

TECHNICAL NOTE

Sensitive Detection and Quantification of Low Abundant Cytokines Using Discovery's Multiplex Immunoassay Services

Introduction

The proteomic service laboratories at Discovery Life Sciences offer services based on the Luminex® xMAP technology for multiplexed immunoassays including assay customization, execution, and data analysis for high-sensitivity protein quantitation. Herein we demonstrate the success of our multiplexed immunoassay services using the ProcartaPlex™ Human High Sensitivity panel and the Luminex xMAP® INTELLIFLEX system to profile and compare plasma cytokine levels in male self-reported tobacco users to those who reported no tobacco use.

Methods

This study analyzed a cohort of male tobacco users (both cigarettes and smokeless tobacco) compared to those who reported no tobacco use (Figure 1). Plasma samples were obtained from Discovery's biospecimen repository from consented donors and categorized according to their self-reported tobacco use: never used, previous use, current use of cigarettes, and current use of smokeless tobacco.

Cytokine levels in the plasma samples were measured using the High Sensitivity 9-Plex Human ProcartaPlex™. The panel analyzes 9 protein targets in a single well using Luminex xMAP® technology for highly sensitive detection of analytes. The assay was run according to the ProcartaPlex™ Multiplex Immunoassays user's guide¹ with an overnight incubation (total time = 20 hours). Each sample was analyzed in duplicate and the average concentration of each cytokine in the panel is shown in Table 2.

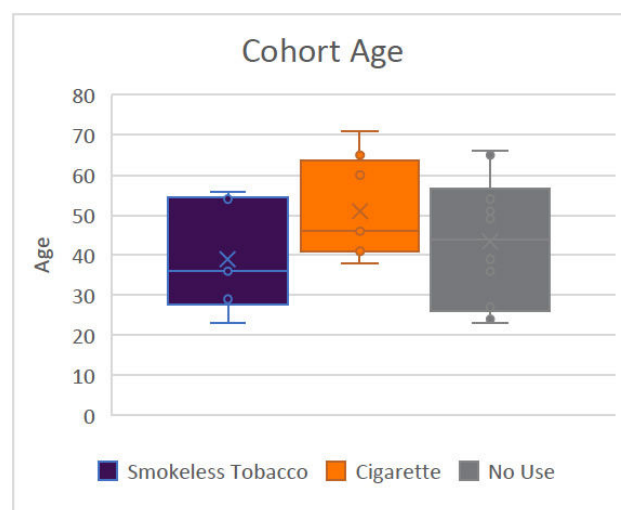


Figure 1. Cohort metrics. Average ages of men who used smokeless tobacco (n = 6), cigarettes (n=8) or were non-smokers (n = 10).

Results:

- Absolute quantification of each target analyte was achieved by using a standard curve, which was generated by incorporating standard analytes into the assay. (Figure 2).
- Most analytes (88%) were measured at values below LLOQ (lower limit of quantification) and above ULOQ (Upper limit of quantification) with Discovery’s established protocol showing that our protocol outperforms the stated kit sensitivity and dynamic range.
- Differences in cytokine levels between tobacco users and non-users were observed (Table 2). In this small cohort study, statistically significant (Mann-Whitney rank test; $p<0.05$) differences for a subset of cytokines between reported cigarette users and non-users were noted (Figure 3).

Table 2: Cytokine measurement results.

Analyte	LLOQ UPLOQ Reported COA	LLOQ UPLOQ Experimental	Average (pg/mL)			
	(pg/mL)		Smokeless Tobacco	Cigarettes	All Tobacco [combined]	Non-Users
IFN Gamma	1.26 1295	0.32 5180	0.9	1.1	1.0	0.6
IL-1 beta	0.28 1130	0.07 1130	1.6	2.0	1.8	0.8
IL-10	0.17 690	0.67 690	ND	ND	ND	ND
IL-12p70	0.77 3170	0.2 3170	6.2	7.2	6.7	3.3
IL-17A (CTLA-8)	0.30 1220	0.07 1220	1.3	1.5	1.4	0.6
IL-2	0.88 3600	0.22 3600	2.6	3.5	3.1	1.8
IL-4	1.29 5270	0.32 5270	1.3	1.8	1.6	0.7
IL-6	1.29 1320	0.32 5280	2.5	6.8	4.8	1.7
TNF alpha	0.62 2520	0.15 2520	2.9	3.7	3.3	1.5

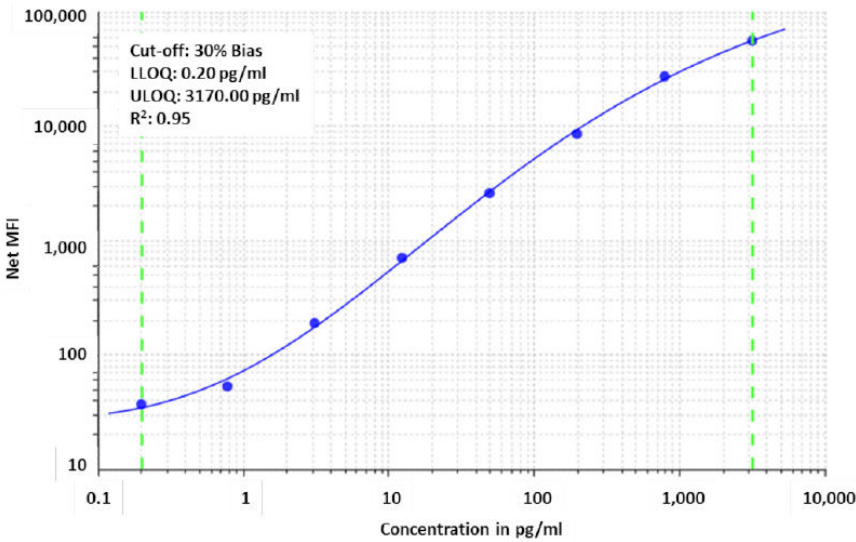


Figure 2. Example calibration curve for IL-12p70.

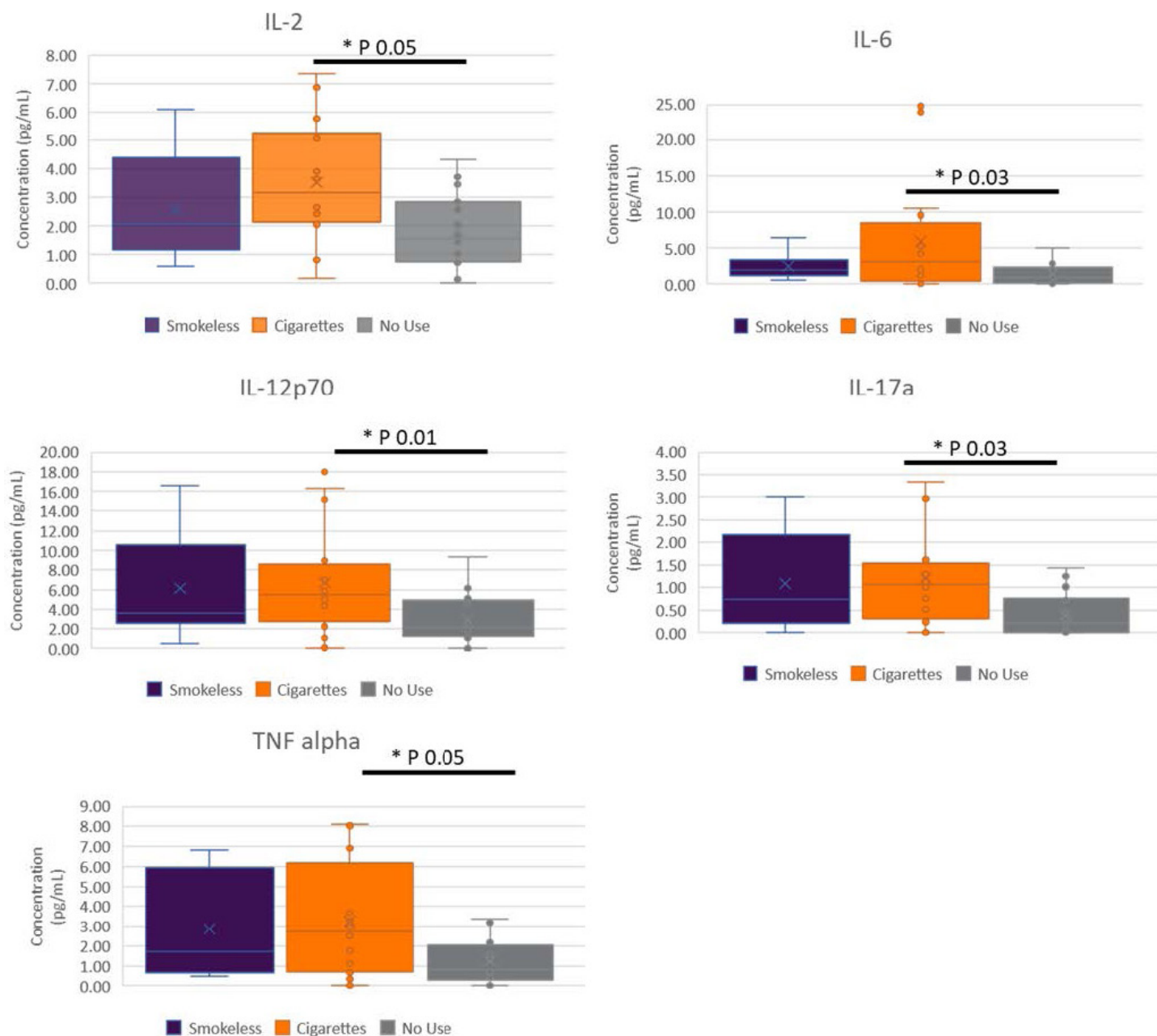


Figure 3. Statistically significant differences among groups for a subset of measure cytokines.

Summary

The results from this small cohort study demonstrate Discovery's ability to profile cytokine levels in plasma samples with highly sensitive detection and qualification using Invitrogen ProcartaPlex™ cytokine assay on the Luminex xMAP® INTELLIFLEX system. The high sensitivity of this assay can accurately measure subtle changes in protein levels, and when combined with multiplexing capabilities, it allows for rapid profiling of cytokine levels in complex biological samples.

Reference

1 ProcartaPlex Multiplex Immunoassay User Guide, Revision A.0 (31.) April 07, 2020.
Publication Number MAN0019074

Discovery offers Luminex®-Based Multiplex Immunoassay Services

Discovery has a full suite of multiplex immunoassays and optimized workflows for proteomic profiling services. Leverage Discovery’s extensive proteomics expertise to deliver accurate and reliable results.

The Luminex xMAP® INTELLIFLEX System is a compact, high throughput flow-based multiplex platform that performs simultaneous measurements on up to 80 protein or RNA targets from a small amount of sample with sensitivity to detect low-level analytes. Each target is compared to a unique standard curve to ensure accurate quantitation that covers 5.5 logs of dynamic range.

The ProcartaPlex™ multiplexed immunoassays make use of the Luminex xMAP® Technology to enable simultaneous analysis of multiple proteins and RNA in a single sample. Select from over 90 preconfigured multiplex panels (2- to 80-plex) in 96 well plate formats or mix and match targets to create custom assays tailored to your needs.

Luminex Description	Sample Types	Deliverables	Applications	Standard Sample Numbers	Standard Sample Volume	Variables for TAT and Price
<ul style="list-style-type: none">• Immunoassay• Bead-based ELISA• Absolute quantification• High sensitivity• High throughput• Preconfigured & customizable	<ul style="list-style-type: none">• Plasma• Serum• CSF• Tissue• Cells• Conditioned media	Absolute quantification of selected protein targets	<ul style="list-style-type: none">• Proteomic profiling• Biomarker profiling	Up to 80 per plate	25 µL	Number of panels Number of samples

About Discovery Life Sciences

Discovery combines the world’s largest commercial biospecimen inventory and procurement network with preeminent multi-omic biomarker services covering genomics, pathology, proteomics, cell biology, and ADME Tox to accelerate new therapies supported by biomarker and companion diagnostic programs for cancer, infectious disease, and other rare and complex conditions. HudsonAlpha Discovery, a division of Discovery, offers a wide spectrum of tools and techniques required for identification and validation of clinical biomarkers, including RNA, whole genome, whole exome, targeted panel, hi-fidelity long-read, single cell, and epigenomic next generation sequencing. Working with Discovery as a single strategic partner helps accelerate biomarker strategies with actionable data and enables the ability to customize study designs as required to take a more targeted and nuanced approach to biomarker discovery and validation.