

Validation Of Highly Specific And Automated Immunoassays For Endogenously Cleaved MTBR-tau243 In CSF: A Comparison With Mass Spectrometry



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Background

- Tau targeting therapies for Alzheimer’s disease (AD) have gained increasing attention in recent years, creating a growing need for reliable and pathology specific tau biomarkers to support their development.
- Among candidate biomarkers, endogenously cleaved MTBR-tau243 (eMTBR-tau243)—specifically two characteristic fragments: the form cleaved at Val256 (V256, Fig.1A) and its deamidated form at Asn255 (V256-dN, Fig.1B) – is increasingly utilized as a promising biomarker that specifically reflects brain tau pathology (Horie *et al.*, *Nat Med*, 2025).
- Previously, our developed antibodies demonstrated high cleavage-site specificity against synthetic peptides (Fig. 2).
- Subsequently, we reported the development of fully automated immunoassays for these fragments on the HISCL™-5000 platform (Fig. 3).
- However, it is necessary to validate whether these assays maintain high specificity for endogenous fragments in actual patient samples, given the presence of structurally similar analogs in complex biological matrices.
- In this study, we validated the analytical specificity of our developed automated immunoassays in cerebrospinal fluid (CSF) by assessing their correlation with a newly established in-house eMTBR-tau243 IP-MS reference method.

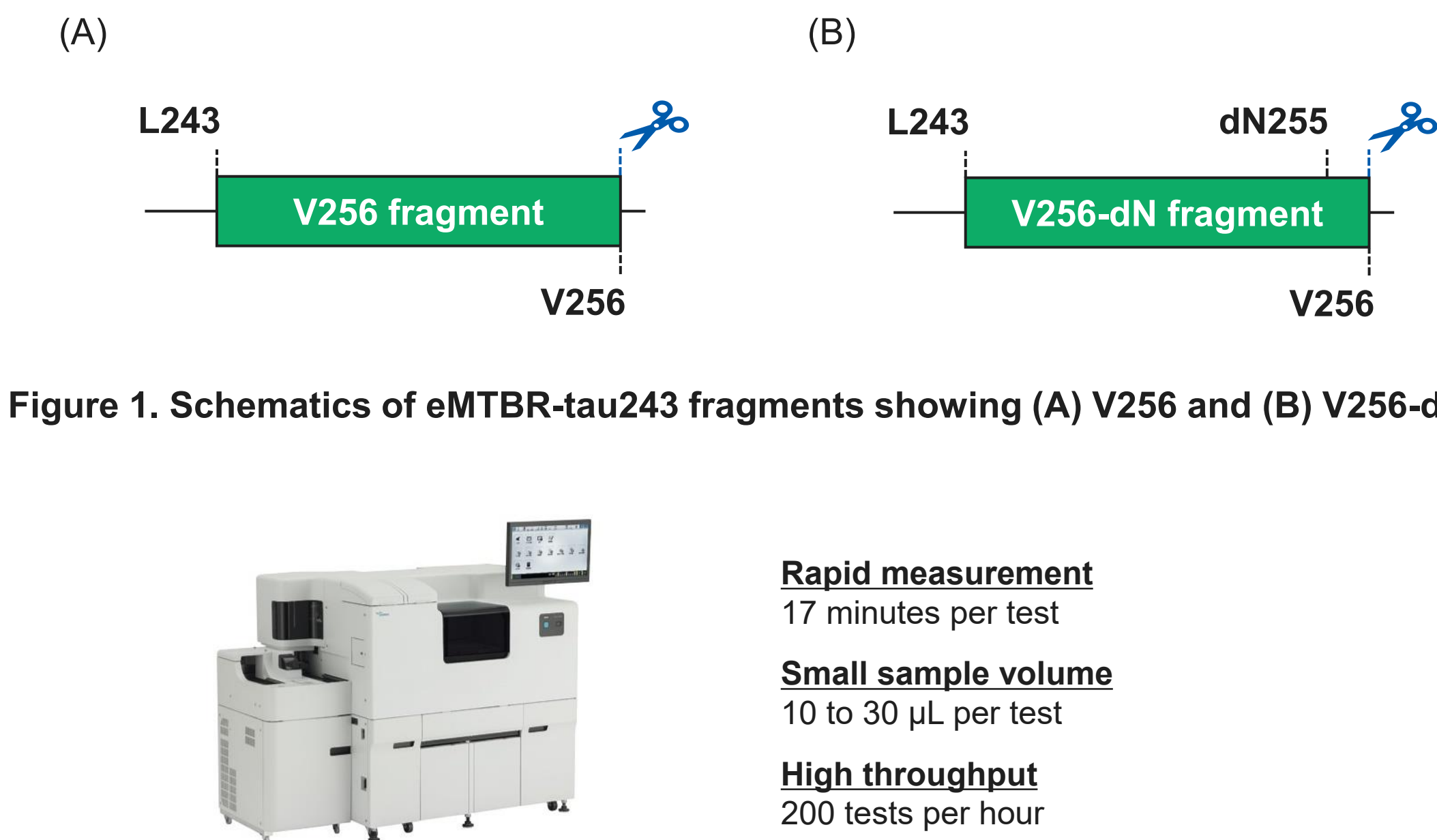


Figure 1. Schematics of eMTBR-tau243 fragments showing (A) V256 and (B) V256-dN.

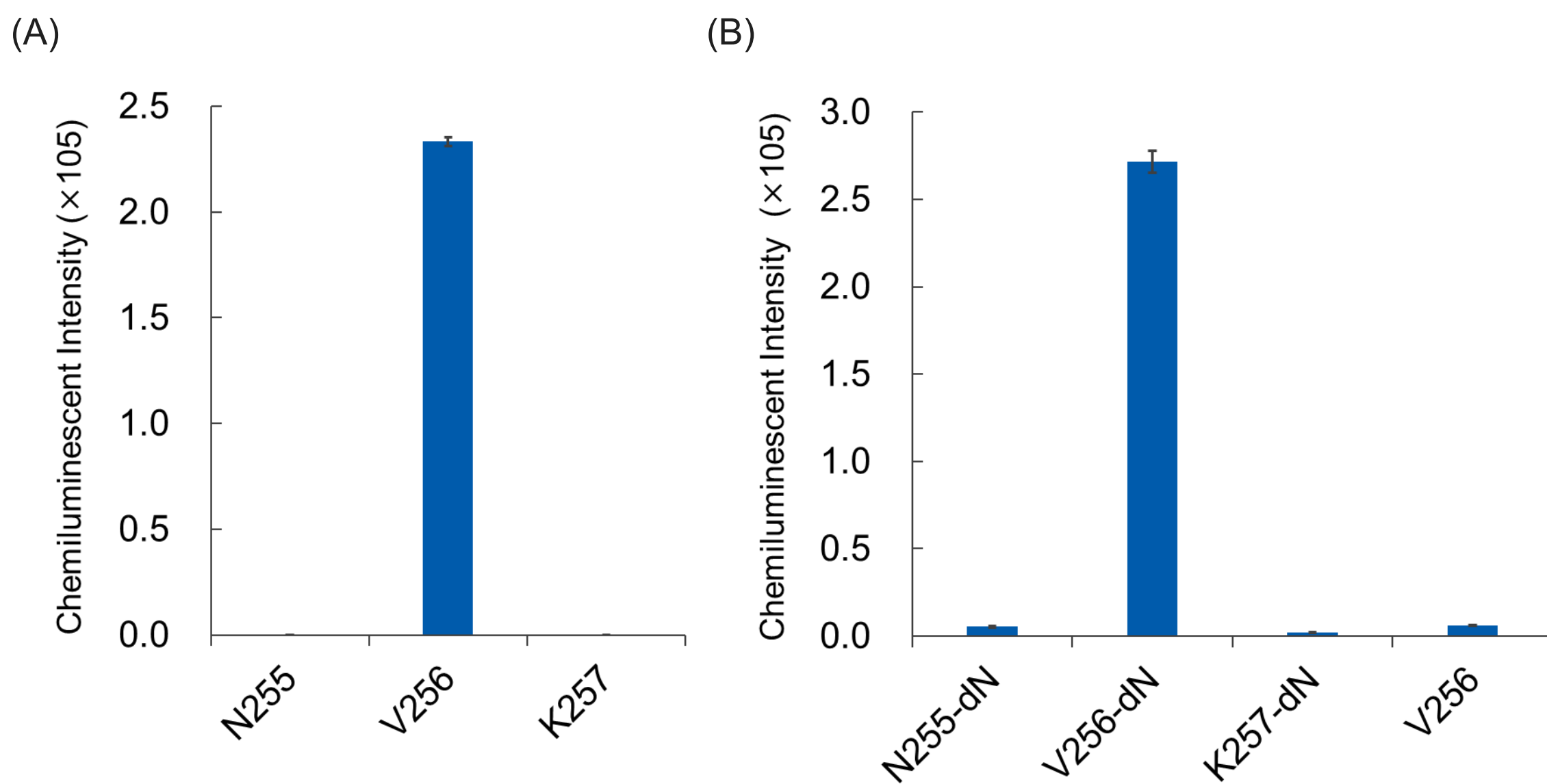


Figure 2. Antibody specificities evaluated by ELISA using synthetic peptides. Reactivity against (A) V256 and (B) V256-dN. Mean ± SD (n = 3).

Methods

- **Assay Development:**
 - IP-MS: An in-house reference method was established based on previous reports (Horie *et al.*, 2023) for precise quantification (Fig. 4A).
 - HISCL: Assays for V256 and V256-dN were developed on the Automated Immunoassay System HISCL™-5000 (Sysmex Corporation) (Fig. 4B).
- **Analytical Validation:**
 - IP-MS: A pilot study was conducted using CSF samples from clinically diagnosed AD dementia (ADD) subjects. Peak characteristics were verified by evaluating chromatogram.
 - HISCL: Analytical performance was characterized by evaluating calibration curve characteristics (dynamic range), within-run imprecision, dilution linearity, and spike recovery.
- **Specificity Validation:**
 - Levels of V256 and V256-dN fragments in commercially available CSF samples (MCI or ADD, n=20) were measured by both in-house IP-MS and HISCL assays. Both measurement values were evaluated using Spearman’s rank correlation (r_s).

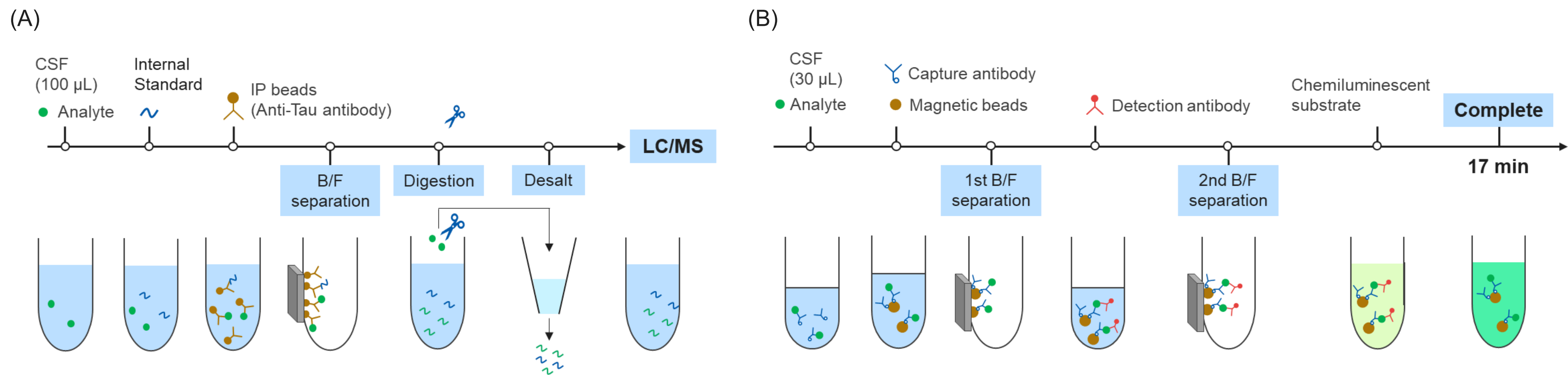


Figure 4. Comparison of assay workflows: (A) IP-MS reference method and (B) fully automated HISCL immunoassay.

Analytical Validation (IP-MS)

- The reference IP-MS method successfully detected distinct and sharp peak profiles for the endogenous fragments and their corresponding internal in the ADD CSF (Fig. 5).

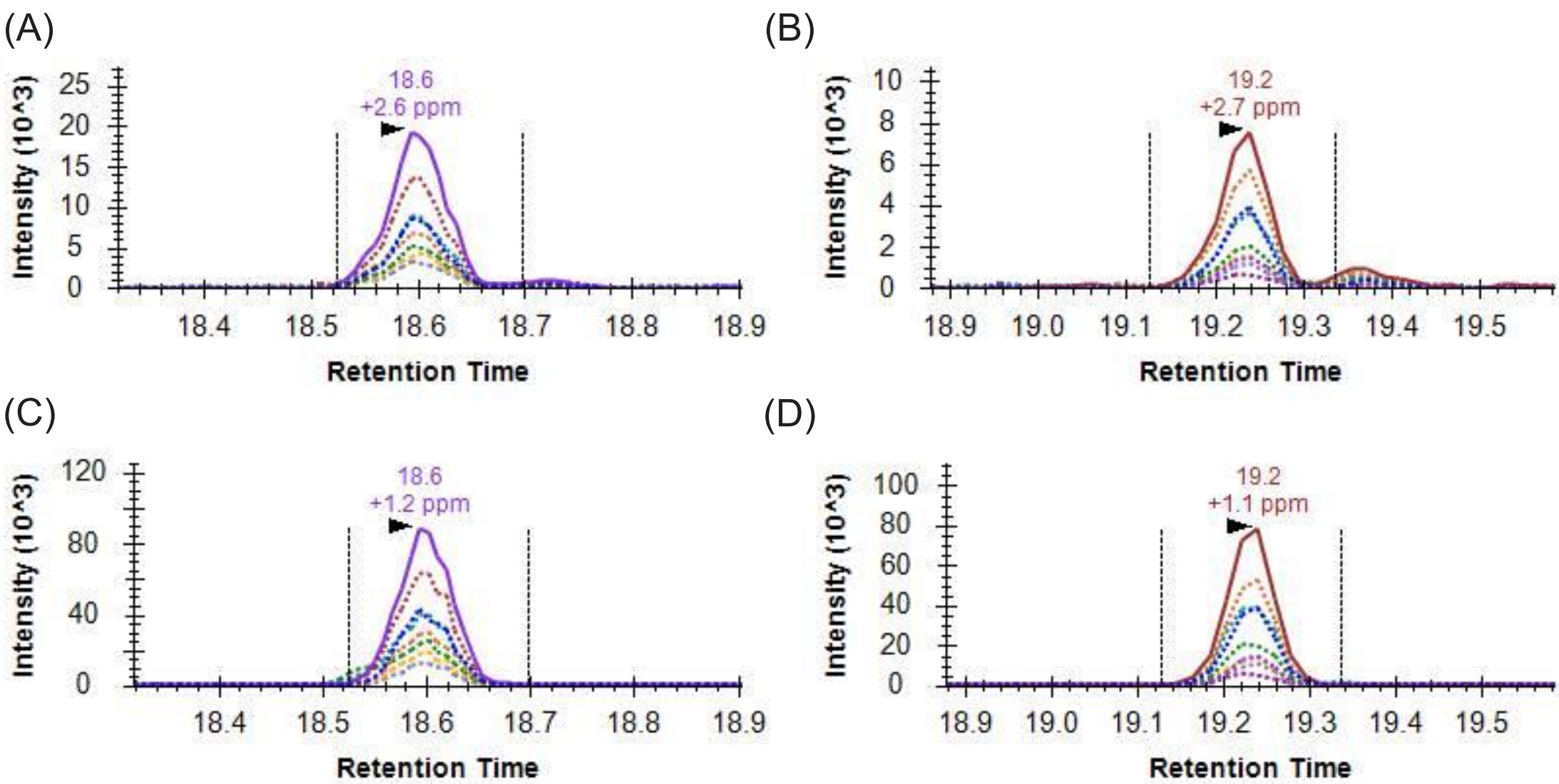


Figure 5. Extracted ion chromatograms of eMTBR-tau243 fragments. Profiles in ADD CSF are shown for endogenous (A) V256 and (B) V256-dN, alongside their respective internal standards (C, D). Colored lines indicate individual product ion transitions; solid lines denote transitions used for quantification.

Analytical Validation (HISCL)

- The developed HISCL immunoassays exhibited broad measurement ranges based on robust calibration curves (V256: 0.023 to 40.00 pg/mL; V256-dN: 0.016 to 10.00 pg/mL) (Table 1).
- Furthermore, they demonstrated high repeatability, excellent dilution linearity, and reliable spike recovery, confirming robust analytical performance in the complex CSF matrices.

Table 1. Summary of analytical parameters

	V256	V256-dN
Dynamic range (pg/mL)	0.023 – 40.00	0.016 – 10.00
Within-run imprecision (%)	≤ 5.6	≤ 1.7
Dilution linearity (%)	90 – 100	100 – 108
Spike recovery (%)	88 – 95	89 – 90

Specificity Validation

- Measurement values from HISCL showed high correlation with the reference IP-MS method for both V256 ($r_s = 0.98$, $p < 0.001$) and V256-dN ($r_s = 0.95$, $p < 0.001$) (Fig. 6).
- This exceptional concordance indicates that the automated immunoassays achieve high specificity comparable to IP-MS.

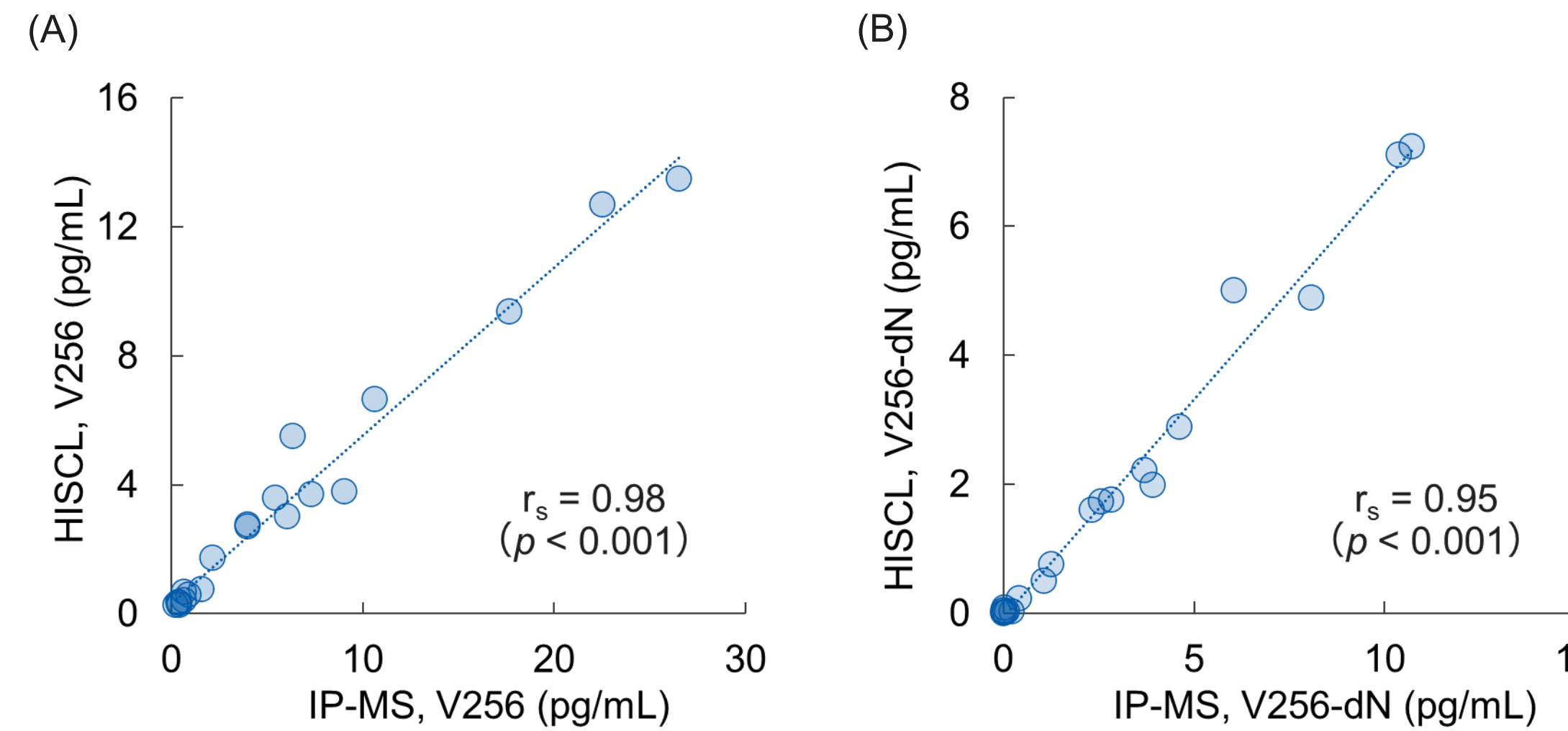


Figure 6. Correlation between HISCL immunoassays and IP-MS measurements for (A) V256 and (B) V256-dN in CSF samples.

Conclusion

- Our fully automated immunoassays exhibited high concordance with the in-house IP-MS method, demonstrating mass spectrometry-level analytical specificity for the detection of endogenous eMTBR-tau243 fragments in the complex CSF matrix.
- Given that IP-MS tau assays have established clinical relevance and predictive utility, our high-throughput and practical HISCL platform—achieving comparable specificity—may enable a more scalable clinical workflow.
- This could support broader implementation, including efficient participant identification, patient stratification, and longitudinal monitoring in tau-targeting therapies.