Simoa® N4PA Assay in Whole Dried Blood Spots

Introduction

Dried blood spots (DBS) have many advantages over traditional sample matrices such as serum, plasma, and even liquid whole blood. Chief among these advantages is simplified sample collection, storage, and shipping. DBS can be collected and shipped without freezing or cold storage, eliminating the need for a centrifuge, freezers, or even a phlebotomist. Ultimately, DBS enable decentralized sample collection, whether in areas lacking laboratory infrastructure, longevity studies that struggle with patient retention, or monitoring of chronic diseases.

In this Application Note, we demonstrate that the Simoa Neuro 4-Plex Assay can measure levels of three neuronal biomarkers – NF-Light (NfL), Tau, and GFAP – in normal, undiseased dried blood spots.

Materials and Methods

Materials and Sample Processing

Liquid human whole blood was collected from four individuals into EDTA collection tubes. Dried blood spots were prepared using 50 µL of whole blood per spot onto a Whatman® protein saver card. Blood was spotted within 5 minutes of collection and air dried at room temperature. Control blood spots were stored frozen at −80°C.

All samples were tested using the Simoa multiplex Neurology 4-Plex “A” (N4PA) assay. Samples were processed and analyzed using the HD-1 Analyzer.

Methods

Stability of the dried spots was assessed by leaving the spots at room temperature for the specified number of days, then storing at −80°C. All spots were thawed at room temperature for 1 hour before testing with the N4PA assay. DBS were eluted in 500 µL N4PA kit sample diluent for 4 hours on an orbital shaker at 500RPM at room temperature, and run neat on the HD-1 Analyzer.

Specificity was determined by immuno-depleting DBS samples of analyte. Eluted DBS samples were incubated at room temperature (RT) rotating, with a 10x number of assay kit capture beads for an hour. Stability testing was performed with spots left at room temperature for either 0, 1, 3, 6, or 8 days to assess for changes in signal with time spent at RT.

Results

Normal Sample Signal

All blood spots measured above kit LOQ for GFAP, NfL, Tau, and UCH-L1, with concentration ranges of 1.70 to 3.90 pg/mL, 0.351 to 3.07 pg/mL, 1.50 to 2.34 pg/mL, and 45.5 to 130 pg/mL, respectively.

For each sample, two blood spots were measured. The coefficient of variation (CV) between the two spots’ analyte concentration was determined. All analyte measurements were found to have CV’s <30%, with CV’s <20% for 81.25% of samples.
Analyte Specificity – Tau

Two blood spots (DBS 1 and DBS 2) were measured for depletion. Tau concentration is depleted by 71.6% and 72.5% in relation to the undepleted values (1.94 to 0.55 pg/mL and 1.49 to 0.41 pg/mL). AEB signal falls to 18.0x and 13.5x the value of calibrator A (blank).

Blood spots left at RT for 21 days were also measured for depletion. Tau concentration is depleted by 82.1% and 80.3% in relation to the undepleted values (2.73 to 0.49 pg/mL and 2.03 to 0.40 pg/mL). AEB signal falls to 15.9 and 13.2x the value of calibrator A (blank).

Analyte Specificity – NfL

NfL concentration is depleted by 100% in both spots. Undepleted spots measure at 1.94 pg/mL and 1.49 pg/mL. Depleted signals fall below LOQ and a concentration can’t be determined. AEB signals fall below calibrator A (blank).

NfL concentration in blood spots left at RT for 21 days is also depleted by 100%. Undepleted signals are elevated, measuring at 2.16 pg/mL and 12.86 pg/mL. Depleted signals fall below LOQ and a concentration can’t be determined. AEB signals fall below calibrator A (blank).
Analyte Specificity – GFAP

GFAP concentration is depleted by 100% in both spots. Undepleted spots measure at 1.50 and 3.13 pg/mL. Depleted signals fall below LOQ and a concentration can’t be determined. AEB signal falls to below the value of calibrator A (blank).

Blood spots left at RT for 21 days are also depleted by 100%. Undepleted spots measure at 1.08 and 2.99 pg/mL. Depleted signals fall below LOQ and a concentration can’t be determined. AEB signals fall below calibrator A (blank).

Analyte Specificity – UCH-L1

None of the blood spots showed depletion of signal for UCH-L1. UCH-L1 concentration is depleted by 10.5%, 33.2%, 63.4%, and 17.2% for DBS 1, DBS 2, and DBS 1 and 2 at RT, respectively.

Stability Testing

Blood spots from all 4 individuals (DBS 1, DBS 2, DBS 3, DBS 4) were left at room temperature for 0, 1, 3, 6 or 8 days prior to measurement to assess changes in signal with time when stored at room temperature. Signal was normalized to signal at Day 0.

Tau was found to be relatively stable for all 8 days at RT, with normalized concentrations ranging from 96.8% – 129.2% of Day 0.
NfL signals were not stable at room temperature. Concentrations ranged from 131.2% – 747.1% of Day 0. NfL signal increased with each consecutive time point, and significant signal elevation was seen as early as Day 1. Surprisingly, elevated signals were still shown to deplete by 100% in specificity testing.

GFAP was found to be stable for all 8 days at RT in three of the four samples. DBS 1, 2, and 4 had normalized concentrations ranging from 95.1% – 114.0% of those measured at Day 0. DBS 3 had normalized concentrations ranging from 237.8% – 280.3% of Day 0.

UCH-L1 was found to be stable for all 8 days at RT, with normalized concentrations ranging from 72.3% – 112.6% of those measured at Day 0. However, since UCH-L1 samples did not show signal depletion, measured concentrations are likely non-specific signal.

**Conclusion/Discussion**

The N4PA kit exhibits feasibility to measure Tau, NfL, and GFAP in dried whole blood. All three analytes are measurable above LOQ in normal dried whole blood. Tau signal depletes by 72% to values above the calibrator blank. Both NfL and GFAP signal deplete by 100% to values below the calibrator blank.
Tau signal is stable for 8 days at room temperature, with signal ranging from 96.8% to 129.2% of Day 0. NfL signal is not stable at room temperature; in the first day at room temperature, signal increases between 131.2% to 355.6%. Use of DBS with NfL is feasible if the spots are put into cold storage within 1 day of collection. Interestingly, this elevated signal depletes to 100%, indicating it is likely specific. It is thought degradation of associated proteins at room temperature may result in increased availability of NfL epitopes; however, the cause of this elevated signal has not been fully elucidated. GFAP signal is stable for 8 days at room temperature in three of four tested samples, with signal ranging from 95.1% to 114.0% of Day 0.

The N4PA kit does not display ability to accurately measure UCH-L1 in dried whole blood. While samples measure above LOQ, signal does not deplete, indicating signal is non-specific.

References