Curriculum Item #1 in Educational curriculum for molecular-genetic laboratories performing NGS-based tests

ATLAS Project
ISO 15189 Accreditation Support Course

“ISO 15189 accreditation program for molecular-genetic laboratories”

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The Committee for Standardization of Molecular-Genetic Testing,
Japanese Committee for Clinical Laboratory Standards (JCCLS)
Japanese National Mirror Committee of ISO/TC212
Tokai University School of Medicine, Department of Laboratory Medicine

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1. Efforts for quality assurance in molecular-genetic tests

Japan Guidelines for Best Practice Guideline for Molecular-Genetic Testing (JCCLS 2012)

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Scope: Quality assurance of testing offered in a clinical context

All three categories of gene-related testing for variations in pathogen, somatic cells, and germ line DNA sequences

Principles and best practices
1) Quality assurance systems
2) Proficiency testing
3) Quality of result reporting
4) Education and training standards for laboratory personnel

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Revolutionary Change ❶

The Dawn of Laboratory Medicine in Japan

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2016	2017	2018	2019	2020	2021

Legislative: Parliament → National Assembly Medical Service Act Amendment → Published →enacted

Associated meetings: Public Comments → Ministerial Ordinance →enacted → Grace Period

Task force → Investigative Research (MHLW) → Questionnaire Survey

Obligation to make effort
Recommendation
(→Mandatory)

JCCLS

Stake holders → Investigative Commission (MHLW)

Lacks EQA/interlaboratory comparison
Third-party accreditation
in genetic testing/LDT

Best Practice Guidelines → ISO 15189 Guidance for Genetic Testing → Accreditation Criteria → Pilot Inspection

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Revolutionary Change ❷

Cancer Genome Medicine in Japan (2019)

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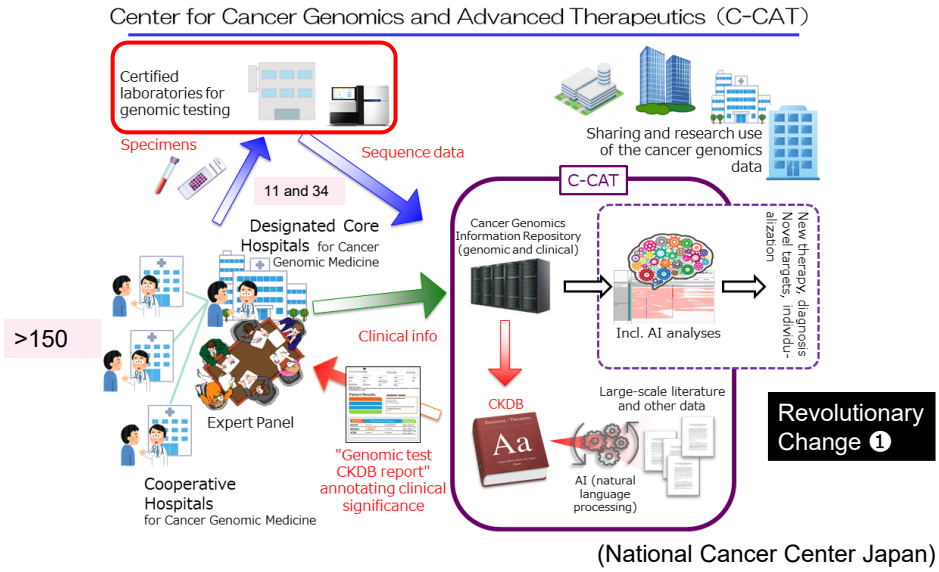

(National Cancer Center Japan)

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Revolutionary Change ❸

Cancer Gene Panel Tests (NGS) Approved for Health Insurance in June 2019

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❶ NCC Oncopanel (Sysmex Co)

Mutation・Amplification (whole exon)					Fusion genes
ABL1	CRKL	IDH2	NF1	RAC2	ALK
ACTN4	CREBBP	IGF1R	NFE2L2/Nrf2	RAD51C	AKT2
AKT1	CTNNB1	IGF2	NOTCH1	RAF1/CRAF	BRAF
AKT2	CUL3	IL7R	NOTCH2	RB1	ERBB4
AKT3	DDR2	JAK1	NOTCH3	RET	FGFR2
ALK	EGFR	JAK2	NRAS	RHOA	FGFR3
APC	ENO1	JAK3	NRG1	ROS1	NRG1
ARAF	EP300	KDM6A/UTX	NTRK1	SETBP1	NTRK1
ARID1A	ERBB2/HER2	KEAP1	NTRK2	SETD2	NTRK2
ARID2	ERBB3	KIT	NTRK3	SMAD4	PDGFRA
ATM	ERBB4	KRAS	NT5C2	SMARCA4/BRG1	RET
AXIN1	ESR1/ER	MAP2K1/MEK1	PALB2	SMARCB1	ROS1
AXL	EZH2	MAP2K2/MEK2	PBRM1	SMO	
BAP1	FBXW7	MAP2K4	PDGFRA	STAT3	
BARD1	FGFR1	MAP3K1	PDGFRB	STK11/LKB1	
BCL2L11/BIM	FGFR2	MAP3K4	PIK3CA	TP53	
BRAF	FGFR3	MDM2	PIK3R1	TSC1	
BRCA1	FGFR4	MDM4	PIK3R2	VHL	
BRCA2	FLT3	MET	POLD1		
CCND1	GNA11	MLH1	POLE		
CD274/PD-L1	GNAQ	MTOR	PRKCI		
CDK4	GNAS	MSH2	PTCH1		
CDKN2A	HRAS	MYC	PTEN	114genes	
CHEK2	IDH1	MYCN	RAC1		

❷ FoundationOne (Chugai Pharm/Foundation Med. CDx)

324 Genes

Covered by health insurance when performed by accredited molecular laboratories.

Cancer Genome Core Hospital reported to Center of Cancer Genomics and Advanced Therapeutics: C-CAT

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Issues and Challenges in ISO 15189 Accreditation in Japan

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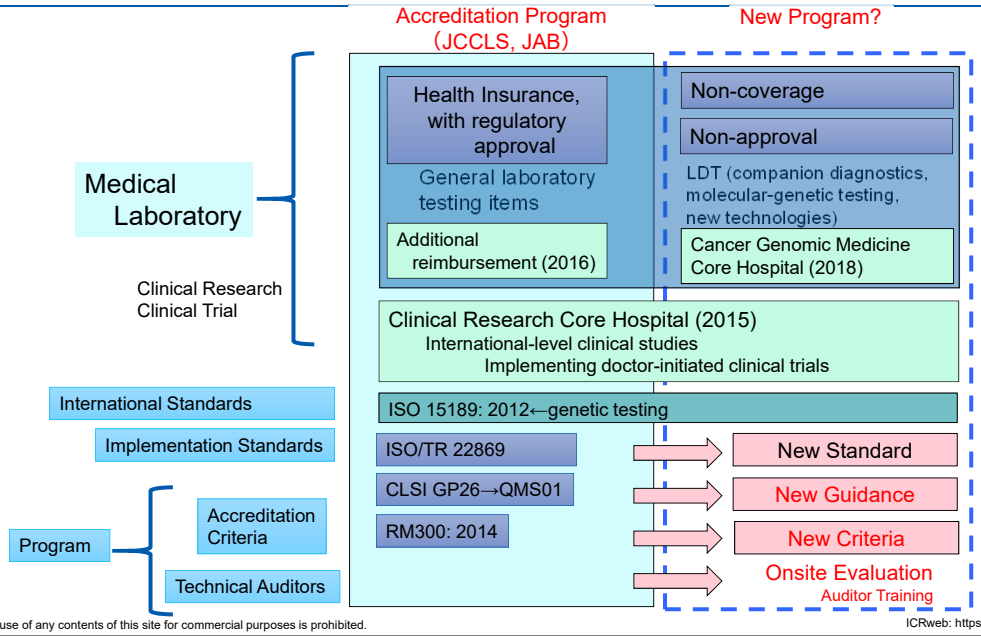

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2. Implementation guidance of ISO 15189 for NGS-based tests

Limited Descriptions of Genetic Testing in ISO 15189: 2012

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5.9 **Release of results** 5.9.1 **General**	NOTE 1: For the results of some examinations (e.g. certain genetic or infectious disease examinations) special counseling may be needed.
5.4.2 **Information for patients and users**	The laboratory shall have information available for patients and users that includes an explanation of the clinical procedure to be performed to enable informed consent. Importance of provision of patient and family information, where relevant (e.g. for interpreting genetic examination results), shall be explained to the patient and user.

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Global Efforts for Standardization of Molecular-Genetic Testing

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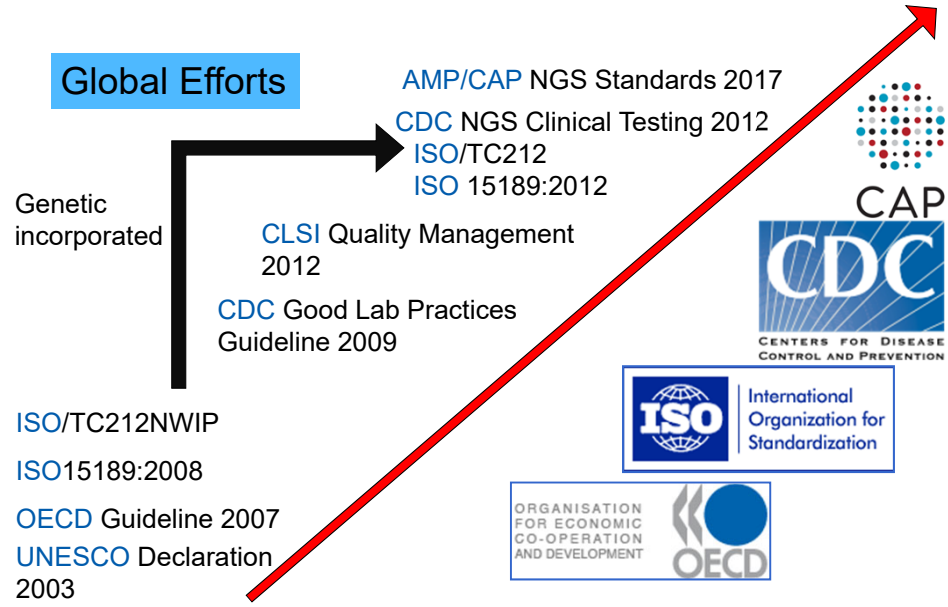

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NGS Guidelines for Oncology

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- Lawrence J, Jennings Marilyn M. Li, et al, “Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer”.

  A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists J Mol Diagn, Vol. 19, 2017.

- Guidelines for Validation of Next-Generation Sequencing-Based Oncology Panels.

  A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists J Mol Diagn, Vol. 19, 2017

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Assuring the Quality of Next-generation Sequencing in Clinical Laboratory Practice (CDC, Nat Biotechnol. 2012)

Requirements for test establishment	NGS-specific recommendation
Validation	Platform validation, test validation, informatics pipeline validation, alternate methods
Quality Control	QC materials, quality metrics, clinically actionable findings
Proficiency Testing	PT challenges, electronic sequence files, consideration of different genomic regions
Reference Materials	Suitable NGS RM

College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests (Aziz N, et al. Arch Pathol Lab Med. 2015;139: 481)

WET BENCH ANALYTIC PROCESS	BIOINFORMATICS PROCESS
Documentation	*Documentation*
Validation	*Validation*
Quality Management Program	*Quality Management Program*
NGS Confirmatory Testing	*Updates*
Laboratory Records	*Data Storage*
Exception Log	*Version Traceability*
Monitoring of Upgrades	*Exception Log*
	NGS Data Transfer Confidentiality Policy
	Sequence Variants—Interpretation/Reporting
	Reporting of Incidental Genetic Findings
	NGS Test Referral Policy

Global and Japan Efforts

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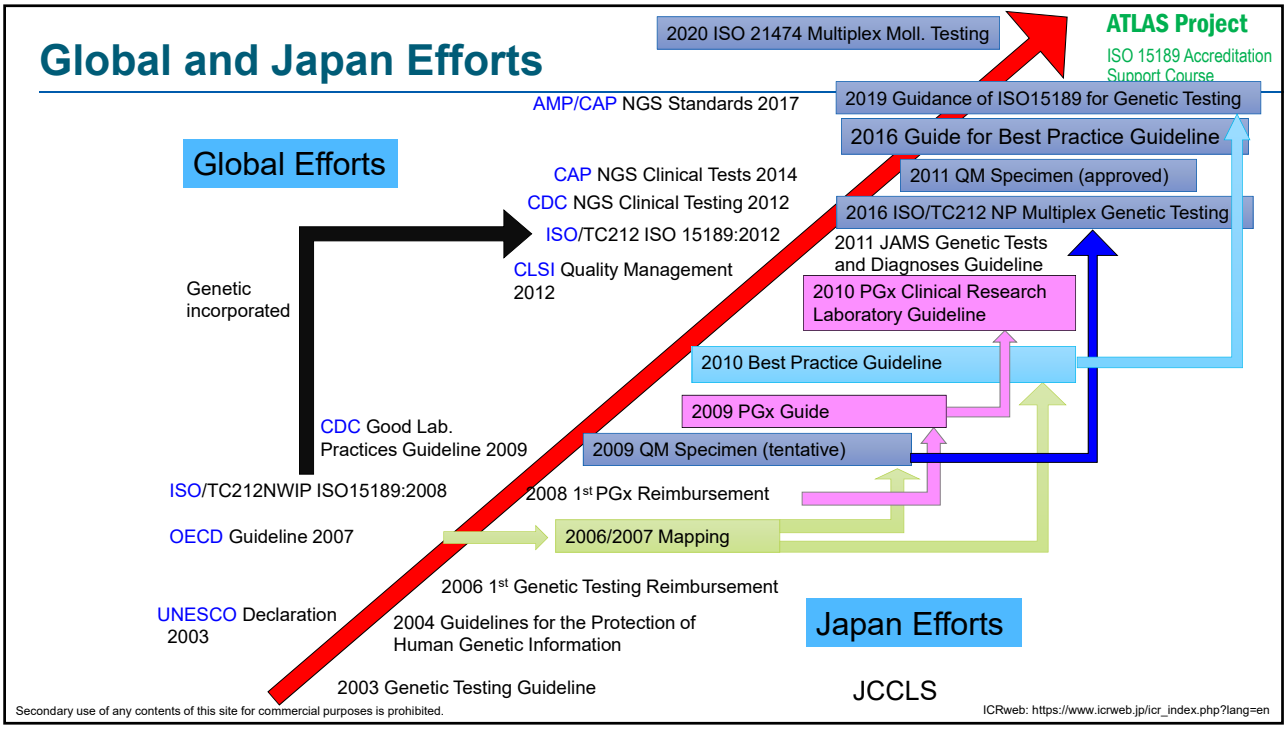

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2019 Guidance of ISO15189 for Mol. Genetic Testing

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QM Specimen
ISO 21474 Multiplex Mol. Testing
ISO 20166 – FFPE
ISO 20184 – Frozen
ISO 20186 - Blood
NGS Clinical Testing (CDC, CAP, AMP)
Guide for Best Practice Guideline

2009 PGx Guide
Genetic Tests and Diagnoses Guideline
2016 Guide for Best Practice Guideline

4 Management requirements
4.1 Organization and management res
4.2 Quality management system
4.3 Document control
4.4 Service agreements
4.5 Examination by referral laboratorie
4.6 External services and supplies
4.7 Advisory services
4.8 Resolution of complaints
4.9 Identification and control of nonconformities
4.10 Corrective action
4.11 Preventive action
4.12 Continual improvement
4.13 Control of records
4.14 Evaluation and audits
4.15 Management review

5 Technical qui
5.1 Personne
5.2 Accommodation and environmental conditions
5.3 Laboratory equipment reagents and consumables
5.4 Pre-examination processes
5.5 Examination processes
5.6 Ensuring quality of examination results
5.7 Post-examination processes
5.8 Reporting of results
5.9 Release of results
5.10 Laboratory information management

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Usage of ISO 15189 Guidance for Mol. Genetic Labs

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Mol. Genetic Labs

- Introduction of quality management
  (validation and verification
  → performance parameters
  → internal quality control)
- Documentation of operational procedures
- Education and training of laboratory staff

Accreditation Body

- Basis for aaccreditation criteria
- Education of auditors
- Harmonization among auditors

Development of a new accreditation program for mol. genetic labs.

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Example of the Content

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Validation

Performance Evaluation

Monitoring Quality Index

Continuous Quality Improvement

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Example of the Content

ATLAS Project
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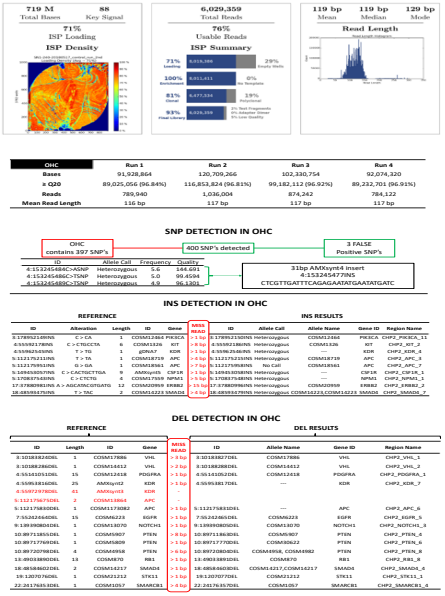
Support Course

Validation

Performance Evaluation

Monitoring Quality Index

Continuous Quality Improvement



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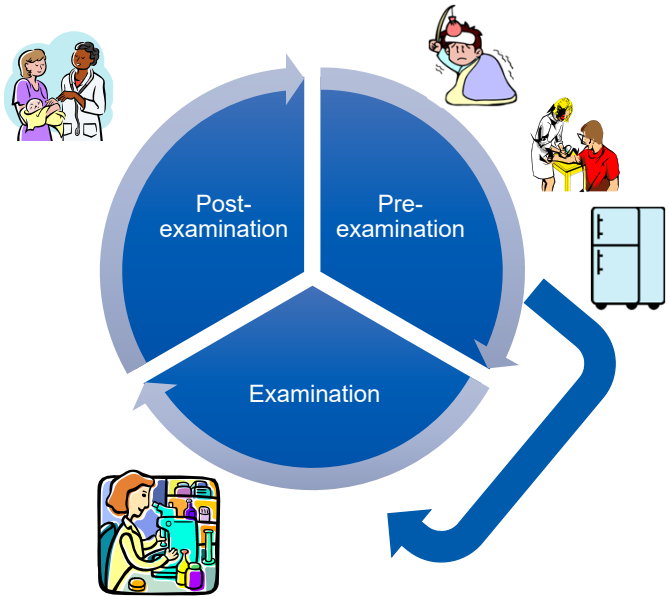
3. Regional and international efforts for standardization of the pre-examination process

Using Tissue Samples of Uncertain Quality



Guideline for Quality Management of Specimens in Molecular Methods- Part 1: Procurement, Transport, and Preparation of Specimens (JCCLS)

Tentative (2009) → Approved (2012)

Guidelines for practical use describing the general principles and basic methods of collection, storage, transport, and preparation of specimens to be evaluated by molecular diagnostic methods

English version: http://www.jccls.org/english/Approved%20QM%20specimesn111201.pdf

Highlights of the Guideline

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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**

PCR of EGFR using DNA from FFPE Lung Tissue: Success Depends on Sample Preparation

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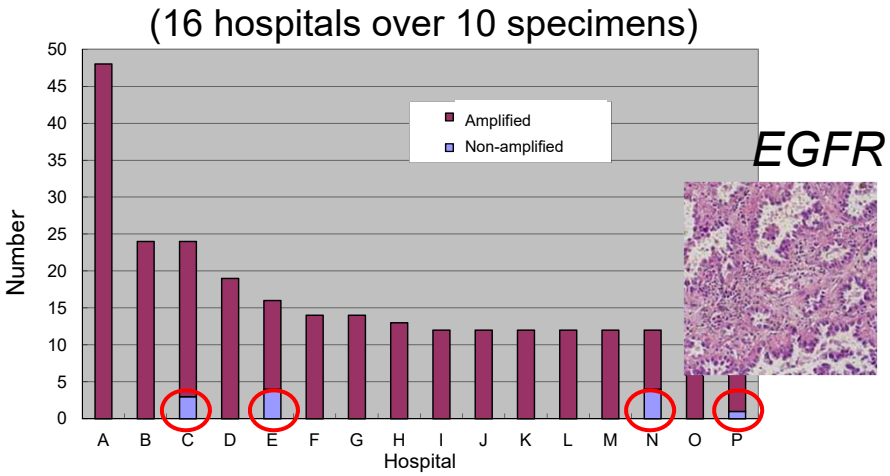

EGFR (190 bp) was not amplified by PCR in **28/521** (5.4%) specimens.

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Optimized Conditions for FFPE Tissue

(Guideline of CLSI. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline.)

- 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.

Approved Guideline for the Quality Management of Specimens for Molecular Methods Part 2 —New Technologies and Sample Quality Control (Highlight of Contents)

(3) Various Examination Technologies/Analytical Sample Quality Control

1. Chromosomal Analysis and FISH
2. Liquid-based Cytology Sample:
   Focusing on Cervical Cytology Examination
3. Array CGH
4. Next-Generation Sequencing (NGS)
5. Circulating Tumor Cell (CTC) Measurement
   (cancer diagnosis/peripheral blood/trace amount cells)
6. miRNA · Exosome
7. Blood Circulating Free Nucleic Acid
8. Mitochondrial DNA

4. Next-generation Sequencing (NGS)

4.1 Sample Quality Control for NGS Analysis
- 4.1.1 Blood
- 4.1.2 Tissues/Cells

4.2 DNA and RNA Quality Control for NGS Analysis
- 4.2.1 General Precautions for Preparation of DNA and RNA
- 4.2.2 DNA Quality Control
- 4.2.3 RNA Quality Control

4.3 Library Quality Control for NGS Analysis
- 4.3.1 General Precautions for Library Preparation
- 4.3.2 Quality Check of Fragmented DNA
- 4.3.3 Confirmation of Success or Failure of Adapter Ligation and Quality Check
- 4.3.4 Quality Check of DNA Library for NGS

Website: https://www.jccls.org/pdf/english/manual_part2_20200127.pdf

Standards with Regards to Pre-examination Process

ISO 20166 – FFPE tissue –
- Part 1: Isolated RNA
- Part 2: Isolated proteins
- Part 3: Isolated DNA
- Part 4: *In situ* detection techniques

ISO 20184 – Frozen tissue –
- Part 1: Isolated RNA
- Part 2: Isolated proteins
- Part 3: Isolated DNA

ISO 20186 - Blood –
- Part 1: Isolated cellular RNA
- Part 2: Isolated genomic DNA
- Part 3: Isolated circulating cell-free DNA from plasma

ISO 3181- Saliva
- Isolated DNA

Scope of ISO 20166-3: Isolated DNA

- This document gives guidelines on the handling, documentation, storage, and processing of formalin-fixed and paraffin-embedded (FFPE) tissue specimens intended for DNA examination during the pre-examination phase before a molecular assay is performed.
- This document is applicable to molecular *in vitro* diagnostic examinations including laboratory-developed tests performed by medical laboratories and molecular pathology laboratories.
- It is also intended to be used by laboratory customers, *in vitro* diagnostics developers and manufacturers, biobanks, institutions and commercial organizations performing biomedical research, and regulatory authorities.

NOTE: International, national, or regional regulations or requirements can also apply to specific topics covered in this document.

Table of Contents (Highlighted)

Requirements for Pre-examination Processes in ISO 15189

- 5.4 Pre-examination processes
  - 5.4.1 General
  - 5.4.2 Information for patients and users
  - 5.4.3 Request form information
  - 5.4.4 Primary sample collection and handling
    - 5.4.4.1 General
    - 5.4.4.2 Instructions for pre-collection activities
    - 5.4.4.3 Instructions for collection activities
  - 5.4.5 Sample transportation
  - 5.4.6 Sample reception
  - 5.4.7 Pre-examination handling, preparation, and storage

Sample Collection Standards (ISO/TS 20658)

ISO/TS 20658:2017
Medical laboratories — Requirements for collection, transport, receipt, and handling of samples

↓ Standard is reviewed every 5 years

Will be replaced by

ISO/AWI 20658
UNDER DEVELOPMENT

1 Scope

- This document specifies requirements and good practice recommendations for the collection, transport, receipt, and handling of samples intended for medical laboratory examinations.
- This document is applicable to medical laboratories and other medical services involved in laboratory pre-examination processes that include examination request, patient preparation and identification, sample collection, transport, receipt, and storage. It may also be applicable to some biobanks.
- This document does not apply to blood and blood products intended for transfusion.

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4. Regional and international efforts to standardize multiplex molecular-genetic tests

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QM Specimen Guideline (JCCLS) Incorporated into Two ISO Standards

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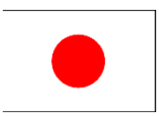
ISO NP 21474
In vitro diagnostic medical devices — general requirements and terminology for multiplex molecular testing



ISO NP 17822 Part 2
In vitro diagnostic test systems — nucleic acid amplification-based examination procedures for detection and identification of microbial pathogens — Part 2: Laboratory quality practice guide

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Trends in Uses of Genome Information

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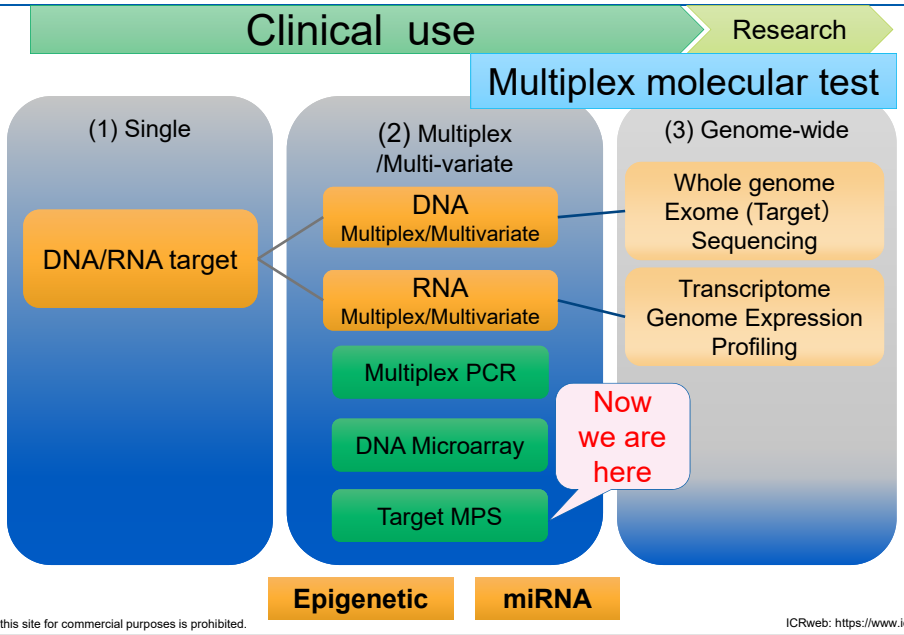

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

Series of Standards for Multiplex Molecular Testing

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ISO 21474-1:2020(en)
In vitro diagnostic medical devices —
Multiplex molecular testing for nucleic acids —
Part 1: Terminology and general requirements for nucleic acid quality evaluation

ISO/CD 21474-2
In vitro diagnostic medical devices —
Multiplex molecular testing for nucleic acids —
Part 2: Validation and verification

ISO/CD 21474-3 (Tentative)
In vitro diagnostic medical devices —
Multiplex molecular testing for nucleic acids —
Part 3: Interpretation and reporting

ICRweb: https://www.icrweb.jp/icr_index.php?lang=en

A Challenge: Multiplex Molecular Testing

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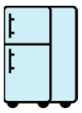
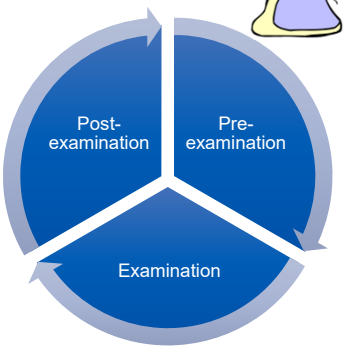


Part 3: Interpretation and reporting (tentative)



Part 1: Terminology and general requirements for nucleic acid quality evaluation

Part 2: Requirements for validation and verification

Scope of Multiplex Molecular Testing for Nucleic Acids, Part 1

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- This document provides the terms and general requirements for the evaluation of the quality of nucleic acids as the analytes for multiplex molecular tests, which simultaneously identify two or more nucleic acid target sequences of interest.
- This document is applicable to all multiplex molecular methods used for examination using *in vitro* diagnostic (IVD) medical devices and laboratory-developed tests (LDTs). It provides information for both qualitative and quantitative detection of nucleic acid target sequences.
- This document is intended as guidance for multiplex molecular assays that detect and/or quantify human nucleic acid target sequences or microbial pathogen nucleic acid target sequences from human clinical specimens.
- This document is applicable to any molecular *in vitro* diagnostic examination performed by medical laboratories.

  It is also intended to be used by laboratory customers, *in vitro* diagnostics developers and manufacturers, biobanks, institutions, and commercial organizations performing biomedical research, and regulatory authorities.
- This document is not applicable to metagenomics.

NOTE: An examination procedure developed for a laboratory's own use is often referred to as a "laboratory-developed test", "LDT", or "in-house test".

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Study Results of NCC Oncopanel, TOP-GEAR Project

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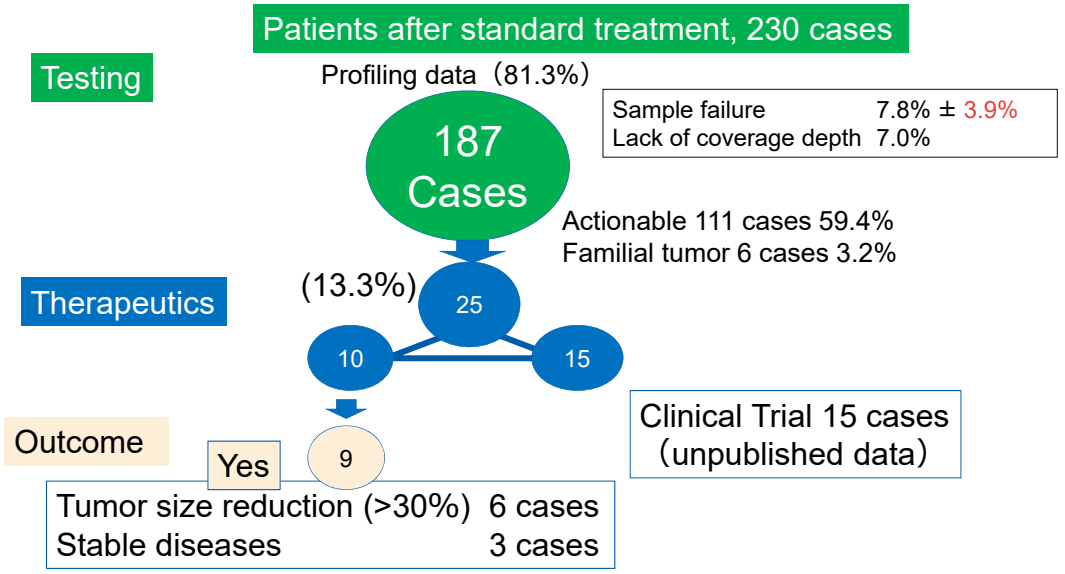
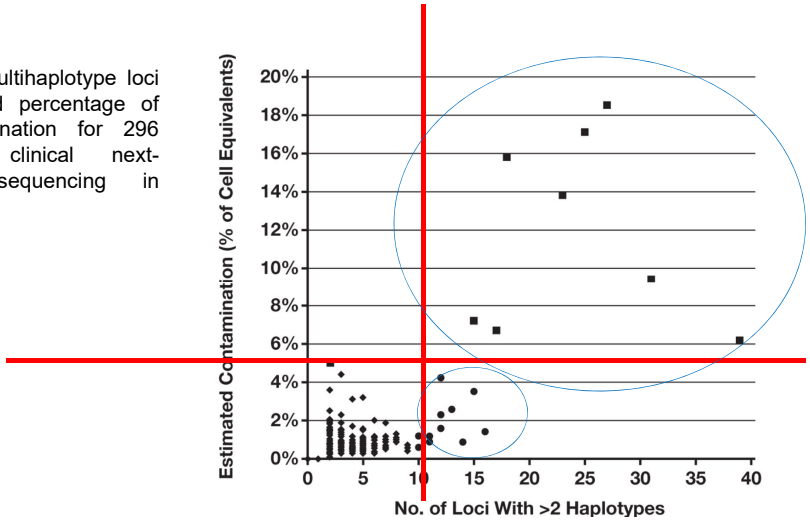


Sunami K, et al. Cancer Science 2019: 110: 1480

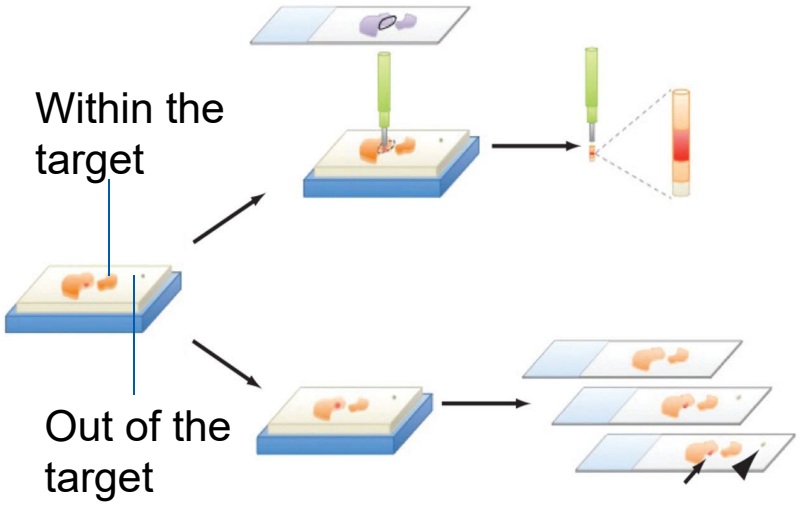
False-positive Results due to Cross Allo-contamination

Figure 1 Number of multihaplotype loci and estimated percentage of DNA contamination for 296 consecutive clinical next-generation sequencing in cancer cases



American Journal of Clinical Pathology, Volume 144, Issue 4, October 2015, Pages 667–674, https://doi.org/10.1309/AJCPR88WDJJLDMBN

Contaminants Originating from Tissue Blocks
Small Bits of Tissue Transferred from One Case to Another



Tissue Contaminant in Histologic Section
This H&E-stained brain biopsy sample shows a small fragment of intestine

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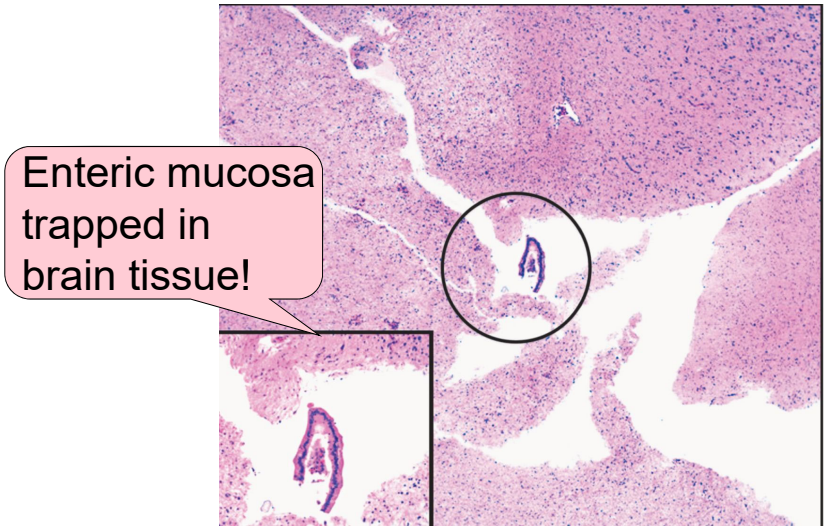

American Journal of Clinical Pathology, Volume 144, Issue 4, October 2015, Pages 667–674, https://doi.org/10.1309/AJCPR88WDJJLDMBN

Scope of Standards for Pre-examination Process (ISO/TC212)

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Collection → Storage → Transport → Preparation → Extraction

Sample collection (ISO/TS 20658)

CCFNA・cellular RNA・genome (ISO 20186)
FFPE (ISO 20166) ・ frozen tissue (ISO 20184)

Nucleic Acid Evaluation → Amplification → Detection

Multiplex Testing

ISO 21474-1:2020
In vitro diagnostic medical devices —
Multiplex molecular testing for nucleic acids —
Part 1: Terminology and general requirements for nucleic acid quality evaluation

A Challenge: Use of Specimens Stored in Clinical Biobanks for Laboratory Tests

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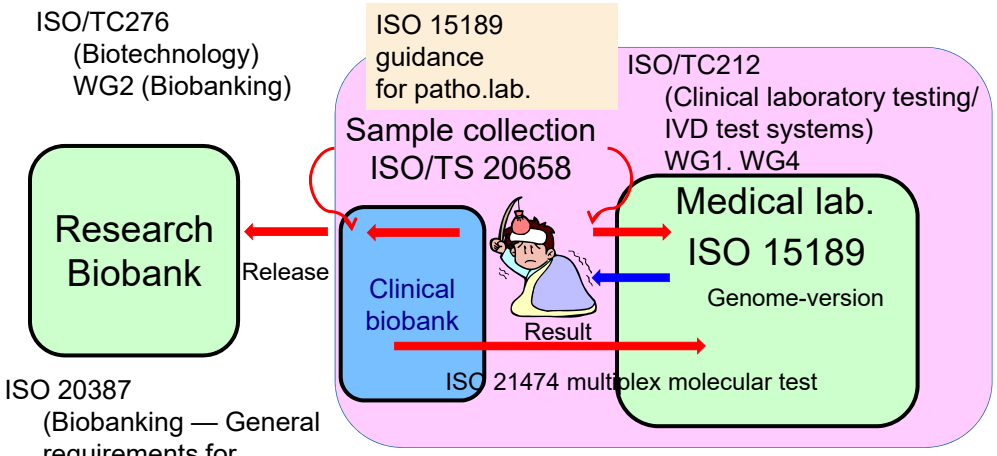

ISO 20387
(Biobanking — General requirements for biobanking)
ISO 22758
(Implementation guide)

ISO 20166 FFPE tissue (RNA, protein, DNA)
ISO 20184 Frozen tissue (RNA, protein)
ISO 20186 Blood (cellular RNA, genome DNA, ccf DNA)

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5. Challenges to the EQA/PT program

5.6.3 Interlaboratory Comparisons

5.6.3 Interlaboratory comparisons

5.6.3.1 Participation

The laboratory shall participate in an interlaboratory comparison program(s) (such as an external quality assessment program or proficiency testing program) appropriate to the examination and interpretation of examination results.

The laboratory shall monitor the results of the interlaboratory comparison program(s) and participate in the implementation of corrective actions when predetermined performance criteria are not fulfilled.

5.6.3.2 Alternative approaches

Whenever an interlaboratory comparison is not available, the laboratory shall develop other approaches and provide objective evidence for determining the acceptability of examination results.

5.6.3.2 ISO 15189: 2012 Alternative Approaches

- Certified reference materials;
- Samples previously examined;
- Material from cell or tissue repositories;
- Exchange of samples with other laboratories;
- Control materials that are tested daily in interlaboratory comparison programs.

External Quality Assessment (EQA) or Proficiency Testing (PT) (IFCC, 2017)

- External quality assessment (EQA) or proficiency testing (PT) describes the process of comparing the laboratory's test results to an outside source.
- There are four methods for EQA/PT:

  rechecking or retesting samples that have previously been tested by a reference laboratory, on-site evaluation, inter-laboratory exchange of samples (usually between a few laboratories), and proficiency testing.
- In proficiency testing, an organization provides unknown samples for testing to a set of laboratories, and the results from all laboratories are analyzed and reported to the laboratories. EQA identifies systematic errors in testing, training needs, and objective evidence of testing quality.

Advantages of On-Site Evaluation

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- Internal process improvement through systematic assessment of laboratory practices such as of analytical and informatics processes to ensure testing quality.
- Promise in NGS-based tests, which use a variety of platforms with complex internal processes.
- Conventionally conducted during the audit of ISO 15189 accreditation.

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Development of On-Site Evaluation in the Audit under ISO 15189 Accreditation

- On-site evaluation in ISO 15189 pilot audit from May to June 2019
- Laboratory accreditation: based on molecular guidance of ISO 15189: 2012
- Participant laboratories: six commercial laboratories (CAP-accredited) providing NGS-based tests such as Cancer Gene Panel Tests

Issues and Challenges

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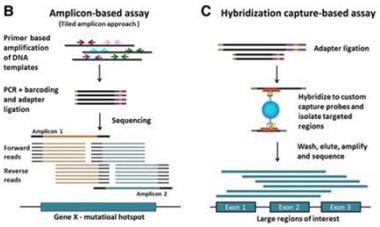
Competence evaluation of labs performing NGS-based tests

Diversity of NGS-based tests

- Purpose of use
- Assay platforms
- Detection of targets of interest

Samples prepared

Genome-based
DNA-based
Cell-based



Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels
J MolDiagn. 2017 May;19(3):341-365

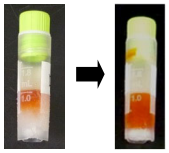
Methods-based Paradigms

Given the number of genes and the wide range of variations for which testing is performed by NGS, it is impractical (if not impossible) to follow an analyte-specific validation approach.

Thus, methods-based paradigms have been developed, which are centered on the method of analysis rather than on the specific analyte being tested.

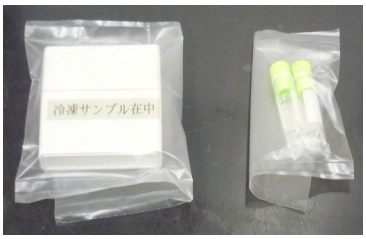
Transport of Samples for NGS Tests for On-site Evaluation

Package



Temp. logger -20° C

Samples at ambient temperature and frozen



Sample tubes

Forms for NGS On-site Evaluation

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Report on self-assessment

Test item
DNA preparation
Library construction results
Sequencing results
Interpretation and discussion
Auditor assessment:

Presentation and discussion
→internal process improvement

Competence of lab. professionals

Instruction for auditors

★1 Diversity of NGS-based tests
Purpose of use
Assay platforms
Detection targets of interest
Three types of samples are delivered.
As necessary, an alternative approach is allowed.

★2 On-site evaluation is conducted to assess routine laboratory work.
Routine lab. practice and competence are to be evaluated.
In the presentation and discussion, information and suggestions can be provided to ensure the quality of the laboratory.

Causes of Detection Failure by NGS-based Cancer Gene Panel Tests

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- Decreased leads due to variants at a primer annealing site.
- Decreased leads due to a panel property.
- Decreased leads due to pseudogenes.
- Filtering of pathogenic variants owing to complex sequences/misalignment.

Plan for correction of internal processes
Inform the limitations to users

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Outcomes of On-site Evaluation

Advantages

- Self-assessment, discussion, and feedback are effective for quality improvement in various NGS-based tests.
- Quality indicators in each process allow to evaluate the appropriateness.
- Each laboratory develops a plan relevant to its own system (dummy RNA, matched pair analysis with reference genome).
- Particularly, this approach works for cancer companion diagnostics.

Limitations and challenges

- Various specimens and applications of NGS-based tests such as circulating cell-free DNA.
- Development and evaluation (assigning values) of all types of samples are costly.
- To cope with diversity, combinations of alternative approaches are accepted.

Issues to be Challenged

- Monitoring the scheme of external quality assessment
- Linkage with clinical (and research) biobank
- Expansion of accredited laboratories
- Qualification and training of auditors
- Training of laboratory professionals

A Solution: Combination Framework of EQA/PT and On-site Evaluation

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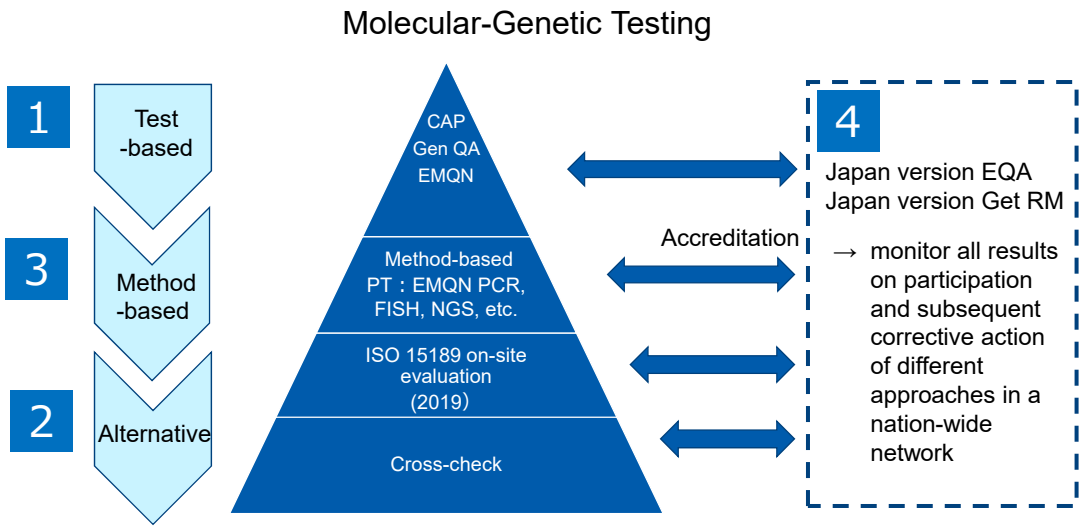

Summary

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1) Efforts for Quality Assurance in Molecular-Genetic Testing were reviewed. The establishment of an accreditation program covering molecular genetic laboratories was underscored.

2) For this purpose, a guidance document to implement ISO 15189 has been developed, incorporating the regional and global efforts towards standards for molecular genetic testing aimed at conforming with global standards.

3) The importance of the pre-examination process in quality assurance was discussed and regional and global efforts reviewed.

4) International efforts have been made towards standardization of multiplex molecular-genetic tests such as NGS.

5) For EQA/PT, on-site evaluation under ISO 15189 in an audit is an effective approach for evaluating labs performing NGS-based tests. A combination framework of EQA/PT and on-site evaluation should be considered.

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