Ensuring Accurate Molecular Genetic Testing

In this issue of Clinical Chemistry, Lutz et al. (1) report on a multicenter evaluation of polymerase chain reaction (PCR) methods for the detection of Factor V Leiden genotypes. Mutations in the Factor V Leiden gene have been shown to result in activated protein C (APC) resistance, which is the most common cause of inherited venous thrombosis in Caucasians (2–4). In particular, a specific mutation in the Factor V Leiden gene, a guanine-to-adenine substitution at nucleotide 1621 that results in a glutamine-to-arginine substitution at position 506 (R506Q), has been shown to be associated with APC resistance (2, 3). Clinically, individuals who are heterozygous for this mutant allele have a 5- to 10-fold increased risk for thrombotic events, and individuals who are homozygous have a 50- to 100-fold increase (2, 5). In the past, patients with APC resistance could be identified only by coagulation-based assays that are limited by decreased reliability of results in a number of conditions (e.g., pregnancy) and in patients receiving certain pharmacologic agents (e.g., anticoagulant therapy) (6). In contrast, molecular testing for the identification of Factor V Leiden gene mutation heterozygotes and homozygotes is not subject to these limitations. This advantage over coagulation-based assays has resulted in the rapid integration of this testing into clinical practice and its widespread availability. For example, in a recent survey of molecular genetic testing laboratory directors, Factor V Leiden mutation detection was available in 29% of 245 responding laboratories (7).

In the study by Lutz et al., six laboratories that offer Factor V Leiden mutation analysis simultaneously tested a set of 62 blinded patient samples to determine the Factor V Leiden genotype. Although each of the participating laboratories had developed PCR-based assays, the exact testing system conditions varied substantially (e.g., PCR primer sequences, restriction endonucleases used). Thus, the purpose of the study was to compare the performance of the different assay systems by assessing whether the same genotype result was obtained for each patient specimen regardless of the methodology employed. In other words, Lutz et al. conducted an interlaboratory proficiency test (PT) for Factor V Leiden mutation detection. The results of the study by Lutz et al. revealed 100% concordance of results among the six laboratories, suggesting that the different methodologies used could each reliably identify the single base pair substitution. However, as with all PTs, these results must be examined carefully because a PT does not provide an assessment of the total testing process. Specifically, the preanalytical phase of testing (e.g., test requisition, accession, and sample preparation) is usually eliminated by the entry of the PT sample into the testing process at the analytical phase. Nevertheless, PT is an important indicator of laboratory performance and provides participating laboratories with the opportunity to identify areas in need of improvement and to develop plans for remedial action.

Indeed, the Clinical Laboratory Improvement Amendments of 1988 (CLIA), which provide for regulation of all clinical laboratories, primarily rely on two performance indicators to evaluate laboratories: periodic on-site inspections and PT performance (8). The latter requirement is dependent on the complexity of the test being performed and the availability of an approved program.

The assignment of a complexity level (i.e., waived, moderate, or high complexity) to each clinical laboratory test is the responsibility of the Centers for Disease Control (CDC), which along with the Health Care Finance Administration (HCFA), implement the CLIA regulations. The criteria used to assess complexity include the knowledge, training, and experience required to perform the test, the complexity of troubleshooting and equipment maintenance, the degree to which test performance relies on interpretation, and other criteria (8). The high complexity category includes nine laboratory specialties for which personnel and quality-control requirements have been developed. PT participation is mandated for all moderate and high complexity testing if an approved program exists. However, although molecular genetic testing falls into the high complexity category, no separate specialty of genetics exists, and a PT program for genetic testing has not been approved and is not required. This has been viewed as a serious deficiency in the CLIA regulations by a number of groups, including the National Institutes of Health-Department of Energy (NIH-DOE) Task Force on Genetic Testing (9). It has been suggested by the Task Force that the CDC should consider the creation of a genetics specialty under CLIA to permit the implementation of a program for the national accreditation of genetic testing laboratories, which would include PT and on-site inspection. In particular, interlaboratory comparison and PTs have been recognized to be especially important for molecular genetic testing because there are no Food and Drug Administration (FDA)-approved kits available for such analyses, requiring most laboratories to rely on so-called “home brew” reagents, and because there is little standardization of methods among laboratories. Last year, the CDC responded to the concerns of the Task Force and others by creating a genetics subcommittee to the Clinical Laboratory Improvement Advisory Committee (CLIAC). This subcommittee is assessing the various clinical laboratory issues that have been identified and what the response should be, if any, in terms of new CLIA regulations.

In the interim, many laboratories that offer clinical molecular genetic testing participate voluntarily in the College of American Pathologists (CAP) survey in molecular genetics. This program has PT specimens available for a limited number of tests including cystic fibrosis, Duchenne muscular dystrophy, RhD, Prader Willi and Angelman Syndromes, Huntington disease, Fragile X, and hemochromatosis. Recently, CAP added Factor V Leiden
to their program. The availability of a PT program for Factor V Leiden now permits laboratories offering this analysis to participate in a formal PT program and makes the need for interlaboratory comparison studies for this particular analysis unnecessary. However, for many other molecular genetic tests, interlaboratory comparison programs such as the one conducted by Lutz et al. will continue to be important in the assessment of laboratory performance, particularly for rare disorders for which it is unlikely that PTs will ever be available. Nevertheless, the requirement for and approval of PT programs by CLIA, especially for common and widely available testing, should be mandated to provide a mechanism for the monitoring of the performance in laboratories offering clinical molecular genetic testing and to secure public confidence in such testing.

In addition to mandating participation in PT programs, the creation of a genetics specialty under CLIA also would provide a mechanism to define personnel standards and to address other aspects of the total molecular genetic testing process (i.e., pre- and postanalytical processes). Under the current CLIA regulations, the director of a laboratory performing high complexity testing must be a pathologist or an MD or DO with two years of clinical laboratory training and one year of experience, or a board-certified PhD (10). In contrast, the Task Force has recommended that all directors of genetic testing laboratories also have formal training in human or medical genetics as evidenced by certification by the American Board of Medical Genetics (ABMG) or a similar organization (9). Some states also have established specific personnel standards for laboratories offering genetic testing. For example, in California legislation has been proposed that requires all directors of clinical genetics laboratories to have ABMG certification. PhD-trained directors also must pass an oral examination. In New York State, directors of genetic testing laboratories must have a doctoral degree and four years experience and training, although ABMG certification is not recognized as a basis for granting a certificate of qualification. Both New York and California require laboratories that accept specimens from residents of their respective states to meet the personnel standards they have established as a condition of licensure. Under the New York regulations, a specimen may be referred to a noncertified laboratory only if the analysis to be performed is not available in any New York State-certified facility. Permission for such referrals must be obtained by the ordering physician by submitting a request to the Division of Laboratories. In California, there is no provision in the proposed regulations for the referral of specimens to noncertified laboratories, a situation that has the potential for limiting access to testing, particularly for rare disorders. The further development of different standards by individual states may present a potential burden to laboratories that offer genetic testing and their directors, who will be forced to comply with many different sets of regulations. Thus the definition of genetics-testing personnel standards through the CLIA mechanism, which takes into consideration the recommendations of the Task Force and other groups, could reduce this burden on laboratories and laboratory directors if it was adopted nationally and would ensure appropriate access to testing for all individuals regardless of the state in which they reside.

In addition to ensuring the quality of the analytical phase of testing, the pre- (e.g., informed consent) and postanalytical (e.g., reporting of results, genetic counseling) issues have been raised. Specifically, it has been noted that the communication of results in a manner that is accessible to non-geneticist physicians is essential to the appropriate clinical use of the information derived from genetic analyses. It has been demonstrated by a number of investigators that non-geneticist providers have limited understanding and knowledge of genetics and the use and application of genetic testing (11). Such gaps in education can result in clinically significant adverse outcomes when ordering physicians misinterpret the results of a molecular-based analysis and provide inaccurate and potentially harmful information to patients (12). Reporting practices also assume great importance for molecular genetic testing systems that require complex analyses and interpretation and when multiple mutant alleles can contribute to the disorder. For example, over 700 mutations have been identified in individuals affected with cystic fibrosis, although a much smaller number of these account for the majority of mutant alleles. To date, testing for this disorder has not been standardized among laboratories, and many different assays have been developed, which vary both in the methodology utilized and the panel of mutations tested for. As a result of the lack of consensus about what constitutes a cystic fibrosis screening test, a patient who undergoes mutation analysis for cystic fibrosis may be tested for different sets of mutant alleles, depending on the laboratory in which the testing is performed (13). Clinically, the result of such differences between laboratories is that an individual who is a carrier for a specific rare mutant allele may have a negative test result at one laboratory but be detected as a carrier in another laboratory. A number of professional societies, including the American Society of Human Genetics, the American College of Medical Genetics, and others, have begun to take a leadership role in formulating policies and recommendations related to the standardization and use of molecular genetics testing (14–17). Their role in defining professional standards in the future will be increasingly important, particularly as presymptomatic and predisposition testing for a larger number of disorders becomes available.

Finally, as Lutz et al. point out in their paper, in contrast to most other medical tests, genetic tests have important implications for family members and for future generations. Genetic testing has implications for future reproduction and may present information about an illness that is beyond the individual’s control and for which there are limited or no medical management options available. For example, the availability of susceptibility testing for breast, ovarian, and colon cancer, as well as the development of other tests for susceptibility to disorders for
which medicine can offer little certain guidance among clinical alternatives, raises a host of demanding choices for individuals as they weigh both the decision about testing and their options if they test positive. In addition, genetic testing raises crucial issues about confidentiality, the impact of the test results on eligibility for health and life insurance, and the potential for social stigma (18). For these reasons, it has been advocated by some that genetic testing must be accompanied by pre- and posttest genetic counseling and that written informed consent should be obtained after individuals contemplating testing have been apprised of all the risks and benefits associated (9, 18). Whose responsibility it is to address these clinical issues, to ensure that informed consent has been obtained, and to provide access to genetic counseling has not yet been determined and remains one of the many challenges associated with the appropriate integration of molecular genetic testing into clinical practice.

References

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