Standardization Activities at the National Center for Clinical Laboratories

Wenxiang Chen, MD, MSc
National Center for clinical Laboratories and Beijing Hospital Institute of geriatrics
chenwenxiang@263.net
About the Center

- An institution and agency of the Ministry of Health
- Mission: to improve the quality of clinical laboratory testing through evaluation, investigation and management
- Organization: EQA Offices and Laboratories specialized in clinical biochemistry, hematology, immunology, microbiology and molecular biology
Scientific activities

- National EQA for clinical laboratories
- Researches and investigations on quality issues
- Reference system development and implementation
Collaborations

- Professional organizations
- Local Centers for Clinical laboratories
- University and institute laboratories
- Metrological institutes
- Clinical laboratories
- Manufacturers
2003-07 National EQA, selected analytes

- All method interlaboratory CV
- Participants ~900, mostly Tier III hospital laboratories

<table>
<thead>
<tr>
<th>Analyte</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
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<tbody>
<tr>
<td>Cholesterol</td>
<td>4.4</td>
<td>4.7</td>
<td>4.6</td>
<td>4.2</td>
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<tr>
<td>Triglycerides</td>
<td>11.3</td>
<td>11.1</td>
<td>17.5</td>
<td>11.4</td>
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<tr>
<td>HDLC</td>
<td>18.7</td>
<td>16.8</td>
<td>14.6</td>
<td>14.3</td>
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<tr>
<td>LDLC</td>
<td>15.1</td>
<td>13.6</td>
<td>13.7</td>
<td>12.9</td>
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<tr>
<td>ALT</td>
<td>11.3</td>
<td>9.7</td>
<td>9.9</td>
<td>7.9</td>
</tr>
<tr>
<td>AST</td>
<td>10.7</td>
<td>9.4</td>
<td>10.1</td>
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<tr>
<td>Sodium</td>
<td>2.4</td>
<td>2.3</td>
<td>2.5</td>
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<tr>
<td>Chloride</td>
<td>3.7</td>
<td>3.3</td>
<td>3.9</td>
<td>3.2</td>
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<tr>
<td>Glucose</td>
<td>4.5</td>
<td>4.8</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Creatinine</td>
<td>10.8</td>
<td>13.8</td>
<td>13.6</td>
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<tr>
<td>Uric acid</td>
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<td>5.6</td>
<td>5.2</td>
</tr>
<tr>
<td>Urea</td>
<td>5.3</td>
<td>5.9</td>
<td>4.7</td>
<td>4.5</td>
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</tbody>
</table>
Beijing 2006 fresh serum HDL-C survey

- Fresh serum pool distributed
- Repeated measurements

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Bias</th>
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<tr>
<td>Lab 1</td>
<td>1.53</td>
<td>24%</td>
</tr>
<tr>
<td>Lab 2</td>
<td>1.22</td>
<td>-1%</td>
</tr>
<tr>
<td>Lab 3</td>
<td>1.23</td>
<td>0%</td>
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<tr>
<td>Lab 4</td>
<td>1.53</td>
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<td>Lab 5</td>
<td>1.58</td>
<td>28%</td>
</tr>
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<td>Lab 6</td>
<td>1.05</td>
<td>-15%</td>
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<td>Lab 7</td>
<td>1.22</td>
<td>-1%</td>
</tr>
<tr>
<td>Median</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>15%</td>
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</table>
Lipid and lipoprotein performance in a clinical study, 1997-2000

- CHD secondary prevention study
- 65 participating laboratories nationwide
- 1997-2000, 8 surveys
- Frozen serum, 3 levels
- Measured by each lab 3 times in triplicate
- ~1500 measurement events, CV and Biases calculated
Lipid and lipoprotein performance in a clinical study, 1997-2000

Total cholesterol, ~50% Bias<3%
Lipid and lipoprotein performance in a clinical study, 1997-2000

Triglycerides, ~40% Bias<5%
Lipid and lipoprotein performance in a clinical study, 1997-2000

HDL cholesterol, ~25% Bias<5%
Situations and issues

- Diverse analytical systems
  - number of manufacturers
  - method combinations and modifications
  - national EQA 2007, chemistry:
    - ~1000 participants
    - ~130 instrument models
    - >90 reagent and calibrator manufacturers
    - hundreds of systems
- EQA difficult
  - peer group impossible or difficult for some analytes
  - pass one, fail another
The Roles of reference systems

- EQA organizers
  - target value assignment
  - property investigations of the materials
- Manufacturers
  - calibration
  - evaluation or verification
- Clinical laboratories
  - verification of the trueness
Reference system activities: lipids and lipoproteins

- Started in the early 80s by Dr. Jianzhai Li
- Candidate reference methods for cholesterol and triglycerides by HPLC
- IFCC apo AI and B calibrations
- CDC Abell-Kendall cholesterol and DCM HDL
- CDC CRMLN member since 2003
- National CRMs (GBWs) for cholesterol and triglycerides (pure substances and serum matrix)
- The CDC CRMLN cholesterol and HDL certifications
- Researches on UC/HPLC lipoprotein cholesterol and ID/MS cholesterol and triglycerides
- Beijing Hospital Institute of Geriatrics in collaboration with NIM
CDC CRMLN bimonthly survey
The cholesterol A-K reference method and HDL DCM activities

- 16 Chol systems of 3 manufacturers certified
- Certification of 2 HDL systems ongoing
- EQAS targeting
- Method evaluations
- Standardization of lipid and lipoprotein measurements in clinical, epidemiological studies
UC/HPLC HDL, LDL, Lp(a) and subclass cholesterol

- Sample volume 0.1 ml (vs. 5 ml in the traditional betaquantification)
- HDL separation by UC [Lp(a) breakdown by mercaptoethanol] (vs. precipitation)
- Type 25 rotor (100 1-ml tubes)
- Lp(a) and subclass cholesterol possible

HDL₃C = BF₁.₁₂₅MEC
HDL₂C = BF₁.₀₆₃MEC − BF₁.₁₂₅MEC
LDLₖC = BF₁.₀₄₄MEC − BF₁.₀₆₃MEC
LDLₐC = BF₁.₀₀₆C − BF₁.₀₄₄C
Lp(a)C = BF₁.₀₄₄C − BF₁.₀₄₄MEC
ID/LC/MS/MS  Serum total cholesterol

- 3,4-¹³C₂ cholesterol as internal standard
- Oxidized to 4-en-3,6-dione
- Positive ion APCI
- SIR and MRM detections
- Total CV <1%
- NIST SRM analysis
ID/LC/MS/MS Serum total glycerides

- 1,2,3-$^{13}$C$_3$ glycerol as internal standard
- Extracted by benzylation
- Positive ion ESI
- MRM detection
- Total CV ~1%
- NIST SRM analysis

![Diagram of chemical reactions and formulas]
Triglycerides: enzymatic vs. ID-LC/MS/MS, correlation

![Graphs showing correlation between enzymatic and ID-LC/MS/MS measurements of triglycerides, with values ranging from 0 to 6 mmol/l on both axes.](image-url)
Triglycerides: enzymatic vs. ID/LC/MS/MS, bias
Triglycerides: commutability, 2007 EQA materials
Reference system activities:

- Transfer of IFCC reference procedures since 2003
- Preliminary laboratory network by CCCLS, Dr. Zhenhua Yang
Reference system activities: hematology and infectious disease

- Hematology
  - ICSH reference procedures for blood cells established
  - platelet and hemoglobin procedures and materials ongoing
  - calibration activities in Beijing area
- Infectious disease
  - Hepatitis B and C nucleic acids RMs (GBWs)
  - Antigen or antibody RMs ongoing
Reference system activities: metabolites, ions and NP hormones

- Multi-organization collaborations
- ID/MS or other reliable principles
- Started and initial progresses made
ID/GC/MS  Serum progesterone

- Total CV ~2%
- IRMM ERM analysis
- Collaborations with NIM

**CCQM-P77b Progesterone in Female Serum**

<table>
<thead>
<tr>
<th>Mass Fraction (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST</td>
</tr>
<tr>
<td>NRCCRM</td>
</tr>
<tr>
<td>PTB</td>
</tr>
<tr>
<td>NMIJ</td>
</tr>
<tr>
<td>New Zealand</td>
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**CCQM-P77b Progesterone in Male Serum**

<table>
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<tr>
<th>Mass Fraction (ng/g)</th>
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<tr>
<td>NIST</td>
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<td>NRCCRM</td>
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<tr>
<td>New Zealand</td>
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Total CV ~2%

IRMM ERM analysis

Collaborations with NIM
<table>
<thead>
<tr>
<th>Sample#</th>
<th>All methods mean</th>
<th>ID/GC/MS</th>
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</thead>
<tbody>
<tr>
<td>200612</td>
<td>50.00 nmol/L</td>
<td>72.22 nmol/L</td>
</tr>
<tr>
<td>200613</td>
<td>36.54 nmol/L</td>
<td>48.71 nmol/L</td>
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</table>
ID/GC/MS serum urea

- Method of Kessler and Siekmann (Clin Chem 1999)
- Total CV <1%
- NIST SRM analysis
ICP/MS serum sodium

- Aluminum as internal standard
- Serum digested with nitric acid
- Total CV ~ 0.2%
- NIST SRM analysis
- Collaborations with the Army’s General Hospital and Laboratory Center
The Nation’s 11th “Five-year Plan”

National research programs supporting reference systems

▪ National Key Technologies R&D Program
  (Project #2007BAI05B09)

  □ Reference or comparison measurement procedures for important metabolites/substrates, electrolytes and metal ions, enzymes, non-peptide hormones, CVD risk factors, hematology and infectious disease tests

▪ National High-tech R&D Program (the 863 Program)
  (Project #2006AA020909)

  □ Reference materials for important chemistry, infectious disease and hematology and genetic tests
Acknowledgements

Colleagues and students at the National Center for Clinical Laboratories

Colleagues from the profession and the metrology institute