

## Physician's Guideline: Cystic Fibrosis Mutation Analysis

### Genetics and Disease Expression

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations in the *cystic fibrosis transmembrane conductance regulator* (*CFTR*, also known as *ABCC7*) gene. Over 1,700 *CFTR* mutations have been described. The most common mutation, F508del (formerly  $\Delta$ F508), accounts for approximately 70–80% of mutant alleles in affected individuals. The remaining alleles are highly heterogeneous, with fewer than 20 mutations occurring at a global frequency above 0.1%. Certain mutations, however, may reach higher frequencies within specific populations due to founder effects among certain ethnic, religious, or geographic groups. In the United States, the estimated pan-ethnic carrier frequency is about 1 in 29, with roughly 30,000 individuals affected nationwide.

*CFTR* mutations are functionally classified as either “**classic**” (**severe**)—encompassing classes I, II, III, and VI—or “**non-classic**” (**mild**)—encompassing classes IV and V. Severe mutations typically result in absent or minimal *CFTR* protein function, while mild mutations produce residual or partial *CFTR* activity. The clinical spectrum of CF is highly variable and depends on the specific mutations present, and the influence of modifier genes and environmental factors.

**Classic CF** typically results from two severe mutations, either in the homozygous state (e.g., F508del/F508del) or as compound heterozygotes (e.g., F508del/G551D or R553X/G542X). This form typically presents in infancy (before one year of age) and involves multiple organ systems. Common manifestations include chronic pulmonary disease, exocrine pancreatic insufficiency (present in > 95% of cases), liver disease (3–5%), elevated sweat chloride levels (> 80 mEq/L), meconium ileus (~20%), and male infertility due to congenital bilateral absence of the vas deferens (CBAVD).

By contrast, **CFTR-related disorders** (CFTR-RDs), which account for approximately 10% of cases, are typically associated with mild or mixed (mild+severe) mutations in homozygous (e.g., R1070W/R1070W) or compound heterozygous (e.g., F508del/R177H-9T or F508del/D1152H) combinations. Individuals with CFTR-RDs often retain pancreatic sufficiency (70–80%), are diagnosed later in life (typically after age 10), have lower sweat chloride levels, lack meconium ileus, and exhibit milder pulmonary disease.

Nonetheless, substantial genotype-phenotype variability exists, even among individuals with identical *CFTR* genotypes. For example, individuals carrying D1152H in combination with a CF-causing mutation such as F508del may present with a broad clinical spectrum, ranging from isolated CBAVD to CF with pancreatic sufficiency but severe pulmonary disease. Similarly, 2–3% of patients homozygous for classic CF mutations are pancreatic sufficient at diagnosis, though many eventually progress to pancreatic insufficiency or an atypical CF phenotype. Even siblings or twins with identical *CFTR* genotypes can exhibit variable disease severity, highlighting that *CFTR* genotype alone is not a reliable predictor of clinical outcome.

Newborn screening (NBS) for CF has been implemented in most U.S. states and is based on measuring immunoreactive trypsinogen (IRT) levels in dried blood spots. This is a screening rather than a diagnostic test; elevated IRT levels alone cannot differentiate between infants with classic CF, those with milder or atypical forms of the disease, or unaffected carriers. A definitive diagnosis requires correlation with clinical findings, sweat chloride testing, and molecular genetic analysis.

Almost all individuals with classic CF exhibit elevated sweat chloride concentrations after the newborn period. While most CF patients will have common *CFTR* mutations identified, the detection rate will vary depending on the testing method used and the ethnic, geographic, and population background of individuals tested. Furthermore, considering the high number of known *CFTR* mutations, even the most comprehensive *CFTR* mutation panels will not detect all possible disease-causing variants.

## Test Method

The **Luminex xTAG® Cystic Fibrosis 39 kit v2** is approved by the U.S. Food and Drug Administration to screen for 39 mutations and 4 variants in the *CFTR* gene associated with CF. The panel includes the 23 mutations/variants recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG)\*, plus 16 other mutations (Table 1). This panel provides approximate *CFTR* mutation detection rates of 90.5% in North American Caucasians, 73.8% in Hispanic Americans, 67.5% in African Americans, 48.9% in Asian Americans, and 94.0% in Ashkenazi Jews.

**Table 1:** Interpretation of *CFTR* mutations/variants detected by the Luminex xTAG® CF 39 kit v2

Mutation (Legacy Name)	cDNA Nomenclature	Protein Change	Mutation Type / Effect	Clinical Significance
ΔF508	c.1521_1523delCTT	p.Phe508del	In-frame deletion	CF-causing (classic CF)
G542X	c.1624G>T	p.Gly542Ter	Nonsense	CF-causing
G551D	c.1652G>A	p.Gly551Asp	Missense (gating defect)	CF-causing (targeted by Ivacaftor)
W1282X	c.3846G>A	p.Trp1282Ter	Nonsense	CF-causing
N1303K	c.3909C>G	p.Asn1303Lys	Missense	CF-causing
621+1G>T	c.489+1G>T	—	Splice site	CF-causing
1717-1G>A	c.1585-1G>A	—	Splice site	CF-causing
R553X	c.1657C>T	p.Arg553Ter	Nonsense	CF-causing
R1162X	c.3484C>T	p.Arg1162Ter	Nonsense	CF-causing
3849+10kbC>T	c.3718-2477C>T	—	Splice alteration	Mild CF or CFRD
2789+5G>A	c.2657+5G>A	—	Splice defect	CF-causing
2184delA	c.2052delA	p.Lys684AsnfsTer38	Frameshift	CF-causing
3659delC	c.3528delC	p.Lys1177SerfsTer9	Frameshift	CF-causing
1898+1G>A	c.1766+1G>A	—	Splice defect	CF-causing
3120+1G>A	c.2988+1G>A	—	Splice defect	CF-causing
R334W	c.1000C>T	p.Arg334Trp	Missense (channel defect)	CF-causing (variable severity)
R347P	c.1040G>C	p.Arg347Pro	Missense	CF-causing
A455E	c.1364C>A	p.Ala455Glu	Missense (processing defect)	CF-causing (mild)
G85E	c.254G>A	p.Gly85Glu	Missense (folding defect)	CF-causing
R560T	c.1679G>C	p.Arg560Thr	Missense	CF-causing

Mutation (Legacy Name)	cDNA Nomenclature	Protein Change	Mutation Type / Effect	Clinical Significance
711+1G>T	c.579+1G>T	—	Splice defect	CF-causing
1078delT	c.946delT	p.Cys316ValfsTer12	Frameshift	CF-causing
I148T	c.443T>C	p.Ile148Thr	Missense	Non-CF-causing (benign or linked to complex allele)
3849+4A>G	c.3717+4A>G	—	Splice site alteration	Mild / variable CF
R117H	c.350G>A	p.Arg117His	Missense	Variable — mild CF or CFRD (depends on polyT)
2789+5G>A	c.2657+5G>A	—	Splice defect	CF-causing
296+1G>A	c.164+1G>A	—	Splice defect	CF-causing
3659delC	c.3528delC	p.Lys1177SerfsTer9	Frameshift	CF-causing
3120+1G>A	c.2988+1G>A	—	Splice defect	CF-causing
3905insT	c.3773_3774insT	p.Leu1258PhefsTer7	Frameshift	CF-causing
W1282X	c.3846G>A	p.Trp1282Ter	Nonsense	CF-causing
Y1092X	c.3276C>A	p.Tyr1092Ter	Nonsense	CF-causing
S549N	c.1646G>A	p.Ser549Asn	Missense	CF-causing
S549R	c.1645A>C	p.Ser549Arg	Missense	CF-causing
G551S	c.1651G>A	p.Gly551Ser	Missense	CF-causing
D1152H	c.3454G>C	p.Asp1152His	Missense	Mild / variable CFTR dysfunction
R117C	c.349C>T	p.Arg117Cys	Missense	Mild / variable CFTR dysfunction
R1070W	c.3208C>T	p.Arg1070Trp	Missense	Mild / variable CF
1717-1G>A	c.1585-1G>A	—	Splice defect	CF-causing
W1282X	c.3846G>A	p.Trp1282Ter	Nonsense	CF-causing

### General Limitations of Test

Results from this test should not be used as the sole basis for clinical diagnosis or patient management. Individuals undergoing testing should receive comprehensive genetic counseling to discuss the significance of the results, residual risks, potential uncertainties, and the reproductive or medical options available to them and their families. This assay detects only a subset of the more than 1,700 known *CFTR* mutations and variants; therefore, a “wildtype” result does not exclude the presence of other *CFTR* mutations or variants. Use of *CFTR* genotype alone to predict disease prognosis is not recommended, as there is considerable clinical variability both among different *CFTR* mutations and among patients with identical genotypes. The variants I506V, I507V, and F508C are considered benign polymorphisms but may cause false-positive calls for I507del or F508del with this assay. Additionally, polymorphisms or mutations within primer or probe binding regions may result in incorrect homozygosity assignments. Confirmatory sequencing should be considered in consultation with genetic counseling. Because NBS programs routinely identify affected infants, a negative result in an unaffected newborn does not provide information about

parental carrier status. Therefore, *CFTR* carrier screening should continue to be offered to women who are planning a pregnancy or are currently pregnant.

### Homozygous or Compound Heterozygous, Classic (Severe) Mutations

Homozygosity or compound heterozygosity with classic mutations results in classic CF. Parental testing is recommended for compound heterozygotes and for homozygotes. If the two mutations are on the same parental *CFTR* gene, they are said to be in *cis*; if each mutation is on a different parental *CFTR* gene, they are said to be in *trans*. When the two mutations are in *cis*, CF may not be confirmed, and the search of another allelic mutation, located in *trans* with the other two, should continue. All homozygous mutations detected should be confirmed by sequencing except for I507del and F508del. For I507del and F508del homozygotes, only sequence if an I506V, I507V, or F508C variant is present.

### Heterozygous, Classic (Severe) Mutation

The presence of one classic mutation in *CFTR* suggests that the individual is a carrier; however, CF cannot be excluded due to the possibility that an additional *CFTR* mutation not targeted by the assay may be present. Absence of a mutation in one or both alleles does not preclude the presence of *CFTR* mutations/variants in the patient's specimen.

### R117H Mutation

The R117H mutation must occur in *cis* (on the same chromosome) with the poly-thymidine 5T (or occasionally 7T) variant to function as a classic CF mutation. For individuals with R117H as the sole mutation detected, CF is unlikely but cannot be excluded due to the possibility that an additional *CFTR* mutation, not targeted by the assay, may be present. The T-tract (5T/7T/9T) polymorphism in intron 8 can modulate the phenotype associated with R117H. The amount of aberrant *CFTR* with missing exon 9 increases with decreasing length of the tract; therefore, the 5T variant produces the highest proportion of non-functional *CFTR* protein, whereas 9T is considered benign. Alone, R117H does not cause sufficient malfunction of the *CFTR* protein to contribute to classical CF disease; however, when 5T is present in *cis* with R117H, R117H acts more like a classic CF mutation with incomplete penetrance. When R117H-5T occurs on one chromosome and is coupled with a classic CF mutation on the other chromosome (e.g., F508del/R117H-5T compound heterozygote), the child may have an elevated or borderline sweat test, mild to moderate lung disease, pancreatitis, and male infertility (i.e., pancreatic sufficient CF). By contrast, when R117H is in *trans* with 5T (or in *cis* or *trans* with 7T or 9T) it acts as a mild CF mutation and when coupled with a classic mutation produces a variable phenotype, including individuals with normal or borderline sweat tests, late-onset, pancreatic sufficient CF, CBAVD alone (esp. F508del/R117H-5T*trans* or F508del/R117H-7T), or no disease at all (esp. F508del/R117H-9T). Accordingly, ACMG/ACOG guidelines indicate that only when R117H is present in *cis* with the 5T should the individual be identified as a CF carrier. Testing of the parents of R117H/5T or R117H/7T positive compound heterozygote infants can be used to determine *cis* vs *trans* positioning of R117H relative to the 5T or 7T variants. F508del invariably segregates with the 9T allele so testing of parents of F508del/R117H 9T/5T or 9T/7T compound heterozygote infants is not recommended. Table 2 indicates some of the clinical interpretations for genotype combinations involving R117H.

**Table 2.** Clinical interpretations involving R117H, other *CFTR* mutations and the 5T/7T/9T variants.

Allele 1	Allele 2	Variants	Clinical Interpretation
R117H	None detected	5T, 7T, 9T any combination	Diagnosis of CF unlikely, but cannot exclude
R117H	F508del or other severe CF mutation	5T included	Consistent with diagnosis of CF (when 5T variant in <i>cis</i> with R117H)
R117H	F508del or other severe CF mutation	7T included	Diagnosis of CF possible (when 7T variant in <i>cis</i> with R117H), but variable phenotype
R117H	F508del or other severe CF mutation	9T/9T	Diagnosis of CF unlikely. Possibility of CF-related disorder (e.g., CBAVD in males)
R117H	Mild CF mutation (i.e., non-classic)	5T or 7T included	Variable phenotype from mild disease to no disease (depends on <i>cis</i> or <i>trans</i> position of R117H relative to T variant)
R117H	Mild CF mutation (i.e., non-classic)	9T/9T	Diagnosis of CF unlikely, but cannot exclude

### No Mutations Detected

When no mutations are detected for both alleles, the diagnosis of CF is unlikely but cannot be excluded due to the possibility that a *CFTR* mutation not targeted by the assay may be present. The approximate carrier residual risk in an individual following a negative *CFTR* mutation test is as follows:

Ethnicity <sup>a</sup>	Risk Prior to Testing	Approx. Risk After Negative <i>CFTR</i> Mutation Analysis
Ashkenazi Jewish	1/24	1/400
North American Caucasian	1/25	1/208
Hispanic American	1/46	1/164
African American	1/65	1/186
Asian American	1/94	1/184

<sup>a</sup> Risk assessments based on ethnicity assuming no family history of CF<sup>4</sup>

### References

1. Patrick R, et al. Applying cystic fibrosis transmembrane conductance regulator genetics and *CFTR*<sub>2</sub> data to facilitate diagnoses. *J Pediatrics* 2017; 181; S27–S32.e1
2. Egan ME. Genetics of Cystic Fibrosis: Clinical Implications. *Clin Chest Med* 2016; 37(1):9-16.
3. Castellani C, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros* 2008; 7(3):179–96.
4. ACOG Committee on Genetics Opinion. Update on carrier screening for cystic fibrosis. *Obstet Gynecol* 2005; 106:1465-8.
5. ACOG Committee on Genetics Opinion No. 486. Update on carrier screening for cystic fibrosis. *Obstet Gynecol* 2011; 117:1028-31.
6. Salvatore F, Scudiero O, Castaldo G. Genotype-phenotype correlation in cystic fibrosis: The role of modifier genes. *Amer J Med Genet* 2002; 111(1): 88-95.
7. Strom CM, et al. Cystic fibrosis testing 8 years on: Lessons learned from carrier screening and sequencing analysis. *Genet Med* 2011; 13:166-72.
8. McGarry ME, et al. Detection of disease-causing *CFTR* variants in state newborn screening programs. *Pediatr Pulmonol* 2023; 58(2):465-74. doi: 10.1002/ppul.26209.