

Assay Summary

CFTR Gene Mutation Analysis

Cystic Fibrosis, CBAVD, Chronic Pancreatitis Assay

Synopsis

Cystic Fibrosis (CF) is the most common lethal genetic disease in Caucasians, with a carrier frequency of about 1 in 25-30, resulting in one affected individual per 3200 live births. This disorder affects the epithelia of the respiratory and male genital tract, as well as the hepatobiliary, exocrine pancreas and exocrine sweat systems. Cardinal manifestations include progressive endobronchial damage secondary to recurrent airway inflammation and fibrosis, pancreatic insufficiency resulting in malabsorption, and male infertility resulting from absent or atrophic Wolffian duct structures. Some men may have isolated infertility due to congenital bilateral absence of the vas deferens (CBAVD), without any of the pulmonary or gastrointestinal complications associated with CF. Additionally, recent studies have shown that compound heterozygosity for mutations in the CFTR gene imparts a 33:1 relative risk for idiopathic chronic pancreatitis (Ref 4). The disease shows autosomal recessive inheritance (two copies of a mutation are necessary for expression of disease) and significant phenotypic variability.

While a mutation panel approach may be suitable for some populations, the detection rates diminish for certain ethnicities. Using the recommended 23 mutation panel test, detection rates are as follows: ~94% for Ashkenazi Jews, ~88% for non-Hispanic Caucasians, ~65% for African Americans, ~72% for Hispanic Caucasians, and ~49% for Asian Americans (Ref 5). Full sequencing analysis of the entire coding region and associated splice site junctions has an estimated detection rate of ~96%. More importantly, this detection rate is the same for any ethnic population. The added certainty of a negative result by full sequencing analysis is particularly important for cases with a clear clinical diagnosis, but for which only one or no mutations have been detected.

Indications for testing

Individuals with a clear diagnosis of Cystic Fibrosis, CBAVD, or pancreatic insufficiency or chronic pancreatitis, with or without a family history of the disease. To help in the diagnosis of suspected Cystic Fibrosis, CBAVD, idiopathic chronic pancreatitis, or pancreatic insufficiency. Once a specific mutation is known in a family, presymptomatic and prenatal testing for appropriate family members can be performed.

Methodology

All coding exons and associated intron junctions of the CFTR gene are analyzed by direct DNA sequence analysis. Additionally, the poly T and poly TG sites are genotyped. We have also designed our assay to look for the deep intronic Highsmith mutation. When a mutation is detected, confirmation is carried out by sequencing in the opposite direction, in a second independent PCR amplification. If no mutation is found, sequence analysis is performed in both directions. At-risk family members can be offered DNA sequence analysis of only the region of the gene with the previously identified mutation.

Performance/Limitations

Full sequencing analysis of the entire coding region and associated splice site junctions has an estimated detection rate of ~96%. This method will not detect mutations located in regions of the genes that are not analyzed (non-coding exon sequences, intron sequences other than the splice junctions, and upstream and downstream sequences). The method also will not detect gross genetic alterations including most large deletions, duplications, and inversions. Some sequence alterations that may be detected (such as those causing uncharacterized missense or silent changes) will be of unknown clinical significance. Interpretation of test results should be in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

(a) Blood samples: 2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes. Keep at ambient temperature and ship by overnight courier. Samples must be received in our laboratory within 72 hours of draw.

Note:

- i) for infants, a minimum of 3 ccs is sufficient.
- ii) we accept DNA; at least 10 micrograms is required.

(b) Prenatal samples: 2 T25 flasks of confluent cells sent padded to arrive on M/Tu/W. A blood sample from the mother maybe required (2 tubes with a total of 6 ccs in ACD (yellow top) or EDTA (lavender top) tubes) for use as positive control. Maternal cell contamination studies are not done here but are required for autosomal disorders and dosage analysis on X-linked disorders. We would be happy to assist in coordinating sending out a specimen for this purpose.

Test Request Form (TRF)

- (a) A completed MDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- (b) [CFTR Patient Information Form](#): Include a completed Cancer Patient Information Form for the proband and a complete pedigree.

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
CFTR-SEQ (CFTR gene, full gene sequencing)	83890, 83898(x30), 83894, 83904(x32), 83912	4 wks
CFTR-CAS (CFTR gene, targeted mutation analysis, known mutation)	83890, 83898, 83894, 83904, 83912	3 wks
CFTR-PD (CFTR gene, known mutation detection, prenatal)	83890, 83898, 83894, 83904, 83912	2 wks

References

1. Rosenstein and Cutting, (1998) J. Pediatr. 132:589-95
2. Chillon, M. et al., (1995) NEJM 332(22):1475-1480
3. Gaia, E. et al., (2002) Dig. Dis. and Sci. 47(11):2416-2421
4. Cohn, J.A. et al. (2005). Hum. Mut 26(4):303-307.
5. Watson et al., (2004) *Genet. Med.* 6:387-91

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc