Challenge of Improved Outcomes in Diabetes, Role of Laboratories

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Contents

- **Laboratory measurements, accuracies and pitfalls**
  - ISO 15189:2013 Medical laboratories. Requirements for quality and competence
  - P and fP-Glucose, also B-HbA1c

- **Point-of-care devices: a field of co-operation**
  - ISO 22870:2016 Point-of-care testing - Requirements for quality and competence

- **Self-monitoring devices**
  - ISO 15197:2013 Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus

- **Continuous glucose monitoring (CGM)**
  - Tissue (interstitial) fluid sample, sensor technology
  - Mean absolute relative difference (MARD), traceability of values?

- **Ketosis** in diabetes: methods of detection and limitations
- **Glucosuria** revisited: methods of detection
Traceability chain of glucose measurements

- **Patient:** Continuous sensor under patient skin, calibration?

- **Patient:** comparisons & factory calibration
  - Nurse vs labtech: comparisons with laboratory
  - EQA and IQC available

- **Lab professionals**
  - Full traceability to primary procedures and EQA/IQC
Plasma (fP/P) Glucose

**Benefits**
- Easy to measure enzymatically, $CV_{(a + pa)} < 5\%$
- Clearly defined traceability in laboratories
- Routine in hospital care
- Reference for other glucose measurements

**Pitfalls**
- **Fasting state** variability in fP-Glucose classification → repeats
- Non-well preserved samples: Test tubes in routine?
- Not for frequent measurement needs (> 6 times/day)
- fP-Glucose fails to detect about 30% of new diabetes cases
WHO and IDF criteria for diabetes, 2006

Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. WHO 2006

NEEDS FOR OGTT

- failures of detection by fP-Glucose

- OGTT is frequently needed to confirm or exclude an abnormality of glucose tolerance in asymptomatic people.

- BUT:
  - bad diagnostic reproducibility
  - needs of reclassifications
  - requires human resource

### Diabetes

<table>
<thead>
<tr>
<th>Test</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>≥7.0 mmol/L (126 mg/dL) or ≥11.1 mmol/L (200 mg/dL)</td>
</tr>
<tr>
<td>2-h plasma glucose*</td>
<td></td>
</tr>
</tbody>
</table>

### Impaired Glucose Tolerance (IGT)

<table>
<thead>
<tr>
<th>Test</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>&lt;7.0 mmol/L (126 mg/dL) and ≥7.8 and &lt;11.1 mmol/L (140 mg/dL and 200 mg/dL)</td>
</tr>
<tr>
<td>2-h plasma glucose*</td>
<td></td>
</tr>
</tbody>
</table>

### Impaired Fasting Glucose (IFG)

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<th>Test</th>
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<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>6.1 to 6.9 mmol/L (110 mg/dL to 125 mg/dL)</td>
</tr>
<tr>
<td>2-h plasma glucose*</td>
<td>and (if measured)</td>
</tr>
<tr>
<td></td>
<td>&lt;7.8 mmol/L (140 mg/dL)</td>
</tr>
</tbody>
</table>

* Venous plasma glucose 2–h after ingestion of 75g oral glucose load

* If 2–h plasma glucose is not measured, status is uncertain as diabetes or IGT cannot be excluded.
B-Haemoglobin A1c

**Benefits**
- IFCC-defined traceability and unit (mmol/mol)
- Cornerstone of diabetes treatment (longterm complications)
- Target of treatment if possible B-HbA1c < 53 mmol/mol (7 % old units)
- Non-fasting, robust sample = minor influence of physiological states

**Diagnostic classification**
- Indirect measure of glycemia; limit 48 mmol/mol (6.5 % old units)

**Pitfalls**
- Disorders of Haemoglobin turnover (autoimmunity etc)
- Blood transfusions
- Hb variants affect glycation reaction
- Diagnostic cut-off in children is unclear, differences between races exist
### Accuracy of results, HbA1c (EQA, Labquality, 5/2017)

<table>
<thead>
<tr>
<th>Sample 2</th>
<th>mean</th>
<th>median</th>
<th>s</th>
<th>CV(%)</th>
<th>min</th>
<th>max</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>52.44</td>
<td>0.2</td>
<td>52.6</td>
<td>2.39</td>
<td>4.6</td>
<td>50.0</td>
<td>66.1</td>
</tr>
</tbody>
</table>

Imprecision (CV) among all laboratories is about 5%, within on method group variable - in one laboratory optimally less than 2 %
Detectable changes in HbA1c ratios

- $D_k = 1.96 \times \sqrt{2} \times u_c$ (combined uncertainty, $p < 0.05$) (Fraser, Harris)

- Laboratory performance, including preanalytical CV = 2%
  - $D_k = 1.96 \times \sqrt{2} \times u_c \approx 3 \times \sqrt{0.0008} = 3 \times 2.8 \% \approx 8 \%$
  - $D_k = 1.96 \times \sqrt{2} \times u_c \approx 3 \times \sqrt{0.0029} = 3 \times 5.4 \% \approx 16 \%$

- Improvement from 90 to 80 mmol/mol = - 11 \%
- Change from 60 to 70 mmol/mol = + 17%  
  or from 60 to 50 mmol/mol = - 17 \%

- Improvement from 60 to 55 mmol/mol = - 8 \% (?)
Point-of-care testing in health care units

ISO 22870:2016  Requirements for quality and competence

- **Co-operation in leadership:** Organised responsibilities, tasks and costs, as defined by hospital or district administration

- Testing and **purchasing** new equipment with public tenders

- Agreement on share of **costs and expenses**, including labour allocation to quality support

- Protocols for measurements and **quality assessment** (IQC and EQA), repeated training practices

- Computerised requisition and possibilities to report into **Lab Information Systems** or Electronic Patient Records
Accuracy of self-monitoring devices

- Requirements of ISO 15197:2013 standard for self-monitoring of blood glucose (SMBG)


<table>
<thead>
<tr>
<th></th>
<th>ISO 15197:2003</th>
<th>ISO 15197:2013</th>
<th>FDA(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative number of results</td>
<td>95%</td>
<td>95%(^b)</td>
<td>95%</td>
</tr>
<tr>
<td>Within</td>
<td>±15 mg/dl</td>
<td>±15%</td>
<td>±15%</td>
</tr>
<tr>
<td>At BG concentrations</td>
<td>&lt;75 mg/dl</td>
<td>≥75 mg/dl</td>
<td>Entire range</td>
</tr>
</tbody>
</table>


\(^b\)In ISO 15197:2013 this acceptance criterion is also applied for the user performance evaluation.
4.4. SKUP’s quality goals in this evaluation
As agreed upon when the protocol was drawn up, the results from the evaluation of Accu-Chek Guide are assessed against the following quality goals:

- Repeatability (CV) .................................................................................................................................. \(\leq 5.0\%\)

- Allowable deviation in the individual result from the comparison method result (according to ISO 15197:2013) *
  - for glucose concentrations \(<5.55\ mmol/L\) .................................................................................. \(\leq 0.83\ mmol/L\)
  - and for glucose concentrations \(\geq 5.55\ mmol/L\) ........................................................................ \(\leq 15\%\)

- Required percentage of individual results within the allowable deviations ......................................................... \(\geq 95\%\)

- User-friendliness, overall rating............................................................................................................. Satisfactory

* The number of results within a stricter Swedish quality goal (allowable deviation in the individual result from the comparison method result \(\leq 0.42\ mmol/L\) at glucose concentration \(<4.2\ mmol/L\) or \(\leq 10\%\) at glucose concentration \(\geq 4.2\ mmol/L\)) will be reported, but not assessed in the report.

A recent example on Roche Accu-Chek tested as case no 2017/112

9.2.2018 T.Kouri, HUSLAB
Figure 5. Accuracy of glucose results on Accu-Chek Guide achieved by the intended users (three lots of test strips). The x-axis represents the mean glucose result of the comparison method. The y-axis represents the glucose deviation in mmol/L of the first measurement on Accu-Chek Guide from the mean result of the corresponding sample of the comparison method. Stippled lines represent quality goal limits set in ISO 15197:2013 (within ±0.83 mmol/L of the results of the comparison method for glucose concentrations <5.55 mmol/L and within ±15% for glucose concentrations ≥5.55 mmol/L). Number of results (n) = 88. Statistical outliers from the calculations of repeatability, are illustrated with a circle around the symbol. An account of the number of samples is given in section 6.1.
Figure 3. Consensus error grid (CEG) analysis of an SMBG system (3 test strip lots, 600 data). According to ISO 15197:2013, 99% of measurement results shall be within CEG zones A and B. The SMBG system displayed shows 99.8% of results within CEG zones A and B.

Freckmann G et al, 2015
Traceability chain of glucose measurements

- Continuous sensor
- Patient comparisons
- Nurse/labtech comparisons
- Laboratories
Continuous glucose monitoring

- Aims at improved glycemia control (lower HbA1c) without suffering from hypoglycaemia
- Most useful in predicting / preventing hypoglycaemia
- Need and risk: about 25% of patients with diabetes have impaired glycemic awareness (US FDA document 2017)
- Search for trueness and traceability of values

- Sensor technologies (commercial patents)
  - Dexcom, Medtronic, Abbott, Senseonics

- Measuring trends rather than accurate concentrations
- Calibration with SMBG by patient,
  - except Dexcom G5 claim! (2017)
Principles of glucose sensors

- **Electrochemical measurement**
  - Glucose oxidase $\rightarrow$ FAD $\rightarrow$ H$_2$O$_2$ $\rightarrow$ /at Pt-electrode/2e$^-$/ + O$_2$ + 2H$^+$
    - Generation of hydrogen peroxide $\rightarrow$ oxygen and electrons
    - Dependence of high pO2 in interstitial fluid

- **Improving accuracy**
  - Direct Ag/Ag+ electrodes
  - Osmium and ferrocene complexes
  - Other principles of measurement
    - optical fluorescence, mini-dialysis etc
CGM systems

Wearable Continuous Glucose Monitoring Sensors: A Revolution in Diabetes Treatment

Giacomo Cappon, Giada Acclaroli, Martina Vettoretti, Andrea Facchinetti and Giovanni Sparacino

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Figure 2. Examples of minimally-invasive CGM systems based on electrochemical sensing technique: (a) A patient wearing a sensor (taken from [27]); (b) Medtronic Enlite sensor with dedicated inserter device (taken from [28]); (c) Dexcom G5 Mobile with Share technology (taken from [29]); (d) Abbott FreeStyle Navigator II. (taken from [30]).
Accuracy estimation: MARD unit

- MARD = mean absolute (+/- removed) relative difference between two measurements (without knowing the true value)

\[
MARD = \frac{1}{N} \sum_{i=1}^{N} \left| \frac{CGM_i - BG_i}{BG_i} \right| \times 100\%
\]

- Closer to Dahlberg’s estimate of coefficient of variation would be PARD measured with two parallel sensors:

\[
PARD = \frac{1}{N} \sum_{i=1}^{N} \left| \frac{CGM_{i,1} - CGM_{i,2}}{(CGM_{i,1} + CGM_{i,2})/2} \right| \times 100\%
\]

- MARD is about 0,8 x CV(%) (Noujaim et al, 2007)
  → Uncertainty, imprecision (u, CV) = about 1,25 x MARD
  Then 2u is about +/- 2,5 x MARD (95% CI of a result)

- Quality specification of ISO15197:2013 is +/- 15% (95% CI)
  → Quality specification to MARD is then < 6% (15%/ 2,5)
Tissue fluid (Tf) = interstitial fluid (If), Challenge for glucose measurement

- Affected by perfusion/ blood pressure and aB-pO₂
  - Dependence of redox reactions on interstitial pO₂
  - Physiology (exercise, nutrition, medication)
- Delayed response as compared to plasma
  - Complicated algorithms needed for prediction (10~20 min ahead)
- Impact of biofilms within subcutaneous tissue?
  - Foreign body response by the patient
  - Replacement after 7/14 days
- Specificity of electrochemical and other reactions?
  - Examples of non-specific molecules: vitamin C, uric acid, dopamine

→ Current recommendation: NOT FOR HOSPITAL USE, BUT YES FOR PROFESSIONAL UNDERSTANDING!
Ketosis: methods of detection

- **Plasma hydroxybutyrate**
  - Major ketone body
  - Measurable with a POC device and in the laboratory

- **Urine acetoacetate (test strip)**
  - Traditional ketone body, Legal’s test
  - Test strip measurements to be traced with Li-AcAc
  - Plasma applications of acetoacetate strip no more recommended
Glucosuria revisited

- Intensive glycemic control may be difficult if glucose is leaking into urine (glucosuria)

- Test strip measurement (U-Glucose) is still reasonable
  - sensitive at 2-3 mmol/l level and
  - linear to allow reports of arbitrary concentrations
THANK YOU!
Minimum change (difference) calculation
(Fraser, Harris)

- \( D_k = 1,96 \times \sqrt{2} \times u_c \) (combined uncertainty, \( p < 0,05 \))

- Combined uncertainty = preanalytical + analytical imprecision
  - \( u(c)^2 = u(\text{preanal})^2 + u(\text{anal})^2 = 0,02^2 + 0,02^2 = 0,0008 \)
    (optimal conditions within one laboratory service)
  - \( u(c)^2 = 0,02^2 + 0,05^2 = 0,0029 \) (average international level)

- \( D_k = 1,96 \times \sqrt{2} \times u_c \sim 3 \times \sqrt{0,0008} = 3 \times 2,8 \% \sim 8 \% \)
- \( D_k = 1,96 \times \sqrt{2} \times u_c \sim 3 \times \sqrt{0,0029} = 3 \times 5,4 \% \sim 16 \% \)