The Molecular Assessment Program (MAP): Evaluation of Newborn Screening Molecular Testing

Newborn screening (NBS) is the process of testing infants for serious developmental, metabolic, and genetic disorders. The Health and Human Services (HHS) Secretary's Advisory Committee on Heritable Disorders in Newborns and Children (SACHDNC) lists 31 core disorders that are recommended to be included in every NBS program in the United States. These core conditions share criteria including that they are prevalent in the U.S. population; can be screened for using a simple, robust test; and a treatment or intervention exists if the disorder is detected early. The majority of screening for these disorders is through a biochemical test. The recommended inclusion of cystic fibrosis (CF) to the core panel of disorders in the mid-2000s resulted in the first wide-spread use of a molecular test in NBS to increase the sensitivity and specificity of biochemical screening results. In addition to CF, several programs adopted second-tier molecular tests for disorders including galactosemia, MCAD, PKU, and CAH. In 2010, the SACHDNC added Severe Combined Immunodeficiency (SCID) to the core screening panel. This disorder signified the first time a molecular assay was used as a primary screening test. The inclusion of SCID will require the expansion of molecular testing activities in NBS programs. Despite the increased use of molecular testing, there are still several public health programs that have yet to introduce molecular testing into their NBS programs.

As NBS laboratories introduce molecular assays into their routine testing, it is imperative that the specific concerns related to molecular testing are addressed to ensure the quality of test performance and laboratory practice. Molecular testing requires specific guidelines that are not covered by CLIA. In response to this, the Newborn Screening Molecular Subcommittee developed a pilot initiative, the Molecular Assessment Program (MAP). MAP will involve the public health laboratory personnel working together with the NSMBB molecular quality improvement program to provide quality management guidance for molecular testing in NBS laboratories. The framework for MAP was modeled after the Newborn Screening System Performance Evaluation Assessment Scheme (PEAS), developed by the National Newborn Screening and Genetics Resource Center and NBS partners as an evaluation tool for quality improvement.

MAP is focused on the NBS program components that have specific concerns for molecular testing. The evaluation criteria can be sub-divided into pre-analytic, analytic, and post-analytic phases of the testing process and are derived from multiple sources including the:

- Newborn Screening Performance Enhancement Assessment Scheme (PEAS)
- CLIA Guidelines for Moderate and High Complexity Tests
- ACMG Standards and Guidelines for Clinical Genetics Laboratories
- CAP Molecular Pathology Checklist
- NYSDOH Clinical Laboratory Evaluation Program
- Good Laboratory Practices for Molecular Genetic Testing for Heritable Diseases and Conditions, MMWR (2009) 58(RR06)

These evaluation criteria have been arranged into the following checklists for the components of the testing process.

MAP will be a broad assessment of categories that have specific molecular testing concerns The proposed components for review include:

Pre-Analytic
- Standard Operating Procedures
- QA/QM Manual
- Assay Validation
- Personnel

Analytic
- Performance of Test Methods
- Proficiency Testing Procedures and Results

Post-Analytic
- Procedures for Results Reporting

These standards and guidelines do not necessarily assure a successful outcome and should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results.

Contents

Part 1: Standard Operating Procedures (Pre-Analytic)

Manuals detailing standard operating procedures should be developed and maintained that describe all procedures that have been validated and approved for use in the laboratory. The standard operating procedure manual should address relevant pre-analytical, analytic, and post-analytical considerations in the laboratory. The manual should be written with sufficient detail to serve as a resource to the technical personnel for required laboratory components such as reagents, quality control procedural steps and interpretation of results, and should be reviewed annually. The manual should be accessible to all staff and may be kept in an electronic version provided that backup systems are present.

The standard operating procedure manual should contain a discussion of the selection of the test procedure. This includes the principle of the procedure, the detection of variants of known clinical validity and utility in the serviced population and the description of indications for performing the molecular test. Relevant literature references should also be included.

1A: A Standard Operating Manual Covers All Procedures Performed in A Laboratory

1A.1: Are all test procedures that are performed in the laboratory documented by a standard operating procedures manual for reference of the test procedure, technical details and test interpretation guidance?

N/A ☐ YES ☐ NO ☐

Comments:

> *Tests that are performed infrequently or only under specific circumstances may be combined into related procedure manuals.*

1B: Sample Management: Receipt and Tracking

1B.1: Are criteria for the collection, handling and identification of specimens defined for the laboratory?

N/A ☐ YES ☐ NO ☐

Comments:

1B.2: Are there criteria to accept or reject specimens for testing?

N/A ☐ YES ☐ NO ☐

Comments:

1B.3: Is specimen identity maintained through all components of sample processing and test procedures?

N/A ☐ YES ☐ NO ☐

Comments:

This includes sample receipt, nucleic acid extraction, nucleic acid quantification, if appropriate, and storage.

1B.4: If aliquoting of specimens is performed, is there a written procedure to maintain sample identification and to prevent cross contamination?

N/A ☐ YES ☐ NO ☐

Comments:

The specimens may be considered by the apparent condition of the sample or if the sample is collected, labeled or handled in a manner that it has become unsatisfactory. This could include an inappropriate time lapse between collection and specimen receipt or temperature range. The description of unsatisfactory specimens should be documented in appropriate quality control/management records.

1B.5: If nucleic acids are extracted, are there guidelines for short-term storage for the use in repeat testing?

N/A ☐ YES ☐ NO ☐

Comments:

Extracted DNA samples may be stored up to a week at 4°C or at -20°C for longer –term storage to minimize degradation of nucleic acids. Samples should be stored in a way to allow prompt retrieval for further or repeat testing.

1B.6: Does the laboratory have defined procedures appropriate to local and state rules and regulations for the term of and storage conditions of residual dried blood spot specimens?
N/A ☐ YES ☐ NO ☐

Comments:

1C: Selection of Test Procedures

1C.1: Does the standard operating procedures manual describe the testing principle for the molecular assay?
N/A ☐ YES ☐ NO ☐

Comments:

1C.2: Does the laboratory perform FDA-approved laboratory tests for targeted genotyping?
N/A ☐ YES ☐ NO ☐

Comments:

1C.3: Does the laboratory perform in-house laboratory developed tests for targeted genotyping?
N/A ☐ YES ☐ NO ☐

Comments:

1C.4: Does the laboratory perform in-house laboratory developed tests for DNA sequence analysis?
N/A ☐ YES ☐ NO ☐

Comments:

1C.5: Does the laboratory perform in-house laboratory developed tests for DNA target quantitative analysis?

N/A ☐ YES ☐ NO ☐

Comments:

1C.6: For quantitative molecular tests, are the reference and reportable ranges of the analyte defined?

N/A ☐ YES ☐ NO ☐

Comments:

The analytic range must be defined by the laboratory and directions should be provided to determine how to handle positive specimen results above or below the analytic measurement range.

1C.7: For any FDA-approved or in-house laboratory developed tests performed by the laboratory, is there documentation in the standard operating procedures manual for verification of test performance for accuracy, specificity, precision and reportable range of results?

N/A ☐ YES ☐ NO ☐

Comments:

Performance specifications determined by validation studies for accuracy, specificity, precision, reportable range of patient results, and analytical sensitivity and specificity for each test should be defined in the standard operating procedure manuals, where applicable. A copy of a validation summary report that is included in the standard operation procedures or a statement that the tests have been validated and a description of where the validation report can be located would be sufficient.

1D: Selection of Test Targets

1D.1: For each molecular test that the laboratory performs, FDA-approved or in-house laboratory developed, are the specific genes/loci targeted by the test clearly documented?
N/A ☐ YES ☐ NO ☐

Comments:

1D.2: For each targeted genotyping molecular test that the laboratory performs, FDA-approved or in-house laboratory developed, are the individual alleles/mutations detected by the test clearly documented?
N/A ☐ YES ☐ NO ☐

Comments:

1D.3: For each DNA sequencing molecular test that the laboratory performs are the gene regions detected by the test clearly documented?
N/A ☐ YES ☐ NO ☐

Comments:

1D.4: For quantitative DNA molecular tests performed by the laboratory, is the target region detected by the test clearly documented?
N/A ☐ YES ☐ NO ☐

Comments:

1D.5: For each molecular test, are the specific locations relative to a reference gene sequence documented for specific mutations, variations, probes and/or oligonucleotide primers documented?
N/A ☐ YES ☐ NO ☐

Comments:

The criteria for the selection of the test targets should be documented. This should include discussion of standard mutation panels for the disorder or if there are population-specific targets that should be included for the test (Hispanic cystic fibrosis, for example). The specific loci, probes, and/or oligonucleotide primers and assay conditions should be detailed. Literature references for mutations may be used or, for in-house generated probes, the reference may be the laboratory's validation studies. If the laboratory is using FDA-approved methods, the procedure manual should reference the package insert.

For PCR –based assays, reaction conditions and the expected size of the PCR product and a description of a positive result should be included. Note that sequence data may not be available for commercially obtained tests if considered proprietary.

1E: Directions for Preparation of Reagents, Standards, and Controls

1E.1: For each molecular assay performed by the laboratory, are the directions for preparation of individual assay reagents clearly described?

N/A ☐ YES ☐ NO ☐

Comments:

1E.2: Are there directions for the proper labeling of chemical, reagents and solutions for compliance with CLIA regulations and good laboratory practices?

N/A ☐ YES ☐ NO ☐

Comments:

1E.3: Does the standard operating procedures manual list the vendor and catalog numbers of purchased chemicals, reagents, solutions and instruments required for each molecular test performed by the laboratory?

N/A ☐ YES ☐ NO ☐

Comments:

1E.4: For each reagent, is it indicated in the standard operating procedures if the test component is to be used in pre-PCR or post-PCR locations in the laboratory to maintain unidirectional flow for molecular tests?

N/A ☐ YES ☐ NO ☐

Comments:

1E.5: For targeted genotyping and DNA sequence analysis molecular assays, does the standard operating procedure manual list the available positive control and quality control specimens for each assay?

N/A ☐ YES ☐ NO ☐

Comments:

1E.6: Are there instructions for the preparation of positive control or quality control specimens for molecular test assays performed by the laboratory?

N/A ☐ YES ☐ NO ☐

Comments:

1E.7: If the laboratory performs quantitative DNA molecular assays, are there directions for the preparation of control materials that will be appropriate for the analytical range of those assays?

N/A ☐ YES ☐ NO ☐

Comments:

All required equipment should be detailed in the standard operating manual, including the vendor and catalog number. All reagents and/or solutions should be appropriately identified with the name and concentration, preparation date, and preparer's name or initials. It should be clearly stated in the manual which reagents and test components need to be prepared in a pre-PCR area and which

components should not be used in pre-PCR areas. Directions for proper labeling of all chemicals, reagents and solutions or mixes should be clearly stated for compliance with applicable CLIA regulations and good laboratory practice requirements. For quantitative assays, the concentrations of controls and calibrators should be clearly described.

Aliquots of reagents or nucleic acids should be performed separately from each other, preferably in separate dedicated pre-amplification areas if possible or in enclosed "PCR hoods" or dead-air boxes. Any reagents involved in a PCR-based assay must be dispensed using aerosol-resistant (filter) tips.

1F: Directions for Storage of Critical Reagents

1F.1: Does the standard operating procedure manual specify the critical reagents and components of the molecular test assays performed by the laboratory?

N/A ☐ YES ☐ NO ☐

Comments:

1F.2: Does the standard operating procedures manual indicate which chemicals and reagents require specific temperature or conditions for storage?

N/A ☐ YES ☐ NO ☐

Comments:

1F.3: Are there directions for the aliquoting of DNA oligonucleotide primers and probes to limit the number of freeze/thaw cycles and to minimize cross contamination?

N/A ☐ YES ☐ NO ☐

Comments:

Critical reagents are determined at the discretion of the laboratory or technical director and may include but are not limited to: DNA extraction reagents, DNA modifying enzymes, probes and probe labeling buffers, PCR reagents and solutions, DNA sequencing reagents and solutions.

Specific directions for temperature-sensitive reagents, such as enzymes or DNA primers or probes, or light-sensitive components should be emphasized. Probes and primers used in PCR should not be frozen and thawed repeatedly. Primers and probes should be stored in small aliquots to minimize the number of freeze/thaw cycles and prevent contamination.

1G: Directions for Performing the Test

1G.1: If the laboratory uses FDA-approved commercial assays, are the directions from the package insert followed as directed?

N/A ☐ YES ☐ NO ☐

Comments:

1G.2: For in-house laboratory developed tests, are the conditions and steps clearly defined in the standard operating procedures manual?

N/A ☐ YES ☐ NO ☐

Comments:

1G.3: Do the laboratory technicians document test reaction conditions on worksheets?

N/A ☐ YES ☐ NO ☐

Comments:

1G.4: Are the appropriate positive and negative amplification controls with each run of specimens being tested?

N/A ☐ YES ☐ NO ☐

Comments:

The positive controls ideally should represent each target allele used in each run. Please note that this may not be practical for highly multiplexed tests, such as

those for Cystic Fibrosis. An approach to address this situation is to rotate different alleles as positive controls in a systematic fashion and frequency as determined by the laboratory director. The controls should be selected based on the population being tested and should be as comprehensive as possible. Based on the rarity of alleles, a heterozygous sample or compound heterozygous samples can be used for individual alleles.

When the PCR amplification product is of varying lengths, such as simple sequence repeats, deletion or insertion alleles, specimens representing large and small amplification products should be used to determine if there is differential amplification.

Assays based on the presence or absence of a PCR product should include an internal positive amplification control to distinguish a true negative result from a false negative result due to failure of DNA extraction or PCR amplification. The internal positive control can be from another target region.

To assure PCR product specificity, reaction conditions and reaction components established from validation should be followed. The different components of the tests that need to be specified include cycle temperature and times, cycle number for the program, concentration of target template and PCR primers, as well as enzyme and buffer conditions.

1G.5: Are the calibration procedures for each applicable quantitative test system performed in accordance with criteria established from validation studies?

N/A ☐ YES ☐ NO ☐

Comments:

For quantitative assays, the manual should describe the criteria for verifying test performance characteristics for the run (e.g., pre-established range for assay sensitivity and linearity, result values that indicate amplification inhibition of the reaction, the calculated value appears reasonable from visual inspection of the raw data, etc.). The range of the assay should be tested with controls in each run, including a negative, a low-positive, and a high-positive control. The effect of partial amplification inhibition on true analyte concentration should also be addressed.

1G.6: Does the laboratory repeat a molecular test assay for any specimen with unexpected genotype results?

N/A ☐ YES ☐ NO ☐

Comments:

1G.7: Is unidirectional workflow specified for molecular test procedures?

N/A ☐ YES ☐ NO ☐

Comments:

The avoidance cross-contamination is imperative. All handling of post-amplification products be in a defined area with defined pipettes, pipette tips using filters or positive displacement pipettes should be used, and all reagents and solutions for PCR procedures should be dedicated to those tests.

1H: Modifications of the Manufacturer's Instructions

1H.1: If the laboratory modifies the test instructions for FDA-approved commercial tests, has the laboratory validated the test performance?

N/A ☐ YES ☐ NO ☐

Comments:

The test validation should demonstrate equal or superior performance to the manufacturer's protocol should be documented and approved by the director.

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1I.1: For FDA-approved tests, are the product insert instructions for test result interpretation included in the standard operation procedures manual?

N/A ☐ YES ☐ NO ☐

Comments:

1I.2: For PCR-based laboratory-developed molecular tests, are the sequences of oligonucleotide primers and probes sufficiently documented relative to a reference gene sequence for the interpretation of test results?

N/A ☐ YES ☐ NO ☐

Comments:

Documented information should include the chromosome location of the gene, the NCBI build and accession number, and literature references. The allele frequencies in each racial or ethnic group for which this information exists should also be included. The type and sequence of probes, if available, and the sequence of and naming scheme of oligonucleotide primers should be referenced to the gene/loci sequence and loci detected should be designated as defined by the Human Gene Mapping Nomenclature committee (http://www.genenames.org/). Alleles that have established names in the literature should have both the recommended nomenclature from HGVS and the traditional allele designations.

1I.3: For genotyping assays involving electrophoretic gel separation of differently-sized PCR products or restriction digest treated PCR products, does the standard operating procedure clearly state interpretive guidelines of test results?

N/A ☐ YES ☐ NO ☐

Comments:

The expected band pattern for gel electrophoretic-based methods for each reference allele and expected PCR product should be clearly defined.

1I.4: For florescent-probe based genotyping assays such as TaqMan or Light-Cycler assays, does the standard operating procedure manual clearly state interpretive guideline for test results?

N/A ☐ YES ☐ NO ☐

Comments:

The melting temperature for high-resolution melt assays, or numeric cutoff of fluorescent-based methods to distinguish the assayed alleles should be detailed. Appropriate allele controls must be included to ensure correct interpretation of results (reference allele and heterozygous or homozygous variant, if available). There should be equal amplification of normal and mutant/variant alleles.

1I.5: For quantitative DNA target analysis, does the standard operating procedures manual clearly state normal reference ranges and interpretive guidelines for specimens that have reading above or below the normal reference range?

N/A ☐ YES ☐ NO ☐

Comments:

1I.6: Are methods for calculating quantitative values and units clearly documented?

N/A ☐ YES ☐ NO ☐

Comments:

1I.7: For DNA sequence based assays, does the standard operating procedure manual provide guidance for interpretation of DNA sequence data?

N/A ☐ YES ☐ NO ☐

Comments:

1I.8: If the laboratory performs gene sequencing assays, is there a database for the identification and location of definitive mutations, such as non-conservative amino acid substitutions, non-sense substitutions (stop codons), insertions, deletions or frameshift mutations as well as normal sequence variations?

N/A ☐ YES ☐ NO ☐

Comments:

1I.9: Is the DNA sequence database maintained and regularly updated by the laboratory after new alleles have been reported or verified in the published literature?

☐ ☐ ☐

N/A YES NO

Comments:

1J: Limits of procedure

1J.1: Does the standard operating procedure manual detail the technical limitations of the molecular test assays performed in the laboratory?

N/A ☐ YES ☐ NO ☐

Comments:

Because of the complexity of genotype-phenotype correlations of many diseases, simply reporting a molecular test as positive or negative may not be sufficient. The manual should discuss known issues of recessive or dominant inheritance, penetrance, severity and other aspects of genotype-phenotype correlations available in the literature. Interpretation of results should be updated in a regular fashion.

Even with gene sequencing, mutation detection is not 100% and a negative test result does not completely rule out the possibility that the patient is a mutation carrier. The portion of the gene that is sequences, (e.g. exon and intron borders, whole gene, bases of upstream and downstream coverage) should be included to define the possibility of mutations occurring in areas that were not sequenced.

Technical limitations of test procedures should include interfering substances as well as the possibility of laboratory error. Literature references that are applicable for the test analysis should be included.

1K: Alternative Methods of Testing

1K.1: If the laboratory performs a primary molecular assay, is a system in place to evaluate and compare those tests results with an alternative method or with alternative methods of evaluation such as sample exchange or sample splitting?

N/A ☐ YES ☐ NO ☐

Comments:

1L: Reporting Guidelines

1L.1: Does the standard operating procedure manual describe a protocol for reporting of results?

N/A ☐ YES ☐ NO ☐

Comments:

1L.2: Is the information in the results report easy to interpret by a non-expert physician?

N/A ☐ YES ☐ NO ☐

Comments:

1L.3: Does the report include an estimation of mutation detection rate and the residual risk of being a carrier for one of the mutations not tested?

N/A ☐ YES ☐ NO ☐

Comments:

1L.4: Does the results report use standard nomenclature that is universally understandable to the genetics community? N/A ☐ YES ☐ NO ☐

Comments:

Refer to the HUGO gene nomenclature committee (http://www.genenames.org/) for naming loci and for mutations refer to den Dunnen and Antonarakis (Hum Mutat 15:7-12, 2000) and den Dunnen and Paalman (Hum Mutat 22:181-182, 2003) or the Human Genome Variation society (http://www.hgvs.org/mutnomen/) for specific guidance. Alleles that have established names in the literature should have both the recommended nomenclature from HGVS and the traditional allele designations.

1L.5: Does the results report include a statement that a positive result is an indication that the individual may be predisposed to or have the specific disease or condition tested for and may want to consider further clinical interpretation and genetic counseling, if appropriate, to explain the implications of the test results and the residual risks and uncertainties?

N/A ☐ YES ☐ NO ☐

Comments:

1M: Critical Alert Procedures

1M.1: Are reporting procedures clearly defined for a prompt communication process to report those test results that require immediate attention?

N/A ☐ YES ☐ NO ☐

Comments:

1N: Literature References

1N.1: Are literature references from all sections of the standard operating procedure maintained?

N/A ☐ YES ☐ NO ☐

Comments:

1O: Documentation of Annual Review

1O.1: Is there documentation of an annual review by the current laboratory director or designee?

N/A ☐ YES ☐ NO ☐

Comments

Part 2: Written Quality Assurance/Management Documentation (Pre-Analytic)

All laboratories should have a manual documenting quality control, quality assurance and quality improvement plans to assure that all reagents, equipment, methodologies and personnel operate at optimum levels. The manual should define the roles and responsibilities of personnel designated to implement the quality systems. It should also make reference to supporting procedures in the laboratory's standard operating procedure manual. The procedures should be capable of detecting problems in the laboratory's systems and identifying opportunities for system improvement.

2A: Control Materials

2A.1: Does the quality assurance manual detail the type and number of control materials for each molecular assay?

N/A ☐ YES ☐ NO ☐

Comments:

The source of QC materials as well as conditions for controls and storage should be included in the manual. The manual should clearly state the interpretive guidelines for quality control results in order to detect errors due to test failure, adverse environmental conditions or operator performance.

There are numerous valid molecular test assays for any given locus. Each laboratory should define its own parameters for assay performance. Furthermore, the types and numbers of controls selected by each laboratory should cover each aspect of the testing process.

2B: Testing of Critical Reagents

2B.1: Are new lots or shipments of reagents that could lead to specimen loss or aberrant results tested prior to or concurrently with the test's introduction?

N/A ☐ YES ☐ NO ☐

Comments:

This includes but is not limited to reagents for DNA extraction, DNA modifying enzymes, PCR reagents and solutions or probes. Further lists of critical reagents are to be determined by the laboratory or technical director as listed in the standard operating procedure manual.

2B.2: Are quality control logs of critical reagent lots maintained for each molecular assay?
N/A ☐ YES ☐ NO ☐

Comments:

2C: Test Performance Monitoring

2C.1: Does the quality assurance manual have a system to periodically monitor test performance as determined by the laboratory director?
N/A ☐ YES ☐ NO ☐

Comments:

2C.2: Are audits of test performance planned and carried out by designated personnel?
N/A ☐ YES ☐ NO ☐

Comments:

Procedures should include the types of audits, frequencies, test methods and documentation. Action plans should be defined to address test results that are not in compliance with stated quality assurance standards.

2C.3: If the same molecular test is run with different instruments, is there a system in place to evaluate and compare those tests results at least twice a year and maintain their results?
N/A ☐ YES ☐ NO ☐

Comments:

This applies to tests performed on the same or different instrument makes or models.

2D: Instrumentation

2D.1: Does the quality assurance manual should have a system in place for documenting ongoing evaluation of instrument performance?

N/A ☐ YES ☐ NO ☐

Comments:

There should be a regular schedule for routine maintenance and inspection of critical operating characteristics for all instruments, especially temperature checks. These functional checks should be documented to detect test assay trends or instrument malfunctions.

2D.2: Do all thermal cyclers have temperature uniformity inspections across the entire sample block, or a representative sample of wells at defined intervals as decided by the laboratory or technical director?

N/A ☐ YES ☐ NO ☐

Comments:

Performance inspections should be monitored by calibrated devices such as system thermometers, internal inspections, or through the use of validated amplification controls for functional checks if other calibration devices are not available. The results of thermal cycler performance inspections should be documented.

2D.3: If the laboratory performs molecular tests that utilize fluorescent probes for allelic discrimination assays or quantitative molecular tests, are periodic background checks performed to test for instrument contamination from the fluorescent dyes?

N/A ☐ YES ☐ NO ☐

Comments:

2E: Laboratory Equipment

2E.1: Is the laboratory equipment used in routine molecular testing checked for accuracy prior to being placed in service and inspected at regular intervals?

N/A ☐ YES ☐ NO ☐

Comments:

Examples include monitoring pipettes for accuracy and verifying that temperatures are within an acceptable range for water baths, heating blocks, refrigerators, and freezers.

2E.2: Are instrument logbooks maintained to document specified maintenance as required by local and regulatory requirements?

N/A ☐ YES ☐ NO ☐

Comments:

2F: Quality Control Results Reporting

2F.1: Does the quality assurance manual include procedures for reporting control results for each run?

N/A ☐ YES ☐ NO ☐

Comments:

The controls of each run should be verified and accepted prior to reporting the test specimen results. If the controls for a run are unacceptable based on defined metrics, the test results of that run should not be reported. Test results from an unacceptable analytic run should be evaluated and corrective actions should be documented.

2G: Maintenance of Records

2G.1: Does the quality assurance/quality management manual have documented guidelines on the retention of laboratory records?

N/A ☐ YES ☐ NO ☐

Comments:

This includes quality control records for laboratory instrumentation and specimen test reports. The terms of record retention should be specified by the laboratory director.

Part 3: Laboratory Space (Pre-Analytic)

3A: Unidirectional Workflow

3A.1: Does the laboratory have clearly defined pre-PCR and post-PCR laboratory spaces?
N/A ☐ YES ☐ NO ☐

Comments:

3A.2: Are pre-PCR and post-PCR laboratory spaces physically separated in different rooms?
N/A ☐ YES ☐ NO ☐

Comments:

3A.3: If pre-PCR and post-PCR areas are in the same room, does the laboratory utilize enclosed dead-air boxes to minimize contamination?
N/A ☐ YES ☐ NO ☐

Comments:

3A.4: Are there dedicated equipment and supplies for pre-PCR areas that are kept separate from post-PCR areas?
N/A ☐ YES ☐ NO ☐

Comments:

3A.5: Does the laboratory use appropriate aerosol barrier pipette tips and disposable plastic ware, where appropriate?
N/A ☐ YES ☐ NO ☐

Comments:

Equipment includes, but is not limited to, items such as pipettes, pipettors, bulbs, tips, pens, cleaning supplies, etc. Positive displacement pipettes or filter (aerosol barrier) pipette tips and only sterile disposable plastic ware should be used when possible.

3A.6: Does the laboratory use dedicated PPE for pre-PCR areas separate from post-PCR areas?
N/A ☐ YES ☐ NO ☐

Comments:

Personal protective equipment (PPE) includes laboratory coats, gloves, safety glasses and other individually worn barriers. If laboratory personnel need to re-enter a pre-amplification area after working with PCR-amplified products, they must use dedicated pre-amplification PPE and additional PPE barriers such as hair nets and shoe covers.

3A.7: Does the laboratory perform contamination checks at appropriate time intervals, i.e. wipe tests of laboratory surfaces for amplified products?
N/A ☐ YES ☐ NO ☐

Comments:

A unidirectional workflow must be maintained for molecular tests. Sufficient space should be allocated to prevent cross-contamination of nucleic acid samples from PCR-amplified products. Laboratory areas for DNA extraction and reagent preparation are referred to as pre-PCR space while areas where PCR-amplified products are present are post-PCR space. Separate rooms are recommended for pre- and post-amplification procedures. Pre-amplification procedures must be performed in a dedicated work area that excludes amplified DNA. Ideally, there should be three separate laboratory areas: pre-amplification, DNA-free reagent preparation; pre-amplification DNA extraction and assay set-up; and post-amplification for test procedures. If separate rooms are not possible, enclosed "PCR hoods" or dead-air boxes and dedicated areas should be defined for each phase of the work, e.g., reagent preparation, specimen preparation, amplification and detection may be used. Reagents used for amplification shall

not be exposed to post-amplification work areas and specimens shall not be exposed to post-amplification work areas.

3B: Laboratory Space

3B.1: Does the laboratory have space suitable for performing molecular screening tasks?

N/A ☐ YES ☐ NO ☐

Comments:

Laboratory space should be suitable for the tasks performed as part of the screening procedure. Sufficient space should be allocated for the workload to be performed without compromising the quality of work and safety of the laboratory personnel.

Part 4: Personnel Qualifications (Pre-Analytic)

4A: Written Job Description Detailing Personnel Responsibilities

4A.1: Are there clearly defined job descriptions that define personnel responsibilities available?
N/A ☐ YES ☐ NO ☐

Comments:

> *Qualifications of the laboratory director, technical supervisor(s) and technologist are to be determined by each program and should be consistent with local and regulatory requirements. The job descriptions of laboratory personnel should specify responsibilities for defined aspects of operations and performance of all testing procedures. Specific guidelines are available from CLIA and CAP.*

4B: Training Program for New Technologists

4B.1: Does the laboratory have an in-house developed training program available for newly hired personnel?
N/A ☐ YES ☐ NO ☐

Comments:

> *Personnel must be trained and their competence assessed in the performance of all tasks for which they are responsible. The policies and procedures for staff training should be established and approved by the laboratory director. The procedures should identify both the methods and materials to be used in training. Criteria to document the effectiveness of training can be assessed through test performance of previously analyzed samples or internal/external proficiency testing procedures.*

4C: Continuing Education Program

4C.1: Does the laboratory have a policy in place for continuing education and training of personnel?

N/A ☐ YES ☐ NO ☐

Comments:

The laboratory director and technical staff should participate in continuing education relevant to the activities of the laboratory. Targeted educational and training opportunities should be appropriate with the scope of the duties of laboratory staff and such training and continuing education shall be documented.

4D: Personnel Levels for Workload to Maintain Assay Quality

4D.1: Does the laboratory have adequate staffing levels required to complete sample processing and analysis in an appropriate time frame?

N/A ☐ YES ☐ NO ☐

Comments:

Sufficient staff should be available to ensure the prompt performance of tests, the accuracy of results and reporting of results.

4E: Competency Assessment

4E.1: Are laboratory personnel assessed on their technical competency on a regular basis?

N/A ☐ YES ☐ NO ☐

Comments:

Personnel should undergo periodic assessment of competency by the technical supervisor. The procedures should include, but are not limited to, direct observation of test performance; monitoring, recording and reporting of test results; review of worksheets, quality control records, PT results and preventative maintenance record; as well as assessment of problem solving skills.

Part 5: 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.

5A: 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:

Part 7: Results Reporting (Post-Analytic)

Procedures for the reporting of results should be defined to ensure the clear understanding of the screening results to a non-expert physician. Discrepancies between preliminary and final reports should be investigated and documented.

7A: Summary of Methods, Loci or Mutations Tested, and Interpretation

7A.1: Does the laboratory have guidelines for reporting of molecular test results?
N/A ☐ YES ☐ NO ☐

Comments:

7A.2: Does the results report clearly describe the gene/loci target of the molecular assay?
N/A ☐ YES ☐ NO ☐

Comments:

7A.3: Does the results report include a summary of the molecular test method?
N/A ☐ YES ☐ NO ☐

Comments:

7A.4: For a targeted genotyping assay, does the results report list all mutations or variations that are detected by the assay?
N/A ☐ YES ☐ NO ☐

Comments:

7A.5: For quantitative molecular assays, does the results report define the normal reference range of the analytes targeted?
N/A ☐ YES ☐ NO ☐

Comments:

7A.6: Does the results report contain an analytic interpretation of the results that is readily interpretable by a non-expert physician?

N/A ☐ YES ☐ NO ☐

Comments:

The report should contain the specific information delineated in the standard operating procedure manual. This should include an appropriate summary of the methods, the loci and mutations, as well as the analytic and clinical interpretation, if appropriate.

7A.7: Does the report include an estimation of mutation detection rate and the residual risk of being a carrier for one of the mutations not tested?

N/A YES NO

Comments:

7B: Technical Limitations

7B.1: Does the results report contain a statement of the technical limitations of the molecular assay?

N/A ☐ YES ☐ NO ☐

Comments:

The technical limitations of an assay defined in the standard operating procedure manual should be included in a statement in the results report. Technical limitations of test procedures should include interfering substances, detection limits of the test (e.g., specific DNA sequences assayed, gene deletions detected) as well as the possibility of laboratory error.

7C: Limitations of Findings

7C.1: Does the results report contain a statement that a screen-negative result does not rule out the possibility that the test subject may be a carrier of a mutation in the tested gene/loci?

N/A☐ YES ☐ NO ☐

Comments:

Testing may not be able to identify all mutations for the disorder. Laboratories should provide the list of mutations in the testing panel and the clinical sensitivities for selected racial/ethnic groups when available and appropriate.

7D: Final Report Review

7D.1: For a screen-positive result, is the final results report reviewed and signed by the laboratory director or designee?

N/A☐ YES☐ NO ☐

Comments:

The final report should be reviewed and signed by the laboratory director or designee if there is a subjective or interpretive component to the test.

7D.2: Does the report include a recommendation that patients receive appropriate genetic counseling for screen positives?

N/A☐ YES☐ NO ☐

Comments:

The final report should contain a statement that a positive result is an indication that the individual may be predisposed to or have the specific disease or condition tested for and may want to consider further genetic counseling to explain the implications of the test results.

7E: Standard Nomenclature Used to Designate Genes and Mutations

7E.1: Does the results report include standard gene/loci and mutation nomenclature as defined by the Human Genome Variation Society?

N/A ☐ YES ☐ NO ☐

Comments:

Gene names are defined by the HUGO gene nomenclature committee (http://www.genenames.org/), and for mutations nomenclature, refer to den Dunnen and Antonarakis (Hum Mutat 15:7-12, 2000) and den Dunnen and Paalman (Hum Mutat 22:181-182, 2003) or the Human Genome Variation society (http://www.hgvs.org/mutnomen/) for specific guidance. Alleles that have established names in the literature should have both the recommended nomenclature from HGVS and the traditional allele designations.

7F: Critical Alert Procedures

7F.1: Does the results report clearly define a prompt communication process to report test results that require immediate attention?

N/A ☐ YES ☐ NO ☐

Comments:

A protocol for reporting imminently life-threatening results, or panic/alert values should be established.

7G: Literature References

7G.1: Do the results contain references and literature citations applicable to test analysis?

N/A ☐ YES ☐ NO ☐

Comments: