SPEAR UltraDetect™ Immunoassays Demonstrate Consistent Biomarker Quantification Across Diverse qPCR Platforms and Laboratories

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Introduction

Neurodegenerative diseases such as Alzheimer's disease (AD) require a highly sensitive and precise biomarker measurement tool for early detection and monitoring. While low-abundant blood-based biomarkers such as pTau 217 and NfL show great promise in disease diagnosis and monitoring, traditional ultrasensitive immunoassay platforms often rely on complicated workflow and proprietary systems that limit the robustness, precision, and scalability of the assays.

Successive Proximity Extension Amplification Reaction (SPEAR) is a homogeneous immunoassay technology with a unique two-factor authentication mechanism, enabling detection of proteins at single-digit copy numbers.

Using pTau 217 and NfL assays as examples, SPEAR UltraDetect™ demonstrates robust performance in measuring low-abundant biomarkers from 1 µL of diluted plasma sample across different qPCR platforms and formats, different operators, and different sites. These results highlight SPEAR technology as a scalable solution for ultra-sensitive protein biomarker measurements for neurology research.

Methods

SPEAR UltraDetect™ Semi-Automated Workflow Overview

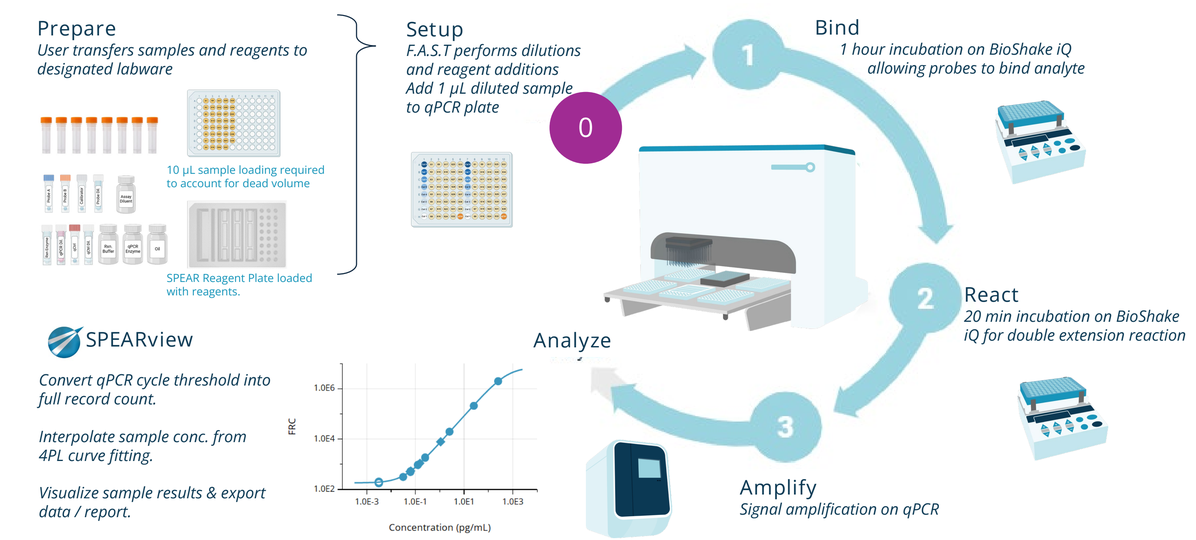

Fig. 1. Semi-Automated SPEAR UltraDetect™ Workflow for High-Throughput, Ultra-Sensitive Immunoassay Detection using Standard qPCR Platforms

The SPEAR UltraDetect™ semi-automated workflow integrates a Formulatrix® F.A.S.T.™ system, a BioShake iQ heater-shaker, and a real-time qPCR system. Users can process up to 39 samples (96-well format) and 174 samples (384-well format) per qPCR plate. After all reagents and samples are loaded to designated plates, F.A.S.T. loads detection probes, prepares and loads the calibration curve, dilutes samples with minimal required dilution (MRD) and loads to qPCR plate. After 1 hour incubation, F.A.S.T. stamps the reaction reagents to qPCR plate and another 20 minute incubation is done on the BioShake iQ for double extension reaction and heat inactivation. At the end, F.A.S.T. adds qPCR mix to the qPCR plate and qPCR amplification is done on qPCR machines. Users then use SPEARview software to process the data and obtain the interpolated concentration of the samples.

QuantStudio™ 12K Flex | QuantStudio™ 7 Pro | QuantStudio™ 5 | QuantStudio™ 3 | Roche LightCycler® Pro | Bio-Rad Opus

Fig. 2. Six qPCR systems were used in this study: QuantStudio™ 12K Flex Real-Time PCR system (96- and 384-well), QuantStudio™ 7 Pro Real-Time PCR system (96- and 384-well), QuantStudio™ 5 Real-Time PCR system (96- and 384-well), QuantStudio™ 3 Real-Time PCR system (96-well), Roche LightCycler® Pro (96- and 384-well), Bio-Rad Opus Real-Time qPCR system (96- and 384-well).

Cross-qPCR Platform Results

Sample reading consistency for 96- and 384- well formats

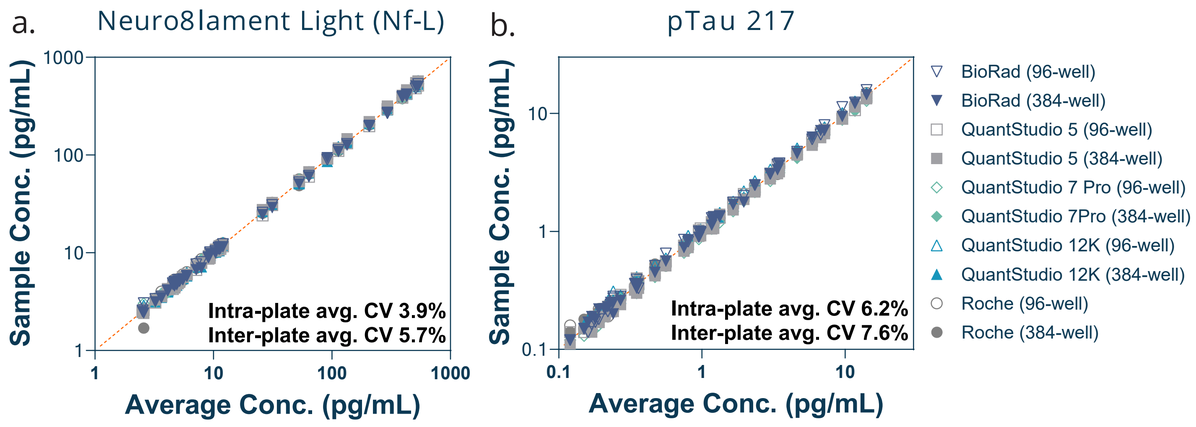

Fig. 3. SPEAR UltraDetect™ (a) Nf-L and (b) pTau 217 assays were performed on five different qPCR models using both 96- and 384-well formats. Average sample concentrations from each format and qPCR model were plotted against the overall mean concentrations across all measurements to assess consistency and platform compatibility. Total 39 plasma samples are included in each run.

Sample readings from both 96- and 384-well plate formats demonstrate strong concordance across 10 different qPCR instruments and formats for the SPEAR UltraDetect™ Nf-L and pTau 217 assays, underscoring the robustness and platform compatibility of the assays. Across all measurements with 1µl diluted plasma sample, the intra-plate coefficient of variation (CV) averaged 3.9% for NfL and 6.2% for pTau 217; inter-plate CVs were 5.7% and 7.6%, respectively, indicating high assay precision and reproducibility.

Acknowledgement: Spear Bio would like to thank our beta-site partners for participation in this study.

Sample Intra-Plate Measurement Precision on Different qPCR Platforms

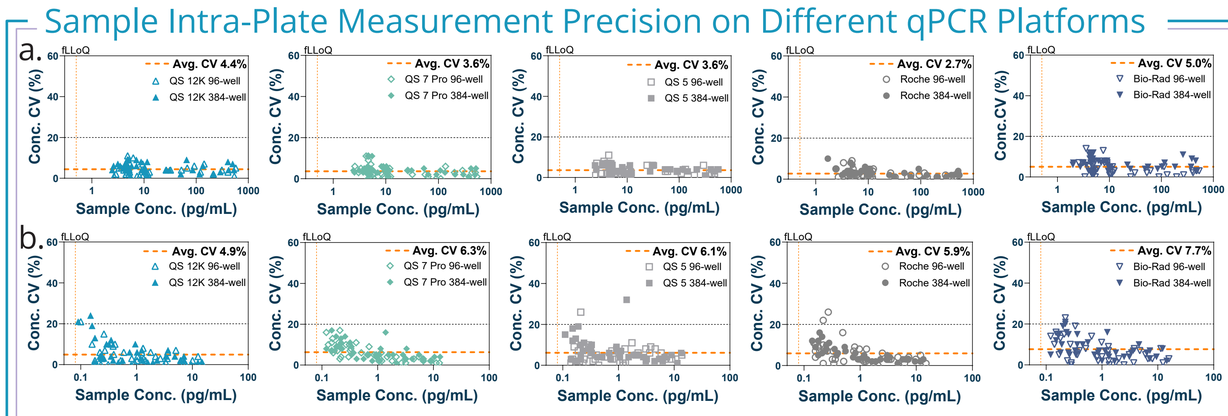

Fig. 4. Intra-plate precision of SPEAR UltraDetect™ (a) Nf-L and (b) pTau 217 assays across qPCR platforms

The SPEAR UltraDetect™ Nf-L assay demonstrated average intra-plate CVs ranging from 2.7% to 5.0% with 1 µl diluted plasma sample, while the pTau 217 assay showed average CVs between 4.9% and 7.7% with 1 µl diluted plasma sample across all tested qPCR models. Slightly elevated CVs were observed on the Bio-Rad qPCR platform for both assays; the results show high precision and reproducibility across a wide range of qPCR instruments.

Sample Cross-Format (96-well and 384-well) Measurement Consistency

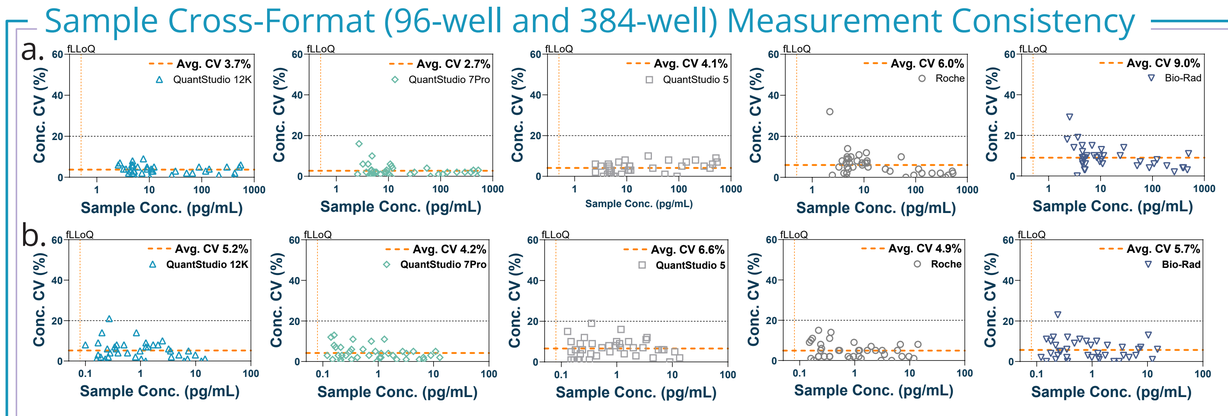

Fig. 5. Inter-plate precision of SPEAR UltraDetect™ (a) Nf-L and (b) pTau 217 assays across qPCR platforms

The SPEAR UltraDetect™ Nf-L assay demonstrated average inter-plate CVs ranging from 2.7% to 9.0% with 1 µl diluted plasma sample when run between 96-well and 384-well formats, while the pTau 217 assay showed average inter-plate CVs between 4.2% and 6.6%.

Cross-Site pTau 217 Results

Sample reading consistency across platforms and sites

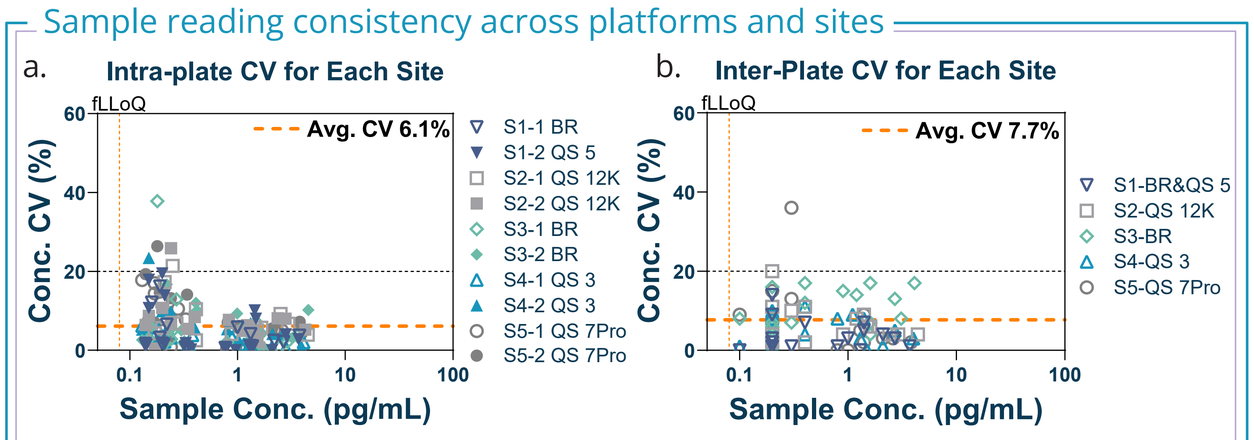

Fig. 6. SPEAR UltraDetect™ pTau 217 assay sample measurement (a) intra-plate and (b) inter-plate performance across five different laboratories and qPCR models. Data represents replicate measurement from two independent runs with 16 plasma samples each performed at each site.

In a cross-site study of the SPEAR UltraDetect™ pTau 217 assay, sixteen samples were tested using the 96-well qPCR plate format across five laboratories worldwide, utilizing five different qPCR platforms. The results demonstrated an average intra-plate CV of 6.1% and average inter-plate CV of 7.7%, confirming high consistency and reproducibility across sites, instruments, and runs.

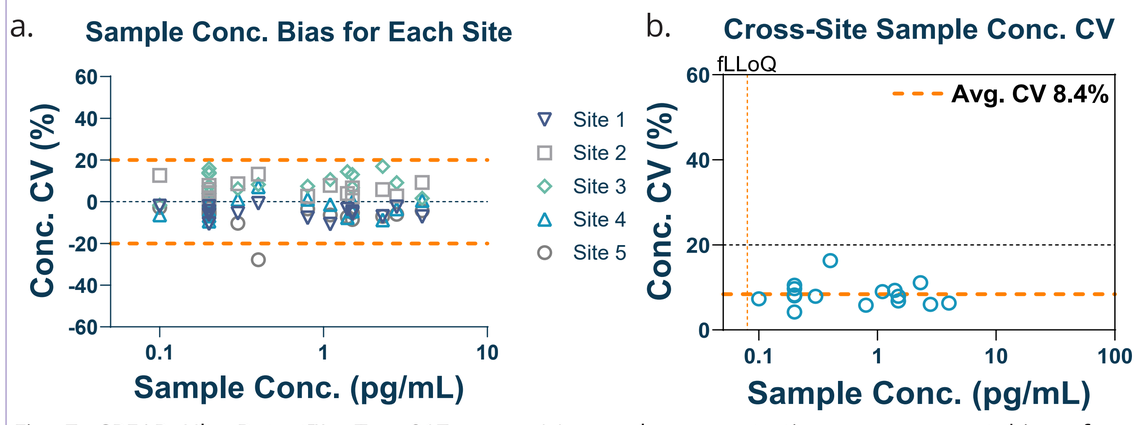

Fig. 7. SPEAR UltraDetect™ pTau 217 assay (a) sample concentration measurement bias of each site compared to average concentration of all the runs, and (b) sample measurement CV performance across five different laboratories and five qPCR models. Total 10 96-well runs are included.

The majority of sample measurements show less than 20% bias compared to the global average, with one exception. The SPEAR UltraDetect™ pTau 217 assay demonstrated an average sample concentration CV of ~8.4% across ten runs in the cross-site study. These cross-site and cross-platform results highlight the scalability of SPEAR as an ultra-sensitive immunoassay platform, delivering consistent and reliable performance using only 1 µL of diluted sample, across different sites and qPCR platforms.

Conclusions

The SPEAR UltraDetect™ assay platform offers a robust and scalable solution for ultra-sensitive biomarker detection. It demonstrates consistent performance across qPCR models, plate formats, and global sites, highlighting its adaptability and precision. By leveraging widely available qPCR instruments, the assay enables streamlined implementation without specialized hardware, supporting high-throughput, reproducible testing in diverse settings.

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