

The Assessment of Cytokines in Dried Blood Spots (DBS)

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Outline

- I. Inflammation, Cytokines and DBS
- II. Uniplex Assay: R&D High Sensitivity ELISA for IL-6
- III. Multiplex Assay: MesoScale Discovery and Quansys



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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



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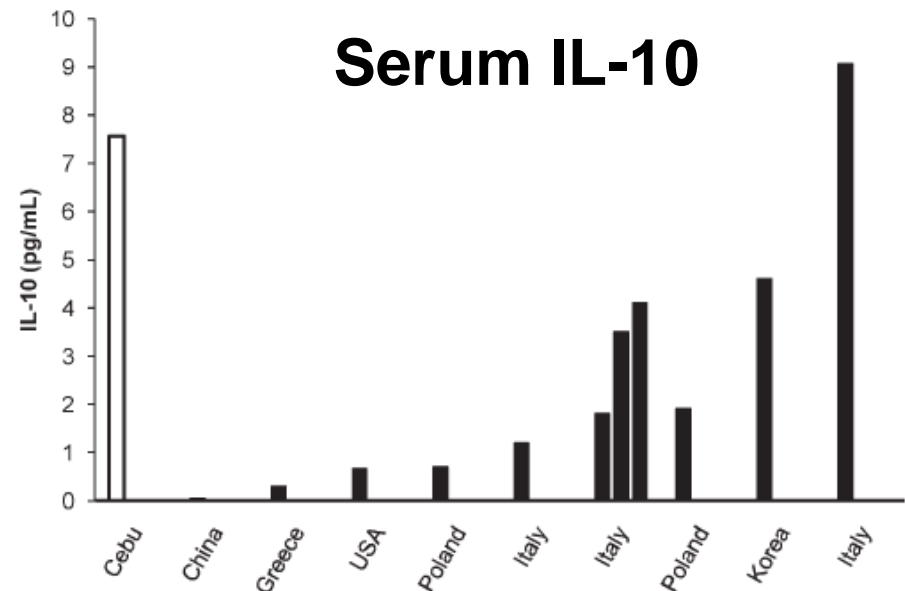
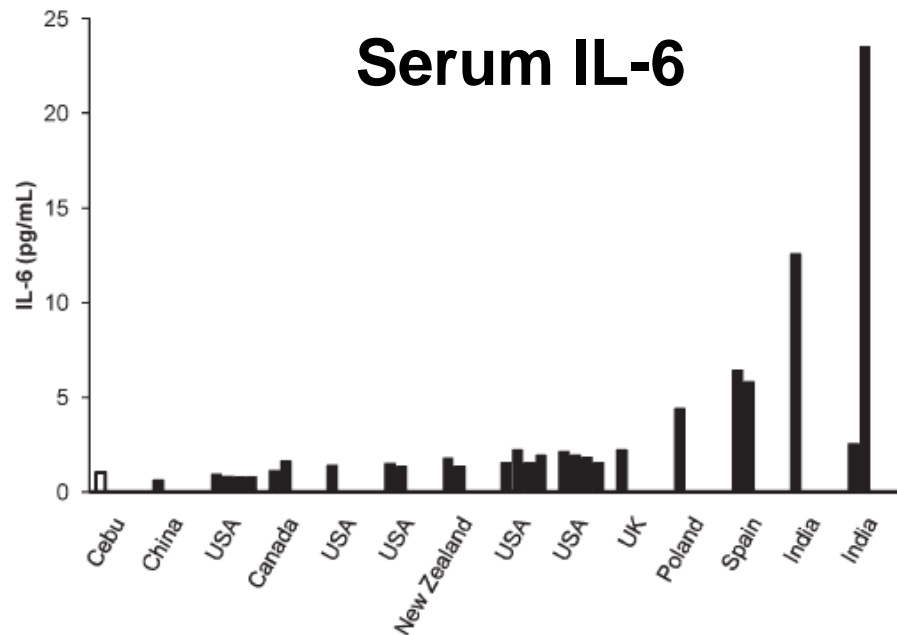
Cytokines

- Cytokines are molecules that allow immune cells to communicate
- Implicated in up- and down-regulation of inflammation
 - IL-6
 - IL-10
 - TNF- α



Cytokine Variation

Inflammatory cytokines vary across populations based on nutritional status, infectious and chronic disease burden, and other factors



From McDade et al. (2011). *Am J Phys Anth*, 146: 373-384.



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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?



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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?





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IL-6 ELISA

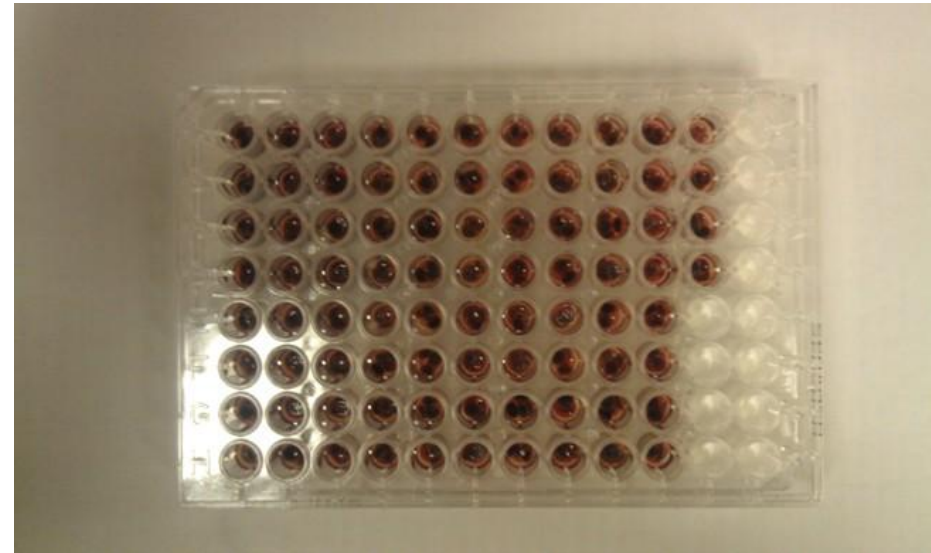
- We have completed validation of a high-sensitivity enzyme-linked immunosorbent assay for IL-6
- Uses DBS calibration materials to control for unique sample matrix
- Innovated the use of filter plates to maximize elution of DBS



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IL-6 ELISA

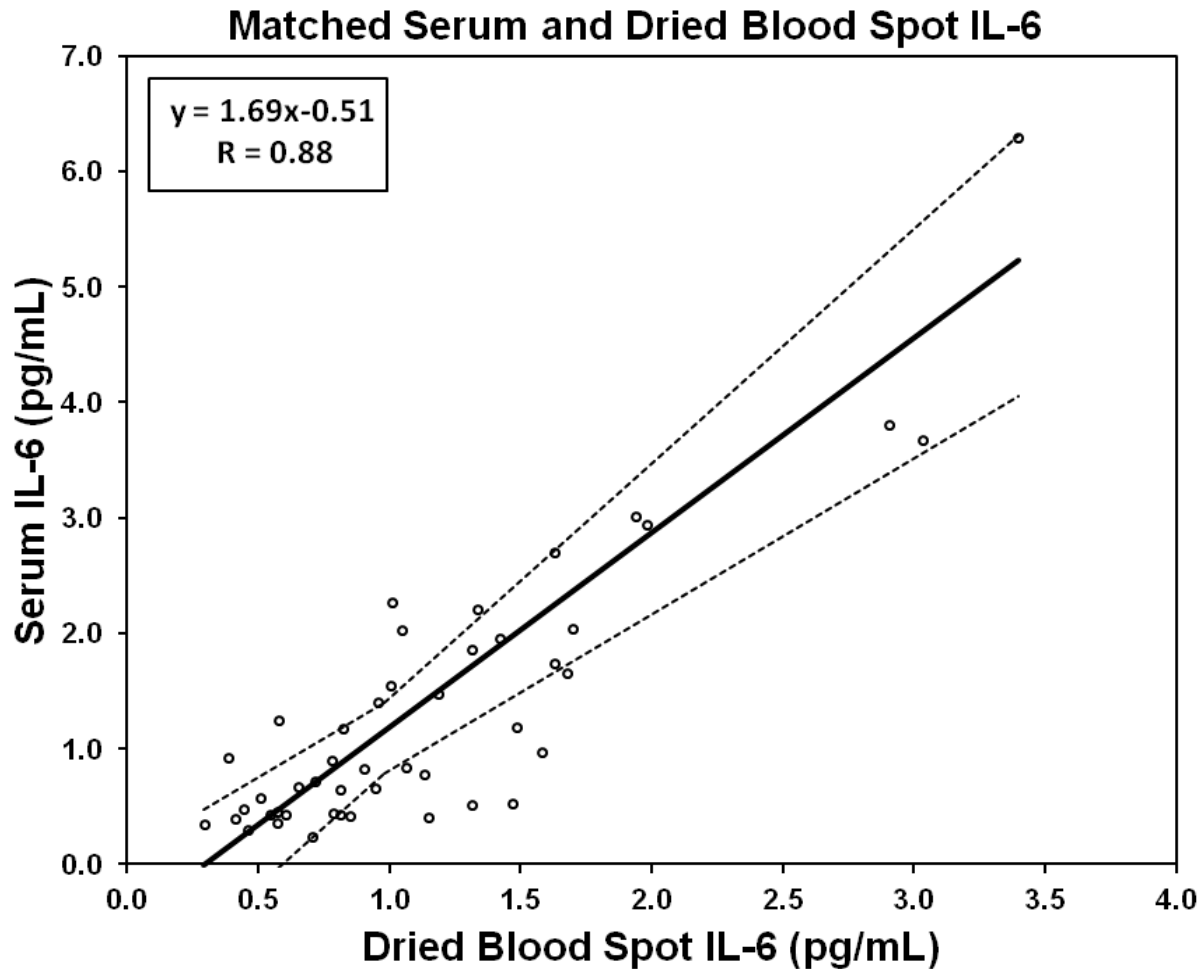
- Sample quantity: 4 x 3.2 mm discs per well
 - 8 discs for duplicate measures
- Lower limit of detection: 0.67 pg/mL
- Precision (within-assay): 6.3%-14.6% across detection range
- Reliability (between assay): 9.4%-15.4% across detection range





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Matched Serum-DBS IL-6





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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



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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



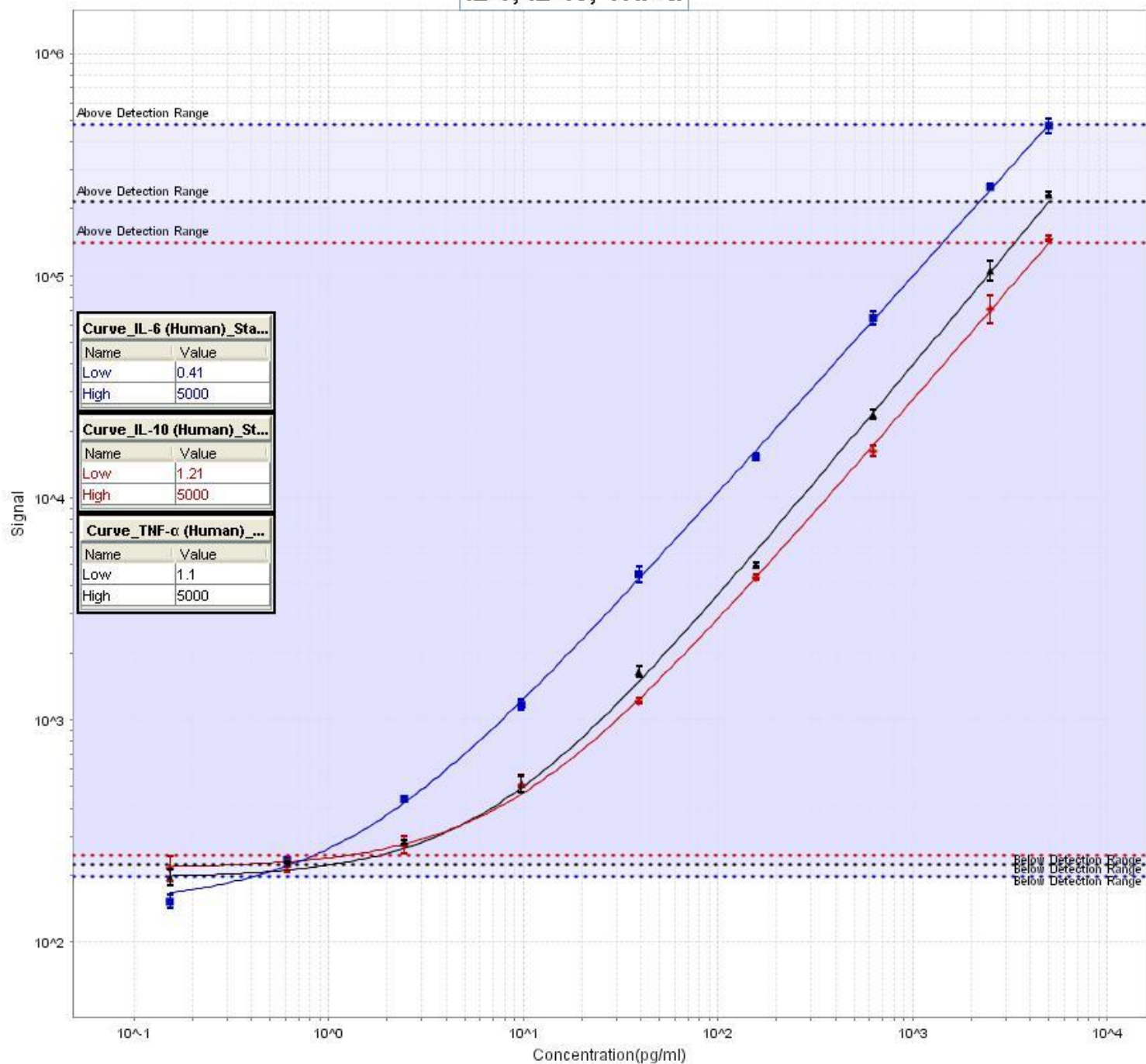
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MSD

- We are finalizing a 7-plex pro-inflammatory cytokine assay protocol on MSD platform
- Lower detection limits
- Very good reliability



IL-6, IL-10, TNF- α



LLD (pg/mL):

IL-6: 0.41

IL-10: 1.21

TNF- α : 1.1



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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