Standardization, harmonization, traceability and transference

Management Module
It is undeniable that the clinical laboratory is experiencing globalization.

It can be argued that the world is “shrinking” Economies are more dependent on multiple countries to market their products.

As a result, people travel more, work in different countries and may find themselves seeking health care in different locations.

There is a need to “standardize” medical practice, including clinical laboratory practice, has increased in importance.
Introduction

History

The Reference Measurement System

Examples of standardization
- Type A analyte: Creatinine
- Type B analyte: HbA₁C

Conclusion: Standardization leads to ????
Introduction

Goal of laboratory medicine is to provide information that is useful in the clinical decision making process and to prevent cases where incorrect interpretation leads to poor patient outcomes

- One of the biggest challenges facing laboratories worldwide is the lack of comparability of analytical results
- The different platforms and the different methodology all contribute to this variability
- 2002 international study conducted by the EU-revealed biases ranging from -60% to +30% for certain analytes
Introduction

• The result:
  ◆ Cannot compare results from different methods/laboratories
  ◆ Cannot define common reference intervals
  ◆ Cannot define common decision limits for a biomarker
  ◆ Difficult application of evidence based medicine, e.g. guidelines established by scientific bodies

• Solution: Reduce the variability through standardization
Introduction

- The definition of STANDARDIZATION
  - Concept whereby agreement of test results is achieved by establishing traceability to higher order reference materials and measurement procedures
  - Achievable when the method base + measurand is clearly defined.
  - Outcome = agreement with established “trueness”
Introduction

- **The definition of HARMONIZATION**
  - Process of making agreement between methods in order to produce a consistent clinical interpretation irrespective of the laboratory in which the samples are analysed
  - No 1\degree reference material/higher order reference measurement procedure exists
  - Measureand cannot be clearly defined
  - Values may not be traceable to SI units
  - Outcome = *not necessarily equated to “trueness”*
Standardization: History

- ISO released 2 documents 17511 and 18153 in response to the lack of standardization - stressed importance of traceability to recognized reference materials and methods thereby constructing ‘traceability chains’

- European directive on in vitro diagnostics (IVD) forces manufacturers to ensure that the systems they market have been calibrated against certificate reference materials and reference measurement procedures

- EU directive was the major driving force for the establishment of the JCTLM in 2002 (Joint Committee for traceability in laboratory medicine)

- The JCTML is tasked with establishing the reference measurement system (identifying reference material, reference method and reference laboratories who would conform to these documentary standards)
Standardization: History

- Implementation of standardization = global task
- Cooperation NB to avoid confusion and waste of resources
- Governmental agencies, metrological institutes, standards organizations, clinical laboratory societies and diagnostics industry work closely together to effectively improve the standardization of tests used in clinical laboratories
In order to achieve standardization, an approach is required that provides a reliable transfer of the measurement values from the highest hierarchical level to methods which are routinely used in the clinical laboratories.

Such a structure is presented by the reference measurement system, based on the concepts of metrological traceability and a hierarchy of analytical measurement procedures.

Key elements of the system:
- Reference measurement procedure
- Reference materials
A detailed definition of the “measurand” constitutes an indispensable part of any analytical reference system.

**Definition of MEASURAND**
- The international Vocabulary of Metrology (VIM) defines measurand as the “quantity intended to be measured”
- Often in laboratory medicine this term is erroneous replaced by the less specific term “analyte”

In laboratory medicine, many hundreds of different analytes are measured.

With regards to implementation of traceability it is however NB to differentiated between **Type A** quantities/analytes and **Type B** quantities/analytes.
Reference Measurement System

Identifying the analyte of interest

- **Type A analytes**
  - Well defined analytes (+/- 65 analytes)
  - Traceable to the SI units i.e. mol/l
  - Results not method dependent
  - Full traceable chains are available

- **Type B analyte**
  - Not well defined
  - Proteins/glycoproteins
  - Usually measured immunochemically
  - Heterogeneous (can be bound/free)
  - Not traceable to SI units
Reference Measurement System

Type A analytes
- Relatively easy to standardise

*Example: Creatinine*

Type B analytes
- make standardization very difficult
- When reference materials /reference procedures are not identified, manufacturers prepare their own calibrators
- Even though a difficult process, a RMS can be achieved by identifying the invariable portion of the molecule, therefore bringing about homogeneity

*Example: IFCC success with HbA1c*
Reference Measurement System

- NB concept in the Reference Measurement System = “commutability”

- Definition of COMMUTABILITY
  - The property of a reference material, demonstrated by the equivalence of the mathematical relationship among the results of different measurement procedures for a reference material and for representative samples of the type intended to be measured

- i.e. ability of reference or control material to show interassay properties similar to those of human procedures
  - Primary reference material = pure analyte
  - Secondary reference material = human serum (pooled to minimize CVg)
Standardization of Creatinine

1° reference material (pure substance)
Reference Procedure (Reference Laboratory)
2° reference material (analyte in human serum)
Calibration of routine methods
Measurement of clinical samples by commercial assays

Crystalline creatinine NIST SRM 914a
Isotope dilution mass spectrometry
Fresh frozen human serum pool with values assigned by IDMS method
Lower reference ranges because of
Reporting of eGFR using IDMS-traceable MDRD formula
Standardization of HbA₁C

- DCCT and UKPDS, landmark trials documenting the benefit of tight glycaemic control (measured as the level of glycated hemoglobin) - to clinical outcome

- A linear correlation was observed between the average HbA₁c values of patients and the risk of diabetic microvascular complications

- It was clear that a minor ↓ of HbA₁c resulted in significant changes in the relative risk of microvascular complications
1993, after publication of DCCT data where HPLC method was used, measurement of HbA1C was placed in the spotlight

- many different assays measuring HbA1C
  - Bayer, Roche, Beckman
- different methods
  - HPLC
  - Cation-exchange
  - Affinity chromatography
  - immunoassays
- Uncalibrated
- unacceptably large biases
- very high imprecision levels

Standardization of HbA1C
# Standardization of HbA1C

<table>
<thead>
<tr>
<th>Glycated proteins</th>
<th>Methods</th>
<th>Some models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin A₁ (HbA₁a+b+c) Unit: %</td>
<td>Ion exchange chromatography</td>
<td>Glycomat</td>
</tr>
<tr>
<td>Electrophoresis (ElectroOsmosis)</td>
<td>Helena HbA₁ REP</td>
<td></td>
</tr>
<tr>
<td>HPLC</td>
<td>Bio-Rad Diamat/Merck L-9100</td>
<td></td>
</tr>
<tr>
<td>Mechanical procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affinity chromatography</td>
<td>GHb Abbott IMx, GLYCO HB, GLYCO-Tek</td>
<td></td>
</tr>
<tr>
<td>Immunological procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZE (Capillary Zone Electrophoresis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other procedures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin A₁c Unit: %</td>
<td>Ion exchange chromatography</td>
<td>Bio-Rad LPLC-DiaSTAT, Glycomat, Helena HbA₁c Column</td>
</tr>
<tr>
<td>Electrophoresis</td>
<td>Beckman Coulter Diatrac HbA₁c, Helena SAS-MX Glyco A₁c, Sebia Hydragel HbA₁c</td>
<td></td>
</tr>
<tr>
<td>HPLC/FPLC</td>
<td>Bio Rad Diamat (HPLC), Bio-Rad DiaSTAT (LPLC), Bio-Rad Variant I (HPLC), Bio-Rad Variant II (HPLC), Bio-Rad D-a0 (HPLC), Merck L-9100 (HPLC), Menarini (HPLC), TOSOH A₁c 2.2 (HPLC)</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Dako HbA₁c</td>
<td></td>
</tr>
<tr>
<td>Turbidimetry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunological procedures</td>
<td>Bayer DCA 2000, Bayer RA HbA₁c-Reagenz, Beckman Coulter Synchron HbA₁c, Dade Behring HbA₁c, Roche Tina-quant® [a] HbA₁c (adapted also to third-party systems), Roche Unimatic</td>
<td></td>
</tr>
<tr>
<td>CZE (Capillary Zone Electrophoresis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCCT Standardization (explicitly declared as a method!)</td>
<td>Roche Tina-quant® [a] HbA₁c, Roche Unimatic</td>
<td></td>
</tr>
<tr>
<td>IFCC Standardization (explicitly declared as a method!)</td>
<td>Roche Tina-quant® [a] HbA₁c, Roche Unimatic</td>
<td></td>
</tr>
<tr>
<td>Other procedures (calculated HbA₁c)</td>
<td>Abbott IMx II, Bio-Rad Micromat II, Glyco-HbA₁c, Cholestech G.D.X, Helena GLYCO-Tek Affinity Column</td>
<td></td>
</tr>
</tbody>
</table>
In 1993 the American Association of Clinical Chemistry established a National Glycohemoglobin Standardization Program (NGSP).

- **Goal**: To harmonize glycohemoglobin test results to the DCCT and UKPDS.

- Consists of a Central primary reference laboratory using HPLC which had the same set points as the DCCT.

- Primary reference laboratories and secondary reference laboratories.

- This involves the assignment of calibrator values or equations used to align themselves to the DCCT set points.
Standardization of HbA₁C

- 2nd step: Precision study → acceptable imprecision < 5%
- In order to maintain and evaluate commercial methods, routine users must submit to the proficiency program conducted by the NGSP
- CAP surveys (2007) revealed that 99% of all labs are using the NGSP certified method
- This harmonization process lead to a significant reduction in inter-laboratory variation from > 12% to < 5%
- Still not truly standardized as different countries (UK vs Sweden vs Japan) harmonized to different methods
IFCC standardization of HbA₁C

- 1995: IFCC HbA₁c working group established

- **Objective:**
  - Define HbA₁c
  - establish a true reference method for international standardization

- **Analyte defined** as adduct of glucose to the N–terminal amino group of the β- chain of HbA₀ named N-[1-deoxyfructosyl] Hb β-chain or DOF-Hb

- **Purity of the analyte:** using endoproteinase, whole blood used to prepare primary materials i.e. glycated Hb and non-glycated Hb by means of 3 stage chromatography process

- **Reference method:** HPLC-MS/ HPLC-CE
IFCC isolation of pure HbA₁C and HbAo

Whole, native blood

Washing/lysis of RBC
Removal of cells debris

Hemoglobin

Cation exchange chromatography

HbA₁C (crude)

GHb

HbA₁C (pure)

HbAo (crude)

Non-GHb

HbAo (pure)

HPLC

Affinity Chromatography
(Boronate; pH 8.0)

Cation exchange chromatography
(Sapharose, pH 6.2)
NGSP versus IFCC

PROBLEM!!

- Clear difference in measurements; IFCC - 1.5 - 2% lower
  - Confusing to dr’s and patients
  - May lead to deterioration of glycaemic control
NGSP versus IFCC

DECISION!!

  - IFCC method is the only valid anchor to implement standardization
  - Results to be reported in mmol/mol [mmol HbA1c/mol of total HbA (HbA1c + HbA0)]
  - Values will be reported in both IFCC (mmol/mol) and DCCT units (%) using the IFCC-DCCT master equation to relate these two values
  - Await results of the ADAG study
Standardization of HbA1C

- Study found a linear relationship between results traceable to the IFCC reference system and previous routine methods allowing the conversion of analytical and clinical data from one system to another.

- In practice it is therefore possible to translate target values generated in previous landmark studies, using methods not traced to the IFCC system, in order to maintain the clinical experience.

\[ y = 0.9148x + 2.152 \]
\[ (\text{NGSP HbA1c}) = 0.9148 (\text{IFCC HbA1c}) + 2.152 \]
\[ (\text{IFCC HbA1c}) = 1.093 (\text{NGSP HbA1c}) - 2.350 \]

rounded: NGSP = 0.915(IFCC) + 2.15
Standardization of HbA1C

In practice it is therefore possible to translate target values generated in previous landmark studies, using methods not traced to the IFCC system, in order to maintain the clinical experience.

<table>
<thead>
<tr>
<th></th>
<th>Current</th>
<th>IFCC traceable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference interval (non-diabetics)</td>
<td>4 – 6%</td>
<td>20 – 42 mmol/mol</td>
</tr>
<tr>
<td>Target for treatment in diabetics</td>
<td>&lt; 7%</td>
<td>&lt; 53 mmol/mol</td>
</tr>
<tr>
<td>Change in Rx in diabetics</td>
<td>&gt; 8%</td>
<td>&gt; 64 mmol/mol</td>
</tr>
</tbody>
</table>
The key components of the process for establishing traceability:

Development and characterization of suitable reference materials and their value assignment in meaningful units using reference measurement procedures

Establishment of commercial routine assays yielding results traceable to higher order reference materials and methods

Availability of appropriate reference intervals and decision limits
Therefore standardization/traceability leads to:

- Comparability of results performed on different platforms
- Standard reference intervals instead of method specific ones
- Supports best care guidelines to be developed + NB in EBLM
- Reduces analytical error and enhances patient safety
- Allows for a more objective comparison through EQA programs and not peer group performances
- Better method comparison studies with reference procedures
- Allows for better laboratory-physician interaction
References

- Standardization –the theory and practice-Jill Tate, M Panteghini

- The JCTML- A global approach to promote standardization of clinical laboratory test results

- Traceability, Reference systems and result comparability- Clin Biochem reviews- 28Aug 2007

- HbA1c-Glycated hemoglobin and Diabetes Mellitus- Science- Prof HR Henrichs

- HbA1c Analysis-from chaos to harmony-DB Sacks- J. Clin Path-2008

- International expert Committee on A1c-Diabetes Care-2009