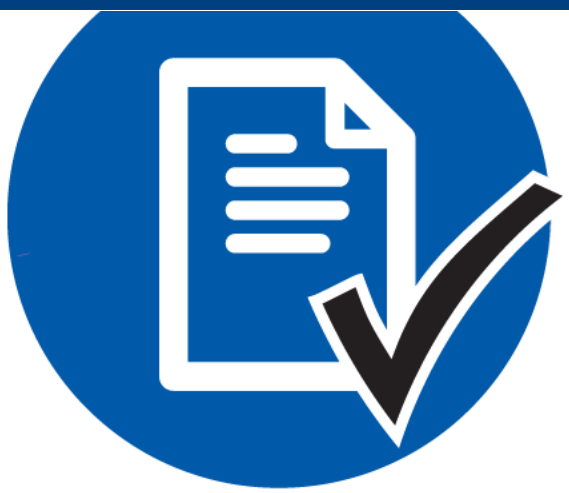
COLLEGE of AMERICAN PATHOLOGISTS

Master

Molecular Pathology Checklist

CAP Accreditation Program



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Molecular Pathology Checklist

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ON-LINE CHECKLIST AVAILABILITY

Participants of the CAP accreditation programs may download the checklists from the CAP website (www.cap.org) by logging into e-*LAB* Solutions. 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.

SUMMARY OF CHECKLIST EDITION CHANGES
Molecular Pathology Checklist
08/21/2017 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 listing of requirements below is 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

Requirement	Effective Date
MOL.31251	08/17/2016
MOL.32427	08/21/2017
MOL.34325	08/17/2016
MOL.36108	08/21/2017
MOL.36118	08/21/2017
MOL.36123	08/21/2017
MOL.36157	08/21/2017
MOL.36435	08/21/2017
MOL.36440	08/21/2017
MOL.36445	08/21/2017
MOL.36450	08/21/2017
MOL.36455	08/21/2017
MOL.36460	08/21/2017
MOL.36470	08/21/2017
MOL.36475	08/21/2017
MOL.36480	08/21/2017

MOL.36490	08/21/2017
MOL.36495	08/21/2017
MOL.37430	08/21/2017
MOL.38600	08/17/2016
MOL.38625	08/17/2016
MOL.38650	08/17/2016
MOL.38675	08/17/2016
MOL.39149	08/17/2016
MOL.49547	08/17/2016

REVISED Checklist Requirements

Requirement	Effective Date
MOL.30440	08/17/2016
MOL.31015	08/21/2017
MOL.32395	08/21/2017
MOL.34516	08/17/2016
MOL.35350	08/17/2016
MOL.35795	08/21/2017
MOL.35840	08/17/2016
MOL.35845	08/17/2016
MOL.35850	08/21/2017
MOL.35865	08/17/2016
MOL.35870	08/17/2016
MOL.36010	08/21/2017
MOL.36015	08/21/2017
MOL.36020	08/17/2016
MOL.36105	08/21/2017
MOL.36115	08/21/2017
MOL.36125	08/17/2016
MOL.36145	08/17/2016
MOL.36155	08/21/2017
MOL.36165	08/21/2017
MOL.37442	08/21/2017
MOL.37726	08/21/2017
MOL.39004	08/17/2016
MOL.39146	08/17/2016
MOL.39155	08/17/2016
MOL.39288	08/17/2016
MOL.39323	08/17/2016
MOL.49575	08/17/2016
MOL.49580	08/21/2017
MOL.49600	08/17/2016
MOL.49615	08/17/2016
MOL.49640	08/17/2016
MOL.49650	08/21/2017
MOL.49655	08/21/2017

DELETED/MOVED/MERGED Checklist Requirements

Requirement	Effective Date
MOL.30670	08/16/2016
MOL.31705	08/16/2016
MOL.37584	08/20/2017
MOL.39398	08/16/2016
MOL.39430	08/16/2016
MOL.44394	08/16/2016

MOL.49660	08/16/2016
MOL.49665	08/16/2016
MOL.58190	08/16/2016
MOL.61050	08/20/2017
MOL.61055	08/20/2017
MOL.61060	08/20/2017
MOL.61065	08/20/2017
MOL.61070	08/20/2017
MOL.61075	08/20/2017
MOL.61080	08/20/2017
MOL.61085	08/20/2017
MOL.61090	08/20/2017

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.

Note for non-US laboratories: Checklist requirements apply to all laboratories unless a specific disclaimer of exclusion is stated in the checklist.

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. Also, the Anatomic Pathology Checklist may instead be used to inspect in situ hybridization (ISH), when ISH testing is performed in the anatomic pathology or cytopathology section.

The Microbiology Checklist must be used to inspect laboratories that perform molecular testing for infectious disease testing (both for FDA-cleared/approved tests, and non-FDA-cleared/approved tests).

QUALITY MANAGEMENT AND QUALITY CONTROL

GENERAL ISSUES

Inspector Instructions:

- Sampling of turnaround time records

MOL.20300 Turnaround Time **Phase I**

There is evidence that the laboratory monitors sample turnaround times and that they are appropriate for the intended purpose of the test.

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

MOL.20550 Test Result Statistics **Phase I**

When appropriate, statistics on molecular pathology test results (*e.g.* 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.

***REVISED** 08/17/2016*
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 (e.g. 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

Validation of a laboratory test requires identifying the purpose 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) and FDA-cleared/approved tests that have been modified by the laboratory. For unmodified FDA-cleared/approved tests, the laboratory need only verify accuracy, precision, reportable range, and reference interval. See the Method Performance Specifications section of the All Common Checklist.

If an FDA-cleared/approved test is modified to meet the needs of the user or if the test 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 (e.g. 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 (e.g. biopsy findings, radiographic and clinical findings, other laboratory results) whenever possible.

Inspector Instructions:

	• Sampling of assay validation studies, including comparisons and appropriate sample types • Sampling of assay validation studies for LDTs introduced since last on-site inspection
	• How does your laboratory validate assay performance prior to test implementation? • How does your laboratory validate clinical claims made by the laboratory about LDTs?

MOL.30785 Validation Summary **Phase II**

For each test there is a validation summary addressing analytical and clinical performance parameters.

NOTE: For modified FDA-cleared/approved tests or laboratory-developed tests (LDTs), the summary 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 (e.g. specimen stability, reagent stability, linearity, carryover, and cross-contamination, etc.), as appropriate and applicable. Clinical performance characteristics must also be addressed. Laboratory director (or designee who meets CAP director qualifications) review and approval before clinical implementation must be recorded.

Evidence of Compliance:

✓ Written summary of validation studies with laboratory director/designee review and approval

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, Wooderchak-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

*****REVISED** 08/21/2017***

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 (*e.g*. blood, fresh/frozen tissue, saliva, paraffin-embedded tissue, prenatal specimens, buccal swabs).

NOTE: For tissue samples, the validation must include representative tissue sources (i.e. 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 (e.g. 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 (e.g. characterized cell lines, cell lines with spiked in nucleic acids).

Evidence of Compliance:

✓ Records of validation studies

MOL.31130 Accuracy **Phase II**

The results of each validation study include a sufficient number of characterized samples to provide a high degree of assurance of the test's accuracy.

NOTE: 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 (e.g. 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 (e.g. 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. The number of samples depends on the intended use of the test.

Evidence of Compliance:

- ✓ Records of comparison of each validation study with a sufficient number of samples comprised of well-characterized reference materials together with appropriate biological matrix **OR** by comparison to another valid test method, such as through specimen exchange

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.

MOL.31145 Precision/Reproducibility **Phase II**

The results of each validation study include a sufficient number of samples with repeated analyses to provide a high degree of assurance of the test's precision or reproducibility.

NOTE: The laboratory must show recorded evidence 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, and for a quantitative test, represented as the coefficient of variation, whereas for a qualitative test, represented as ratios of concordant results. Laboratories are encouraged to review the cited references for guidance and provide confidence intervals to estimated performance characteristics.

Evidence of Compliance:

- ✓ Records of precision/reproducibility studies throughout the reportable range

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.

MOL.31245 Reportable Range **Phase II**

The results of each validation study include a sufficient number of samples to confirm or establish the test's reportable range.

NOTE: The reportable range encompasses the full range of reported values. For qualitative tests that would include all reportable outcomes (e.g. 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. The laboratory must also determine how to handle positive patient results below or above the AMR, since numerical values outside the AMR may be inaccurate. For example, these may be reported as <x or >y, or they may be reported as low positive or high positive along with an explanation that values outside the linear range cannot be quantified, or the sample may be concentrated or diluted and rerun to calculate an accurate value within the reportable range.

Evidence of Compliance:

- ✓ Records of validation studies to confirm or establish each test's reportable range

REFERENCES
1) American College of Medical Genetics and Genomics. Laboratory Standards and Guidelines for Clinical Genetics Laboratories, 4th ed. Bethesda, MD: ACMG, 2008. Available at: http://www.acmg.net Accessed 2006
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) 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)]

*****NEW** 08/17/2016***

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

MOL.31255 Reference Interval **Phase II**

The results of each validation study include a sufficient number of samples to verify or establish the test's reference interval.

NOTE: The reference interval is the range of results expected in the normal population. For some qualitative tests (e.g. 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 validation studies to verify or establish each test's reference interval

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.31360 Analytical Sensitivity/Lower Limit of Detection **Phase II**

For modified FDA-cleared/approved tests or LDTs, the results of each validation study include a sufficient number of samples to establish the test's lower limit of detection.

NOTE: The analytical sensitivity corresponds to the lower limit of detection. It 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 (e.g. pathogens, rare variants, chimerism, mosaicism, tumor-normal admixtures).

Evidence of Compliance:
- ✓ Records of validation studies to establish lower limits of detection

REFERENCES
1) Clinical and Laboratory Standards Institute (CLSI). Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory - Approved Guideline- Third Edition - CLSI Document EP28-A3C. (ISBN 1-56238-682-4) Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 2500, Wayne, PA, 19087-1898, USA, 2010.
2) 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)]
3) 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.
4) 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.31375 Analytical Specificity/Interfering Substances **Phase II**

For modified FDA-cleared/approved tests or LDTs, the results of each validation study include a sufficient number of samples to establish the test's analytical specificity.

NOTE: 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.

Evidence of Compliance:
- ✓ Records of validation studies to establish analytical specificity

REFERENCES

1) Clinical and Laboratory Standards Institute. *Interference Testing in Clinical Chemistry; Approved Guideline.* 2nd ed. CLSI Document EP07-A2. Clinical and Laboratory Standards Institute. Wayne, PA; 2005.
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 (e.g. 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:

	• 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
	• Processing of molecular pathology specimens
	• 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 racial/ethnicity, when appropriate (*e.g.* 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** 08/21/2017***

MOL.32395 Neoplastic Cell Content **Phase II**

For paraffin-embedded tumor specimens from which DNA or RNA is extracted for analysis (*e.g.* 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 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 (e.g. 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.

*****NEW** 08/21/2017***

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

MOL.32430 Nucleic Acid Quantity **Phase II**

The quantity of nucleic acid is measured, when appropriate.

NOTE: The quantity of nucleic acid must be measured prior to use in a procedure whose success generally depends on accurately determining the concentration/quantity of the nucleic acid.

Evidence of Compliance:

- ✓ Written policy defining conditions under which quantity of nucleic acid is measured **AND**
- ✓ Records of nucleic acid measurement

MOL.32435 Nucleic Acid Quality **Phase II**

The integrity and purity of nucleic acid is assessed, when appropriate.

NOTE: RNA in specimens is highly labile because RNase is ubiquitous and difficult to inhibit. For human RNA targets, RNA quality must be assessed. An appropriate "housekeeping" mRNA should be assessed as an internal control for RNA integrity. However, depending on the target, it may not be necessary for all specimens to be assessed for RNA quality.

Evidence of Compliance:

- ✓ Records of nucleic acid quality assessment

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

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 (*i.e.* 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 LMPI 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 maintained 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, state or local laws and/ or 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 AND CALIBRATION VERIFICATION

CALIBRATION is the set of operations that establish, under specified conditions, the relationship between reagent system/instrument response and the corresponding concentration/activity values of an analyte. 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 denotes the process of confirming that the current calibration settings for each analyte remain valid for a test system. If calibration verification confirms that the current calibration settings for each analyte are valid, it is not necessary to perform a complete calibration or recalibration of the test system. Each laboratory must define limits for accepting or rejecting tests of calibration verification. Calibration verification can be accomplished in several ways. If the manufacturer provides a calibration validation or verification process, it should be followed. Other techniques include (1) assay of the current method calibration materials as unknown specimens, and determination that the correct target values are recovered, and (2) assay of matrix-appropriate materials with target values that are specific for the test system.

REQUIRED FREQUENCY OF CALIBRATION VERIFICATION
Laboratories must calibrate a test system when it is first placed in service and perform calibration verification at least every six months thereafter. However, a laboratory may opt to recalibrate a test system (rather than perform calibration verification) at least every six months. If a test system has been recalibrated then it is NOT necessary to also perform calibration verification sooner than six months following recalibration. In addition to

this six-month schedule, calibration verification or recalibration is required (regardless of the length of time since last performed) immediately if any of the following occurs:

1. *A change of reagent lots for chemically or physically active or critical components, 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 materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fails to identify and correct the problem*
3. *After major maintenance or service. The Laboratory Director must determine what constitutes major maintenance or service.*
4. *When recommended by the manufacturer*

MATERIALS SUITABLE FOR CALIBRATION VERIFICATION
Materials for calibration verification must have a matrix appropriate for the clinical specimens assayed by that method and target values appropriate for the measurement system. Suitable materials may include, but are not limited to:

1. *Calibrators used to calibrate the analytical system*
2. *Materials provided by the analytical measurement system vendor 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 of calibration following reagent lot changes if the material is listed in the package insert or claimed by the method manufacturer to be commutable with patient specimens for the method. A commutable reference material is one that gives the same numeric result as would a patient specimen containing the same quantity of analyte in the analytic method under discussion; i.e. matrix effects are absent. Commutability between a reference material and patient specimens can be demonstrated using the protocol in CLSI EP14-A3.*
6. *Proficiency testing material or proficiency testing validated material with matrix characteristics and target values appropriate for the method*

In general, routine control materials are not suitable for calibration verification, except in situations where the material is specifically designated by the method manufacturer as suitable for verification of the method's calibration.

ANALYTICAL MEASUREMENT RANGE

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

LINEARITY AND THE AMR
An important concept in verifying the AMR is that a plot of measured values from test samples vs. their actual (or expected) concentration or relative concentrations must be linear within defined acceptance criteria over the AMR. Verifying linearity using such a plot verifies the AMR. Beyond the limits of the AMR, there may not be a linear relationship between measured and actual analyte concentrations, and test results may therefore be unreliable. For patient samples, only measured values that fall within the AMR (or can be brought into the AMR by sample dilution or concentration) should be reported. Values that fall outside the AMR may be reported as "less than" or "greater than" the limits of the AMR (see the note below, Patent Samples with Unusually High Concentrations of Analyte).

AMR VERIFICATION
Minimum requirements for AMR verification can be met by using matrix appropriate materials, which include the low, mid and high concentration or activity range of the AMR and recovering appropriate target values, within defined acceptance criteria. Records of AMR verification must be available.

The best practice for AMR verification is to demonstrate a linear relationship, within defined acceptance criteria, between measured concentrations of analytes and expected values for a set of four or more matrix-appropriate samples that cover the AMR.

AMR verification may be accomplished through calibration under certain circumstances. It is not necessary to perform a separate AMR verification if calibration of an assay includes calibrators that span the full range of the AMR, with low, midpoint and high values (i.e. three points) included. A one-point or two-point calibration does not include all of the necessary points to validate the AMR.

REQUIRED FREQUENCY OF AMR VERIFICATION
When initially introducing a new method, it is necessary to verify the AMR independently from calibration. In this situation, suitable materials for the AMR verification include those listed below (see OTHER MATERIALS SUITABLE FOR AMR VERIFICATION). Additionally, when multipoint calibration that spans the AMR is utilized, a set of calibrators from a different lot number than that used to calibrate the system may be suitable for independent AMR verification.

The AMR must be verified at least every six months after a method is initially placed in service and following the criteria defined in the checklist. If multipoint calibrators that span the AMR are used for calibration/calibration verification, it is not necessary to independently verify the AMR, as long as the system is calibrated at least every six months.

OTHER MATERIALS SUITABLE FOR AMR VERIFICATION
The materials used for AMR verification must be known to have matrix characteristics appropriate for the method. The matrix of the sample (i.e. 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. The verification must include specimens, which at a minimum, are near the low, midpoint, and high values of the AMR. Suitable materials for AMR verification include the following:

1. *Linearity material of appropriate matrix*
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*

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 method manufacturer's instructions for verifying the AMR should be followed, when available. The Laboratory Director must define limits for accepting or rejecting verification tests of the AMR.

Inspector Instructions:

	• Sampling of calibration and AMR policies and procedures • Sampling of calibration/calibration verification records • Sampling of AMR verification records
	• Sampling of calibration materials (quality)
	• 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?
	• Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration and unacceptable calibration verification

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 and criteria for acceptable performance.

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) Kroll MH, Emancipator K. A theoretical evaluation of linearity. *Clin Chem.* 1993;39:405-413
4) 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
5) Miller WG. "Quality control." Professional Practice in Clinical Chemistry: A Companion Text, ed. DR Dufour. Washington, DC: AACC Press, 1999:12-1 to 12-22
6) Kroll MH, *et al*. Evaluation of the extent of non linearity in reportable range studies. *Arch Pathol Lab Med.* 2000;124:1331-1338

MOL.33696 Calibration Materials **Phase II**

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

Evidence of Compliance:
- ✓ Written policy defining the use of appropriate calibrators **AND**
- ✓ Records of calibration

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 — 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 vendor, 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]

MOL.33860 Calibration/Calibration Verification Criteria — Phase II

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

NOTE: Criteria typically include:

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 materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem*
3. *After major maintenance or service*
4. *When recommended by the manufacturer*
5. *At least every six months*

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." Professional Practice in Clinical Chemistry: A Companion Text, ed. DR Dufour. Washington, DC: AACC Press, 1999:12-1 to 12-22

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)]

MOL.33942 AMR Verification — 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, appropriate acceptance criteria are defined, and the process is recorded and reviewed.

NOTE: If the materials used for calibration or for calibration verification include low, midpoint, and high values that are near the stated AMR, and if calibration verification data are within the laboratory's acceptance criteria, the AMR has been verified; no additional procedures are required. If the calibration and/or calibration verification materials do not span the full AMR, or the laboratory extends the AMR beyond the manufacturer's stated range, the AMR must be verified by assaying materials reasonably near the lowest and highest values of the AMR.

The materials used for verification must be known to have matrix characteristics appropriate for the method. The test specimens must have analyte values that as a minimum are near the low, midpoint, and high values of the AMR. Guidelines for analyte levels near the low and high range of the AMR should be determined by the laboratory director. Factors to consider 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 method manufacturer's instructions for verifying the AMR should be followed, when available. Specimen target values can be established by comparison with peer group values for reference materials, by assignment of reference or comparison method values, and by dilution ratios of one or more specimens with known values. Each laboratory must define limits for accepting or rejecting verification tests of the AMR.

Evidence of Compliance:

- ✓ Written procedure for AMR verification defining the types of materials used and acceptability criteria consistent with manufacturer's instructions

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]

MOL.33983 AMR Verification Criteria — Phase II

Criteria are established for verifying the analytical measurement range, and compliance is recorded.

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. *A change of reagent lots for chemically or physically active or critical components, 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 materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem*
3. *After major preventive maintenance or change of a critical instrument component*
4. *When recommended by the manufacturer*

Evidence of Compliance:

- ✓ Written policy defining the method, frequency and acceptability criteria for AMR verification

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 tests 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 (e.g. amplification), methods, and instrument systems used (e.g. 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:

	• Sampling of QC policies and procedures • Sampling of QC records, including monthly monitoring of imprecision
	• Sampling of control material (storage)
	• 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?
	• 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 (e.g. pathogens, chimerism, mosaicism, tumor-normal admixtures).

If an internal quality control process (e.g. 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 to address the use of the alternative control system. 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 Individualized Quality Control Plan 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 (e.g. 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 to address the use of the alternative control system. 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 Individualized Quality Control Plan 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.

MOL.34311 Tolerance Limits - Controls **Phase II**

Tolerance and 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:

- ✓ Records of defined tolerance limits for control range verification of each lot

*****NEW** 08/17/2016***

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.

NOTE: It is implicit in quality control that patient test results will not be reported when controls are unacceptable.

Evidence of Compliance:

- ✓ Written policy stating that controls are reviewed and acceptable prior to reporting patient results **AND**
- ✓ Evidence of corrective action taken when QC results are not acceptable

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 (e.g. 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, recognizing that pre-analytic and post-analytic variables may differ from those encountered with patients.

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) Mukherjee KL. Introductory mathematics for the clinical laboratory. Chicago, IL: American Society of Clinical Pathology, 1979:81-94
2) Barnett RN. Clinical laboratory statistics, 2nd ed. Boston, M; Little, Brown, 1979
3) Weisbrodt IM. Statistics for the clinical laboratory. Philadelphia, PA: JB Lippincott, 1985
4) Matthews DF, Farewell VT. Understanding and using medical statistics. NY, NY: Karger, 1988
5) 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)
6) 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
7) 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.
8) 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 (e.g. trending for repeat failures, etc.).

Evidence of Compliance:

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

*****REVISED** 08/17/2016***

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 (e.g. 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 (e.g. 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:

- Sampling of restriction endonuclease digestion records

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, i.e. 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:

	• Sampling of electrophoresis policies and procedures
	• Autoradiographs/gel photographs (sufficient resolution/quality)
	• 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 Autoradiograph/Electrophoretic Gel Interpretation **Phase I**

Autoradiographs or electrophoretic gels are interpreted using objective criteria.

Evidence of Compliance:

✓ Written procedure including interpretive criteria for autoradiographs or 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 Autoradiograph/Gel Photograph Resolution **Phase II**

The autoradiographs and gel photographs 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:

	• Sampling of amplification/PCR policies and procedures
	• Physical containment practices (frequent glove change, separate manipulation of pre- and post-specimens, dedicated pipettes)
	• How does your laboratory distinguish a true negative from a false negative result?

***REVISED** 08/17/2016*

MOL.35350 Carryover **Phase II**

Nucleic acid amplification procedures (*e.g.* PCR) are designed to minimize carryover (false positive results) 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) Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989;339:237-238
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.35360 Internal Controls - NAA **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 interrogate 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:

- Sampling of array quality verification and lot-to-lot comparison records

MOL.35722 Integrity/Labeling Verification **Phase I**

Patient nucleic acid integrity and labeling are verified.

NOTE: In many current applications, the sample is labeled during a PCR or RT-PCR reaction. Some arrays include a control feature that targets an endogenous positive target. Other possible controls include visualizing the material on electrophoretic gels/capillaries or by detection of label. Addition of an exogenous spiked control during labeling will monitor efficiency of labeling but will not control for quality of sample nucleic acid.

Evidence of Compliance:

- ✓ Written procedure for verifying nucleic acid integrity/labeling **AND**
- ✓ Records of verification

REFERENCES
1) Clinical and Laboratory Standards Institute. *Diagnostic Nucleic Acid Microarrays: Approved Guideline*; CLSI Document MM12-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2006.

MOL.35766 Array Quality **Phase I**

The quality of the arrays is verified and new lots and shipments are checked for acceptability prior to use.

NOTE: Manufacturer quality control specifications should be available as a reference. Additional verification by the laboratory should include:

- *Verification of each probe for each lot and shipment. This can be achieved in part by using labeled oligonucleotides that hybridize to all probes, a mixture of labeled oligonucleotides specific for each probe, or a mixture of control samples that hybridize to each probe.*
- *For quantitative assays such as gene dosage, pathogen load, or expression levels, a positive control near limiting dilution (low positive) for one or more probes should be included in each run. These controls should be rotated to include all analytes. A separate array for template blanks (i.e. no RT control or water) should also be included to monitor for inadvertent contamination.*
- *Function checks on software used to analyze array data points.*

Evidence of Compliance:

✓ Written procedure for verifying array quality and the acceptability of new lots and shipments prior to use **AND**

✓ Records of verification and acceptability of new lots and shipments

REFERENCES

1) Clinical and Laboratory Standards Institute. *User Evaluation of Between Reagent Lot Variation; Approved Guideline.* CLSI Document EP26-A. Clinical and Laboratory Standards Institute. Wayne, PA; 2013.
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.35785 Cytogenomic Microarray Report Elements — Phase II

Reports for cytogenomic microarrays include the following elements:

1. **Platform used**
2. **Genome build used**
3. **Methods**
4. **Resolution**
5. **Current ISCN-compliant nomenclature**
6. **References to any databases used**
7. **A statement on the need for genetic counseling when indicated**
8. **A statement recommending further testing when indicated**
9. **All disclaimers required by federal guidelines**
10. **Clinical significance of DNA copy number changes**

NOTE: Resolution includes but is not limited to, the number of probes on the array, approximate distance between probes and threshold levels for determining a copy number change.

REFERENCES

1) Shaffer LG, Beaudet AL, Brothman AR, Hirsch B, Levy B, Martin CL, Mascarello JT, Rao KW; Working Group of the Laboratory Quality Assurance Committee of the American College of Medical Genetics. Microarray analysis for constitutional cytogenetic abnormalities. *Genet Med.* 2007 Sep;9(9):654-662
2) 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
3) ISBN 2009. An International System for Human Cytogenetic Nomenclature. Shaffer LG, Slovak ML, Campbell LJ (eds); S. Karger, Basel, 2009
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):1043-1044 [42CFR493.1276], 1047-1048 [42CFR493.1291]
5) 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.

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 (e.g. 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.

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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 proportions (e.g. 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 proportions 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, e.g. 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.* 20105;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 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:

	• Sampling of next generation sequencing policies and procedures • Records of wet bench and bioinformatics dry bench validations • QM program records with corrective action for component failure • Sampling of exception log records
	• What is your course of action when processes deviate from written procedures?

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** 08/17/2016***

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 (e.g. 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 (e.g. 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 (e.g. 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.

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.

*****REVISED** 08/17/2016***

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 (e.g. 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 (e.g. 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** 08/21/2017***

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 (e.g. 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 NGS-identified organisms, markers of drug resistance pathogenicity, and host response should be performed. The methods described above are acceptable and may also include species-specific PCR.

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

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** 08/17/2016***
MOL.35865 NGS Data Transfer Confidentiality **Phase I**

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 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 (e.g. 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 local, state, and/or federal requirements, as applicable (e.g. HIPAA).

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 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.

*****REVISED** 08/17/2016***

MOL.35870 NGS Data Storage **Phase I**

The laboratory retains NGS data necessary to support primary results generated and re-analysis for a minimum of two years or as required by local, state or federal laws.

NOTE: The data retained must include the files necessary to re-review cases as originally performed for original results reporting. Examples include specimen tracking and quality metrics data/files, sequencing run quality metrics reports, log or configuration file information regarding bioinformatics pipeline parameters, sequence read alignments, exception log information, variants undergoing manual review, and files containing filtered and/or interpreted variants. The retained files and records must also 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.

Examples of retained files may include FASTQ, BAM, VCF, and derivatives thereof. The policy must be in accordance with local, state, and federal requirements for storage of data, as applicable.

Evidence of Compliance:

- ✓ Written policy that describes files, type of data to be retained and length of retention

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** 08/21/2017***

MOL.36010 NGS Analytical Wet Bench Procedure **Phase II**

The laboratory has 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 (e.g. 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 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 (e.g. 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, and fungi), including a description of the minimal specimen requirements to perform the assay*
- *Methods and reagents used for isolating 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 (e.g. multiplex PCR based or oligonucleotide based capture), if applicable*
- *Methods and reagents used for depletion of host or unwanted nucleic acids (e.g. 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 (e.g. 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 (e.g. flow cells, chips)*
- *Instrument software and version used to generate on-instrument (primary) data and output format (e.g. 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** 08/21/2017***

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 (e.g. blood, fresh/frozen tissue, paraffin embedded tissue, prenatal specimens, saliva, culture isolates). Validations can be augmented by, but not supplanted with, additional reference standards (e.g. cell lines such as NIST NA12878).

- *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 (e.g. diagnostic 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 (e.g. 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 (e.g. HIV reverse transcriptase K103N or CMV UL97 M460V/I) or expect to identify common resistance genes (e.g. 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 (e.g. 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.

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 (e.g. samples with known sequence variants or reference standards such as NIST NA12878) 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

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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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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.
8) Thomas M, Sukhai MA, Zhang T, et al. Integration of Technical, Bioinformatic, and Variant Assessment Approaches in the Validation of a Targeted Next-Generation Sequencing Panel for Myeloid Malignancies. *Arch Pathol Lab Med.* 2017; 141(6):759-775.
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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.

*****REVISED** 08/17/2016***

MOL.36020 Wet Bench Process - Quality Management Program Phase I

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 (e.g. 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

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)

MOL.36035 Monitoring of Upgrades **Phase I**

The laboratory has a 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. 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** 08/21/2017***
MOL.36105 NGS Analytical Bioinformatics Procedure **Phase II**

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

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

The written procedure must include:
- *Individual applications and databases used with versions and appropriate command line flags, or other configuration items needed to compile, install, and run the pipeline*
- *Additional scripts or steps used to connect discrete applications in the pipeline*
- *Source code including version and evidence of using a source versioning system (e.g. CVS, SVN or git), test case descriptions (e.g. 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 (e.g. inclusion/removal of 'ChrUn,' 'ChrN random' 'and/or chrM from the file)

- *Description of input and output data files or information (e.g. parameters/flags and values) in each process step*
- *Criteria and specific thresholds used for inclusion of variants in classification and interpretation steps following variant calling (e.g. minimum read coverage depth, base or variant quality scores, variant read percentage)*
- *Criteria and specific thresholds used for inclusion of a sequence for organism identification*
- *Bioinformatics processes and thresholds applied for prioritizing and identifying causal or candidate variants or genes (especially important for, but not limited to, exome or genome sequencing, and evaluation of drug resistance mutations), if applicable. Processes may include, but are not limited to, filtering variants based on population frequency, predicting variant impact on gene production or function (e.g. derived from PolyPhen, SIFT), identifying regions of high homology or pseudogenes. In family studies, process steps are documented for prioritization of variants based on parameters including, but not limited to, 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 should be documented when applicable for exome/genome or panels.*
- *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 water control, 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) percentage of reads mapping to the target, 3) duplicate read rate, 4) 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, and 7) 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*
- *Written procedures for any portion of the NGS bioinformatics process performed by a* referral *laboratory, if applicable*

Evidence of Compliance:

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

*****NEW** 08/21/2017***

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** 08/21/2017*

MOL.36115 NGS Analytical Bioinformatics Process Validation **Phase II**

The laboratory validates the analytical bioinformatics process (also termed 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. This information is 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 (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.

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 and/or representative spectrum of the types of organisms the test is designed to detect (e.g. single nucleotide variants, indels, copy number variants, and other structural variants such as translocations and inversions). When using the same wet bench process for more than one test designed to detect the same types of variants (e.g. the same targeted enrichment chemistry for different diagnostic gene 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 variants must be used for validation and the validation can be augmented by, but not supplanted with, reference materials (e.g. NIST Standard NA12878). Additional samples 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 (e.g. indels and CNVs).*
- *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 genes with common pathogenic variants (e.g. CFTR p.Phe508del, a mutation hotspot of therapeutic relevance in an oncology gene, HIV reverse transcriptase K103N, or CMV UL97 M460V/I) or expect to identify common resistance genes (e.g. S, aureus mecA gene), specimens with common pathogenic variants as defined by the scientific literature and/or relevant experts (e.g. published guidelines) should be included to demonstrate the test's ability to detect them. 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 of transversion (ts/tv) ratio in exome and genome.

- *Provide a description of the analytical target (e.g. exons, genes, or targeted regions) and bioinformatics pipeline used for analysis, including algorithms, test scripts, and test or training datasets*
- *Description of 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 when sample pooling methods (e.g. indexes or barcodes) are used*
- *Establish criteria and thresholds for target calling (e.g. minimum coverage of the target, position-wise read depth, base or variant quality scores, variant read percentage), and variation from the target sequence. Criteria may be differently defined based on application (e.g. detection of germline versus somatic mutations, or pathogen detection versus drug resistance mutation identification)*
- *Determine and record how interference by highly homologous regions (e.g. pseudogenes) is mitigated or avoided. This may include custom approaches and/or orthogonal methods (e.g. Sanger sequencing) to confirm test results in these regions.*
- *Determine test performance characteristics for all variant types to be detected by the test (e.g. 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 for performance characteristics (e.g. analytical sensitivity=100%, CI=98.3-100%).*
- *Determine test performance characteristics for all microbial target types to be detected by the test (e.g. 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 and the data are either re-processed or not further processed.*

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

REFERENCES
1) Schrijver *et al*. Opportunities and Challenges Associated with Clinical Diagnostic Genome Sequencing; A Report of the AMP. *J Mol Diagn*. 2012, 14(6):526-540
2) Gargis A, *et al.* Assuring the Quality of Next Generation Sequencing in Clinical Laboratory Practice. *Nature Biotechnology.* 2012; 30(11):1033-1036
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) 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.
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*****NEW** 08/21/2017***

MOL.36118 NGS Sequencing 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: Determination of the limit of detection (LOD) of NGS 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 aneuploidy, detection of antiviral drug resistance mutations, and identification of pathogens by targeted or metagenomic approaches.

Lower limit of detection may vary based on variant type (e.g. single nucleotide variants, indels, copy number variants and other structural variants, such as translocations and inversions) or target characteristics (e.g. organism genome size). During validation, determination of LOD is required for each variant and 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.

*****NEW** 08/21/2017***

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 (e.g. 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 (e.g. 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.

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.

*****REVISED** 08/17/2016***

MOL.36125 NGS Bioinformatics Process/Pipeline - Quality Management Program Phase I

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 (e.g. 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 (e.g. 30X, 100X, 2000X) for germline or somatic variant tests compared to a reference average that is determined during assay validation*
- *Determination of test reproducibility (e.g. identification of the same variants in a specimen) over time (e.g. 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 (e.g. 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.

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. Revalidation/confirmation may cover all or a subset of steps in bioinformatics pipeline depending on the type of upgrade implemented (see MOL.36115). This procedure must designate specific monitoring intervals and address when such updates will be implemented.

Evidence of Compliance:

- ✓ Procedure for monitoring patch-releases, upgrades and updates **AND**
- ✓ Records of monitoring activities **AND**
- ✓ Records of revalidation/confirmation data including the type of upgrade, 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** 08/17/2016***

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 (pipeline) are typically comprised of a combination of different software packages, scripts, and databases. The versions and configuration of each component in the bioinformatics pipeline (e.g. 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 a laboratory-specific designation (e.g. NGS Pipeline v1.01) and/or include log files if generated with each analysis of a patient dataset. Laboratory-specific designations must be unique to each version of pipeline components and configurations. Any changes to software packages, scripts, databases, configuration files or other pipeline components require tracking in the pipeline version control system and updating to a new version.

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** 08/21/2017***

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 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 (e.g. REFSeq Accession Number, Ensembl Transcript/Protein ID, or CCDS ID) that allows unambiguous mapping of the variant. The reference genome (if applicable) assembly and version number used for alignment and variant calling must be reported. Variant chromosomal position (i.e. genomic coordinate) should be reported.

The ACMG guidelines should be used for classification and interpretation of germline variants in inherited disorders. The ACMG guidelines were not developed for the classification and interpretation of pharmacogenetic variants or somatic variants. For classification and interpretation of somatic variants (e.g. 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 (e.g. 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 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 (e.g. 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 (e.g. the Stanford HIV Drug Resistance Database, https://hivdb.stanford.edu/).

The written procedure describes the frequency of variant reassessments (can differ by clinical significance, e.g. 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 (e.g. 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.

***NEW** 08/21/2017*

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 (e.g. in the context of metagenomic sequencing) or between gene and phenotypic resistance (e.g. in the context of antimicrobial resistance), if applicable.

The written procedure describes the frequency of reassessments (can differ by clinical significance, e.g. 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 (e.g. 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

***REVISED** 08/21/2017*

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 (e.g. 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 ANEUPLOIDY

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

MOL.36310 Requisitions - Gestational Age **Phase I**

Requisitions include the gestational age estimate (based on ultrasound measurements), first day of the last menstrual period (LMP) or the estimated date of confinement (EDC).

NOTE: Relevant clinical validation studies included samples drawn only over a specific gestational age range (e.g. 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 22 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 (e.g. 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

MOL.36320 Requisitions - Maternal Birth Date/Age **Phase I**

Requisitions include 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 aneuploidies.

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

MOL.36330 Requisitions - Maternal Weight **Phase I**

Requisitions include 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. BMI may be a suitable replacement for maternal weight, but this has not yet been demonstrated.

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

MOL.36340 Requisitions - Parentage Information **Phase I**

Requisitions include 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) Blanchi 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 include clinical evidence of multiple gestations (e.g. 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

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

Requisitions include patient or family history of chromosomal abnormality (e.g. 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 include 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 (e.g. Down syndrome risk reported after a first trimester combined test) or collect information that the laboratory can use to derive a prior risk (e.g. maternal age and measurement of nuchal translucency).

MOL.36380 Quality Control Monitoring **Phase II**

Test performance limits and quality control parameters (e.g. 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 (e.g. maternal blood screening for fetal aneuploidy) 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) Blanchi 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

MOL.36390 Quality Control — Phase II

Positive and negative controls are included in each analytical run.

NOTE: Within each run, appropriate positive control DNA (e.g. a z-score of 7.5 for chromosome 21) and negative control DNA (e.g. a z-score of +0.3 for chromosome 13) are included.

Evidence of Compliance:

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

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. 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

MOL.36410 Monitoring of Targeted Disorders — Phase I

The percentages of women with positive results for each targeted disorder (e.g. Down syndrome, Turner syndrome), test failure rates (e.g. low fetal fraction) and 'inconclusive' (e.g. 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 (e.g. 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 (e.g. low risk, high risk). In many instances, the pregnancy is at high risk for only one or two of the aneuploidies, 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 (e.g. 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 (e.g. < 1:20,000 or > 10:1). If possible, the final interpretation should include, or refer to, previous relevant test results.

MOL.36430 Patient Report Information **Phase I**

The patient report includes the following information as appropriate: 1) a recommendation for follow-up diagnostic testing for all pregnancies with a positive test result; 2) a statement that this test is not intended to identify pregnancies at risk for open neural tube defects; and 3) recommendations regarding next steps for women with uninformative results and/or test failures.

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

STEM CELL ENGRAFTMENT MONITORING

Inspector Instructions:

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

*****NEW** 08/21/2017***

MOL.36435 Stem Cell Engraftment **Phase II**

For stem cell engraftment, the polymorphic nature and independent segregation (e.g. 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.

***NEW** 08/21/2017*
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.

***NEW** 08/21/2017*
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.

***NEW** 08/21/2017*
MOL.36450 Sensitivity Control **Phase II**

A sensitivity control is used and evaluated with each run.

***NEW** 08/21/2017*
MOL.36455 Internal Controls **Phase II**

For stem 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

***NEW** 08/21/2017*
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.

***NEW** 08/21/2017*
MOL.36470 Stem Cell Engraftment Testing **Phase II**

For stem 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 stem cell engraftment testing

***NEW** 08/21/2017*

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 stem cell engraftment testing **AND**
- ✓ Records of stem cell engraftment testing

***NEW** 08/21/2017*

MOL.36480 Preferential Allele Amplification **Phase II**

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

***NEW** 08/21/2017*

MOL.36490 Minimal Number of Informative Loci **Phase II**

For stem 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.

***NEW** 08/21/2017*

MOL.36495 Result Reporting **Phase II**

For stem 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. March 1, 2016.

RELATIONSHIP AND FORENSIC IDENTITY TESTING

Inspector Instructions:

	• 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
	• Limited-access secured area for specimens and records
	• What is your laboratory's course of action when evaluating closely spaced alleles?

***NEW** 08/21/2017*

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.

***REVISED** 08/21/2017*

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.

***REVISED** 08/21/2017*

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 maintained in a limited access, secured area and appropriate records of chain-of-custody maintained.

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 (SWGDAM). 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.

Inspector Instructions:

READ	• Sampling of ISH policies and procedures • Sampling of probe validation records • Sampling of QC records • Sampling of patient reports
ASK	• How are ISH cut-off values established? • How does your laboratory validate assay performance prior to test implementation? • What is your course of action if a probe does not produce an internal control signal? • What is your laboratory's course of action when negative HER2 (ERBB2) and/or negative results by IHC are obtained and the fixation time was not appropriate?

- Review a sampling of ISH cases and controls. Evaluate signal, background and morphology.

***NEW** 08/17/2016*

MOL.38600 ISH Probe Validation **Phase II**

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

NOTE: Refer to MOL.39323 for specific validation requirements for HER2 testing in breast carcinoma. Additional requirements for test method validation are in the All Common Checklist.

Evidence of Compliance:

✓ Written procedure for validation of ISH probes

REFERENCES

1) American College of Medical Genetics, Standards and Guidelines for Clinical Genetics Laboratories, 2009 edition, Revised 01/2010; Accessed 2/3/2011 at http://www.acmg.net/StaticContent/SGs/Section_E_2011.pdf
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.

***NEW** 08/17/2016*

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, 2009 edition, Revised 01/2010; Accessed 2/3/2011 at http://www.acmg.net/StaticContent/SGs/Section_E_2011.pdf
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.

***NEW** 08/17/2016*

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

***NEW** 08/17/2016*

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

***REVISED** 08/17/2016*

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: Refer to MOL.39393 for specific requirements on the scoring criteria for HER2 testing in breast carcinoma.

REFERENCES

1) American College of Medical Genetics and Genomics. Laboratory Standards and Guidelines for Clinical Genetics Laboratories, 4th ed. Bethesda, MD: ACMG, 2008.
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.

***REVISED** 08/17/2016*

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 (e.g. 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 and Genomics. Laboratory Standards and Guidelines for Clinical Genetics Laboratories, 4th ed. Bethesda, MD: ACMG, 2008.
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.

***NEW** 08/17/2016*

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** 08/17/2016*

MOL.39155 ISH Interpretation **Phase II**

If an *in situ* hybridization (ISH) study requires consultation with a 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.

***REVISED** 08/17/2016*

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: ISH assays for neoplastic disorders must be retained for 10 years; images 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 and Genomics. Laboratory Standards and Guidelines for Clinical Genetics Laboratories, 4th ed. Bethesda, MD: ACMG, 2008.

***REVISED** 08/17/2016*

MOL.39323 HER2 (ERBB2) Assay Validation **Phase II**

If the laboratory tests for HER2 (ERBB2) gene amplification by *in situ* hybridization (*e.g.* FISH, CISH, SISH, etc.), the laboratory has records of validation for the assay(s).

NOTE: This requirement applies to both new and existing assays. Test validation must be performed on a minimum of 20 positive and 20 negative samples for FDA-cleared/approved assays; or 40 positive and 40 negative samples for laboratory-developed tests (LDTs). Equivocal samples need not be used for validation studies. If the initial validation of existing assays does not meet the current standard, it must be supplemented and brought into compliance. It is permissible to do this retroactively by review of performance on past proficiency testing challenges or by sending unstained slides from recent cases to a referral laboratory for correlation. If there are no records of the initial validation, the assay must be fully revalidated and records retained.

Validation may be performed by comparing the results of testing with a validated alternative method (i.e. IHC vs. ISH) either in the same laboratory or another laboratory, or with the same validated method performed in another laboratory; validation testing must be done using the same set of cases in both labs. The validation records should identify the comparative test method(s) used.

The validation data should clearly show the degree of concordance between methods, e.g. for IHC: 0, 1+, 3+; for FISH, CISH, SISH: positive, negative, as defined by the cut-offs listed in the latest version of the CAP/ASCO guideline.

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

Samples used for validation must be handled in conformance with the guidelines in this checklist. If specimens are fixed in a medium other than 10% neutral buffered formalin, the validation study must show that results are concordant with results from formalin-fixed tissues.

If significant changes are made in testing methods (e.g. probe, pretreatment protocol), revalidation is required.

This checklist requirement applies to laboratories that perform the technical testing of specimens for HER2 (ERBB2) amplification. Patient specimens should be fixed in the same manner as the specimens used for the validation study(ies).

Evidence of Compliance:

✓ Records of validation data including criteria for concordance

MOL.39358 HER2 (ERBB2) by ISH - Fixation **Phase I**

If the laboratory assesses HER2 (ERBB2) gene amplification by *in situ* hybridization (*e.g.* FISH, CISH, SISH), there is a written procedure to ensure appropriate specimen length of fixation time.

NOTE: Specimens subject to HER2 (ERBB2) testing should be fixed in 10% neutral buffered formalin for at least six hours and up to 72 hours. The volume of formalin should be at least 10 times the volume of the specimen. Decalcification solutions with strong acids should not be used.

Laboratories must communicate the following fixation guidelines to clinical services:

1. *Specimens should be immersed in fixative within one hour of the biopsy or resection*
2. *If delivery of a resection specimen to the pathology department is delayed (e.g. specimens from remote sites), the tumor should be bisected prior to immersion in fixative. In such cases, it is important that the surgeon ensure that the identity of the resection margins is retained in the bisected specimen; alternatively, the margins may be separately submitted.*
3. *The time of removal of the tissue and the time of immersion of the tissue in fixative should be recorded and submitted to the laboratory*

Communication may be through memoranda, website, phone, face-to-face meetings, or other means. The laboratory should consider monitoring compliance and contacting clients when these guidelines are not met.

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

Laboratories testing specimens obtained from another institution should have a policy that addresses time of fixation. Information on time of fixation may be obtained by appropriate requirements on the laboratory's requisition form.

Reports should qualify any negative results for specimens not meeting the above guidelines.

MOL.39393 HER2 (ERBB2) by ISH - Scoring **Phase II**

If the laboratory interprets HER2 (ERBB2) gene amplification by *in situ* hybridization (*e.g.* FISH, CISH, SISH), results are reported using either the ASCO/CAP scoring criteria or the manufacturer's instructions.

NOTE: The table below contains the ASCO/CAP scoring criteria used to determine HER2 (ERBB2) gene status by in situ hybridization.

Careful attention should be paid to the recommended exclusion criteria for performing or interpreting in situ hybridization for HER2 (ERBB2) (e.g. signal obscured by background; for FISH, difficulty in defining areas of invasive carcinoma under UV light).

Variable ISH positivity (heterogeneity) must also be considered when analyzing ISH studies. ISH slides are scanned at low power prior to counting to determine if there is a discrete population of amplified cells representing more than 10% of the invasive tumor cells in that area; such cases are reported as HER2 (ERBB2) positive (amplified).

For FDA-cleared or approved test systems that use different scoring criteria, the manufacturer's instructions may be followed.

Method	Result	Ratios of HER2 (ERBB2) to CEP17**	Average HER2 (ERBB2) Copy Number (Signals/ Cell)**
HER2 (ERBB2) ISH - Test systems with internal control probe	Positive (amplified)	≥2.0	N/A
		<2	≥6.0
	Negative	<2.0	<4.0
	Equivocal	<2.0	≥4.0 and <6.0
HER2 (ERBB2) ISH - Test systems without an internal control probe	Positive (amplified)	N/A	≥6.0
	Negative	N/A	<4.0
	Equivocal	N/A	≥4.0 and <6.0

***Criteria in both columns must be met for tests with internal reference probes. For example, for a result to be negative, the ratio must be <2.0 and the average copy number must be <4.0.*

REFERENCES
1) Wolff AC, Hammond ME, Hicks DG, *et al.* Recommendations for human epidermal growth factor receptor 2 testing in breast cancer; American Society of Clinical Oncology/College of American Pathologists. *Arch Pathol Lab Med* 2014;138(2):241-256

SPECTROPHOTOMETERS

Inspector Instructions:

READ	• Spectrophotometer policies/procedures • Sampling of manufacturer required system checks
ASK	• 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:

- Sampling of background checks

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:

	• Sampling the film processing maintenance records

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:

	• Pipette calibration procedure • Sampling of pipette/dilutor checks • Sampling of thermocycler monitoring records
	• How does your laboratory ensure the individual wells of the thermocycler are maintaining accurate temperature?

MOL.48588 Pipette Accuracy **Phase II**

Pipettors that are used for quantitative dispensing of material are checked for accuracy and reproducibility before being placed in service and at defined intervals (at least annually), and records maintained.

NOTE: Pipette checks must be performed following manufacturer's instructions, at minimum, and as defined in laboratory procedure. Such checks are most simply done gravimetrically. This consists of transferring a number of measured samples of water from the pipette to a balance. Each weight is recorded, the weights are converted to volumes, then means (for accuracy), and

SD/CV (for imprecision) are calculated. Alternative approaches include spectrophotometry or (less frequently) the use of radioactive isotopes, and commercial kits are available from a number of vendors. Computer software is useful where there are many pipettes, and provides convenient records.

REFERENCES
1) Curtis RH. Performance verification of manual action pipets. Part I. *Am Clin Lab. 1994*;12(7):8-9
2) Curtis RH. Performance verification of manual action pipets. Part II. *Am Clin Lab*. 1994;12(9):16-17
3) Perrier S, *et al*. Micro-pipette calibration using a ratiometric photometer-reagent system as compared to the gravimetric method. *Clin Chem.* 1995;41:S183
4) Clinical and Laboratory Standards Institute. *Laboratory Instrument Implementation, Verification, and Maintenance; Approved Guideline.* CLSI Document GP31-A. Clinical and Laboratory Standards Institute, Wayne, PA; 2009.
5) Johnson B. Calibration to dye for: Artel's new pipette calibration system. *Scientist.* 1999;13(12):14
6) Connors M, Curtis R. Pipetting error: a real problem with a simple solution. Parts I and II. *Am Lab News.* 1999;31(13):20-22
7) Skeen GA, Ashwood ER. Using spectrophotometry to evaluate volumetric devices. *Lab Med.* 2000;31:478-479

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.

*****NEW** 08/17/2016***
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

Inspector Instructions:

- Sampling of molecular genetic test reporting policies and procedures
- Sampling of patient test reports for completeness
- Sampling of patient test reports performed with Class I ASRs including appropriate disclaimer

- 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?

- Follow a molecular genetic test report from specimen receipt and processing to evaluation, interpretation, identification and reporting

MOL.49555 Preliminary Reports **Phase I**

Preliminary reports are promptly generated, when indicated.

MOL.49560 Preliminary/Final Report Discrepancies **Phase II**

Discrepancies between preliminary and final reports are investigated, corrective action taken, as necessary, and records maintained.

MOL.49565 Discrepant Results **Phase I**

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.

MOL.49570 Final Report Criteria **Phase II**

The final report includes an appropriate summary of the methods, the loci or variants tested and the analytic interpretation (*i.e.* test result). When appropriate, the final report includes the clinical interpretation.

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 or quantity of the analyte. Limitations of testing should be incorporated in the report, including a statement citing that variants not detected by the assay that was performed 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. Braziel, Kevin C. Halling (2007) Clinical Laboratory Reports in Molecular Pathology. *Archives of Pathology & Laboratory Medicine*: Vol. 131, No. 6, pp. 852-863

***REVISED** 08/17/2016*

MOL.49575 Variant Database **Phase I**

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

***REVISED** 08/21/2017*

MOL.49580 ASR Disclaimer **Phase II**

If patient testing is performed using Class I analyte-specific reagents (ASRs) obtained or purchased from an outside vendor, the patient report includes the disclaimer statement required by federal regulations.

NOTE: ASRs are antibodies, both polyclonal and monoclonal, specific receptor proteins, ligands, nucleic acid sequences, and similar reagents which, through specific binding or chemical reaction with substances in a specimen, are intended for use in a diagnostic application for identification and quantification of an individual chemical substance or ligand in biological specimens.

An ASR is the active ingredient of a laboratory-developed test system. Class I ASRs are not subject to preclearance by the US Food and Drug Administration (FDA) or to special controls by the FDA.

If the laboratory performs patient testing using Class I ASRs, federal regulations require that the following disclaimer accompany the test result on the patient report: "This test was developed and its performance characteristics determined by (laboratory name). It has not been cleared or approved by the US Food and Drug Administration."

The CAP recommends additional language, such as "The FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing."

The disclaimer is not required for tests using reagents that are sold in kit form with other materials and/or an instrument, and/or with instructions for use, and/or when labeled by the manufacturer for in vitro diagnostic use (IVD), Class II IVD, or Class III IVD.

The laboratory must establish the performance characteristics of tests using Class I ASRs in accordance with the Method Performance Specification section of the All Common Checklist and the Assay Validation section of this checklist.

The laboratory may put a single ASR disclaimer on the patient report for all studies collectively used in a particular case. Separately tracking each reagent used for a case and selectively applying the disclaimer to only the Class I ASRs is unnecessary.

REFERENCES

1) Department of Health and Human Services, Food and Drug Administration. Medical devices; classification/reclassification; restricted devices; analyte specific reagents. Final rule. *Fed Register.* 1997(Nov 21);62243-45 [21CFR809, 21CFR864]
2) Caldwell CW. Analyte-specific reagents in the flow cytometry laboratory. *Arch Pathol Lab Med.* 1998;122:861-864
3) Graziano. Disclaimer now needed for analyte-specific reagents. Northfield, IL: College of American Pathologists *CAP Today*. 1998;12(11):5-11

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

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 (e.g. 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.

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

*****REVISED** 08/17/2016***

MOL.49600 Report Criteria **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

*****REVISED** 08/17/2016***

MOL.49615 Report Criteria **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 http://www.abmg.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 (e.g. ERBB2) should be used, along with any colloquial names (e.g. 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/mutnomen/ accessed 2/8/2012
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:

	• Record retention policy and procedures
	• Autoradiographs/gel photographs/*in situ* 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** 08/17/2016***
MOL.49640 Record Retention **Phase II**

A copy of each final report, all records of results, membranes, autoradiographs, gel photographs, and *in situ* hybridization slides, are retained in compliance with applicable 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.

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

***REVISED** 08/21/2017*

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.

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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:

	• Records of biological safety cabinet certification
	• 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
1) 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
1) 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
2) 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
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.