Test Verification and Validation for Molecular Diagnostic Assays

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• With our ever-increasing understanding of the molecular basis of disease, clinical laboratories are implementing a variety of molecular diagnostic tests to aid in the diagnosis of hereditary disorders, detection and monitoring of cancer, determination of prognosis and guidance for cancer therapy, and detection and monitoring of infectious diseases. Before introducing any new test into the clinical laboratory, the performance characteristics of the assay must be ''verified,'' if it is a US Food and Drug Administration (FDA)–approved or FDA-cleared test, or ''validated,'' if it is a laboratory-developed test. Although guidelines exist for how validation and verification studies may be addressed for molecular assays, the specific details of the approach used by individual laboratories is rarely published. Many laboratories, especially those introducing new types of molecular assays, would welcome additional guidance, especially in the form of specific examples, on the process of preparing a new molecular assay for clinical use.

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In 2009, Jennings et al and the College of American Pathologists (CAP) Molecular Pathology Resource Committee[1] published a detailed article in *Archives of Pathology & Laboratory Medicine* describing the established principles of test validation and relevant regulations in the United States as they pertain to molecular diagnostic assays. In the present issue of the ARCHIVES, 4 CAP resource committees involved in molecular diagnostics present specific examples of approaches used by individual laboratories in introducing molecular tests into the clinical laboratory. These articles are examples of approaches taken by individual laboratories for verification or validation of molecular assays. They are not meant to imply that they are the best or only approach for establishing performance characteristics, or that they are endorsed guidelines. However, we believe presenting specific examples of approaches of validation or verification of new molecular assays will be very useful for laboratories initially introducing molecular diagnostic assays. In addition, these examples may provide experienced molecular laboratories with alternative approaches for improvement of their current validation and verification procedures.

Before introducing a molecular assay into the clinical laboratory, the laboratory director must be familiar with current federal and state regulations and with the FDA approval status of the assay. The articles in this special section do not address these critical issues in depth. Therefore, it is important to briefly review the FDA status of assays and other regulations affecting molecular diagnostics.

Some molecular assays have been commercially produced and have gone through an FDA 510(k) clearance or PMA (premarket approval) process. These assays are referred to as ''in vitro diagnostics'' or ''IVDs.'' In contrast, most new molecular diagnostic assays are developed within individual clinical laboratories and are referred to as ''laboratory-developed tests'' or ''LDTs.'' Many of these clinically important LDTs are unlikely to ever become FDA cleared or approved because low test volumes make it economically unfeasible to go through the current FDA 510(k) or PMA approval process.

In vitro diagnostics that have gone through and passed a 510(k) trial are considered FDA cleared. 510(k) trials are designed to show that an IVD is substantially equivalent to a legally marketed predicate device. 510(k) trials are less involved and less expensive than PMA trials. An IVD that has gone through and passed a PMA trial is considered FDA approved. Premarket approval trials are required for class III medical devices or if there is no predicate device available for comparison. They are significantly more expensive to perform than 510(k) trials.

Molecular diagnostic assays are classified by Clinical Laboratory Improvement Amendments of 1988 (CLIA) as nonwaived testing. As such, the quality system requirements defined by CLIA state that the clinical laboratory performing the FDA-cleared or FDA-approved test must ''verify'' that the manufacturer's performance characteristics, which were established during the FDA trial, can be obtained or exceeded by the laboratory. Specifically, for FDA-cleared/approved tests, a laboratory must document its verification of the following 4 performance characteristics: accuracy, precision, reference range, and reportable range. These performance characteristics are published in the manufacturer's package insert.

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CLIA requires that laboratories performing LDTs, and modified FDA-cleared/approved tests, ''establish'' the same 4 performance characteristics that are required for FDA-cleared/approved tests, as well as determine analytic sensitivity, analytic specificity, and any additional performance characteristics that may be important to establish (eg, specimen stability, linearity). Verification of FDA-cleared/approved tests generally entails smaller studies with fewer samples than are required to validate an LDT or a modified FDA-cleared/approved test because the manufacturer has already established the performance characteristics of the FDA-cleared/approved tests. A laboratory must document that it has adequately verified or validated test performance characteristics for all clinically offered tests. It should be noted that the term *validation* is widely used by laboratorians to mean establishing the performance characteristics of an LDT, but the term *validation* never appears anywhere within the CLIA regulations.

A useful mnemonic for remembering the performance characteristics that need to be verified for FDA-cleared/approved tests is **PARR** (**p**recision, **a**ccuracy, **r**eportable range, and **r**eference range). For LDTs or modified FDA-cleared/approved tests it is **PARR+AS+AS** (**p**recision, **a**ccuracy, **r**eportable range, **r**eference range, **a**nalytic **s**ensitivity, and **a**nalytic **s**pecificity). It should be noted that there may be additional parameters that a laboratory needs to address beyond the 4 or 6 respective performance characteristics. For example, the laboratory may find it important to assess specimen stability, linearity, and carryover.

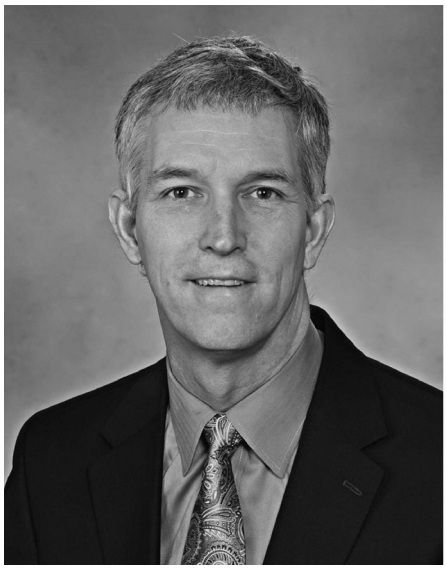
Although not required by CLIA, knowledge of the clinical validity (ie, clinical sensitivity and specificity) and clinical utility of these assays is also good laboratory practice. It is often difficult for an individual laboratory to thoroughly assess these parameters (eg, clinical sensitivity) because there may not be enough patients with an uncommon disease at an institution to do a large study. However, patients without disease can easily be identified to determine clinical specificity. It is currently considered permissible to cite scientific literature that established clinical sensitivity and specificity. The CAP and various laboratory organizations are beginning to work with the FDA to discuss what will be required by individual laboratories for evidence of clinical validity and utility.

While a laboratory must, at a minimum, address all the performance characteristics required by CLIA, the specifics of how to verify or establish these is left by CLIA to the judgment of the laboratory director. The scientific approach used to verify or validate the performance characteristics of a test is one of the more challenging aspects of being a laboratory director. CLIA '67 was revised and expanded in 1988, well before genetic testing became widely used. For this reason, the meaning and relevance of some performance characteristics, such as reportable range, reference range, and analytic specificity for molecular diagnostic tests, is still being discussed and refined. Organizations such as the Clinical and Laboratory Standards Institute (CLSI) provide excellent guidance on how to approach test verification and validation for a wide variety of test types. In addition to CLSI, other organizations such as the Centers for Disease Control and Prevention and the American College of Medical Genetics have published recommendations on approaches to validation for certain types of molecular pathology testing. The CAP Molecular Pathology Checklist also provides some guidance on test validation.

Nonetheless, laboratories across the country are looking for further guidance and specific examples on how to verify FDA-approved tests and especially how to validate LDTs. The goal of the set of special section articles included in this issue of *Archives of Pathology & Laboratory Medicine* is to provide examples of how individual laboratories have verified or validated the analytical performance characteristics for certain FDA-approved/cleared or laboratory-developed molecular diagnostic assays. Two examples of validating and implementing FDA-cleared or FDA-approved tests (cystic fibrosis and *Clostridium difficile* cytotoxin) are provided, as well as 4 examples of validating and implementing LDTs. The LDT examples include validation of (1) a *KRAS* test for colorectal cancer treatment decisions, (2) a *BCR/ABL1* minimal residual disease assay for guiding treatment of chronic myelogenous leukemia patients, (3) a sequencing assay for germline *PTEN* mutations, and (4) a fluorescence in situ hybridization assay for the detection of *MLL* rearrangements in leukemia. Although the primary goal of these articles is to discuss verification and validation, the articles also address other aspects of the total process of introducing a new molecular assay in the clinical laboratory, starting from familiarization/characterization and proceeding to validation and test implementation.

References

1. Jennings L, Van Deerlin VM, Gulley ML; for the College of American Pathologists Molecular Pathology Resource Committee. Recommended principles and practices for validating clinical molecular pathology tests. *Arch Pathol Lab Med.* 2009;133(5):743–755.



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