Evaluation of Potential Biomarkers For Anti-Influenza A Drug Development Programs

Poster # Day3-P09

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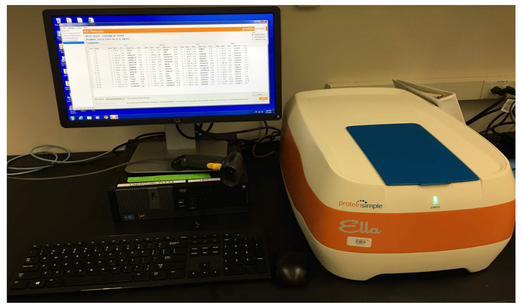
INTRODUCTION

Purpose:

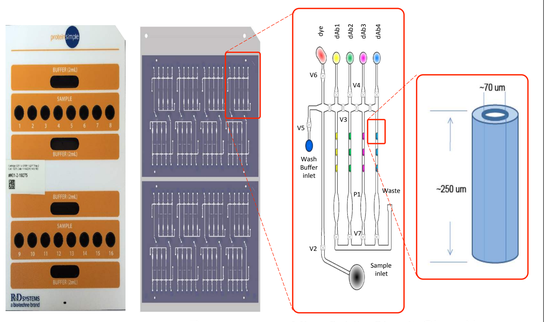
- Influenza is a highly contagious respiratory infection that can cause severe illness, potentially leading to hospitalization or death. The majority of human influenza infections are caused by the Influenza A (Flu A) virus. In conjunction with the clinical development of anti-Flu A therapies, we evaluated a new Simple Plex assay technology for the detection and measurement of 8 inflammatory biomarkers of potential interest in the context of this disease. These biomarkers include IL-6, IL-8, IL-10, MCP-1, IFN-γ, IP-10, sTNF-α-R1, and TNF-α.

Evaluation: Platform & Samples

Simple Plex Technology



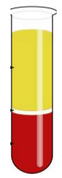
Parallel microfluidics channels eliminates any cross-reactivity between analytes



Front | Rear | Microfluidic Circuit P1 = Pump V2...V7 = Valves | Glass Nano Reactor Coated with Capture Antibody

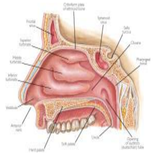
Simple Plex Cartridge

Sample Matrices



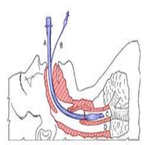
Serum

- Clinical Study (A)
- Commercial



Nasal Swab (NS)

- Clinical Study (B)
- Commercial



Tracheal Aspirate (TA)

- Clinical Study (B) (limited panel)

Methods

- Assay performance was assessed in serum, NS, and TA samples. Our assessments included analyte detectability, assay reproducibility, and sample dilution linearity. Data from a panel of serum and NS samples from Flu A-infected patients, previously tested with an orthogonal assay platform, was used for comparison. Commercial multiplex assay kits were purchased from ProteinSimple. Serum, NS, and TA samples were diluted at minimum required dilution (MRD), or tested serially diluted, following the procedure described by the vendor. Biomarker concentrations were derived using vendor's data analysis software. Vendor's Standard curves, LLOQ & ULOQ values were used in data analysis.

Biomarker Evaluation Strategy

Step 1

- Detectability
- Correlation with archival data (Clinical A study)

Step 2

- Reproducibility
- Dilution Linearity

RESULTS & DISCUSSION

Simple Plex Assay Control Performance

- High, Low, and Endogenous QCs performed adequately for all biomarkers, with accuracy within Vendor's determined ±25% range for High and Low QCs, and had %CV of 18% or less.

Table I. Reproducibility

- Serum, NS, & TA samples were diluted 1/2 & experiments were performed on two different days.
- % difference (Diff) was calculated from biomarker concentrations measured on two different days.
- Fresh sample aliquots were used for each run

	IL-6	IL-8	IL-10	MCP-1	IFN-γ	IP-10	TNF-α	TNF-α-R1
Samples	% Diff	% Diff	% Diff	% Diff	% Diff	% Diff	% Diff	% Diff
S2	-20	-9	-28	-10	*NA	-9	-24	-7
S3	-20	-2	-21	-1	NA	1	-27	-9
S4	-14	-20	-21	-9	NA	-13	-14	-1
NS3	-30	-13	NA	-11	NA	-14	NA	-16
NS4	-7	-3	-15	-12	-8	NA	NA	-37
NS8	-13	-35	NA	4	NA	-18	NA	5
TA1	-77	-31	-16	-17	1	-22	-10	-14
TA6	-64	-49	-67	-34	-23	-44	-66	-55
TA7	-1	1	-29	-8	-53	22	-47	52

Highlighted values are outside the ±30% Diff acceptable criteria
* NA = Result was either GTR (greater than range) or LTR (less than range)

Conclusions: Acceptable reproducibility in Serum & in most analytes in NS samples (2 biomarkers in NS not acceptable); reproducibility not acceptable in TA samples.

Table II. Linearity of Dilution

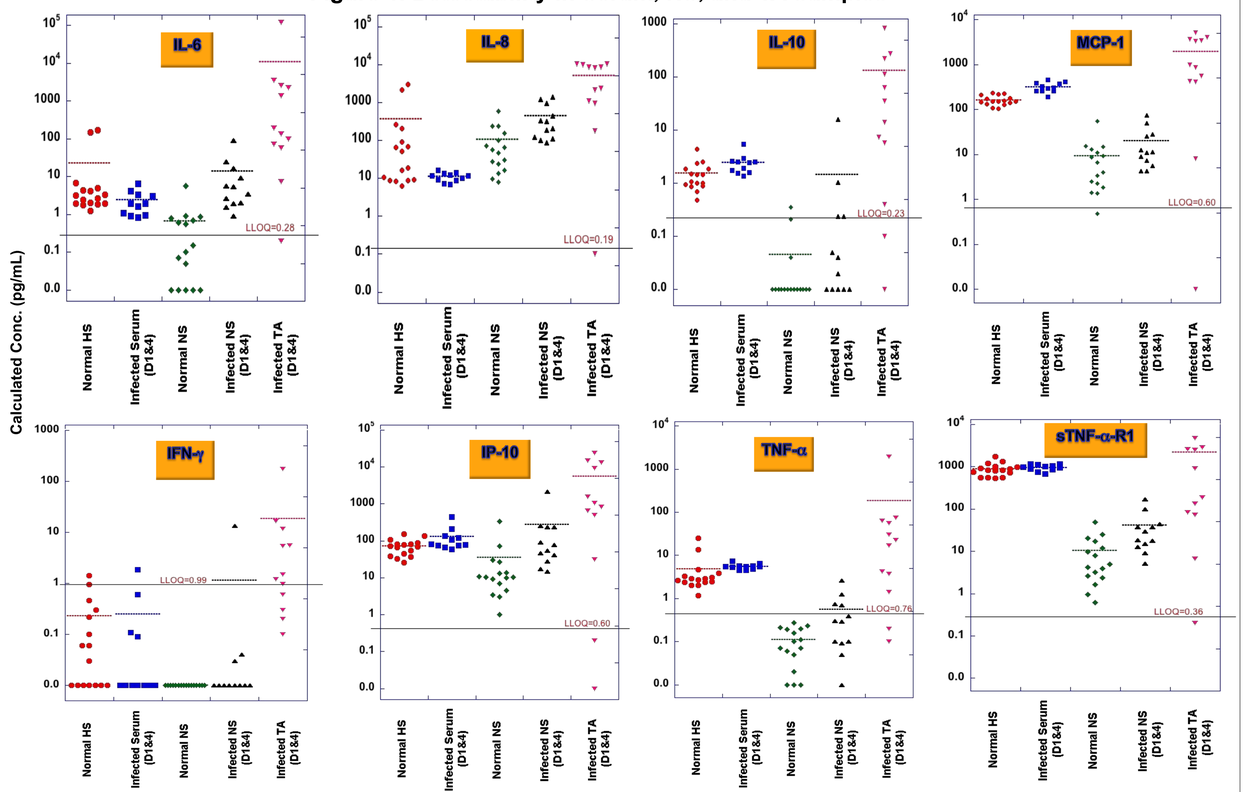
- Serum, NS, & TA samples were serially diluted 1/2 &1/4.
- % Diff for each sample was calculated from quantitated biomarker concentrations of the two dilutions.

	IL-6	IL-8	IL-10	MCP-1	IFN-γ	IP-10	TNF-α	TNF-α-R1
Samples	% Diff	% Diff	% Diff	% Diff	% Diff	% Diff	% Diff	% Diff
S2	*NA	-3	-8	-5	NA	-4	36	3
S3	52	23	-18	-6	NA	40	NA	6
S4	20	9	-12	-1	NA	27	NA	-1
NS3	17	16	NA	-16	NA	7	NA	7
NS4	4	-4	-2	-2	31	NA	NA	7
NS8	17	13	NA	-18	NA	10	NA	-1
TA1	63	NA	-12	NA	0	NA	28	NA
TA6	12	97	-2	NA	-4	NA	-6	-6
TA7	3	-10	-6	-14	NA	-10	-10	4

Highlighted values are outside the ±30% Diff acceptable criteria
* NA = Result was either GTR or LTR

Conclusions: Acceptable linearity in Serum, NS, and TA samples except 3, 1, and 2 biomarkers in serum, NS, and TA respectively.

Figure 1. Detectability in Serum, NS, and TA Samples



Conclusions: IL-6, IL-8, IL-10, MCP-1, IP-10, and sTNF-α-R1 are detectable in most serum samples, NS, and TA samples; however, TNF-α is only detectable in serum and TA samples while IFN-γ is predominantly detectable in most TA samples.

Table III. Correlation: Archival data x Simple Plex in Infected Serum and NS Samples

Matrix	IL-6	IL-8	IL-10	MCP-1	IFN-γ	IP-10	TNF-α
Serum	No	Yes	No	Yes	No	Yes	No
NS	Yes	Yes	Yes	Yes	No	Yes	Yes

Conclusions: Good Correlation with archival data observed for IL-8, MCP-1, and IP-10 in sera from Flu A infected individuals; also for IL-6, IL-8, IL-10, MCP-1, IP-10, and TNF-α in NS samples.

SUMMARY

- **Various inflammatory biomarkers that may potentially be used during clinical development of anti-Flu A therapeutic molecules were evaluated.**
- **Our work demonstrated that the new Simple Plex platform is sensitive, reproducible, and multiplexable, with a simple assay procedure, limited hands-on requirements, and short total assay time (1 hour); however, our results also indicated that not all analytes meet our performance requirements and not all matrices are suitable for this technology.**
- **The Simple Plex 4-Plex assay performed adequately and we will further qualify the assay to support clinical studies.**
- **Detectability, reproducibility, dilution linearity, as well as Simple Plex's correlation with archival data were criteria considered for final selection of biomarker panel to support clinical studies.**
- **Additional work is needed to improve reproducibility of biomarker quantitation in TA samples.**

ACKNOWLEDGEMENTS

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