UNITY

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BILLIONTOONE

PATIENT		SAMPLE		PROVIDER	
First Name	N/A	Sample Type	N/A	Provider	N/A
Last Name	N/A	Date Collected	N/A	Clinic Address	N/A
DOB	N/A	Date Received	N/A	Phone Number	N/A
Ethnicity	N/A	Accession ID	N/A	Fax Number	N/A
Gender	N/A	Requisition ID	N/A		
Gestational Age	N/A	Date Reported	N/A		
Medical Record #	N/A				

UNITY Screen™ **Carrier Screen** with **Reflex sgNIPT**

POSITIVE CARRIER

LOW RISK FETUS

CONDITIONS SCREENED	MATERNAL CARRIER STATUS	FETAL RISK BY sgNIPT
Alpha-Thalassemia ***(HBA1, HBA2)***	**Negative**	
Sickle Cell Disease / Beta-Thalassemia / Hemoglobinopathies ***(HBB)***	**Negative**	
Cystic Fibrosis ***(CFTR)***	**POSITIVE** c.1521_1523del (p.Phe508del)	**LOW RISK** *See Results Below*
Spinal Muscular Atrophy ***(SMN1)***	**Negative** 2 *SMN1* copies, SNP not present	

sgNIPT RESULT DETAILS

CONDITIONS SCREENED	FETAL RISK	Risk *Before* sgNIPT	Risk *After* sgNIPT	Fetal Fraction
Cystic Fibrosis	**LOW**	**1 in 96 - 1 in 376**	**< 1 in 5000**	**9.0%**
		Fetal Risk Before sgNIPT 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.	**Fetal Risk After sgNIPT** assumes the paternal carrier status is unknown.	

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 Screen™ carrier screening evaluates for cystic fibrosis (*CFTR*), hemoglobinopathies (*HBB, HBA1 and HBA2*), and spinal muscular atrophy (*SMN1*). Reflex sgNIPT is performed to evaluate fetal risk when a pregnant patient is identified as a carrier for these conditions.

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RECOMMENDED FOLLOW-UP

GENETIC COUNSELING is **recommended** 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.

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

Interpretation *next page* ›

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Patient Name N/A	DOB N/A	Gestational Age N/A	Medical Record # N/A

INTERPRETATION

UNITY Screen™ Carrier Screen

This patient has the c.1521_1523del (p.Phe508del) pathogenic variant in the *CFTR* gene (NM_000492.4) and is a CARRIER for cystic fibrosis.

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. If this patient's reproductive partner is a carrier, each pregnancy has a 25% risk for a child to inherit both the maternal and paternal *CFTR* variants and be affected with cystic fibrosis. If both variants are inherited, the phenotype would depend on the severity of the specific maternal and paternal variants inherited.

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 Screen™ sgNIPT for Cystic Fibrosis

The fetus is LOW RISK to be affected with cystic fibrosis. The estimated fetal fraction was 9.0%.

sgNIPT was performed to evaluate for fetal *CFTR* variants and concluded the fetus is LOW RISK to be affected with cystic fibrosis. No paternally inherited *CFTR* variants were detected in the cell-free DNA.

This result significantly reduces, but does not eliminate, the risk for cystic fibrosis in the fetus. The fetal risk after sgNIPT is based on the residual risk of the fetus being either homozygous for the maternal variant or compound heterozygous with a distinct paternal variant not assessed by this screen. The fetal risk after sgNIPT assumes paternal carrier status is unknown. If the father of the pregnancy is a known carrier, please contact BillionToOne at (650) 460-2551.

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

Prenatal diagnosis via chorionic villus sampling or amniocentesis can be considered if the patient desires additional information. UNITY sgNIPT is not diagnostic. No irreversible decisions regarding the pregnancy should be made without confirmatory invasive prenatal testing. Genetic testing can also be performed postnatally.

Patient Name N/A	DOB N/A	Gestational Age N/A	Medical Record # N/A

INTERPRETATION

UNITY Screen™ Carrier Screen

No other reportable gene variants were found.

Sickle Cell Disease/Beta-Thalassemia/Hemoglobinopathy *HBB* (NM_000518.5)	**Negative**
Alpha-Thalassemia *HBA1* (NM_000558.5), *HBA2* (NM_000517.6)	**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 Screen™ 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 next generation sequencing (NGS). Results were aligned and examined on a custom bioinformatics pipeline and compared to the published human genome build GRCh37/hg19 reference sequence. NGS performance of small nucleotide variants (SNV) was confirmed via Sanger sequencing for any variants with sequencing coverage less than 100x and for rare variants in genes that have not yet been confirmed by Sanger sequencing 10 times. Large copy number changes were confirmed using digital Multiplex Ligation Probe Amplification (dMLPA) except where 50 confirmations had previously been obtained for the copy number change. Large copy number changes were confirmed using digital Multiplex Ligation Probe Amplification (dMLPA) for all New York State samples. Pathogenic and likely pathogenic variants were reported. Incidental findings may not be reported. Variant classifications are determined by the information and data available at the time of reporting. Internal reanalysis of variants is performed every 24 months. A change in classification may occur over time as additional information becomes available.

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 Screen™ 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 Screen™ sgNIPT

Cell-free DNA (cfDNA) was isolated from 2-4mL of plasma from whole blood collected in a cell-free DNA tube. Single-gene NIPT (sgNIPT) was performed as multiplex PCR & next-generation sequencing on (i) common single nucleotide variants (SNVs) to measure the fraction of cell-free DNA of fetal origin (i.e., fetal fraction), (ii) amplicons for all *CFTR* and *HBB* exons and critical introns for paternal exclusion analysis of pathogenic alleles, and breakpoint PCR & sequencing for exclusion of the SEA deletion (i.e., paternal exclusion analysis), and (iii) common *HBB* and *CFTR* variants and *SMN1* copy number for determining maternal inheritance by Relative Mutation Dosage and Quantitative Counting Template (QCT) molecular counting technology, when indicated (i.e., maternal dosage analysis). Fetal fraction, paternal mutation analysis, and maternal dosage analysis are combined in a statistical algorithm with prior risk to report a post-test fetal risk for each condition.

Test Limitations: Single-gene NIPT may not be reported when the amount of cell-free DNA in the blood sample is below the limit of detection. Results from this test are highly accurate; however, discordant results may occur. Potential causes of discordant results include: laboratory-specific variant classifications, low fetal fraction, vanishing twin, maternal bone marrow transplant, laboratory error, or other reasons. The sgNIPT result is valid only for a singleton pregnancy achieved without egg donation or gestational carrier. This test 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), sgNIPT may not detect a homozygous affected fetus. While this limitation is accounted for in the post-test sgNIPT risk for the general population, the reported sgNIPT post-test risk may not adequately represent the residual risk for consanguineous couples.

Test sensitivity and mutation spectrum: Next generation sequencing of all exons and critical introns in *HBB* and *CFTR* was performed. The *HBB* sgNIPT is designed to detect >99% of affected sickle cell disease and beta-hemoglobinopathy cases, and *CFTR* sgNIPT is designed to detect >97% of affected cystic fibrosis cases. When performed on double deletion carriers in *cis* (--/αα) or in *trans* (-α/-α), or on non-deletion variant carriers, including Hb Constant Spring, *HBA* sgNIPT detects or excludes paternal inheritance of a double gene deletion in *cis* (SEA, SA, BRIT, FIL or THAI) using breakpoint PCR and inheritance of a paternal haplotype in the *HBA1-HBA2* locus. Additionally, *HBA* sgNIPT detects paternal inheritance of the Hb Constant Spring allele for double deletion carriers in *cis* (--/αα) and non-deletion variant carriers with a variant other than Hb Constant Spring. When performed on Alpha Thalassemia single gene deletion (-α/αα) carriers, the *HBA* sgNIPT detects paternal inheritance of the SEA deletion using breakpoint PCR. The SMA sgNIPT detects inheritance of *SMN1* copy number.

Carrier screen genotypes excluded from sgNIPT analysis: *SMN1* sgNIPT is not performed for two copy, SNP positive individuals.

This 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. This test was performed using BillionToOne's patented technology (www.billiontoone.com/patents).

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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}: 1 in 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
Sickle Cell Disease, Beta-Thalassemia, Hemoglobiopathies	*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 37,300
		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%	<1 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 – LOW 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 may have a shortened life-expectancy.

The type and severity of symptoms vary from one person to another. In some cases, knowing the specific genetic change may help clarify the severity of the disease expected.

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 Screen™ uses advanced technology to determine if you are a carrier and if your current pregnancy is at risk.

YOUR 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) 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: LOW CHANCE TO BE AFFECTED WITH CYSTIC FIBROSIS

The testing performed by UNITY Screen™ evaluated your baby's risk to have cystic fibrosis. The results show your baby's chance of being affected with cystic fibrosis is very low, but not zero. Please reference your report to review personalized risk figures for this pregnancy.

No further testing is recommended for this pregnancy. 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.

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