Establishment of a sustainable measurement infrastructure for standardised measurement of cardiovascular disease biomarkers

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Background

- Cardiac diseases, with 11.3 million new cases and 1.8 million deaths per year, are one of the main challenges for health care in the EU
- Estimated to cost the EU economy: € 210 billion per year
- Quantification of cardiac biomarkers for diagnosis is very difficult and challenging, the residual risk of undiagnosed cases is high and can be lethal
- Regulation (EU)2017/746 requires the metrological traceability of medical test results
- Large between-methods variability due to a lack of reference measurement procedures (RMPs) / traceability chains

Biomarkers used in patient stratification and long-term CVD risk assessment apolipoproteins

Development of a reference measurement system for a panel of apolipoproteins (ApoA-I, B, C-I, C-II, C-III, E and apo(a)) in close collaboration with IFCC WG-ApoMS

https://www.ifcc.org/ifcc-scientific-division/sd-working-groups/wg-apo-ms/

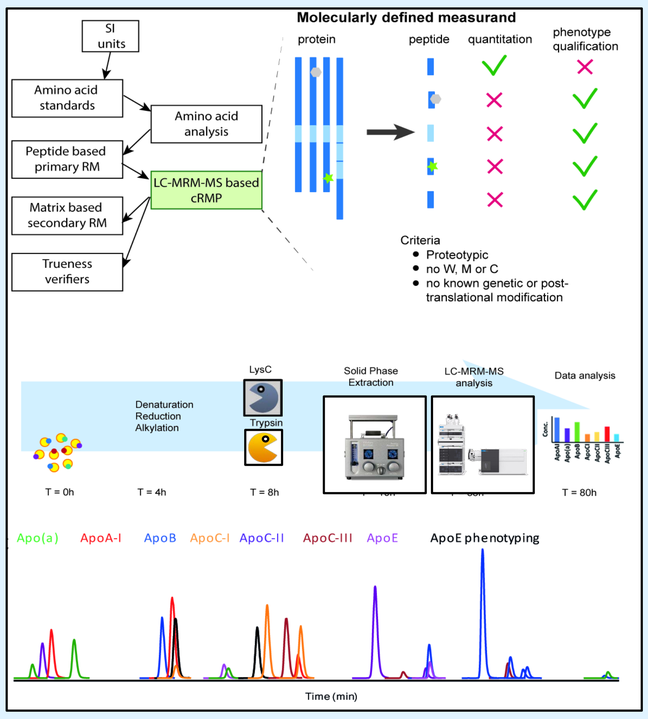
Goal: To establish a traceability chain to the SI units for results of a panel of apolipoproteins consisting of:

➢ **Peptide-based primary reference materials**
- Purity evaluated by high resolution mass spectrometry
- Concentration in solution certified by amino acid analysis

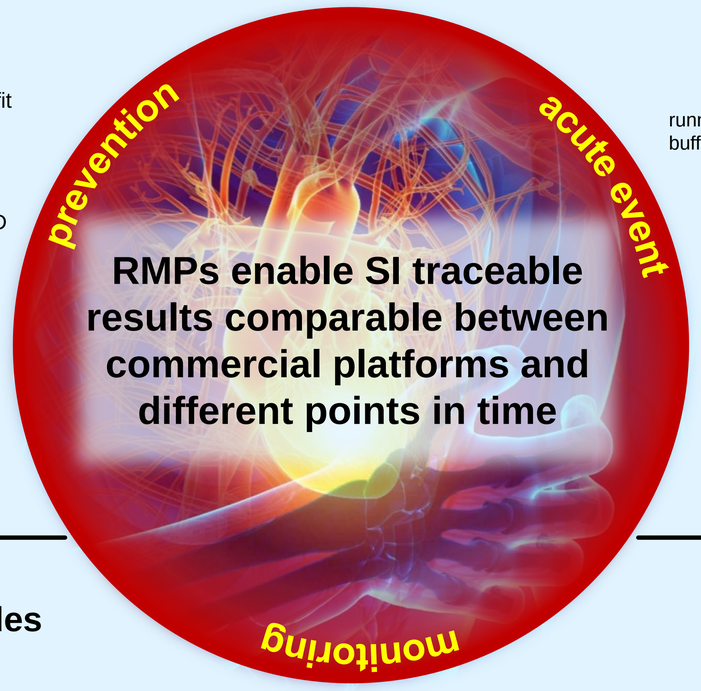
➢ **A candidate RMP using a bottom-up approach by IDMS**
- Target peptides chosen
- Digestion and LC-MS conditions optimised
- Digestion completeness and equimolar release of the proteotypic peptides is currently verified
- Protocol for isolation of proteotypic peptides established

➢ **A sustainable network of calibration laboratories**
- SOP tested among three calibration laboratories
  ⇒ results in good agreement but further comparisons are needed to demonstrate the comparability of results provided by different calibration laboratories in the long run

➢ **Secondary certified reference materials developed to**
- Recalibrate immunoassays in close cooperation with assay manufacturers to ensure the actual implementation of a new reference measurement system
- Evaluate the accuracy and comparability of results provided by the different immunoassays before and after standardisation



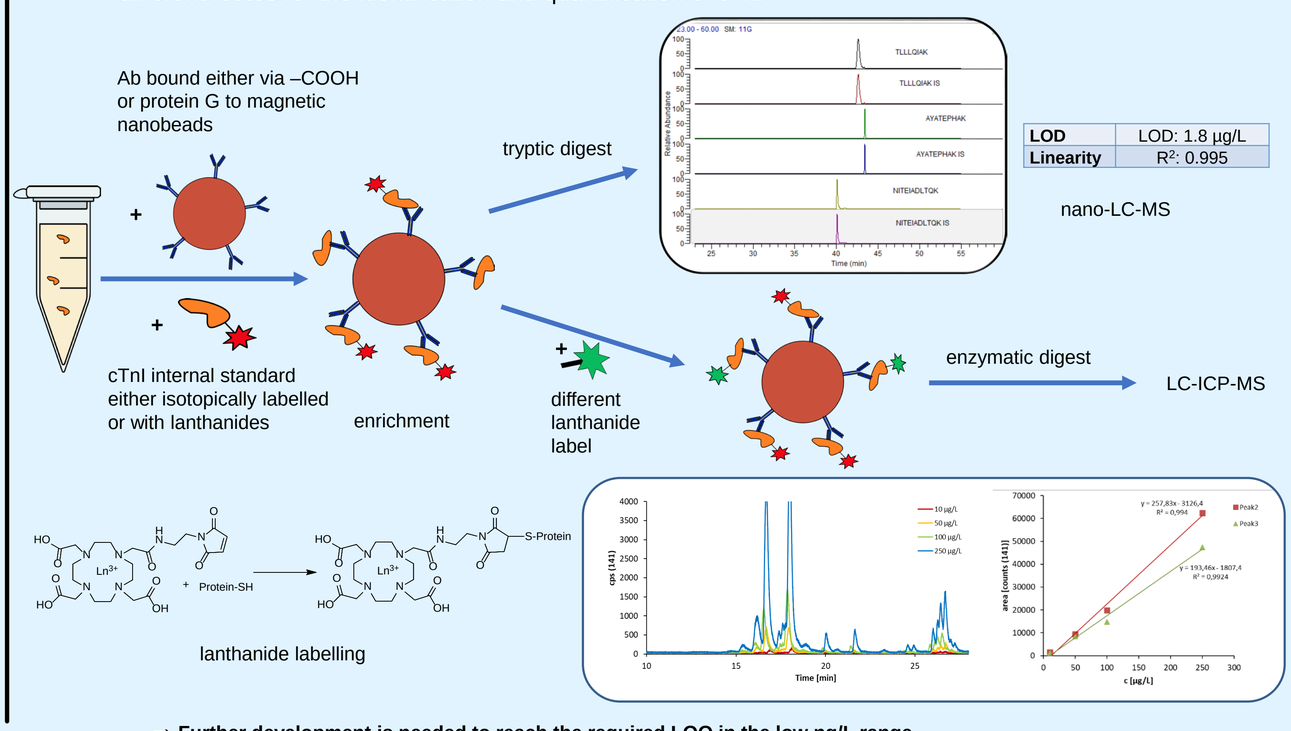
➢ **Evaluate metrology needs for estimating long term CVD risk**
- Extensive data analysis (62 048 patients with myocardial infarction) using conventional biomarkers with a special focus on patients lacking classical risk factors conducted using the SWEDEHEART cardiac registry to identify subgroups of patients who would most profit from using lipoproteins as additional biomarkers
  ⇒ **14.9 % of patients with myocardial infarction had no standard modifiable cardio-vascular risk factors (hypertension, diabetes, hypercholesterolaemia, smoking)**
- An EQA scheme is being organised to further document the state of the art regarding CVD risk using diagnostic tests based on conventional biomarkers (e.g. LDL-c, HDL-c, TG, …) currently used in everyday clinical practice and determine the measurement uncertainty needed for routine methods to accurately stratify patients. It is anticipated that introducing refined and adequate EQA-designs in line with the current state of science will help demonstrating the undisputable clinical fitness for purpose of serum apolipoproteins.
  ⇒ **accuracy and comparability of direct LDL-c assays will be evaluated**
- Participation in CDC's WG on lipid analysis performance criteria for conventional biomarkers currently used to estimate long-term CVD risk
  ⇒ **recommendations are currently discussed with assay manufacturers and are expected to be published before end 2022**



Acute rule out diagnosis: Cardiac troponin

RMPs as basis of a future reference measurement system for cTnI in cooperation with IFCC WG-TNI

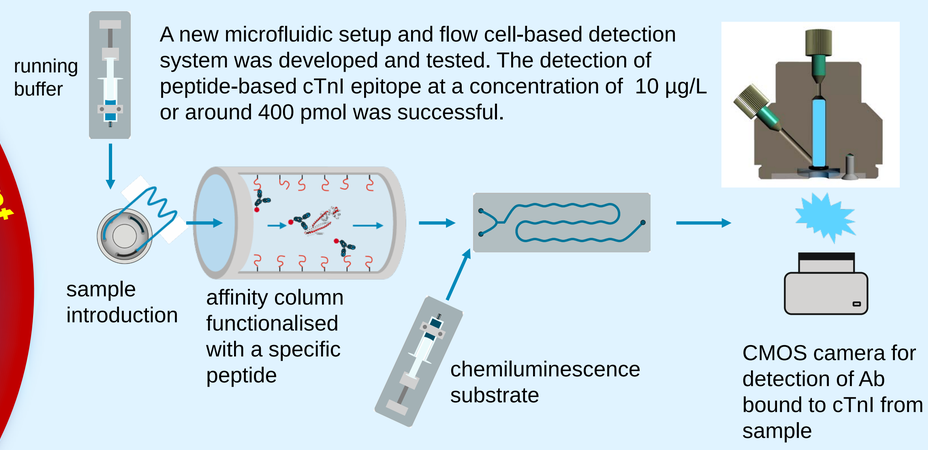
Goal: To develop a RMP based on IDMS capable of determining cTnI at clinical relevant levels investigating different routes for the identification and quantification of cTnI



LOD	LOD: 1.8 µg/L
Linearity	R^2: 0.995

⇒ **Further development is needed to reach the required LOQ in the low ng/L range**

Biosensor for cTnI



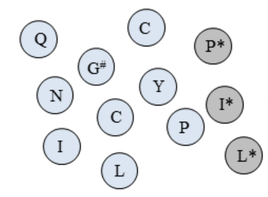
Diagnosis and outcome prediction: Natriuretic peptides

NT-proBNP primary calibrator - Quantification via amino acid analysis and double exact matching IDMS

Hytest NT-proBNP
No significant impurities

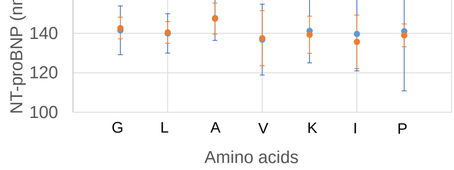
MHPLGSPGSASDLETSGLQEQRNHL
QGKLSELQVEQTSLEPLQESPRPTGV
WKSREVATEGIRGHRKMVLYTLRAPR

Microwave assisted
Acid hydrolysis (6M HCl)
~ 3 hours [1]



Derivatisation

GC-MS/MS
SIM experiments



Average nmol/g	*u*	*U* (*k*=2)	RSU %
140.697	2.045	4.089	2.906

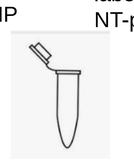
Average value of selected primary calibrator:
(1207.9 ± 17.6) mg/L (2.9 % RSU)

Pritchard et al. *Analytical Biochemistry, 412,* 2011, 40-46

NT-proBNP method development for quantification in serum

1. Spike human serum with native NT-proBNP and add labelled

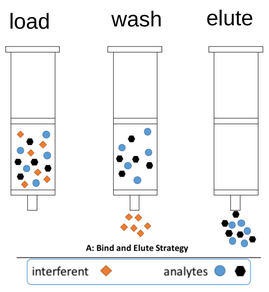
native NT-proBNP ●
labelled NT-proBNP* ●



500 µL human serum

Strata X C18 column

2. Solid phase extraction (SPE) vacuum centrifuge



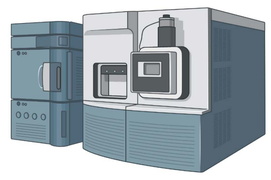
Wash 1: 0.1 % FA
Wash 2: 20 % ACN + 0.1 % FA
Elution: 65 % ACN + 0.1 % FA

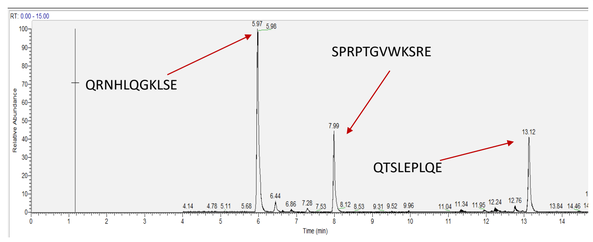
3. GluC digestion (50 µg)
- Reconstitution in ammonium bicarbonate buffer pH 7.8
- Addition of GluC (50 µg)

HPLGSPGSASDLETSGLQE**QRNHLQGKLSE**LQ
VE**QTSLEPLQE** | **SPRPTGVWKSRE**VATEGIR
GHRKMVLYTLRAPR

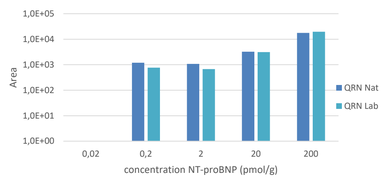
QRNHLQGKLSE
QTSLEPLQE
SPRPTGVWKSRE

4. Capillary flow TQ-XS and MRM Data Analysis





Sensitivity in human serum
QRN peptide



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