American College of Medical Genetics and Genomics (ACMG)

TECHNICAL STANDARDS FOR CLINICAL GENETICS LABORATORIES
(2021 Revision)

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C: GENERAL POLICIES

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

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), incubators, hybridization chambers, water baths, PCR equipment, etc. must be maintained and monitored such that the optimal temperature(s) for the storage or handling of each type of reagent or sample is met. Monitoring must be performed and documented as outlined by the Clinical Consultant (Section Director/Technical Supervisor), at their discretion. 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 and federal regulations.

C1.5 All laboratory equipment should be maintained and undergo preventive maintenance at appropriate intervals. The records of such maintenance and repair must be kept for at least two years. Some laboratories may choose to maintain these records for a longer period to facilitate troubleshooting.

C1.6 Adequate facilities and/or server space for record storage must be available to the laboratory. If patient information is stored in “the cloud”, effective systems such as firewalls and data encryption must be in place.

C1.7 A laboratory may engage the services of another laboratory. In this event, the subcontracting laboratory must meet all applicable guidelines and standards outlined by CLIA '88. Additionally, it is recommended that the subcontracting laboratory meet the standards stated in this document. 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 Primary specimen containers arriving in the lab must include two identifiers, which may include the patient's name, date of birth, hospital number, lab number or another unique identifier. The date of specimen collection and, when appropriate, the time of collection, should be included.

C2.2 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.

C2.3 Intake information to accompany the specimen must include sufficient clinical information to ensure appropriate and accurate testing and interpretation of results.

C2.3.1 When appropriate, intake records should include the date each specimen was obtained and received in the laboratory, as well as the quantity and qualitative condition of the specimen. All specimens for cell culture should be received within one day of being obtained, whenever possible.

C2.3.2 Patient information to accompany the specimen must include the name, unique identifier, date of birth, biological sex, time and date of collection, type of specimen, name of physician requesting the test (with address, phone, fax or pager numbers), the type of test requested, the indication for testing, and if relevant, race/ethnicity and pedigree information.

C2.4 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, based on established guidelines.

C2.5 Specimen collection, handling, and processing methods should preclude contamination, tampering or substitution.

C2.6 The laboratory should retain the original patient sample until all testing is completed and the report has been signed out. Retention of a patient's processed specimen material such as DNA, cell pellets or slides, should be in compliance with state and federal laws, as well as with professional standards.

C2.7 De-identified patient specimens can be reused for quality control, quality assurance, and

test development purposes as allowed by the relevant Institutional Review Board (IRB).

C3 Patient Information and Sample Collection
C3.1 Requisition forms, intake information and informed consent should contain sufficient information to carry out the desired test. [See also Sections C2.3 and C2.4]

C3.2 Acceptable sample types, collection procedures, and transport protocols, based on published or in-house experience, must be defined for the assay and readily accessible (*i.e.*, printed on test requisition forms or published online). [See also Section C2]

C3.3 Written criteria for acceptance or rejection of specimens must 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).

C4 Specimen Processing and Storage
C4.1 The testing protocol for sample preparation must be documented and include criteria for adequate quality, quantity, storage conditions, and stability. [See also Section C3]

C4.2 The laboratory must establish policies regarding specimen retention and appropriate storage conditions. Conditions may vary depending on anticipated expectations for repeat or reflex testing. [See also Sections C2.6 and C2.7]

C5 Patient Records
C5.1 Records of prior patient testing performed within the laboratory must be accessible to the Clinical Consultant (Section Director/Technical Supervisor).

C5.2 Records should be retrievable by both patient name and a second unique identifier (*e.g.*, laboratory accession number).

C5.3 Records must be maintained in a manner that will ensure privacy, security, integrity and access, as required by law and professional standards.

C5.4 Records should be released only with appropriate authorization for release.

C5.5 Records that are reviewed as part of inspection or regulatory practices should be treated in such a way as to maintain patient confidentiality.

C5.6 The various record components for each case should be maintained for the time periods indicated in specialty sections (E, F or G) or as required by specific state laws and other professional standards. 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.

C5.7 The laboratory computer system must be validated to ensure proper functioning in all aspects of the laboratory, include a security system that safeguards patient confidentiality, have

sufficient back-up and verification to allow uninterrupted functioning of the laboratory and prevent loss of data. Both hardware and software must be properly maintained and updated as needed to maintain laboratory functions. The Clinical Consultant (Section Director/Technical Supervisor) is responsible for the selection or development of the appropriate computer system for the laboratory and must annually review its performance. The laboratory must maintain documentation of annual review and all upgrades.

C6 Quality Control/Assurance/Improvement

C6.1 Each laboratory should have a documented quality management (QM) plan that encompasses quality control (QC), quality assurance (QA) and quality improvement (QI) to ensure that all reagents, equipment, methodologies and personnel operate at optimum levels.

*C6.*2 Each laboratory must participate in at least one external proficiency testing (PT) program for each relevant testing methodology used. If not available commercially, the laboratory must participate in at least one alternative PT program for each relevant methodology. Examples of alternative testing methods include split sample analysis with referral or other laboratories, split sample analysis with an established in-house method, or use of blinded, previously assayed materials. [See also Section C12]

C6.3 The Clinical Consultant (Section Director/Technical Supervisor) and technical staff must participate in continuing education relevant to the activities of the laboratory. Continuing education activities must be documented.

C6.4 Personnel must follow manufacturers' directions for FDA-approved commercial kits. The Clinical Consultant (Section Director/Technical Supervisor) must validate any procedural changes from manufacturers' instructions. This validation must be available for external review during inspection.

C6.5 Reagents and/or solutions must be appropriately identified with content and concentration information, preparation date, storage conditions, and the expiration date. If appropriate, information regarding storage and expiration dates should also be stated in the protocol manual.

C6.5.1 Reagents should be monitored for contamination and, if applicable, functionality. Cell culturing reagents should be tested to ensure they support growth of the particular cells for which they are to be used.

C7 Standard Operating Procedure Manuals

Manuals outlining the laboratory’s procedures and policies must be established, and then reviewed at least every two years by the Clinical Consultant (Section Director/Technical Supervisor) or a designee. Additionally, all new procedures and substantial changes to existing procedures must be reviewed.

C8 Nomenclature

When appropriate, laboratories must use accepted standard nomenclature to describe the genetic test results. See individual sections (E, F and G) for the recommended nomenclature specific to those areas of testing.

C9 Levels of Development of a Diagnostic Test [Leeflang et al., 2019]
C9.1 **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 the analytical validation and performance characteristics (sensitivity, specificity, and reproducibility) of the new application, regardless of the anticipated research or clinical use. Anonymized specimens from a variety of biorepositories may be used without IRB approval to generate validation results at this level of test development.

C9.2 **Investigational Studies**: This is the second level of testing that some clinical genetics laboratories may undertake to establish clinical utility, as well as acceptance of the technology and/or its application by ordering physicians. Studies conducted at this level should have IRB approval and any reported results should meet the standards of clinical test reporting and include information regarding the investigational nature of the testing. [See also Sections E8, F8, and G17]

C9.3 **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 and/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. All reported results must meet the requirements of Sections E8, F8 or G17, as appropriate. Tests in this category should be reimbursable.

C10 Test Validation
Overview
In accordance with CLIA 1988, each laboratory is responsible for validating each new test before introduction into clinical use. Validation is necessary for laboratory-developed tests (LDTs) or FDA-approved/cleared materials that have been locally modified. FDA-approved kits may only require limited verification data if manufacturers' directions are followed without modification [CAP COM.40300, 2020]. LDTs may include homemade reagents or customized methodology. The College of American Pathologists (CAP) provides additional resources for validation of LDTs and necessary documentation for accreditation [CAP COM.40350, 2020; CAP MOL.30785, 2020]. The first step in the development of an LDT is to define the clinical disorder being tested, 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. Individual laboratories need to verify the analytic performance of testing using the sample type(s), reagents, and protocols that will be used clinically.

C10.1 Clinical test validation should include:
- Reviewing professional guidelines and relevant literature.
- Performing and evaluating analytic and clinical correlation studies within the laboratory to establish validity.
- Defining the limitations of the test.
- Determining the variables that must be monitored to maintain a high level of performance.

- Collecting information about the clinical utility of the test to inform patients and providers about appropriate test usage.
- Identifying and addressing relevant ethical, legal and social issues.

C10.2 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.

C10.3 **Analytic Validity**
C10.3.1 The analytic validity of a genetic test defines its ability to measure a specific analyte or identify pathogenic variants of interest in the sample type(s) to be used clinically. Each laboratory is responsible for in-house validation of each test methodology. Information from the literature on test performance can be used as supplementary supporting evidence only if the laboratory can demonstrate the methodology is essentially identical.

C10.3.2 It is important that assay quality control procedures be established, followed, and documented when assessing analytic validity.

C10.4 **Analytic Sensitivity**
Analytic sensitivity is **the proportion of biological samples that have a positive test result (*i.e.*, abnormal finding) that are correctly classified as positive**. Analytic sensitivity is determined using samples with known test results or variant status, either by comparison with another methodology or by consensus findings (*e.g*., proficiency testing samples). Estimates should include confidence intervals. Each laboratory should establish a reliable methodology for determining confidence intervals.

For example, 25 individuals with clinically defined cystic fibrosis have been assayed by another laboratory using a panel of 25 pathogenic variants. A total of 45 pathogenic variants were identified. Your laboratory identifies these same 45 variants. Analytic sensitivity is therefore 100% (95% confidence interval of 92.1% to 100%). Determining analytic sensitivity can be more difficult in certain areas of testing (*e.g.*, very rare or newly identified disorders with limited positive controls). It is not yet clear how to establish validity when a proportion of tests cannot be classified as either positive or negative (*e.g.*, sequencing that identifies unclassified variants, partially reduced enzyme activity, or noncanonical metabolite patterns).

C10.5 **Analytic Specificity**
Analytic specificity is **the proportion of biological samples that have a negative test 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 a reference method as being true positives. Estimates should include confidence intervals. Each laboratory should establish a reliable methodology for determining confidence intervals.

For example, the laboratory tests a total of 100 apparently normal individuals for 25 pathogenic cystic fibrosis variants. Five pathogenic variants 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. The analytic specificity in this scenario is 95/96 or 99.0% (95% confidence interval 94.3% to 99.9%).

C10.6 **Clinical Validity**
The clinical validity of a genetic test defines its **ability to identify individuals who have (or will develop) the disorder or phenotype of interest**. For this assessment, it is necessary to define the disorder of interest along with the clinical setting in which the test is to be applied. Many individual laboratories may not be able to 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.

Two example scenarios: when offering prenatal testing for cystic fibrosis (CF), identifying carriers is part of a process aimed at identifying a fetus with two pathogenic CF variants who will develop the phenotype of interest. In contrast, individuals who are heterozygous or homozygous for the pathogenic factor V Leiden variant are the individuals of interest because both are at risk of developing venous thrombosis and may benefit from preventive action.

C10.7 **Clinical Sensitivity**
The clinical sensitivity of a genetic test 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 clinically significant variants cannot identify all fetuses that will develop cystic fibrosis (CF). In both 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. Each laboratory should establish a reliable methodology for determining confidence intervals.

C10.8 **Clinical Specificity**
The clinical specificity of a genetic test 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 and target population. Confidence intervals should be included in these estimates. Each laboratory should establish a reliable methodology for determining confidence intervals.

C10.9 **Predictive Values**
C10.9.1 The predictive values of genetic tests may depend on prevalence, analytic sensitivity, clinical specificity, clinical presentation and history, ethnicity/race and other factors (*e.g.*, the model used for a screening test). Calculation of predictive values requires data on prevalence, the frequency of individuals with the disorder (or with clinically significant variants causing the disorder) in the population of interest. The positive and negative predictive values of testing in

the target population measure the ability of the test to give accurate clinical information.

C10.9.2 The **positive predictive value** of a genetic test 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).

C10.9.3 The **negative predictive value** of a genetic test 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).

C10.10 It is important to understand any genetic, environmental or other modifying **factors that impact testing**. Examples might include the effect of a recent blood donation or transfusion, alcohol use, or dietary supplements on biochemical testing for hereditary hemochromatosis, the significance of intron 8 poly-T status for a carrier of the pathogenic Arg117His *CFTR* gene variant, or the significance of the Ile1307Lys germline variant in the *APC* gene. Also important are genotype/phenotype associations that may occur between some pathogenic variants and disease phenotypes.

C11 Assay Methodology

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, CLSI standards and guidelines provide information on documenting assay methodology that includes formatting as well as specific essential requirements and discretionary elements [MM1-A, 2000]. Also, the CAP all common checklist includes required test validation documentation for laboratory accreditation [CAP COM. Test Method Validation and Verification - Nonwaived Tests, 2020].

C11.1 **Detailed Analytic Procedures** should include the following information and be maintained to reflect current laboratory practices.

- Principles of testing methodology.
- Information on sources, preparation and storage of key reagents.
- Calibrators and calibration procedures where applicable.
- A description of how results are to be calculated and reported (*e.g.*, as a specific pathogenic variant(s), positive versus negative, a continuous variable).
- Quality control (QC) parameters and acceptable limits.
- Preparation, characterization and use of controls.
- Type and frequency of QC assessments.
- Measures of reproducibility both within and between runs.
- Long-term measures of variability (*e.g.*, reagent lot-to-lot variability, QC variability, instrumentation trends).
- Definitions of ranges and cutoffs (*e.g.*, gender- and age-specific ranges).
- Description of positive, negative and indeterminate results, including discussion of nomenclature and complex issues such as unclassified variants when appropriate.
- Technical limitations of the methodology for the intended use.
- Failure rates for different sample types.

- Routine equipment calibration and preventive maintenance.

C11.2 **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 it 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 or negative test result in determining the necessity or type of confirmatory testing.

C11.3 **Assay robustness** measures how resistant testing is to small changes in pre-analytic and analytic variables. 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 four days or more, then the protocol should dictate rejection of specimens that are/have been frozen or are received more than three days after the sample date.

C12 **External Proficiency/QC Testing**
The laboratory is obligated to participate in 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 use other recommended methods such as interlaboratory comparisons (split sample analysis with another laboratory), split samples with another established in-house method, or repeat analysis of previously assayed materials (*i.e.*, repeat testing of a patient with a known diagnosis).

C13 Ordering and Reporting
C13.1 **Educational Materials for Patients**: Laboratories may 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).

C13.2 **Informational Materials for Providers**: Laboratories should provide materials that include the following:

C13.2.1 Detailed information about the type of sample(s) acceptable for specific tests, and how samples should be obtained, stored and transported to the laboratory.

C13.2.2 Samples of test requisitions and/or other documents that provide information about the patient and specimen needed for accurate test interpretation.

C13.2.3 General information on such issues as turnaround time and costs.

C13.2.4 Information that assists providers in understanding test performance, test interpretation, and report formats.

C13.3 **Report Formats**
Report formats must be developed for all expected results and reviewed for consistency with any clinical cytogenetics [See also Section E8; Section E9.5; Riggs et al., 2019; Mikhail et al., 2019], clinical biochemical genetics [Section F8], and clinical molecular genetics [Section G17], as well as disorder-specific guidelines [*e.g.*, Deignan et al., 2020]. In general, elements to be considered include:

- Repetition on the report of key interpretive information.
- Clear presentation of the result (with ranges or cutoffs as appropriate).
- Interpretive statement that explains the result in the context of the test purpose (may include an estimate of risk).
- Disclaimer or explanation of test limitations (*e.g.*, analytic and clinical validity, misattributed parentage).
- Investigational test statement if appropriate.
- Information used for risk assessment calculations.
- Relevant published references including appropriately vetted websites.
- Final laboratory reports must be signed.

References

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