PREANALYTICAL AND ANALYTICAL ASPECTS AFFECTING CLINICAL RELIABILITY OF PLASMA GLUCOSE RESULTS

Sara Pasqualetti
TOTAL VARIABILITY OF LABORATORY TEST RESULTS

\[ V_{TOT} = (V_P^2 + V_A^2 + V_I^2)^{1/2} \]

**PREANALYTICAL**
- Patient preparation
- Sample collection
- Delivery to the laboratory
- Handling
- Storage

**BIOLOGICAL**
- Within-subject biological variation (Fluctuation of analyte concentrations in a body fluid around the homeostatic setpoint)

**ANALYTICAL**
- Systematic error
- Random error

**SOURCES OF VARIABILITY AFFECTING TEST RESULT**
Pre-analitical sources of variation in glucose testing

\[ V_{TOT} = (V_P^2 + V_A^2 + V_I^2)^{1/2} \]
CRITICAL ISSUE: TO PREVENT *in-vitro* GLYCOLYSIS

GLUCOSE @ physiological concentrations in sample stored at room temperature IS LOST through an average rate of 5-7% per hour


GOLD STANDARD FOR SAMPLE COLLECTION

- NATIONAL ACADEMY OF CLINICAL BIOCHEMISTRY (NACB) GUIDELINES FOR LABORATORY ANALYSIS IN DIABETES
- WORD HEALTH ORGANIZATION

1. SEPARATE plasma from blood cells IMMEDIATELY after sample collection

2. PLACE the sample tube immediately in an ICE-WATER SLURRY and SEPARATE plasma from the cells WITHIN 30 MIN

**OR**

3. USE OF AN EFFECTIVE GLUCOSE STABILIZER

- Tubes with only *enolase inhibitors*, such as FLUORIDE, should not be relied on to prevent glycolysis
- Tube containing a *rapidly effective glycolysis inhibitor*, such as CITRATE BUFFER, should be used

*CIRME* 10th International Scientific Meeting. November 17-18, 2016
**in-vitro GLYCOLYSIS STABILIZERS**

**CITRATE BUFFER**
- Acidification to pH 5.3-5.8
- Inhibition of HE and PFK which act earlier in the glycolytic pathways
- Prompt stabilizing effect, guaranteed for ~10 h at room temperature

NO LOSS OF GLUCOSE AFTER 2h
LOSS OF GLUCOSE ~1.2% AFTER 24h

**FLUORIDE (and oxalate mixture)**
- It forms a complex with enolase in the presence of P and Mg
- Inhibition of ENO which acts downstream in the glycolytic pathway
- Complete stabilizing effect achieved after 4 h from withdrawal

LOSS OF GLUCOSE DURING THE FIRST HOURS

**CIRME 10**
CELEBRATING **10 Years**

10th International Scientific Meeting, November 17-18, 2016
Effectiveness and Reliability of citric/citrate to prevent in-vitro glycolysis

Table 1. Effect of collection tube type and additives on stability of glucose.

<table>
<thead>
<tr>
<th>Sample type, postdraw storage</th>
<th>NACB Reference</th>
<th>Mean delta, mmol/L(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid plasma, 2 h at 37 °C</td>
<td>Heparin plasma, 30 min at 0 °C</td>
<td>(6.393 - 6.414 = -0.021 (0.3))</td>
</tr>
<tr>
<td>Citric acid plasma, 24 h at 37 °C</td>
<td>Heparin plasma, 30 min at 0 °C</td>
<td>(6.393 - 6.316 = 0.07 (1.2))</td>
</tr>
<tr>
<td>Fluoride plasma, 2 h at 37 °C</td>
<td>Heparin plasma, 30 min at 0 °C</td>
<td>(6.393 - 6.099 = 0.294 (4.6))</td>
</tr>
<tr>
<td>Fluoride plasma, 24 h at 37 °C</td>
<td>Heparin plasma, 30 min at 0 °C</td>
<td>(6.393 - 5.943 = 0.450 (7.0))</td>
</tr>
<tr>
<td>Plasma, 30 min, ambient</td>
<td>Serum, 30 min, ambient</td>
<td>(5.589 - 5.638 = -0.049 (0.9))</td>
</tr>
<tr>
<td>Barrier serum, 24 h at 37 °C</td>
<td>Barrier serum, 30 min, ambient</td>
<td>(5.826 - 5.819 = 0.007 (0.1))</td>
</tr>
</tbody>
</table>


Postdraw storage

\(T 20-24^\circ C\)

4 h

Mean Delta %, 0.95%

(95% CI, 0.44–1.46)

Bonetti G et al, Prim Care Diabetes 2016;10:227-32
## VENOSAFE GRANULAR *citric/citrate buffer* (TVG) vs. fluoride

<table>
<thead>
<tr>
<th>AUTHORS</th>
<th>GLUCOSE mmol/L</th>
<th>MEAN DIFFERENCE</th>
</tr>
</thead>
</table>
| Szőke D et al  
*Clin Chem Lab Med* 2014;52:e87-9 | Range 4.5 to 11.1 vs. 4.1 to 10.7 | +6.7% |
| Bonetti G et al  
*Biochemia Medica* 2016;26:68-76 | Median (range) 5.60 (5.47 - 5.73) vs. 5.21 (5.05 - 5.32) | +6.8% |

## GLUCOMEDICS LIQUID *citric/citrate buffer* (GLD) vs. fluoride

<table>
<thead>
<tr>
<th>AUTHORS</th>
<th>GLUCOSE mmol/L</th>
<th>MEAN DIFFERENCE</th>
</tr>
</thead>
</table>
| Dimeski et al  
*Ann Clin Biochem* 2014;52:270-5 | Mean 5.7 vs. 5.3 | +7.5% |
| Juricic G et al  
*Clin Chem Lab Med* 2016;54:363-71 | Mean (±SD) 6.2 (±1.1) vs. 5.7 (±1.0) | +9.9% |
| Juricic G et al  
*Clin Chem Lab Med* 2016;54:411-8 | Mean (±SD) 6.0 (±0.8) vs. 5.5 (±0.8) | +8.5% |
| Carta M et al  
*Ann Clin Biochem* 2016 doi:10.1177/0004563216645621 | Median (95%CI) 5.6 (5.5-5.9) vs. 5.1 (4.8-5.3) | +8.9% |
The difference between **LIQUID** vs. **GRANULAR** citric/citrate buffer

<table>
<thead>
<tr>
<th>AUTHORS</th>
<th>GLUCOSE mmol/L</th>
<th>MEAN DIFFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juricic G et al</td>
<td>Mean (±SD) 6.0 (1.0) vs. 5.8 (0.9)</td>
<td>+3.4%</td>
</tr>
<tr>
<td>Pasqualetti S et al</td>
<td>Median (95% CI) 5.6 (5.5-5.9) vs. 5.4 (5.1-5.7)</td>
<td>+3.7%</td>
</tr>
<tr>
<td>Carta M et al</td>
<td>Mean (±SD) 6.0 (1.0) vs. 5.8 (0.9)</td>
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</tbody>
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### Table 1. Effect of collection tube type and additives on stability of glucose.

<table>
<thead>
<tr>
<th>Sample type, postdraw storage</th>
<th>Comparator, postdraw storage</th>
<th>Mean delta, mmol/L&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Delta (%)</th>
<th>95% CI</th>
<th>P (n)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma, 2 h at 37 °C</td>
<td>Heparin plasma, 30 min at 0 °C</td>
<td>6.393 − 6.414 = −0.021 (0.3)</td>
<td>−0.07−0.02</td>
<td>0.33 (30)</td>
<td></td>
</tr>
<tr>
<td>Plasma, 24 h at 37 °C</td>
<td>Heparin plasma, 30 min at 0 °C</td>
<td>6.393 − 6.316 = 0.077 (1.2)</td>
<td>−0.002−0.06</td>
<td>0.05 (30)</td>
<td></td>
</tr>
<tr>
<td>Plasma, 30 min, ambient</td>
<td>Serum, 30 min, ambient</td>
<td>5.589 − 5.638 = −0.049 (0.9)</td>
<td>0.021−0.077</td>
<td>&lt;0.001 (90)</td>
<td></td>
</tr>
<tr>
<td>Barrier serum, 24 h at 37 °C</td>
<td>Barrier serum, 30 min, ambient</td>
<td>5.826 − 5.819 = 0.007 (0.1)</td>
<td>−0.011−0.025</td>
<td>0.45 (66)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean delta, mmol/L.<br> <sup>b</sup>P values and n values in parentheses.

**Reference**<br>Venosafe Granula Citrate

**NACB Reference**
The difference between LIQUID vs. GRANULAR citric/citrate buffer: why?

1. INCORRECT DILUTION CORRECTION FACTOR

<table>
<thead>
<tr>
<th></th>
<th>GRANULAR</th>
<th>LIQUID (Dilution Factor, 1.16)</th>
<th>LIQUID (Dilution Factor, *1.10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAN</td>
<td>5.4 mmol/L</td>
<td>5.6 mmol/L</td>
<td>5.4 mmol/L</td>
</tr>
</tbody>
</table>


2. IMPRECISE VACUUM ACTION

Perfect correction factor may become incorrect when tubes are not exactly filled as intended

... our experience

- well trained phlebotomists,
- tubes underfilled considered indicative of human error

...we speculated some problems in tubes manufacturing
The introduction of granular citrate tubes determined a 'shift to the right' in the FPG distribution.

**FASTING PLASMA GLUCOSE DISTRIBUTION**

**CLINICAL CLASSIFICATION OF SUBJECTS UNDERGONE FASTING PLASMA GLUCOSE (FPG) TEST AFTER INTRODUCTION OF GRANULAR CITRATE**

**NaF**
- FPG ≥7.00 mmol/L (diabetes)
- FPG ≥5.60-<7.00 mmol/L (impaired glycaemia)
- FPG <5.60 mmol/L (normoglycaemia)

**CITRATE**
- FPG ≥7.00 mmol/L (diabetes)
- FPG ≥5.60-<7.00 mmol/L (impaired glycaemia)
- FPG <5.60 mmol/L (normoglycaemia)
IADPSG GDM criteria:
- implementation of NACB & WHO protocols
- or tube types that yields compatible results

Table 1. Comparison of mean glucose concentrations between research and usual conditions for each test.

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Research conditions</th>
<th>Usual conditions</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>90.0 (12.6)</td>
<td>81.0 (12.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>5.0 (0.7)</td>
<td>4.5 (0.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>1-h</td>
<td>140.4 (43.2)</td>
<td>133.2 (41.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>7.8 (2.4)</td>
<td>7.4 (2.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2-h</td>
<td>102.6 (32.4)</td>
<td>99.0 (32.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>5.7 (1.8)</td>
<td>5.5 (1.8)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

a Data are mean (SD).
b Paired Student t test.


Table 2. Comparison of the incidence of GDM between research and usual conditions for each test.

<table>
<thead>
<tr>
<th>Glucose</th>
<th>Research conditions</th>
<th>Usual conditions</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>75 g OGTT GDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5.1 mmo/L</td>
<td>51 (32.9)</td>
<td>10 (6.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>&gt;10.0 mmo/L</td>
<td>20 (13.3)</td>
<td>17 (11.0)</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;8.5 mmo/L</td>
<td>4 (2.6)</td>
<td>4 (2.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>59 (38.1)</td>
<td>22 (14.2)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* According to the HAPO study performed under well controlled preanalytical conditions for glucose testing

Screened subjects, 155

- To rightfull classify subjects as diabetics
- To receive the needed treatments that will deprived from in presence of preanalytical invalid conditions.
The introduction of citrate in clinical practice: which caveat?

Evidence 1 - data about the performance of different “citrate tubes” are confused

Caveat 1 – selection of tubes containing citrate requires caution

Evidence 2 - reliable tubes that promptly inhibit *in vitro* glycolysis may lead to a different clinical classification of subjects

Caveat 2 – which decision limits should be applied to plasma glucose?

- should these be redefined when tubes are used that promptly inhibit *in vitro* glycolysis
- or
- should they be maintained, so that more subjects at increased risk for diabetes will be identified earlier?

Letter to the Editor

Sara Pasqualetti*, Dominika Szőke, Sarah Birindelli, Alberto Dolci and Mauro Panteghini

Optimal collection tubes for plasma glucose determination: confusion reigns supreme

FROM EU MARKET

✓ Terumo Venosafe™ Glycaemia – citrate buffer/NaF/Na₂EDTA - GRANULAR FORM
✓ Grainer Bio-one GLUCOMEDICS – NaF/EDTA & citrate – LIQUID FORM
✓ Sarstedt GlucoEXACT - NaF/citrate – LIQUID FORM
✓ Grainer Bio-one Vacuette® FC Mix tube – citrate buffer/NaF/Na₂EDTA - GRANULAR FORM

..... A MESSY STATE OF AFFAIRS

Need for a well-designed clinical study comparing the suitable options using blood acidification offered by the market

..... IN THE MEANIME

Staying (returning) to tubes containing sodium fluoride only as these have been used in the majority of studies generating the current glucose cut-points for diabetes diagnosis

10th International Scientific Meeting. November 17-18, 2016
Plasma Glucose and its Biological Variation

\[ V_{TOT} = \left( V_P^2 + V_A^2 + V_I^2 \right)^{1/2} \]
The concentrations of *measurands* in body fluids are physiologically **variable** as they fluctuate around the individual homeostatic set point - of each individual **Within-subject** \((CV_i)\) - random fluctuation of setting points among individuals **Between-subject** \((CV_g)\).

### Application of Biological Variation Data

**“Result interpretation”**

- **INDEX OF INDIVIDUALITY**
  - To select the right criteria for results interpretation (reference interval, longitudinal variation)

- **REFERENCE CHANGE VALUE (RCV)**
  - Clinically significative change in two consecutive results

- **SPECIMENS NEEDED TO ESTABLISH INDIVIDUAL’S HOMEOSTATIC SET POINT**

**BIOLOGICAL VARIATION**

**ANALYTICAL PERFORMANCE SPECIFICATIONS**

**“Reliability of test results”**
Published data are of varying quality and quite heterogeneous
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)

**Plasma**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year of Publication</th>
<th>CV&lt;sub&gt;i&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;g&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cummings</td>
<td>1988</td>
<td>4.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Godsland</td>
<td>1985</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Davie</td>
<td>1993</td>
<td>13.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Rohlfing</td>
<td>2002</td>
<td>5.7</td>
<td>5.8</td>
</tr>
<tr>
<td>Lacher</td>
<td>2005</td>
<td>8.3</td>
<td>12.5</td>
</tr>
<tr>
<td>Lacher</td>
<td>2010</td>
<td>7.5</td>
<td>11.7</td>
</tr>
<tr>
<td>Bailey</td>
<td>2013</td>
<td>11.4</td>
<td>9.1</td>
</tr>
<tr>
<td>Loh</td>
<td>2014</td>
<td>12.2</td>
<td></td>
</tr>
</tbody>
</table>

**Diabetic**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year of Publication</th>
<th>CV&lt;sub&gt;i&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;g&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlsen</td>
<td>2011</td>
<td>30.5</td>
<td>16.8</td>
</tr>
</tbody>
</table>

**Serum**

<table>
<thead>
<tr>
<th>First Author</th>
<th>Year of Publication</th>
<th>CV&lt;sub&gt;i&lt;/sub&gt;</th>
<th>CV&lt;sub&gt;g&lt;/sub&gt;</th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harris</td>
<td>1970</td>
<td>5.6</td>
<td>7.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>1971</td>
<td>6.6</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Williams</td>
<td>1978</td>
<td>11.5, 6.1, 6.3, 6.6, 7.8, 7.8, 6.9</td>
<td>12.9, 5.6, 6.7, 8.3, 6.8, 10, 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costangs</td>
<td>1985</td>
<td>13.3; 7.9; 12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraser</td>
<td>1989</td>
<td>4.7</td>
<td>5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ricos</td>
<td>1989</td>
<td>10.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eckfeldt</td>
<td>1994</td>
<td>4.2</td>
<td>10.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carlsen</td>
<td>2011</td>
<td>5.4</td>
<td>5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pineda-Tenor</td>
<td>2013</td>
<td>5.5</td>
<td>8.2</td>
<td>&gt;80</td>
<td>♂</td>
</tr>
<tr>
<td>Pineda-Tenor</td>
<td>2013</td>
<td>3.7</td>
<td>8.8</td>
<td>19-42</td>
<td>♂</td>
</tr>
<tr>
<td>Pineda-Tenor</td>
<td>2013</td>
<td>6.8</td>
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<td>&gt;80</td>
<td>♀</td>
</tr>
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<td>2013</td>
<td>4.5</td>
<td>7.5</td>
<td>19-42</td>
<td>♀</td>
</tr>
<tr>
<td>Loch</td>
<td>2015</td>
<td>8.5; 10.4</td>
<td>16.2; 16.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Issues with (Glucose) BV data**

- Heterogeneity of protocols for derive biological variation data
- CV<sub>i</sub> and CV<sub>g</sub> values possibly dependent from different biological matrices
- CV<sub>i</sub> and CV<sub>g</sub> values different for healthy and diseased individuals
## Quantifying Biological Variation

### How do you do the experiment?

<table>
<thead>
<tr>
<th>✓ Subjects</th>
<th>How many?</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Collect specimens</td>
<td>Number? Frequency?</td>
</tr>
<tr>
<td>✓ Analyse specimens</td>
<td>Minimise analytical variation?</td>
</tr>
<tr>
<td>✓ Analyse data</td>
<td>Outliers? Statistics?</td>
</tr>
</tbody>
</table>

Biological variation from patients
Should they be used?

Inherent biological variability

\[ A \]

Inherent biological variability
+ disease (and treatment) related variability

\[ B \]


10th International Scientific Meeting, November 17-18, 2016
A checklist for critical appraisal of studies of biological variation

Within-subject biological variation of glucose and HbA$_{1c}$ in healthy persons and in type 1 diabetes patients

Siri Carlsen$^{1,2,9}$, Per Hyltoft Petersen$^2$, Svein Skeie$^{1,2}$, Øyvind Skadberg$^1$ and Sverre Sandberg$^2$

1 Department of Medicine, Stavanger University Hospital, Stavanger, Norway
2 Norwegian Center for Quality Improvement of Primary Care Laboratories (NOKLUS), Section for General Practice, Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway

<table>
<thead>
<tr>
<th>CV$_i$</th>
<th>CV$_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4%</td>
<td>5.6%</td>
</tr>
</tbody>
</table>
Assessing the number of specimens \((n)\) required to estimate the individual’s homeostatic setpoint of plasma glucose.

\[
n = 1.96^2 \times \left( \frac{CV_A^2 + CV_i^2}{D^2} \right)
\]

- \(CV_A\), Analytical coefficient of variation
- \(CV_i\), Within-subject biological coefficient of variation
- \(D\), desired percentage of closeness (usually, 95%)

<table>
<thead>
<tr>
<th>Glucose</th>
<th>HbA\textsubscript{1c}</th>
<th>CV\textsubscript{A}</th>
<th>CV\textsubscript{i}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2%</td>
<td>1.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose = 5.4%</td>
<td>HbA\textsubscript{1c}  = 2.5%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Glucose \(n = 4.7\)
- HbA\textsubscript{1c} \(n = 1.2\)

**Table 2.1—Criteria for the diagnosis of diabetes**

- FPG \(\geq 126\) mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
- 2-h PG \(\geq 200\) mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
- A1C \(\geq 6.5\% (48\) mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.

*Diabetes Care 2016; s1-112

**10th International Scientific Meeting, November 17-18, 2016**
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).
1. The measurand has a central role in diagnosis and monitoring of a specific disease ⇒ outcome model → Plasma Glucose

2. The measurand has a high homeostatic control ⇒ biological variability model

3. Neither central diagnostic role nor sufficient homeostatic control ⇒ state-of-the-art model
Workflow for allocation of laboratory measurands to different models for performance specifications

1. Has the measurand a central role in a specific disease?
   - YES: Do valid outcome data exist?
     - YES: Assign to outcome model
     - NO: Temporarily
   - NO: Has the measurand a steady state?
     - YES: Do valid biological variability data exist?
       - YES: Assign to biological variability model
       - NO: Temporarily
     - NO: Temporarily

2. Assign to the state-of-the-art model

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Ferruccio Ceriotti*, Pilar Fernandez-Calle, George G. Klee, Gunnar Nordin, Sverre Sandberg, Thomas Streichert, Joan-Lluis Vives-Corrons and Mauro Panteghini, on behalf of the EFLM Task and Finish Group on Allocation of laboratory tests to different models for performance specifications (TFG-DM)

10th International Scientific Meeting. November 17-18, 2016
Analytical performance specifications for plasma glucose based on data of biological variability of the analyte

**Minimum**

\[ CV_A < 0.75 \times CV_I \quad 4.05\% \]

\[ B < 0.375 \times (CV_I^2 + CV_G^2)^{0.5} \quad 3.0\% \]

\[ TE < [1.65 \times 0.75 \times CV_I + 0.375 \times (CV_I^2 + CV_G^2)^{0.5}] \quad 9.6\% \]

**Desirable**

\[ CV_A < 0.50 \times CV_I \quad 2.7\% \]

\[ B < 0.250 \times (CV_I^2 + CV_G^2)^{0.5} \quad 1.95\% \]

\[ TE < [1.65 \times 0.50 \times CV_I + 0.250 \times (CV_I^2 + CV_G^2)^{0.5}] \quad 6.4\% \]

**Optimum**

\[ CV_A < 0.25 \times CV_I \quad 1.35\% \]

\[ B < 0.125 \times (CV_I^2 + CV_G^2)^{0.5} \quad 1.0\% \]

\[ TE < [1.65 \times 0.25 \times CV_I + 0.125 \times (CV_I^2 + CV_G^2)^{0.5}] \quad 3.2\% \]
Defining analytical performance specifications using *indirect outcome data* (Model 1b)

- Impact of analytical performance of test on clinical classifications or decisions and thereby on probability of outcomes (simulation or decision analysis).
- To model the clinical outcomes of misclassification requires clinical evidence about the consequences for patients.
- Where clinical evidence about these consequences is not available, the model estimates will be based on *assumptions* drawn from what evidence there is about disease prognosis, treatment benefits, harms, etc.
A subject with a FPG of 117.5 mg/dL must be differentiate from healthy condition (from one side) and a frank diabetes diagnosis (from the other side). Therefore, TE of FPG measurement should be kept $< \frac{7.5}{117.5} = 6.38\%$, so that a subject with an IFG cannot be misclassified as diabetic (FPG >125 mg/dL) or healthy (FPG <110 mg/dL).

Model 2 - $TE_a < [1.65 \times 0.50 \times CV_i + 0.250 \times (CV_i^2 + CV_g^2)^{0.5}]$ 6.4%
Impact of measurement error of plasma glucose on clinical classification

Model 1b simulation analysis

<table>
<thead>
<tr>
<th>PG distribution</th>
<th>Reference</th>
<th>@ -6.38%</th>
<th>@ +6.38%</th>
</tr>
</thead>
<tbody>
<tr>
<td>n 6537</td>
<td>( \bar{X} = 109 \text{ mg/dL} )</td>
<td>( \bar{X} = 102 \text{ mg/dL} )</td>
<td>( \bar{X} = 116 \text{ mg/dL} )</td>
</tr>
<tr>
<td>IQR: 99-128</td>
<td>IQR: 93-120</td>
<td>IQR: 105-136</td>
<td></td>
</tr>
</tbody>
</table>

- 6.38% TE
- Reference
+ 6.38% TE

Outpatients

Cumulative distribution

Glucose, mg/dL

- 6.38% TE
- 12.6% IFG misclassified as healthy
- 6.2% DM misclassified as IFG

@ - 6.38% TE
@ + 6.38% TE

- 6.2% DM misclassified as IFG
- 18.1% Healthy misclassified as IFG
- 7.7% IFG misclassified as DM

Pasqualetti S, Braga F, Panteghini M
Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Italy

10th International Scientific Meeting, November 17-18, 2016
Analitical aspects of glucose testing

\[ V_{\text{TOT}} = (V_p^2 + V_A^2 + V_I^2)^{1/2} \]
Laboratory customers (i.e., doctors and patients) expect lab results to be equivalent and interpreted in a reliable and consistent manner.

**Unbroken Traceability Chain**
Definition of higher order references in order to implement the appropriate trueness transfer process to commercial calibrators.

**STANDARDIZATION**
To achieve metrological traceability of patient results to higher-order references.

**Measurement uncertainty budget**
Definition of allowable limits for uncertainty.

**Post-market surveillance**
Survey - suitability of assays and laboratory performances.

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“Non-negative parameter characterizing the dispersion of the quantity values being reasonably attributed to a measurand, based on the information used”

**Bias**, systematic measurement error

**Uncertainty of calibrator**

**Bias correction**, realignment of measuring system by adjusting the value assigned to the calibrator

**Uncertainty**
Three main components of uncertainty:
1. **Uncertainty of references** - reference materials, reference procedures;
2. **Uncertainty of commercial system calibrators** - manufacturer’s calibrator values [transfer process];
3. **Uncertainty of random sources** – system imprecision, individual lab performance.

\[
\text{Measurement uncertainty budget} = \sqrt{u_{\text{ref}}^2 + u_{\text{cal}}^2 + u_{\text{random}}^2}
\]

---

**ALLOWABLE UNCERTAINTY BUDGET**

---

**Uncertainty of references**

**System calibration uncertainty**

**System imprecision**

**Individual lab performance (IQC safety margin)**

---

**For Plasma Glucose**

\[\text{From MODEL 2} \]

\[u_{\text{ref}}\]

\[\sqrt{u_{\text{ref}}^2 + u_{\text{cal}}^2 + u_{\text{random}}^2}\]

**Mesurand definition**

**Patient result**

---

**Measurement uncertainty goal**

[for unbiased results]

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**10th International Scientific Meeting. November 17-18, 2016**
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.

- The allowable limit for the combined uncertainty of manufacturer’s commercial calibrators @ 50% of the goals

Measurand definition

Measurement uncertainty budget

System calibration (combined) uncertainty

System imprecision

Individual lab performance (IQC safety margin)

Patient result

CIRME

10

CELEBRATING

Years

10th International Scientific Meeting, November 17-18, 2016
IVD MANUFACTURERS MAY SPEND DIFFERENT AMOUNTS OF THE TOTAL UNCERTAINTY BUDGET TO ALLOW TRACEABILITY OF THEIR ANALYTICAL SYSTEM TO HIGHER ORDER REFERENCES.


10th International Scientific Meeting. November 17-18, 2016
Are the analytical system commercially available for glucose determination able to achieve the desirable limit for combined uncertainty in a clinical setting (fit for purpose)?

<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 NIST SRM 965</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>NIST SRM 965</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>NIST SRM 917a</td>
<td>B</td>
<td>1.60–3.00%</td>
<td></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 ND</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 ND</td>
<td>B</td>
<td>1.70%</td>
</tr>
<tr>
<td></td>
<td>Modular</td>
<td>Hexokinase</td>
<td>C.f.a.s.</td>
<td>0.84%</td>
<td>IDMS ND</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 NIST SRM 917a</td>
<td>C</td>
<td>1.88–3.26%</td>
</tr>
<tr>
<td></td>
<td>GOD</td>
<td>Chemistry calibrator</td>
<td>Chemistry calibrator</td>
<td>0.80%</td>
<td>Hexokinase NIST SRM 917a</td>
<td>C</td>
<td>1.88–3.26%</td>
</tr>
</tbody>
</table>

\[
\text{Measurement uncertainty budget} = \left( u^2_{\text{ref}} + u^2_{\text{cal}} + u^2_{\text{random}} \right)^{\frac{1}{2}}
\]

\[
\text{Uncertainty of references} = u_{\text{ref}}
\]

\[
\text{System calibration uncertainty} = (u^2_{\text{ref}} + u^2_{\text{cal}})^{\frac{1}{2}}
\]

\[
\text{System imprecision} = (u^2_{\text{cal}} + u^2_{\text{random}})^{\frac{1}{2}}
\]

\[
\text{Patient result} = \text{uncertainty budget + system imprecision + measurement imprecision}
\]

\[
\text{Chain A} = 1.45\% \text{ vs. Chain C} = 3.26\%
\]

\[
\text{4.05\% minimum}
\]

\[
\text{2.70\% desirable}
\]

\[
\text{1.35\% optimum}
\]


10th International Scientific Meeting. November 17-18, 2016
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 reference 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 (EQAS category 1/2A or 1/2B)</td>
<td>To verify the suitability of laboratory measurements in clinical setting</td>
</tr>
</tbody>
</table>

Infusino I et al., Clin Chem Lab Med 2010;48:301
Trueness-Based EQAS – Example 1

Analytical performance of 17 general chemistry analytes across countries and across manufacturers in the INPUs project of EQA organizers in Italy, the Netherlands, Portugal, United Kingdom and Spain

References (materials and procedure)
- frozen human serum
- GC-IDMS reference procedure

Performance specifications for TEa derived from biological variation

<table>
<thead>
<tr>
<th>Analyte</th>
<th>ES</th>
<th>IT</th>
<th>PT</th>
<th>UK</th>
<th>All</th>
<th>NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>7.7</td>
<td>6.8</td>
<td>8.3</td>
<td>8.0</td>
<td>7.5</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Glucose TE
9.6% minimum
6.4% desirable
3.2% optimum

Between laboratory CV, %

EQAS Category 1/2A

10th International Scientific Meeting. November 17-18, 2016
Trueness-Assessment for serum glucose measurement in different Commercial Systems through the preparation of Commutable Reference Materials

ChangYu et al., Ann Lab Med 2012;32:243-9

References (materials and procedure)
- Pooled sera
- US Centers for Disease Control (CDC) reference procedure

Most BUT NOT ALL of the measurement systems met the minimum quality specifications for bias.

Table 1. Relative bias for glucose measurement using 6 commercial systems

<table>
<thead>
<tr>
<th>System code</th>
<th>Manufacturer</th>
<th>Method</th>
<th>Stated traceability for the reference method</th>
<th>Analyzer type</th>
<th>Relative bias (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>RM1</td>
</tr>
<tr>
<td>GOD01</td>
<td>Beckman</td>
<td>GOD-oxygen electrode</td>
<td>HK</td>
<td>DxC800 (N=2), DxC20 (N=1)</td>
<td>2.88</td>
</tr>
<tr>
<td>GOD02</td>
<td>Roche</td>
<td>GOD-POD</td>
<td>ID-MS</td>
<td>Modular P800 (N=3)</td>
<td>3.19</td>
</tr>
<tr>
<td>GOD03</td>
<td>Ortho</td>
<td>GOD-dry chemistry</td>
<td>HK</td>
<td>Vitros 250 (N=3)</td>
<td>1.92</td>
</tr>
<tr>
<td>HK01</td>
<td>Beckman</td>
<td>HK-G6PD</td>
<td>HK</td>
<td>DxC800 (N=2), DxC20 (N=1)</td>
<td>-1.92</td>
</tr>
<tr>
<td>HK02</td>
<td>Roche</td>
<td>HK-G6PD</td>
<td>ID-MS</td>
<td>Modular P800 (N=3)</td>
<td>-3.83*</td>
</tr>
<tr>
<td>HK03</td>
<td>Behring</td>
<td>HK-G6PD</td>
<td>ID-MS</td>
<td>RXL-MAX (N=3)</td>
<td>-1.28</td>
</tr>
</tbody>
</table>

From MODEL 2

3.0% minimum
2.0% desirable
1.0% optimum

10th International Scientific Meeting. November 17-18, 2016
Verification of the accuracy of three glucose point-of-care testing (POCT) devices for their use in a hospital setting

Elena Aloisio, Erika Frusciante, Alberto Dolci, Mauro Panteghini
Research Centre for Metrological Traceability in Laboratory Medicine (CIRME), University of Milan, Italy

- Comparison with a standardized automated system (Abbott, ref. n. 3L82, mean bias 0.2% vs CDC ref. procedure performed @CIRME)
- CLSI acceptability criteria (POCT12-A3)

...DESPITE MANY EFFORTS BY THE PROFESSION...

...QUANTIFICATION OF A SIMPLE MOLECULE LIKE GLUCOSE IS NOT SIMPLE...

...BUT WE ARE WELL ON THE WAY!
Thank you for Your kind attention!!