SPECIMEN TYPE VALIDATION AND ESTABLISHEMENT OF NORMAL CYTOKINE REFERENCE INTERVALS IN CEREBROSPINAL FLUID

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INTRODUCTION AND OBJECTIVE

Cerebrospinal fluid (CSF) is a critical body fluid to examine in attempts to discover potential biomarkers for neuroinflammatory and other disorders of the central nervous system (CNS). Serum and/or plasma cytokine levels have been associated with a variety of inflammatory conditions, with some shown to be actionable therapeutic targets. However, less is known about cytokine levels in CSF. Similarly, serum/plasma cytokine testing is widely available in clinical laboratories, with cytokine testing in CSF extremely limited and if performed, accompanied by a disclaimer as an unvalidated specimen type. In this study, we validate CSF as a specimen type and determine normal reference intervals for multiple cytokines and markers.

MATERIALS AND METHODS

CSF was validated as a specimen type for testing using a laboratory developed magnetic bead based multiplexed cytokine assay previously validated to measure 13 cytokines/markers in serum and plasma. The assay can quantitate concentrations for TH1 type cytokines: IFNγ, IL12, IL2; TH2 type: IL4, IL5, IL10, IL13; Monokines: IL1β, IL6, IL8, TNFα; as well as IL17 and IL2 receptor in only 25 uL of specimen. Performance parameters including specimen dilution, heterophile antibody interference, linearity and precision were examined. Reference intervals were established using 197 normal and control CSF specimens by non-parametric quantile-based statistics according to the CLSI C28-A guidelines.

Demographics of Reference Range Specimens

Source	Sex	N	Mean Age (y)	STDV Age (y)
Innovative Research	Male	39	41.7	24.8
	Female	56	39.3	23.0
	Combined	95	40.3	23.7
Normal Controls	Male	9	41.7	16.7
	Female	17	37.4	12.6
	Combined	26	38.8	14.0
OCB	Male	30	49.7	17.9
	Female	46	49.0	17.6
	Combined	76	49.3	17.6
Total	Male	78	44.8	21.7
	Female	119	42.8	20.3
	Combined	197	43.6	20.8

Innovative Research samples purchased as normal CSF specimens. Normal controls obtained from a collaborating neurologist. Oligoclonal Band Profile testing (OCB) specimens had normal CSF results for all tests in the panel including Immunoglobulin G, Albumin, Albumin Index, CSF IgG/Albumin Ratio, CSF Oligoclonal Bands and IgG Synthesis Rate.

RESULTS

Reference intervals were established using CSF from 197 controls

Analyte	Reference interval
IL13	≤ 7.3 pg/mL
IL17	≤ 4.6 pg/mL
IL4	≤ 5.2 pg/mL
IL2r	≤ 26.8 pg/mL
IL6	≤ 7.5 pg/mL
TNFα*	≤ 1.7 pg/mL
IL12*	≤ 1.9 pg/mL
IL2*	≤ 2.1 pg/mL
IL10	≤ 12.7 pg/mL
IL5*	≤ 2.1 pg/mL
IL1β*	≤ 6.5 pg/mL
IFNγ*	≤ 4.2 pg/mL
IL8	4.6 - 283.5 pg/mL

*Indicates Reference interval was less than the lower limit of Quantitation for that analyte.

Linearity was confirmed throughout the AMR of the assay according to CLSI EP06-A guidelines for all 13 analytes

	Range Confirmed (pg/mL)			Nonlinearity	
Analyte	Low	High	Fit	Lowest	Highest
IL13	5.0	3942.2	3rd Poly	-7.2%	14.4%
IL17	4.8	3535.5	Linear	NA	NA
IL4	3.6	2624.9	3rd Poly	-4.0%	4.5%
IL2r	8.9	6490.2	Linear	NA	NA
IL6	5.8	3505.5	Linear	NA	NA
TNFα	4.2	2368.3	Linear	NA	NA
IL12	6.4	4341.7	3rd Poly	-3.8%	7.0%
IL2	2.9	2823.1	3rd Poly	-4.8%	9.5%
IL10	7.0	3678.7	3rd Poly	-3.0%	3.1%
IL5	4.9	3673.7	Linear	NA	NA
IL1β	5.9	3020.6	3rd Poly	-6.8%	13.7%
IFNγ	4.6	3112.6	2nd Poly	-8.8%	8.8%
IL8	16.8	3501.0	Linear	NA	NA

Linearity was performed by proportionally mixing high and low CSF pools spiked with recombinant human cytokines at 20% dilutions for a total of 6 points. If results were not linear, bias due to nonlinearity was measured as the difference between the linear fit and the best polynomial fit allowing for 25% nonlinearity.

Accuracy studies showed good correlation with 15 CSF specimens spiked with known concentrations of recombinant cytokines

Analyte	R^2	Slope
IL13	0.993	0.940
IL17	0.999	0.943
IL4	0.992	0.851
IL2R	0.993	0.905
IL6	0.994	0.975
TNFα	0.992	0.856
IL12	0.998	1.035
IL2	0.995	0.888
IL10	0.996	0.960
IL5	0.998	0.954
IL1β	0.997	0.919
IFNγ	0.986	0.900
IL8	0.997	1.014

Correlation determined by linear regression with observed results compared to the expected results based on the concentration of cytokine spiked into the sample adjusting for endogenous concentrations.

Acceptable results were obtained for additional performance characteristics included in the validation

	Parameters	Low	Medium	High
		Range	Range	Range
Precision	Within Run	1.3% to 10.4%	1.0% to 5.6%	0.8% to 5.6%
	Between Run	3.2% to 19.5%	1.8% to 8.3%	1.2% to 10.7%
		%Difference	%Difference	%Difference
Interference	Hemoglobin	-17.6% to 19.1%	-12.1% to 12.0%	-7.8% to 15.4%
Stability	2 Day 4ºC	-26.9%* to 39.3%	-29.7%* to 10.6%	-41.1%* to 6.1%
	30 days -20ºC	-21.9%^ to 2.1%	TBD	-14.6% to 0.3%
	3X Freeze/Thaw	-18.3% to 11.2%	-14.7% to 6.6%	-14.9% to 6.9%

Precision data based on replicates of 5 on three separate runs. Hemoglobin interference based on 1500 mg/dL. The range from the lowest to highest %CV or difference of the 13 analytes is shown. *Two day 4°C storage showed instability for TNFα. ^IFNγ demonstrated a percent difference just out of our 20% target.

DISCUSSION AND CONCLUSIONS

- CSF demonstrated optimal analyte recover and minimal matrix effect at a 1:4 specimen dilution.
- Heterophile antibody interference was observed in CSF, but was eliminated or mitigated by the heterophile blocking reagent incorporated in the specimen diluent.
- Patient specimens for cytokine testing should be submitted as critical frozen, up to 3 freeze-thaw cycles, however, did not affect results. If repeat testing is required after the initial thaw and cannot be performed the following day, the specimen should be re-frozen.
- CSF as a specimen type demonstrated acceptable performance in our multiplexed cytokine assay.
- By establishing normal reference intervals for cytokine concentrations in CSF, their potential as biomarkers for infectious, autoimmune and other inflammatory CNS disorders can be more appropriately investigated.