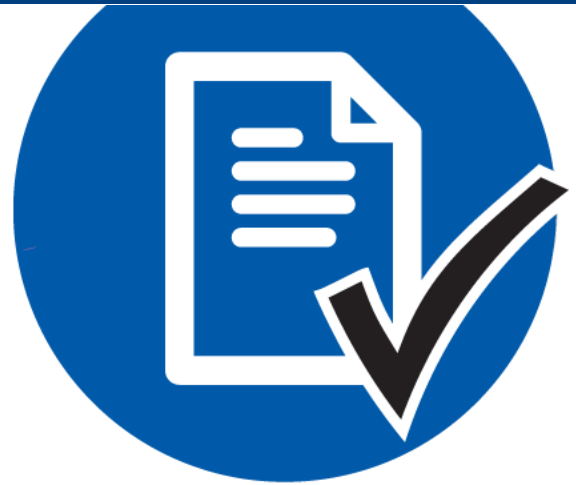


Molecular Pathology Checklist

CAP Accreditation Program



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ON-LINE CHECKLIST AVAILABILITY AND RESOURCES

Participants of the CAP accreditation programs may download the checklists from the CAP website (cap.org) by logging into e-LAB Solutions Suite. They are available in different checklist types and formatting options, including:

- Master — contains ALL of the requirements and instructions available in PDF, Word/XML or Excel formats
- Custom — customized based on the laboratory's activity (test) menu; available in PDF, Word/XML or Excel formats
- Changes Only — contains only those requirements with significant changes since the previous checklist edition in a track changes format to show the differences; in PDF version only. Requirements that have been moved or merged appear in a table at the end of the file.

A repository of questions and answers and other resources is also available in e-LAB Solutions Suite under Accreditation Resources, Checklist Requirement Q & A.

SUMMARY OF CHECKLIST EDITION CHANGES

Molecular Pathology Checklist

06/04/2020 Edition

The information below includes a listing of checklist requirements with significant changes in the current edition and previous edition of this checklist. The list is separated into three categories:

1. New
2. Revised:
 - Modifications that may require a change in policy, procedure, or process for continued compliance; or
 - A change to the Phase
3. Deleted/Moved/Merged:
 - Deleted
 - Moved — Relocation of a requirement into a different checklist (requirements that have been resequenced within the same checklist are not listed)
 - Merged — The combining of similar requirements

NOTE: The requirements listed below are from the Master version of the checklist. The customized checklist version created for on-site inspections and self-evaluations may not list all of these requirements.

NEW Checklist Requirements

<u>Requirement</u>	<u>Effective Date</u>
MOL.35400	09/17/2019
MOL.35420	09/17/2019
MOL.35430	09/17/2019
MOL.35440	09/17/2019
MOL.35450	09/17/2019
MOL.36117	09/17/2019
MOL.39295	09/17/2019
MOL.39315	09/17/2019
MOL.39365	09/17/2019
MOL.39410	09/17/2019
MOL.39420	09/17/2019
MOL.39440	09/17/2019
MOL.39480	09/17/2019
MOL.39490	09/17/2019

REVISED Checklist Requirements

<u>Requirement</u>	<u>Effective Date</u>
MOL.20300	09/17/2019
MOL.30785	06/04/2020
MOL.31015	09/17/2019
MOL.31130	06/04/2020
MOL.31255	09/17/2019
MOL.32395	06/04/2020
MOL.32430	09/17/2019
MOL.33655	09/17/2019
MOL.33696	09/17/2019
MOL.33860	09/17/2019
MOL.33942	09/17/2019
MOL.33983	09/17/2019
MOL.34311	09/17/2019
MOL.35785	09/17/2019
MOL.35840	09/17/2019
MOL.35850	09/17/2019
MOL.35865	06/04/2020
MOL.35870	09/17/2019
MOL.36010	09/17/2019
MOL.36015	06/04/2020
MOL.36035	09/17/2019
MOL.36105	09/17/2019
MOL.36115	06/04/2020
MOL.36118	06/04/2020
MOL.36135	09/17/2019
MOL.36145	09/17/2019
MOL.36155	09/17/2019
MOL.36310	09/17/2019
MOL.36320	09/17/2019
MOL.36330	09/17/2019
MOL.36380	09/17/2019
MOL.36390	09/17/2019
MOL.36400	09/17/2019
MOL.36410	09/17/2019
MOL.36430	09/17/2019
MOL.36450	09/17/2019
MOL.38600	09/17/2019
MOL.39155	06/04/2020
MOL.39323	09/17/2019
MOL.39358	09/17/2019
MOL.39470	06/04/2020
MOL.49575	06/04/2020
MOL.49590	09/17/2019
MOL.49640	09/17/2019

DELETED/MOVED/MERGED Checklist Requirements

<u>Requirement</u>	<u>Effective Date</u>
MOL.31145	09/16/2019
MOL.31245	09/16/2019
MOL.31360	09/16/2019
MOL.31375	09/16/2019
MOL.32435	09/16/2019

MOL.35722	09/16/2019
MOL.35766	09/16/2019
MOL.39393	09/16/2019
MOL.48588	09/16/2019

INTRODUCTION

This checklist is used in conjunction with the All Common and Laboratory General Checklists to inspect a molecular pathology laboratory section or department.

Molecular pathology inspectors must be actively practicing molecular scientists familiar with the checklist and possessing the technical and interpretive skills necessary to evaluate the quality of a laboratory's performance. If the team leader's laboratory performs similar molecular pathology services as the inspected lab, the inspecting laboratory's molecular pathology section director or section supervisor is a qualified inspector. If the team leader has no such resource, the list of qualified regional inspectors included in the Inspector's Inspection Packet should be consulted.

Laboratories not subject to US regulations: *Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist. When the phrase "FDA-cleared/approved test (or assay)" is used within the checklist, it also applies to tests approved by an internationally recognized regulatory authority (eg, CE-marking).*

APPLICABILITY


The Molecular Pathology Checklist covers clinical molecular testing in the areas of oncology, hematology, inherited disease, HLA typing, forensics and parentage applications. The inspection of laboratories performing such molecular testing requires the Molecular Pathology Checklist, except that the Cytogenetics or Anatomic Pathology Checklist (as appropriate) may instead be used to inspect in situ hybridization (ISH), when such testing is performed in the cytogenetics, cytopathology or anatomic pathology section.

With the exception of next generation sequencing, the Microbiology Checklist is used alone to inspect laboratories that perform molecular testing for infectious disease testing.

QUALITY MANAGEMENT AND QUALITY CONTROL

GENERAL ISSUES

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of turnaround time records
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****REVISED** 09/17/2019**

MOL.20300 Turnaround Time

Phase I

The laboratory has defined sample turnaround times that are appropriate for the intended purpose of the test and performs ongoing monitoring for compliance.

NOTE: Appropriate turnaround times will vary by test type and clinical application. There are certain clinical situations in which rapid completion is essential. For example, inappropriate

delays in completing a prenatal diagnosis test can cause unacceptable emotional stress for the parents, make ultimate pregnancy termination (if chosen) much more difficult, or even render the results of the test unusable.

Evidence of Compliance:

- ✓ Written procedure defining turnaround time and mechanism for monitoring **AND**
- ✓ Records showing that defined turnaround times are routinely met

REFERENCES

- 1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2018 edition, Revised 01/2018.
- 2) Cooley LD, Lebo M, Li MM, Slovak ML, Wolff DJ, Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders. *Genet Med.* 2013; 15(6):484-94.

MOL.20550 Test Result Statistics

Phase I

When appropriate, statistics on molecular pathology test results (eg, percentages of normal and abnormal findings) are maintained, and appropriate comparative studies performed.

NOTE: Periodic review of test result statistics can be used to identify changes in test performance. This process may detect systemic errors.

Evidence of Compliance:

- ✓ Written procedure for calculating statistics **AND**
- ✓ Records of statistical data, evaluation and corrective action if indicated

PROCEDURE MANUAL

Inspector Instructions:



- Representative sample of policies and procedures for completeness. Current practice must match policies and procedures.

MOL.30440 Calculations for Quantitative Tests

Phase II

For quantitative molecular tests, methods for calculating quantitative values are adequately described, and units clearly stated, in the procedure manual.

NOTE: Quantitative testing requires that the dynamic range of the assay be defined and assay performance tested with controls in each run, including a negative, low positive, and high positive control.

MOL.30555 Analytic Interpretation Guidelines

Phase II

There are written guidelines for analytic interpretation of results.

NOTE: For a qualitative assay, the procedure manual should describe, for example, the expected band pattern, melting temperature, or numeric cutoff to distinguish a positive from a negative result. For a quantitative assay, the manual should describe, for example, the criteria for verifying test performance characteristics of the run (eg, assay sensitivity and linearity are within pre-

established range, there is no significant inhibitor of the patient reaction, the calculated value appears reasonable from visual inspection of raw data) prior to releasing the quantitative result.

ASSAY VALIDATION - MODIFIED FDA-CLEARED/ APPROVED TESTS AND LABORATORY-DEVELOPED TESTS

Validation of a laboratory test requires identifying the intended use of the test and establishing demonstrated evidence that provides a high degree of assurance that a test will consistently perform as expected.



This section applies to:

- *Laboratory-developed tests (LDTs)*
- *FDA-cleared/approved tests that have been modified by the laboratory*
- *For laboratories not subject to US regulations, tests approved by an internationally recognized regulatory authority (eg, CE-marking) that have been modified by the laboratory.*

These requirements are used in conjunction with the All Common Checklist requirements in the Test Method Validation and Verification - Nonwaived Tests section for inspection (eg, COM.40830, COM.40850). For unmodified, non-LDTs, refer to the Test Method Validation and Verification - Nonwaived Tests section of the All Common Checklist. Additional requirements for validation in other sections of the Molecular Pathology Checklist, such as in the Next Generation Sequencing sections, also apply based on the methods used.

If a test is modified to meet the needs of the user or is developed by the laboratory (LDT), both analytical and clinical performance parameters need to be established. Analytical performance parameters include accuracy, precision, reportable range, and reference interval, as well as analytical sensitivity, analytical specificity, and any other parameter that is considered important to assure the analytical performance of a particular test (eg, specimen stability, reagent stability, linearity, carryover, cross-contamination, as appropriate and applicable). The clinical validity, which includes clinical performance characteristics, such as clinical sensitivity, clinical specificity, positive and negative predictive values in defined populations or likelihood ratios, and clinical utility should also be considered, although individual laboratories may not be able to assess these parameters within their own patient population, especially for rare diseases. However, patients without disease can typically be tested to assess clinical specificity. If clinical validity cannot be established within a laboratory, it is appropriate to cite scientific literature that established clinical sensitivity and specificity. Clinical performance characteristics should be determined relative to clinical data (eg, biopsy findings, radiographic and clinical findings, other laboratory results) whenever possible.

Inspector Instructions:

	<ul style="list-style-type: none"> • Policies and procedures for the introduction of new tests, methods, or instruments • Sampling of assay validation studies, including comparisons and appropriate sample types • Sampling of assay validation studies with emphasis on tests introduced in the past two years
	<ul style="list-style-type: none"> • Which laboratory tests or instruments have been implemented in the past two years? • How does your laboratory validate assay performance prior to test implementation? • How does your laboratory verify or establish reference intervals? • How does your laboratory validate clinical claims made by the laboratory about LDTs?

DISCOVER

- Select at least one validation study performed for each type of instrument or method introduced during the past two years.
- In addition, select assays for evaluation if recurrent problems have been identified in proficiency testing results, quality control, competency assessment, or physician complaints regardless of how long the assay has been in place.
- Review validation records to confirm that appropriate studies were performed using an adequate number of cases, and a written assessment of the data was performed. If the data showed discordances or unacceptable variations, investigate how they were resolved. If a study was not performed or is missing required components, cite the appropriate related requirement(s) (eg, MOL.31130).
- Confirm that the written assessment of each component (accuracy, precision, etc.) of the validation studies has been approved by the laboratory director (or qualified designee) prior to the initiation of clinical testing. If the study assessment was not signed by the laboratory director or designee, cite MOL.30785.
- Review examples of patient reports for laboratory-developed tests and modified FDA-cleared/approved tests to identify clinical claims being made by the laboratory for the testing. For laboratories not subject to US regulations, review would include tests approved by an internationally recognized regulatory authority modified by the laboratory. Confirm that studies were performed.

****REVISED** 06/04/2020**

MOL.30785 Validation Study Approval

Phase II

Prior to clinical use of each modified FDA-cleared/approved test or laboratory developed test (LDT), the laboratory director, or designee meeting CAP director qualifications, has signed the laboratory's written assessment of the validation study addressing analytical and clinical performance parameters to confirm the acceptance of the study data and written assessment, and to approve each test for clinical use.

NOTE 1: For laboratories not subject to US regulations, this requirement also applies to tests approved by an internationally recognized regulatory authority that have been modified.

NOTE 2: The approval must include: 1) review of the written assessment of the validation study, including the acceptability of the data and investigation of discordant results; 2) signed approval statement, such as, "I have reviewed the validation data for accuracy, precision, reportable range, analytical sensitivity (LOD), analytical specificity, reference interval, and clinical performance parameter studies (insert other components, as required) for the (insert instrument/test name), and the performance of the method is considered acceptable for patient testing."

The validation study must address accuracy, precision, reportable range, reference interval, analytical sensitivity (LOD), analytical specificity, and any other parameter that is considered important to validate the analytical performance of a test (eg, specimen stability, reagent stability, linearity, carryover, and cross-contamination, etc.), as appropriate and applicable (MOL.31130, MOL.31255). Clinical performance characteristics must also be addressed (MOL.31590).

Evidence of Compliance:

- ✓ Records of approved validation studies and approval of clinical use

REFERENCES

- 1) Jennings L. *et al.* Recommended practices and principles for validating clinical molecular pathology tests. *Arch Pathol Lab Med.* 2009; 133(5):743-755
- 2) Halling KC, *et al.* Test verification and validation for molecular diagnostic assays. *Arch Pathol Lab Med.* 2012, 136(1):11-13
- 3) Saxe DF, Persons DL, Wolff DJ, Theil KS; Cytogenetics Resource Committee of the College of American Pathologists. Validation of fluorescence in situ hybridization using an analyte-specific reagent for detection of abnormalities involving the mixed lineage leukemia gene. *Arch Pathol Lab Med.* 2012. Jan;136(1):47-52
- 4) Pont-Kingdon G, Gedge F, Woederchak-Donahue W, Schrijver I, Weck KE, Kant JA, Oglesbee D, Bayrak-Toydemir P, Lyon E; Biochemical and Molecular Genetic Resource Committee of the College of American Pathologists. Design and analytical validation of clinical DNA sequencing assays. *Arch Pathol Lab Med.* 2012. Jan;136(1):41-6

- 5) Jennings LJ, Smith FA, Halling KC, Persons DL, Kamel-Reid S; Molecular Oncology Resource Committee of the College of American Pathologists. Design and analytic validation of BCR-ABL1 quantitative reverse transcription polymerase chain reaction assay for monitoring minimal residual disease. *Arch Pathol Lab Med.* 2012. Jan;136(1):33-40
- 6) Kamel-Reid S, Zhang T, Persons DL, Nikiforova MN, Halling KC; Molecular Oncology Resource Committee of the College of American Pathologists. Validation of KRAS testing for anti-EGFR therapeutic decisions for patients with metastatic colorectal carcinoma. *Arch Pathol Lab Med.* 2012. Jan;136(1):26-32
- 7) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24); [42CFR493.1253]

****REVISED** 09/17/2019**

MOL.31015 Validation Studies - Specimen Types

Phase II

Validation studies with an adequate number and representative distribution of samples are performed for each type of specimen expected for the assay (eg, blood, fresh/frozen tissue, saliva, paraffin-embedded tissue, prenatal specimens, buccal swabs).

NOTE: For tissue samples, the validation must include representative tissue sources (ie, organ/site) that are expected to be tested by the assay. This should include commonly tested tissue sources, as well as those with potentially interfering substances (eg, melanin or mucin). It is not expected for the laboratory to include every tissue source that could be examined by the assay.

Specimen processing and fixation conditions can significantly influence the quality and integrity of nucleic acids extracted from a specimen. Consequently, laboratories must include specimens handled with significantly different fixation and processing methods in the validation. As an example, a laboratory validating a test that will utilize nucleic acids from FFPE tissue, FFPE cell blocks, or decalcified tissue should include specimens handled with these types of fixation and processing methods in the validation.

Validations can be augmented by, but not supplanted with, additional reference materials (eg, characterized cell lines, cell lines with spiked in nucleic acids).

For validation of different specimen types for DNA-based copy number arrays, refer to MOL.35400 in the Arrays section.

Evidence of Compliance:

- ✓ Records of validation studies

****REVISED** 06/04/2020**

MOL.31130 Validation of Test Performance Specifications

Phase II

Prior to clinical use of each modified FDA-cleared/approved test and laboratory-developed test (LDT), the laboratory has performed a validation study and prepared a written assessment of each of the following test method performance specifications, as applicable, using a sufficient number of characterized samples:

- **Analytical accuracy**
- **Analytical precision/reproducibility**
- **Reportable range**
- **Analytical sensitivity (lower detection limit)**
- **Analytical specificity**
- **Any other performance characteristic required to ensure analytical test performance (eg, specimen stability, reagent stability, linearity, carryover, and cross-contamination)**

NOTE 1: For laboratories not subject to US regulations, this requirement also applies to tests approved by an internationally recognized regulatory authority that have been modified.

NOTE 2: Accuracy is validated by comparing results to a definitive or reference method, or an established comparative method. For a quantitative test, accuracy refers to 'closeness to true' whereas for a qualitative test it refers to correlation to a comparative test or tests that are used to establish 'true'. Accuracy can be assessed using well-characterized reference material together with appropriate biological matrix or by comparison to another valid test method, such

as through specimen exchange. Assays for genetic disorders with a limited number of possible genotypes (eg, hereditary hemochromatosis) should confirm the ability of the assay to detect these genotypes. Assays for genetic disorders with considerable allelic heterogeneity and/or significant numbers of private pathogenic variants (eg, cystic fibrosis or Lynch Syndrome) should confirm the accuracy of the methodology used to provide a high degree of assurance that the assay will detect targeted genotypes. Various sample types may affect the analytical performance of a test. Therefore, laboratories may need to establish sample-specific analytical and clinical performance characteristics.

For laboratory-developed tests, an appropriate number of samples to demonstrate analytical accuracy is defined as the following:

- For quantitative tests, a minimum of 20 samples with analyte concentrations distributed across the analytical measurement range should be used. Proportionate mixtures of samples may be used to supplement the study population.
- For qualitative tests, a minimum of 20 samples, including positive, negative, and, when appropriate, low-positive samples with concentrations near the lower level of detection should be used; equivocal samples should not be used

If the laboratory uses fewer samples, the laboratory director must record the criteria used to determine the appropriateness of the sample size. In many cases, a validation study with more samples is desirable and/or recommended.

NOTE 3: Records for validation of precision/reproducibility must show that a test will return the same result regardless of minor variations in testing conditions that can cause random error, such as different technologists, instruments, reagent lots, days, etc. This is usually determined by repeated measures of samples throughout the reportable range. For a quantitative test, it is represented as the coefficient of variation, whereas for a qualitative test, it is represented as ratios of concordant results. Laboratories are encouraged to provide confidence intervals to estimated performance characteristics.

NOTE 4: The reportable range encompasses the full range of reported values.

- For qualitative tests that would include all reportable outcomes (eg, homozygous wild type, heterozygous or homozygous variant).
- For quantitative tests, the laboratory must define the analytical measurement range (AMR) as described in the Quantitative Assays; Calibration and Standards section of the checklist.

NOTE 5: The analytical sensitivity (lower limit of detection) refers to the ability of a test to confidently or consistently detect a minor allele or variant in a background of assay relevant biological matrix (eg, pathogens, rare variants, chimerism, mosaicism, tumor-normal admixtures).

NOTE 6: The analytical specificity refers to the ability of a test or procedure to correctly identify or quantify an entity in the presence of interfering or cross-reactive substances that might be expected to be present.

NOTE 7: If multiple identical instruments or devices are in use, there must be records (data and written assessment) showing that the method performance specifications have been separately validated for each test and instrument or device.

Evidence of Compliance:

- ✓ Written procedure for validating test method performance specifications **AND**
- ✓ Records of validation study and written assessment of each component of the test method performance specifications

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI Document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.
- 2) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2018 edition, Revised 01/2018.
- 3) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.
- 4) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1253(b)(2)]

- 5) Clinical and Laboratory Standards Institute. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline*. 3rd ed. CLSI Document EP28-A3C. Clinical and Laboratory Standards Institute. Wayne, PA; 2008.
- 6) Clinical and Laboratory Standards Institute. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline*. 2nd ed. CLSI Document EP17-A2. Clinical and Laboratory Standards Institute. Wayne, PA; 2012.
- 7) Clinical and Laboratory Standards Institute (CLSI). *Interference Testing in Clinical Chemistry*. 3rd ed. CLSI guideline EP07. Clinical and Laboratory Standards Institute. Wayne, PA; 2018.
- 8) Jennings LJ, Arcila MD, Corless C, et al. Guidelines for Validation of Next Generation Sequencing-Based Oncology Panels: A Joint Commission Recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn*. 2017; 19(3):341-65.

MOL.31251 Qualitative Cut-Off Validation**Phase II**

For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is established initially using a sufficient number of samples.

NOTE: The threshold value that distinguishes a positive from a negative result must be established when the test is initially placed in service.

Evidence of Compliance:

- ✓ Written procedure for initial establishment of the cut-off value **AND**
- ✓ Records of initial establishment of the cut-off value

****REVISED** 09/17/2019****MOL.31255 Reference Interval****Phase II**

The laboratory verifies or establishes its reference intervals.

NOTE: The reference interval is the range of results expected in the normal population. For some qualitative tests (eg, HLA genotyping), the reference interval may include all genotypes. If the reference value depends on the clinical situation, then a plan for interpreting the patient result must be defined. If published data are used to determine the reference interval, it must be carefully verified, with records of the evaluations retained.

Evidence of Compliance:

- ✓ Records of reference interval study

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline*. 3rd ed. CLSI Document EP28-A3C. Clinical and Laboratory Standards Institute. Wayne, PA; 2008.
- 2) Clinical and Laboratory Standards Institute. *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI Document MM19-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2011.

MOL.31590 Clinical Performance Characteristics**Phase II**

The clinical performance characteristics of each assay are determined and recorded, using either literature citations or a summary of internal study results.

NOTE: The clinical performance characteristics of a test relate to its diagnostic sensitivity and specificity, and its positive and negative predictive values in the (various) target population(s) or likelihood ratios, and clinical utility. Issues that affect the clinical interpretation of a test which should be considered include (1) the clinical setting in which the test is used, (2) genotype/phenotype associations when these vary with particular variants, and (3) genetic, environmental or other factors which modify the clinical expression of the genetic alteration detected.

Clinical performance characteristics should be determined relative to a combination of clinical data (eg, biopsy findings, radiographic and clinical findings, other laboratory results, etc.). Establishing clinical validity may require extended studies and monitoring that go beyond the purview or control of the individual laboratory. The laboratory should perform clinical validation in-house, except in the case of very rare conditions, in which case data from the literature can be used, or in the case of very common conditions for which the clinical validity is well-established in the literature. It is essential that the laboratory director or designee use professional judgment




in evaluating the results of such studies and in monitoring the state-of-the-art worldwide as it applies to newly discovered gene targets and potential new tests, especially those of a predictive or incompletely penetrant nature.

Evidence of Compliance:

- ✓ Records of validation studies to establish clinical performance and/or appropriate cited literature

COLLECTION, TRANSPORT, PREPARATION, AND STORAGE OF SPECIMENS

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of requisition forms for completeness • Sampling of nucleic acid extraction policies and procedures • Sampling of nucleic acid measurement records • Sampling of RNA assessment records/false negative rate records • Sampling of molecular pathology specimen processing, handling, aliquoting, storage, and retention policies and procedures
	<ul style="list-style-type: none"> • Processing of molecular pathology specimens
	<ul style="list-style-type: none"> • What is your course of action when you receive unacceptable molecular pathology specimens? • How does your laboratory ensure RNase-free conditions are maintained? • How does your laboratory ensure specimen adequacy?

MOL.32350 Requisition Information

Phase II

Test requests are accompanied with a pedigree and/or race/ethnicity, when appropriate (eg, for linkage analysis).

Evidence of Compliance:

- ✓ Specimen requisitions/collection forms

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7162 [42CFR493.1241(c)]

MOL.32360 Specimen Handling

Phase II

There are written procedures to prevent specimen loss, alteration, or contamination.

NOTE: Because of the high sensitivity and potential for contamination in molecular testing involving amplification of DNA, the laboratory must be alert to the possibility of commingled specimens. An example of a potentially commingled specimen is one that is received after the specimen container was entered by a sampling device that enters multiple samples, albeit with rinses in between specimens. If such samples must be tested by molecular methods, the results should be interpreted with caution, considering the potential for contamination.

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.32365 Specimen Preservation/Storage**Phase II**

There is a written procedure describing methods for specimen preservation and storage before testing, consistent with good laboratory practice.

REFERENCES

- 1) Schultz CL, *et al.* A lysis, storage, and transportation buffer for long-term, room-temperature preservation of human clinical lymphoid tissue samples yielding high molecular weight genomic DNA suitable for molecular diagnosis. *Am J Clin Pathol.* 1999;111:748-752
- 2) Makowski GS, *et al.* In situ PCR amplification of Guthrie card DNA to detect cystic fibrosis mutations. *Clin Chem.* 1996;41:471-479
- 3) Farkas DH, *et al.* Specimen stability for DNA-based diagnostic testing. *Diagn Mol Pathol.* 1996;5:227-235
- 4) Kaul K, *et al.* Amplification of residual DNA sequences in sterile bronchoscopes leading to false-positive PCR results. *J Clin Microbiol.* 1996;34:1949-1951
- 5) Tsui NMY, *et al.* Stability of endogenous and added RNA in blood specimens, serum, and plasma. *Clin Chem.* 2002;48:1647-1653
- 6) Rainen L, *et al.* Stabilization of mRNA expression in whole blood samples. *Clin Chem.* 2002;48:1883-1890
- 7) Pahl A, Brune K. Stabilization of gene expression profiles in blood after phlebotomy. *Clin Chem.* 2002;48:2251-2253
- 8) Clinical and Laboratory Standards Institute (CLSI), *Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline*. CLSI document MM13-A (ISBN 1-56238-591-7). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2005.

MOL.32375 Physician Notification**Phase II**

The submitting physician (or requester) is promptly notified when a specimen is inadequate or if insufficient nucleic acid is isolated.

Evidence of Compliance:

- ✓ Records of physician notification of inadequate specimen in patient record or log

MOL.32385 Specimen Aliquots**Phase II**

If aliquoting of specimens is performed, there is a written procedure to prevent any possible cross-contamination of the specimens.

NOTE: Although in some cases it may be appropriate to aliquot a specimen, the laboratory must have a policy that no aliquot is ever returned to the original container.

MOL.32390 Specimen Processing/Storage**Phase II**

Patient samples are processed promptly or stored appropriately to minimize degradation of nucleic acids.

Evidence of Compliance:

- ✓ Written procedure for processing and storage of specimens

REFERENCES

- 1) Farkas DH, Kaul KL, Wiedbrauk DL, *et al.* Specimen Collection and Storage for Diagnostic Molecular Pathology Investigation. *Arch Pathol Lab Med.* 1996;120:591-596
- 2) Kiechle FL, Kaul KL, Farkas DH. Mitochondrial Disorders: Methods and Specimen Selection for Diagnostic Molecular Pathology. *Arch Pathol Lab Med.* 1996;120:597-603
- 3) Farkas DH, Drevon AM, Kiechle FL, *et al.* Specimen Stability for DNA-based Diagnostic Testing. *Diag Molec Pathol.* 1996;5(4):227-235

****REVISED** 06/04/2020****MOL.32395 Neoplastic Cell Content****Phase II**

For paraffin-embedded tumor specimens from which DNA or RNA is extracted for analysis (eg, microsatellite instability, *KRAS* or *KIT* analysis), there is a record of histological assessment of neoplastic cell content.

NOTE: In addition to confirming the presence or absence of neoplastic cells by a qualified pathologist, it may be necessary for some assays to estimate and consider neoplastic cellularity in relation to the lower limit of detection of the assay.

A corresponding H&E section from the same tissue block used for nucleic acid extraction may be used to assess sample adequacy. Alternatively, a stain such as toluidine blue may be used to stain the slide that is being used for nucleic acid extraction. When assessment of sample adequacy is performed outside of the testing laboratory, a record of such assessment must accompany the sample.

This requirement is applicable to all molecular methods for the detection of sequence variants (eg, Sanger sequencing, NGS, PCR).

MOL.32425 Nucleic Acid Extraction/Isolation/Purification Phase II

Nucleic acids are extracted, isolated, and purified by methods reported in the literature, by an established commercially available kit or instrument, or by a validated method developed by the laboratory.

NOTE: Extraction procedures may combine purification or isolation of nucleic acids according to the level of purity needed for downstream applications.

Evidence of Compliance:

- ✓ Records to support nucleic acid extraction/isolation/purification is performed by a validated method

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.
- 2) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed.* CLSI guideline MM21-ED1. Clinical and Laboratory Standards Institute, Wayne, PA, 2015.

MOL.32427 Extracted Nucleic Acid Specimens Phase II

If extracted nucleic acid is accepted as a specimen type, the laboratory has a written policy that isolation of nucleic acids for clinical testing occurs in a CLIA-certified laboratory or a laboratory meeting equivalent requirements as determined by the CAP and/or the CMS. This policy is clearly displayed to ordering clients.

NOTE: All clinical testing must be performed in CLIA-certified laboratories or laboratories meeting equivalent requirements (refer to GEN.41350 and MOL.35840). This includes all components of testing that may impact the quality of the test result, including isolation or extraction of nucleic acids. Laboratories may choose to have referring clients formally attest that extracted nucleic acid submitted for testing has been isolated or extracted in an appropriately qualified laboratory.

Evidence of Compliance:

- ✓ Written statement on the test requisition, test catalog, or policy available to referring clients stating that the laboratory only accepts isolated or extracted nucleic acids for which extraction or isolation is performed in an appropriately qualified laboratory

****REVISED** 09/17/2019**

MOL.32430 Nucleic Acid Quantity and Quality Determination Phase II

The quantity and quality of nucleic acids are determined when appropriate.

NOTE: The quantity and quality of nucleic acids (DNA or RNA) must be measured prior to use in a procedure whose success depends on accurately determining the quantity, concentration, integrity, and/or purity of the nucleic acids. Techniques commonly used to assess nucleic acid

quantity and/or quality include electrophoresis, UV/VIS spectrophotometry, and fluorescence spectroscopy.

Evidence of Compliance:

- ✓ Written policy defining conditions under which quantity and/or quality of nucleic acid are measured **AND**
- ✓ Written procedure for determining nucleic acid quantity and/or quality **AND**
- ✓ Records of nucleic acid quantity and/or quality determinations

REFERENCES

- 1) Tsui NBY, Ng EKO, Lo YMD. Stability of Endogenous and Added RNA in Blood Specimens, Serum and Plasma. *Clin Chem* 48:1647-1653,2002
- 2) Farrell R. Gel electrophoresis based assessment of cellular RNA quality may also be used (RNA Isolation Strategies). In: RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, 1998
- 3) Clinical and Laboratory Standards Institute. *Diagnostic Nucleic Acid Microarrays: Approved Guideline*; CLSI Document MM12-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.

MOL.32440 Ribonuclease-Free Conditions

Phase I

Ribonuclease-free conditions are maintained for all assays that detect RNA or use an RNA probe.

NOTE: RNA is extremely susceptible to degradation by ribonucleases that are ubiquitous in the environment. To ensure preservation of target RNA or RNA probes, special precautions are needed.

Evidence of Compliance:

- ✓ Written procedure defining environmental requirements for RNase-free conditions **AND**
- ✓ Records that RNase-free conditions are maintained (ie, wipe test in event of contamination incident) with corrective action if conditions are not met

REFERENCES

- 1) Gulley ML, et al. Guidelines for interpreting EBER *in situ* hybridization and LMP1 immunohistochemical tests for detecting Epstein-Barr virus in Hodgkin lymphoma. *Am J Clin Pathol.* 2002;117:259-267

MOL.32445 Concentration Techniques

Phase I

Concentration techniques for quantitative tests are verified.

NOTE: Techniques used to concentrate specimens for analysis must be verified at specified, periodic intervals (not to exceed one year or manufacturer's recommendations).

Evidence of Compliance:

- ✓ Written procedure for verifying the accuracy of concentration techniques **AND**
- ✓ Records of concentration technique verification at defined frequency

MOL.33150 Specimen Storage

Phase II

Stored specimens are retained in a way to allow prompt retrieval for further testing.

MOL.33250 Specimen Retention

Phase II

Specimens are retained in compliance with applicable laws and regulations.

NOTE: CAP retention guidelines may be found in the Quality Management section of the Laboratory General checklist, and also at <http://www.cap.org/>. However, national, federal, state (or provincial), or local laws and regulations may be more stringent than CAP guidelines.

Retention of fluorochrome-stained slides should be defined in a laboratory policy.

Evidence of Compliance:

- ✓ Written retention policy

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.
- 2) Clinical and Laboratory Standards Institute (CLSI). *Molecular Methods for Clinical Genetics and Oncology Testing; Approved Guideline—Third Edition*. CLSI document MM01-A3 (ISBN 1-56238-793-6). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2012.

QUANTITATIVE ASSAYS: CALIBRATION AND STANDARDS

CALIBRATION: *The process of adjusting an instrument or test system to establish a relationship between the measurement response and the concentration or amount of the analyte that is being measured by the test procedure.*

During the validation of a quantitative assay, calibrators are used to generate a calibration curve that spans the analytical measurement range (AMR) to assess accuracy, linearity, limit of detection (LOD) and limit of quantification (LOQ). Unlike standards used to generate a standard curve, calibrators must have a matrix appropriate for the clinical specimens assayed by that method. For example, an assay that measures copies of RNA transcript would require calibrators that consist of RNA target in an appropriate matrix such as total RNA.

CALIBRATION VERIFICATION: *The process of confirming that the current calibration settings for each analyte remain valid for a test system.*

Each laboratory must define limits for accepting or rejecting results of the calibration verification process. Calibration verification can be accomplished in several ways. If the manufacturer provides a calibration validation or verification process, it must be followed. Other techniques include (1) assay of the current method calibration materials as unknown specimens, and (2) assay of matrix-appropriate materials with target values that are specific for the test system.

ANALYTICAL MEASUREMENT RANGE (AMR): *The range of analyte values that a method can directly measure on the specimen without any dilution, concentration, or other pretreatment that is not part of the usual assay process.*

LINEARITY AND THE AMR

Linearity is a fundamental characteristic of many analytic measurement methods, whereby there is a straight-line relationship between "true" analyte concentrations and measured concentrations. In this context, linearity refers to the relationship between the predicted and observed measurement results and not to the relationship between instrument signal output and analyte concentration. For most assays, this relationship is linear within the AMR.

AMR VERIFICATION

Laboratories are required to verify that the appropriate relationship is maintained over the AMR. Laboratories may verify and use an AMR that is narrower than the range defined by the manufacturer. This may be appropriate when materials available for method validation and/or AMR verification are not available to verify the full range claimed by the manufacturer, or reporting values across the full range defined by the manufacturer is not clinically relevant. For some types of assays, results beyond the AMR can be reported through dilution studies.





Minimum requirements for AMR verification can be met by using matrix appropriate materials, which include low, mid and high concentration or activity range of the AMR with recovery of results that fall within a defined range of the target value. Records of AMR verification must be available.

CLOSENESS OF SAMPLE CONCENTRATIONS OR ACTIVITIES TO THE UPPER AND LOWER LIMITS OF THE AMR

When verifying the AMR, it is required that materials used are near the upper and lower limits of the AMR. Factors to consider in verifying the AMR are the expected analytic imprecision near the limits, the clinical

impact of errors near the limits, and the availability of test specimens near the limits. It may be difficult to obtain specimens with values near the limits for some analytes. In such cases, reasonable procedures should be adopted based on available specimen materials. The closeness of sample concentrations and activities to the upper and lower limits of the AMR are defined at the laboratory director's discretion. The method manufacturer's instructions for verifying the AMR must be followed, when available. The laboratory director must define limits for accepting or rejecting verification tests of the AMR.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of calibration and AMR policies and procedures • Sampling of calibration/calibration verification records • Sampling of AMR verification records
	<ul style="list-style-type: none"> • Sampling of calibration materials (quality)
	<ul style="list-style-type: none"> • What is your course of action if calibration is unacceptable? • When was the last time you performed calibration and how did you verify the calibration? • What is your course of action when you receive calibration materials for non-FDA cleared/approved assays? • What is your course of action when preparing controls and calibrators in-house?
	<ul style="list-style-type: none"> • Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration and unacceptable calibration verification

****REVISED** 09/17/2019**

MOL.33655 Calibration Procedures

Phase II

Calibration procedures for each test system are appropriate, and the calibration records are reviewed for acceptability.

NOTE: Calibration must be performed following manufacturer's instructions, at minimum, including the number, type, and concentration of calibration materials, frequency of calibration, and criteria for acceptable performance. Calibration procedures are typically specified in the manufacturer's instructions but may also be established by the laboratory.

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 1992(Feb 28):7165 [42CFR493.1217]
- 2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]
- 3) Clinical and Laboratory Standards Institute. *Evaluation of Matrix Effects; Approved Guideline*. 3rd ed. CLSI Document EP14-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014
- 4) Miller WG. Quality control. In: Henry's Clinical Diagnostic and Management by Laboratory Methods, 21st Edition, ed McPherson RA, Pincus MR, Saunders Elsevier, 2007:99-111.

****REVISED** 09/17/2019**

MOL.33696 Calibration and Calibration Verification Materials

Phase II

High quality materials with test system and matrix-appropriate target values are used for calibration and calibration verification whenever possible.

NOTE: Calibration and calibration verification must have defined analysis target values and appropriate matrix characteristics for the clinical specimens and specific assay method. Many instrument systems require calibration materials with system-specific target values to produce accurate results for clinical specimens.

Suitable materials for calibration verification include, but are not limited to:

1. *Calibrators used to calibrate the analytical system*
2. *Materials provided by the manufacturer for the purpose of calibration verification*
3. *Previously tested unaltered patient/client specimens*
4. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
5. *Third party general purpose reference materials that are suitable for verification*

In general, routine control materials and proficiency testing materials are not suitable for calibration verification, except in situations where the material has been shown to be suitable (eg, specifically designed by the method manufacturer) or no other materials are available.

Evidence of Compliance:

- ✓ Written policy defining the use of appropriate calibration and calibration verification materials

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Evaluation of Matrix Effects; Approved Guideline*. 3rd ed. CLSI Document EP14-A3. Clinical and Laboratory Standards Institute, Wayne, PA; 2014
- 2) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]

MOL.33737 Calibration Materials - Non-FDA Cleared/Approved Assays

Phase II

The quality of all calibration materials used for non-FDA cleared/approved assays is evaluated and recorded.

NOTE: Commercial standards used to prepare calibrators require certificates of quality from the manufacturer, or a quality check as part of the initial assay validation. The laboratory must ensure the accuracy of a new lot of calibrators by checking the new lot against the current lot.

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]

****REVISED** 09/17/2019**

MOL.33860 Recalibration/Calibration Verification Criteria

Phase II

Criteria are established for frequency of recalibration or calibration verification, and the acceptability of results.

NOTE: Laboratories must either recalibrate or perform calibration verification at least every six months and if any of the following occur:

1. *At changes of reagent lots, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client results*
2. *If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range*
3. *After major maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

Single use devices, and other test devices that do not allow user calibration, do not require calibration verification.

Evidence of Compliance:

- ✓ Written policy defining the method, frequency and limits of acceptability of calibration verification for each instrument/test system **AND**
- ✓ Records of calibration verification at defined frequency

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3707[42CFR493.1255(b)(3)]
- 2) Miller WG. Quality control. In: Henry's Clinical Diagnostic and Management by Laboratory Methods, 21st Edition, ed McPherson RA, Pincus MR, Saunders Elsevier, 2007:99-111.

MOL.33901 Recalibration**Phase II**

The system is recalibrated when calibration verification fails to meet the established criteria of the laboratory.

Evidence of Compliance:

- ✓ Written policy defining criteria for recalibration **AND**
- ✓ Records of recalibration, if calibration or calibration verification has failed

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255(a)(3)]

****REVISED** 09/17/2019****MOL.33942 AMR Verification Materials****Phase II**

Verification of the analytical measurement range (AMR) is performed with matrix-appropriate materials that include the low, mid and high range of the AMR, and appropriate acceptance criteria are defined.

NOTE: The matrix of the sample (ie, the environment in which the sample is suspended or dissolved) may influence the measurement of the analyte. In many cases, the method manufacturer will recommend suitable materials. Other suitable materials for AMR verification include the following:

1. *Linearity material of appropriate matrix, eg, CAP CVL Survey-based or other suitable linearity verification material*
2. *Previously tested patient/client specimens, that may be altered by admixture with other specimens, dilution, spiking in known amounts of an analyte, or other technique*
3. *Primary or secondary standards or reference materials with matrix characteristics and target values appropriate for the method*
4. *Patient samples that have reference method assigned target values*
5. *Control materials, if they adequately span the AMR and have method specific target values*

Evidence of Compliance:

- ✓ Written policy for AMR verification defining the types of materials used and acceptability criteria

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]
- 2) Shah VP, Midha KK, Dighe S, et al. Bioanalytical Method Validation - *Pharm Res*. 1992; 9(4):588-92.
- 3) Hartmann C, Smeyers-Verbeke J, Massart DL, McDowall RD. Validation of bioanalytical chromatographic methods. *J Pharm Biomed Anal*. 1998; 17(2):193-218.
- 4) Findlay JW, et al. Analytical Methods Validation - Bioavailability, Bioequivalence and Pharmacokinetic Studies. *Pharm Res*. 2000; 17(12):1551-7.
- 5) Killeen AA, Long T, Souers R, Styler P, Ventura CB, Klee GG. Verifying Performance Characteristics of Quantitative Analytical Systems: Calibration Verification, Linearity, and Analytical Measurement Range. *Arch Pathol Lab Med*. 2014; 138(9):1773-81.

****REVISED** 09/17/2019**

MOL.33983 AMR Verification**Phase II**

Verification of the analytical measurement range (AMR) is performed at least every six months and following defined criteria. Records are retained.

NOTE: The AMR must be verified at least every six months after a method is initially placed in service and if any of the following occur:

1. *At changes of reagent lots, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient/client test results, and the range used to report patient/client test data*
2. *If QC shows an unusual trend or shift or is outside acceptable limits, and the system cannot be corrected to bring control values into the acceptable range*
3. *After major preventive maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

It is not necessary to independently verify the AMR if the calibration of an assay includes calibrators that span the full range of the AMR, with low, midpoint and high values (ie, three points) and the system is calibrated at least every six months. A one-point or two-point calibration does not include all of the necessary points to validate the AMR.

Evidence of Compliance:

- ✓ Written policy for AMR verification defining the frequency performed **AND**
- ✓ Records of AMR verification at least every six months

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare & Medicaid Services. Medicare, Medicaid and CLIA Programs; Laboratory Requirements Relating to Quality Systems and Certain Personnel Qualifications; final rule. *Fed Register*. 2003(Jan 24):3707 [42CFR493.1255]

MOL.34024 Calibrator Preparation**Phase II**

Calibrators and controls are prepared separately.

NOTE: In general, calibrators should not be used as QC materials. If calibrators are used as controls, then different preparations should be used for these two functions. For example, when using commercial calibrators and controls, the lot number for calibration should be different than the lot number used for QC, whenever possible.

Evidence of Compliance:

- ✓ Written policy and procedure for the use and in-house preparation of controls and calibrators

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):3708 [42CFR493.1256(d)(9)]

REAGENTS**Inspector Instructions:**

- Sampling of probe/primer information

Additional requirements are in the REAGENTS section of the All Common Checklist.

MOL.34188 Probe Characteristics**Phase II**

Information regarding the nature of any probe or primer used in an assay is sufficient to permit interpretation and troubleshooting of test results.

NOTE: Items of importance where appropriate include: the type (genomic, cDNA, oligonucleotide or riboprobe) and origin (human, viral, etc.) of the probe or sequence; the oligonucleotide sequence and complementary sequence or gene region recognized; an appropriate restriction enzyme map of the DNA; known polymorphisms, sites resistant to endonuclease digestion, and cross-hybridizing bands; the labeling methods used and standards for adequacy of hybridization or amplification. For linkage analysis, recombination frequencies and map positions must be recorded. Loci should be designated as defined by the Human Gene Mapping Nomenclature Committee. For inherited disease tests, additional information such as chromosomal location of the target, allele frequencies of the variant in various ethnic groups, and recombination frequencies (for linkage probes) may be required. Sequence and size data may not be available for commercially-obtained probes and primers when this information is considered proprietary.

REFERENCES





- 1) McAlpine PJ, et al. The Catalog of mapped genes and report of the nomenclature committee. Human gene mapping. Cytogenet Cell Genet. (most recent version)

CONTROLS

Controls are samples that act as surrogates for patient/client specimens. They are processed like a patient/client sample to monitor the ongoing performance of the entire analytic process in every run. This section of the checklist is applicable to the different steps of the testing process (eg, amplification), methods, and instrument systems used (eg, sequencing, PCR, arrays).

Molecular tests typically include positive and negative controls and, in some instances, a sensitivity control to show that low level target is detectable. An internal control, extraction control, and a contamination control may be indicated. A single control may be able to serve multiple purposes. Quantitative tests typically include at least two levels of control at relevant decision points to verify that calibration status is maintained within acceptable limits.

Inspector Instructions:

 <p>READ</p>	<ul style="list-style-type: none"> • Sampling of QC policies and procedures • Sampling of QC records, including monthly monitoring of imprecision
 <p>OBSERVE</p>	<ul style="list-style-type: none"> • Sampling of control material (storage)
 <p>ASK</p>	<ul style="list-style-type: none"> • How do you determine when QC is unacceptable and corrective action is needed? • How does your laboratory verify the cut-off value used to distinguish positive from negative results? • What is your course of action when monthly statistical data changes significantly from the previous month's data?
 <p>DISCOVER</p>	<ul style="list-style-type: none"> • Review a sampling of QC data over the previous two-year period. Select several occurrences in which QC is out of range and follow records to determine if the steps taken follow the laboratory procedure for corrective action

MOL.34229 Controls - Qualitative Assays

Phase II

For qualitative tests, positive, negative and sensitivity controls are included for each assay, when appropriate, in every run and as specified in the manufacturer's instructions (as applicable) and laboratory procedure.

NOTE: Ideally, one should use a positive control for each analyte in each run. However, in some circumstances such as in a large targeted panel for detection of cystic fibrosis pathogenic variants, this is not practical. One way to address this situation is to rotate positive controls in a systematic fashion and at a frequency defined in the laboratory procedure. A sensitivity control may be required if the molecular assay is being used to detect low-level target sequences (eg, pathogens, chimerism, mosaicism, tumor-normal admixtures).

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director. The quality control plan must include the monitoring of the extraction and amplification phases based on the risk assessment performed by the laboratory and the manufacturer's instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

Evidence of Compliance:

- ✓ Written QC procedures **AND**
- ✓ Records of QC results including external and internal control processes **AND**
- ✓ Manufacturer's product insert or manual, as applicable

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical Laboratory Improvement Amendments of 1988; Final Rule. *Fed Register*. 2003(Jan 24);7166 [42CFR493.1256(D)(3)(II)]
- 2) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.34270 Controls - Quantitative Assays**Phase II**

For quantitative tests, control materials at more than one concentration (level) are included in every run and as specified in the manufacturer's instructions (as applicable) and laboratory procedure.

NOTE: Controls should verify assay performance at relevant analytic and clinical decision points.

If an internal quality control process (eg, electronic/procedural/built-in) is used instead of an external control material to meet daily quality control requirements, the laboratory must have an individualized quality control plan (IQCP) approved by the laboratory director. The quality control plan must include the monitoring of the extraction and amplification phases based on the risk assessment performed by the laboratory and the manufacturer's instructions. Please refer to the IQCP section of the All Common Checklist for the eligibility of tests for IQCP and requirements for implementation and ongoing monitoring of an IQCP.

Evidence of Compliance:

- ✓ Written QC procedures **AND**
- ✓ Records of QC results including external and internal control processes **AND**
- ✓ Manufacturer product insert or manual, as applicable

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline*. 4th ed. CLSI document C24-ED4. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.
- 2) Ye JJ, et al. Performance evaluation and planning for patient/client-based quality control procedures. *Am J Clin Pathol*. 2000;113:240-248.

****REVISED** 09/17/2019****MOL.34311 Control and Standard Acceptability Limits****Phase II**

Acceptability limits are defined for all control procedures, control materials and standards.

NOTE: These controls must be appropriate for the range of sensitivities tested and should, ideally, focus on result ranges that are near clinical decision points.

Evidence of Compliance:

- ✓ Written policy defining acceptability limits

MOL.34325 Alternative Control Procedures**Phase II**

If the laboratory performs test procedures for which control materials are not commercially available, there are written procedures for an alternative mechanism to detect immediate errors and monitor test system performance over time. The performance of alternative control procedures must be recorded.

NOTE: "Performance" includes elements of accuracy, precision, and clinical discriminating power. Examples of alternative procedures may include split sample testing with another method or with another laboratory, the testing of previously tested patient specimens in duplicate, testing of patient specimens in duplicate, or other defined processes approved by the laboratory director.

Evidence of Compliance:

- ✓ Written procedures for alternative quality control **AND**
- ✓ Records of alternative control procedures

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(h)].

MOL.34352 QC Confirmation of Acceptability**Phase II**

The results of controls are reviewed for acceptability before reporting of results.**Evidence of Compliance:**

- ✓ Written policy stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Records of control result approval

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 1992(Feb 28):7166 [42CFR493.1218(e)]

MOL.34393 QC Corrective Action**Phase II****There are records of corrective action when control results exceed defined acceptability limits.**

NOTE: Patient test results obtained in an analytically unacceptable test run or since the last acceptable test run must be evaluated to determine if there is a significant clinical difference in patient results. Re-evaluation may or may not include re-testing patient samples, depending on the circumstances.

Even if patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results. For example, evaluation could include comparison of patient means for the run in question to historical patient means, and/or review of selected patient results against previous results to see if there are consistent biases (all results higher or lower currently than previously) for the test(s) in question.

The corrective action for tests that have an Individualized Quality Control Plan (IQCP) approved by the laboratory director must include an assessment of whether further evaluation of the risk assessment and quality control plan is needed based on the problems identified (eg, trending for repeat failures, etc.).

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Oct 1):1046[42CFR493.1282(b)(2)]

MOL.34434 QC Handling**Phase II****Control specimens are tested in the same manner and by the same personnel (including specimen preparation) as patient samples.**

NOTE: It is implicit in quality control that control specimens be tested in the same manner as patient specimens. Moreover, QC specimens must be analyzed by personnel who routinely perform patient testing. This does not imply that each operator must perform QC daily, so long as each instrument and/or test system has QC performed at required frequencies, and all analysts participate in QC on a regular basis. To the extent possible, all steps of the testing process must be controlled.

For newborn screening testing, good laboratory practice is to punch controls and patient blood spot samples with the same equipment.

Evidence of Compliance:

- ✓ Records reflecting that QC is run by the same personnel performing patient testing

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical laboratory improvement amendments of 1988; Final rule. *Fed Register*. 1992(Feb 28):7166 [42CFR493.118(c)]

MOL.34475 QC Statistics**Phase I****For quantitative assays, quality control statistics are calculated and reviewed at least monthly to define analytic imprecision and to monitor trends over time.**

NOTE: The laboratory must use statistical methods such as calculating SD and CV at specified intervals to evaluate variance in numeric QC data.

Evidence of Compliance:

- ✓ QC records showing monthly monitoring and corrective action, as applicable

REFERENCES

- 1) Rifai N, Horvath AR, Wittwer CT, eds. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. 6th ed. St. Louis, MO: Elsevier; 2018.
- 2) Department of Health and Human Services, CMS. Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24):7146 [42CFR493.1256(d)(10)(i)]
- 3) Ross JW, Lawson NS. Analytic goals, concentrations relationships, and the state of the art of clinical laboratory precision. *Arch Pathol Lab Med*. 1995;119:495-513
- 4) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline*. 4th ed. CLSI document C24-ED4. Clinical and Laboratory Standards Institute, Wayne, PA, 2016.
- 5) Brooks ZC, et al. Critical systematic error used of varied QC rules in routine chemistry. *Clin Chem*. 2000;46:A70

MOL.34495 Monthly QC Review

Phase II

Quality control data are reviewed and assessed at least monthly by the laboratory director or designee.

NOTE: The review of quality control data must be recorded and include follow-up for outliers, trends, or omissions that were not previously addressed.

The QC data for tests performed less frequently than once per month should be reviewed when the tests are performed.

The review of quality control data for tests that have an Individualized Quality Control Plan (IQCP) approved by the laboratory director must include an assessment of whether further evaluation of the risk assessment and quality control plan is needed based on the problems identified (eg, trending for repeat failures, etc.).

Evidence of Compliance:

- ✓ Records of QC review including follow-up for outliers, trends or omissions

MOL.34516 Qualitative Cut-Off Verification

Phase II

For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is verified with every change in lot or at least every six months.

NOTE: The threshold value that distinguishes a positive from a negative result must be verified with every change in lot (eg, new master mix), instrument maintenance, or at least every six months. Note that a low-positive control that is close to the threshold value can satisfy this checklist requirement, but must be external to the kit (eg, weak-positive patient sample or reference material prepared in appropriate matrix).

Evidence of Compliance:

- ✓ Written procedure for verification of the cut-off value **AND**
- ✓ Records of verification of cut-off value at defined frequency

MOL.34557 Control Storage


Phase I

Controls are stored in a manner that maintains their integrity.

PROCEDURES AND TEST SYSTEMS

RESTRICTION ENDONUCLEASES

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of restriction endonuclease digestion records
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MOL.34580 Restriction Endonuclease Digestion Confirmation

Phase II

The completeness and accuracy of restriction endonuclease digestion are confirmed, when appropriate.




NOTE: The treatment of DNA with restriction endonucleases (RE) must be performed for an appropriate amount of time and under appropriate reaction conditions, ie, to guard against non-specific activity. The efficacy of RE digestion must be established for each new lot of enzyme and in each run. Buffers must be used before their expiration date and properly stored.

Evidence of Compliance:

- ✓ Written policy defining conditions under which RE should be used **AND**
- ✓ Records of confirmation of efficacy of RE digestion with each new lot of enzyme and in each run

ELECTROPHORESIS

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of electrophoresis policies and procedures
	<ul style="list-style-type: none"> • Gel images (sufficient resolution/quality)
	<ul style="list-style-type: none"> • How does your laboratory prevent degradation of the nucleic acid sample used for electrophoresis?

MOL.34990 Loading Nucleic Acids **Phase I**

Standard amounts of nucleic acid are loaded on analytical gels, when possible.

MOL.35050 Molecular Weight Markers **Phase II**

Known molecular weight markers that span the range of expected bands are used for each electrophoretic run.

Evidence of Compliance:

- ✓ Records of appropriate markers with each run

MOL.35100 Visual/Fluorescent Markers **Phase II**

Visual or fluorescent markers are used to determine the endpoint of gel electrophoresis.

MOL.35150 Electrophoretic Gel Interpretation **Phase I**

Electrophoretic gels are interpreted using objective criteria.

Evidence of Compliance:

- ✓ Written procedure including interpretive criteria for gels

REFERENCES




- 1) American Association of Blood Banks. Standards for parentage testing laboratories. Bethesda, MD: AABB, 1998:7.100, 7.500
- 2) Cossman J, *et al.* Gene rearrangements in the diagnosis of lymphoma/leukemia. Guidelines for use based on a multiinstitutional study. *Am J Clin Pathol.* 1991;95:347-354

MOL.35175 Gel Image Resolution **Phase II**

The gel images are of sufficient resolution and quality (low background, clear signal, absence of bubbles, etc.) to permit the reported interpretation.

TARGET AMPLIFICATION/POLYMERASE CHAIN REACTION (PCR)

Inspector Instructions:

 <p>READ</p>	<ul style="list-style-type: none"> • Sampling of amplification/PCR policies and procedures
 <p>OBSERVE</p>	<ul style="list-style-type: none"> • Physical containment practices (frequent glove change, separate manipulation of pre- and post-specimens, dedicated pipettes)
 <p>ASK</p>	<ul style="list-style-type: none"> • How does your laboratory distinguish a true negative from a false negative result?

MOL.35350 Carryover

Phase II

Nucleic acid amplification procedures (eg, PCR) minimize carryover (false positive results) by using appropriate physical containment and procedural controls.

NOTE: This item is primarily directed at ensuring adequate physical separation of pre- and post-amplification samples to avoid amplicon contamination. The extreme sensitivity of amplification systems requires that the laboratory take special precautions. For example, pre- and post-amplification samples should be manipulated in physically separate areas; gloves must be worn and frequently changed during processing; dedicated pipettes (positive displacement type or with aerosol barrier tips) must be used; and manipulations must minimize aerosolization. Enzymatic destruction of amplification products is often helpful, as is real-time measurement of products to avoid manual manipulation of amplification products.

Evidence of Compliance:

- ✓ Written procedure that defines the use of physical containment and procedural controls as applicable to minimize carryover

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.35360 Internal Controls - Nucleic Acid Amplification

Phase II

In all nucleic acid amplification procedures, internal controls are run to detect a false negative reaction secondary to extraction failure or the presence of an inhibitor, when appropriate.

NOTE: The laboratory should be able to distinguish a true negative result from a false negative due to failure of extraction or amplification. Demonstration that another sequence can be successfully amplified in the same specimen should be sufficient to resolve this issue. For quantitative amplification assays, the effect of partial inhibition must also be addressed.

The internal control should not be smaller than the target amplicon.

Evidence of Compliance:

- ✓ Written procedure defining use of internal controls **OR** records of assay validation and monitoring statistics for test result trends

MOL.35370 Melting Temperature




Phase I

For tests that generate a result based on a T_m , appropriately narrow temperature ranges (+/- 2.5 °C) are defined and recorded each day of use.

ARRAYS

Arrays include a variety of reverse and forward hybridization formats. Reverse hybridization arrays use multiple unlabeled probes on a solid support to investigate a patient sample that carries a label, either direct (fluorescent or radioactive) or indirect (affinity labels such as biotin, digoxigenin, etc.). Another form of array involves multiple real-time amplification assays to measure multiple targets simultaneously. Controls for arrays monitor those steps carried out by the laboratory (sample preparation and labeling, hybridization and detection) and by the manufacturer (assay preparation, detection and hybridization reagents). Manufacturers also contribute to QC by producing products under good manufacturing procedures (GMP), providing control material for each analyte, and by providing sequence information or confirmatory tests to resolve ambiguous results.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of array procedures, including analytical wet bench and bioinformatics processes • Sampling of array performance monitoring records • Sampling of patient test reports
	<ul style="list-style-type: none"> • How does your laboratory validate assay performance prior to test implementation? • What processes are used to monitor ongoing assay performance?
	<ul style="list-style-type: none"> • Review records of assay performance monitoring. If any problems are found during review of performance monitoring records, or when asking questions, further evaluate the laboratory's investigation and resolution.

****NEW** 09/17/2019**

MOL.35400 Validation Studies for DNA-Based Copy Number Array - Specimen Types

Phase II

Validation studies for DNA-based copy number arrays are performed for each specimen type that can be affected by different preanalytic variables, that requires different processes for DNA extraction, and for those specimens with potentially interfering substances (eg, FFPE tissue, decalcified tissue, tissue containing melanin or mucin).

NOTE: A number of preanalytic and analytic processing variables can significantly influence the quality and integrity of nucleic acids extracted from a specimen. Commonly tested tissue sources must be included in the validation, but it is not expected for the laboratory to include every tissue source that could be examined by the assay.

It is the responsibility of the laboratory director or designee meeting CAP director qualifications to determine when a separate validation is needed versus a limited study to demonstrate that the DNA obtained from the specimen performs the same. For example, an array platform that has been originally validated to detect constitutional copy number abnormalities from peripheral blood will require a separate full validation to detect somatic alterations but may only require a more limited study to allow for a different specimen such as saliva to be used.

If an array has been validated for constitutional copy number alterations from fresh/frozen tissue, every potential tissue source (lung, liver, kidney, etc.) does not require separate validation, unless they potentially include interfering substances (eg, mucin).

Validations can be augmented by, but not supplanted with, additional reference materials (eg, characterized cell lines, cell lines with spiked in nucleic acids). Matrix-appropriate samples must be included.

Evidence of Compliance:

- ✓ Records of validation studies

****NEW** 09/17/2019**

MOL.35420 Assay Performance Monitoring

Phase I

Assay performance is monitored for each run and quality metrics are verified prior to reporting results.

NOTE: The monitoring of assay performance includes the review and recording of the quality metrics of each run. This may include:

- *DNA labeling verification (using detection of label, purification and quantitation of labeled DNA fragments, or electrophoretic techniques)*
- *Review of DLRs (Derivative Log Ratio)*
- *Genotyping performance (SNP arrays only)*
- *Number of suboptimal samples*
- *Monitoring the number of copy number alterations per sample*
- *Other quality metrics provided by the array software*

Criteria for acceptable performance must be defined. This includes hardware and analytical software.

Evidence of Compliance:

- ✓ Written procedure for monitoring assay performance with defined acceptability criteria **AND**
- ✓ Records of verification

REFERENCES

- 1) South ST, Lee C, Lamb AN, Higgins AW, Kearney HM, Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG standards and guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med.* 2013; 15(11):901-9.
- 2) Cooley LD, Lebo M, Li MM, Slovak ML, Wolff DJ, Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. [American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders.](#) *Genet Med.* 2013; 15(6):484-94.
- 3) Vermeesch JR, Fiegler H, de Leeuw N, Szuhai K, Schoumans J, Ciccone R, Speleman F, Rauch A, Clayton-Smith J, Van Ravenswaaij C, Sanlaville D, Patsalis PC, Firth H, Devriendt K, Zuffardi O. Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet.* 2007 Nov;15(11):1105-14
- 4) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications.* 1st ed. CLSI guideline MM21-ED1. Clinical and Laboratory Standards Institute, Wayne, PA; 2015.

****NEW** 09/17/2019**

MOL.35430 Array Analytical Wet Bench Procedure

Phase II

There is a written procedure for performing the analytical wet bench process.

NOTE: The procedure must include:

- *A description of the analytical target regions (eg, targeted or genome-wide)*
- *A description of acceptable sample types (see MOL.35400)*
- *Methods and reagents used for isolating, labeling, and hybridization of nucleic acids, as applicable*
- *Controls (including in silico)*
- *Instrument software and version*
- *Acceptance and rejection criteria for the results generated by the wet bench. These should include criteria for determining when the wet bench process has failed or is suboptimal.*
- *Written procedure for any portion of the wet bench process performed by a referral laboratory, if applicable*

Evidence of Compliance:

- ✓ Written procedure(s) that describes the analytical wet bench process, including sections noted above, as applicable

****NEW** 09/17/2019**

MOL.35440 Array Analytical Bioinformatics Procedure

Phase II

The laboratory has a written procedure that describes the steps in recording the bioinformatics process (also termed pipeline) used to analyze, interpret, and report array findings.

NOTE: A bioinformatics pipeline includes all algorithms, software, scripts, parameters, reference sequences, and databases, whether in-house, vendor-developed, or open source.

The written procedure must describe the bioinformatics process(es) including, where applicable:

- Individual software applications (open source, proprietary, and custom scripts) and versioning
- Description of input and output data files for each step of the pipeline, including in silico control files or sources
- Annotations and their sources (eg, public or private databases, with versions used)
- Criteria and thresholds for detection of array findings (eg, minimum number of probes or genomic size for copy number variants)
- Determination of the limits of detection
- Additional scripts or steps used to connect discrete applications in the pipeline
- Quality control metrics, including batch or sample-specific metrics and acceptance and rejection criteria for the results generated by the analytical bioinformatics process. Criteria must be based on metrics and quality control parameters established during test optimization and utilized during validation
- Required corrective actions when results fail to meet the laboratory's acceptance criteria
- Limitations in the test methodology
- Written procedures for any portion of the array bioinformatics process performed by a referral laboratory or a commercial service provider, if applicable. This should include a written description of how the security of identifiable patient information (eg, HIPAA compliance) is ensured during transmission and storage of data by the referral laboratory or commercial service provider.

Evidence of Compliance:

- ✓ Written procedure that describes the analytical bioinformatics process, including sections noted above, as applicable

****NEW** 09/17/2019**

MOL.35450 Interpretation and Reporting of Array Findings

Phase I

There is a written policy for interpreting and reporting of array findings.

NOTE: The laboratory must have a written algorithm for classifying and interpreting the clinical significance of identified findings. The ACMG guidelines can be used for classification and interpretation of copy number variants in inherited disorders.

Genome-wide array analysis may yield genetic findings unrelated to the clinical presentation for which the patient is undergoing testing. The laboratory policy must describe which, if any, and for what reasons, findings unrelated to the clinical purpose for testing are reported and the method of communication to the ordering physicians and patients, as applicable.

The written policy must include indications for confirmatory testing. The laboratory must determine by confirmation studies during validation if and when confirmatory testing of identified findings should be performed.

Evidence of Compliance:

- ✓ Written procedure that describes the process used for classification, interpretation, and reporting of array findings **AND**
- ✓ Records of compliance with procedure for classification, interpretation, and reporting of findings **AND**
- ✓ Laboratory database of findings identified and/or reported **AND**
- ✓ Written policy that describes which, if any, and for what reasons, findings unrelated to the clinical purpose for testing are reported

REFERENCES

- 1) South ST, Lee C, Lamb AN, Higgins AW, Kearney HM, Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG standards and guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med.* 2013; 15(11):901-9.
- 2) Cooley LD, Lebo M, Li MM, Slovak ML, Wolff DJ, Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. [American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders.](#) *Genet Med.* 2013; 15(6):484-94.

- 3) Rehder CW, David KL, Hirsch B, Toriello HV, et al. American College of Medical Genetics and Genomics: standards and guidelines for documenting suspected consanguinity as an incidental finding of genomic testing. *Genet Med.* 2013; 15(2):150-2.
- 4) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed.* CLSI guideline MM21-ED1. Clinical and Laboratory Standards Institute, Wayne, PA; 2015.

****REVISED** 09/17/2019**

MOL.35785 DNA Copy Number Array Report Elements

Phase II

Reports for DNA copy number analysis using arrays include the following elements:

1. Platform used
2. Genome build used
3. Analysis and/or reporting strategy: Detection and/or reporting criteria for copy number and homozygosity (if applicable), including number of probes and/or size limitations
4. ISCN-or HGVS-compliant nomenclature for reported findings (see note)
5. References to any databases used
6. A statement on the need for genetic counseling when indicated
7. A statement recommending further testing when indicated
8. Clinical significance of DNA copy number changes, when applicable

NOTE: Platform information includes, but is not limited to manufacturer, array version, number of probes, average probe spacing, SNP content, and targeted regions. Analysis strategy includes the copy number controls methodology: comparative/competitive or in silico.

Complex findings should be reported using ISCN-compliant nomenclature (eg, table format, "cth" for chromothripsis), when possible. HGVS nomenclature can be used to report copy number variants, including whole exon or whole gene copy number changes.

REFERENCES

- 1) South ST, Lee C, Lamb AN, Higgins AW, Kearney HM, Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG standards and guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med.* 2013; 15(11):901-9.
- 2) Cooley LD, Lebo M, Li MM, Slovak ML, Wolff DJ, Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders. *Genet Med.* 2013; 15(6):484-94.
- 3) Vermeesch JR, Fiegler H, de Leeuw N, Szuhai K, Schoumans J, Ciccone R, Speleman F, Rauch A, Clayton-Smith J, Van Ravenswaaij C, Sanlaville D, Patsalis PC, Firth H, Devriendt K, Zuffardi O. Guidelines for molecular karyotyping in constitutional genetic diagnosis. *Eur J Hum Genet.* 2007 Nov;15(11):1105-1114
- 4) McGowan-Jordan J, Simons A, Schmid M, eds; International Standing Committee on Human Cytogenomic Nomenclature. *ISCN: An International System for Human Cytogenomic Nomenclature (2016)*. Basel, New York: Karger; 2016.
- 5) Shaffer LG, McGowan-Jordan J, Schmid M, eds; International Standing Committee on Human Cytogenomic Nomenclature. *ISCN 2013: An International System for Human Cytogenomic Nomenclature*. Basel, New York: Karger; 2013.
- 6) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. *Fed Register.* 2003(Jan 24):1043-1044 [42CFR493.1276], 1047-1048 [42CFR493.1291]
- 7) Clinical and Laboratory Standards Institute. *Genomic Copy Number Microarrays for Constitutional Genetic and Oncology Applications. 1st ed.* CLSI guideline MM21-ED1. Clinical and Laboratory Standards Institute, Wayne, PA, 2015.
- 8) Sequence Variant Nomenclature. <http://www.HGVS.org/varnomen/> accessed 4/9/2018.

SANGER SEQUENCING AND PYROSEQUENCING

Inspector Instructions:



- Sampling of sequencing policies and procedures



- How does your laboratory ensure individual nucleotides are visualized adequately?
- How does your laboratory interpret sequence variation?

MOL.35790 Sequencing Lower Limit of Detection

Phase I

Testing is performed during assay validation to establish the approximate lower limit of detection for sequencing performed on mixed populations of cells (eg, in tumor samples), and the limit of detection is included in the laboratory report.

NOTE: Detection of 20% variant allele proportion, which is typically equivalent to a 40% proportion of heterozygous positive cells, is commonly cited as the LOD for Sanger Sequencing. For tumor samples, consideration of the percentage of tumor cells present in conjunction with the analytical LOD of the assay is essential for proper interpretation of a negative test result.

MOL.35795 Analysis of Tumor Cell Percentage and Sequencing Lower Limit of Detection

Phase II

For sequencing assays involving analysis of tumor cells, the laboratory considers the tumor cell percentage in cells, tissues, or the area of the slide from which the DNA is extracted and the analytical sensitivity of the assay when interpreting sequencing procedures, and conveys that information in the report and to the ordering provider as appropriate.

NOTE: Consideration of the percentage of tumor cells in light of the lower limit of detection of the sequencing procedure is essential for proper interpretation of a negative test result.

MOL.35800 Gene Information

Phase I

There is adequate information about the gene being tested regarding the reference sequence and reported pathogenic and benign variants.

NOTE: DNA sequencing assays should be reserved for those genes that have been adequately characterized in the literature and in genomic databases so that the complete reference sequence of the target region is known, as well as the identity and location of both clinically silent and clinically important sequence variants.

Evidence of Compliance:

- ✓ Records of literature references or databases for reference sequence and reported pathogenic and benign variants

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline*. 2nd ed. CLSI Document MM09-A2. Clinical and Laboratory Standards Institute. Wayne, PA; 2014.

MOL.35805 Sequencing Assay Optimization

Phase I

Sequencing assays are optimized to minimize background noise and achieve high signal to noise ratios to ensure a readable signal throughout the length of the target region and ready detection of sequence variants, especially those with low variant allele fraction (eg, from mixed cellularity tumors) near the stated limit of detection of the assay.

NOTE: Sequencing assays differ from most other molecular pathology assays in that many targets (individual nucleotides) are examined at once, rather than addressing a discrete

nucleotide site. Assay procedures must assure that each of these targets is visualized adequately to produce an unequivocal sequence readout, whether this is done by manual or automated methods. Single nucleotide variants with low allele fraction in particular may be overlooked if the signals are low or unequal. Approaches to prevent this problem include performing bidirectional sequencing of both sense and antisense strands or unidirectional coverage by replicate independent reads.

For sequence-based testing on mixed cellular populations, eg, tumor/normal, it is extremely important to distinguish low-level signals from analytical background noise. Therefore, special care must be taken to optimize the assay to minimize background noise, and to preserve adequate signal strength. In addition, because of formalin-induced DNA crosslinking, sequencing performed on DNA derived from FFPE tissue is prone to artifacts that could potentially lead to false positive results. Bidirectional sequencing is necessary to consistently achieve required accuracy in somatic applications.

Evidence of Compliance:

- ✓ Written procedure for performing sequencing assays detailing criteria for interpretation of heterozygous variants from mixed cell populations, as relevant **AND**
- ✓ Records of validation for sequencing assay optimization for the relevant specimen types

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline*. 2nd ed. CLSI document MM09-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.

MOL.35815 Sequencing Data Criteria

Phase I

Criteria are established for the acceptance and interpretation of primary sequencing data.

NOTE: Criteria for acceptance and interpretation of sequencing data must include correct assignments for non-polymorphic positions, definition of the sequencing region, criteria for peak intensity, baseline fluctuation, signal-to-noise ratio and peak shapes.

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline*. 2nd ed. CLSI document MM09-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.

MOL.35820 Sequence Interpretation Guidelines

Phase I

The laboratory follows professional guidelines for interpretation of sequence variation.

NOTE: The laboratory should have an algorithm for decision-making in interpretation of pathogenic variants, benign variants and variants of uncertain clinical significance. The ACMG guidelines for classification of variants should be used for interpretation of germline variants associated with inherited diseases. For clinical interpretation of somatic variants, such as in tumor samples, the laboratory should have a written protocol for variant interpretation that considers variant and patient-specific clinical/pathological factors.




REFERENCES

- 1) Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-24. doi: 10.1038/gim.2015.30.
- 2) COSMIC: Catalog of Somatic Mutations in Cancer. *Nucl. Acids Res*. gkq929 first published online October 15, 2010 doi:10.1093/nar/gkq929
- 3) Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19(1):4-23.

NEXT GENERATION SEQUENCING

Next Generation Sequencing (NGS) testing is comprised of two major analytical processes: (1) a wet bench process, including specimen handling, NGS library preparation and sequence generation; and (2) a bioinformatics ("dry bench") process, including base calling, sequence alignment or assembly, variant calling, variant annotation, and variant prioritization and/or interpretation performed with the aid of algorithms and software. The bioinformatics process may also include assignment of sequences for specific organisms or taxonomic groups, assignment of drug resistance sequences and variants, assignment of pathogenicity markers, and assignment of host response markers. In contrast to standard molecular tests where bioinformatics requirements are minimal, the large volumes of data produced by NGS, as well as the accompanying complex computational analyses, have created a need for new requirements specific to bioinformatics for record keeping, validation, quality control, quality monitoring, data storage, as well as assessment and implementation of new technology and software releases. Despite having separate requirements, the bioinformatics and wet bench processes are inextricably linked, and their combination is needed to achieve optimization and validation of the total NGS analytical testing process. The results from the total NGS analytical testing process are used for subsequent data analysis, interpretation and clinical reporting by the laboratory director.

Inspector Instructions:

<p>READ</p> 	<ul style="list-style-type: none"> • Sampling of next generation sequencing policies and procedures • Written policies and procedures for result reporting, including the reporting of secondary or incidental findings • Records of wet bench and bioinformatics dry bench validations and revalidations, including review of validations for components performed at referral laboratories • QM program records, including quality control and quality metrics, with corrective action when results fail to meet defined acceptable criteria • Sampling of exception log records • Sampling of patient reports for adherence to professional guidelines
<p>ASK</p> 	<ul style="list-style-type: none"> • What is your course of action when processes deviate from written procedures? • If all components of the testing process are not done at your laboratory, explain the distributive testing model used. • What are the software tools that comprise the NGS bioinformatics process? • As applicable, how and when does your laboratory perform confirmatory testing by an orthogonal method? • How does your laboratory ensure the security of internal and external storage and transfer of NGS data? • How can the reagents, methods, and instruments for processing and analyzing specimens be identified and traced? • How are versions of software used in the bioinformatics process traced? • How does the laboratory know when NGS data bioinformatics processing fails and at what step?
<p>DISCOVER</p> 	<ul style="list-style-type: none"> • Review computer log of NGS data processing for a sample to determine integrity of sequential processing from FASTQ to VCF file generation. Confirm that data is accessible and is being retained as defined in the written retention policy. • Review runs that were accepted and rejected. Follow records to determine if the steps taken follow the written procedure for corrective action.

PRIMARY/REFERRING LABORATORY REQUIREMENTS FOR NGS

This section of the checklist is used to inspect laboratories that perform overall assay design, validation, data analysis, interpretation and reporting of NGS testing, but also includes requirements that pertain to laboratories that send out, or refer portions, of the total NGS analytical testing process to referral laboratories.

****REVISED** 09/17/2019**

MOL.35840 Next Generation Sequencing (NGS) Referral Laboratory Selection

Phase II

The laboratory has a written policy for selection and evaluation of referral laboratories for NGS testing.

NOTE: The laboratory director, in consultation with the institutional medical staff or physician clients (where appropriate), is responsible for the selection and evaluation of referral laboratories.

Referral may include the total NGS analytical testing process or portions of the process (eg, only the wet bench or bioinformatics portions).

For laboratories subject to US regulations referring the total NGS analytical testing process, or portions of the process (eg, only the wet bench or bioinformatics portions), referrals must be made to a CLIA-certified laboratory or a laboratory meeting equivalent (or more stringent) requirements as determined by the CAP and/or the Centers for Medicare and Medicaid Services (CMS).

For non-US CAP accredited laboratories, referral of the total NGS analytical testing process, or portions of the process (eg, only the wet bench or bioinformatics portions) must be sent to a laboratory accredited by the CAP, or a laboratory meeting equivalent requirements as determined by the CMS, or accredited by an established international standard from a recognized organization, or certified by an appropriate government agency. The inspector may need to exercise judgment in determining the acceptability of referral laboratory accreditation.

The certification or accreditation of referral laboratories must include all applicable portions of the NGS testing process performed to meet the intent of the requirement.

Evidence of Compliance:

- ✓ Records of evaluations of referral laboratories for NGS referral testing **AND**
- ✓ Copies of valid CLIA certificates from CLIA-certified referral laboratories **OR**
- ✓ Copies of valid CAP accreditation certificates from CAP accredited referral laboratories **OR**
- ✓ Copies of valid accreditation equivalency as determined by CMS **OR**
- ✓ Copies of valid accreditations and certifications from established international organizations and/or government agencies

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services, Clinical laboratory improvement amendments of 1988; final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1242(c)]
- 2) Clinical and Laboratory Standards Institute. *Quality Management System: Qualifying, Selecting and Evaluating a Referral Laboratory; Approved Guideline*. 2nd ed. CLSI document QMS05-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2012.

MOL.35845 Tracking of Specimens Referred for NGS Testing

Phase I

The laboratory has records for the tracking of each specimen referred to other laboratories as part of NGS testing.

NOTE: Specimens referred for NGS testing may undergo total NGS analytical testing, or portions of the testing (eg, only the wet bench portion or the bioinformatics portions) or sub-portions therein. For example, a laboratory may convert a specimen into high quality DNA and then send the DNA sample to a referral laboratory for sequencing. A referral laboratory may convert

the DNA into an NGS library and perform sequencing to generate file formatted sequencing reads (eg, FASTQ files) and send it to another referral laboratory to perform bioinformatics to align reads to a reference sequence and identify and annotate variants. There must be records of each of these transfer steps between the primary/referring and recipient referral laboratories to describe unambiguously when and how specimens and data (including file formats) are transferred and/or exchanged. Labeling of the sample, material, or file must comply with COM.06200.

Evidence of Compliance:

- ✓ Records of testing workflow and methods for specimen handling, chain of custody, and data transfer starting from initial NGS test order to the final report

****REVISED** 09/17/2019**

MOL.35850 NGS Confirmatory Testing

Phase I

The laboratory has a written policy that describes the indications for confirmatory testing of reported variants, organisms, and markers of drug resistance, pathogenicity, and host response.

NOTE: The laboratory must determine by confirmation studies during validation if and when confirmatory testing of NGS-identified variants should be performed. While Sanger sequencing is commonly used for confirmation, other methods (eg, allele-specific PCR, melting curve analysis, alternative NGS chemistries) are acceptable.

The laboratory must also determine by confirmation studies during validation if and when confirmatory testing of detected organisms, significant sequence variants, markers of drug resistance or pathogenicity, and host response should be performed. Confirmatory methods could include PCR, alternative sequencing methods, culture, or other methods.

If the laboratory concludes during validation that confirmatory testing is not necessary, the rationale for, and data supporting this must be recorded. It is recognized that the need for confirmation may change over time (for example, as a result of changes in technology) and the rationale for, and data supporting a change in confirmation policy must be recorded.

Evidence of Compliance:

- ✓ Policy that describes the indications for confirmatory testing **AND**
- ✓ Records of compliance with confirmatory testing policy **AND**
- ✓ Records of review of correlation of NGS test results and confirmatory test results over time

REFERENCES

- 1) Lincoln SE, Truty R, Lin CF, et al. A rigorous interlaboratory examination of the need to confirm next generation sequencing - detected variants with an orthogonal method in clinical genetic testing. *J Mol Diag.* 2019;21(2):318-29.

GENERAL REQUIREMENTS FOR NGS

This section of the checklist is used to inspect laboratories performing any component of NGS testing.

MOL.35860 Exception Log/Record

Phase I

The laboratory maintains an exception log or record for patient specimens for which steps deviate from written procedures.

NOTE: The exception log or record should retain links to the patient case and must include records of each deviation, reason(s) for the deviation, and records of review of the exception by the laboratory director or designee(s) with comment on any issues or corrective action taken as a result of these reviews.

Evidence of Compliance:

- ✓ Records of review of the exception log/record by the laboratory director or designee **AND**
- ✓ Records of any issues and corrective action taken as a result of these reviews

****REVISED** 06/04/2020****MOL.35865 NGS Data Transfer Confidentiality****Phase II****The laboratory ensures that internal and external storage and transfer of NGS data maintains patient confidentiality, security, and data integrity.**

NOTE: It is recognized that laboratories may transfer NGS sequencing data, by physical shipment or electronic means, to referral laboratories for analysis and/or to external companies for storage, including through cloud-based computing.

Procedures must be in place to ensure confidentiality of patient data, including data encryption, use of secure and encrypted protocols for electronic data transfer (eg, SFTP, HTTPS, FTPS), system and user authentication, activity logs, access restrictions, and appropriate data backups. These procedures must ensure that patient confidentiality is maintained and meets national, federal, state (or provincial), or local laws and regulations as applicable (eg, HIPAA).

Methods to check data integrity, such as a hash/checksum (eg, MD5) pre and post-transfer of files during NGS workflow, must be implemented to prevent modification/deletion of data and to ensure that complete and intact unmodified data have been transferred.

Evidence of Compliance:

- ✓ Records of security parameters and protocols for NGS data transmission and storage locations, including encryption, control of physical and virtual access to data, system backups and redundancy **AND**
- ✓ Records showing data integrity of transferred files **AND**
- ✓ Records of audit trails maintained on sent NGS data where the audit trail includes the associated data files, date/time stamp and, as applicable, user ID and sending and receiving systems **AND**
- ✓ Copies of any valid HIPAA Business Associate Agreements for referral laboratories or companies storing datasets

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Technology Security of in vitro Diagnostic Instruments and Software Systems; Approved Standard. 2nd ed.* CLSI document AUTO11-A2. Clinical and Laboratory Standards Institute, Wayne, PA, 2014.
- 2) Roy S, Coldren C, Karunamurthy A, et al. Standards and Guidelines for Validating Next Generation Sequencing Bioinformatics Pipelines: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn.* 2018;20(1):4-27.

****REVISED** 09/17/2019****MOL.35870 NGS Data Storage****Phase II****There is a written policy for retention of NGS data necessary to support primary results generation and re-analysis.**

NOTE: The data retained must include the files used to analyze and generate reports. Specifically, sequence read files (eg, FASTQ, uBAM, BAM, CRAM) and variant calling files (eg, vCF, gVCF) must be retained for a minimum of two years. Other files that can be retained include specimen tracking and quality metrics data/files, sequencing run quality metrics reports, log or configuration file information regarding bioinformatics pipeline parameters and versions, exception log information, variants undergoing manual review, and files containing filtered and/or interpreted variants.

The retained files and records must be structured to facilitate inter-laboratory replication of the original analyses, annotations and/or interpretation, whether initiated by the laboratory or at the request of the referring physician or patient tested.

Importantly, longer lengths of retention may be necessary for specific clinical scenarios. For example, sequence read files on pediatric exome sequencing cases may require storage for several years for availability for re-analysis.

The written policy must include:

- *A description of the storage practices and retention times within each type of storage. This must include location of the storage (eg, physical location), accessibility, time for disaster recovery, and storage redundancy.*
- *A description of the file types being retained, including any compression that is used and whether compression is lossless or not. Evidence that compression does not affect the integrity of the data must be present in the validation.*

The policy must be in accordance with local, state, and federal requirements for storage of data, as applicable. A party external to the laboratory can prepare this policy if the laboratory outsources the NGS data storage.

Evidence of Compliance:

- ✓ Written policy that describes the NGS data storage procedures

ANALYTICAL WET BENCH PROCESS FOR NGS

This section of the checklist is used to inspect laboratories performing analytical wet bench processing of NGS specimens.

****REVISED** 09/17/2019**

MOL.36010 NGS Analytical Wet Bench Procedure

Phase II

There is a written procedure for performing the analytical wet bench process used to generate next generation sequencing data.

NOTE: The written procedure must include:

- *A description of the analytical target regions (eg, genes or organisms in a panel, exome, genome or other targeted regions, such as introns or promoter sites) or whether the procedure utilizes a metagenomic approach.*
- *A description of the evidence supporting the inclusion of the genes or targets analyzed and interpreted in an individual NGS test. An evidence based method to establish the strength of gene-disease associations is critical to facilitate an informed approach to the interpretation of genomic variants in panels, as well as exomes/genomes. The genes selected for analysis and interpretation in an individual NGS test should be based on the diagnostic purpose of the test and their relevance should be substantiated by evidence in the scientific literature and/or as established by expert consensus guidelines.*
- *A description of acceptable sample types for which the assay has been validated (eg, primary specimens, such as plasma, whole blood, body fluids, stool, tissue, saliva, and FFPE, or the type of cultured isolate, such as viruses, bacteria, mycobacteria, fungi, or positive broth culture), including a description of the minimal specimen requirements*
- *Methods and reagents used for isolating and quantitating nucleic acids and conversion into an NGS library, if applicable (including reverse transcription for RNA targets)*
- *Methods and reagents used for enrichment of target regions (eg, multiplex PCR based or oligonucleotide based capture), if applicable*
- *Methods and reagents used for depletion of host or unwanted nucleic acids (eg, oligonucleotide based depletion), if applicable*
- *Methods and reagents used for molecular indexing/barcoding of pooled samples, if applicable*
- *Controls used during analytical wet bench process, as applicable (eg, control to demonstrate limits of detection, controls to ensure adequate nucleic acid extraction and*

- detection of specific taxonomic classes, such as viruses, bacteria, mycobacteria, and fungi, or a control(s) with known variant(s))
- Sequencing platform and manufacturing versions of sequencing reagents and disposables (eg, flow cells, chips)
 - Instrument software and version used to generate on-instrument (primary) data and output format (eg, FASTQ files)
 - Acceptance and rejection criteria for the results generated by the wet bench analytical process. Criteria must be based on metrics and quality control parameters established during test optimization and utilized during validation. These should include criteria for determining when the analytical wet bench process has failed and the specimen is not processed further. Depending on the enrichment and sequencing approach used, these may include, but are not limited to: 1) post-fragmentation nucleic acid size distribution, 2) pre-capture library concentration and size distribution, 3) post-capture library concentration and size distribution, 4) final library quantification, 5) flow cell cluster density, 6) sequence read base quality scores, 7) sequence reads passing instrument quality filters, 8) total numbers of sequence reads generated, and 9) error rates.
 - Required corrective actions when results fail to meet the laboratory's acceptance criteria
 - Limitations in the test methodology
 - Complete written procedure for any portion of the NGS analytical wet bench process performed by a referral laboratory, if applicable

Evidence of Compliance:

- ✓ Written procedure(s) that describes the analytical wet bench process, including sections noted above, as applicable
- ✓ Written evidence base for genes analyzed and interpreted for each NGS test

****REVISED** 06/04/2020**

MOL.36015 NGS Analytical Wet Bench Process Validation

Phase II

The laboratory validates the analytical wet bench process and revalidates the entire process and/or confirms that the performance of the components of the process is acceptable when modifications are made.

NOTE: The output of the NGS analytical wet bench process is a collection of sequence data that requires additional bioinformatics processing and analysis to determine whether the sequence is of sufficient quality and quantity for the intended test. To determine this, and to ensure acceptable beginning-to-end test performance, validation of the NGS analytical wet bench process must be integrated with the bioinformatics process validation for the intended test (see MOL.36115).

The analytical wet bench process and the bioinformatics process for a test may occur within a single laboratory, or in a combination of primary and referral laboratories (see MOL.35840). Whether performed in a single laboratory, or in a distributive model involving primary and referral laboratories, the validations of the wet bench and bioinformatics processes for an intended test must be integrated to ensure acceptable beginning-to-end test performance. It is the responsibility of the laboratory director or designee meeting CAP director qualifications to review and approve all validations relevant to the intended test for processes performed within their laboratory, and to review all validations relevant to the intended test for processes performed in referral laboratories, if applicable.

Laboratories validating NGS tests must comply with MOL.31015 and/or MIC.64770, as applicable, and meet the requirement that validations are performed with samples for each type of specimen expected for the assay (eg, blood, fresh/frozen tissue, paraffin embedded tissue, prenatal specimens, saliva, culture isolates). Validations can be augmented by, but not supplanted with, additional reference standards (eg, cell lines such as NIST NA12878 or ATCC isolates). For assays in which detection of hotspot mutations is clinically meaningful, samples representing major hotspots must be included in the validation cohort.

- *Analytical validations must consist of a baseline methods-based validation that establishes the test's general performance for the detection of the sequence variant type(s) that the test is designed to identify. For tests that are offered for specific clinical indications (eg, diagnostic or antibiotic resistance gene panels) the laboratory should investigate whether any analyte, disease or gene specific needs exist that necessitate inclusion of additional specimens or reference samples in the validation. For example, this may include samples containing prevalent pathogenic variants, especially if they are technically difficult to accurately detect (eg, large indels). For detailed requirements on validation see MOL.36115 (NGS Analytical Bioinformatics Process Validation).*
- *Due to extensive microbial genetic variation and diversity, it is not possible to perform an NGS test validation that would assess the ability of the test to accurately and reliably detect every possible organism or variant that may be present in a specimen. To address this limitation, a methods-based approach can be used for validation wherein the specimens used for validation contain a representative spectrum of the types of organisms, resistance variants, pathogenic factors, and host-response markers that the test is designed to detect. For tests that are designed for organism detection, common pathogens found in a particular specimen type should be included, when feasible, in the validation to ensure their accurate detection. Similarly, for tests that analyze genes with common pathogenic mutations (eg, HIV reverse transcriptase K103N or CMV UL97 M460V/I) or expect to identify common resistance genes (eg, *S. aureus* *mecA* gene), specimens with those common mutations should be included, when feasible, in the validation. For broad-range methods, organisms of all significant taxonomic classes (eg, viruses, bacteria, mycobacteria, and fungi) should be included, when feasible, in the validation.*

In addition to determining analytical performance characteristics, metrics and quality control parameters must be established and used to assess wet bench process performance during validation. Metrics and parameters will vary between technology platforms and tests and typically include, but are not limited to: 1) NGS library preparations show the expected size distribution of fragments and are of adequate concentration for sequencing, 2) NGS library dilution procedures result in adequate cluster generation, 3) NGS instrument runs generate sufficient sequence reads with acceptable base qualities and error rates for the intended test.

Example worksheets for test design, optimization, validation, quality management, and bioinformatics for inherited disorder testing are available on cap.org in Member Resources - Precision Medicine (<https://www.cap.org/member-resources/precision-medicine>).

Validations, revalidations, and/or confirmations require written approval by the laboratory director (or designee that meets CAP director qualifications). Revalidation and/or confirmation may cover all or a subset of steps in the process depending on the extent of the modification. An example of a minor change is the introduction of a new lot of a previously validated capture reagent where equivalency can be established by sequencing previously tested samples and comparing all relevant performance metrics and parameters. Examples of major changes requiring more extensive revalidation are changing the sequencing platform or target enrichment method. The laboratory must demonstrate that equivalent or acceptable performance metrics are met when modifications in the wet bench process are made.

Evidence of Compliance:

- ✓ Records of validation and revalidation and/or confirmation studies, including documented sources of validation samples and/or reference materials (eg, samples with known sequence variants or reference standards such as NIST NA12878 or ATCC stains) used for validation, metrics and QC parameters used to establish and assess wet bench performance **AND**
- ✓ Written approval of validations, revalidations and/or confirmation studies **AND**
- ✓ Records of review of referral laboratory validations, if applicable

REFERENCES

- 1) Gargis, A, *et al.* Assuring the Quality of Next Generation Sequencing in Clinical Laboratory Practice. *Nature Biotechnology*. 2012; 30(11):1033-1036
- 2) Schrijver, I, *et al.* Opportunities and Challenges Associated with Clinical Diagnostic Genome Sequencing: A Report of the AMP. *J Mol Diagn*. 2012; 14(6):525-540
- 3) Rehm, HL, *et al.* ACMG clinical laboratory standards for clinical next generation sequencing. *Genet Med*. 2013; 15(9):733-747

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- 5) Richards CS, Palomaki GE, Lacbawan FL, *et al.* CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Three-year experience of a CAP/ACMG methods-based external proficiency testing program for laboratories offering DNA sequencing for rare inherited disorders. *Genet Med*. 2014;16:25-32.
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- 9) Schlberg R, Chui CY, Miller S, Procop GW, Weinstock G. The Professional Practice Committee and Committee on Laboratory Practices of the American Society for Microbiology, and the Microbiology Resource Committee of the College of American Pathologists. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection. *Arch Pathol Lab Med*. 2017; 141(6):776-786.
- 10) Santani A, Murrell J, Funke B, *et al.* Development and Validation of Targeted Next-Generation Sequencing Panels for Detection of Germline Variants in Inherited Diseases. *Arch Pathol Lab Med*. 2017; 141(6):787-797.
- 11) Hedge M, Santani A, Mao R, Ferreira-Gonzalez A, Weck KE, Voelkerding KV. Development and Validation of Clinical Whole-Exome and Whole-Genome Sequencing for Detection of Germline Variants in Inherited Disease. *Arch Pathol Lab Med*. 2017; 141(6):798-805.
- 12) Gandhi MJ, Ferriola D, Huang Y, Duke JL, Monos D. Targeted Next-Generation Sequencing for Human Leukocyte Antigen Typing in a Clinical Laboratory: Metrics of Relevance and Considerations for Its Successful Implementation. *Arch Pathol Lab Med*. 2017; 141(6):806-812.
- 13) Santani A, Simen BB, Briggs M, *et al.* Designing and Implementing NGS Tests for Inherited Disorders - a Practical Framework with Step-by-Step Guidance for Clinical Laboratories. *J Mol Diag*. December 18, 2018.
- 14) Jennings LJ, Arcila MD, Corless C, *et al.* Guidelines for Validation of Next Generation Sequencing-Based Oncology Panels: A Joint Commission Recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn*. 2017; 19(3):341-65.

MOL.36020 Wet Bench Process - Quality Management Program**Phase II**

The laboratory follows a written quality management (QM) program for the NGS analytical wet bench process.

NOTE: The laboratory records all deviations from the standard operating procedure, and assesses their impact on quality of the clinical assay. Corrective actions and exceptions must be recorded for every step that fails performance standards as set by the laboratory. The QM program must specify the controls, metrics, and quality control parameters that are used to monitor and assess each analytical run, as well as those that are monitored on a regular basis as decided by the laboratory (eg, weekly, monthly, quarterly). Metrics and QC parameters may include, but are not limited to: 1) NGS library fragment size distribution, 2) NGS instrument cluster densities, and 3) NGS instrument sequence output, base quality and error rates. All exceptions to standard operating procedures must be approved by the laboratory director or designee.

Evidence of Compliance:

- ✓ Written QM plan **AND**
- ✓ Records of QM program monitoring, including deviations and corrective action taken **AND**
- ✓ Records of review and approval of exceptions by the laboratory director or designee

REFERENCES

- 1) Gargis A, *et al.* Assuring the Quality of Next Generation Sequencing in Clinical Laboratory Practice. *Nature Biotechnology*. 2012; 30(11):1033-1036
- 2) Santani A, Simen BB, Briggs M, *et al.* Designing and Implementing NGS Tests for Inherited Disorders - a Practical Framework with Step-by-Step Guidance for Clinical Laboratories. *J Mol Diag*. December 18, 2018.

MOL.36030 Laboratory Records**Phase II**

Methods, instruments, and reagents used for processing and analyzing a sample (or batch of samples) can be identified and traced in the laboratory's records.

Evidence of Compliance:

- ✓ Records identifying methods, instruments, and reagents used throughout the entire testing process for a given sample (or batch of samples)

****REVISED** 09/17/2019****MOL.36035 Monitoring of Upgrades****Phase I**

There is a written procedure for monitoring, implementing, and recording upgrades to instruments, sequencing chemistries, and reagents or kits used to generate NGS data.

NOTE: The laboratory must have a written procedure to periodically monitor and implement upgrades. Congruent with that procedure, the laboratory must demonstrate that acceptable performance specifications are met when a procedure is changed. The extent of revalidation and/or confirmation is modification dependent. This should include on-board software that may affect downstream processing or QC of the resulting data. Revalidation/confirmation may cover all or a subset of steps in the process depending on the type of upgrade (see MOL.36015).

Evidence of Compliance:

- ✓ Procedure defining process for monitoring upgrades **AND**
- ✓ Records of monitoring activities **AND**
- ✓ Records of revalidation/confirmation data including the type of upgrade, and metrics and quality control (QC) parameters monitored to assess analytical run performance **AND**
- ✓ Approval of revalidation/confirmation data and of the change in protocol by the laboratory director **AND**
- ✓ Date of implementation

ANALYTICAL BIOINFORMATICS PROCESS FOR NGS

This section of the checklist is used to inspect laboratories performing the analytical bioinformatics process of NGS testing.

****REVISED** 09/17/2019**

MOL.36105 NGS Analytical Bioinformatics Procedure

Phase II

There is a written procedure that describes the steps included in recording the bioinformatics process (also termed bioinformatics pipeline) used to analyze, interpret, and report NGS test results.

NOTE: A bioinformatics pipeline includes all algorithms, software, scripts, parameters, reference sequences, and databases, whether in-house, vendor-developed, or open source.

The written procedure must describe the pipeline architecture and data flow including:

- *Individual software applications (open source, proprietary, and custom scripts) and databases used with versions and appropriate command line flags, or other configuration items needed to compile, install, and run the pipeline. Vendor/service provider of one or more components of the pipeline and their contact information, if applicable, should also be documented.*
- *Additional scripts or steps used to connect discrete applications in the pipeline*
- *Source code including version and evidence of using a source versioning system (eg, CVS, SVN, mercurial, or git), test case descriptions (eg, unit tests, positive tests, negative tests, stress tests, integration tests), and software validation execution results for laboratory-developed tools*
- *For applications that require sequence read alignment to a reference sequence, recording of the reference sequence assembly, version number, source, and URL from where the reference assembly was downloaded, and details of any modifications made to the reference file (eg, inclusion/removal of 'ChrUn,' 'ChrN random' 'and/or chrM from the file)*
- *Description of input and output data files and/or content (eg, parameters/flags and values) for the pipeline*

- Description of how provenance tracking is maintained from the beginning to the end of the bioinformatics procedure, including unique tracking of the testing location, patient sample, and run
- Description of database or files to capture "meta-data" or run and sample specific information (eg, samples on a run, bar code(s) assigned to a sample, run date)
- Criteria and thresholds used for determining the failure of a sample and/or terminating the downstream analytical process (eg, total coverage per sample, coverage distribution across target regions, average coverage, completeness of coverage, contamination of sample, failure of identity check)
- Criteria and specific thresholds used for inclusion or exclusion of variants in classification and interpretation steps following variant calling (eg, minimum read coverage depth, base or variant quality scores, variant allele fraction)
- Criteria and specific thresholds used for assigning a sequence to an organism at an appropriate level of taxonomic resolution (eg, CLSI MM18)
- Bioinformatics processes and thresholds applied for prioritizing and identifying reportable/clinically significant variants, biomarkers or genes, if applicable. Processes may include, but are not limited to, filtering variants based on population frequency, predicting variant impact on gene production or function using in silico algorithms (eg, derived from PolyPhen, SIFT), identifying regions of high homology or pseudogenes, variant prevalence and pathogenicity interpretations in databases, and variant functional class (eg, missense, inframe, nonsense, frameshift, etc.). In family studies, process steps are documented for prioritization of variants based on parameters including shared genomic segments, regions of identity by descent, inheritance patterns, and/or co-segregation of variants with patient phenotype. Processes where variants are prioritized or filtered based on phenotype or tumor histologic subtype should be documented.
- Bioinformatics processes and thresholds applied for prioritizing and identifying causal or candidate pathogens and resistance genes, if applicable. Processes may include, but are not limited to, filtering commensal organisms, filtering organisms present in the negative controls, and filtering organisms known to be laboratory, environmental, or reagent contaminants.
- Acceptance and rejection criteria for the results generated by the analytical bioinformatics process. Criteria must be based on metrics and quality control parameters established during test optimization and utilized during validation. These should include criteria for determining when the bioinformatics process has failed and the data are either re-processed or not further processed. These may include, but are not limited to: 1) base and mapping quality scores, 2) total number of reads and percentage of reads mapping to the target per sample, 3) duplicate read rate, 4) distribution of read coverage of recognized target(s) regions, 5) target regions with inadequate sequence due to mapping qualities and/or coverage below thresholds, 6) numbers and types of variants from reference, 7) contamination of sample, 8) failure of identity check, and 9) transition to transversion ratio in exome and genomes.
- Required corrective actions when results fail to meet the laboratory's acceptance criteria
- Limitations in the test methodology including those for each variant type assessed by the assay (eg, length of indels, copy number variant differences detectable by the assay)
- Written procedures for any portion of the NGS bioinformatics process performed by a referral laboratory if the primary laboratory outsources bioinformatics services (eg, a copy of the bioinformatics procedure used by the referral bioinformatics laboratory)

Evidence of Compliance:

- ✓ Written procedure that describes the analytical bioinformatics process, including sections noted above, as applicable

MOL.36108 Analysis of Tumor Cell and Sequencing Lower Limit of Detection

Phase II

For NGS assays involving analysis of tumor cells, the laboratory considers the tumor cell percentage in cells, tissues, or the area of the slide from which the DNA is extracted

and the analytical sensitivity of the assay when interpreting sequencing test results, and conveys that information in the report and to the ordering provider, as appropriate.

NOTE: Estimation and consideration of the percentage of tumor cells in conjunction with the lower limit of detection of the sequencing test is essential for proper interpretation of a negative test result.

****REVISED** 06/04/2020**

MOL.36115 NGS Analytical Bioinformatics Process Validation

Phase II

The laboratory validates the analytical bioinformatics process (also termed bioinformatics pipeline) and revalidates the entire process and/or confirms the performance of the components of the process as acceptable when modifications are made.

NOTE: The outputs of the NGS analytical bioinformatics process are data files containing information such as target read coverage, and numbers and types of variants.

The output of the NGS analytical bioinformatics process are used to determine if the sequence generated by the wet bench process is of sufficient quality and quantity for the intended test. To ensure acceptable beginning-to-end test performance, validation of the bioinformatics process must be integrated with the wet bench process validation for the intended test (see MOL.36015).

The analytical wet bench process and the bioinformatics process for a test may occur within a single laboratory, or in a combination of primary and referral laboratories/commercial service providers (see MOL.35840). Whether performed in a single laboratory, or in a distributive model involving primary and referral laboratories, the validations of the wet bench and bioinformatics processes for an intended test must be integrated to ensure acceptable beginning-to-end test performance. The validation should closely emulate the real-world environment of the laboratory in which the test is performed. It is the responsibility of the laboratory director or designee meeting CAP director qualifications to review and approve all validations relevant to the intended test for processes performed within their laboratory, and to review all validations relevant to the intended test for processes performed in referral laboratories or by commercial service providers, if applicable.

Methods-based validation:

- *Due to the diversity of human genetic variation (including germline and somatic mutation) and extensive microbial genetic variation and diversity, it is not possible to perform an NGS test validation that would assess the ability of the test to accurately and reliably detect every possible variant or organism that may be present in a specimen. To address this limitation, a methods-based approach can be used for validation wherein the specimens used for validation contain a sufficiently high number of variants that are representative of the different variant types (eg, single nucleotide variants, indels, copy number variants, and other structural variants such as translocations and inversions) and/or representative spectrum of the types of organisms the test is designed to detect. When using the same wet bench process for more than one test designed to detect the same types of variants (eg, the same targeted enrichment chemistry for different diagnostic gene panels, including whole exome panels), a single methods-based validation can be conducted combining results from more than one test. This approach allows for maximizing the number of variants tested and therefore maximizing statistical confidence in the assay's analytical performance.*
- *Specimens with known, well-characterized variants or targets within diagnostic genes that will be reported in one or more panels must be used for validation and the validation can be augmented by, but not supplanted with, reference materials (eg, NIST Genome in a Bottle samples). Additional samples/variants should be added for variant types that are under-represented in the specimens and reference materials used, particularly if they are difficult to accurately detect and identify by NGS (eg, indels and CNVs) or require specialized algorithms and software for accurate detection (internal tandem duplications, large structural alterations).*

- *Specimens used for analytical bioinformatics process validation can be complemented with, but not supplanted by, synthetic or in silico generated datasets containing variants*
- *For tests that analyze well established disease-associated genes with common pathogenic/clinically significant variants (eg, CFTR p.Phe508del, a mutation hotspot of clinical relevance in an oncology gene, HIV reverse transcriptase K103N, or CMV UL97 M460V/I) or expect to identify common resistance genes (eg, *S. aureus* mecA gene), specimens with common pathogenic variants as defined by the scientific literature and/or relevant experts (eg, published guidelines) should be included in the validation to demonstrate the test's ability to detect them. Merely including benign variants in the validation cohort is not sufficient to establish performance characteristics of the assay. Laboratories should record efforts to obtain specimens with common pathogenic variants.*

In addition, validations must:

- *Encompass the entire beginning-to-end bioinformatics process. Metrics and quality control parameters must be established and used to assess the bioinformatics process performance during validation. Metrics and parameters will vary between tests and typically include, but are not limited to: 1) base and mapping quality scores, 2) percentage of reads mapping to recognized target(s), 3) duplicate read rate, 4) read coverage of target regions, 5) target regions with inadequate sequence due to mapping qualities and/or coverage below thresholds, 6) numbers and types of variants from reference, and 7) transition to transversion (ts/tv) ratio in exome and genome, 8) evidence of demultiplexing (percent of reads that contain expected sample bar code index), 9) mean insert size and 10) ensuring integrity of identity. Per variant parameters include, but are not limited to: 1) depth of coverage, 2) allele fraction, 3) strand bias, 4) quality score, 5) number of distinct variants at the same position (multiple alternate alleles), and 6) number of variants within a prescribed cluster window size in base pairs.*
- *Provide a description of the analytical target (eg, exons, genes, targeted regions) and bioinformatics pipeline used for analysis, including algorithms, test scripts, and test or training datasets*
- *Be performed in an environment (eg, hardware, operating system, virtual machine, or cloud service) within which the pipeline is expected to be executed in clinical production*
- *Describe the sequence database containing the sequences and identifiers of the target organisms, resistance and pathogenicity markers, and host-responsive markers. Depending on the application, the database description may include representation of taxonomic groups, rare species, potentially confounding identical genotypes in clinically dissimilar organisms, potentially contaminating environmental and microbiota organisms, and the degree of taxonomic curation of the database. Resistance databases must be documented as to drugs and drug classes represented, and pathogen strains and/or genotypes, as clinically indicated.*
- *Ensure that individual sample identity is maintained throughout the bioinformatics pipeline. This includes indexes and barcodes when sample pooling methods are used, as well as identifiers for all file types used in the pipeline. Identifiers may be different depending on the laboratory workflow are used. Examples of identifiers that can be used individually or in combination include a unique patient identifier, a unique sample identifier, a unique run identifier, and/or a laboratory/location identifier to ensure provenance of sample identity.*
- *Establish criteria and thresholds for target calling (eg, minimum coverage of the target, position-wise read depth, base or variant quality scores, variant allelic fraction), and variation from the target sequence. Criteria may be differently defined based on application (eg, detection of germline versus somatic mutations, or pathogen detection versus drug resistance mutation identification)*
- *Determine and record how interference by specific sequence characteristics, such as highly homologous regions (eg, pseudogenes), low complexity regions (eg, GC rich regions) is mitigated or avoided. This may include custom approaches and/or orthogonal methods (eg, Sanger sequencing) to confirm test results in these regions.*

- Determine test performance characteristics for each variant type to be detected by the test (eg, single nucleotide variants, indels, copy number and other structural variants). Performance characteristics include, but are not limited to, test sensitivity, precision (reproducibility), and the percentage of false positive calls (which can be calculated from the number of true positives divided by the number of true positives plus the number of false positives). It is recommended that confidence intervals (CI) be established and recorded for performance characteristics (eg, analytical sensitivity=100%, CI=98.3-100%). The desired CI determines the number of samples needed for validation.
- Determine test performance characteristics for all microbial target types to be detected by the test (eg, organisms, resistance genes, pathogenicity markers, and host-response markers), as applicable. Performance characteristics include, but are not limited to, test sensitivity, specificity, and precision (reproducibility).
- Establish acceptance and rejection criteria for the results generated by the analytical bioinformatics process. Criteria must be based on metrics and quality control (QC) parameters established during test optimization and utilized during validation for the intended test (see above). These should include criteria for determining when the bioinformatics process has failed whether the data should be either re-processed or not further processed.
- Calculate true positives, false positives, true negatives, false negatives for each variant type.

Example worksheets for test design, optimization, validation, quality management, and bioinformatics for inherited disorder testing are available on cap.org in Member Resources - Precision Medicine (<https://www.cap.org/member-resources/precision-medicine>).

Validations, revalidations, and/or confirmations require written approval by the laboratory director or designee meeting CAP director qualifications. Revalidation and/or confirmation may cover all or a subset of steps in the process depending on the extent of the modification. For example, when implementing an upgrade of a previously validated application in the bioinformatics process, equivalency can be established by processing and analyzing datasets of previously tested specimens and comparing all relevant performance metrics. Examples of major changes requiring more extensive revalidation are changing the alignment algorithm or software for variant calling. The laboratory must demonstrate that equivalent or acceptable performance metrics are met when modifications in the bioinformatics process are made.

Evidence of Compliance:

- ✓ Records of validation and revalidation and/or confirmation studies, including metrics and QC parameters used to establish and assess bioinformatics performance **AND**
- ✓ Written approval of validations, revalidations and/or confirmation studies **AND**
- ✓ Records of review of referral laboratory validations, if applicable **AND**
- ✓ Records of software release notes and factory default settings for pipeline, if applicable

REFERENCES

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- 2) Gargis A, *et al.* Assuring the Quality of Next Generation Sequencing in Clinical Laboratory Practice. *Nature Biotechnology.* 2012; 30(11):1033-1036
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- 4) Gargis AS, *et al.* Good laboratory practice for clinical next generation sequencing informatics pipelines. *Nat Biotechnol.* 2015;33(7):689-93. doi: 10.1038/nbt.3237.
- 5) Mandelker D, Schmidt RJ, Ankala A, *et al.* Navigating highly homologous genes in a molecular diagnostic setting: a resource for clinical next generation sequencing. *Genet Med.* 2016;18(12):1282-1289.
- 6) Weck KE. The Next Generation of Molecular Pathology is Here: Validation of Next-Generation Sequencing Technology for Clinical Molecular Testing Across Multiple Different Disciplines. *Arch Pathol Lab Med.* 2017; 141(6):749-750.
- 7) Garcia EP, Minkovsky A, Yonghui J, *et al.* Validation of OncoPanel: A Targeted Next-Generation Sequencing Assay for the Detection of Somatic Variants in Cancer. *Arch Pathol Lab Med.* 2017; 141(6):751-758.
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- 9) Schlaberg R, Chui CY, Miller S, Procop GW, Weinstock G. The Professional Practice Committee and Committee on Laboratory Practices of the American Society for Microbiology, and the Microbiology Resource Committee of the College of American Pathologists. Validation of Metagenomic Next-Generation Sequencing Tests for Universal Pathogen Detection. *Arch Pathol Lab Med.* 2017; 141(6):776-786.
- 10) Santani A, Murrell J, Funke B, *et al.* Development and Validation of Targeted Next-Generation Sequencing Panels for Detection of Germline Variants in Inherited Diseases. *Arch Pathol Lab Med.* 2017; 141(6):787-797.

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- 13) Roy S, Coldren C, Karunamurthy A, et al. Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn.* 2018; 20(1):4-27.
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- 15) Santani A, Simen BB, Briggs M, et al. Designing and Implementing NGS Tests for Inherited Disorders - a Practical Framework with Step-by-Step Guidance for Clinical Laboratories. *J Mol Diag.* December 18, 2018.

****NEW** 09/17/2019**

MOL.36117 Infrastructure for the NGS Analytical Bioinformatics Pipeline

Phase I

There is a written procedure that describes the information technology (IT) infrastructure of the NGS analytical bioinformatics pipeline and its management.

NOTE: The analytical bioinformatics processes and pipelines for NGS are typically operated within a larger clinical IT infrastructure. The infrastructure includes the hardware, software, networks, data centers, facilities, and related equipment used to operate and/or support IT services. The IT infrastructure of the bioinformatics pipeline and computer environment must be tested during validation and continuously monitored during clinical testing.

- *The test validation should be performed within an environment (eg, hardware, operating system, virtual machine, or cloud service) where the pipeline is expected to be executed in clinical production.*
- *Records containing the level of service (eg, uptime, monitoring, technical support availability) and redundancy (ie, for data storage and computers) provided by the IT infrastructure must be available.*
- *A disaster recovery procedure for the bioinformatics pipeline and files generated by the pipeline must be established, recorded, and tested (see GEN.43946)*

The written procedure must include:

- *Description of the IT infrastructure (eg, hardware, operating system, virtual environment, cloud service, or pipeline software dependencies, as applicable) where the pipeline is executed in clinical production. For example, number and internet protocol (IP) addresses of servers used to run the pipeline and provision data storage, processor and memory specifications of the services, operating system versions, server access protocol, and security policies. The information captured should be enough to allow for redeployment in an equivalent environment.*
- *Description of the disaster recovery plan and the downtime procedures.*
- *Description of the process to grant access to the pipeline environment and associated data and the process to ensure uninterrupted access by authorized users.*
- *Description of the process to ensure file integrity after electronic transmission (over network or via portable storage). For example, using md5 checksums to demonstrate data equivalency.*

Evidence of Compliance:

- ✓ Written procedure that describes the IT infrastructure and its management **AND**
- ✓ Records of testing the disaster recovery procedure

****REVISED** 06/04/2020**

MOL.36118 NGS Lower Limit of Detection

Phase II

Testing is performed during assay validation to establish the lower limit of detection for sequencing performed on mixed populations.

NOTE: The NGS limit of detection (LOD) for variants consists of two data points: 1) the minimum required depth of coverage at the variant site and 2) minimum variant allele fraction. Determination of the LOD is relevant to several clinical diagnostic scenarios. These include, but are not limited to, detection of somatic variants in tumor samples and cell free DNA, germline variant detection in chimerism and mosaicism, maternal blood screening for fetal trisomy, detection of antimicrobial resistance mutations, microbiome analyses, identification of pathogens by targeted or metagenomic approaches, and identification of the presence/absence of clinically relevant microbial genes.

Lower limit of detection for variants may vary based on variant type (eg, single nucleotide variants, indels, copy number variants and other structural variants, such as translocations and inversions) or target characteristics.

In the case of microbial testing, LOD may be influenced by organism genome size. During validation, determination of LOD is required for each variant and microbial target type that the assay is intended to detect. For antiviral drug resistance testing, determination of LOD must take into account the virus load and variant allele fraction.

Validation of LOD requires inclusion of samples whose variant allele fraction or percentage has been determined by orthogonal methods. Cell line mixtures, plasmid spike in studies, and the use of in silico NGS data sets may augment, but not supplant, the use of patient samples.

Evidence of Compliance:

- ✓ Records of validation used to establish lower limit of detection for sequencing performed on mixed populations **AND**
- ✓ Written approval of validations, revalidations and/or confirmation studies **AND**
- ✓ Records of review of referral laboratory validations, if applicable

REFERENCES

- 1) Roy S, Coldren C, Karunamurthy A, et al. Standards and Guidelines for Validating Next-Generation Sequencing Bioinformatics Pipelines: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn.* 2018;20(1):4-27.

MOL.36123 Identification of Causal Germline Genetic Variants by Exome and Genome Sequencing Phase II

The validation study demonstrates the ability to use exome or whole genome sequencing to identify causal germline genetic variants in patients suspected of having a heritable disorder.

NOTE: Strategies for identification of causal genetic variants include combinations of variant filtration and/or prioritization based on population frequency, predicted biological impact of a variant, variant segregation within affected and unaffected family members, evidence for genotype-phenotype correlation, presence within variant databases and patient phenotype. These strategies may be conducted by manual and/or software assisted approaches.

The validation study must demonstrate the ability to identify causal genetic variants in patient samples with known modes of inheritance (eg, dominant, recessive, X linked, and de novo patterns).

If testing and analysis will be performed jointly on sequencing data from a proband and biological relatives (eg, trio or quad analysis) the validation must include samples from biologically related family members with known phenotypes, genotypes, and inheritance patterns.

The use of cell lines and in silico data sets may augment, but not supplant, the use of patient samples.

Evidence of Compliance:

- ✓ Records of validation used to establish the ability of exome and genome sequencing to identify causal germline variants in patient samples with known modes of inheritance **AND**
- ✓ Written approval of validations, revalidations and/or confirmation studies **AND**
- ✓ Records of review of referral laboratory validations, if applicable

REFERENCES

- 1) Yang Y, Muzny DM, Reid JC, et al. Clinical Whole-Exome Sequencing for the Diagnosis of Mendelian Disorders. *N Engl J Med.* 2013;369(16):1502-1511.
- 2) Posey JE, Harel T, Liu P, et al. Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. *N Engl J Med.* 2017;5:376(1):21-31.

MOL.36125 NGS Bioinformatics Process/Pipeline - Quality Management Program**Phase II****The laboratory follows a written quality management (QM) program for the NGS bioinformatics process/pipeline.**

NOTE: The laboratory records all deviations from the standard operating procedure, and assesses their impact on quality of the clinical assay. Corrective actions and exceptions must be recorded for every step that fails performance standards as set by the laboratory. The QM program must specify the controls, metrics, and quality control parameters that are used to monitor and assess each analytical run, as well as those that are monitored on a regular basis as decided by the laboratory (eg, weekly, monthly, quarterly). Metrics and QC parameters may include, but are not limited to:

- Total reads generated for each sample compared to a reference average that is determined during assay validation
- Percent of reads aligned to target compared to a reference average that is determined during assay validation
- Percent of unique reads aligned to target (for targeted-capture assays) compared to a reference average that is determined during assay validation
- Average coverage of targeted bases compared to a reference average that is determined during assay validation
- Percent of bases covered at specific read depths (eg, 30X, 100X, 2000X) for germline or somatic variant tests compared to a reference average that is determined during assay validation
- Determination of test reproducibility (eg, identification of the same variants in a specimen) over time (eg, monthly, quarterly) by re-testing a subset of specimens
- For somatic cancer assays, monitoring of limit of detection controls for determination of assay sensitivity over time (eg, weekly, monthly, quarterly)

Evidence of Compliance:

- ✓ Written QM plan **AND**
- ✓ Records of QM program monitoring, including deviations and corrective actions taken **AND**
- ✓ Records of review and approval of exceptions by the laboratory director or designee

REFERENCES

- 1) Gargis A, et al. Assuring the Quality of Next Generation Sequencing in Clinical Laboratory Practice. *Nature Biotechnology.* 2012; 30(11):1033-1036
- 2) Gargis AS, et al. Good laboratory practice for clinical next generation sequencing informatics pipelines. *Nat Biotechnol.* 2015;33(7):689-93. doi: 10.1038/nbt.3237.
- 3) Santani A, Simen BB, Briggs M, et al. Designing and Implementing NGS Tests for Inherited Disorders - a Practical Framework with Step-by-Step Guidance for Clinical Laboratories. *J Mol Diag.* December 18, 2018.

****REVISED** 09/17/2019****MOL.36135 Bioinformatics Process/Pipeline - Updates****Phase I****The laboratory has a procedure for monitoring, recording, and implementing patch-releases, upgrades, and other updates to the bioinformatics pipeline.**

NOTE: NGS bioinformatics pipelines are comprised of multiple components - open source or commercial software packages, additional scripts, and databases for managing content and aspects of analysis and reporting. Due to the ongoing evolution of the field, laboratories need to establish a procedure for regular monitoring of updates, patch-releases, and other upgrades for each component of the pipeline. Congruent with that procedure, the laboratory must demonstrate that acceptable performance specifications are met when a change to the bioinformatics pipeline is implemented. The extent of revalidation and/or confirmation is modification dependent. For example, a small modification to the bioinformatics pipeline may include adding error logging

function to the pipeline, which would not be expected to change the output of the update software component or the global pipeline. In contrast, examples of a large modification include transition to a new sequence alignment algorithm or variant calling software, which would be expected to have a significant impact on the output of the pipeline and clinical test results.

Revalidation/confirmation may cover all or a subset of steps in bioinformatics pipeline depending on the type of upgrade implemented and where in the pipeline it is implemented (see MOL.36115). All processes of the bioinformatics pipeline downstream to the implemented update/change must be included in the revalidation. This procedure must designate specific monitoring intervals and address when such updates will be implemented. In addition, when a laboratory uses a referral laboratory or vendor for any component of the pipeline, there must be a procedure to report and record patch-releases, upgrades and updates made to the outsourced components.

Evidence of Compliance:

- ✓ Procedure for monitoring patch-releases, upgrades and updates, including outsourced components **AND**
- ✓ Records of monitoring activities **AND**
- ✓ Records of revalidation/confirmation data including the type and scale of upgrade, performance metrics, and quality control (QC) parameters monitored to assess analytical run performance **AND**
- ✓ Approval of revalidation/confirmation data by the laboratory director **AND**
- ✓ Dates of implementation

****REVISED** 09/17/2019**

MOL.36145 Version Traceability

Phase I

The specific version(s) of the bioinformatics process (pipeline) used to generate NGS data files are traceable for each patient report.

NOTE: NGS bioinformatics processes (pipelines) are typically comprised of a combination of different software packages, scripts, and databases. The versions and configuration of each component in the bioinformatics pipeline (eg, command line flags or other configuration items) must be traceable for each patient report. Records of each pipeline component do not need to appear in the patient report. Rather, it is acceptable to refer to the pipeline as a whole, using appropriate version nomenclature (eg, NGS Pipeline v1.0.1) that is maintained by the laboratory or provided by the pipeline vendor and/or included in log files generated with each analysis of a patient dataset. Version nomenclature designations must be unique to each version of pipeline components and configurations and can be provisioned using version control software (MOL.36105). Any changes to software packages, scripts, databases, configuration files or other pipeline components require tracking in the pipeline version control system (MOL.36105) and updating to a new version nomenclature (eg, NGS Pipeline v1.0.1. to v1.0.2).

Evidence of Compliance:

- ✓ Records identifying software packages, scripts, and databases with associated version numbers and configuration items for a given patient report, as appropriate

INTERPRETATION AND REPORTING OF NGS RESULTS

This section of the checklist is used to inspect laboratories that are responsible for the final interpretation and reporting of NGS test results.

****REVISED** 09/17/2019**

MOL.36155 Sequence Variants - Interpretation and Reporting

Phase I

Interpretation and reporting of sequence variants follows professional organization recommendations and guidelines.

NOTE: The laboratory must have a written algorithm for classifying and interpreting the clinical significance of identified targets or variants. This may include an algorithm for determining the strength of gene-disease (typically in the context of panels/exome/genome sequencing) or variant resistance relationships (typically in the context of infectious diseases testing), if applicable.

Human sequence variants must be reported using HGVS nomenclature and include the HUGO Gene Nomenclature Committee (HGNC) gene name, and a standard versioned reference identifier to the transcript/protein (eg, RefSeq Accession Number, Ensembl Transcript/Protein ID, or CCDS ID) that allows unambiguous mapping of the variant. The reference genome assembly and version number used for alignment and variant calling must be reported. Variant chromosomal position (ie, genomic coordinate) should be reported.

The CAP/ACMG guidelines should be used for classification and interpretation of germline variants in inherited disorders. The CAP/ACMG guidelines were not developed for the classification and interpretation of risk for pharmacogenetic variants. Separate guidelines developed by AMP/ASCO/CAP have been developed for the classification and interpretation of somatic variants. For classification and interpretation of somatic variants (eg, in tumors), the laboratory should have a written procedure that considers factors such as variant allele frequency in the tumor type and its evidence in the specific cancer (eg, as documented in the COSMIC database), gene specific functional data, evaluation of population-specific minor allele frequencies given the patient's race and ethnicity, availability of targeted therapy, and other relevant patient specific clinical pathological factors. For interpretation of germline variants, the laboratory should record the list of databases used, which may include, but is not limited to, the Human Gene Mutation Database (HGMD), ClinVar and any disease specific LOVDs used as reference. For pharmacogenetic variants, standardized nomenclature for reporting variants and predicted phenotypes should be adopted, and interpretive algorithms should be referenced appropriately.

Microbial variants should be reported according to the convention of the field (eg, HIV variants are reported using single letter amino acid codes, for example K103N). As in human genetics, the reference genome assembly and version number used for alignment and variant calling must be reported. For classification and interpretation of drug resistance variants, the laboratory should utilize an interpretive database that considers factors such as genotype-phenotype, genotype-therapy, and genotype-clinical outcome (eg, the Stanford HIV Drug Resistance Database, <https://hivdb.stanford.edu/>).

The written procedure describes the frequency of variant reassessments (can differ by clinical significance, eg, variants of uncertain significance may be assessed more frequently than pathogenic variants) and what actions are taken when reassessment results in a change in classification (eg, potential retro-active notification of the ordering physician).

Evidence of Compliance:

- ✓ Procedure that describes the process used for classification, interpretation, and reporting of sequence variants **AND**
- ✓ Records of compliance with procedure for classification, interpretation, and reporting of variants **AND**
- ✓ Laboratory database of variants identified and/or reported **AND**
- ✓ Records demonstrating adherence to procedure on the frequency of variant reassessments **AND**
- ✓ Records of actions taken when variants are reclassified

REFERENCES

- 1) ACMG Standards and Guidelines for Clinical Laboratories, <http://www.acmg.net>
- 2) Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomics in the Catalog of Somatic Mutations in Cancer. *Nucl. Acids Res.* Jan 2011;39 (Database issue):D945-50.
- 3) Human Genome Variation Society. Sequence Variant Nomenclature. <http://varnomen.hgvs.org/>. Last accessed March 13, 2017.

- 4) Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-24. doi: 10.1038/gim.2015.30.
- 5) Kalman LV, et al. Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting. *Clin Pharmacol Ther.* 2016;99(2):172-85.
- 6) Lauschke VM and Ingelman-Sundberg M. Requirements for comprehensive pharmacogenetics genotyping platforms. *Pharmacogenomics.* 2016;17(8):917-24.
- 7) Li MM, Datto M, Duncavage EJ, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017;19(1):4-23.

MOL.36157 Identification of Infectious Diseases - Interpretation and Reporting

Phase I

The laboratory has a procedure for the interpretation and reporting of organisms, resistance genes, markers of pathogenicity, and host-response markers.

NOTE: The laboratory must have a written algorithm for classifying and interpreting the clinical significance of identified organisms, genes associated with resistance and/or pathogenicity, and host-response markers. This may include an algorithm for determining the strength of correlation between organism and disease (eg, in the context of metagenomic sequencing) or between gene and phenotypic resistance (eg, in the context of antimicrobial resistance), if applicable.

The written procedure describes the frequency of reassessments (can differ by clinical significance, eg, organisms of uncertain clinical significance may be assessed more frequently than organisms known to be causative agents of particular infectious diseases) and what actions are taken when reassessment results in a change in classification (eg, potential retro-active notification of the ordering physician).

Evidence of Compliance:

- ✓ Procedure that describes the process used for classification, interpretation, and reporting of organisms, resistance genes, markers of pathogenicity, and host-response markers **AND**
- ✓ Records of compliance with procedure for classification, interpretation, and reporting of these targets **AND**
- ✓ Laboratory database of targets identified and/or reported **AND**
- ✓ Records demonstrating adherence to procedure on the frequency of result reassessments **AND**
- ✓ Records of actions taken when results are reclassified

MOL.36165 Reporting of Incidental or Secondary Findings

Phase I

The laboratory has a policy for reporting findings unrelated to the clinical purpose for testing (eg, incidental or secondary findings).

NOTE: Gene panel(s), exome, genome, and transcriptome sequencing may yield genetic findings unrelated to the clinical presentation for which the patient is undergoing testing. The laboratory policy must describe which, if any, and for what reasons, genetic findings unrelated to the clinical purpose for testing are reported and the method of communication to the ordering physicians and patients, as applicable.

Laboratories may follow ACMG recommendations for reporting a set number of genes or develop their own policy for reporting. Limiting sequence analysis to a panel of genes or organisms that are relevant to the diagnosis of a particular disease state (either with targeted sequencing or targeted bioinformatics analysis) may limit, but not eliminate the potential for identifying genetic findings unrelated to the clinical purposes for testing.

If applicable, the laboratory consent form should clearly describe the categories of secondary findings with a description of each category such as carrier status, adult onset and pharmacogenetic findings.

Evidence of Compliance:

- ✓ Policy that describes which, if any, and for what reasons, genetic findings unrelated to the clinical purpose for testing are reported **AND**

- ✓ Informed consent records for patients, if applicable




REFERENCES

- 1) Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2017;19(2):249-255.
- 2) Hedge M, et al. Reporting incidental findings in genomic scale clinical sequencing—a clinical laboratory perspective: a report of the Association for Molecular Pathology. 2015;17(2):107-17. doi: 10.1016/j.jmoldx.2014.10.004.

NEXT GENERATION SEQUENCING OF MATERNAL PLASMA TO IDENTIFY FETAL TRISOMY

This section applies to laboratories performing maternal blood screening to detect fetal trisomy using NGS technologies.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of test requisitions for completeness • Quality control records (positive and negative) for each analytical run • Records of test performance limits and quality control parameters • Sampling of patient reports for adherence to professional guidelines • Records of longitudinal monitoring and monitoring of targeted disorders • Test failure records and the procedures used to provide a clinically useful result
	<ul style="list-style-type: none"> • Describe the quality control materials that are included with each analytical run • How are the percentages of screen positive results for each disorder monitored? • What actions are taken if the rates significantly increase or decrease?
	<ul style="list-style-type: none"> • Review runs that were accepted and rejected. Follow records to determine if the steps taken follow the written procedure for corrective action. • Review long term monitoring trends. Follow trends to determine if the steps taken follow the written procedure for corrective action.

****REVISED** 09/17/2019**

MOL.36310 Requisitions - Gestational Age

Phase I

Requisitions require the gestational age estimate (based on ultrasound measurements), first day of the last menstrual period (LMP) or the estimated date of delivery (EDD).

NOTE: Relevant clinical validation studies included samples drawn only over a specific gestational age range (eg, 10 to 20 completed weeks gestation). Knowing the estimated gestational age allows for the exclusion of samples collected too early in gestation where the test has not been validated (or is not valid). Fetal fraction increases slightly between 10 and 20 weeks gestation, but this increase is not sufficiently large to require gestational age specific test interpretations. Although less data are available for late second trimester or third trimester pregnancies, they strongly suggest that these tests will be reliable later in gestation. Laboratories can modify risk estimates to be specific to the pregnancy's gestational age (eg, trisomies are more common in the first trimester than in the second trimester or term).

REFERENCES

- 1) Palomaki GE, Kloza EM, Lambert-Messartian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. *Genet Med* 2011; 13:913-920

- 2) Zimmermann B, Hill M, Gemelos G, *et al.* Noninvasive prenatal aneuploidy testing of chromosomes 13,18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn* 2012; 32:1233-1241
- 3) Norton ME, Brar H, Weiss J, *et al.* Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; 207; 137: e131-138
- 4) Rose NC, Mercer BM, Committee on Practice Bulletins - Obstetrics, Committee on Genetics, and the Society for Maternal-Fetal Medicine. Practice Bulletin No. 163: Screening for Fetal Aneuploidy. *Obstet Gynecol.* 2016; 127(5):e123-37.

****REVISED** 09/17/2019**

MOL.36320 Requisitions - Maternal Birth Date/Age

Phase I

Requisitions require maternal birth date or maternal age at estimated date of delivery.

NOTE: Maternal age is a useful patient identifier and is used as the primary information to establish the prior risk for common trisomies.

REFERENCES

- 1) Morris JK, Wald NJ, Watt HC. Fetal loss in Down syndrome pregnancies. *Prenat Diagn* 1999; 19:142-145
- 2) Morris JK, Savva GM. The risk of fetal loss following a prenatal diagnosis of trisomy 13 or trisomy 18. *Am J Med Genet A* 2008; 146:827-832
- 3) Savva GM, Walker K, Morris JK. The maternal age-specific live birth prevalence of trisomies 13 and 18 compared to trisomy 21 (Down syndrome) *Prenat Diagn* 2010; 30:57-64

****REVISED** 09/17/2019**

MOL.36330 Requisitions - Maternal Weight

Phase I

Requisitions require maternal weight.

NOTE: Maternal weight has a strong impact on fetal fraction (higher weight women have lower fetal fractions). This can reduce analytical sensitivity due to inadequate levels of fetal DNA. This can also result in lower separation between disomic and trisomic fetuses, thereby reducing analytical specificity.

REFERENCES

- 1) Palomaki GE, Kloza EM, Lambert-Messartian GM, *et al.* DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. *Genet Med* 2011; 13:913-920, (Supplementary Materials)
- 2) Ashoor G, Syngelaki A, Poon LC, *et al.* Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. *Ultrasound Obstet Gynecol* 2013; 41:26-32
- 3) Haghiaç M, Vora NL, Basu S, *et al.* Increased death of adipose cells, a path to release cell-free DNA into systemic circulation of obese women. *Obesity (Silver Spring).* 2012; 20:2213-2219.

MOL.36340 Requisitions - Parentage Information

Phase I

Requisitions require parentage information for analytical methods that use parental genotypes for interpretation or whose interpretation may be influenced by IVF techniques.

NOTE: Parentage information should include all biological scenarios including, but not limited to, IVF with surrogate egg donation, other IVF procedures, and use of a surrogate mother.

REFERENCES

- 1) Zimmermann B, Hill M, Gemelos G, *et al.* Noninvasive prenatal aneuploidy testing of chromosomes 13,18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn* 2012; 32:1233-1241
- 2) Bianchi DW, Platt LD, Goldberg JD, *et al.* Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012; 119:890-901

MOL.36350 Requisitions - Multiple Gestation

Phase I

Requisitions require clinical evidence of multiple gestations (eg, twins).

NOTE: For some testing methods, insufficient information may be available to provide interpretations for these pregnancies and the test should not be performed. Insufficient data are currently available to interpret results in triplet or higher number of multiple gestations. It might be useful to solicit information regarding demise of a co-twin, but data are currently insufficient to provide reliable guidance on the interpretation.

REFERENCES

- 1) Committee opinion no 545: noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol* 2012; 120:1532-1534
- 2) Canick JA, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn* 2012; 32:730-734
- 3) Le Conte G, Letourneau A, Jani J, et al. Cell-free fetal DNA analysis in maternal plasma as screening test for trisomies 21, 18, and 13 in twin pregnancy. *Ultrasound Obstet Gynecol*. 2018; 52(3):318-324.
- 4) Sarno L, Revello R, Hanson E, et al. Prospective first trimester screening for trisomies by cell-free DNA testing of maternal blood in twin pregnancy. *Ultrasound Obstet Gynecol*. 2016; 47(6):705-711.

MOL.36360 Requisitions - Family History**Phase I**

Requisitions require patient or family history of chromosomal abnormality (eg, translocation carrier, offspring with Down syndrome).

NOTE: All tests make the assumption that the mother is euploid for each of the autosomal chromosomes examined. In rare instances, this is not the case. Laboratories should collect this information to determine whether testing is appropriate, or whether the interpretation of results might need to be modified.

REFERENCES

- 1) Committee opinion no 545: noninvasive prenatal testing for fetal aneuploidy. *Obstet Gynecol* 2012; 120:1532-1534

MOL.36370 Requisitions - Prior Pregnancy Risk**Phase I**

Requisitions require prior pregnancy risk for aneuploidies for analytical methods that report odds, risks, or probabilities of being euploid or trisomic.

NOTE: Requisitions may collect prior risk (eg, Down syndrome risk reported after a first trimester combined test) or collect information that the laboratory can use to derive a prior risk (eg, maternal age and measurement of nuchal translucency).

****REVISED** 09/17/2019****MOL.36380 Quality Control Monitoring****Phase II**

Test performance limits and quality control parameters (eg, minimum number of acceptable reads, range of acceptable fetal fractions) are monitored.

NOTE:

- *These parameters are likely to be test-specific, but might include a range of acceptable fetal fraction, minimum amount of fetal DNA, minimum number of matched reads, and minimum read quality scores. Written procedures providing necessary actions (re-extracting, re-sampling, re-sequencing, and reporting a failed test) should accompany each quality control parameter.*
- *Analytical sensitivity or limits of detection of the assay for heterogeneous genotype samples (eg, maternal blood screening for fetal trisomy) must be determined. Please refer to MOL.36015.*
- *Any SNP based genotyping approaches using PCR should follow applicable requirements for performance of PCR as in the Target Amplification/Polymerase Chain Reaction (PCR) section of the Molecular Pathology Checklist.*

Evidence of Compliance:

- ✓ Records for performance limits and quality control parameters, with corrective action when defined limits are not met

REFERENCES

- 1) Zimmermann B, Hill M, Gemelos G, et al. Noninvasive prenatal aneuploidy testing of chromosomes 13,18, 21, X, and Y, using targeted sequencing of polymorphic loci. *Prenat Diagn* 2012; 32:1233-1241
- 2) Bianchi DW, Platt LD, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012; 119:890-901
- 3) Ehrich M, Declu C, Zwiefelhofer T, et al. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011; 204: 205 e201-211
- 4) Sparks AB, Struble CA, Wang ET, et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood; evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol* 2012; 206:319 e311-319

****REVISED** 09/17/2019****MOL.36390 Quality Control****Phase II**

Positive and negative controls are included in each analytical run.

NOTE: Within each run, separate positive and negative sample controls must be run. These may be patient derived or artificial. The positive control could be for a single disorder (eg, trisomy 21) and the target disorder should vary over time (eg, trisomy 13).

Evidence of Compliance:

- ✓ Records for ongoing results for positive and negative controls, with corrective action when defined limits are not met

****REVISED** 09/17/2019****MOL.36400 Longitudinal Monitoring of Assays****Phase I**

The laboratory performs longitudinal monitoring of assay characteristics.

NOTE: For quality management, laboratories should monitor assay performance parameters which are applicable to the specific method, such as median fetal fraction and proportion of samples falling above and below specified clinical cut-off levels, median z-score or normalized value for each tested chromosome, or proportion of male or female results for methods that assess fetal sex, or separation of median fetal fraction for singleton and twin pregnancies. Departures from the expected or routinely observed assay performance parameters should be investigated to identify potential causes for the change.

Evidence of Compliance:

- ✓ Records for ongoing longitudinal monitoring of test characteristics, with corrective action for assays that are outside of the assay performance parameters

REFERENCES

- 1) Knight GJ, Palomaki GE. Epidemiologic monitoring of prenatal screening for neural tube defects and Down Syndrome. *Clin Lab Med* 2003; 23:531-551, xi
- 2) Palomaki GE, Knight GJ, McCarthy J, et al. Maternal serum screening for fetal Down Syndrome in the United States: a 1992 survey. *Am J Obstet Gynecol* 1993; 169:1558-1562

****REVISED** 09/17/2019****MOL.36410 Monitoring of Targeted Disorders****Phase I**

The percentages of women with positive results for each targeted disorder (eg, Down syndrome), test failure rates (eg, low fetal fraction) and 'inconclusive' (eg, grey zone) test results are calculated and reviewed at least quarterly.

NOTE: Since this type of testing may be performed in a mixed risk population (eg, high or low risk woman in the general population), the proportion of women with positive results will likely vary by laboratory. If possible, laboratories should stratify test results and rates by indication of testing (eg, low risk, high risk). In many instances, the pregnancy is at high risk for only one or two of the trisomies, offering the opportunity to establish relatively robust general population positive rates (both initial positive and false positive) for at risk and not at risk for specific chromosome abnormalities. These rates may be compared to the expected positive rates based on prevalence and clinical sensitivity and specificity. Monitoring test failure and inconclusive rates may be chromosome-specific or combined.

Evidence of Compliance:

- ✓ Records for ongoing monitoring of relevant test characteristics, with corrective action when indicated

MOL.36420 Maternal Plasma DNA Reporting**Phase I**

Reports include qualitative and/or quantitative test results for each target chromosome (eg, z-score, fetal fraction, likelihood ratio), reference intervals or cutoff values as appropriate, and a summary set of risks/categorical interpretations.

NOTE: Extremely high (or low) risks should be avoided by "capping" risks that are high, or low values beyond which clinical decision-making is unlikely to be impacted (eg, < 1:20,000 or > 10:1). If possible, the final interpretation should include, or refer to, previous relevant test results.

****REVISED** 09/17/2019**

MOL.36430 Patient Report Information

Phase I

The patient report includes the following information:

- Screening result for each condition tested
- Recommendation for follow-up diagnostic testing for all pregnancies with a screen positive test result
- Recommendations regarding next steps for women with uninformative screen results and/or test failures.

NOTE: Due to the historical association of prenatal screening for Down syndrome and open neural tube defects, laboratories could consider adding a statement such as "This test is not intended to identify pregnancies at risk for open neural tube defects."

REFERENCES

- 1) Committee opinion no. 545: Noninvasive Prenatal Testing for Fetal Aneuploidy. *Obstet Gynecol* 2012; 120:1532-1534

HEMATOPOIETIC PROGENITOR CELL ENGRAFTMENT MONITORING

Inspector Instructions:



- Sampling of hematopoietic progenitor cell engraftment monitoring policies and procedures
- Sampling of hematopoietic progenitor cell engraftment testing records
- Sampling of QC records
- Sampling of hematopoietic progenitor cell engraftment reports for completeness

MOL.36435 Hematopoietic Progenitor Cell Engraftment

Phase II

For hematopoietic progenitor cell engraftment, the polymorphic nature and independent segregation (eg, location on separate chromosomes) of the DNA system used is detailed and recorded in the literature.

REFERENCES

- 1) Clark JR, Scott SD, Jack AL, et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br J Haematol.* 2015;168(1):26-37.

MOL.36440 Chimerism

Phase II

There are records of the accuracy of quantitative methods used to measure chimerism.

NOTE: The accuracy of quantitative methods used to measure chimerism must be verified at least annually by controlled blood mixing or other suitable method. If results on cell

subpopulations are reported, there must be records of periodic testing of the purity of such cell subsets.

MOL.36445 Negative Control Phase II

A negative control is used and evaluated for non-specific background with each run.

REFERENCES

- 1) Clark JR, Scott SD, Jack AL, et al. (2015), Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of Short Tandem Repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br J Haematol.* 2015;168:26-37.

****REVISED** 09/17/2019**

MOL.36450 Sensitivity Control Phase II

A sensitivity control is used and evaluated with each run.

NOTE: A low positive control may be used to meet this requirement.

MOL.36455 Internal Controls Phase II

For hematopoietic progenitor cell engraftment assays, internal controls are used to determine appropriate genotypes or at least to distinguish patient from donor(s) with each run.

NOTE: There must be criteria for the acceptance and rejection of the amplification of a particular genetic locus or individual sample.

Evidence of Compliance:

- ✓ Written procedure defining criteria for acceptance/rejection of amplification results

MOL.36460 Preferential Amplification Phase II

Reactions are optimized to avoid preferential amplification. The minimum amount of DNA is determined to obtain optimal sensitivity.

NOTE: Method validation must include a dilution study to evaluate the concentration of DNA to determine minimum sensitivity of the assay.

MOL.36465 Cell Subset Purity Phase II

If cell subset enrichment is performed, the patient report includes the actual or approximate purity of the cell subset.

NOTE: The determination of the actual or approximate purity of the cell subset does not imply that the purity determined in validation studies can be used without further evaluation. An actual measurement may be performed at the time of sample testing. Some isolation methods and cell subpopulations (eg, CD56) may not produce enough cells to test purity and run the monitoring engraftment test. At a minimum, the purity can be determined for each lot of reagent used to isolate the cell subset and then be reported as an approximate purity for that specific lot.

MOL.36470 Hematopoietic Progenitor Cell Engraftment Testing Phase II

For hematopoietic progenitor cell engraftment, samples from pre-transplant patient (recipient), pre-transplant donor(s), post-transplant patient, and an appropriate control are analyzed concurrently.

NOTE: Previously generated data from pre-transplant specimens may be used to compare to post-transplant results if a validated system is used to identify and link the appropriate data files for concurrent analysis.

Evidence of Compliance:

- ✓ Written procedure for hematopoietic progenitor cell engraftment testing

MOL.36475 Engraftment Analysis

Phase II

Prior to evaluating post engraftment specimens, the laboratory evaluates a specimen from the donor(s) and a pre-transplant specimen from the patient to determine the number of informative loci to test in order to meet the minimum number of loci needed for calculations.

Evidence of Compliance:

- ✓ Written procedure for hematopoietic progenitor cell engraftment testing **AND**
- ✓ Records of hematopoietic progenitor cell engraftment testing

MOL.36480 Preferential Allele Amplification

Phase II

Preferential allele amplification is considered in the interpretation of hematopoietic progenitor cell engraftment tests.

MOL.36490 Minimal Number of Informative Loci

Phase II

For hematopoietic progenitor cell engraftment testing, a minimum of three informative loci are routinely used in the calculations.

NOTE: There are exceptions to this rule. Informative loci refer to loci that can distinguish between donor(s) and recipient. An exception for the number of informative loci used may occur in syngeneic twins (donor(s) and recipient) and rarely in closely related donor(s) and recipient.

MOL.36495 Result Reporting

Phase II




For hematopoietic progenitor cell engraftment, the final report includes an appropriate summary of the methods, the loci tested, the number of informative loci used, the percent donor cells, an indication of any trace cells, and the sensitivity of the assay.

REFERENCES

- 1) Clark JR, Scott SD, Jack AL, et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br J Haematol.* 2015;168(1):26-37.
- 2) Organ Procurement and Transplantation Network (OPTN) Bylaws. Appendix C. Membership Requirements for Histocompatibility Laboratories. US Department of Health and Human Services. November 20, 2018.

RELATIONSHIP AND FORENSIC IDENTITY TESTING

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of relationship and forensic identity testing policies and procedures • Sampling of relationship and forensic identity testing data for completeness • Sampling of requisition/collection forms (include transfusion/transplant history) • Sampling of information/label verification records • Sampling of patient reports for completeness
	<ul style="list-style-type: none"> • Limited-access secured area for specimens and records
	<ul style="list-style-type: none"> • What is your laboratory's course of action when evaluating closely spaced alleles?

MOL.37430 Relationship and Forensic Identity Testing Specimen Collection

Phase II

Specimens collected for relationship and forensic identity testing are collected and processed meeting the following criteria:

1. Collections are performed by an unbiased third party, individual with no interest in the outcome of the case.
2. Collection materials are not in the possession of the tested parties at any time prior to, during, or following the collection procedure.
3. The specimens and accompanying documents are shipped to the testing laboratory directly by the collector.

Evidence of Compliance:

- ✓ Policies and procedures for specimen collection

REFERENCES

- 1) Standards for Relationship Testing Laboratories. 12th ed. American Association of Blood Banks. Bethesda, MD: 2016.

MOL.37442 Relationship and Forensic Identity Testing Specimen Collection Data

Phase II

For relationship and forensic identity testing, the following data are obtained during specimen collection for each person to be tested:

1. Printed name of person being tested
2. Alleged relationship, if applicable
3. Date of birth
4. Race/ethnic background with the exception of a child being tested
5. Place and date of specimen collection
6. Printed name, signature, and contact information of person(s) collecting and/or witnessing (if different) the specimen collection
7. Photograph or legible photocopy of a picture identification card for each individual tested (government issued ID or other photograph suitable for identification)

8. **History of transfusion in preceding three months or any history of allogeneic hematopoietic progenitor cell transplantation**
9. **Synopsis of case history/investigation, sample source, if applicable for forensic purposes**
10. **Record of informed consent from the individual being tested or individual with legal authority**

NOTE: If the laboratory uses prepackaged kits for specimen collection, any additional instructions that accompany the kit must be followed.

Evidence of Compliance:

- ✓ Policies and procedures for specimen collection **AND**
- ✓ Records of specimen collection for relationship and forensic identity testing

REFERENCES

- 1) Standards for Relationship Testing Laboratories. 12th ed. American Association of Blood Banks. Bethesda, MD: 2016.

MOL.37726 Relationship and Forensic Identity Testing Specimen Labeling Phase II

For relationship and forensic identity testing, information about each individual and the accuracy of the specimen label are verified by that individual or the legal guardian. The affixed label on each specimen contains the following:

1. **At least two unique identifiers, such that each specimen can be unmistakably identified from other specimens in the same case**
2. **Date of specimen collection**
3. **Initials or signature of the collector verifying the specimen integrity**

Evidence of Compliance:

- ✓ Records of information and label verification by patient or legal guardian

REFERENCES

- 1) Standards for Relationship Testing Laboratories. 12th ed. American Association of Blood Banks. Bethesda, MD: 2016.

MOL.37868 Specimen Verification Phase II

The condition of the specimen is recorded upon receipt in the laboratory including any evidence of tampering, adequacy of volume, and a firmly attached label bearing a unique identification.

MOL.38010 Secured Relationship and Forensic Records Phase II

For relationship and forensic identity testing, specimens are retained in a limited access, secured area and appropriate records of chain-of-custody retained.

Evidence of Compliance:

- ✓ Written policy addressing restricted access to relationship and forensic identity specimens and records **AND**
- ✓ Records of authorized personnel with access **AND**
- ✓ Records of chain-of-custody on patient reports

MOL.38152 Report Content Phase II

The report includes the individual paternity index for each genetic system, the combined paternity index, the probability of paternity as a percentage, prior probability of paternity used in calculations and the population used for comparison.

MOL.38294 DNA Results Interpretation Phase II

For relationship testing, DNA results (RFLP, STR, SNP) are interpreted twice, independently.

Evidence of Compliance:

- ✓ Written policy stating the requirement for a second, independent interpretation of DNA results **AND**
- ✓ Patient records/worksheets

MOL.38436 Exclusion Evaluation

Phase II

For relationship testing, exclusions based on closely spaced alleles (usually defined as less than one tandem repeat apart) are evaluated by co-electrophoresis or other methods.

Evidence of Compliance:

- ✓ Written procedures for the evaluation of closely spaced alleles **AND**
- ✓ Records of evaluation by secondary method

MOL.38578 Forensic Identity Testing Requirements

Phase II

For forensic identity testing, laboratory methods, test validation, personnel qualifications, interpretation, and reporting of results meet current guidelines.

NOTE: For laboratories subject to US regulations, these guidelines are provided by the DNA Advisory Board and the Scientific Working Group on DNA Analysis Methods (SWGDM). In the case of forensic identity testing, the appropriate degree, training, or experience in forensic science is required.

REFERENCES



- 1) DNA Advisory Board. Quality assurance standards for Forensic DNA testing laboratories. Federal Bureau of Investigations. 2011; <https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view>
- 2) Short tandem repeat (STR) interpretation guidelines. Ibid
- 3) Guidelines for mitochondrial DNA (mtDNA) nucleotide sequence interpretation. Ibid. 2003:5


IN SITU HYBRIDIZATION (ISH)

The use of the term in situ hybridization (ISH) in this section applies to all ISH methods, including fluorescence (FISH), chromogenic (CISH), silver (SISH), and brightfield (BRISH) in situ hybridization.

Please refer to the Definition of Terms section in the All Common (COM) Checklist for definitions of analytical validation and analytical verification.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of ISH policies and procedures • Sampling of probe validation/verification records • Sampling of QC records • Sampling of patient reports • Sampling of predictive marker assay validation, verification, and revalidation/verification studies • Records of annual benchmark comparison for breast predictive markers
	<ul style="list-style-type: none"> • How are ISH cut-off values established? • How does your laboratory validate/verify assay performance prior to test implementation? • How did you validate/verify the most recently added predictive marker on your test menu?

	<ul style="list-style-type: none"> • What is your course of action if a probe does not produce an internal control signal? <hr/> <ul style="list-style-type: none"> • Review a sampling of ISH cases and controls. Evaluate signal, background and morphology.
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****REVISED** 09/17/2019**

MOL.38600 ISH Probe Validation/Verification

Phase II

There are policies, procedures, and records of validation/verification of all *in situ* hybridization (ISH) probes.

NOTE: Refer to MOL.39323 for specific validation/verification requirements for tests that provide independent predictive information (eg, HER2 predictive marker testing in breast carcinoma). Additional requirements for test method validation/verification are in the All Common Checklist.

Evidence of Compliance:

- ✓ Written procedure for validation/verification of ISH probes

REFERENCES

- 1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2018 edition, Revised 01/2018.
- 2) Clinical and Laboratory Standards Institute. *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline*. 2nd ed. CLSI Document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.
- 3) Wiktor AE, Van Dyke DL, Stupca PJ, et al. Preclinical validation of fluorescence in situ hybridization assays for clinical practice. *Genetics in Medicine* 8:16-23, 2006.
- 4) Weremowicz S, Sandstrom DJ, Morton CC, Miron PM. Validation of DNA probes for preimplantation genetic diagnosis (PGD) by fluorescence in situ hybridization (FISH) R1. *Prenat Diagn*. 2006 Nov;26(11):1042-50.
- 5) Lawrence Jennings, Vivianna M. Van Deerlin, Margaret L. Gulley (2009). Recommended Principles and Practices for Validating Clinical Molecular Pathology Tests. *Archives of Pathology & Laboratory Medicine*: Vol. 133, No 5, pp. 743-755.
- 6) Saxe DF, Persons DL, Wolff DJ, Theil KS; Cytogenetics Resource Committee of College of American Pathologists. Validation of fluorescence in situ hybridization using an analyte-specific reagent for detection of abnormalities involving the mixed lineage leukemia gene. *Arch Pathol Lab Med*. 2012; 136(1):47-52.

MOL.38625 Interphase ISH - Cut-off Value

Phase II

For interphase *in situ* hybridization (ISH), the laboratory establishes a normal cut-off value for results for each probe used, when applicable.

NOTE: Refer to the All Common Checklist for specific test method validation requirements. Cut-off values are usually required when ISH testing uses locus-specific probes against nuclear DNA.

REFERENCES

- 1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2018 edition, Revised 01/2018.
- 2) Clinical and Laboratory Standards Institute. *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline*. 2nd ed. CLSI Document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.

MOL.38650 New Reagent Lot - ISH Probes

Phase II

Each lot of *in situ* hybridization (ISH) probe(s) is checked for acceptable performance.

Evidence of Compliance:

- ✓ Written procedure for the verification of new lot of ISH probes prior to use **AND**
- ✓ Records of verification

MOL.38675 ISH Assay Performance

Phase I

There are records of *in situ* hybridization (ISH) performance for each assay.

NOTE: Assay performance should include monitoring hybridization efficiency, probe signal intensity and overall assay results, including controls, as applicable.

Evidence of Compliance:

- ✓ Written procedure defining acceptance criteria for ISH assay performance **AND**
- ✓ Records of QC monitoring of ISH assay performance at defined frequency

MOL.39004 ISH Scoring

Phase II

When applicable, there are written procedures for scoring *in situ* hybridization (ISH) results, including the number of cells scored, and all analyses are scored according to these procedures.

NOTE: For predictive marker testing, refer to MOL.39295 for requirements on reporting of the scoring method used.

REFERENCES

- 1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2018 edition, Revised 01/2018.
- 2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.

MOL.39146 ISH Controls

Phase II

Controls (internal and/or external) are used and recorded for each *in situ* hybridization (ISH) analysis.

NOTE: What functions as a control depends on the specific assay, signal pattern present, and sample type. For example, assays designed to detect deletions may use internal controls that include both the probe of interest and a control locus probe, both of which map to the same chromosome. In this situation, there are two internal controls, the signal for the probe of interest on the normal homolog and the control locus signals on both the normal and deleted homolog. For a dual fusion assay, the probe signals on each of the normal homologs function as internal controls. If a probe is used that does not produce an internal control signal (eg, a Y chromosome probe in a female), another sample that is known to have the probe target must be run in parallel as an external control with the patient sample. In addition, many ISH assays use an external control(s). For FDA-cleared or approved ISH assays, laboratories must follow manufacturer's instructions for quality control at minimum.

Evidence of Compliance:

- ✓ Written policy defining use of control loci with each ISH analysis **AND**
- ✓ Records of QC results

REFERENCES

- 1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2018 edition, Revised 01/2018.
- 2) Clinical and Laboratory Standards Institute (CLSI). *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline—Second Edition*. CLSI document MM07-A2 (ISBN 1-56238-885-1] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087-1898 USA, 2013.
- 3) Stupca P, Meyer RG, Dewald GW. Using controls for molecular cytogenetic testing in clinical practice. *J Assoc Genet Tech*. 2005;31:4-8.

MOL.39149 ISH Probe Intended Target

Phase I

There is a system in place to ensure that the *in situ* hybridization (ISH) probe used is for the intended target.

NOTE: Examples can include (but may not be limited to): 1) concurrent analysis of any available metaphase cells in an interphase cell analysis; 2) inclusion of an internal or external target that results in a positive signal for each hybridization; 3) written protocols that ensure the respective probe is applied to the intended specimen.

Evidence of Compliance:

- ✓ Written policy defining the system for ensuring use of the appropriate ISH probe **AND**
- ✓ Records confirming intended target

****REVISED** 06/04/2020****MOL.39155 ISH Interpretation****Phase II**

If an *in situ* hybridization (ISH) study requires consultation with a qualified pathologist and/or a cytogeneticist for accurate interpretation, the appropriate expert is consulted and their involvement is recorded.

REFERENCES

- 1) Clinical and Laboratory Standards Institute. *Fluorescence In Situ Hybridization Methods for Clinical Laboratories; Approved Guideline*. 2nd ed. CLSI Document MM07-A2. Clinical and Laboratory Standards Institute, Wayne, PA; 2013.

MOL.39288 Retention - Images and Slides**Phase II**

Photographic or digitized images or permanent slides are retained of all *in situ* hybridization (ISH) assays for an appropriate period.

NOTE: Images or permanent slides of ISH assays for neoplastic disorders must be retained for 10 years; images or permanent slides of ISH assays for constitutional disorders must be retained for 20 years. For an ISH assay with a normal result, retain an image of at least one cell illustrating the normal probe signal pattern. For an ISH assay with an abnormal result, retain images of at least two cells illustrating each relevant abnormal probe signal pattern.

There is no retention requirement for retaining images of slide preparations when the source slides remain readable for the required retention period.

Evidence of Compliance:

- ✓ Written retention policy

REFERENCES

- 1) American College of Medical Genetics, *Standards and Guidelines for Clinical Genetics Laboratories*, 2018 edition, Revised 01/2018.

PREDICTIVE MARKERS

The term predictive marker used within this section refers to ***in situ* hybridization (ISH) tests** used to predict responsiveness to a specific treatment independent of other histopathologic findings. Rather than confirming a specific diagnosis, these tests should differentiate predicted responsiveness to a targeted therapy among cases of the same diagnosis.

The current CAP guidelines ([CAP Guidelines](http://www.cap.org)) relating to predictive marker testing (eg, ASCO/CAP HER2 in breast cancer) may be found at <http://www.cap.org> in the *Protocols and Guidelines* section. The guidelines are periodically updated based on new evidence. Laboratories should review updated predictive marker guidelines and promptly implement changes for items relating to requirements in the checklists (eg, validation, fixation, scoring criteria).

If digital image analysis is used, additional requirements in the *Digital Image Analysis* section also apply.

****NEW** 09/17/2019****MOL.39295 Report Elements****Phase I**

For *in situ* hybridization (ISH) tests that provide independent predictive information, the patient report includes information on specimen processing, the probe, and the scoring method used.

NOTE: The following information must be included in the patient report:

1. *The type of specimen fixation and processing (eg, formalin-fixed paraffin-embedded sections, air-dried imprints).*
2. *The probe and, if applicable, the detection system used (ie, LSAB, polymer, proprietary kit, vendor name, etc.; information on the type of equipment used is not necessary)*
3. *Criteria used to determine a positive vs. negative result and scoring system (eg, manual or automated)*
4. *Laboratory interpretation of predictive marker testing (ISH) is reported according to the manufacturer's instructions, or when available, following the structure, format, and criteria set forth in the current [CAP guidelines](#) relating to predictive marker testing (eg, ASCO/CAP HER2 testing in breast cancer and CAP/ASCP/ASCO HER2 in gastroesophageal carcinoma).*

Evidence of Compliance:

- ✓ Written procedure for scoring and reporting ISH results for tests involving predictive markers **OR** report template containing all required elements **AND**
- ✓ Copies of patient reports confirming inclusion of the required elements **AND**
- ✓ Established guidelines used by the laboratory

REFERENCES

- 1) Wolff AC, Hammond EH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Pathol Lab Med.* 2018; 142(11):1364-82.
- 2) Bartley AN, Washington MK, Ventura CB, et al. HER2 Testing and Clinical Decision Making in Gastroesophageal Adenocarcinoma: Guideline from the College of American Pathologists, American Society for Clinical Pathology, and American Society of Clinical Oncology. *Arch Pathol Lab Med.* 2016;140(12):1345-1363.

****NEW** 09/17/2019**

MOL.39315 Annual Result Comparison - Breast Carcinoma

Phase II

For *in situ* hybridization (ISH) tests performed on breast carcinoma that provide independent predictive information, the laboratory at least annually compares its patient results with published benchmarks and evaluates interobserver variability between individuals performing the technical component of ISH testing (ie, scoring of ISH slides).

NOTE: For HER2 studies, the overall proportion of HER2 positive breast cancers in patients with breast cancer is 10-25%; however, the proportion of HER2 positive results within an individual laboratory may differ based on the case mix of specimens received for testing. The laboratory should monitor its overall trends in positive results in light of this information.

Individuals performing the technical component of ISH testing must have their concordance compared with each other at least annually. Interobserver concordance for positive vs. negative ISH results should be at least 95%.

Evidence of Compliance:

- ✓ Records of annual result comparison and evaluation of interobserver variability

REFERENCES

- 1) Wolff AC, Hammond EH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Pathol Lab Med.* 2018; 142(11):1364-82.
- 2) Rüschoff J, Lebeau A, Kreipe H, et al. Assessing HER2 testing quality in breast cancer: variables that influence HER2 positivity rate from a large, multicenter, observational study in Germany. *Mod Pathol.* 2017;30:217-26.

****REVISED** 09/17/2019**

MOL.39323 Predictive Marker Testing - Validation/Verification

Phase II

Predictive marker testing by *in situ* hybridization (eg, FISH, CISH, SISH, etc.) is validated/verified and records of validation/verification are retained.

NOTE: For HER2 (ERBB2) breast predictive marker testing, the following **minimum** numbers of cases must be used:

- FDA-cleared/approved tests - 40 cases (20 positive and 20 negative samples)
- Laboratory-developed tests (LDTs) - 80 cases (40 positive and 40 negative samples)

For **other predictive markers**, the laboratory director must determine the appropriate number of positive and negative samples to be used to adequately validate/verify the test. In general laboratories should consider using higher numbers of test cases when assessing laboratory-developed tests or modified FDA-cleared/approved tests than is necessary for unmodified FDA-cleared/approved tests for the same analyte. For genetic abnormalities where positive cases are rare, the laboratory director may determine that fewer validation cases are necessary. However, the rationale for using fewer cases must be recorded.

The validation/verification data should clearly show the degree of concordance between the assay or methods. Acceptable concordance levels should be defined by the laboratory and follow the current CAP guidelines if available.

The characteristics of the cases used for validation/verification should be similar to those seen in the laboratory's patient population (ie, core biopsy vs. open biopsy, primary vs. metastatic tumor, etc.).

Samples used for validation/verification must be handled in conformance with the guidelines in this checklist. Laboratories should use tissues that have been processed by using the same fixative and processing methods as cases that will be tested clinically.

If changes are made to the testing method (eg, probe, pretreatment protocol), the laboratory director is responsible for determining the extent of the performance verification or revalidation needed based on the scope of the changes in the test method.

This requirement is applicable to both new and existing assays. If review of the initial validation/verification does not meet the current standard, it must be supplemented and brought into compliance. It is possible to do this retroactively by review and documentation of past proficiency testing challenges or by sending unstained slides from recent cases to a referral laboratory for correlation. If no records exist from the initial validation/verification, the assay must be fully revalidated/verified.

This checklist requirement applies to laboratories that perform the technical portion of the testing process.

Evidence of Compliance:

- ✓ Records of validation/verification data including criteria for concordance

REFERENCES

- 1) Wolff AC, Hammond EH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Pathol Lab Med.* 2018; 142(11):1364-82.

****REVISED** 09/17/2019**

MOL.39358 Fixation - HER2 (ERBB2) Breast Predictive Marker Testing

Phase I

If the laboratory assesses HER2 (ERBB2) gene amplification by *in situ* hybridization (eg, FISH, CISH, SISH) for breast predictive marker testing, there is a written procedure to ensure appropriate specimen fixation time.

NOTE: Specimens subject to HER2 (ERBB2) testing should be placed in fixative within one hour of biopsy or resection (cold ischemia time) and remain in 10% neutral buffered formalin for at least six hours and up to 72 hours (formalin fixation time). Decalcification solutions with strong acids should not be used.

Laboratories testing specimens obtained from another institution must have a policy that addresses cold ischemia time and time of fixation. This information may be obtained using the laboratory requisition form. Laboratories must communicate with the submitting service to

facilitate appropriate specimen handling and proper recording of fixation parameters (refer to ANP.22983 for details).

Reports should qualify any negative results for specimens not meeting the above guidelines (including a disclaimer statement). In addition, any treatment that may potentially alter results, such as decalcification, must be included.

If specimens are fixed in a medium other than 10% neutral buffered formalin, the laboratory must perform a validation study showing that results are concordant with results from formalin-fixed tissues.

REFERENCES

- 1) Wolff AC, Hammond EH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. *Arch Pathol Lab Med*.2018; 142(11):1364-82.

****NEW** 09/17/2019**

MOL.39365 Predictive Marker Testing - Decalcified Specimens

Phase I

If the laboratory performs *in situ* hybridization (ISH) for predictive markers on decalcified specimens, the assay was validated for decalcified specimens or the results include a disclaimer noting that these assays have not been validated on decalcified specimens.

NOTE: Decalcification may adversely affect patient results. If the assay has not been validated for decalcified specimens, a disclaimer must be included in the patient report, such as, "This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens."

Using acid decalcified tissues is not recommended.

REFERENCES



- 1) Darvishian F et al. Impact of decalcification on receptor status in breast cancer. *The Breast Journal* 2011. 17:689-91.
- 2) Hanna W et al. Testing for HER2 in breast cancer: current pathology challenges faced in Canada. *Curr Oncol* 2012. 19:315-323.
- 3) Gertych A et al. Effects of tissue decalcification on the quantification of breast cancer biomarkers by digital image analysis. *Diag Pathol* 2014. 9:213.

DIGITAL IMAGE ANALYSIS

This section applies to laboratories using digital image analysis to evaluate specific features in a specimen or tissue section image following enhancement and processing of that image, including but not limited to morphometric analysis, ISH and cytogenetics (evaluation of metaphase chromosomes).

If predictive marker testing is performed, additional requirements in the Predictive Markers section also apply.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of validation and calibration policies and procedures • Sampling of validation/calibration records • Sampling of specimen analysis policies and procedures • Sampling of patient digital image analysis reports for completeness
	<ul style="list-style-type: none"> • What is your course of action if calibration is unacceptable?

DISCOVER



- Select a representative case and follow the entire process from receipt to final reporting

****NEW** 09/17/2019**

MOL.39410 Preanalytic Testing Phase Validation

Phase II

There are records showing that the preanalytic phase of the test system has been validated for each assay, including fixation and processing.

NOTE: Applicable requirements under the "Test Method Validation and Verification-Nonwaived Tests" section of the All Common Checklist must be followed.

REFERENCES

- 1) Hipp J, Bauer TW, Bui MM, et al. CAP Pathology Resource Guide: Digital Pathology. Version 5.0(1). Northfield, IL: College of American Pathologists; 2014.

****NEW** 09/17/2019**

MOL.39420 Calibration

Phase II

Each instrument is calibrated in accordance with the specifications of the instrument.

REFERENCES

- 1) Hipp J, Bauer TW, Bui MM, et al. CAP Pathology Resource Guide: Digital Pathology. Version 5.0(1). Northfield, IL: College of American Pathologists; 2014.

****NEW** 09/17/2019**

MOL.39440 Quality Control - Digital Image Analysis

Phase II

Control materials are run concurrently with patient specimens to ensure appropriate functionality of the digital image system.

NOTE: Controls are samples that act as surrogates for patient/client specimens. They are periodically processed like a patient/client sample to monitor the ongoing performance of the analytic process. Controls should check test performance at relevant decision points for the digital image analysis system.

For qualitative tests, a positive and a negative control may be sufficient. For quantitative or semiquantitative tests, controls at more than one level should be used.

Evidence of Compliance:

- ✓ Written QC policy **AND**
- ✓ Records of QC results

REFERENCES

- 1) Department of Health and Human Services, Centers for Medicare and Medicaid Services. Medicare, Medicaid and CLIA programs; CLIA fee collection; correction and final rule. *Fed Register*. 2003(Jan 24): [42CFR493.1256(d)(3)(i)].
- 2) Clinical and Laboratory Standards Institute (CLSI). *Statistical Quality Control for Quantitative Measurement Procedures: Principles and Definitions; Approved Guideline. 4th ed.* CLSI Document C24-ED4. Clinical and Laboratory Standards Institute, Wayne, PA; 2016.

****NEW/REVISED** 06/04/2020**

MOL.39470 Area of Analysis

Phase II

A qualified pathologist selects or confirms the appropriate areas for analysis, as applicable.

NOTE: Specimens that do not represent "in situ" samples embedded in paraffin may not require pathologist review. Examples include cultured preparations and direct preparations of liquid specimens including blood, urine, pleural fluid, etc.

****NEW** 09/17/2019**

MOL.39480 Analysis Guidelines and Procedures

Phase II

There are written guidelines for identification of appropriate areas and cells for analysis.

NOTE: Evaluation of heterogeneous cell populations requires use of specific guidelines and procedures to ensure analysis of the appropriate areas and/or cells, particularly if there is background or nonspecific staining, or if there is cell debris, endogenous pigment, and/or artifacts of aging, sectioning or preparation.

Test results may be affected by fixation parameters, including time of fixation, type of fixative used, hemorrhage, necrosis, and autolysis of tissue.

****NEW** 09/17/2019**



MOL.39490 Final Report Elements - Digital Image Analysis

Phase II

The final report includes the specimen source, name of the vendor and imaging system used, probe, and the detection method, as well as any limitations of the test result, if applicable.

SPECTROPHOTOMETERS

Inspector Instructions:

	<ul style="list-style-type: none"> • Spectrophotometer policies/procedures • Sampling of manufacturer required system checks
	<ul style="list-style-type: none"> • How does your laboratory verify calibration curves?

MOL.44860 Wavelength Calibration

Phase II

Spectrophotometer wavelength calibration is checked with appropriate solutions, filters or emission line source lamps, at least annually (or as often as specified by the manufacturer).

Evidence of Compliance:

- ✓ Records of wavelength calibration at defined frequency

MOL.45326 Calibration Curves

Phase II

For procedures using calibration curves, all the curves are rerun at defined intervals and/or verified after servicing or recalibration of instruments.

NOTE: Calibration curves must be run following manufacturer's instructions, at minimum, and as defined in laboratory procedure.

Evidence of Compliance:

- ✓ Records of calibration curves rerun and/or verification at defined frequency

SIGNAL DETECTION INSTRUMENTS

The following requirements apply to scintillation counters, luminometers, densitometers, etc.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of background checks
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MOL.46258 Background Level Criteria

Phase II

Background levels are compared on each day of use with established criteria for acceptability.

Evidence of Compliance:

- ✓ Records of background checks and corrective action when levels are unacceptable

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments: CLSI document MM19-A* (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.46491 Bleed-Through Signal

Phase I

For test platforms measuring multiple fluorochromes, precautions are taken to identify and correct for bleed-through signal from one channel to another.

Evidence of Compliance:


- ✓ Written procedure defining steps taken to identify and correct bleed-through

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments: CLSI document MM19-A* (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

FILM PROCESSING/PHOTOGRAPHIC EQUIPMENT

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling the film processing maintenance records
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MOL.46724 Film-Processing

Phase II

Film-processing (developing) equipment is serviced, repaired, and appropriately replenished with reagents if maintained by the laboratory.

NOTE: If the laboratory uses another department's film processing equipment, the quality of the autoradiographs produced must be monitored and the appropriate personnel notified if corrective action is required.



Evidence of Compliance:

- ✓ Records of maintenance at defined frequency
- ✓ Records of service or repair

INSTRUMENTS AND EQUIPMENT

The checklist requirements in this section should be used in conjunction with the requirements in the All Common Checklist relating to instruments and equipment.

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of thermocycler monitoring records
	<ul style="list-style-type: none"> • How does your laboratory ensure the individual wells of the thermocycler are maintaining accurate temperature?

MOL.49520 Thermocycler Temperature Checks

Phase II

Individual wells (or a representative sample thereof) of thermocyclers are checked for temperature accuracy before being placed in service and at least annually thereafter.

NOTE: A downstream measure of well-temperature accuracy (such as productivity of amplification) may be substituted to functionally meet this requirement. For closed systems this function should be performed as a component of the manufacturer-provided preventive maintenance.

Evidence of Compliance:

- ✓ Written procedure for verification of thermocycler accuracy **AND**
- ✓ Records of thermocycler verification

REFERENCES

- 1) Saunders GC, *et al.* Interlaboratory study on thermal cycler performance in controlled PCR and random amplified polymorphic DNA analyses. *Clin Chem.* 2001;47:47-55
- 2) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.49547 ISH Slide Processing Temperature Checks

Phase II

Individual slide slots (or a representative sample thereof) of *in situ* hybridization (ISH) temperature controlled slide processing systems are checked for temperature accuracy before being placed in service and at least annually thereafter.




Evidence of Compliance:

- ✓ Written procedure for verification of temperature accuracy **AND**
- ✓ Records of equipment verification

POST ANALYSIS**RESULTS REPORTING**

Reporting requirements for use of analyte-specific reagents and other reagents used in laboratory-developed tests are included in the All Common Checklist (COM.40850).

Inspector Instructions:

	<ul style="list-style-type: none"> • Sampling of molecular genetic test reporting policies and procedures • Sampling of patient test reports for completeness
	<ul style="list-style-type: none"> • What is your laboratory's course of action when discrepancies exist between the preliminary and final reports? • What is your laboratory's course of action when molecular results are discrepant with other clinicopathologic findings? • How does your laboratory ensure patient confidentiality when releasing/transmitting patient reports?
	<ul style="list-style-type: none"> • Follow a molecular genetic test report from specimen receipt and processing to evaluation, interpretation, identification and reporting

MOL.49555	Preliminary Reports Preliminary reports are promptly generated, when indicated.	Phase I
MOL.49560	Preliminary/Final Report Discrepancies Discrepancies between preliminary and final reports are investigated, corrective action taken, as necessary, and records retained.	Phase II
MOL.49565	Discrepant Results Discrepancies between the molecular pathology laboratory's final results, other laboratory findings, and the clinical presentation are investigated and recorded, along with any necessary corrective action.	Phase I
MOL.49570	Final Report Criteria The final report includes an appropriate summary of the methods, the loci or variants tested and the analytic interpretation (ie, test result). When appropriate, the final report includes the clinical interpretation.	Phase II

NOTE: Laboratory reports should be designed to convey patient results effectively to a non-expert physician. This includes a record of the analytic procedure used or the commercial kit version accompanied by an interpretation of the findings.

“Analytic interpretation” means examining the raw data to reach a conclusion about the quality and/or quantity of the analyte. Limitations of testing should be incorporated in the report. These factors are likely to be test specific, but include variants not detected by the assay that may impact the phenotype. “Clinical interpretation” means reaching a conclusion about the implications of the result for the patient. The clinical interpretation may be stated in general terms, or may be based on specific knowledge of the patient's situation. For pharmacogenetic tests, the phenotype prediction provided on a report should be accompanied by a statement citing that undetected genetic and/or non-genetic factors such as drug-drug interactions, may impact the phenotype.

REFERENCES

- 1) Margaret L. Gulley, Rita M. Brazier, Kevin C. Halling (2007) Clinical Laboratory Reports in Molecular Pathology. *Archives of Pathology & Laboratory Medicine*: Vol. 131, No. 6, pp. 852-863

****REVISED** 06/04/2020**

MOL.49575 Variant Database

Phase I

The laboratory's database for the clinical significance of variants is recorded and updated as needed, when applicable.

NOTE: Laboratory databases must be well annotated to enable tracking of variants identified and to provide consistent variant annotations. Variant categorizations must be evaluated based on evolving data and modified to reflect changes in clinical impact. Databases must be versioned to document the timeline of updates made.

Evidence of Compliance:

- ✓ Laboratory database of targets identified and/or reported

MOL.49585 Report Sign-off

Phase II

The final report is reviewed and signed by the section director (or designee who meets section director qualifications) if there is a subjective or an interpretive component to the test.

NOTE: When diagnostic reports are generated by computer or telecommunications equipment, the actual signature or initials of the section director need not appear on the report. Nevertheless, the laboratory must have a procedure that ensures that the report has been reviewed and approved before its release, and that records exist of the review and approval.

REFERENCES

- 1) Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:6.4
- 2) College of American Pathologists, Commission on Laboratory Accreditation. Standards for laboratory accreditation; Standard I. Northfield, IL: CAP, 1998

****REVISED** 09/17/2019**

MOL.49590 Patient Confidentiality

Phase II

Molecular genetic test reports are released and transmitted in a manner adequate to maintain patient confidentiality at a level appropriate for the particular test.

NOTE: In view of the recognized risks of genetic discrimination and stigmatization, confidentiality of molecular test results is an important consideration. Results should be communicated only to the referring physician, genetic counselor, the medical record, the patient or personal representative upon request. Potentially non-confidential media (eg, FAX) should be used with caution. Some patients, aware of the insurability risks, will choose to pay for testing out-of-pocket and request that the results not be recorded in their medical record; such requests

should be honored by the laboratory to the extent allowable under applicable laws. Under no circumstances should results be provided to outside parties such as employers, insurers or other family members, without the patient's express consent, despite the fact that there will be cases in which such action would appear to be in the best interest of the patient, family, or society. Laboratory workers must even use caution when publishing or publicly presenting the results of such studies, as some family members have recognized their own pedigrees in published material and thereby derived otherwise confidential information. National, federal, state (or provincial), or local laws and regulations may have specific reporting requirements that must be followed (eg, reporting to law enforcement agencies in cases of suspected child abuse).

Evidence of Compliance:

- ✓ Written procedures for release and transmittance of genetic test results

REFERENCES

- 1) Health Insurance Portability and Accountability Act, 1996

MOL.49595 Linkage Analysis Criteria**Phase II**

When linkage analysis is performed, the molecular inherited disease testing report includes an estimate of the risk of false negatives and false positives arising from recombination between the linked probe(s) and the disease allele or pathogenic variant.

REFERENCES

- 1) Keats BJB, et al. Guidelines for human linkage maps. An international system for human linkage maps (ISLM, 1990). *Ann Hum Genet.* 1991;55:1-6

MOL.49600 Report Criteria - Detection Rate and Residual Risk**Phase II**

In genetic testing for complex heritable disease genes with multiple possible pathogenic variants, the report includes (when appropriate) an estimate of the detection rate and the residual risk of being a carrier for a pathogenic variant not tested for.

NOTE: Many disease genes, such as those for cystic fibrosis and familial breast/ovarian cancer, are extremely heterogeneous at the molecular level, with hundreds of different pathogenic variants reported in different patients and families. Even with gene sequencing, the detection rate is not 100%, since sequencing of the coding regions will typically not detect pathogenic intronic variants, large exonic deletions/duplications or whole gene deletions/duplications. A negative test result, therefore, does not completely rule out the possibility that the patient is a carrier. The test report should convey this information in a fashion understandable to the physician and, when appropriate, the patient. A calculated value for residual risk, based on the known population allele frequencies in the patient's ethnic group, is recommended.

REFERENCES

- 1) Gulley et al. Clinical laboratory reports in molecular pathology. *Arch Pathol Lab Med.* Vol 131, June 2007

MOL.49615 Report Criteria - Limitations of Findings**Phase II**

At a level appropriate for the particular test, the report includes a discussion of the limitations of the findings and the clinical implications of the detected variant (or negative result) for complex disorders with regard to recessive or dominant inheritance, recurrence risk, penetrance, severity and other aspects of genotype-phenotype correlation.

NOTE: Because of the complexity of genotype-phenotype correlations for many genetic diseases and pharmacogenetic associations, simply reporting a molecular genetic test as positive for a variant is not acceptable since it conveys no information to the referring physician and patient as to the clinical or pharmacological ramifications of the result. Since major and often irreversible interventions may be initiated based on the test result, it is essential that the report convey the most current and accurate understanding of the clinical relevance of the variant identified, penetrance, phenotype predictions, and recurrence risks.

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.49620 Counseling Recommendation**Phase I**

The report includes a recommendation that patients receive appropriate genetic consultation to explain the implications of the test result, its residual risks and uncertainties, and the reproductive or medical options it raises for the patient, where appropriate.

NOTE: Molecular genetic test results are often extremely complex since they impart a probabilistic risk of disease rather than an objective positive/negative or quantitative answer. Physicians and counselors may require guidance to convey such subtle and emotionally charged information to patients in an understandable manner. In order to derive the most meaningful benefit from this testing, it is recommended that the results and subsequent options from these complex genetic tests be discussed with patients by a trained genetics professional.

REFERENCES

- 1) American Board of Medical Genetics and Genomics <http://www.abmgg.org/>
- 2) National Society of Genetic Counselors <http://www.nsgc.org/>
- 3) American Board of Genetic Counseling <http://www.abgc.net/>

MOL.49625 Result Correlation**Phase I**

For assays performed on histology/cytology samples, the interpretive report includes correlation with the morphologic findings, as applicable.

MOL.49630 Standard Nomenclature**Phase I**

Standard nomenclature is used to designate genes and variants.



NOTE: Whenever possible, human genes, loci and mutations should be designated according to standard nomenclature as defined in the references below. Where a common name is also in wide use in the medical literature, it may also be given in the report to improve clarity and prevent misunderstanding. Official symbols (eg, ERBB2) should be used, along with any colloquial names (eg, HER2, HER-2/neu, TKR1) to communicate results accurately and unequivocally.

REFERENCES

- 1) Wain HM, et al. Guidelines for Human Gene Nomenclature. *Genomics*. 2002;7:464-470
- 2) den Dunnen JR, et al. Mutation Nomenclature Extensions and Suggestions to Describe Complex Mutations: A Discussion. *Human Mutation*. 1999;15:7-12
- 3) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.
- 4) <http://www.HGVS.org/varnomen/> accessed 4/9/2018
- 5) Ogino S, Gulley ML, den Dunnen JT, Wilson RB, Association for Molecular Pathology Training and Education Committee. Standard mutation nomenclature in molecular diagnostics: practical and educational challenges. *J Mol Diagn* 2007;9:1-6
- 6) Kalman LV, et al. Pharmacogenetic Allele Nomenclature: International Workgroup Recommendations for Test Result Reporting. *Clin Pharmacol Ther*. 2016;99(2):172-85.

RECORDS

Inspector Instructions:

	<ul style="list-style-type: none"> Record retention policy and procedures
	<ul style="list-style-type: none"> Autoradiographs/gel photographs/<i>in situ</i> hybridization slides (adequately labeled/cross-referenced?)

MOL.49635 Laboratory Records

Phase II

The laboratory record includes sufficient information regarding the individual specimen and assay conditions.

NOTE: Appropriate information may include the quantity and quality of nucleic acid isolated and the amount used in the assay; the lot numbers of the restriction endonucleases, probes or primers used and any assay variables.

****REVISED** 09/17/2019**

MOL.49640 Record Retention

Phase II

A copy of each final report, all records of results, membranes, autoradiographs, gel photographs/images, and *in situ* hybridization slides, and array data files are retained in compliance with applicable national, federal, state (or provincial), and local laws and regulations.

NOTE: CAP requires that test reports for neoplastic conditions be retained for 10 years, and that test reports for constitutional disorders be retained for 20 years. Electronic versions are acceptable.

The retention of slides stained with fluorochromes may be defined in laboratory policy.

Retention of array data files from bioinformatics analysis must include the original data to support primary results generated and re-analysis for a minimum of two years.

MOL.49645 Cross-Referenced

Phase II

All autoradiographs, gel photographs and *in situ* hybridization slides are adequately cross-referenced in the case records.

Evidence of Compliance:

- ✓ Records for cross-reference

PERSONNEL

As applicable, the personnel requirements in the Laboratory General Checklist should be consulted. For optimal patient care, only qualified personnel may be involved with molecular pathology testing.

Inspector Instructions:



- Records of education and experience

MOL.49650 Section Director/Technical Supervisor Qualifications

Phase II

The section director/technical supervisor of the molecular pathology laboratory is a pathologist, board-certified physician in a specialty other than pathology, or doctoral scientist in a chemical, physical, or biologic science, with specialized training and/or appropriate experience in molecular pathology.

NOTE: If more stringent state or local regulations are in place for supervisory qualifications, including requirements for state licensure, they must be followed.

Evidence of Compliance:

- ✓ Records of qualifications including diploma, transcript(s), primary source verification record, equivalency evaluation, board-certification, or current license (if required) **AND**
- ✓ Records of work history in related field

REFERENCES

- 1) Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.

MOL.49655 Molecular Pathology General Supervisor Qualifications

Phase II

The molecular pathology general supervisor is qualified as one of the following.

1. **Person who qualifies as a section director/technical supervisor; or**
2. **Bachelor's degree in a chemical, physical, biological, or clinical laboratory science or medical technology with at least four years of experience (at least one of which is in molecular pathology methods) under a qualified section director**

Evidence of Compliance:



- ✓ Records of qualifications including diploma, transcript(s), primary source verification record, equivalency evaluation, board-certification, or current license (if required) **AND**
- ✓ Records of work history in related field

LABORATORY SAFETY

The inspector should review relevant requirements from the Safety section of the Laboratory General checklist, to assure that the molecular pathology laboratory is in compliance. In particular, the Inspector should review the use of universal precautions and the handling and disposal of hazardous chemicals such as ethidium bromide,

acrylamide, and organic reagents. If radioactive materials are used or stored, appropriate requirements are found in the Radiation Safety section of the Laboratory General Checklist.

Inspector Instructions:

	<ul style="list-style-type: none"> Records of biological safety cabinet certification
	<ul style="list-style-type: none"> Fume hood/chemical filtration unit Use of UV protective shielding, if applicable

MOL.52760 Fume Hood

Phase II

A properly functioning fume hood (or chemical filtration unit) is available for any procedures using volatile chemicals.

REFERENCES

- Clinical and Laboratory Standards Institute. *Clinical Laboratory Safety; Approved Guideline*. 3rd ed. CLSI Document GP17-A3. Clinical and Laboratory Standards Institute. Wayne, PA; 2012.

MOL.54570 Biological Safety Cabinet

Phase II

A biological safety cabinet (or hood) is available, when appropriate, and is certified at least annually to ensure that filters function properly and that airflow rates meet specifications.

Evidence of Compliance:

- ✓ Maintenance schedule of BSC function checks **AND**
- ✓ Records of testing and certification

REFERENCES

- Classification of etiologic agents on the basis of hazard; US Department of Health, Education, and Welfare, PHS, Centers for Disease Control, Office of Biosafety. Atlanta, GA. Reprinted September, 1976
- Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington Publishing No. (CDC) 21-1112; December 2009, DC: Publishing No. (CDC) 21-1112, December 2009 HHS.

MOL.54580 UV Protection

Phase II

If ultraviolet light sources are used, proper protective shielding is available to users.

Evidence of Compliance:

- ✓ Written policy including precautionary measures when UV light source are utilized

REFERENCES

- Clinical and Laboratory Standards Institute (CLSI). *Establishing Molecular Testing in Clinical Laboratory Environments*: CLSI document MM19-A (ISBN 1-56238-773-1). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2011.