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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
07/28/2015 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

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MOL.49545  04/20/2014
MOL.49550  04/20/2014
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 fluorescence in situ hybridization (FISH), 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

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:

READ

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

MOL.30440 Calculations for Quantitative Tests Phase II

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

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. When melting curves are generated, there should be criteria for interpreting results.

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.

MOL.30670 Intended Use of Assay Phase II

The procedure manual describes the intended use of the assay for ordering the tests in patient management, with pertinent literature references.
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 range. 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 range, 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, etc., 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, etc.) 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
- Sampling of laboratory-developed patient test reports including methodology, statement and performance characteristics

- How does your laboratory validate assay performance prior to test implementation?
- How does your laboratory validate clinical claims made by the laboratory about LDTs?

**REVISED** 07/28/2015

MOL.30785 Validation Summary

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 range, 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

MOL.31015 Validation Studies - Specimen Types Phase II
Validation studies with an adequate number and representative (reasonable) distribution of samples are performed for each type of specimen expected for the assay (e.g. blood, fresh/frozen tissue, paraffin-embedded tissue, prenatal specimens).

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 mutations (e.g. cystic fibrosis or hereditary nonpolyposis colorectal cancer) 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

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

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 mutant). 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

**REVISED** 07/28/2015

MOL.31255 Reference Range

Phase II

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

NOTE: The reference range is the range of results expected in the normal population. For some qualitative tests (e.g. HLA genotyping), the reference range 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 is used to determine the reference range, it must be carefully evaluated, with records of the evaluations retained.

Evidence of Compliance:
✓ Records of validation studies to confirm or establish each test's reportable range

REFERENCES

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 appropriate biological matrix (e.g. pathogens, rare mutants, chimerism, mosaicism, etc.).

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

**REFERENCES**

**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**

**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 mutations or polymorphisms, 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

MOL.31705 LDT Report Phase I

Reports for laboratory-developed tests (LDTs) contain a description of the method, a statement that the assay was developed by the laboratory, and appropriate performance characteristics.

NOTE: General guidelines for reports are given in the Results Reporting section of this checklist. Particularly with respect to newly implemented assays, careful attention should be given to including in the report the analytical and clinical performance specifications.

Evidence of Compliance:
✓ Patient reports for LDTs with required information

REFERENCES

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
**REVISED** 07/28/2015

**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


**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


**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

MOL.32395 Neoplastic Cell Content

For paraffin-embedded tumor specimens from which DNA 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 assess neoplastic cellularity to ensure that the percentage of neoplastic cells exceeds the limit of detection for the assay.

A corresponding H&E section from the same tissue block used for DNA 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 DNA extraction. When assessment of sample adequacy is performed outside of the testing laboratory, a record of such assessment must accompany the sample.

MOL.32425 Nucleic Acid Extraction/Isolation/Purification

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

MOL.32430 Nucleic Acid Quantity

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

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

**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**

**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/](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**
QUANTITATIVE ASSAYS: CALIBRATION AND STANDARDS

DEFINITIONS:

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

DEFINITIONS:

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 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:

- **READ**
  - Sampling of calibration and AMR policies and procedures
  - Sampling of calibration/calibration verification records
  - Sampling of AMR verification records

- **OBSERVE**
  - Sampling of calibration materials (quality)

- **ASK**
  - 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?

- **DISCOVER**
  - Further evaluate the responses, corrective actions, and resolutions for unacceptable calibration and unacceptable calibration verification

**REVISED** 07/28/2015
MOL.33655 Calibration Procedures

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
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.33696 Calibration Materials

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
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

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 should 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

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

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

**REVISED** 07/28/2015

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
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

MOL.34024 Calibrator Preparation
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

REAGENTS

Inspector Instructions:
- Sampling of probe/primer information

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

MOL.34188 Probe Characteristics
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

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

**REVISED** 07/28/2015

**MOL.34229 Controls - Qualitative Assays**

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 mutation panel for cystic fibrosis, 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 is required if the molecular assay is being used to detect low-level target sequences.

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**
Controls - Quantitative Assays

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

Tolerance Limits - Controls

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

QC Confirmation of Acceptability

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
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

**REVISED** 04/21/2014

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

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
**REVISED** 07/28/2015

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

MOL.34516  Qualitative Cut-Off  Phase II

For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is established initially, and 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 established or verified when the test is initially placed in service, and verified with every change in lot (e.g. new master mix), instrument maintenance, or at least every six months thereafter. 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 initial establishment and verification of the cut-off value AND
✓ Records of initial establishment and verification of cut-off value at defined frequency

MOL.34557  Control Storage  Phase I

Controls are stored in a manner that maintains their integrity.

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METHODS AND INSTRUMENT 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

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

MOL.35050  Molecular Weight Markers

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

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

MOL.35150  Autoradiograph/Electrophoretic Gel Interpretation

Autoradiographs or electrophoretic gels are interpreted using objective criteria.

Evidence of Compliance:
✓ Written procedure including interpretive criteria for autoradiographs or gels

REFERENCES

MOL.35175  Autoradiograph/Gel Photograph Resolution

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?

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. In a given run, specimens should be ordered in the following sequence: patient samples, positive controls, negative controls (including “no template” controls in which target DNA is omitted and therefore no product is expected). 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

MOL.35360 Internal Controls - NAA

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

For tests that generate a result based on a Tm, 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

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**MOL.35722** Integrity/Labeling Verification

**Phase I**

**Integrity/Labeling Verification**

**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**


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**MOL.35766** Array Quality

**Phase I**

**Array Quality**

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**


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**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  

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 mutation 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.

**REVISED** 07/28/2015  
MOL.35795 Analysis of Tumor Cell Percentage and Sequencing Lower Limit of Detection  
Phase I  
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

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

NOTE: DNA sequencing assays should be reserved for those disease 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

**REVISED** 04/21/2014

MOL.35805 Sequencing Assay Optimization

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 mutant 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 mutation 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 mutation 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
MOL.35815 Sequencing Data Criteria

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

**REVISED** 04/21/2014

MOL.35820 Sequence Interpretation Guidelines

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 unknown 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 frequency in the affected tumor (e.g. as reported in the COSMIC database), gene-specific functional data, availability of targeted therapy, and other patient-specific clinical/pathological factors.

REFERENCES
1) ACMG Standards and Guidelines for Clinical Laboratories, http://www.acmg.net
2) COSMIC: Catalog of Somatic Mutations in Cancer. Nucl. Acids Res. gkq929 first published online October 15, 2010 doi:10.1093/nar/gkq929

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. 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 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 reference laboratories.

**REVISED** 07/28/2015

MOL:35840 Next Generation Sequencing (NGS) Reference Laboratory Selection Phase II

The laboratory has a written policy for selection and evaluation of reference 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 reference laboratories.

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

When utilizing a reference laboratory for NGS testing, the laboratory director must ensure that the reference laboratory's validation, quality, and turnaround times are acceptable for the clinical needs for which testing is being done. This requirement does not apply if the reference laboratory issues the final interpretive report.

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 or a laboratory meeting equivalent requirements as determined by the Centers for Medicare and Medicaid Services (CMS). CAP accreditation standards must meet or exceed those of the CLIA regulations.

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, 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 reference laboratory accreditation.

Evidence of Compliance:
✓ Records of evaluations of reference laboratories for NGS referral testing AND
✓ Copies of valid CLIA certificates from CLIA-certified reference laboratories OR
✓ Copies of valid CAP accreditation certificates from CAP accredited reference 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)]

**NEW** 07/28/2015
MOL.35845  Tracking of Specimens Referred for NGS Testing  Phase I

The laboratory has records for the tracking of specimens 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 reference laboratory for sequencing. A reference 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 reference 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 reference laboratories to describe unambiguously when and how specimens and data (including file formats) are transferred and/or exchanged.

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** 07/28/2015
MOL.35850  NGS Confirmatory Testing  Phase I

The laboratory has a written policy that describes the indications for confirmatory testing of reported variants.

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 to confirm variants, other methods (e.g. allele-specific PCR, melting curve analysis, alternative NGS chemistries) are acceptable. 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.

**REVISED** 07/28/2015
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 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** 07/28/2015
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 to reference laboratories for analysis or to external companies for storage, including through cloud-based computing.

Procedures to ensure confidentiality can include message security, use of secure and encrypted protocols for data transfer (e.g. SFTP, HTTPS, FTPS), system and user authentication, activity logs, encryption, 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 reference laboratories or companies storing datasets

**REVISED** 07/28/2015
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.

NOTE: The data retained must include the files necessary to re-review cases in the exact manner that was conducted for original results reporting. Examples include specimen tracking and quality metrics data/files, sequencing run quality metrics reports, sequence read alignments, variants manually reviewed and files containing filtered and/or interpreted variants. The files retained must facilitate inter-laboratory interoperability to permit between laboratory re-analysis, re-annotation or reinterpretation 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** 07/28/2015
MOL.36010  NGS Analytical Wet Bench Process Records  Phase II

The laboratory has a written procedure for performing the analytical wet bench process used to generate next generation sequencing data.

NOTE: The procedure must include:

- A description of the analytical target regions (e.g. genes in a panel, exome, genome or other targeted regions, such as introns or promoter sites)
- A description of acceptable sample types for which the assay has been validated (e.g. blood, saliva, FFPE specimens), 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
- 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 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 or a control with known mutation(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. These may include, but are not limited to: 1) post-fragmentation on nucleic acid size distribution, 2) NGS library concentration on and size distribution, 3) flow cell cluster density, 4) sequence read base quality scores, 5) sequence reads passing instrument quality filters, 6) total numbers of sequence reads generated, and 7) error rates.
- Written procedure for any portion of the NGS analytical wet bench process performed by a reference laboratory, if applicable

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

**REVISED** 07/28/2015
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 the performance of the components of the process are 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 reference laboratories (see MOL.35840). Whether performed in a single laboratory, or in a distributive model involving primary and reference 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 primary laboratory director 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 reference laboratories, if applicable.

Due to the diversity of human genetic variation (including germline and somatic mutation), 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 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 variants that the test is designed to detect. When using the same wet bench process for more than one test designed to detect the same types of variants (e.g. the same targeted enrichment chemistry for different diagnostic gene panels), a single methods-based validation can be conducted including results from more than one test. For tests that analyze genes with common pathogenic mutations (e.g. CFTR p.Phe508del or a mutation hotspot of therapeutic relevance in an oncology gene), specimens with those common mutations should be included, when feasible, in the validation to ensure their accurate detection.

Metrics and quality control parameters must be established and used to assess wet bench process performance during validation. Metrics and parameters will vary between 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. 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 metrics and QC parameters used to establish and assess wet bench performance AND
- Written approval of validations, revalidations and/or confirmation studies by the laboratory director AND
- Records of review of reference laboratory validations by the primary laboratory director, if applicable

REFERENCES

**REVISED** 07/28/2015
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 any deviations from the standard operating procedure, together with their impact on clinical testing. Corrective actions must be recorded for any steps failing laboratory determined standards. The QM program must include metrics and quality control parameters that are used to monitor and assess analytical run performance on a per run basis and those that are monitored on a periodic basis (e.g. 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, quality and error rates. Exceptions must be reviewed and 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

MOL.36030 Laboratory Records Phase II

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

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

**REVISED** 07/28/2015

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.
The laboratory has a written procedure for 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.

Records 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
- Records of the code, tests performed and 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 on 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 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, allelic read percentage)
- 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), 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), correlating with patient phenotype, 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 phenotype
- 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) read coverage of target regions, 4) target regions with inadequate sequence due to mapping qualities and/or coverage below thresholds, 5) numbers and types of variants from reference, and 6) transition to transversion ratio in exome and genomes.
- Written procedures for any portion of the NGS bioinformatics process performed by a reference laboratory, if applicable

**Evidence of Compliance:**

✓ Written procedure that describes the analytical bioinformatics process, including sections noted above, as applicable
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 reference laboratories (see MOL.35840). Whether performed in a single laboratory, or in a distributive model involving primary and reference 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 primary laboratory director 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 reference laboratories, if applicable.

Due to the diversity of human genetic variation (including germline and somatic mutation), 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 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 variants that the test is designed to detect. When using the same bioinformatics pipeline to process sequence generated by tests that use the same wet bench process (e.g. the same targeted enrichment chemistry for different diagnostic gene panels), a single methods-based validation can be conducted including results from more than one test. For tests that analyze genes with common pathogenic mutations (e.g. CFTR p.Phe508del or a mutation hotspot of therapeutic relevance in an oncology gene), specimens with those common mutations should be included, when feasible, in the validation to ensure their accurate detection.

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:

- Description of the analytical target (e.g. exons, genes, or targeted regions, such as introns or promoter sites) and bioinformatics pipeline used for analysis, including algorithms, test scripts, and test or training datasets
- Validation of sample pooling methods to ensure that individual sample identity is maintained throughout the bioinformatics pipeline, if applicable
- Criteria and thresholds for variant calling (e.g. minimum read coverage depth, base or variant quality scores, allelic read percentage). Criteria may be differently defined based on application (e.g. detection of germline versus somatic mutations)
- Determination of target regions where variant calls may be affected by highly homologous regions (e.g. pseudogenes). The laboratory must document how interference by homologous regions is mitigated or avoided. This may include custom approaches to confirm test results in these regions.
- Determination of test performance characteristics for 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 variant calls (which can be calculated from the number of true positives divided by the number of true positives plus the number of false positives). Specimens containing examples of the different variant types to be detected by the test must be included in the validation in order to determine test performance characteristics. This can be complemented, but not supplanted, by the use of synthetic or in silico generated datasets.
- Determination of the limits of detection for variants in samples with heterogeneous genotypes (e.g. tumor sample or maternal blood screening for fetal aneuploidy), if applicable
- Determination of the detection rate for causal variants (i.e. mutations) by bioinformatics processes and software designed for their detection in exome and genome sequencing datasets. This is primarily applicable to exome and genome sequencing data analysis.
for undiagnosed patients suspected of having a genetic etiology. Approaches for
determination of detection rates may include, but are not limited to, analysis of samples
with known genetic etiologies (e.g. previously characterized samples with known
autosomal recessive, dominant or de novo variants).

- 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. 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) read coverage of target regions, 4) target regions with
inadequate sequence due to mapping qualities and/or coverage below thresholds, 5)
numbers and types of variants from reference, and 6) transition to transversion (ts/tv)
ratio in exome and genome.

Validations, revalidations, and/or confirmations require written approval by the laboratory director. 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 wet bench 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 by the laboratory director AND
✓ Records of review of reference laboratory validations by the primary laboratory director, if applicable

REFERENCES
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 composed 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 specify at what interval the monitoring will occur and also 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, and metrics and quality control (QC) parameters monitored to assess analytical run performance AND
✓ Approval of revalidation/confirmation data and of the change of protocol by the laboratory director AND
✓ Dates of implementation

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

NOTE: The bioinformatics process (pipeline) used to generate and analyze NGS data is typically comprised of a combination of different software packages, scripts, and databases. The specific versions of each component and associated configurations (e.g. command lines or other configuration items) of the bioinformatics pipeline used to generate NGS data must be traceable for each patient report. Records of each component of the pipeline 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 a single combination of pipeline components and configurations. Therefore, any change to a different version of a software package, script, or internal or external database or change to this configuration of any software would require a new unique laboratory-specific designation.

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** 07/28/2015
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 relationships, if applicable (typically in the context of exome/genome sequencing).

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 sequence genome 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. For classification and interpretation of somatic variants (e.g. in tumors), the laboratory should have a written procedure that considers factors such as variant frequency in the tumor type (e.g. as documented in the COSMIC database), gene specific functional data, availability of targeted therapy, and other relevant patient specific clinical pathological factors.

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
2) COSMIC: Catalog of Somatic Mutations in Cancer. Nucl. Acids Res. gkq929 first published online October 15, 2010 doi:10.1093/nar/gkq929
3) HGVS. http://www.hgvs.org/mutnomen

**REVISED** 07/28/2015
MOL.36165  Reporting of Genetic Findings  Phase I

The laboratory has a policy for reporting genetic 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 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.

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, as applicable

REFERENCES

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

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
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**

**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**

**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

**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**


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

MOL.36410 Monitoring of Targeted Disorders

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

Reports include qualitative and/or quantitative test results for each target chromosome (e.g. z-score, fetal fraction, likelihood ratio), reference ranges 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

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
PARENTAGE AND FORENSIC IDENTITY TESTING

Inspector Instructions:

- Sampling of parentage/forensic policies and procedures
- Sampling of paternity/forensic 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

- What is your laboratory's course of action when evaluating closely spaced alleles?

MOL.37442 Paternity/Forensic Data

For paternity/forensic identity testing, the following data are obtained.

1. Place and date of specimen collection
2. Identity of person collecting the specimen
3. Photograph or photocopy of a picture identification card for each individual tested
4. Signed record of information (including name, race, relationship) for each individual tested
5. Date of birth of child
6. Synopsis of case history/investigation, sample source
7. Record of informed consent

REFERENCES
1) Standards for Parentage testing laboratories. American Association of Blood Banks. Standards for parentage testing laboratories. Bethesda, MD: 2003:5.2.4

MOL.37584 Transfusion/Transplantation History

For paternity/forensic identity testing, the laboratory seeks a history of transfusion in the preceding three months or a history of allogeneic hematopoietic progenitor cell transplantation at any time.

Evidence of Compliance:
✓ Specimen requisition/collection forms with transfusion/transplant history

REFERENCES
1) Standards for Parentage testing laboratories. American Association of Blood Banks. Standards for parentage testing laboratories. Bethesda, MD: 2003:5.2.4.6

MOL.37726 Specimen Labeling - Paternity/Forensic ID

Phase II
For paternity/forensic identity testing, the information about each individual and the accuracy of the sample label are verified by that individual or the legal guardian.

Evidence of Compliance:
✓ Records of information and label verification by patient or legal guardian

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 Parentage/Forensic Records Phase II
For legal parentage and forensic 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 parentage and forensic 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.

REFERENCES

MOL.38294 DNA Results Interpretation Phase II
For parentage 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

REFERENCES
1) Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:5.4.2.1-3

MOL.38436 Exclusion Evaluation Phase II
For parentage 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

REFERENCES
1) Standards for parentage testing laboratories. Bethesda, MD: AABB, 2003:5.4.2.1-2

**REVISED** 04/21/2014
MOL.38578 Forensic ID 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 Standards and 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 DNA testing laboratories. Forensic Science Communications 2000:2.2
2) Short tandem repeat (STR) interpretation guidelines. Ibid
3) Guidelines for mitochondrial DNA (mtDNA) nucleotide sequence interpretation. Ibid. 2003:5

**FLUORESCENCE AND NON-FLUORESCENCE**

**IN SITU HYBRIDIZATION (FISH, ISH)**

Inspector Instructions:

- Sampling of FISH/ISH policies and procedures
- Sampling of HER2 (ERBB2) assay validation studies
- Sampling of probe validation records
- Sampling of QC records
- Sampling of patient reports for completeness including ASCO/CAP scoring when applicable

- 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?

MOL.39004  FISH Scoring  Phase II

There are written procedures for scoring FISH results, including the number of cells scored, and all analyses are scored according to these procedures.

REFERENCES

**REVISED** 07/28/2015

MOL.39146  FISH Controls  Phase II

A hybridization control, internal or external, is used with each FISH analysis.

NOTE: When normal chromosome targets are expected to be present within a sample, an internal control for that target should be used during each hybridization (i.e. a locus specific probe at a different site on the same chromosome and/or a normal locus on the abnormal homolog). 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 with the patient sample.

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. 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 policy defining use of control loci with each FISH analysis AND
✓ Records of QC results

REFERENCES

MOL.39155  Morphologic Interpretation  Phase II

For in situ hybridization studies, the morphologic interpretation and correlation of results are performed by a qualified anatomic or clinical pathologist as appropriate.

NOTE: In situ hybridization requires evaluation of the histopathology or cytopathology in the hybridized slide to ensure that the hybridization signal is located to the appropriate lesional cells. For example, for HER2 (ERBB2), FISH or ISH, a corresponding H&E slide must be evaluated by a pathologist to ensure that the studies are performed on invasive tumor cells.

REFERENCES

MOL.39288  Retention - Images  Phase II

Photographic or digitized images are retained of all FISH assays (at least one cell for assays with normal results and at least two cells for assays with abnormal results).

NOTE: Images of FISH assays for neoplastic disorders must be retained for 10 years; images of FISH assays for constitutional disorders must be retained for 20 years. Brightfield ISH slides or images must be retained for the same time periods.

Evidence of Compliance:
✓ Written retention policy

REFERENCES

**REVISED** 04/21/2014
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. Initial 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 reference 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. FISH) 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).

(*CISH = chromogenic in-situ hybridization; SISH = silver-enhanced in-situ hybridization)

Evidence of Compliance:
✓ Records of validation data including criteria for concordance

**REVISED** 07/28/2015
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 buffered 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.
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/approved test systems that use different scoring criteria, the manufacturer’s instructions may be followed.

<table>
<thead>
<tr>
<th>Method</th>
<th>Result</th>
<th>Ratios of HER2 (ERBB2) to CEP17**</th>
<th>Average HER2 (ERBB2) Copy Number (Signals/Cell)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 (ERBB2) ISH - Test systems with internal control probe</td>
<td>Positive (amplified)</td>
<td>≥2.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;2</td>
<td>≥6.0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>&lt;2.0</td>
<td>&lt;4.0</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>&lt;2.0</td>
<td>&gt;4.0 and &lt;6.0</td>
</tr>
<tr>
<td>HER2 (ERBB2) ISH - Test systems without an internal control probe</td>
<td>Positive (amplified)</td>
<td>N/A</td>
<td>≥6.0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>N/A</td>
<td>&lt;4.0</td>
</tr>
<tr>
<td></td>
<td>Equivocal</td>
<td>N/A</td>
<td>≥4.0 and &lt;6.0</td>
</tr>
</tbody>
</table>

**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

**ISH Interpretation**

Appropriate interpretation of in situ hybridization results are provided in the report.

NOTE: ISH reports should include both an analytical and clinical interpretation.

REFERENCES
BRIGHTFIELD *IN SITU* HYBRIDIZATION

**Inspector Instructions:**

- Sampling of brightfield ISH policies and procedures
- Sampling of QC records

**MOL.39430 Assay Conditions/Tissue Pretreatment Verification**

**Phase II**

Assay conditions and conditions of tissue pretreatment are verified for each sample, using an appropriate positive control probe(s) against endogenous targets.

**NOTE:** The laboratory must demonstrate and record that assay conditions and tissue pretreatment allow for detection of the intended target sequence. Nucleic acid preservation and accessibility vary with fixation and processing. Adjusting assay conditions to demonstrate the signal for an endogenous target with a positive control probe allows negative results with the test probe to be interpreted. Negative results with the endogenous positive control probe allow inadequately preserved samples to be eliminated. The positive control probe may be directed to any target known to be in the sample.

**Evidence of Compliance:**
- Records of positive control probes for endogenous targets for each sample

**REFERENCES**


SPECTROPHOTOMETERS

**Inspector Instructions:**

- Spectrophotometer policies/procedures
- Sampling of manufacturer required system checks
- How does your laboratory verify calibration curves?

**MOL.44394 Filter Photometers**

**Phase II**

Filters (filter photometers) are checked at least annually to ensure they are in good condition (*e.g.* clean, free of scratches).

**Evidence of Compliance:**
- Records of filter checks at defined frequency
**REVISED** 07/28/2015
**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

**REVISED** 07/28/2015
**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

**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
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?

**REVISED** 07/28/2015  
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

**REVISED** 07/28/2015

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

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

Preliminary reports are promptly generated, when indicated.

**REVISED** 07/28/2015

MOL.49560 Preliminary/Final Report Discrepancies

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

MOL.49565 Discrepant Results

Discrepancies between the molecular pathology laboratory's final results, other laboratory findings, and the clinical presentation are investigated and recorded, along with any necessary corrective action.

**REVISED** 07/28/2015

MOL.49570 Final Report Criteria

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


MOL.49575 Mutation Database

The laboratory’s database for known mutations, benign polymorphisms and variants of undetermined significance is recorded and updated as needed, when applicable.

MOL.49580 ASR Disclaimer

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 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.

This checklist requirement concerns Class I ASRs. Class I ASRs are not subject to preclearance by the US Food and Drug Administration (FDA) or to special controls by FDA. Most ASRs are Class I. Exceptions include those used by blood banks to screen for infectious diseases (Class II or III), or used to diagnose certain contagious diseases (e.g. HIV infection and tuberculosis) (class III).

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 "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 or an instrument, nor reagents sold with instructions for use, nor reagents labeled “for in vitro diagnostic use” (IVD) by the manufacturer.

The laboratory must establish the performance characteristics of tests using Class I ASRs in accordance with the Assay Validation section of this checklist.

REFERENCES

**REVISED** 07/28/2015
MOL.49585 Report Sign-off

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

**REVISED** 07/28/2015
MOL.49590 Patient Confidentiality

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 mutation.

REFERENCES

MOL.49600 Report Criteria Phase II

In genetic testing for complex heritable disease genes with multiple possible mutations, the report includes (when appropriate) an estimate of mutation detection rate and the residual risk of being a carrier for one of the mutations 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 mutations reported in different patients and families. Even with gene sequencing, mutation detection is not 100% sensitive, since sequencing will not detect large exons or deletions/duplications or whole gene deletions/duplications. A negative test result, therefore, does not completely rule out the possibility that the patient is a mutation 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

**REVISED** 07/28/2015

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 mutation (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 mutation 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 penetrance, phenotype predictions, and recurrence risks.

REFERENCES

MOL.49620 Counseling Recommendation

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

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

MOL.49630 Standard Nomenclature

Standard nomenclature is used to designate genes and mutations.

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
RECORDS

Inspector Instructions:

- Record retention policy and procedures
- Autoradiographs/gel photographs/in situ hybridization slides (adequately labeled/cross-referenced?)

MOL.49635 Laboratory Records

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.

MOL.49640 Record Retention

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/genetic conditions be retained for 20 years. Electronic versions are acceptable.

MOL.49645 Cross-Referenced

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
- Continuing education policy
- Sampling of continuing education records

**MOL.49650 Section Director/Technical Supervisor Qualifications**

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.

**Evidence of Compliance:**

✓ Records of qualifications including degree or transcript, board-certification, current license (if required) and work history in related field

**REFERENCES**


**REvised** 04/21/2014

**MOL.49655 Bench Testing Supervision**

The person in charge of bench testing /section supervisor of the molecular pathology laboratory 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 4 years of experience (at least 1 of which is in molecular pathology methods) under a qualified section director

**Evidence of Compliance:**

✓ Records of qualifications including degree or transcript, board-certification, as applicable, current license (if required) and work history in related field

**REvised** 04/21/2014

**MOL.49660 Testing Personnel Qualifications**

Personnel performing the technical work of molecular pathology have appropriate experience in molecular pathology methods and qualify as high complexity testing personnel with a minimum of the following:

1. Bachelor's degree in a chemical, physical, biological or clinical laboratory science or medical technology; or
2. Associate degree in a laboratory science or medical laboratory technology from an accredited institution, or equivalent laboratory training and experience meeting the requirements defined in the CLIA regulation 42CFR493.1489. The qualifications to perform high complexity testing can be assessed using the following link: CAP Personnel Requirements by Testing Complexity.

**NOTE:** Persons gaining experience in the field must work under the direct supervision of persons who are qualified.

**Evidence of Compliance:**

✓ Records of qualifications including degree or transcript, current license (if required), and work history in related field
MOL.49665  Training/CME

**Phase I**

**There is an adequate training program for new technologists, and there is a continuing medical laboratory education program.**

**Evidence of Compliance:**
- ✓ Written training and continuing education program AND
- ✓ Records of training by the institution or appropriate outside organization AND
- ✓ Records of continuing education

**REFERENCES**

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**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.

**Inspector Instructions:**

- Records of biological safety cabinet certification
- Fume hood/chemical filtration unit
- Use of UV protective shielding, if applicable

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MOL.52760  Fume Hood

**Phase II**

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

**REFERENCES**

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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

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

MOL.58190 Refrigerators Phase II

Refrigerators are free of improper materials (such as food, externally contaminated specimens or unsealed volatile materials).

RADIATION SAFETY

Inspector Instructions:

• Sampling of radiation safety policies and procedures
• Sampling of radiation area surveys/wipe tests records
• Sampling of radioactive waste disposal records
• Sampling of personnel records of radionuclide training

• Radionuclide storage areas (properly shielded)
• Appropriate signage where radioactive materials are used/stored

• How does your laboratory check the effectiveness of workbench decontamination?

MOL.61050 Radiation Safety Manual Phase II

There is an up-to-date radiation safety manual that includes sections on decontamination and radioactive waste.

NOTE: A radiation safety manual providing procedures for the safe handling of radioactive substances in both routine and emergency situations is required by the Nuclear Regulatory Commission (NRC). Requirements for laboratory safety in nuclear medicine can be found in several references.

REFERENCES
**REVISED** 07/28/2015
MOL.61055  Workspace Decontamination  Phase II

Workbenches and sinks are decontaminated each day of use, and the effectiveness tested at least monthly.

NOTE: If the laboratory uses only iodine-125, either a wipe test or a portable scintillation probe can be used.

Evidence of Compliance:
✓ Records of daily workbench /sink decontamination AND
✓ Records of monthly effectiveness checks

REFERENCES

**REVISED** 07/28/2015
MOL.61060  Radionuclides Handling  Phase II

There are written policies regarding the authorization or restriction of personnel handling radionuclides.

NOTE: These policies should be incorporated into the department's radiation safety manual.

MOL.61065  Radionuclide Leak  Phase II

There are written procedures for notification if a damaged or leaking radionuclide shipment is received.

NOTE: Procedures must include inspection, monitoring of shipments, and instructions for notification, if leakage or damage is noted in a radionuclide shipment.

Evidence of Compliance:
✓ Records of inspections and notifications

**REVISED** 07/28/2015
MOL.61070  Radionuclide Storage  Phase II

Radionuclide storage and decay areas are properly shielded, if required for specific isotopic materials.

NOTE: Radionuclide storage and decay areas must be properly shielded, if required for specific isotopic materials, to avoid excessive exposure to personnel and interference with counting procedures.

Evidence of Compliance:
✓ Written procedure defining shielding requirements for radionuclide storage and decay areas

**REVISED** 07/28/2015
MOL.61075  Radiation Surveys  Phase II

There are regular radiation area surveys and wipe tests, with records maintained.

NOTE: Routine radiation surveys and wipe tests to determine exposure rates and detect contamination must be performed and recorded at defined frequencies.
Evidence of Compliance:
✓ Written procedure for radiation survey and wipe tests to determine exposure rates and detect contamination

**REVISED** 07/28/2015
MOL.61080 Radioactive Material Sign Phase I

All areas or rooms where radioactive materials are being used or stored are posted to indicate the presence of radioactive materials.

NOTE: For US laboratories, all areas or rooms where radioactive materials are being used or stored must be posted to indicate the presence of radioactive materials, consistent with 10CFR20, Appendix C.

REFERENCES

MOL.61085 Radionuclide Training Phase II

Personnel are instructed in decontamination routines and in the safe handling and proper disposal of radionuclides (wastes, syringes, needles, and sponges).

Evidence of Compliance:
✓ Records of radionuclide training in personnel file

**REVISED** 07/28/2015
MOL.61090 Radioactive Waste Phase II

Radioactive waste is stored separately, under required conditions, and appropriately discarded, with records maintained.

NOTE: For US laboratories, NRC regulations specify that separate areas be established for the receipt of radioactive waste and that these areas be properly shielded to reduce radiation levels below those maximum permissible limits specified in 10CFR20.

Evidence of Compliance:
✓ Written procedure defining criteria for proper storage and disposal of radioactive waste

REFERENCES