Global harmonization in laboratory medicine

Mauro Panteghini
Drivers for global harmonization in laboratory medicine

- Patient safety, empowerment and public confusion
- Clinical governance and guidelines
- Laboratory accreditation, consolidation and networking
- Advances in IT and electronic health records

Appropriate test in the right context
Right sample handling
Result equivalence and harmonization of QC practice
Result utilization and intervention
Uniform communication

Clinical-laboratory interface
HARMONIZATION
Request
Collection
Analysis
Interpretation
Report

CIRME
Università degli Studi di Milano
Lab-related causes of diagnostic error

- Inappropriate test ordered (20%)
- Appropriate test not ordered (45%)
- Appropriate test result inaccurate
- Appropriate test result not used properly
  - Knowledge deficit
  - Failure of synthesis (no results integration)
  - Misleading result (unaware of test limitations)
- Appropriate test result delayed/missed

Promoting clinical and laboratory interaction by harmonization

Mario Plebani a,*, Mauro Panteghini b

Harmonization at the clinical-laboratory interface

Harmonization of test demand (pre-pre-analytical phase)
- Practice guidelines and their local implementation
- Common laboratory test profiles
- Periodicity of (re)testing
- Reflex testing and algorithms
- Policy of introducing new tests and discontinuing obsolete tests

Harmonization of result interpretation (post-post-analytical phase)
- Information on quality of laboratory tests
- Traceable reference intervals and decision thresholds
- Critical value definition and communication
- Interpretative comments: when and how to interpret

Harmonization of consultant advisory services (both on demand and interpretation)
NHS Atlas of Diagnostic Variation

- Large variations in clinician requesting that cannot easily be explained by differences in disease prevalence
Annual rate of use for CA125

From 0.11 to 9.0 per 1000 practice population
→ 80-fold variation
or
(after excluding 5 outliers)
from 0.92 to 8.4
→ 9-fold variation

Contributory factors?

- Differences in professional practice
- Differences in uptake of innovation post-NICE guidelines
Implementing practice guidelines on correct use of tumor marker largely decrease the number of ordered tests and reagent costs, without any impact on marker clinical role.
Removal from the test menu of obsolete and useless tests

- Removing tests that offer little incremental information would save money, avoid additional investigations arising from incidental and clinically irrelevant abnormalities, and improve the risk to benefit ratio.

- For instance, deleting myoglobin, total creatine kinase (CK) and CK MB isoenzyme determinations from laboratory order forms in patients admitted to ED leads to significant cost saving and reduces possible confusion in data interpretation and patient management. Overall testing costs were reduced by €104,871 per annum.

Plebani M & Panteghini M, Clin Chim Acta 2014;432:15

Markers still used for the diagnosis of AMI in addition to troponin

The Cardiac Marker Guideline Uptake in Europe (CARMAGUE) Study of the EFLM WG Cardiac Markers
The "famous pairs" in Laboratory Medicine

- Serum creatinine and urea
- ESR and C-reactive protein
- AST and ALT
- Amylase and lipase
- PT and aPTT
- fT3 and fT4
- Ferritin and transferrin saturation
Periodicity of (re)testing:
How often should tests be requested

- As often as necessary (very small group)
- Once in a lifetime (genetic test for hereditary disorders)
- Never ordered on inpatients (lipoproteins as CV risk factors)
- Never ordered again once a positive result has been obtained (TPPA)
- Not ordered more frequently than daily or longer (C-reactive protein)
- Not ordered more frequently than monthly (HB/HCV Abs)
- Not ordered more frequently than every 3 months (HbA1c)
- Ordered no more frequently than annually (renal function in diabetics)
- Never be ordered (vitamin D screening)
National Minimum Re-testing Interval Project:

A final report detailing consensus recommendations for

minimum re-testing intervals for use in Clinical Biochemistry

Prepared for the Clinical Practice Group of the

Association for Clinical Biochemistry and Laboratory Medicine and

supported by the Royal College of Pathologists.

Report Author: Dr Tim Lang – Project Lead
C-reactive protein requests analysed for June 2006–June 2013

48-h minimum re-testing interval introduced
• Patients are unaware of importance of proper collection of urine specimen and how this may affect test results

• A substantial proportion of patients do not come properly prepared for lab testing and are not well informed about the fasting requirements
Standardization of collection requirements for fasting samples
For the Working Group on Preanalytical Phase (WG-PA) of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

A.M. Simundic a,b,⁎, M. Corones b,c, K. Grankvist b,d, G. Lippi b,e, M. Nybo b,f

1. Existing guidelines for phlebotomy need revision. Revised recommendations should include the exact definition of requirements for patient preparation for laboratory testing. Blood for all blood tests should be drawn preferably in the morning from 7 to 9 a.m. [30]. Fasting should last for 12 h, during which water consumption is permitted. Alcohol should be avoided for 24 h before blood sampling. In the morning before blood sampling, patients should refrain from cigarette smoking and caffeine containing drinks (tea, coffee, etc.).

2. Professional associations (IFCC, EFLM and other) should support harmonization efforts by disseminating standardized recommendations for fasting.

3. Laboratories worldwide should implement standardized procedures for blood sampling and patient preparation.

4. Laboratories should have policies for sample acceptance criteria related to fasting samples. Blood samples for routine testing should not be taken if a patient has not been appropriately prepared for sample collection. ‘No sample is better than a bad sample’ should always be the leading principle.
CLSI GP33-A Accuracy in Patient and Sample Identification

- to minimize the error risk:
  - generate labels at the time and site of collection
  - label the sample in the presence of the patient

An observational study by the EFLM Working Group for the preanalytical phase (WG-PRE)

Question: Were the tubes labelled in the presence of the patient?
Reasons for Rejecting Chemistry and Hematology Specimens

- **CAP Q-Probe 95-02, Chemistry Specimen Acceptability (n=461 labs)**
  - 60% Hemolyzed
  - 11% Insufficient quantity
  - 7% Inadequately labeled
  - 3.5% Improper collection tube
  - 2% Clotted

- **CAP Q-Probe 92-05, Hematology Specimen Acceptability (n=604 labs)**
  - 65% Clotted
  - 10% Insufficient quantity
  - 5% Unacceptable variance (delta check)
  - 5% Inadequately labeled
  - 2% Platelet clumps
  - 2% Hemolyzed
### Evidence Review

**Straight Needle Venipuncture vs. IV Catheter Starts**

**Conclusions and Recommendations**

<table>
<thead>
<tr>
<th>Strength of evidence rating</th>
<th><strong>High:</strong> A sufficient number of well-designed and well conducted studies with substantial effect size are available. These studies provide consistent evidence of improvement with respect to rates of hemolysis and associated healthcare problems as a result of this practice.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LMBP Recommendation Statement</strong></td>
<td>Straight needle venipuncture must replace the use of intravenous catheters as the primary method of collecting blood samples in the Emergency Department in order to significantly reduce rates of hemolysis.</td>
</tr>
</tbody>
</table>

**Clinical Biochemistry** 2012; 45:1012-32
Visual handling of hemolyzed samples increases the risk of reporting inaccurate results for cTnT, K and bilirubin, possibly affecting the clinical decision and patient outcome.


Courtesy of A-M Simundic
Advantages

- Overcoming visual inspection and arbitrary judgment about sample quality
- Automatic transmission of hemolysis degree to the laboratory information system (LIS)
- Assessment of sample quality in high-volume clinical laboratories where preanalytical and analytical workstations are integrated
- Surrogate measure to judge phlebotomists’ performance
Good agreement was obtained between hemoglobin concentrations measured using the reference method and HI, for the most of studied analyzers, particularly those giving quantitative HI.

Harmonization in hemolysis detection and prevention. A working group of the Catalanian Health Institute (ICS) experience

Table 4 κ of Cohen index and coefficients of intra-class correlation.

<table>
<thead>
<tr>
<th></th>
<th>Beckman</th>
<th>Siemens</th>
<th>Siemens</th>
<th>Roche</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AU 5400</td>
<td>Vista</td>
<td>Advia</td>
<td>Cobas-Modular</td>
</tr>
<tr>
<td>Synchron Lxi725 – DXC800</td>
<td>0.7895</td>
<td>0.8209</td>
<td>CCI</td>
<td>0.973</td>
</tr>
<tr>
<td>κ</td>
<td>0.8326</td>
<td>0.8209</td>
<td>0.982</td>
<td></td>
</tr>
<tr>
<td>CI 95%</td>
<td>0.6899–0.8891</td>
<td>0.7278–0.9374</td>
<td>0.910–0.996</td>
<td>0.863–0.995</td>
</tr>
</tbody>
</table>
Harmonization of automated hemolysis index assessment and use: Is it possible?

Alberto Dolci a,*, Mauro Panteghini a,b

a Clinical Chemistry Laboratory, University Hospital "Luigi Sacco", Milan, Italy
b Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

Table 1
Characteristics of hemolysis index [HI] test parameters on different commercial platforms.

<table>
<thead>
<tr>
<th>Company/platform</th>
<th>Interferent material used</th>
<th>Maximum concentration of hemoglobin tested [g/l]</th>
<th>Sample volume for HI testing [µl]</th>
<th>Diluent type [volume] [µl]</th>
<th>Read wavelengths [nm]</th>
<th>HI report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott Architect</td>
<td>Fresh erythrocyte hemolysate</td>
<td>20</td>
<td>5.3</td>
<td>Saline [200]</td>
<td>572/604; 628/660</td>
<td>5 levels</td>
</tr>
<tr>
<td>Beckman Coulter AU</td>
<td>Fresh erythrocyte hemolysate</td>
<td>5</td>
<td>2.0–1.6</td>
<td>Saline [150]</td>
<td>410/480; 600/800</td>
<td>6 levels</td>
</tr>
<tr>
<td>Beckman Coulter Synchron</td>
<td>Fresh erythrocyte hemolysate</td>
<td>5</td>
<td>14</td>
<td>Tris buffer pH 7.6 [200]</td>
<td>340, 410, 470, 600, 670</td>
<td>11 levels</td>
</tr>
<tr>
<td>Ortho Vitros</td>
<td>Fresh erythrocyte hemolysate</td>
<td>5–10</td>
<td>35*</td>
<td>Undiluted</td>
<td>522/750</td>
<td>Concentration units</td>
</tr>
<tr>
<td>Roche Cobas &amp; Integra</td>
<td>Fresh erythrocyte hemolysate</td>
<td>10</td>
<td>6</td>
<td>Saline [150]</td>
<td>570/600</td>
<td>Absolute numbers [range: 1–1000]</td>
</tr>
<tr>
<td>Siemens Advia</td>
<td>Fresh erythrocyte hemolysate</td>
<td>5.25</td>
<td>5</td>
<td>Saline [100]</td>
<td>571/596</td>
<td>5 levels</td>
</tr>
<tr>
<td>Siemens Dimension</td>
<td>Fresh erythrocyte hemolysate</td>
<td>10</td>
<td>10</td>
<td>Water [150]</td>
<td>405/700</td>
<td>8 levels</td>
</tr>
<tr>
<td><strong>Recommended</strong></td>
<td>Fresh erythrocyte hemolysate</td>
<td>10</td>
<td>The lowest yielding an accurate measurement</td>
<td>Not giving rise to paraprotein precipitation</td>
<td>Detection methods should account for the absorbance spectrum overlap of hemoglobin, bilirubin and lipemia/turbidity</td>
<td>Concentration unit or absolute number</td>
</tr>
</tbody>
</table>

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*a* HI analysis does not consume the sample.

*b* According to the Clinical and Laboratory Standards Institute document C56-A [5].
### Hemolysis limits based on biological variation (A) vs. manufacturer’s recommended (B)

<table>
<thead>
<tr>
<th>Test</th>
<th>Hemolysis interference limit, g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Advia 2400 (Siemens)</td>
</tr>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>ALT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>AST&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6</td>
</tr>
<tr>
<td>CK&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>COL</td>
<td>&gt;6.9</td>
</tr>
<tr>
<td>P</td>
<td>2.4</td>
</tr>
<tr>
<td>FAL</td>
<td>2.4</td>
</tr>
<tr>
<td>FE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.8</td>
</tr>
<tr>
<td>GLU&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4</td>
</tr>
<tr>
<td>GGT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>K</td>
<td>0.6</td>
</tr>
<tr>
<td>LD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16</td>
</tr>
<tr>
<td>PT&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.9</td>
</tr>
<tr>
<td>TG</td>
<td>6.9</td>
</tr>
</tbody>
</table>
Harmonize management of unreliable samples: the most challenging issue?

- Do not process specimen and contact the ward: 27%
- Provide comment and no result: 16%
- Adjust sample result using algorithms: 3%
- Perform analysis and associate comment: 54%
Summary of other practice comments:
- If haemolysed - repeat sample requested.
- A & E samples - repeat requested urgently.
- All samples rejected and requestor contacted.

Roche users
- Haemolysis - TnT-hs < 100 ng/L = result deleted; if TnT-hs > 100 ng/L reported as > 100 ng/L.
- TnT-hs < 14 ng/L not issued. All haemolysed requests carry a comment suggesting repeat.

- Our policy varies depending on level of troponin and degree of haemolysis.
Heterogeneity of manufacturers' declarations for lipemia interference — An urgent call for standardization

Nora Nikolac a,*,1, Ana-Maria Simundic a, Manuela Miksa a, Gabriel Lima-Oliveira b,1, Gian Luca Salvagno b, Beatrice Caruso c, Gian Cesare Guidi b,c

Comparison of declared and measured data on lipemia interference.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beckman Coulter AU 680</th>
<th>Cobas® 6000 &lt; c501&gt;</th>
<th>Dimension Vista System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Potassium</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Chlorides</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Lipase</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Iron</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>ALT</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>AST</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bilirubin, direct</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Urea</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Creatinine</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Glucose</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Phosphates</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Albumin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CK-MB</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CK</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>LD</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>AMY</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>ALP</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>GGT</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Magnesium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Calcium</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Total proteins</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CRP</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>
Clinical impact of direct HDLc and LDLc method bias in hypertriglyceridemia. A simulation study of the EAS-EFLM Collaborative Project Group

Michel R. Langlois a,b,c, *, Olivier S. Descamps d, Arnoud van der Laarse e, Cas Weykamp f, Hannsjörg Baum a,g, Kari Pulkki a,h, Arnold von Eckardstein i, Dirk De Bacquer j, Jan Borén k, Olov Wiklund k, Paivi Laitinen a, Wytze P. Oosterhuis b, Christa Cobbaert l, for the EAS-EFLM Collaborative Project

a European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group (WG) Cardiac Markers

b WG Guidelines, EFLM
Dutch EQA survey (n=197 labs) of hypertriglyceridemic serum (TG ~600 mg/dL)
Clinical impact of biased HDLc-risk multipliers, simulated in men with initial SCORE of 4%

<table>
<thead>
<tr>
<th>Method</th>
<th>Labs (n)</th>
<th>HDL-C median (range) (mg/dL)</th>
<th>Error (mean bias)</th>
<th>SCORE &gt;5% n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1</td>
<td>42 [HDL multiplier, 1; SCORE = 4%]</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Overall</td>
<td>197</td>
<td>35 (24-48)</td>
<td>-15%</td>
<td>84 (43%)</td>
</tr>
<tr>
<td>Abbott</td>
<td>18</td>
<td>41 (38-42)</td>
<td>-3%</td>
<td>0</td>
</tr>
<tr>
<td>Beckman</td>
<td>39</td>
<td>39 (31-45)</td>
<td>-7%</td>
<td>2 (5%)</td>
</tr>
<tr>
<td>Roche</td>
<td>113</td>
<td>36 (26-48)</td>
<td>-19%</td>
<td>71 (63%)</td>
</tr>
<tr>
<td>Siemens</td>
<td>14</td>
<td>31 (24-46)</td>
<td>-22%</td>
<td>10 (71%)</td>
</tr>
</tbody>
</table>

Langlois MR et al. The EAS-EFLM Collaborative Project. Atherosclerosis 2014; 233:83
The choice of anticoagulant

PLASMA GLUCOSE DETERMINATION: GOLD STANDARD FOR SAMPLE COLLECTION

NATIONAL ACADEMY OF CLINICAL BIOCHEMISTRY (NACB) GUIDELINES FOR LABORATORY ANALYSIS IN DIABETES

- tubes with only enolase inhibitors, such as NaF, should not be relied on to prevent glycolysis
- tube containing a rapidly effective glycolysis inhibitor, such as citrate buffer, should be used for collecting the sample

(Rating scale for the quality of evidence, B moderate) Clin Chem 2011;57:e1-47

The shift from fluoride to citrate blood collection tubes for glucose testing: is the clinical impact carefully considered?
CLINICAL CLASSIFICATION OF SUBJECTS UNDERWENT FPG TEST

DIABETOLOGIST ASSOCIATIONS SHOULD CLARIFY IF:

1. Decisional limits for FPG should be redefined with the use of tubes that promptly inhibit the in vitro glycolysis

2. Current cut-offs should be maintained, so that the “higher” FPG results could more effectively and early identify subjects at increased risk for diabetes

Pasqualetti S et al., Clin Chem Lab Med 2015;53:S666
The Report

The product that underpins the effectiveness of the laboratory product

A synthesis of:

- data
- knowledge
- information
Challenges reported by US primary care physicians when using lab test results

<table>
<thead>
<tr>
<th>Receiving results</th>
<th>% of respondents reporting factor is very or extremely problematic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results not received in a timely manner</td>
<td>34</td>
</tr>
<tr>
<td>Previous results are not easily available</td>
<td>32</td>
</tr>
<tr>
<td>Errors in results are suspected</td>
<td>25</td>
</tr>
<tr>
<td>Results are inconsistent with patient’s symptoms</td>
<td>24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Report format</th>
<th>% of respondents reporting factor is very or extremely problematic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lab-to-lab variation in normal range</td>
<td>22</td>
</tr>
<tr>
<td>Lab-to-lab variation in report formats</td>
<td>21</td>
</tr>
<tr>
<td>Lab report format is difficult to understand</td>
<td>18</td>
</tr>
<tr>
<td>Not enough information in lab report</td>
<td>16</td>
</tr>
</tbody>
</table>

Potentially affecting 13 million pts/yr, raising significant concerns about the safety and efficient use of lab tests

Urine Albumin Measurement

SIBioC Survey 2015: post-analytical phase

How do you define the analyte in the report?

- Microalbumin: 42%
- Urine Albumin: 11%
- U-Albumin: 19%
- Albuminuria: 28%

CIRME
Università degli Studi di Milano
Adoption of SI units @ national level
Decimal numbers and safe interpretation of clinical pathology results

Michael Sinnott,¹,² Robert Eley,¹,² Vicki Steinle,³ Mary Boyde,⁴ Leanne Trenning,¹ Goce Dimeski⁵

Take-home messages

► Poor comprehension of decimal numbers was illustrated by many laboratory and clinical staff.
► Resultant misinterpretation of test results is a potential source of medical errors.
► Whenever possible, pathology results should be presented as whole numbers.
To be interpreted results should be compared with:

- a population reference interval (transversal evaluation - biological level)
- a decision limit (transversal evaluation - nosological level)

Two fundamental issues drive improvement in defining and using reference intervals in clinical practice:

1) There is the need to link the analytical standardization based on the principles of metrological traceability with the identification of appropriate reference intervals.
2) The ISO 15189:2012 states that “biological reference intervals shall be periodically reviewed” and they should be verified every time a variation in analytical and/or pre-analytical procedures occurs.
Lack of proper reference intervals may hamper the implementation of standardization

- The implementation of standardization can modify the analyte results
- Without adequate R.I. this situation can impair the interpretation of the results and, paradoxically, worsen the patient’s outcome
- The absence of reliable R.I. for the newly standardized commercial methods hampers their adoption

Until today

Method-dependent results

Method-dependent reference intervals

From today

Standardized methods that provide traceable results

Traceable reference intervals
Reference Intervals for Serum Creatinine Concentrations: Assessment of Available Data for Global Application

Ferruccio Ceriotti,1* James C. Boyd,2 Gerhard Klein,3 Joseph Henny,4 Josep Queraltó,5 Veli Kairisto,6 and Mauro Panteghini,7 on behalf of the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL)

<table>
<thead>
<tr>
<th>Age (gender) group</th>
<th>Percentile value, mg/dL&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.5th</td>
</tr>
<tr>
<td>Cord blood</td>
<td>0.52</td>
</tr>
<tr>
<td>Preterm neonates 0–21 d</td>
<td>0.32</td>
</tr>
<tr>
<td>Term neonates 0–14 d</td>
<td>0.31</td>
</tr>
<tr>
<td>2 m–&lt;1 y</td>
<td>0.16</td>
</tr>
<tr>
<td>1 y–&lt;3 y</td>
<td>0.17</td>
</tr>
<tr>
<td>3 y–&lt;5 y</td>
<td>0.26</td>
</tr>
<tr>
<td>5 y–&lt;7 y</td>
<td>0.29</td>
</tr>
<tr>
<td>7 y–&lt;9 y</td>
<td>0.34</td>
</tr>
<tr>
<td>9 y–&lt;11 y</td>
<td>0.32</td>
</tr>
<tr>
<td>11 y–&lt;13 y</td>
<td>0.42</td>
</tr>
<tr>
<td>13 y–&lt;15 y</td>
<td>0.46</td>
</tr>
<tr>
<td>Adult (males)</td>
<td>0.72</td>
</tr>
<tr>
<td>Adult (females)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<sup>a</sup>To express creatinine values in μmol/L, multiply the values by 88.4. d, days; m, months; y, years.
Research Article

Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and \( \gamma \)-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study

Ferruccio Ceriotti\(^1\)*, Joseph Henny\(^2\), Josep Queraltó\(^3\), Shen Ziyu\(^4\), Yeşim Özarda\(^5\), Baorong Chen\(^6\), James C. Boyd\(^7\) and Mauro Panteghini\(^8\) on behalf of the IFCC Committee on Reference Intervals and Decision Limits (C-RIDL) and Committee on Reference Systems for Enzymes (C-RSE)
Validation of traceable reference intervals

The validation can be done according to the CLSI document C28-A3, paragraph 11.2, by examining 20 reference individuals from a laboratory’s own subject population.

If no more than 2 (10%) of the 20 tested values fall outside the TRI, this can be adopted.
Critical value definition and communication

- Laboratories are responsible for communicating critical values, a key issue in maximizing patient safety
- However, the reported variations between procedures and policies used by different laboratories in the same country and by those in different countries emphasize the need for harmonization
Laboratory tests considered important in published surveys to be included in alert lists

Need for harmonized policies and procedures

- Definition of critical tests
- Identification of critical values
- Notification procedures of critical values:
  - data validation
  - timeliness of reporting
  - communication tools (phone, informatics, call centers)
  - personnel responsible for data transmission and receiving results
  - acknowledgment of results and feed-back
  - data recording
- Procedures for evaluating and monitoring outcomes of critical results management practices
The result standardization issue: an absolute priority for public health

→ Our customers (i.e., clinicians and patients) expect laboratory results to be equivalent and interpreted in a reliable and consistent manner.
Effect of analytic bias in creatinine on the distribution of estimated GFR values

Klee GG et al., Clin Chem Lab Med 2007;45:737
**Economic impact**

- **$60M/yr wasted**
- **$199M/yr wasted**

![Graph showing economic impact](image)

*Source: NIST Planning Report 04-1, 2004*
In short: the lack of standardization may become an ethical issue

“Standardization of laboratory tests has an ethical dimension as it aims to affect the way diagnostic tests are used in order to guarantee optimal care for patients in a global world.”

Analytical improvements are matter of patient safety and key to future
Basic requirements to establish traceability

- Establishment of a calibration hierarchy
- Establishment of the metrological traceability for the measurement results (understand the measurements)
- Elimination of measurement bias
- Adequate estimation of measurement uncertainties
Role of IVD manufacturers

IVD manufacturers should define a calibration hierarchy to assign traceable values to their system calibrators and to fulfil during this process uncertainty limits, which represent a proportion of the uncertainty budget allowed for clinical laboratory results.
Thus, clinical laboratories need to rely on the manufacturers who must ensure traceability of their analytical system to the highest available level.

[Adapted from Braga F & Panteghini M, Clin Chim Acta 2014;432:55]

[Adapted from Kallner A, Scand J Clin & Lab Invest 2010; 70(Suppl 242): 34]
The manufacturer must indicate the *combined* uncertainty associated with calibrators when used in conjunction with other components of the analytical system (platform and reagents). Such uncertainty estimates provided by the manufacturer should include the uncertainty associated with higher levels of the metrological traceability chain.
Need to define criteria for manufacturers that can be achieved for their calibrators leaving enough uncertainty budget for the laboratories to produce clinically acceptable results.

Allowable limit for the combined uncertainty of manufacturer’s commercial calibrators @ 50% of the goals

[Note that these are goals for random variability, as at the calibrator level the systematic error (bias), in agreement with the metrological traceability theory, must be corrected if present in a non negligible amount]
The calibrator value assignment protocol of the Abbott enzymatic creatinine assay is inadequate for ensuring suitable quality of serum measurements.

Note: For serum creatinine measurements on patient samples, the acceptable limits for expanded uncertainty derived from its CV are 6.0% (desirable) and 9.0% (minimum quality level), respectively.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Uncertainties for each contributing factor in determination of serum creatinine with Abbott enzymatic assay on Architect c16000 platform after calibration with two different lot of system calibrator. Data obtained by measurements of NIST SRM 967a reference material (certified value ± expanded uncertainty: L1, 0.847 mg/dL ± 0.018 mg/dL and L2, 3.877 mg/dL ± 0.082 mg/dL).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SRM 967a level 1</td>
</tr>
<tr>
<td></td>
<td>0.47%</td>
</tr>
<tr>
<td>Imprecision (u_RM)</td>
<td>3.57%</td>
</tr>
<tr>
<td>Bias (u_{bias})</td>
<td>3.60%</td>
</tr>
<tr>
<td>Relative combined standard uncertainty [u_c = (u_{bias}^2 + u_{RM}^2)^{0.5}]</td>
<td>7.20%</td>
</tr>
<tr>
<td>Expanded uncertainty (U = k \times u_c)</td>
<td>8.10%</td>
</tr>
</tbody>
</table>

*Multigent Clin Chem Calibrator lot no. 40043Y600*

|         | 0.53%             | 0.42%             |                                                                                                                                                                                                                       |
| Imprecision (u_RM)                | 4.02%             | 1.71%             |                                                                                                                                                                                                                       |
| Bias (u_{bias})                    | 4.05%             | 1.76%             |                                                                                                                                                                                                                       |
| Relative combined standard uncertainty [u_c = (u_{bias}^2 + u_{RM}^2)^{0.5}] | 8.10%             | 3.52%             |                                                                                                                                                                                                                       |
The role of the Laboratory Profession: “check”

- Availability and quality of information about IVD metrological traceability and uncertainty
- Daily surveillance of IVD system traceability
Currently, the full information about calibration is usually not available.

Manufacturers only provide the name of higher order reference material or procedure to which the assay calibration is traceable, without any description of implementation steps and their corresponding uncertainty.
Opinion Paper

Federica Braga*, Ilenia Infusino and Mauro Panteghini

Performance criteria for combined uncertainty budget in the implementation of metrological traceability

Table 2: The information that in vitro diagnostics manufacturers should provide to laboratory users about the implementation of metrological traceability of their commercial systems. Adapted from [7].

- An indication of higher order references (materials and/or procedures) used to assign traceable values to calibrators;
- Which internal calibration hierarchy has been applied by the manufacturer, and
- A detailed description of each step;
- The (expanded) combined uncertainty value of commercial calibrators, and
- Which, if any, acceptable limits for uncertainty of calibrators were applied in the validation of the analytical system.
Types of metrological chains that can be used to implement the traceability of blood glucose results*

A  NIST SRM 917
   GC-IDMS @ NIST
   NIST SRM 965
   (glucose in human serum)
   Manufacturer’s internal procedure
   Commercial calibrator
   Commercial system
   Patient’s sample results

B  NIST SRM 917
   GC-IDMS
   [accredited reference laboratory]
   Comparison on biological samples
   Manufacturer’s internal procedure
   Commercial calibrator
   Commercial system
   Patient’s sample results

C  NIST SRM 917
   CDC Hexokinase
   [accredited reference laboratory]
   Comparison on biological samples
   Manufacturer’s internal procedure
   Commercial calibrator
   Commercial system

D  NIST SRM 917
   Manufacturer’s internal procedure
   Commercial calibrator
   Commercial system
   Patient’s sample results

*all JCTLM recognized

Verification of in vitro medical diagnostics (IVD) metrological traceability: Responsibilities and strategies

Federica Braga *, Mauro Panteghini
Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Milan, Italy

Table 1
Metrological traceability and uncertainty information derived from calibrator package inserts of commercial systems measuring blood glucose marketed by four IVD companies.

<table>
<thead>
<tr>
<th>Company</th>
<th>Platform</th>
<th>Principle of commercial method</th>
<th>Calibrator</th>
<th>Declared standard uncertainty</th>
<th>Higher-order reference employed</th>
<th>Type of traceability chain used</th>
<th>Combined standard uncertainty associated with the used chain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>Architect</td>
<td>ND</td>
<td>Multiconstituent calibrator</td>
<td>2.70%</td>
<td>IDMS</td>
<td>A</td>
<td>1.22-1.45%</td>
</tr>
<tr>
<td>Beckman</td>
<td>AU</td>
<td>Hexokinase</td>
<td>System calibrator</td>
<td>ND</td>
<td>ND</td>
<td>A</td>
<td>1.22-1.45%</td>
</tr>
<tr>
<td>Synchron</td>
<td>Hexokinase</td>
<td>Synchron multicalibrator</td>
<td>ND</td>
<td>ND</td>
<td>NIST SRM 917a</td>
<td>D</td>
<td>1.60-3.00%</td>
</tr>
<tr>
<td>Roche</td>
<td>Cobas c</td>
<td>Hexokinase</td>
<td>C.f.a.s.</td>
<td>0.84%</td>
<td>IDMS</td>
<td>B</td>
<td>1.70%</td>
</tr>
<tr>
<td></td>
<td>Integra</td>
<td>Hexokinase</td>
<td>C.f.a.s.</td>
<td>0.62%</td>
<td>IDMS</td>
<td>B</td>
<td>1.70%</td>
</tr>
<tr>
<td></td>
<td>Modular</td>
<td>GOD</td>
<td>C.f.a.s.</td>
<td>0.84%</td>
<td>IDMS</td>
<td>B</td>
<td>1.70%</td>
</tr>
<tr>
<td>Siemens</td>
<td>Advia</td>
<td>Hexokinase</td>
<td>Chemistry calibrator</td>
<td>1.30%</td>
<td>Hexokinase</td>
<td>C</td>
<td>1.88-3.26%</td>
</tr>
<tr>
<td></td>
<td>GOD</td>
<td>GOD</td>
<td></td>
<td>0.80%</td>
<td>Hexokinase</td>
<td>C</td>
<td>1.88-3.26%</td>
</tr>
</tbody>
</table>
Profession (e.g., JCTLM, EFLM):

Define analytical objectives: reference measurement systems (traceability chain) and associated clinically acceptable uncertainty (fitness for purpose)

Diagnostic manufacturers:

Implement suitable analytical systems (platform, reagents, calibrators, controls) fulfilling the above established goals

Post-marketing surveillance of IVD metrological traceability

End users (clinical laboratories):

Survey assay and laboratory performance through IQC and EQA redesigned to meet metrological criteria

Adapted from Panteghini M, Clin Chem Lab Med 2010;48:7
Internal Quality Control (Component I)

Acceptance/rejection of the analytical run in "real time"

Any "out of control" signal must be made available with sufficient time to allow immediate corrective actions to bring again the situation under control (virtually "unbiased") and before reports related to the samples analyzed in the affected analytical run are issued.

Internal Quality Control
(Component II)

System stability at medium/long term

Estimating the measurement uncertainty due to random effects (“imprecision”)

This program provides, through mechanisms of retrospective evaluation, data useful to the knowledge of variability of the analytical system and of its use by the individual laboratory.

Monitoring the reliability of the analytical system through IQC: Component II. Evaluate the system + individual lab imprecision

- System calibration (combined) uncertainty
- System imprecision
- Individual lab performance (IQC safety margin)

Cardiac troponin T high sensitive
Monthly monitoring of imprecision by IQC material

Cumulative mean, 17 ng/L
[adopted cut-off for myocardial necrosis >15 ng/L]
Requirements for the applicability of EQAS results in the evaluation of the performance of participating laboratories in terms of traceability of their measurements

<table>
<thead>
<tr>
<th>Feature</th>
<th>Aim</th>
</tr>
</thead>
<tbody>
<tr>
<td>EQAS materials value-assigned with reference procedures by an accredited ref. laboratory</td>
<td>To check traceability of commercial system to reference systems</td>
</tr>
<tr>
<td>Proved commutability of EQAS materials</td>
<td>To allow transferability of participating laboratory performance to the measurement of patient samples</td>
</tr>
<tr>
<td>Definition and use of the clinically allowable measurement error</td>
<td>To verify the suitability of laboratory measurements in clinical setting</td>
</tr>
</tbody>
</table>

Panteghini M, CCLM 2010;48:7
Infusino I et al., CCLM 2010;48:301
Braga F & Panteghini M, CCLM 2013;51:1719
Unique benefits of EQAS that meet metrological criteria

• Giving objective information about quality of individual laboratory performance
• Creating evidence about intrinsic standardization status/equivalence of the examined assays
• Serving as management tool for the laboratory and IVD manufacturers, forcing them to investigate and eventually fix the identified problem
• Helping manufacturers that produce superior products and systems to demonstrate the superiority of those products
• Identifying analytes that need improved harmonization and stimulating and sustaining standardization initiatives that are needed to support clinical practice guidelines
• Abandonment by users (and consequently by industry) of nonspecific methods and/or of assays with demonstrated insufficient quality
EQAS materials with physiologic (88.4 µmol/L) and borderline (123.8 µmol/L) creatinine concentrations vs. the desirable goal for TE (±8.9%). The vast majority (87%) of laboratories using systems employing enzymatic assays were able to fulfill the desirable performance, while only one third of laboratories using picrate-based systems were able to meet the target.

Enzymatic assays (n=23)

Alkaline picrate assays (n=296)
http://users.unimi.it/cirme/home/index.php

Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME)

under the auspices of

The Joint Committee for Traceability in Laboratory Medicine

CIRME
Università degli Studi di Milano

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www.mzcongressi.com

9th International Scientific Meeting
STRUCTURING EQAS FOR MEETING METROLOGICAL CRITERIA:
READY FOR PRIME TIME

MILANO, ITALY
November 27th, 2015

AULA MAGNA - SETTORE DIDATTICO COLOMBO
Università degli Studi
Via L. Mangiagalli 25, Milano
Steps of the process and different responsibilities in implementing traceability of patient results and defining their uncertainty

Profession (e.g., JCTLM, IFCC, EFLM):

Define analytical objectives: reference measurement systems (traceability chain) and associated clinically acceptable uncertainty (fitness for purpose)

Diagnostic manufacturers:

Implement suitable analytical systems (platform, reagents, calibrators, controls) fulfilling the above established goals

End users (clinical laboratories):

Survey assay and laboratory performance through IQC and EQA redesigned to meet metrological criteria

Adapted from Panteghini M, Clin Chem Lab Med 2010;48:7
Analytical performance specifications: definition

• Criteria that specify (in numerical terms) the quality required for analytical performance in order to deliver laboratory test information that would satisfy clinical needs for improving health outcomes.
The Essential Question...

“What amount of medical harm due to analytical error is it ok to let go undetected?”

Fit for purpose?
1st EFLM Strategic Conference
Defining analytical performance goals
15 years after the Stockholm Conference
8th CIRME International Scientific Meeting

Milan (IT)
24-25 November 2014
Although the essence of the hierarchy established in Stockholm was supported, new perspectives have been forwarded prompting simplification and explanatory additions.

The most innovative aspect of the new consensus is that it is recognized that some models are better suited for certain measurands than for others; the attention is therefore primarily directed towards the measurand and its biological and clinical characteristics.
Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine

Model 1: Based on the effect of analytical performance on clinical outcomes

a. Done by direct outcome studies – investigating the impact of analytical performance of the test on clinical outcomes;

b. Done by indirect outcome studies – investigating the impact of analytical performance of the test on clinical classifications or decisions and thereby on the probability of patient outcomes, e.g., by simulation or decision analysis.

Model 2: Based on components of biological variation of the measurand.

Model 3: Based on state of the art of the measurement (i.e., the highest level of analytical performance technically achievable).
Model 1. Based on the effect of analytical performance on clinical outcomes

- **Advantage**: to address the influence of analytical performance on clinical outcomes that are relevant to patients and society.

- **Disadvantage**: it is only useful for examinations where the links between the test, clinical decision-making and clinical outcomes are straightforward and strong.
Demonstrating the value of lab tests on health outcomes is reliant on linking the test with processes that directly impact outcomes.
Clinical outcomes of patients with only hs troponin positive before and after the introduction of a sensitive troponin assay

3-months outcome

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>MI</td>
<td>27</td>
<td>20</td>
</tr>
<tr>
<td>Death/MI</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

12-months outcome

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>MI</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>Death/MI</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

P < 0.05

Mills NL et al, JAMA 2011;305:1210
APS based on clinical needs may be defined in terms of allowable misclassification rates

Table. Recommended analytical performance goals for cardiac troponin measurement for definition of the limit of quantitation of assays.

<table>
<thead>
<tr>
<th>Quality level</th>
<th>Imprecision goal (as CV)</th>
<th>Bias goal(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outcome-based</td>
<td>Biological variability(^a)</td>
</tr>
<tr>
<td>Minimum</td>
<td>&lt;13(^b)%</td>
<td>&lt;7.3%</td>
</tr>
<tr>
<td>Desirable</td>
<td>&lt;10(^c)%</td>
<td>&lt;4.9%</td>
</tr>
<tr>
<td>Optimum</td>
<td>&lt;6(^d)%</td>
<td>&lt;2.4%</td>
</tr>
</tbody>
</table>

\(^a\) Calculated according to Fraser CG,  Hyltoft Petersen P, Libeer JC, Ricos C. Proposal for setting generally applicable quality goals solely based on biology. Ann Clin Biochem 1997;34:8-12.

\(^b\) Assuming a diagnostic misclassification of 1.8%, \(^c\) 1.0%, and \(^d\) 0.5%.
Intra-individual (within-subject) variation: represents the random fluctuation around an individual’s own homeostatic set-point.

Inter-individual (between-subject) variation: represents differences in homeostatic set-points among different individuals.

Data on biological variation can be used to derive allowable limits for analytical imprecision and bias.
Model 2. Based on components of biological variation of the measurand

- **Advantage**: it can be applied to most measurands for which a “steady state” biologic model can be established.

- **Disadvantage**: need to carefully assess the relevance of biological variation data.
Generation of estimates of CV₁ and CV₂ using the MEDIAN of all data compiled.
Within-subject biological variation (CVI)

What is the meaning of this dispersion?

What are the consequences?

ALT, AST and γGT

The arrows show the values currently present in the Ricos' database

Quantifying Biological Variation

How do you do the experiment?

- Subjects
  - How many?

- Collect specimens
  - Number? Frequency?

- Analyse specimens
  - Minimise analytical variation?

- Analyse data
  - Outliers? Statistics?
Experimental Protocol for Biological Variability Estimate

• Select healthy subjects (n=20)*
  (subjects must be representative of the population)

• Specimens taken at set time intervals

• Specimens processed & stored frozen @ –80 °C

• When ALL specimens are available:
  analysis of all samples in a single run in duplicate

*Conditions should minimise pre-analytical variables:
✓ Usual life style
✓ No drugs (alcohol, smoking?)
✓ Phlebotomy by same person at the same time of day
✓ Optimal protocol for sample transport, processing & storage
Analysis of Data

OUTLIER TEST
- OBSERVATIONS, S^2 > A (COCHRAN)
  "MEAN VALUES OF SUBJECTS"
  (REED’S CRITERION)

NORMALITY TEST:
- ON SET OF RESULTS FROM EACH
  INDIVIDUAL
  (SHAPIRO WILK)

REMOVE THE SUBJECT WITH AN
OUTLIER AND REPEAT THE TEST

NORMALITY TEST:
- ON MEAN VALUES OF SUBJECTS
  (SHAPIRO WILK)

NATURAL LOG-TRANSFORMATION

ANOVA

NORMALITY TEST:
- ON MEAN VALUES OF SUBJECTS
  (KOLMOGOROV-SMIRNOV)

NATURAL LOG-TRANSFORMATION

ANOVA

NORMALITY TEST ON Ln DATA:
- ON SET OF RESULTS FROM EACH
  INDIVIDUAL
  (SHAPIRO WILK)

STOP

ANOVA

NORMALITY TEST ON Ln DATA:
- ON MEAN VALUES OF SUBJECTS
  (SHAPIRO WILK)

STOP
Is available information on biological variability reliable?

The majority of studies assayed a measurand different from that defined by IFCC, like total glycated hemoglobins, also including hemoglobins glycated on other sites differing from N-terminal valine of the β-chain.

### Table 4
Summary of the characteristics of studies on biological variability of HbA\textsubscript{1c} evaluated in this systematic review.

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Method specificity as per HbA\textsubscript{1c} measurand definition</th>
<th>Recruitment of healthy subjects</th>
<th>Optimal study duration</th>
<th>Optimal protocol of sample analysis</th>
<th>Statistical analysis described</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>Yes</td>
<td>±</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>±</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>±</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>±</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Yes</td>
<td>Yes</td>
<td>±</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>Yes (M only)</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>±</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Assay selectivity is an important biological variation qualifier

If the used methodology has different specificity for the measured analyte, one can expect that also the biological variability, a property closely associated with the characteristics of the analyte itself, significantly changes. And, if the biological variability changes, the analytical goals derived from it may be different.
Biological variation from patients
Should they be used?

Healthy subjects

Diabetics

Stable clinical control, short and long term
Good, acceptable, and poor clinical control

Intra-individual variation in pathology >> CV₁ of healthy individuals

Braga F et al, Chim Clin Acta 2010;411:1606
Invited critical review

Biologic variability of C-reactive protein: Is the available information reliable?

Federica Braga *, Mauro Panteghini

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Assay sensitivity</th>
<th>Recruitment of healthy subjects</th>
<th>Optimal study duration and sampling frequency</th>
<th>Appropriate sample type</th>
<th>Optimal protocol of sample analysis</th>
<th>Statistical test for outliers</th>
<th>Testing normal distribution of data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3a</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3b</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>Yes</td>
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</tbody>
</table>

NA, information not available.
Is available information on biological variability of troponin T reliable?

Vasile VC et al., Clin Chem 2010;56:1086
Invited critical review

Biologic variability of C-reactive protein: Is the available information reliable?

Federica Braga *, Mauro Panteghini

Table 2
Summary of the characteristics of studies on biologic variability of C-reactive protein (CRP) evaluated in this systematic review.

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Assay sensitivity</th>
<th>Recruitment of healthy subjects</th>
<th>Optimal study duration and sampling frequency</th>
<th>Appropriate sample type</th>
<th>Optimal protocol of sample analysis</th>
<th>Statistical test for outliers</th>
<th>Testing normal distribution of data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>Yes</td>
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<td>3b</td>
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<tr>
<td>5</td>
<td>Yes</td>
<td>± a</td>
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<td>No</td>
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<td>6</td>
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<td>No</td>
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<tr>
<td>9</td>
<td>Yes</td>
<td>± b</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
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<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NA, information not available.
Have data not normally distributed been appropriately transformed?

1) This is a critical aspect! Studies using statistical parametric approach on data not normally distributed should be excluded from database! Otherwise we will continue to have CV>>33%!!

2) When a not normal data distribution is present, a log-transformation of data is recommended, but this approach does not always solve the distribution problems: Check normality after log-transformation!!
http://www.westgard.com/biodatabase1.htm

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Number of papers</th>
<th>Biological Variation</th>
<th>Desirable specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>C reactive protein</td>
<td>3</td>
<td>42.2</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>56.6</td>
</tr>
</tbody>
</table>


25.  

serum samples

CVw = 63%;  
CVg = 76.3%


plasma samples

CVw = 42.2%;  
CVg = 92.5%


serum samples

CVw = 36.8%;  
CVg = 62.2%
Summary

- BV data are complex reference data
- Published data are of varying quality
- Safe application requires prior critical appraisal
- Need for standards (i.e., a minimum set of attributes to enable the data to be effectively transmitted and applied)
A checklist for critical appraisal of studies of biological variation

BioVar Checklist

1. Title
2. Abstract
3. Introduction
   - Context
   - Previous studies
4. Study Design
   - Analyte
   - Subjects
   - Analytical Method
   - Length of Study
   - Samples
   - Conditions for the Analysis of Specimens Adequately Described
5. Data Analysis
   - Evidence of Outlier Analysis Undertaken?
   - Heterogeneity of within subject biological variation tested and excluded
   - Statistical methods described and appropriate
6. Results
   - Terminology
   - Results Clearly Presented and Managed
7. Discussion

This relates to the highest level of analytical performance technically achievable. [Alternatively, it could be defined as the analytical performance achieved by a certain percentage of laboratories]

- Advantage: numbers are readily available.
Problems with the state-of-the-art concept

- No scientific reasoning
- Often based on “old“ data which may be outdated
- Lack of transparency
- Lack of neutrality (dependency on industry)
- No relationship between what is achievable and on what is needed clinically
Possible criteria for allocation of laboratory tests to different models for performance specifications

1. The measurand has a central role in diagnosis and monitoring of a specific disease ⇒ outcome model

2. The measurand has a high homeostatic control ⇒ BV model

3. Neither central diagnostic role nor sufficient homeostatic control ⇒ state-of-the-art model
Defining analytical performance specifications: Consensus Statement from the 1st Strategic Conference of the European Federation of Clinical Chemistry and Laboratory Medicine

Performance specifications for pre- and post-analytical phases

It is acknowledged that, for patient care, optimizing the quality of the total (pre-analytical/analytical/post-analytical) examination process is the ultimate goal and therefore it would be desirable to go beyond setting analytical performance specifications and to establish examination performance specifications. In principle, the performance specifications for the pre- and post-analytical laboratory processes should follow the same models as for analytical performance specifications. When components of these additional phases can be expressed in numerical terms, they should be added in defining examination performance specifications. In other situations, pre- and post-analytical performance specifications will be best represented by separate quality indicators that should reflect models 1 and 3 listed above.
## Performance criteria

<table>
<thead>
<tr>
<th>Models for performance specifications</th>
<th>Analytical Phase</th>
<th>Pre/Post-Analytical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defined</td>
<td></td>
<td>Not defined</td>
</tr>
<tr>
<td></td>
<td>Possibly based on the State-of-the-Art and on Outcome measures</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metrics</th>
<th>Analytical Phase</th>
<th>Pre/Post-Analytical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well defined</td>
<td></td>
<td>Proposed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Percentage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Parts per million (ppm)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Six sigma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tools of measures</th>
<th>Analytical Phase</th>
<th>Pre/Post-Analytical Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well defined</td>
<td></td>
<td>Recently defined</td>
</tr>
<tr>
<td>Internal Quality Control (IQC)</td>
<td></td>
<td>Quality Indicators (QI)</td>
</tr>
<tr>
<td>External Quality Assessment (EQA)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1
Summary of features and requirements for achieving harmonization in laboratory testing.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Requirements</th>
<th>Vested Stakeholders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-pre-analytical</td>
<td>1. Use of evidence-based guidelines for appropriate test selection,</td>
<td>1. Clinicians; the laboratory community; guideline organizations.</td>
</tr>
<tr>
<td></td>
<td>2. Plan for implementation and educational phases.</td>
<td>2. Professional societies; the laboratory community.</td>
</tr>
<tr>
<td>Pre-analytical</td>
<td>1. Standardize pre-laboratory/external pre-analytical processes.</td>
<td>1. Healthcare practitioners e.g., phlebotomist; laboratory personnel.</td>
</tr>
<tr>
<td></td>
<td>2. Implement SOPS to reduce error and ensure patient safety.</td>
<td>2. WHO World Alliance for Patient Safety; CLSI; IFCC WG-LEPS.</td>
</tr>
<tr>
<td>Analytical</td>
<td>1. Harmonize patient results through a standardization and/or harmonization process.</td>
<td>1. JCTLM; national metrology institutes; reference material providers; IFCC; IVD manufacturers; EQAS organizers; clinical laboratories.</td>
</tr>
<tr>
<td></td>
<td>2. Harmonize laboratory test names and units.</td>
<td>2–4. Clinical terminology and information systems providers; IUPAC; IFCC C-NPU; Governments; patient safety groups.</td>
</tr>
<tr>
<td></td>
<td>3. Standardize test requesting and reporting for the EHR.</td>
<td>5. IVD manufacturers; EQAS organizers; clinical laboratories.</td>
</tr>
<tr>
<td></td>
<td>4. Harmonize report formats where there are patient safety issues.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Monitor reliability of analytical systems and analytical quality of measurements.</td>
<td></td>
</tr>
<tr>
<td>Post-analytical</td>
<td>1. Harmonize reference intervals and clinical decision limits.</td>
<td>1–2. Professional societies; IFCC C-RIDL; the laboratory community; clinicians.</td>
</tr>
<tr>
<td></td>
<td>2. Plan for implementation and educational phases.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Report critical patient values according to an agreed critical test list.</td>
<td>3. Laboratory personnel; clinicians; GPs.</td>
</tr>
<tr>
<td>Post-post-analytical</td>
<td>1. Educate users about the meaning of laboratory tests.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2. Develop an on-going laboratory-clinical systems provider working relationship for long-term sustainability of pathology harmonization.</td>
<td></td>
</tr>
</tbody>
</table>

Harmonization of laboratory testing — Current achievements and future strategies

Jillian R Tate a,*, Roger Johnson b, Julian Barth c, Mauro Panteghini d

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