The Next Generation Sequencing Quality Initiative

The Next Generation Sequencing (NGS) Quality Initiative is a collaboration between the Centers for Disease Control and Prevention (CDC), the Association of Public Health Laboratories (APHL), and state and local public health laboratories (PHLs) to address the many challenges laboratories encounter when implementing NGS-based assays. The Initiative is developing an NGS-focused quality management system (QMS) to assure foundational quality during the development and implementation of sequencing-based tests by providing customizable, ready-to-implement tools and resources that laboratories can use to standardize and institute quality management practices and procedures. The NGS Quality Initiative has published additional tools and resources, including templates and procedures, that may be of assistance to laboratories throughout their NGS workflow. Please visit the following website to access these resources: https://www.cdc.gov/labquality/qms-tools-and-resources.html.

This document is intended to be used as a tool for implementing, improving, or maintaining an NGS QMS. Blue text provides examples for appropriate input and can be changed, deleted, or augmented as needed for the laboratory's specific requirements.

These documents and tools are not controlled files; format and content **must** be modified as needed to meet the document control, QMS, or regulatory requirements within your laboratory. It is the responsibility of your laboratory to take any necessary actions to ensure the information within these documents remains applicable.

Disclaimer:
Use of trade names and commercial sources is for identification only and does not imply endorsement by the U.S. Centers for Disease Control and Prevention or by the U.S. Department of Health and Human Services.

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Scope

The Centers for Disease Control and Prevention and the Association of Public Health Laboratories' Next Generation Sequencing (NGS) Quality Initiative (QI) developed "A Pathway to Quality-Focused Testing" (Pathway). The Pathway is intended to support laboratory personnel responsible for validation, continued testing, and maintenance of NGS workflows. It provides an interactive method to monitor progress and track task completion during a method validation and could be presented to laboratory leadership (or designee) when reviewing validation plans or monitoring progress. The Pathway is intended as one option for approaching method validation. Use does not ensure a successful validation. This Pathway is not intended as a stand-alone document for developing an NGS Quality Management System (QMS) but as a supplement to other published tools and resources that address these topics in more depth. This document identifies additional NGS-related tools and resources that provide additional information and examples (e.g., precision, accuracy). Please visit https://www.cdc.gov/labquality/qms-tools-and-resources.html. It is expected that each laboratory will use this Pathway, in whole or in individual phases, to guide NGS method validation according to their needs. The phases and resources can help to address considerations and recommend quality checkpoints from pre- through post-analytic phases of the total testing process and are intended to be used as resources for method validation from preparation through continual maintenance. The Pathway is based on guidance from the Clinical and Laboratory Standards Institute (CLSI), the College of American Pathologists (CAP), and others.

NOTE: **Additional resources are included in the references section.**

Introduction

NGS has revolutionized clinical and public health practice, with clinical and public health laboratories relying on it for numerous applications. Despite the availability and continuing development of guidance for establishing performance specifications and supporting test validations for NGS, challenges remain due to the absence of a standardized quality framework. To help address this, the Pathway can be used to support NGS assay validation, testing implementation, and maintenance of the test system(s). This Pathway is based on the twelve Quality System Essentials (QSEs) developed by CLSI and was cross-walked to the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations as well as guidelines for NGS developed by professional organizations. The Pathway provides a framework that accommodates the complexities of NGS, integration into clinical and public health workflows, and the need to maintain a reliable platform that delivers high-quality results.

The Pathway provides a step-by-step framework, including suggestions and links to existing guidance, as well as resources and references to specific, relevant regulations for laboratories to consider when preparing for and completing method validations.

The Pathway is divided into five phases:

- Phase 1 – Prepare for Validation
- Phase 2 – Review and Finalize Procedures
- Phase 3 – Perform Validation
- Phase 4 – Post Validation-Train and Authorize Personnel
- Phase 5 – Test and Maintain

The five phases are comprised of multiple sections, with each section comprised of multiple steps. For example, the first phase, "Prepare for Validation," is comprised of sections for "Personnel Performing Validation," "Equipment," "Reagents, Media and Supplies," and "Laboratory Space and Safety," with the "Personnel Performing Validation" section including "Job Descriptions and Role-Based Responsibilities," "Qualification Requirements," "Personnel Training," and "Approval of Personnel." Each step is further broken down into a checklist with brief descriptions, cross-references to relevant standards and regulatory requirements, and relevant published NGS QI resources.

For clinical and public health laboratories, this tool can contribute to the development of NGS workflows in diagnostic, environmental, surveillance, and research settings that include the necessary components of a QMS, documentation of quality control, integration of quality practices for development and maintenance of bioinformatics pipelines, and compliance with CLIA requirements. ***NOTE:*** **Personnel may use the references pages for more information on the guidance provided.**

Disclaimer:
This tool is not inclusive of all existing guidance and resources currently available, but rather incorporates relevant published NGS QI resources that are agnostic and customizable for laboratories to adopt for their intended use.

Intended Use

The Pathway to Quality-Focused Testing serves as an interactive tool for monitoring progress and tracking task completion during a method validation. It can be presented to laboratory leadership for review. It is designed to be flexible and adaptable, allowing each laboratory to utilize it in its entirety or select individual phases based on specific needs.

The Pathway is not meant to be a stand-alone document for developing an NGS QMS but rather a supplement to other published tools and resources that provide more in-depth information on the topic.

The Pathway to Quality-Focused Testing is available in two formats, a webtool and a fillable PDF. To access the webtool, click the following link.

To use the checkboxes in either version, simply click on the checkbox next to each desired option to select or deselect it. For the webtool, selections are saved automatically; to clear all selections, click the "Reset Progress" button at the bottom of the webpage.

***NOTE*: It is recommended that users not open the PDF in a web browser. Please download and open using a PDF reader prior to populating with laboratory-specific information to ensure that progress is saved successfully.**

Pathway to Quality-Focused Testing

Phase 1: Prepare for Validation

Phase 1 outlines considerations for how to proceed in preparing for validation. This preparation is vital to the validation process, as it forms the basis for validation itself. In this phase, the laboratory should be meticulous and thorough in identifying and documenting all the criteria needed to prepare for testing. These criteria should include personnel, equipment, reagents/media, and laboratory safety policies and procedures involved in the validation process as defined below.

Prepare for Validation: Personnel Performing Validation

Personnel developing, overseeing, and performing testing should be qualified, trained, and competent within the year that testing is performed. "Personnel" refers to laboratory staff possessing the skills, knowledge, and credentials necessary to perform the validation.

NOTE: There are multiple references to CLIA personnel requirements throughout each phase of the Pathway. Additional aspects of personnel training will be mentioned in subsequent phases.

Job descriptions and role-based responsibilities

- □ Job description and role-based responsibilities are defined and documented.
  - ○ Resulting documents/records
    - Standardized job description and responsibilities
    - Organizational chart
- □ Responsibilities are reviewed by staff designated to assign and perform duties.
  - ○ Resulting documents/records
    - A form documenting personnel roles and responsibilities must be populated and signed.

Related requirements: CLIA: 42 C.F.R. §§ 493.1441, 493.1489, 493.1423; CAP: GEN.54400

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the responsibilities section from the following resource for examples of position descriptions.

- NGS Method Validation SOP

Qualification requirements

- □ Regulatory and accreditation qualification requirements (e.g., CLIA for clinical testing) are defined and documented based on roles and responsibilities.
  - ○ Resulting documents/records
    - Job aid with role-based qualification requirements

☐ Qualified personnel are identified.
- Resulting documents/records
  - Review form for personnel qualification documents
  - Maintenance of employee qualifications on file

  Related requirements: CLIA: 42 C.F.R. §§ 493.1445(e)(10) - 493.1445(e)(11); CAP:COM.04100

Personnel training

☐ Required training is outlined and defined for each role in the method validation process.
☐ Personnel training includes topics of safety, equipment, and record management.
☐ Personnel are trained to perform the activities of their assigned position.
- Resulting documents/records
  - Procedure that outlines training steps and that can be used as a guide
  - Form to capture the completion of training

  Related requirements: CLIA: 42 C.F.R. § 493.1451(b)(7); CAP: GEN.55450

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of forms that outline training steps related to bioinformatics and that capture completion of training.

- Training forms

Approval of personnel

☐ Laboratory personnel positions and responsibilities, including qualifications, training, and competency, must be confirmed in writing by laboratory leadership or an authorized designee.
- Resulting documents/records
  - Log of approved testing personnel

  Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(15); CAP: DRA.11300

Prepare for Validation: Equipment

Proper equipment management is essential in ensuring quality results for laboratory testing. Equipment should be maintained per the manufacturer's guidelines to ensure operation at optimum conditions and generation of accurate and reliable test results. Proper equipment maintenance also helps to ensure optimal equipment performance and longevity.

Selection of equipment

☐ Selection Qualification (SQ)
- Criteria for selecting equipment are established with consideration of laboratory needs, total cost of ownership (including service contracts, as applicable), laboratory environment requirements (humidity, temperature), turnaround time, complexity, physical space, and necessary resources for operating equipment.

- The laboratory has a system that periodically re-evaluates the NGS equipment for the potential to onboard alternative or additional equipment.
- Resulting documents/records
  - Procedure that outlines the criteria for selection of test equipment

Related requirements: CLIA: 42 C.F.R. § 493.1101(b)

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of checklists that include information related to the selection of test equipment.

- Key Criteria for Selection of NGS Equipment
- Pre-Installation Checklists

Equipment management

☐ Process for capturing and managing all required equipment information is developed, including tracking equipment location, routine maintenance, preventive maintenance, equipment records, and manufacturer manuals.

- Resulting documents/records
  - Procedure that outlines the performance of equipment management and maintenance schedules

Related requirements: CLIA: 42 C.F.R. § 493.1254; CAP: COM.04200

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for examples of equipment management.

- Summary of Inventory Management Processes

Equipment qualification

☐ Equipment qualification is a process put in place to provide documented, objective evidence of whether the equipment operates within the manufacturer's specifications and is functional for use by the laboratory.

☐ The laboratory has a system that periodically evaluates and defines the relationship between/among test results using the different equipment.

- Resulting documents/records
  - Laboratory equipment qualification checklist, which includes a section for equipment comparison
  - Form that captures personnel performing testing and results of the comparison

☐ Installation Qualification (IQ)

- Equipment installation according to the vendor's specifications is confirmed; the laboratory environment is suitable for the proper functioning of the equipment.
- The manufacturer's service engineer often performs this process.
- Resulting documents/records
  - Vendor-provided installation documentation

□ Operational Qualification (OQ)

- Confirmation of equipment as operational for intended use and location
- OQ confirms the instruments' basic operational specifications, as established by the manufacturer, before implementation in the laboratory.
- This process is sometimes performed by the manufacturer's service engineer alongside the laboratory staff.

○ Resulting documents/records

- Vendor-provided operational qualification documentation

□ Performance Qualification (PQ)

- Verification that the method and equipment meet established performance specifications using the laboratory's staff and documents
- PQ provides confirmation that the equipment produces acceptable results under normal operating conditions by testing both the device and the ability of the process to manage the work in the anticipated period and meet stated requirements. The PQ should evaluate the full range of intended use (e.g., number of samples, number of runs).
- This process should be performed by the laboratory staff to validate their method/assay performance.

○ Resulting documents/records

- Validation records of method and equipment meet established performance specifications (accuracy, precision, analytical sensitivity, analytical specificity, interferences, reference interval[s], and reportable range) of any test that has been modified.

Related requirements: CLIA: 42 C.F.R. §§ 493.1253, 493.1281; CAP: COM.40250, COM.40300, COM.40350, COM.40475

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below to attach to the documentation provided by the vendor.

- Vendor-Performed IQ-OQ Coversheet

Equipment calibration

□ Development and approval of a procedure defining the process for maintaining laboratory equipment and ensuring performance of equipment calibration according to manufacturer requirements

□ Procedure defining the process of equipment comparison performed by following manufacturer recommendations, guidelines, and applicable regulations (**e.g., calibration verification every 6 months).** Laboratories should consider performing a recalibration when a procedure's reagents change completely, after performing a major preventive maintenance or replacing critical parts that could affect test performance, when control materials indicate an unusual deviation or fall outside acceptable limits, and/or if a

laboratory's established schedule for verifying the reportable range for patient test results requires more frequent calibration verification.

- Resulting documents/records
  - Procedure that outlines the step-by-step process of equipment calibration and maintenance
  - Form that captures equipment calibration and maintenance
  - Procedure that outlines the step-by-step process of equipment comparison
  - Form that captures equipment comparison

Related requirements: CLIA: 42 C.F.R. §§ 493.1254 (a)(1), 493.1255; *CAP: COM.30600*

Preventive maintenance

☐ Preventive maintenance is defined and documented in the form of scheduled, periodic work on equipment to ensure that the system functions according to the manufacturer's established criteria required to produce quality results.

- Resulting documents/records
  - Form to capture performance of manufacturer-required instrument preventive maintenance

Related requirements: CLIA: 42 C.F.R. § 493.1254; CAP: COM.04200, COM.30550

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of logs and procedures that capture the performance of preventive and routine maintenance.

- Maintenance Logs and SOPs
- Error Log for Illumina Sequencers
- Error Log for Thermo Fisher Sequencers

Prepare for Validation: Reagents, Media, and Supplies

The laboratory should define processes and establish procedures for the handling, storage, and quality assessment of reagents, materials, and supplies.

Selection of reagents, media, and supplies

☐ Criteria for selecting reagents, media, and supplies are established with consideration of laboratory needs, total cost, and storage requirements.

- Resulting documents/records
  - Procedure for the selection of reagents, media, and supplies
  - Form to capture information about reagents and media that are prepared, stored, and discarded after expiration

Related requirements: CLIA: 42 C.F.R. §§ 493.1101 (b), 493.1252 (b) (1-4); CAP; COM.30350

Quality Control (QC)

☐ Development and documentation of procedures necessary to ensure that new and in-use reagents and media lots can produce acceptable quality results

- Resulting documents/records
  - Procedure that outlines QC for reagents, media, and supplies
  - Form to track lot numbers and QC results of new and in-use reagents and media

Related requirements: CLIA: 42 C.F.R. § 493.1256 (a)(b)(c) (1-2); CAP: COM.30450

Labeling

☐ All reagents, solutions, culture media, control materials, and calibration materials are labeled with their identity, receipt date, storage requirements, expiration date, titer, strength or concentration (as applicable), and other relevant information.

- Resulting documents/records
  - Procedure that outlines labeling of reagents, solutions, culture media, control materials, and calibration materials

Related requirements: CLIA: 42 C.F.R. § 493.1252(c); CAP: COM.30300

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for examples of inventory management, such as labeling of reagents.

- Summary of Inventory Management Processes

Storage

☐ Environmental conditions for properly storing reagents, specimens, and samples are defined and documented.

- Resulting documents/records
  - Procedure that outlines proper storage of inventory
  - Form to capture tracking of freezer and refrigerator temperatures where inventory is being stored

Related requirements: CLIA: 42 C.F.R. § 493.1252(c); CAP: COM.30250

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for examples of inventory management, such as proper storage and tracking temperatures.

- Summary of Inventory Management Processes

Inventory management

☐ A reliable method for inventory management is developed to ensure accurate procurement, receipt, expiration, and tracking of use for reagents, media, and supplies.

☐ Specific instructions are provided that prohibit the use of reagents, solutions, culture media, control and calibration material, and other supplies after their expiration date and in the event of observed evidence of deterioration.

- Resulting documents/records
  - Standard Operating Procedure (SOP) that outlines procurement, receipt, expiration, and tracking of use for inventory
  - Method to track and manage inventory in the form of logs or checklists

  Related requirements: CLIA: 42 C.F.R. § 493.1252; CAP: GEN.61900

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of inventory management outlining procurement, receipt, tracking, and use of inventory.

- Summary of Inventory Management Processes
- Inventory Management Database
- Inventory Management Database User Guide
- Inventory Management Database Video Tutorials
  - Inventory Tool Tutorial 1 - Introduction and Items (V3)
  - Inventory Tool Tutorial 2 - Orders (V3)
  - Inventory Tool Tutorial 3 - Inventory Checks (V3)

Environmental condition monitoring

☐ Environmental conditions are monitored and documented, including temperature, water quality, and humidity.

- Resulting documents/records
  - Procedure that outlines how to monitor and document laboratory environmental conditions
  - Form to track laboratory temperature, water quality, and humidity

  Related requirements: CLIA: 42 C.F.R. § 493.1252 (b); CAP: GEN.60250

Prepare for Validation: Laboratory Space and Safety

The laboratory should have appropriate and sufficient space to conduct all phases of laboratory testing. Safety procedures, including risk assessments, should be established, accessible, and observed to protect from physical, chemical, biochemical, and electrical hazards, as well as biohazardous materials.

Laboratory space

☐ Conditions for laboratory safety, laboratory personnel safety, and work quality are designed and arranged.

☐ The laboratory has considerations for a unidirectional workflow that are intended to reduce testing error and promote testing quality.

- Resulting documents/records
  - Laboratory safety procedure and manual
  - Form to capture laboratory safety orientation training

  Related requirements: CLIA: 42 C.F.R. § 493.1101; CAP: GEN.60000

Safety documentation

☐ Identification and evaluation of laboratory hazards are performed and documented in the form of a safety manual and associated procedures.

- Resulting documents/records
  - Chemical hygiene and hazard plan
  - Procedure or manual that outlines general laboratory safety rules and personnel responsibilities

Related requirements: CLIA: 42 C.F.R. § 493.1101(d); CAP: GEN.76000, GEN.77800, GEN.73200

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for considerations related to laboratory and personnel safety.

- Waste Disposal SOPs

Risk assessments

☐ A risk assessment is performed and documented to minimize risks and provide a safe work environment. This allows the laboratory to evaluate what could go wrong by determining the likelihood of an undesirable incident (e.g., injury, exposure) and its possible consequences (e.g., infection, disease).

☐ Risk Assessments should include evaluation for physical, biological, chemical, and radiological threats.

☐ Risk Assessments evaluate risk of error due to data entry errors, loss, and corruption.

- Resulting documents/records
  - Procedure that outlines the process for risk assessment (including physical, biological, chemical, and radiological hazards)
  - Form for risk assessment

Related requirements: CLIA: 42 C.F.R. § 493.1101(c); CAP: GEN.76000

Personnel safety training

☐ Safety training is provided to personnel who have been assigned to perform validation.

☐ Personnel receive safety training on personal protective equipment (PPE) that is appropriate for specific laboratory tasks and/or methods.

☐ Personnel are trained on evacuation procedures and crisis response techniques.

- Resulting documents/records
  - Procedure that outlines testing personnel safety training
  - Form to capture testing personnel safety training

Related requirements: CLIA: 42 C.F.R. §§ 493.1451(b)(7), 493.1445(e)(12); CAP: GEN.54400

Phase 1 Notes

NOTE: **Personnel may use the references pages for more information on the guidance provided.**

End of Phase 1: Prepare for Validation

Phase 2: Review and Finalize Procedures

The laboratory should develop and implement procedures necessary to perform testing and report results. Developing clear procedures for testing and reporting results contributes to the foundation of the quality management system (QMS). The testing procedure is drafted prior to validation and is then finalized, signed, and approved following the validation, as the laboratory may need to adjust based on the performance of the validation.

Review and Finalize Procedures: Procedure Planning

The laboratory test procedure should be well-written and detailed for validated methods.

Document draft

- ☐ An individual who has a robust understanding of the method and its requirements should be designated to finalize the testing procedure.
  - Resulting documents/records
    - Procedure that outlines personnel responsibilities, identifying individuals responsible for the development of the document draft

  Related requirements: CLIA: 42 C.F.R. § § 493.1253, 493.1251

Document review

- ☐ A subject matter expert in the area related to the method is identified and designated to review the testing procedure draft.
  - Resulting documents/records
    - Procedure that outlines personnel responsibilities, identifying individuals responsible for the review of the document

  Related requirements: CLIA: 42 C.F.R. § 493.1251; CAP: COM.40475

Review and Finalize Procedures: Finalize a Test Procedure

A method and data analysis test procedure should be developed before validation is executed. The SOP should be in its final format, and any necessary changes are expected to be corrected during method design, development, and optimization before this point (e.g., in-house controls and their expected values are established via concurrent, repetitive testing).

- ☐ Develop worksheets to capture information about samples, reagents, and equipment used during testing. Include variations in manufacturers and kits.
- ☐ Use different lots of key reagents to verify reproducibility.
- ☐ Procedure controls are defined and documented.
  - Resulting documents/records
    - Final method SOP
    - Final worksheets
    - Control qualification documentation

Review and Finalize Procedures: Template Development

Developing a testing procedure template establishes the information necessary for all methods. The template should include the method section headings and a description of the information to be included in each section. As new methods are drafted, the template should be used and revised as appropriate for the specific method.

Template development

☐ A method template document is developed and maintained according to the laboratory document control policy.

- Resulting documents/records
  - Procedure template that outlines the information necessary for all methods

Related requirements: CLIA: 42 C.F.R. § 493.1251; CAP: COM.40475

Template sections

☐ The following sections are considered and clearly defined for method template development.

- Purpose and Principle
- Scope
- Definitions
- Responsibilities
- Performance Specifications
- Limitations and Potential Sources of Variation
- Equipment
- Reagents, Media, and Materials
- Safety
- Sample Acceptance Criteria
- Procedure Steps
- Quality Control
- Calculations, if applicable
- Interpretation of Results
- Data Review and Reporting Results
- Reflex Testing, if applicable
- Sample and Data Retention
- Related Documents
- References
- Resulting Documents/Records
- Procedure that outlines and defines the sections necessary to develop a method template

Related requirements: CLIA: 42 C.F.R. § 493.1251

Review and Finalize Procedures: Procedure Development

Testing procedures should include specified sections that establish the document's groundwork. These sections should be included in all documents unless deemed unnecessary in certain situations.

Procedure sections

- □ The purpose of the test is defined and documented.
- □ The principles behind the test method (e.g., information about the theory, background, and methodology) are briefly defined and documented.
- □ The scope of the sample matrices, target organisms and/or analyses, and related restrictions are defined and documented.
- □ Relevant terms and definitions are defined and documented.
- □ The roles and responsibilities of the individual(s) responsible for the test method are defined and documented.
- □ Method performance specifications (e.g., accuracy, precision, specificity, sensitivity, reporting range, the limit of detection, the limit of quantitation) are defined and documented and/or the validation report is referenced.
- □ Limitations and potential sources of variation (e.g., significant cross-reactivities, interfering substances, other conditions known to affect test results, including atypically rigorous requirements for sample collection or sample handling [time, temperature, etc.]) are defined and documented and/or the validation report is referenced.
- □ Equipment with sufficient specificity required to carry out the procedure is documented (e.g., open-platform, general use).
- □ Reagents, media, supplies, and disposables with sufficient specificity required to carry out the procedure (e.g., source, item number, grade) are identified and documented.
- □ PPE necessary to perform the method is identified and documented.
- □ Hazardous organisms, chemicals, carcinogens, mutagens, and teratogens are identified and documented.
- □ Criteria for accepting samples (e.g., chain of custody, receipt, short-term storage, retention, type, volume, shipment, and rejection of specimens) and sample management are identified and documented.
- □ Methods for the disposal of samples once the procedure has been completed are identified and documented.
- □ The test procedure is identified and documented step-by-step and in detail.
- □ Quality control samples required for the procedure (e.g., positive, negative, blank, process, internal controls) are identified and documented.
- □ Initial and/or continuing calibration verification (ICV and CCV, respectively) reference materials are identified and documented.

- □ Equations, calculation instructions for solving equations, and examples for solving equations are identified and documented.
- □ For quantitative tests, where relevant, an estimate of the measurement of uncertainty associated with results is identified and documented.
- □ Procedures for interpreting results are identified and documented, if needed (e.g., records with clinical significance normal range information).
- □ Procedures for interpreting unexpected clinical results (e.g., critical/panic values, immediate values that are outside normal range, and values that could cause immediate risk to patient, as applicable) are identified and documented.
- □ Procedures for data review and reporting results are identified and documented if they are not addressed in a related reporting results procedure.
- □ Procedures for bioinformatics pipeline version control are identified and documented.
- □ Procedures and requirements for storage and retention are identified and documented.
    - Resulting documents/records
        - Draft laboratory method and/or procedure

    Related requirements: CLIA: 42 C.F.R. §§ 493.1105 (a)(2), 493.1242(a) (1-8), 493.1251, 493.1253, 493.1291(f)

Review and Finalize Procedures: Procedure Review

Standard procedures should be well-written and detailed for validated methods.

Document review

- □ Draft procedure is submitted to laboratory leadership or authorized designee previously identified for review and feedback.
- □ Feedback received during the review process is considered.
- □ Draft procedure is revised accordingly.
- □ Bioinformatic pipelines are reviewed when pipeline software is implemented, if existing software is updated, or if any changes are made to the pipeline analysis steps.
    - Verification and validation processes should be included in the updates to bioinformatic pipelines.
    - Resulting documents/records
        - Draft laboratory method and/or procedure

    Related requirements: CLIA: 42 C.F.R. § 493.1251

Document approval

- □ Laboratory leadership or authorized designee provides document approval upon successful review and revision of the method.
- □ Timeline for the authorized designee's periodic assessment of procedures is determined (e.g., annual or biennial review).

□ Documents and/or forms necessary to support the validated method should be identified. There should also be procedures in place that support the QMS.

- Resulting documents/records
  - Approved laboratory method and/or procedure

Related requirements: CLIA: 42 C.F.R. §§ 493.1251, 493.1445, 493.1451; CAP: COM.10100, GEN.20375

Accessioning

□ Procedure that defines the minimum requirements for specimen accessioning, including input of specimen metadata, is developed and approved.

- Resulting documents/records
  - Procedure that outlines sample accessioning criteria

Related requirements: CLIA: 42 C.F.R. § 493.1283(a)(1-4)(b); CAP: COM.06100, COM.06200, GEN.40502

Reporting

□ Procedure that defines the process of reporting laboratory results is developed and approved.

□ The laboratory procedure, in addition to the test report, includes parameters, guidelines for interpreting results, and any necessary disclaimers.

- Resulting documents/records
  - Procedure that outlines reporting of laboratory results

Related requirements: CLIA: 42 C.F.R. § 493.1291; CAP: GEN.41096

Phase 2 Notes

NOTE: **Personnel may use the references pages for more information on the guidance provided.**

End of Phase 2: Review and Finalize Procedures

Phase 3: Perform Validation

The laboratory should ensure test methods, test data, and results are reproducible, accurate, and precise by validating test procedures. Validation is essential, as the results provide confidence in analytical and diagnostic results' accuracy, reliability, and reproducibility. Validations provide objectively determined evidence that an assay performs as intended and complies with applicable regulatory, accreditation, and organizational requirements. The reliability of the reported test result is essential to patient care and informed public health decisions. The following phase outlines the steps necessary for a validation.

Validation: Planning and Development

The first step in starting the validation process is to create a Method Validation Plan. This document provides details of the entire validation plan and serves as a record of the intended specifications for determining performance characteristics. The plan should be reviewed by an authorized designee (general supervisor, clinical consultant, quality manager, lab director, or CLIA laboratory director) for approval before starting validation. A good validation plan consists of the following sections:

Purpose of method

- □ The test procedure and its purpose have been defined and documented.
  - ○ Resulting documents/records
    - • Draft method validation plan

    Related requirements: CLIA: 42 C.F.R. § 493.1253

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for an example template and procedure related to method validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP

Establishing performance specifications

- □ Parameters for testing, establishing performance characteristics, and acceptance criteria for the validation plan are documented.
- □ Laboratories establish criteria for defining test results (e.g., a sample is determined to be a True Negative if, by defined metric thresholds, there is no detection of signal).
- □ Strategy for resolution of discrepant/inconclusive test results is defined and documented.
  - ○ Resulting documents/records
    - • Draft method validation plan

    Related requirements: CLIA: 42 C.F.R. §§ 493.1251, 493.1253; CAP: COM.40350

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for an example template and procedure related to method validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP

Sample requirements

☐ Sample requirements for the validation plan are defined and documented.

☐ Validation controls and reference materials are defined and documented.

- Resulting documents/records
  - Draft method validation plan
  - Documents verifying results of test controls and reference materials

Related requirements: CLIA: 42 C.F.R. § 493.1253(b)(2); CAP: COM.40350

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for example templates and procedures related to method validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP
- NGS QC Guidance for Illumina Workflows
- NGS QC Guidance for MinION 1D Workflows
- NGS QC Guidance for MinION Rapid Sequencing Workflows

Roles and responsibilities during validation

☐ The individuals responsible for performing the validation procedure and associated tasks are identified and documented.

- Resulting documents/records
  - Draft method validation plan
  - Standardized job description and responsibilities

Related requirements: CLIA: 42 C.F.R. §§ 493.1445(e)(11), 493.1445(e)(15CAP: GEN.54400

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for example roles and responsibilities related to method validation.

- NGS Method Validation SOP

Equipment used during validation

☐ The equipment used as part of the test method and its associated performance criteria are identified and documented.

- Resulting documents/records
  - Draft method validation plan

Related requirements: CLIA: 42 C.F.R. §§ 493.1101(b), 493.1281; CAP: COM.40350

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for an example template and procedure related to equipment used during validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP

Bioinformatics pipeline

A bioinformatics pipeline is a computational workflow that processes raw NGS data and associated metadata through one or more computational tools in a sequential manner.

- □ Verify that the components of the bioinformatics pipeline to be used as part of the test method are identified and defined. Key components to record during the planning and execution of this process include:
  - Operating system
  - Computational
  - Verifying file integrity
  - Name of pipeline and version number
  - Database
  - Input file(s) and file extension(s)
  - Output file(s) produced because of the pipeline and file extension(s)
  - Pipeline workflow manager, if used
  - Version of reference genomes, if used
  - Version of reference database, if used
  - List of computational tools used, including:
    - Name and version of tool used
    - The parameters used in the implementation, whether they are default or modified
    - External files, if any, needed for running the tool
- □ Establish acceptance and rejection criteria for the data as it runs through the pipeline.
- □ Establish parameters for QC metrics.
  - ○ Resulting documents/records
    - Draft method validation plan

Related requirements: CLIA: 42 C.F.R. § 493.1291(a); CAP: GEN.43450

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for example templates and procedures related to the bioinformatics pipeline during validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP
- Assembly QC SOP
- Bioinformatics QC Workflows
- Pre-Analysis QC SOP

- SanitizeME Host DNA Removal SOP
- Sequencing QC SOP
- Singularity Overview
- Package and Environment Management Systems for Bioinformatics

Approval of validation plan

☐ Laboratory leadership or authorized designee reviews and approves the validation plan before performing validation testing. As applicable, an addendum document describing any issues or proposed changes should be submitted for approval.

- Resulting documents/records
  - Draft method validation plan
  - Validation plan addendum
  - Approved validation plan

  Related requirements: CLIA: 42 C.F.R. § 493.1445

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for an example template and procedure related to method validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP

Test performance

☐ Once the method validation plan is approved, perform the validation plan and analyze the data. This ensures the acceptability and accuracy of the evaluated test procedure.

- Resulting documents/records
  - Method Validation Report
  - Testing Documentation

  Related requirements: CLIA: 42 C.F.R. § 493.1253; CAP: COM.40350

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for an example template and procedure for completion of the method validation plan.

- NGS Method Validation Plan Template
- NGS Method Validation SOP

Data analysis

☐ Data from the validation tests are analyzed and evaluated against the established method validation acceptance criteria. Corrective actions are performed as needed.

- Resulting documents/records
  - Method Validation Report
  - All files generated during the validation (raw sequencing data, intermediate and final bioinformatics pipeline outputs)

Related requirements: CLIA: 42 C.F.R. § 493.1253(b)(2); CAP: COM.40350

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for an example template and procedure for the analysis and evaluation of data resulting from the validation test against the acceptance criteria.

- NGS Method Validation Plan Template
- NGS Method Validation SOP

Validation: Reporting and Implementation

Once validation testing and analysis are complete, all the results are reported and summarized for approval. The results are then assessed to ensure that they meet criteria for acceptability prior to laboratory implementation of the method. This document provides guidance to summarize and report results of validation testing.

Summary of results

☐ A summary for the method validation is recorded, including observations, raw data, a summary of results, an interpretation of results, and a statement of suitability.

- Resulting documents/records
  - Method Validation Summary Report

Related requirements: CLIA: 42 C.F.R. § 493.1253, CAP: COM.40350

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for a summary report of the method validation.

- NGS Method Validation Summary Report Template

Approval of method validation report

☐ The method validation summary report is submitted for final approval to the authorized designee assigned to approve validation reports.

☐ Once approved, the method validation summary report is maintained according to laboratory records retention procedures.

- Resulting documents/records
  - Final method validation plan
  - Approved method validation summary report

Related requirements: CLIA: 42 C.F.R. § 493.1445

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for example templates and a procedure related to method validation.

- NGS Method Validation Plan Template
- NGS Method Validation SOP
- NGS Method Validation Summary Report Template

Phase 3 Notes

NOTE: **Personnel may use the references pages for more information on the guidance provided.**

End of Phase 3: Validation

Phase 4: Post Validation-Train and Authorize Personnel

The laboratory should train and authorize testing personnel to perform the test procedure. Following validation and completion of procedure development, training of testing personnel is essential to ensure that staff can demonstrate the skills and knowledge necessary to perform all procedures. The personnel are then authorized by laboratory leadership or an authorized designee to perform testing.

Post Validation: Train and Authorize: Develop and Submit Training Plan

Once validation is complete, a training plan should be developed and submitted to laboratory leadership or an authorized designee for approval. All testing personnel should follow the training plan for initial training and re-training, as applicable. Per applicable regulatory requirements, a competency assessment should be performed for all trained personnel. This is essential for accurate and reliable testing and reporting for the test procedure.

Identify training needs and goals

- ☐ The type of training provided is determined based on laboratory needs and goals (e.g., addressing training gaps and/or opportunities for continuing education).
- ☐ A training schedule is created to meet needs and goals.
  - Resulting documents/records
    - Procedure that outlines the development of a training plan

    Related requirements: CLIA: 42 C.F.R. §§ 493.1251, 493.1253, 493.1445; CAP: GEN.55450

Develop training procedures

- ☐ Training requirements, procedures, and/or courses that are followed by all testing personnel are defined and developed.
- ☐ Requirements and procedures for initial training and re-training, as applicable, to assess testing personnel performance levels are determined and developed.
  - Resulting documents/records
    - Procedure that outlines training plan and training of testing personnel
    - Form that captures training of testing personnel

    Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(12); CAP: GEN.55450

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of forms and SOPs that outline training steps related to equipment, bioinformatics, and the capture of completed training.

- Bioinformatics Employee Training SOP
- iSeq 100 Sequencer Employee Training SOP
- MiniSeq Sequencer Employee Training SOP
- MiSeq Sequencer Employee Training SOP
- NextSeq 500/550 Sequencer Employee Training SOP
- Ion PGM™ Sequencer Employee Training SOP

- MinION Sequencer Employee Training SOP
- CLC Genomics Workbench Employee Training Form
- De novo Genome Assembly Employee Training Form
- Operating in Linux Environment Employee Training Form
- Preprocessing Read Quality Control Employee Training Form
- Read Trimming of Next Generation Sequencing Data Employee Training Form
- Short Read Alignment Employee Training Form

Submit training plan

☐ Training plan is then submitted to laboratory leadership or an authorized designee for approval and authorization.

- ○ Resulting documents/records
  - Procedure that outlines the development of a training plan

Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.55450

Post Validation: Train and Authorize: Implement Training Plan

Once the training plan is approved, it should be implemented and followed by all testing personnel for initial training and re-training, as applicable, and in the event of procedural changes. This step is essential for maintenance of training documentation related to the approved method validation plan.

Implement training plan

☐ A successful training plan is implemented and followed by all personnel.

- ○ Resulting documents/records
  - Procedure that outlines the development of a training plan

Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(12); CAP: GEN.55450

Post Validation: Train and Authorize: Training Method

Once the training plan is implemented, it is necessary to train all testing personnel on all mandatory and laboratory-specific test procedures and supporting procedures by an authorized trainer. This ensures that the laboratory possesses staff with the knowledge and technical skills required to perform the test procedure.

Roles and responsibilities

☐ The trainee(s) within the laboratory who will be performing the validated testing procedure is/are identified and documented.

☐ The trainer, a competent individual authorized to perform and train on the test system procedure, is identified and documented.

☐ The authorizer, who is either a member of laboratory leadership or an authorized designee, is responsible for signing off on the performance of specific test systems for everyone's testing personnel form. The authorizer is identified and documented.

- Resulting documents/records
  - Standardized job description and responsibilities
  - Form to capture training of testing personnel

  Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.53625

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the responsibilities sections from the resources below for examples of position descriptions.

- MiSeq Sequencer Trainer Designation Form
- Ion PGM™ Sequencer Trainer Designation Form

TELL: Base knowledge

☐ All necessary documents, SOPs, and work instructions relevant to the test procedure are provided to the trainee by the trainer.

☐ All the laboratory SOPs, work instructions, and other supporting documents relevant to the test procedure are read and understood by the trainee.

- Resulting documents/records
  - Procedure that outlines training of testing personnel
  - Form to capture training of testing personnel

  Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.55450

SHOW: Observation

☐ Trainee observes a demonstration of the test procedure performed by an approved trainer.

- Resulting documents/records
  - Procedure that outlines training of testing personnel
  - Form to capture training of testing personnel

  Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.55450

DO: Performance under supervision

☐ Trainee performs the test procedure independently while being observed by the trainer.

- Resulting documents/records
  - Procedure that outlines training of testing personnel
  - Form to capture training of testing personnel

  Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.55450

APPLY: Post-training assessment

☐ Trainee has successfully performed the post-training assessment.

☐ Trainer has reviewed the trainee's results.

☐ Trainer has compared trainee's results to the expected results to assess the trainee's performance.

- Resulting documents/records
  - Procedure that outlines training of testing personnel

- Form to capture training of testing personnel

Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.55450

Documentation of training

☐ Personnel training is documented using a developed training form and SOP.

- Resulting documents/records
  - Procedure that outlines training requirements and its frequency for testing personnel
  - Signed training form

Related requirements: CLIA: 42 C.F.R. § 493.1445 (e)(13); CAP: GEN.54400, GEN.55500

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of SOPs and training forms related to documentation of training.

- Training documents

Post Validation: Train and Authorize: Authorization

Once training is complete, laboratory leadership or an authorized designee gives approval by signing the authorization form indicating that the testing personnel is competent to perform assigned tasks.

Authorizer signs training form

☐ The laboratory leadership or authorized designee verifies that testing personnel have successfully completed training by reviewing and signing the training form.

- Resulting documents/records
  - Procedure that outlines training of testing personnel
  - Form to capture training of testing personnel

Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: COM.50500

Phase 4 Notes

NOTE: **Personnel may use the references pages for more information on the guidance provided.**

End of Phase 4: Post Validation: Train and Authorize

Phase 5: Test and Maintain

Once the laboratory has established a QMS with processes in place to monitor the accuracy and precision of testing and has begun implementation, it is necessary to continue quality practices that maintain the approved procedure and overall QMS. This ensures that both testing and results reporting remain accurate, reliable, and assist in detecting and correcting errors.

Test and Maintain: Competency Assessment

Competency assessments are used to measure and document laboratory testing personnel's ability to perform testing accurately. There should be defined policies/procedures in place to determine personnel competency. The following sections focus on competency assessments for laboratory testing personnel and ensure that the testing personnel apply their skills, knowledge, and experience to perform the laboratory tasks accurately.

Roles and responsibilities

□ Personnel who are responsible for conducting competency assessments are identified and documented. These personnel should be individuals previously evaluated and deemed competent to perform the task.

NOTE: CLIA requires that annual competency assessment is performed by the Technical Supervisor (TS). The General Supervisor (GS) may also complete the assessment if delegated in writing by the TS or Laboratory Director.

- ○ Resulting documents/records
  - • Standardized job description and responsibilities

Related requirements: CLIA: 42 C.F.R. §§ 493.1235, 493.1451(b)(8); CAP: GEN.53400, GEN.53600, GEN.53625, GEN.55510

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the responsibilities sections from the resources below for examples of position descriptions.

- ▪ MiSeq Sequencer Competency Assessment SOP
- ▪ Ion PGM™ Sequencer Competency Assessment SOP
- ▪ Bioinformatician Competency Assessment SOP

Establish competency assessment for specific procedure or process, as applicable.

□ Assessment process and criteria are defined.

- ○ Resulting documents/records
  - • Procedure that outlines competency assessment (tasks/procedures to be assessed, assessment process, and criteria)

Related requirements: CLIA: 42 C.F.R. §§ 493.1235, CAP: GEN.55499, GEN.55500

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of forms and SOPs related to competency assessment.

- MiSeq Sequencer Competency Assessment SOP
- Ion PGM™ Sequencer Competency Assessment SOP
- MiSeq Sequencer Competency Assessment Form
- Ion PGM™ Sequencer Competency Assessment Form
- Bioinformatician Competency Assessment SOP
- Bioinformatician Competency Assessment Form

Frequency

- □ Schedule for regular and/or periodic assessment of personnel is determined.
- □ Evaluation timeline and frequency for nonwaived testing is determined by the approved laboratory designee.
- □ These evaluations should occur during a testing personnel's first year; competency should be assessed at least semiannually. After an individual performs their duties for one year, competency should be assessed at least once per calendar year.
- □ Timeframe for training and assessment of all personnel is implemented.
  - ○ Resulting documents/records
    - Procedure that outlines competency assessment

Related requirements: CLIA: 42 C.F.R. §§ 493.1235, 493.1451; CAP: GEN.55499, GEN.55500

Verification of competency

- □ Elements of testing personnel competency assessment are documented and verified, including but not limited to:
  - Direct observation of routine patient test performance, including, as applicable, patient identification and test preparation, specimen collection, handling, processing, and testing
  - Monitoring the recording and reporting of test results, including, as applicable, reporting critical results
  - Review of intermediate test results or worksheets, quality control records, proficiency testing results, and preventive maintenance records
  - Direct observation of the performance of instrument equipment maintenance and function checks
  - Assessment of test performance via the testing of previously analyzed specimens, internal blind testing samples, or external proficiency testing samples
  - Evaluation of problem-solving skills
  - ○ Resulting documents/records
    - Procedure that outlines training competency assessment of testing personnel
    - Form to capture verification of testing personnel competency by the competency assessor

Related requirements: CLIA: 42 C.F.R. §§ 493.1445I(13), 493.1451(b)(8); CAP: GEN.55450

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for examples of forms and SOPs related to competency assessment.

- MiSeq Sequencer Competency Assessment SOP
- Ion PGM™ Sequencer Competency Assessment SOP
- MiSeq Sequencer Competency Assessment Form
- Ion PGM™ Sequencer Competency Assessment Form
- Bioinformatician Competency Assessment SOP
- Bioinformatician Competency Assessment Form

Re-training for competency

- □ Trainer performs and documents competency-specific re-training when the performance level of the trainee is unsatisfactory or when there have been changes in the developed procedures.
- □ Trainer then re-verifies and documents the trainee's competency level/level of performance for the given test procedure.
- □ Document remedial action for unsatisfactory performance.
- □ For more information about this process, reference the CMS brochure What Do I Need to Do to Assess Personnel Competency?
  - ○ Resulting documents/records
    - Procedure that outlines training of testing personnel
    - Form to capture additional training of testing personnel

    Related requirements: CLIA: 42 C.F.R. § 493.1445(e)(13); CAP: GEN.55450, GEN.55500

Documentation after competency

- □ Standard competency assessment forms are developed and used for all personnel.
- □ A record of all competency assessments, including the date and results, is created.
  - ○ Resulting documents/records
    - Form to capture competency assessment

    Related requirements: CLIA: 42 C.F.R. §§ 493.1445, 493.1451; CAP: GEN.55499, GEN.55500

Test and Maintain: Proficiency Testing

The accuracy and reliability of laboratory test systems are evaluated using Proficiency Testing (PT) or Alternative Assessments (AA). The laboratory should participate in an appropriate PT program and/or establish an AA procedure when PT is unavailable.

NOTE: CLIA requires that laboratories enroll in a PT program for their test or perform an AA when PT is not available. For more information on proficiency testing, please reference CLIA Requirements for Proficiency Testing and PT Referral.

Roles and responsibilities

☐ Testing personnel responsible for carrying out PT/AA are identified and documented.

☐ Laboratory leadership or authorized designee is responsible for reviewing and approving PT/AA reports.

- Resulting documents/records
  - Standardized job description and responsibilities

Related requirements: CLIA: 42 C.F.R. §§ 493.1445, 493.1445(4); CAP: GEN.53400, GEN.53625

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the responsibilities section from the resource below for examples of position descriptions.

- Proficiency Testing and Alternative Assessment SOP

Establish a PT/AA plan

☐ A procedure describing the laboratory's process to perform PT/AA is developed.

☐ AA procedures are used for all other test systems or when a PT program is inadequate to cover testing performed for a regulated analyte.

- Resulting documents/records
  - Procedure that outlines PT/AA plan

Related requirements: CLIA: 42 C.F.R. §§ 493.801(b), 493.1236; CAP: COM.01000, COM.01300

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for an example procedure related to PT/AA.

- Proficiency Testing and Alternative Assessment SOP

Frequency

☐ A frequency for evaluating each test system is established. These evaluations should occur periodically and include low-volume testing for accuracy and reliability using either external PT or AA procedures.

- Resulting documents/records
  - Procedure that outlines PT/AA plan

Related requirements: CLIA: 42 C.F.R. §§ 493.801(b), 493.911-959, 493.1236; CAP: COM.01500

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for an example procedure related to PT/AA.

- Proficiency Testing and Alternative Assessment SOP

Documentation

☐ A record of all proficiency testing, which includes the date and results, is available.

- ☐ The test system being evaluated is clearly indicated on all documentation.
- ☐ Handling, preparation, processing, testing, and reporting of PT/AA (every stage of the PT process, from sample arrival to receipt of the evaluation report) are all documented.
- ☐ A summary of the PT/AA result evaluation is documented.
- ☐ Attestation statement signed by the Laboratory Director or authorized designee and by all Testing Personnel who participated in the PT/AA sample testing is documented.
- ☐ A documentation retention plan for all PT/AA testing and results is available.
  - ○ Resulting documents/records
    - Form to capture proficiency testing

    Related requirements: CLIA: 42 C.F.R. §§ 493.801(b), 493.911-959; CAP: COM.01000, COM.01400, COM.01700

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for an example procedure related to PT/AA.

- Proficiency Testing and Alternative Assessment SOP

Reporting PT/AA results

- ☐ A routine laboratory procedure for submitting test results (with worksheets and equipment printouts) is followed by testing personnel.
- ☐ Test results are reviewed and approved by laboratory leadership (Laboratory Director, Technical Supervisor) or authorized designee.
- ☐ Once results are approved, laboratory procedures for PT/AA result reporting are followed.
  - ○ Resulting documents/records
    - Completed PT/AA result report

    Related requirements: CLIA: 42 C.F.R. §§ 493.801, 493.8–3 - 493.865; CAP: COM.01700

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for an example procedure related to PT/AA.

- Proficiency Testing and Alternative Assessment SOP

Failures and corrective actions

- ☐ Depending on the significance of the failure, a corrective action plan may be required. Failed PT/AA results are documented in a nonconforming event (NCE) report.
  - ○ Resulting documents/records
    - Form to capture corrective action

    Related requirements: CLIA: 42 C.F.R. §§ 493.803, 493.8–3 - 865, 493.1445; CAP: COM.01700, COM.01950

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for example procedures related to PT/AA.

- Proficiency Testing and Alternative Assessment SOP
- Identifying and Documenting Nonconforming Events

- Root Cause Analysis Procedure
- Corrective Action Prevention Procedure

Test and Maintain: Sample Management

The laboratory should establish a QMS with processes in place to monitor and ensure the accuracy and reliability of testing being performed on samples, as well as to detect and correct errors. This might include passing quality controls when samples and/or data are shared between/among laboratories.

Roles and responsibilities

- □ The role of laboratory personnel in sample management is identified and documented.
- □ A sample management procedure is developed.
- □ Training should accompany the sample management procedure to ensure that all laboratory staff know how samples should be managed within the laboratory.

  Related requirements: CLIA: 42 C.F.R. §§ 493.1445, 493.1451, 493.1495

Sample storage and retention

- □ Samples are stored to protect against cross-contamination, deterioration, loss, or damage.
- □ Samples are transferred to appropriate long-term storage according to laboratory retention requirements. The laboratory describes this process, as applicable.
- □ Sample storage locations are recorded.
- □ Storage equipment and/or space is/are monitored to verify and record compliance with individualized requirements (e.g., sample and reagent storage temperature requirements).
- □ Samples are retained according to laboratory retention requirements. Project-specific retention requirements are recorded (e.g., study- or outbreak-specific) in the project documentation. Laboratory to edit as appropriate.
  - Resulting documents/records
    - Procedure that outlines sample management processes

  Related requirements: CLIA: 42 C.F.R. §§ 493.1242, 493.1105, 493.1252; CAP: GEN.20377

Test and Maintain: Quality Control

The laboratory should maintain a QMS with processes in place to monitor the accuracy and precision of the laboratory methods. The laboratory may consider an Individualized Quality Control Plan (IQCP) as part of its quality control processes. Within its IQCP, the laboratory can incorporate guidelines for reagent, run, and data analysis QC.

NOTE: For more IQCP-related information, please reference Individualized Quality Control Plan (IQCP) on the Laboratory Quality Tools and Resources Webpage.

Process control

- □ Process control of activities is employed across the laboratory, including control of sample handling and examination processes to ensure accurate and reliable testing.

- □ Established criteria for acceptability of samples, controls, frequency of control testing, and results are developed and documented.
- □ Establish the steps to be taken in the event of a QC failure.
  - Resulting documents/records
    - Procedure that outlines process control
    - Procedure to monitor control materials' performance over time

    Related requirements: CLIA: 42 C.F.R. § 493.1256, 493.1445(e)(5)

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for guidance related to laboratory processes, organization, and quality control.

- Identifying and Monitoring NGS Key Performance Indicators SOP
- Key Performance Indicator Development Form
- NGS QC Guidance for Illumina Workflows
- NGS QC Guidance for MinION 1D Workflows
- NGS QC Guidance for MinION Rapid Sequencing Workflows

Test and Maintain: Quality Assurance

The laboratory should have a QMS with processes in place to monitor accuracy and precision within every step of the laboratory process. This system should be designed to assure the accuracy and reliability of the testing being performed and to detect and correct errors.

Procedures

- □ SOPs for each step of the laboratory testing process, ranging from specimen handling to QC for data input, as well as equipment performance validation to quality control of bioinformatic pipeline outputs, are established.
- □ Reproducibility studies and correlation testing are performed and documented for duplicate equipment for specific test methods.
  - Resulting documents/records
    - Procedure that outlines quality assurance measures and troubleshooting steps

    Related requirements: CLIA: 42 C.F.R. §§ 493.1200(b), 493.1239, 493.1249, 493.1289, 493.1299; CAP: GEN.13806

Quality management

- □ Administrative requirements, such as mandatory recordkeeping, data evaluation, and internal audits to monitor adherence to SOPs, are defined and documented.
  - Resulting documents/records
    - Procedure that outlines administrative requirements

    Related requirements: CLIA: 42 C.F.R. § 493.1200; CAP: GEN.13806

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for guidance related to quality management.

- QMS Assessment Tool

Corrective action

☐ Corrective actions, documentation, and the persons responsible for performing corrective action procedures are identified.
- ○ Resulting documents/records
  - Procedure that outlines corrective actions

Related requirements: CLIA: 42 C.F.R. § 493.1282; CAP: GEN.20310

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for example procedures related to corrective action.

- Identifying and Documenting Nonconforming Events
- Root Cause Analysis Procedure
- Corrective Action Prevention Procedure
- Nonconforming Event Report Closeout and Trending

Data and Reporting

NOTE: CLIA requires that patient data be accessed only by authorized individuals.

☐ Considerations for how information from the validation is shared with external partners are defined and documented.

☐ Considerations for transmission and reporting of test results (e.g., Electronic Library Information Management System, STARLIMS, patient, provider) are defined and documented.

☐ Considerations for data retention are defined and documented.

☐ Mechanisms for data utilization and exchange are evaluated and documented.

☐ Mechanisms for links to other databases are evaluated and documented.
- ○ Resulting documents/records
  - Procedure that outlines information exchange and data reporting

Related requirements: CLIA: 42 CFR 512.275

Test and Maintain: Document and Record Management

The laboratory should establish a QMS with procedures, methods, and records to monitor laboratory processes, accuracy, and precision. These documents should be developed, approved, and controlled by qualified, designated personnel. This ensures the accuracy and reliability of the testing being performed and helps detect and correct errors.

Document and record control

☐ The process for document control is developed, documented, and approved. This should include instructions for developing, reviewing, approving, and revising all the laboratory procedures and methods.

☐ The process for record control is developed, documented, and approved. Record control should include results from audit reports, management reviews, observations, calculations, test reports, and any other laboratory records in the form of operating logs.

- Resulting documents/records
  - Procedure that outlines document and record control processes
  - Form to capture document and record control process

Related requirements: CLIA: 42 C.F.R. §§ 493.1251(e), 493.1105, 493.1283; CAP: GEN.20375

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for processes related to document and record management.

- Documents and Records Management

Document and record retention

☐ The process for retaining documents and records within the laboratory is developed and approved. Key components to record during the execution of this process include:

- Equipment (e.g., IQ/OQ/PQ, maintenance [user-performed, manufacturer-/service tech-performed, repair/service records, calibrations, etc.])
- Test procedures (e.g., validation; procedures [nucleic acid extraction and purification SOPs and documents, library prep and quantification SOPs and documents, sequencer-specific and corresponding reagents SOPs, worksheets or documents that include quantity of kits, lot #, expiration dates, etc.]; proficiency testing)
- Testing records (e.g., test requisitions, testing worksheets/run logs, control results, test reports [copy, final, preliminary, and corrected reports])
- Quality systems (e.g., NCEs, compliance records, management reviews, turnaround time tracking)
- Personnel records (e.g., qualifications, initial training, six-month competency, annual competency)
- Reagents, media, and supply records (e.g., quality control)
- Laboratory computer services
- Data analysis (verification) (e.g., version of reference database, updates to bioinformatic pipeline [e.g., software versions, pipeline versions], pipeline changes to ensure traceability and reproducibility)
- Data (e.g., raw reads, FASTQ files, integrity files)
- Computer system validation records
- Test library (e.g., SNP detection tool, external reference databases)
- Major functions of laboratory information systems, ongoing computer system checks

- Resulting documents/records
  - Procedure that outlines the process for document and record retention within the laboratory

Related requirements: CLIA: 42 C.F.R. §§ 493.1251(d), 493.1105; CAP: GEN.20377

Available products on NGS QI Tools and Resources Webpage:

- Documents and Records Management

Document, database, and record maintenance

- □ Document and record management procedure is integrated into the laboratory QMS.
- □ Bi-annual database review to check for new species discovered and novel resistance genes.
- □ Records are comprised of anything that contains information about and evidence of laboratory activities.
  - Documents and records are easily accessible when needed and properly disposed of when the retention period is over according to the established records management procedure.
  - Resulting documents/records
    - Procedure that outlines record maintenance

Related requirements: CLIA: 42 C.F.R. § 493.1105; CAP: GEN.20375, GEN.20377

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for processes related to records management.

- Documents and Records Management

Record medium

- □ The medium for the official records is determined. Records can exist in many different media types. Sometimes, the original medium of a record may not be the best medium for long-term records retention. In some cases, a record may exist in multiple forms, such as electronic and hard copy.
  - Resulting documents/records
    - Procedure that outlines record medium/media

Related requirements: CLIA: 42 C.F.R. § 493.1105

Auditing records

- □ Internal audits are conducted regularly and documented as a part of the laboratory's internal audit program. Internal record audits vary based on the type of record and how/where the record is stored.
- □ An audit of laboratory workspace records is conducted to ensure that only the required records are retained, and unneeded records are properly disposed of according to the laboratory's established records management procedure.
  - Resulting documents/records
    - Procedure that outlines auditing records

Related requirements: CAP: GEN.23584

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for processes related to records management.

- Documents and Records Management

Test and Maintain: Equipment Maintenance

The laboratory should ensure that the appropriate equipment is installed and that personnel are properly trained to use it. Equipment should be calibrated and/or have preventive maintenance performed as indicated by the manufacturer or per laboratory policy (e.g., twice annually). Equipment manuals should be available in the laboratory area for easy reference. An equipment inventory and maintenance log should be maintained and stored, including maintenance, service, movement, and repair records.

Standard maintenance

- □ Maintenance and function checks have been performed as defined by the manufacturer and with the frequency specified by the manufacturer for all unmodified, FDA-cleared test systems.
- □ For modified test systems, methods developed in-house, or methods without a protocol provided by the manufacturer, a maintenance and function check protocol has been established and performed.
- □ All equipment maintenance activities have been documented.
  - Resulting documents/records
    - Forms to capture standard equipment maintenance

    Related requirements: CLIA: 42 C.F.R. § 493.1254(a)(1); CAP: COM.30600

Calibration verification

- □ The calibration of a test system should be verified by following manufacturer recommendations, guidelines, and applicable regulations (e.g., every 6 months per CLIA). Laboratories should consider performing a recalibration when a procedure's reagents change completely, after performing a major preventive maintenance or replacing critical parts that could affect test performance, when control materials indicate an unusual deviation or fall outside acceptable limits, and/or if a laboratory's established schedule for verifying the reportable range for patient test results requires more frequent calibration verification.
- □ Documentation of calibration is retained for two years. If calibration verification results are unacceptable, the test system's calibration procedure should be repeated. If there are still issues after recalibration, the laboratory should take corrective action(s) and document this activity.
  - Resulting documents/records
    - Forms to capture calibration verification

Related requirements: CLIA: 42 C.F.R. §§ 493.1251(b)(5), 493.1255; CAP: COM.30675, COM.50500

Test and Maintain: Internal Assessments

The laboratory should utilize assessments as a tool for examining laboratory performance. Internal assessment is a critical aspect of laboratory quality management, modeling defined criteria for how external assessors investigate the laboratory. The assessor usually leads this type of assessment. These assessments should include documents and records involved in and resulting from laboratory testing.

Procedure

- □ Procedure for internal assessments is established.
- □ Foundation for robust assessments of the laboratory utilizing internal resources is detailed in the procedure.
- □ Roles necessary to complete regular assessments and provide a report are defined and documented.
  - ○ Resulting documents/records
    - Procedure that outlines the process for internal assessments

    Related requirements: CAP: GEN.23584

Roles and responsibilities

- □ The Lead Assessor, a qualified, designated individual usually responsible for directing internal assessment activities and providing consultation, is identified.
- □ Additional individuals who will serve as assessors are also identified.
  - ○ Resulting documents/records
    - Standardized job description and responsibilities

    Related requirements: CAP: GEN.23584

Planning and initiating internal assessments

- □ Timeframe for starting and completing the internal assessment is determined.
- □ Schedule of dates during which the assessors will be performing internal assessments is developed and documented.
- □ Assessments are scheduled, ensuring adequate time to conduct a thorough assessment.
- □ Assessment teams are assigned.
- □ Lead Assessor is designated to be responsible for coordinating assessment activities.
  - ○ Resulting documents/records
    - Procedure that outlines the process for internal assessments
    - Form to capture observations of laboratory activities

    Related requirements: CAP: GEN.23584

Scheduling and preparation

- □ Lead Assessor sends e-mail invitation ahead of scheduled internal assessment to confirm dates and provide information necessary to prepare for the assessment, including:
  - Date and time for the opening and closing meetings
  - Timeframe planned for the duration of the assessment
  - Documents and records to be available for review
  - Personnel required to be available
  - Estimates of time commitment
  - Plan for assessors to tour laboratory space
  - ○ Resulting documents/records
  - Procedure that outlines the process for internal assessments

  Related requirements: CAP: GEN.23584

Performing the assessment

- □ Opening meeting is conducted during the beginning of the assessment timeframe.
- □ Closing meeting is conducted during the end of the assessment timeframe.
- □ Meetings identify and include leadership and laboratory points of contact before the assessment.
- □ Assessors review laboratory policies, processes, and procedures.
- □ Assessors tour the laboratory to observe safety practices, equipment, and current logs/forms.
- □ Any observed noncompliance is documented.
  - ○ Resulting documents/records
  - Procedure that outlines the process for internal assessments
  - Form to capture observations of laboratory activities

  Related requirements: CAP: COM.50600, GEN.23584

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below to perform an internal assessment and determine if quality requirements are met.

- ▪ QMS Assessment Tool

Reports and corrective action

- □ Corrective action procedures and policies are made available to the laboratory for all test processes and procedures.
- □ Necessary personnel complete and submit a corrective action plan for each finding of noncompliance and observed risk reported within an acceptable period of receiving the assessment report.

- ☐ Assessors provide the laboratory with a report within an acceptable length of time following the closing meeting; the report should include detailed documentation related to findings of noncompliance as well as observed risks that require corrective actions.
- ☐ Each assessor sends their observations of findings and observed risks to the Lead Assessor.
- ☐ Lead Assessor reviews all observations and consults with assessors if needed.
- ☐ Consensus agreement is made by members of the assessment team.
- ☐ Lead Assessor creates the assessment report draft (.doc) and sends it to the internal assessment team for preliminary review.
- ☐ Draft report is finalized.
- ☐ Report is sent to the Laboratory Director.
- ☐ Laboratory Director reviews and consults with internal assessment team and Lead Assessor, if needed, regarding questions and then provides any revisions required.
- ☐ Lead Assessor makes required revisions and creates a final report (.pdf).
- ☐ Lead Assessor signs the final report.
- ☐ Lead Assessor returns the final report to Laboratory Director.
- ☐ Laboratory Director signs the final report and sends it to the necessary personnel.
    - ○ Resulting documents/records
        - Finalized internal assessment report
        - Form to capture responses to the findings of the internal assessment

    Related requirements: CLIA: 21 CFR 117.150; CAP: GEN.23584

Test and Maintain: Nonconforming Event Management

The laboratory should establish a procedure and process for identifying, documenting, and investigating NCEs. The laboratory should also have a procedure for developing corrective action plans. The corrective action should be the resulting action that is taken to eliminate the cause(s) of a detected nonconformance or other quality issue.

Procedure

- ☐ Procedure for identifying, documenting, reviewing, and closing NCEs is developed and documented.
- ☐ Procedure outlines how NCEs are tracked and trended over time.
- ☐ Procedure for issuing corrective reports is established and documented.
    - ○ Resulting documents/records
        - Procedure that outlines the process for NCEs

    Related requirements: CLIA: 42 C.F.R. §§ 493.1239, 493.1249, 493.1289, 493.1299, 493.1282; CAP: GEN.23584, GEN.30000, COM.04000, GEN.20318

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for procedures related to NCEs.

- Identifying and Documenting Nonconforming Events

- Nonconforming Event Report Closeout and Trending

Roles and responsibilities

- □ Staff are equipped to identify and document NCEs by the established NCE procedure. In some cases, taking remedial (immediate) action may be all that is necessary to address an NCE. A report should still be written.
- □ Laboratory leadership or an authorized designee who is responsible for reviewing and closing NCEs according to the established procedure is identified and documented.
  - Resulting documents/records
    - Standardized job description and responsibilities

    Related requirements: CLIA: 42 C.F.R. § 493.1282; CAP: GEN.20208, GEN.30000

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the responsibilities section from the resources below for examples of position descriptions.

- Identifying and Documenting Nonconforming Events
- Root Cause Analysis Procedure
- Nonconforming Event Report Closeout and Trending

Identification

- □ Remedial (immediate) action is taken once an NCE is identified.
- □ Steps are taken to ensure the safety and preservation of equipment and materials.
- □ If needed, testing and reporting have been discontinued until the issue has been resolved.
- □ If test results were released, it is determined whether the results need to be recalled or the person receiving the results needs to be notified; the procedure for issuing corrected reports is followed.
- □ Anyone affected by the event and their supervisor are notified, as needed.
  - Resulting documents/records
    - Procedure that outlines the process for NCEs

    Related requirements: CLIA: 42 C.F.R. § 493.1282; CAP: GEN.20208, GEN.20310

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for a procedure related to NCEs.

- Identifying and Documenting Nonconforming Events

Investigation

- □ Form or another medium for documenting NCEs is developed.
- □ Personnel identifying an NCE properly document the occurrence according to the laboratory's procedure.
- □ At minimum, the following information has been captured:
  - Name of NCE initiator and date initiated
  - Date and time the NCE occurred

- Date and time the NCE was discovered
- Affected area/assay, specimen number, equipment type/ID, lot numbers
- Indication of whether the NCE is a result of a complaint and inclusion of necessary information
- Description of the occurrence in detail, including how it was identified
- If applicable, reference to the procedure or standard that was deviated from
- Notation of what was expected as well as what occurred
- Documentation of immediate actions taken to remediate the initial impact of the NCE. This may include troubleshooting, verifying that controls were in range, identifying test results that were potentially affected, and/or product containment and segregation.
- Description of possible causes of the NCE. There is often more than one underlying cause of an NCE.

□ An informal investigation to identify possible causes is conducted.

□ Any impact on patient samples, reagents/supplies/materials, test results, reports, etc., is evaluated.

□ Actions taken are explicitly stated.

○ Resulting documents/records

- Document that outlines the NCE

Related requirements: CLIA: 42 C.F.R. § 493.1282; CAP: COM.50600, GEN.20208, GEN.20310, GEN.20318

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for procedures related to NCEs.

- Identifying and Documenting Nonconforming Events
- Nonconforming Event Types and Common Root Causes
- Root Cause Analysis Procedure
- Root Cause Analysis Job Aid

Corrective action and preventive action (CAPA)

□ CAPA plan to address either a systemic, complex, or cross-functional quality issue is developed and documented.

□ Process to resolve severe NCEs requiring management oversight is established and documented.

□ Records containing periodic tracking and trending of NCEs or other quality system metrics indicating a negative trend, potential future issue, or opportunity for improvement are established and documented.

○ Resulting documents/records

- Form to capture CAPA
- Procedure that outlines steps to follow in the event of a CAPA

Related requirements: CLIA: 42 C.F.R. § 493.1282; CAP: COM.50600, GEN.20318, GEN.20325, GEN.23584, GEN.40492

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resource below for an example procedure related to CAPA.

- Corrective Action Prevention Procedure

Test and Maintain: Continuous Improvement

The laboratory QMS should be designed with the primary goal of continuous improvement in a systematic manner. Implementing multiple layers of quality processes that identify errors improves the quality and efficiency of the laboratory.

Process improvement

- □ Potential sources of any system weakness or error are identified.
- □ Process improvement plans are developed and implemented in the laboratory.
- □ Procedure for continuous review of the process improvement plan is established.
  - ○ Resulting documents/records
    - Procedure that outlines process improvement plan

    Related requirements: CLIA: 42 C.F.R. §§ 493.1239, 493.1249, 493.1289, 493.1299; CAP: COM.50400, COM.50600

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for guidance related to continual improvement.

- Identifying and Monitoring NGS Key Performance Indicators SOP
- Key Performance Indicator Development Form

Management review

- □ Internal assessments are performed regularly as part of the laboratory's internal assessment program.
- □ All the information gathered through internal assessment and other process improvement activities is documented and reviewed.
- □ Management review of laboratory records (e.g., quality control, inventory management, and equipment maintenance) is performed regularly to provide information about areas for improvement.
- □ The QMS, in accordance with the review and assessment results, is modified.
  - ○ Resulting documents/records
    - Forms to capture internal assessment records
    - Procedure that outlines the laboratory's internal assessment and review process

    Related requirements: CLIA: 42 C.F.R. §§ 493.1239, 493.1249, 493.1289, 493.1299; CAP: COM.04000, GEN.23584

Customer focus

- ☐ Needs of laboratory's internal and external customers are identified.
- ☐ Capability to meet customer expectations is established.
- ☐ Customer needs are identified regularly.
- ☐ Customer complaints are recorded, managed, and addressed regularly.
- ☐ Customer feedback is captured, and changes are implemented accordingly.
    - Resulting documents/records
        - Forms to capture customer needs
        - Forms to capture customer complaints

        Related requirements: CLIA: 42 C.F.R. § 493.1233; CAP: GEN.20316, GEN.20335

Available products on NGS QI Tools and Resources Webpage:

Personnel may use the resources below for guidance related to customer focus.

- NGS Result Report Interpretation Guide
- NGS Result Report Template
- NGS Training Resource Repository

Phase 5 Notes

NOTE: **Personnel may use the references pages for more information on the guidance provided.**

End of Phase 5: Test and Maintain

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