

Overview

The Ambry Tests:[™] CF AMPLIFIED[™] & 508 FIRST[™]

The Ambry Test: CF AMPLIFIED is a sequence analysis of the CFTR gene with reflex to deletion/duplication testing that detects ~99% of CF mutations in all ethnic groups. It is the most thorough test available to establish or rule out a molecular diagnosis of cystic fibrosis and to detect mutations for appropriate counseling of potential carrier relatives. 508 FIRST is an option to screen for the deltaF508 mutation before reflex to CF AMPLIFIED, which allows diagnosis of deltaF508 homozygotes inexpensively in less than one week.

When

- **Usual or atypical respiratory and/or GI symptoms of CF**
- **Meconium ileus**
- **Abnormal or borderline sweat chloride values**
- **Infertility caused by CBAVD**
- **Follow-up of positive newborn screening**
- **Retesting if mutation panel detected <2 mutations**

Why

- **Mutation panels are designed for carrier testing, not diagnostic testing**
- **Uncommon mutations also cause severe CF**
- **Ambry Genetics has unprecedented experience with over 10,000 patients tested and offers an extensive menu of CF test options**

A Comparison of Ambry Genetics' Full Gene Sequence Analysis to Common Mutation Testing in the Diagnosis of Cystic Fibrosis

Cystic fibrosis is one of the most common life-shortening inherited disorders, affecting approximately 1 in 3,500 children born in the U.S. each year. Common symptoms include chronic cough, recurrent lung infections leading to diminished pulmonary function, pancreatic insufficiency, elevated sweat chlorides, and poor growth. The median life-expectancy has improved to just under 37 years, and currently 43% of known CF patients are over age 18.¹ Older children and adults with CF are subject to a host of other possible complications including diabetes, bone disease, cirrhosis, portal hypertension, and numerous gastrointestinal ailments.

Diagnosis can be difficult due to the variability in clinical symptoms, wide range in age of presentation, and the limited availability of expert sweat chloride testing.

DNA testing has an important role in CF diagnosis as it offers a more direct analysis of the potential cause of symptoms than does a clinical exam. A molecular diagnosis of two CF mutations satisfies the requirement for evidence of abnormal CFTR function in consensus diagnostic criteria.²

In 2001, the American College of Medical Genetics (ACMG) recommended a standard panel of 25 CF mutations for screening the U.S. population for CF carrier status. This panel was designed in response to a 1997 NIH guideline that CF carrier screening be offered to those with a family history of CF, couples seeking prenatal care, or couples planning a pregnancy. In 2004 the ACMG updated their recommended screening panel by removing two of the 25 mutations. Detection rates of the current 23-mutation panel range from 49% to 88% in the major American ethnic groups (see top chart on other side).³ Various clinical tests are available with different versions of this panel, including just these 23 mutations or up to nearly 100 mutations. **Limited-mutation panels were developed to accommodate carrier screening guidelines, not diagnostic testing needs.**

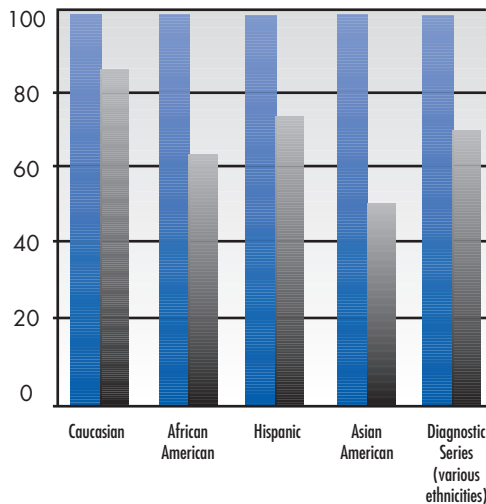
More than 1,300 mutations in the CFTR gene have been described so far. Only seven of these occur in CF patients with a frequency of greater than 1%.⁴ Most CF mutations are rare; more than 97% of mutations occur with a frequency of less than 1 in 1,000. Ambry Genetics has detected over 700 mutations.

The identity of a mutation doesn't consistently determine the phenotype, nor does the frequency of a mutation correlate with its severity. Rare mutations cause classical CF as well as atypical disease. Using a carrier screening mutation panel for diagnostic testing has an inherently limited yield.

There are >1,300 known CFTR mutations. Only 7 of these occur with a frequency of >1%.

Genetic Counselors are available for a thorough explanation of results and to address any questions or concerns about an abnormal report.

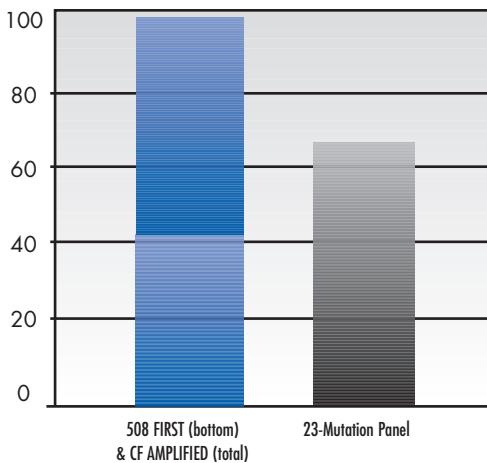
Ambry Genetics
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Percent Mutation Detection by the Ambry Test (blue)

vs.

ACMG 23-Mutation Panel (grey)



Affected Patients Diagnosable by the Ambry Tests (blue)

vs.

ACMG 23-Mutation Panel (grey)

Common mutation panels do not adequately diagnose the spectrum of CF mutations found in affected patients. In a series of 877 samples from CF Centers that use Ambry Genetics' full gene analysis for all new patients, 31% of the mutations detected by the Ambry Test would have been missed by the 23-mutation panel. There was only a 5% increase in mutation detection using a 97-mutation panel.⁵

This equates to 34% of two-mutation patients that would not be conclusively diagnosed if tested by the 23-mutation panel instead of the Ambry Test.

Forty-one percent of these affected CF patients had homozygous deltaF508 mutations (see bottom chart). These patients could have been diagnosed inexpensively in less than one week using Ambry Genetics' 508 FIRST test, which is appropriate for all suspected CF patients who have not had previous DNA testing. This option combines a screen for the deltaF508 mutation with reflex to CF AMPLIFIED if homozygous deltaF508 is not detected. Analysis shows 508 FIRST testing is more cost-effective than mutation-panel testing followed by full gene analysis for panel-negative patients.

In a separate series of 114 patients with congenital absence of the vas deferens (CBAVD), 70% patients had at least one mutation. Full gene analysis detected non-panel mutations in 35% of patients. In 13 patients, the Ambry Test detected second mutations in patients positive for one of the panel mutations.⁶ Correct diagnosis in this group is critical for accurate risk assessment and CF carrier testing of the partner before the couple pursues invasive and expensive fertility treatments.

The Ambry Tests: CF AMPLIFIED and 508 FIRST should be the CF diagnostic tests of choice. Common mutation panels miss causative mutations and leave patients without a conclusive diagnosis while the Ambry Test: CF AMPLIFIED detects approximately 99% of mutations in all ethnic groups. Ambry Genetics has unprecedented knowledge and experience with CF disease-causing mutations, having analyzed the complete CF gene in more than 10,000 patient samples. This provides the largest single-laboratory result database in the world from which to draw interpretations for your patient. 508 FIRST with reflex to CF AMPLIFIED is the testing pathway of choice for diagnosis of cystic fibrosis.

A 23-mutation panel would have missed 31% of mutations identified by the Ambry Test.

1 Cystic Fibrosis Foundation, 2005 data, personal communication.

2 Rosenstein BJ, Cutting GR. *J Pediatr.* 1998;132:589-595.

3 ACMG Technical Standards and Guidelines for CFTR Mutation Testing, 2006 edition.

Available at: http://www.acmg.net/Pages/ACMG_Activities/stds-2002/cf.htm. Accessed September 27, 2006.

4 Watson MS et al. *Genet Med.* 2004;6:387-391.

5 Data on file. Excludes novel mutations >30 base pairs into intron.

6 Data on file. Consistent with our previous publication: Danziger KL et al. *Hum Reprod.* 2004;19:540-546.