

Emerging Approaches to Improve the Quantification of Huntingtin Protein in Human Cerebrospinal Fluid

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Background

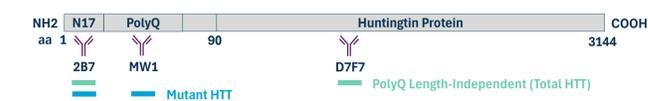
- Immunoassays designed to quantify mutant (mHTT) and polyQ length-independent huntingtin protein (total HTT or tHTT) in human cerebrospinal fluid (CSF) using Single Molecule Counting (SMC) technology¹⁻³ have served as useful tools in supporting the clinical development of HTT-lowering therapies such as RNA interference (RNAi) therapeutics for the treatment of Huntington's disease (HD).
- Sensitivity is a common challenge when utilizing these assays, as baseline mHTT levels in some patient samples fall near the lower limit of quantitation (LLOQ)^{1,4}.
- Two recent advancements in addressing these sensitivity challenges are summarized, including the validation of off-the-shelf SMCxPRO assays with 15.2 fM LLOQs, and introduction of the proximity ligation NULISA™ platform⁵ which offers a potential breakthrough in sensitive biomarker quantification.

Methods

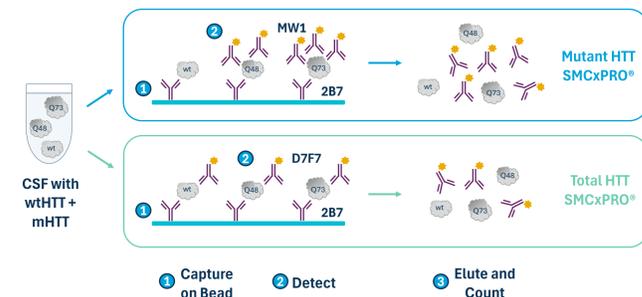
SMCxPRO® vs. NULISA™

- Both methods are designed to quantify CSF containing heterogeneous polyQ lengths (wtHTT, mHTT, tHTT with somatic expansion), with signal calibrated against recombinant Q48 1-3144 HTT produced in HEK 293 cells.
- NULISA can offer more consistency across polyQ lengths, with signal limited by the number of 2B7 antibodies bound.

Antibodies



Approach 1: SMCxPRO®



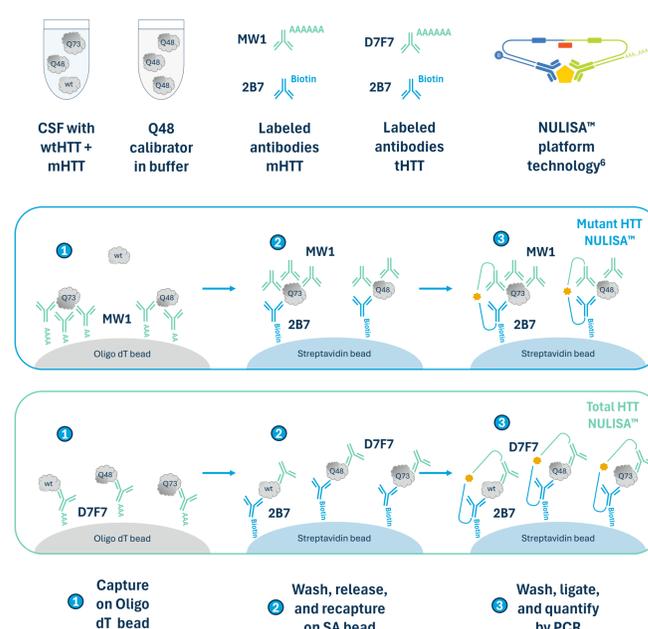
Features of recently implemented SMCxPRO® method

- Harmonized calibration standard (Q48 1-3144 HEK 293)
- Pooled HD CSF endogenous QC (eQC) for trending
- Off-the-shelf, validated, GCLP

mHTT and tHTT SMCxPRO Method Comparison

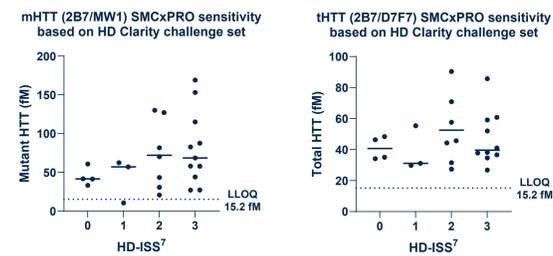
Assay	Platform	Calibrator	Antibody Pair	Calibrator Range	LLOQ Buffer	LLOQ Matrix
mHTT	SMCxPRO	Q48 1-3144 HEK 293	2B7-MW1	750-3.53 fM	7.59 fM	15.2 fM
tHTT	SMCxPRO	Q48 1-3144 HEK 293	2B7-D7F7	750-3.53 fM	7.59 fM	15.2 fM

Approach 2: NULISA™



Results

Sensitivity of SMCxPRO® assays with harmonized calibration curves demonstrates quantification at baseline in ≥96% of individual HD CSF samples

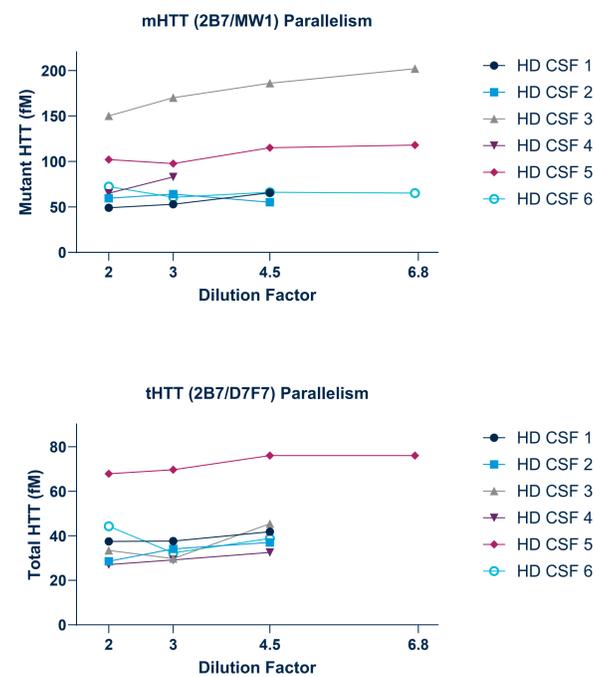


% CSF Samples Quantifiable

	Observed Results in HD Clarity CSF		Theoretical 50% Reduction		Theoretical 80% Reduction	
	mHTT	tHTT	mHTT	tHTT	mHTT	tHTT
# Quantifiable	25	25	22	22	9	2
# Analyzed	26	25	26	25	26	25
% Quantifiable	96	100	85	88	35	8

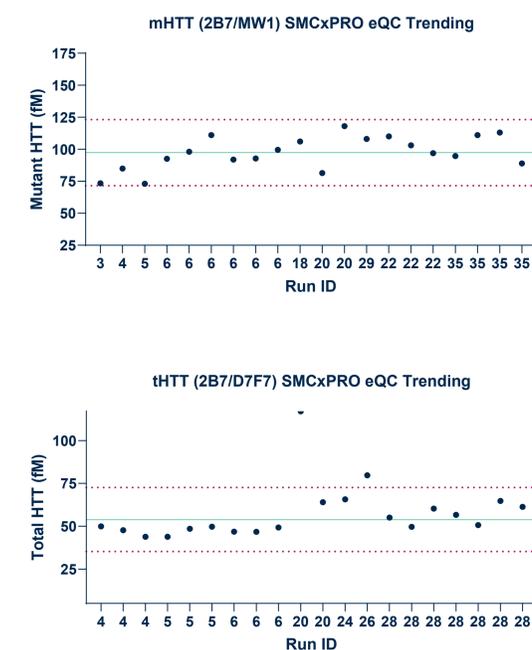
Parallelism in SMCxPRO® assays shows suitable performance relative to 2-fold Minimum Required Dilution (MRD)

- Recovery relative to MRD within 20% in ≥80% of samples



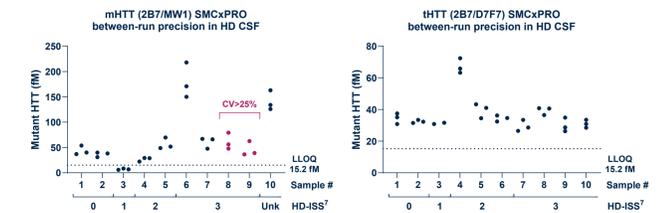
Introduction of pooled HD CSF endogenous Quality Control (eQC) enables trending in matrix

- Allows monitoring of assay drift and provides valuable information during critical reagent bridging



Results

Between-run precision in SMCxPRO® assays is within 25% CV in ≥80% individual HD CSF samples



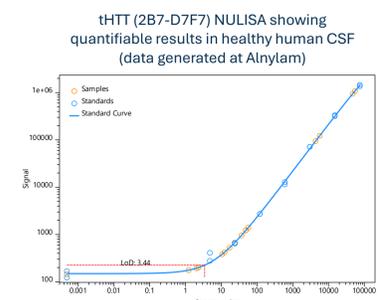
Peripheral hemolyzed whole blood shows false-high assay interference

- Hemolyzed whole blood from healthy donors spiked into pooled HD CSF at ≥0.0005% showed ≥25% bias relative to unspiked control, with no apparent contribution to signal from HD Clarity plasma

% Spike into Pooled HD CSF	mHTT Blood Interference		tHTT Blood Interference	
	HD Patient Plasma	Hemolyzed Whole Blood	HD Patient Plasma	Hemolyzed Whole Blood
0.0	108	N/A	80	N/A
0.0001	106	-1.9	62	-22.2
0.0005	100	-7.4	69	-13.3
0.005	103	-4.6	60	-24.8
0.05	108	0.0	63	-21.1
0.5	107	-0.9	77	-3.0

Opportunity to improve sensitivity: promising performance of NULISA™ based on initial method development and qualification runs

- Homebrew NULISA™ method using 2B7 and MW1 or D7F7-labeled antibodies and Q48 calibrator shows potentially improved sensitivity and robustness
- Targeting sensitivity of ≥90% HD CSF samples quantifiable with theoretical 80% reduction from baseline



Summary

SMCxPRO® Assay Improvements

- Off-the-shelf SMCxPRO® using Q48 1-3144 calibrators and eQC demonstrated suitable performance for measuring mHTT or tHTT in human CSF
- Blood interference results reinforce importance of peripheral blood cell removal from CSF samples prior to freezing for mHTT or tHTT biomarker analysis
- Room for improvement in assay sensitivity for supporting clinical development of HTT-lowering therapies beyond 50% reduction from baseline

Opportunities to improve sensitivity

- Possible breakthrough in sensitive biomarker quantification offered by NULISA™ technology
- Application of NULISA™ in HTT-lowering clinical studies will be evaluated for its ability to inform target engagement

Abbreviations and References

Abbreviations
 CSF: cerebrospinal fluid; CV: coefficient of variation; eQC: endogenous quality control; fM: femtomolar; GCLP: Good Clinical Laboratory Practice; HD: Huntington's disease; HD-ISS: Huntington's Disease Integrated Staging System; LLOQ: lower limit of quantitation; mHTT: mutant huntingtin protein; MRD: minimum required dilution; NULISA: Nucleic Acid Linked Immuno-Sandwich Assay; polyQ: polyglutamine; SMC: Single Molecule Counting; tHTT or "total HTT": polyQ length-independent huntingtin protein; wtHTT: wild-type huntingtin protein

References

- Wild, et al. *J. Clin. Invest.* 2015
- Fodale, et al. *J. Huntington's Dis.* 2017
- Vauleon, et al. *Sci. Rep.* 2023
- Rodrigues, et al. *Sci. Transl. Med.* 2020
- Feng, et al. *Nat. Commun.* 2023
- Alamar Biosciences Inc., Fremont, CA
- Tabrizi, et al. *Lancet Neurol.* 2022

