An Effort for Standardization of Pre-analytic Process of Laboratory Testing in Japan

2013 JCCLS Symposium
4, April/2013
Tokyo, JAPAN

Midori Ishibashi
New Tokyo Hospital
Positioning of Laboratory Testing in a Health Care Setting

- Clinical diagnosis
- Evaluation of therapeutic effect and prognostic prediction
- Health control in preventive medical care
- Assessment of safety and efficacy in clinical trials
- Assessment of efficacy in clinical research

Each of the above items requires high-quality data and consistent assessment criteria
1. **Inter-individual variation:** Hereditary, environmental, and temporal factors

2. **Intra-individual variation:** Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)

3. **Sample collection:** Blood sample collection sites and tubes

4. **Custody of blood samples from time of collection to measurement:** Pretreatment, storage temperature, etc.

5. **Factors affecting analysis:** Management of procedural precision, reagents, instruments, etc.

6. **Laboratory error:** Sample mix-ups, data entry errors, etc.

7. **Factors affecting interpretation of results:** Reference interval/cut-off values, comments, etc.
<table>
<thead>
<tr>
<th><strong>Hereditary factors</strong></th>
<th><strong>Sex, race, genetic polymorphism/gene mutation, etc.</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine, CK, uric acid ・・・</td>
</tr>
<tr>
<td><strong>Environmental factors</strong></td>
<td><strong>Eating, drinking, and smoking habits</strong></td>
</tr>
<tr>
<td></td>
<td>TP, ALB, γGT, TG, CEA ･･･</td>
</tr>
<tr>
<td><strong>Temporal factors</strong></td>
<td><strong>Age, etc.</strong></td>
</tr>
<tr>
<td></td>
<td>TC, ALB, HbA1c, ALP ･･･</td>
</tr>
</tbody>
</table>
ALP Isozyme

Serum ALP ratio (%)

Liver | Bone | Intestine

Blood type B, O
Age Profiling

The JCCLS Committee for Commoditizing Reference Interval
1. **Inter-individual variation:** Hereditary, environmental, and temporal factors

2. **Intra-individual variation:** Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)

3. **Sample collection:** Blood sample collection sites and tubes

4. **Custody of blood samples from time of collection to measurement:** Pretreatment, storage temperature, etc.

5. **Factors affecting analysis:** Management of procedural precision, reagents, instruments, etc.

6. **Laboratory error:** Sample mix-ups, data entry errors, etc.

7. **Factors affecting interpretation of results:** Reference interval/cut-off values, comments, etc.
Physiological variations

Food and drink intake, exercise, posture, circadian rhythm, menstrual cycle, pregnancy, inter-seasonal variation, age, etc.
### After meal | Exercise | Standing position | Circadian rhythm | Sex differential | Infant | Third trimester
--- | --- | --- | --- | --- | --- | ---
TP |  | ↑↑ |  | ↓↓ |  |
UA |  |  |  | M | ↑↑ |  |
CA |  |  |  |  | ↑ |  |
IP |  |  |  |  | ↓↓ |  |
LDH |  | ↑↑ |  |  |  |  |
AST,ALT |  | ↑↑ |  |  |  |  |
FE |  |  |  | Morning↑↓Night |  |  |
TG | ↑↑ |  |  | ↑ |  |  |
FFA |  |  |  |  | ↓↓ |  |
Circadian rhythm of ACTH and Cortisol
Variation of Index for Glycemic Control in Normal Pregnancy

- HbA1c
- GA
- Blood glucose

Gestational age

Gestational age

Gestational age
1. Inter-individual variation: Hereditary, environmental, and temporal factors
2. Intra-individual variation: Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)
3. Sample collection: Blood sample collection sites and tubes
4. Custody of blood samples from time of collection to measurement: Pretreatment, storage temperature, etc.
5. Factors affecting analysis: Management of procedural precision, reagents, instruments, etc.
6. Laboratory error: Sample mix-ups, data entry errors, etc.
7. Factors affecting interpretation of results: Reference interval/cut-off values, comments, etc.
<table>
<thead>
<tr>
<th>Blood sample collection site</th>
<th>Venous blood, Arterial blood, Blood capillary, etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood tube type</td>
<td>Blood test tubes, anticoagulant agents, separating agents, etc.</td>
</tr>
<tr>
<td>Tourniquet pressure and duration</td>
<td>Clenching</td>
</tr>
<tr>
<td>Order of blood collection</td>
<td>Additive agents contained in the test tube and tissue fluid contamination</td>
</tr>
</tbody>
</table>
A, B: NaF + Citrate + Citric Na + EDTA-2Na
C, D: NaF + Heparin Na + EDTA-2Na
Serum > Plasma
1. Test tubes containing citric acid for coagulation testing
2. Serum test tubes
3. Test tubes containing heparin
4. Test tubes containing EDTA
5. Test tubes containing glycolytic inhibitor
6. Others

JCCLS Guidelines for Standard Blood Collection Method
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Inter-individual variation:</td>
<td>Hereditary, environmental, and temporal factors</td>
<td></td>
</tr>
<tr>
<td>2. Intra-individual variation:</td>
<td>Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)</td>
<td></td>
</tr>
<tr>
<td>3. Sample collection:</td>
<td>Blood sample collection sites and tubes</td>
<td></td>
</tr>
<tr>
<td>4. Custody of blood samples from time of collection to measurement:</td>
<td>Pretreatment, storage temperature, etc.</td>
<td></td>
</tr>
<tr>
<td>5. Factors affecting analysis:</td>
<td>Management of procedural precision, reagents, instruments, etc.</td>
<td></td>
</tr>
<tr>
<td>6. Laboratory error:</td>
<td>Sample mix-ups, data entry errors, etc.</td>
<td></td>
</tr>
<tr>
<td>7. Factors affecting interpretation of results:</td>
<td>Reference interval/cut-off values, comments, etc.</td>
<td></td>
</tr>
</tbody>
</table>
Transportation of sample 1
  Time before, temperature during, air shooters, etc.

Centrifugal separation
  Time before, speed, temperature, time, etc.

Storage
  Temperature, time, etc.

Transportation of sample 2 (to an external laboratory)
  Time before, temperature during, management until measurement, etc.
Stability of Endotoxin (stored whole blood)
Stability of NSE in Serum at Storage Temperature

Room Temperature

-20 °C

-80 °C

~ 205 %

Room Temperature

-20 °C

-80 °C

~ 205 %
1. Inter-individual variation: Hereditary, environmental, and temporal factors

2. Intra-individual variation: Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)

3. Sample collection: Blood sample collection sites and tubes

4. Custody of blood samples from time of collection to measurement: Pretreatment, storage temperature, etc.

5. Factors affecting analysis: Management of procedural precision, reagents, instruments, etc.

6. Laboratory error: Sample mix-ups, data entry errors, etc.

7. Factors affecting interpretation of results: Reference interval/cut-off values, comments, etc.
Factors affecting Analysis

- Management of reagents
- Maintenance of instruments
- Detection of abnormal reactions
- Internal quality control
- External quality control
Japan Medical Association Quality Assessment Surveillance
changes observed over 12 years

- Method: Recommended by Japan Society of Clinical Chemistry
- ERM
- Investigative research committee
- Ad hoc committee
- Survey 2000
- Survey 2003
- PSA
- γ-GT

Intra-method (CV [%])

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><strong>Inter-individual variation:</strong></td>
<td>Hereditary, environmental, and temporal factors</td>
</tr>
<tr>
<td>2.</td>
<td><strong>Intra-individual variation:</strong></td>
<td>Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)</td>
</tr>
<tr>
<td>3.</td>
<td><strong>Sample collection:</strong></td>
<td>Blood sample collection sites and tubes</td>
</tr>
<tr>
<td>4.</td>
<td><strong>Custody of blood samples from time of collection to measurement:</strong></td>
<td>Pretreatment, storage temperature, etc.</td>
</tr>
<tr>
<td>5.</td>
<td><strong>Factors affecting analysis:</strong></td>
<td>Management of procedural precision, reagents, instruments, etc.</td>
</tr>
<tr>
<td>6.</td>
<td><strong>Laboratory error:</strong></td>
<td>Sample mix-ups, data entry errors, etc.</td>
</tr>
<tr>
<td>7.</td>
<td><strong>Factors affecting interpretation of results:</strong></td>
<td>Reference interval/cut-off values, comments, etc.</td>
</tr>
</tbody>
</table>
6. Laboratory Errors

- Labeling errors
- Sampling errors
- Recording and data entry errors
- Failure to verify results

• • • etc.
Patient at initial consultation

Leukemia patient in remission

WBC 4,200
PLT 250,000

Return home

WBC 20,500
PLT 32,000

Emergency hospitalization
Scheduled for bone marrow aspiration

Patients with Same Surname – Labeling Error
1. **Inter-individual variation:**  
   Hereditary, environmental, and temporal factors

2. **Intra-individual variation:**  
   Physiological variations (food and drink intake, exercise, posture, seasonal/circadian rhythm, etc.)

3. **Sample collection:**  
   Blood sample collection sites and tubes

4. **Custody of blood samples from time of collection to measurement:**  
   Pretreatment, storage temperature, etc.

5. **Factors affecting analysis:**  
   Management of procedural precision, reagents, instruments, etc.

6. **Laboratory error:**  
   Sample mix-ups, data entry errors, etc.

7. **Factors affecting interpretation of results:**  
   Reference interval/cut-off values, comments, etc.
7. Factors affecting Results Interpretation

- Reference interval
- Method for measurement
- Interpretation of comments
Measurement methods are unclear (HDL-C, LDL-C, VLDL)

Units are unclear

Reference interval and criteria are neither clear nor coordinated with the measurement method

Reference interval is not clear for pediatrics

The measurement method is hanged during the clinical trial period

Different measurement units for HbA1c from those in global trials were unknowingly used

Cell components were measured by a centralized system and required a long storage time → Unsuitable

Trials require different storage conditions for the same test
### Commoditizing Reference Interval

#### The JCCLS Committee for Commoditizing Reference Interval

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit</th>
<th>Difference index between the sexes</th>
<th>Men and Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower limit</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Intermediate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upper limit</td>
</tr>
<tr>
<td>WBC</td>
<td>1000/μL</td>
<td>0.13</td>
<td>3.3</td>
</tr>
<tr>
<td>RBC</td>
<td>10000/μL</td>
<td>1.17</td>
<td>5.4</td>
</tr>
<tr>
<td>Hb</td>
<td>g/dL</td>
<td>1.49</td>
<td>8.6</td>
</tr>
<tr>
<td>Ht</td>
<td>%</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>fL</td>
<td>0.21</td>
<td>83.6</td>
</tr>
<tr>
<td>MCH</td>
<td>pg</td>
<td>0.11</td>
<td>4077</td>
</tr>
<tr>
<td>MCHC</td>
<td>%</td>
<td>0.19</td>
<td>27.5</td>
</tr>
<tr>
<td>PLT</td>
<td>10000/μL</td>
<td>0.18</td>
<td>31.7</td>
</tr>
<tr>
<td>TP</td>
<td>g/dL</td>
<td>0.02</td>
<td>15.8</td>
</tr>
<tr>
<td>Alb</td>
<td>g/dL</td>
<td>0.19</td>
<td>23.6</td>
</tr>
<tr>
<td>Glb</td>
<td>g/dL</td>
<td>0.08</td>
<td>34.8</td>
</tr>
<tr>
<td>A/G</td>
<td></td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>UN</td>
<td>mg/dL</td>
<td>0.35</td>
<td>5179</td>
</tr>
<tr>
<td>CRE</td>
<td>mg/dL</td>
<td>1.62</td>
<td>8</td>
</tr>
<tr>
<td>UA</td>
<td>mg/dL</td>
<td>1.25</td>
<td>12</td>
</tr>
<tr>
<td>Na</td>
<td>mEq/L</td>
<td>0.44</td>
<td>5019</td>
</tr>
<tr>
<td>K</td>
<td>mEq/L</td>
<td>0.24</td>
<td>5178</td>
</tr>
<tr>
<td>Cl</td>
<td>mEq/L</td>
<td>0.18</td>
<td>5001</td>
</tr>
<tr>
<td>Ca</td>
<td>mg/dL</td>
<td>0.32</td>
<td>4923</td>
</tr>
<tr>
<td>IP</td>
<td>mg/dL</td>
<td>0.36</td>
<td>5188</td>
</tr>
<tr>
<td>Glu</td>
<td>mg/dL</td>
<td>0.35</td>
<td>2972</td>
</tr>
<tr>
<td>TG</td>
<td>mg/dL</td>
<td>0.61</td>
<td>3142</td>
</tr>
<tr>
<td>TCho</td>
<td>mg/dL</td>
<td>0.00</td>
<td>3397</td>
</tr>
<tr>
<td>HDL-C</td>
<td>mg/dL</td>
<td>0.63</td>
<td>5152</td>
</tr>
<tr>
<td>LDL-C</td>
<td>mg/dL</td>
<td>0.24</td>
<td>3068</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
HbA1c values in Japanese unit are lower in the efficacy assessment of global clinical trials for antidiabetic.

Japan: JDS unit
Europe and America: NGSP unit

NGSP (%) unit = JDS unit + 0.4 (%)
### Conversion of HbA1c Results

<table>
<thead>
<tr>
<th>JDS (%)</th>
<th>NGSP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5%</td>
<td>6.92%</td>
</tr>
<tr>
<td>7.0%</td>
<td>7.43%</td>
</tr>
<tr>
<td>8.0%</td>
<td>8.45%</td>
</tr>
</tbody>
</table>

**IFCC unit: mmol/mol**

**NGSP unit: %**

**JDS unit: %**

\[
\text{NGSP unit (\%)} = 1.02 \times \text{JDS unit (\%)} + 0.25
\]

\[
\text{JDS unit (\%)} = 0.98 \times \text{NGSP unit (\%)} - 0.245
\]
Japan: JDS units → NGSP units
Scandinavian countries: Mono-S units
US, Australia, Asia, other countries: NGSP units
Japan: JDS units → NGSP units
Scandinavian countries: Mono-S units
US, Australia, Asia, other countries: NGSP units
Diabetes management guidelines: HbA1c

ADA (US)
HbA1c < 7%

IDF (Europe)
HbA1c ≤ 6.5%

CDA (Canada)
HbA1c ≤ 7%

NICE (UK)
HbA1c 6.5–7.5%

AACE (US)
HbA1c ≤ 6.5%

ALAD (Latin America)
HbA1c < 6–7%

APPG (Asia Pacific)
HbA1c < 6.5%

JDS (Japan)
HbA1c ≤ 6.5% + Glu.

Australia
HbA1c ≤ 7%

6 ALAD. Rev Asoc Lat Diab 2000; Suppl. 1.
8 NSW Health Department. 1996.
Diabetic Medicine has adopted dual reporting of glycated haemoglobin (HbA1c) measurement. HbA1c measurements must be reported in IFCC units (mmol/mol – no decimal point) in addition to derived NGSP units (% - one decimal). IFCC units should be listed first followed by NGSP units in parentheses.

Units should conform to the SI convention.

Both traditional DCCT-derived units (as %) and IFCC-recommended units (as mmol/mol)

Beginning with manuscripts submitted after Jan. 1, 2013, Diabetes Care requires authors to report HA1c levels in both traditional DCCT-derived units (as %) and SI, IFCC-recommended units (as mmol/mol).
Standardization of Pre-analytic Process
• Pre-analytical variation
• Analytical variation
• Within-subject variation (biological sources)
Variation in the Pre-analytic Phase

- Time of day
- Fed/fasting state
- Previous and recent exercise
- Previous and recent use of stimulants
- Posture
- Source of sample
- Anticoagulant or preservative or stabilizer
- Tourniquet application period
- Transport time and temperature
- Centrifugation time and force
- Storage conditions
1. Specify the durations among sample collection time to pretreatment time and measurement time.

2. Specify the transportation method.

3. Specify the centrifugation conditions (temperature, speed, time).

4. Specify the storage temperature.

5. Indicate the reference interval.

6. Indicate the measurement method.

7. Indicate the regression equation of the previous method when the measurement method is changed.

8. Clearly indicate the measurement units.
Standardize the conditions of patient preparation including time of day and posture in particular

Adopt standard procedures for phlebotomy including types of samples collected and containers used, and tourniquet application time

Adhere to standard types of sample transport, handling, and centrifugation.

Pre-analytical variation can be minimized by standardizing procedures and all other aspects relevant to sample collection.
Thank you for your attention.

This presentation is supported by the National Cancer Center Research and Development Fund (23-A-1), Japan.