Novel Multiplex Immunoassay Tools for Characterizing Neurological Disease Autoantibody Profiles

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Introduction

By binding to common autoantigens including synaptic proteins, neuronal antigens, cell surface receptors, and aggregation-prone proteins, autoantibodies contribute to the pathogenesis of several neurological diseases by disrupting normal neural function and promoting inflammation and tissue damage in the nervous system. Detection of autoantibodies as biomarkers present a promising approach to enhance our understanding of neuroinflammation and to facilitate the preclinical development of therapeutic strategies.

The MILLIPLEX® Human Neurodegenerative Autoantibody Panel (Cat. No. **HNDGAB-220K**) is a configurable 25-plex kit to be used for the qualitative analysis of any or all of the following antibodies in serum, plasma, and cerebrospinal fluid (CSF) samples: anti-α-synuclein, anti-AChR (acetylcholine receptor), anti-AGNA (Anti-Glial Nuclear Antibody, SOX1), anti-Ago2 (Su), anti-CRYAB (α B-crystallin), anti-AMPA-R, anti-Amphiphysin, anti-ANNA2 (Ri)/NOVA1, anti-AQP4 (aquaporin-4), anti-Aβ42, anti-CRMP5 (Collapsin Response Mediator Protein 5), anti-DPPX, anti-DRD2 (Dopamine D2 Receptor), anti-ELAVL4 (HuD), anti-GFAP (Glial Fibrillary Acidic Protein), anti-MBP (Myelin Basic Protein), anti-C1q, anti-mGluR1, anti-MOG (Myelin Oligodendrocyte Glycoprotein), anti-NF-L (Neurofilament light chain), anti-NMDAR1, anti-NRXN3 (neurexin-3), anti-PCA1 (Yo)/CDR2, anti-PNMA1 (Ma1), and anti-tTau.

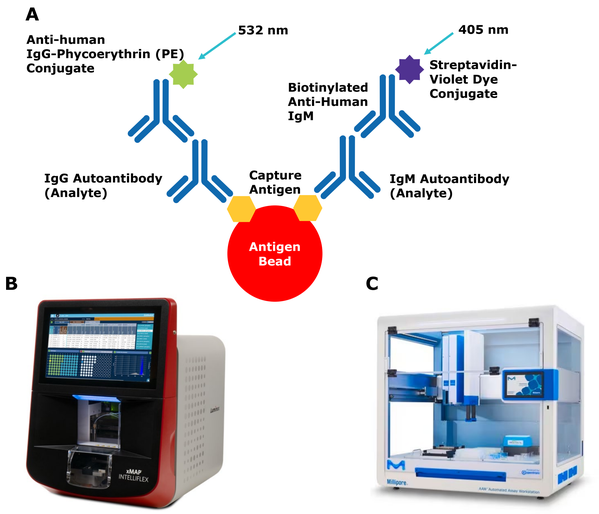

Figure 1. The MILLIPLEX® Human Neurodegenerative Autoantibody Panel Assay utilizes a capture antigen method and can be paired with the MILLIPLEX® Dual Reporter Autoantibody IgG/IgM Reagent Pack and AAW™ Automated Assay Workstation. The xMAP® INTELLIFLEX® DR-SE Instrument is required for detection.

MILLIPLEX® autoantibody kits are for the qualitative measurement of autoantibodies in a variety of biofluid samples including serum, plasma, and CSF. **A**) Simultaneous detection of human neurodegenerative IgG and IgM autoantibodies leveraging the dual reporter functionality of the MILLIPLEX® Dual Reporter Autoantibody IgG/IgM Reagent Pack (Cat. No. **DRAABPK-240K**) and the xMAP® INTELLIFLEX® DR-SE instrument (Cat. No. **INTELLIFLEX-DRSE**). The dual reporter autoantibody IgG/IgM reagent pack contains the anti-IgG-PE and biotinylated anti-IgM antibody along with Streptavidin-violet dye conjugate as the detection components. **B**) xMAP® INTELLIFLEX® DR-SE instrument equipped with green and violet laser to allow simultaneous excitation of two fluorophores. **C**) MILLIPLEX® Human Neurodegenerative Autoantibody Panel leverages the AAW™ assay-ready workstation (Cat. No. **99900256**), a robotic platform designed for automating assays and sample preparation for a broad range of applications.

Samples

Matched serum and plasma samples from presumed healthy donors and individuals with multiple sclerosis and stroke were sourced from commercial suppliers for testing. Serum, plasma, and cerebrospinal fluid (CSF) samples from those with Alzheimer's and Parkinson's diseases were also tested. Sample preparation followed the kit protocol, with serum and plasma diluted 1:1000 and CSF diluted 1:2 in assay buffer. Results were generated overnight as per the protocol.

Autoantibodies in Biological Samples

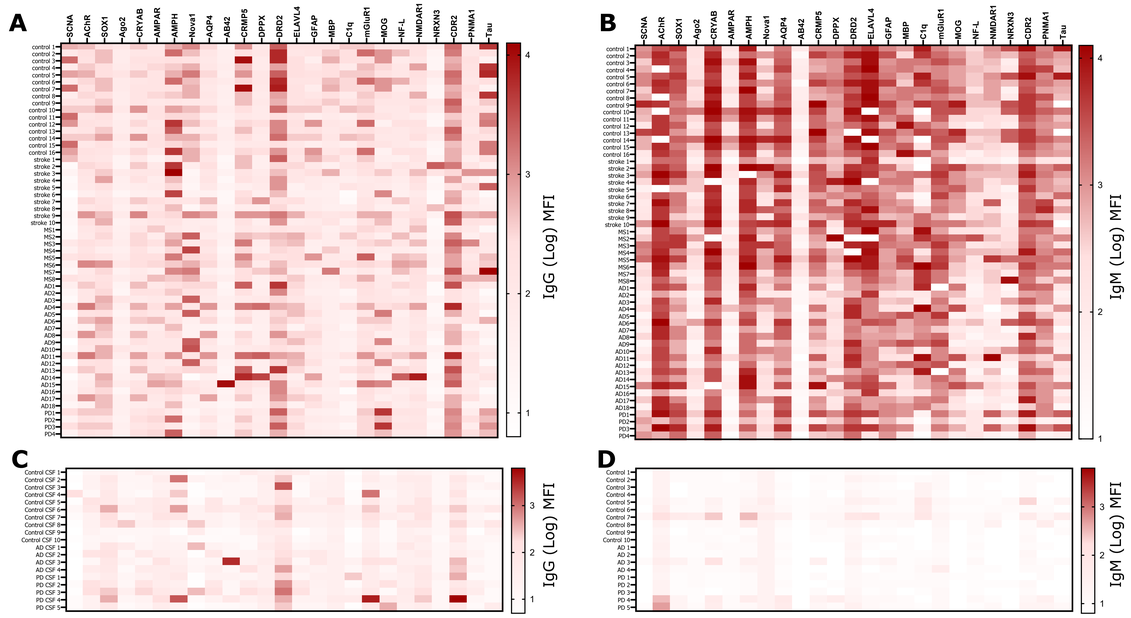

Figure 2. Measurement of IgG and IgM autoantibodies in human serum, plasma, and CSF samples.

Heatmaps illustrating the concentration of **A**) IgG and **B**) IgM autoantibodies in serum and plasma samples, and **C**) IgG and **D**) IgM autoantibodies in CSF samples. Each cell in the heatmaps represents the logarithm of the detected analyte's mean fluorescence intensity (MFI). Each row corresponds to an individual sample, while each column represents the specified analyte. (**A** & **B**) 56 serum and plasma samples were analyzed, including 16 samples from presumed healthy donors (Control 1 – 16), 10 serum stroke samples (Stroke 1 – 10), 8 serum multiple sclerosis samples (MS 1 – 8), 18 serum and plasma Alzheimer's disease samples (AD 1 – 18), and 4 serum and plasma Parkinson's disease samples (PD 1 – 4). (**C** & **D**) 19 CSF samples were analyzed including 10 from presumed healthy donors (Control CSF 1 – 10), 4 Alzheimer's disease CSF (AD CSF 1 – 4), and 5 Parkinson's disease CSF (PD CSF 1 – 5).

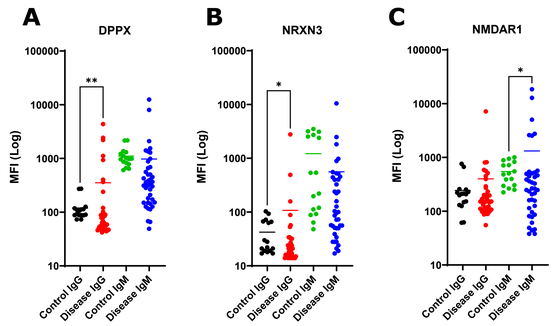

Figure 3. Comparison of autoantibody levels between presumed healthy and diseased samples.

IgG and IgM autoantibody MFIs for selected analytes were plotted, comparing serum and plasma samples from presumed healthy donors (control) and those with disease (stroke, MS, AD, and PD). The plots include **A**) anti-DPPX, **B**) anti-NRXN3, and **C**) anti-NMDAR1 autoantibodies (n = 16 control, 40 disease; Mann-Whitney test: * P < 0.05, ** P < 0.01).

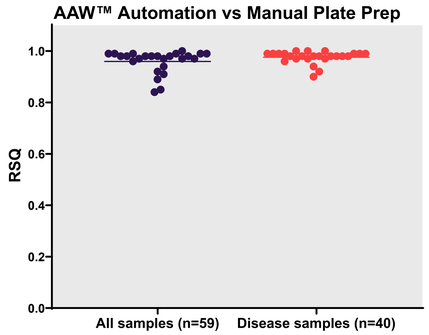

Figure 4. Correlation between assay plate preparation by automated AAW™ Workstation and manual pipetting.

Correlation analyses were performed and plotted as R-squared (RSQ) values for each IgG autoantibody in the panel, based on the 59 total samples and the 40 disease samples. Each RSQ value is represented as a data point.

Methods

Immunoassays and Data Analysis: The MILLIPLEX® Human Neurodegenerative Autoantibody Panel (Cat. No. **HNDGAB-220K**) and MILLIPLEX® Dual Reporter Autoantibody IgG/IgM Reagent Pack (Cat. No. **DRAABPK-240K**) were used according to the protocol. The assay plate was prepared with the AAW™ Automated Assay Workstation – Assay Ready (Cat. No. **99900256**). Assays were read on the xMAP® INTELLIFLEX® DR-SE (Cat. No. **INTELLIFLEX-DRSE**) instrument and data was acquired via xPONENT® v. 4.3 software. Figures were prepared in GraphPad Prism and Microsoft Excel.

Summary

The MILLIPLEX® Human Neurodegenerative Autoantibody Panel enables simultaneous qualitative measurement of up to 25 autoantibodies relevant in various neurodegenerative autoimmune disorders. When combined with the MILLIPLEX® Dual Reporter Autoantibody IgG/IgM Reagent Pack and the INTELLIFLEX® DR-SE instrument, this panel can assess both IgG and IgM antibody levels in the same sample.

Our results establish a comprehensive autoantibody profile in biological samples from various neurological disease states. While the detection of autoantibodies does not necessarily indicate disease, significantly elevated IgG and IgM levels were observed in certain disease samples (**Figure 3**). The IgM autoantibody profile was generally higher (**Figure 2 A-B**) than that of IgG in serum and plasma, likely due to IgM's greater avidity and broader specificity. Conversely, IgM autoantibodies were predominantly absent in CSF, consistent with their limited ability to cross the blood-brain barrier due to their larger size and restricted diffusion (**Figure 2 C-D**).

Additionally, our results highlight the advantages of automation, as the AAW™ Automated Assay Workstation demonstrated a strong correlation with manual assay preparation (**Figure 4**) across all analytes in a mixed set of samples.

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