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APPLICATION NOTE

ROBUST AND REPRODUCIBLE NEUROFILAMENT LIGHT QUANTITATION IN SERUM AND PLASMA SAMPLES USING ELLA

INTRODUCTION

Neurofilament light (NF-L) has emerged as a biomarker for diagnosing and monitoring the progression of a variety of neuropathies such as Huntington's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and multiple sclerosis. High levels of NF-L in the blood and cerebrospinal fluid (CSF) indicate axonal damage, a hallmark of the aforementioned neurodegenerative disorders. From a sample source perspective, quantifying the amount of NF-L in the blood is less invasive than taking a CSF sample, which also increases the availability of research working material and therefore the utility of NF-L as a biomarker. However, the level of NF-L present in blood derivatives such as plasma and serum is typically in the low picogram per milliliter range, which is considerably lower than levels in CSF[1]. Therefore, a sensitive, robust and reproducible assay is needed to provide the high-quality data required to characterize and monitor neurodegenerative disease progression and treatment reliably.

The Simple Plex NF-L Assay on Ella delivers the sensitivity required to measure NF-L with a high level of reproducibility in serum, plasma and CSF sample types. Here, we demonstrate the Simple Plex NF-L Assay's ability to distinguish between normal and disease state samples, compare our NF-L measurements to published reports and demonstrate that Ella is a reproducible and reliable platform for conducting NF-L research.

HOW SIMPLE PLEX ASSAYS WORK ON ELLA

Simple Plex Assays are run on a microfluidic cartridge that automates all steps of the immunoassay. Each cartridge is composed of channels that contain glass nano reactors (GNRs), which are the core of a Simple Plex immunoassay. Each channel of the cartridge contains three GNRs coated with a capture antibody so that each sample is automatically processed in triplicate. The GNRs in the NF-L assay cartridge are coated with an Uman Diagnostics anti-NF-L light monoclonal antibody responsible for capturing the native protein. A second monoclonal antibody, also developed by Uman Diagnostics, is lyophilized in the cartridge to act as the detection reagent.

Setting up a Simple Plex assay takes just 10 to 15 minutes. First, samples are diluted by mixing 25 µL of the sample with 25 µL of the Sample Diluent provided with the Simple Plex NF-L Assay Kit. Next, the diluted samples are loaded onto the NF-L assay cartridge alongside the Wash Buffer, also provided with the kit. Ella then automatically reads, processes and analyzes the cartridge of samples in just 75 minutes after pressing Start. Each cartridge also comes with a factory-calibrated standard curve, saving you the time and cost of setting up your own curve. This automated workflow delivers an assay with a wide dynamic range and single-digit coefficient of variation (CV) values.

ELEVATED LEVELS OF NF-L MEASURED IN ALS SAMPLES

The Simple Plex NF-L Assay is validated to quantify NF-L levels in serum, plasma and CSF sample types. To assess its applicability as a biomarker for brain pathology, we used ALS patient samples as a model system to quantify NF-L levels, since previous reports have established NF-L levels in ALS CSF and serum samples to be elevated[2,3]. In line with the published literature, our results indicate an elevated level of NF-L in serum and CSF when compared with healthy control samples (**FIGURE 1**).

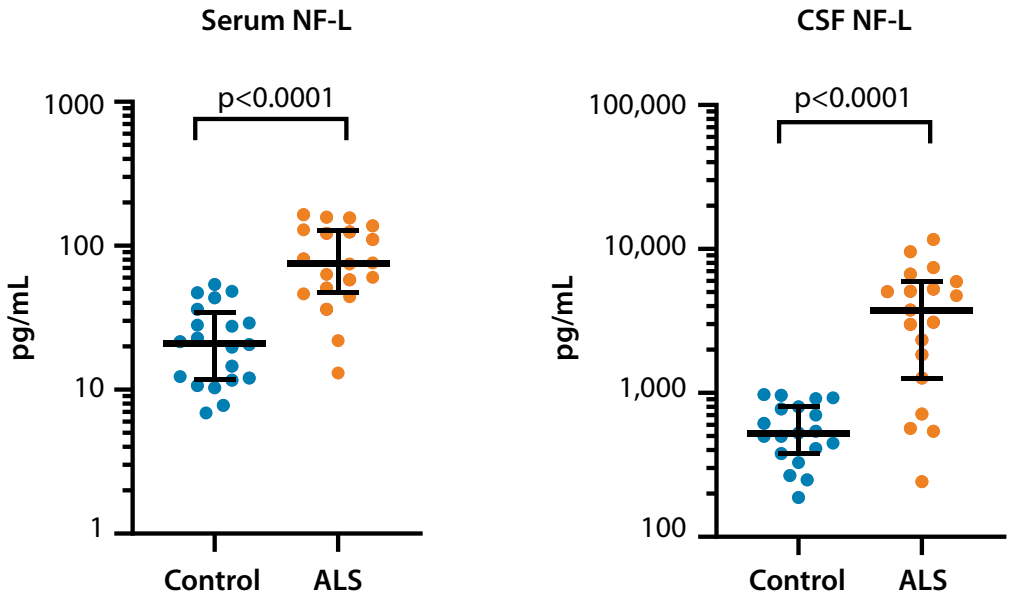

FIGURE 1. Quantitation of NF-L in serum and CSF samples with Ella. Healthy samples were analyzed on a 72x1 Simple Plex NF-L Cartridge, and increased NF-L levels were observed in the ALS samples tested. Bars represent median NF-L level ± the interquartile range. The statistical difference was evaluated using the nonparametric Mann-Whitney test with 19 to 20 samples per group.

RELIABLE NF-L DETECTION AND QUANTITATION

Signal-to-background ratio (S/B) is often used to evaluate the reliability of an assay when detecting low-abundance analytes such as NF-L. In this study, cohorts of healthy serum and plasma samples were run alongside blanks consisting of the assay Sample Diluent (FIGURE 2). The median S/B ratio for the plasma and serum samples was 4.6, with an interquartile range of 3.8 to 6.4. Consistent quantitation of levels in normal samples is critical as is it helps researchers distinguish between healthy controls and samples with a given pathology.

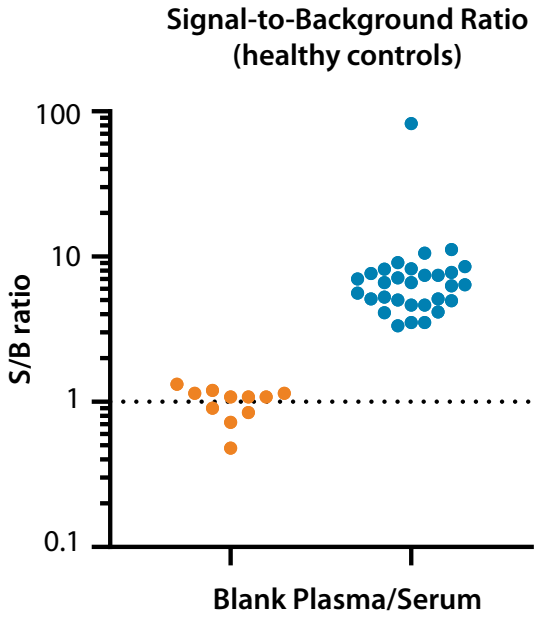

FIGURE 2. The S/B ratio of a healthy sample cohort using Simple Plex immunoassays on Ella. The signal intensity of each sample is expressed as a measure of mean relative fluorescent units (RFU). The S/B ratio was calculated by dividing the sample RFU to the mean blank value for each sample.

NF-L SIMPLE PLEX ASSAY CORRELATES TO OTHER QUANTITATIVE METHODOLOGIES

The antibodies developed by Uman Diagnostics and applied within the Simple Plex NF-L cartridge have been used in several other commercially available immunoassays for quantifying NF-L, including a bead-based assay and a standard plate-based ELISA kit. We analyzed cohorts of healthy and ALS samples and compared the results of the Simple Plex assay on Ella with those obtained using the Simoa® bead-based assay and the Uman Diagnostics plate-based ELISA (**FIGURE 3**). The plasma and serum sample levels of NF-L measured using Ella and the Simoa assay were consistent with a coefficient of determination (R^2) value of 0.95. The levels of NF-L measured in CSF samples also correlated (R^2 = 0.94) when the samples were run using Ella and the Uman ELISA. Serum and plasma samples could not be quantitated using the plate-based ELISA, as the values fell below the lower limit of quantitation for the assay.

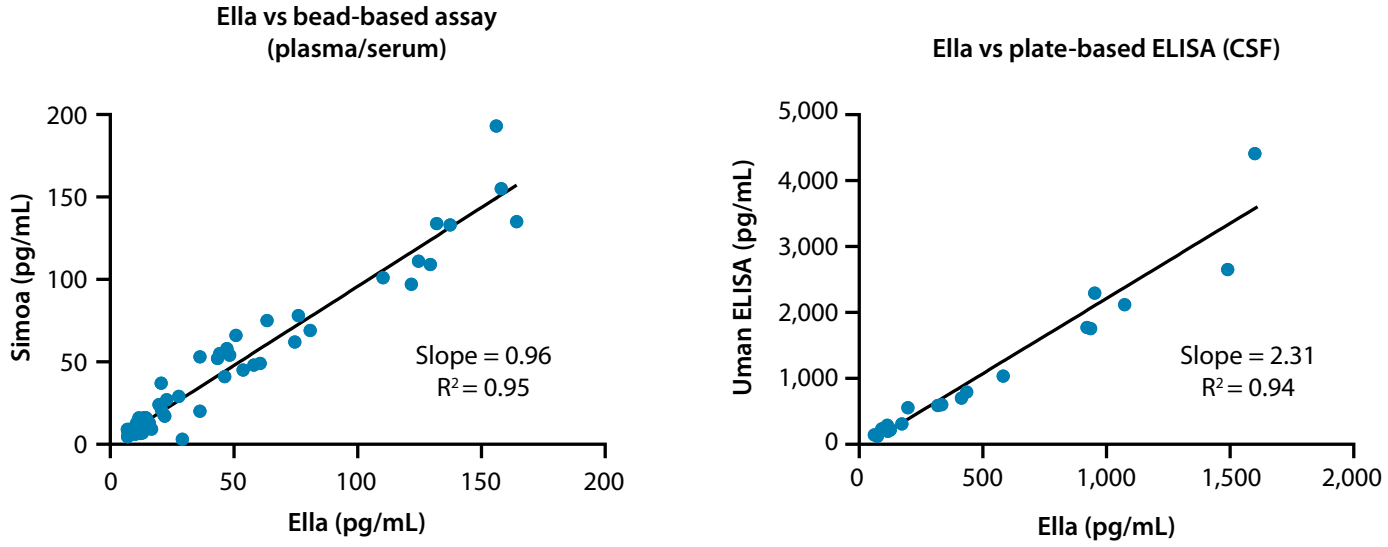

FIGURE 3. Linear correlation of serum and plasma samples on Ella versus other commercial platforms. NF-L levels measured using the Simple Plex Assay correlated with those quantified by the Simoa assay and the Uman Diagnostics ELISA. Samples were prepared as per the recommendations of the respective kit manufacturer, and levels of NF-L were measured within the linear range of each assay. The dilution factors were used to back-calculate the original concentration in the samples. These values were then plotted against each other, and a linear curve fitting algorithm was applied.

A HIGHLY REPRODUCIBLE PLATFORM METHOD

Biomarker measurements need to be precise and reproducible across different experiments, operators and sites to offer useful data for research and clinical applications. The hands-off workflow and factory-calibrated cartridges that come with Ella help reduce immunoassay complexity, thereby improving data precision and reproducibility. To evaluate this, healthy donor plasma and serum samples were run by three different operators of Ella, at three different sites, all assessing NF-L levels. The precision rate for measurements taken even in three different laboratories remained high, with a single-digit CV of 9.7% (**FIGURE 4**).

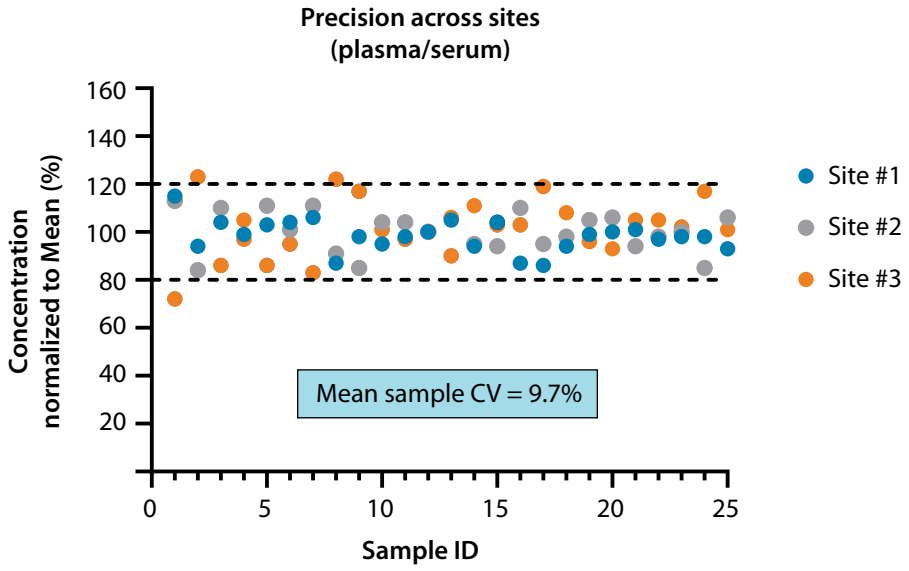

FIGURE 4. Inter-assay reproducibility. The same plasma and serum samples, obtained from healthy donors, were prepared according to the Simple Plex NF-L Assay instructions and measured at three different sites.

CONCLUSION

NF-L is a promising biomarker for diagnosing and monitoring a variety of neurological conditions. Here, we have demonstrated that the Simple Plex NF-L Assay has the sensitivity to produce high-quality data necessary to provide researchers with robust datasets from both blood derivatives and CSF samples. The workflow is hands-off and gives you the same result, regardless of operator or location, which is critical for ensuring NF-L as a biomarker that can positively impact human health. Ella also comes with a small footprint and gets you the assay data in just 75 minutes, giving you actionable results without sacrificing time and space. This combination of assay performance, reproducibility and ease-of-use make Ella perfect for translational NF-L research laboratories.

REFERENCES

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