2013 JCCLS Symposium

「An Approved Guideline for the Quality Management of Specimens for Molecular Methods; The Procurement, Transport, and Preparation of Specimens」

Hayato Miyachi, MD, PhD
Department of Laboratory Medicine, Tokai University School of Medicine. Japan
Japanese Committee of Clinical Laboratory Standards
Japanese National Committee for ISO/TC212
Outline of Presentation

• Trends of molecular genetic testing
• Global and regional efforts in standards
• Current status and issues
• Standards for Quality Management of Specimens
• Evidence based in the standards
• Challenges with issues and standards
Expanded Use and Global Standards

• Ongoing expansion: Research → Clinical
• Sequencing and biological significance of human genome → individual drug responses or future disease risks → Genome-based medicine (Individualized, Preventive)
• Entry of clinical laboratories into service
• Entry of molecular/genetic scientists into service
• Genetic information service
  Medicine → Health industry
• Regional → Global
• Untrained care providers

Needs for global standards:
OECD → ISO, CDC, CLSI etc
JCCLS:
Committee of Standardization of Gene-related testing （2006～）

Difficult to standardize due to special complexity

Government
Industry
Professionals

Ministry of Economy, Trade and Industry
Ministry of Health, Labor and Welfare

Sysmex Co.
Jap Bioindustry Assoc.
ISO/TC212 Japan

Jap. Soc. Gene Diag Ther
Jap. Soc. Hum Genetics
Jap. Assoc. Med. Technologists
Efforts for Global and Regional Efforts

Global

UNESCO
Genetic information

OECD
Guideline Draft

OECD
Guideline Issue

ISO TC212
NWIP

CDC
guideline

SPIDIA
project

CLSI
guideline

2003 2004 2005 2006 2007 2008 2009 2010 2011

Guideline of genetic testing

Guideline of privacy protection

Insurance Coverage of solid tumor And inherited diseases

Insurance coverage of human gen PGx

Guideline of specimens or genetic testing

Guideline best practice

Guidelines genetic tests and diagnoses

Japan

Guideline of genetic testing

Insurance Coverage of solid tumor And inherited diseases

Mapping of genetic testing

Guideline PGx testing

Issued by JCCLS:
Japanese Committee of Clinical Laboratory Standards (NPO)
Gene-related tests (Biological materials)

(exogenous)

① Pathogen Molecular tests (nucleic acid tests)
  - Virus • bacteria
  - hepatitis virus
  - Mycobacterium tuberculosis
  - Chlamydia trachomatis
  - N.gonorrhoeae

② Somatic Cells
  - Leukemia
  - Malignant lymphoma
  - Solid tumors

③ Germ line cells
  - Drug metabolism and response
  - Monogenic diseases
    • Hereditary diseases
    • familial tumors
  - Disease susceptibility
  - Body constitution
    • alcohol
    • obesity
    • Personal identification

(endogenous)

Confirmatory diagnostic tests
Carrier tests
Preclinical tests
<table>
<thead>
<tr>
<th>Items</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>I) Drafting of best practice guideline</td>
<td>1) Education and Training of personnel and Qualification, Accreditation, and Audit · Directive, 2) Proficiency testing, 3) Proper use, testing and report, 4) Feedback of test utilization</td>
</tr>
<tr>
<td>II) Development of technology for standardization</td>
<td>QC of specimens, application kits/automated system, reference materials</td>
</tr>
<tr>
<td>III) Proficiency testing</td>
<td>Domestic and CAP survey, new survey</td>
</tr>
<tr>
<td>IV) Proper use, test performance and report</td>
<td>Clinical utility, indication, labeling, QC methods, reporting requirement</td>
</tr>
<tr>
<td>V) Feedback of testing</td>
<td>Collection of record and report of outcomes, analysis of test utilization, evidence for coverage decisions</td>
</tr>
<tr>
<td>VI) Education of physicians and consumers</td>
<td>Media? School?</td>
</tr>
</tbody>
</table>
Quality Assurance of Total Process of Testing

- Patient monitored
- Right test ordered
- Right specimens procured
- Quality of analytic process

Preanalytic
- Right test ordered
- Right specimens procured

Analytic
- Test performed correctly
- Results tracked and returned - Clinician

Postanalytic
- Correct response to results
- Patient notified of results
## Quality Assurance in Nucleic Acid Tests

<table>
<thead>
<tr>
<th>Process</th>
<th>Major factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target</td>
<td>target loads</td>
</tr>
<tr>
<td></td>
<td>sequences (variations)</td>
</tr>
<tr>
<td>Sampling</td>
<td>specimens variety</td>
</tr>
<tr>
<td></td>
<td>(compatibility and stability)</td>
</tr>
<tr>
<td></td>
<td>inhibitors</td>
</tr>
<tr>
<td>Extraction</td>
<td>Collection, transport, storage</td>
</tr>
<tr>
<td></td>
<td>sample preparation, reagents</td>
</tr>
<tr>
<td>Amplification</td>
<td>nucleic acid degradation</td>
</tr>
<tr>
<td>Detection</td>
<td>contamination, internal control</td>
</tr>
<tr>
<td>Result</td>
<td>Methods</td>
</tr>
<tr>
<td>Report</td>
<td>Clinical validity</td>
</tr>
<tr>
<td></td>
<td>Interpretation</td>
</tr>
</tbody>
</table>
HCV Ab and RNA (5,395 samples)

507 Pos. for Ab.

8 false-Neg. for HCV RNA

Because of heparin or subtypes
Pre-analytical Process

Collection → Storage → Transport → Pretreatment → Extraction of Nucleic acids

Specimen types, characteristics, interference:
Biological, physical, and chemical

Professional with manual techniques:
Collection and thereafter

Laboratory and personnel:
Procedures and techniques

Issue: Standards for the process and quality assurance of testing
A Guideline for Quality Management of Specimens in Molecular Methods: Procurement, Transport and Preparation of Specimens

• The guideline for a practical use on general principle and basic methods of collection, storage, transport and preparation of specimens for molecular diagnostic methods
Scope

• The principles and basic methods of specimen procurement: namely, the collection, storage, transport, and preparation of specimens for methods of molecular diagnosis to measure specific sequences for pathogens, somatic cells, and germ line cells.
A Tentative Guideline for Quality Management of Specimens in Molecular Methods: Procurement, Transport and Preparation of Specimens

<table>
<thead>
<tr>
<th>Proper methods to assure specimen conditions</th>
<th>Inappropriate conditions of specimens</th>
<th>Possible causes of inaccurate results</th>
<th>How to avoid these problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatic cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germ line cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Published studies
2. The experience of expertise
3. Recommendations from manufactures
Highlights of the Guideline

1. Introduction
2. Scope
3. Storage and Transport of Specimens for Molecular Methods
   3.1 for Pathogens
      3.1.1 Serum · Plasma
      3.1.2 Urine
      3.1.3 Sputum
   3.2 for Somatic cells
      3.2.1 Tissue · Tissue Slice Fragments
      3.2.2 Whole Blood (WBC)
      3.2.3 Urine · Stool
   3.3 for Germ Line Cells
4. Preparation of Specimens for Molecular Methods
5. Collection of Specimens for Molecular Methods
## Selected Contents in the Guideline

<table>
<thead>
<tr>
<th>Categories</th>
<th>Sampling</th>
<th>Storage and transport</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens</td>
<td>Target lesions</td>
<td>Avoidance of degradation of nucleic acids</td>
<td>Avoidance of contamination Washing</td>
</tr>
<tr>
<td></td>
<td>Avoidance of heparin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatic cells</td>
<td>Target lesions</td>
<td>Avoidance of degradation of nucleic acids</td>
<td>Separation of malignant cells.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fixation with 10% NBF</td>
<td></td>
</tr>
<tr>
<td>Germ line cells</td>
<td>Face-to-face</td>
<td>Privacy protection</td>
<td></td>
</tr>
</tbody>
</table>
### 3.2 Storage and Transport of for Somatic cells

#### 3.2.2 Whole Blood Cells (WBC)

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Storage in RT</th>
<th>Alternative methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>① RNA quantitation</td>
<td>&lt;2 h</td>
<td>RT 1 wk after RNA denature (Guanidine isothiocyanate)</td>
</tr>
<tr>
<td>② DNA variation</td>
<td>&lt;3 days</td>
<td>Freeze whole blood</td>
</tr>
<tr>
<td>③ High molecular DNA analysis</td>
<td>&lt;24 h</td>
<td>Freeze after cell separation (−70°C)</td>
</tr>
<tr>
<td>(Southern blotting)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## New Evidence in the Guideline

<table>
<thead>
<tr>
<th>Analysis of basic properties of specimens</th>
<th>Interference of properties of specimens on measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Sputum</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Tissue</strong></td>
<td></td>
</tr>
</tbody>
</table>

1. New experimental studies
2. Analysis of exiting results
3. Published studies and In-house data
Gene-related tests (human-derived specimens)

(exogenous)

① Pathogen Molecular tests (nucleic acid tests)
  - Virus • bacteria
  - hepatitis virus
  - Mycobacterium tuberculosis
  - Chlamydia trachomatis
  - N. gonorrhoeae

② Somatic Cells
  - Leukemia
  - Malignant lymphoma
  - Solid tumors
  - Drug metabolism and response

③ Germ cell line
  - Monogenic diseases
  - Hereditary diseases
  - familial tumors
  - Disease susceptibility
  - Body constitution
    • alcohol
    • obesity
    • Personal identification

Pharmacogenonomic/Companion Diagnostic tests

Confirmatory diagnostic tests
Carrier tests
Preclinical tests
## Companion Diagnostics

<table>
<thead>
<tr>
<th>CD function</th>
<th>Therapeutic</th>
<th>Cancer type</th>
<th>Diag. target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>Herceptin</td>
<td>Breast cancer</td>
<td>Her2/neu</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen, Aromasin</td>
<td>Breast cancer</td>
<td>E/P receptor</td>
</tr>
<tr>
<td></td>
<td>Erlotinib/Tarceva</td>
<td>NSCL lung cancer</td>
<td>EGFR</td>
</tr>
<tr>
<td></td>
<td>Erbitux</td>
<td>Colorectal cancer</td>
<td>EGFR</td>
</tr>
<tr>
<td></td>
<td>Erbitux</td>
<td>Colorectal cancer</td>
<td>KRAS</td>
</tr>
<tr>
<td></td>
<td>Gleevec</td>
<td>CML</td>
<td>BCR-ABL</td>
</tr>
<tr>
<td></td>
<td>Gleevec</td>
<td>GIST</td>
<td>CKIT</td>
</tr>
<tr>
<td></td>
<td>Rituxan</td>
<td>NHL</td>
<td>CD20</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen</td>
<td>Breast cancer</td>
<td>CYP450</td>
</tr>
<tr>
<td></td>
<td>Gemzar</td>
<td>NSCLC, Breast, Ovarian, Pancreatic</td>
<td>RRMI</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>NSCLC, Colorectal cancer</td>
<td>ERCC1</td>
</tr>
<tr>
<td></td>
<td>Cisplatin</td>
<td>NSCLC, Colorectal cancer</td>
<td>TS</td>
</tr>
<tr>
<td>Safety</td>
<td>Camptosar</td>
<td>Colorectal cancer</td>
<td>UGT1A1</td>
</tr>
<tr>
<td></td>
<td>Purinethol</td>
<td>Leukemia</td>
<td>TPMT</td>
</tr>
<tr>
<td></td>
<td>5-FU</td>
<td>Colorectal cancer</td>
<td>DHPD</td>
</tr>
<tr>
<td></td>
<td>Elitek</td>
<td>Leukemia, Lymphoma</td>
<td>G6PD</td>
</tr>
</tbody>
</table>
Monitoring during Imatinib Therapy

- **CCR**: Complete cytogenetic response
- **MMR**: Major molecular response (>3-log reduction)
- **CMR**: Complete molecular response (BCR-ABL-negative by nested PCR)

**Imatinib**

- Within 12 M.
  - **CCR**
  - Within 18 M.
    - **MMR**
    - nested PCR (+) ↓ (-)
    - **CMR**

- **PCR(PB)**
  - Each 3M
  - Each 6M

- **Karyotype(BM) or FISH**
  - Each 3-6M
  - Karyotype Each 12M
  - ± FISH (18M～)

CCR: Complete cytogenetic response
MMR: major molecular response (>3-log reduction)
CMR: complete molecular response (BCR-ABL-negative by nested PCR)
Isolation of Leucocytes from Blood for RNA

- Remove erythrocytes by a hypotonic buffer
- Use of a buffy coat
- Isolation by density-gradient centrifugation
- Enrichment based on density (Erutriation)
- Enrichment using antibodies
Estimate the integrity of total RNA samples

- RNA Integrity Number (RIN) determined by Agilent 2100 bioanalyzer.
- Separated by electrophoretic separation on microfabricated chips, and subsequently detected via laser induced fluorescence detection.
  → Software algorithm allows the classification of total RNA, based on a numbering system from 1 to 10.
Cell Separation Methods for Leukemia on Quality of Extracted RNA

**Electrophoresis patterns on chip and data analysis**

### Cell separation methods for leukemia on quality of extracted RNA

<table>
<thead>
<tr>
<th>Methods</th>
<th>A260/A280</th>
<th>RIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Hemolysis</td>
<td>1.78</td>
<td>2.2</td>
</tr>
<tr>
<td>2: Ficoll-Hypac (Ficoll layer)</td>
<td>1.30</td>
<td>5.6</td>
</tr>
<tr>
<td>3: Ficoll-paque (Upper layer)</td>
<td>1.31</td>
<td>5.4</td>
</tr>
<tr>
<td>4: Buffy-coat</td>
<td>1.58</td>
<td>6.1</td>
</tr>
<tr>
<td>5: Ficoll-paque (whole blood)</td>
<td>1.84</td>
<td>7.1</td>
</tr>
<tr>
<td>11. K562 cells</td>
<td>2.03</td>
<td>9.5</td>
</tr>
</tbody>
</table>

### Effects on quality of RNA on ABL expression

<table>
<thead>
<tr>
<th>Specimen 1</th>
<th>BM</th>
<th>A260/A280</th>
<th>RIN</th>
<th>RNA</th>
<th>ABL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>1.95</td>
<td>2.3, 2.3</td>
<td>1 µg</td>
<td>2.83E+03</td>
<td></td>
</tr>
<tr>
<td>Ficoll</td>
<td>1.95</td>
<td>9.2, 9.2</td>
<td>1 µg</td>
<td>4.31E+04</td>
<td></td>
</tr>
<tr>
<td>Buffy coat</td>
<td>2.01</td>
<td>8.0, 8.2</td>
<td>1 µg</td>
<td>4.74E+04</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Specimen 2</th>
<th>BM</th>
<th>A260/A280</th>
<th>RIN</th>
<th>RNA</th>
<th>ABL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>1.79</td>
<td>N/A, 1.1</td>
<td>100ng</td>
<td>7.22E+02</td>
<td></td>
</tr>
<tr>
<td>Ficoll</td>
<td>1.99</td>
<td>8.1, 8.9</td>
<td>100ng</td>
<td>8.37E+03</td>
<td></td>
</tr>
<tr>
<td>Buffy coat</td>
<td>1.96</td>
<td>7.6, 7.3</td>
<td>100ng</td>
<td>1.35E+04</td>
<td></td>
</tr>
</tbody>
</table>
Optimized Conditions for FFPE Tissue

- Fixation with 10% neutral buffered formalin.
- Even short-term treatment induces degradation of DNA.
- DNA segments of less than 200 base pairs can be amplified efficiently.
- FFPE tissue cannot be used for Southern blotting.

(The Guideline of CLSI. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.)
PCR for EGFR using DNA from FFPE lung tissue: Success Depends on Sample Preparation (16 Hospitals over 10 specimens)

EGFR (190bp) was not amplified by PCR in 28/521 specimens.
PCR Amplification and DNA Recovery from FFPE Tissue (n=521)

All of DNA with a concentration below 16ng/µL showed a failure of PCR (190bp) in 28/521 specimens.
RNA Extraction from FFPE Tissue by AGPC or Column Method

Modified AGPC

Column

AGPC: Acid Guanidinium-Phenol-Chloroform
<table>
<thead>
<tr>
<th>Categories</th>
<th>Sampling</th>
<th>Storage and transport</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogens</td>
<td>Target lesions</td>
<td>Avoidance of degradation of nucleic acids</td>
<td>Avoidance of contamination Washing</td>
</tr>
<tr>
<td>Somatic cells</td>
<td>Target lesions</td>
<td>Avoidance of degradation of nucleic acids Fixation with 10% NBF</td>
<td>Leukemia cell separation: other than hemolysis FFPE : Column method DNA purity (OD260/280&gt;1.8) DNA recovery (&gt;16ng/μL)</td>
</tr>
</tbody>
</table>
The first formalin-fixed, paraffin-embedded (FFPE) KRAS process controls (AcroMetrix)

- manufactured by mixing KRAS mutation-positive cells with a copolymer, creating a synthetic tissue, which is then formalin-fixed and paraffin-embedded.
Comparison of KRAS Process Controls

- **Deparaffinization**
- **Extraction**
- **Amplification**
- **Molecular Analysis**

**AcroMetrix® KRAS FFPE Process Controls**

- **Cultured Cells**
- **Purified gDNA**
- **Plasmid DNA**

- The KRAS FFPE Process Controls enable laboratories to assess the entire FFPE section process workflow
CAP Proficiency Testing Program for Molecular Oncology

- KRAS
- BRAF
- Epidermal Growth Factor Receptor (EGFR)
- In Situ Hybridization for HER2
- KIT/PDGFRA
- Molecular Hematological Oncology
- Minimal Residual Disease (MRD)
- Microsatellite Instability (MSI)
- Sarcoma Translocation

**Diagram:**

- Deparaffinization
- Extraction
- Amplification
- Molecular Analysis
Quality assurance for preanalytic process

Collection → Storage → Transport → Preparation

Sites Timing → Temp., Time, Fixation → Cell Separation

A Guideline for Quality Management of Specimens

Deparaffinization → Extraction → Amplification → Molecular Analysis

AcroMetrix® KRAS FFPE Process Controls

CAP Proficiency Testing Program
## Efforts to Address Issues and Standards

<table>
<thead>
<tr>
<th></th>
<th>EU</th>
<th>Japan</th>
<th>USA/Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global</strong></td>
<td></td>
<td>ISO TC212, OECD</td>
<td></td>
</tr>
<tr>
<td>Pre-analytic process</td>
<td>SPIDIA project</td>
<td>Guideline for Quality Management</td>
<td>CLSI MAQCII</td>
</tr>
</tbody>
</table>

ISO/TC212 PWI: Nucleic acid based multiplex analysis – definition and requirement
**Expected Effects and Outcomes**

**JCCLS WG-2**
Specimen Quality Guideline
(Tentative, 2009)
↓
Evidence
↓
Approved (2011)

**ISO/TC212 WG1**
Clinical Laboratory Testing and IVD Test Systems

**Standards for Management and Assessment**

**Analytical Validity**

**Analytical System Robustness, Evaluation, Development**

**Standardized Technology**

**QC of Analytical System**

**Clinical Validity**

**Clinical Utility**

**Evidence**

**Specimen Quality Evaluation**

**Propose**

**Verify**

**Reflect**

**Complementary**

**Standardization of System**

**Quality of Medical Practice**

**Best-Practice Guideline (2011)**

**JCCLS WG-1**

**Apply**
Conclusions

1) Expanded use, penetrating into society and globalization need the standards. JCCLS has been making efforts with standards. Domestic issues in Japan with respect to global standards have been raised.

2) We discussed importance of pre-analytic process in quality assurance and the JCCLS guideline for procurement of specimens.

3) Evidence-based approaches are required in drafting the guidelines.

4) Standards for pre-analytic process would be a key point not only clinical use but also development of the system.

5) All of these activities for the global standardization of molecular-genetic testing should lead to ensure minimum international requirements for quality assurance of a total process of the laboratory systems and practices, allowing for the appropriate diagnosis and effective control of diseases.