MAP Analytic Checklist

Contents

Part 1: Assay Procedure Validation Documentation (Analytic)

Each laboratory is responsible for validating each test method before introducing it into routine use. For every molecular test performed by the laboratory, the analytical performance characteristics, such as the sensitivity, specificity, and reproducibility of the method for each gene or analyte should be determined, where applicable. For methods that are cleared or approved by the FDA as safe and effective for *in vitro* diagnostic use, the laboratory should verify the performance specifications as part of method validation for accuracy, precision, and reportable range of results established by the manufacturer

The overall process involves reviewing the relevant literature and professional guidelines. It also requires decisions regarding the variables that must be monitored for test performance, the limitations of the test, establishing clinical and laboratory validity. The performance characteristics that should be determined testing include: defining the analytical sensitivity, analytical specificity, precision, linearity for quantitative results, the reportable range of test results, and reference values for the test. Analytic sensitivity refers to the ability to detect a given analyte and is determined by using samples with known test results either by comparison with another methodology or by consensus findings. Analytic specificity refers to the degree to which interfering substances are not detected by the assay, such as related gene families or pseudogenes. Precision refers to the reproducibility of a test result. Precision refers to repeatability. In some instances, testing may not be able to identify all mutations for the disorder. Clinical sensitivities, the ability to detect causal mutation, for selected racial/ethnic groups should be determined when available and appropriate. Confidence intervals should be estimated.

For any given test procedure, there are numerous acceptable variations of molecular testing methodologies. The accuracy and dependability of the molecular testing procedure, with specific emphasis on polymerase chain reaction (PCR) detection and validation for in-house developed assays, should be documented in each laboratory.

1A: Assay Methodology

5A.1: Is there documentation that the laboratory has performed validation studies to establish the performance characteristics of FDA-approved tests?

N/A ☐ YES ☐ NO ☐

Comments:

5A.2: Is there documentation that the laboratory has performed validation studies to establish the performance characteristics of laboratory-developed assays?

N/A ☐ YES ☐ NO ☐

Comments:

Laboratory developed assays include tests developed in-house, tests that are used for research-use only and have commercially-available reagents as well as FDA-cleared kits that have been modified by the laboratory.

5A.3: Does the validation documentation for each molecular test define the principles of the testing methodology and include a description of the positive, negative and indeterminate test results?

N/A ☐ YES ☐ NO ☐

Comments:

This should also include test result nomenclature. Documentation of qualitative assays should include the reference value, screen negative and screen positive definitions, and the reportable outcomes for the alleles (homozygous reference, homozygous mutant, heterozygous mutant or compound heterozygous). For quantitative assays, the reference and reportable ranges should be defined.

5A.4: Are the technical limits of the measurement range defined and verified by the laboratory?

N/A ☐ YES ☐ NO ☐

Comments:

Since there are numerous acceptable variations of molecular testing methodologies, the accuracy and dependability of all molecular testing procedures should be documented. Guidance for developing protocols can be found in CLSI MM01-A2 Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline-Second Edition (2006).

5B: Detailed Procedures

5B.1: Are there detailed procedures for each test method including manufacturer's detailed protocol procedures for FDA-approved commercially available kits?

N/A ☐ YES ☐ NO ☐

Comments:

5B.2: If the laboratory modifies an FDA-cleared assay, is validation performed and documented to demonstrate equal or superior performance to the original protocol?

N/A ☐ YES ☐ NO ☐

Comments:

5B.3: Are quality control parameters, required controls, acceptable assay limits reportable range of results and cutoffs defined?

N/A ☐ YES ☐ NO ☐

Comments:

Specific instructions for equipment calibration and maintenance should be noted.

5B.4: For PCR amplification, are conditions of time, temperature, cycle number and concentration documented for each set of primers using known controls?

N/A ☐ YES ☐ NO ☐

Comments:

5B.5: Is there documentation of no amplification bias of normal or mutant alleles in allelic discrimination tests?

N/A ☐ YES ☐ NO ☐

Comments:

5B.6: For tests based on T_m, such as allele-specific amplification or ARMS-PCR, are appropriately narrow temperature ranges defined and monitored?
N/A☐ YES☐ NO☐

Comments:

5B.7: For multiplex PCR amplification, are all targets present and equally amplified?
N/A☐ YES☐ NO☐

Comments:

5C: Documentation of DNA Probes, Primers and Other Nucleic Acid Reagents

5C.1: Do validation studies verify that the size of the expected positive result for primers and PCR conditions is consistent with predicted results from documented reference sequences?
N/A☐ YES☐ NO☐

Comments:

5C.2: For DNA sequencing assays, is it made clear that the sequence from the amplification primers is not considered in the assignment of alleles?
N/A☐ YES☐ NO☐

Comments:

> *All loci targeted by the test should be well documented with standard nomenclature. Documentation of primers and probes used in an assay should be sufficient to permit interpretation and troubleshooting of test results.*

5D: DNA Probe and Primer Design

5D.1: For in-house laboratory developed tests, do test validation results demonstrate that there are not competing targets of similar DNA sequence compositions for oligonucleotide primers or probes which can be found related to gene families or pseudogenes?

N/A ☐ YES ☐ NO ☐

Comments:

5D.2: For in-house laboratory developed tests, is DNA nucleotide variation in target regions extensively defined to identify polymorphisms that could potentially interfere with primer or probe hybridization and consequently allele dropout or assay failure?

N/A ☐ YES ☐ NO ☐

Comments:

5E: Specimens Representing DBS Matrix and Reportable Results

5E.1: Are the materials used for method verification derived from the dried blood spot matrix?

N/A ☐ YES ☐ NO ☐

Comments:

5E.2: Do validation studies include specimens representing each of the reportable results of the molecular test assay?

N/A ☐ YES ☐ NO ☐

Comments:

5E.3: For quantitative molecular assays, is the analytical measurement range validated with matrix appropriate materials that include the low, mid and high range analytical values.

N/A ☐ YES ☐ NO ☐

Comments:

For newborn screening assays, the method validation should be performed with dried blood spots, if available. The validation process should define criteria for the acceptance or rejection of specimens and acceptable specimen types and variables that can affect acceptability (e.g., unsatisfactory dried blood spot, insufficient quantity of nucleic acid).

Validation of molecular tests should confirm as many of known reportable genotypes as possible. Assays for genetic disorders with considerable allelic heterogeneity should confirm the ability of the assay to detect a high percentage of the possible genotypes. However, it will not be possible to document that such assays can detect every possible genotype.

For commonly performed tests, relevant published literature may be used as a laboratory reference (normal) range. Results from the population served should be periodically reviewed *and compared to these ranges to determine if these prevalence values are appropriate. If the population served represents specific sub-sets of the overall population, special care may be needed in establishing reference allelic panels.*

5F: Validation of Test Results

5F.1: Are In-house developed tests compared to another valid assay, where available?
N/A ☐ YES ☐ NO ☐

Comments:

Test results should be compared with an existing "gold standard" assay. Alternatively, accuracy may be evaluated through split-sample comparisons within the laboratory or with another laboratory (known positive and/or quality control samples), with another validated method, or blind testing of specimens with known results. Additional guidance can be found in CLSI Approved Guideline Assessment of Laboratory Tests when Proficiency Testing is Not Available, GP29-A2 (2008).

5F.2: Are measures of test sensitivity and specificity compared in a blinded fashion within single runs?

N/A ☐ YES ☐ NO ☐

Comments:

5F.3: Are measures of test sensitivity and specificity compared in a blinded fashion between multiple runs?

N/A ☐ YES ☐ NO ☐

Comments:

5G: Documentation of Test Results

5G.1: Is there documentation of test accuracy, analytical sensitivity, analytical specificity and reproducibility?

N/A ☐ YES ☐ NO ☐

Comments:

5G.2: Has validation documentation been approved by a laboratory supervisor or designee?

N/A ☐ YES ☐ NO ☐

Comments:

> *Determining analytic sensitivity can be difficult with methods that can identify unclassified variants, such as DNA sequencing.*
>
> *The true-positive and true-negative rates of detection should be stated. These values are dependent on prevalence, analytic sensitivity, and clinical specificity and may not be easily obtainable.*

Part 6: Proficiency Testing (Analytic)

6A: Participation In An Appropriate Proficiency Testing Program

6A.1: Does the laboratory participate in at least one external proficiency testing (PT) program intended for evaluating ongoing molecular tests?

N/A ☐ YES ☐ NO ☐

Comments:

6A.2: If an external PT program is not available, does the laboratory participate in at least one inter-laboratory comparison program?

N/A ☐ YES ☐ NO ☐

Comments:

6A.3: Is PT for each test performed at least every 6 months?

N/A ☐ YES ☐ NO ☐

Comments:

> *If PT testing programs are not available, an internal PT program that may include split-sample comparisons with another validated method, blind testing of specimens with known results or sample exchange with another program. Further guidance can be found in CLSI Approved Guideline Assessment of Laboratory Tests when Proficiency Testing is Not Available, GP29-A2 (2008).*

6B: PT samples Incorporated Into Routine Laboratory Workflow

6B.1: Are PT samples introduced into the routine workflow process with the same procedures that are applied to test specimens?

N/A ☐ YES ☐ NO ☐

Comments:

6B.2: Does the technical staff that performs routine testing perform with the same assay, test kit or instrument as the primary method for the PT event?

N/A ☐ YES ☐ NO ☐

Comments:

> *Repeated analysis of the proficiency testing samples should not be permitted unless the laboratory performs the same repeat analysis in the routine processing of screening specimens. If the laboratory uses multiple methods for a target analyte, PT samples should be analyzed and reported by the primary method. After the PT result submission deadline, PT samples may be used for other purposes such as competency testing or quality control samples.*

6C: Procedures Prohibiting Inter-Laboratory Communication

6C.1: Does the laboratory have policies in place to prohibit inter-laboratory communication or discussion pertaining to the results of testing PT samples until after the date the laboratories are required to report the results?

N/A ☐ YES ☐ NO ☐

Comments:

6C.2: Does the laboratory have policies in place prohibiting the laboratory from sending or sharing PT materials with any other laboratory for analysis until after the date the laboratories are required to report PT results to the submitter?

N/A ☐ YES ☐ NO ☐

Comments: