Cystic Fibrosis

Cystic fibrosis (CF), or mucoviscidosis, is a genetically inherited multisystem disorder that affects the respiratory, gastrointestinal and reproductive systems. More than 1,800 different mutations have been discovered since the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene responsible for CF was discovered in 1989 [1]. According to the CF Foundation's National Patient Registry, the median age of survival for a person with CF is currently 33.4 years [2], with respiratory failure as the most common cause of death. Approximately 15% of individuals with CF have a mild form of the disease with a median survival of 56 years. Only thirty years ago, a CF patient was not expected to reach adulthood. Now, with the current diagnostic and treatment options, nearly 50% of CF patients live longer than 28 years [3].

The National Institute of Health (NIH), the American College of Medical Genetics (ACMG), and the American College of Obstetricians and Gynecologists (ACOG), along with other professional associations have released recommendations and developed guidelines for the application of genetic screening tests to clinical practice [4,5,6]. In 2011, ACOG updated its guidelines for prenatal and preconception carrier screening for CF. It has been recognized that while CF is more common among the non-Hispanic Caucasian and Ashkenazi Jewish population groups, the disease is becoming more prevalent in other ethnic groups and the population at large. Therefore, the updated guidelines recommend offering CF carrier screening to all women in pregnancy and for preconception evaluation [4,5,6,7,8].

Epidemiology

- Approximately 30,000 people in the United States and 70,000 people worldwide have CF [2,3].
- Approximately 1,000 new CF cases are diagnosed each year [2].
- More than 10 million Americans are asymptomatic carriers of the defective *CFTR* gene [4].
- CF is the most common, life-threatening, autosomal-recessive condition in the non-Hispanic Caucasian population [2].
- CF is diagnosed equally in males and females [2].
- More than 75% of people with CF are diagnosed by age 2.
- Currently, ACOG recommends offering CF carrier screening to all women in pregnancy and for preconception evaluation. If a patient has been screened previously, CF screening results should be documented, but the test should not be repeated [5].
- NIH recommends screening as part of the newborn screening panels. The newborn screening panels that include CF screening do not replace maternal carrier screening [4].

Table 1: Incidence Rate and Carrier Risk for Cystic Fibrosis based on ethnicity [2,4].

Ethnic Group	Carrier Risk	Incidence Rate
Caucasian American	1/29	1/ 3,200
Ashkenazi Jewish	1/24	1/3,000
Hispanic American	1/46	1/ 8,000 – 9,000
African American	1/65	1/ 15,000 - 17,000
Asian American	1/94	1/ 31,000 - 100,000
Overall US population	1/31	1/ 3,500

Pathogenesis

- CF is an autosomal recessive inherited disease: a person must inherit two copies of the defective gene, one from each parent in order to have CF. Carriers have only one copy of the defective gene and do not have symptoms of the disease. However, they can pass their mutation on to their children.
- In 1989, the *CFTR* gene associated with CF was identified and mapped to the long arm of chromosome 7.
- More than 1,800 known mutations of the *CFTR* gene have been discovered.
- The first mutation identified was ΔF508. This major mutation has been determined to account for almost 70% of the CF cases in the United States.
- The W1282X mutation occurs more frequently in the Ashkenazi Jewish population compared with other ethnicities [2,3,5].

Clinical Significance

- CF is a multisystem disease with morbidity and mortality resulting from chronic obstructive pulmonary disease and respiratory failure being the primary cause of death among > 90% of CF patients.
- CF does not affect intelligence or mental capacity and there are no physical features attributed to the disease.
- Symptoms may include very salty tasting skin, persistent coughing, frequent lung infections, wheezing or shortness of breath, excessive appetite but poor growth/weight gain, and greasy, bulky stools.
- More than 95% of males with CF have primary infertility with obstructive azoospermia due to congenital bilateral absence of the vas deferens.

- Pancreatic insufficiency is present at diagnosis in almost 80% of patients and progresses with age to 90%.
- Thickened secretions can cause blockages in bile ducts resulting in irreversible liver damage such as cirrhosis [1,2,3,4,5,6].

Laboratory Diagnosis

- The sweat test (pilocarpine iontophoresis), is a standard diagnostic test for CF. Sweat chloride levels > 60mEq/L are considered diagnostic for CF [7,8].
- Molecular approaches for the detection of *CFTR* mutations include two main options: 1) targeted mutation analysis or 2) sequencing of the *CFTR* gene.
- Initially, the American College of Medical Genetics Cystic Fibrosis Carrier Screening Working Group recommended that laboratories use a pan-ethnic panel of 25 mutations that were present in at least 0.1% of patients with classic CF. The current ACOG and ACMG guidelines approved December 16, 2010 use a 23-mutation panel for CF screening [5,6].
- Complete analysis of the *CFTR* gene by DNA sequencing is valuable for patients with CF, patients with a family history of CF, males with congenital bilateral absence of the vas deferens, or newborns with positive newborn screening, but negative by targeted analysis.
- Genetic counseling is important to discern whether the combination of mutations and variants would cause classic or atypical CF.
- The MDL **Cystic Fibrosis Core Test** is a *CFTR* gene sequence analysis screen, utilizing Next Generation DNA sequencing technology, for the 23 major mutations approved by ACOG and ACMG for CF screening [5,6,9].
- The MDL **Cystic Fibrosis Comprehensive Test** is an expanded *CFTR* gene sequence analysis screen, utilizing Next Generation DNA sequencing technology, for 191 variants, including the set of 23 major mutations approved by ACOG and ACMG for CF screening and 9 mutations recommended by the FDA for determining Ivacaftor treatment efficacy [5,6,10].
- The MDL **Cystic Fibrosis Site Specific Analysis** screens for known family CF mutations previously identified in blood relatives.

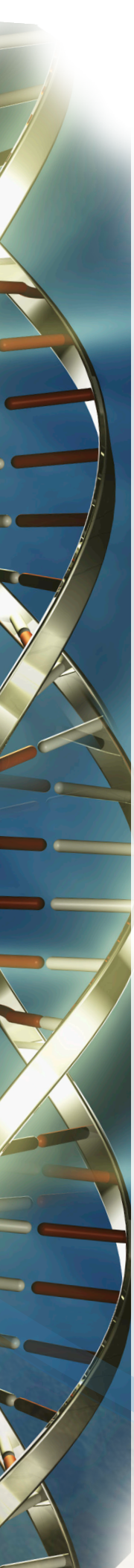
Table 2: MDL Cystic Fibrosis Core Panel: 23 mutations approved by ACOG and ACMG.

ΔF508	**R553X**	**R1162X**	**2184delA**	**ΔI507**
621+1G>T	**G85E**	**3849+10kbC>T**	**G542X**	**R117H**
R334W	**2789+5G>A**	**G551D**	**1717-1G>A**	**R347P**
3659delC	**W1282X**	**A455E**	**711+1G>T**	**3120+1G>A**
N1303K	**R560T**	**1898+1G>A**		

Table 3: MDL Cystic Fibrosis Comprehensive Panel: 191 variants, including the set of 23 major mutations approved by ACOG/ACMG and the 9 mutations recommended by the FDA for determining Ivacaftor treatment efficacy.

ΔF508 ♦	**R553X** ♦	**R1162X** ♦	**2184delA** ♦	**ΔI507** ♦
621+1G>T ♦	**G85E** ♦	**3849+10kbC>T** ♦	**G542X** ♦	**R117H** ♦
R334W ♦	**2789+5G>A** ♦	**G551D** ♦ *	**1717-1G>A** ♦	**R347P** ♦
3659delC ♦	**W1282X** ♦	**A455E** ♦	**711+1G>T** ♦	**3120+1G>A** ♦
N1303K ♦	**R560T** ♦	**1898+1G>A** ♦	1078delT	1154insTC
1248+1G>A	1288insTA	1471delA	1213delT	1259insA
1341+1G>A	1461ins4	1525-1G>A	1548delG	1677delTA
1717-8G>A	1782delA	1811+1.6kbA>G	1811+1G>C	1812-1G>A
1824delA	1898+1G>C	2105-2117del13insAGAAA	2118del4	1898+3A>G
2556insAT	2055del9>A	2143delT	2183AA>G	2184insA
2307insA	2347delG	2585delT	2622+1G>A	2896insAG
2789-2insA	2711delT	2790-1G>C	2869insG	297-1G>A
3007delG	306insA	3120G>A	3121-1G>A	394delTT
3132delTG	3667ins4	3737delA	3791delC	3272-26A>G
3821delT	3850-1G>A	3876delA	4015delA	4016insT
4326delTC	3905insT	457TAT>G	4005+1G>A	405+1G>A
406-1G>A	4209TGTT>AA	663delT	4374+1G>T	4382delA
4428insGA	444delA	574delA	675del4	712-1G>T
711+3A>G	711+5G>A	852del22	A561E	A46D
A559T	C276X	D110H	D579G	D614G
D1152H	D1270N	E1371X	E1104X	E56K
E585X	E60X	E92X	E822X	E831X
E92K	F1074L	F1052V	**G551S** *	G970R
G1061R	**G1244E** *	**G178R** *	G330X	**G1349D** *
H199Y	G1069R	H1054D	I1234V	I336K
K710X	L558S	L1077P	L1065P	L227R
L206W	L732X	L927P	L467P	M1V
M1101K	P205S	P67L	Q359K	Q1313X
Q1412X	Q39X	Q525X	Q890X	Q220X
Q414X	Q493X	Q552X	Q98X	R352Q
R1066C	R1066H	R560K	R709X	R75X
R764X	R1070W	R1158X	R117C	R1070Q
R347H	R792X	R851X	R764X	R74W
S1251N *	S466X	S489X	**S1255P** *	S1255X
S341P	S492F	**S549R** *	S977F	S912X
S945L	**S549N** *	S1196X	ST	T338I
T360K	V520F	W1204X	W1089X	W401X
W846X	Y122X	Y849X	Y913X	Y1092X
Y569D				

♦ The major 23 mutations recommended by ACOG and ACMG are indicated in bold font.
* The 9 mutations recommended by the FDA for determining Ivacaftor treatment efficiency.



Benefits of Minimally Invasive Testing

- Utilizing innovative Next Generation DNA Sequencing technologies, MDL provides an extremely high accuracy of genetic testing.
- Specimens collected using the MDL non-invasive kits yields sufficient material for the DNA sequencing technology.
- MDL Cystic Fibrosis tests are designed in full compliance with all official guidelines for cystic fibrosis screening and diagnosis [5,6,7,8].

Diagnostic Considerations

- The ACOG Committee Opinion 486, released in April 2011, recommends preconception CF carrier screening for all women of reproductive age. “It is becoming increasingly difficult to assign a single ethnicity to individuals. It is reasonable, therefore, to offer CF carrier screening to all patients” [5].
- If a patient has been screened previously, CF screening results should be documented, but the test should not be repeated.
- Newborn screening panels that include CF screening do not replace maternal carrier screening. Newborn screening for CF can be performed as early as 2 days after birth.
- For couples in which both partners are carriers, genetic counseling is recommended to review prenatal testing and reproductive options.

Treatment Options

- As with many other genetic disorders, CF has been considered a disease with no available cure.
- While there is no cure for CF, patients must follow a regular treatment routine to maintain optimal lung function, prevent infections and support pancreatic function.
- Pharmaceutical companies continue to research in order to develop treatment options for CF.
- Ivacaftor (Kalydeco™) was approved by the FDA in 2012 for the treatment of CF patients with the G551D mutation in the *CFTR* gene. On February 21, 2014, the FDA approved the expanded use of Kalydeco™ for additional mutations underlying CF: G551D, G1244E, G1349D, G178R, G551S, S1251N, S1255P, S549N, and S549R [10].

Frequently Asked Questions

Q: How many mutations does the MDL CF Test detect?

A: The MDL CF Core Panel detects the major 23 mutations (including ΔF508) that have been determined to account for the majority of cases in the United States. The MDL CF Comprehensive Panel screens for 191 variants, including a set of 23 mutations approved by ACOG and ACMG guidelines for CF screening and 9 mutations recommended by the FDA for determining Ivacaftor (Kalydeco™) treatment efficacy. The MDL CF Site Specific Analysis is performed specifically for known family *CFTR* gene mutations.

Q: What should I do if a patient tests positive for CF?

A: A “positive” carrier test for CF means that a person has one mutation of the CF gene. This result is more than 99% accurate. Carriers of single CF mutations are generally asymptomatic and experience no discernible health problems. ACOG guidelines recommend offering CF screening to both parents before/during pregnancy, because in order to pass on the disease CF disease, a child must inherit one copy of a mutation of the CF gene from each parent.

Q: What should I do if a patient tests negative for CF?

A: With more than 1,800 different mutations of the CF gene, there are some rare disease-causing mutations that the test may not find. If your test is negative for a mutation of the *CFTR* gene, there is still a small chance you could be a carrier of one mutation. The chance depends on your race or ethnic group and the number of mutations the test was validated to detect.

Q: Do I need to test the patient every pregnancy?

A: No. Every patient should be asked if she has been already tested. According to ACOG guidelines, the test should not be repeated if the patient has been screened before. The result of the previous test should be noted in the patient’s medical record for each subsequent pregnancy.

Q: Should I screen the patient if her newborn CF screening results are available?

A: Yes. According to ACOG/ACMG guidelines, newborn screening panels that include CF screening do not replace maternal carrier screening.

Q: Traditionally, I have only tested certain ethnic groups for CF. Should I reconsider this policy?

A: ACOG guidelines recommend offering CF carrier screening to all patients of reproductive age due to the difficulty of assigning a single ethnicity to individuals.

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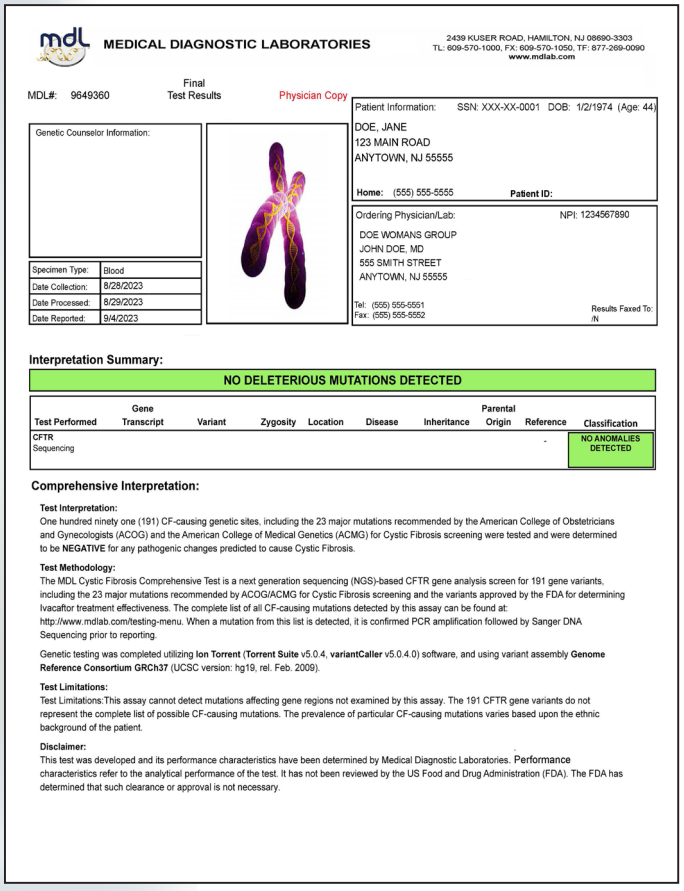


MEDICAL DIAGNOSTIC LABORATORIES

2439 KUSER ROAD, HAMILTON, NJ 08690-3303
TL: 609-570-1000, FX: 609-570-1050, TF: 877-269-0090
www.mdlab.com

MDL#: 9649360 Final Test Results Physician Copy

Patient Information: SSN: XXX-XX-0001 DOB: 1/2/1974 (Age: 44)
DOE, JANE
123 MAIN ROAD
ANYTOWN, NJ 55555
Home: (555) 555-5555 Patient ID:

Genetic Counselor Information:

Ordering Physician/Lab: NPI: 1234567890
DOE WOMANS GROUP
JOHN DOE, MD
555 SMITH STREET
ANYTOWN, NJ 55555
Tel: (555) 555-5551
Fax: (555) 555-5552
Results Faxed To: /N

Specimen Type:	Blood
Date Collection:	8/28/2023
Date Processed:	8/29/2023
Date Reported:	9/4/2023

Interpretation Summary:

NO DELETERIOUS MUTATIONS DETECTED

Test Performed	Gene Transcript	Variant	Zygosity	Location	Disease	Inheritance	Parental Origin	Reference	Classification
CFTR Sequencing								-	NO ANOMALIES DETECTED

Comprehensive Interpretation:

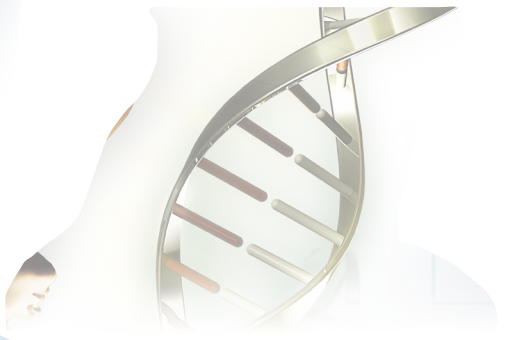
Test Interpretation:
One hundred ninety one (191) CF-causing genetic sites, including the 23 major mutations recommended by the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) for Cystic Fibrosis screening were tested and were determined to be **NEGATIVE** for any pathogenic changes predicted to cause Cystic Fibrosis.

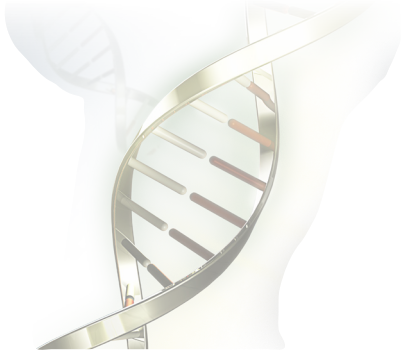
Test Methodology:
The MDL Cystic Fibrosis Comprehensive Test is a next generation sequencing (NGS)-based CFTR gene analysis screen for 191 gene variants, including the 23 major mutations recommended by ACOG/ACMG for Cystic Fibrosis screening and the variants approved by the FDA for determining Ivacaftor treatment effectiveness. The complete list of all CF-causing mutations detected by this assay can be found at: http://www.mdlab.com/testing-menu. When a mutation from this list is detected, it is confirmed PCR amplification followed by Sanger DNA Sequencing prior to reporting.

Genetic testing was completed utilizing **Ion Torrent** (**Torrent Suite** v5.0.4, **variantCaller** v5.0.4.0) software, and using variant assembly **Genome Reference Consortium GRCh37** (UCSC version: hg19, rel. Feb. 2009).

Test Limitations:
Test Limitations:This assay cannot detect mutations affecting gene regions not examined by this assay. The 191 CFTR gene variants do not represent the complete list of possible CF-causing mutations. The prevalence of particular CF-causing mutations varies based upon the ethnic background of the patient.

Disclaimer:
This test was developed and its performance characteristics have been determined by Medical Diagnostic Laboratories. Performance characteristics refer to the analytical performance of the test. It has not been reviewed by the US Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.





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ANYTOWN, NJ 55555
Tel: (555) 555-5551
Fax: (555) 555-5552
Results Faxed To: /N

Specimen Type:	Blood
Date Collection:	8/28/2023
Date Processed:	8/29/2023
Date Reported:	9/4/2023

Interpretation Summary:

CARRIER OF A CF-CAUSING MUTATION;

Test Performed	Gene Transcript	Variant	Zygosity	Location	Disease	Inheritance	Parental Origin	Reference	Classification
CFTR Sequencing	NM_000492.3	c.350G>A (p.Arg117His)	Heterozygous	Exon4	Cystic Fibrosis	Autosomal recessive	Unknown	-	CF-Causing

Comprehensive Interpretation:

Test Interpretation:
The 23 Cystic Fibrosis-causing genetic sites recommended by the American College of Obstetricians and Gynecologists (ACOG) and the American College of Medical Genetics (ACMG) were tested and were determined to be **POSITIVE for the R117H**. This individual is a carrier of a CF-Causing Mutation.

Test Methodology:
The MDL Cystic Fibrosis Comprehensive Test is a next generation sequencing (NGS)-based CFTR gene analysis screen for 191 gene variants, including the 23 major mutations recommended by ACOG/ACMG for Cystic Fibrosis screening and the variants approved by the FDA for determining Ivacaftor treatment effectiveness. The complete list of all CF-causing mutations detected by this assay can be found at: http://www.mdlab.com/testing-menu. When a mutation from this list is detected, it is confirmed PCR amplification followed by Sanger DNA Sequencing prior to reporting.

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Mail: Yes USPS All Yes
Fax: Yes All Manual No

Medical Director: Jing-Jing Yang, M.D.

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