

Commentary

Quest for Controls in Molecular Genetics

Wayne W. Grody

From the Departments of Pathology and Laboratory Medicine, Pediatrics, and Human Genetics, UCLA School of Medicine, Los Angeles, California

The rapid growth in scope and volume of molecular testing for inherited single-gene disorders, accelerated by the success of the Human Genome Project, has engendered both great excitement and significant trepidation. With the explosion of gene sequence data and the ready adaptability of modern molecular diagnostic techniques, it is now possible to develop and market a test within weeks or even days of the published identification of a new genetic disease gene. And with the potential targets for testing, particularly in autosomal recessive diseases, being vast numbers of healthy people in high-risk ethnic groups or even the whole population—numbers far exceeding those for diagnostic testing in symptomatic patients—a strong financial incentive has added further impetus to the rapid translation of research results to clinical testing. While this growth is gratifying for a segment of molecular diagnostics that had formerly represented a small niche or esoteric market, it has raised concerns in some quarters that the transition from gene discovery to diagnostic reagent may be moving ahead too quickly, without sufficient attention to issues of clinical utility, test validation, and quality assurance. Indeed, at least three major federally funded task forces have been appointed over the last several years to examine this very question (the Institute of Medicine Committee on Assessing Genetic Risks, the NIH-DOE Task Force on Genetic Testing, and the HHS Secretary's Advisory Committee on Genetic Testing), and the conclusion of all has been to push for increased regulatory oversight of quality assurance in molecular genetic testing.¹

Quality assurance in laboratory medicine is multifaceted, encompassing everything from basic analytic quality control to clinical predictive value and pre- and post-test delivery and reporting issues. At the most fundamental level, any laboratory setting up a new test must establish the analytic sensitivity and specificity of the assay: its ability to detect the target analyte when present and not detect it when it is absent. This requirement is as old as laboratory testing itself, is embedded in the Clinical Laboratory Improvement Amendments (CLIA) regulations that all clinical laboratories must follow, and long predates the birth of molecular genetic testing. Dur-

ing test validation these parameters must be established by analysis of positive and negative samples in parallel with a predicate method or through sample exchange with another expert laboratory. Once brought on-line, continued monitoring of test sensitivity and specificity is accomplished through the use of well-characterized positive and negative controls, and CLIA mandates that a positive control be available and run for each analyte tested. This is rather straightforward for routine clinical chemistry analytes and antigenic targets of immunohistochemical assays, but what is the "analyte" in molecular genetic testing? Is it the DNA, the gene, or the individual mutation?

Most people would interpret the target analyte of a direct mutation test to be the particular mutation being probed, whether this is done by allele-specific oligonucleotide hybridization, DNA sequencing, real-time PCR, Invader assay, microarray, dHPLC, or any of the variety of other current and emerging techniques. Thus, the required positive control must be some cellular or DNA sample that contains the mutation of interest in heterozygous and/or homozygous form. How does one obtain such controls? For common mutations that account for most or all of the disease-related alleles, such as factor V Leiden or the Glu→Val β -globin mutation of sickle cell disease, positive samples are readily available from regular patient specimens that come through the lab or from other clinical sources. But for diseases with heterogeneity of point mutations, some of which may be infrequently encountered or even quite rare, and for diseases with many possible variations of single mutation mechanisms, such as different lengths of trinucleotide-repeat expansions or the deletions of the dystrophin gene in Duchenne muscular dystrophy, the desired positive samples may be hard to obtain or even totally unavailable. This situation has recently been brought to the fore with the launch of universal cystic fibrosis carrier screening using a mandated core panel of 25 mutations.² As a number of vendors have come forward with robust analyte-specific reagent (ASR) systems for performing the test itself, the burden of setting up a complicated multiplex home-brew

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Address reprint requests to Wayne W. Grody, M.D., Ph.D., Department of Pathology and Laboratory Medicine, UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, CA 90095-1732. E-mail: wgrody@mednet.ucla.edu.

assay has been lifted from the laboratories themselves. What has not been forthcoming, however, is access to positive controls for all 25 mutations. Though chosen for their prevalence, some of these alleles have never been seen even by large reference laboratories, and the cystic fibrosis (CF) test reagent manufacturers have been disinclined to provide positive controls because of the nature of the ASR market and its regulatory constraints. With some effort, the Coriell Institute for Medical Research, a prominent mutant cell bank repository funded by the National Institute of General Medical Sciences (NIGMS), has collected the majority of them to distribute as a package, but several are still unobtainable. This situation is emblematic of many complex genetic tests, and fulfills the prophecy of a Centers for Disease Control (CDC)-sponsored consensus study that the lack of positive mutation controls represented the single most pressing deficiency impacting quality assurance and further progress of molecular genetic testing.³ This dearth also impacts proficiency testing programs, most prominently the one administered jointly by the College of American Pathologists and the American College of Medical Genetics, which face exactly the same hurdle in trying to obtain mutation samples providing a sufficient variety of challenges.⁴

Aside from rarity, there are several other factors that represent obstacles to procurement of human mutation samples. These include the effort and expense required for identification, collection, shipment, and distribution of candidate patient samples; onerous informed consent procedures; ethical dilemmas regarding sample ownership and patient privacy; and simple lack of awareness among the treating physicians as to this need in the laboratories. Years ago, the NIH-DOE Task Force on Genetic Testing recognized this need and recommended the establishment of a central repository of human mutant cell lines or DNA samples.⁵ The Coriell Institute comes closest to filling this role, though many of its deposited samples remain uncharacterized at the molecular level, carrying only a referring clinical diagnosis, and there are many desired genes and mutations not represented.

Stepping into this void, and building on the lessons learned from its earlier consensus meetings, the CDC has competitively funded two projects aimed at exploring novel methods for accruing human mutation samples for use in quality control and proficiency testing. The initial report of one of these projects, from Stenzel's group⁶ at Duke, exploring more efficient and convenient routes to patient sample collection by using residual clinical laboratory blood samples as substrates for establishing lymphoblastoid cell cultures, appears in this issue of *The Journal of Molecular Diagnostics*. The other project, directed by this author at UCLA, avoids patient-derived samples altogether and instead is aimed at constructing artificial mutation specimens through recombinant DNA techniques; its initial findings are forthcoming (Jarvis et al, manuscript in preparation).

Recognizing that it will usually be the laboratory, rather than the referring physician, that sees the value in archiving a particular patient sample, and that the potential value of that sample often does not become apparent

until lengthy testing is completed, Bernacki et al⁶ sought to determine the limits and parameters for successful Epstein-Barr virus (EBV)-transformation of peripheral blood lymphocytes in residual clinical testing blood samples for creation of immortalized cell lines. These could then be made available as renewable genomic controls or deposited in existing cell bank repositories such as Coriell. They observed an overall transformation success rate of 63% for acid citrate dextrose (ACD)- or ethylenediamine tetraacetic acid (EDTA)-treated blood samples stored at room temperature for up to 1 week or at 4°C for up to 2 weeks. Samples older than that showed a dramatic drop-off in viability. While, of course, one would wish for success rates approaching 100% in order not to lose rare mutation samples, these results probably represent a realistic expectation and offer new hope that many samples of interest can now be captured without having to go back to the patient for a fresh blood sample. Moreover, this approach allows for sample anonymization and may alleviate some of the informed consent burdens.

As a result of this study, we thus have a promising new avenue for expanding the existing repositories of human cell lines containing infrequent or rare genetic disease mutations. Forthcoming reports from this group will describe the particular mutations, for cystic fibrosis and other disorders, uniquely collected through this process, along with their characterization for stability in culture and clinical performance.

Finally, it seems appropriate, though not often done in this context, to pay tribute to the funding agency that inspired and underwrote this study. The need for validated mutation controls in diagnostic genetic testing and proficiency surveys has been achingly apparent for years, and while many have paid lip service to it, no major governmental or professional agency had yet stepped up to the plate, other than, with a somewhat different focus, the aforementioned NIGMS effort at Coriell. The National Institute of Standards and Technology (NIST) has long provided validated DNA standards for forensic analysis,⁷ and even flirted with the idea of doing the same thing for genetic diseases (particularly the trinucleotide repeat disorders, since those alleles resemble the polymorphic short-tandem repeats they were already offering in forensics), though nothing in that area has yet emerged. Meanwhile, the research and development required to evolve such resources would likely be considered too clinical/applied to be a valid candidate for NIH funding. So it was quite laudable to see the CDC, an agency formerly not widely identified with genetic disease, fund first the exploratory consensus process to see what the needs were,³ and then literally put its money where its mouth is and launch funded projects to develop the needed materials. Moreover, the two-pronged approach taken with the two awarded contracts, immortalization of patient-derived blood cells and creation of artificial recombinant constructs, served to maximize the chances that useable materials applicable to the broad variety of genetic disease targets would emerge: the idea being that if one approach did not work optimally for a certain class of mutations, perhaps the other one would. It is

hoped that the success of this program, the first example of which is the study by Bernacki et al⁶ published here, will inspire other government-academic-industry partnerships to address the demanding quality assurance needs of this burgeoning field.

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