Analytical Validation of the Disease Activity Score and 4 Disease Pathway Scores for a Multivariate Proteomic Multiple Sclerosis Disease Activity Test

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P006

Background

- Validated biological tools to quantitatively measure the level of disease activity in MS patients will help address a significant unmet need.
- One barrier to having a validated blood-based assay available to neurologists has been the lack of accurate, precise, and robust methods that ensure consistency in reported results over time.
- A Multiple Sclerosis Disease Activity (MSDA) Test that measures the concentrations of 18 proteins, and utilizes an algorithm to determine 4 disease pathways scores (immuno modulation, neuroinflammation, myelin biology, and neuroaxonal integrity) and an overall disease activity score was previously analytically validated for each of the individual protein biomarkers (P010 ACTRIMS 2021).
- The algorithm used to determine the 5 scores was finalized in a clinical validation study and was shown to significantly associate with radiographic and clinical disease activity assessments (P574 ECTRIMS 2021).

Objective

- Analytically validate the overall disease activity score and the 4 disease pathway scores for the MSDA Test focusing on the following parameters: Precision, Accuracy, and Serum sample stability (stressed temperature and freeze-thaw cycles)

Methods

- The MSDA Test was developed using Proximity Extension Assay (PEA) methodology on the Olink™ platform.
- The 18 proteins on the MSDA Test are associated with four biological pathways and each factor in one or more disease pathway models which are then used as inputs into an overall Disease Activity Score algorithm (Figure 1).
- During the analytical validation of the MSDA Test, the 5 scores were calculated in a series of experiments to establish robustness and reproducibility at the algorithm score level, in addition to the individual protein biomarkers.
- To establish precision, 48 Multiple Sclerosis (MS) samples were assayed repeatedly across 10 immunoassay plates, varying parameters including: Laboratories (2), Reagent lots (2), Operators (4).
- Each of the 5 scores was evaluated across the 48 samples for precision and robustness across all the runs.
- Accuracy was evaluated by mixing 4 individual samples at varying ratios (n=20 sample mixes).
  - Ratios of sample blends for mixtures with 2 samples were 25%:75%, 50%:50% & 75%:25%.
  - Ratios of sample blends for mixtures with 4 samples were 25%:25%:25%:25% & 40%:10%:40%:10%.
- Accuracy was assessed by comparing scores that were calculated using the algorithm between the measured and expected concentrations of the individual biomarkers based on the mixture ratios.
- For Freeze Thaw stability, 4 MS samples were subjected to 5 freeze thaw cycles and compared to the fresh sample. All 5 scores were calculated for the freeze thawed samples and compared back to the fresh sample scores.
- Sample stability at various temperatures (critical for sample handling logistics in a clinical setting) was tested by analyzing 4 MS samples that were aliquoted and placed in 4 temperature conditions (≤ -65°C, -20°C, 4°C, and room temperature) for multiple timepoints up to 28 days. Disease activity scores were calculated to determine the duration of time that samples would remain stable.
- Additionally, a follow up experiment was performed focused on 4°C with 14 samples and 4 timepoints (1 day, 2 days, 3 days, and 7 days) to account for the maximum duration of time expected from sample draw at the clinic to assaying samples.

Results

- Scores for the Disease Activity and Disease Pathway scores range from 1.0 - 10.0, with 0.5 increments. The Disease Activity scores are assigned into Low (1.0 - 4.0), Moderate (4.5 - 7.0), and High (7.5 - 10.0) categories.Thresholds were established in the clinical validation study to reflect the count of gadolinium enhanced lesions on an associated MRI.
- In the precision experiment, the 48 samples were stratified into the three groups for the overall DA Score as shown in Table 1. Data is representative of 9 out of 10 runs (1 run failure was observed based on established quality control criteria and was removed from the analysis).
- 0.5 (equal to one interval on the DA score scale) is the minimum score increment and was treated as 1 Standard Deviation (SD), rounding up from the 0.4 SD calculated for all samples.
- In subsequent stability experiments where Disease Activity scores were evaluated, comparing stressed conditions to controls, 3 SDs (e.g. ± 1.5 Disease Activity Score units) was the acceptability criteria applied.
- The Disease Activity score and the four Disease Pathway scores demonstrated reproducible results throughout the full range of scores evaluated in the experiment (Figure 2).
- For accuracy, expected individual biomarker concentrations and algorithm scores of the mixed samples were calculated by applying the targeted ratios of the unmixed samples.
- Accuracy at the score level was assessed by correlating all 5 scores using expected concentrations vs. observed concentrations of the 20 sample mixes.
- $R^2 \geq 0.90$ was observed for each correlated score (see Figure 3).
- For serum sample stability (freeze thaw and stressed temperature), the DA score was calculated for each stressed condition and compared to a control condition.
- As established in the precision experiment, the allowable score difference from the stressed sample condition is 3 SDs (±1.5 score difference).
- For freeze thaw stability, the 4 samples all returned scores within the acceptable range for up to 5 Freeze Thaw cycles (Table 2).
- For stressed temperature sample stability, the 4 samples all returned scores within the acceptable range for all temperature conditions up to 28 days (Table 3).
- For the follow up 4°C Sample Stability temperature study, the 14 samples all returned scores within the acceptable range for up to 7 days (Table 4).

Conclusions

- The results of this score level analytical validation analysis complement the previously reported individual protein biomarker analytical validation results.
- The Disease Activity and Pathway scores from the MSDA Test were observed to be accurate, precise, robust, and stable for sample processing and storage conditions expected in real world clinical conditions.
- The results of this analytical validation study complement the completed clinical validation study.
- A validated multivariate proteomic blood-based assay for objective MS disease assessments can serve as a quantitative and objective tool to enhance the standard of care for MS patients and their physicians.

References

- Hu et al. 2021: Analytical Validation of a Multivariate Proteomic Serum Based Assay for Disease Activity Assessments in Multiple Sclerosis, P010 ACTRIMS 2021 W. Hu[1], L. Loh[1], H. Patel[1], M. DeGuzman[1], M. Becich[1], F. Rubio da Costa[1], V. Gehman[1], E. Assarsson[2], S.Ohlsson[2], M. Lundberg[2], J. Bergman[2], N. Nordberg[2], F. Qureshi[1] [1]Octave Bioscience, Inc., Menlo Park, CA, USA; [2]Olink Proteomics, Uppsala, Sweden
- Chitnis et al. 2021: Clinical Validation Study Results of a Multivariate Proteomic Serum Based Assay for Disease Activity Assessments in Multiple Sclerosis, P574 ECTRIMS 2021 T. Chitnis[1], J. Foley[5], C. Ionete[2], N. El Ayoubi[3], S. Saxena[1], P. Gaitan-Walsh[1], H. Lokhande[1], A. Paul[1], F. Saleh[1], H. Weiner[1], F. Qureshi[4], M. J. Becich[4], F. Rubio da Costa[4], V. M. Gehman[4], S. J. Khoury[3] [1]Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [2]University of Massachusetts Medical School, Worcester, MA; [3]American University of Beirut, Beirut, Lebanon; [4]Octave Bioscience, Menlo Park, CA; [5]Rocky Mountain Multiple Sclerosis Clinic, Salt Lake City, UT.

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Table 1. DA Score Sample Stratification

DA Score Level	Sample Count	Average SD
Low (1.0 - 4.0)	14	0.3
Moderate (4.5 - 7.0)	22	0.5
High (7.5 - 10.0)	12	0.3
All (1.0 - 10.0)	48	0.4

Table 2. Freeze Thaw Stability Disease Activity Scores

DA SCORE	Fresh (Control Condition)	FT1	FT2	FT3	FT4	FT5
A	5	5	4.5	5	4	5.5
B	6	6	5.5	6	6	6
C	3	3	3	3	3	3
D	2	2	2	2	2	2.5

Legend
Low DA Score
Moderate DA Score
High DA Score

Table 3. Stressed Temperature Sample Stability Study Disease Activity Scores

DA SCORE	-80°C (Control Condition)	RT 4H	RT D1	RT D3	RT D7	RT D14	RT D28
A	3	2.5	3	3	2.5	2.5	2
B	4	4.5	4	4.5	4.5	4	3
C	6	5.5	5	6	6	5.5	5
D	8.5	8.5	8.5	8.5	9	8.5	8

DA SCORE	-80°C (Control Condition)	4°C 4H	4°C D1	4°C D3	4°C D7	4°C D14	4°C D28
A	3	3	2.5	2.5	3	3	2.5
B	4	4	5	4.5	4.5	5	5
C	6	6	6.5	6.5	6	6.5	6
D	8.5	8.5	8.5	8.5	8.5	9	8.5

DA SCORE	-80°C (Control Condition)	-20°C D1	-20°C D3	-20°CD7	-20°C D14	-20°C D28
A	3	2.5	2.5	3	2.5	2.5
B	4	4.5	4	4.5	4.5	4.5
C	6	5	5.5	6	6	6
D	8.5	8.5	8.5	8.5	8.5	8

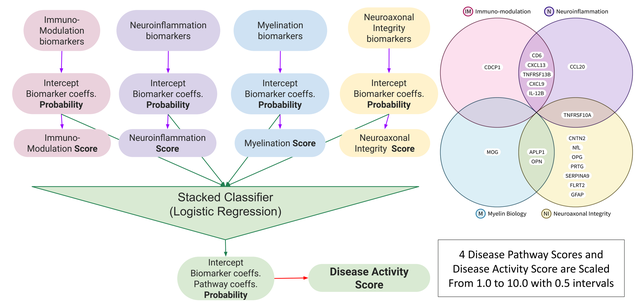
Legend
Low DA Score
Moderate DA Score
High DA Score

Table 4. 4°C Sample Stability Study Disease Activity Scores

DA SCORE	-80°C (Control Condition)	4°C 1 Day	4°C 2 Days	4°C 3 Days	4°C 7 Days
A	8	8	8.5	8	8.5
B	5	4.5	4.5	4.5	4.5
C	7.5	6	7.5	7.5	6.5
D	6	6.5	6	5.5	7
E	5	4.5	4.5	4.5	5
F	6.5	6.5	6.5	6	7
G	4	3.5	3.5	3.5	4
H	6	5.5	7	7	7
I	6	5.5	6.5	6	6.5
J	3	2.5	2.5	3	2.5
K	1.5	1	1.5	1.5	1.5
L	2.5	2.5	2	2.5	2
M	2.5	2.5	2.5	2	2.5
N	7	7	8	7.5	8

Legend
Low DA Score
Moderate DA Score
High DA Score

Figure 1. MSDA Stacked Classified Meta-Feature Algorithm



4 Disease Pathway Scores and Disease Activity Score are Scaled From 1.0 to 10.0 with 0.5 intervals

Figure 2. Precision - Score Distributions

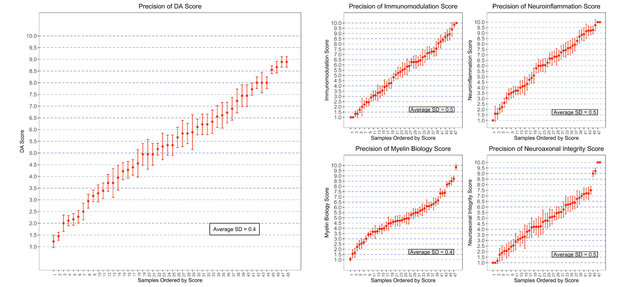


Figure 3. Accuracy - Score Correlations