ISO 15189 Application Guidance Document for Molecular-Genetic Examination

Japanese Committee for Clinical Laboratory Standards

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* ISO 15189 requirement itself has not been cited so that the original document should be referred.

■Foreword

To ensure the objectivity and reliability of medical laboratories performing molecular-genetic examinations, it is necessary to meet the requirements of the Japanese version of "The Best Practice Guidelines for Molecular-Genetic Examinations". Medical laboratories should perform tests in compliance with ISO 15189 requirements, particularly when those are not covered by NHI (National Health Insurance) such as laboratory-developed tests (LDTs).

In establishment of the accreditation program under ISO 15189, the outcome of activities related to quality assurance and standardization should be reflected to ensure the required level of the Japanese version of "Best Practice Guidelines for Molecular-Genetic Tests'.

Based on these, a research project of the Japan Agency for Medical Development (AMED) was conducted to investigate fundamental structures to establish the ISO 15189 accreditation program for molecular-genetic examination as part of ISO/TC212 research project from FY 2017 to FY 2019.

The research subject was summarized as follows:

1) The development of a guidance document to clarify a basis of the criteria for accreditation;
2) The development of proficiency tests for on-site evaluation;
3) The development of external quality assessment program to monitor the performance of accredited laboratories.

A basic policy for the development of the guidance document was assignment of relevant descriptions of guidelines and academic literatures dedicated to molecular-genetic examinations to each of requirements of ISO 15189. The references were added with their sources and put as notes, as necessary.

Regarding molecular-genetic examinations including NGS, the following recommendations and requirements are described in this guidance document.

1) personnel competences,
2) validation and verification of procedures,
3) internal quality control methods,
4) use of quality control materials,
5) external quality assessment program (such as an interlaboratory comparison program or proficiency testing program),
6) handling of patient specimens and confidentiality of patient genome information.

It would be useful to develop ISO 15189 application guidance on molecular-genetic tests for both accreditation bodies and medical laboratories.

Namely, the merits for the accreditation body include a guide for preparation of accreditation criteria, the training of auditors and standardization of audit.

Meanwhile, the merits for medical laboratories include the implementation and improvement of quality management system and technical management system.

As mentioned above, this guidance document would be useful for quality improvement of medical laboratories performing molecular-genetic examinations.

Consequently, we sincerely hope that this guidance document will contribute to the promotion of high-quality practice of genome-based medicine through appropriate medical laboratory service.

To use this guidance document as a supplement teaching aid in ISO 15189 Accreditation Support Course of the Training Program, as part of AMED Asian Cancer Clinical Trials Network (ATLAS) Project, the Japanese version was translated to English and edited in research project from FY 2020 to FY 2021.

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ISO 15189 Application Guidance Document for Molecular-Genetic Examination

1 Scope

・ This guidance document specifies the quality and competence requirements in medical laboratories that perform molecular and cytogenetic examinations that are used for medical care or whose results are to be reported to patients.

A medical laboratory is a facility for the purpose of providing information on disease for diagnosis, management, prevention, and treatment, or for health assessment. It performs biological, microbiological, immunological, chemical, immunohematological, hematological, biophysical, cytological, pathological, genetic, molecular, or other examinations using a biological sample derived from the human body, and can provide consultation and advisory services covering all aspects of medical laboratory work, including the interpretation of results and advice on further appropriate examinations.

【Note】

・ Molecular/cytogenetic examination includes those approved and non-approved under the Pharmaceutical and Medical Device Law. At present, research facilities are not covered in the scope of this guidance document in Japan. Internationally, research facilities are included in the ISO 15189 accredited facilities.

・ Molecular examination is classified and defined as follows.

① Nucleic acid testing for pathogen: testing to detect and analyze the nucleic acid (DNA or RNA) of exogenous pathogens (viruses, bacteria, etc.) that cause infectious diseases in humans.

② Molecular-genetic testing for human somatic gene alterations: molecular examination to clarify transient genetic information that can be changed with the disease state, confined to disease lesions/tissues, such as to detect structural abnormalities of genes unique to cancer cells and gene expression analysis.

③ Human genetic testing (molecular-genetic tests for human germline alterations): testing to reveal the genetic information inherent in the individual (germline alterations information revealed by genetic analysis) that does not change throughout the genome and mitochondria that the individual naturally holds, such as monogenic diseases, multifactorial diseases, effects, side effects, metabolism of drugs, or genetic testing related to individual identification.

2 Normative references＊

3 Terms and definition

3.7 Examination

- The term “molecular-genetic examination” used herein refers to molecular examination and includes the following three generic terms.

① Nucleic acid testing for pathogen: testing to detect and analyze nucleic acids (DNA or RNA) of pathogens (microorganisms such as viruses and bacteria) that cause infectious diseases in humans

② Molecular-genetic testing for human somatic gene alterations: testing that reveals transient genetic information that is limited to diseased lesions/tissues, and can change with the symptoms, such as detection of structural abnormalities of genes unique to cancer cells and gene expression profiling.

③ Human genetic testing (Molecular-genetic tests for human germline alterations): testing that reveals genetic information inherent to the individual that does not change throughout the genome and mitochondria that the individual naturally holds (germline alterations information revealed by genetic analysis) such as monogenic diseases, multifactorial diseases, effects/adverse effects/metabolism for drugs, and genetic testing related to individual identification.

④ Cytogenetic examination: “Molecular/cytogenetic examination” is generally referred to as “Molecular examination”. “Molecular examination” basically includes “Cytogenetic examination” and the name is used only when the content is specialized for “Cytogenetic examination”.

4 Management Requirements

4.1 Organization and Management Responsibility

4.1.1.3 Ethical conduct

a) there is no involvement in any activities that would diminish confidence in the laboratory’s competence, impartiality, judgement or operational integrity.

- When conducting molecular examination, legal and ethical aspects should be taken into account and performed in a scientifically and technically correct manner.

e) confidentiality of information is maintained.

- In the case of adding detailed information on gene variants by receiving the gene sequence data obtained from the molecular/cytogenetic examination performed at medical institutions or registered clinical laboratories and analyzing the sequence data of the gene using dedicated software, programs, or databases, as it does not correspond to clinical laboratory tests as defined by the act on clinical laboratory technicians, it is not necessary to register as a registered clinical laboratory.
- However, the laboratory should not only inform relevant parties such as medical institutions or registered clinical laboratories requesting analysis in advance about the conditions for submitting gene sequence data and the reliability of additional information, but also refer to Registered

Clinical Laboratory Instruction Guidelines about preparation of standard operating procedures and information security measures, if necessary.

4.1.2.7 Quality Manager

c) ensuring the promotion of awareness of users' needs and requirements throughout the laboratory organization.

- A person responsible for ensuring quality shall be appointed. It should be noted that the law that partially amends the Medical Care Act, etc., allows the concurrent appointment of a person in charge of ensuring the quality of overall clinical laboratory tests. Regarding qualifications, expertise in all medical laboratory quality controls with clinical tests, including molecular examination, appropriate experience, and quality, is required.

【Note】

Persons familiar with the quality control of both clinical and molecular examination are desirable.

4.2 Quality Management System

4.2.1 General Requirements

【NGS Wet Bench Process—Quality Management Program】

- The laboratory shall follow a documented quality management program for the NGS analytical wet bench process.
- All molecular examinations shall be carried out under a quality assurance framework, and the laboratory shall be responsible for ensuring that the test results meet the purpose and that the method used is appropriate by setting and maintaining the quality of the examination procedure. Therefore, whether the quality assurance framework can be applied to laboratories that provide molecular examination should be considered accordingly.

(Explanation)

- The quality assurance framework for molecular examination includes all works that directly or indirectly affect the quality of the examination work, such as training of technologists and experts, promotion of quality assurance plans for standardization of protocols, acquisition of reference materials, promotion of proficiency testing systems for testing laboratories, and provision of information on the adaption and interpretation of molecular examination. In other words, not only the technical aspects of the testing but also the assurance of safety, validity, and usefulness in terms of medical and health care related to testing should be comprehensively considered.

【NGS bioinformatics pipeline quality management program】

- The laboratory shall have a documented quality management program for the NGS bioinformatics pipeline.

4.2.2 Documentation Requirements

4.2.2.1 General

e) copies of applicable regulations, standards and other normative documents.

- As NGS-based clinical tests are emerging technologies and are much more complex than traditional Sanger sequencing-based testing, new regulatory standards are being developed for laboratories offering these tests.

4.3 Document Control

- The laboratory shall create standard operating procedures (SOPs), strive to standardize operations, and perform all operations in accordance with the standardized SOPs. In addition, the laboratory should maintain operation records and/or logs, history of non-conformities, errors, and corrections, and conduct continuous improvements. The laboratory shall create and operate appropriate report formats for these documents. Additionally, they shall review them regularly and manage and store them appropriately.

f) Changes to documents are identified.

【Edition Traceability (Version etc.)】

- The specific version(s) of the bioinformatics pipeline used to generate NGS data files shall be traceable for each patient report.
  - ① The specific versions of each component and, where available, associated configurations (e.g. command line parameters or other configuration items) of the bioinformatics pipeline used to generate NGS data shall be traceable for each patient report. The bioinformatics pipeline for analyzing NGS data, especially when based primarily on open-source software, is often composed of a combination of different software packages, scripts, and databases.
  - ② The performance of a single software package or script and the composition of an internal or external database can significantly impact the overall performance of the bioinformatics pipeline. As a result, it is important for the laboratory to be able to connect each patient report to the particular bioinformatics pipeline used to generate the report.
  - ③ For in-house scripts and software packages, changes in the script or software shall also be documented, but documentation of each component of the pipeline does not need to appear in the patient report. Rather, it is acceptable to refer to the entire pipeline using laboratory-specific designations (e.g. NGS pipeline v1.0.1).
  - ④ Laboratory-specific designations are unique to a single combination of pipeline components and configurations. Therefore, any change to a different version of a software package, script, or internal or external database, or change to the configuration of any software, requires a new unique laboratory-specific designation and assay re-validation.

j) At least one copy of an obsolete controlled document is retained for a specified time period or in accordance with applicable specified requirements.

Monitoring of Upgrades

- The laboratory shall have a policy for monitoring, implementing, and documenting upgrades to instruments, sequencing chemistries, and reagents or kits used to generate NGS data.

① Laboratories should be aware of upgrades to ensure that they do not use obsolete methods.

② The laboratory shall implement a policy to monitor and implement upgrades to instruments, sequencing chemistries, and reagents or kits used to generate NGS data. The policy should address how laboratories performing NGS-based testing can ensure that they are using the most up-to-date sample library preparation as appropriate for that assay, clone fragment amplification, and sequencing methods in this rapidly evolving environment, provided that these newer methods have been validated by the laboratory to improve the quality, reproducibility, and accuracy of the assay.

- The policy should also address the methods used to monitor upgrades, and when a relevant upgrade(s) will be implemented and validated before productive clinical use. For example, the laboratory's policy can be to monitor and implement upgrades at specified intervals (e.g. quarterly, semiannual, or annually) depending on the relevance of the new upgrade for enhancing assay performance. Additionally, since the implementation of upgrades can require revalidation of the entire wet bench process or at least the relevant steps, it can be convenient to set time intervals accordingly.

4.4 Service agreements *

4.5 Examination by Referral Laboratories

4.5.1 Selecting and Evaluating Referral Laboratories and Consultants

NGS testing referral policy

- The laboratory shall have a policy for the selection of referral laboratories and other service providers for NGS testing. Referral can include the entire NGS testing process or only the wet bench or bioinformatics process.

① The laboratory director or the designee is responsible for the selection of an external referral laboratory or other service provider.

② The laboratory director or the designer ensures the quality of performance of the external provider for NGS wet bench and/or bioinformatics services.

③ Some of the specific aspects that the laboratory director needs to consider when selecting referral laboratories or service providers are ensuring that

(1) Turnaround times are acceptable for the clinical needs for which testing is being performed.

(2) The referral laboratory providing the analytic wet bench information (i.e., sequence generation) meets the selection criteria as per CAP requirements.

(3) The quality of the results from the external bioinformatics service provider is verified to be

accurate at a high level.

④ For evidence of compliance, copies of valid certificates are required for those who outsource the wet bench sequencing workflow.

⑤ Copies of in-house validation of laboratories providing bioinformatics analysis are required for laboratories that outsource the bioinformatics workflow.

b) Arrangements with referral laboratories and consultants are reviewed and evaluated periodically to ensure that the relevant parts of this International Standard are met.

- When a part of the process is referred, the laboratory accredited by ISO 15189 is responsible for not only carrying out the process before and after the process (preparation of specimen, transportation, release of result report, etc.), but also for selecting and evaluating the referral laboratories.
- To satisfy international standards for molecular and cytogenetic examination, the laboratory should maintain the system necessary for obtaining third-party accreditation (e.g. ISO 15189) by implementing documentation.

4.6 External services and supplies＊

4.7 Advisory services＊

4.8 Resolution of Complaints

- In branch laboratories and registered clinical laboratories, it is necessary to clarify the procedures for resolution of complaints received from medical institutions that ordered the test, the recording method, and the reporting method to medical institutions and governments.

Document name: Standard Operating Procedure for Resolution of Complaint

Major matters to be included.

① Complaint resolution system (including the role of the supervising doctor)

② Complaint resolution procedure

③ Matters concerning reporting to the medical institution that ordered the test and the government

④ How to complete the complaint resolution log

4.9 Identification and Control of Nonconformities

h) each episode of nonconformity is documented and recorded, with these records being reviewed at regular specified intervals to detect trends and initiate corrective action.

Exception Log

The laboratory maintains an exception log for patient samples (specimens), where steps used in the NGS analytic wet bench process deviate from standard operating procedures (SOPs).

① The laboratory shall document any deviation from the SOP along with an explanation of the deviation and the resulting outcome. Examples of anticipated deviations can include altered processing upon receipt of a suboptimal specimen, changes to library preparation, and sequencing of libraries with suboptimal concentrations.

② Exceptions can pertain to the specimen quality and the examination process. At the time of specimen accessioning, an assessment is made as to whether or not a sample is in optimal condition for testing. If there is a concern, this shall be documented on the worksheet or on a pending log and communicated to a supervisor or laboratory director.

③ The laboratory director can decide to proceed with the testing, but should communicate the issue to the ordering physician and document this communication electronically or on the worksheet.

One example of such a scenario is a specimen that was not transported under the optimal conditions.

A decision can be made to process the sample and proceed with subsequent testing only if the DNA sample is found to be adequate. (See Section 4.4.1 e).)

④ Issues related to specific steps of the wet bench procedure shall be reported to the laboratory supervisor or director. This allows the evaluation of whether the testing itself has been compromised and whether the test can be completed.

⑤ If, after troubleshooting, the testing is evaluated as satisfactory, and the results can be interpreted by the laboratory director, provided that the quality controls of the test and sample results were deemed adequate, all aspects of the testing issue(s) shall be thoroughly documented in an "exception log," including the troubleshooting, resolution, and pertinent communications (especially regarding who was involved and who was informed by whom and on what date), and can be also incorporated into the monthly quality assurance report.

⑥ On occasion, the laboratory SOP itself may have to be revised to improve phrasing, make process steps clearer, or remove small inaccuracies in order to optimize the protocol. In such cases, the proposed correction should ideally be supported by at least two additional individuals, including the laboratory supervisor and either the technologist who developed the assay or a reference technologist. Any such corrections shall be approved, signed, and dated by the director of the laboratory. This is not an exception log issue, but rather a correction in the manner in which the assay is described. (See Section 4.3 (i).)

Exception Log-Bioinformatics pipeline

- The laboratory shall maintain an exception log for patient cases in which the examination steps used in the bioinformatics pipeline deviate from standard operating procedures (SOPs).

① Deviations from the laboratory SOP during any examination step used in the bioinformatics pipeline shall be documented in an exception log file, including any alterations in software packages, script, version number, database, command line, or parameters.

③ Any failures arising during the bioinformatics process shall also be recorded in the exception log, including documentation of the issues, the results of any investigations of these issues, any corrective actions taken, and pertinent communications, with sign-off by the laboratory director or designee.

④ The exception log is also required to retain links to the patient reports, and the laboratory director can choose to communicate any clinically relevant SOP deviations to the ordering physician. (See 4.4.1 e).)

⑤ Exception log documentation can also be incorporated into the monthly quality assurance report.

⑥ Deviations, such as needing to rerun the analytic pipeline due to network, computer, storage failure, or memory issues, in which a particular step is performed with different parameters or cutoffs than those used to validate the assay shall be documented along with the final report and explanation.

For example, a laboratory can need to alter settings on specific tools or components of its bioinformatics pipeline to adequately analyze particular regions or variants in a given patient case.

⑦ The reason for the deviation shall be described in the exception log, as well as the specific components of the deviation. Each deviation should be linked to the associated patient case and reviewed by the laboratory director or appropriate design (s). The deviation or aspects of it can be included in the final report or in specific communication with the ordering physician according to agreements.

⑧ Deviations related to bugs or failures in the bioinformatics pipelines also need to be recorded in the exception log.

⑧ The bug, affected cases, and proposed corrective action shall be approved, signed, and dated by the laboratory director or designee.

⑨ Outright failures of the bioinformatics pipelines, which might have resulted from hardware as well as software or operator error, shall also be recorded in the exception log to document errors that can have occurred in analyzing individual patient cases.

⑩ Evidence of compliance for the exception log requires the ability to demonstrate appropriate documentation of review of the exception log by the laboratory director, demonstration that the laboratory records any issue arising during the bioinformatics procedure, and adequate documentation of subsequent corrective actions taken as a result of these reviews.

4.10 Corrective Action

- The program addresses common problems that arise during testing. “Problems” include events that can affect the test result or its clinical use, as well as nonconformities with the laboratory’s own policies and procedures. Documentation includes both a review of the effectiveness of corrective

actions taken and the revision of policies and procedures intended to prevent recurrence.

4.11 Preventive action *

4.12 Continual improvement *

4.13 Control of Records

Laboratory Records

Methods, instrument(s), and reagents used for processing and analyzing samples (specimens) (or batches of samples) can be identified and traced in the laboratory records.

① Comprehensive records of laboratory assay "runs" are essential to document the conditions and events associated with the complex processes and algorithms involved in the performance and interpretation of NGS-based analyses. Accordingly, such archived information shall be maintained within a comprehensive framework where all reagents, primers, sequencing chemistries, and platforms used for the analysis of each patient sample are traceable.

② Such records shall contain a description of the test performed, including the nature of the target sequence (e.g. genome, exome, specific genes for target panels, transcriptome, or methylome) and depth of coverage (e.g. range and average).

③ It is also necessary to cite details of the analysis, including any publications or websites (with dates accessed) describing the pertinent parameters or other information and/or notations relative to the testing and reporting processes.

④ While all details of the analysis need not be included in the patient report, it is critical that the laboratory maintain a documentation system from which detailed information regarding the analysis of individual patient specimens can be obtained.

Data Storage

The laboratory shall have a policy regarding the storage of input, intermediate, and final data files generated by the bioinformatics pipeline.

① The laboratory shall establish and follow a procedure for the storage of data files generated by the bioinformatics pipeline.

② Large data files are generated by NGS and the associated data analysis, including flow cell imaging files, sequence read files containing base calls and associated quality scores, other intermediate files generated after subsequent analysis steps, and variant text files.

③ It is generally not practical to retain all such files for an extended period, so this checklist requirement mandates that the laboratory establish a policy for data storage that specifies data file retention times and which files will be retained after a final report has been generated. It is recommended that, if feasible, the laboratory retain sequence files with corresponding quality scores (e.g. FASTQ file) or retain an archival format from which these files can be regenerated

(e.g. BAM file).

④ For genome or other large-scale sequencing data, retention of FASTQ files or standard archival formats for long periods of time can be cost-prohibitive with current storage technologies. The period for which such files shall be stored is a more complicated decision that depends on numerous issues, including the size of the data set, laboratory storage capacity, and medicolegal considerations, as well as other institutional, local, or national requirements for data storage.

⑤ It is emphasized that the laboratory's policy for data storage and file retention times should be in accordance with local and national requirements for data storage.

- The retention period of all information related to reports should be at least five years in accordance with the Medical Care Act, but as long as possible, depending on the circumstances of the laboratory and technical requirements.

h) examination results and reports.

- The laboratory records and maintains all information related to the report. The period should be at least five years in accordance with the Medical Care Act, but as long as possible, depending on the circumstances of the laboratory and technical requirements.

4.14 Evaluation and Audit

4.14.4 Staff Suggestions

- The program should also encourage laboratory personnel to communicate concerns about the quality of laboratory testing. The investigation of personnel complaints and suggestions is part of the quality assurance program.

4.14.7 Quality Indicators

- Quality indicators can be specified and defined based on the validation process and results.

4.15 Management review *

5 Technical Requirements

5.1 Personnel

5.1.1 General

- The laboratory that performs molecular examination and a place that performs similar tests shall establish a system that guarantees genetic expertise. This is equivalent to that applied in other areas of clinical laboratory testing. In addition, it includes a system to confirm the validity of requirements for education/training, skills/qualifications specific to molecular examination, and procedures to confirm the validity of requirements for testing equipment and operations.

5.1.2 Personnel Qualifications

- The laboratory shall be directed by personnel with clear qualifications and responsibilities regarding the services provided.

Laboratory Director

Licensed Doctor of Osteopathic Medicine or Orthopedic Surgery (DO; Medical qualification similar to MD in the United States) or Doctor of Preventive Medicine (DPM) and certification or equivalent in anatomical or clinical pathology. Or trained residency/fellowship in a medical laboratory for at least one year, or at least two years of experience supervising high-complexity testing.

【Note 1】

- FDA defines "high-complexity testing" as the scores (e.g. knowledge, training and experience, preparation of reagents and materials, and characteristics/automation/QC in operational steps).
- Evidence of Compliance;
  Resume, degree, license, certificate by expert advisors, records of education and training, and work experience in related fields.

【Note 2】

- The "laboratory director" corresponds to ISO 15189.
- The "manager" of the registered clinical laboratory and the "responsible persons for ensuring accuracy" of medical institutions shall be medical doctors or clinical laboratory technicians.
- In the US, medical genetic doctors signs the final report.
- Assignment of a person responsible for ensuring accuracy: When conducting molecular-genetic examination, a person responsible for ensuring accuracy shall be appointed. In addition, it is appropriate to allow a responsible person to ensure the accuracy of the overall testing and a responsible person to ensure the accuracy of molecular-genetic examination to serve concurrently.

Responsible for ensuring accuracy of molecular and cytogenetic testing

① A manager or a responsible person to ensure accuracy can concurrently serve.

【Note 1】

- The practical experience required for the responsible person to ensure accuracy of molecular and cytogenetic testing can be met in at least three years by performing examination and quality control operations at the same time.

【Note 2】

- It is desirable that the person responsible for ensuring the accuracy of molecular and cytogenetic testing be a full-time employee, but the laboratory can decide whether or not the responsible person is to be a full-time employee in consideration of its actual situation.

② Examples of a person who has expertise and experience in molecular and cytogenetic testing shall include those who meet the following conditions: with more than three years' experience in examination and more than three years' experience in quality control (if served concurrently, their experience can be applied to meet both requirements despite the overlap), and who have taken molecular biology-related subjects (such as molecular biology, genetic analysis, cytogenetics, human genetics, microbiology, biochemistry, immunology, hematology, physiology, anatomic pathology, anatomy, animal cell engineering, or biological science) at graduate schools, universities, colleges, vocational schools, or colleges of technology.

③ When a medical doctor or a clinical laboratory technician is appointed as the person responsible for ensuring the accuracy of molecular and cytogenetic testing, it is desirable to refer to ② above.

- The management system for conducting molecular and cytogenetic testing and the quality assurance system should be established and maintained. ① In order to provide appropriate testing reports and related information in response to requests from customers, not only to conduct the essential confirmation but also to instruct and supervise testing persons in charge. ② To strive to ensure appropriate accuracy by sharing with the person responsible for quality assurance. ③ To conduct proper assignment based on the competency performance of testing persons in charge and provide continuous education, training, and skill evaluation.
- Appropriate experience and capabilities required for the responsible person: For the capability, it is appropriate to have expertise in clinical testing, including molecular examination. As a general rule, it is desirable to have a certain amount of experience as practical experience related to molecular examination, following the requirements of the quality control manager at the registered clinical laboratory.
- The person responsible for ensuring accuracy in molecular examination (medical doctor/clinical laboratory technician) should have appropriate experience and capability. For experience, as a general rule, it is desirable to require a certain level of practical experience related to molecular examination. For capability, it is necessary to have expertise in clinical testing, including molecular examination.
- In the case of dental institutions, the person responsible for ensuring the accuracy of molecular

examination shall be a dentist or a clinical laboratory technician. In the case of medical institutions, the head of the department that conducts clinical laboratory tests does not necessarily have to be responsible for ensuring the accuracy of the overall testing.

- The person responsible for ensuring the accuracy of clinical laboratory tests other than molecular-genetic tests can concurrently serve.
- The occupation of the responsible person to ensure accuracy of registered clinical laboratory tests shall be a medical doctor or a clinical laboratory technician (a dentist or a clinical laboratory technician at a dental medical institution, and a midwife at a maternity hospital). Although no special requirements have been set for practical experience, it is advisable to refer to the requirements for the quality control manager in the registered clinical laboratory (appoint a person with more than 6 years of practical experience related to clinical laboratory tests and more than 3 years of practical experience related to quality control).

【Note 3】

- If there is no suitable medical doctor or clinical laboratory technician at a medical institution, this shall not prevent people in other occupations who have expertise and experience in molecular-genetic examination from becoming the head of the department that conducts clinical laboratory tests.
- A person responsible for ensuring accuracy shall be appointed. In addition, under the law that partially amended the Medical Care Act, the responsible person to ensure the accuracy of all clinical laboratory tests is allowed to serve concurrently. Regarding capability, expertise in quality control of all clinical testing (including clinical laboratory tests), including molecular examination and appropriate experience and capability, is required.

【Note 4】

- Familiarity with the quality control of both clinical testing and molecular examination is desirable.

Technical Supervisor

A pathologist and/or a medical doctor (MD) or a doctor of osteopathy (DO) certified as a clinical pathologist, or possess qualifications equivalent to those required by a panel of experts (e.g. medical doctors). The technical supervisor, delegated by the laboratory director, is responsible for supervising all laboratory practices, including technical and scientific aspects, and for conducting and recording competence assessments in high-complexity testing.

Evidence of Compliance

- Records of qualifications, including diploma, transcript(s), or current license, if required.
- Certification/registration.

【Note】

- This corresponds to the "technical personnel" of ISO 15189.
- The qualification of an anatomic pathologist is a clinical pathologist in Japan, and the qualification of a clinical pathologist is a licensed doctor of a clinical laboratory physician.

- High-complexity testing refers to anything other than a molecular examination system consisting of a series of pharmaceutically approved reagents and equipment. Examples include pathological specimens, multiplex analysis, and sequencing.

Clinical Consultant

- Medical doctor or holder of a PhD in science. The details of this are described below.

Evidence of compliance

- Qualification record of clinical consultant.

General Supervisor

- Medical doctor, holder of a PhD in science or of a bachelor's degree in a chemical, physical, biological, clinical laboratory science, or medical technology with at least one year of experience in high-complexity testing; or

  Associate degree in laboratory science or medical technology with at least two years of experience in high-complexity testing; or

  Laboratory director or technical supervisor of a laboratory that performs high complexity testing, or medical doctor (MD), doctor of osteopathy (DO), doctor of preventive medicine (DPM), PhD, master's degree, or bachelor's degree holder in clinical laboratory science with at least one year of experience in high-complexity testing.

Evidence of compliance

- Records of qualifications, including diploma, transcript(s), or current license.
- Certification/registration.

【Note 1】

- An associate degree is a clinical laboratory technician who has graduated from a vocational school.
- The manager of the molecular department shall be properly educated and trained to ensure the quality of the department's molecular examination.

  At the least, the manager of the molecular department should have the following expertise and skills:

  ① can consider whether the request for testing is appropriate,
  ② can confirm the validation of the testing,
  ③ can perform the examination and interpret the results,
  ④ can communicate to the medical institutions about the testing result and related information, whether expert or non-expert,
  ⑤ can take responsibility for the operation of molecular examination facilities on a daily basis, and
  ⑥ can establish and maintain a quality control system.

Personnel in the molecular department

For testing that requires advanced medical expertise and skills, in order to ensure the appropriateness of the testing, it is desirable that those who have expertise and skills such as clinical laboratory technicians perform the testing.

【Note】

- The main qualifications related to molecular examination technologies are as follows:
  Certified qualifications for molecular and cytogenetic testing include Genetic Analysis Science Certification (beginner/first grade) (Japan Institute of Genetic Analysis Science), Clinical Cytogenetics Certification (Japan Society of Human Genetics), certified qualifications for Clinical Cytogenetics (Japanese Society of Cytogenetics/Japanese Association of Medical Technologists), Biotechnologists (Japan Association of Biotechnology Education), Genetic Experts (Japanese Society for Gene Diagnosis and Therapy), and Bioinformatics Engineers (Japanese Society for Bioinformatics).
- Examination should be performed by bachelor-level personnel, personnel recognized as equivalent in the laboratory, or personnel under their control. In addition, if relevant professional qualifications are required, they shall be done by qualified and experienced personnel.
- Personnel certified as bachelor-level are usually considered to be experienced personnel with about two years of relevant operation experience.
- Personnel who are undergoing education/training or do not have the relevant qualifications can conduct an examination if they have been clearly educated/trained at the appropriate level and have appropriate supervision.

5.1.5 Training

- The laboratory should recognize that education and training in clinical genetics is essential for conducting molecular examination.
- Testing department managers should ensure that personnel conducting molecular examination continue to participate in education and training programs.

b) assigned work processes and procedures

- The laboratory shall ensure that all personnel are educated and trained to carry out examinations and operate equipment properly.
- Appropriate education/training standards and professional qualifications should be established for persons in charge of the molecular department. Educational requirements should include systematic training in molecular biology, molecular genetics in clinical tests, and other specialized subjects in related fields.

c) the applicable laboratory information system

- All related personnel to operate the laboratory information system shall be trained initially, after system modification, and after the installation of a new system.

d) health and safety, including the prevention or containment of the effects of adverse incidents

- Training (education/training) on understanding the concept of biosafety based on biorisk evaluation shall be implemented.

e) ethics;

・ Training (education/training) to understand ethics shall be implemented.

【Note】

・ Ethics shall include medical ethics: International declarations and agreements on medical ethics including the "World Declaration on Human Genome and Human Rights," "International Declaration on Human Genetic Information," "World Declaration on Bioethics and Human Rights," "Guidelines for Genetic Tests and Diagnosis in Medical Practice" (The Japanese Association of Medical Sciences. February 2011), "Ethical Guidelines for Human Genome/ Genetic Analysis Research" (Ministry of Education, Ministry of Health, Labor and Welfare, Ministry of Economy, Trade and Industry), "Ethical, Legal and Social Implications" (CDC: American Center for Disease Control and Prevention), and "Ethical Guideline for Medical Research in Humans" (Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labor and Welfare, Ministry of Economy, Trade and Industry).

f) Confidentiality of patient information

・ The patient's personal genetic information shall be protected in accordance with the Personal Information Protection Law and guidelines based on it.

・ To properly protect the personal data handled, it is necessary to raise awareness about the protection of personal information through education and training for personnel.

・ It is necessary to consider the implementation of education and training regarding the handling of personal information, based on the guideline that "for temporary workers, the laboratory should also strive to provide education and training as needed."

5.1.6 Competence Assessment

・ The laboratory should evaluate the degree of skill acquisition during education and training using an objective scale. For example, the laboratory shall monitor that personnel competencies have been maintained using quality control techniques.

【Note】

・ The laboratory director shall ensure that all testing personnel are properly trained and have appropriate competencies before performing molecular examinations.

f) examination of specially provided samples, such as previously examined samples, interlaboratory comparison materials, or split samples.

【NOTE】

・ NMIJ CRM 6204-b and 6205-a are examples of interlaboratory comparison substances (see Section 5.6.3.2).

5.1.8 Continuing Education and Professional Development

・ The laboratory manager is responsible for ensuring proper education and training. The importance of self-edification should be emphasized, especially for expert analysts.

5.1.9 Personnel Records

- The laboratory shall maintain the latest records of education and training conducted by personnel. Records typically include the following:

a) educational and professional qualifications

- Educational background and professional qualifications;

b) copy of certification or license, when applicable:

- Transcript, certification/registration certificate;

f) training in current job tasks:

- Records of attendance of training courses inside and outside the laboratory,
- Related OJT and re-education/re-training conducted as needed;

h) records of continuing education and achievements:

- Published technical papers and presentations in academic societies.

5.2 Accommodation and Environmental Conditions

5.2.1 General

- It is necessary to establish a safety management plan for pathogens based on biosafety and strive to prevent unintentional exposure or leakage of pathogens and toxins (see Section 5.2.6).

5.2.2 Laboratory and Office Facilities

a) Access to areas affecting the quality of examinations is controlled.

- Depending on the operation to be carried out, it can be necessary to restrict access to specific areas of the laboratory. If restricted access is in effect, personnel should be informed of the following:
  - ① Purpose of using a specific area
  - ② Operational restrictions on the area.
  - ③ Reasons for the restrictions
  - ④ Actions taken when such restrictions are violated.
- The laboratory shall establish a safety management plan for pathogens based on the concept of biosafety and strive to prevent unintentional exposure or leakage of pathogens and toxins.

b) Medical information, patient samples, and laboratory resources are safeguarded from unauthorized access.

- To prevent theft or loss of personal data, the laboratory shall take the following physical security management actions:
- Entrance/exit (room) management, preventive actions against theft (for example, filming with a camera/video), and physical protection such as fixing of equipment and/or devices.
- Based on the operational needs to prevent unauthorized operations, the functions given to terminals that handle personal data are restricted as follows: connection restrictions and response to device updates for devices with recording functions such as smartphones and personal computers.

e) Safety facilities and devices are provided and their functioning regularly verified.

- During UV irradiation, UV-blocking protective glasses or protective shield glass shall be used to prevent direct exposure of the eyes to UV rays.

5.2.6 Facility Maintenance and Environmental Conditions

- Deviations from the limits of environmental conditions can be identified by monitoring the system or quality assurance of certain analyses. The impacts of deviations from the environment can be assessed as part of the robustness test through validation. Emergency operating procedures should be established as appropriate.
- All temperature-controlled equipment and environmental conditions shall be checked daily.
- In the biobank, there shall be evidence that samples (specimens) are stored at the temperature required by the protocol.
- In the biobank, the temperature shall be checked daily with thermometers for all temperature-dependent equipment and environment.
- In the biobank, when the temperature is out of the acceptable range, appropriate corrective action should be taken, and then the temperature should be checked again and recorded.
- The identity of the individual recording the temperature(s) shall be documented (recording the initials of the individual is adequate).
- The use of automated (including remote) temperature monitoring systems is acceptable, and the functionality of the system shall be documented daily.
- In the biobank, temperature-controlled storage equipment shall have an emergency power supply, if necessary.
- There are documented procedures to follow if there are deviations in the storage temperature limits.
- Alarm systems will continue to function if power is interrupted.
- Temperature-acceptable limits for the alarm system are established considering the anticipated response time.
- Molecular examination laboratories should be divided into at least two areas, a nucleic acid extraction/amplification reagent preparation area and an amplification/detection area, to avoid contamination by nucleic acid amplification products. In the case of fully automated molecular examination equipment, it is necessary to ensure that contamination measures are taken according to the internal structure of the equipment.
- A clean bench A clean bench that can keep the inside clean should be used in the nucleic acid extraction/amplification reagent preparation area. A clean bench shall be used as the area of reagent preparation for amplification reagents.
- Under the Act on Clinical Laboratory Technicians (before the 2018 amendment), registered clinical laboratories that carry out molecular examination in the classification of clinical laboratory

tests are required to have a safety cabinet as machinery and equipment for testing.

- If the room cannot be divided, the laboratory should separate the nucleic acid extraction area and the amplification reagent preparation area using a desktop biosafety cabinet with ultraviolet rays. If there is no hood equipped with an ultraviolet irradiation device, it is also effective to change the measurement location and wipe the laboratory bench top and pipette thoroughly with a 0.5% sodium hypochlorite aqueous solution before and after use.
- Pipettes, tips with filters, and tubes shall be dedicated to each area and irradiated with ultraviolet rays to destroy DNA. It is desirable to prepare two sets of pipettes, one for the reagents and one for the samples.

【Note】

- Use a clean bench when preparing reagents and a biosafety cabinet when handling infectious specimens. The biosafety cabinet requirement was removed from the amended Act on Clinical Laboratory Technicians.
- When selecting a designated area for a new operation, previous use of that area should be considered.

5.3 Laboratory equipment, reagents, and consumables

5.3.1.2 Equipment Acceptance Testing

- The laboratory shall perform the following applicable activities to verify that the equipment meets the required specifications and any other specifications related to the testing.

Installation Qualification (IQ)

After the equipment is installed in the actual operating location, it is necessary to verify and document whether the equipment configuration is appropriate and whether the equipment, including safety features, is installed correctly. The installation qualification and operator training for all equipment used for testing should be completed.

Operational Qualification (OQ) and Performance Qualification (PQ)

It is necessary to verify that the equipment and its measurement subsystem meet the required functional and performance specifications through various tests.

【Note】 OQ and PQ shall be carried out on a regular basis.

5.3.1.4 Equipment Calibration and Metrological Traceability

b) recording the metrological traceability of the calibration standard and the traceable calibration of the item of equipment.

【Note】

- The laboratory shall have a thermometer with a calibration certificate (calibration certificate with JCSS mark issued by JCSS calibration company, calibration certificate with international mutual approval MRA marked, calibration with calibration certificate by NIST or PTB) used to measure

temperature as the standard.

d) recording the calibration status and date of recalibration.

・ Equipment that requires calibration, such as temperature measuring devices, shall be recalibrated and recertified prior to the date of expiration of the guarantee of calibration; documentation of recalibration/recertification shall be maintained for review.

e) ensuring that, where calibration gives rise to a set of correction factors, the previous calibration factors are correctly updated.

・ An appropriate calibrated thermometer shall be used for measuring temperature.

【Note】

・ All non-certified thermometers shall be checked against an appropriate thermometric standard device guaranteed by the JCSS calibration certificate, MRA marked calibration certificate, and NIST or PTB calibration certificate before initial use.

・ The laboratory should work with relevant national and international institutes to collect, develop, validate, and make available reference standards for molecular-genetic examination.

・ The laboratory should use reference standards and family specific mutation references whenever available.

g) safeguards to prevent adjustments or tampering that might invalidate examination results.

・ The laboratory should work with relevant national and international institutes to collect, develop, validate and make available reference standards for molecular-genetic examination.

・ The laboratory should use reference standards and family-specific mutation references wherever available.

【Note】

・ Although it is a “standard material” in the literature, standard materials have not been developed for many tests. Therefore, it is paraphrased as “reference standard.”

5.3.1.5 Equipment Maintenance and Repair

【Note】

・ For equipment maintenance, refer to JAB RL358 Annex A2, guidelines for equipment validation, and performance verification.

・ For unmodified manufacturers’ devices, equipment, or testing systems, the laboratory shall conduct the following:

① The laboratory shall perform and document the maintenance specified by the manufacturer.

② The laboratory shall perform and document the functional checks specified by the manufacturer at the specified frequency. The functional confirmation shall be within the tolerances set by the manufacturer before performing the patients’ tests.

・ For the instruments, equipment, and testing systems developed by the laboratory, commercially available, and modified by the laboratory, the laboratory shall conduct the following if maintenance

and functional verification protocols are not provided by the manufacturer:

① The maintenance protocols for instruments, equipment, or testing systems required for accurate and reliable testing results and testing result reporting shall be established, implemented, and documented.

② The functional verification protocols that ensure the performance of instruments, equipment, or testing systems required for accurate and reliable testing results and testing result reporting shall be defined, implemented, and documented. Functional verification shall be within the established tolerances of the laboratory before performing the patients' tests.

5.3.2.7 Reagents and Consumables — Records

- Prepared reagents and reference standards shall be labeled to indicate substance name, concentration, solvent (if not purified water), special precautions or hazards, expiration date, preparation date, and/or expiration date. The person responsible for preparing the reagent shall be identified from the label or record.

【Note】

- Although it is a "standard material" in the literature, standard materials have not been developed for many molecular-genetic tests. Therefore, it is paraphrased as "reference standard."
- A reference standard is defined as "a substance that is sufficiently homogeneous and stable with respect to one or more specific characteristics established to suit the intended use in the measurement process."
- The characteristics shall be determined using different procedures to minimize the bias relevant to a particular method. For NGS, different NGS techniques or characterization using orthogonal methods, such as Sanger sequencing and quantitative real-time PCR (qPCR), can be required. In this way, consensus values, such as genotypes, can be assigned to the reference standard.
- To fit the intended use, it shall function as well as the samples (specimens).
- Commutability requires clinical DNA standards to react similarly to a patient's genomic DNA (gDNA) during library preparation, sequencing, and analysis. Using reference standards with less commutability causes biases in the calibrated measurement values and makes the diagnosis inaccurate. Once commutability is established, reference standards can be used to calibrate measurements from patient specimens. The uncertainty of DNA sequences in patient specimens can be determined by comparison with the reference standards for known abundances, along with the uncertainties relevant to this measurement. This makes it possible to standardize the measurement values across multiple specimens and fix the diagnostic threshold to the reference standards.

5.4 Pre-examination processes

5.4.2 Information for Patients and Users

g) instructions for patient-collected samples.

- The person responsible for sample collection shall be informed of how to handle and store the various collected samples (specimens).

l) availability of clinical advice on ordering of examinations and on interpretation of examination results.

- Molecular examination laboratory should disclose information about the characteristics and limitations of the tests provided, including analytical validity, clinical validity, and usefulness.
- The laboratory facilities should ensure that service users understand the latest evidence of the clinical validity and usefulness of the provided tests.

n) the laboratory's complaint procedure.

- The laboratory shall ensure that consent is obtained from the patient with sufficient explanation regarding the implementation of the test and before and after the test.
- If necessary, medical doctors, licensed doctors of clinical geneticists, or licensed doctors of clinical laboratory specialists, and personnel (such as pharmacists, nurses, and clinical laboratory technicians) with expertise in pharmacogenomics testing shall explain the procedure to the subjects.
- There shall be an explanation procedure for the subject when conducting molecular-genetic tests.
- Because the items to be explained to the subject differ depending on the product of the pharmacogenomics testing and the content of the testing, information on the characteristics of the product/testing shall be obtained from the providing company (diagnostic drug manufacturer and registered clinical laboratory). The same information shall be provided to the subject when a medical institution performs tests using the home-brew method in its own facilities.
- The sample provider shall notify the biobank on how to share the obtained data.
- There shall be procedures and records of informed consent when conducting molecular-genetic tests.
- Documents include the following: ① period of storage of specimens; ② management of residual specimens; ③ disposal method; ④ possibility of re-examination with specimens (for example, due to significant advances in knowledge and technology), ⑤ possibility of secondary use under anonymity for the purpose of quality control, ⑥ possibility of access to the specimen by a third party, and ⑦ confidentiality protection methods (symbolization/anonymization).

【Note】

- International declarations and agreements on informed consent include the "Universal Declaration on Human Genome and Human Rights," "International Declaration on Human Genetic Information," and "Universal Declaration on Bioethics and Human Rights."
- The analysis results found in the next-generation sequencer include the "primary findings" that are the main purpose of the test and "secondary findings," as described below. The main purpose of the test needs to be explained in detail to the subject with sufficient time provided for clarification,

but it is also necessary to provide an explanation beforehand and get understanding about secondary findings.

5.4.3 Request Form Information

e) clinically relevant information about the patient and the request, for examination performance and result interpretation purposes.

- The laboratory shall confirm that the testing request is appropriate, and if necessary, inform the subject that information on the subject/family is required to correctly interpret the test result.

【Note】

- The significance of test results often depends on the accuracy and appropriateness of the information provided to the laboratory. All information necessary to carry out the testing, including the status of transportation of the samples (specimens) to the laboratory, shall be sent to the laboratory with the samples (specimens).

5.4.4 Primary Sample Collection and Handling

5.4.4.1 General

- According to the purpose of the testing, the procedures of collection and storage to ensure quality for each type of primary sample (specimen) should be clarified.

【Note 1】

Typical primary samples are shown according to the purpose of testing.

① Nucleic acid tests for pathogens: serum, plasma, urine, sputum, feces, etc.

② Molecular-genetic tests for human somatic gene alterations: tissue, blood (white blood cells), plasma, bone marrow, urine (sediment), sputum

③ Human genetic testing: blood (white blood cells), oral mucosa, hair, nails, blood stains, umbilical cord

【Note 2】

Recommendation (draft): Report of a test result to patients using stored samples in a biobank.

① Report of a test result to the patients using biobank samples is premised on a quality assurance mechanism.

② There shall be documented policies and procedures to ensure the quality of the sample.

③ Refer to the following for the quality control and assurance of biobanks for clinical use:
Domestic standard documents (such as Japanese Society of Pathology, JCCLS);
International standards: ISO 15189 standard documents related to pre-measurement processes, ISO 20658 related to collection, ISO 20166, ISO20184, ISO 20186, and ISO 21474 related to specimen/nucleic acid (ISO 15189 guidance document).

④ An anatomical laboratory with a clinical biobank should undergo a bank function audit by third-party accreditation (e.g. ISO 15189) as a mechanism for quality assurance.

⑤ When returning samples and information to patients at the research biobank, it shall be considered whether or not to comply with 2 and 3 above, how to deal with them, and reflect on the report (e.g. impact on the test results, test limits) in cooperation with the third-party accredited laboratories specified in 4 above.

- In genetic testing for the purpose of diagnosing monogenic diseases, personal information shall be protected by anonymization and confidential reporting.

5.4.4.2 Instructions for Pre-Collection Activities

c) type and amount of the primary sample to be collected with descriptions of the primary sample containers and any necessary additives.

- The containers of the primary samples (specimens) for molecular-genetic tests and necessary additives shall be appropriately selected according to the purpose of the tests (nucleic acid tests for pathogens, molecular-genetic tests for human somatic gene alterations, human genetic test), and sample type (e.g. blood, plasma, urine, and tissue).

d) special timing of collection, where needed.

- When collecting liquid-based cytology specimens, an appropriate collection method that can sufficiently collect the target cells shall be specified and explained.

e) clinical information relevant to or affecting sample collection, examination performance or result interpretation (e.g. history of administration of drugs).

- While the intended use of specimens is not always known, the specimens shall typically be stored for use in a wide range of molecular-genetic tests based on an understanding of the variable factors that affect the quality of the specimen.
- In the cytogenetic testing of tumor cells, there shall be instructions on the timing of sample (specimen) collection, such as collection before administration of anticancer drugs.

5.4.4.3 Instructions for Collection Activities

b) verification that the patient meets pre-examination requirements [e.g. fasting status, medication status (time of last dose, cessation), sample collection at predetermined time or time intervals, etc.].

- In nucleic acid tests for pathogens (virus tests), it is important to collect appropriate specimen materials at the appropriate stage in order to perform the testing accurately.

c) instructions for collection of primary blood and non-blood samples, with descriptions of the primary sample containers and any necessary additives.

- The containers of the primary samples (specimens) for molecular-genetic tests and necessary additives shall be appropriately selected according to the purpose of the tests (nucleic acid tests for pathogens, molecular-genetic tests for human somatic gene alterations, human genetic test), and specimen type (e.g. blood, plasma, urine, and tissue).

d) in situations where the primary sample is collected as part of clinical practice, information and instructions regarding primary sample containers, any necessary additives and any necessary processing and sample transport conditions shall be determined and communicated to the appropriate clinical staff.

- The container of the primary samples (specimens), necessary additives, and transportation conditions shall be documented, specified and explained.

e) instructions for labelling of primary samples in a manner that provides an unequivocal link with the patients from whom they are collected.

- Specimen storage containers should be uniquely identified and managed using barcodes (1D, 2D, etc.) or RFID to accurately track and guarantee specimens. When barcodes are unique and easy to read, they are expected to automate many downstream processes and avoid the risk of human error.

g) instructions for proper storage conditions before collected samples are delivered to the laboratory.

- The person responsible for specimen collection shall be notified of how to handle and store the various collected specimens.
- The appropriate storage conditions (temperature and storage container) for the primary samples (specimens) for molecular-genetic tests shall be established. The extracted RNA is particularly unstable. The storage containers shall be considered to have the potential for the adsorption of nucleic acids.

5.4.5 Sample Transportation

- The appropriate transport methods and transport temperature control (freezing, refrigeration, room temperature) shall be established and documented according to molecular-genetic test samples (specimens).

b) within a temperature interval specified for sample collection and handling and with the designated preservatives to ensure the integrity of samples.

- The appropriate storage conditions and storage temperatures for samples for molecular-genetic tests shall be established.

5.4.6 Sample Reception

- The received sample shall be evaluated to determine whether it is suitable for the intended testing.

a) Samples are unequivocally traceable, by request and labelling, to an identified patient or site.

- The history of specimen collection, storage, and transportation shall be traceable.

b) Laboratory-developed and documented criteria for acceptance or rejection of samples are applied.

- The criteria for rejection of samples (specimens), such as confirming inappropriate collection containers or storage conditions that affect the results, shall be established.

d) All samples received are recorded in an accession book, worksheet, computer or other comparable

system. The da te and time of receipt and/or registration of samples shall be recorded. Whenever possible, the identity of the person receiving the sample shall also be recorded.

- The receipt of samples shall be recorded reliably.

5.4.7 Pre-Examination Handling, Preparation, and Storage

- There shall be a documented procedure to follow if there are deviations in the storage temperature limits.
- When a medical institution refers molecular-genetic tests using a pathological specimen to a registered clinical laboratory, the medical institution can gross the specimen or the registered clinical laboratory can gross it. In the case of grossing in a registered clinical laboratory, when the medical institution submits the pathological specimen to the registered clinical laboratory, the medical doctor shall give specific instructions on the part to be grossed. Based on this, the registered clinical laboratory shall make the grossing and confirm that the grossing is performed according to the instructions.

5.5 Examination processes

5.5.1.1 General

- The laboratory shall select a measurement procedure that meets users' needs, including sampling methods, and is suitable for the testing performed. The laboratory shall select one of the appropriate methods published as international standards, regional or national standards, publications of well-established technical institutions, and relevant scientific literature or periodicals.

5.5.1.2 Verification of Examination Procedures

【Note】

- NGS-based tests regulatory-approved by pharmaceutical affairs correspond with this section.
- The following verification is required before reporting the test results to the patient.

Accuracy

- Closeness of agreement between the measured quantity value including the accuracy and precision and the true quantity value of the measurand.
- The definition in NGS is the closeness of agreement between the nucleic acid sequence derived from the assay and a reference sequence.

Precision

- Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Measurement precision is usually expressed as a measure of imprecision; that is, it is calculated as the standard deviation of the measurement.
- The definition in NGS is the closeness to which repeated sequence analyses give the same

repeatability and reproducibility.

Reportable range

- The definition in NGS is the region of the genome in which sequences of acceptable quality can be detected by testing.

Reference range

- The definition in NGS is the range of base sequence variations detected in the unaffected population.

5.5.1.3 Validation of Examination Procedures

- Platform validation
- Test validation: Disease-associated variants are correctly identified in targeted regions of the genome.

【Note】

- Validation is conducted to confirm by investigation that individual requirements are adequate for a specific intended use and to provide objective evidence.
- The basis for validation is the establishment of a reliable sequence analysis across the genomic regions targeted by the test.
- Test validation is conducted to establish an accurate identification system that links diseases to mutant genomic regions.
- Bioinformatics pipeline validation is used to establish the algorithm(s) by reliably analyzing platform data to produce an accurate sequence.

NGS confirmation testing

- The laboratory shall have a policy that documents indications for confirmatory testing of the reported variants.
  - ① Confirmation tests using other methods can be required to achieve the desired confidence in the variants that are reported.
  - ② Some laboratories can decide to perform re-evaluation at a later stage and perform confirmation tests on variants for a predetermined trial period.
  - ③ Each laboratory performing NGS shall have a policy in place that clearly documents indications for confirmation test and/or documents how they determined that confirmation testing was unnecessary through their assay validation.
  - ④ The laboratory shall document compliance with their confirmation testing policy and show the evidence of ongoing monitoring of their NGS assay(s) to ensure that the benchmarks achieved during the validation process are maintained during the routine work of NGS-based clinical testing and variant reporting. (See 5.5.1.2.)
- Measurement systems (e.g. analytical instruments and reagents) used in clinical testing shall be guaranteed to meet the intended use and to provide reliable results that meet the requirements.

Therefore, prior to the implementation of panel testing, specifications suitable for the purpose of the test shall be determined, and instruction manuals, package insert documentation of analytical instruments, in-vitro diagnostic drugs (template DNA preparation reagents), medical equipment (e.g. DNA sequencer and analysis program), and servers obtained that meet the respective requirements, various SOPs created, and Installation Qualification(IQ) and Operation Qualification (OQ) for each equipment performed, as well as Performance Qualification (PQ) as necessary.

b) laboratory designed or developed methods.

- The method developed or selected in the laboratory shall be suitable for the intended use and can be used if validated.
- The laboratory shall validate the analytical wet-bench process. In addition, if the process is modified, the entire process should be revalidated and/or confirmation made that the process components perform satisfactorily. The degree of revalidation shall be dependent on the modification. The analytical performance of NGS testing should be internally validated before clinical implementation.
- Next-generation sequencing analysis is a complex procedure involving many steps within the wet bench workflow. Each step needs to be optimized individually to empirically determine the optimal assay conditions and analysis settings. Once those are in place, an analytic validation shall be performed for the whole test in a "beginning-to-end" fashion, including the entire wet bench process.
- Important performance characteristics that need to be determined during the validation are analytical sensitivity, analytical specificity, accuracy, precision, and limit of detection. For any molecular assay, validation should also be conducted independently for each received specimen type.

① **Analytical sensitivity**

Analytical sensitivity can be assessed using a method-based approach that aims to maximize the number of sequence variants that are compared to a gold standard method to increase the confidence of analytic performance. It is important to determine this baseline performance by using as many different genomic regions as possible, since sequence context can be an important influence.

② **Analytical specificity**

- Validation requires a combination of analyte-specific methods and methods based on procedure because NGS permits the detection of novel as well as known sequence variants and necessitates a comprehensive approach to determine test performance with adequate confidence. It is important to include conventional positive controls with patient samples, including relevant variants for analyte-specific validation.
- Analytical specificity is often calculated by using "negative" samples (i.e., samples that have no

pathogenic variant) to determine the fraction that is correctly identified as negative.

- A method-based approach can be leveraged to calculate analytical specificity across the assayed region, for example, by determining the false-positive rate (fraction of variants detected as incorrect calls). It is also useful to determine the average number of false-positive calls for the regions tested in a clinical specimen. Note that the analytical specificity accounts for numerous sources of type I errors, including base-calling errors, errors due to misalignment, and variant-calling errors.
- Homologous sequences significantly affect the analysis of affected genes because pseudogenes can interfere with accurate variant calling. If such genes are included in the NGS test, the laboratory shall devise a method to ensure that the identified variants are not due to pseudogene sequences and shall document the accuracy of the method. When pooled sequencing of bar-coded samples is performed, the laboratory shall document that individual sample identity is maintained throughout the wet bench process.

③ **Precision**

Precision (inter-run and intra-run variability) shall be determined using at least three samples.

④ **Limit of Detection**

【Note】

- Regarding the detection of somatic mutations, the limit of detection can be determined by panel tests with fixed analysis targets, but it is difficult to determine the range for molecular-genetic tests for human germline alterations when using genome-wide analysis.
- Determining the limit of detection is important for assays that interrogate samples with heterogeneous genotypes. Mixing sample experiments can be used to assess the limit of detection for each variant type.
- The total number of samples that needs to be run to appropriately validate the NGS test needs to be evaluated by the size of the test (larger assayed regions include more variants available for deriving their technical performance), by the number of specific analytes (variants) that need to be assessed, by the possible requirement to determine the limit of detection across a range of allele frequencies, and by the number of runs and samples needed to determine precision.
- For bioinformatics analysis of NGS data, it is necessary to validate the ① robustness, ② accuracy, and ③ reproducibility at each step. There are 17 best practice consensus recommendations for validating NGS bioinformatics from the Molecular Pathology Association, which is the organizational representative of the College of American Pathologists and the American Medical Informatics Association.

Recommendation 1: Medical laboratories should validate their bioinformatics pipeline when conducting NGS-based testing.

Recommendation 2: Qualified healthcare professionals with appropriate training and certification in interpreting NGS should be involved in monitoring the validation process.

Recommendation 3: Validation shall be performed after the design, development, optimization,

and proficiency of the bioinformatics pipeline and component have been completed.

Recommendation 4: Validation of the bioinformatics pipeline should be adapted to the actual environment of the laboratory in which testing is performed.

Recommendation 5: Validation includes the individual components of all bioinformatics pipelines used in the analysis. Each component shall be reviewed and approved by a qualified clinical molecular expert and laboratory director.

Recommendation 6: The design and implementation of the bioinformatics pipeline shall ensure the security of patient information and comply with all applicable laws and regulations.

Recommendation 7: Validation of the bioinformatics pipeline is appropriate and applicable to the variants detected by intended clinical use, specimens (samples), and NGS testing.

Recommendation 8: The laboratory shall ensure that the design, implementation, and validation of the bioinformatics pipeline comply with applicable laboratory accreditation standards and regulations.

Recommendation 9: The bioinformatics pipeline is a part of the testing procedure. Its components and processes shall be documented in accordance with the laboratory accreditation standards and regulations.

Recommendation 10: Specimen identification should be maintained at each step of the NGS bioinformatics pipeline with at least four unique identification indicators, including location identification created by the pipeline or characteristics of each datafile read content.

Recommendation 11: Special quality control and quality assurance parameters should be evaluated for validation and used to determine satisfactory specifications for the bioinformatics pipeline.

Recommendation 12: Methods of modifying or filtering sequence reads at any point in the bioinformatics pipeline should be validated prior to implementation to ensure that they are presented in the data interpretation. All these documents are stored as test documents according to the laboratory accreditation standards and regulations.

Recommendation 13: The medical laboratory should have special measures to maintain the integrity of each datafile created in the bioinformatics pipeline and to warn or prevent the use of data files that have been tampered with or unintentionally modified.

Recommendation 14: The validation of the bioinformatics pipeline can be complemented by in silico analysis, but it cannot be used as a substitute for the validation of the entire bioinformatics pipeline using human samples.

Recommendation 15: Validation of bioinformatics pipelines includes the confirmation of representative variant sets with high-quality independent data. Appropriate validation metrics for each variant type should be reported.

Recommendation 16: The medical laboratory should guarantee the accuracy of the nomenclature and interpretation of software-created HGVS variants. If they need to be reviewed or corrected manually, a warning shall appear, and the documents shall be maintained for any corrections.

Recommendation 17: Supplementary validation is required when significant changes are made to the components of the bioinformatics pipeline.

- Validation results will be approved by the laboratory director.

[Note]

SOP (see 5.5.3)

- Document all steps for sample preparation, data analysis, and judgment.
- Document the selection and confirmation method of the target of a protocol that confirms detection.

QC (see 5.6.2)

- The quality of the base score should be at least 20 or equivalent per base.
- Establish depth and coverage uniformity, that is, minimum read count, across all target areas. A minimum of 500 reads is recommended.
- Define the minimum coverage required for target regions (e.g. amplicon, exons) where the state of mutation in the region cannot be confidently defined. The minimum coverage required can vary between mutation and non-mutation.
- Define the minimum percentage of variant reads in the background of normal reads required to call a "detected" variant with default reliability and sensitivity.
- Define the maximum strand bias allowed, if applicable.
- When changing to a new reagent lot, validation/confirmation of analytical sensitivity is required to ensure that low positives are not missed with the reagents of the new lot. This applies to all reagents and includes coating depth and uniformity to detect potential dropout of the target area.
- All QC metrics should be tracked and documented to ensure that there is no performance degradation.
- All software updates that affect critical parameters such as base call and alignment shall be reconfirmed by repeating at least 3–5 runs of previous analyses. This verification also verifies that the coverage depth and readability of the variants differ significantly between the two software versions. The reverse process is clearly documented in the SOP.

Control (see 5.6.2.2)

- No template control (NTC) shall be included in all amplification steps to ensure that there is no contamination between the samples and reagents. This control can be analyzed in any suitable way before proceeding with actual sequencing.
- Negative controls such as DNA from the HapMap cell line (e.g. NA12878 and/or NA19240) should be included to verify the accuracy and specificity of the analysis for the detection of mutations during the initial confirmation and periodically thereafter.
- Positive/sensitivity controls shall be included in each run. It is suggested that these controls be individually barcoded low-positive DNA samples containing multiple known variants of each type, detected near analytic sensitivity to verify that a low percentage of variants is consistently

identified. If not all variants, and if all target areas can be included in a single control sample, then a defined rotation schedule should be used.

Report (see 5.8.1)

- The variants detected shall be reported, whether or not the purpose is to detect all somatic variants, whether the clinical significance is known or unknown. In addition, its clinical significance needs to be explained.
- Secondary findings of potential germline variants: These should be included separately in the report to warn treating physicians of potential clinical relevance.
- Indicate in the report that the analysis does not find everything. Due to the limitations of the analysis, the areas that the analysis could not cover well and where the sensitivity was low should be included in the report.

Validation

- Because the performance characteristics are different for each variant, it is necessary to verify the performance (e.g. sensitivity) and perform a validation based on it.
- Along with a demonstration of the quality sequence of all target areas without sample type bias, the performance characteristics of each sample type (e.g. FFPE, FF, WB, BM, FNA) need to be established and verified.
- Minimal data needed to establish important performance characteristics (prevalence, i.e., total number of variants detected in all studies, normal and variant readings, tables, and graphs (histograms) of reading depth for each target area are recommended.)
  - ① Accuracy: At least three well-characterized reference samples (e.g. HapMap DNA NA12878, NA19240, or genomes in a bottle) should be sequenced to determine robust laboratory-specific error rates across all target regions (specificity). This error rate is expected to be < 2%.
  - ② Initial validation: At least 50 patient samples shall be included, including all intended and tumor-type samples. However, if FFPE is included, at least 75% of the samples should be derived from FFPE to ensure that the assay is robust when utilizing the most degraded sample input sources. These samples contain a representative distribution of reportable variants across all target regions (including GC-rich sequences) and shall be verified by an independent reference method. Independent reference methods cannot utilize the same technology as the NGS platform unless they are implemented in different laboratories.
  - ③ Complete validation: Ten positive samples shall be sequenced and verified for each type of intended variant in each target region. The reported variants of clinical significance identified during clinical testing for which ongoing validation has not yet been fully validated, that is, confirmation by an independent reference method, should be verified using at least 10 positive samples per intended type by an independent reference method.
  - ④ Accuracy (in progress): At least three positive patient samples containing variants close to the assay's specified sensitivity should be analyzed in three lines in the same manner using different

barcodes.

⑤ Reproducibility (between runs): For each type of variant, at least three positive patient samples containing variants close to the assay's specified sensitivity shall be analyzed by two different technicians, if possible, three times separately using different barcodes (from original DNA to sequencing and data analysis) on different days.

⑥ Analytical sensitivity: The analytical sensitivity of the assay for each type of variants shall be established. This can be initially established with a defined mixture of cell line DNA (not a plasmid), but should be verified with samples from 3–5 patients.

⑦ When multiplexing the sample with different barcodes, it should be ensured that there is no crosstalk between the samples and the barcodes, and that the running patient/barcode combination provides reproducible results for all target areas and variant types regardless of the patient/barcode.

- The accuracy and reproducibility of the bioinformatics process should be verified for both the detection of all types of variants and the identification and classification of individually barcoded and multiplexed patient samples, if applicable.
- Initial validation should be performed using a single version of all analytical software.

c) standard methods used outside their intended scope.

- The laboratory shall perform validation to ensure that the methods specified in the standard for use outside the intended scope and the extensions and modifications to the methods specified in the standard are suitable for their (new) intended use.

d) validated methods subsequently modified.

- Validation should be performed based on scientific evidence and clinical usefulness. Scientific evidence is a validation record.

・Quality metrics and continuous quality control for validation.

① Core quality metrics: quality and quantity of nucleic acids

1) Validation parameters

The requirements for DNA quality and quantity differ depending on the tissue source, extraction method, and NGS measurement method.

The laboratory shall establish the following:

(1) Minimum criteria to ensure accurate variant detection and reproducible results, depending on the measurement requirements and established sensitivity.

(2) Standard procedures for adjusting DNA input depending on sample quality.

2) Continuous QC

Therefore, a plan for ongoing monitoring should be established. Any changes in the extraction protocols should be followed by close monitoring of all downstream processes to ensure adequate measurement performance.

② Core quality metrics: library qualification and quantification

1) Validation parameters

The laboratory shall standardize protocols for library qualification and quantification.

As a recommendation for library quantification, each laboratory shall validate a method that is suitable for its needs.

2) Continuous QC

The fragment sizes of the library shall be measured to ensure that they fall within the expected narrow molecular weight range.

Continuous methods should be used to minimize primer dimers, adapter dimers, and broader bands of higher molecular weights.

③ Core quality metrics: Depth of coverage

1) Validation parameters

Requirements vary depending on the platform used and the application.

Coverage is defined as achieving adequate sensitivity and specificity in the regions of interest.

The laboratory shall establish the minimum criteria for the depth of coverage characteristic of a particular region under standard assay conditions (coverage threshold).

2) Continuous QC

Methods should be used to monitor the overall coverage and region coverage in each run. If the coverage thresholds are outside the validated range, the sample should be subjected to reanalysis. If only local regions are affected, testing of that region can be performed using an alternate method.

④ Core quality metrics: uniformity of coverage

1) Validation parameters

The required level of coverage across the targeted regions shall be defined during the validation stage.

2) Continuous QC

The uniformity of coverage shall be monitored and compared to the levels established during validation. If the coverage uniformity profile falls outside the expected profile as established by the validation, this can be indicative of errors during the testing process.

⑤ Core quality metrics: GC bias

1) Validation parameters

GC content affects the sequencing efficiency and uniformity of coverage of the targeted regions. The extent of GC bias in all parts of the genome included in the assay should be determined during validation.

2) Continuous QC

The GC bias should be monitored in every run to detect changes in measurement performance or sample quality issues.

⑥ Core quality metrics: cluster density and alignment rate

1) Validation parameters

The laboratory shall define the right balance between overclustering and underclustering, and outline the steps to prevent and resolve both instances. As a general guideline, the percentage of clusters passing the filter should be > 80%, and the alignment rate should be > 95%.

2) Continuous QC

Cluster density and alignment rate shall be monitored in every run.

⑦ Core quality metrics: transition/transversion ratio

1) Validation parameters

An important parameter for whole-genome or whole-exome sequencing is not required for targeted sequencing. The transition/transversion ratio shall be comparable to published values.

2) Continuous QC

The transition/transversion ratio shall be monitored for every sample of each run to evaluate the performance. Ratios lower or higher than expected can indicate that the quality of the base calls was low.

⑧ Core Quality Metrics: base call quality scores

1) Validation parameters

The laboratory shall establish acceptable raw base call quality score thresholds for the assay during validation. Preprocessing methods to remove low-quality base calls should be established to reduce the false-positive rate.

2) Continuous QC

Quality scores and the quality of the signal/noise ratio shall be monitored in every run. Low-quality scores can lead to increased false-positive variant calls; thus, the results should be interpreted with caution, and repeat tests can be indicated.

⑨ Core quality metrics: Mapping quality

1) Validation parameters

Parameters for mapping quality should be established during validation, and should demonstrate that the test only analyzes reads that map to the regions targeted by the assay. Steps should be established to filter reads that map to non-targeted regions.

2) Continuous QC

The proportion of reads that do not map to target regions shall be monitored in each run. Poor mapping quality can be a result of non-specific amplification, capture of off-target DNA, or contamination.

⑩ Core quality metrics: duplication rate.

1) Validation parameters

The acceptable parameters for the maximum duplication rate should be established for each assay. Filtering of duplicate reads by the analysis pipeline should be established to increase the

amount of usable sequencing data and prevent skewing of allelic fractions.

2) Continuous QC

The duplication rate should be monitored in every run and independently for each sample to monitor library diversity.

⑪ Core quality metrics: Strand bias

1) Validation parameters

Strand bias occurs when the genotype inferred from the information presented by the forward and reverse strands disagrees. The laboratory shall define the tolerance level for strand bias and determine specific criteria for the adoption of alternate testing.

2) Continuous QC

Closeness of strand bias shall be monitored in all samples.

【Note】

- Accuracy, precision, analytical sensitivity, analytical specificity, reportable range, reference range, reference interval, or alternatives shall be measured to clarify analytical/clinical validity and clinical usefulness.
- The performance characteristics of the testing procedure obtained by the validated method shall meet the customer's needs in the evaluation for the intended use.
- When conducting testing, it is necessary to clarify the scientific basis, such as analytical validity, clinical validity, and clinical usefulness, of the testing.
- Since molecular examination, especially gene panel testing, is often implemented as a laboratory developed test (LDT), both the validity of the entire testing and the performance characteristics of the testing procedure need to be evaluated and documented, including the entire testing procedure.

【Terminology】

Accuracy:

- Closeness of agreement between a measured quantity value and the true quantity value of a measurand
- The definition in NGS is the closeness of agreement between the nucleic acid sequence derived from the assay and a reference sequence.

Precision:

- Closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions. Measurement precision is usually expressed as a measure of imprecision; that is, it is calculated as the standard deviation of the measurement.
- The definition in NGS is the closeness to which repeated sequence analyses give the same repeatability and reproducibility.

Repeatability:

- To measure all operations of the analytical method multiple times in a short time under the same conditions using the quality control material specified in 5.6.2.2.

Reproducibility:

- To measure all operations of the analytical method multiple times by changing the measurement conditions between different facilities.

Analytical sensitivity:

- Sensitivity a) performance of the analytical method or instrument represented by the lower limit of detection of a certain quantity value in measurement, b) performance of the analytical method represented by the slope of the calibration curve. The minimum or change quantity value that the instrument can detect or the percentage of response to changes in the measurand.
- The definition for NGS is the expectation of detecting the targeted sequence variations by analysis, if present.

Analytical specificity:

- Ability to clearly distinguish the measurement target components that are expected to exist in various components.
- NGS is defined as the probability that sequence variation will not be detected by analysis when none is present (the false positive rate is a useful measured value for sequencing assays).

Reportable range:

- NGS is defined as the region of the genome in which a sequence of acceptable quality can be detected by the testing.

Reference range:

- The definition of NGS is the region of sequence variations that can be detected in an unaffected population.

Robustness:

- Confirmation by measurement that the ability of the analytical procedure is not affected, even if there are fluctuations in analytical method parameters.

Analytical validity:

- The definition in genetic testing is that the testing method is established and quality control is properly performed, such as obtaining highly reproducible results. It is evaluated based on information such as the positive rate when there is a mutation, the negative rate when there is no mutation, the existence of a quality control program, and the confirmatory testing methods.

Clinical validity:

- The definition of genetic testing is that the testing results are well defined. It is evaluated based on information such as the sensitivity (the positive rate with diseases), specificity (the negative rate without disease), disease prevalence, positive predictive rate, negative predictive rate, and the relationship between genetic type and phenotype.

Clinical usefulness:

- The definition for genetic testing is that there are clinical merits, such as the ability to obtain information on future prospects or to link it to appropriate preventive and therapeutic methods, to diagnosing the disease being tested. It is evaluated based on information such as the impact of the testing results on the subject and the existence of effective countermeasures.
- The laboratory shall have written information about the analytical validity, clinical validity, and clinical usefulness of the performed tests.
- As method development proceeds, regular reviews should be carried out to verify that the needs of the customer are still being fulfilled. Any change in requirements requiring modifications to the development plan should be approved and authorized.

5.5.3 Documentation of Examination Procedures

【NGS Wet Bench Process Documentation】

The laboratory shall use a documented standard operating procedure (SOP) of the analytic wet bench process used to generate the NGS data.

① The detailed documentation of wet bench processes is a critical part of quality assessment in medical laboratories.

② All standard operating protocols of DNA/RNA sample preparation, fragmentation, library preparation, barcoding (molecular indexing), sample (specimen) pooling, and sequence generation shall be documented to track each step and subsequent manipulations.

③ Medical laboratories that handle different types of samples (e.g. blood, formalin-fixed paraffin-embedded specimens) should develop standard operating procedures (SOPs) for each validated sample (specimen) type.

④ Commonly used metrics include the percentage of reads mapped to the target region, the fraction of bases meeting specified quality and coverage thresholds, and average coverage/base and target region.

⑤ The laboratory shall define and document acceptance and rejection criteria for the wet bench process, including sample preparation and sequencing.

⑥ It is critical to determine and summarize regions that failed analysis (e.g. due to inadequate coverage) if they are not covered by orthogonal technologies (such as Sanger sequencing).

【NGS Bioinformatics Pipeline Documentation】

The laboratory shall use a documented SOP for the bioinformatics pipeline used to analyze, interpret, and report the NGS results.

① The laboratory shall document all algorithms, software, and databases (referred to as components) used in the analysis, interpretation, and reporting of NGS results.

② The versions of each of these components in the overall bioinformatics pipeline shall be recorded and traceable for each patient result (Version Control).

③ For each component, the laboratory can use a baseline, default installation, or can customize

the pipeline by using alternate configuration parameters in deploying individual bioinformatics tools or in running specific algorithms.

④ In either case, the laboratory shall document any customizations that vary from default configuration, namely which parameters, cutoffs, and values are used.

⑤ Most NGS bioinformatics analyses are conducted by aligning sequence reads to a reference sequence. The reference sequence version number and assembly details shall also be identified.

⑥ When describing the bioinformatics pipeline, the laboratory should document the overall workflow of the data analysis and include the input and output files for each process step.

⑦ For each step, the laboratory should develop and document quality control parameters for optimal performance. For example, a laboratory shall determine acceptable criteria, such as the number of reads passing instrument-specific quality filters in the primary step.

⑧ Criteria for variant calling are essential and called parameters include thresholds for read coverage depth, variant quality scores, and allelic read percentages. Each of these requirements applies to multigene panel applications, as well as exome and genome sequencing.

⑨ The laboratory should also document the bioinformatics processes that are used for reducing a large variant data set to a list of causal relation and/or candidate genes and/or variants. For example, in inherited disease assays, the laboratory should document approaches used to identify recessive (latent), dominant (overt), and new variants.

⑩ Evidence of compliance for this requirement should be demonstration of appropriate documentation and that the laboratory follows its outlined procedures.

【Note】

- Variant calling shall be named according to HGVS nomenclature, allowing explicit mapping to standardized reference IDs.
- A complete procedure manual shall be available on the workbench or in the work area.

5.6 Ensuring Quality of Examination Results

5.6.1 General

【NGS Wet Bench Process—Quality Management Program】

- The quality assurance program follows the workflow path. The programs should assess the pre-examination process of NGS, examination processes, and post-examination processes used in sequence analysis through reporting.
- The NGS quality program should be integrated within the institution's overall quality assurance program.
- The overall goal of the quality program is to ensure that testing is clinically relevant. This is particularly important for tests such as NGS, for which no comparative analytical results of greater sensitivity can exist. The appropriateness of test orders and analytic decisions should be grounded in medical science and evidence.

【NGS Bioinformatics Pipeline—Quality Management Program】

- The laboratory shall maintain a record of deviations from expected results, and document the root cause investigative measures and the corrective action taken.
- Evidence of compliance includes documentation of monitoring quality control metrics as well as records describing any divergences, including appropriate investigative measures and subsequent corrective actions.
- Selection of biopsy specimens in the preanalytic process is important for the success or failure of NGS-based testing. The appropriate requirements shall be set according to the platform being used.

【Tissue processing and storage methods】

FFPE tissue:

- This is a standard for pathological evaluation and can be stored for a long time.
- There are concerns about nucleic acid fragmentation due to formalin fixation.
- Tumor fractions of unstained FFPE sections can be evaluated from H&E-stained sections.

Frozen tissue:

- There is little concern regarding nucleic acid fragmentation.
- The presence of tumor tissue and the tumor fraction cannot be accurately evaluated.
- Can be stored for a long time at −80 °C.

【Amount of tissue and percentage of tumor cells】

Amount of tissue:

- If sufficient tissue is obtained, various NGS library preparation methods can be applied.
- If only fine tissue is obtained, only the NGS library preparation methods that require a small amount of nucleic acid can be applied.

Percentage of tumor cells:

- If the proportion of tumor cells is high, various NGS library preparation methods can be applied.
- If the proportion of tumor cells is low, more unstained sections are needed for nucleic acid extraction.

【Tissue collection methods】

Surgical biopsy:

- A sufficient amount of tumor tissue is often obtained.

Biopsy with core needle:

- Only a limited amount of tumor tissue will be obtained.

【Ratio of surviving tumor cells】

Surviving cell tissue:

- Ideal for pathological evaluation and NGS library preparation by PCR

  Necrotic cell tissue:
- Not suitable for NGS library preparation by PCR

【Amount of nucleic acid harvested and required amount】

Amount of harvest:

- Depends on the amount of tissue and the proportion of tumor cells

Required amount:

Depends on NGS library preparation method

【Note 1】

For the handling of FFPE specimens, the "Guidelines on the handling of pathological tissue samples for genomic medical treatment" can be referred to.

【Note 2】

It is necessary to set appropriate criteria for cytological specimens.

Analyzing FASTQ file and BAM file information, which are digital data obtained from NGS analysis, is a dry-lab process, but it is the basis for ensuring the quality and accuracy of proper execution of the wet-lab process. Therefore, the following items need to be referred to, the appropriate criteria set in advance according to the platform being used, and a check made as to whether the wet-lab process meets the criteria.

Depth of coverage:

Depth of coverage characteristics of a particular region under standard examination conditions shall be confirmed. It is critical to define adequate coverage to achieve the proper sensitivity and specificity in the region(s) of interest. When the coverage threshold is outside the specified range, the region should be subjected to analysis by an alternate method (e.g. Sanger sequencing) or require additional evaluation.

Uniformity of coverage:

The coverage across the targeted regions should be established to produce reliable sequencing results. The uniformity of coverage (targeted regions) should be monitored and compared to the established values.

GC bias:

GC bias affects the efficiency of sequencing reactions and uniformity of coverage of the targeted regions. The amount of GC bias in all parts of the genome included in the assay should be determined during verification. GC bias should be monitored in every run to detect changes in test performance.

Transition/transversion ratio (Ti/Tv ratio):

The ratio of transitions to transversions (Ti/Tv) should be comparable to published values. The Ti/Tv ratio should be monitored in every run to detect changes in test performance.

Base call quality scores:

An acceptable raw-base-call quality-score threshold should be established during validation. Informatics filters should be established to eliminate any reads with raw base calls lower than the established quality score (for a Q score of 10, the probability that the base is incorrect is 10%, 1%

for a Q score of 20, and 0.1% for a Q score of 30). In long-read technologies, when the detection of larger indels is of interest, alignments can tolerate lower base call quality because the sequence length and accuracy at the base level are less critical. The quality of the signal-to-noise ratio should be monitored by examining the quality scores and quality of the signal-to-noise ratio across a read for each run. Quality scores among the existing instruments are not readily comparable from one to another.

Mapping quality:

Mapping quality is an indicator of the uncertainty that a read is properly mapped to the genomic position. During the validation, it should be demonstrated that the test analyzes only the reads that map only to specific target regions. Informatics filter programs should be established to eliminate any reads that map to non-target regions and remove duplicate reads. The proportion of reads that did not map to the target regions should be monitored during each run. When reads do not match the reference sequence, this is an indication that the sample does not perform within normal parameters, and those reads should be excluded from analysis. For applications that involve enrichment steps, poor mapping quality can be a result of non-specific amplification, capture of non-target DNA, or contamination.

Duplicate read success rate and removal of duplicate reads:

Informatics filter programs should be established to eliminate duplicate reads resulting from clonal amplification (all but one with the highest quality score) during alignment. This should be monitored to prevent skewing of allelic fractions.

First-base read success:

Some platforms allow the early intra-assay evaluation of sequence reads to determine quality scores and the number of reads that pass the established quality filters. The number of reads that passed the established quality filters should be established during assay validation. Evaluation of quality scores and the number of reads that pass the established quality filters early in the sequencing process can be used to monitor for contamination, confirm proper sample loading, and ultimately assess the likelihood of a successful run. Some platforms allow a run to be prematurely terminated if the established quality parameters are not met.

Decline in signal intensity:

During assay validation, the expected signal intensity across a read should be evaluated to establish normal measurement performance ranges and the expected decline in signal intensity. (Signal intensity across the read length depends on the platform.) Signal intensity attenuation should be monitored on a run-by-run basis. A sudden reduction or increase in signal intensity indicates an error in sequencing chemistry.

Strand bias:

Bias between DNA strands occurs when the data obtained from the forward and reverse strands are different. It is necessary to check the degree of bias that is acceptable to the results. Interchain

bias should be monitored for all samples.

5.6.2 Quality Control

5.6.2.1 General

- The testing facility shall establish policies and procedures for regularly evaluating quality assurance in the facility, and document the evaluation results and measures taken for improvement.
- Routine application of validated bioinformatics pipelines should be accompanied by monitoring laboratory-determined quality control metrics.
- Divergence from expected quality metrics during the analysis of clinical samples (specimens) requires investigation and resolution.

【Note】

- It is necessary to monitor the quality indicator to ensure that the analysis is always performed correctly. Sampling and nucleic acid extraction are also important factors in all molecular examination, so regular monitoring is needed with proper quality control materials close to the specimens.
- Quality control procedures shall be implemented to monitor the performance of the examination process. Control procedures are designed to detect test system failures, environmental disadvantages, and immediate errors due to operator performance, and to monitor test performance accuracy and precision over time. Although sequence analysis is usually considered a qualitative analysis, NGS has both qualitative and quantitative aspects to consider when devising effective control and control procedures.
- NGS quality control materials and metrics should be established during validation. QC materials and metrics should be established for each component of the NGS testing process, including DNA extraction, library preparation, DNA sequencing, and informatics analysis pipelines. The laboratory shall use appropriate quality control procedures to ensure all aspects of the sequencing process, including instrument sample performance, base calls, alignments, and variant calls.

5.6.2.2 Quality Control Materials

- A combination of multiple quality control materials should be used as much as possible. It is necessary to regularly use quality control materials that are the reference standards or traceable to reference standards, not limited to in-kit control.
- The level and type of quality control depends on the importance, content of the analysis, frequency of the analysis, size of the batch, degree of automation, and difficulty and reliability of the testing.
- As the nucleic acid amplification test is highly sensitive, it is necessary to pay attention to cross-contamination. Negative controls shall be used. Parallel runs are desirable for negative controls, but in the case of instruments for single assays, it is necessary to avoid the risk of cross-contamination by using alternatives.

- To ensure a negative result, it is necessary to ensure that the nucleic acid amplification reaction is performed correctly using an internal control.
- For regular quantitative tests, at least two concentrations of positive and negative controls shall be used. For qualitative tests, one concentration of positive and negative controls shall be used. In either case, it is necessary to establish and monitor quality control procedures, including nucleic acid extraction steps. Quality control materials that can closely examine the steps of nucleic acid extraction that are close to the specimens should be used. Internal positive controls should also be regularly used.
- Negative controls shall be added to every amplification step to ensure that there was no contamination between the samples (specimens) and the reagents. This control can be analyzed in an appropriate way before actual sequencing.
- It is necessary to verify the accuracy and specificity of the analysis to detect variants, including reference genomes recommended by NIST or equivalent institutions, such as NA12878 and/or GM24385 and/or NA19240, as negative controls during and thereafter on a regular basis.
- Positive/sensitivity controls shall be included in each run. It is suggested that these controls be individually barcoded, low-positive DNA samples containing multiple known variants of each type that were detected near analytical sensitivity to verify that a low percentage of variants is consistently identified. If not whole variants, and if all target areas can be included in a single control sample, then a defined rotation schedule should be used.

【Note】

- Although it is a “standard material” in the literature, standard materials have not been developed for many tests. Therefore, it is paraphrased as “reference standard”
- Products approved by pharmaceutical affairs are marketed by guaranteeing negative results without the use of internal controls.
- The level of selected quality controls shall be sufficient to ensure the validity of the results. Various quality controls can be used to monitor various fluctuations during the process. By analyzing the quality control materials for each batch of samples, the fluctuation tendency of the system can be understood. The use of various blanks reveals the contribution to the risk, as well as the contribution from the components being analyzed.

5.6.2.3 Quality Control Data

- Internal quality control takes various forms, including blanks, reference standards, spiked samples, blind materials, iterative analysis, and the use of quality control materials. In particular, X-R control charts, twin plots, and multi-rule control methods should be used for monitoring with quality control materials.

5.6.3 Interlaboratory Comparisons

5.6.3.1 Participation

【Note 1】

- Interlaboratory comparison means "organization, performance, and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions."
- The proficiency test refers to the "evaluation of the performance of participating laboratories against a predetermined standard by comparison between laboratories."

【Note 2】

- International large-scale external accuracy evaluation programs for molecular-genetic tests include CAP (College of American Pathologists) and GenQA/EMQN (European Molecular Genetics Quality Network).
- The facility proficiency testing system should be structured to evaluate all stages of the examination process performed in the laboratory, including reporting results.

5.6.3.2 Alternative approach

- If a facility proficiency testing system is not available, testing facilities should implement alternative methods.
- Alternative methods include exchanging samples among laboratories, repeating tests of samples, conducting tests by different independent methods, and comparison with other parameters. Such alternative methods can also include multiple proficiency tests that examine individual steps in the analytical process (e.g. sequence).

【Note】

- Note that alternative approaches are different for molecular-genetic examination.
- Measured values can differ between facilities; therefore, this point should be taken into consideration in the evaluation method.
- Currently, external quality assesment surveys for molecular and cytogenetic tests established by domestic institutions are a qualitative test for the tubercle bacillus complex, and quantitative tests for hepatitis B virus and hepatitis C virus provided by the Japanese Association of Medical Technologists.

5.7 Post-examination processes

5.7.2 Storage, Retention, and Disposal of Clinical Samples

- The biobank has a policy for handling submitted clinical specimens: ① Purpose of intended use of specimen, ② Required specimen data, ③ Safety procedures considering the types of clinical specimens/pathogen and required biosafety/risk level, ④ Duration of storage.
- Procedures include the duration of sample storage, the control method of remaining samples,

disposal method of samples, possibility of being re-contacted when sample retests are required (e.g. due to significant advances in knowledge or technology), the possibility of secondary use for quality control under anonymity, the possibility of access to the samples by a third party, and the method of confidentiality protection (symbolization/anonymization).

- It is also necessary to coordinate the handling of the sample after the test in advance with the parties concerned.
- The administrator is responsible for discarding the tested samples after a specified period of storage to prevent unintended use.
- The sample processing standard operating procedure includes the following items: It is necessary to clarify the acceptable criteria for handling the return, retest, and additional tests of the sample (hereinafter referred to as "return, etc.") from the referring customers by setting standards for storage, return, and disposal of each sample and standardizing these operations.
  - ① Regarding the setting of the storage period for each sample, it should be set appropriately based on the nature of the sample and the frequency of return and display the storage conditions such as room temperature, refrigeration, and freezing. In addition, it is desirable that a specimen for which an emergency report has been made should be stored for a certain period of time after the report has been submitted to the referring customer.
  - ② It is necessary to describe the confirmation items and return procedure when the referring customer requests a return, the confirmation items before disposal of the sample whose storage period has expired, and disposal procedure.
- The period of sample storage shall be set considering the characteristics of the sample (specimen) and the appropriate storage environment.
- DNA quality declines over time
- Biobanks shall store the appropriate volume of sample, as freeze/thaw cycles for the sample (specimen) can be deleterious to sample quality.

【Note】

- For example, properly stored FFPE specimens can be used within five years.
- The use, storage, transfer, and disposal of samples (specimens) taken for molecular examination shall be in accordance with legal, ethical, and professional standards.

5.8 Reporting of Results

5.8.1 General

- The report shall be able to convey the information properly, considering that the recipient can not be a professional healthcare personnel. (This is common to ① nucleic acid tests for pathogens, ② molecular-genetic tests for human somatic gene alterations, and ③ human genetic testing, but, as a general rule, mainly applies to ③ human genetic testing.)
- The report should be accurate, concise, and comprehensive, and should include all essential

information so that the subject experts can make appropriate decisions.

【Reporting of Scondary Genetic Findings】

The laboratory has a policy for reporting secondary genetic findings unrelated to the clinical purpose of testing.

① Clinically significant genetic findings that are unrelated to the phenotype for testing can occur when performing single gene, gene panel, exome, and whole genome sequencing.

② Restricted sequence analysis of a panel of genes that are relevant to the diagnosis of a particular disease state (either with targeted sequencing or targeted bioinformatics analysis) can limit, but not eliminate, the potential for secondary findings. This can include identification of variants relevant to autosomal dominant (overt) diseases, carrier status for recessive (latent) diseases, predisposition to adult-onset dominant (overt) conditions (including cancer and neurodegenerative conditions), and drug response alleles commonly known as pharmacogenetic markers.

③ The laboratory starting to use NGS for clinical testing should be aware of the potential for finding incidental, clinically significant results and should have a policy in place for whether these results will be reported for those assays where such incidental findings are expected (e.g. exome).

④ The recently published ACMG recommendations for reporting medically actionable incidental findings include a minimum gene list for which, if a known mutation is found, it should be reported. Laboratories can choose to follow the ACMG recommendations but are not necessarily expected to report only the findings of these genes.

⑤ Laboratories can also develop their own policies regarding the return of incidental results. If the laboratory's policy is not to report incidental findings or to limit reporting to a subset of variants related to a particular disease state, this should be clearly stated in the laboratory report for assays where incidental findings are expected.

⑥ Ethical considerations should also be taken into account when deciding whether to reveal certain genetic information to patients.

⑦ The level of risk associated with disclosing incidental findings depends on the severity of the disease, clinical actionability, and other risk-benefit indicators. For example, common disease risk alleles, such as for type 2 diabetes or cardiovascular disease, which have a small effect size (low relative risks), or pharmacogenetic risk information, can have different consequences than genetic information indicating a predisposition to cancer or a Mendelian disorder that can or cannot be medically treatable. All these aspects should be considered before returning the results to patients.

【Note】

It should be desirable to refer to the "Proposals concerning the information transmission process in genomic medicine-regarding comprehensive tumor genomic panel tests and germline whole

genome / whole exome analysis" in the description of the secondary genetic findings report.

5.8.3 Report Content

a) a clear, unambiguous identification of the examination including, where appropriate, the examination procedure.

The report should use internationally accepted terminology and nomenclature, such as using standard methods for describing nucleotide sequences.

b) the identification of the laboratory that issued the report.

- Name and address of testing performed facility
- Contact information for the testing facility

c) identification of all examinations that have been performed by a referral laboratory.

- When conducting testing at a referral facility, the name and location of the referral facility

d) patient identification and patient location on each page.

- Subject identification information

e) name or other unique identifier of the requester and the requester's contact details

- Name and contact information of medical doctors and/or other test requesters.

f) date of primary sample collection (and time, when available and relevant to patient care).

- Sample (specimen) collection date and receipt date

g) type of primary sample.

- Sample (specimen) type

i) examination results reported in SI units, units traceable to SI units, or other applicable units.

- Testing results (when making an interim report of testing results, it is necessary to include the contents in the final report)

k) interpretation of results, where appropriate.

- Information necessary for interpreting testing results (reports should endeavor to provide sufficient information to requesters, including appropriate medical interpretations with clinical significance)
- At the least, the reports should include the following information:
  - ① Subject identification information
  - ② Name of medical doctors and/or other test requesters and its contact information
  - ③ Reasons for applying the test and medical information of the subject required to perform the testing.
  - ④ Testing and methods performed (including scope of analysis, test limitations, and analysis sensitivity and specificity)
  - ⑤ Specimen type
  - ⑥ Specimen collection date and receipt date
  - ⑦ Information on the condition of the specimen (additives, transportation, and storage conditions, if necessary)

⑧ Name and address of the laboratory where the specimen was actually tested (including the referral laboratory if re-referred)

⑨ Testing results (when making an interim report of testing results, it is necessary to reflect the contents in the final report)

⑩ Information necessary for interpreting testing results (reports should endeavor to provide sufficient information to requesters, including appropriate medical interpretations with clinical significance)

⑪ Affiliation, title, and name of the report author and final administrator

⑫ Laboratory contact information

⑬ Date of the report release

【Note】

- The information necessary for interpreting the results is the subject's clinical data, pedigree information, race, clinical sensitivity, and specificity of the specimen. The report shall be clearly stated so that the recipient can understand the clinical usefulness and limitations of the test results. If the quantity and quality of the specimen received can affect the results, it shall be stated in the report.
- As a general rule, the following information should be included in the testing report: The main subject of the report is genetic testing, but in some cases, nucleic acid tests for pathogens and molecular-genetic tests for somatic gene alterations are also assumed.
  ① The need for genetic counseling by qualified genetic counseling professionals
  ② Potential impact on family
  ③ Information such as necessary additional testing
- All information regarding the interpretation of the results should be attached to the final report, including when the testing was re-outsourced.

l) other comments such as cautionary or explanatory notes (e.g. quality or adequacy of the primary sample which may have compromised the result, results/interpretations from referral laboratories, use of developmental procedure).

- Information on the condition of the sample (specimen) (e.g. additives, transportation, and storage conditions, if necessary)

n) identification of the person(s) reviewing the results and authorizing the release of the report (if not contained in the report, readily available when needed).

- Affiliation and name of the final administrator of the report

o) date of the report, and time of release (if not contained in the report, readily available when needed).

- Date of the report release

5.9 Release of Results

5.9.1 General

【Note1】

- Since genetic counseling is important not only for providing information but also for psychosocial support that enables autonomous selection of patients and subjects, it is desirable that medical doctors with abundant medical experience of the disease and persons who are proficient in genetic counseling cooperate and carry out medical care as a team.
- If necessary, medical doctors, medical doctors licensed as clinical geneticists, doctors licensed as clinical laboratory specialists, and persons in charge (such as pharmacists, nurses, and clinical laboratory technicians) who have specialized knowledge about pharmacogenomics testing should explain the results to the subjects (see 5.4.2).

【Note2】

- Genetic counseling includes the following:
  ① Interpretation of family history and medical history to assess the occurrence and recurrence of the disease
  ② Education on genetic phenomena, testing, management, prevention, resources, and research
  ③ Informed choice (autonomous choices after obtaining sufficient information) and counseling to facilitate adaptation to risks and situations

【Note3】

Handling genetic information when using it for medical treatment:

- Genetic information obtained by genetic testing includes information on the constitution and onset of illness due to changes in a person's genes and chromosomes, as well as information on relatives. Since this information does not change over a person's life, if it leaks, it can cause serious damage and suffering to the person and relatives. Therefore, regarding the handling of genetic information obtained by genetic testing, it is necessary to pay particular attention to the guidelines stipulated in the UNESCO International Declaration and the guidelines stipulated by related organizations.
- Even if a person agrees to carry out the testing, the person and the family members often have great anxiety, because it is difficult for them to understand the meaning of the test result accurately and how to deal with the future predictability of the disease. Therefore, when a medical institution conducts genetic testing, it is necessary to provide psychosocial support to the person and his/her family, such as genetic counseling by a person with expertise in clinical genetics.

5.10 Laboratory Information Management

5.10.1 General

- Computer access codes (security codes, user codes) shall be in place, and the security of the access codes shall be maintained (inactivated when the employees leave).
- Personal genetic information shall comply with the Personal Information Protection Law and guidelines based on it (see 5.4.2).

- Information security measures should comply with relevant laws and regulations such as the Act on the Protection of Personal Information. In addition, the laboratory should refer to "Guidelines for Safety Management of Medical Information Systems" (issued by the MHLW Health Policy Bureau on March 31, 2005, No.0331009, PFSD Notification No. 0331020, Insurance Notification No. 0331005 Director of Medical Affairs Bureau, Ministry of Health, Labor and Welfare Bureau, Director of Pharmaceutical and Food Safety Bureau, Insurance Director Notification attached).

【NGS data transfer confidentiality policy】

The laboratory shall have a policy and procedures describing processes to ensure that patient confidentiality and security are maintained during internal and external storage and transfer of sequencing data.

① Next-generation sequencing generates significant amounts of data, particularly of gene sequences that, with other information such as name, date of birth, medical record number, and other components of protected health information, can potentially be used to identify individual patients.

② The laboratory shall establish rigorous processes to ensure the protection and privacy of this information.

③ The laboratory shall have robust policies regarding the transfer of genomic information to other health care entities and third-party vendors, such as those providing cloud-based computing resources or registered clinical laboratory services. Procedures to ensure confidentiality include data encryption, secure data transfer, user authentication with controlled access to protected health information, and audit trails that track the transmission of data as well as the receiving entities and/or users.

④ The laboratory performing large-scale genomic sequencing analysis for clinical testing should be aware of efforts to study the medical and ethical implications of returning incidental results of NGS and consider these when developing their reporting policies.

- Regarding personal information protection and information security, it shall clearly state its compliance with the relevant laws and regulations, such as the Personal Information Protection Law and the "Guidelines for Safety Management of Medical Information Systems" prepared by the Ministry of Health, Labor and Welfare.
- There shall be policies and procedures for protecting the confidentiality of personal information obtained from samples (specimens) and testing results.
- When referring to molecular-genetic tests, various safety management measures (such as organizational, human, physical, and technical safety management measures) should be taken from the viewpoint of personal information protection, patient names shall be anonymized, and special attention should be paid to the handling of samples (specimens) and testing information through all processes from sample (specimen) collection to reporting of test results.
- In genetic tests aimed at diagnosing monogenic diseases, personal genetic information should be

protected by anonymization and confidential reports.

- Regarding the handling of biological samples (specimens) after testing, the administrator is responsible for discarding the biological samples after a clear storage period, even if they have been used in other studies.

【Note 1】

- As a general rule, the results of genetic tests performed to diagnose patients who have already developed a disease should be recorded in medical records as information shared by medical professionals related to the diagnosis of patients, similar to the results of other clinical tests. The genetic information of an individual obtained by genetic testing, such as all medical information, is subject to confidentiality and should not be disclosed to third parties, including relatives, without the consent of the subject. With the consent of the subject, if it is considered that the diagnosis result of the subject is useful for the health management of the relatives and cannot lead to effective prevention or treatment without the information, consideration shall be given to disclosing it to the relatives. Genetic counseling is provided at the right time as needed for genetic testing and diagnosis.
- Genetic tests performed for the purpose of non-onset carrier diagnosis, presymptomatic diagnosis, and prenatal diagnosis shall be performed after appropriate genetic counseling has been performed in advance. Genetic counseling helps people understand and adapt to the genetic, psychological, and family impacts of the genetic involvement of a disease. This process includes ① family history and medical history interpretation to assess the development and recurrence of the disease; ② education on genetic phenomena, testing, management, prevention, resources, and research; ③informed choice (autonomous choices after obtaining sufficient information); and ④ counseling to facilitate adaptation to risks and situations.
- The subjects of genetic testing shall be divided into patients who had already developed the disease and others. PGx is equivalent to patients who have already developed the disease. Pharmacology genomics tests included in pharmacogenomics tests deal with genetic information of germline lines, but unlike genetic information of monogenic diseases, in clinical practice, it can be treated in the same way as normal medical information after referring to the related guidelines.

【Note 2】

- When conducting genetic testing, the medical doctor in charge shall obtain informed consent regarding genetic testing from the subject in advance.
- Samples for genetic testing shall be stored strictly in accordance with safeguards, and the confidentiality of personal identification information and personal genetic information as test results should be protected.
  - ① As a general rule, general medical information and genetic information linked to a specific individual should be stored separately.
  - ② Personal identification information and individual genetic information are subject to

confidentiality obligations, and the medical doctor in charge, genetic counseling staff, and the person in charge of the medical institution shall strictly protect and manage them to prevent leakage to third parties.

③ When a part of genetic testing is referred to another testing institution/facility, the sample shall be anonymized in advance, and the confidentiality of personal identification information shall be maintained.

【Note 3】

- Personal information is "information that can identify a specific individual by the name, date of birth, or other description contained in the information (including those that can be easily collated with other information and thereby identify a particular individual)" (JIS Q 15001)
- " Personal identification code" refers to characters, numbers, symbols, and other codes stipulated by law as being able to identify a specific individual from the information alone, and information that includes such information is personal information. Among the characters, numbers, symbols, and other codes converted to use any of the physical characteristics listed in ① to ⑦ for use in computers, "those that meet the standards stipulated by the rules of the Personal Information Protection Commission as sufficient to identify a specific individual" are said to correspond to personal identification codes.

① Concerning DNA, those pieces of information that authenticate the person by genotype information such as whole-nucleus genome sequence data, whole exome sequence data, whole-genome single nucleotide polymorphism (SNP) data, sequence data consisting of 40 or more independent SNPs, and a short tandem repeat (STR) of four bases above nine loci.

② Those that can authenticate the person using a device or software that aims to authenticate the person with the characteristic information extracted from the skeleton and color of the face and the position and shape of the eyes, nose, mouth, and other parts of the face.

③ Those that can authenticate a person with a device or software that aims to authenticate the person with the characteristic information extracted from the linear pattern formed by the undulations on the surface of the iris using infrared light or visible light.

④ Those that can authenticate the person with a device or software that aims to authenticate the person, such as a speaker recognition system, for characteristic information related to vocal cord vibration, glottis opening/closing, vocal tract shape, and voice changes.

⑤ Those that can authenticate the person using a device or software that aims to authenticate the person with characteristic information extracted from the posture, the movement of both arms, stride length, and other walking modes during walking.

⑥ Those that can authenticate the person using a device or software that aims to authenticate the person with the characteristic information extracted from the shape of the vein determined by the branch and the end point of the vein under the skin of the palm, back of the hand, or finger using infrared light or visible light.

⑦ (fingerprint) Those that can authenticate the person using a device or software that aims to authenticate the person with the characteristic information extracted from a fingerprint formed by a ridge on the surface of a finger.

(palm print) Those that can authenticate the person using a device or software that aims to authenticate the person with the characteristic information extracted from the palm print formed by ridges and wrinkles on the surface of the palm.

⑧ Combination

Those that can authenticate the person by a device or software that aims to authenticate the person by combining the characteristic information extracted from ① to ⑦.

- "Personal information requiring special consideration" refers to information that requires special consideration in handling so as not to cause unreasonable discrimination, prejudice, or other disadvantages. The information that corresponds to the personal information requiring special consideration in medical institutions and care-related businesses is the medical history recorded in medical records or care-related records, medical information and dispensing information, results of health examinations and contents of health guidance, facts of disabilities (such as physical disabilities, intellectual disabilities, mental disorders), and the facts of being harmed by crimes that medical professionals could know about the patient's physical condition, medical condition, treatment in the process of medical treatment, and dispensing.

5.10.3 Information System Management

a) validated by the supplier and verified for functioning by the laboratory before introduction, with any changes to the system authorized, documented and verified before implementation.

- Computer programs should be checked for proper performance after installation of new systems or modifications of existing systems.
- Any changes or modifications to the system should be documented and approved.
- Documentation shall be retained for at least two years beyond the service life of the system in the biobank.

6 Reference

1) ISO 15189, Medical laboratories - Requirements for quality and competence
2) Aziz N, Zhao Q, Bry L, Driscoll DK, et al. College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests, Arc Pathol Lab Med 2014; 1-13.
3) Code of Federal Regulations 42 Part493, Laboratory Requirements, Subpart M - Personnel for Nonwaived Testing. 1992.
4) College of American Pathologists. Biorepository Accreditation Program Checklist. 2019; 1-64.
5) ISO 15190, Medical laboratories - Requirements for safety.
6) ISO 35001, Biorisk management for laboratories and other related organizations.
7) ISO 20658, Medical laboratory examinations - Requirements for collection, transport, receipt and handling of samples.
8) Smith J, Johnson P, Jacoby R, et. al. Quality Management Systems - Process Validation Guidance. GHTF/SG3/N99-10: 2004. 1-36.
9) ISO/IEC Guide 99, International vocabulary of metrology - Basic and general concept and associated terms (VIM).
10) Hardwick SA, Deveson IW, Mercer TR. Reference standards for next-generation sequencing, Nat Rev Genet 2017; 18(8): 473-484.
11) ISO TS 20658, Medical laboratory examinations - Requirements for collection, transport, receipt and handling of samples.
12) ISO 17822, In vitro diagnostic test systems – Quantitative nucleic acid based in vitro examination procedure for detection and identification of microbial pathogens – Quality practices for nucleic acid amplification.
13) ISO 20166-1, Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for formalin-fixed and paraffin - embedded (FFPE) tissue - Part 1: Isolated RNA.
14) ISO 20166-2, Molecular in-vitro diagnostic examinations - Specifications for pre-examination processes for formalin-fixed and paraffin - embedded (FFPE) tissue - Part 2 : isolated proteins.
15) ISO 20166-3, Molecular in-vitro diagnostic examinations - Specifications for pre-examination processes for formalin-fixed and paraffin-embedded (FFPE) tissue - Part 3 : isolated DNA.
16) ISO 20184-1, Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for frozen tissue - Part 1: Isolated RNA.
17) ISO 20184-2, Molecular in-vitro diagnostic examinations - Specifications for pre-examination processes for frozen tissue – Part 2: Isolated proteins.
18) ISO 20186-3, Molecular in vitro diagnostic examinations - Specifications for pre-examination processes for frozen tissue - Part 3: Isolated DNA.

19) ISO 21474-1, In vitro diagnostic medical devices - Multiplex molecular testing for nucleic acid - Part 1: Terminology and general requirements for nucleic acid quality evaluation.
20) ISO 21474-1, In vitro diagnostic medical devices - Multiplex molecular testing for nucleic acid - Part 1: Terminology and general requirements for nucleic acid quality evaluation.
21) ISO 20387, Biotechnology-Biobanking - General requirements for biobanking.
22) Jennings LJ, Arcila ME, Corless C, et al. Guidelines for Validation of next - generation sequencing - Based oncology panels, J Mol Diagn 2017, 19(3), 341-365
23) ISO/IEC 17025, General requirements for the competence of testing and calibration laboratories.
24) Gargis AS, Kalman L, Berry MW, et al. Assuring the quality of next-generation sequencing in clinical laboratory practice, Nat Biotechnol 2021; 30(11): 1033-1036.
25) Roy S, Coldren C, Karunamurthy A, et al. Standards and Guidelines for validating next-generation sequencing bioinformatics pipelines, J Mol Diagn 2018; 20(1): 4-27.
26) Zucker HA, Dreslin S. “Next Generation” Sequencing (NGS) guidelines for somatic genetic variant detection, New York State of Opportunity. 2018; Mar. 1-6.