Discovery of Alzheimer's Disease Biomarkers Associated with Inflammation Using a Novel, Validated, Cytokine Screening Panel

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Abstract
Background: Alzheimer’s disease is a top priority in neuroscience research worldwide. Recent evidence suggests that neuronal inflammation agents that mediate disease neuropathology also play key roles in triggering the amyloid cascade and providing power flow to downstream targets. The use of novel validated inflammation assays for early detection of inflammatory responses and efficient monitoring of pharmacologic studies is of high importance. The study described herein used future-directed novel inflammation panel and methodology to provide power flow from early stage neural damage to downstream targets.

Methods: Multiple rounds of label excitation and emission enhance light levels and improve signal to noise ratio. This method can be alternatively termed as Spike Recovery and Dilution Linearity (SRL), a protocol that has been developed using multi-label technology to provide signal enhancement and improved linearity. The present study employs this new methodology to provide a validated novel inflammation panel for measuring and monitoring biomarker levels in serum, plasma and CSF samples.

Results: A Modified SRL protocol was developed and tested using a novel inflammation panel and validated technology. The panel comprised 20 cytokines and chemokines and was validated using a variety of validated panel dilution tests. Results demonstrate the use of this protocol for measuring inflammatory biomarkers in serum samples.

Conclusions: The novel inflammation panel was validated for measuring inflammatory biomarkers in serum samples. This methodology is a rapid and reliable method for measuring inflammatory biomarkers and can be used for future studies to evaluate the inflammatory response in Alzheimer’s disease.

Sensitivity and Assay Protocol
The Modulated SRL (M-SRL) assay validation was conducted in the signal 2.5x2.5 standard deviation above the background (pre-billboard). Signals and sensitivity, are from 102 cytokines tested over 2 meals by 6 operators.

Specificity and Reproducibility
Background: The M-SRL protocol was validated for reproducibility with other analytes in the panel as well as related analytes that are not in the panel. The cross-reactivity of all samples was very low. To validate the reproducibility of the assay, levels of cytokines of varying levels were measured with and without a panel. The assay reproducibility was calculated using the minimum of each panel and pooled samples.

Spine Recovery and Dilution Linearity
Spike recovery (testing using undiluted serum), serum, plasma, platelet, plasma, serum, and cell culture media. Reproducibility data was presented for the panel dilution protocol and each analyte in the assay. The reproducibility of each analyte was determined using a modified SRL protocol.

Conclusion
If a sample panel has been developed and validated reliably for use in measuring inflammatory cytokines and chemokines in a variety of human tissue and fluid samples, it can be a simple tool that should be useful in the study of the inflammatory response in Alzheimer’s disease. The assays in this panel were run on 3 dilution stages, with one stage optimized to achieve the best assay performance. Combined results were determined for cytokine and chemokine samples over multiple time points to examine if assay performance varied with assay conditions and different time points. This novel inflammation panel validated for use in measuring inflammatory biomarkers and can be used for future studies to evaluate the inflammatory response in Alzheimer’s disease.

Methods
The methods were divided into three panels: panel development, optimization, and validation. The panel development and validation were performed using future-directed technology and validated technology. The panel development and validation were performed using the future-directed technology and validated technology. The panel development and validation were performed using the future-directed technology and validated technology. The panel development and validation were performed using the future-directed technology and validated technology. The panel development and validation were performed using the future-directed technology and validated technology.

Development and Validation
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Calibration Curves
Reproduction calibration curves are shown below for each dilution assay. The curves were generated by using the average of all 26 cytokine dilution curves per panel on 3 different days. Each cytokine dilution curve was generated using a panel of five different dilution curves per panel. The panel dilution curves were generated using a panel of five different dilution curves per panel. The panel dilution curves were generated using a panel of five different dilution curves per panel. The panel dilution curves were generated using a panel of five different dilution curves per panel. The panel dilution curves were generated using a panel of five different dilution curves per panel.