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International Multi-Center Training and Validation of CSF Alzheimer's Biomarker Assays

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1 Abstract

Objectives: To maximize the power of longitudinal studies, Alzheimer's biomarker assays must exhibit strong, consistent analytical performance with minimal variability from analytical factors. We conducted a multi-center study to assess intra- and inter-center variability of results from validated MSD® Human Aβ42 and Human Total Tau Kits, and we implemented a training program to minimize variability due to inconsistent execution of the assay protocol. We will present the multi-center study results and provide a review of the training program.

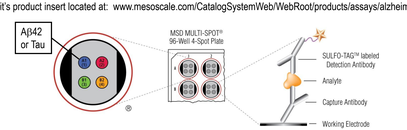
Methods: Forty-eight operators at 19 sites on 3 continents were trained by expert scientists prior to conducting the validation experiments. Eight of the sites participated in the validation, which included generating standard curves and testing quality controls and 12 blinded cerebrospinal fluid (CSF) sample pools in at least 4 discrete runs per assay over at least 3 days. Matrix tolerance was evaluated by dilution linearity using 10 CSF sample pools diluted 2- to 32-fold in 2 discrete runs per assay over 2 days. All results were evaluated against pre-defined acceptance criteria.

Results: The Aβ42 and Total Tau assays produced standard curves within 20% of expected signals across all testing sites. The average intra-run CVs were <5% for quality controls at most sites and <10% for samples. Testing blinded technical replicates suggested that pre-analytical and sample handling issues may contribute to increased inter-run variability of Aβ42 in CSF samples compared to QC samples. Total Tau inter-run CVs were <15% for most sites, but systematic differences between sites were identified, whereas the differences between sites for Aβ42 were random.

Conclusions: These multi-center testing and training programs have proven valuable for simultaneously assessing and maximizing the robustness of the validated Aβ42 and Total Tau assays. The assays consistently quantified CSF samples across testing sites and are suitable for longitudinal studies. The multi-site study identified sample handling as a critical factor for the Aβ42 assay but not for Total Tau. These programs should be extended to other validated assays to demonstrate utility prior to release into the community.

2 Methods: MSD Assays

- MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels, which emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.
- The Human Aβ42 and Human Total Tau Kits have been internally validated by MSD. Data from the multi-lot validation can be found in each kit's product insert located at: www.mesoscale.com/CatalogSystemWeb/WebRoot/products/assays/alzheimers.aspx.



Assay Protocol

1. Add 150 µL/well of Diluent 35. Incubate 1 hour at room temperature (RT).
2. Wash with PBS-T. Add 50 µL/well of calibrator or diluted sample. Incubate 1 hour at RT.
3. Wash with PBS-T. Add 25 µL/well of detection antibody. Incubate 1 hour at RT.
4. Wash with PBS-T. Add 150 µL/well of Read Buffer T.* Read on MSD instrument.
   *For the Human Total Tau Kit, MSD recommends an 8–10 min incubation after adding Read Buffer T.

3 Methods: Assay Performance Training and Validation

Assay Performance Training

Goal: To assess the effectiveness of operator training in reducing site-to-site variation of assay results.

1. An MSD scientist reviews best laboratory practices, MSD product inserts, and assay performance acceptance criteria (see below) at each site.
2. Individual sites generate standard curves, measure controls, and assess dilution linearity for controls.
3. If performance acceptance criteria are met, a training certificate is awarded to operator(s).

Performance Acceptance Criteria

- ✓ **Standard Curve (STD001-005):** Signals within 3-fold of certificate of analysis value, CV of signals <20%, recovery between 80% and 120%, and concentration CV <20%.
- ✓ **Controls:** Mean concentration of each control must be between 75% and 125% of the expected concentration. The concentration CV must be <20%.
- ✓ **Dilution of the Controls:** Must be linear when the signals are within the linear range of the assay.

Validation

Goal: To evaluate the analytical performance of MSD's Human Aβ42 and Human Total Tau Kits across multiple sites.

After completion of assay performance training, trained operators at 8 sites participated in validation experiments that used quality controls (QC) and a common set of 10–12 blinded human CSF samples to generate calibration curves and dilution linearity data for multiple plates. MSD provided testing materials and supporting documentation to each center. Data analysis was performed at MSD and final results were shared with all participating centers.

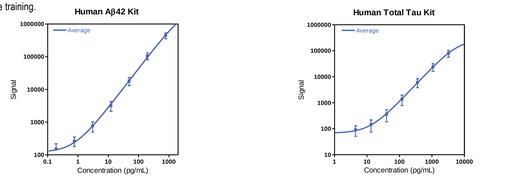
4 Assay Performance Training: Participants

- BioAgilytix Labs
- Bristol-Myers Squibb†
- Covance-Greenfield
- Edith Cowan University†
- Frontage Laboratories
- Hospices Civils de Lyon
- ICON Labs†
- KC Analytical Services
- Medtox
- Meso Scale Discovery†
- MPI Research
- PharmOptima
- Provista Diagnostics
- QPS Delaware†
- Tandem Labs (LabCorp) †
- University College London†
- University of Erlangen
- University of Gothenburg†
- WuXi AppTec

†*Validation participant*
NOTE: Sites are listed in alphabetical order.

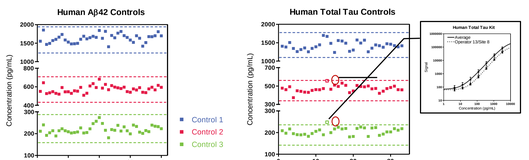
5 Assay Performance Training: Standard Curves

Average standard curve signals were plotted for operators who met performance acceptance criteria for the Human Aβ42 and Human Total Tau Kits. Average signals from the calibrators were used to generate the standard curve using a 4-parameter logistic model with $1/y^2$ weighting. Error bars=1 standard deviation (SD). A total of 40 operators (19 sites) for Aβ42 and 33 operators (18 sites) for Total Tau successfully completed assay performance training.



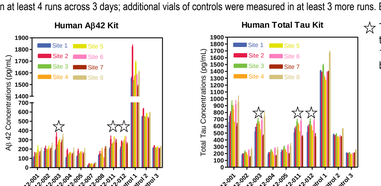
6 Assay Performance Training: Multi-Operator Control Tracking

Control tracking data is shown for all operators that met performance acceptance criteria, representing at least 165 replicates for each control. The horizontal dashed bars indicate ±25% recovery of each control. The inter-operator CVs for controls 1–3, respectively, were: Aβ42=7.1%, 6.6%, and 8.7%; Total Tau=7.8%, 6.8%, and 7.2%. Seven out of 45 operators for Aβ42 and 10 out of 40 operators for Total Tau did not initially meet specifications for the assay performance training. Of the operators that did not meet specifications, 2 operators for Aβ42 and 3 operators for Total Tau elected to re-train and successfully completed assay performance training. Open data points (circled) from operator 13 (site 8 in the validation study) indicate that several controls were out of specification for Total Tau. Upon review of the training records, we noted that the nonconformity was related to a pipette issue that led to a lower standard curve for the Total Tau assay (inset). This example demonstrates the efficacy of the training program in identifying assay protocol issues and implementing corrective actions.



7 Validation: Intra-Site Sample Levels

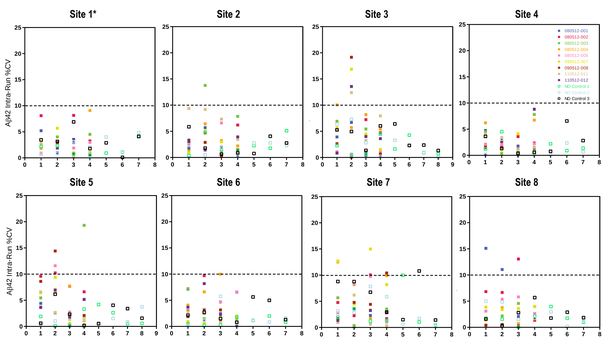
Data is shown below for the sites that participated in the multi-site validation study. Twelve blinded CSF samples were prepared from pooled human CSF. Three samples were below the lower limit of quantitation (LLOQ) for Aβ42, and 5 samples were below the LLOQ for tau. These samples were omitted from the analysis. Controls were prepared in diluent that mimicked human CSF. Individual vials of CSF and controls were measured in at least 4 runs across 3 days; additional vials of controls were measured in at least 3 more runs. Error bars=1SD.



☆ Technical replicates prepared from the same CSF pool; samples 110512-011 and 110512-012 were built several days after 080512-003.

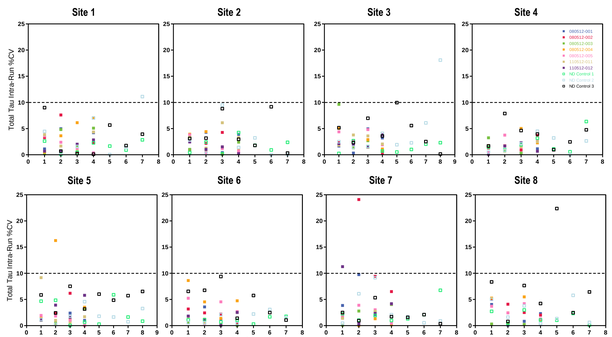
8 Validation: Intra-Run CV for the Human Aβ42 Kit

Each site collected data for 4 runs of CSF samples (solid squares) and at least 7 runs of QC samples (open squares). For Aβ42, the intra-run concentration CVs were typically <10% for both the CSF samples and controls.
*NOTE: Data for the following samples are not shown on the graph for Site 1. Run 1: 110512-012, CV=39.2%; 090512-007 and 090512-008 were excluded due to an analytical error; Run 2: 080512-002, CV=39.7%.



9 Validation: Intra-Run CV for the Human Total Tau Kit

Each site collected data for 4 runs of CSF (solid squares) and at least 7 runs of QC samples (open squares). For Total Tau, the intra-run concentration CV was typically <10% for both the CSF samples and controls.



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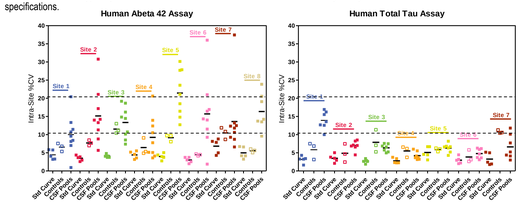
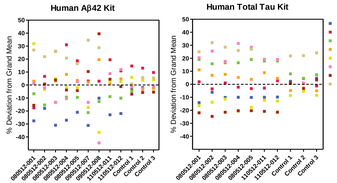
10 Validation: Comparison of Intra-Site Variation

The intra-site sample CV values are plotted below for calibrators 1–5, controls, and CSF samples. Intra-site CVs for calibrators and controls were generally <5% and <10%, respectively. For Aβ42, the intra-site variation for CSF samples was generally <20%. Some samples showed higher variability than others. A marked difference was observed for the Human Total Tau assay, as the intra-site variation for the CSF samples was typically <10%. Site 8 was excluded from the Total Tau assay analysis due to several controls that did not meet specifications.



11 Validation: Comparison of Site-to-Site Variations

For each site, we assessed the degree of bias from the overall mean concentration for each biomarker. The grand mean biomarker concentration was calculated for each sample using data from all testing sites. For both assays, the overall bias for the controls was much lower than observed for the CSF samples, indicating that CSF handling is a potential source of variability between sites. The Total Tau assay demonstrates consistent patterning of site bias, whereas the Aβ42 assay exhibits a more random pattern.

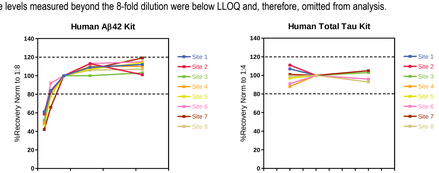


Human Aβ42 Assay	
Sample ID	Inter-Site %CV
080512-001	27.7%
080512-002	17.1%
080512-003	21.0%
080512-004	22.2%
080512-005	17.8%
090512-006	Below LLOQ
090512-007	23.3%
090512-008	33.8%
090512-009	Below LLOQ
090512-010	Below LLOQ
110512-011	18.7%
110512-012	19.2%
Control 1	8.8%
Control 2	8.6%
Control 3	8.9%
Inter-technical replicates	19.6%

Human Total Tau Assay	
Sample ID	Inter-Site %CV
080512-001	17.8%
080512-002	17.6%
080512-003	15.4%
080512-004	19.2%
080512-005	18.9%
090512-006	Below LLOQ
090512-007	Below LLOQ
090512-008	Below LLOQ
090512-009	Below LLOQ
090512-010	Below LLOQ
110512-011	15.8%
110512-012	16.4%
Control 1	6.6%
Control 2	6.7%
Control 3	7.7%
Inter-technical replicates	15.7%

12 Validation: Dilution Linearity

Each site assessed dilution linearity for 10 CSF sample pools for each assay. Each sample was diluted 2-fold, 4-fold, 8-fold, 16-fold, and 32-fold. The percent recovery at each dilution was normalized to the recommended dilution for the respective assay. Representative results are shown below for CSF sample 081512-002. All sites demonstrated comparable dilution linearity profiles for both assays. For the Total Tau assay, analyte levels measured beyond the 8-fold dilution were below LLOQ and, therefore, omitted from analysis.



13 Conclusion

On-site operator training and use of control tracking proved a powerful tool to identify and minimize variability attributed to the assay protocol and, in some cases, identified operators for retraining. Compared to the Total Tau assay, the Aβ42 assay was more sensitive to intra-site variation for CSF samples. Intra-run CV values were <10% for the CSF samples and <5% for QC samples for both assays, indicating that the observed intra-site variability is not likely due to issues with running the assay. Testing blinded technical replicates prepared in discrete builds revealed differences in Aβ42 levels suggesting that pre-analytical factors and sample handling could account for the increased intra- and inter-site variability. Additional studies have been initiated to identify and evaluate pre-analytical factors that may contribute to the site-to-site variations observed for the Aβ42 assay.

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