0.8 | -10.0 | 4.7 | 8.2 |
| Cal6 | 0.14 | 0.421 | 0.679 | 1.5 | -1.9 | -8.7 | -12.9 | 4.6 | 3.7 |
| Cal7 | 0.21 | 0.584 | 0.788 | -2.6 | 0.2 | -0.5 | -1.2 | -4.6 | -4.1 |
| Cal8 | 0.29 | 0.751 | 1.062 | 1.1 | 5.3 | 0.3 | -0.3 | -2.3 | -4.5 |
| Cal9 | 0.42 | 1.087 | 1.393 | 0.9 | -0.6 | 3.9 | 5.3 | -0.4 | -0.6 |
| Cal10 | 0.60 | 1.222 | 2.083 | 0.6 | 0.6 | -0.4 | 1.4 | 0.7 | 3.6 |
| Cal11 | 0.86 | 1.836 | 2.921 | 3.6 | 5.8 | -0.1 | 2.5 | 4.5 | 5.8 |
| Cal12 | 1.23 | 2.922 | 3.838 | -1.9 | -2.7 | -0.7 | 1.5 | -3.4 | -3.9 |
| Cal13 | 1.75 | 3.870 | 5.431** | 0.0 | -0.2 | 0.3 | -1.7 | -0.4 | 0.7 |

After applying a linear fit across a range of nominal concentrations that yield the best calculated accuracy of the IBP4 concentration, a range of nominal concentrations was found that: yielded a good correlation for all instruments; covered the apparent concentration values for QC1 and QC2 (0.16 to 0.67 ug/mL); covered the range of response ratios expected for the intended use patient population; and yielded bias values ≤ 20%. There was no systematic bias in the accuracy values. Accordingly, the assay was deemed to yield an acceptable linearity for IBP4 across all instruments.

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# Performance Characteristics

**Table 3.** Signal to background ratio at lower limit of quantitation

| Protein | Sample | Instrument 1 | | | Instrument 2 | | |
|---|---|---|---|---|---|---|---|
| | | Average Signal (RR) | Background (UTAK Blank Serum) | Ratio | Average Signal (RR) | Background (UTAK Blank Serum) | Ratio |
| SHBG | Cal2 | 0.106 | 0.001 | **106** | 0.100 | 0.001 | **100** |
| IBP4 | Cal1 | 0.245 | 0.001 | **245** | 0.232 | 0.001 | **232** |

The ratio obtained by dividing the signal obtained at the LLOQ by the background signal was found to be ≥5 times the analyte response at the zero calibrator. Accordingly, the assay was deemed to yield acceptable results across all instruments.

**Table 4.** Imprecision at lower limit of quantitation

| Protein | Sample | Concentration (ug/mL) | INST 1 Day 1 (% CV) | INST 2 Day 1 (% CV) | INST 1 Day 2 (% CV) | INST 2 Day 2 (% CV) | INST 1 Day 3 (% CV) | INST 2 Day 3 (% CV) |
|---|---|---|---|---|---|---|---|---|
| SHBG | Cal2 | 0.69 | 5% | 7% | 9% | 9% | 5% | 7% |
| IBP4 | Cal1 | 0.02 | 5% | 4% | 10% | 10% | 8% | 4% |

**Table 5.** Linearity test linear fit results for SHBG

Both instruments yielded linear correlation coefficients that met acceptance criteria for SHBG linearity.

| Instrument | Correlation Coefficient ($R^2$) | Pass/Fail ($R^2 > 0.99$) |
|---|---|---|
| Instrument 1 | 0.9997 | PASS |
| Instrument 2 | 0.9995 | PASS |

**Table 6.** Linearity test linear fit results for IBP4

Both instruments yielded linear correlation coefficients that met acceptance criteria for IBP4 linearity.

| Instrument | Correlation Coefficient ($R^2$) | Pass/Fail ($R^2 > 0.99$) |
|---|---|---|
| Instrument 1 | 0.9995 | PASS |
| Instrument 2 | 0.9988 | PASS |

## Assay Reportable Range

The lowest and highest reportable individualized risks 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 20141, contemporaneous with the clinical studies. The upper bound is truncated to the highest score observed in clinical validation studies.

**DRIED WHOLE BLOOD SAMPLES**
using Antibody Capture Methodologies with LCMS Analysis

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# Performance Characteristics

## Analytical Specificity/Interference

Interference and specificity were evaluated by spiking a pooled serum sample derived from our intended use population with two levels of Triglyceride Rich Lipoproteins, conjugated and unconjugated bilirubin, and hemolysate at levels considered clinically high and pathological. No significant shift in the results ($p \geq 0.05$) was detected at any level of interference. Additionally, no level of interference shifted the retention time in a way that prevented specific identification of the target analyte or changed its qualitative to quantitative transition ratio significantly.

## Precision – Intrabatch (Repeatability)

Intrabatch precision was evaluated by testing samples (N = 32 for each sample type) that represented high, low and threshold test results from our intended use population. These samples were analyzed repeatedly within a day to demonstrate that the assay could yield CVs ≤ 20%. Samples were tested for both response ratios and proteomic score, but the score result is used in clinical decision making.

**Table 7.** Within-batch imprecision (repeatability) of analytes by batch

| SAMPLE | Analyte/Score | N | % CV | Pass/Fail |
|---|---|---|---|---|
| High | IBP4 | 31* | 1.3 | Pass |
| | SHBG | 31* | 0.9 | Pass |
| | Score | 31* | 4.2 | Pass |
| Low | IBP4 | 32 | 4.4 | Pass |
| | SHBG | 32 | 3.3 | Pass |
| | Score | 32 | 4.0 | Pass |
| Threshold | IBP4 | 32 | 9.0 | Pass |
| | SHBG | 32 | 1.7 | Pass |
| | Score | 32 | 3.3 | Pass |

* One replicate dropped owing to liquid handling issue

The method demonstrated acceptable repeatability.

## Precision – Interbatch (Reproducibility)

Interbatch precision was evaluated by testing samples (N = 294 for each sample type) that represented high, low and threshold test results from the intended use population. These samples were analyzed repeatedly across 21 batches spread over more than 20 days across all test systems and personnel, to demonstrate that the assay could yield overall CVs of ≤ 20% across that time frame.

**Table 8.** Interbatch imprecision (reproducibility) of analytes

| SAMPLE | Analyte/Score | N | % CV | Pass/Fail |
|---|---|---|---|---|
| High | IBP4 | 95* | 5.3 | Pass |
| | SHBG | 95* | 4.8 | Pass |
| | Score | 95* | 5.5 | Pass |
| Low | IBP4 | 96 | 6.0 | Pass |
| | SHBG | 96 | 5.1 | Pass |
| | Score | 96 | 5.1 | Pass |
| Threshold | IBP4 | 96 | 5.3 | Pass |
| | SHBG | 96 | 2.1 | Pass |
| | Score | 96 | 1.2 | Pass |

* One replicate dropped owing to liquid handling issue

The method demonstrated acceptable reproducibility.

**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 spontaneous 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.

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