[A technical review]

Genetic Testing for Cystic Fibrosis

Comprehensive testing for routine carrier screening, high-risk carrier screening, and diagnosis

Introduction

Cystic fibrosis (CF) is one of the most common genetic diseases in the Caucasian population. Approximately 1 in every 25 Caucasians is a carrier for this recessive condition, and 1 in 2500 is clinically affected.[1-2] Cystic fibrosis can occur in any ethnic group, but the carrier frequency and disease incidence can vary greatly.[1-2] Cystic fibrosis has a broad clinical presentation ranging from chronic lung disease to pancreatic insufficiency, meconium ileus, failure to thrive, and infertility.[1-2]

Cystic fibrosis is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) chloride channel gene on chromosome 7.[1] More than 1300 mutations have been identified in the *CFTR* gene to date; however, the majority of mutations are rare.[3]

Routine Carrier Screening

In 2001, the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) recommended that routine cystic fibrosis carrier screening be offered to all Caucasian couples who are pregnant or considering pregnancy.[4] Moreover, they recommended that carrier screening be made available to other ethnic populations with the understanding that the likelihood of being a CF carrier and the mutation detection rate may be much lower in non-Caucasian populations.[4]

The current pan-ethnic panel recommended by ACOG/ACMG includes 23 mutations for routine carrier screening. This panel includes known CF-causing mutations with an allele frequency of ≥0.1% in CF patients.[5] Table 1 provides the carrier risk rate by racial or ethnic group and the detection rate of the ACOG/ACMG panel of 23 mutations.[2,3,5]

Table 1.— Cystic Fibrosis Carrier Rate and Detection Rate by Racial/Ethnic Group

Racial/Ethnic Group	Carrier Rate[2]	Detection Rate
Ashkenazi Jewish	1/25	97%[2]
Caucasian (non-Hispanic)	1/25	90%[2]
Hispanic American	1/46	72%[5]
African American	1/65	69%[2]
Asian American	1/90	55%[5]

LabCorp offers a panel of 32 of the most common CF mutations (Table 2) appropriate for routine carrier screening—including the 23 mutations recommended by ACOG and ACMG.

Table 2—LabCorp Panel of 32 Common CF Mutations

ΔF508*	ΔI507*	G542X*	G551D*
N1303K*	S549R	R553X*	621+1G>T*
1717-1G>A*	A455E*	R560T*	394delTT
G85E*	R334W*	R347P*	711+1G>T*
3905insT	2184delA*	1078delT	3849+10kbC>T*
3659delC*	2183AA>G	3120+1G>A*	R347H
S549N	3876delA	V520F	2789+5G>A*
1898+1G>A*	R1162X*	W1282X*	R117H*†
*ACOG-/ACMG-recommended mutations			
†5T variant analysis is performed automatically if R117H is positive.			
Polymorphisms F508C, I506V, and I507V are included in this panel to rule out false-positive ΔF508 homozygotes.			

Expanded Mutation Screening

In some clinical situations, there may be a benefit in offering testing with an expanded CF mutation panel. Such situations may include the following[1-3]:

- Diagnostic testing
- Carrier testing in individuals with a family history of CF
- Carrier testing for the partner of a person who is a CF carrier or has CF
- Prenatal diagnosis for a fetus with echogenic bowel in the second trimester
- Carrier screening for a person with non-Caucasian ancestry
- Individuals who request additional mutation information

LabCorp also offers an expanded panel of 70 mutations (Table 3). The detection rate for the 70-mutation panel by ethnic/racial group is represented in Table 4.

Table 3 — LabCorp Expanded Panel of 70 CF Mutations			
G85E*	R347H	G542X*	R560T*
621+1G>T*	2184delA*	3876delA	2183AA>G
E60X	R117C	G330X	1677delTA
Q890X	R117H*†	A455E*	S549N
R1162X*	711+1G>T*	2789+5G>A*	3905insT
2307insA	R75X	G178R	R352Q
1812-1G>A	2869insG	ΔI507*	S549R
W1282X*	1078delT	3120+1G>A*	Y122X
Y1092X	405+3A>C	L206W	S364P
2055del9>A	3120G>A	3199del6	R334W*
ΔF508*	G551D*	N1303K*	1717-1G>A*
3659delC*	A559T	M1101K	406-1G>A
935delA	G480C	2143delT	R1066C
R347P*	V520F	R553X*	394delTT
1898+1G>A*	3849+10kbC>T*	1898+5G>T	S1255X
444delA	ΔF311	Q493X	K710X
G622D	W1089X	D1152H	R1158X
3791delC	S1196X		

*ACOG-/ACMG-recommended mutations

†5T variant analysis is performed automatically if R117H is positive. Polymorphisms F508C, I506V, and I507V are included in this panel to rule out false-positive ΔF508 homozygotes.

Table 4 — CF Mutation Detection Rate: 70 Mutations	
Racial/Ethnic Group	**Detection Rate**
Ashkenazi Jewish[2]	97%
Caucasian (non-Hispanic)[5,6]	91%
African American[6]	81%
Hispanic[5,7]	81%
Native American[6]	81%
Asian[5]	55%

Diagnostic Testing for CF

CF diagnostic testing is appropriate for individuals with a certain or suspected diagnosis of CF. When the familial mutations are known, testing may be performed using the routine or expanded profile if the known mutations are included in those profiles. LabCorp also offers full-gene sequencing for cystic fibrosis through its Correlagen Diagnostics laboratory. ACOG recommendations state that complete analysis of the *CFTR* gene by DNA sequencing is not appropriate for routine carrier screening, but has value in selected diagnostic scenarios, including "patients with cystic fibrosis, a family history of cystic fibrosis, infertile males with congenital bilateral absence of the vas deferens, or a positive newborn screening result when mutation testing using an expanded panel of mutations has a negative result."[3] Full-gene sequencing may be performed, or in the case of a known mutation, a specimen can be tested for the specific mutation. Sequencing for a known mutation requires a result report indicating the family member's mutation.

Cystic Fibrosis 5T Allele (*IVS8-5T*)

It is estimated that the 5T allele is found in approximately 5% of alleles in the population and causes reduced production of functional CF gene (*CFTR*) product.[1,4] The 5T allele is known to modify the expression of the R117H mutation when it is present on the same chromosome as the R117H mutation (in cis).[1-2] In addition, when *IVS8-5T* is found on the chromosome opposite another CF mutation (in trans), or when a person inherits two copies of the 5T allele, the phenotype is highly variable.[1] Clinical symptoms range from having no symptoms to male patients with congenital bilateral absence of the vas deferens (CBAVD) or individuals with mild respiratory symptoms.[1]

Reflex testing for the 5T variant is appropriate and recommended when the R117H mutation is positive.[1,3,4] Testing for 5T may also be of value in evaluating individuals with male infertility or atypical cystic fibrosis.[1,3] If the patient is positive for the 5T variant, family studies are recommended to determine cis or trans status. In the absence of an R117H mutation or male infertility, the prognostic and diagnostic value of 5T testing is low and does not represent standard of care.[3,5] Apart from R117H reflex testing and male infertility, 5T analysis is not recommended.[3,5]

Diagnostic Testing for CBAVD

LabCorp offers full-gene sequencing for CBAVD through its Correlagen Diagnostics laboratory. ACOG recommendations state that complete analysis of the *CFTR* gene is appropriate in specific instances, including that of "infertile males with congenital bilateral absence of the vas deferens."[3] Full-gene sequencing may be performed, or in the case of known mutations, a specimen can be tested for those specifically. Sequencing for a known mutation requires a result report indicating the family member's mutation.

Cystic Fibrosis Profile, DNA Analysis 480533

CPT 83891; 83900; 83901(x14); 83909; 83912; 83914(x32)
Test Includes Detection of 32 CF mutations
Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.
Specimen 7 mL whole blood in a lavender-top (EDTA) tube or yellow-top (ACD) tube, or two buccal swabs in a LabCorp buccal swab kit
Storage Instructions Maintain specimen at room temperature.
Use Help determine affected or carrier status for the 32 most common CF mutations.
Limitations This assay detects as many as 90% of all the mutations that cause cystic fibrosis. Within specific ethnic groups, there may be higher or lower detection efficiency.
Methodology Polymerase chain reaction (PCR) and oligonucleotide ligation assay (OLA)

Cystic Fibrosis, Fetal Analysis 480541

CPT 83891; 83900; 83901(x14); 83909; 83912; 83914(x32)
Test Includes Detection of 32 CF mutations
Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.
Specimen 10 mL amniotic fluid or 20 mg chorionic villus sample (CVS) (submission of maternal blood is required for fetal testing)in a sterile plastic conical tube or two confluent T25 flasks
Storage Instructions Maintain specimen at room temperature.
Use Determine carrier or affected status for the 32 most common cystic fibrosis mutations.
Limitations This assay detects as many as 90% of all the mutations that cause cystic fibrosis. Within specific ethnic groups, there may be higher or lower detection efficiency.
Methodology Polymerase chain reaction (PCR) and oligonucleotide ligation assay (OLA)

Cystic Fibrosis Profile, DNA Analysis and 5T Allele Genotyping 480555

CPT 83891; 83900; 83901(x14); 83909; 83912; 83914(x35)

Test Includes Detection of 32 CF mutations, plus 5T/7T/9T variants.

Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.

Specimen 7 mL whole blood in a lavender-top (EDTA) tube or yellow-top (ACD) tube, or two buccal swabs in a LabCorp buccal swab kit

Storage Instructions Maintain specimen at room temperature.

Use Determine affected or carrier status for the 32 most common CF mutations; determine the presence of the 5T allele.

Limitations This assay detects as many as 90% of all the mutations that cause cystic fibrosis. Within specific ethnic groups, there may be higher or lower detection efficiency. Routine screening for the 5T allele in the cystic fibrosis (CF) gene as part of standard CF carrier screening is not recommended since the goal of carrier screening is to identify couples at risk for having children with cystic fibrosis. The 5T allele is associated primarily with male infertility or atypical symptoms, although many individuals are asymptomatic. Analysis of the 5T allele may be a useful adjunct to the standard CF panel of 32 mutations in patients with male infertility or idiopathic pancreatitis. Genetic counseling is recommended for any person testing positive for the 5T variant. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients.

Methodology Polymerase chain reaction (PCR) and oligonucleotide ligation assay (OLA)

Cystic Fibrosis, 5T Allele Genotyping 480970

CPT 83891; 83898; 83909; 83912; 83914(x3)

Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.

Specimen 7 mL whole blood in a lavender-top (EDTA) tube or yellow-top (ACD) tube, or two buccal swabs in a LabCorp buccal swab kit

Storage Instructions Maintain specimen at room temperature.

Use Detect congenital bilateral absence of the vas deferens (CBAVD) (OMIM 277180); refine the clinical impact of an identified R117H CF mutation

Limitations Routine screening for the 5T allele in the cystic fibrosis (CF) gene as part of standard CF carrier screening is not recommended since the goal of carrier screening is to identify couples at risk for having children with cystic fibrosis. The 5T allele is associated primarily with male infertility or atypical symptoms, although many individuals are asymptomatic. Analysis of the 5T allele may be a useful adjunct to the standard CF panel of 32 mutations (test 480533) in patients with male infertility or idiopathic pancreatitis. Genetic counseling is recommended for any person testing positive for the 5T variant. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients.

Methodology Polymerase chain reaction (PCR) plus oligonucleotide ligation assay (OLA)

Cystic Fibrosis Expanded Profile 480700

CPT 83891; 83892(x2); 83900; 83901(x19); 83912; 83914(x70)

Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.

Specimen 7 mL whole blood in a lavender-top (EDTA) tube or yellow-top (ACD) tube, or two buccal swabs in a LabCorp buccal swab kit

Storage Instructions Maintain specimen at room temperature, or refrigerate at 4°C.

Use LabCorp offers an expanded mutation panel of 70 mutations for cystic fibrosis for diagnostic testing and for testing in those persons whose family history or ethnicity requires testing for less common mutations.

Limitations This assay detects as many as 91% of all the mutations that cause cystic fibrosis. Within specific ethnic groups, there may be higher or lower detection efficiency.

Methodology DNA analysis of the *CFTR* gene is performed on the Tm Bioscience/Luminex Universal Array Platform using primer extension chemistry. Multiplex PCR amplifies DNA fragments containing the mutations. Primer extension then generates a biotin-labeled product that hybridizes to complementary, bead-immobilized probes to permit flow-sorted detection of both normal and mutation sequences.

Cystic Fibrosis Expanded Fetal Profile 480760

CPT 83891; 83892(x2); 83900; 83901(x19); 83912; 83914(x70)

Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.

Specimen 10 mL amniotic fluid or 20 mg chorionic villus sample (CVS) (submission of maternal blood is required for fetal testing) in a sterile plastic conical tube or two confluent T-25 flasks for fetal testing

Storage Instructions Maintain specimen at room temperature, or refrigerate at 4°C.

Use LabCorp offers an expanded mutation panel of 70 mutations for cystic fibrosis for prenatal testing, diagnostic testing, and for testing in those persons whose family history or ethnicity require testing for less common mutations.

Limitations This assay detects as many as 91% of all the mutations that cause cystic fibrosis. Within specific ethnic groups, there may be higher or lower detection efficiency.

Methodology DNA analysis of the *CFTR* gene is performed on the Tm Bioscience/Luminex Universal Array Platform using primer extension chemistry. Multiplex PCR amplifies DNA fragments containing the mutations. Primer extension then generates a biotin-labeled product that hybridizes to complementary, bead-immobilized probes to permit flow-sorted detection of both normal and mutation sequences.

Cystic Fibrosis (CF): *CFTR* (Full Gene Sequencing). . 252763

CPT 83894; 83898 (x90); 83909 (x60); 83912

Test Includes This test covers all coding nucleotides of gene *CFTR*, plus at least two and typically 20 flanking intronic nucleotides upstream and downstream of each coding exon, covering the conserved donor and acceptor splice sites, as well as typically 20 flanking nucleotides in the 5′ and 3′ UTR. In addition, the TG tract adjacent to the 5T/7T/9T polymorphism near the intron 9 acceptor site and the intronic region covering position c.3718-2477 are also analyzed (note that intron 9 is referred to as intron 8, if exon 6a/b nomenclature is used, and that c.3718-2477 is commonly known as 3849+10kb).

Special Instructions For all Correlagen tests, specimens must be accompanied by a completed consent form.

Specimen 2 mL whole blood in a lavender-top (EDTA) tube; DNA is accepted. (Call Correlagen at 781-647-0604 for DNA collection information.)

Use Confirm a clinical diagnosis of CF; predict risk of CF in blood relatives

Limitations This method does not reliably detect mosaic variants; large deletions; large duplications, inversions, or other rearrangements; or deep intronic variants. It may be affected by allele dropout, it may not allow determination of the exact numbers of T/A or microsatellite repeats, and it does not allow any conclusion as to whether two heterozygous variants are present on the same or on different chromosome copies.

Cystic Fibrosis (CF): CFTR (Known Mutation). . . . 252760

CPT All specimens are forwarded to Correlagen for testing, reporting, and billing.

Special Instructions For all Correlagen tests, specimens must be accompanied by a completed consent form.

Test Includes This test covers all coding nucleotides of gene CFTR, plus at least two and typically 20 flanking intronic nucleotides upstream and downstream of each coding exon, covering the conserved donor and acceptor splice sites, as well as typically 20 flanking nucleotides in the 5′ and 3′ UTR. In addition, the TG tract adjacent to the 5T/7T/9T polymorphism near the intron 9 acceptor site and the intronic region covering position c.3718-2477 are also analyzed (note that intron 9 is referred to as intron 8, if exon 6a/b nomenclature is used, and that c.3718-2477 is commonly known as 3849+10kb).

Specimen 2 mL whole blood in a lavender-top (EDTA) tube; DNA is accepted. (Call Correlagen at 781-647-0604 for DNA collection information.)

Storage Instructions Maintain specimen at room temperature.

Use Confirm a clinical diagnosis of CBAVD; predict risk of CF in blood relatives

Limitations This method does not reliably detect mosaic variants; large deletions; large duplications, inversions, or other rearrangements; or deep intronic variants. It may be affected by allele dropout, it may not allow determination of the exact numbers of T/A or microsatellite repeats, and it does not allow any conclusion as to whether two heterozygous variants are present on the same or on different chromosome copies.

Congenital Bilateral Absence of the Vas Deferens (CBAVD) (Full Gene Sequencing) 252766

CPT 83891; 83898; 83909; 83912; 83914(x3)

Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.

Specimen 7 mL whole blood in a lavender-top (EDTA) tube or yellow-top (ACD) tube, or two buccal swabs in a LabCorp buccal swab kit

Storage Instructions Maintain specimen at room temperature.

Use Detect congenital bilateral absence of the vas deferens (CBAVD) (OMIM 277180); refine the clinical impact of an identified R117H CF mutation

Limitations Routine screening for the 5T allele in the cystic fibrosis (CF) gene as part of standard CF carrier screening is not recommended since the goal of carrier screening is to identify couples at risk for having children with cystic fibrosis. The 5T allele is associated primarily with male infertility or atypical symptoms, although many individuals are asymptomatic. Analysis of the 5T allele may be a useful adjunct to the standard CF panel of 32 mutations (test 480533) in patients with male infertility or idiopathic pancreatitis. Genetic counseling is recommended for any person testing positive for the 5T variant. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients.

Methodology Polymerase chain reaction (PCR) plus oligonucleotide ligation assay (OLA)

Congenital Bilateral Absence of the Vas Deferens (CBAVD) (Known Mutation) 252770

CPT 83891; 83898; 83909; 83912; 83914(x3)

Special Instructions A completed Cystic Fibrosis Screening Questionnaire must accompany specimens. Call 800-345-4363 to request forms.

Specimen 7 mL whole blood in a lavender-top (EDTA) tube or yellow-top (ACD) tube, or two buccal swabs in a LabCorp buccal swab kit

Storage Instructions Maintain specimen at room temperature.

Use Detect congenital bilateral absence of the vas deferens (CBAVD) (OMIM 277180); refine the clinical impact of an identified R117H CF mutation

Limitations Routine screening for the 5T allele in the cystic fibrosis (CF) gene as part of standard CF carrier screening is not recommended since the goal of carrier screening is to identify couples at risk for having children with cystic fibrosis. The 5T allele is associated primarily with male infertility or atypical symptoms, although many individuals are asymptomatic. Analysis of the 5T allele may be a useful adjunct to the standard CF panel of 32 mutations (test 480533) in patients with male infertility or idiopathic pancreatitis. Genetic counseling is recommended for any person testing positive for the 5T variant. This procedure may be considered by Medicare and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients.

Methodology Polymerase chain reaction (PCR) plus oligonucleotide ligation assay (OLA)

References

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