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# Mutant Huntingtin (mHTT) Protein Quantitative Assay: Clinical Sample Analysis Support


**FOR FURTHER INFORMATION:**

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## SUPPORT THERAPEUTIC APPROACHES AIMED TO LOWER MUTANT HTT EXPRESSION THROUGH ANALYSIS OF CSF SAMPLES FROM CLINICAL TRIALS

- mHTT in CSF samples is a pivotal efficacy biomarker in the characterization of HD, both as a disease progression biomarker and a pharmacodynamic readout for HTT-lowering therapeutic approaches
- mHTT assay is based on the single molecule counting (SMC) method using the innovative SMCxPRO platform
- SMCxPRO is a robust, reproducible and ultrasensitive laser-based instrument designed to perform digital counting of low concentrated biomarkers (pM – fM range)

## 2B7 AND MW1 ANTIBODIES USED FOR MUTANT HTT ASSAY VALIDATION


## SUMMARY OF THE MUTANT HTT ASSAY PARAMETERS VALIDATED FOLLOWING EMA AND FDA BIOANALYTICAL GUIDELINES

| Validation parameter | Results available |
|---|---|
| Calibration Model | 5 parameter logistic (5PL) model |
| Validated Quantification Range (in artificial CSF) | 1.95 to 250 pg/mL |
| Validation Samples/Quality Controls (in artificial CSF) | LLQ: 1.95 pg/mL, LVS/LQC: 5.86 pg/mL, MVS/MQC: 84.6 pg/mL, HVS/HQC: 175 pg/mL, ULQ: 250 pg/mL |
| Precision (%) Within-Run (in artificial CSF) | ≤13.3% |
| Precision (%) Between-Run (in artificial CSF) | ≤13.1% |

| Validation parameter | Results available |
|---|---|
| Accuracy (%) (in artificial CSF) | 90.9% ≤ Accuracy ≤101% |
| Spike Recovery<br>(in artificial and pooled non-HD human CSF) | %CV ≤ 8.1<br>103.4% ≤ Recovery ≤110.8% |
| Specificity<br>(in artificial and pooled non-HD human CSF) | No cross-reactivity between mHTT and wild type HTT-Q19 concentrations corresponding to LLQ, MQC and HQC levels |
| Selectivity<br>(10 individual non-HD human CSF matrices) | No significant matrix interference: pass rate 80% at LLQ, 100% at HQC and 100% unspiked matrices BLQ |
| Interference with human Hemoglobin (Hb)<br>(in pooled non-HD human CSF) | No interference up to 2.4 µg/mL Hb; interference observed at 3.4 µg/mL Hb for mHTT at LLQ level (not observed for mHTT at HQC level) |
| Prozone/Hook Effect | Not observed |
| Stability in artificial CSF | Storage up to 4 hours at room temperature and at 2-8°C; storage up to 31 days at -20°C and at -80°C |
| Freeze-Thaw Stability | Up to 2 cycles from -20°C and from -80°C to room temperature |
| Stability of labelled antibodies | Storage up to 94 days at 2-8°C for capture and detection labelled antibodies |

## FURTHER PARAMETERS TO BE VALIDATED

- Long term stability of mHTT in artificial CSF (at least 3, 6, 12, 24 months)
- Additional long term stability timepoints of the labelled antibodies used for the assay
- Further Hb interference: Hb spiked between 2.4–3.4 µg/mL and mHTT at additional levels
- Dilution Parallelism in human HD CSF samples
- Short/Long Term Stability endogenous mHTT in human HD CSF samples

## ADDITIONAL ASSAYS ON PIPELINE FOR VALIDATION

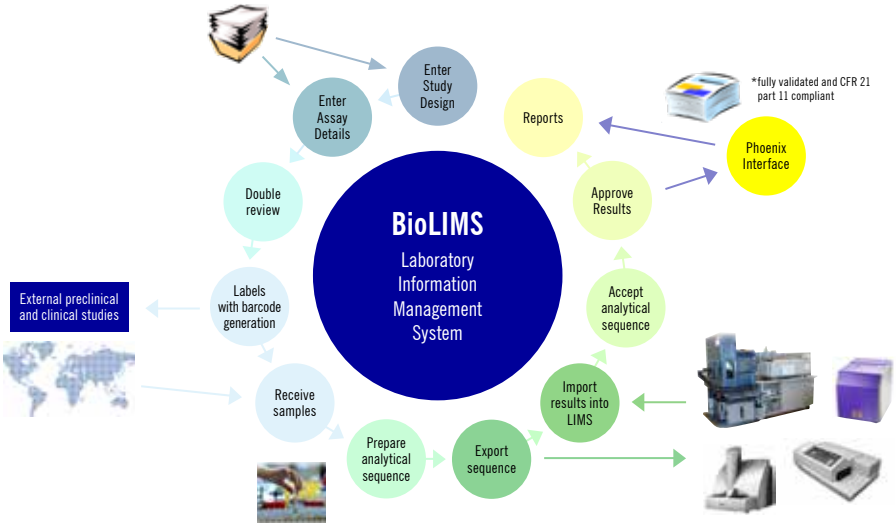
- mHTT assay partial validation to lower quantification range (0.977–125 pg/mL) in CSF
- Total soluble human HTT protein in CSF
- Human Neurofilament light chain (NFL) in CSF and plasma

## SERVICES AVAILABLE AT EVOTEC VERONA IN SUPPORT OF CLINICAL STUDIES

- Provision of kits for samples collection:
  - Collection tubes and storage tubes appropriate to the type of matrix, provided in duplicate to allow clinical sites to retain an aliquot of each sample onsite
  - Tube labels for specimen univocal identification for each subject, with and without barcode
  - Laboratory study manual detailing sample collection, storage and shipment instructions to reflect the study protocol

- Samples receipt from different locations in the world

- Sample storage:
  - Dedicated sample management team
  - 2 storage rooms (one for long-term storage)
  - 27 freezers (–20°C and –80°C storage)
  - Storage capacity: >280,000 samples
  - Fully validated temperature monitoring system
  - Sample storage options after 3 months from report finalization (disposal of samples, return to sponsor, charge for additional storage)


- LIMS validated system for high quality quantitative bioanalysis
- Sample analysis performed within the GLP testing facility regularly inspected by the MoH GLP Monitoring Unit every 2 years
- Authorization to carry out analysis of samples from clinical trials according to Determina AIFA 19 Jun 2015.

Agenzia Italiana del Farmaco AIFA

Ministero della Salute