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Current Landscape and New Paradigms of Proficiency Testing and External Quality Assessment for Molecular Genetics

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● **Context.**—Participation in proficiency testing (PT) or external quality assessment (EQA) programs allows the assessment and comparison of test performance among different clinical laboratories and technologies. In addition to the approximately 2300 tests for individual genetic disorders, recent advances in technology have enabled the development of clinical tests that quickly and economically analyze the entire human genome. New PT/EQA approaches are needed to ensure the continued quality of these complex tests.

Objectives.—To review the availability and scope of PT/EQA for molecular genetic testing for inherited conditions in Europe, Australasia, and the United States; to evaluate the successes and demonstrated value of available PT/EQA programs; and to examine the challenges to the provision of comprehensive PT/EQA posed by new laboratory practices and methodologies.

Data Sources.—The available literature on this topic was reviewed and supplemented with personal experiences of several PT/EQA providers.

Conclusions.—Proficiency testing/EQA schemes are available for common genetic disorders tested in many clinical laboratories but are not available for most genetic tests offered by only one or a few laboratories. Provision of broad, method-based PT schemes, such as DNA sequencing, would allow assessment of many tests for which formal PT is not currently available. Participation in PT/EQA improves the quality of testing by identifying inaccuracies that laboratories can trace to errors in their testing processes. Areas of research and development to ensure that PT/EQA programs can meet the needs of new and evolving genetic tests and technologies are identified and discussed.

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Recent advances in genetic testing technologies and an increased understanding of the role of DNA variations in health and disease have produced expansion of molecular diagnostics and led to an increased role for clinical genetic testing in patient management. Currently, molecular genetic, biochemical, and cytogenetic tests for approximately 2300 inherited genetic diseases are offered in clinical laboratories for diagnosing disease, screening carriers, predicting clinical disease susceptibility, assessing risk, and prognosticating on the course of disease.¹ Because of the rapid growth and the potential effect of genetic testing results on clinical management or reproductive decisions, quality management practices are essential at all stages of the testing process to ensure the accuracy and utility of these tests.

Quality management is defined as an ongoing effort that includes policies and procedures established and implemented for the purpose of providing accurate laboratory test results.² Quality management of the analytic component encompasses a variety of quality assurance processes designed to ensure the performance of a test in the clinical laboratory.^{3,4} This assurance requires a system that includes both internal and external procedures that are described in national and international guidance and regulatory documents.^{4–15} Internal quality assurance processes include measures to maintain analytic accuracy, such as quality control and personnel competency. External quality assess-

ment measures include examination of laboratory procedures by a third party accreditation process and participation in proficiency testing (PT) or external quality assessment (EQA) programs. Most of the current quality assurance practices commonly used in genetic testing laboratories are designed for well-established technologies, such as targeted mutation analysis that detect limited sequence variations in one or a few genes associated with a particular disorder or condition. Newer technologies, such as next-generation DNA sequencing and chromosomal microarrays, allow detection of more sequence variations or gene expression levels. These new tests exhibit a higher level of complexity owing to the sophistication of chemistry, hardware, and software innovations.^{16,17} Therefore, it is unclear whether traditional methods of quality assurance and assessment will suffice for this higher level of complexity or whether new paradigms must be developed.

Proficiency testing is defined in International Organization for Standardization (ISO) 17043 as: "evaluation of participant performance against preestablished criteria by means of interlaboratory comparisons."¹⁸ This can be achieved through participation in a formal PT program. Proficiency testing programs usually focus on the analytic results. External quality assessment also provides evaluation of laboratory performance on examination of external samples but focuses more on the preanalytic (preexamination) and postanalytic (postexamination) activities than PT does.¹⁸ In the absence of a formal PT or EQA program, laboratories can assess their performance through alternative assessment activities, such as exchanging samples with another laboratory performing similar tests, or by internal assessment.¹⁹

Proficiency testing/EQA is an important component of clinical laboratory quality assurance. It provides a mechanism to compare analytic test performance among different laboratories, which is important for determining the consistency of test results for a common analyte. Participants in formal PT/EQA programs periodically receive specimens (with a genotype unknown to them), which are tested in a manner similar to procedures used for their regular clinical samples. Participating laboratories return the results of the requisite analyses to the PT/EQA program, which then compiles the data and provides summarized results and educational insights to the participants. These programs provide an independent measure of laboratory performance in comparison with an external standard or a mean value obtained by other participating laboratories. Participation in PT/EQA allows laboratories to recognize analytic and interpretive errors that may indicate internal problems with quality control, calibration, assay design, or test interpretation. This is important because most clinical molecular genetic tests are developed by individual laboratories and are not available as commercial test kits that are manufactured and evaluated in a consistent manner. Also, unlike molecular tests for infectious diseases, such as HIV and tumor markers, which are typically used for patient monitoring and may be performed repeatedly, most molecular genetic tests for inherited disease are performed only once in a patient's lifetime. In this situation, errors may not be noticed through discrepancy with subsequent testing.

An international survey of molecular genetic testing laboratories determined that 74% of responding laboratories participate in PT/EQA, which was suggested to correlate with higher quality assurance scores.^{20,21} The most common reason cited by laboratories for failure to participate in PT

was a lack of programs relevant to the clinical laboratory services offered.²¹ In the United States, formal molecular genetic PT programs are available from the College of American Pathologists (CAP)²² for 27 tests for inherited diseases, representing only a small fraction of the 1739 (US) or 2247 (worldwide) molecular genetic tests currently available.¹ However, the 27 CAP PT surveys are for tests with the greatest frequency of use, including factor V Leiden, prothrombin 20210A variant, cystic fibrosis, and fragile X syndrome. The CAP and other PT/EQA providers also offer schemes for many other molecular genetic tests, including cytogenomic microarray analysis, pharmacogenetics, paternity testing, and human leukocyte antigen typing.

Another survey of clinical genetic laboratories in the United States indicated that increased participation in PT correlated directly with fewer PT failures and fewer incorrect patient test reports, as reported by laboratories.²³ Published perspectives about the ability of PT to accurately measure routine laboratory performance are conflicting; therefore, this correlation may be difficult to prove.^{24–30} Formal PT/EQA provides interlaboratory comparison of specific samples and does not always examine the entire testing process or day-to-day quality management issues that may affect laboratory performance. Proficiency testing represents a "snapshot" and is not intended to provide a comprehensive evaluation of a laboratory's quality assurance processes. Nonetheless, it has documented value for identifying problems that compromise the quality of laboratory test results.

Regulatory authorities and professional organizations recognize that PT/EQA is an essential component of quality assurance and have developed policies and recommendations for inclusion of PT/EQA, where feasible, into laboratory practice. Several international organizations, such as the Clinical and Laboratory Standards Institute and the ISO, have published guidelines and standards related to PT for molecular diagnostic methods. The Clinical and Laboratory Standards Institute protocol documents MM14-A, *Proficiency Testing (External Quality Assessment) for Molecular Methods*,³¹ GP27-A2, *Using Proficiency Testing to Improve the Clinical Laboratory*,³² and GP29-A2, *Assessment of Laboratory Tests When Proficiency Testing is Not Available*,¹⁹ offer guidance for the management and operation of PT/EQA for PT providers as well as molecular genetic diagnostic laboratories. The ISO/International Electrotechnical Commission standard 17 043:2010, *Conformity Assessment—General Requirements for Proficiency Testing*,¹⁸ specifies the requirements for the competence of PT providers and for the development and operational aspects of providing PT schemes. This guidance also describes methods of PT testing. The ISO document 15189, *Medical Laboratories—Particular Requirements for Quality and Competence*,³ recommends that laboratories participate in EQA and that these EQA schemes should provide clinically relevant challenges that mimic clinical samples and encompass all steps of the testing process, including preanalytic and postanalytic components.

In the United States, the Secretary's Advisory Committee on Genetics, Health, and Society was mandated by the Department of Health and Human Services to report on the adequacy of genetic testing oversight and regulation and to identify gaps that could affect patient safety. In their 2008 report, *U.S. System of Oversight of Genetic Testing: A Response to the Charge of the Secretary of Health and Human Services*,³³

| Method Used | % of Diseases Tested |
|--------------------------------------|----------------------|
| Sequencing ^a | 93 (90 of 97) |
| Transcription-mediated amplification | 23 (22 of 97) |
| Deletion/duplication analysis | 27 (26 of 97) |
| Mutation scanning | 18 (18 of 97) |
| Methylation analysis | 2 (2 of 97) |

^a Analysis of a random sample (~10%) of diseases for which molecular genetic testing methods are used (February 26, 2009 report of ~970 diseases obtained from GeneTests), determined that testing for ~93% (90 of 97) of these diseases used DNA sequencing methods in at least some of the laboratories that offered testing; 49% (48 of 97) of the diseases in the sample were tested using only sequencing techniques.

the Secretary's Advisory Committee on Genetics, Health, and Society formulated recommendations to address the main gaps in genetic testing oversight. One recommendation addressed the absence of formal PT programs for all genetic tests, particularly rare genetic disorders, and suggested that the Department of Health and Human Services promote the development of new PT products by investigating other performance assessment approaches, including method-based processes.

In this article, we review PT/EQA as it applies to molecular genetic testing for inherited conditions in the United States, Europe, and Australasia. We consider the successes and demonstrated value of available PT/EQA programs and examine the challenges posed by evolving laboratory practices and testing technologies. Proficiency testing/EQA programs are often unable to assess the total laboratory testing process, focusing primarily on the analytic phase of testing (often neglecting the preanalytic and postanalytic phases) nor are they able to provide PT/EQA for all available tests. This critical evaluation suggests the need for research and development of targeted efforts to meet future PT/EQA needs.

PT/EQA—THE CURRENT SITUATION

Resources Describing the Scope of Genetic Testing

No formal mechanism exists either to capture information, such as disorders and genes tested, or analytic methods about all genetic tests offered in the United States or worldwide or to identify all laboratories that perform these tests. Data are limited to voluntary registries, such as the GeneTests³⁴ and Orphanet.³⁵ These resources do not obtain information from laboratories that either decline to analyze samples submitted from outside of their own institutions or that decline to register for other reasons. Thus, the true scope of molecular genetic testing and the laboratories that perform such testing is unknown. The National Institutes of Health in the United States has recently developed another registry³⁶ to collect information about genetic tests offered in the United States, although it may not be more comprehensive than the registries that currently exist.

According to the GeneTests Web site, the number of disorders with available genetic testing worldwide has increased more than 20-fold between 1993 and 2012 (from 100–2300 tests).³⁴ Nucleic acid–based testing is available for about 2000 of those genetic disorders; other diagnoses use biochemical, chromosomal, or other genetic tests.¹

The Orphanet database, also a voluntary registry, has catalogued 5954 rare diseases, but only some of those diseases have diagnostic testing available. The database lists

5424 laboratories that offer testing (both research and clinical), mostly located in Europe.³⁵

Molecular Methods Used for Genetic Testing

Many molecular methods can be used to detect mutations, depending on the disorder and the associated molecular defects. For example, many common mutations cause cystic fibrosis; testing for that disorder is usually performed by targeted mutation analysis using a panel of the more frequent mutations (single or a few nucleotides) associated with severe disease phenotype. Duchenne muscular dystrophy is most often caused by deletions and duplications in the *DMD* gene; therefore, testing for that disorder includes deletion/duplication analysis. Other commonly used molecular testing techniques include DNA sequence analysis, mutation scanning, and methylation analysis. To estimate the percentage of tests using each method, we chose a random sample (10%) of diseases with available molecular genetics diagnostic methods (February 26, 2009, report of ~970 diseases obtained from GeneTests) and tabulated the method or methods used to detect mutations for each. Whole-gene or targeted-exon DNA sequence analysis was used by the laboratories to analyze 93% (90 of 97) of the disorders in our sample (Table 1). Testing for 49% (48 of 97) of the disorders in our random sample was performed using only DNA sequencing techniques. That fraction may increase as next-generation DNA sequencing technologies transition to clinical laboratories. Testing for approximately 23% (22 of 97) of the disorders in our sample used targeted mutation analysis, and testing for 27% (26 of 97) of the disorders used deletion/duplication analysis, usually as a follow-up to sequencing assays, which may miss large deletions and duplications (Table 1).

Molecular Genetic Tests Evaluated by Formal PT/EQA Programs

In the past 2 decades, formal PT and EQA programs have become an integrated part of clinical genetics laboratory practice. There are many regional, national, and international PT/EQA programs. We describe here some of the larger programs that serve national and international participants; however, smaller programs that serve a more-limited base or programs without publicly available information were not included. A comprehensive list of available molecular genetic PT/EQA programs can be found on the Eurogentest Web site (<http://www.eurogentest.org/laboratories>).¹⁴ In the United States, CAP is the largest provider of molecular genetic PT challenges.²² In partnership with the American College of Medical Genetics, CAP offers PT to laboratories worldwide for 27 inherited genetic disorders, with 5 pharmacogenetic loci and methods-based PT for cytogenomic microarray analysis and for postanalytic Sanger DNA sequence analysis (Table 2). The samples distributed in the PT challenges are typically highly purified nucleic acids extracted from human cell lines. This program provides participants with 3 samples per disorder twice per year. The methods-based challenge for Sanger DNA sequencing assays is currently focused on interpretation of electronic data files but will evolve to include a “wet” challenge using extracted DNA and including DNA sequence analyses. The Centers for Disease Control and Prevention's Newborn Screening Quality Assurance Program³⁷ offers PT for molecular cystic fibrosis screening. The Newborn Screening Quality Assurance Program requires

Table 2. Proficiency Testing/External Quality Assessment (PT/EQA) Provider Schemes for Inherited Disorders

| PT/EQA Provider Source, y | Analytes Covered |
|---|--|
| CAP, ²² 2012 | <i>Disease-specific schemes:</i> factor V Leiden, fragile X syndrome, hemochromatosis, <i>MTHFR</i> , Prader-Willi/Angelman syndrome, prothrombin, CF, Huntington disease, Friedreich ataxia, hemoglobin SC, Duchenne muscular dystrophy, myotonic dystrophy, RhD, spinal muscular atrophy, spinocerebellar ataxia, <i>BRCA1</i> and <i>BRCA2</i> , connexin 26, multiple endocrine neoplasia type 2, Canavan disease, familial dysautonomia, Tay-Sachs disease, mucopolidosis IV, Bloom syndrome, Fanconi anemia, Gaucher disease, glycogen storage disease type 1A, Niemann-Pick disease type A. <i>Pharmacogenetic markers. Methods-based schemes:</i> DNA sequencing, microarray genomic copy number assay. |
| EMQN, ³⁸ 2012 | <i>Disease-specific schemes:</i> Y-chromosome microdeletions, <i>BRCA1</i> and <i>BRCA2</i> , CAH, CMT, Familial Adenomatous Polyposis Colon Cancer, <i>GJB2</i> and <i>GJB6</i> , HNPCC, monogenic diabetes, Marfan syndrome, porphyria, hereditary recurrent fevers, myotonic dystrophy, Duchenne muscular dystrophy, fragile X syndrome, Friedreich ataxia, Huntington disease, hemochromatosis, multiple endocrine neoplasia type 2A, PKU, Prader-Willi/Angelman syndromes, retinoblastoma, short stature homeobox gene testing, spinocerebellar ataxia, spinal muscular atrophy, Von Hippel Lindau disease, Wilson disease. <i>Methods-based schemes:</i> Sanger DNA sequencing, array-CGH, next-generation sequencing (pilot). |
| UKNEQAS, ⁴⁰ 2012 | <i>Molecular genetics:</i> Angelman syndrome, Becker/Duchenne muscular dystrophy, <i>BRCA1</i> and <i>BRCA2</i> , CF, factor V Leiden, familial adenomatous polyposis, fragile X syndrome, hereditary and motor sensory neuropathy, Huntington disease, HNPCC, hereditary neuropathy with liability to pressure palsies, MCADD, mitochondrial disorders, molecular rapid aneuploidy testing, myotonic dystrophy, Prader-Willi syndrome, spinal muscular atrophy, spinocerebellar ataxia. <i>Molecular testing on dried blood spots:</i> CF, MCADD. <i>Microarray CGH.</i> |
| HGSA, ⁴³ 2012 | 23 disease-specific PT modules in collaboration with EMQN and the CF Network. Y-chromosome deletions, familial breast cancer (<i>BRCA</i>), CAH, CF, CMT, connexin 26, myotonic dystrophy, Becker/Duchenne muscular dystrophies (BMD/DMD), familial adenomatous polyposis coli, fragile X syndrome, Friedreich ataxia, Huntington disease, HNPCC, hereditary recurrent fevers (pilot), multiple endocrine neoplasia Type 2, mitochondrial myopathy, monogenic diabetes, PKU, porphyria, Prader-Willi/Angelman syndromes, retinoblastoma, spinocerebellar ataxias, spinal muscular atrophy, Von Hippel Lindau syndrome, Wilson disease. Also methods-based DNA sequence and analysis scheme and cytogenetics are also offered. |
| ISS, ⁴¹ 2012 DGKL, ⁴² 2012 | Cystic fibrosis, β thalassemia, fragile X syndrome, adenomatous polyposis of colon. Molecular Biology schemes: Factor V-Leiden, prothrombin, <i>MTHFR</i> , PAII (<i>SERPINE1</i>), factor XIII (<i>F13A1</i>), GPIIIa (<i>ITGB3</i>), β Fib (<i>FGB</i>), <i>VKORC1</i> , Factor XII (<i>F12</i>), a1 PI, <i>APOE</i> , <i>APOB</i> , <i>ACE</i> , <i>CETP</i> , <i>TPMT</i> , <i>CYP2C19</i> , <i>CYP2D6</i> , <i>CYP2C8</i> , <i>CYP2C9</i> , <i>UGT1A1</i> , DPD (<i>DPYD</i>), <i>BCHE</i> , <i>ALDOB</i> , <i>HFE</i> , <i>LCT</i> , <i>NOD2</i> , <i>ATP7B</i> , <i>FSAP</i> (<i>HABP2</i>), <i>ITGA2</i> , <i>KRAS</i> . Method schemes: DNA sequencing, DNA isolation + Factor V genotyping. |
| CDC NSQAP, ³⁷ 2012 | Dried blood-spot testing for CF mutations. |

Abbreviations: β -Fib, Fibrinogen-Beta polypeptide chain; CAH, congenital adrenal hyperplasia; CAP, College of American Pathologists; CDC, Centers for Disease Control and Prevention; CD-ROM, compact disk-read-only memory; CF, cystic fibrosis; CGH, comparative genomic hybridization; CMT, Charcot-Marie-Tooth disease; DGKL, Reference Institute for Bioanalytics; DPD, Dihydropyrimidine dehydrogenase; EMQN, European Molecular Genetics Quality Network; GPIIIa, platelet glycoprotein IIIa; FASP, Factor VII-Activating Protease; HGSA, Human Genetics Society of Australasia; HNPCC, hereditary nonpolyposis colorectal cancer (Lynch syndrome); ISS, Istituto Superiore di Sanita; MCADD, medium chain acyl-coenzyme A dehydrogenase deficiency; *MTHFR*, methylenetetrahydrofolate reductase; NSQAP, Newborn Screening Quality Assurance Program; PAII, Plasminogen Activator Inhibitor I PI, proteinase inhibitor; PKU, phenylketonuria; RhD, Rhesus (hemolytic) disease; SC, sickle cell; UKNEQAS, United Kingdom National External Quality Assessment Service.

laboratories to extract DNA from blood spotted on filter-paper collection devices and sends 5 blood spots to participating laboratories on a quarterly basis, which allows laboratories to perform all phases of the testing process, including DNA extraction from the appropriate matrix, within the PT context.

European PT/EQA challenges are provided by both national and international organizations (Table 2) with additional participation by laboratories outside Europe. The European Molecular Genetics Quality Network (EMQN),³⁸ which is supported by user subscriptions, offers disease-specific EQA for a variety of genetic disorders as well as methods-based EQA for Sanger DNA sequencing, molecular cytogenomic analysis, and mutation scanning. The EMQN EQA programs provide 3 lyophilized DNA samples per disease to participants once each year. Participants are assessed on their ability to correctly genotype, interpret, and report the results using their usual laboratory report format. The Cystic Fibrosis (CF) European Network³⁹ offers an

external quality assessment scheme for CF molecular genetic testing to more than 200 laboratories worldwide. This program "aims to evaluate the entire analytical process, from DNA sample receipt and genotyping up to the written report with the final interpretation of the data as it is normally being sent to the clinician who requested the genetic test." The United Kingdom National External Quality Assessment Service (UKNEQAS)⁴⁰ provides external quality assessment for a range of inherited diseases currently tested in diagnostic molecular genetic testing laboratories by providing challenges using lyophilized DNA or dried blood spots. This program examines the analytic and postanalytic stages, including evaluation of laboratory reports of testing and provides 3 samples per disorder per year to participants worldwide. The Italy-based Istituto Superiore di Sanità addresses current practice, problems, and future directions of interlaboratory comparisons. Their focus is PT/EQA for molecular genetic testing of a few diseases. The PT materials used are DNA samples extracted

Table 2. Extended

| Molecular Genetic Challenges/y | Sample Types | Phases of Testing Covered |
|---|---|---|
| <p><i>Disease-specific schemes:</i> 3 DNA samples 2 times/y; cystic fibrosis: 2 samples 2 times/y <i>Pharmacogenetic markers:</i> 2 DNA samples 2 times/y. <i>Postanalytic DNA sequencing scheme:</i> 3 electronic challenges 2 times/y. <i>Microarray genomic copy number assay:</i> 2 DNA samples + 1 paper challenge 2 times/y 3 DNA samples + mock clinical data 1 time/y</p> | <p><i>Disease-specific, pharmacogenetic, and microarray genomic copy number assay schemes:</i> extracted DNA. <i>Postanalytic DNA sequencing scheme:</i> 1 CD-ROM containing DNA sequence electropherogram files</p> <p>Lyophilized DNA</p> | <p><i>Disease-specific, pharmacogenetic and microarray genomic copy number assay schemes:</i> genotyping and interpretation <i>Postanalytic DNA sequencing scheme:</i> Interpretation</p> <p>Genotyping, interpreting and reporting</p> |
| <p>Includes most disease-specific schemes: 3 samples 1 time/y; molecular testing on blood spots: 3 samples 4 times/y</p> | <p>Lyophilized DNA, whole blood spotted on filter paper and dried</p> | <p>Genotyping, interpreting and reporting</p> |
| <p>3 samples are sent out per disease module, 1 times/y</p> | <p>Extracted DNA or RNA, lyophilized DNA (through EMQN); amplified DNA products and sequencing primers</p> | <p>Analytic, postanalytic</p> |
| <p>6 samples/disease <i>Molecular biology schemes:</i> 2 times/y. <i>Sequencing-based scheme:</i> 2 times/y. <i>DNA isolation:</i> 2 times/y.</p> | <p>DNA from lymphoblastoid cells <i>Molecular biology schemes:</i> lyophilized DNA. <i>Sequencing-based scheme:</i> 2 lyophilized DNA samples, DNA isolation: 2 tubes whole blood</p> | <p>Analytic and postanalytic <i>Molecular biology schemes:</i> analytic. <i>Method-based schemes:</i> sequence result and interpretation. <i>DNA isolation:</i> determination of concentration of DNA, ratio 260:280, method of identification, defined genotypes</p> |
| <p>5 dried blood spots 4 times/y</p> | <p>Blood from patient with CF spotted on filter paper and dried</p> | <p>Preanalytic, analytic, interpretation</p> |

from lymphoblastoid cell lines. This program covers both the analytic and postanalytic phases of testing and provides 6 samples per disease annually.⁴¹ The Deutsche Vereinte Gesellschaft für Klinische Chemie und Laboratoriumsmedizin eV⁴² in Germany offers a variety of test modules as well as methods-based PT for DNA sequence analysis and DNA isolation for factor V Leiden genotyping. Lyophilized DNA is provided for all its PT challenges, except the DNA isolation scheme, for which, whole-blood samples are sent.

Since 2008, the Human Genetics Society of Australasia Molecular Genetics Quality Assurance Program⁴³ has offered 23 disease-specific PT modules in collaboration with EMQN and the CF European Network. This program also offers 1 to 3 generic modules, per annum, which are based on methods and techniques to enable wider participation in the program. In particular, the generic modules are aimed at laboratories that do not participate in any of the disease-specific modules but use the same methods or technologies in their testing. One such module was an audit-based assessment of result reporting, for which laboratories were asked to provide deidentified copies of reports for one positive, one negative, and one not-tested result received for analysis within a specific period. Another module was "Mutation Detection and Biological Interpretation," for

which, laboratories were provided with amplified products and sequencing primers and were required to genotype the fragment, detect the variation, and report a biologic interpretation of the results. Laboratories were assessed for measures of the quality of data provided and result interpretation. Laboratories from Australia, Hong Kong, Indonesia, and Saudi Arabia participate in this program, which has amalgamated with the Royal College of Pathologists of Australasia quality assurance programs (RCPA QAP Pty/Ltd, Surry Hills, New South Wales, Australia) in 2010. There is also a PT program for cytogenetic testing offered through the Human Genetics Society of Australasia.

These formal PT/EQA programs include some of the more-common genetic disorders that are tested in many laboratories (Table 2). However, these tests represent only a small fraction of the more than 2300 disorders with available genetic tests. Proficiency testing/EQA providers select disorders, such as CF, which are tested by multiple laboratories to make the programs economically feasible and usually perform customer surveys to assess needs before developing a new disease challenge. In 2003, EQA was available for 8.4% and 4.2% of available genetic tests in the United Kingdom and the Netherlands, respectively.

However when these data are considered in the context of how frequently a particular test was performed, that represented 63% of the total cases reported in the United Kingdom and 48% of the cases reported in the Netherlands at that time.⁴⁴

The availability of appropriate and diverse materials, such as blood from affected patients or characterized cell lines from which PT/EQA materials are derived, also affects the availability of PT/EQA programs. It is not logistically or economically possible to provide a formal PT/EQA challenge for disorders that are tested in only one or a few laboratories or for those without a supply of available materials.

In the absence of formal PT/EQA schemes, laboratories in the United States and elsewhere must evaluate the performance of their assays using alternative methods (alternative assessment). This can be accomplished by blinded retesting of previously tested samples, by sample exchanges with laboratories performing similar tests, or by internal evaluation of data. Methods for alternative assessment are described in numerous guidance documents.^{3,19,31,45,46} Proficiency testing/EQA providers, such as CAP and UKNEQAS, facilitate sample exchanges among laboratories for tests without formal PT/EQA schemes. The effect of alternative assessment on laboratory quality has not been evaluated.

Phases of Testing and Their Evaluation by PT/EQA

To have the greatest value, PT/EQA challenges should evaluate performance in the preanalytic, analytic, and postanalytic (preexamination, examination, and postexamination) stages of the testing process. Laboratories should process and analyze PT/EQA samples in the same manner as routine clinical patient specimens to the fullest extent possible. Successful PT/EQA participation should demonstrate proficiency in all examined phases of testing.

Preanalytic Phase.—The preanalytic testing phase includes the receiving, accessioning, labeling, and initial processing of the sample. When PT/EQA results are evaluated across laboratories, a common type of error is a sample switch or mislabeling that is probably caused by a clerical error in the preanalytic phase. Even though the analytic process may be performed correctly, the clerical mistake made earlier in the testing process produces incorrect test results and reporting (Table 3). This type of error is of particular concern because laboratory workers are frequently aware that the sample was provided for PT/EQA purposes.⁴⁷ However, because PT samples do not enter the laboratory workflow in the same way that routine patient samples do, there may be hidden, uncontrollable factors in the acquisition of PT specimens that could make such errors more likely.

The next step in the testing process is nucleic acid isolation, which can be performed using laboratory-developed methods or a variety of commercially available reagent kits and instruments. The isolated DNA or RNA obtained through these extractions may be derived from blood; bone marrow; formalin-fixed, paraffin-embedded tissue; or fresh tissues. The quality of the isolated nucleic acids is central to the success of molecular genetic testing methods.

The ideal PT/EQA sample would be a clinical specimen because it would most closely represent what is actually tested in a clinical laboratory and would permit the evaluation of all phases of the testing process, including the DNA extraction step.⁴⁷ It is, however, often difficult or

impossible for formal PT programs to obtain sufficient quantities of appropriate, high-quality, safe, homogeneous, and stable clinical samples to supply all of the laboratories participating in the PT event with the exact same specimen material.^{44,47} For many genetic disorders, it is difficult to recruit enough patients to reflect the variety of mutations that would be required for a comprehensive PT/EQA program, especially for rare diseases or mutations. Institutional review board and informed consent requirements may also present an obstacle to the use of clinical specimens. Clinical samples are often incompletely characterized and may yield unexpected genotypes, including patterns of mosaicism, minor clonal populations,⁴⁸ or previously unidentified alleles.^{49,50} It is also difficult to transport potentially infectious whole blood across international borders.⁵¹ The DNA extracted from cell lines, which is available in almost unlimited supply, is often substituted as PT/EQA samples. The disadvantage of this practice is that the nucleic acid isolation phase is not performed by the participating laboratory and cannot be evaluated as part of the PT process. In addition, sources of potential PT materials are limited in the range of diseases and mutations represented. For these reasons, most PT/EQA samples are not directly comparable to the samples usually received and cannot be processed in the same way as actual clinical samples.⁴⁷ Anecdotal evidence also indicates that some analytic techniques, such as next-generation sequencing and cytogenomic microarrays, often do not perform optimally with DNA isolated by some methods used by external sources.

Analytic Phase.—The analytic phase encompasses the actual testing of the analyte. Each participating laboratory performs the testing using its own validated method or methods. In surveys with many subscribers, such as for CF, it is not uncommon for participants to use a wide variety of commercially available assays as well as laboratory-developed tests. Although PT/EQA performance is usually excellent for molecular genetic tests,^{52,53} analytic errors do occur. Some errors may be associated with the design of the assay. For example, previously unrecognized polymorphic variants located in the binding sites of the amplification primer may preclude effective amplification and detection of the actual mutation. One PT/EQA scheme for CF determined that a particular laboratory-developed test could not accurately detect the 621+1G>T mutation in the PT sample. The laboratory subsequently removed this mutation from its clinical assay.⁵⁴ In a similar case, a method-specific artifact produced a false result in a hereditary hemochromatosis PT challenge.⁵⁵ Poor performance on a PT/EQA challenge alerted another laboratory that the primer binding site in a *BRCA* assay was too close to the target mutation nucleotide, which hindered the detection of the variant.⁴⁴ Proficiency testing/EQA testing is one mechanism to alert laboratories to such problems and to indicate changes to avoid future errors. The results of PT/EQA can also be used to compare laboratory performance with different assay methods among laboratories.⁵⁴ This has been especially useful when assessing the accuracy of trinucleotide repeat sizing. For example, it is very important to accurately determine the size of fragile X premutation expansions to correctly predict the risk of allelic expansion, premature ovarian failure, and fragile X-associated tremor/ataxia syndrome. Inaccurate sizing, by even a few triplet repeats, could affect prenatal diagnosis and the risk of expansion estimates or cause conflicting reports among different family members with

similar repeat sizes tested in different laboratories. If laboratories cannot accurately determine the size of the fragile X triplet repeat, when compared with results from the other PT/EQA survey participants or from the previously measured repeat size of the sample, additional calibration of the assay is warranted. Poor performance by several laboratories in the 2002 and 2003 UKNEQAS fragile X PT/EQA scheme resulted in the development of consensus testing and reporting guidelines in the United Kingdom.⁴⁴ The American College of Medical Genetics assesses results from a subset of the CAP PT surveys; if a particular problem occurs at a high frequency, disease-specific testing practice guidelines are established and published.⁴⁷ American College of Medical Genetics guidelines for fragile X testing⁵⁶ were written in response to suboptimal performance on the CAP fragile X proficiency survey.

Postanalytic Phase.—During the postanalytic phase of testing, the test results are reviewed and interpreted. Most of the molecular genetic PT modules offered by the CAP have an analytic, as well as a clinical interpretation, component, which are graded separately.⁴⁴ Participants provide an interpretation of the detected genotype within the context of a described clinical scenario (presentation of the patient), such as whether the identified mutations are consistent with the diagnosis of the disorder or whether the genotype indicates a genetic carrier. However, other components of the laboratory report, such as compliance with existing guidance or recommendations¹⁵ describing required information elements, accuracy about the indications for testing, test performed, results obtained, and appropriateness of follow-up guidance, are not evaluated. In European EQA programs, such as UKNEQAS, the CF European Network, and the EMQN, the participants are required to submit results in their usual clinical reporting format. Those reports are assessed for accuracy of genotyping, the appropriateness of the interpretation, and clerical accuracy.^{38–40,44,51,57} That requirement allows the laboratory's interpretation of the analytic results to be evaluated in the context of the mock clinical data supplied with the DNA samples and permits assessment of other important elements in the laboratory report, such as residual risk calculations, recommendations for further testing, use of proper genetic nomenclature, and accurate inclusion of patient identification such as name, gender, and birth date. Correct interpretation of the analytic result is essential because patient management will, in many cases, be based on a combination of the analytic result and the final interpretation. This is especially important for genetic testing, where the test may only be performed once in a patient's lifetime. Because a genotype is not informative, per se, proper result interpretation requires integration with other information that may include family history, ancestry, and knowledge of genotype/phenotype associations, which is important in clinical genetics because insights about the causes of disease and the effects of various mutations or combinations changes rapidly and may affect the clinical management of the patient.

Another important component of the postanalytic analysis is laboratory reporting of the identified mutation using the appropriate gene mutation nomenclature, which makes clear to the physician interacting with the patient which sequence change has been identified. This may also include a reference to "common" nomenclature, such as "factor V Leiden," which is not consensus nomenclature but may be helpful in the report because it is most familiar

to clinicians.^{44,51} Use of incorrect or ambiguous nomenclature can lead to errors in interpretation, treatment selection, and testing other family members or their result interpretations, especially if testing is performed in different laboratories.

Grading

Methods of grading proficiency test results vary with the provider, the specific scheme, and the analyte. Some providers, such as the CAP, grade survey results on the accuracy of the genotypic result and the interpretation. For example, the CAP has recently started to grade the sizing of Huntington disease and myotonic dystrophy repeats in all size categories but has graded interpretation at 80% consensus for many years. Many of the CAP surveys are graded based on the consensus of 80% of participants; if that level of consensus is not reached, the challenge remains ungraded and "educational." Other providers, such as EMQN, provide numeric scores based on genotyping, interpretation, and reporting.³⁸ Each EMQN participant receives an individualized report with their scores and comments from the evaluators, including areas of their test report that need improvement. Scoring of EMQN schemes depends on the analyte and the scheme. For example, there are 2 EQA schemes for *BRCA* gene testing (breast cancer risk). The *BRCA* Full scheme assesses genotyping, biologic, and clinical interpretation, whereas the EMQN *BRCA* Geno scheme assesses only genotyping and biologic interpretation. Other EMQN schemes, such as congenital adrenal hyperplasia and hereditary deafness (*GJB2/GJB6* mutations), score genotyping only. These differences reflect the reporting practices of the participating laboratories. Finally, it is often challenging for PT programs to compare results because of the use of different methods, different calibration standards, and different cutoff values by participants. These issues do not usually affect molecular genetic testing for inherited genetic disease because most results are qualitative, but they are important for tests such as *BCR/ABL1* or viral load, where the results are quantitative. Proficiency testing/EQA programs cannot effectively grade some quantitative surveys because of the lack of calibration materials, standardized values for reference ranges, and cutoff values.

The Role of Laboratory PT in Quality Management

Participation in PT/EQA allows laboratories to compare their performance against the range of responses provided by a group of peer laboratories. These comparisons are most effectively used to influence laboratory practice when they are systematically integrated into a *Quality Management System* (QMS; defined by the ISO as "a management system to direct and control an organization with regard to quality").³ As part of QMS, a laboratory must participate in relevant PT/EQA schemes and ensure that the cost is integrated into the budget with sufficient time and staff resources assigned to support participation. In addition, the laboratory must ensure that the results of PT/EQA are properly considered, disseminated, and implemented to improve laboratory testing. Participation in appropriate PT/EQA and/or alternative assessment is a requirement within the CAP, the ISO, and the Australian accreditation processes.

The quality policy implemented through a QMS defines the overall approach to PT/EQA and how PT/EQA challenges are appropriately handled in the laboratory. The QMS should include clearly defined laboratory proce-

Table 3. Disease-Specific Proficiency Testing Performance (as Assessed From Select, Published Literature)

| Source, y | Disease | Participation | General Performance |
|---|---|---|---|
| Palomaki and Richards (CAP), ⁵³ 2012 | Huntington disease | 33 US and 23 international laboratories | Analytic sensitivity, 99.5%, during 6 surveys |
| Weck et al (CAP), ⁵² 2012 | Fragile X syndrome | Average of 90 laboratories during study period; US and international | Analytic sensitivity for detection of full mutations 99% (males), 96% (females); premutations 98% |
| Seneca et al (EMQN), ⁷⁰ 2008 | Spinocerebellar ataxia | Only 28.8% (15 of 52) laboratories participated in all consecutive y | 97.5% of reports correctly identified genotype as normal or pathogenic |
| Mueller et al (EMQN), ⁵⁷ 2004 | <i>BRCA1</i> and <i>BRCA2</i> | | Error rate during 3 y, 7.4% (9 of 121)–3.7% (4 of 108) |
| Dequeker, Cassiman (European Concerted Action on Cystic Fibrosis framework), ⁵⁴ 1998 | Cystic fibrosis | 135 European laboratories, 1 Australia laboratory | 35% (48 of 136) of laboratories incorrectly genotyped ≥ 1 of 12 <i>CFTR</i> alleles in study |
| Ramsden et al, ⁴⁴ 2006; Perry et al (UK NEQAS), ⁵¹ 2006 | Several diseases | | Poor performance was sporadic |
| Falbo et al (ISS), ⁷¹ 2008 | Fragile X syndrome | 65% (22 of 34) of all Italian laboratories that perform testing participated. 15 laboratories participated in each of the 5 y | Overall success rate, 76% (458 of 601). |
| Taruscio et al (ISS), ⁷² 2004 | Cystic fibrosis, fragile X syndrome, others | | Interpretive performance varied by disease |
| Tosto et al (ISS), ⁷³ 2009 | β thalassemia | 11–18 laboratories during 5 y | 98.8% (889 of 900) of alleles correctly identified |
| Hertzberg et al (RCPA), ⁷⁴ 2006 | <i>HFE</i> | 37 laboratories currently | 99.47% (3000 of 3016) success during 10 surveys |

Abbreviations: CAP, College of American Pathologists; EMQN, European Molecular Genetics Quality Network; ISS, Istituto Superiore di Sanita; PT, proficiency training; QAP, quality assurance program; RCPA, Royal College of Pathologists of Australasia; UK NEQAS, United Kingdom National External Quality Assessment Service.

Table 3. Extended

| Analytic Errors Identified | Interpretation Errors Identified | Lessons Learned |
|--|--|--|
| <p>Repeat length errors occurred in 2.6% (28 of 1060) allelic challenges. Most errors were minor and occurred in a small subset of the laboratories. Non-US participants had the higher error rate (17.5%, (107 of 612)). Measurement of repeat length was more accurate with smaller samples.</p> | <p>3 interpretation errors were made in US laboratories during the 6 surveys.</p> | <p>Performance was very good. An analytic method with a high error rate was identified.</p> |
| <p>Large interlaboratory variation seen in allele sizing. Gross diagnostic errors occurred every year (false-negatives, false-positives, and sample misidentification). Not all laboratories reported allele size.</p> | <p>In samples with repeat sizes of 20–33, during 18 surveys, 3 interpretation errors made. Many laboratories incorrectly identified samples with 42 repeats as gray-zone or premutation.</p> <p>Wide range of allele sizing but without incorrect diagnoses. Clerical errors observed.</p> | <p>Accuracy of sizing and interpretation of larger repeats improved over time.</p> <p>(1) One-third (5 of 15) of laboratories with inaccurate sizing did not participate the following year, and for those that did, >50% (6 of 10) continued to report wrong sizes, showing importance of participation in PT every year; and (2) allele sizing should always be reported: it is a measure of technical quality and may give clinically important information.</p> |
| <p>Missed mutations (53% (9 of 17) of false results). Problems with incorrect nomenclature.</p> <p>Mistyping, misinterpretation of data, erroneous technical results.</p> | <p>Misinterpretation of observed sequence changes.</p> | <p>Horizontal comparison among laboratories allows for better understanding of the state of the field and for detection of systematic technical failures.</p> <p>Regular participation in PT schemes would be beneficial for laboratories. Laboratory personnel should participate in regular training sessions. Need consensus strategies for diagnostic testing. Centralized mutation analysis facilities would be useful for identified rare mutations.</p> |
| <p>Technical errors and wrong nomenclature.</p> | <p>Incomplete reports; wrong clinical interpretations.</p> | <p>(1) Any poor performance causes immediate correspondence between the organizer and the laboratory; and (2) scheme located test-validity problems as well as clinical practice problems, with wider implications for the genetic testing community.</p> <p>(1) Importance of reports for patient care; and (2) importance of secondary screening methods.</p> |
| <p>Overall, 5% (30 of 601) of samples tested were incorrectly genotyped, and 1.5% (9 of 601) of that was due to 1 sample, a male mosaic normal/full mutation, which was incorrectly genotyped 9 times (3 by the same laboratory). Some technical failures caused by prescreening methods.</p> <p>7% (2 of 28) of laboratories incorrectly genotyped 2 mutations, although their commercial kit detects those mutations. Many laboratories did not include clinically relevant cystic fibrosis mutations in their panels.</p> | <p>Reports showed considerable variation. Starting in 2004, a model was issued for reports.</p> <p>Written reports were inadequate for >50% of laboratories. The most common error was lack of risk calculation when a mutation was not detected.</p> | <p>(1) Even commercial kits must be validated in the laboratory before use for clinical testing; (2) laboratories need guidance on the kinds of mutations to include in their panels; (3) complete reports are crucial for patient care; and (4) laboratory errors were disease specific and were not indicative of overall laboratory analytic performance.</p> <p>Laboratories did a good job of reporting some aspects of the test, such as diagnostic sensitivity; however, many reports did not identify the mutations tested, indication for genetic counseling, or interpretation of results.</p> |
| <p>Most errors were due to sample handling or result transcription. A few errors caused by technical issues.</p> | <p>Incorrect results because of transcriptional errors.</p> | <p>Performance was very good, but laboratories should continue to participate in large quality assurance programs to maintain high testing quality.</p> |

Table 4. Methods-Based Proficiency Testing (PT) Schemes and Performance

| Source, y | Technology Assessed | Laboratories Provided With | PT protocol | Laboratory Performance |
|--|----------------------------------|---|--|---|
| Brothman et al (CAP), ⁷⁵ 2011 (ongoing PT scheme) | aCGH | Extracted DNA from cell lines derived from individuals with a constitutional abnormality. | Laboratories must (1) analyze specimens and identify and interpret results for clinically significant copy number abnormalities, and (2) identify and characterize gains or losses and the cytogenetic location of any abnormalities detected using standard nomenclature. | 95.7% (472 of 493) of responses were concordant for an abnormality involving a particular chromosomal location. Errors included designating an opposite change, reporting a clinically insignificant copy number change, and failure to report a clinically significant abnormality. |
| (CAP), ²² (ongoing PT scheme) | Postanalytic DNA sequencing | One CD-ROM containing DNA sequence electropherogram files. | Laboratories must interpret and report DNA sequence variants for inherited disease using standard nomenclature. | No published data available. |
| Orlando et al (EQUAL), ⁶⁵ 2007 (pilot) | DNA extraction and amplification | (1) Two preextracted DNA samples, (2) whole blood samples, and (3) primer sets. | Laboratories must (1) perform DNA extraction by routine procedures, (2) estimate DNA quality and quantity of both preextracted and laboratory-extracted DNA, (3) perform PCR with 100 ng of DNA from all samples, (4) submit raw data from DNA quantification and post-PCR interpretation, and (5) send aliquot of DNA extracted from whole blood back to EQUAL. | 25% (42 of 165) of laboratories performed poorly in quantification of ≥ 1 of the 2 preextracted samples; 27% (46 of 166) of laboratories had questionable results for quality and/or quantity of blood sample extractions. High degree of variability seen with PCR performance of all samples. |
| Ramsden et al (EQUAL), ⁶⁶ 2006 (pilot) | Real-time PCR | (1) <i>ABL</i> gene primers, (2) 5'-FAM/3'-TAMRA-labeled probes, (3) 5 <i>ABL</i> standard plasmids (10E2-10E5), (4) 3 test cDNA samples, and (5) 2 cell samples. | Laboratories must (1) construct a calibration curve; (2) estimate cDNA copy numbers in 3 cDNA samples; (3) perform RNA extraction, real-time PCR, and cDNA quantification of the 2 cell samples; and (4) provide Ct values for NTC, calibrators, and unknowns, plus details of the testing platforms used. | For the cDNA samples, 80% (74 of 93) of laboratories provided accurate values for all 3. For cell samples, approximately 35% (27 of 75, 25 of 74) of laboratories provided 95% CI limits that fell outside the range of standard dilutions. Wide variations in laboratory results were observed. |
| Ahmad-Nejad et al (EQUAL), ⁶² 2006 (pilot) | Sequencing | (1) Primer sets, (2) 2 plasmid DNA samples (1, a commercial cloning vector; 1, a mixture of 2 different plasmids generated to provoke comments about template quality), (3) a purified PCR product, and (4) a finished sequencing reaction to be purified and analyzed. | Laboratories must (1) perform sequencing and provide longest possible sequence without any mistakes (primer-walking not allowed) for DNA plasmids and purified PCR product and (2) purify and analyze the finished sequencing reaction. | For the cloning vector plasmid, the mean sequence stretch (of the 2355-bp insert) was 537 bases and only 14 of 43 participants (33%) identified the insert correctly. For the mixture of 2 different plasmids, 21% (9 of 43) of laboratories did not register any kind of difficulty; however, some excellent specific comments were received. For the finished PCR product, 57% (24 of 43) of laboratories made no effort at identification. |

dures for receipt of performance results from a PT/EQA agency or other assessment protocols, such as alternative assessment. This procedure will normally follow 3 phases. First, the laboratory director is required to disseminate the data to staff immediately. This conveys to staff the

importance of participating in PT/EQA schemes and enhances the educational content of the exercise. The performance data must be reviewed for any indication of deficient performance that requires immediate corrective action to avoid errors. Proficiency testing/EQA data should

Table 4. Continued

| Source, y | Technology Assessed | Laboratories Provided With | PT protocol | Laboratory Performance |
|--|---|---|---|--|
| Patton et al (EMQN), ⁶³ 2006 (ongoing EQA scheme) | Sequencing | (1) 450-bp, PCR-amplified fragments of <i>CFTR</i> covering all main types of sequence changes, (2) a wild-type control, (3) primers (with location in sequence), (4) reference sequence, and (5) protein translation | Laboratories must (1) return form with genotyping results and (2) return color copies of electropherograms and electronic copies of sequence data files. | 59% (36 of 61 laboratories) scored the maximum (12.0 marks); 19 genotyping errors were made from 346 genotypes analyzed (5%); 10 of 19 (53%) were false-negative results, and 9 of 19 (47%) were false-positive results; 59% (27 of 46) of errors were made in naming mutations. Most laboratories produced data with acceptable diagnostic quality. |
| Raggi et al (ISS), ⁶⁴ 2003 (pilot) | DNA extraction, PCR performance, interpretation | (1) Three primer pairs, (2) 1 reference DNA, (3) 3 DNA calibrators, and (4) 1 blood sample | Laboratories must (1) extract DNA from blood sample and measure quality and quantity, (2) measure quantity and quality of 3 standard DNA samples, (3) perform PCR, and (4) return PCR results, extracted DNA aliquot, and PCR product aliquots. | Variability was high for DNA quantification (range, 0.012–0.54 µg/µL; CV, 82%). The 260:280 ratios varied (range, 0.8–2.5; CV, 21%). Large variability seen in yield and quality of PCR performance. |
| (HGSA-ASOC), ⁴³ (ongoing scheme) | Microarray analysis | Extracted DNA from an individual tested for a constitutional abnormality | Assessment of laboratory processing and/or analytic skills, as well as interpretive comments. | No published data available. |
| (HGSA-MGSA), ⁴³ (ongoing scheme) | DNA sequencing and interpretation | (1) Amplified products and (2) sequencing primers | Laboratories must (1) perform Sanger sequencing using PCR products and primers provided, and (2) analyze and report results using standard nomenclature and provide biologic interpretation. | No published data available. |
| Birch et al, ⁷⁶ 2004 | DNA extraction, PCR amplification | (1) Tubes containing high, medium, and low concentrations of bacteria in buffer; (2) control DNA in buffer; (3) PCR reagents (dNTPs, primers, Taq polymerase, reaction buffer); and (4) 100-bp DNA ladder, gel loading dye. | Laboratories must (1) extract DNA from suspended bacteria, (2) PCR-amplify extracted DNA according to protocol provided, and (3) analyze using agarose gel electrophoresis. | Identified analytic issues such as inappropriate extraction or amplification procedures, PCR inhibition or contamination, poor labeling or poor-quality gel photography, and failure to record results correctly. |
| (DGKL), ⁴² (ongoing scheme) | DNA sequencing and interpretation | Lyophilized DNA | DNA sequencing and corresponding diagnostic interpretation | No published data available. |
| (DGKL), ⁴² (ongoing scheme) | DNA isolation, quantitation (and factor V genotyping) | Whole blood | Laboratories must (1) isolate DNA, (2) determine the concentration of DNA, (3) report 260:280 ratio, and (4) identify the factor V genotype of the sample. | No published data available. |

be routinely discussed in the most appropriate meetings of management, the quality team, and staff so that lessons learned can be discussed and, if necessary, changes in standard operating procedures can be quickly integrated into the QMS. Proficiency testing/EQA data provides an opportunity for a laboratory to compare its performance

with peers as well as to celebrate and praise the staff when performance is exemplary. Second, PT performance data should be presented during management review meetings to examine any recurrent deficiencies that require correction or improvement. Third, PT/EQA records should be stored and formatted for external audit by an accrediting agency.

Table 4. Continued

| Source, y | Technology Assessed | Laboratories Provided With | PT protocol | Laboratory Performance |
|--|----------------------|--|--|--|
| Raggi et al (ISS), ⁷⁷ 2005 (pilot) | TaqMan real-time PCR | (1) Standard cDNA solution obtained by in vitro transcription of a fragment of hTERT cloned into a plasmid vector, (2) mix of primers, (3) 3 unknown cDNA samples, and (4) PCR conditions. | Laboratories must (1) prepare dilutions of the provided standard, (2) perform PCR and analyze results, (3) report Ct values for each well, and (4) report concentrations of unknown samples. | Only 12 of 42 laboratories (29%) gave results that were both concise and accurate for all samples tested; 17 of 42 laboratories (40%) reported inaccurate data for ≥ 1 result. Inaccuracy showed an inverse dose-dependent trend; 12 of 42 laboratories were unable to measure a sample with low concentration. |

Abbreviations: aCGH, array-comparative genomic hybridization; ASOC, Australasian Society of Cytogeneticists; bp, base pair; CAP, College of American Pathologists; cDNA, complementary DNA; CI, confidence interval; Ct, cycle threshold; CV, coefficient of variation; dNTP, deoxynucleotide triphosphate; EMQN, European Molecular Genetics Quality Network; EQA, external quality assessment; HGSA, Human Genetics Society of Australasia; hTERT, human telomerase reverse transcriptase; ISS, Istituto Superiore di Sanita; MGQAP, Molecular Genetics Quality Assurance Program; NTC, no template control; PCR, polymerase chain reaction.

Laboratories may experience a lapse or error in PT/EQA performance. Single occurrences of poor performance should be logged as an incident and used as an opportunity to review procedures and make improvements. A careful evaluation of the error may determine whether there is a system failure that may require redesign of a test, more-frequent instrument calibration, or adjustments to training procedures. However, PT/EQA may detect serial or persistent failures that the laboratory is obligated to address with a more fundamental review. Some PT/EQA providers report that laboratories have discontinued testing services following instances of poor performance in PT/EQA.⁴⁴ That indicates the importance that laboratory directors assign to this external comparison and emphasizes the role of PT/EQA as an educational tool and mechanism to improve performance in clinical laboratories.

In some countries, PT/EQA providers are required to report poor and recurrent poor performance to an official monitoring agency. That agency may have a role in ensuring that corrective actions are designed to address poor PT/EQA performance. Ultimately, they may have the power to escalate their intervention to involve the host institution of the laboratory concerned as well as regulatory agencies.

Evidence of the Value of PT/EQA in Ensuring Good Laboratory Performance

Although few studies have addressed whether participation in PT/EQA programs directly improved laboratory performance, there is empirical evidence showing that the educational aspect of PT/EQA does help laboratories detect errors in their testing protocols and identify problems associated with their assays.² One study reviewed 3 rounds of PT data from 2002 (any provider) from approximately 6300 CAP-accredited laboratories.⁵⁸ The study evaluated whether laboratories corrected deficiencies identified by PT or whether they continued to have unsuccessful PT performance. The analysis indicated that about 90% of the PT problems were resolved after the first round of PT and 99% were resolved by the third round, suggesting that the laboratories had successfully corrected mistakes identified by PT performance. Other studies have shown that participation in the CAP "Calibration Verification/Linearity Surveys," which examine calibration verification and analytic measurement range of several nongenetic analytes (chemistry, immunology, hematology, among others), is

associated with fewer PT failures.^{59,60} A UKNEQAS PT/EQA scheme for hemophilia A from 2003 failed 4 laboratories based on poor performance in the evaluation of their clinical report. In subsequent surveys, only 1 laboratory failed to report properly, suggesting that the educational value and improvement of testing practices were derived from that exercise.⁵¹ Another value of PT/EQA is the opportunity for laboratories to analyze unusual samples not often encountered in their service, such as, mosaicism of variants in fragile X syndrome.

The UKNEQAS PT/EQA program emphasizes interpretation of data and their implications for both the patient and the family. One summary of data indicates that poor PT/EQA performance because of interpretation errors has decreased between 1997 and 2006.⁴⁴ This may be derived from continued participation in PT/EQA.

Analysis of PT/EQA results has also revealed inconsistent results stemming from the lack of uniformity in practices among laboratories. Once these issues were identified, steps were implemented to harmonize practices among the laboratories. Poor and inconsistent performance of laboratories participating in a PT/EQA scheme for fragile X syndrome led to consensus testing and reporting guidelines development in the United Kingdom⁴⁴ and the United States.⁵⁶ Because of ambiguities in the ways laboratories report sequence variations, PT schemes (such as UKNEQAS) strongly suggested that genotypic PT/EQA results should be reported using the Human Genome Variation Society standard nomenclature.^{44,51} Participants in the CAP's disease-specific surveys typically report results using the common allele names; however, CAP requires the use of the Human Genome Variation Society nomenclature for participants in its PT scheme on interpretation of sequence variants in rare disorders and is moving toward integration of the consensus nomenclature for all schemes.

Limitations of PT

To assess a given test, PT/EQA should evaluate the capability of a particular assay to identify a range of possible test results or alleles, either during an individual challenge or during the course of several challenges. Considering that, PT for a given disease or genetic variant may be limited by the following:

1. Issues related to the limited availability of resources, including appropriate samples and reference materials;

2. Logistical and practical difficulties encountered, including the inability to offer PT/EQA for all genetic disorders, especially those tests for rare disorders or those performed by a few laboratories only; and
3. Limitations in the PT process for evaluation of laboratory performance.

Ideally, PT/EQA should evaluate the ability of the laboratory to identify all genotypes of interest for a particular disorder, which requires the availability of appropriate samples. For simple tests, such as factor V Leiden or prothrombin 20210A, very few alleles are tested in a clinical setting; thus, relatively few PT/EQA samples are required to represent the population variant affecting patients. For other disorders, such as CF, there is a large variation in the number and composition of alleles included in clinical assays. Some assays only examine the 23 alleles recommended for carrier screening by the American College of Medical Genetics and the American College of Obstetricians and Gynecologists,⁶¹ whereas other laboratories offer assays that test more than 100 alleles, some of which are unique to particular patient populations. Many of the alleles included in the more comprehensive CF assays are very rare, and it is often difficult to obtain samples from patients or cell lines for PT/EQA. Another difficulty is the lack of highly characterized reference materials or calibrators with which to evaluate potential PT/EQA materials. This is especially important for DNA fragment-sizing assays, such as triplet repeat testing but may also be a factor for qualitative assays.

Proficiency testing programs also encounter logistic and practical problems. Most of the 2300 clinical genetic tests are offered in only one or a few laboratories, making provision of a formal proficiency survey logistically and economically difficult. Many multiplex genetic tests may simultaneously detect dozens of alleles (eg, CF or pharmacogenetic loci). It is logistically impossible for PT/EQA programs distributing 3 samples 1 or 2 times a year to provide enough different samples to adequately challenge the variety of genotypes represented in all patients or all assays for a particular genetic disorder. The DNA sequence analysis identifies mutations in any part of a gene and can pose a unique challenge in interpretation. In addition, PT/EQA typically targets well-characterized conditions and mutations. However, new disease associations are discovered regularly and genotype-phenotype correlations are often not fully elucidated until years after the initial discovery. Allelic heterogeneity and the clinical effect of some sequence variants, such as missense mutations and complex variants, pose considerable challenges for interpretation when functional studies have not yet been performed or were inconclusive. An example of this situation is pharmacogenetic testing for *CYP2D6* variants. Many tests for this gene include polymorphisms with undefined effects on *CYP2D6* enzyme activity. These less-well-characterized variants are challenging for developing of reference materials, reporting, and interpreting PT/EQA results. Formal PT/EQA might become available for more inherited conditions in the future, but it will probably not be feasible for such programs to cover all conditions and available tests.

Approaches to Address Limitations of Proficiency Testing: Methods-Based PT/EQA

Proficiency testing that is designed to evaluate the performance of analytic methods, rather than to evaluate specific genotypic assays, has been proposed as a comple-

mentary mechanism to more-broadly assess laboratory performance.^{45,62-64} Methods-based PT/EQA examines technologies common to many genetic tests, such as DNA purification, polymerase chain reaction (PCR) amplification, or DNA sequencing, and allows evaluation of critical analytic steps for individual assays that are not directly assessed by traditional PT/EQA schemes. It also permits interlaboratory comparisons and can highlight analytic practices, such as DNA quantification, which could be optimized for improved performance.⁶²

The European Commission funded a project (EQUAL) (Full program title: "Multinational External Quality Assay [EQA] Programmes in Clinical Molecular Diagnostics Based on Performance and Interpretation of PCR Assay Methods Including Dissemination and Training") to develop and evaluate the utility of methods-based EQA to address methodologic procedures and analytic proficiency in molecular diagnostic test performance independent of the target. Three EQUAL pilot projects for qualitative analysis, quantitative PCR, and DNA sequencing (EQUAL-Qual, EQUAL-Quant, and EQUAL-Seq) were initiated.^{62,65,66} The results from those pilot studies identified many areas of laboratory performance that varied considerably between participants (Table 4) and suggested areas that could be targeted for improvement.

Because of relatively poor laboratory performance in the EQUAL-Seq project, specific training highlighting analytic and methodologic skills were subsequently offered to the participating laboratories. A significant improvement of technical and interpretative skills was demonstrated in a confirmatory second round of EQA.⁶⁷

Currently, methods-based PT is available for a variety of assays (Table 4). The EMQN offers a methods-based scheme for Sanger sequencing. Participants receive amplicons to characterize, identify, and report the sequence variants using the proper nomenclature. They also provide their raw sequence data, which is evaluated by EMQN for quality scores, quality read length, and quality read overlap.⁶³ In the United States, the CAP has launched an electronic DNA sequence-analysis survey. In 2012, that survey will send a set of primers and 3 DNA specimens to participants. Participants will identify all variants and report on them with accepted nomenclature. In the future, both the CAP and the EMQN plan to offer methods-based surveys to address the performance of next-generation DNA sequencing.

Synthetic Samples

Synthetic DNA samples can be used for PT/EQA. These samples may be composed of plasmid DNA containing specific sequences or PCR amplicons. The CF Network tested the usefulness of a synthetic reference material containing 6 homozygous mutations in the cystic fibrosis gene (*CFTR*) and one polymorphism for suitability as PT material.⁶⁸ Most of the laboratories in the study successfully identified the mutations, although some technical difficulties, such as incorrect genotyping (10 of 197 participants; 5%), or absent, incorrect, or insufficient interpretation (33 of 197 participants; 17%) regardless of genotype, were reported. This synthetic sample was supplied in a bloodlike matrix, which also permitted evaluation of the DNA extraction step. In the United States, artificially constructed CF mutation samples designed to mimic extracted human genomic DNA produced similar results.⁶⁹

COMMENT

Proficiency testing has demonstrated value as an important laboratory quality assurance tool and has helped laboratories identify issues related to test design and performance. In addition, the ability to compare laboratory performance with others using the same or different methods on identical samples can highlight issues related to test methodology or interpretation or may inform development of best-practice guidelines and standard policy.

Adoption of new and complex testing technologies, such as next-generation sequencing assays, will require modifications to PT/EQA design and provision. In contrast to traditional genetic tests, which identify only a few mutations or perhaps the whole sequence of a well-characterized gene, next-generation sequencing has the capacity to examine the sequence of large gene panels, the exome, or the entire human genome, with an almost infinite variety of possible variants. Proficiency testing programs for next-generation sequence assays need to monitor the ability of the laboratory to detect mutations in any part of the genome included in their validated test. In addition to the analytic phase of the testing process, the data analysis and interpretation of next-generation DNA sequencing is considerably more complicated than that of sequencing tests with smaller scopes. Proficiency testing/EQA can be used to compare performance among laboratories during all phases of next-generation DNA sequencing testing and may provide important indicators of which steps of the testing process are problematic. That information may not be readily discernible through daily quality-control practices, and PT/EQA could be an important tool to assess whether the testing algorithm is sufficient to detect a loss of sensitivity or specificity for the detection of sequence variations that may only be evident when comparing results among laboratories. Interpretation of the analytic test result of such large-scale analyses is difficult and uncertain because the effect of individual mutations, the function of each gene and its interaction with other genes in the genome has not yet been determined. Proficiency testing/EQA schemes to assess the ability of the laboratory to interpret and report complex data could be quite informative. Novel and innovative PT/EQA challenges will need to be developed to ensure the quality of these new tests.

Proficiency testing or EQA should include sufficient analytes to provide a reasonable estimate of interlaboratory comparability. For example, PT for factor V Leiden testing assesses the laboratory's capacity to identify 1 of 3 possible genotypes per sample (wild type, factor V Leiden heterozygote, and factor V Leiden homozygote). Developing a PT program to gauge interlaboratory comparability in this example is fairly straightforward. Proficiency testing for disorders with many disease-associated alleles, such as CF, or a next-generation sequencing test presents additional challenges. Current PT can only assess a subset of possible mutations per challenge. Is the current process sufficient for achieving a credible interlaboratory comparison for very complex tests? The limited availability of characterized DNA materials and the cost associated with increasing the number of PT samples can be significant barriers for a PT/EQA program with a goal to offer comprehensive challenges.

Significant research, needs assessment, and pilot testing should be performed to ensure that PT addresses the

changing needs of genetic testing technology and an evolving knowledge base and continues to be a relevant and useful quality assurance tool. Some of the research topics may include ways to provide PT/EQA for the increasing number of new tests, many of which are offered in only one or a few laboratories; to include all phases of the testing process, rather than just the analytic phase; and to develop novel approaches for effective multiplex genotype-testing challenges, as well as PT/EQA strategies to assess new technologies, such as next-generation sequencing. Additional research will define PT/EQA improvements to promote the quality of laboratory testing, interpreting, and reporting.

Most genetic tests are done in only one or a few laboratories. Many of these tests use the same technology, such as Sanger or next-generation sequencing to examine a particular gene or set of genes. Many other tests share similar methodology, such as DNA and RNA purification, PCR amplification, or multiplex ligation-dependent probe amplification. Other aspects, such as result reporting, are also common across tests. Research to develop and evaluate novel method- or technology-based PT/EQA schemes may simultaneously assess the performance of many tests. Schemes that evaluate the quality of laboratory reports, for example, those of the CF Network, UKNEQAS, and the EMQN, could also be developed more broadly. In addition, research should be conducted to assess the effectiveness and relationship of the commonly used methods of alternative assessment, such as sample exchange or blinded retesting of previously tested specimens, to the accuracy of routine laboratory test results. Information gleaned from such studies might provide guidance to laboratories on effective methods of alternative assessment.

Much work needs to be done to ensure that PT/EQA programs can meet the needs of new and evolving genetic tests and technologies. An important first step would be to design a scientific approach to collecting data on the effect of PT/EQA on laboratory testing quality. Such studies could measure the relationship between PT performance and the accuracy of routine test results in that same laboratory and could be expanded to include PT/EQA for the newer testing technologies, including microarrays and next-generation sequencing. Information from these studies might inform the development of improved PT programs, professional guidelines, and regulations.

A variety of research and development projects will address some of the current limitations of PT/EQA, including the shortage of characterized reference materials, both naturally occurring and synthetic, that can be used as PT/EQA samples. Cell lines can be created from patients with genetic disorders who are currently part of PT/EQA programs and also from those with disorders for which PT/EQA would be useful but for which programs do not yet exist. Consideration should be given to each disorder to ensure that a comprehensive set of reference materials, containing as many clinically significant alleles as possible, will be developed. In addition, development of synthetic reference materials containing many alleles for a given disorder simultaneously should also be considered. These materials must be evaluated by testing in several clinical laboratories using a variety of assays and technologies before they are used as PT/EQA samples.

Proficiency testing and external quality assessment have been and should remain an integral part of laboratory quality assurance. In the next few years, we hope to conduct

and facilitate these and other research projects to evaluate and improve the quality of PT for molecular genetic testing.

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