

American College of Medical Genetics and Genomics STANDARDS AND GUIDELINES FOR CLINICAL GENETICS LABORATORIES

2008 Edition, Revised 02/2007

(For a general overview of these Standards and Guidelines, including purpose and disclaimer, see Section A)

C	GENERAL POLICIES
C1	Resources and Facilities
C1.1	Laboratory space, equipment, facilities and supplies must be sufficient to ensure safe, accurate and acceptable standards of performance.
C1.2	Temperature-dependent equipment such as refrigerators, freezers (standard, ultralow or liquid nitrogen) and incubators must be maintained at temperatures optimal for the storage or handling of each type of reagent or sample. They must be monitored and documented at appropriate intervals as determined at the discretion of the laboratory director. Similarly, equipment requiring modified atmospheres must be monitored and documented for gas concentrations (i.e., CO ₂ , O ₂ , N ₂) at appropriate intervals as determined at the discretion of the laboratory director.
C1.3	The laboratory must be in compliance with all relevant safety codes to ensure safe handling of chemicals, radiation, recombinant DNA biologicals, blood samples or other human tissues/fluids and to ensure their proper disposal as stipulated by the Occupational Safety and Health Administration (OSHA) and the local institution.
C1.4	All laboratories are required to participate in mandated laboratory inspection as required by state or federal regulations.
C1.5	All laboratory equipment should be maintained at appropriate intervals. The records of such maintenance and repair must be kept as long as the equipment is in use.

C1.6	Adequate facilities for record storage must be available to the laboratory.
C1.7	A laboratory may engage the services of another laboratory to provide service for test systems not available to the primary laboratory or to divert excess volume. In this event, the subcontracting laboratory also must meet all applicable guidelines and standards stated in this document, as well as those of CLIA '88. The identity of the subcontracting laboratory and that portion of the study for which it is responsible must be noted clearly on the report.
C2	Specimens and Intake Information
C2.1	Specimen containers arriving in the lab must include two identifiers, which may be the patient's name, date of birth, hospital number, lab number or other unique identifier. The date of specimen collection and, when appropriate, the time of collection, should be included.
C2.2	When appropriate, specimen collection methods must not compromise aseptic technique.
C2.3	Specimen transport and handling must be in accordance with OSHA guidelines with the express understanding that any human tissues and fluids may harbor infectious agents.
C2.4	Intake information to accompany the specimen must include sufficient clinical information to ensure appropriate and accurate testing and interpretation of results.
C2.4.1	When appropriate, intake records should include the dates the specimen is obtained and received in the laboratory, and the quantity and qualitative condition of the specimen. All specimens for cell culture should be received within 1 day of being obtained, if at all possible.
C2.4.2	Patient information to accompany the specimen must include name, unique identifier on specimen container, date of birth, sex, time and date of collection, type of specimen, name of physician requesting test (with address, phone, fax or beeper numbers), type of test requested, indication for testing, race/ethnicity (if needed), and pedigree (if needed).
C2.4.3	Informed consent should be obtained as required by law and professional standards. The laboratory should be available to assist in determining the appropriate level of informed consent, which can be obtained from established guidelines.

C2.5	Specimen handling and processing methods should preclude contamination, tampering or substitution.
C2.6	Laboratory or General Supervisor
C2.7	The laboratory should retain the original patient sample until all testing is completed and the report has been signed out. The retention of a patient's DNA should be in compliance with state and federal laws.
C2.8	De-identified patient specimens can be reused for quality control and quality assurance, and for test development as allowed by the Institutional Review Board (IRB).
C3	Records
C3.1	All patient test records of the patient's laboratory testing must be accessible to the laboratory director.
C3.2	Files should be retrievable by both patient name and a second unique identifier (e.g., laboratory accession number).
C3.3	Records must be maintained in a manner that will ensure privacy, security, integrity and access, as required by law and professional standards
C3.4	Laboratory records should be released only with appropriate authorization for release .
C3.5	Laboratory records that are reviewed as part of inspection or regulation should be treated in such a way as to maintain patient confidentiality.
C3.6	The various components of records of each case should be maintained for time periods as shown in the specialty sections or as required by specific state laws. In general, critical records of genetic testing are kept for 1 generation (20 years). If copied, there should be a policy statement made that the duplicate record is a complete copy of the original. Additional specialty-specific recommendations can be found in those sections.
C3.7	The Laboratory Computer System must be validated to ensure proper functioning in all aspects of the laboratory, include a

	<p>security system that safeguards patient confidentiality issues, have sufficient back-up and verification to allow uninterrupted functioning of the laboratory and prevention of loss of data. Both hardware and software must be properly maintained and updated as needed to maintain the functioning of the laboratory. The laboratory director is responsible for the selection or development of the appropriate computer system for the laboratory and must annually review the performance. The laboratory must maintain documentation of all upgrades.</p>
C4	Quality Control/Assurance/Improvement
C4.1	All laboratories should have documented quality control (QC), quality assurance (QA) and quality improvement (QI) plans to assure that all reagents, equipment, methodologies and personnel operate at optimum levels.
C4.1.1	Cell culture reagents should be tested for contamination and support of growth of the particular cells for which they are to be used.
C4.1.2	The laboratory must participate in at least one external proficiency testing (PT) program evaluating its subspecialty. If not available, the laboratory must participate in at least one interlaboratory comparison program covering this subspecialty.
C4.2	The laboratory director and technical staff must participate in continuing education relevant to the activities of the laboratory
C4.3	All reagents and/or solutions must be appropriately identified with name and concentration, preparation date, and preparer's name. Storage conditions and expiration date, if appropriate, should be stated in the protocol manual and on the solution or reagent.
C4.4	All personnel must follow manufacturers' directions for FDA-approved commercial kits . The laboratory director must validate any procedural changes from manufacturers' instructions. This validation must be available for external review during inspection.

C5	<p>Standard Operating Procedure Manuals Manuals detailing procedures and policies must be developed and maintained, and must be reviewed annually by the director. All changes must be initialed and dated.</p>
C6	<p>Nomenclature Laboratories must use accepted standard nomenclature to describe the genetic testing done. See individual sections for the recommended nomenclature specific to those areas of testing.</p>
C7	<p>Levels of Development of a Diagnostic Test</p>
C7.1	<p>Research and Development: When a laboratory undertakes the development of a new test, the first level of testing should be a structured, stepwise process to fulfill the need to document right to use, analytical validation and performance characteristics (sensitivity, specificity) of the new application, regardless of the anticipated research or clinical use. Anonimized specimens from a variety of bio-repositories can be used without IRB approval to generate validation results not utilized for direct patient care.</p>
C7.2	<p>Investigational Studies: This is the second level of testing that some clinical genetic laboratories may undertake to seek clinical utility and acceptance of the technology and/or application by ordering physicians.. Studies conducted at this level should have IRB approval and the results given to the patients with caveats about the investigational nature of the testing (G17.3).</p>
C7.3	<p>Accepted Clinical Test: This is the third and final level of testing provided by the clinical genetics laboratory. At this level, the test has been validated by peer review publication or has been deemed acceptable by outside review, as may be necessary for tests for rare diseases. The methods are clearly stated and widely utilized. Reports or results from tests at this level must meet the requirements of Sections E8, F8 or G17, as appropriate. Tests in this category should be reimbursable.</p>
C8	<p>Test Validation (<i>Revised November 2003</i>)</p>
C8.1	<p>Overview In accordance with CLIA 1988, each laboratory is responsible for validating each new test before introduction into clinical use. This includes tests performed with FDA-approved kits, as well as "home brew" tests developed in the laboratory (reagents homemade or purchased under</p>

	<p>analyte-specific reagent rules). A necessary first step is to define the clinical disorder being tested for, and the intended use or clinical setting of the test (e.g., diagnostic testing, screening), because clinical validity can vary based on the clinical setting. Validation of each test in a specific clinical setting is focused on the collection of data to establish analytic validity, clinical validity, and clinical utility.</p> <p>The process involves:</p> <ol style="list-style-type: none"> 1 reviewing professional guidelines and relevant literature. 2 performing and evaluating analytic and clinical correlation studies within the laboratory to establish validity. 3 defining the limitations of the test. 4 determining the variables that must be monitored to maintain a high level of performance. 5 identifying and addressing relevant ethical, legal and social issues. 6 collecting information about the clinical utility of the test in order to inform patients and providers about appropriate test usage. <p>For some test applications, gaps in knowledge may exist, and these gaps should be identified. The laboratory should provide justification for offering the test in a clinical setting based on the information and data currently available.</p>
C8.2	Patient Information and Sample Collection
C8.2.1	Requisition Forms, Intake Information and Informed Consent: See Section C2.4 .
C8.2.2	Specimen Collection and Transportation: Collection and transport protocols based on published or in-house experience obtaining acceptable results for this assay with different sample types. See also Sections C2.1 through C2.3 .
C8.2.3	Specimen Processing and Storage
C8.2.3.1	Written criteria for acceptance or rejection of specimens should specify optimal and acceptable specimen types (may differ by intended use of test) and variables that can affect acceptability (e.g., insufficient quantity, exposure to

	extreme temperatures, inappropriate blood anticoagulant). See also Sections C2.4 through C2.6 .
C8.2.3.2	Protocol for preparation of samples for testing, with criteria for adequate quality and quantity and storage conditions, and information on stability. See Sections C2.5 and C2.6 .
C8.2.3.3	Establishment of laboratory policies regarding specimen retention and appropriate storage conditions. See Sections C2.7 and C2.8 .
C8.3	<p>Assay Methodology</p> <p>To ensure validation of the assay methodology, laboratories must address a number of issues that are outlined in the following sections. Guidance on developing assay protocols is available. For example, NCCLS standards and guidelines provide information on documenting assay methodology that includes formatting as well as specific essential requirements and discretionary elements (e.g., MM1-A. <i>Molecular Diagnostic Methods for Genetic Diseases; Approved Guideline</i>. 2000; Vol. 20, No. 7. http://www.nccls.org).</p>
C8.3.1	Detailed Analytic Procedures (See also Sections C5 and C6 .)
C8.3.1.1	Principles of testing methodology
C8.3.1.2	Information on sources, preparation and storage of key reagents. See Section C4.3 .
C8.3.1.3	Calibrators and calibration procedures where applicable
C8.3.1.4	Calculation of results and/or procedure for reporting as identified mutation(s), positive/negative, or continuous variable
C8.3.2	Quality control parameters and acceptable limits
C8.3.2.1	Preparation, characterization and use of controls

C8.3.2.2	Type and frequency of QC assessments
C8.3.2.3	Measures of repeatability both within and between runs
C8.3.2.4	Routine equipment calibration and preventive maintenance
C8.3.2.5	Long-term measures of variability (e.g., reagent lot-to-lot variability)
C8.3.2.6	Definitions of ranges and cutoffs (including gender- and age-specific ranges)
C8.3.2.7	Description of positive, negative and indeterminate results, including discussion of nomenclature and complex issues such as unclassified variants when appropriate
C8.3.2.8	Technical limitations of the methodology for the intended use
C8.3.2.9	Failure rates for different sample types
C8.3.3	External Proficiency/QC Testing: In most cases, the laboratory is obligated to participate in external quality control/proficiency testing. It is recommended that laboratories identify and take part in available external proficiency testing programs sponsored by professional or regulatory organizations that include the test/methodology being validated. However, particularly when this option is not available, the laboratory can utilize other recommended methods, such as scheduled inter-laboratory comparisons, split sample analysis with another laboratory, split samples with another established in-house method, or use of assayed materials.
C8.4	Analytic Validity The analytic validity of a genetic test defines its ability to accurately and reliably measure a specific analyte, or identify a mutation of interest in the sample type(s) to be used clinically. Each laboratory is responsible for in-house

validation of a test methodology. Information from the literature on test performance can be used as supplementary supporting evidence only if the laboratory can demonstrate that the methodology is essentially identical.

C8.4.1

Analytic sensitivity is the proportion of biological samples that have a positive test result or known mutation and that are correctly classified as positive (assumes mutation is tested for). Analytic sensitivity is determined using samples with known test results or mutation status, either by comparison with another methodology or by consensus findings (e.g., proficiency testing samples). Estimates should include confidence intervals. For example, 25 individuals with clinically defined cystic fibrosis have been run by another laboratory using a panel of 25 mutations. A total of 45 mutations were identified. Your laboratory agrees with all 45 mutations. Analytic sensitivity is 100% (95% confidence interval of 92.1% to 100%). (A confidence interval calculator is available at http://www.swogstat.org/stat/public/binomial_conf.htm.) Determining analytic sensitivity can be more difficult in certain areas of testing (e.g., genome scanning methods that can identify unclassified variants). It is not yet clear how to confidently establish validity when a proportion of tests cannot be classified as either positive or negative.

C8.4.2

Analytic specificity is the proportion of biological samples that have a negative test result or no identified mutation (being tested for) and that are correctly classified as negative. Analytic specificity is also determined using samples with known test results. Alternatively, samples from the target population could be tested with all positive results confirmed by referent method as being true positives. Estimates should include confidence intervals. For example, your laboratory tests a total of 100 apparently normal individuals for 25 cystic fibrosis mutations. Five mutations are found and four of these are confirmed by another laboratory using a different methodology. The fifth was found to be a false positive due to contamination. Analytic specificity is 95/96 or 99.0% (95% confidence interval 94.3% to 99.9%).

C8.4.2.1	<p>Confirmatory testing is a repeated test aimed at corroborating an earlier positive test result. Depending on circumstances, the confirmatory test may use the same sample or a newly processed or collected sample, and may utilize the same or a different technology. Laboratories should consider factors such as clinical setting, estimated analytic specificity, and the clinical impact of a false positive test result in determining the necessity or type of confirmatory testing.</p>
C8.4.3	<p>Assay robustness measures how resistant testing is to small changes in pre-analytic and analytic variables. In an attempt to define performance requirements and minimize possible impact on assay performance (e.g., analytic validity, reproducibility, failure rates), laboratories should consider the effects of common variables. Such variables may include sample type, sample handling (e.g., transit time or conditions), sample quality, reagent lots, or minor changes in assay conditions (e.g., timing or temperature). For example, if sample degradation sufficient to decrease assay performance is observed when samples are frozen, or are in transit 4 days or more, then the protocol should include rejection of specimens that are/have been frozen or are received more than 3 days after the sample date.</p>
C8.4.4	<p>Individual laboratories need to verify the analytic performance of testing using the sample type(s), reagents and protocols that will be used clinically.</p>
C8.4.5	<p>It is important that assay quality control procedures be established, followed and documented when assessing analytic validity.</p>
C8.5	<p>Clinical Validity The clinical validity of a genetic test defines its ability to accurately and reliably identify individuals who have (or will develop) the disorder or phenotype of interest. For this assessment, it is necessary to clearly define the disorder of interest along with the clinical setting in which the test is to be applied. For example, when offering prenatal testing for cystic fibrosis, identifying carriers is part of a process aimed at identifying a fetus with two CF mutations who will develop the phenotype of interest. In contrast, carriers</p>

	<p>identified with the factor V Leiden mutation are the individuals of interest, are at risk of developing venous thrombosis, and may benefit from preventive action. Many individual laboratories may not be able to directly quantify clinical performance through their own studies. However, through use of the literature and in-house estimates of analytic performance, laboratories must provide reliable information about the clinical validity of the tests they offer.</p>
C8.5.1	<p>The clinical sensitivity is the proportion of individuals who have (or will develop) the phenotype of interest and who have a positive test result. Clinical sensitivity can be directly determined by applying the test to an unbiased selection of individuals affected with the disorder. When testing is shown to be comparable, the literature can be used to support laboratory evidence of clinical sensitivity. In some instances, testing may not be capable of identifying all gene/chromosome/biochemical defects associated with the phenotype of interest. For example, not all breast cancer caused by defects in the BRCA1 gene can be identified by sequencing (e.g., large deletions). A panel of 25 mutations cannot identify all fetuses that will develop CF. In both of these instances, the estimate of clinical sensitivity should reflect the fact that a proportion of the individuals of interest will not be identified. Laboratories should provide clinical sensitivities for selected racial/ethnic groups, when available and appropriate. Confidence intervals should be included in these estimates.</p>
C8.5.2	<p>The clinical specificity is the proportion of all unaffected individuals identified by the proposed test as being negative. Laboratories should provide estimates of clinical specificity that are tailored to their test, model, and target population. Confidence intervals should be included in these estimates.</p>
C8.5.3	<p>The positive and negative predictive values of testing in the target population measure the ability of the test to give accurate clinical information.</p>
C8.5.3.1	<p>Calculation of predictive values requires data on prevalence, the frequency of individuals with the</p>

	disorder (or with mutations causing the disorder) in the general population in the United States. For some disorders, prevalence may vary by race/ethnicity.
C8.5.3.2	The positive predictive value is the proportion of positive test results that correctly identify an individual who has the phenotype of interest (number of true positives / true positives + false positives).
C8.5.3.3	The positive predictive value is dependent on prevalence, analytic sensitivity, clinical specificity, and other factors (e.g., the model used for a screening test).
C8.5.3.4	The negative predictive value is the proportion of negative tests that correctly identify an individual who does not have the phenotype of interest (number of true negatives / true negatives + false negatives).
C8.5.3.5	The negative predictive value is dependent on prevalence, analytic and clinical sensitivity, and other factors (e.g., the model used for a screening test).
C8.5.4	It is important to understand any genetic, environmental or other modifying factors that impact testing. Examples might include the effect of blood donation, alcohol use, or dietary supplements on biochemical testing for hereditary hemochromatosis, the significance of intron 8 poly-T status for a carrier of the R117H CF mutation, or I307K and somatic APC gene mutations. Also important are genotype/phenotype associations that may occur between mutations and disease phenotype (e.g., probability of expansion in trinucleotide repeat expansion disorders, or the relationship of the reading frame to predicting disease phenotype as Duchenne or Becker muscular dystrophy).
C8.5.5	Ordering and Reporting
C8.5.5.1	Educational Materials for Patients Laboratories can often utilize materials that have been developed and appropriately evaluated by professional organizations or pilot programs. If developing materials in-

	house, laboratories should consider not only accurate content, but also the target audience, needed translations, reading level and presentation (e.g., type size, length and visual aids).
C8.5.6	<p>Informational Materials for Providers</p> <p>Laboratories should give providers materials that include the following:</p>
C8.5.6.1	Detailed information about the type of sample(s) acceptable for specific tests, and how samples should be obtained, stored and transported to the laboratory.
C8.5.6.2	Samples of test requisitions and/or other documents that provide information about the patient and specimen needed for accurate test interpretation.
C8.5.6.3	General information on such issues as turn-around time and reimbursement.
C8.5.6.4	Information that assists providers in understanding test performance, test interpretation, and report formats.
C8.5.6.7	<p>Report Formats</p> <p>Report formats must be developed for all expected results and reviewed for consistency with any existing regulatory requirements or established guidelines. See appropriate sections for clinical cytogenetics (E9.7, E10.7, E11.4), clinical biochemical genetics (F8), and clinical molecular genetics (G17), as well as disorder-specific guidelines (e.g., "Technical Standards and Guidelines for CFTR Mutation Testing"). In general, elements to be considered include:</p> <ol style="list-style-type: none"> 1 repetition on the report of key interpretive information. 2 clear presentation of the result (with ranges, cutoffs as appropriate). 3 interpretive statement that explains the result in the context of the test purpose (may include an estimate of risk). 4 disclaimer or explanation of test limitations (for example, analytic and clinical validity, non-paternity).

- 5 investigational test statement if appropriate.
- 6 information used for risk assessment calculations.
- 7 Final laboratory reports must be signed