IFCC Scientific Division activities on standardization and traceability

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Chair IFCC Scientific Division
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Scientific Division

- **Mission**: To advance the science of Clinical Chemistry and Laboratory Medicine and to apply it to the clinical practice
- Transfer of research results to the clinical laboratory
- Analytical standardization: reference systems, new techniques
- Post-analytical standardization: establish diagnostic strategies for new biomarkers
- Standards for good laboratory practice
- Collaborations: BIPM, CLSI, ILAC, IRMM, IUPAC, NIST, WHO, ADA, NKDEP
Quality Specifications for Cardiac Troponin Assays

International Federation of Clinical Chemistry and Laboratory Medicine (IFCC)

IFCC Scientific Division
Committee on Standardization of Markers of Cardiac Damage

Prepared for publication by

Mauro Pantechnini, Willie Gerhardt, Fred S. Apple, Francesco Dati, Jan Ravikilde, and Alan H. Wu


Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program

Gary L. Myers, W. Greg Miller, Josef Ceresi, James Fleming, Neil Greenberg, Tom Greene, Thomas Hostetter, Andrew S. Levey, Mauro Pantechnini, Michael Welch, and John H. Eckfeldt for the National Kidney Disease Education Program Laboratory Working Group

National Academy of Clinical Biochemistry and IFCC Committee for Standardization of Markers of Cardiac Damage Laboratory Medicine Practice Guidelines: Analytical Issues for Biochemical Markers of Acute Coronary Syndromes

NACB VP/Chief of Staff, Tom Green; Ross Jeckel, Joel Kesten, Robert H. Christenson, and Michael Welch

Approved IFCC recommendation on reporting results for blood glucose

Standards for good laboratory practice

Circulation

MMITTEE ON STANDARDIZATION OF MARKERS OF CARDIAC DAMAGE (C-SMCD) MEMBERS

Fred S. Apple, Chair; Robert H. Christenson; Allan S. Jaffe, Rochester, MN; Johannes Mair, Innsbruck, Austria; Jordi Ordóñez-Llanos, Barcelona, Spain; Brescia, Italy; Mauro Pantechnini, Milan, Italy; Jillian Tate, Brisbane, Australia; and


Recommendation for measuring and reporting chloride by ISEs in undiluted serum, plasma or blood
STANDARDS IN COOPERATION WITH CLSI:

- Analysis of Body Fluids in Clinical Chemistry; Proposed Guideline (C49-P)
- Body Fluid Analysis for Cellular Composition; Approved Guideline (H56-A)
- Point-of-Care Connectivity; Approved Standard (POCT1-A2)
- Metrological Traceability and Its Implementation; A Report (X05-R)
- Measurement of Free Thyroid Hormones; Approved Guideline (C45-A)
- Application of Biochemical Markers of Bone Turnover in the Assessment and Monitoring of Bone Diseases; Approved Guideline (C48-A)
- Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (EP17-A)
- Performance of Single Cell Immune Response Assays; Approved Guideline (I/IA26-A)
- Diagnostic Nucleic Acid Microarrays; Approved Guideline (MM12-A)
- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline (MM13-A)
- Proficiency Testing (External Quality Assessment) for Molecular Methods; Approved Guideline (MM14-A)
- Use of External RNA Controls in Gene Expression Assays; Approved Guideline (MM16-A)
SD Activities

- IMPLEMENTATION OF STANDARDIZATION IN LABORATORY MEDICINE
  - Reference Materials
  - Reference Measurement Procedures
  - Networks of Reference Laboratories
  - Reference Intervals & Decision Limits

- PUBLICATIONS

- COLLABORATIONS
IFCC standardisation activities are currently accomplished by 8 Committees (theme-oriented) and 13 Working Groups (task-oriented)
CRM 470 - Human Serum Proteins
Effect of CRM 470 on plasma protein results

- **Very Good but Could be Better!**
  - $\alpha_1$-Antitrypsin
  - Haptoglobin
  - Transferrin
  - IgA, G, and M

- **Not Good**
  - Ceruloplasmin
  - C-Reactive Protein

- **Too Few Labs**
  - $\alpha_1$-Acid Glycoprotein
  - $\alpha_2$-Macroglobulin

- **Not Evaluated Yet**
  - $\alpha_1$-Antichymotrypsin
Effect of CRM 470 on plasma protein results

Transferrin

Effect of CRM 470 on plasma protein results

Transferrin

International Federation Of Clinical Chemistry And Laboratory Medicine
Effect of CRM 470 on plasma protein results

C-Reactive Protein

For CRP 1993, samples from Beckman, Boehringer, Roche, and Abbott are shown.

For CRP 2002, samples from Ortho, Ilab, DadeBehring, Dako, Bayer, Olympus, Kone, Beckman, and Orion are shown.
Main reasons for remaining variability

- Inadequate transfer of values by manufacturers from CRM 470 to working calibrators
- Commutability problems (CRM 470 is based on a delipidated and lyophilized serum pool)
COMMITTEE ON PLASMA PROTEINS

ONGOING ACTIVITIES

• standardization of protein measurement in biological fluids:
  - scientific and technical support to IRMM for the preparation of the new reference material for plasma proteins [ERM-DA470]
  - serum collection, completed;
  - selection of laboratories participating in the value transfer, completed;
  - feasibility study, completed;
  - value transfer campaign (Autumn 2007);
  - assignment of values for additional proteins:
    → β2-microglobulin
COMMITTEE ON PLASMA PROTEINS

ONGOING ACTIVITIES

• standardization of protein measurement in biological fluids:
  - scientific and technical support for the preparation of the new ERM
  - value transfer protocol for serum proteins

*Protocol for Transfer of Values from CRM 470 to Manufacturers’ Reference Materials*

A. Myron Johnson, M.D.*; University of North Carolina School of Medicine, Chapel Hill, NC USA 27599-7516, and
Soren Blirup-Jensen, DVM, PhD*; DakoCytomation a/s, Productionsvej 42, DK-2600 Glostrup, Copenhagen, Denmark
Committee on Reference Systems for Enzymes

Reference Measurement Procedure

✓ ALT
✓ CK
✓ LDH
✓ GGT
✓ Amylase
✓ AST*

*Reference material under certification

Evaluation of transferability of ref meas procedure: Alkaline phosphatase
Under discussion: Pancreatic lipase
Establishing Traceability of ALT Results

Alanineaminotransferase (ALT)
Serum or Plasma
SI-Unit: katal/l

Definition of the measurandum

Primary calibrator
Panel of Native Human Samples

IFCC Reference Procedure (2002) - based on physicochemical constants, non-calibrated

Procedure similar to reference measurement procedure but less stringent control of measurement conditions and larger uncertainty

Procedure applying same chemistry and equipment as routine procedure, but more precisely controlled conditions and more replicates to reduce uncertainty

Commercially available system including product reagent and calibrator lot

Product calibrator
Lot of Commercial Product Calibrators

Manufacturer’s master calibrators, e.g. Set of Human Serum Pools

Manufacturer’s selected measurement procedure

Manufacturer’s standing measurement procedure

End User’s Routine Measurement Procedure

Routine sample – Human Patient Specimens, e.g. Serum or Plasma

RESULT
ALT in katal/l

Adapted from CLSI/IFCC document X5-R: Metrological traceability and its implementation
To support reference laboratories in the context of complete reference systems by establishing an EQAS (ring trials) for reference laboratories in order to monitor their competence.
Traceability in Laboratory Medicine

Results from IFCC laboratory network

Results of IFCC ring trials are available at: http://www.dgkl-rfb.de:81
C-RI DL
Reference Intervals and Decision Limits

- Preparation of a protocol for collaborative experiments on the establishment of reference values using assays traceable to reference systems
- Production of “standardized” reference intervals for AST, ALT, and γGT
C-RIDL

Reference Intervals and Decision Limits

Multicenter Reference Interval study for AST, ALT & GGT

Three phases

1. Distribution of commutable trueness materials, collection of information on methods characteristics and on the analytical quality of the group of participants [completed]

2. Collection of reference samples according to a well defined protocol established for each analyte following the principles indicated in CLSI C28 standard [ongoing]

3. Centralized data reduction according to C28.
International assessment of IVD devices for enzyme measurements

✓ A commutable Trueness Control Material (TCM) was developed in the Calibration 2000 project of The Netherlands

✓ This TCM was targeted by 3 IFCC reference labs for ALT, AST, CK, GGT, LDH, and amylase

✓ 70 European laboratories employing the 6 most commonly used instruments/companies (all CE marked) were requested to measure TCM and their results evaluated
International assessment of IVD devices for enzyme measurements

Fig. 1. Target value (fat line), mean = SD₀ (U/L) for each company system, and the area (dashed) of maximum allowable SD₀ in absence of significant bias.
Apolipoprotein B survey

<table>
<thead>
<tr>
<th>Method</th>
<th>No. labs</th>
<th>Survey CHM-01</th>
<th>Survey CHM-02</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>CV</td>
</tr>
<tr>
<td>Beckman INA</td>
<td>20</td>
<td>73.0</td>
<td>3.8%</td>
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<tr>
<td>Dade INA</td>
<td>31</td>
<td>71.0</td>
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<tr>
<td>IT assays</td>
<td>53</td>
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</tr>
<tr>
<td>All</td>
<td>122</td>
<td>71.1</td>
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<tr>
<td>Method</td>
<td># Labs</td>
<td>LP-01</td>
<td>LP-02</td>
</tr>
<tr>
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<td>--------</td>
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</tr>
<tr>
<td><strong>“Direct”</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>1605</td>
<td>108.2 (14.7)</td>
<td>82.0 (17.3)</td>
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<tr>
<td>Surfactant</td>
<td>301</td>
<td>134.5 (5)</td>
<td>105.3 (5.8)</td>
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<tr>
<td>Deterg/Select</td>
<td>219</td>
<td>105.6 (7.6)</td>
<td>77.9 (9.5)</td>
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<tr>
<td>Liquid Select</td>
<td>992</td>
<td>99.9 (6.6)</td>
<td>75.0 (8.8)</td>
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<tr>
<td><strong>Calculated</strong></td>
<td>2931</td>
<td>134.4 (4.7)</td>
<td>106.6 (4.4)</td>
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<tr>
<td><strong>CDC ‘target’ value</strong></td>
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<td>136.9</td>
<td>106.8</td>
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</table>
# Apolipoprotein A-I survey

<table>
<thead>
<tr>
<th>Method</th>
<th>No. labs</th>
<th>Survey CHM-01</th>
<th>Survey CHM-02</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>CV</td>
</tr>
<tr>
<td>Beckman INA</td>
<td>19</td>
<td>129.2</td>
<td>5.0%</td>
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<tr>
<td>Dade INA</td>
<td>26</td>
<td>134.9</td>
<td>5.4%</td>
</tr>
<tr>
<td>IT assays</td>
<td>52</td>
<td>124.5</td>
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</tr>
<tr>
<td>All</td>
<td>115</td>
<td>128.0</td>
<td>6.6%</td>
</tr>
<tr>
<td>Method</td>
<td># Labs</td>
<td>LP-01</td>
<td>LP-02</td>
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<tr>
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<tr>
<td><strong>“Homogeneous”</strong></td>
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<tr>
<td>All</td>
<td>3954</td>
<td>52.4 (8.9)</td>
<td>38.3 (8.1)</td>
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<tr>
<td>Accel Select Deter</td>
<td>616</td>
<td>57.7 (3.9)</td>
<td>41.5 (4.9)</td>
</tr>
<tr>
<td>Liqu Deterg/Selec</td>
<td>1607</td>
<td>53.2 (8.0)</td>
<td>38.5 (7.3)</td>
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<tr>
<td>Modif Enzymatic</td>
<td>890</td>
<td>49.8 (5.1)</td>
<td>37.1 (4.9)</td>
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<tr>
<td><strong>“Precipitation”</strong></td>
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<td></td>
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<tr>
<td>Dex S04 50K</td>
<td>171</td>
<td>48.9 (6.0)</td>
<td>35.7 (5.9)</td>
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<tr>
<td>Magnet Dex S04</td>
<td>213</td>
<td>48.1 (6.4)</td>
<td>35.3 (6.2)</td>
</tr>
<tr>
<td>CDC ‘target’ value</td>
<td></td>
<td>49.3</td>
<td>35.7</td>
</tr>
</tbody>
</table>
Standardization of apolipoprotein measurements

❖ PT data provide very good assessment of state of accuracy and allows direct comparisons of the lipoprotein and apolipoprotein measurement reliability in “real” life.

❖ CAP survey data indicates Apos performance is clearly better than for direct LDL & HDL cholesterol measurements.

❖ Apolipoprotein B & A-I standardization is now available and achievable.
Reference System for HbA1c

Primary reference materials (IRMM 466 and 467)

IFCC reference measurement procedure (HPLC-CE or HPLC-MS)

Secondary reference materials (blood panels)

Manufacturer’s internal reference measurement procedure

Manufacturer’s working calibrator

Manufacturer’s standing measurement procedure

Manufacturer’s product calibrator

Routine measurement procedure

Patient Sample

IFCC Network

Manufacturer

Individual laboratory
Recommendations on the unit and nomenclature for HbA1c:

- **Systematic name** → Haemoglobin beta chain(Blood) — N-(1-deoxyfructos-1-yl)haemoglobin beta chain; substance fraction
- **Trivial name** → Hemoglobin A1c (HbA1c)
- **SI unit** → mmol/mol
Metrological vs. “Clinical” Traceability

- The knowledge about the clinical validity of HbA1c and the decision-making criteria used by physicians in different parts of the world are based on data which are generated with routine tests which are not standardized.

- Tracing back the calibration of these routine tests to the IFCC reference system may then invalidate the clinical decision-making criteria currently used.

- In order to maintain the clinical experience, the quantitative relationship to the previous calibration system should be established and, if necessary, the clinical decision-making criteria should be adjusted accordingly.
In the case of HbA1c, reliable linear relationships between results traceable to the IFCC reference system for HbA1c and previous national and regional recommended methods have been demonstrated, allowing the conversion of analytical and clinical data from one system to another.
Suggested units and target values for HbA1c when measured with methods traceable to the IFCC reference system. A comparison with the current figures is also given.

<table>
<thead>
<tr>
<th></th>
<th>Current&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IFCC traceable methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference interval (non-diabetics)</td>
<td>4-6%</td>
<td>20-42 mmol/mol</td>
</tr>
<tr>
<td>Target for treatment in diabetics&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;7%</td>
<td>&lt;53 mmol/mol</td>
</tr>
<tr>
<td>Change of therapy in diabetics&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;8%</td>
<td>&gt;64 mmol/mol</td>
</tr>
</tbody>
</table>

<sup>a</sup> refer to methods aligned to the U.S. National Glycohemoglobin Standardization Program.

<sup>b</sup> as recommended by American Diabetes Association.

Panteghini M & John G, CCLM 2007; 45: 942
Advantages

The use of a completely different unit (mmol/mol instead of %) avoids confusion when recalculating old HbA1c targets to the new IFCC standardized values if clinical laboratories wish to implement HbA1c results traceable to the IFCC reference system.

A positive impact of changing of scale of reported HbA1c results is expected, allowing clinicians and diabetic patients to better understand the marker changes (currently they may perceive small changes in percentage values – although linked to large health effects – as unimportant).

Supposed increased potential for future use of HbA1c as diagnostic tool.
1. The HbA1c results should be standardized worldwide, including the reference system and results reporting.

2. The IFCC reference system for HbA1c represents the only valid anchor to implement standardization of the measurement.

3. The HbA1c assay results are to be reported worldwide in IFCC units (mmol/mol) and derived NGSP units (%), using the IFCC-NGSP master equation.

4. If the ongoing “average plasma glucose study” fulfills its a priori specified criteria, an HbA1c-derived average glucose (ADAG) value will also be reported as an interpretation of the HbA1c result.

5. Glycemic goals appearing in clinical guidelines should be expressed in IFCC units, derived NGSP units, and as ADAG.
Standardization of GFR assessment

International Initiatives

- Program to standardize and improve serum creatinine measurements
- Development of a modified MDRD-like equation that is appropriate for standardized creatinine
- Routinely report estimated GFR
The Reference Measurement System for Creatinine

Primary reference material
(pure substance)
NIST SRM 914

calibrate

Ref. procedure
(GC-IDMS or LC-IDMS)
Reference laboratories

certify

calibrate

Secondary reference material
(creatinine in human serum)
NIST SRM 967

calibrate

certify

Measurement of clinical samples by commercial assays

Panteghini M et al., Clin Chem Lab Med 2006
Trueness verification study of creatinine measurements

- 172 laboratories from Belgium, Finland, France, Germany, Italy & The Netherlands

Delanghe J et al., 2007
2006 trueness verification study of creatinine measurements in the Australasian region

- 16 serum samples analysed by 9 instrument/methods in 6 different laboratories
4-Variable Equation for Estimating GFR

(Levey AS et al., Clin Chem 2007)

GFR (ml/min 1.73 m²) =
175 x (s-Cr)^{-1.154} x (Age)^{-0.203} x (0.742 if Female)
 x (1.210 if African American)

By using this equation and a standardized creatinine assay, clinical laboratories can report estimated GFR more uniformly and accurately.
Reference System for Troponin I

Primary reference material

Ref. procedure (high level consensus method)

Secondary ref. material
(cTnI in human serum)

Measurement of clinical samples by immunoassays

Calibration of routine methods

M. Panteghini, Clin Chem 2005
Designated Higher-Order Reference Procedures for Cardiac Troponin I

- Not commercial cTnI immunoassay based on monoclonal antibodies (1x1 or 2x2 MAbs)
- Directed to an invariant part of the molecule
- Comparable antibody specificity with the last-generation commercial assays
- Calibrated with NIST SRM 2921

Thorough definition of assay characteristics including:
- Antibody specificity
- Immunoreactivity to cTnI forms present in plasma
- Detection limit and imprecision
Candidate cTnI Commutable Secondary Reference Materials

Panel consisting of:

- Three (3) pools of positive cTnI serum samples from MI subjects with clinically relevant cTnI concentrations
- Production of at least an estimated 5-year supply for each level.
New IFCC Reference Systems in Development

a) Enzymes: ALP, Lipase

b) Proteins: Cystatin C, Carbohydrate-Deficient Transferrin (CDT), Albumin (urine), HbA2, Myoglobin

c) Hormones: freeT4 & totalT4, hCG, GH, Insulin
Thanks to more than 500 specialists in Laboratory Medicine, coming from diverse organisations around the world (Hospitals, Universities, Manufacturers, Regulatory and Governmental Bodies) who contribute to the IFCC SD activities.