A Multi-Site Validation of the MESO SCALE DISCOVERY® Human Aβ42 and Human Total Tau Kits for Use in Human CSF

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1 Abstract

Objective: To conduct an external validation of MSD's Human Aβ42 and Human Total Tau Kits by independent testing sites, including academic institutions, biopharmaceutical institutions, and CROs.

Background: The MSD® Human Aβ42 and Human Total Tau Kits have been validated internally for the detection of their respective analytes in human cerebrospinal fluid (CSF) using fit-for-purpose criteria. Here we extend the validation of both kits to 8 independent testing sites to assess their functional performance and intra- and inter-center variability.

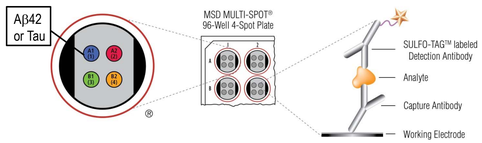
Design/Methods: All testing sites were trained by an MSD field application scientist (FAS) prior to conducting the validation experiments. Each site tested standard curves, quality controls (QC), and 10–12 blinded CSF samples in 4 discrete runs per assay over 3 days. Matrix tolerance was evaluated by dilution linearity using 10 CSF sample pools diluted 2- to 32-fold in 2 discrete runs per assay over 2 days. All samples were evaluated against pre-defined acceptance criteria such that if the %CV of any of the calibrator points exceeded 20%, that value was excluded from curve fitting.

Results: The Aβ42 and 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 sampling handling issues may contribute to the increased inter-site variability observed for CSF samples compared to QC samples.

Conclusions: Biomarker levels measured in CSF samples correlate across all testing sites with inter-site %CV values ranging from 17.1 to 33.8% for Aβ42 and 15.4 to 19.2% for tau. We report that certain CSF sample pools exhibit increased inter-site variability; ongoing studies are underway to determine the cause of sample-specific variability.

2 Methods

- MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that 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.



Protocol

1. Add 150 µL of Diluent 35. Incubate for 1 hour at RT.
2. Wash with PBS-T. Add 50 µL of calibrator or diluted sample. Incubate for 1 hour at RT.
3. Wash with PBS-T. Add 25 µL of detection antibody. Incubate for 1 hour at RT.
4. Wash with PBS-T. Add 150 µL of Read Buffer T. Read on SECTOR® Imager.
   Note: The Human Total Tau Kit recommends an 8–10 minute incubation after adding Read Buffer T.

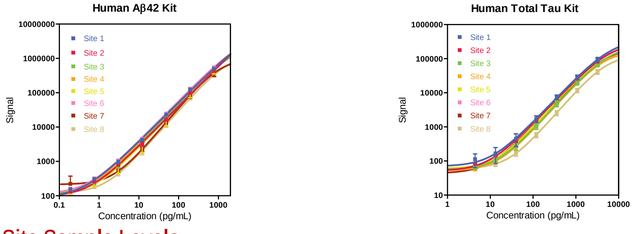
List of Participating Testing Sites*

- Meso Scale Discovery
- Bristol-Myers Squibb
- University of Gothenburg
- QPS Delaware
- University College London
- Tandem Labs (LabCorp)
- ICON Labs
- Edith Cowan University

*Sites are listed in no particular order

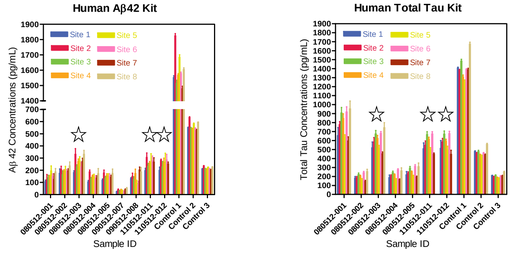
3 Standard Curves

Average standard curve signals for each of the independent testing sites are plotted below for the Human Aβ42 and Human Total Tau Kits. Signals from the calibrators were used to generate the standard curve using a 4-parameter logistic model with $1/y^2$ weighting. For calibrators within the quantitative range of the assay, the average concentration %CV for testing sites 1–8, respectively, was: Aβ42 = 4.4, 3.5, 4.1, 4.4, 4.0, 3.0, 6.8, 5.0%; tau = 3.2, 3.5, 2.7, 2.8, 5.1, 3.0, 3.3, 3.7%. Error bars = 1SD.



4 Intra-Site Sample Levels

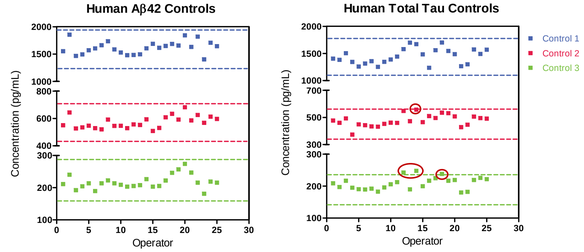
Twelve CSF samples were prepared from pooled human CSF. Test sites received blinded samples. Three samples were below the lower limit of quantitation (LLOQ) for Aβ42, and 5 samples were below LLOQ for tau. QC samples were prepared in diluent that mimicked human CSF. Samples with analyte levels below the limit of quantification (BLQ) were omitted from the analysis. Individual vials of CSF and QC samples were measured in at least 4 runs across 3 days; additional vials of QC samples 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.

5 Multi-Operator Training

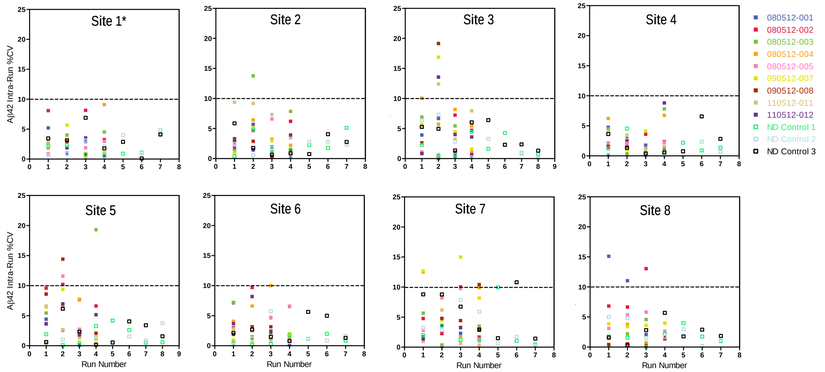
In addition to the 8 sites that participated in the multi-site sample testing, a total of 15 sites and 25 operators were trained on each assay. Data is shown for all 15 sites (25 operators), representing a total of 146 replicates for each control. The horizontal dashed bars indicate ± 25% recovery of each control. The inter-operator %CVs for QC samples 1–3, respectively, were: Aβ42 = 8.7, 8.8, and 9.6%; tau = 7.3, 7.1, and 8.0%.



6 Intra-Run %CVs: Human Aβ42 Kit

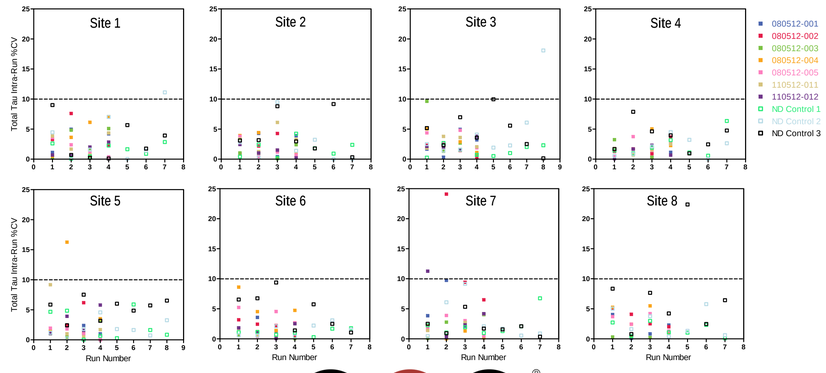
Each site collected data for 4 runs of CSF samples (solid squares) and at least 7 runs of QC samples (open squares). For Aβ42, the intra-run concentration %CVs were typically <10% for both the CSF and QC samples.

*NOTE: Sample data not shown for Site 1; Run 1: 110512-012 %CV = 39.2%; 90512-007 and 090512-008 were excluded due to an analytical error. Run 2: 080512-002 %CV = 39.7%.



7 Intra-Run %CVs: Human Total Tau Assay

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 %CVs were typically <10% for both the CSF and QC samples.



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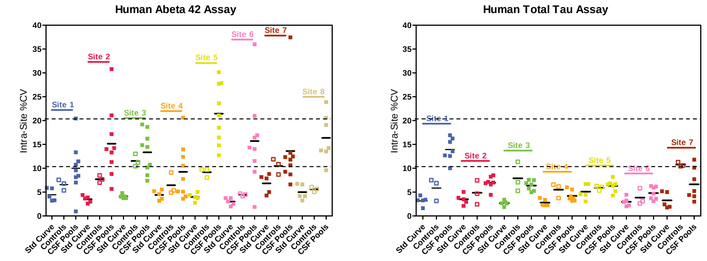
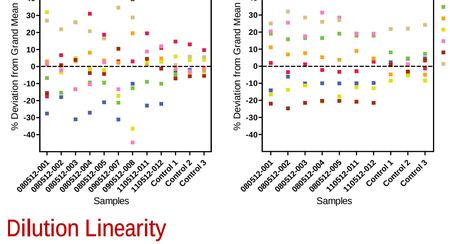
8 Comparison of Intra-Site Variation

The intra-site sample %CV values are plotted below for calibrators 1–5, QCs, and CSF samples. Intra-site %CVs for calibrators and QC samples were generally less than 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 tau assay as the intra-site variation for the CSF samples was typically <10%. Site 8 was excluded from the tau assay analysis due to several controls that did not meet specification.



9 Comparison of Site-to-Site Variations

For each site, we assessed the degree of bias from the overall mean concentration for each biomarker. We calculated the grand mean concentration of Aβ42 and total tau for the CSF and QC samples using data from all testing sites. For CSF samples, the average % deviation from the grand mean for sites 1–8, respectively, was: Aβ42 = -23.4, 11.3, -11.9, 5.1, -2.0, -6.6, 3.8, 25.1%; tau = -10.0, -0.9, 17.5, 6.9, -15.0, 23.1, -21.6, 27.7%. For QC samples, the average % deviation from the grand mean for sites 1–8, respectively, was: Aβ42 = -2.0, 12.5, -3.1, -2.4, 4.6, -1.4, -6.0, 4.1%; tau = 3.8, 0.1, 6.7, -4.2, -7.5, 1.8, 0.5, 22.7%. The tau assay demonstrates consistent patterning of site bias, whereas the Aβ42 assay exhibits a more random pattern. For both assays, overall bias for the QC samples was much lower than observed for the CSF samples, indicating that CSF handling is a potential source of variability between sites.

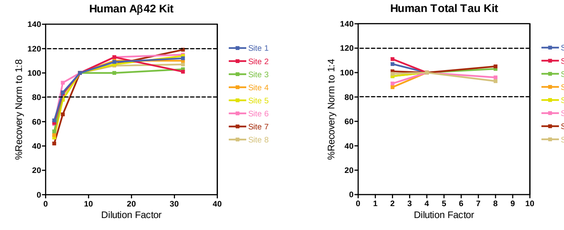


Human Aβ42 Assay	
Sample ID	Inter-Site %CV
080512-001	27.7%
080512-002	17.1%
080512-003	21.0%
080512-004	22.2%
080512-005	17.8%
090512-006	BLQ
090512-007	23.3%
090512-008	33.8%
090512-009	BLQ
090512-010	BLQ
110512-011	18.7%
110512-012	19.2%
Control 1	8.8%
Control 2	8.6%
Control 3	8.9%
Inter-technical replicate	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	BLQ
090512-007	BLQ
090512-008	BLQ
090512-009	BLQ
090512-010	BLQ
110512-011	15.8%
110512-012	16.4%
Control 1	6.6%
Control 2	6.7%
Control 3	7.7%
Inter-technical replicate	15.7%

10 Dilution Linearity

Dilution linearity was measured for 10 CSF sample pools on each assay at each site. 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 tau assay, analyte levels measured beyond the 8-fold dilution were BLQ and, therefore, were omitted from analysis.



11 Conclusion

On-site operator training and use of control tracking proved useful to identify and minimize variability contributions from the assay protocol and, in some cases, identified operators for retraining. Compared to the 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 of blinded technical replicates prepared in discrete builds revealed differences in Aβ42 levels suggesting that pre-analytical and sample handling could account for the increased intra- and inter-site variability. Studies are ongoing to identify and evaluate pre-analytical factors that represent potential sources of assay variability.

Acknowledgements

MSD: Jennifer Lewis, Daisy Roy, Qian Ning; Bristol-Myers Squibb: Steve Piccoli, Flora Berisha, Suk Kwok; University of Gothenburg: Henrik Zetterberg, Kaj Blennow, Lotta Olofson; QPS Delaware: Susan Carr Zondlo, Sally Wheeler, Antonio Polley; University College London: Viki Worthington, Jamie Toombs, Miles Chapman; Tandem Labs: Paul Rhyne, Erika Hess, Ketal Shah; Icon Labs: Michael Brown, Mason Brown, Tyler Allen; Edith Cowan University: Ralph Martins, Steve Pedrini, Veer Gupta, Eugene Hone; BioAgilytix Labs: Kathie Lindley; Medtox: Marya Awker, Sarah Flies, Sherry Brutt; MPI Research: Dipika Gemani, Hoan Nguyen; WuXi App Tec: Hong Ke, Dongzhao Chen; Covance-Greenfield: Marci Copeland; Provista Diagnostics: Susan Yeh, Tony Lamothe; Frontage Laboratories: Fengping Li, Robin Wakshlag.

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