

# Validation of the U-PLEX<sup>®</sup> Human Alpha-Synuclein Assay for Use with Human CSF, Saliva, and Whole Blood Samples Across Multiple Sites

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## 1 Abstract

**Objectives:** An analytically validated assay for human alpha-synuclein was used in a round-robin study to assess robustness across multiple laboratory sites.

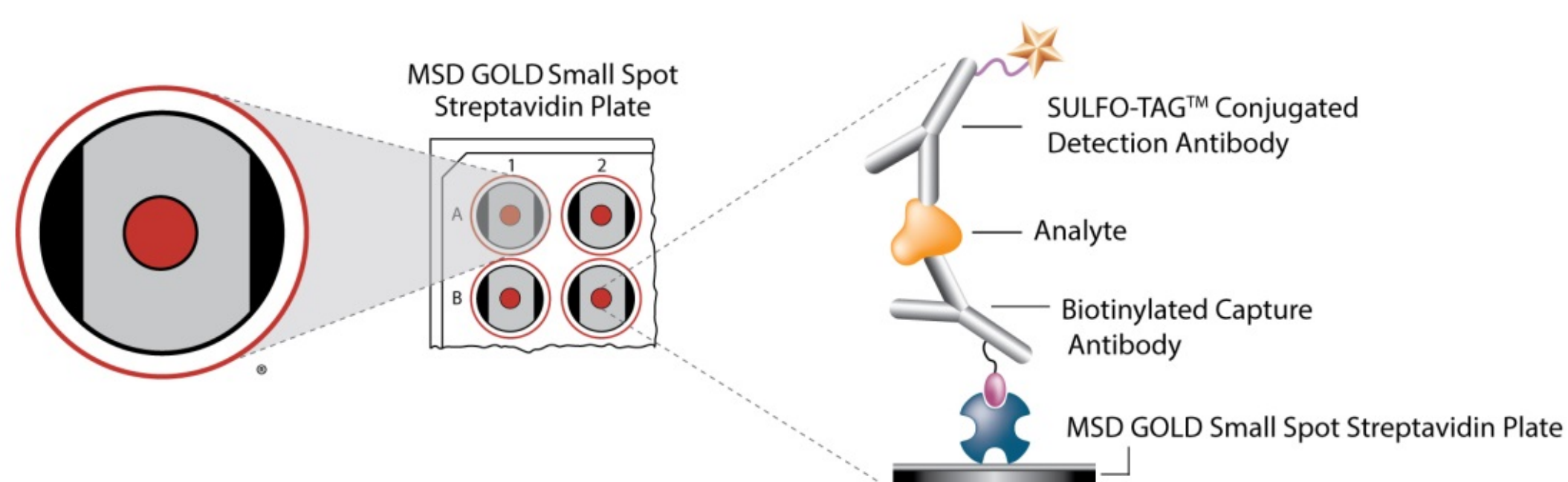
**Methods:** The U-PLEX Human Alpha-Synuclein Kit was developed using MSD's MULTI-ARRAY<sup>®</sup> technology and validated to confirm consistency and precision. Assay components were supplied to all sites along with a common set of samples. The samples included cerebral spinal fluid (CSF), saliva, and whole blood and were a matching set inasmuch as the fluids were derived from the same individuals. Laboratory technicians were trained in the pre-analytical handling of samples as well as the execution of the assay. Four laboratories participated including sites in the USA, Sweden, and Germany. Each sample was analyzed in duplicate across three production lots of the kits over nine days.

**Results:** The human alpha-synuclein assay exhibited robustness across four sites and three kit lots. The observed variability within plates, across kit lots, and across sites is reported here.

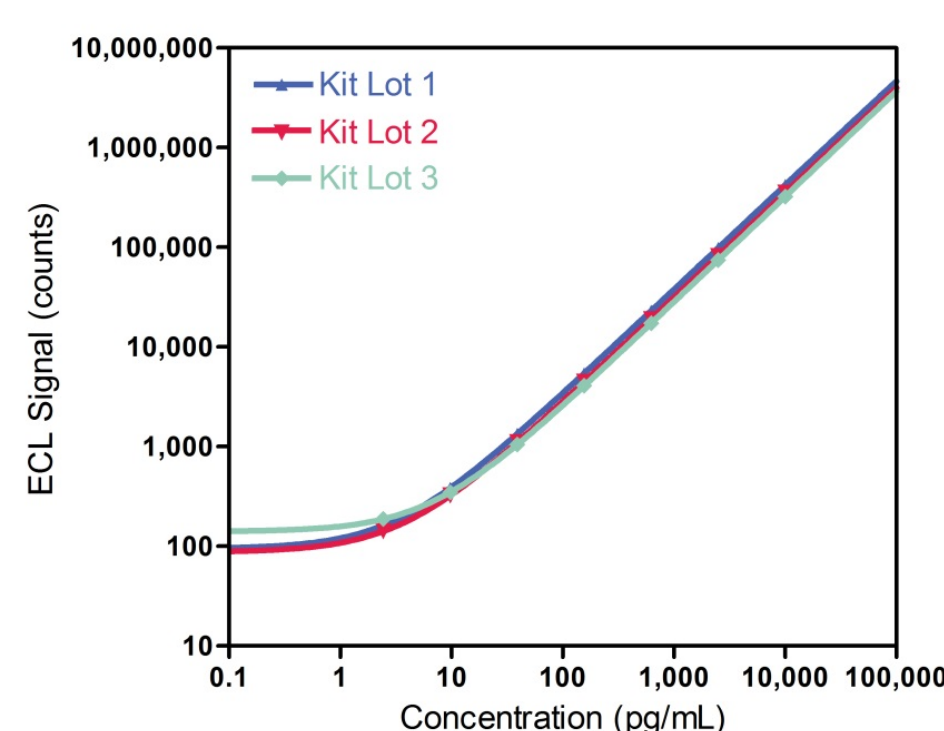
**Conclusions:** MSD's validated human alpha-synuclein assay has proven robust in measuring the protein in CSF, saliva, and whole blood samples across multiple manufactured lots and multiple laboratories. The results demonstrate that the kit is suitable for long-term studies and research involving multiple international sites. Development of this assay was supported by funding from The Michael J. Fox Foundation for Parkinson's Research (MJFF).

## 2 Methods

The U-PLEX Human Alpha-Synuclein Assay is a sandwich immunoassay. MSD provides a pre-coated MSD GOLD<sup>™</sup> Small Spot Streptavidin Plate. Biotin-conjugated anti-human alpha-synuclein capture antibody is added to the plate, followed by the addition of sample, and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD<sup>®</sup> SULFO-TAG). Analyte in the sample binds to capture antibodies immobilized on the working electrode surface, then recruitment of the detection antibodies by the bound analytes completes the sandwich. Detection is enabled with the addition of MSD Read Buffer, a solution containing reactants required for electrochemiluminescence. The plate is loaded into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The intensity of emitted light is measured, and signal values are back-fitted to a standard curve, providing a quantitative measure of analyte in the sample.



## 3 Standard Curve and Assay Protocol



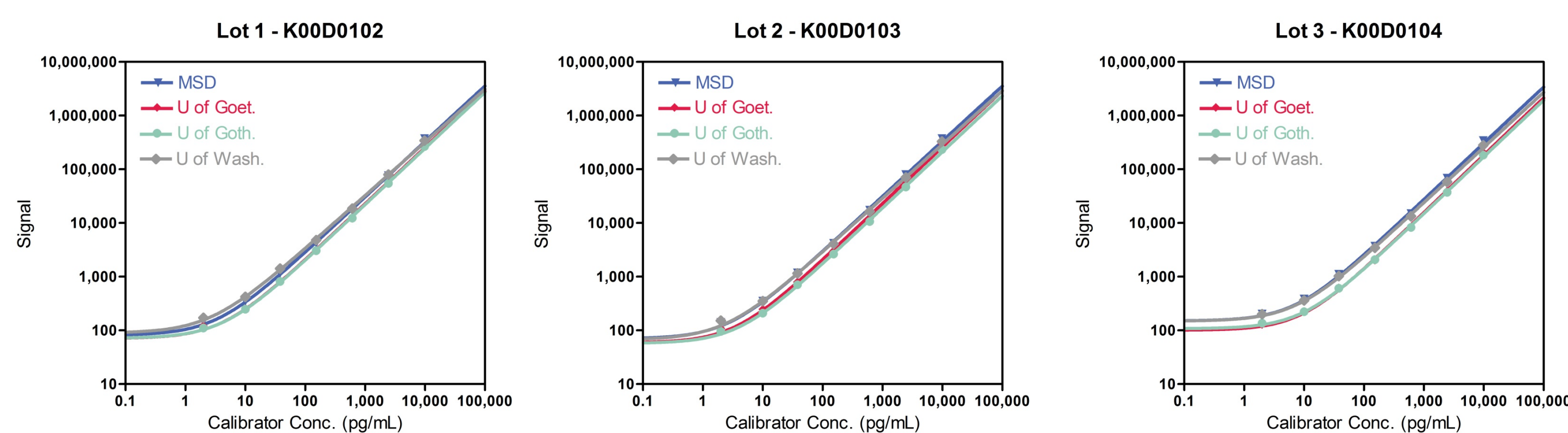
| Human Alpha-Synuclein |                |     |
|-----------------------|----------------|-----|
| Conc. (pg/mL)         | Average Signal | %CV |
| 10,800                | 410,627        | 4.3 |
| 2,700                 | 86,788         | 4.3 |
| 675                   | 19,405         | 3.6 |
| 169                   | 4,689          | 2.7 |
| 42.2                  | 1,242          | 2.5 |
| 10.5                  | 364            | 2.9 |
| 2.64                  | 143            | 3.7 |
| 0                     | 68             | 7.0 |

### Protocol

1. Add capture antibody solution (25  $\mu$ L per well). Incubate 1 hour at RT.
2. Wash with PBS-T. Add detection antibody solution (25  $\mu$ L per well). Add 25  $\mu$ L of standard or diluted sample. Incubate 2 hours at RT.
3. Wash and add Read Buffer (150  $\mu$ L per well). Analyze with MSD instrument.

Left: Standard curves from the validation of three independently manufactured kit lots are presented, illustrating the wide dynamic range of the assay and the highly reproducible standard curve signals. Each curve represents the average signals from a multi-run, multi-analyst, multi-day data set. Middle: Representative data from the three kit lots. Right: The human alpha-synuclein assay protocol is described.

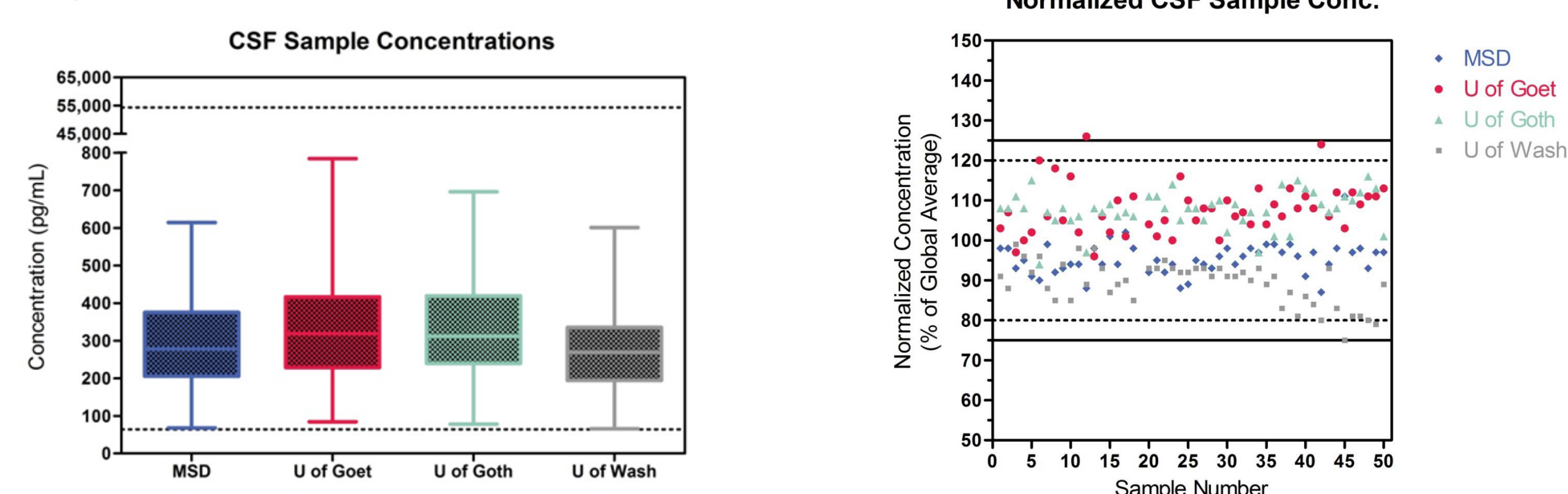
## 4 Multi-Site Test Results



| Site                     | Saliva (n=31)      |                    |                | CSF (n=49)         |                    |                | Whole Blood (n=50) |                    |                |
|--------------------------|--------------------|--------------------|----------------|--------------------|--------------------|----------------|--------------------|--------------------|----------------|
|                          | Avg. Intra-run %CV | Avg. Inter-lot %CV | Inter-Site %CV | Avg. Intra-run %CV | Avg. Inter-lot %CV | Inter-Site %CV | Avg. Intra-run %CV | Avg. Inter-lot %CV | Inter-Site %CV |
| MSD                      | 3.4                | 10.9               | 13.2           | 2.3                | 7.6                | 10.5           | 2.3                | 10.1               | 9.2            |
| University of Goettingen | 4.2                | 10.8               |                | 3.6                | 15.2               |                | 3.3                | 12.2               |                |
| University of Gothenburg | 2.6                | 16.0               |                | 2.4                | 11.4               |                | 2.3                | 8.0                |                |
| University of Washington | 2.8                | 6.2                |                | 2.6                | 9.8                |                | 2.7                | 7.1                |                |
| Average                  | 3.3                | 11.0               |                | 2.7                | 11.0               |                | 2.7                | 9.4                |                |

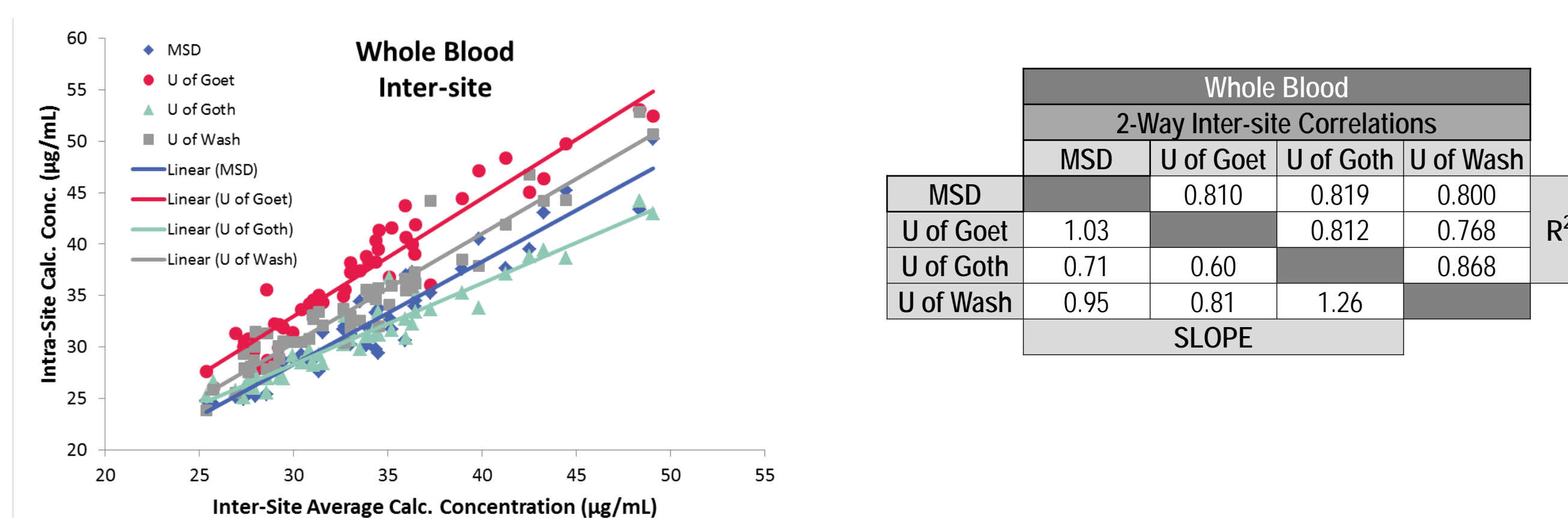
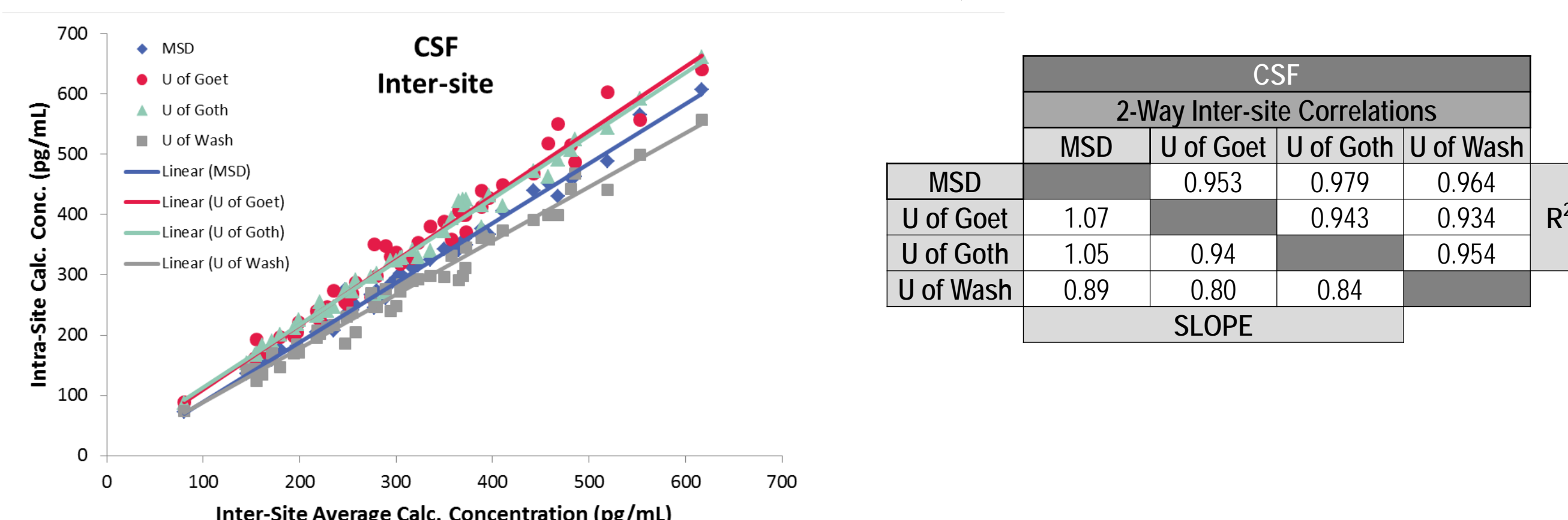
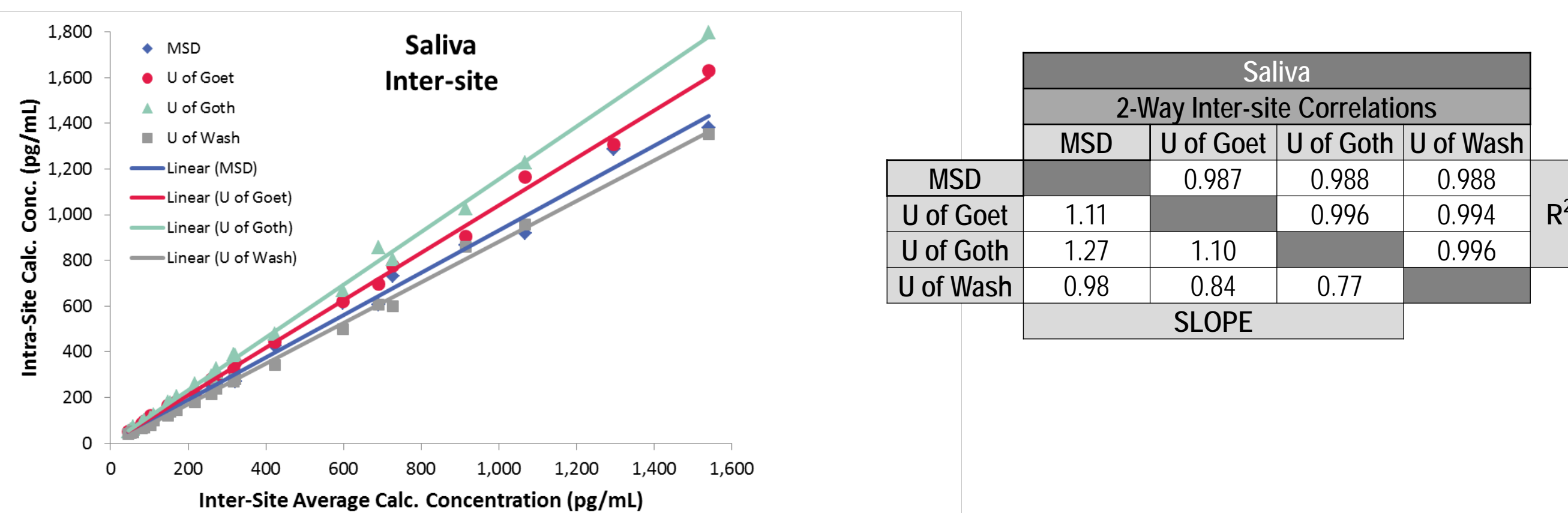
The U-PLEX Human Alpha-Synuclein Assay provided consistent results within and between kit lots, as well as within and between laboratory sites. Top: For each kit lot, calibrator curves were consistent across test sites. Only limited signal biases ( $\leq 35\%$ ) were observed at any given calibrator point. Each curve represents the average of five runs, across three days, by a single operator. Bottom: Across all kit lots, the quantification of human saliva (n=31), CSF (n=49), and whole blood (n=50) samples was highly reproducible. Intra-plate, i.e. intra-run, CVs were typically  $< 5\%$ . Inter-lot CVs were  $\leq 16\%$ . Inter-site CVs were less than 14%.

## Sample Quantification - CSF



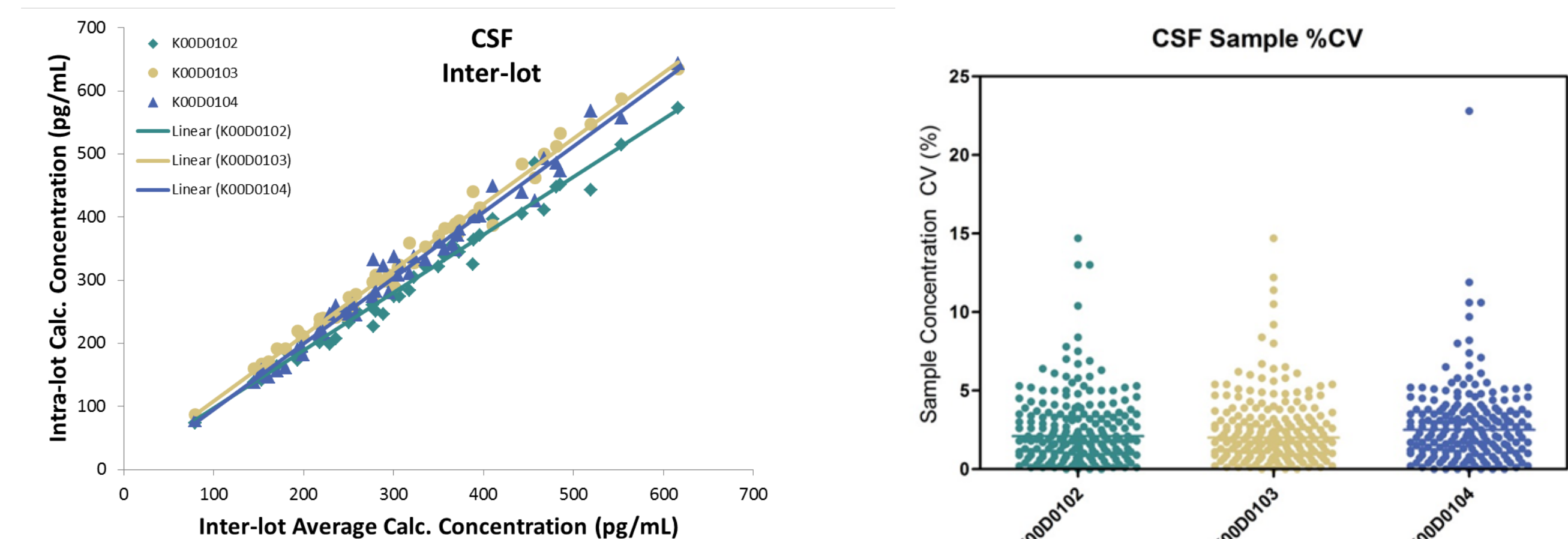
Quantification of CSF samples (n=49) across sites. Left: 99% of all sample concentration measurements fell within the quantifiable range of the assay, represented by horizontal dotted lines. Right: Measured concentration at each site normalized to the average concentration across all sites. Horizontal lines represent guard bands of 20% (dotted) and 25% (solid) above and below the global average. Data represents the average of three runs across three kit lots. Samples were run in duplicate. Similarly consistent results were obtained for saliva (n=31) and whole blood (n=50) samples.

## Inter-site Sample Correlations



Sample quantification was consistent across sites. Left: Linear relationship between the measured concentration at a given site and the average measured concentration across all sites. Right: Summary of the slopes and R<sup>2</sup> values of all pairwise comparisons between the test sites.

## Intra- and Inter-lot Consistency



Quantification of CSF samples (n=49) was consistent within runs and across kit lots. Left: Linear relationship between the measured concentration from a given kit lot and the average measured concentration across all kit lots. Data represents the average of four runs across all four test sites. Right: Intra-plate, i.e. intra-run, calculated concentration CVs were consistently low across all kit lots. Plots show four individual runs of each sample, one at each site. Similar results were obtained for saliva (n=31) and whole blood (n=50) samples.

## 5 Conclusions

The MSD U-PLEX Human Alpha-Synuclein Assay is a robust method for the quantification of native protein in human CSF, saliva, and whole blood. Consistent results were achieved across four international laboratories and across three production lots of assay kits. The consistency across kit lots supports that the assay is useful for longitudinal studies. The inter-site reproducibility demonstrates that multi-site studies can be supported with the assay. The results presented here satisfy a key goal of the Parkinson's disease research community, namely the uniform quantification of alpha-synuclein with an immunoassay.

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