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Immunoassay Performance of a Preterm Birth Classifier

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Abstract

Introduction: We developed a mass spectrometry (MS)-based proteomics approach that identified two serum protein biomarkers predictive of spontaneous preterm birth (sPTB) risk. Here we present sPTB classification performance derived from the immunoassay measure of these proteins using specimens collected within the 19-21 weeks gestational age window of the Proteomic Assessment of Preterm Risk (PAPR) study. Performance is based on a predefined logarithmic classifier score derived from measures of IBP4 and SHBG.

Methods: A set of 30 individual clinical specimens and pooled serum standards were analyzed by our peptide-based LC-MRM-MS workflow and in parallel by ELISA to screen antibody pairs for MS-ELISA correlation and for reproducibility. In a nested case:control (1:2) study, representative samples from the PAPR trial were analyzed by ELISAs using the highest performing antibody pairs. sPTB classification performance was calculated using the 57 subjects in the cohort and 34 subjects within a Body Mass Index (BMI) restricted subset. Sensitivity and specificity were calculated from the point on the ROC curve where the sum of each value was maximal.

Results: Correlation between ELISA and MS-based measurements of IBP4 and SHBG were calculated for several antibody pairs, with the best performing sets giving Pearson r values of 0.86 and 0.84 respectively. These antibodies also gave highly reproducible ELISA data; intraplate CVs for IBP4 and SHBG were 4.4% and 7.0% respectively. ELISA-based classifier scores resulted in AUCs of 0.82 and 0.72 with and without the BMI restriction [table1].

	AUC	p-value	Sensitivity	Specificity
All BMI	0.72	0.0030	84.21%	60.53%
BMI restricted	0.82	0.0014	92.31%	66.67%

Conclusions: We demonstrate a two protein ELISA platform that is highly correlative with LC-MRM-MS results and generates sPTB risk scores with excellent classification performance. An immunoassay-based test may improve throughput relative to the technically complex LC-MRM-MS workflow, and thereby enable scaling required to address the need for risk assessment for preterm birth in both developed and developing countries where sPTB rates are very high.

Keywords: Preterm birth, Biomarkers, Immunoassay

Proteomic Assessment of Preterm Risk (PAPR) Clinical Trial

Purpose: to collect blood specimens and corresponding clinical data from asymptomatic pregnant women to develop a noninvasive test for prediction of preterm delivery

Primary Outcome Measures: Spontaneous Preterm Birth (SPTB)

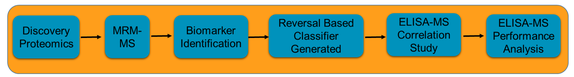
Enrollment: 5,501 pregnant women who were receiving prenatal care

Inclusion Criteria:
- Subject is 18 years or older.
- Subject has a singleton pregnancy.
- Subject is able to provide consent.

Exclusion Criteria:
- Subject is pregnant with more than one fetus.
- There is a known or suspected fetal anomaly.

ClinicalTrials.gov Identifier: NCT01371019

Preterm Immunoassay Strategy

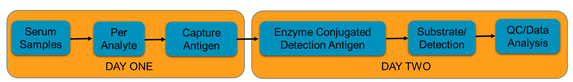


MRM-MS Work Flow

MS Derived Reversal Based Classifier

Figure 1. sPTB (orange) versus term (blue). **A)** IBP4 higher in sPTB *vs*. term in GA week window 17-23; **B)** SHBG lower in sPTB *vs.* term; **C)** Averaged classifier scores [ln(IBP4/SHBG)] across GA week windows, higher scores in sPTB compared to term births. Generating the "reversal" of upregulated IBP4/downregulated SHBG increases the separation between sPTB cases and controls relative to each analyte.

Immunoassay (ELISA) Work flow



IBP4 and SHBG Plate CVs

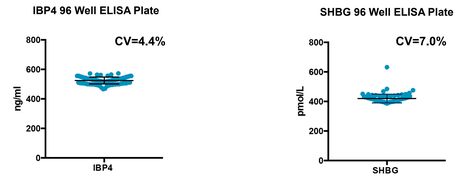


Figure 2. Immunoassay intraplate CVs for IBP4 and SHBG were 4.4% and 7.0%.

Analyte Correlation: Immunoassay vs. MS

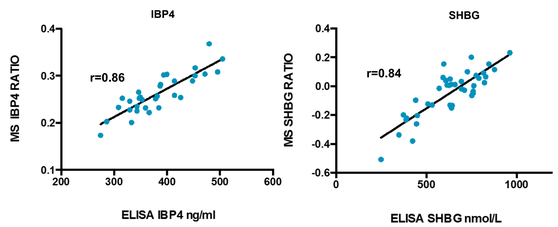


Figure 3. The relative values generated by MS and immunoassay for the individual analytes IBP4 and SHBG correlated with a Pearson's r value of 0.86 and 0.84, respectively.

Assay Correlation: Immunoassay vs. MS

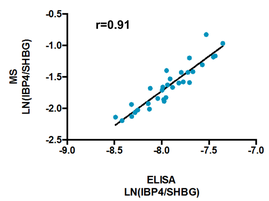


Figure 4. The multi-analyte algorithmic classifier [ln(IBP4/SHBG)] generated by both MS and immunoassay correlated with a Pearson's r value of 0.91.

Ability to Separate Term from sPTB: Immunoassay vs. MS

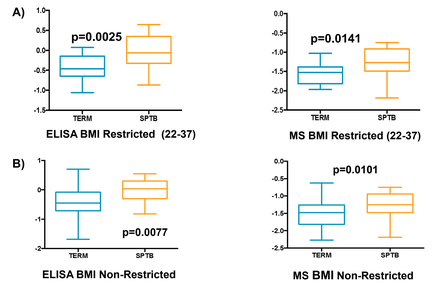


Figure 5. A) Term and sPTB samples from patients with a BMI score between 22 and 37 were separated with p-values of 0.0025 and 0.0141 by ELISA and MS respectively. **B)** Term and sPTB samples without any BMI restrictions were separated with p-values of 0.0077 and 0.101 by ELISA and MS. All samples were acquired from women at $19^{0/7}$-$21^{6/7}$ weeks GA.

Performance: Immunoassay vs. MS

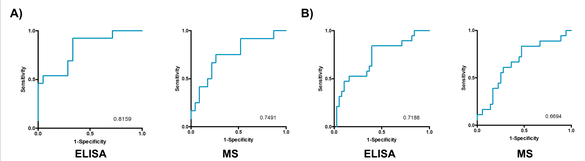


Figure 6. A) The AUC for ELISA and MS performance with samples from patients with a BMI between 22 and 37 was 0.8159 and 0.7491, respectively. **B)** The AUC for ELISA and MS classifier performance was from samples from patients with no BMI restrictions was 0.7188 and 0.6694, respectively. All samples were acquired from women at $19^{0/7}$-$21^{6/7}$ weeks GA.

Conclusions

We demonstrate a two protein ELISA platform for the prediction of preterm birth that is highly correlative with published LC-MRM-MS results[1].

Our immunoassay generates sPTB risk scores with excellent classification performance.

An immunoassay-based test may enable the scaling required for preterm birth risk assessment in both developed and developing countries where sPTB rates are very high.

Stratifying risk in populations may enable the exploration of intervention strategies.

[1]Saade GR, Boggess KA, Sullivan SA, Markenson GR, Iams JD, Coonrod DV, Pereira LM, Esplin MS, Cousins LM, Lam GK, Hoffman MK, Severinsen RD, Pugmire T, Flick JS, Fox AC, Lueth AJ, Rust SR, Mazzola E, Hsu C, Dufford MT, Bradford CL, Ichetovkin IE, Fleischer TC, Polpitiya AD, Critchfield GC, Kearney PE, Boniface JJ, Hickok DE. **Development and validation of a spontaneous preterm delivery predictor in asymptomatic women**. Am J Obstet Gynecol. 2016 Feb 11. pii: S0002-9378(16)00284-2. doi: 10.1016/j.ajog.2016.02.001. [Epub ahead of print]