

# Physician Insert: Cystic Fibrosis 139-Variant Assay

FOR IN VITRO DIAGNOSTIC USE

## Genetic Testing and Cystic Fibrosis

Cystic fibrosis (CF) is a chronic disease that affects multiple organ systems, in particular the pulmonary and digestive systems. It is the most common life-threatening autosomal recessive disorder in the United States and results from inheriting a defective copy of the cystic fibrosis transmembrane conductance receptor (*CFTR*) gene from each parent that contains genetic mutations<sup>1,2</sup>. Typically CF individuals are diagnosed through newborn screening programs and confirmed with sweat chloride testing by the age of two<sup>3</sup>. To date, over 1,900 variants in the *CFTR* gene have been identified; however, only a relatively small subset of those variants has been clinically and functionally verified and determined to cause cystic fibrosis<sup>4</sup>. A variant (sometimes called a “mutation” when it causes disease) is a genetic change that is identified as different from the normal (or “wild-type”) reference sequence to which it is being compared. *CFTR* variants can be disease-causing, of varying clinical consequence, benign, or of unknown significance. Those that are of varying clinical consequence may cause CF only under certain circumstances or be associated with CF-related conditions.

## What is the Life Expectancy of Patients with Cystic Fibrosis in the United States?

The length of time and quality of life an individual can live with CF is highly dependent on many different factors including the severity of the disease and the timing of initiation of treatment. It is important to note that not all *CFTR* mutations will result in severe disease. Many people have a mild case of CF, while others can have moderate or severe cases. Data from the CF Foundation Patient Registry, which tracks health statistics from patients treated at CF Foundation-accredited care centers, shows that more than 47% of all people with CF in the US are 18 years or older and the current overall median survival is 38.3 years<sup>5</sup>.

## Epidemiology of Cystic Fibrosis

CF is one of the most common autosomal recessive genetic disorders<sup>6,7</sup>. It has a disease incidence estimated at one in 2,000 to 4,000 live births and a prevalence of approximately 30,000 individuals in the US population<sup>8</sup>. CF occurs in different ethnic and racial populations at various frequencies: one in 3,000 Caucasians; one in 9,200 Hispanic Americans; one in 10,900 Native Americans; one in 15,000 African Americans; and one in 31,000 Asian Americans<sup>8,9</sup>.

## Genetic Testing and Carrier Screening for Cystic Fibrosis

Carrier testing can be used to determine if someone has a genetic variant in his or her *CFTR* gene. Testing is limited to those variants known to be disease-causing. If both parents have a disease-causing variant, then the child has a one in four chance of inheriting both genes and thus developing the disease. Carrier detection rates are dependent on the individual’s race and ethnicity because many mutations are observed with only certain ethnic groups. More than 10 million Americans are carriers of one mutation of the CF gene. Current estimates of *CFTR* mutation carrier frequency by ethnicity in the US, based on a cohort of 364,890 individuals referred for carrier testing with no family history of CF are provided in [Table 1](#).

Table 1 General Cystic Fibrosis Mutation Carrier Frequency in Different Ethnic Groups in the US<sup>10</sup>

Ethnic Group	Observed Carrier Frequency
African American	1 in 84
Ashkenazi Jewish	1 in 29
Asian	1 in 242
Caucasian	1 in 28
Hispanic	1 in 59
Jewish	1 in 32
Middle Eastern	1 in 91
Native American	1 in 70
South Asian	1 in 118
Other Ethnicity	1 in 111
Other Ethnicity: > 1 Ethnicity	1 in 34
Other Ethnicity: Part African American	1 in 56
Other Ethnicity: Part Caucasian	1 in 32
Other Ethnicity: Part Hispanic	1 in 51
Not provided	1 in 37
All individuals	1 in 38

## Genetic Testing Panels

CF testing for genetic mutations can vary greatly between different laboratories and depends on the specific test used by the laboratory. Some limit their coverage to the 23 CF pan-ethnic variants recommendation by the 2004 American College of Medical Genetics (ACMG)<sup>11</sup> and 2011 American College of Obstetricians and Gynecologists (ACOG)<sup>12</sup>, while others include additional common and rarer variants, which are found in more ethnically diverse populations<sup>2,5,10,13</sup>. The variants included in the ACMG recommended panel were originally selected based on their prevalence in the general U.S. population and known association with moderate to severe disease.

The clinical validity of those variants included in the Cystic Fibrosis 139-Variant Assay was based on information collected and published by the CFTR2 Project<sup>14,15</sup>. The CFTR2 Project links to the original Cystic Fibrosis Mutations Database (now called CFTR1) which is devoted to the collection of variants identified in the *CFTR* gene for the international CF research community. CFTR2 is an international collaboration between cystic fibrosis researchers, clinicians, and registries working towards the goal of categorizing all variants found in a database of 39,696 patients with CF according to their disease-causing status: disease-causing, mutations of varying clinical consequence (MVCC), mutations of unknown significance, and non-CF causing (i.e., benign or neutral)<sup>14,15</sup>. The classification of these variants is based on clinical data (i.e., sodium chloride levels, lung function, and pancreatic function), in vitro functional studies (i.e., CFTR protein synthesis, maturation, expression, function, and chloride conductance), and penetrance studies (using apparently healthy and fertile fathers of CF patients to study any *CFTR* variants occurring on the allele not transmitted to their affected sons)<sup>14</sup>. As of September 2013, the CFTR2 Project has identified over 160 variants that occur with a frequency of > 0.01% in individuals with CF, of which 134 unique variants (based on nucleotide-level changes, and corresponding to 129 variants in the CFTR2 database) have been classified as CF-causing<sup>14,15</sup>.

## Cystic Fibrosis 139-Variant Assay Features

Testing with the Cystic Fibrosis 139-Variant Assay is performed on DNA extracted from a whole blood specimen. The assay tests for: 134 CF-causing variants; one ACMG recommended panel variant (R117H, classified as a

Mutation of Varying Clinical Consequence, MVCC, by CFTR2); one conditionally reported modifying variant (PolyTG/PolyT); and three conditionally reported benign variants (I506V, I507V, F508C)<sup>16</sup>; for a total of 139 reported variants.

The 134 CF-causing variants correspond to 129 CF-causing variants in the CFTR2 database. The CFTR2 database includes five CF-causing variants for which the same protein level change can arise from two distinct nucleotide changes [e.g., S466X(C>A) and S466X(C>G)]. These five variants are listed according to the amino acid codon in the CFTR2 database (e.g., S466X) while the assay reports each individual variant [e.g., S466X(C>A) and S466X(C>G)]. The list of 139 variants reported by the Cystic Fibrosis 139-Variant Assay is provided in [Table 2](#).

Table 2 Cystic Fibrosis 139-Variant Assay Summary of Variants

[Listed in genomic coordinate order; **Bold**=ACMG-23; *Italics*=Conditionally reported; \*\*=Validated with synthetic samples]

M1V**	T338I**	Q552X	3121-1G>A**
CFTR dele2,3	1154insTC	<b>R553X</b>	3272-26A>G
Q39X**	S341P**	A559T	L1065P**
E60X	R347H	<b>R560T</b>	R1066C
P67L	<b>R347P</b>	R560K**	R1066H
R75X	R352Q	1811+1.6kb A>G**	L1077P**
<b>G85E</b>	1213delT**	1812-1 G>A	W1089X
394delTT	1248+1G>A**	E585X**	Y1092X(C>A)
405+1 G>A**	1259insA**	<b>1898+1G&gt;A</b>	Y1092X(C>G)**
406-1G>A	W401X (c.1202G>A)**	1898+3A>G**	M1101K
E92X	W401X (c.1203G>A)**	2143delT	E1104X**
E92K**	1341+1G>A**	R709X	R1158X
Q98X**	<i>PolyTG/PolyT</i>	K710X	<b>R1162X</b>
457TAT>G**	1461ins4**	2183delAA>G	<b>3659delC</b>
D110H	<b>A455E</b>	2184insA	S1196X
R117C	1525-1G>A**	<b>2184delA</b>	W1204X (c.3611G>A)**
<b>R117H</b>	S466X (C>A)**	2307insA	W1204X (c.3612G>A)**
Y122X	S466X (C>G)	L732X**	3791delC
574delA**	L467P**	2347delG**	<b>3849+10kbC&gt;T</b>
<b>621+1G&gt;T</b>	1548delG†	R764X	G1244E**
663delT	S489X**	2585delT**	3876delA
G178R	S492F**	E822X**	S1251N
<b>711+1G&gt;T</b>	Q493X	2622+1G>A**	3905insT
711+3A>G**	<b>I507del</b>	E831X**	<b>W1282X</b>
711+5 G>A**	<b>F508del</b>	W846X	4005+1G>A**
712-1 G>T**	1677delTA	R851X**	<b>N1303K</b>
H199Y**	V520F	2711delT**	4016insT**
P205S	Q525X**†	<b>2789+5G&gt;A</b>	Q1313X**
L206W	1717-8G>A	Q890X	4209TGTT>AA**
Q220X**	<b>1717-1G&gt;A</b>	L927P**	CFTRdele22,23
852del22**	<b>G542X</b>	S945L**	4382delA**
1078delT	S549R (c.1645A>C)	3007delG**	<i>I506V</i>
G330X	S549R (c.1647T>G)	G970R**	<i>I507V</i>
<b>R334W</b>	S549N	3120G>A	<i>F508C</i>
I336K	<b>G551D</b>	<b>3120+1G&gt;A</b>	

† Classified in the CFTR2 database<sup>15</sup> as a CF-causing variant, while the Sosnay paper<sup>14</sup> classifies the variant as indeterminate. The database classification is more current and reflects the completed functional testing, which was not available at the time of the Sosnay publication.

The use of a test that detects all of these variants is expected to detect at least 95.4% of CF-causing alleles in patients with CF in the CFTR2 Project patient cohort. Also, the rate of detection of couples at risk of having a child with cystic fibrosis should increase to ~91% of couples with use of this panel, compared to 72% of couples with use of the ACMG recommended panel of 23 variants<sup>14</sup>. These estimates however are dependent upon variant distribution and frequency according to variability in geography and ethnicity.

## Test Indication

- ▶ This test is intended for the evaluation of carrier status for 139 clinically relevant variants of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, including variants recommended in 2004 by ACMG<sup>11</sup> and in 2011 by ACOG<sup>12</sup>.
- ▶ This test is intended for use in adults of reproductive age.
- ▶ This test is intended for confirmatory diagnostic testing of newborns and children.
- ▶ This test is intended as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis.



### CAUTION

This test is not indicated for newborn screening, fetal diagnostic testing, pre-implantation testing, or for stand-alone diagnostic purposes.

- ▶ The Cystic Fibrosis 139-Variant Assay is for prescription use only.

## Test Performance Characteristics

Test performance was based on comparisons to two reference methods, Sanger bi-directional sequencing and a validated PCR assay, to verify the accuracy of the assay for the detection of the 139 variants. Due to the rarity of many of the variants included in the assay, it was not feasible to obtain clinical specimens for all variants. Therefore, the accuracy of detection for certain variants was established using synthetic specimens consisting of complex plasmid constructs mixed with wild type DNA in order to simulate heterozygous specimens. The assay was able to accurately identify variants present in all samples with > 99.99% overall accuracy. Through a reproducibility study conducted at three user sites, the assay was demonstrated to be reproducible for both positive variant (99.77%) and negative variant (99.88%) detection.

## Guide to Interpreting Results

Test results should be interpreted in the context of clinical findings, family history, and other laboratory data. Molecular testing may not detect all possible mutations leading to CF. A negative result does not rule out the possibility that the individual has an unidentified mutation in the *CFTR* gene. This test should be used with other available laboratory and clinical information. All clinical interpretations of the variants detected should be made by a board-certified molecular pathologist, clinical molecular geneticist, or equivalent. It is recommended that the physician ordering the test consult with a board-certified clinical medical geneticist or genetic counselor. Patients are also advised to seek counseling from a genetic counselor. Additional information may be obtained from the Cystic Fibrosis Foundation ([www.cff.org](http://www.cff.org)), the Clinical and Functional Translation of CFTR (CFTR2) ([www.cftr2.org](http://www.cftr2.org)), and the American College of Medical Genetics ([www.acmg.net](http://www.acmg.net)).

## Test Limitations

- ▶ The results obtained should be used and interpreted in the context of a full clinical evaluation.
- ▶ The product is designed to identify a specific subset of known variants in the *CFTR* gene, but does not include all variants identified in the *CFTR* gene. Therefore, if a variant is not identified, it does not guarantee that other *CFTR* variants are not present in the samples being analyzed.

- ▶ Conditional reporting has been recommended by ACMG/ACOG for four variants that are considered problematic in interpretation due to the complexity of their association with other variants. The conditionally reported variants in the Cystic Fibrosis 139-Variant assay consist of the polyTG/polyT region (which is reported when the R117H variant is identified) and benign variants I506V, I507V, and F508C<sup>16</sup> (reported when a homozygous F508del or I507del are identified).

**NOTE**

Because this is a sequencing-based assay, there is no interference to F508del or I507del reporting on account of the three benign polymorphisms. Hence, no corrections will be made to the detected result.

- ▶ The assay cannot determine whether the orientation of the PolyTG/PolyT variant is in cis/trans to the R117H variant. For patients with an R117H variant, additional testing to determine whether a PolyTG/PolyT variant, which may affect the clinical phenotype (e.g., 12-13(TG) or 5T), is in a cis/trans orientation to the R117H variant should be performed.
- ▶ Variants identified by this assay vary in frequency among different populations.
- ▶ While much is known about the severity of the disease for some of the variants, for others, information is limited and is based on a limited number of reported clinical cases.
- ▶ For those variants that were validated only through synthetic specimens (Table 2), it is recommended that the laboratory verify the presence of such variants through another validated method prior to reporting results. Contact your testing laboratory to establish their testing procedure.
- ▶ Laboratory errors are rare but can occur. Underlying differences in a patient's DNA or other analytical factors can affect the performance of the assay, and consequently result in calls being made or missed.

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## Revision History

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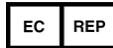
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