Poster 285

Proteomics: Clinical Applications

ASMS 2014

June 19, 2014

Baltimore, MD

Development and Validation of an Analytical Method for Discovery of Biomarkers of Preterm Birth

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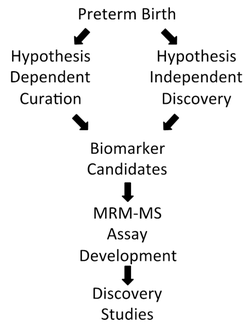
SERA PROGNOSTICS

Introduction

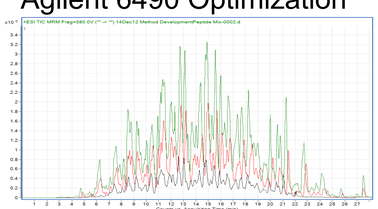
- PTB is multifactorial with etiologies that include: infection, inflammation, placental hemorrhage, uterine distention and stress.
- Existing tests have poor performance and are limited in utility, especially in nulliparous patients.
- There is a compelling need to identify biomarkers of PTB to enable interventions for patients at risk.
- The complex etiology of PTB indicates that a prerequisite of good test performance will be the coverage of multiple biological pathways.
- We used both hypothesis dependent and independent approaches to identify candidate analytes of broad biological relevance.
- **We report the development of a MS-based highly multiplexed assay and its use in the discovery of biomarkers of PTB.**



Process Workflow

- Human gold standards (HGS) and clinical samples were processed through the workflow above in a 96-well format
- Each step was optimized to reduce variability and to improve throughput
- Data was acquired on an Agilent 6490 in dMRM mode and peak areas were calculated using Mass Hunter software.
- Data was corrected for run-order and batch effects.

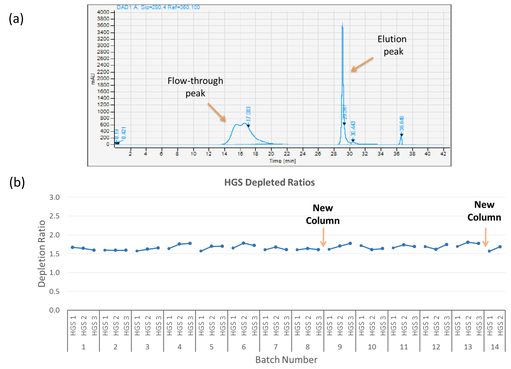
Agilent 6490 Optimization



Total ion chromatogram showing improved performance after source conditions (red) and dMRM parameters (green) were optimized. For dMRM optimization, the delta RTs were minimized in the middle of the elution profile where transition concurrency was high and increased in the sparse portions of the chromatogram.

Optimization of mass spectrometer-specific parameters increased sensitivity and the number of analytes able to be detected.

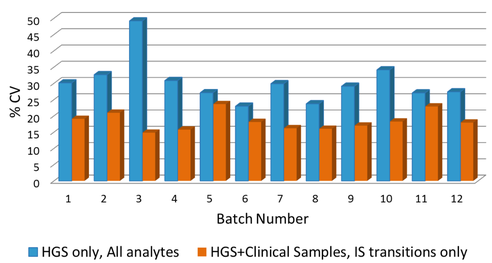
Mars-14 Depletion Variability



(a) MARS-14 depletion chromatogram defining the flow through (depleted serum sample) and elution (depleted proteins) peaks. (b) Ratio of the areas of the flow through peak to the elution peak for HGS replicates across 14 batches. For each batch, the HGS samples were interspersed with clinical samples (not shown).

The %CVs for HGS replicates on the MARS-14 column ranged from 0.2% to 5% per batch, and was 3.1% across all 14 batches and 3 different columns.

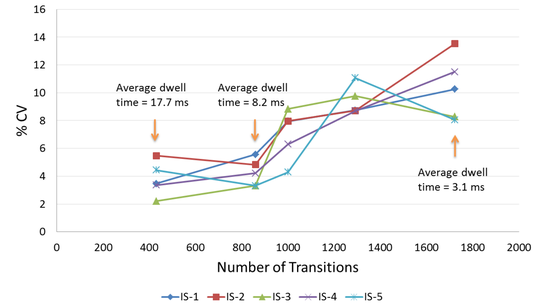
Initial Round of Discovery Variability



270 clinical serum samples and 36 HGS samples were run on the Agilent 6490 using a dMRM assay comprised of 1722 transitions. The coefficient of variation was calculated for all detectable transitions in just the HGS samples (blue), and averaged per batch. The %CVs were also calculated for 5 IS peptides that were added just prior to LC-MS/MS (orange), and averaged per MS batch.

The variability within a MS batch as estimated by the IS peptides was 17.3% (average of all the batch averages) and the variability of the entire process averaged 30.3%.

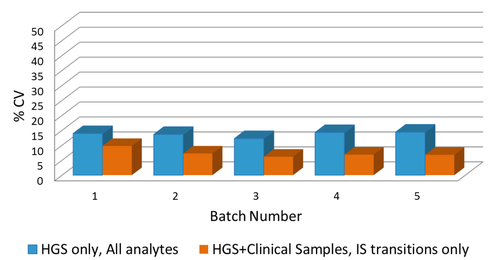
Reducing Transition Numbers Reduces CV



A neat solution of the 5 IS peptides was analyzed using the full dMRM method (1722 transitions) and compared to similar methods containing a reduced number of total transitions. The %CVs were calculated for each IS peptide for each method, as well as the average dwell times.

%CVs improved as the number of transitions decreased to about 900, after which no improvement was seen, despite a continued increase in dwell times. With ~900 transitions, dwell times averaged 8.2 ms.

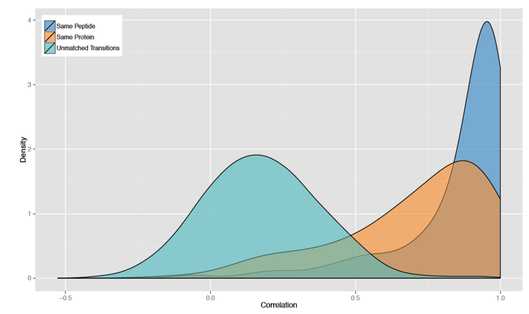
Improved Performance with Truncated Assay



The dMRM assay was reduced to 690 transitions by removing redundancy and poorly behaving analytes. The first five batches were re-run with the truncated assay and the % CVs were calculated for HGS samples (blue), and 5 IS peptides (orange), averaged per batch.

Reducing the number of transitions significantly reduced the assay variability. MS batch variability as estimated by the IS peptides in all samples dropped by more than half to 7.4%. Variability in the entire process as estimated by all transitions in HGS only also was reduced by more than half to 13.7%.

Transition Correlations



Graph showing the correlations between transitions to the same peptide (blue), same protein (orange), or for unmatched proteins (aqua) in the dMRM assay.

Transitions to the same peptide and peptides to the same protein were highly correlated, indicative of a robust assay.

PTB Significant Hit Classification

Functional Category	%	Enrichment P-Value
Immunity and defense	38	<0.001
Complement-mediated immunity	10	<0.001
Proteolysis	22	<0.001
Macrophage-mediated immunity	10	<0.01
Lipid and fatty acid transport	8	<0.01
Cytokine/chemokine signaling	10	<0.01
Ligand-mediated signaling	12	<0.05
Cell communication	20	<0.05
Blood clotting	6	<0.05

Univariate analysis of the MRM discovery study identified analytes amongst multiple pathways and functional categories of relevance to PTB, with immune response highly represented (DAVID Bioinformatics Resources).

Conclusions

- **We evaluated the performance and feasibility of a large MRM assay for biomarker discovery and subsequent clinical diagnostic development.**
- **Our highly complexed dMRM assay was effective in screening large numbers of candidate biomarkers to prioritize peptides and transitions.**
- **Reducing the total number of transitions to less than 900 dramatically improved reproducibility.**
- **Using the "advanced" MRM method, we identified biomarkers across multiple pathways of relevance to PTB.**
- **We are currently building and verifying multivariate classifiers for PTB.**