Poster # W1230-02-10

Qualification of a 48-Plex Cytokine Olink Panel to Support Rare Disease and Pediatric Clinical Studies

Jaymes Farrell Ph.D. Scott Allen Ph.D. Thomas Schneider Ph.D.

BioAgilytix, Boston MA

CONTACT INFORMATION: Jaymes.Farrell@BioAgilytix.com, Scott.Allen@BioAgilytix.com

aaps National Biotechnology CONFERENCE

INTRODUCTION

Measuring pathway-specific, physiologically relevant biomarkers is critical for successful development, testing, and approval of therapeutics. However, selection of appropriate biomarkers for novel modalities and/or rare disease indications can be challenging. Biomarker analysis for rare diseases and pediatric clinical studies can benefit from multiplexed platforms that can look at numerous analytes with minimal sample volume. Here, we describe the qualification of a multiplex Olink method which simultaneously measures a panel of 45 cytokine biomarkers in human serum using extremely low sample volumes (1µL human serum). The assay demonstrated high levels of precision and accuracy, even at low concentrations.

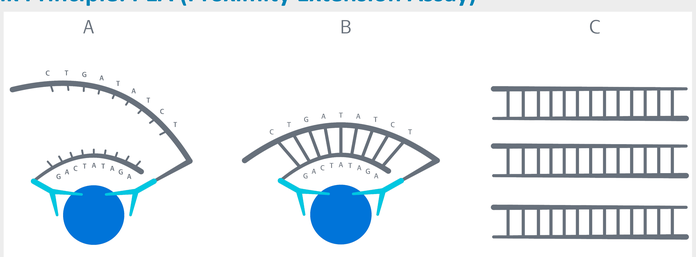
PURPOSE

- Evaluate a multiplex Olink panel as an alternative to traditional biomarker immunoassays
- Test inter- and intra- assay precision across operators and across days
- Test accuracy, parallelism, specificity, and stability

METHOD

- 10 healthy human serum donor samples commercially obtained
- 3-4 replicate samples per donor (or a pool of all 10) tested
- 4 precision runs across two operators on two different days performed
- Accuracy and parallelism assessed with kit control (human plasma) spike into serum
- Parallelism samples diluted in non-human matrix (Rat plasma) with little to no detectable background signal (not shown).

Olink Principle: PEA (Proximity Extension Assay)



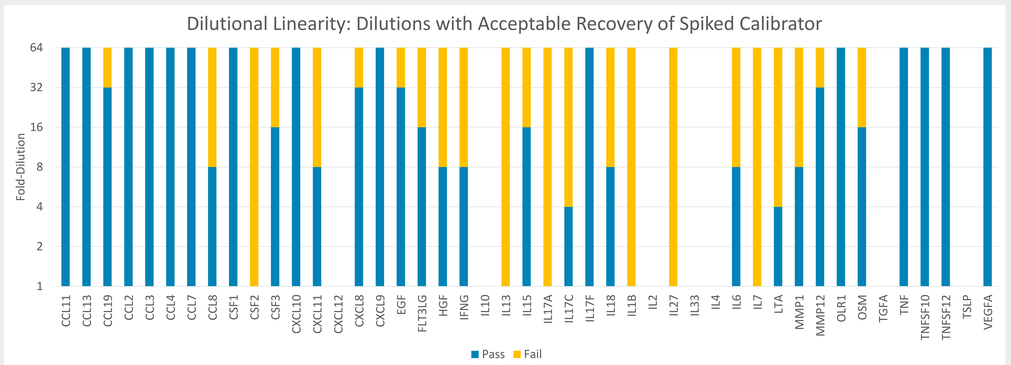
A. Two distinct antibodies, each with complementary oligonucleotide sequences, bind to target analytes overnight
B. The proximity of these sequences allow for binding and subsequent PCR, converting protein signal to DNA signal
C. DNA sequences can then undergo qPCR, allowing for back-calculation of starting concentrations of protein

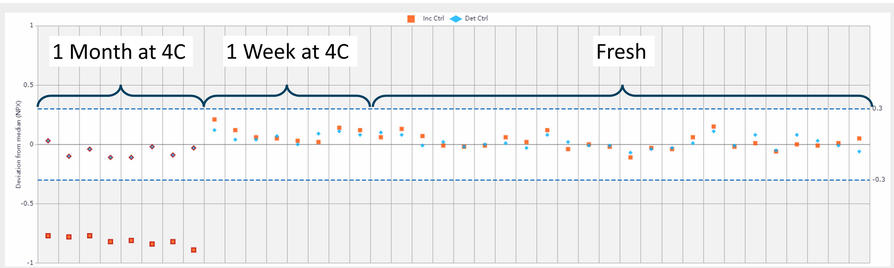
RESULTS

- **Dilutional Linearity/LLOQ:** Normal human serum mixed 1:1 with sample control then diluted serially in a non-human matrix (Rat plasma). Passing result defined as 70%-130% recovery of neat signal. Lowest passing dilution and lowest passing signal (pg/mL) shown.
- **Accuracy:** Normal human serum mixed 1:1 with Olink-provided sample control (human plasma). Recovery (%recovery) based on expected values (sample control signal plus endogenous signal, not shown).
- **Precision:** Ten normal human donors tested in replicates of 3-4 across two different operators and two different days. Coefficients of variance (%cv) shown.
- **Stability:** Stability of intermediate PCR products stored at 4˚C for 1 month, 1 week, or made fresh.
- **Bold** indicates NA, Red indicates outside acceptance criteria

	CCL11	CCL13	CCL19	CCL2	CCL3	CCL4	CCL7	CCL8	CSF1	CSF2	CSF3	CXCL10	CXCL11	CXCL12	CXCL8	CXCL9	EGF	FLT3LG	HGF	IFNG	IL10	IL13	IL15
Olink LLOQ (pg/mL)	0.47	0.06	0.95	0.24	0.05	0.12	0.12	0.03	0.15	0.29	10.38	0.44	0.12	54.75	0.30	0.20	0.48	0.95	0.22	0.04	1.32	0.50	0.78
Lowest Passing Dilution	1/64	1/64	1/32	1/64	1/64	1/64	1/64	1/8	1/64	Neat	1/16	1/64	1/8	**NA**	1/32	1/64	1/32	1/16	1/8	1/8	**NA**	Neat	1/16
Lowest Passing signal (pg/mL)	3.73	1.87	1.79	16.20	0.32	2.23	4.37	5.02	123.32	0.35	10.91	3.39	8.20	**NA**	5.15	2.70	1.19	9.35	43.63	0.14	**NA**	0.75	1.60
Accuracy (% Recovery)	99.4	97.7	99.0	101.1	103.4	107.1	107.1	104.2	103.8	117.9	104.6	100.6	102.3	115.0	95.3	99.5	95.2	100.1	103.1	101.6	95.7	94.7	102.1
Precision (Mean %CV)	12.1	13.3	11.9	9.8	9.1	11.7	12.7	11.3	6.2	**NA**	8.0	11.9	11.2	2.0	9.2	11.7	14.5	9.0	9.9	16.6	19.0	18.7	12.2

	IL17A	IL17C	IL17F	IL18	IL1B	IL2	IL27	IL33	IL4	IL6	IL7	LTA	MMP1	MMP12	OLR1	OSM	TGFA	TNF	TNFSF10	TNFSF12	TSLP	VEGFA
Olink LLOQ (pg/mL)	0.23	0.73	0.30	0.46	0.35	0.09	0.47	0.21	0.04	0.25	0.27	0.95	3.56	7.63	0.95	0.13	1.09	3.81	1.69	3.81	0.36	0.48
Lowest Passing Dilution	Neat	1/4	1/64	1/8	Neat	**NA**	Neat	**NA**	**NA**	1/8	Neat	1/4	1/8	1/32	1/64	1/16	**NA**	1/64	1/64	1/64	**NA**	1/64
Lowest Passing signal (pg/mL)	1.07	0.96	0.30	54.89	0.64	**NA**	4.06	**NA**	**NA**	28.17	0.42	7.95	138.63	10.85	2.12	0.16	**NA**	13.03	7.13	10.43	**NA**	4.34
Accuracy (% Recovery)	113.9	102.6	**137.3**	97.7	114.1	94.7	97.6	126.0	**NA**	91.3	117.1	111.0	80.3	100.0	102.8	110.0	109.8	128.8	97.2	99.3	101.5	104.1
Precision (Mean %CV)	13.5	13.0	10.7	9.7	14.3	**NA**	29.0	13.7	**NA**	10.5	11.1	10.6	10.2	8.5	12.0	10.8	12.1	4.2	9.3	10.5	16.1	11.0





CONCLUSIONS

- The Olink Target 48 panel is appropriate for highly multiplexed analysis, and can detect low levels (many below 1pg/mL) from low volumes of precious sample (rare diseases or pediatric studies)
- Excellent precision across multiple operators and multiple days
- Excellent accuracy when spiking with sample control material
- Acceptable dilutional linearity, variable depending on analyte: large detection range means diluting into the range of the assay is likely not necessary
- Short-term storage of PCR product at 4˚C possible to avoid using sample volume on re-tests

REFERENCES

BioAgilytix Boston: 1320 Soldiers Field Rd, Boston MA 02135
Olink Press Library, https://olink.com/about-us/press-image-library/
"PEA – a high-multiplex immunoassay technology with qPCR or NGS readout." (2020).
"Validation data: Target 48 Cytokine." (2023)

BioAgilytix