NULISAseq™ Analysis of Samples Collected by Microsampling and Dried Blood and Plasma Spot Methods

TECH NOTE

Introduction and Experimental Design

Proteomics is quickly gaining traction as a powerful tool for elucidating biomarkers in biofluids. Despite state-of-the-art molecular techniques only requiring microliter-volume samples, phlebotomy techniques dating to the 1940s continue to dominate human biofluid collection protocols. Venipuncture-based sample matrices (e.g., whole blood, plasma, and serum) require professional collection and cold chain storage to ensure biomolecule stability, limiting their geographical and socioeconomic utility **(Figure 1A)**.[1,2]

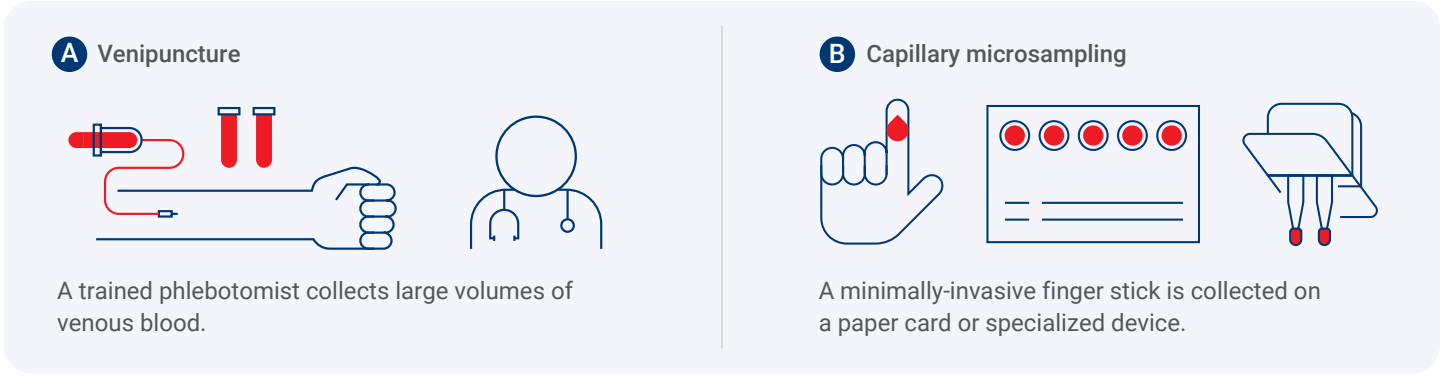


Figure 1. Overview of blood collection methodology involving either (A) venipuncture, whereby a phlebotomist collects several milliliters of blood, or (B) capillary microsampling, whereby the subject can self-collect blood from a fingerstick and apply to a device, such as a paper card or metered device.

The collection of capillary blood through dried blood spot (DBS), quantitative DBS (qDBS), dried plasma spot (DPS), and other microsampling devices represents viable and time-tested alternative methods that circumvent the complexities of phlebotomy-based venipuncture. These systems can be self-administered and maintain analyte stability at ambient temperature during shipping and short-term storage.[3] Due to the simplicity, cost-effectiveness, and logistics advantages, capillary blood microsampling has been leveraged in diverse applications, including population health, infant screening, patient selection and drug monitoring, and environmental exposure research while facilitating longitudinal study design and at-home specimen collections.[1,2,4,5]

In capillary blood collection, a minimally invasive skin piercing technique (e.g., a lance, microneedles, or liquid jet) induces a drop of blood, typically at the fingertip **(Figure 1B)**. Extraction may be facilitated through vacuum, microfluidics, or capillary action.[6] DBS involves directly applying capillary blood to filter paper, whereby several spots are qualitatively filled on a card and allowed to air dry. By contrast, microsampling devices are designed to collect a specific amount (i.e., metering) of whole blood on a sponge or using microfluidics. These systems may further separate cells from plasma and apply anti-coagulation mechanisms to generate microsampled plasma. In either case, the blood residual is extracted in a buffer and analyzed for biomarkers.

Nucleic acid-linked immunosandwich assay (NULISA™) provides unparalleled access to a wide dynamic range of proteins in human biofluids like blood, CSF, and urine.[7–9] By including a unique immunocomplex purification step, NULISA significantly reduces the background relative to other affinity-based protein methods, lowering the effective limit of detection (LoD) for protein targets.[10]

Multiplexing hundreds of validated, pan-application protein targets enables comprehensive and interpretable functional data measured from each sample and study. Furthermore, the NULISAseq assay is fully automated and optimized for the high-throughput analysis of proteins in low volumes of biofluid (25 µl). Taken together, NULISA provides the ideal platform for extracting value from capillary blood microsampling initiatives while consistently measuring low-abundance targets, such as the blood-based biomarker pTau-217 for Alzheimer's disease (AD).[11]

A recent preprint showcased the utility of NULISAseq proteomics in self-collected qDBS samples. In this COVID-19 study involving over 400 individuals, NULISAseq offered insights into cell-mediated immunity and vaccination within a heterogeneous population, enhancing clinical investigations.[12] Routine analyte extractions were performed to gather the data, and here, we extended the study's promising results to other capillary microsampling methodologies.

To test NULISA performance, several low-volume and dried spot collection systems available on the market were analyzed and compared to matched plasma **(Figure 2)**. Each system comprises different sampling protocols and input volumes, which were followed according to the manufacturer's recommendations. The NULISAseq Inflammation Panel 250 was evaluated in the Mitra microsampling device system. The NULISAseq CNS Disease Panel 120 was assessed in the Capitainer®B whole blood, the TelImmune™ Duo, and TASSO+® plasma systems.

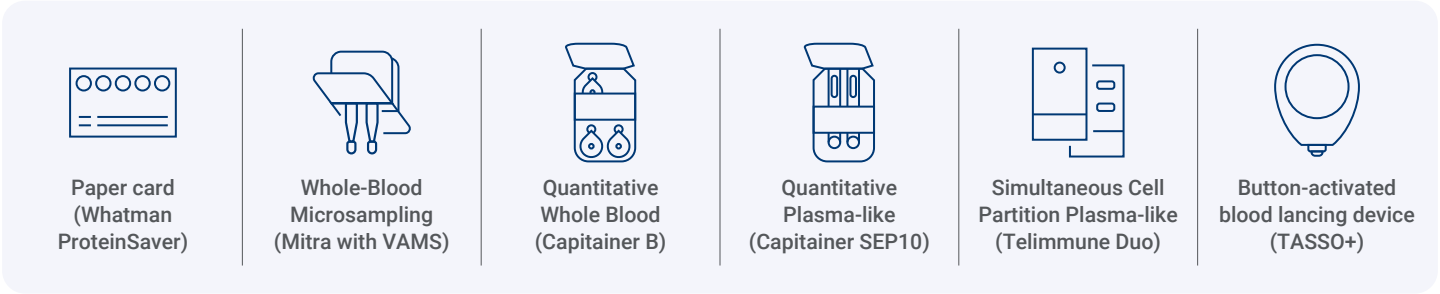


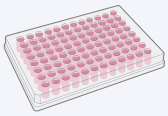
Figure 2. Capillary blood collection systems tested with NULISA in this study.

Alamar collected samples from volunteers to evaluate the feasibility of NULISA-based proteomics using capillary blood microsampling devices as matrices **(Table 1)**. Following specimen collection, the sample preparation for NULISA analysis was accomplished by a unified protocol (Figure 3). Extraction volumes were optimized depending on the input volume (e.g., a lower extraction volume was ideal for a lower input volume). The extracted samples were applied to plates with the NULISAseq Inflammation Panel 250 and CNS Disease Panel 120, followed by automated analysis on an ARGO HT instrument. Protein levels were normalized using internal and inter-plate controls, $\log_2$-transformed, and reported in units of NULISA Protein Quantification (NPQ).

Table 1. Collection and processing parameters for devices tested in this study.

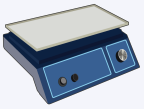
Sampling Device	Final Dried Product	Capacity (µl)	Volumetric capability
Whatman™ ProteinSaver	Whole Blood	75–80	No
Mitra Microsampling	Whole Blood	10, 20, 30	Yes
Capitainer® B	Whole Blood	2 x 10	Yes
Capitainer® B50	Whole Blood	2 x 50	Yes
Capitainer® -SEP10	Plasma	2 x 10	Yes
TelImmune™ Duo	Plasma	2 x 3	Yes
TASSO+ Device	Whole Blood or Plasma	200–600	Yes

1. Collect 10–50 µl of whole blood or plasma with the preferred device, place on a 96-well plate, and allow to dry overnight.



2. Elute in 100–300 µl of NULISA Sample Diluent buffer spiked with 1:100 protease inhibitor and 1:100 phosphatase inhibitor.

3. Gently shake the plate on a shaker for 1 hour at RT.



4. Centrifuge the plate at 3000 rpm (1278 g) for 30 min at RT.

5. Collect supernatant, which can be immediately analyzed by NULISA or stored at -80°C.

Figure 3. Sample preparation procedure for analyzing DBS/DPS collections using NULISA.

Results

Greater than 93% detectability of inflammatory proteins observed from blood collected using microsampling device

Using the Mitra Microsampling Device, 233 of 250 NULISAseq Inflammation Panel proteins (93.2%) were above the LoD in more than 50% of samples, and most targets achieved close to 100% detectability **(Figure 4A)**. Microsampling detectability was similar to paired plasma samples, which showed 99% detectability. Prominent targets were present at levels well above the background (i.e., NPQ – LoD >> 0), including NAMPT, IL18, GZMA, LCN2, and MIF **(Figure 4B)**.

The Inflammation Panel 250 encompasses a wide dynamic range of proteins, with low-abundance targets such as IL20, GDF2, SCG2, CXCL12, and KITLG detected in the majority of microsampled capillary blood samples. Therefore, multiplexed NULISAseq effectively captures immunoproteins found in microsampled whole blood in a manner comparable to conventionally collected venipuncture-based plasma samples.

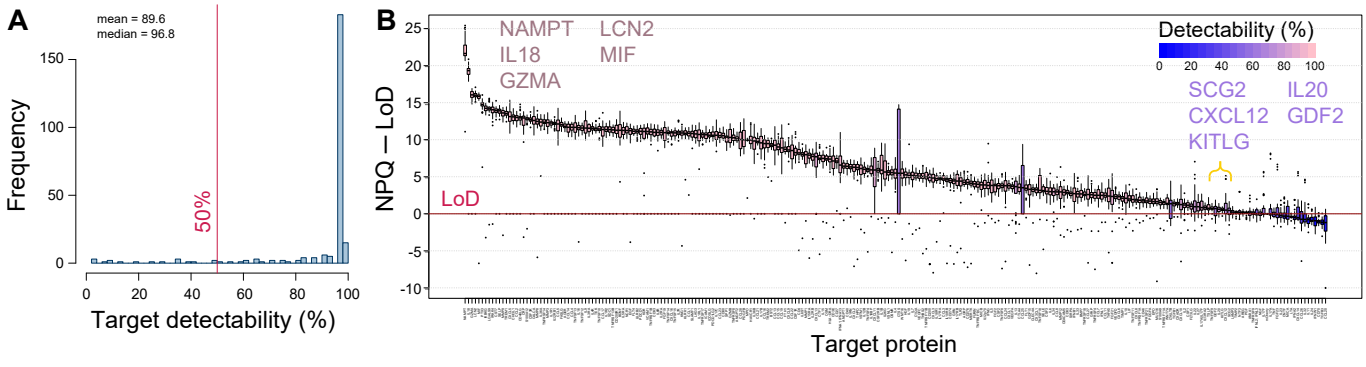


Figure 4. NULISAseq Inflammation Panel targets show good detectability in microsampled capillary whole blood. (A) Most protein targets are detectable in >50% of samples (red line). (B) Detectability plot for each protein in the panel; low and high detectability are shaded blue and pink, respectively. These data demonstrate the detection of a broad range of targets in microsampled whole blood including low-abundance targets labeled in blue.

High CNS protein target detectability observed in microsampled whole blood and plasma

Capitainer-B devices were tested with the NULISAseq CNS Disease Panel 120. Of the 120 targets, 102 (85%) were above the LoD in more than 50% of samples, approaching the 89% observed detectability in matched plasma samples (Figure 5A). Like the Inflammation Panel with Mitra Microsampling Devices, most CNS targets achieved 100% detectability. Important tau biomarkers, including microtubule-associated protein tau (MAPT) and the phosphorylated tau proteins pTau-231, pTau-181, and pTau-217, achieved 100% detectability and low variation among all 31 relatively healthy subjects **(Figure 5B)**.

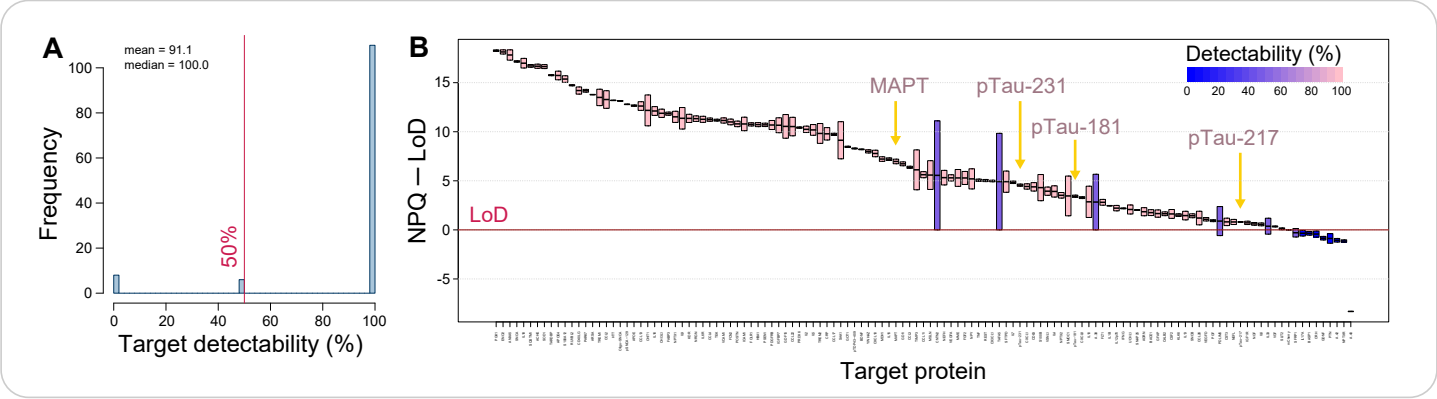


Figure 5. NULISAseq CNS Disease Panel targets show good detectability in microsampled capillary whole blood. (A) Most protein targets are detectable in >50% of samples (red line). (B) Detectability plot for each protein in the panel; low and high detectability are shaded blue and pink, respectively. Most targets show low variability among samples and are consistently above the limit of detection (LoD). Tau protein biomarkers are indicated with yellow arrows.

The TelImmune Duo microsampling system collects whole blood from a finger stick and separates a plasma-like component on the device. In these plasma-like samples, 108 of the 120 NULISAseq CNS Disease Panel targets (90%) were detected above the LoD in at least 50% of samples, approaching the 95% detectability observed in matching plasma samples **(Figure 6A)**.

Similar to the results of the Capitainer-B whole blood system, tau biomarkers MAPT and the phosphorylated tau analytes pTau-231 and pTau-181 achieved 100% detectability and low variation among the 31 subjects in the plasma-like microsamples **(Figure 6B)**. At the same time, pTau-217 was detected in most samples.

Plasma-like NULISAseq data were also collected from TASSO+ devices, which utilize a button-activated blood lancet to collect a specific amount of whole blood. The plasma component was subsequently extracted for analysis using standard methods. Overall, 117 out of 120 CNS Disease Panel analytes (97.5%) were detected above the LoD in at least 50% of samples **(Figure 6C)**.

The consequential biomarkers TREM1, TREM2, NfL, GFAP, and the three phosphorylated Tau variants were among the proteins achieving 100% detectability **(Figure 6D)**. Furthermore, target detectability with the TASSO+ devices showed excellent agreement with matched venous-collected plasma samples. In fact, pTau-217, IL1B, and CXL8 were among the CNS proteins that attained 100% detectability with TASSO+ but not in matched venous-collected plasma, while PTN and SNCB showed low detectability in both collection methods.

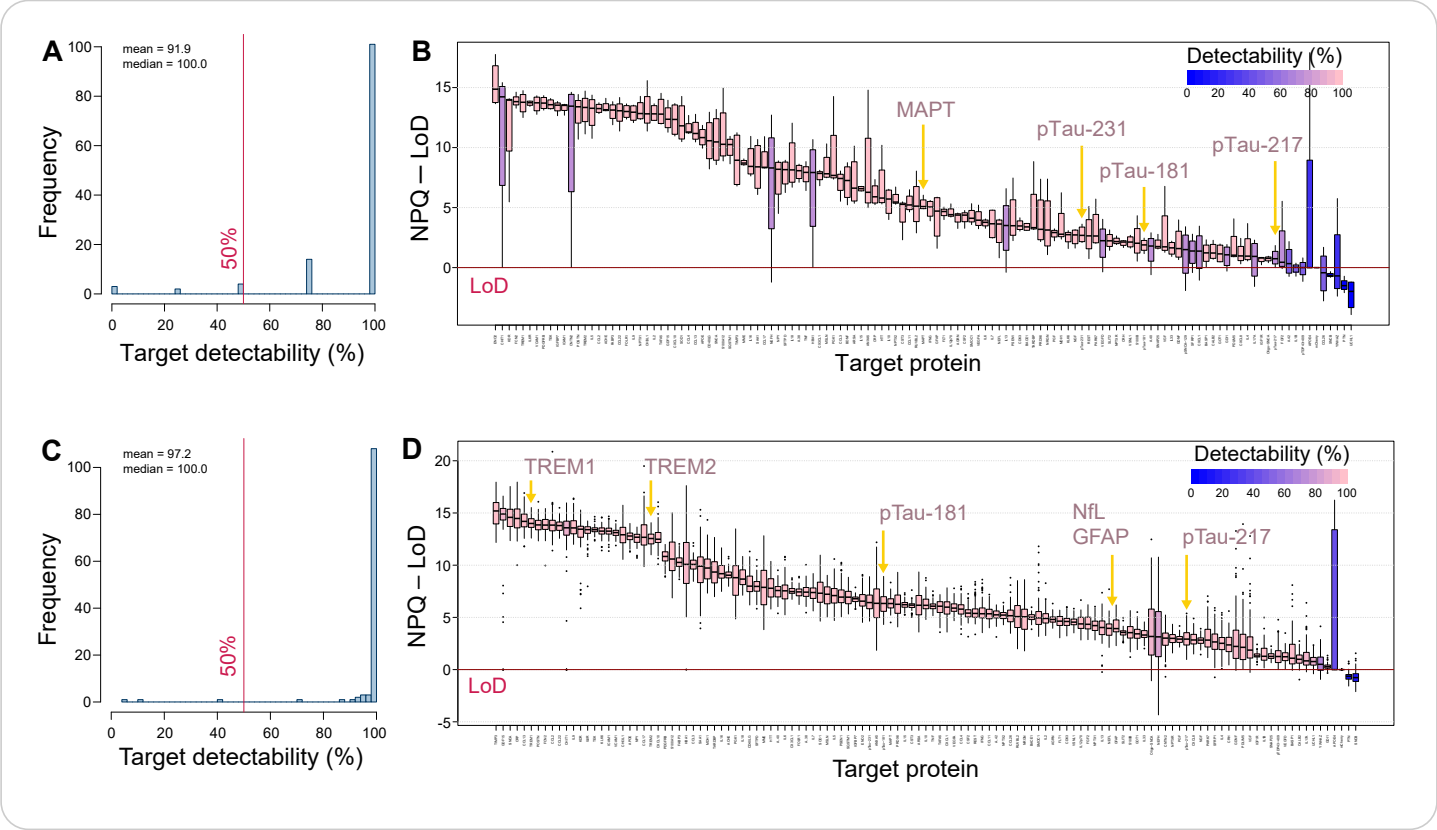


Figure 6. NULISAseq CNS Panel targets show good detectability in microsampled capillary plasma. Most protein targets are detectable in >50% of samples (red line) in both the (A) TelImmune Duo system and (C) TASSO+ device. Detectability plots for each protein in the panel for samples collected with (B) TelImmune Duo system and (D) TASSO+ device. Low and high detectability are shaded blue and pink, respectively. Most targets show low variability among samples and are consistent above the limit of detection (LoD). Tau protein and other consequential CNS protein biomarkers are indicated with yellow arrows.

Taken together, the NULISA platform can be easily adapted to both whole-blood and plasma-like microsampling systems for elucidating biomarkers from capillary blood **(Table 2)**. Overall, the data suggest that NULISA technology enables the adoption of microsampling devices in immunological and neurological biomarker studies that circumvent collection, storage, and transportation challenges associated with venipuncture-dependent protocols. Combining NULISA proteomics with capillary microsampling systems can contribute to cost reductions and diversification of populations under investigation.

Table 2. Advantages of capillary blood microsampling and NULISA proteomics.

Capillary blood microsampling	NULISA
• Minimally invasive	• Low-volume input, ~25 µl
• Small volumes and small footprint	• High-throughput automation
• Little or no professional training	• Industry-leading sensitivity
	• Multiplexing hundreds of relevant protein biomarkers

Tips and Best Practices

While capillary microsampling represents an alternative blood collection method for proteomics studies, these sample matrices are not analogous to venipuncture-based biofluids. The following points should be considered when designing a blood collection study:

- The biophysical properties of certain proteins (e.g., amyloid beta peptides) render them “sticky," complicating their extraction. Consequently, reliably measuring these proteins can be challenging, regardless of the proteomics technique employed (e.g., affinity or mass spectrometry). If specific markers are crucial to your study, optimization of the extraction buffers and conditions may need to be determined.
- Each microsampling device is unique. Some devices include additives for protein preservation, while others separate the plasma-like component from the cells. We recommend considering these differences when designing your study and selecting a single method for all collections instead of combining them.
- The similarity of each microsampling technique to classical methods (e.g., EDTA plasma-like separations) is still under investigation. Therefore, we recommend each study uniformly using either venipuncture or capillary collected samples and avoid combining collection methods into one dataset.
- The role that the timing between collection and analysis plays in the final results currently needs to be defined. For example, the impact of how long samples are kept at ambient temperatures during storage and shipping on each analyte and its specific stability is unknown. Therefore, we recommend minimizing variability in the time from sample collection to analysis as much as possible.
- Currently, we do not have any robust methodology for normalizing proteins collected via DBS on paper cards; therefore, results from these collection systems will be qualitative.

Conclusions

We have assessed the feasibility of employing NULISA proteomics technology in various capillary blood microsampling systems. Overall, the detectability of NULISAseq analytes in both the Inflammation Panel and CNS Disease Panel is comparable to matched plasma samples obtained from whole blood and plasma-like collection devices. These findings suggest the consistent and robust compatibility of NULISA with metered (i.e., when the volume of the collected sample is known) capillary blood microsampling systems and offer valuable biomarker information with high sensitivity and precision.

The low-volume characteristic of capillary blood microsampling inherently calls for the industry-leading proteomics sensitivity with multiplexing that NULISA provides. These capabilities can also be extended to other low-volume sampling applications, including pediatric cohorts and preclinical rodent studies. Future work on standardizing extraction and analytical protocols aims to establish these less invasive and logistically straightforward collection methods as viable alternatives to venipuncture-based collections in supporting diverse and longitudinal population biomarker research studies.

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