Abstract # 98094

Novel Immunoassays for TDP-43 Detection in Plasma and CSF

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❶ Abstract

Background

TAR DNA-binding protein-43 (TDP-43) has an important role in the pathogenesis of several neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and limbic predominant age-related TDP-43 encephalopathy (LATE). Improved immunoassays for detecting TDP-43 and its disease-associated modifications are needed, based on the hypothesis that proteins involved in disease pathology can serve as effective biomarkers.

Methods

Antibodies were screened against purified TDP-43 proteins and fragments, brain lysate, cerebrospinal fluid (CSF) and plasma from individuals with ALS (provided by MGH) and healthy controls (commercially obtained) to develop and preliminarily evaluate research use only (RUO) standard and ultrasensitive S-PLEX assays for TDP-43 and pTDP-43.

Results

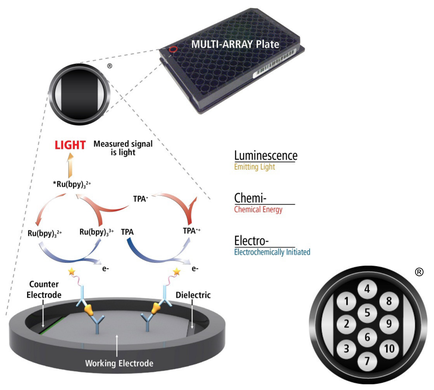
The full length TDP-43 assay in standard format had a quantitative range of 10-275,000 pg/mL and sufficient sensitivity to quantitate 100% of the tested plasma samples. The assay displayed excellent dilution linearity. Whole blood, plasma and platelet-rich plasma show higher concentrations than red blood cells, serum and platelet-poor plasma, suggesting a need for good control over efficiency and timing of the separation of plasma from platelets and blood cells to avoid pre-analytical effects. To measure the lower TDP-43 levels in CSF samples, the more sensitive S-PLEX TDP-43 assay was used. It had a quantitative range of 5-34,000 pg/mL and was able to quantitate 48% of the tested CSF samples. The S-PLEX pTDP-43 assay was used to measure TDP-43 phosphorylated at S409/410 in plasma samples. It had a quantitative range of 1-50,000 pg/mL. Specificity was confirmed by testing phosphorylated and non-phosphorylated purified protein. To preliminarily evaluate the assays, a small set of plasma and CSF samples from individuals with ALS was tested. Relative to the control samples, the ALS samples had higher median plasma TDP-43 levels (ratio = 4.8, p = 0.0018), lower median CSF TDP-43 levels (ratio = 0.49, p = 0.01).

Conclusions

Newly developed immunoassays for TDP-43 and pTDP-43 provide powerful tools for research on neurodegeneration biomarkers.

❷ Methods

MSD® 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 improved sensitivity of S-PLEX® assays is due to the proprietary TURBO-TAG® and TURBO-BOOST® reagents.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

❸ Antibody screening

A set of TDP-43 antibodies were screened to identify optimal antibody pairs based on binding to recombinant calibrator materials and relevant biological samples. The antibodies and calibrator materials included reagents generated at MSD or sourced commercially. Materials used for screening included human whole brain lysate (no indication), full length recombinant E. coli-produced TDP-43, overexpression HEK cell lysate, pooled normal plasma, and recombinant TDP-43 fragments 1-399 (yeast), 8-192 and 104-266 (E. coli). Candidate antibody pairs which 1) recognized the recombinant full length calibrator with excellent linearity (Hill Slope ~ 1.0), 2) showed a low limit of detection, and 3) recognized native and recombinant samples were shortlisted for further testing.

TDP-43 Assay:		Antibody Pair 1	Antibody Pair 2	Antibody Pair 3
Sample	Concentration (pg/mL)	ECL Signal		
Cal 01	275,000	549,381	939,374	1,037,394
Cal 02	68,750	152,267	244,929	279,893
Cal 03	17,188	38,166	62,835	69,726
Cal 04	4,297	10,139	14,719	17,532
Cal 05	1,074	2,666	4,108	4,648
Cal 06	269	782	1,057	1,250
Cal 07	67.1	310	344	442
Cal 08	0	142	99	159
Hill Slope		0.99	1.00	1.00
Estimated LOD (pg/mL)		17.43	9.70	11.33
Brain lysate		9,263	5,653	19,462
Diluent 100		161	125	179
Full length recombinant; E. coli		70,649	75,877	99,617
O/E HEK lysate		457	571	18,017
Pooled Plasma		2,411	965	5,493
Recombinant AA 1-399; Yeast		169	127	160
Recombinant AA 8-192; E. coli		119	127	173
Recombinant TDP-43 AA 104-262; E. coli		91	124	159

Table 1: Signals from the top three candidate total TDP-43 antibody pairs in standard MSD immunoassay format.

❹ Standard Format Total TDP-43 Assay

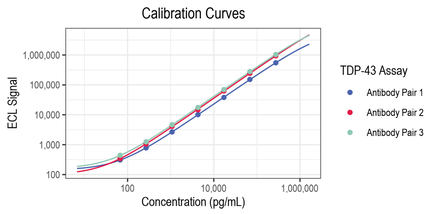
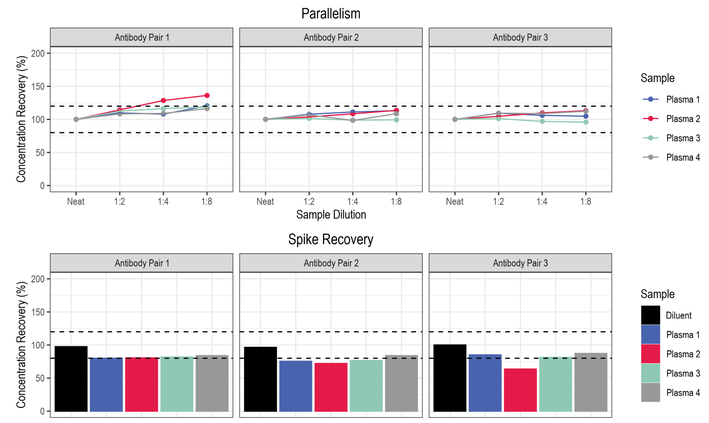


Figure 1: Calibration curves of top three candidate antibody pairs.

Protocol

1. Coat with capture antibody solution at 25 µL/well. Incubate 1 hour at room temperature (RT) with shaking.
2. Add assay diluent and calibrator, control, or sample (25 µL+ 25 µL/well). Incubate 1 hour at RT with shaking.
3. Wash and add detection antibody solution (50 µL/well). Incubate 1 hour at RT with shaking.
4. Wash and add read buffer (150 µL/well). Analyze with MSD instrument.

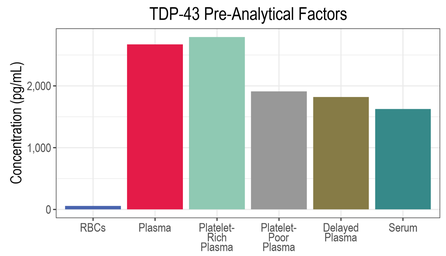
Figure 2: (top) Parallelism of the three candidate total TDP-43 assays with four plasma samples diluted through 8-fold. (bottom) Recovery of spiked calibrator into diluent or individual plasma samples, then diluted 2-fold before testing.



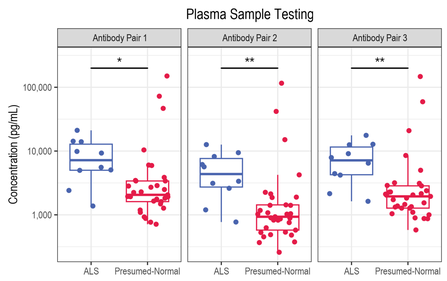
❺ Pre-analytical Factors

Matched pre-analytical samples from apparently healthy individuals including whole blood, red blood cells (RBCs), EDTA plasma, platelet-rich plasma, platelet-poor plasma, delayed plasma and serum were tested on the standard format total TDP-43 assay to determine the effect of sample handling on concentration.

Figure 3: Standard format TDP-43 testing normal human matched pre-analytical samples.



❻ Human and Rodent Native Sample Testing

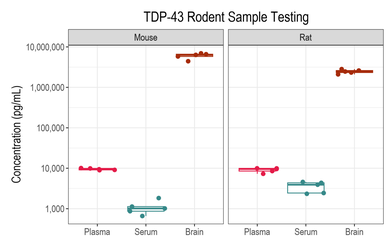


Plasma samples from individuals with ALS (provided by MGH) and commercially-obtained normal plasma were tested in a preliminary experiment to determine plasma TDP-43 differences. The data indicate a moderate concentration increase in ALS by all three candidate antibody pairs.

Figure 4: ALS (n=10) and normal (n=33) human EDTA plasma sample testing in the top three candidate standard format total TDP-43 assays.

TDP-43 is highly conserved between rodent and human proteins. To confirm cross-reactivity, samples from healthy mice and rats were tested on the assay. Data indicate the assay is capable of quantitating rodent samples.

Figure 5: Normal mouse and rat plasma (n=4), serum (n=5) and brain (n=5) sample testing on the standard format total TDP-43 assay.



❼ Ultrasensitive Total TDP-43 Assay

Candidate antibody pairs were screened in the ultrasensitive S-PLEX format, and Antibody Pair 2 was selected to move forward based on optimal performance.

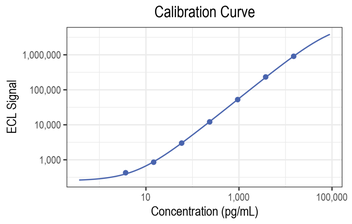


Figure 6: TDP-43 S-PLEX calibration curve.

Protocol

1. Coat with capture antibody solution at 25 µL/well. Incubate 1 hour at RT with shaking.
2. Add assay diluent and calibrator, control, or sample (25 µL+ 25 µL/well). Incubate 1 hour at RT with shaking.
3. Wash and add detection antibody solution (50 µL/well). Incubate 1 hour at RT with shaking.
4. Wash and add Enhance Solution (50 µL/well). Incubate 30 minutes at RT with shaking.
5. Wash and add Detect Solution (50 µL/well). Incubate 1 hour at 27°C with shaking.
6. Wash and add read buffer (150 µL/well). Analyze with MSD instrument.

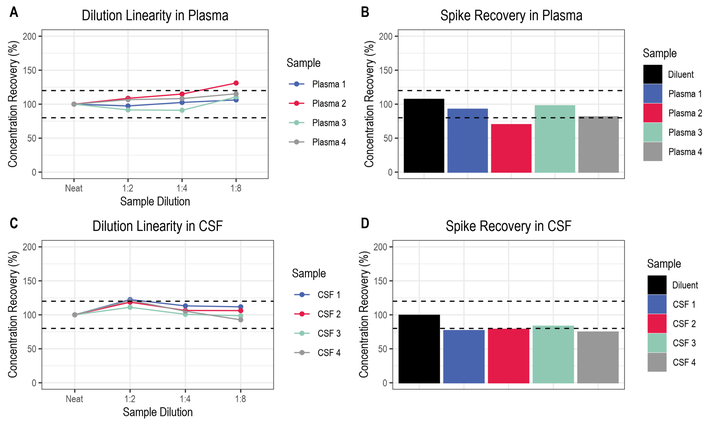


Figure 7: (A) Dilution linearity of S-PLEX TDP-43 assay in plasma samples. **(B)** Recovery of spiked analyte in plasma samples. **(C)** Dilution linearity of S-PLEX TDP-43 assay in CSF samples. **(D)** Recovery of spiked analyte in CSF samples.

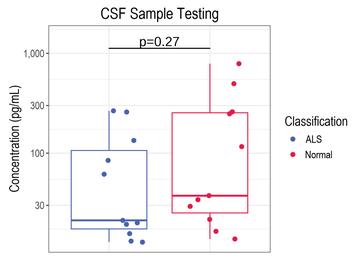


Figure 8: ALS (n=10) and presumed normal (n=10) CSF sample testing on the S-PLEX total TDP-43 assay.

❽ Ultrasensitive phosphorylated (S409/410) TDP-43 Assay

pTDP-43 Assay Calibration Curve		
Sample	Concentration (pg/mL)	ECL Signal
Cal 01	10,000	733,107
Cal 02	2,500	162,346
Cal 03	625	33,064
Cal 04	156	10,867
Cal 05	39.1	2,886
Cal 06	9.8	775
Cal 07	2.44	494
Cal 08	0	337
Hill Slope		1.04
Estimated LOD (pg/mL)		1.75
Unphosphorylated TDP-43	550,000	3,830

Candidate antibodies against TDP-43 phosphorylated at S409/S410 were screened in both capture and detect orientations. The top pair was selected based on lowest background, lowest LOD and highest specific signal. Analytical specificity was tested against full length recombinant unphosphorylated calibrator. Cross reactivity was 0.01% for unphosphorylated TDP-43 on the pTDP-43 assay.

Table 2: S-PLEX pTDP-43 calibration curve and cross reactivity testing. Calibrator is recombinant phosphorylated TDP-43.

❾ Conclusions

Here we present newly developed immunoassays for TDP-43 and pTDP-43 to provide powerful tools for research on neurodegeneration biomarkers. These assays display excellent analytical sensitivity and specificity, and good accuracy based on parallelism, dilution linearity and spike recovery data. Preliminary sample testing results with a small number of test samples show a significant difference between ALS and normal plasma. The standard format full length TDP-43 assay can be used for serum, plasma and brain tissue, and cross reacts with mouse and rat TDP-43 for quantitation in rodent serum and plasma. The ultrasensitive assay can detect the much lower concentrations of TDP-43 found in cerebrospinal fluid.

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❿ Acknowledgements

This work was supported by the Assistant Secretary of Defense for Health Affairs endorsed by the Department of Defense, in the amount of $704,431.23, through the FY22 Amyotrophic Lateral Sclerosis Research Program/ Clinical Biomarker Development Award under Award No. W81XWH-22-ALSRP-CBDA. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Assistant Secretary of Defense for Health Affairs endorsed by the Department of Defense.

We would like to thank our collaborators at the Department of Neurology Massachusetts General Hospital for providing ALS plasma and CSF samples for assay development purposes.

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