The Assessment of Cytokines in Dried Blood Spots (DBS)

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I. Inflammation, Cytokines and DBS

II. Uniplex Assay: R&D High Sensitivity ELISA for IL-6

III. Multiplex Assay: MesoScale Discovery and Quansys
Inflammation

• Inflammation is implicated in a host of biological functions, including aging

• C-reactive protein is a broad marker of general inflammation commonly used in population studies

• More complex underlying mechanisms are involved in etiology of inflammation
Cytokines

- Cytokines are molecules that allow immune cells to communicate

- Implicated in up- and down-regulation of inflammation
  - IL-6
  - IL-10
  - TNF-α
Inflammatory cytokines vary across populations based on nutritional status, infectious and chronic disease burden, and other factors.

Limitations

• What is the range of inflammatory states across populations and how is it regulated?

• How do we measure the low concentrations of inflammatory cytokines associated with chronic, as opposed to acute, levels of activity?
Dried Blood Spots

• Dried blood spots (DBS) are a population-friendly alternative to venipuncture blood collection

• A wide range of biomarkers can be assayed in DBS
  – Can we add cytokines to the list?
We have completed validation of a high-sensitivity enzyme-linked immunosorbent assay for IL-6.

Uses DBS calibration materials to control for unique sample matrix.

Innovated the use of filter plates to maximize elution of DBS.
• Sample quantity: 4 x 3.2 mm discs per well
  – 8 discs for duplicate measures
• Lower limit of detection: 0.67 pg/mL
• Precision (within-assay): 6.3%-14.6% across detection range
• Reliability (between assay): 9.4%-15.4% across detection range
Matched Serum-DBS IL-6

Matched Serum and Dried Blood Spot IL-6

\[ y = 1.69x - 0.51 \]
\[ R = 0.88 \]
IL-6 ELISA

• This method can be performed by most wet labs with basic enzyme immunoassay equipment
  – Requires specialized equipment for filter plates

• This method only quantifies one biomarker of inflammation

• We would like lower detection limits
Multiplex Assays

• Assay multiple inflammatory cytokines at once

• Requires specialized equipment

• We are currently evaluating multiplex assay platforms for DBS
  – MesoScale Discovery (MSD) electrochemiluminescence
  – Quansys
We are finalizing a 7-plex pro-inflammatory cytokine assay protocol on MSD platform

- Lower detection limits
- Very good reliability
LLD (pg/mL):

**IL-6**: 0.41

**IL-10**: 1.21

**TNF-α**: 1.1
Conclusion

• Cytokines help us understand the regulation of inflammation across the lifespan and in disease

• DBS cytokine assays will facilitate this research across populations

• We are making exciting headway in assay methodology

• We would like to thank the Biomarker Network for supporting this project