Qualification of a Novel Multiplex Assay for the Quantitation of Chemokine Markers in Human Plasma and CSF Samples

Poster Number: M1330-02-09

Christian Braithwaite, Robert Beck, Roman Wernyj, Franck Grall

BioAgilytix Labs, 1320 Soldiers Field Rd, Brighton, MA 01235

CONTACT INFORMATION: Christian.Braithwaite@bioagilytix.com.

aaps National Biotechnology CONFERENCE

PURPOSE

Multiplex assays have become an increasingly valuable tool in biomarker analyses. They offer the ability to evaluate multiple targets or analytes simultaneously, while using less sample volume, with decreased time and labor in generating impactful clinical results. Platforms which offer the flexibility of combining multiple biomarkers of interest for a particular disease on the same assay can be even more valuable. Additionally, assays which have been characterized in multiple matrices, such as plasma, serum, CSF, urine, etc. can provide additional value of disease state or drug effect between the various physiological compartments, which may improve understanding of biological interactions and target mediated effects. Here we have optimized and qualified a method to measure 7 individual chemokines, implicated in various autoinflammatory, oncological, and neurodegenerative indications, in both plasma and cerebrospinal fluid (CSF). The successful use of this method will generate useful data for multiple purposes, including characterization of disease state, definition of the relationship between peripheral and CNS, as well as measurement of drug effects.

OBJECTIVE(S)

The objective of this project was to qualify a custom 7-biomarker MSD "U-Plex" multiplex assay for the quantitation of Fractalkine (CX3CL1), GRO-a (CXCL1), IL-18, IL-1RA, IL-8 (CXCL8), SDF-1α (CXCL12), and MCP-1(CCL2) in Human Plasma and CSF samples. Additionally, samples were assessed to determine the utility of the specific 7-analyte set for discerning differences between normal and disease states.

METHOD(S)

Reagents and kit components, including plates, calibrators, and labeled capture and detection antibody reagents were purchased from MSD, (Gaithersburg, MD). Normal and Parkinson disease (PD) Plasma and CSF samples were procured from BioIVT, (Westbury, NY). Assay performance assessments included calibrator and sample inter and intra-precision, accuracy, dilutional linearity short term and freeze-thaw stability. Spiked samples in pooled and individual plasma and CSF samples were prepared at multiple concentrations and stored in multiple aliquots at the beginning of the Qualification and stored at -80°C until used for each experiment during the Qualification.

RESULT(S)

Qualification Results: Intra-and inter-assay precision and accuracy, dilutional linearity, and short-term stability results are presented in Table 1. The target acceptance was based on 25% CV and ±25% RE (30% at LLOQ). Results outside of the target acceptance would be considered as fit for purpose based on the context of use of the individual study (See Table 1).

Normal vs Parkinson's disease samples: 10 individual donors each for PD and normal CSF and plasma (unmatched) results are presented below. Distinct differences in analyte concentrations are apparent between disease and normal state, with elevated levels in all 7 analytes for both CSF and plasma donors.

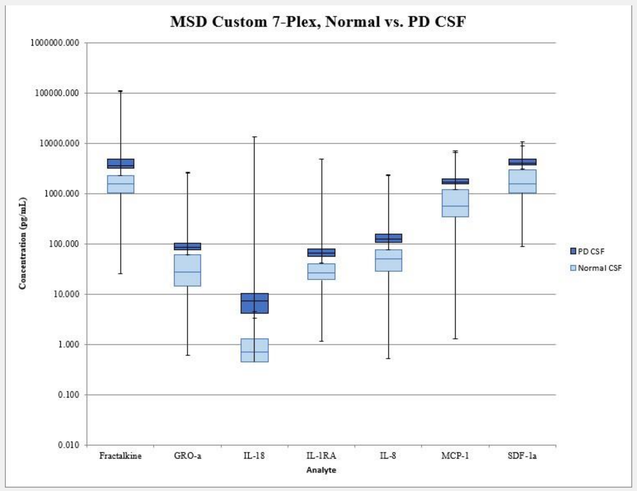
Biomarker	Calibrator Ranges	Matrix	Intra Precision (%CV)	Inter Precision (%CV)	Accuracy (%RE)	Linearity	Stability
Fractalkine	105,000 pg/mL to 25.63 pg/mL	Plasma	6.0% to 57.4%	13.7% to 55.2%	-22.6% to 6.5%	1:4 - 1:32	PASS
		CSF	9.6% to 19.8%	18.4% to 43.5%	3.2% to 33.8%	1:1 - 1:32	PASS
GRO-a	2,510 pg/mL to 0.61 pg/mL	Plasma	2.0% to 12.0%	11.9% to 21.3%	-10.3% to -7.7%	1:1 - 1:32	PASS
		CSF	3.1% to 24.7%	7.7% to 24.8%	-16.4% to -2.6%	1:1 - 1:32	PASS
IL-18	13,400 pg/mL to 3.27 pg/mL	Plasma	3.1% to 13.7%	4.7% to 27.3%	-3.5% to 1.0%	1:1 - 1:32	PASS
		CSF	5.6% to 19.4%	6.4% to 19.6%	-10.4% to 3.5%	1:1 - 1:32	PASS
IL-1RA	4,810 pg/mL to 1.17 pg/mL	Plasma	2.7% to 40.8%	7.5% to 60.9%	8.4% to 47.1%	1:1 - 1:32	PASS
		CSF	3.8% to 72.7%	5.5% to 64.1%	4.8% to 18.9%	**	PASS
IL-8	2,160 pg/mL to 0.52 pg/mL	Plasma	1.3% to 7.9%	5.6% to 29.0%	-10.3% to -6.1%	1:1 - 1:32	PASS
		CSF	1.1% to 9.6%	5.3% to 22.1%	-14.5% to -4.1%	1:1 - 1:32	PASS
MCP-1	5,220 pg/mL to 1.27 pg/mL	Plasma	1.9% to 16.5%	12.2% to 24.7%	-3.8% to 0.7%	1:1 - 1:32	PASS
		CSF	4.40% to 37.3%	7.8% to 22.6%	-9.5% to -2.2%	1:1 - 1:32	PASS
SDF-1a	5,725 pg/mL to 89.45 pg/mL	Plasma	2.0% to 44.7%	13.9% to 72.9%	3.7% to 48.5%	1:1 - 1:32	*
		CSF	5.5% to 46.4%	15.1% to 106.2%	-5.7% to 93.8%	1:1 - 1:32	*

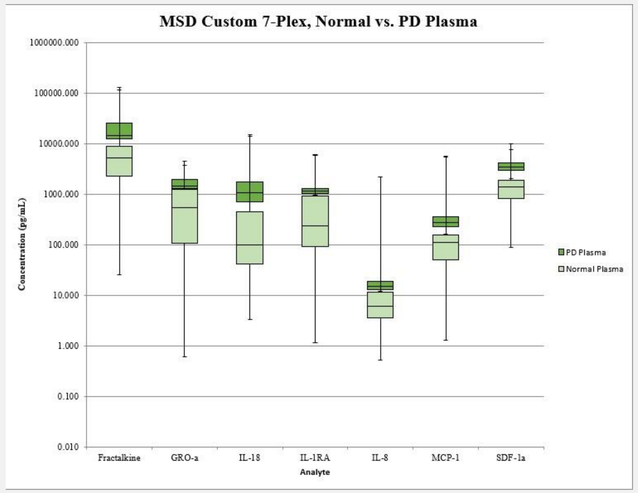
Red = outside of 25% target acceptance: Fit for Purpose

** Short term stability: 24 hours at room temperature and at 5° C did not meet acceptance, although freeze-thaw stability was acceptable*

***Control acceptance was not met, however linearity was observed from 1:1 to 1:32*

10 normal and PD CSF individuals were assessed on the 7 analyte panel. Darker boxes = PD ranges lighter boxes = normal ranges. Assay quantifiable ranges are indicated by the error bars.



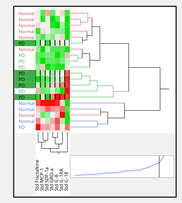


COMPARISON BETWEEN POPULATIONS

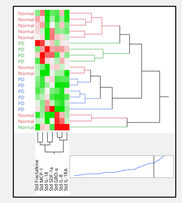
Marker	CSF SigMean Diff	CSF Sig Var Diff	Plasma SigMean Diff	Plasma Sig Var Diff
Fractalkine	Same	Same	Same	Same
GRO-a	Same	Same	DIFFERENT	Same
IL-18	DIFFERENT	Same	DIFFERENT	Same
IL-1RA	Same	Same	Same	Same
IL-8	Same	Same	DIFFERENT	Same
MCP-1	Sam	Same	Same	Same
SDF-1a	DIFFERENT	Same	Same	Same

Initial comparison of total measurements indicate that IL-18 was significantly different between normal and PD patients in both CSF and plasma matrix. In CSF, SDF-1a was also significantly different. Plasma matrix identified GRO-a and IL-8 to be significantly different. Variance between all comparison were shown to be similar. Heirarchical clustering was used to discern patterns and potential groupings of the markers between the normal and diseased groups. Prior to analysis, marker measurements were scaled using the z-score normalization. Within-cluster variance was measured using Ward method. Clustering information is visualized as a heat map, green is low values and red higher values. Dendrograms identify the groupings branches captured for this dataset.

Hierarchical Clustering Matrix: CSF



Hierarchical Clustering Matrix: Plasma



CONCLUSIONS

A custom 7-biomarker multiplex assay was qualified in human plasma and CSF. This assay may provide rapid and reliable results to support clinical studies for various autoinflammatory, oncological, and neurodegenerative indications. Additionally, by assessing assay performance in both CSF and plasma, clinical studies may be designed to collect samples from the appropriate CNS or peripheral compartments where biological activity is targeted. This multiplex assay is considered fully qualified for quantitation of 7 chemokines within the concentrations meeting target acceptance criteria and fit for purpose for exploratory use within the full range of calibrators.

The 7-analyte panel also demonstrated utility in detecting higher levels of all 7 analytes in Parkinson's Disease CSF as well as plasma. Statistical analysis of the markers between disease state and healthy individuals showed some differences for key analytes. These results may support use of the method for disease progression in PD as well as other disorders.

BioAgilytix