

# Current Challenges in Cystic Fibrosis Screening

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• **Content.**—This article gives an overview of the symptoms and mutations associated with classic and atypical cystic fibrosis (CF). Current testing methods for mutation detection in CF are discussed.

**Objectives.**—Review testing for CF, including American College of Medical Genetics and American College of Obstetrics and Gynecology guidelines and recommendations regarding population screening for CF. Describe symptomatic and mutational differences between patients with classic CF and atypical CF, including monosymptomatic conditions such as congenital bilateral absence of the vas deferens, idiopathic pancreatitis, and chronic sinusitis. Explain the concern about predicting the phenotypic expression of the condition from the genotype. Discuss the challenges of CF testing, including the preanalytic, analytic, and postanalytic phases. List the current methods for detecting CF transmembrane conductance regulator gene mutations, specifying the advantages and disadvantages of

each. Describe the basic patient information necessary for laboratories to provide accurate risk assessments, such as ethnicity and family history, and reasons for the test being conducted (carrier or affected status).

**Results.**—The technical challenges of detecting the 25 recommended mutations are being met by commercially available reagents. Challenges remain for the preanalytic and postanalytic phases. Only with accurate patient information can laboratories provide specific risk reductions on the basis of a negative genetic test result.

**Conclusion.**—As health care providers become better informed about the recommendations for CF testing and laboratories continue to increase the sensitivities of their assays, patients will benefit from increased screening efficiency and accuracy. This will allow affected individuals to receive prompt and effective treatment and carriers to enjoy an expanded number of reproductive options.

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Classic cystic fibrosis (CF) is a progressive disorder characterized by frequent respiratory infections and pancreatic insufficiency. Mortality is related to respiratory insufficiency in 90% of the cases. The median age of diagnosis is 7 months, with 66% of the affected persons diagnosed by 1 year of age. CF is no longer considered just a childhood illness, as the life expectancy of affected individuals has increased to a median of 30 years. This is because vigorous treatment of the condition by optimizing nutrition, daily chest physiotherapy, use of bronchodilators, nonsteroidal anti-inflammatory drugs, oxygen, deoxyribonuclease, antibiotics, and lung transplantation will prolong life. Although gene therapy may be helpful, it is not a permanent treatment.

The CF transmembrane conductance regulator (*CFTR*) gene was cloned in 1989.<sup>1</sup> It is located on chromosome 7q31.2. The *CFTR* protein is an epithelial ion and water transporter. It is expressed in the apical membrane of epithelial exocrine cells. More than 1000 different disease-causing mutations are distributed throughout the *CFTR* gene in its membrane-spanning and adenosine triphosphate-binding domains.<sup>2</sup> The molecular consequences of

various *CFTR* mutations are classified in the following 5 ways: (1) no synthesis (resulting from nonsense, frameshift, and splice junction mutations), (2) block in processing (missense and amino acid deletion mutations), (3) block in regulation (missense mutations), (4) altered conductance (missense mutations), and (5) reduced synthesis (missense and alternate splicing mutations).<sup>3</sup> Various mutations may result in little or no functional *CFTR* protein at the apical cell membrane. Mutations are classified as severe or mild, depending on the effect on the functional protein. Generally, severe mutations result in no synthesis or blocked processing, whereas mild mutations show altered conductance or reduced synthesis. However, genotypes vary in severity and are influenced by additional genetic or environmental factors.

The most commonly reported mutation is the F508del, responsible for 70% of the mutated alleles in white individuals. It is caused by a 3-bp deletion in the *CFTR* gene, resulting in the loss of amino acid 508 of the *CFTR* protein. This common mutation is considered severe, with homozygosity resulting in both pulmonary and pancreatic disease.

CF is an autosomal recessive disorder. Affected individuals typically have inherited one mutated allele from each parent. Uniparental disomy, the inheritance of 2 mutated alleles from one parent and none from the other, has rarely been reported as a cause of CF. Maternal uniparental disomy 7 screening is recommended only in CF cases when the affected child also has an unusually short stature.<sup>4</sup> Therefore, couples who have had one affected offspring are likely to be CF carriers with a 25% recurrence

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risk in each future pregnancy. Healthy siblings of an affected person have a two-thirds chance of being CF carriers. Second- and third-degree relatives of affected individuals have a 50% and 25% risk of being carriers, respectively.

### DIAGNOSTIC TESTING

DNA diagnostic testing should be offered to individuals who have symptoms of CF. The symptoms include meconium ileus (a bowel obstruction present in 5%–10% of affected infants), failure to thrive, large foul-smelling yellow stools, recurrent pneumonia, enlarged spleen and liver, thick sputum, pancreatic insufficiency, diabetes, and infertility.<sup>5</sup> Mutation detection can be useful for diagnostic confirmation of affected individuals as well as for documentation of the specific familial *CFTR* mutations. Prenatal diagnosis is available for couples with a previously affected child as well as for those who may learn they are carriers.<sup>6</sup> Prenatal diagnosis may be performed by chorionic villus sampling in the first trimester or by amniocentesis in the second or third trimester. If the specific *CFTR* mutations the couple carry can be identified, then direct DNA mutation analysis can be performed. If the couple has an affected child who does not have 2 detectable mutations, prenatal diagnosis may still be possible through DNA linkage analysis.<sup>7</sup> Some persons may undergo prenatal diagnosis to prepare for the birth of an affected infant, while others may choose pregnancy termination. CF DNA diagnostic testing has been added to the newborn screening programs in 8 states. In fact, about 10% of affected infants in the United States are now diagnosed at birth either by prenatal diagnosis (3%) or newborn screening (7%).<sup>8</sup> Evidence suggests that treating the condition from birth may improve disease management and lung function in affected individuals.<sup>9,10</sup>

### CARRIER TESTING

Since the cloning of the *CFTR* gene, carrier testing has been recommended for the reproductive partners and relatives of affected individuals as well as for the relatives of known CF carriers. In 1997, a Consensus Development Conference on Cystic Fibrosis, convened by the National Institutes of Health, recommended CF population screening.<sup>11</sup> A second National Institutes of Health conference was convened in 1998 to develop physician and laboratory guidelines before carrier screening was implemented. We will review many of the difficult issues involved in CF population carrier screening. There are more than 1000 different documented *CFTR* mutations, most of them very rare familial mutations. CF disease incidence and mutation detection vary significantly with patient ethnicity. A wide spectrum of clinical variability exists within the disorder, ranging from infertility or mild pulmonary symptoms in healthy individuals to life-threatening bowel obstruction and severe pulmonary compromise in others. *CFTR* genotypes show variable expression, making it difficult, if not impossible, to predict the phenotype for some genotypes. Limited numbers of appropriately trained genetic counselors are available nationally to address the multitude of issues arising from mass-scale CF carrier screening.

In 2001, the American College of Obstetrics and Gynecology and the American College of Medical Genetics (ACMG) recommended that CF carrier screening be extended to white and Ashkenazi Jewish individuals who

are expecting or planning a pregnancy.<sup>12</sup> They also recommended that CF carrier screening be made available to others. CF population screening is currently designed to identify at-risk couples prior to the birth of an affected child. This allows couples who are identified as CF carriers to choose whether or not to conceive biological children and provides them the opportunity to pursue prenatal diagnosis or preimplantation genetic diagnosis. A multiethnic standard screening panel of 25 *CFTR* mutations with a US population frequency equal to 1 in 1000 or greater is recommended. Additional *CFTR* mutations, mild or severe, will be added to the panel if they reach this frequency. Depending on the methodology used, reflex tests for the benign variants I506V and I507V and the mild variant F508C may need to be performed to distinguish between these and *CFTR* mutations. Furthermore, when the R117H mutation is identified, the 5T reflex test must be performed. Near the 3' region of intron 8 in the splice acceptor site, there is a tract of 5, 7, or 9 thymidine residues. Usually 7 or 9 thymidines are present, which allows normal gene splicing and production. When 5 thymidine residues are present—the 5T variant—abnormal splicing occurs, which leads to the skipping of exon 9 and the presence of only 5% of the normal *CFTR* messenger RNA. If the 5T allele is identified, a family study must be performed to determine whether the 5T is in trans (opposite chromosome) or in cis (same chromosome) to the R117H mutation. If it is in cis, the individual is a classic CF mutation carrier. If it is in trans, the individual carries a mild *CFTR* mutation and is unlikely to have offspring with classic CF. The 5T variant is present on 5% of the alleles in the general population and is associated with male infertility. Since the purpose of population screening is to identify CF carriers and not infertility, the ACMG recommends that this variant be tested only in the presence of R117H.

### ATYPICAL CF

Although classic CF is diagnosed in infancy or early childhood, atypical cases of CF may be diagnosed in adults with mild monosymptomatic diseases such as congenital bilateral absence of the vas deferens (CBAVD), chronic pancreatitis, sinusitis, diffuse bronchiectasia, acute and recurrent pancreatitis, and nasal polyps.<sup>13</sup> Individuals with atypical CF phenotypes are often compound heterozygotes carrying one severe and one mild *CFTR* mutation, or they are homozygous for the 5T variant. Several cases of compound heterozygotes (F508del/R347H) have been reported. Affected males have CBAVD and respiratory tract symptoms, whereas an affected female was described with mild pulmonary symptoms, paranasal polypsis, and a normal sweat chloride test.<sup>14</sup> A study of patients with chronic sinusitis showed an increased frequency of compound heterozygosity of the M470V polymorphism in combination with a severe mutation.<sup>13</sup> Individuals with atypical CF should be given several examinations, including sweat chloride testing; DNA mutation analysis for mild and severe *CFTR* alleles, including the 5T variant; and nasal potential difference testing to evaluate the physiologic functioning of the *CFTR*. Although patients with chronic rhinosinusitis, allergic bronchopulmonary aspergillosis, and asthma are more likely to have a non-CF genetic or environmental reason for their conditions, there remains an increased occurrence of *CFTR* mutations.<sup>15,16</sup>

CBAVD causes 2% to 6% of male infertility and is believed to represent a genital form of CF.<sup>17,18</sup> Nearly 75% of men with CBAVD have at least one detectable common *CFTR* mutation.<sup>19</sup> About 20% of men with CBAVD have a single detectable *CFTR* mutation, while another 20% have 2 common *CFTR* mutations, usually one classic and one mild. The most frequent genotype, found in 33% of patients with CBAVD, is the presence of one *CFTR* mutation and one 5T variant. Approximately 1% of individuals with CBAVD have two 5T alleles. In an intensive study of 327 French men with CBAVD, the entire *CFTR* coding/flanking sequences were scanned. Seventy-one percent carried 2 *CFTR* mutations, and 16% had 1 identifiable mutation, leaving only 13% with no detectable *CFTR* mutation.<sup>20</sup> Men with CBAVD with 2 mutations had either a severe/mild (88%) or a mild/mild (12%) mutation combination, whereas individuals with classic CF had 2 severe (88%) or a severe/mild (12%) mutation combination. Therefore, individuals with CBAVD should be offered CF carrier screening prior to undergoing assisted reproductive techniques such as microsurgical epididymal spermatozoa aspiration for the purpose of intracytoplasmic sperm injection.<sup>21</sup> Of equal importance, their reproductive partner should undergo *CFTR* mutation screening.<sup>22</sup> If she is a CF carrier, extensive mutation analysis should be performed on the individual with CBAVD. If both partners have identifiable mutations, intracytoplasmic sperm injection with preimplantation genetic diagnosis will increase their chances for healthy biological offspring. The couple should also be counseled regarding their other reproductive options, including adopting and using a sperm or egg donor who is not a CF carrier.

Some *CFTR* mutations appear to have a variable expression, depending on their association with additional cis-acting mutations. For example, the I148T mutation has been detected at a higher frequency in CF carriers than in affected individuals. A second mutation, 3199delC, in cis with I148T, appears to be necessary to create a severe mutation.<sup>23</sup> As stated earlier, R117H is considered a mild mutation unless it is found in cis with the 5T variant.

Genotype-phenotype correlations are strongest for pancreatic status and weakest for pulmonary function.<sup>24,25</sup> Mutations leading to classic CF and pancreatic insufficiency are usually more severe. Predicting the effect that a mild mutation will have on *CFTR* function is more difficult. Pulmonary status appears to be influenced by additional genetic and environmental modifiers. The clinical condition of affected individuals often does not closely mirror their specific genotype. Yet the ability to secrete chloride may be closely associated with phenotype.<sup>26</sup> Affected individuals with a poor prognosis, on the basis of a low mean forced expiratory volume and a poor pulmonary radiologic score, show a lower apical *CFTR* protein and a lower chloride secretion than do affected individuals with an apical *CFTR* protein and a higher chloride secretion.

#### SWEAT CHLORIDE AND NASAL POTENTIAL DIFFERENCE

Obtaining 2 sweat chloride values on different occasions that are >60 mEq/L has been the "gold standard" for diagnosing CF since 1959; however, the sensitivity of sweat chloride testing is reportedly only 90%.<sup>27</sup> Some atypically affected individuals have a normal or borderline sweat chloride value. In such cases, it may be diagnostically useful to perform *CFTR* mutation analysis as well as

to measure transepithelial bioelectric properties. The measurement of nasal potential differences provides an additional means of diagnosing CF.<sup>28</sup> CF patients show a markedly negative nasal potential difference across respiratory epithelia. The mean nasal potential difference in affected persons is -46 mV, whereas the mean for unaffected individuals is -19 mV. Several case reports describe compound heterozygosity and abnormal nasal potential differences in patients with lung disease but normal sweat chloride values.<sup>29,30</sup> Therefore, if mild CF symptoms are present with normal or borderline sweat chloride levels, nasal potential difference testing and *CFTR* mutation analysis may confirm *CFTR* dysfunction. If 2 mutations are not found and sweat chloride measurements are in the normal or borderline range, abnormal nasal potential difference measurements on 2 separate days can serve as evidence of *CFTR* dysfunction. Most individuals with CBAVD will have normal nasal potential difference values, suggesting normal *CFTR* activity.<sup>31</sup> Occasionally, males with CBAVD will have abnormal nasal potential difference values, abnormal sweat chloride values, and mild CF symptoms.

An accurate diagnosis of CF can be important, enabling individuals with severe lung disease to obtain aggressive therapy in specialized centers. Yet genotype should not be used as the sole diagnostic criterion of CF.<sup>32</sup> A healthy compound heterozygote (F508del/R117H) infant was diagnosed with CF, which led to emotional, financial, and employment distress in her parents. Follow-up sweat chloride testing, bronchoscopy, and nasal potential difference values were all in the normal range, and the infant did not have the 5T variant. The infant was thought to have a sufficient amount of functional *CFTR* protein.<sup>33</sup> Therefore, asymptomatic or mildly monosymptomatic patients and their families may benefit from counseling regarding the extensive phenotypic variability in CF. This may enable such individuals to avoid unnecessary aggressive treatment and the psychosocial implications that surround a diagnosis with CF.

### CURRENT TESTING CHALLENGES

#### Pretesting

The first challenge in offering the *CFTR* mutation screen to the general population is the clear and accurate relaying of information to the patients. The discussion should include an explanation of CF, its inheritance pattern, the risks and benefits of learning one's carrier status, and the limitations and strengths of the test. Health care providers should determine whether there is a family history of CF and the couple's ethnicity, as this will affect their carrier risks. The couple should understand that the DNA test cannot detect all mutations within the gene; therefore, a negative mutation screen reduces, but does not eliminate, the risk of being a carrier. However, DNA testing is the only way to determine one's CF carrier status. If mutations are found in both members of a couple, testing is straightforward and definitive for a woman's pregnancy or other family members. CF patient brochures are available through several laboratories and the American College of Obstetrics and Gynecology.

Informed consent is recommended for all genetic testing and is required by New York and several other states. Informed consent documents that disease-specific concepts, risk of employment and insurance discrimination, and ac-

curacy of testing methods have been reviewed and that the patient has had the opportunity to have questions answered. The consent forms are kept with the patient's medical or laboratory records. Informed consent often includes the option to allow or refuse the use of residual samples for research.

When ordering CF DNA testing, the laboratory should be provided with the following information: whether the test is being ordered to determine affected or carrier status, whether there is a family history of CF, and the patient's ethnicity. By providing the reason for CF testing, the laboratory will know whether to look for 1 mutation (CF carrier) or 2 mutations (classic or atypical CF such as CBAVD). If there is a positive family history, the relationship of the affected family member to the patient and the specific mutations involved should be indicated. Ethnicity is important, since the carrier rate, mutation frequencies, and detection rate for the panel vary for different populations. This information will enable the laboratory to calculate accurate patient-specific risks.

### Technologies

The number of mutations to be analyzed makes CF testing a high mutation spectrum test. Traditionally, laboratories have developed their own assays, and the numbers and types of mutations have varied significantly between laboratories. Following the ACMG/American College of Obstetrics and Gynecology recommendations for population screening, commercial manufacturers are developing *CFTR* reagents as analyte-specific reagents that conform to the recommended testing panel. Several different platforms are available, and more are being developed. In choosing an assay platform, considerations include laboratory work flow, technical time or automation potential, and test volume. Most analyte-specific reagents require polymerase chain reaction (PCR) and post-PCR allele detection. Analyte-specific reagents vary from one to several PCRs needed for the panel. They differ in the number of steps and the amount of technical time for post-PCR. Ease of test interpretation is another consideration, especially as the volume of CF testing increases. Cost considerations include labor, reagents, and instrumentation. To lower costs, laboratories may develop their own methods, although development costs initially are higher. Some analyte-specific reagents, or laboratory-developed tests, include more than the 25 recommended mutations, but according to the CF guidelines of the ACMG, these laboratories should clarify that testing for additional mutations results in only a small increase in detection rate. Summaries of some commercially available methods are provided in the paragraphs that follow.

Reverse-line blot assays are available from several companies. One PCR is required for these assays; then, probes linked to a filter paper are hybridized.<sup>34,35</sup> Multiple washes are necessary for an optimal signal-noise ratio. Washes increase the manual labor time, and the results are read visually. The main advantage to line blots is that no expensive instrumentation is required.

The oligo ligation assay requires one PCR and then a ligation step that necessitates an additional enzyme reaction and incubation. Ligation products are separated by gel or capillary electrophoresis.<sup>35-37</sup> Advantages include minimal technical time, automation capability, and electronic data storage.

Array technologies are being developed for *CFTR* test-

ing. Array assays may be solid-based assays with probes linked to chips<sup>38,39</sup> or liquid-based assays with probes linked to beads in a solution.<sup>40,41</sup> These arrays use natural DNA bases or chemically modified bases that may increase signal and specificity. PCR products hybridize to probes complementary to the mutation or wild-type sequence on the array. An advantage to arrays is flexibility in adding additional ethnic-specific mutations.

Melting curve analysis uses separate reactions for each mutation but in a high-throughput, highly automated format. After PCR, a 384-well plate is placed in a fluorescent monitoring instrument that requires 10 minutes for analysis. Although multiple PCRs will increase reagent costs, these costs may be offset by minimal technical time for post-PCR handling and rapid detection.

Amplification refractory mutation scanning is also known as allele-specific amplification using sequence-specific primers.<sup>42,43</sup> The 3' end of the PCR primer must match exactly to allow primer extension. Targets are amplified with a wild-type and a mutant-specific primer in separate reactions. PCR products are analyzed by electrophoresis, and the presence or absence of amplicons determines the genotype. Advantages include minimal equipment and flexibility.

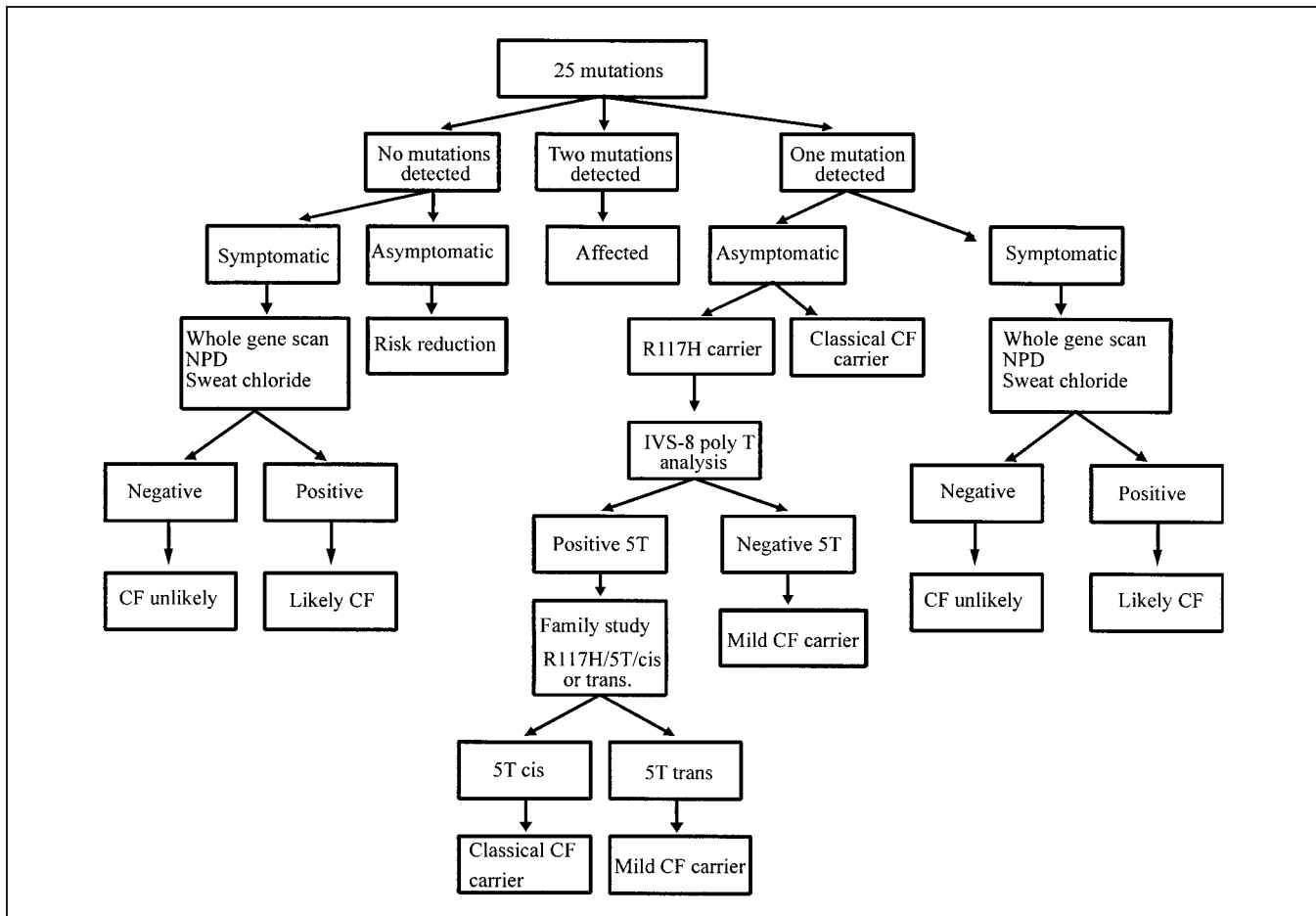
### Mutation Scanning

Technologic advances will increase the availability of whole-gene scanning. The entire coding region and intron/exon boundaries of the gene are amplified and analyzed to search for any alteration. Since *CFTR* mutations outside the recommended panel are rare, most patients possessing such a mutation will be heterozygous (except in cases of consanguinity). Whole-gene scanning methods include single-strand conformational polymorphisms,<sup>44,45</sup> denaturing gradient gel electrophoresis,<sup>46-48</sup> and denaturing high-performance liquid chromatography.<sup>48,49</sup> These methods rely on conformation of heteroduplex formation or melting domains of the PCR product. If an alteration is detected, the portion of the gene containing the alteration is sequenced to confirm the mutation. The reported sensitivity of these methods ranges from 75% to 100%. Some of these detection rates have been determined with relatively small sample sizes, since large numbers of samples previously characterized by complete gene sequencing are difficult and expensive to obtain. Without such studies, the sensitivity of the technology is only estimated.

### Test Validation

Since DNA analysis for CF is considered a "high-complexity" test, the laboratory must be certified as such under the Clinical Laboratory Improvement Act. Food and Drug Administration-approved *CFTR* test kits are not available yet. Instead, in-house validation is required, usually by comparing sample results with a different technology and/or results from another laboratory. Commercially available technologies are usually sold under an analyte-specific reagent designation. An analyte-specific reagent is provided as the reagent only, without controls or procedures. The performing laboratories are responsible for developing and evaluating assays with analyte-specific reagents equivalent to an in-house-developed assay.

Obtaining characterized samples for the validation and/or evaluation of *CFTR* assays is a challenge, particularly for laboratories that have not previously performed *CFTR* testing. However, a sample *CFTR* panel is available for 22



Algorithm for cystic fibrosis (CF) testing. This algorithm depicts a testing pattern for symptomatic and asymptomatic individuals. It also depicts the circumstances when further testing for additional mutations is warranted. NPD indicates nasal potential difference.

of the 25 mutations from Coriell Cell repositories, a National Institutes of Health–funded, nonprofit collection of characterized cell lines. The proposed use, along with an agreement not to share samples (unless approved by the repository), must be sent with the order request.

### CFTR Testing Algorithms

Laboratories may categorize and offer *CFTR* testing in several ways. These categories may include diagnostic testing versus carrier testing, atypical CF versus classic CF, and high-risk testing (for individuals with a positive family history) versus population screening. A separate test may be available for only the F508del, if this mutation has been documented, versus the full mutation panel. The mutation panel is used to identify mutations in affected individuals or their families or in individuals suspected of having CF when sweat testing is inconclusive or unable to be performed. Although no recommendations have been made by the ACMG for newborn CF screening, some states may supplement immunoreactive trypsin tests with the 25-mutation panel.<sup>49,50</sup> States may decide to add ethnic-specific mutations appropriate for the population. When performing carrier testing for individuals with a family history of CF, the familial mutations should be documented if possible. The specific mutations tested by the laboratory should be examined to ensure that the familial mutation is tested in its panel. The 25-mutation panel is rec-

ommended for population screening when no family history is present. Carrier screening also includes reproductive partners of patients with atypical CF such as CBAVD.

Atypical CF patients, identified by conditions such as CBAVD, should be tested for the 25 mutations plus the IVS-8 variant. In contrast to testing IVS-8 variants only when the R117H mutation is present (as recommended for population carrier screening), the IVS-8 variant is routinely analyzed in every CBAVD patient whether or not R117H is present, since the 5T variant is present in a high percentage of CBAVD men. A flow chart for CF testing is shown in the Figure.

### RESULT INTERPRETATION

Laboratories can provide an accurate risk assessment if sufficient information is given. The laboratory must be provided with the indication for testing (symptomatic individual, family history of CF, or population screen).

Diagnostic testing requires the laboratory to identify 2 mutations. If only one is found, further testing may be warranted, depending on symptoms and ethnicity. For example, if no mutation is found in a symptomatic infant who is white or of Ashkenazi Jewish descent, the chance that the infant has CF is low. If one mutation is detected, searching for the potential second mutation may be warranted. Since the mutation detection rate is lower for pop-

**Table 1. Estimated Risk for a Child to Have Cystic Fibrosis (CF) After a Negative Mutation Test in One Parent\***

Ethnic Background	Chance for Child to Have CF Before a Test	Detection Rate, %	Chance for Child to Have CF After a Normal Test
Ashkenazi Jewish	1 in 2800	97	1 in 93 000
White American	1 in 2500	90	1 in 25 000
African American	1 in 15 000	69	1 in 48 000
Hispanic American	1 in 8000	57	1 in 18 000
Asian American	1 in 32 000	30	1 in 46 000

**Table 2. Estimated Risk for Individuals With CBAVD to Carry at Least One CFTR Mutation After a Negative Mutation Result\***

Ethnic Group	Pretest Risk	Detection Rate, %	Posttest Risk
Ashkenazi Jewish	3:4	97	~1 in 44
White American	3:4	90	~1 in 13
African American	3:4	69	~1 in 4
Hispanic American†	3:4	57	~1 in 3
Asian American	3:4	.. .‡	.. .

\* CBAVD indicates congenital bilateral absence of the vas deferens; CFTR, cystic fibrosis transmembrane conductance regulator.

† This is a pooled set of data and requires additional information to accurately predict risk for specific Hispanic populations.

‡ Ellipses indicate no data available.

ulations exclusive of whites, detecting one mutation in such populations increases the possibility of CF more than it would in populations of white individuals, where it may be a coincidental finding.

For population screening, the risk reduction after a negative result depends on the patient's ethnicity. Table 1 shows how a negative screen reduces the risk of having an affected child for various ethnicities.<sup>51</sup> If a mutation is found in one member of a couple, then that individual's partner should be tested. If the partner is positive, the couple should undergo genetic counseling and be offered several options, mainly preimplantation genetic diagnosis, adoption, egg or sperm donor (who is not a CF carrier) usage, and prenatal diagnosis. Additionally, carriers should be encouraged to share this information with family members, including the specific mutation, so they can be tested. By testing negative for the familial mutation, one's carrier risk is reduced to his/her ethnic-specific risk. Without knowing the familial mutation, only the ethnic-specific risk is modified. If one partner is positive and the other negative for the population screen, some individuals may desire testing for additional mutations. This is not recommended by the ACMG, since the detection rate is minimally increased by testing for a greater number of mutations and will not give much more assurance that a mutation is not present.

For CBAVD testing, the mutation panel will be tested with the IVS-8, since the poly(5T) variant is often associated with CBAVD. Even if no mutations are found in these men, their reproductive partners should be tested, since men with CBAVD may still have undetected CFTR mutations. The risk of CBAVD patients carrying a CFTR mutation before and after a negative mutation test is shown in Table 2.

Testing for additional mutations is sometimes warranted, but the extent to which laboratories should go to find a mutation is not determined. Some laboratories offer a greater number of mutations than the standard panel, but

a negative test still leaves uncertainties. Mutation scanning of the entire gene may show additional mutations, but even mutation scanning cannot guarantee that any alteration can be detected. If a mutation is found, the additional information can be included in the risk assessment, but if no mutation is identified, uncertainty will still exist. A mutation scan is not recommended for the general population, since it may detect DNA alterations that have not been described in CF; therefore, it would be unknown whether the alteration was a true mutation or a harmless variant. This might cause additional worry. However, certain cases may warrant additional mutation testing or scanning. If the reproductive partner of a man with CBAVD is a CF carrier and 2 mutations are not found in the individual with CBAVD, additional mutation testing is recommended, as the couple's offspring will be at high risk for CF. Identifying a mutation in both will clarify the risk for CF and expand their reproductive options. Expanded mutation analysis should also be considered in children with CF symptoms and one identified mutation, especially with a positive sweat chloride test. This is particularly relevant for individuals other than whites, as their mutation detection rate is low.

The challenges in CFTR mutation testing range from pretest evaluation to analytic issues to proper result interpretation. The expanded research into the CFTR gene and classic and atypical CF adds to the challenges of appropriate testing. Knowledge of certain CFTR mutations may indicate a severe or mild phenotype; however, predicting the severity of the disease from the genotype is still not practical. As we gain a better understanding of the CFTR gene and its function, we can improve CF testing by technologic advances in the laboratory, but the issues surrounding CF testing and interpretation remain complex.

#### References

1. Kerem B, Rommens JM, Buchanan A, et al. Identification of the cystic fibrosis gene: genetic analysis. *Science*. 1989;245:1073-1080.
2. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*. 1989;245:1060-1072.
3. Cystic Fibrosis Mutation Database [database on-line]. Toronto, Ontario, Canada: Cystic Fibrosis Genetic Analysis Consortium; 2002. Available at: <http://www.genet.sickkids.on.ca/cftr>. Updated March 14, 2003.
4. Hannula K, Lipsanen-Nyman M, Kristo P, et al. Genetic screening for maternal uniparental disomy of chromosome 7 in prenatal and postnatal growth retardation of unknown cause. *Pediatrics*. 2002;109:441-448.
5. Oliveira MC, Reis FJ, Monteiro AP, Penna FJ. Effect of meconium ileus on the clinical prognosis of patients with cystic fibrosis. *Braz J Med Biol Res*. 2002;35:31-38.
6. Lemna WK, Feldman GL, Kerem B, et al. Mutation analysis for heterozygote detection and the prenatal diagnosis of cystic fibrosis. *N Engl J Med*. 1990;322:291-296.
7. Feldman GL, Lewiston N, Fernbach SD, et al. Prenatal diagnosis of cystic fibrosis by using linked DNA markers in 138 pregnancies at 1-in-4 risk. *Am J Med Genet*. 1989;32:238-241.
8. US National Screening Status Report [database on-line]. Austin, Tex: National Newborn Screening and Genetics Resource Center; 2003. Available at: <http://genes-r-us.uthsesa.edu/resources/newborn/screenstatus.htm>. Updated March 18, 2003.

9. Cystic Fibrosis Foundation. Living with CF resources page. Available at: <http://www.cff.org/living-with-cf/>. Accessed September 10, 2002.
10. Merelle ME, Schouten JP, Gerritsen J, Dankert-Roelse JE. Influence of neonatal screening and centralized treatment on long-term clinical outcome and survival of CF patients. *Eur Respir J*. 2001;18:306–315.
11. NIH Consensus Development Conference Statement. Genetic testing for cystic fibrosis. April 14–16, 1997. *Arch Intern Med*. 1999;159:1529–1539.
12. Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med*. 2001;3:149–154.
13. Noone PG, Knowles MR. “CFTR-opathies”: disease phenotypes associated with cystic fibrosis transmembrane regulator gene mutations. *Respir Res*. 2001;2:328–332.
14. Kosztolányi G, Malik N, Rutishauser M. Mild CF in a  $\Delta F508/R347H$  compound heterozygote woman: does the manifestation of this genotype differ in the two sexes. *Clin Genet*. 1996;49:103–105.
15. Raman V, Clary R, Siegrist KL, Zehnbauer B, Chatila TA. Increased prevalence of mutations in the cystic fibrosis transmembrane conductance regulator in children with chronic rhinosinusitis. *Pediatrics*. 2002;109:E13.
16. Babinski D. Rhinitis in patients with cystic fibrosis. *Otolaryngol Pol*. 2002;56:23–30.
17. Jequier AM, Ansell ID, Bullimore NJ. Congenital absence of the vasa deferentia presenting with infertility. *J Androl*. 1985;6:15–19.
18. Holsclaw DS, Lober B, Jockin H, Schwachman H. Genital abnormalities in male patients with cystic fibrosis. *J Urol*. 1971;106:568–574.
19. Chillón M, Casals T, Mercier B, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. *N Engl J Med*. 1991;332:1475–1480.
20. Claustres M, Guittard C, Bozon D, et al. Spectrum of CFTR mutations in cystic fibrosis and in congenital absence of the vas deferens in France. *Hum Mutat*. 2002;16:143–156.
21. Viville S, Warter S, Meyer J, et al. Histological and genetic analysis and risk assessment for chromosomal aberration after ICSI for patients presenting with CBAVD. *Hum Reprod*. 2000;5:1613–1618.
22. Lewis-Jones, DI, Gazvani MR, Mountford R. Cystic fibrosis in infertility: screening before assisted reproduction. *Hum Reprod*. 2000;15:2415–2417.
23. Rohlf s EM, Zhou Z, Sugarman EA, et al. The I148T CFTR mutation confers a variable phenotype and occurs on multiple haplotypes. *Am J Hum Genet*. 2001;69(suppl):623.
24. Salvatore F, Scudiero O, Castaldo G. Genotype-phenotype correlation in cystic fibrosis: the role of modifier genes. *Am J Med Genet*. 2002;111:88–95.
25. Zielenski J. Genotype and phenotype in cystic fibrosis. *Respiration*. 2000;67:117–133.
26. Ho LP, Samways JM, Porteous DJ, et al. Correlation between nasal potential difference measurements, genotype and clinical condition in patients with cystic fibrosis. *Eur Respir J*. 1997;10:2018–2022.
27. Stewart B, Zabner J, Shuber AP, Welsh MJ, McCray PB Jr. Normal sweat chloride values do not exclude the diagnosis of cystic fibrosis. *Am J Respir Crit Care Med*. 1995;151(3 pt 1):899–903.
28. Alton EW, Currie D, Logan-Sinclair R, Warner JO, Hodson ME, Geddes DM. Nasal potential difference: a clinical diagnostic test for cystic fibrosis. *Eur Respir J*. 1990;3:922–926.
29. Lebecque P, Leal T, Godding V. Cystic fibrosis and normal sweat chloride values: a case report. *Rev Maladies Respir*. 2001;18(4 pt 1):443–445.
30. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr*. 1998;132:563–565.
31. Pradal U, Castellani C, Delmarco A, Mastella G. Nasal potential difference in congenital bilateral absence of the vas deferens. *Am J Respir Crit Care Med*. 1998;158:896–901.
32. Chmiel JF, Drumm ML, Konstan MW, Ferkol TW, Keresmar CM. Pitfall in the use of genotype analysis as the sole diagnostic criterion for cystic fibrosis. *Pediatrics*. 1999;103(4 pt 1):823–826.
33. Lotem Y, Barak A, Mussaffi H, et al. Reaching the diagnosis of cystic fibrosis—the limits of the spectrum. *Isr Med Assoc J*. 2000;2:94–98.
34. Cuppens H, Buyse I, Baens M, Marynen P, Cassiman JJ. Simultaneous screening for 11 mutations in the cystic fibrosis transmembrane conductance regulator gene by multiplex amplification and reverse dot-blot. *Mol Cell Probes*. 1992;6:33–39.
35. Strom CM, Huang D, Buller A, et al. Cystic fibrosis screening using the College panel: platform comparison and lessons learned from the first 20,000 samples. *Genet Med*. 2002;4:289–296.
36. Grossman PD, Bloch W, Brinson E, et al. High-density multiplex detection of nucleic acid sequences: oligonucleotide ligation assay and sequence-coded separation. *Nucleic Acids Res*. 1994;22:4527–4534.
37. Gasparini P, Arbustini E, Restagno G, et al. Analysis of 31 CFTR mutations by polymerase chain reaction/oligonucleotide ligation assay in a pilot screening of 4476 newborns for cystic fibrosis. *J Med Screen*. 1999;6:67–69.
38. Gilles PN, Wu DJ, Foster CB, Dillon PJ, Chanock SJ. Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. *Nat Biotechnol*. 1999;17:365–370.
39. Heller MJ, Forster AH, Tu E. Active microelectronic chip devices which utilize controlled electrophoretic fields for multiplex DNA hybridization and other genomic applications. *Electrophoresis*. 2000;21:157–164.
40. Dunbar SA, Jacobson JW. Application of the Luminex LabMAP in rapid screening for mutations in the cystic fibrosis transmembrane conductance regulator gene: a pilot study. *Clin Chem*. 2000;46:1498–1501.
41. Taylor JD, Briley D, Nguyen Q, et al. Flow cytometric platform for high-throughput single nucleotide polymorphism analysis. *Biotechniques*. 2001;30:661–666, 668–669.
42. Feldmann D, Guittard C, Georges MD, et al. Genetic testing for cystic fibrosis: evaluation of the Elucigene CF20 kit in blood and buccal cells. *Ann Biol Clin (Paris)*. 2001;59:277–283.
43. Bradley LA, Johnson DA, Chaparro CA, Robertson NH, Ferrie RM. A multiples ARMS test for 10 cystic fibrosis (CF) mutations: evaluation in a prenatal CF screening program. *Genet Test*. 1998;2:337–341.
44. Wine JJ, Kuo E, Hurlock G, Moss RB. Comprehensive mutation screening in a cystic fibrosis center. *Pediatrics*. 2001;107:280–286.
45. Ellis LA, Taylor CF, Taylor GR. A comparison of fluorescent SSCP and denaturing HPLC for high throughput mutation scanning. *Hum Mutat*. 2000;15:556–564.
46. Bombieri C, Giorgi S, Carles S, et al. A new approach for identifying non-pathogenic mutations. An analysis of the cystic fibrosis transmembrane regulator gene in normal individuals. *Hum Genet*. 2000;106:172–178.
47. Cremonesi L, Carrera P, Fumagalli A, et al. Validation of double gradient denaturing gradient gel electrophoresis through multigenic retrospective analysis. *Clin Chem*. 1999;45:35–40.
48. De Braekeleer M, Mari C, Verlingue C, et al. Complete identification of cystic fibrosis transmembrane conductance regulator mutations in the CF population of Saguenay Lac-Stain-Jean (Quebec, Canada). *Clin Genet*. 1998;53:44–46.
49. Ravnik-Glavac M, Atkinson A, Glavac D, Dean M. DHPLC screening of cystic fibrosis gene mutations. *Hum Mutat*. 2002;19:374–383.
50. Bobadilla JL, Macek M, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. *Hum Mutat*. 2002;19:575–606.
51. Richards CS, Bradley LA, Amos J, et al. Standards and guidelines for CFTR mutation testing. *Genet Med*. 2002;4:379–391.