18HLT10 CardioMet

Providing the measurement infrastructure to allow quantitative diagnostic methods for biomarkers of coronary heart diseases

A Joint Research Project within the European Metrology Research Programme EMPIR.
- 11.3 million new cases of cardiac diseases and 1.8 million deaths/year in the EU with estimated costs of € 210 billion/year

- Large between-methods variability due to a lack of traceability chains required by IVD Regulation 2017/746

- Measurement of cardiac biomarkers is challenging due to the low concentrations, structural heterogeneity & complex biological matrix

### WP1: Biomarkers for long term CVD risk assessment
- Define metrology needs and performance specifications for conventional biomarkers
- Standardization of ApoA-I, B, C-I, C-II, C-III, E & apo(a) through the development of an IDMS RMP & CRMs
- Document the clinical utility of apolipoprotein profiling advanced lipoprotein testing

### WP2: Biomarkers for acute myocardial infarction
- Development of a candidate reference method for cTnI
- Application to patient samples & EQA materials
- Development of a biosensor for quasi-continuous monitoring of cardiac biomarkers

### WP3: Biomarkers for acute and chronic heart failure
- Reference a measurement procedure for NT-proBNP
- Application of reference measurement procedure for NT-proBNP to EQAS
- Understanding issues for 1-32 BNP measurements in clinics
WP1: Biomarkers for patient stratification and long-term CVD risk assessment

Objective: provide the metrological support needed for clinicians to accurately predict CVD risk and properly stratify patients to select the best therapy

✓ Task 1.1: document the state of the art in terms of CVD risk assessment and patient stratification based on conventional biomarkers with the goal of defining metrology needs and performance specifications for accurately estimating long-term CVD risk.

✓ Task 1.2: standardization of a panel of apolipoproteins (apo) A-I, B, C-I, C-II, C-III, E and apo (a) through the development of an IDMS reference method and matrix CRMs.

✓ Task 1.3: document the clinical utility of alipoprotein profiling vs currently used lipid markers and determine performance specification of advanced lipoprotein testing methods for accurate CVD risk assessment and patient stratification.
Why reliable lipid / lipoprotein testing is important

Table 6 Risk factor goals and target levels for important cardiovascular risk factors

<table>
<thead>
<tr>
<th>Smoking</th>
<th>No exposure to tobacco in any form.</th>
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</thead>
<tbody>
<tr>
<td>Diet</td>
<td>Low in saturated fat with a focus on wholegrain products, vegetables, and fruit and fish.</td>
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<tr>
<td>Physical activity</td>
<td>At least 150 minutes a week of moderate aerobic PA (30 minutes for 5 days/week) or 75 minutes a week of vigorous aerobic PA (15 minutes for 5 days/week) or a combination thereof.</td>
</tr>
<tr>
<td>Body weight</td>
<td>BMI 20–25 kg/m². Waist circumference &lt;94 cm (men) or &lt;80 cm (women).</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>&lt;140/90 mmHg¹</td>
</tr>
<tr>
<td>Lipids¹</td>
<td>Very high-risk: &lt;1.8 mmol/L (&lt;70 mg/dL), or a reduction of at least 50% if the baseline is between 1.8 and 3.5 mmol/L (70 and 135 mg/dL)²</td>
</tr>
<tr>
<td>LDL¹ is the primary target</td>
<td>High-risk: &lt;2.6 mmol/L (&lt;100 mg/dL)³, or a reduction of at least 50% if the baseline is between 2.6 and 5.1 mmol/L (100 and 200 mg/dL).</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>No target but &gt;1.7 mmol/L (&lt;150 mg/dL) in men and &gt;1.2 mmol/L (&lt;45 mg/dL) in women indicates lower risk. Higher levels indicate a need to look for other risk factors.</td>
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</table>


¹ Blood pressure levels are expressed in mmHg and are age-adjusted.
² Some evidence suggests that patients with diabetes may benefit from even lower levels of LDL cholesterol.
³ In patients with a history of coronary heart disease (CHD) and diabetes, lowering LDL cholesterol further or attempting to achieve lower levels of LDL cholesterol can significantly reduce the risk of heart attack and stroke.
Task 1.1: Defining metrology needs for estimating long-term CVD risk with current biomarkers

Objective: document the state of the art regarding CVD risk prediction on the basis of diagnostic tests currently used in day to day clinical practice and determine what is the measurement uncertainty needed for routine methods to accurately stratify patients based on concentration of conventional biomarkers (e.g. LDL-C, non-HDL-C, TG, …).
Task 1.1: conventional biomarkers

Using the Swedish cardiac registry, establish the relationship between the concentration of conventional biomarkers (e.g. LDL-C, non-HDL-C, ApoB…) and CVD events with the objective to estimate the rate of residual CVD risk during high intensity secondary preventive treatment.
Organize an EQA scheme to document accuracy and between-methods agreement of assays relying on conventional biomarkers with a focus on direct LDL-C assays in presence of elevated concentrations of triglycerides.

Propose recommendations for analytical performance criteria for assays relying on conventional biomarkers currently used to estimate long-term CVD risk.
Task 1.1: conventional biomarkers

Lipids Analytical Performance Criteria Work Group

- Nader Rifai, PhD (Boston Children's Hospital)
- Mariko Harada-Shiba, MD, PhD (National Cerebral and Cardiovascular Center)
- Vincent Delatour, PhD (Laboratoire National de Métrologie et d'Essais)
- Jacques Genest, MD, FRCP®, FAHA (McGill University Health Centre - Division of Cardiology)
- Greg Miller Jr, PhD (Virginia Commonwealth University)
- Anette Varbo, MD, PhD (Region Hovedstaden - Copenhagen University Hospital)
- Børge Nordestgaard, MD, DMSc (University of Copehagen)
- John Chapman, PhD (Pierre and Marie Curie - Sorbonne Université)

Centers for Disease Control and Prevention

- Hubert Vesper, PhD, Director – CDC’s Clinical Standardization Programs
- Uliana Danilenko, PhD
- Nasim Khoshnam, BS, MS
- Fidelia Pokuah, BS, MPH
Lipids Analytical Performance Criteria Work Group

Background
- Accurate blood lipid measurements are critical for correct patient classification and monitoring the success of treatments
- Accuracy and precision requirements for blood lipid tests were defined by the NCEP over 20 years ago
- Findings about the accuracy and reliability of tests results from patients with certain conditions and new target goals for blood lipids outlined in new clinical practice guidelines warrant the review and revision of current performance criteria

Objective
- Review current analytical performance criteria in light of analytical performance of blood lipid testing
- Develop recommendations for new analytical performance goals where needed
- Publish recommendations in peer reviewed journal

Scope
- The working group will focus on currently used performance criteria for total cholesterol, total glycerides, HDL-cholesterol, LDL-cholesterol and will investigate the possibility to establish performance goals for other CVD markers such as non-HDL-cholesterol and apoB.
Beyond LDL-C in CVD risk assessment

Cardiovascular Risk Tracks With Particles, Not Cholesterol

More Particles
Higher Risk

LESS RISK
FOR HEART DISEASE

LESS PARTICLES
SAME CHOLESTEROL LEVELS
LESS TRAFFIC

MORE PARTICLES
SAME NUMBER OF PASSENGERS
MORE TRAFFIC

MORE RISK
FOR HEART DISEASE

LDL-C=100 mg/dl

Large LDL Particles

Small LDL Particles

LDL Cholesterol Balance
Apolipoprotein B and Cardiovascular Disease Risk: Position Statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices

John H. Contois,¹¹ Joseph P. McConnell,² Amar A. Sethi,³ Gyorgy Csako,³ Sridevi Devaraj,⁴ Daniel M. Hoefner,⁵ and G. Russell Warnick⁶

“In light of the mounting evidence, the members of this working group of the Lipoproteins and Vascular Diseases Division of the AACC believe that apoB and alternate measures of LDL particle concentration should be recognized and included in guidelines, rather than continuing to focus solely on LDL-C.”
Association of Apolipoprotein B and Nuclear Magnetic Resonance Spectroscopy–Derived LDL Particle Number with Outcomes in 25 Clinical Studies: Assessment by the AACC Lipoprotein and Vascular Diseases Division Working Group on Best Practices

Thomas G. Cole,1* John H. Contois,2 Gyorgy Csako,3 Joseph P. McConnell,4 Alan T. Remaley,3 Sridevi Devaraj,5 Daniel M. Hoefner,4 Tonya Mallory,4 Amar A. Sethi,6 and G. Russell Warnick4

CONCLUSIONS: In most studies, both apo B and LDL-P were comparable in association with clinical outcomes. The biomarkers were nearly equivalent in their ability to assess risk for CVD and both have consistently been shown to be stronger risk factors than LDL-C. We support the adoption of apo B and/or LDL-P as indicators of atherogenic particle numbers into CVD risk screening and treatment guidelines. Currently, in the opinion of this Working Group on Best Practices, apo B appears to be the preferable biomarker for guideline adoption because of its availability, scalability, standardization, and relatively low cost.

Michel R. Langlois,1* M. John Chapman,2 Christa Cobbaert,3 Samia Mora,4 Alan T. Remaley,5 Emilio Ros,6 Gerald F. Watts,7 Jan Borén,8 Hannsjörg Baum,9 Eric Bruckert,10 Alberico Catapano,11 Olivier S. Descamps,12 Arnold von Eckardstein,13 Pia R. Kamstrup,14 Genovefa Kolovou,15 Florian Kronenberg,16 Anne Langsted,14 Kari Pulkki,17 Nader Rifai,18 Grazyna Sypniewska,19 Olov Wiklund,8 and Børge G. Nordestgaard,14 for the European Atherosclerosis Society (EAS) and the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Joint Consensus Initiative

Despite the overwhelming evidence that LDL-C-targeted strategies effectively reduce CVD, there is substantial between-subject variability in the response to lipid-lowering therapies and the reduction of CVD risk (7). Furthermore, accumulating evidence indicates that a focus solely on the assessment and management of LDL-C is not an optimal strategy for all patients.

Clearly, additional biomarkers beyond LDL-C are needed to identify and treat more persons at high CVD risk.

Consensus-based recommendation. Non-HDL-C and apoB tests are more accurate than dLDLC and cLDLC, especially for measurements in samples that are hypertriglycerideremic, nonfasting, or at low LDL-C concentrations.
Beyond LDL-C in CVD risk assessment

Plasma concentrations of LDL cholesterol (LDL-C) are positively associated with increased risk of atherosclerotic cardiovascular disease. There is a variety of robust evidence indicating that this association is causal in nature. First, rare and common genetic variants that specifically influence LDL-C concentrations are also strongly associated with cardiovascular risk. Second, interventions that reduce LDL-C, especially but not exclusively statin therapy, reproducibly reduce cardiovascular events. In fact, the data with statins are so strong that they are often used in patients whose LDL-C concentrations are not particularly increased, a setting in which statins have still been shown to reduce cardiovascular risk. Thus there is substantial interest in lipoprotein-related biomarkers that provide information about future cardiovascular risk above and beyond LDL-C itself.

Several methods have emerged that allow a more direct quantification of the number of LDL particles. Because an LDL particle contains a single molecule of apo B, it is possible to directly estimate the number of particles through a simple measurement of apo B concentration (particularly when expressed in molar units). apo B is typically measured by immunonephelometry or immunoturbidimetry, and reagents are available from a wide variety of manufacturers. Standardization of these measurements has been facilitated by the availability of WHO-IFCC reference materials (SP3–07, SP3–08) (4, 5). apo B analytical measurements have shown good reproducibility across laboratories (6%–8% CV in 2012 College of American Pathologists survey), although a number of preanalytical biological confounders, including diurnal and seasonal effects, have been described.

How do the different ALT methods compare with each other?
Comparability of non-HDL-P measurements by IN, LC-MS/MS, NMR, ES-DMA and VAP
Comparability of Lipoprotein Particle Number Concentrations Across ES-DMA, NMR, LC-MS/MS, Immunonephelometry, and VAP:
In Search of a Candidate Reference Measurement Procedure for apoB and non-HDL-P Standardization


- The different candidate reference methods for non-HDL-P do not yet provide equivalent results
- LC/MS/MS is the most suitable candidate RMP to standardize ApoB as it would provide results traceability to the SI
- Primary calibrators are needed to calibrate the IDMS RMP
Apolipoproteins by Mass Spectrometry (WG-APO MS)

### Membership

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<td>NL</td>
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<td>2017 01 - 2019 12</td>
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<td>V. Delatour</td>
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<td>J. Dittrich</td>
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<td>C. Hirtz</td>
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<td>A. Hoofnagle</td>
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<td>Z. Kuklenyik</td>
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<td>L.R. Ruhaak</td>
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<td>H. Althaus</td>
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<td>G.M. Kostner</td>
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<tr>
<td>I. Zegers</td>
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### Terms of Reference

- To achieve standardization of a panel of clinically relevant serum apolipoproteins (apo) A-I, B, C-I, C-II, C-III, E and apo (a) (including qualitative phenotyping where needed). Standardization is done in such a way that measurement results are traceable to SI as outlined in ISO 17511. Other traceability chains will be used in cases where traceability to SI cannot be achieved.
- To evaluate clinical performance and clinical utility of serum apolipoprotein panel(s) for CVD risk stratification and treatment, in comparison to or together with contemporary blood lipids.
Objective: set up an MS-based reference measurement system for standardization of a panel of clinically relevant apolipoproteins

✓ Production and characterization of primary calibrators
✓ Development of an IDMS-based reference measurement procedure for the multiplexed analysis of apoA-I, B, C-I, C-II, C-III, E and Apo(a)
✓ Production and characterization of commutable CRMs and EQA materials
✓ Evaluation of the clinical performance and clinical utility of apolipoprotein profiling for CVD risk stratification and treatment
Objectives:

- Document the clinical utility of advanced lipoprotein testing methods for accurate CVD risk assessment and patient stratification;
- Propose performance specification and suitable routes for standardization

- Identify theoretical subgroups of patients with acute coronary syndrome and specific lipid disorders for which apolipoprotein profiling and other advanced lipoprotein testing methods are expected to have the highest value added for CVD risk assessment and patient stratification compared with conventional makers.

- Establish a patients cohort and measure samples by apolipoprotein profiling and other available advanced lipoprotein testing methods with the objective to:
  - Document the clinical utility of advanced lipoprotein testing to unravel residual CVD risk that cannot be diagnosed with conventional markers,
  - Establish standardized reference ranges for apolipoproteins.
Task 1.3: Towards advanced lipoprotein testing?

Are advanced lipoprotein testing and subfractionation clinically useful?

Advanced Lipoprotein Testing and Subfractionation Are Not (Yet) Ready for Routine Clinical Use

Samia Mora, MD, MHS

Cardiac troponin (cTn): Complex of three regulatory proteins

- **Troponin T**
  - Specific marker
  - 35 924 Da
  - Binds to Tropomyosin

- **Troponin I**
  - Specific marker
  - 24 008 Da
  - Binds to Actin

- **Troponin C**
  - Cardiac and slow skeletal same
  - 18 403 Da
  - Ca binding part

⇒ Diagnosis of acute coronary syndrome in combination with electrocardiograms
After release into the blood:

- Trimer ICT
- Dimer IC
- Monomers I, C, T
- Fragments
- Phosphorylation
- Acetylation
- Glycosylation

For diagnosis, the increase of cTn over 3 h is important

Wu A. Analytical Issues for Clinical Use of Cardiac Troponin. Cardiovascular Biomarkers: Pathophysiology and Disease Management, Morrow DA (ED.), 2006.
Proficiency test for cardiac markers CM 4/19 organised by RfB

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<th>Sample</th>
<th>A/µg/L</th>
<th>B/µg/L</th>
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<td>Mean</td>
<td>0.915</td>
<td>0.36</td>
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<td>CV</td>
<td>57.201</td>
<td>55.458</td>
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<tr>
<th>Sample</th>
<th>A/µg/L</th>
<th>B/µg/L</th>
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<tr>
<td>Mean</td>
<td>1.099</td>
<td>0.334</td>
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<tr>
<td>CV</td>
<td>14.104</td>
<td>10.327</td>
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<td>Participants</td>
<td>590</td>
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WP2: State of the Art

Results from the French mandatory EQA scheme in 2015

Between-lab CV : 79%!
Task 2.1: Preparation and characterisation of reference and spike for cardiac troponin

Task 2.2: Development and validation of a suitable quantification method

Task 2.3: Application to patient samples

Task 2.4: Development of a biosensor for the quasi-continuous monitoring of cardiac biomarkers
### WP2: connection with IFCC WG-cTnI

**Standardisation of Troponin I (WG-TNI)**

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<th>Position</th>
<th>Country</th>
<th>Term</th>
<th>Time in Office</th>
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<td>Chair</td>
<td>US</td>
<td>1st</td>
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<td>D. Armbruster</td>
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<td>J. Barth</td>
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<td>A. Katrulka</td>
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<td>M. Panteghini</td>
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<tr>
<td>L. Wang</td>
<td>Member</td>
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</tbody>
</table>

**Terms of Reference**

- Development of a candidate secondary reference measurement procedure and candidate secondary reference material for cardiac troponin I (cTnI)
- Testing for cTnI standardization and clinical validation by comparison with validated commercial assays in a round robin study

**Current Projects**

- Preparation of a secondary reference material for cTnI consisting of three cTnIpositive serum pools (Phase 2)
- Validation of cTnI standardization through a round robin after a value transfer using the secondary reference material as common calibrator (Phase 3)

**NIST**

- Produced certified reference material SRM 2921 (pure cardiac troponin complex)
- Developed an ID-LC-MS/MS method for cTnI: *Analytical and Bioanalytical Chemistry (2018) 410:2805–2813*
WP3: Biomarkers for acute & chronic heart failure

NT-proBNP ≥125pg/mL
BNP ≥35pg/mL
Brain Natriuretic Peptides

NT-proBNP
- 76 amino acid glycosylated protein
- Not active
- Stability 60-120min

Pro-BNP
- 108 amino acid protein

BNP
- 32 amino acid peptide
- Active form
- Stability 20min
- Bio-therapeutic
WP3 : Need for BNP standardization!

Poor agreement between the different available immunoassays!
### Results from the French mandatory EQA scheme in 2014

**Tableau II : BNP (ng/L) – résultats, échantillon C6**

<table>
<thead>
<tr>
<th>Techniques ou appareils</th>
<th>Effectif</th>
<th>%</th>
<th>Moyenne (ng/L)</th>
<th>CV (%)</th>
<th>Moyenne +/- 2ET</th>
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<td>EIA, fluorimétrie</td>
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<td>45,9</td>
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<td>27,8</td>
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<td>0,8</td>
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<td>TOSOH Bioscience, AIA série I ST AIA Pack BNP</td>
<td>28</td>
<td>5,4</td>
<td>26,6</td>
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<td>EIA, luminéométrie</td>
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<td>BECKMAN Coulter, Triage BNP w/Access, Dx &amp; LXI systems</td>
<td>127</td>
<td>24,6</td>
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<td>BECKMAN Coulter UniCel DxC 600/600i</td>
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<tr>
<td>ABBOTT, ARCHITECT® ii® systems BNP, 8K28</td>
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**Between-lab CV : 27%!**
1-32 BNP degradation patterns

Firendo 2: The known main degradation sites of BNP by the action of DPP IV, NEP and IDE

NEP

IDD

DPP IV

BNP(1-32) SPKMQVQGSGCFGRKMDRISSSSSLGCKVLRHH
BNP(3-32) KMQVQSGCGFRKMDRISSSSSLGCKVLRHH
BNP(4-32) MVQSGCFCGRKMDRISSSSSLGCKVLRHH
BNP(5-32) VQGSCGFGRKMDRISSSSSLGCKVLRHH
BNP (5-31) VQGSCGFGRKMDRISSSSSLGCKVLRHH
BNP (5-27) VQGSCGFGRKMDRISSSSSLGCK
BNP (5-26) VQGSCGFGRKMDRISSSSSLG

JCTLM Members’ and Stakeholders’ meeting - December 2nd & 3rd, 2019 – BIPM
<table>
<thead>
<tr>
<th>Assay</th>
<th>Antibody</th>
<th>Standard materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alere Triage BNP</td>
<td>capture detection</td>
<td>Recombinant BNP</td>
</tr>
<tr>
<td>Beckman Coulter</td>
<td>capture detection</td>
<td>Recombinant BNP</td>
</tr>
<tr>
<td>Abbott</td>
<td>capture detection</td>
<td>Synthetic BNP</td>
</tr>
<tr>
<td>Siemens (Bayer)</td>
<td>capture detection</td>
<td>Synthetic BNP</td>
</tr>
<tr>
<td>Siemens (Dade Behring)</td>
<td>capture detection</td>
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</tr>
<tr>
<td>ET Healthcare</td>
<td>capture</td>
<td>proBNP glycosylated</td>
</tr>
</tbody>
</table>

The different available immunoassays do not target the same epitope and rely on different types of calibrators.
A candidate liquid chromatography mass spectrometry reference method for the quantification of the cardiac marker 1-32 B-type natriuretic peptide

- SI traceable primary calibrator fully characterized
- Stabilisation protocol for BNP in plasma
- Candidate RMP for quantification of BNP in plasma
- Reference method target values assigned to 40 EQA materials
- Correlation between IDMS and immunoassays established
- Method available for monitoring metabolites
- Interlaboratory comparisons LGC / NIST
NT-proBNP immunoassay detection

Objective: enhance BNP measurements and improve patient outcomes through provision of reference measurement procedures & standardization of EQAS:

- development of a candidate reference measurement procedure for NT-proBNP
- application to EQA schemes and clinical samples
- Definition of procedures for the calculation of measurement uncertainty within EQAS
- Definition of commutability requirements for EQA materials for NT-proBNP
- Monitor BNP circulating forms to understand issues for 1-32 BNP measurement
Stakeholders & Partners

Medical associations, national & international organizations

- IFCC (International Federation of Clinical Chemistry and Laboratory Medicine)
- DGKL (Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin e.V.)
- SFBC
- AACC (American Association for Clinical Chemistry)
- EFLM (European Federation of Clinical Chemistry and Laboratory Medicine)
- JCT/LM
- NIH

Health Authorities

- CDC (Centers for Disease Control and Prevention)
- NHS (Greater Glasgow and Clyde)

EQA Providers

- UK NEQAS (Cardiac Markers)
- EQUALIS
- RfB (Referenzinstitut für Bioanalytik)

Manufacturers

- Quest Diagnostics®
- promise DIAGNOSTICS
- VAP PROTEOMICS
- LabCorp (Laboratory Corporation of America)
- Solomon Park
- SUN DIAGNOSTICS

Hospitals

- CHOI (Children's Hospital Oakland Research Institute)
- UCSF Benioff Children’s Hospitals
- Hôpitaux Universitaires Pitie Salpêtrière Charles Foix
- NWRL
Acknolegements