A panoramic view of the accuracy of molecular genetic testing

Karen E. Weck, MD^{1,2} and Iris Schrijver, MD³

With the significant advancements in molecular diagnostic capabilities over the past several decades, it has become standard practice for a growing number of genetic diagnoses to be made on the basis of molecular genetic test results. Indeed, given the common occurrence of phenotypic overlap among genetic diseases, molecular testing may often be the only way to reach an accurate clinical diagnosis. Moreover, establishing an underlying molecular etiology increasingly influences how genetic conditions are managed.

Our ability to make accurate genetic diagnoses using molecular methods is only as good as the diagnostic tools that are currently available. These continue to improve and drive advances in our field. Analysis of the landscape of laboratory performance in the context of molecular genetic testing is important to inform the clinical and laboratory communities regarding what their expectations should be in terms of analytic performance of different methodologies. It can also identify areas where improvements in standardization, methodology, or interpretation guidelines are needed.

The paper by Richards et al., “Results From an External Proficiency Testing Program: Eleven Years of Molecular Genetics Testing for Myotonic Dystrophy Type 1,”¹ is the latest in a series of articles in this journal summarizing the performance of laboratories engaged in the College of American Pathologists (CAP) proficiency testing (PT) surveys.^{1–8} In each of these articles, approximately 10 years of PT data were analyzed for overall performance by molecular genetics laboratories and for global performance over time; these data were not provided to individual participating laboratories and are not available outside of these publications. It is not feasible to publish all PT survey data at once because there are so many data points to analyze and there are different aspects that are important to address for each clinical disease/test. In this commentary, we provide a summary of the overall landscape of laboratory performance for molecular genetic testing.

For clinical laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 and accredited by the CAP, external PT is required. In the realm of inherited genetic diseases, the CAP offers analyte-specific PT surveys that are targeted to specific genes or mutations for many molecular

genetics tests, in addition to methods-based PT for Sanger dideoxy sequencing and next-generation sequencing for germline variants. Methods-based PT is an external quality assessment approach based on methodology rather than targeted to individual analytes. It is especially helpful for large-scale genetic testing because it enables laboratories to assess their performance in detecting the range of mutation types and to closely monitor overall technical performance of evolving sequencing approaches. With the growing number of tests offered for rare genetic conditions, a combination of methods-based PT for genomic sequencing assays and, when available, analyte-specific PT offers thorough and efficient quality assessment and helps ensure optimal preanalytic, analytic, and postanalytic laboratory performance.⁹ The joint CAP/ACMG Biochemical and Molecular Genetics (BMG) committee oversees the CAP PT surveys for molecular genetic testing and has worked to summarize the longitudinal data from the PT surveys for seven of the most commonly tested genetic diseases and for methods-based PT for Sanger sequencing.^{1–8} The data from individual rounds of PT, which are offered two times per year, are not made publicly available by CAP, except to laboratories enrolled in their PT programs.

The surveys analyzed by the CAP/ACMG BMG committee encompass a variety of molecular methodologies used for single mutation testing, mutation panels, and three different trinucleotide repeat disorders. Overall, there has been excellent performance by clinical laboratories participating in the CAP molecular genetics PT programs, with >95% analytical sensitivity and >99% specificity for all tests analyzed by the CAP/ACMG BMG committee (Table 1). This compares quite favorably with analytical accuracy parameters for other laboratory analytes (as summarized elsewhere⁸). This is encouraging because the educational nature of these surveys tends to include particularly challenging genotypes at a much higher frequency than would be expected to be observed in a clinical molecular diagnostic laboratory. There was more variance in the clinical interpretation of results, indicating that there may be a need for improvement or guidelines in this area, although it is noted that the limited clinical description and the multiple-choice format of the PT surveys may not completely assess interpretation by laboratories in their

¹Department of Pathology & Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina, USA; ²Department of Genetics, University of North Carolina, Chapel Hill, North Carolina, USA; ³Department of Pathology, Stanford University School of Medicine, Stanford, California, USA. Correspondence: Karen E. Weck (kweck@unch.unc.edu)

Submitted 30 June 2016; accepted 7 July 2016; advance online publication 18 August 2016. doi:10.1038/gim.2016.116

Table 1 Summary of analytical performance on the College of American Pathologists molecular genetics proficiency surveys

	Analytical sensitivity (95% CI)	Analytical specificity (95% CI)
Fragile X syndrome		99.9% (99.7–99.96)
Males, full mutation	99.0% (98.1–99.7)	
Females, full mutation	95.5% (91.5–98.3)	
Premutation	98.0% (97.1–98.2)	
Huntington disease	99.5% (97.3–99.9)	99.2% (97.2–99.9)
<i>BRCA1/2</i> ^a	99.0% (97.6–99.7)	99.9% (99.4–99.9)
Ashkenazi Jewish panel ^b	97.2% (94.7–98.7)	99.8% (99.1–99.9)
Cystic fibrosis	99.3% (98.8–99.5)	99.6% (99.3–99.8)
Hereditary hemochromatosis ^c	99.3% (99.0–99.6)	99.6% (99.4–99.7)
Myotonic dystrophy type 1	99.2% (97.7–99.8)	99.2% (98.6–99.5)

^a*BRCA1* and *BRCA2* Ashkenazi Jewish founder mutations associated with hereditary breast and ovarian cancer syndrome; ^bTay–Sachs disease, Canavan disease, familial dysautonomia; ^cHFE p.C282Y and p.H63D variants.

clinical reports. In addition, there was consistently better performance of US laboratories compared to non-US laboratories. Similarly, during the first 3 years of a methods-based survey for Sanger sequencing, in which laboratories were asked to analyze electropherograms and provide variant interpretations, 98.3% of US laboratories had acceptable performance compared to 88.9% for international participants.⁴ Finally, there was consistent demonstration of improvement over time for both analytical accuracy and clinical interpretation, which could be due to improvement in methodologies and/or to participation in the PT program, which can provide valuable feedback to laboratories regarding test accuracy and interpretation.

It is worth noting that virtually all of the molecular genetics assays used by laboratories participating in these surveys are laboratory-developed tests or procedures, because there are almost no US Food and Drug Administration (FDA)-approved tests available for molecular genetic conditions. The data from the CAP PT program indicate that molecular genetic testing using laboratory-developed tests/laboratory-developed procedures is quite accurate. This is critically important information in light of the recent FDA draft guidance for regulatory oversight of laboratory-developed tests.¹⁰ For most molecular genetic tests, laboratories develop their own analytical assays using standard molecular methods to test for a clinical indication that is either considered standard of care or has documented clinical validity. The molecular laboratorians who oversee this testing are well-trained medical professionals who

are expert and nimble at developing and validating molecular tests for clinical use. The results of the CAP PT surveys clearly indicate that the majority of diagnostic molecular genetics laboratories have been accomplishing this quite capably for many years and that clinical molecular genetic testing is safe and accurate. This bodes well for the genetics community and, more importantly, for the patients and families it serves.

DISCLOSURE

K.E.W. and I.S. are the present and past chairs of the CAP Biochemical and Molecular Genetics Committee.

REFERENCES

- Richards CS, Palomaki GE, Hegde M; on behalf of the Biochemical and Molecular Genetics and Genomics Resource Committee, College of American Pathologists/American College of Medical Genetics and Genomics. Results from an external proficiency testing program: 11 years of molecular genetics testing for myotonic dystrophy type 1. *Genet Med* e-pub ahead of print 2 June, 2016.
- Palomaki GE, Richards CS. Assessing the analytic validity of molecular testing for Huntington disease using data from an external proficiency testing survey. *Genet Med* 2012;14:69–75.
- Weck KE, Zehnbauser B, Datto M, Schrijver I; CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Molecular genetic testing for fragile X syndrome: laboratory performance on the College of American Pathologists proficiency surveys (2001–2009). *Genet Med* 2012;14:306–312.
- Richards CS, Palomaki GE, Lacbawan FL, Lyon E, Feldman GL; CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Three-year experience of a CAP/ACMG methods-based external proficiency testing program for laboratories offering DNA sequencing for rare inherited disorders. *Genet Med* 2014;16:25–32.
- Feldman GL, Schrijver I, Lyon E, Palomaki GE; CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Results of the College of American Pathology/American College of Medical Genetics and Genomics external proficiency testing from 2006 to 2013 for three conditions prevalent in the Ashkenazi Jewish population. *Genet Med* 2014;16:695–702.
- Tafe LJ, Datto MB, Palomaki GE, Lacbawan FL; CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Molecular testing for the *BRCA1* and *BRCA2* Ashkenazi Jewish founder mutations: a report on the College of American Pathologists proficiency testing surveys. *Genet Med* 2015;17:58–62.
- Lyon E, Schrijver I, Weck KE, Ferreira-Gonzalez A, Richards CS, Palomaki GE; CAP/ACMG Biochemical and Molecular Genetics Committee. Molecular genetic testing for cystic fibrosis: laboratory performance on the College of American Pathologists external proficiency surveys. *Genet Med* 2015;17:219–225.
- Press RD, Eickelberg G, McDonald TJ, et al.; for the CAP/ACMG Biochemical and Molecular Genetics Resource Committee. Highly accurate molecular genetic testing for HFE hereditary hemochromatosis: results from 10 years of blinded proficiency surveys by the College of American Pathologists. *Genet Med*; e-pub ahead of print 20 April 2016.
- Schrijver I, Aziz N, Jennings LJ, Richards CS, Voelkerding KV, Weck KE. Methods-based proficiency testing in molecular genetic pathology. *J Mol Diagn* 2014;16:283–287.
- US Food and Drug Administration. *Framework for regulatory oversight of laboratory developed tests (LDTs)—draft guidance*, 3 October 2014. <http://www.fda.gov/downloads/medicaldevices/deviceregulationandguidance/guidancedocuments/ucm416685.pdf>. Accessed 25 May 2016.