225 Global Standardization and Quality Services of Molecular-Genetic Testing

Hayato Miyachi MD, PhD

2011 Annual Meeting – Las Vegas

AMERICAN SOCIETY FOR CLINICAL PATHOLOGY
33 W. Monroe, Ste. 1600
Chicago, IL 60603
225 Global Standardization and Quality Services of Molecular-Genetic Testing

This session will present current status of the globalization of molecular-genetic testing, and associated quality issues of the testing used in daily practice. The presentation will include the global and regional efforts for standardization of molecular genetic testing and its use. Discussion will emphasize issues in quality services of the testing in situations where specimens are being transported regionally and even across borders of countries, and their routine use in care of patients with infectious, hematopoietic, neoplastic and genetic diseases. A case-based approach will demonstrate quality issues of the testing, focusing on pre-analytic processes such as design, set-up and selection of the tests, and procurement, storage, transport and preparation of specimens. Participants will recognize appropriate quality management tools and best practices in molecular-genetic testing, and eventually will be able to implement them into their daily practice.

- Participants will identify the current status of the globalization of molecular-genetic testing, and associated quality issues of the testing used in daily practice.
- Participants will recognize importance of the pre-analytic processes of molecular-genetic testing.
- Participants will recognize appropriate quality management tools and the best practices in molecular-genetic testing.

FACULTY:

Hayato Miyachi MD, PhD

Practicing Pathologists
Molecular Pathology
Molecular Pathology
1.0 CME/CMLE Credit

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Global Standardization and Quality Services of Molecular-genetic Testing

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
Presenter disclosure information

Global Standardization and Quality Services of Molecular-genetic Testing

- I will not discuss off label use or investigational use in my presentation.
- I have no financial relationships to disclose, including Employee, Consultant, Stockholder, Research support, and Honoraria.
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
(OECD: Organization for Economic Cooperation and Development)

Scope:
Quality assurance of testing offered in a clinical context
Genetic testing for variations in germ line DNA sequences

<table>
<thead>
<tr>
<th>Principles and best practices</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) General principles and best practices</td>
</tr>
<tr>
<td>2) Quality assurance systems</td>
</tr>
<tr>
<td>2) Proficiency testing</td>
</tr>
<tr>
<td>3) Quality of result reporting</td>
</tr>
<tr>
<td>4) Education and training standards for laboratory personnel</td>
</tr>
</tbody>
</table>
OECD Guideline: Intended users

Principles are directed primarily at governments and those involved in the regulation of genetic services.

Best Practices primarily are aimed at professional associations and directors of molecular genetic testing laboratories and others involved in the provision of molecular genetic testing.
**Highlights in Methods and Best Practice**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Principle</th>
<th>Best Practice (selected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2) Quality assurance systems</td>
<td>Accreditation or equivalent recognition</td>
<td>Accredited or hold an equivalent recognition.</td>
</tr>
<tr>
<td></td>
<td>Regulation and incentives</td>
<td>Internationally accepted standard terminology and nomenclature</td>
</tr>
<tr>
<td></td>
<td>Monitoring and specific actions to ensure compliance and maintenance of</td>
<td>Policies and procedures to document the analytical validity of all tests performed</td>
</tr>
<tr>
<td></td>
<td>performance improvements.</td>
<td></td>
</tr>
<tr>
<td>2) Proficiency testing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Quality of result reporting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) Education and training</td>
<td></td>
<td></td>
</tr>
<tr>
<td>standards for laboratory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>personnel</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Highlights in Methods and Best Practice

<table>
<thead>
<tr>
<th>Methods</th>
<th>Best Practice (selected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Quality assurance systems</td>
<td></td>
</tr>
<tr>
<td>2) Proficiency testing</td>
<td>Acceptable performance levels</td>
</tr>
<tr>
<td></td>
<td>Timely corrective actions</td>
</tr>
<tr>
<td></td>
<td>Assess all phases</td>
</tr>
<tr>
<td></td>
<td>Scheme for every disease or alternative methods</td>
</tr>
<tr>
<td>3) Quality of result reporting</td>
<td>Effectively communicable Information with non-specialist health care professional</td>
</tr>
<tr>
<td>4) Education and training standards for</td>
<td>Measures to assure professional competence, directors: MD or PhD or equivalent</td>
</tr>
<tr>
<td>laboratory personnel</td>
<td>Continuing education and training program</td>
</tr>
</tbody>
</table>

- Need for specific guidelines for compliance with CLIA requirements
- Need for specific recommendation for good laboratory practices to ensure quality
Highlights in the Content

• Good Laboratory Practices for Total Testing Process –
  – Pre-analytic testing phase:
    • Laboratory responsibilities for providing test information to users
    • Documentation of informed consent
    • Test requests
    • Specimen submission, handling and referral
    • Pre-analytic systems assessment
  – Analytic testing phase
    • Establishment and verification of performance specifications
    • Documentation of clinical validity
    • Quality control procedures
    • Proficiency testing and alternative performance assessment
  – Post-analytic testing phase
    • Test reports (including providing updates or revisions)
    • Retention of records, reports and tested specimens

Others: Confidentiality
  Test authorization - laboratory responsibility
  Factors to consider before introducing tests
  Benefits of a quality management system
Proficiency testing

• The Best Practices encourage laboratories to make use of these alternative methods. Alternative methods include blind sample exchanges and review of results between laboratories, blind repeat testing, testing by different independent methods, and correlation of results to clinical and laboratory parameters. If practicable, blind sample exchanges between laboratories is the preferred approach.
CLSI Guidelines – Molecular Genetic Testing

- Molecular Diagnostic Methods for Genetic Diseases (MM1)
- Molecular Diagnostic Methods for Infectious Diseases (MM3)
- Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline (MM5)
- Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine (MM9)
- Diagnostic Nucleic Acid Microarrays (MM12)
- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods (MM13)
- Use of External RNA Controls in Gene Expression Assays (MM16)
- Verification and Validation of Multiplex Nucleic Acid Assays (MM17)
CLSI: New Molecular Genetic Testing Projects

• Quality Management for human Molecular Genetic Testing for inherited or acquired conditions.
• Laboratory Practices for Ensuring Quality and Competence in Molecular Genetics Testing

- Based upon recommendations published in the Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions issued by CDC in Morbidity and Mortality Weekly Report (MMWR)
ISO/TC212
WG1 (Quality management in the clinical laboratory)

• 2005–Japanese National Committee proposal for new standard (Resolution No.209)
• 2007–ISO 15189 : Medical laboratories – Particular requirements for quality and competence
• 2008–Incorporation unique features
• 201X–ISO 15189
ISO/TC212 and OECD

Clinical laboratories

Hematology
Biochemistry
Immunology

Molecular
Genetic tests
- Medical use
- Via physicians

Other laboratories

Food
Environment
Farm products

Genetic info. services

ISO/TC212
OECD
ELSI
Efforts to Address Molecular-Genetic Testing Issues

Regional (Japan)  Global

JCCLS  OECD

Committee of Standardization of Gene-based testing (2006)

Japanese National Committee  OECD guideline draft (2006)

ISO/TC212

International Organization for Standardization
JCCLS:
Committee of Standardization of Gene-related testing （2006〜）

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

Government

Industry

Professionals

Difficult to standardize due to special complexity

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
- CLSI guideline

2003 2004 2005 2006 2007 2008 2009 2010 2011

Japan

- 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

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

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

Somatic Cells
- Leukemia
- Malignant lymphoma
- Solid tumors

Drug metabolism and response
- Monogenic diseases
- Hereditary diseases
- familial tumors

Germ line cells
- Disease susceptibility
- Body constitution
- alcohol
- obesity
- Personal identification

Confirmatory diagnostic tests
- Carrier tests
- Preclinical tests
Current Issues in Japan (2006)

- No best-practice framework responding to OECD guideline.
- Lack for technological standards.
- Lack for evidence for clinical utility.
- Failure to systematically accumulate record and outcome of the testing.
- Failure to educate physicians and customers.
- Lack for coverage decisions.
<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

- **Preanalytic**
  - Right test ordered
  - Right specimens procured

- **Analytic**
  - Test performed correctly

- **Postanalytic**
  - Results tracked and returned - Clinician
  - Correct response to results
  - Patient notified of results
  - Patient monitored

Quality of analytic process
# Quality Assurance in Nucleic Acid Tests

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

507 Pos. for Ab.

8 false-Neg. for HCV RNA
Pre-analytical Process

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 Tentative 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></th>
<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>
<td></td>
</tr>
<tr>
<td>Somatic cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germ line cells</td>
<td></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 and washing</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>Germ line cells</td>
<td>Face-to-face</td>
<td>Privacy protection</td>
<td></td>
</tr>
</tbody>
</table>
3.2 Storage and Transport of 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>RNA quantitation</td>
<td>&lt;2 h</td>
<td>RT 1,\text{w} after RNA denature (Guanidine isothiocyanate)</td>
</tr>
<tr>
<td>DNA variation</td>
<td>&lt;3 days</td>
<td>Freeze whole blood</td>
</tr>
<tr>
<td>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></th>
<th>Analysis of basic properties of specimens</th>
<th>Interference of properties of specimens on measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td></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)

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

Somatic Cells
  - Leukemia
  - Malignant lymphoma
  - Solid tumors

Drug metabolism and response

Germ cell line
  - Monogenic diseases
  - Hereditary diseases
  - familial tumors

Disease susceptibility

Body constitution
  - alcohol
  - obesity
  - Personal identification

Pharmacogenomic/Companion Diagnostic tests

Confirmatory diagnostic tests
- Carrier tests
- Preclinical tests

(exogenous)

(endogenous)
## 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></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herceptin</td>
<td>Breast cancer</td>
<td></td>
<td>Her2/neu</td>
</tr>
<tr>
<td>Tamoxifen, Aromasin</td>
<td>Breast cancer</td>
<td></td>
<td>E/P receptor</td>
</tr>
<tr>
<td>Erlotinib/Tarceva</td>
<td>NSC lung cancer</td>
<td></td>
<td>EGFR</td>
</tr>
<tr>
<td>Erbitux</td>
<td>Colorectal cancer</td>
<td></td>
<td>EGFR</td>
</tr>
<tr>
<td>Erbitux</td>
<td>Colorectal cancer</td>
<td></td>
<td>KRAS</td>
</tr>
<tr>
<td>Gleevec</td>
<td>CML</td>
<td></td>
<td>BCR-ABL</td>
</tr>
<tr>
<td>Gleevec</td>
<td>GIST</td>
<td></td>
<td>CKIT</td>
</tr>
<tr>
<td>Rituxan</td>
<td>NHL</td>
<td></td>
<td>CD20</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Breast cancer</td>
<td></td>
<td>CYP450</td>
</tr>
<tr>
<td>Gemzar</td>
<td>NSCLC, Breast, Ovarian, Pancreatic</td>
<td></td>
<td>RRMI</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>NSCLC, Colorectal cancer</td>
<td></td>
<td>ERCC1</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>NSCLC, Colorectal cancer</td>
<td></td>
<td>TS</td>
</tr>
<tr>
<td>Safety</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camptosar</td>
<td>Colorectal cancer</td>
<td></td>
<td>UGT1A1</td>
</tr>
<tr>
<td>Purinethol</td>
<td>Leukemia</td>
<td></td>
<td>TPMT</td>
</tr>
<tr>
<td>5-FU</td>
<td>Colorectal cancer</td>
<td></td>
<td>DHPD</td>
</tr>
<tr>
<td>Elitek</td>
<td>Leukemia, Lymphoma</td>
<td></td>
<td>G6PD</td>
</tr>
</tbody>
</table>
Monitoring during Imatinib Therapy

CCR: Complete cytogenetic response
MMR: major molecular response (>3-log reduction)
CMR: major 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

Effects on quality of RNA on ABL expression

<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>

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

<table>
<thead>
<tr>
<th>BM Specimen 2</th>
<th>A260/A280</th>
<th>RIN</th>
<th>RNA量</th>
<th>ABL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysis</td>
<td>1.79</td>
<td>N/A</td>
<td>100ng</td>
<td>7.22E+02</td>
</tr>
<tr>
<td>Ficoll</td>
<td>1.99</td>
<td>8.1</td>
<td>100ng</td>
<td>8.37E+03</td>
</tr>
<tr>
<td>Buffy coat</td>
<td>1.96</td>
<td>7.6</td>
<td>100ng</td>
<td>1.35E+04</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 can not be used for Southern blotting
  (The Guideline of CLSI. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.)
PCR amplification for EGFR using DNA extracted from FFPE lung tissue from 16 Hospitals (over 10 specimens)

EGFR (190bp) was not amplified by PCR in 28/521 specimens.
All of DNA with a concentration below 16ng/µL showed a failure of PCR (190bp) in 28/521 specimens.
DNA with a lower purity showed a failure of PCR (190bp).
RNA Extraction from FFPE Tissue by AGPC or Column Method

AGPC: Acid Guanidinium-Phenol-Chloroform
## Examples in the Guideline for Approved Version

<table>
<thead>
<tr>
<th>Categories</th>
<th>Sampling</th>
<th>Storage and transport</th>
<th>Pretreatment</th>
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<tr>
<td>Pathogens</td>
<td>Target lesions</td>
<td>Avoidance of degradation of nucleic acids</td>
<td>Avoidance of contamination Washing</td>
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<tr>
<td>Somatic cells</td>
<td>Target lesions</td>
<td>Avoidance of degradation of nucleic acids</td>
<td>Leukemia cell separation: other than hemolysis</td>
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<td>Fixation with 10% NBF</td>
<td>FFPE : Column method</td>
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<td></td>
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<td></td>
<td>DNA purity (OD260/280&gt;1.8)</td>
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<td>DNA recovery (&gt;16ng/µL)</td>
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</tbody>
</table>
Process in Development

- Basic study
  - New genes responsible for a disease

- Application Study
  - Analytical validity
  - Clinical validity
  - Clinical utility

- Public Welfare Study
  - Cost-effectiveness
  - Pilot study
  - Policy

Policies and procedures to document the analytical validity of all tests performed
(Best practice for quality assurance systems in OECD Guideline)

1. Rare disease
2. Emerging tests (IVD)
3. Genetic services
ACCE Project: Model Process for Collection, Evaluation, Interpretation, and Reporting

Disorder/Setting

- Analytic Validity
- Clinical Validity
- Clinical Utility
- ELSI

Importance of assuring analytic validity
↑ pre-analytic phase

http://www.cdc.gov/genomics/gtesting/ACCE/fbr.htm
Emerging Systems

Disease symptoms and management

- Symptoms
- Latent
- Disease risk
- Healthy

Pharmacogenomics

Prognosis

Monitoring

MammaPrint (Agilent/Agendia): Array

Oncotype DX (Genomic Health): RT-PCR

VeriGene System (Nanopore): PCR

eSencor XT-8 System (Osmectch): Array

Screening

- Genome profile
  - 23andMe
  - deCODE genetics
  - Navigene

Diagnosis

- AmpliChip CYP (Affymetrix/Roche): Array

Treatment

Efficacy

Importance of pre-analytic process

Cure

2005 year 7000 tests
2006 year 14,500 tests
2010 total 175,000 tests
Process in Development

Basic study
- New genes responsible for a disease

Application Study
- Analytical validity
- Clinical validity
- Clinical utility
- Cost-effective ness
- Pilot study

Public Welfare Study
- Policy
- General use

MAQC: FDA
MAQCII: FDA

MammaPrint (Agilent/Agendia)
0ncotype DX (Genomic Health)

Quality of sample
Oncotype DX Development

Step 1. Optimization of methods for quantifying gene expression in formalin-fixed, paraffin-embedded tissue

Step 2. Selection of 250 candidate genes from the human genome

Importance of sample quality

Step 3. Testing of candidate genes to identify an optimal gene panel for clinical validation

Step 4. Prospective clinical validation of the 21-gene panel and Recurrence Score calculation
The SPIDIA Project launched by EU (Jan., 2009)

• The SPIDIA project: Standardisation and improvement of generic Pre-analytical tools and procedures for In-vitro DIAGnostics
• QIAGEN led-consortium to develop standards for patient sample processing in order to facilitate the discovery and prediction of diseases
  – scheduled to run for 4 years
  – a total budget of over 13 million Euros.
  – The consortium consisting a total of 16 companies and research institutions
  – from 11 countries
## Efforts to Address Issues and Standards

<table>
<thead>
<tr>
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<th>EU</th>
<th>Japan</th>
<th>USA</th>
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<tbody>
<tr>
<td><strong>Global</strong></td>
<td></td>
<td>ISO TC212, OECD</td>
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<tr>
<td>Pre-analytic</td>
<td>SPIDIA project</td>
<td>Guideline for Quality Management of Specimens</td>
<td>CLSI MAQCII</td>
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<tr>
<td>process</td>
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<tr>
<td>Entire process</td>
<td>EuroGenTest</td>
<td>ISO15189 Resolution No.209 (2005→2008)</td>
<td>CDC ClSI CETT GeTRM</td>
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<tr>
<td>of molecular</td>
<td>Orphanet EPPOSI</td>
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<tr>
<td>genetic testing</td>
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</table>
A variety of Scopes

Pathogens  Somatic cells  Germline cells

Pre-analytic process

Analytic process

ISO15189
JCCLS
Best practice Guideline

CLSI QM Guideline

Post analytic process

JCCLS Specimen Quality Guideline
CLSI Specimen Collection Guideline

OECD QA Guideline
CDC Best practice Guideline
Guideline and Quality

Quality Management

Structure studies
- Environment
- Organization
- Professionals
- Record keep

Process studies

Outcome studies

Standards/guidelines
- Compliance

Regulation
- Incentive

Baseline Quality

PDSA cycle

Continuous improvement

Surveillance of quality
- Provider performance
- Data feedback
- Interventions

Accreditation/Certification

Case Audit
- Peer review
Challenges with Issues and Standards

Leadership and oversight in policy:
Regulation, incentive and budget

Government

Industry

Professionals

Global standards and evidence-based in development, implement and clinical use

Guidance

Society

Public

Education of users
Media, School

Care provider
Training and qualification

Quality practice
Global Standardization and Quality Services of Molecular-genetic Testing (Summary)

1) Expanded use, penetrating into society and globalization need the standards.

2) OECD issued the guideline for quality assurance in molecular-genetic testing for clinical uses in 2007. Other international bodies such as ISO/IEC TC212, CDC and CLSI are also engaged with the standards.

3) JCCLS has been making efforts with standards. Domestic issues in Japan with respect to global standards have been raised.

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

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

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

7) 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.
Thank you any questions?