aaps PharmSci 360

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Strategy for Assessing Stability in Multiplexed Cytokine Panels

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Session Description and Objectives

Abstract

Clinical trials requiring analysis of more than one biomarker can greatly benefit from implementation of multiplex biomarker panels. This is a key strategy for pediatric studies, studies with limited sample volumes, or early phase studies exploring diverse drug effects. However, there are challenges associated with developing and validating these methods. Parameters that are particularly challenging for multiplex panels are the assessment of parallelism and evaluation of analyte stability. It can be difficult to source samples with either high or low concentrations of all analytes across the panel and oftentimes these experiments are performed by using samples with spiked reference material as a surrogate which is not ideal due to potential discordance between recombinant kit calibrators and endogenous proteins. In this presentation, these challenges will be discussed, along with strategies for conducting stability using ex-vivo stimulation of whole blood and sample admixing to better assess stability during method validation.

Learning Objectives

1. Upon completion, participants will be able to appreciate the challenges with determining analyte stability of several biomarkers in a multiplex panel.
2. Upon completion, participants will gain understanding of the relationship between kit calibrators and endogenous proteins.
3. Upon completion, participants will be able to understand different strategies for assessing analyte stability in multiplex panels.

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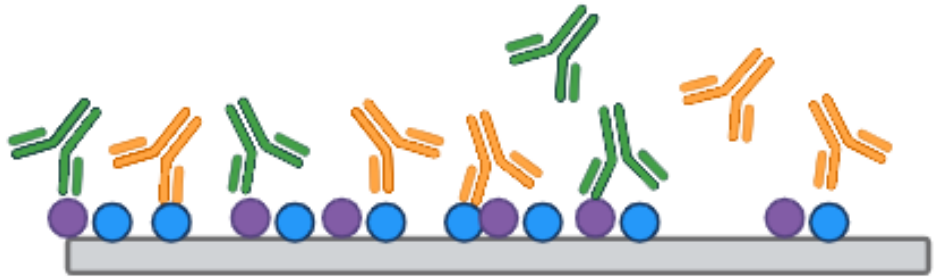
- Christian is the Associate Director of Scientific Services for the Biomarker/Cytokine group at BioAgilytix in Boston, MA.

- He has over 20 years in the pharmaceutical industry, specializing in the translational research and biomarkers, as well as ADA/PK space.

- He received his Bachelors degree in Biology from the University of Massachusetts Boston in 2002, and his Master of Science degree in biotechnology from Worcester State University in 2009.

Multiplexed Biomarker Analysis

- Allows simultaneous measurement of multiple analytes from a single sample
  - Advantage for studies with limited sample volume such as pediatric studies or limited matrices (vitreous humor, CSF)
- Challenges
  - Parallelism
  - Analyte Stability
  - Obtaining samples with endogenous levels of high and low concentrations of all analytes
    - Potential discordance between calibrators (recombinant) and endogenous proteins



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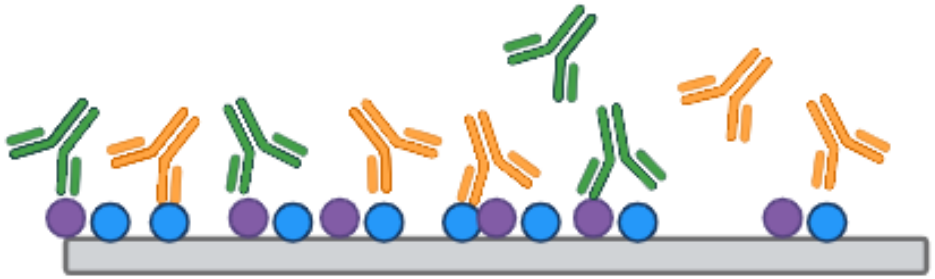

Utilize ex vivo stimulation of whole blood to generate endogenous analyte pool

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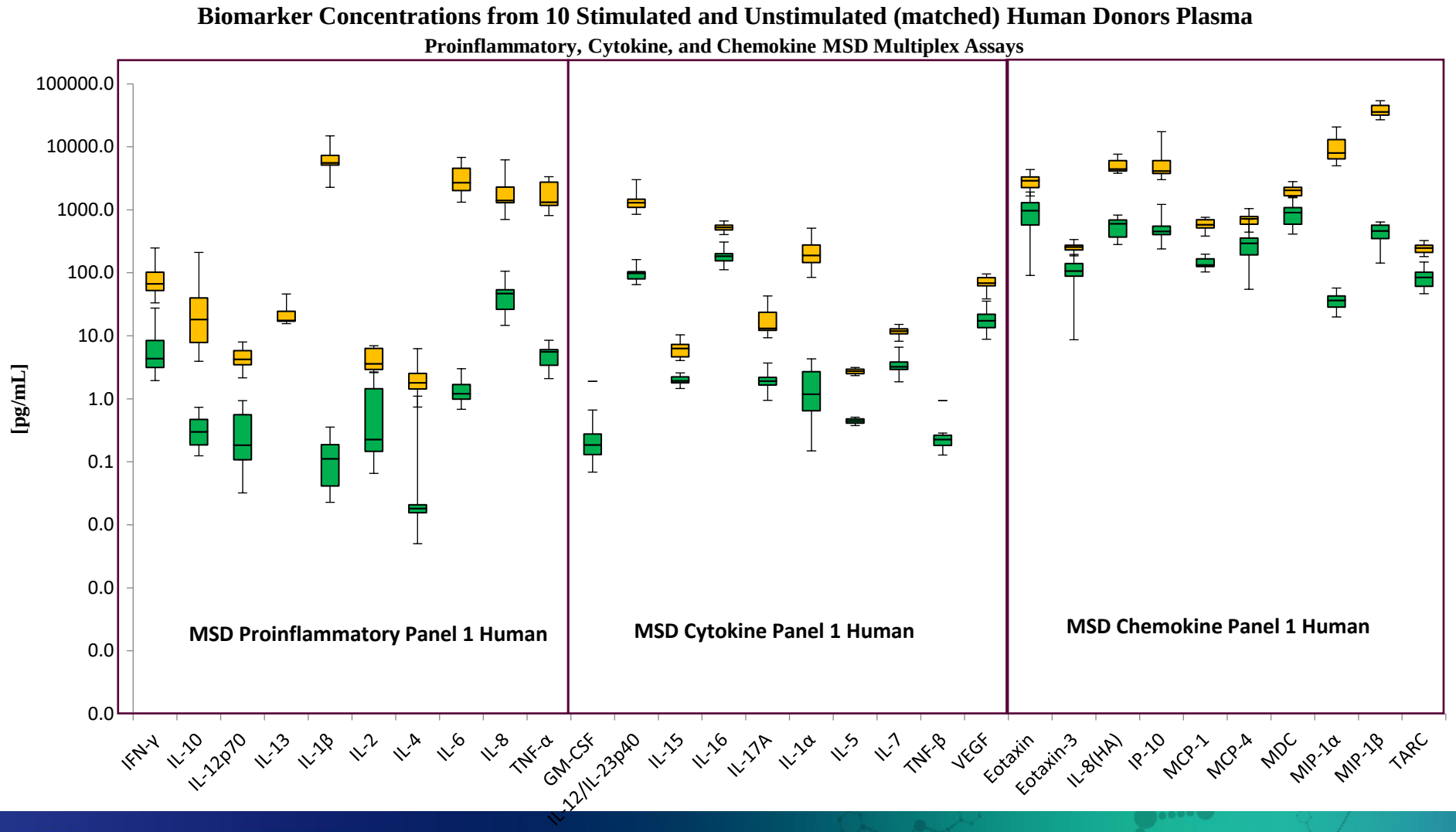
Methodology

- Stimulated 10 whole blood donors with LPS/ATP
  - 0.25 ng/mL LPS, 3.5 hr. @ 37ºC
  - 30µL/mL of 100mM ATP, 0.5 hr. @ 37ºC
  - Used matched unstimulated samples as negative control
- Isolated plasma after stimulation
- Screened samples on MSD Proinflammatory, Cytokine, and Chemokine v-plex panels and assess levels of 30 biomarkers
- Assessed parallelism, selectivity, and LTS (baseline)

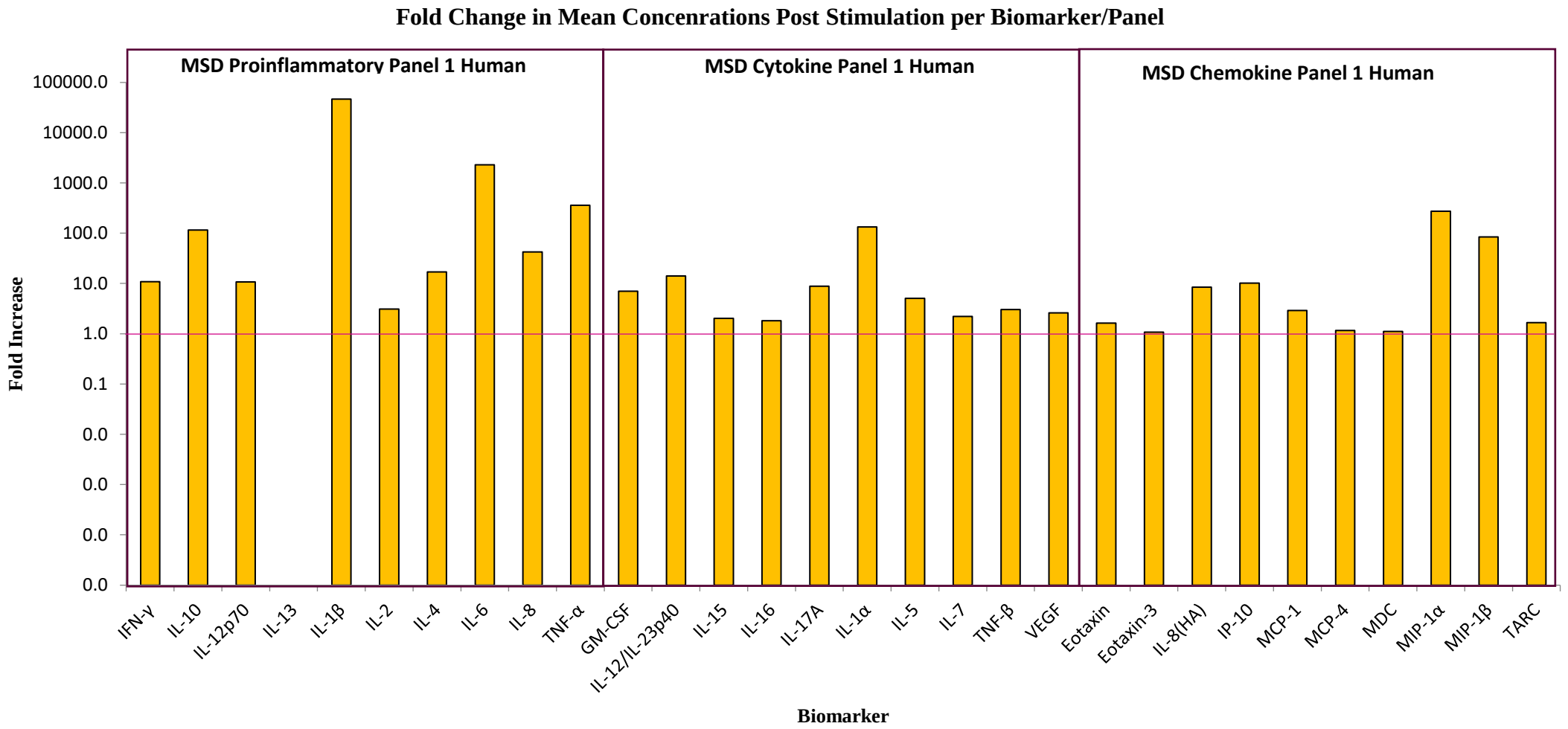
LPS/ATP-induced Cytokine Production



Orange = Stimulated Pool

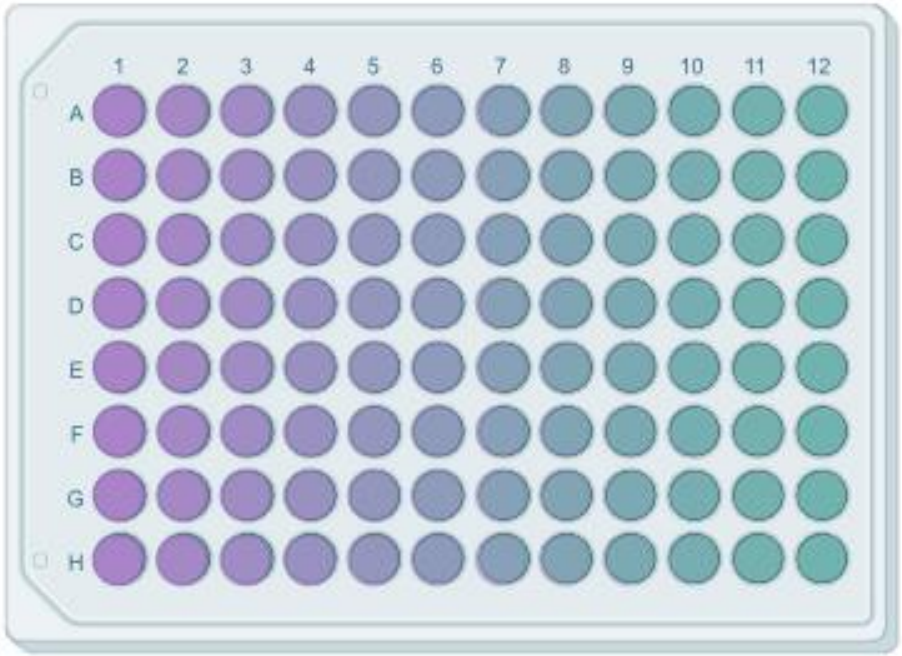
Green = Unsimulated Pool

LPS/ATP-induced Cytokine Production



Parallelism

Evaluation of serial dilutions of the calibrator material against dilutions of the endogenous analyte to ensure that the standard curve in the assay is appropriate and accurate for determining concentrations in samples

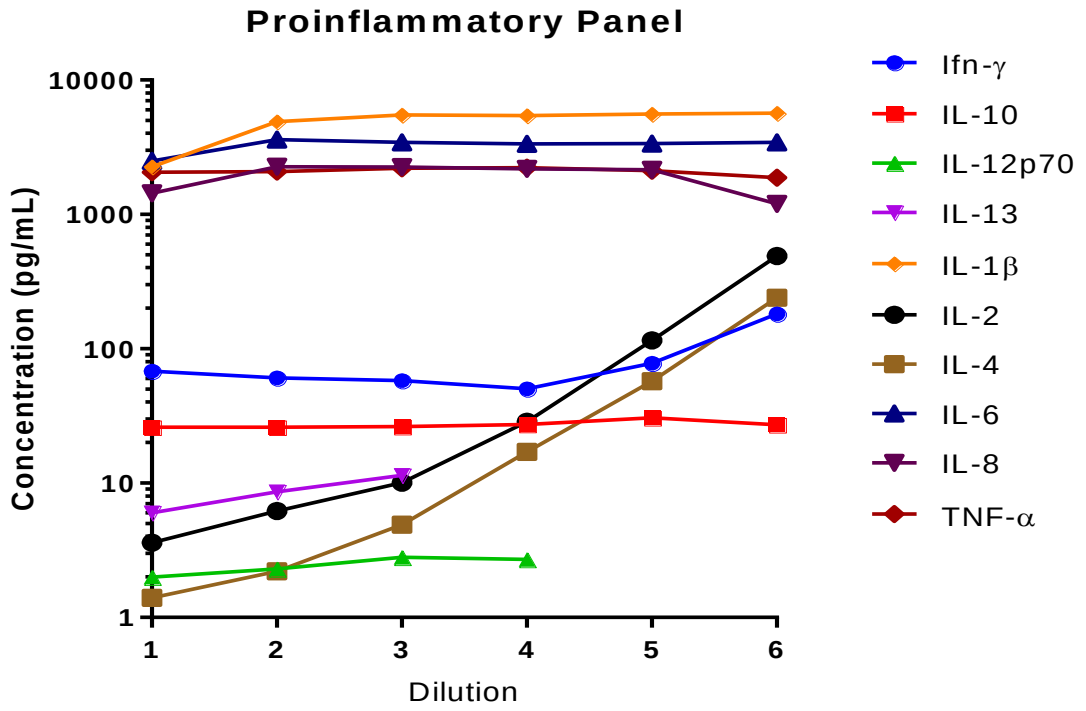


Experiment: Dilute stimulated individuals into unspiked control plasma and run 2-fold serial dilutions

Parallelism in the Proinflammatory Panel

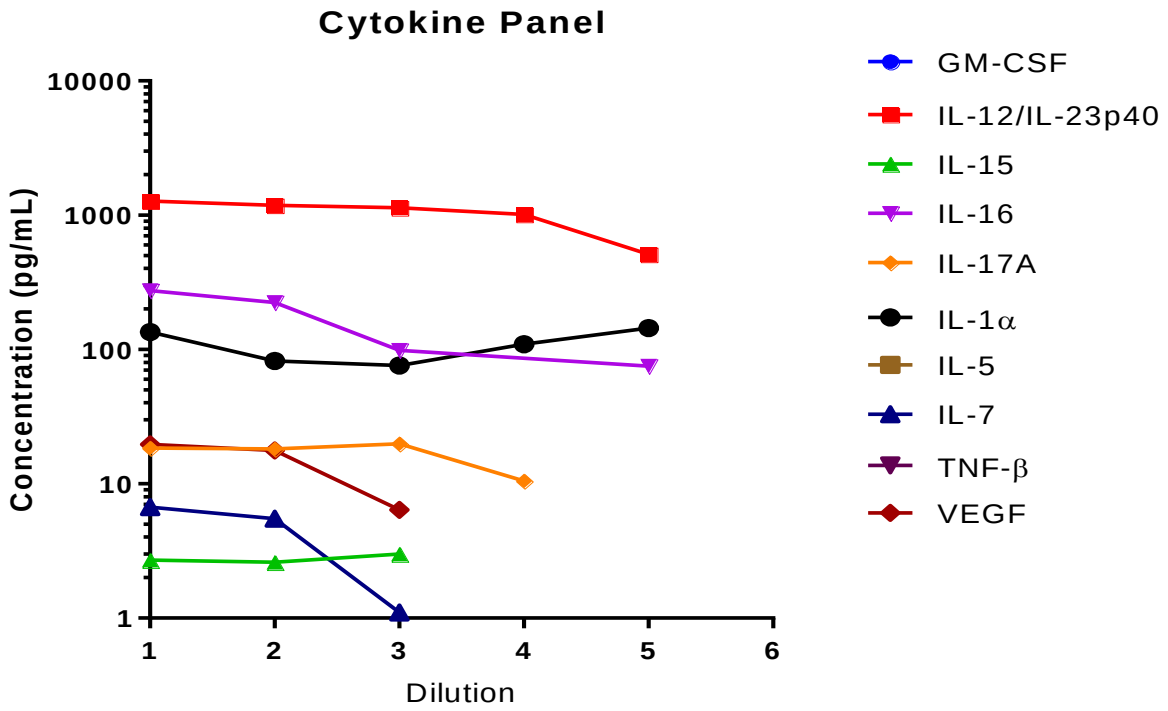
Analyte	LLOQ (Parallelism)	LLOQ (Validation)	Difference
	Concentration (pg/mL)		
Ifn-γ	7.9	4.6	3.3
IL-10	0.4	0.2	0.2
IL-12p70	0	0.3	0.3
IL-13	1.6	7	5.4
IL-1β	5.5	0.3	5.3
IL-2	1	0.8	0.3
IL-4	1	0.1	0.9
IL-6	4.8	0.6	4.2
IL-8	47.9	0.8	47.1
TNF-α	6.8	0.5	6.3

Sample did not dilute to LLOQ



Parallelism in the Cytokine Panel

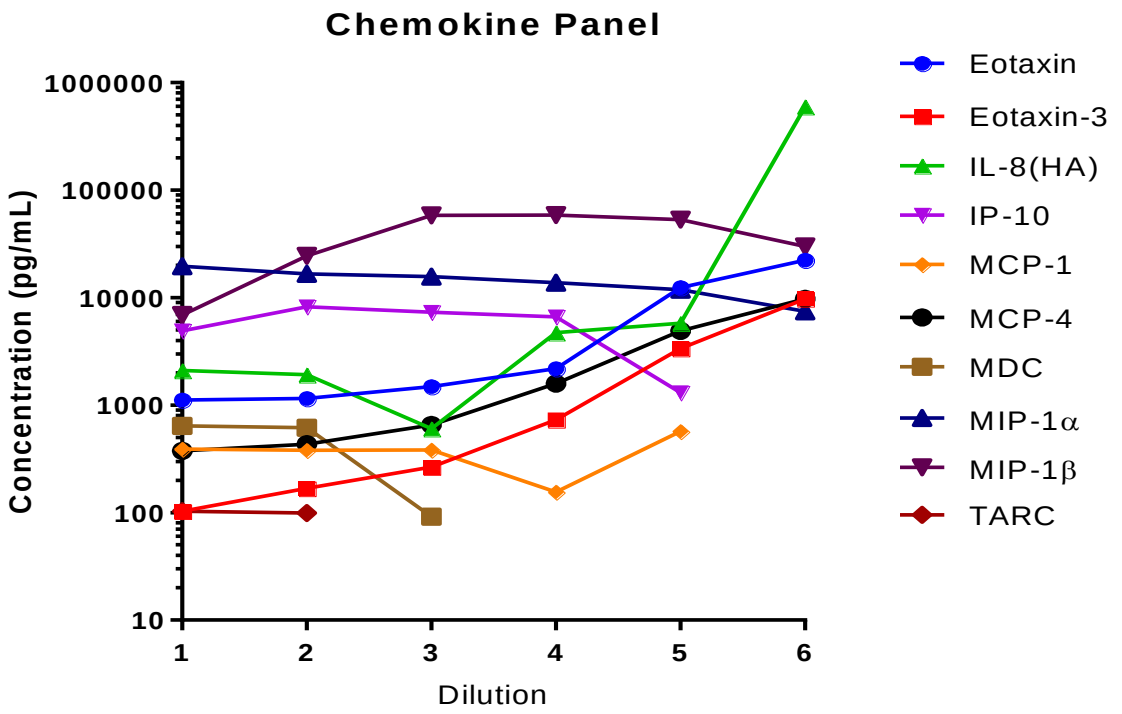
Analyte	LLOQ (Parallelism)	LLOQ (Validation)	Difference
	Concentration (pg/mL)		
GM-CSF	0.2	0.7	0.4
IL-12/IL23p40	100.8	37.2	63.7
IL-15	2	1	1
IL-16	179	47.3	131.8
IL-17A	2	3.4	1.3
IL-1α	4.2	1.3	2.9
IL-5	0.2	0.4	0.2
IL-7	3.4	1.6	1.8
TNF-β	0.3	0.4	0.1
VEGF	6.8	10.2	7



Parallelism in the Chemokine Panel

Analyte	LLOQ (Parallelism)	LLOQ (Validation)	Difference
	Concentration (pg/mL)		
Eotaxin	1026.7	N/A	N/A
Eotaxin-3	96.2	3.4	92.8
IL-8(HA)	679.7	N/A	N/A
IP-10	528.2	3.9	524.4
MCP-1	147.2	0.7	146.4
MCP-4	323.5	1.5	322
MDC	796.8	66.6	730.1
MIP-1α	44.3	3.4	40.8
MIP-1β	6905.9	1.2	6904.7
TARC	6.8	1.4	79.9

Sample did not dilute to LLOQ



Conclusions

- Stimulation of donor blood successfully increased endogenous levels of analytes for 29/30 biomarkers.
  - Parallelism evaluation showed positive correlation to calibrators
    - Suitability of calibrators for endogenous quantitation supported
    - Assay LLOQs correlated to validated LLOQs for most analytes
    - Selectivity confirmed for most analytes
  - Baseline levels established for LTS evaluation

Next Steps for Endogenous Analyte Analysis in Multiplexed Panels

- Test near and long-term stability samples
  - Establish -80°C stability using endogenous analyte
- Evaluate additional matrices
  - Serum, CSF
- Evaluate additional platforms
  - Luminex, O-Link
- Evaluate use of stimulated matrices for future validations
  - Parallelism for MRD, Selectivity, LLOQ

Acknowledgments

- Thank you to Robert Beck for conducting the donor stimulation and cytokine assay runs!

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Questions

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