Abstract # 1803

Characterizing the neuroinflammatory profile in neurodegenerative conditions using rapid, multiplexed assays with enhanced sensitivity

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1 Abstract

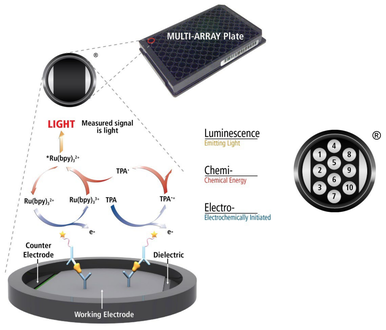
Neurological disorders affect approximately 15% of the world's population, with neurodegenerative conditions representing the most significant burden. Complex interactions between lymphocytes and resident cells in the central nervous system (CNS) can determine the progression and severity of symptoms. Excessive or dysregulated expression of cytokines released by these immune cells may lead to neuronal cell death and synaptic plasticity dysfunction. Subsequent impacts on learning, memory, and other cognitive deficits pose significant challenges to healthcare systems, highlighting the need for better tools to understand their pathogenesis. However, sample volumes are limited, and there is a dearth of assay methods that provide the required sensitivity for detecting analytes during the pre-symptomatic phase. Moreover, the need for higher sensitivity assays to correlate animal models to human disease remains unmet.

A one-incubation assay protocol using the MESO SCALE DISCOVERY electrochemiluminescent (ECL) platform provides a faster, simpler workflow for detecting biomarkers of interest. Using this platform, we examined circulating levels of GM-CSF, Granzyme B, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, IL-17A, Perforin, and TNF-α in commercially sourced samples with Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, as well as expression profiles in human glioblastoma cells and rodent samples. The addition of a proprietary signal enhancement process complemented the platform, resulting in an average three-fold increase in the number of samples within the detection limits across all analytes. The increase in sensitivity provides the ability for higher dilution, thus preserving valuable samples. This holds particular importance for rodent studies due to the limited quantity of material that researchers can extract from a single animal. Initial testing showed distinct concentrations of markers associated with individual diseases, suggesting unique phenotypic profiles for each condition.

Our data successfully show a reliable multiplex solution with ECL detection and accelerated time-to-result on the MSD platform, offering increased sensitivity and throughput without the need for large sample volumes. Identifying the immune landscape and onset of cell death pathways using such tools can improve our understanding of disease progression.

2 Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates. The MSD® S-PLEX Proinflammatory Panel 1 is a nine-analyte ultrasensitive panel. The S-PLEX® platform uses ECL technology, retaining its well-known advantages and superior analytical performance.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Strategy

Neurodegeneration is associated with dysfunctions in synaptic plasticity, breakdown of the blood-brain barrier, activation of resident immune cells, release of inflammatory mediators, and neuronal cell death, all of which may contribute to symptoms of cognitive and motor deficits. The challenge of achieving effective treatment interventions is compounded by the time-consuming nature of sample screening and low abundance of analytes leading to poorly understood, variable timing of these cellular activities across different disease conditions. We sought to use two detection methods, SULFO-TAG (ST) and TURBO-BOOST® (TB) reporters, on the MSD technology to tease out unique, underlying pathologies of neurodegenerative diseases.

EDTA plasma and serum samples from apparently healthy individuals, and individuals with Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), or glioblastoma (GBM) were commercially sourced and tested to characterize the expression profile of markers of neuroinflammation and cell death. The SULFO-TAG reporter panel co-incubated samples and detection reagents, allowing for a faster time-to-result for more high throughput testing. Equal representation of gender, as well as age range, were taken into account. Those with mild to moderate cognitive decline were rated on the Mini-Mental State Examination (MMSE), and multiple sclerosis samples were grouped into newly diagnosed, relapsing-remitting MS (RRMS), primary progressive MS (PPMS), and secondary progressive MS (SPMS).

The relationship between these analytes and stages of disease progression was evaluated using simple correlation. All analysis was conducted on R version 4.3.1 (2023-06-16 ucrt) -- "Beagle Scouts", and R Studio 2024.04.1 Build 748. MSD assay kits were used according to protocol for all testing, and samples were sourced from external vendors.

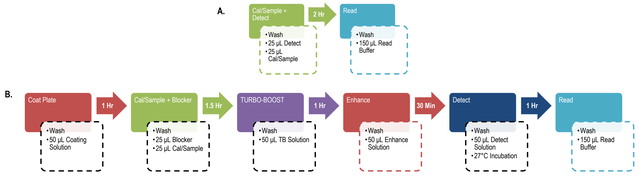
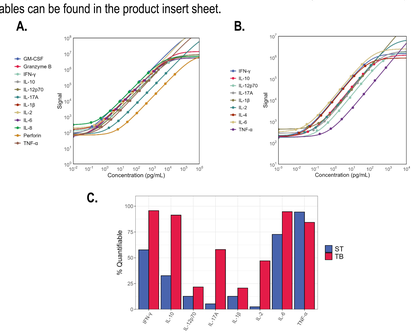


Figure 1. Assay protocols for SULFO-TAG (A) and TURBO-BOOST (B) reporter systems.

4 Calibrator Curves and Sensitivity Tables

Due to calibrator assignment differences between panels, analyte concentrations were evaluated against NIBSC/WHO international standards except for Granzyme B and Perforin, where standards are not available. High abundance analytes were run on the SULFO-TAG panel only. NIBSC/WHO conversion tables can be found in the product insert sheet.



D.

Analyte	LLOD	ULOD	Units
GM-CSF	0.116	642	pg/mL
IFN-γ	0.232	11200	pg/mL
IL-1β	0.100	926	pg/mL
IL-2	0.465	888	pg/mL
IL-6	0.108	1370	pg/mL
IL-8	0.410	565	pg/mL
IL-10	0.0884	942	pg/mL
IL-12p70	0.101	1790	pg/mL
IL-17A	0.778	7560	pg/mL
TNF-α	0.449	2590	pg/mL
Granzyme B	0.0966	1200	pg/mL
Perforin	3.04	27800	pg/mL

E.

Analyte	LLOD	ULOD	Units
IL-6	0.0241	36.9	pg/mL
IL-10	0.0518	168	pg/mL
IL-12p70	0.107	529	pg/mL
IL-4	0.0180	55.3	pg/mL
TNF-α	0.343	747	pg/mL
IL-2	0.056	112	pg/mL
IL-1β	0.162	161	pg/mL
IFN-γ	0.018	49.3	pg/mL
IL-17A	0.282	340	pg/mL

Figure 2. Typical standard curve representation for panels with SULFO-TAG (A) and TURBO-BOOST (B) reporters. The number of quantifiable samples with CV ≤ 20% were normalized to the total number of samples tested and are represented per reporter system and analyte (C). The upper and lower limit of detection are represented in Tables D and E, respectively.

5 Matrix Differences

Initial overview of native sample testing showed matrix differences with commercially sourced healthy and diseased samples, regardless of detection method.

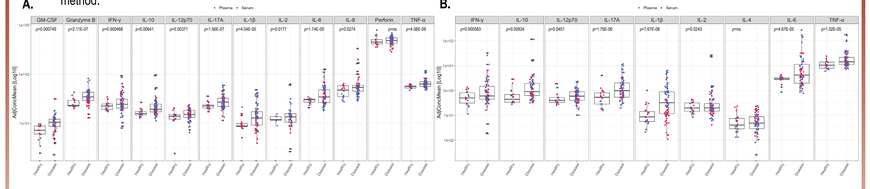
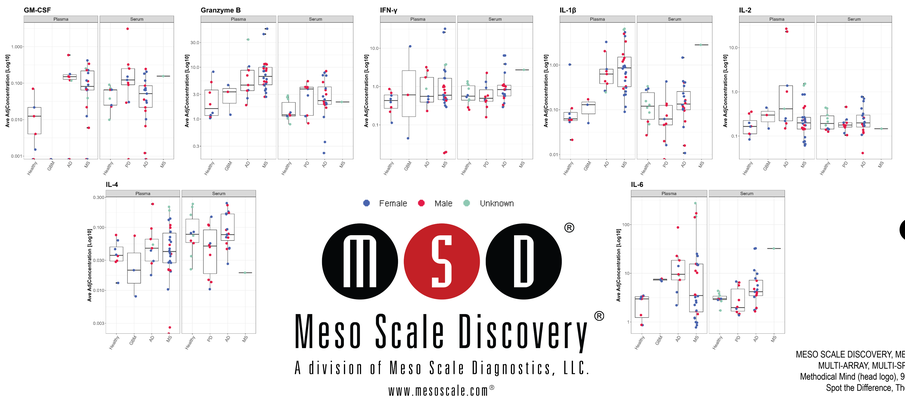


Figure 3. Dilution-corrected sample concentrations of plasma (blue, N=32), and serum (red, N=37) are represented on a log axis for each analyte with SULFO-TAG (A) or TURBO-BOOST (B) reporter. Due to sample volume limitations, four serum samples are not represented in the dataset for Figure B. Healthy and Disease groups were compared using Student's t-test, and p values were reported for all significant observations.

6 Analytes and Neurodegenerative Diseases

Samples for each analyte were further broken down into multiple groups. Commercially sourced healthy (serum N=8, plasma N=6) samples were tested neat, and diseased samples including Alzheimer's (AD; serum N=20, plasma N=9), multiple sclerosis (MS; serum N=1, plasma N=14), Parkinson's (PD; serum N=10), and glioblastoma (GBM; plasma N=3) samples were tested at 2-fold dilution. Samples were analyzed using One Way ANOVA and Tukey's Post Hoc test to determine significance between groups.

GM-CSF was significant between PD and AD (p=0.0257) and Healthy and PD (p=0.0466) serum groups. Granzyme B was significant between Healthy and AD (p=0.00676) serum groups. IL-1β expression was significant between Healthy and MS (p=0.00874) plasma groups. IL-2 was significant between Healthy and AD (p=0.00207), GBM and AD (p=0.0465), and MS and AD (p=8.64E-05) plasma groups. TNF-α expression was significant between Healthy and AD (p=0.0189) and PD and AD (p=2.68E-05) serum groups, and Perforin was significant between Healthy and AD (p=0.00960), PD and AD (p=5.91E-08), and Healthy and PD (p=0.0356) serum groups. Color visualization for each sample provided an insight to potential sex differences within each group.



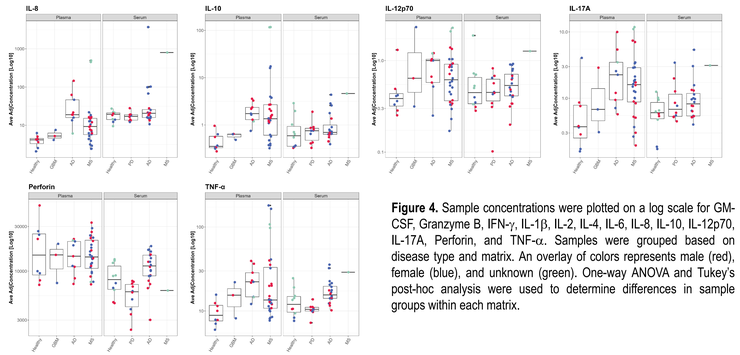
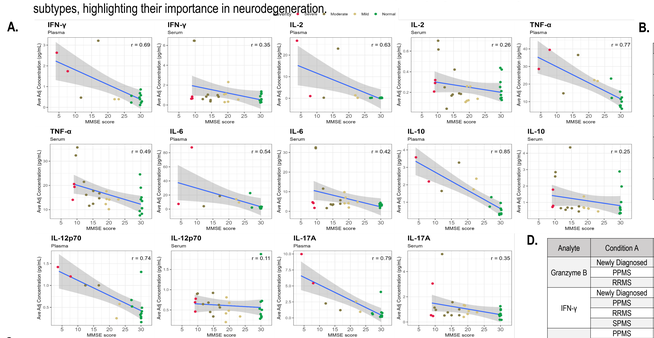


Figure 4. Sample concentrations were plotted on a log scale for GM-CSF, Granzyme B, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-17A, Perforin, and TNF-α. Samples were grouped based on disease type and matrix. An overlay of colors represents male (red), female (blue), and unknown (green). One-way ANOVA and Tukey's post-hoc analysis were used to determine differences in sample groups within each matrix.

7 Analyte Correlation to Disease Progression

The figure below shows the correlation of analytes with cognitive decline, determined based on MMSE scores, as well as different categories of MS. In commercially sourced samples of cognitive decline, we found that there were distinct matrix differences for most analytes, but especially for IFN-γ, IL-10, IL-12p70, IL-17A and TNF-α. Similar trends were observed with SULFO-TAG reporter for IFN-γ and IL-10. These analytes show the interplay of neuroprotection and inflammation in later stages of the disease. Moreover, a majority of these analytes are consistent with distinguishing MS disease subtypes, highlighting their importance in neurodegeneration.



D.

Analyte	Condition A	Condition B	p value
Granzyme B	Newly Diagnosed	Healthy	0.000576
	PPMS	Newly Diagnosed	0.0249
	RRMS	Newly Diagnosed	0.0101
IFN-γ	Newly Diagnosed	Healthy	0.000950
	PPMS	Newly Diagnosed	0.00608
	RRMS	Newly Diagnosed	0.00104
	SPMS	Newly Diagnosed	0.00479
IL-1β	PPMS	Healthy	0.000134
	SPMS	Healthy	2.41E-05
	PPMS	Newly Diagnosed	0.00179
	SPMS	Newly Diagnosed	0.000409
	RRMS	PPMS	0.00165
	SPMS	RRMS	0.000338
IL-6	PPMS	Healthy	4.25E-05
	PPMS	Newly Diagnosed	0.000124
	RRMS	PPMS	0.000212
	SPMS	PPMS	0.000415
IL-8	PPMS	Healthy	0.0195
	SPMS	Healthy	2.92E-06
	SPMS	Newly Diagnosed	0.0392
	SPMS	RRMS	0.00318
IL-10	Newly Diagnosed	Healthy	0.00103
	RRMS	Newly Diagnosed	0.0104
	SPMS	Newly Diagnosed	0.00520
IL-17A	PPMS	Healthy	0.000199
	PPMS	Newly Diagnosed	0.0197
	RRMS	PPMS	0.0174
	SPMS	PPMS	0.000604
TNF-α	Newly Diagnosed	Healthy	0.00156
	PPMS	Newly Diagnosed	0.0475
	RRMS	Newly Diagnosed	0.0114
	SPMS	Newly Diagnosed	0.00942

Figure 5. Correlation between analytes (y-axis) and MMSE scores (x-axis) using Pearson's correlation for the TURBO-BOOST reporter system. Pearson's r values are summarized in (B). Samples were grouped based on severity on the MMSE scale: Normal (≥24, green), Mild (19-23, yellow), Moderate (10-18, brown), and Severe (≤9, red). The cutoff was set to plasma r ≥ 0.50. Correlation summary (D) between plasma levels of analytes (y-axis) and MS progression was analyzed with One-way ANOVA and Tukey's post-hoc test (C), and results summarized in (D). MS plasma samples were categorized into healthy (N=4, blue), newly diagnosed (N=3, red), RRMS (N=4, gray), SPMS (N=3,brown), and PPMS (N=3, teal).

8 Summary and Conclusion

The capability to detect analytes using two different methods provides flexibility when designing experiments. By combining the two methods, we are able to show disease and matrix-specific changes in markers associated with inflammation and cell death. Additionally, we are able to show analyte correlation to the progression of cognitive decline and subtypes of MS. Faster protocols, and highly sensitive assays for biomarkers offer great promise for understanding the unique underlying pathology of biomarkers associated with neurodegenerative diseases.

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