Development of an Electrochemiluminescence-Based Pharmacokinetics Assay for an Antisense Oligonucleotide Drug for the Treatment of Amyotrophic Lateral Sclerosis

Abstract #6201

Annamaria Szabolcs, Isaac Shin, Timothy J. Break, Seth B. Harkins, Jeff Debad, and Jacob N. Wohlstadter

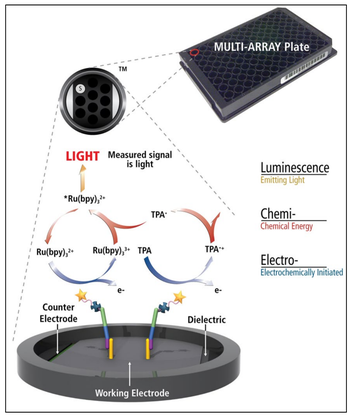
sharkins@meso-scale.com; Meso Scale Discovery, Rockville, Maryland, USA

❶ Objective

Antisense oligonucleotide (ASO) drugs provide treatment options for some debilitating diseases that were previously considered undruggable. One example of such an ASO drug is tofersen, which is used in the treatment of Amyotrophic Lateral Sclerosis. With the emergence of this new class of drugs comes the challenge of building highly sensitive, reliable, and high-throughput pharmacokinetics (PK) assays. Here, we demonstrate the feasibility of developing a sensitive, extraction-free, plate-based assay for detection of the ASO drug tofersen in biological fluids and tissue samples.

❷ Methods

MSD's electrochemiluminescence (ECL) detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



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.

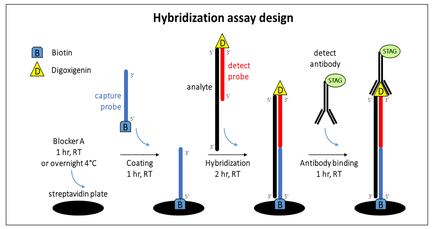
❸ Assay Design

A dual-probe hybridization assay with an antibody-based detection method was developed using MSD's platform to achieve high sensitivity. In brief, a biotin-labeled DNA probe, complementary to a portion of the analyte, was coated on the surface of a streptavidin plate. A digoxigenin (DIG)-labeled detection probe was hybridized to the analyte in the biological matrix using a thermal cycler or heat block. Samples were then added to the plate, where the analyte-detection probe complexes were bound by the coated capture probes. An anti-DIG antibody conjugated with an MSD GOLD™ SULFO-TAG label was used for signal generation (with the addition of read buffer) and the plate was read on an MSD® instrument. The emitted light measured by the instrument is proportional to the amount of analyte present in the sample and provides a quantitative measure of the analyte in the sample. In addition to this standard assay format, an augmented reporter system was established (N-PLEX ULTRA™) to further improve assay sensitivity through signal amplification.

During protocol development, consideration was given to avoid hook effects and free-biotin interference that can present challenges for PK assays. A specially-formulated lysis buffer was also developed for applications targeting oligonucleotide analytes.

Protocol

1. Block plates with **Blocker A**. (1 hr at RT)
2. Wash plate and add **capture probe**. (1 hr at RT w. shaking). During this incubation time, add **detect probe** to samples and heat to 95°C for 5 minutes then cool down to RT.
3. Wash plate and add **samples**. (2 hrs at RT w. shaking)
4. Wash plate and add **SULFO-TAG labeled anti-DIG antibody**. (1 hr at RT w. shaking)
5. Wash plate, add **read buffer** and read on MSD instrument



❹ Probe Design

Tofersen is a highly-modified antisense oligonucleotide gapmer containing DNA bases, 2'-O-methoxyethyl-modified RNA bases and phosphorothioate (*) bonds for increased uptake and nuclease resistance (Takakusa et al. PMID: 36735616). The relatively low melting temperature of this analyte makes the development of a strictly hybridization-based, room temperature assay challenging without the use of locked nucleic acids (LNAs) in the probe design.

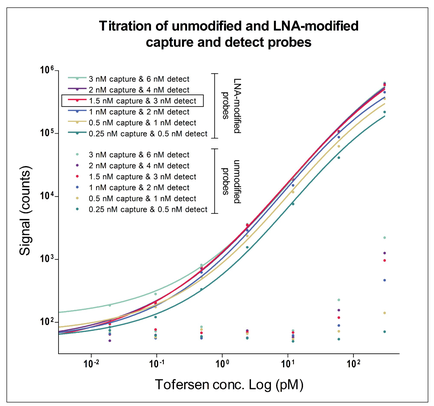
To demonstrate the beneficial effect of LNAs on assay sensitivity, we designed and evaluated a set of unmodified capture and detect probes, as well as LNA-modified versions of these probes in our hybridization assay.

Synthetic Oligonucleotides used in Assay Design

Oligo Type	Oligo Name	Sequence and Modifications	T_m (at 50 mM NaCl)
Analyte	Tofersen (ref.:PMID: 30010620)	/52MOErC/*/i2MOErA/ /i2MOErG/*/i2MOErG/ /i2MOErA/*/T*A*C*A*T*T*T*C*T*A*/i2MOErC/ /i2MOErA/*/i2MOErG/ /i2MOErC/*/32MOErT/	49.2°C
Probe	Unmodified capture	/5Biosg/AGCTGTAGAA	25.2°C
	Unmodified detect	/5Phos/ATGTATCCTG/3Dig_N/	22.2°C
	LNA capture	/5Biosg/A+GCTGTA+GAA	32.3°C
	LNA detect	/5Phos/AT+GTAT+C+CTG/3Dig_N/	31.0°C

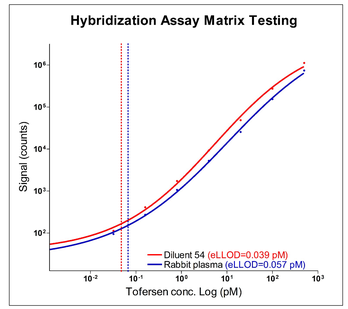
❺ Probe Titration

A probe titration experiment was designed to identify the optimal capture and detect probe concentrations for the assay. Increasing concentrations of unmodified or LNA-containing capture probes were coated on the assay plates. Unmodified probes generated very low signal levels. The use of LNA-containing probes significantly increased signal generation and assay sensitivity. When using 1.5 nM LNA for capture and 3 nM for detection probes, the assay was approaching its binding capacity. This condition was selected for further experiments.



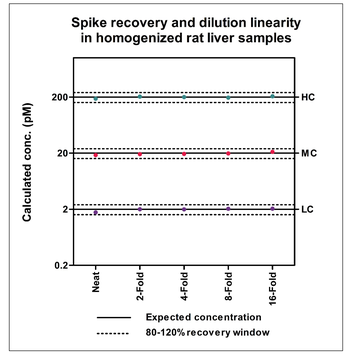
❻ Matrix Performance

Following the selection of the ideal probe type and concentrations, we evaluated assay sensitivity in diluent versus rabbit plasma. The assay showed good linearity in both matrices. The estimated lower limit of detection (eLLOD) was 39 fM (0.2 pg/mL) in diluent and 57 fM (0.5 pg/mL) in rabbit plasma. By pre-binding the biotinylated capture probe to the plate we minimized free-biotin interference, which can be a concern in sample testing.



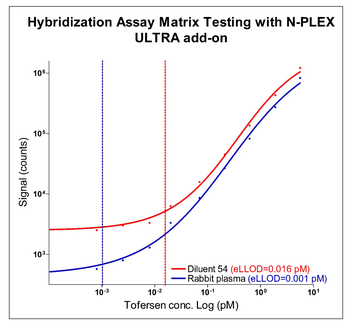
❼ Spike Recovery and Dilution Linearity

The analyte was spiked into rat liver lysate at 200 (high control, HC), 20 (mid control, MC) or 2 pM (low control, LC). Samples were then tested undiluted or after 2, 4, 8 or 16-fold dilutions in diluent. Quantification was performed using an eight-point calibration curve in plasma matrix. All sample recoveries were between 88 and 105% of the expected concentration and consistent linearity of dilution was observed over the tested range (0.125-200 pM).



❽ N-PLEX ULTRA Performance

The feasibility of the proprietary N-PLEX ULTRA signal amplification method was evaluated in both diluent and rabbit plasma. This application requires two additional steps in the assay protocol that extend the assay time by 90 minutes. This assay format provides excellent results in complex matrices such as plasma. We achieved an approximately 50-fold increase in assay sensitivity in rabbit plasma compared to the non-amplified method and an eLLOD of 1 fM (7 fg/mL).



❾ Conclusion

This ECL-based assay method offers a simple, highly sensitive, extraction-free workflow for PK assay development for ASO drugs. The 96-well format and the short on-instrument time makes this assay compatible with high-throughput screening. The assay formats are compatible with highly-modified oligonucleotide drugs, and the improvement in sensitivity with the N-PLEX ULTRA format sets a new standard for ultrasensitive oligonucleotide PK assay development.

DOWNLOAD POSTER

Meso Scale Discovery®
A division of Meso Scale Diagnostics, LLC.
www.mesoscale.com®

221-CM-1023