UNITY

1035 O'Brien Drive
Menlo Park, CA 94025
unityscreen.com

T 650.460.2551
F 833.915.0146
E support@unityscreen.com

POWERED BY Billion To One

PATIENT		SAMPLE		PROVIDER	
First Name	Jane	**Sample Type**	Blood	**Name**	Dr. Jane Smith
Last Name	Doe	**Date Collected**	05/01/2021	**Address 1**	1234 Street Name
DOB	10/20/1990	**Date Received**	05/02/2021	**Address 2**	Suite 120
Gender	Female	**Sample ID**	123-123-123	**City**	San Francisco
Ethnicity	Caucasian	**Requisition ID**	11223344	**State Zip**	CA, 94102
Gestational Age	12W	**Date Reported**	05/16/2021	**Phone**	555-555-5555
Medical Record #	12344321			**Fax**	555-555-5555

UNITY™ **Five Gene Carrier** Screen with **Reflex NIPT**

POSITIVE CARRIER **HIGH RISK** FETUS

CONDITIONS SCREENED	MATERNAL CARRIER STATUS	FETAL RISK BY NIPT
Alpha-Thalassemia ***(HBA1, HBA2)***	**Negative**	
Sickle Cell Disease / Beta-Thalassemia / Hemoglobinopathies ***(HBB)***	**Negative**	
Cystic Fibrosis ***(CFTR)***	**POSITIVE** c.1521_1523delCTT (p.Phe508delPhe)	**HIGH RISK** *See results below*
Spinal Muscular Atrophy ***(SMN1)***	**Negative** 2 *SMN1* copies, SNP not present	

NIPT RESULT DETAILS

CONDITIONS SCREENED	FETAL RISK	Risk *Before* NIPT	Risk *After* NIPT	Fetal Fraction
Cystic Fibrosis	**HIGH**	**1 in 96 – 1 in 376**	**9 in 10**	**6.2%**
		Fetal Risk Before NIPT is dependent on paternal ethnicity and assumes paternal carrier status is unknown. See disease carrier frequencies based on ethnicity on the last page of the report.		

Recommended Follow-Up *next page*

The ACOG Committee on Genetics (co486 and co691) recommends cystic fibrosis, hemoglobinopathy, and spinal muscular atrophy carrier screening for all patients who are planning a pregnancy or seeking prenatal care. UNITY™ carrier screening evaluates for cystic fibrosis (*CFTR*), hemoglobinopathies (*HBB, HBA1* and *HBA2*), and spinal muscular atrophy (*SMN1*). Reflex NIPT is performed to evaluate fetal risk when a pregnant patient is identified as a carrier.

Patient Name	Jane Doe	DOB	10/20/1990	Gestational Age	12W	Medical Record #	12341234

RECOMMENDED FOLLOW-UP

PRENATAL DIAGNOSIS via chorionic villus sampling or amniocentesis is **RECOMMENDED.**

GENETIC COUNSELING is **recommended** for this patient to review the implications of this result.

The patient may contact BillionToOne at (650) 460-2551 to schedule an appointment for a complimentary telephone genetic consultation to review these results. A genetic counselor can also be found at www.nsgc.org.

CARRIER SCREENING for cystic fibrosis for the patient's reproductive partner is recommended prior to a future pregnancy.

Interpretation *next page* ›

Patient Name	Jane Doe	DOB	10/20/1990	Gestational Age	12W	Medical Record #	12341234

INTERPRETATION

UNITY™ Five Gene Carrier Screen

This patient has the c.1521_1523delCTT (p.Phe508delPhe) pathogenic variant in the *CFTR* gene (NM_000492.3) and is a CARRIER for cystic fibrosis.

If this patient's reproductive partner is a carrier for cystic fibrosis, there is a 25% risk for an affected child with each pregnancy. Carrier screening for cystic fibrosis for the patient's reproductive partner is recommended prior to a future pregnancy to clarify the risks for an affected child.

This patient's first-degree relatives each have a 50% chance to be a carrier for cystic fibrosis as well. We recommend these results be shared with blood relatives, especially those of reproductive age.

UNITY™ NIPT for Cystic Fibrosis

The fetus is HIGH RISK to be affected with cystic fibrosis. The estimated fetal fraction was 6.2%.

NIPT was performed to evaluate for fetal *CFTR* variants and concluded the fetus is high risk to be homozygous for the c.1521_1523delCTT (p.Phe508delPhe) pathogenic variant in the *CFTR* gene. Therefore, the fetus is HIGH RISK to be affected with cystic fibrosis.

This NIPT result is valid only for a singleton pregnancy achieved without egg donation or gestational carrier.

Prenatal diagnosis via chorionic villus sampling or amniocentesis is recommended. UNITY™ NIPT is not diagnostic. No irreversible decisions regarding the pregnancy should be made without confirmatory invasive prenatal testing. Genetic testing can also be performed postnatally.

Genetic counseling is recommended for this patient to review the implications of this result. The patient may contact BillionToOne at (650) 460-2551 to schedule an appointment for a complimentary telephone genetic consultation to review these results. A genetic counselor can also be found at www.nsgc.org.

Patient Name	Jane Doe	DOB	10/20/1990	Gestational Age	12W	Medical Record #	12341234

INTERPRETATION

UNITY™ Five Gene Carrier Screen

No other reportable gene variants were found.

Alpha-Thalassemia *HBA1* (NM_000558.5), *HBA2* (NM_000517.6)	**Negative**
Sickle Cell Disease/Beta-Thalassemia/Hemoglobinopathy *HBB* (NM_000518.5)	**Negative**
Spinal Muscular Atrophy *SMN1* (NM_000344.3) • *SMN1* Copy Number • SMA Region Informative SNP (rs143838139)	**Negative** • **2 copies (most common)** • **Not present (most common haplotype)**

Carrier frequencies both before and after screening vary by ethnicity and assume no personal or family history of the condition. See Pre- and Post-Test Carrier Frequencies tables on the last page of the report.

Comprehensive genetic counseling is recommended for a patient with a family history of a genetic disorder so that carrier risks can be accurately discussed, as well as potential reproductive risks and additional testing options that may be available.

Carrier screening does not evaluate for all genetic conditions. In addition, carrier screening is not able to identify all possible variants in the genes analyzed. As a result, a negative result significantly reduces the probability of being a carrier; it does not eliminate the risk.

METHODS AND LIMITATIONS

UNITY™ Five Gene Carrier Screen

DNA was extracted and purified from leukocyte enriched peripheral blood. The resulting DNA was subjected to a Custom Amplicon Panel PCR that utilized Spikein DNA technology to detect both small nucleotide variants and large copy number changes. The DNA was sequenced by synthesis on an Illumina NextSeq. Results were aligned and examined on a custom bioinformatics pipeline and compared to the published human genome build GRCh37/hg19 reference sequence. NGS performance was confirmed via 10x validation of small nucleotide variants (SNV) in each gene via Sanger sequencing. In addition, SNVs were confirmed using Sanger Sequencing for any variants with sequencing coverage less than 100x. Large copy number variants were confirmed using digital Multiplex Ligation Probe Amplification (digitalMLPA). Pathogenic and likely pathogenic variants were reported.

Test limitations: A negative result significantly reduces but does not eliminate the chance of being a carrier. Additional carrier screening may be indicated for individuals of Ashkenazi Jewish, French Canadian, or Cajun descent, as these patients are at higher risk of diseases that we do not test in our panel.

Test sensitivity and mutation spectrum: UNITY™ is designed to maximize detection of pathogenic alleles for cystic fibrosis, spinal muscular atrophy, and hemoglobinopathies (alpha-thalassemia, beta-thalassemia, and sickle cell disease). We sequence all exons, exon-intron junctions and select intronic regions of *CFTR*, *HBA1*, *HBA2*, and *HBB*. Copy number analysis is performed on *CFTR*, *SMN1*, *HBA1*, *HBA2*, and *HBB*. This includes all *CFTR* variants recommended by the American College of Medical Genetics (ACMG), all common *HBB* variants including HbS, HbC, HbE, IVS1-1, and 41/42-TTCT, the HBA2 Constant Spring variant and the SMN1 silent carrier linked SNP g.27134T>G (rs143838139) when two copies of SMN1 are present. The alpha-thalassemia carrier screen also reports single and double gene deletions including alpha3.7, alpha4.2, SEA, MED-I, SA, 20.5, BRIT, FIL or THAI.

UNITY™ NIPT

Cell-free DNA (cfDNA) was isolated from 2-4mL of plasma from whole blood collected in a Streck cell-free DNA tube. A paternal inheritance NIPT was performed as a multiplex PCR on common single nucleotide variants (SNVs) to measure the fraction of cell-free DNA of fetal origin. The paternal inheritance NIPT also contains amplicons for *CFTR* and *HBB* for paternal exclusion analysis of pathogenic alleles. Recessive inheritance of certain common maternal variants, i.e., fetal inheritance of the same pathogenic allele from both parents, was determined by a separate PCR on cfDNA to perform Relative Mutation Dosage analysis using BillionToOne's QCT molecular counting technology as available. When multiple blood tubes are analyzed for NIPT (e.g., for redraws), we report the combined reported fetal fraction by taking the weighted average of fetal fractions across different tubes based on the total number of molecules identified via QCTs. Due to the tube-to-tube assay variability, the reported fetal fraction for the same patient can differ between single-gene NIPT and aneuploidy NIPT.

Test Limitations: Single gene NIPT may not be reported when the amount of cell-free DNA in the blood sample is too low. Single gene NIPT is not performed on genes that are not covered on the UNITY™ Five Gene Carrier Screen panel (e.g., Tay-Sachs, Canavan, familial dysautonomia). The NIPT result is valid only for a singleton pregnancy achieved without egg donation or gestational carrier. UNITY is designed and optimized as a general population screening tool, and additional information regarding maternal and paternal mutations should be supplied to the laboratory for appropriate risk adjustment in cases where the test is being used for high-risk couples where paternal carrier status is known. Relative Mutation Dosage analysis for the identification of homozygosity is not available for all variants. Therefore, in rare cases (less than 1% of affected pregnancies), NIPT may not detect a homozygous affected fetus. While this limitation is accounted for in the post-test NIPT risk for the general population, the reported NIPT post-test risk may not adequately represent the residual risk for consanguineous couples.

Test sensitivity and mutation spectrum: Next generation sequencing of critical exons and introns in *HBB* and *CFTR* was performed. The *HBB* NIPT detects >99% of pathogenic alleles and the cystic fibrosis NIPT detects >94% of pathogenic alleles. When performed on double deletion in cis (--/αα) carriers, the *HBA* NIPT detects or excludes paternal inheritance of the double gene deletion in cis (SEA, SA, BRIT, FIL or THAI) through a combination of detection of common paternal SNVs in the *HBA1-HBA2* locus and breakpoint PCR. Additionally, *HBA* NIPT detects paternal inheritance of the Hb Constant Spring allele. When performed on Alpha Thalassemia single gene deletion (-α/αα) carriers, double gene deletion carriers in trans (-α/-α) or Hb Constant Spring carriers (α_{cs}α/αα), the HBA NIPT detects paternal inheritance of the SEA deletion using breakpoint PCR. When performed on Alpha Thalassemia non-deletion variant (α_{nd}α/αα) carriers with a variant other than Hb Constant Spring the HBA NIPT detects paternal inheritance of the SEA deletion via breakpoint PCR and detects paternal inheritance of the Hb Constant Spring allele. The SMA NIPT detects inheritance of *SMN1* copy number.

Carrier screen genotypes excluded from NIPT analysis: *SMN1* NIPT is not performed for two copy, SNP positive individuals of non-Ashkenazi Jewish ancestry.

This five gene carrier screen and NIPT was developed and its performance characteristics determined by the BillionToOne laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The BillionToOne laboratory is regulated under CLIA. This test is used for clinical purposes. It should not be regarded as investigational or for research

BillionToOne Laboratory	**Laboratory Director**	Joseph Michael Anderson, MD	**Phone**	(650) 460-2551
1035 O'Brien Drive	**CLIA ID**	05D2167800	**Fax**	(833) 915-0146
Menlo Park, CA 94025	**CLF**	00353741	**Email**	support@billiontoone.com

Joseph Michael Anderson, MD
Laboratory Director
California License: A 98379

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UNITY™ CARRIER SCREEN: CARRIER FREQUENCIES

Disease	Gene	Ethnicity	Carrier Frequency *Before* Testing	Detection Rate	Carrier Risk *After* Negative Testing
Alpha-Thalassemia Alpha thalassemia silent carrier includes the single allele deletion and trans double allele deletion. Double deletion includes cis double deletion only. CS means Constant Spring mutation	*HBA1, HBA2*	African American	aa/a-: 1 in 3 aa/--: 1 in 5,000 aa/aa^{CS}: 1 in 10,000	>95%	aa/a-: <1 in 60 aa/--: <1 in 100,000 aa/aa^{CS}: <1 in 200,000
		Asian	aa/a-: 1 in 16 aa/--: 1 in 93 aa/aa^{CS}: 1 in 93	>95%	aa/a-: <1 in 320 aa/--: <1 in 1860 aa/aa^{CS}: <1 in 1860
		Northern European	aa/a-: 1 in 44 aa/--: 1 in 3807 aa/aa^{CS}: 1in 10,000	>95%	aa/a-: <1 in 880 aa/--: <1 in 76,140 aa/aa^{CS}: <1 in 200,000
		General Population	aa/a-: 1 in 16 aa/--: 1 in 570 aa/aa^{CS}: 1 in 10,000	>95%	aa/a-: <1 in 320 aa/--: <1 in 11,400 aa/aa^{CS}: <1 in 200,000
Beta-Thalassemia, Hemoglobinopathies	*HBB*	African American	1 in 8	>99%	<1 in 800
		Ashkenazi Jewish	1 in 49	>99%	<1 in 4900
		Asian	1 in 54	>99%	<1 in 5400
		Northern European	1 in 373	>99%	<1 in 37300
		Hispanic	1 in 17	>99%	<1 in 1700
		Mediterranean	1 in 28	>99%	<1 in 2800
		General Population	1 in 49	>99%	<1 in 4900
Cystic Fibrosis	*CFTR*	African American	1 in 61	>99%	<1 in 6100
		Ashkenazi Jewish	1 in 24	>99%	<1 in 2400
		Asian	1 in 94	>99%	<1 in 9400
		Northern European	1 in 25	>99%	<1 in 2500
		Hispanic	1 in 58	>99%	<1 in 5800
		General Population	1 in 45	>99%	<1 in 4500
Spinal Muscular Atrophy	*SMN1*	African American	1 in 72	>90.3%	< in 375 (2 copies, SNP absent) <1 in 4200 (3+ copies)
		Ashkenazi Jewish	1 in 67	>92.8%	<1 in 900 (2 copies, SNP absent) <1 in 5400 (3+ copies)
		Asian	1 in 59	>93.6%	<1 in 900 (2 copies, SNP absent) <1 in 5600 (3+ copies)
		Northern European	1 in 47	>95%	<1 in 900 (2 copies, SNP absent) <1 in 5600 (3+ copies)
		Hispanic	1 in 68	>92.6%	<1 in 900 (2 copies, SNP absent) <1 in 5400 (3+ copies)
		General Population	1 in 54	>91.2%	<1 in 525 (2 copies, SNP absent) <1 in 5400 (3+ copies)

CYSTIC FIBROSIS CARRIER – HIGH RISK FETUS

CYSTIC FIBROSIS

Cystic fibrosis is an inherited condition that causes thick and sticky mucus to build up and damage many of the organs in the body. Symptoms often begin in early childhood and may include lifelong problems with the digestive system and frequent lung infections. Digestive issues may cause diarrhea, poor growth, malnutrition, and weight loss. Recurrent lung infections often cause permanent lung damage, lung failure, and the need for lung transplant. Infertility in men is also common. Cystic fibrosis does not affect intelligence. There is no cure for the condition; however, treatments and medications may help lessen the symptoms of the disease. Even with treatment, individuals with cystic fibrosis have a shortened life-expectancy.

The type and severity of symptoms varies from one person to another. Knowing the specific genetic change may help clarify the severity of disease expected, but not in all cases.

WHAT CAUSES CYSTIC FIBROSIS?

Everyone has two copies of the *CFTR* gene. Cystic fibrosis is caused when a child inherits two non-working copies of the *CFTR* gene, one from their mother and one from their father. If someone has one non-working copy of the *CFTR* gene, they are called a carrier. When both parents are carriers of cystic fibrosis, there is a 25% (1 in 4) chance to have an affected child with each pregnancy. UNITY™ uses advanced technology to determine whether you are a carrier and whether your current pregnancy is at risk.

YOUR CARRIER STATUS: CARRIER OF CYSTIC FIBROSIS

You were identified to be a carrier of cystic fibrosis. Carriers of cystic fibrosis are typically healthy and do not have any symptoms. Carrier screening for cystic fibrosis for the father of your children is recommended prior to a future pregnancy to clarify the risk for an affected child. Your first-degree relatives (e.g., brothers, sisters, children, and parents) also have a 50% chance to be a carrier of cystic fibrosis. More distant relatives also have a chance to be a carrier. We recommend that you share these results with blood relatives, especially those of reproductive age.

YOUR BABY'S RISK: HIGH CHANCE TO BE AFFECTED WITH CYSTIC FIBROSIS

The testing performed by UNITY™ shows your baby's chance of being affected with cystic fibrosis is **significantly increased**. Please reference your report to review personalized risk figures. **No irreversible pregnancy decisions, such as pregnancy termination, should be considered based on UNITY™ results alone.**

Follow-up testing and genetic counseling is strongly recommended. You may contact BillionToOne at (650) 460-2551 to schedule an appointment for a complimentary telephone genetic consultation to review these results. A local genetic counselor can also be found at www.nsgc.org.

CYSTIC FIBROSIS CARRIER – HIGH RISK FETUS

CONFIRMATORY TESTING

Although UNITY™ is a highly accurate screening test, diagnostic testing is strongly recommended to determine whether your baby is affected with cystic fibrosis. UNITY™ uses an advanced technology to determine whether your baby's risk to have cystic fibrosis is high or low. Confirmatory genetic testing during pregnancy or after birth can determine whether or not your baby is actually affected.

Before Birth

Testing during pregnancy can be performed by obtaining a sample of placenta (chorionic villus sampling or CVS) or fluid around your baby (amniocentesis) which both contain DNA that is representative of your baby. A CVS is ideally performed between 10 to 13 weeks pregnancy while an amniocentesis is performed between 15 to 20 weeks pregnancy. Both procedures are safe, but have a small risk for pregnancy complications, including miscarriage. The exact risk depends on the experience of the doctor performing the procedure.

After Birth

Testing for your baby can be performed after birth through a blood test. All babies born in a hospital in the United States are automatically screened for cystic fibrosis. The majority of babies with cystic fibrosis will be diagnosed through this screening process. In rare cases, a diagnosis may be missed or a baby is incorrectly diagnosed.

RESOURCES

Cystic Fibrosis Foundation: https://www.cff.org/
Cystic Fibrosis Research Inc: http://cfri.org/
Baby's First Test: https://www.babysfirsttest.org/newborn-screening/conditions/cystic-fibrosis-cf
American College of Obstetricians and Gynecologists Guide to Prenatal Diagnosis: https://www.acog.org/Patients/FAQs/Prenatal-Genetic-Diagnostic-Tests?IsMobileSet=false