Metrology to enable rapid and accurate clinical measurements in acute management of sepsis

SEPTIMET: metrology developments to improve identification and treatment of sepsis – Focus on Procalcitonin activities

Dr Amandine Boeuf, LNE
In 2017, WHA and WHO made sepsis a global health priority. Resolution to improve the prevention, diagnosis and management of sepsis.

https://www.worldsepsisday.org/
Patient outcomes and therapy: **the sooner the better!**

Diagnostic == critical factor in managing sepsis, but **still a challenge** for clinicians in ICU and emergency departments.

From Kumar et al. Crit Care Med 2006; 34: 1589=96
Metrology to enable rapid and accurate clinical measurements in acute management of sepsis

Coordinator: Jim Huggett (LGC)
Start: September 2019

Objective:
Improve the speed, accuracy and reproducibility of diagnostic tests for the identification and treatment of sepsis

WP1. Patients recruited from hospital & community settings from four European institutions
Routine test measurements submitted to project database
Samples for clinical metrology research and biomarker evaluation

WP2. Reference methods for fast accurate diagnosis of sepsis
Improved identification of ‘at risk’ patients

WP3. Metrological framework for rapid pathogen identification
Faster/near patient treatment guidance

WP4. Investigation of the metrological framework needed to underpin new & innovative approaches for sepsis
Novel methods/new diagnostic targets

WP5. Data analysis & evaluation of metrological support required for fast/point of care sepsis testing
Consortium database development and evaluation of measurement uncertainty

WP6. Impact/European Network of Excellence: Clinicians, Industry, NMIs, Standards Bodies, Legislators, Academics & Non-profit organisations
Procalcitonin (PCT)

- 116 amino acid polypeptide
- Precursor of calcitonin
- ↑ when bacterial inflammation
- Peak level achieved rapidly
- ↓ rapidly after end of injury

→ PCT concentration may rise to 100 ng/mL
→ Low PCT concentration (0.05 ng/mL)

Specific biomarker for bacterial infection

From Lindsheid et al, Endocrinology, 2003;144(12):5578-5584

Procalcitonin assays

- Routine assays → Immunoasays
- No higher order reference measurement procedures
- No Certified Reference Materials

EQA program performed by ANSM in France in 2014 and 2015

M. Dipalo et al. / Practical Laboratory Medicine 2 (2015) 22–28

→ Need of standardisation?

→ Evaluate the needs and feasibility

→ 17-20% between-methods RSD observed

→ Good correlation at low PCT level (clinically relevant level)
1/ Develop and validate a reference measurement procedure for PCT absolute quantification by IDMS to establish metrological traceability of results to the SI Units

2/ Document and understand the variability of results provided by the different commercially available PCT assays

3/ Evaluate the feasibility for standardisation of PCT assays through common calibration with commutable calibrators

4/ If standardisation of PCT assays appears desirable and feasible:
   - Produce commutable calibrators value assigned with the IDMS reference method
   - Effectively recalibrate PCT assays
   - Assess accuracy and comparability of PCT assays
   - Evaluate the impact of assays recalibration
Calibrators: Synthetic peptides

POSTER P-12: Candidate reference method to establish traceable PCT measurement results
Huu Hien Huynh et al.
Procalcitonin

Calibrators: recombinant protein, supplied in 180 aliquots of 50 µL

Characterisation of primary calibrator:

- Quantification by Amino Acid Analysis
- Impurity identification and quantification
- Correction of Amino Acid Analysis results
**Amino Acid Analysis**

*first results*

(Secondment of H.H. Huynh in July 2019)

<table>
<thead>
<tr>
<th></th>
<th>Test 1 (from one aliquot)</th>
<th>Test 2 (from two aliquots)</th>
<th>Test 3 (from two aliquots)</th>
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<tbody>
<tr>
<td>Concentration</td>
<td>865.3</td>
<td>721.4</td>
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<td>(µg/mL)</td>
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<td>Mean concentration</td>
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<td>(µg/mL)</td>
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<td>CV%</td>
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</tbody>
</table>

→ Homogeneity study on-going
Purity analysis (LC-MS)

first results

U3000 / Q Exactive Focus (Thermo scientific)
Pepmap100 C18 column, 3µm, 1 x 100 mm
Mobile phase A: H2O, 0.1% Formic Acid
Mobile phase B: ACN, 0.1% Formic Acid
T column: 25°C
Purity analysis (LC-MS) first results

(secondment of A. Boeuf in Sept 2019)

Vanquish / Q Exactive Plus (Thermo scientific)
ACE 3 C4-300, 2.1 x 100 mm
Mobile phase A: H2O, 0.1% Formic Acid
Mobile phase B: ACN, 0.1% Formic Acid
T column: 25ºC
Procalcitonin

Calibrators: recombinant protein, supplied in 180 aliquots of 50 µL

Characterisation of primary calibrator:

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