LCMS methods and traceability of CSF biomarker measurements

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Reliability of medical tests is a major public health challenge

- 60 to 70% of medical decisions are based on an in-vitro diagnostic test
- Results are not always traceable to internationally recognized references

- Results may depend on the method used!!

- **Consequences:**
  1. **Patient’s Health**: risk of inappropriate medical decision
  2. **Economical**: repetition of measurements - 25 to 30% of costs are due to test repetitions, prevention and error detection instead of diagnostic itself (15-30 billion $ / year in the US)
  3. Lack of reliable data for **epidemiological studies and clinical trials**
Reform of medical biology in France

By 2016-2020, accreditation according to ISO 15189 is mandatory for ALL clinical laboratories (both public and private)

In vitro diagnostic Directive on medical devices 2017/746

« The metrological traceability of values assigned to calibrators and/or control materials shall be assured through suitable reference measurement procedures and/or suitable reference materials of a higher metrological order »

LNE’s mission: help clinical labs & IVD industrials to meet regulatory requirements regarding metrological traceability of results

- Development of reference methods for the main biomarkers used in clinical biology: creatinine, glucose, HbA1c, TCh, LDL-C, HDL-C, TG, …
- Production of Certified Reference Materials
- Assignment of reference values to calibration & quality control materials
WP3: Establishing traceability of AD and PD biomarkers measurements

Fit for purpose reference methods and materials for the measurements of tau protein (AD) and α-synuclein (PD) in CSF
Terms of Reference

- To develop an international reference material for cerebrospinal fluid (CSF)

Current Projects

- Collection of CSF material
- Preparation of the reference material
- Establishment of reference methods for the key measurands for assignment of values to the reference material

Calibration of immunoassays for Tau quantification
Principal Biomarkers of Alzheimer’s disease

**Alzheimer Disease:** deposit of plaques and tangles in the brain

Peptide $\alpha$-42

Tau protein

Why measurements matter?

1. Early diagnosis
2. Monitoring of disease progression
3. Monitoring of therapeutic effects (e.g. clinical trials)
Tau (46 kDa):
- Exists in CSF as 6 isoforms of varying length
- Contains many PTMs, especially phosphorylations.
- Low concentration in CSF (around 5 pM).

- Lack of inter-method comparability (immunoassays and LC-MS)
The importance of the definition of the measurand

Tau quantification: definition of the measurand

Routine immunoassays vs. LC-MS

- Primary calibrator for inter-method comparability!
SI-traceable primary calibrator: recombinant Tau 441

1) Intact mass analysis and impurity profile by high resolution mass spectrometry
**SI-traceable primary calibrator:** recombinant Tau 441

2) Tau mass fraction by amino acid analysis ID-LCMS

**Protein hydrolysis**

Tau protein → Labeled amino acids solution → 6N HCl, 130°C, 60h → ID-LCMS (SIR)

**Amino acid analysis – Recombinant Tau**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Tau Mass Fraction (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe</td>
<td>330±10</td>
</tr>
<tr>
<td>Ile</td>
<td>340±10</td>
</tr>
<tr>
<td>Pro</td>
<td>350±10</td>
</tr>
<tr>
<td>Val</td>
<td>360±10</td>
</tr>
<tr>
<td>Leu</td>
<td>370±10</td>
</tr>
</tbody>
</table>

**Standardization curve**

**Ratio aa/aa**

**Peak areas extraction for labelled and unlabelled amino acids**


**Tau LC-MS reference method**

**Measurand:** peptide GAAPPGQK (156-163)

1. **Protein precipitation**
   - 500 µL CSF + Labelled Tau
   - 25 µL perchloric acid (70%)
   - Centrifugation
   - 50 µL 1% TFA on supernatant

2. **SPE purification**

3. **Digestion**
   - Reconstitution in Trypsin 1 µg/mL + labelled Internal Standard
   - Incubation at 37°C for 24h
   - Stop digestion 10% formic acid

4. **LC-MS²**
   - UPLC separation (C18)
   - Detection in a Thermo Q-Exactive MS (quadrupole orbitrap in PRM mode)

Bros et al. 2015
*Antibody-free quantification of seven tau peptides in human CSF using targeted mass spectrometry*
Quantification of the target protein in a complex matrix by exclusively following its peptides and their transitions in predefined m/z and retention time ranges.

Fragments detection at high resolution.
Tau LC-MS reference method

Quantification using a primary calibrator

- Selection of the most appropriate labelled internal standard
- Production of secondary calibrators (commutable materials)
- Metrological calibration of immunoassays

![Graph showing ratio GAA/GAA* vs. Tau conc.](image)

- GAAPPGQK + GAAPPGQK* (IS)
- aCSF
- Tau + GAAPPGQK* (IS)
- aCSF
- Ratio GAA/GAA* vs. Tau conc.
- R² = 0.9974

- Peak area ratio vs. Tau conc. (pM)
- CSF pools
- Tau conc. (PM)
- LCMS
- ELISA
- x 30

5 December 2017
JCTLM Members’ and Stakeholders’ meeting
Next steps

- Primary calibrator
- Validation of the reference measurement procedure
- Secondary calibrators
- Commutability study in 2018

- Submission of a new EMPIR project on NDD diseases (beginning 2018)
- Any suggestions? Partners?
Conclusions: take-home messages...

- The importance of the measurand definition: ELISA vs MS

- Difficulty of validation of the reference measurement procedures: sensitivity problems, structural heterogeneity, matrix complexity, small sample volumes

- Importance of industrial cooperation
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