Introduction to Molecular Cystic Fibrosis Testing

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Technology improvements are rapidly bringing molecular diagnostics into routine laboratories. Recent recommendations for cystic fibrosis carrier testing by the American College of Medical Genetics (ACMG) have led to commercial test kit development and increased testing volumes. Molecular testing of genetic diseases presents a variety of challenges and situations that may be unfamiliar to laboratories with limited molecular genetic experience. We will briefly review the disease and discuss mutation testing indications, methodologies, quality assurance, and reporting issues associated with cystic fibrosis testing.

ABBREVIATIONS: ACMG = American College of Medical Genetics; ACOG = American College of Obstetrics and Gynecology; ASRs = analyte specific reagents; cAMP = cyclic adenosine-5'-monophosphate; CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; CLSI = Clinical and Laboratory Standards Institute; FDA = United States Food and Drug Administration; IRT = immunoreactive trypsinogen; KB = kilo bases; NHGRI = National Human Genome Research Institute. PKA = protein kinase A; QC = quality control

INDEX TERMS: asthma; congenital bilateral absence of the vas deferens; cystic fibrosis; cystic fibrosis trans-membrane regulator; mutations; pancreatitis.

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MUTATIONS ASSOCIATED WITH CYSTIC FIBROSIS AND DISEASE OUTCOMES

Cystic fibrosis (CF) is one of the most common inherited diseases in the United States with an incidence of about 1 in 3,700 births.[1] CF is more common among Caucasians (1 in 2,500, with a carrier frequency of 1 in 25) than among other groups such as African Americans (1 in 15,000) or Asian Americans (1 in 31,000).[2]

Cystic fibrosis, inherited in an autosomal recessive manner, arises from mutations in the gene encoding the 1,480 amino acid-long cystic fibrosis transmembrane conductance regulator (CFTR) protein that functions as a membrane chloride channel. As a recessive disorder, an individual must inherit two defective CF genes, one from each parent, to have CF. The CF patient need not inherit two copies of the same mutation but may inherit two different CFTR mutations, a condition referred to as compound heterozygosity. A person having just one mutant gene will produce functional CFTR protein and will not have symptoms of CF but is considered a carrier. Each time two carriers conceive, there is a 25% chance that their child will have CF; a 50% chance that the child will be a carrier of a CF mutation; and a 25% chance that the child will be a non-carrier. Healthy siblings of patients with CF have a two-thirds chance of being a carrier and therefore, a one-third chance of not being a carrier.

The CFTR gene has been mapped to chromosome seven; it is about 230 KB long, contains 27 exons and encodes a mature mRNA of 6.5 KB. Mutations in the CFTR gene include insertions, deletions, and single nucleotide polymorphisms that may alter mRNA splicing, expression or stability and activity, or localization of the protein. More than 1,000 disease-causing mutations have been identified; however, a single mutation, F508del, is found in almost two-thirds of CF patients worldwide.[3] An up-to-date list of known CFTR mutations is available in the CF Mutation Database found at http://www.genet.sickkids.on.ca. F508del represents a three base pair deletion that causes the loss of a single amino acid (phenylalanine) residue at position 508 in the amino acid sequence, which results in misfolding and inappropriate localization of the protein. The frequency of this mutation among CF patients ranges from 70% to 80% in Northern

Europeans to 30% to 50% in Southern Europeans and other racial/ethnic groups. The frequencies of other CF mutations also differ significantly among various racial/ethnic and geographically distributed groups.

Cystic fibrosis is a phenotypically heterogeneous disorder with the clinical presentation ranging from relatively mild to severe. The underlying defect in CF is reduced chloride ion transport across epithelial cell membranes. Normally, the CFTR chloride channels open when cyclic adenosine-5'-monophosphate (cAMP) levels increase and activate protein kinase A (PKA) within mucus-producing epithelial cells. Enzyme-mediated phosphorylation of the membrane chloride channels enhances secretion of chloride. In some cases of CF, abnormalities in the CFTR protein result in epithelial chloride channels that cannot be activated by either cAMP or cAMP-dependent protein kinase. In other cases, normal CFTR protein expression is simply absent or significantly reduced. CF is a multi-organ disorder, most commonly affecting the lungs, pancreas/intestine/liver, male genital tract, and sweat glands. In the lungs, the inability of epithelial cells to secrete chloride ions leads to a dehydration of the pulmonary secretions.[4] The lung secretion becomes thick and viscous, making it difficult to be removed by ciliary action. This thick, static mucus serves as a growth environment for opportunistic bacterial pathogens such as mucoid *Pseudomonas aeruginosa* and allows chronic, difficult-to-treat infections to persist. Repeated cycles of infections and host inflammatory responses result in permanent damage to the airways and, ultimately, bronchectasis and death.

The classic CF patient develops chronic pulmonary disease, exocrine pancreatic insufficiency with malabsorption, and infertility in males. Other manifestations of CF may include nasal polyps, chronic sinusitis, liver disease, and chronic pancreatitis.[5,6] Classic CF patients often display symptoms shortly after birth. Meconium ileus (a surgical emergency caused by the stoppage of intestinal motility in a newborn due to the bowel being plugged with thick, dehydrated meconium) occurs in 15% to 20% of all CF patients. Pancreatic insufficiency, resulting in malabsorption, occurs in about 85% of patients. By 18 years of age, 17% of CF patients require insulin for treatment of diabetes mellitus.[7] With improved therapies such as antibiotics, physical therapy, anti-inflammatories, pancreatic enzyme replacement, and dietary supplements, the median lifespan of CF patients is now 32.9 years.[7]

Prediction of CF disease course based on inherited mutations is challenging but some genotype/phenotype correlations have been established. One such example is the correlation of pancreatic sufficiency or insufficiency in CF with specific CFTR mutations. Pulmonary function is more difficult to predict because environmental and other extragenic factors have a greater effect on lung function than pancreatic function. Patients with sufficient pancreatic function, defined as those who do not require pancreatic enzyme supplementation (pancreatic sufficiency), carry at least one "mild" mutation, such as one causing reduced expression of a fully functional protein. Examples of this type of mild mutation include 2789+5 G>A and 3849+10kb C>T. Other types of mild mutations include those that result in normal expression of a CTFR channel with suboptimal activity, such as R117H or R347P. Pancreatic insufficient patients with malabsorption have lost more than 98% of their enzyme secretary function. Such patients are either homozygotes or compound heterozygotes with severe mutations that produce a nonfunctional protein or no protein at all. A number of mutations fall into this severe category, including the common F508del. Pulmonary function usually parallels pancreatic sufficiency or insufficiency, but with greater variability in range of function.

Male CF patients are almost always infertile due to lack of formation of the vas deferens during embryonic development. This congenital bilateral absence of the vas deferens (CBAVD) is typical of classic CF; however, it can occur alone or with very mild respiratory or pancreatic manifestations. A number of CFTR mutations present solely as CBAVD.[8,9] One of the best-studied alleles is a complex allele where two sequence variations are present in the same CFTR gene. As noted above, the R117H mutant protein retains some ability to transport chloride. A polymorphic stretch of thymidine bases in the splice acceptor site of intron 8 (a chromosome can have 5, 7, or 9 Ts at this position) has been shown to affect splicing efficiency of the CFTR mRNA. The 5T allele is associated with a decreased amount of correctly spliced CFTR mRNA.[10] When 5T allele is found in the same CFTR gene (i.e., in cis) as a mutation that decreases the activity of the protein, such as R117H, the combination leads to a severe lack of CFTR. When the 5T/R117H complex allele is inherited with a classic CF mutation such as F508del on the other chromosome 7, a CF phenotype, typically pancreatic sufficient, results. However, when the R117H is in cis with a 7T allele, the decrease in CFTR function is much less severe, and when 7T/R117H alleles are inherited with a CF mutation on the opposite chromosome, the clinical phenotype is quite variable. It may be pancreatic sufficient CF or even CBAVD alone.

The observation that the R117H mutation occurs on different chromosome backgrounds (5T or 7T) indicates that this mutation has arisen more than once in human evolutionary history. In addition, when the R117H mutation is detected during CF genetic testing, reflex testing of intron 8 poly-T allele is indicated. The decreased CFTR production from the 5T allele is not sufficient to give clinical symptoms by itself, even in homozygous form. Even when the 5T allele is inherited with a classic CF mutation on the opposite chromosome, the decrease in CFTR function is not sufficient to cause classic CF. It is, however, sufficient to result in CBAVD in males. Thus, the status of the poly-T region should only be reported in select circumstances, when the R117H mutation is detected, or in a work-up of a male whose infertility is a result of the absence of the vas deferens. Inappropriate reporting of the poly-T alleles early in the implementation of population carrier screening gave rise to significant concern.[11]

TESTING INDICATIONS

In addition to confirming the diagnosis on an individual suspected of having CF, molecular testing plays a role in newborn screening and carrier testing for this disease. Due to the lack of chloride ion secretion, CF patients have three to five times the normal concentrations of salt in their sweat. Sweat chloride testing, which has a sensitivity of greater than 95%, has been the gold standard for CF diagnosis; however, this test may not be positive in infants with CF until three to five weeks of age. In 2004, the Centers for Disease Control and Prevention released National Guidelines (http://www.guideline.gov) recommending that states consider including CF testing with routine newborn screening.[12] Newborn screening algorithms typically begin with measurement of immuno-reactive trypsinogen (IRT) levels from dried blood spots followed by repeat IRT levels or molecular testing which are then confirmed with sweat chloride testing. Newborn screening often occurs in large state-financed laboratories and presents unique concerns and caveats that are beyond the scope of this paper.

In 2001, the American College of Medical Genetics (ACMG), the American College of Obstetrics and Gynecology (ACOG), and the National Human Genome Research Institute (NHGRI) recommended that physicians offer prenatal/preconception CF carrier testing for all Caucasian couples. They also recommended that testing be made available to individuals and couples of other racial/ethnic backgrounds. In the Northern European Caucasian population, a panel of 25 mutations was estimated to be sufficient to detect 90% of CF carriers. The panel includes the following mutations:

F508del; R334W; G542X; R560T; W128X; R1162X; G551D; 3569delC; 621+1G>T; A455E; N1303K; G85E; R553X; 2184delA; del507; 1898+1G>A; 3849+10kbC>T; I148T; 3120+1G>T; 1078delT; R117H; 2789+5G>A; 1717+1G>T; R347P; and 711+1G>T.

Even though this panel detects a smaller percentage of carriers in racial/ethnic populations other than Caucasian, it was recommended for population-based carrier screening. In 2004, ACMG revised its recommended prenatal/preconception testing mutation panel based on new data available from US population screening. Two mutations, I148T and 1078delT, were dropped from the recommended panel.[13] The first was dropped because further investigations revealed that it was unlikely to be an authentic CF mutation, the second because it did not meet the 0.1% overall frequency in the population, which is the criteria for inclusion in the panel.[13-15] Using this revised panel, the sensitivity for known mutations in the overall US pan-racial/ethnic population is 84%. The mutations in this panel detect 94% of known mutations in Ashkenazi Jewish populations, 88% of known mutations in non-Hispanic Caucasian populations, 65% of known mutations in African American populations, and 49% of known mutations in Asian American populations.[13] Given this racial/ethnic heterogeneity in mutation frequencies it is imperative that accurate information regarding a patient's racial/ethnic heritage be available and considered when reporting residual risk. The residual risk is defined as the risk that an individual is a carrier given a negative test result. Even in the Northern European Caucasian population, the recommended panel does not identify 100% of carriers. The residual risk is determined using the carrier frequency in a given population along with the percentage of carriers detected with any given panel of mutations, and it can vary widely from racial/ethnic group to group. Table 1 lists the residual risks for major racial/ethnic groups frequently encountered in the United States using the revised, ACMG endorsed, 23-mutation panel. Local demographics may require the inclusion of other mutations to increase the sensitivity for specific racial/ethnic groups; fourteen additional mutations are suggested by ACMG for consideration of inclusion in such instances.

Carrier testing of minors is not recommended unless performed as part of prenatal care.[13] Prenatal testing to identify fetuses affected with CF is also possible from specimens obtained from amniocentesis and chorionic villus sampling. Such testing is indicated for couples that are both known carriers. In addition to fetal cell testing, both parents should be tested and

the possibility of maternal cell contamination of the fetal specimen must be ruled out. Like newborn screening, prenatal testing of fetal samples has unique concerns as well as ethical issues that have been addressed in detail at the Human Genome Project: Ethical, Legal, and Social Issues (web site (http://www.ornl.gov/sci/techresources/Human_Genome/elsi/elsi.shtml). Molecular testing for CF mutations is also indicated in cases of male infertility when CBAVD is considered to be a possibility in the presence or absence of other CF related symptoms. For male infertility testing, the inclusion of intron 8 polyT alleles is considered essential.

TEST METHODS

There are currently a number of commercial test methods available for CF testing. Mutation scanning assays (for example, sequencing of all 27 exons) that are capable of detecting any deviation from a standard gene coding sequence will not be discussed here, as they often require significant expertise and resources not available in most routine clinical laboratories. Those methods most applicable to laboratories with limited molecular testing experience detect a limited number, or panel, of the most common CF causing mutations. As discussed earlier, the primary concern with such assays is the fact that none of the current assays detect all known or possible mutations. Mutations included in the ACMG recommendations should be included in every assay and additional racial/ethnic specific mutations may be added to address the most common patient populations. Other factors to consider when choosing a method include vendor expertise and technical service; instrumentation needs; compatibility, cost and flexibility; space requirements; throughput or scalability; turnaround time; reproducibility; precision; laboratory personnel expertise requirements; and reagent and control costs. As of December 2005 the FDA has approved only one commercial CF test method for clinical use. Commercial test methods for CF testing are typically sold as analyte specific reagents (ASRs), whose producers comply with good manufacturing practice. The clinical validity and utility, as well as the analytical performance characteristics of ASRs, must be validated "in house" by each laboratory using such a method. Further review of assay validation will be discussed in the quality assurance section. Commercial test kits for CF testing utilize a variety of methodology strategies. A representative list of commercial CF test methods available is provided in Table 2.

QUALITY ASSURANCE

As early as 1989, the College of American Pathologists established the Molecular Pathology Resource Committee to develop guidelines for clinical laboratories performing molecular diagnostic tests. Other organizations and agencies have also established useful guidelines including the ACMG, New York State Department of Health, CLSI (formerly National Committee for Clinical Laboratory Standards), and the Clinical Laboratory Improvement Amendments of 1988. ASRs do not include controls or FDA validated procedures. Laboratories using ASRs must be certified as capable of performing high complexity tests, write a comprehensive procedure and complete "in-house" validation of performance characteristics. Validation of the entire testing process includes pre- and post-analytical procedures as well as analytic and clinical validation studies.

Pre-analytic steps include collection of patient information such as family history, race/ethnicity, indication for testing, and informed consent (required for all CF genetic tests). If there is a family history it is very helpful to determine whether molecular testing has been done previously and if the proband mutations have been identified. Such information will allow for the most clinically relevant interpretation of results. Gathering this information may require the generation of specialized test requisition forms. Education for patients and healthcare providers is also a key process requiring validation. Such education is necessary to ensure the successful implementation of any genetic test, because such tests require true informed consent and collection of accurate patient information to provide the best possible results. Open lines of communication with laboratory directors and/or genetic counselors can help to facilitate pre-analytic procedures

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Table 1. Estimated carrier risk when molecular testing finds no mutations

Racial/ethnic group	Detection rate*	Carrier frequency†	Post-test residual carrier risk
European American	88%	1 in 25	1 in 208
Ashkenazi Jewish	94%	1 in 25	1 in 417
African American	65%	1 in 65	1 in 186
Hispanic American	72%	1 in 46	1 in 164

* Detection rates are based on the 2004 ACMG recommended 23-mutation panel
† Carrier frequencies are based on those reported by Bobadilla J and others 2002.[3]

and test reporting. In addition to pre-test information and education, general specimen collection processing, transport and storage procedures must also be validated. State-specific laws must be adhered to regarding the storage, archiving, or reuse of genetic specimens.

Analytic sensitivity, specificity, precision, accuracy, and sample stability must be documented with each potential specimen type. If possible, each potential mutation genotype should be included in validation studies. The frequency of rare mutations in the population makes obtaining representative samples for each genotype problematic. A molecular genetics proficiency test program from CAP/ACMG has included material for CF testing since 1995.

Control materials present another hurdle in CF testing. Negative controls containing no target DNA should always be run to rule out amplicon contamination and false positives. The Coriell Institute for Medical Research Cell Repository (http://cimr.umdnj.edu), funded by the National Institutes of Health, has developed an extensive collection of cell lines both for research and for use as control material that includes all of the mutations in the ACMG recommended panel. While highly desirable, it is not economically feasible for laboratories to test individual positive controls for all 23 of the recommended mutations on every run or each day. Rotating controls has been the most common solution to this problem. With this system, control materials containing no DNA (e.g., a water blank), F508del, and a negative (e.g., a DNA sample from a non-carrier of any of the mutations for which the tests are run) are analyzed with every run. In addition, a DNA containing one or two additional mutations from the particular test's menu is included. The particular DNA, or control, is rotated every few runs, such that after a period of time all the mutations being tested for are subjected to quality control (QC) investigation. Clearly, this system is not optimal; should the performance of the test for a given allele or mutation fail or degrade over time, it will not be detected until that specific control material is rotated into the assay. When laboratories adopt this QC strategy, it is critical that every mutation be tested as part of the validation of each new lot of reagents.

Table 2. Commercial molecular cystic fibrosis test systems

Test name	Manufacturer	Mutations detected	Technology used
InnoLiPA CFTR	Innogenetics	36	PCR, reverse hybridization
Elucigene™ CF 29, CF 30	Tepnel	29 or 30	Amplification Refractory Mutation System (ARMS™)
CF V3.0ASR	Abbott/Celera	33	PCR and oligonucleotide ligation assay (OLA®)
Tag-It™ Cystic Fibrosis Kit	TM Bioscience	44	PCR and ASPE
Multicode Plx™	Eragen	29	PCR, ASPE using AEGIS™ technology
Invader™	Third Wave	25	Linear signal amplification
SNP Capture™ mutation screening	Panomics	Screens all 27 exons for mutations	PCR, agarose electrophoresis for Holiday Junction

Note: This is not a comprehensive list of commercially available tests but is intended to be representative of available methods.

Several novel solutions to the problem of adequately controlling multi-allele mutation assays have been developed. One such solution has been developed by the Molecular Controls Project (Sacred Heart Medical Center, Spokane, WA, http://www.molecularcontrols.com), which is currently offering a recombinant control material that contains 32 CF mutations on a set of synthetic oligonucleotides that can serve as a comprehensive control material. This material is inserted into each run at the PCR step and is available for the Abbott/Celera OLA® (CF32 Control™) and the Tm Biosciences Tag-It™ (CF40+4 Control™) platforms. Maine Molecular Quality Controls, Inc. (http://www.mmqci.com) offers a multi-allele control (CF Panel I) that is manufactured in a blood-mimic matrix, and thus is intended to control the entire assay. The Centers for Disease Control and Prevention has recently convened a series of workshops and meetings and assembled a group of experts, the Quality Control Materials for Genetic Testing Group, to attempt to build a national, ongoing process for the provision of control materials for molecular genetic testing. A manuscript describing the group's deliberations has been recently published.[16] Among the resources this group is making available to the molecular genetics community as a result of the Centers for Disease Control and Prevention's efforts is a website with resource information, and a National QC Materials Coordinator. Both of these are likely to prove invaluable for laboratories carrying out tests of increasing complexity.

REFLEX TESTING
Reflex testing for intron 8 polyT alleles is appropriate during diagnostic and carrier testing if the R117H mutation is found. F508del mutations should trigger reflex testing of I507del, I506V, and F508C polymorphisms to rule out cross reactivity unless these variants have previously been demonstrated not to interfere with the test method.

REPORTING CONSIDERATIONS
Confidentiality of results is critical for genetic testing because of patient concerns regarding insurability and discrimination. Accurate reporting of genetic tests requires translating technical results into understandable information for medical providers and the patient. Significant misconceptions are common among patients regarding the concept and implications of carrier status or recessive inheritance of genetic disease. A negative result is often mistakenly interpreted as having no risk.

Report Forms
Each report should contain a description of the testing method and a list of the detectable mutations. Reports should also include sensitivity for various racial/ethnic populations based on allele frequencies and list populations for which this information is not known. Reports must clearly state that the test does not detect all mutations, and should provide the race/ethnicity-specific residual risk if available. Laboratories must also include an FDA-required disclaimer on reports for tests using ASRs, whether obtained commercially or produced "in-house". The required language is: "This test was developed and its performance characteristics determined by [laboratory name]. It has not been cleared or approved by the US Food and Drug Administration".[17]

It should be emphasized that in very few instances is it appropriate for a genetic test to be simply reported as positive or negative without further explanation or interpretation. Reports must include information collected prior to testing such as the indication for testing, patient's reported race/ethnicity, and family history with proband mutation information if provided, as this information has implications for test interpretation. Reports should use concise terms such as "homozygous", "heterozygous", "compound heterozygous", and "no mutations found". Further explanatory information is essential to convey the full meaning and implications of test results for utilization by non-geneticist healthcare providers.

What does a negative result mean?
The residual risks calculated based on a patient's racial/ethnic background should be calculated and explained thoroughly in the report. This residual risk calculation must take into account the specific testing scenario such as carrier testing, newborn screening, and potential diagnosis of CF or CBAVD because the calculation may be different for each scenario. Such calculations require accurate information regarding the patient's racial/ethnic background, which is essential for estimating carrier frequencies and test sensitivity (based on allele frequencies) for a given test mutation panel. Family history, if collected with sufficient confidence of its accuracy, must also be considered when reporting residual risk for a patient with a negative test result. Scenario-specific Bayesian calculations combining the pre- and post-testing probabilities should be provided in such instances. An example of a Bayesian calculation is provided in Figure 1. Reports for carrier testing should include, if known, the test results of the reproductive partner and include calculation of the couple's combined risk of having a child affected with CF. Carrier test reporting is simplified somewhat because de novo mutations and uniparental disomy are very rarely implicated in CF.

What does a positive test result mean?
Positive or unusual test reports should include a recommendation of referral to a medical geneticist or genetic counselor for follow-up and further counseling. They should also include recommendations such as confirmatory tests or the testing of a reproductive partner. Education of healthcare

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Figure 1. Example Bayesian calculation

What is the carrier risk for a Caucasian American patient (II-2) that has tested negative for CFTR mutations using the ACMG recommended panel who has a cousin (II-1) that is affected with CF?

We must first determine the patient's pretest carrier risk. Assuming that everyone except the cousin is asymptomatic for CF, we know that the cousin's parents (I-1 and I-2) must be carriers. There is a 50% chance that the patient's parent (I-3) who is a sibling of the cousin's parent is a carrier and therefore a 25% risk that the patient is a carrier. To calculate the post-test risk we must now take the test sensitivity into account. If the test sensitivity is 88%, there is a 12/100 risk that the patient tested negative because the disease-causing mutation was not included in the testing panel.
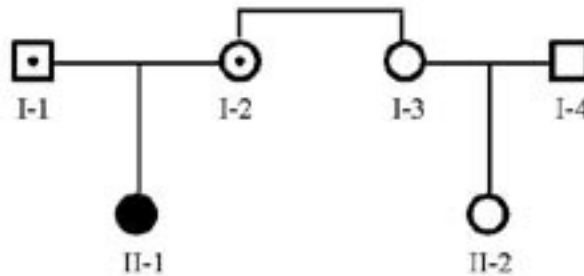
There are two potential outcomes, either the patient is a carrier or the patient is not a carrier.

	Probability the patient is a carrier	Probability the patient is not a carrier
Prior probability	1/4	3/4
Conditional [test] probability	12/100	1
Joint probability	1/4 x 12/100 = 12/400	3/4 x 1 = 3/4 or 300/400
Posterior probability	$\frac{12/400}{12/400 + 300/400} = 1/26$	$\frac{300/400}{12/400 + 300/400} = 25/26$

The post-test probability that the patient is not a carrier is one in 26, so while we cannot say the patient is definitely not a carrier we have reduced the patient's carrier risk from 1/4 to 1/26. If the cousin has had molecular testing performed and the mutations have been identified, test interpretation may be different. If the mutations identified in the cousin are included in laboratory's test panel and they are not found in the patient's sample, the above calculations are not necessary and the report should state that the patient is not a carrier of the mutations identified in the affected family member. The patient's residual risk is then the same as that found for any random individual from that ethnic group (Table 1).

personnel is critical to ensure that the reporting of any results be accurately conveyed to the healthcare provider and ultimately to the patient. Laboratory contact information should be provided to enhance the communication process.

CONCLUSION

Molecular testing for cystic fibrosis mutations may well represent the introduction of genetic testing to many laboratorians unfamiliar with this realm of diagnostics along with its challenges, pitfalls, and benefits. Given the research efforts and progress seen in genetic testing, it clearly requires significant effort to keep abreast in this rapidly evolving field. This brief introduction to the challenges in pre- and post-analytic procedures, as well as the analytic methods, serves as a starting point for laboratorians considering implementation of clinical molecular CF testing. Given the education and counseling considerations, it should be clear that such test implementation represents a significant departure from other traditional laboratory test methods and will require a "paradigm shift" that includes various healthcare personnel from many areas of an institution.

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