UNITY

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POWERED BY Billion To One

PATIENT		SAMPLE		PROVIDER	
First Name	JANE	Sample Type	BLOOD	Name	DR. JANE SMITH
Last Name	DOE	Date Collected	5/1/2019	Address 1	1234 STREET NAME
DOB	1/1/1985	Date Received	5/16/2019	Address 2	SUITE 123
Gender	FEMALE	Sample #	1234567890	City	SAN FRANCISCO
Ethnicity	ETHNICITY	Case ID	1234567890	State, Zip	CA, 90000
Pregnant	YES	Date Reported	5/22/2019	Phone	555-555-5555
Gestational Age	10 WEEKS			Fax	555-555-5555
Medical Record #	1234567890			Email	INFO@CLINIC.COM

UNITY™ Five Gene Carrier Screen with Reflex NIPT

POSITIVE CARRIER **LOW RISK FETUS**

SUMMARY OF RESULTS

FETAL FRACTION 8%

POSITIVE CARRIER
for **Cystic Fibrosis**

This patient has the **c.1519_1521delATC (p.I507del) variant** in the *CFTR* gene and **is a carrier for cystic fibrosis.**

LOW RISK FETUS
for **Cystic Fibrosis**

NIPT 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.

This result significantly reduces but does not eliminate the risk for cystic fibrosis in the fetus.

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

NIPT RESULT DETAILS

Conditions Screened	Results	Fetal Risk *Before* NIPT	Fetal Risk *After* NIPT
Cystic Fibrosis	**LOW Fetal Risk**	1/96 - 1/376	**<1/9600**
		Fetal Risk Before NIPT is dependent on paternal ethnicity and assumes paternal carrier status is unknown. See disease carrier frequencies based on ethnicity on page 5.	Fetal Risk After NIPT assumes the paternal carrier status is unknown

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	1/1/1985	Gestational Age	10W	Medical Record #	1234567890

RECOMMENDED FOLLOW-UP

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	1/1/1985	Gestational Age	10W	Medical Record #	1234567890

INTERPRETATION

UNITY™ Five Gene Carrier Screen

This patient has the c.1519_1521delATC (p.I507del) 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 up to 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. Please see the NIPT result below to determine the fetal risk for cystic fibrosis for the current pregnancy.

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.

No other reportable gene variants were found.

Alpha-Thalassemia *HBA1* (NM_000558.5), *HBA2* (NM_000517.6)	**Negative**
Beta-Thalassemia/Hemoglobinopathies *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* on page 5.

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.

UNITY™ NIPT For Cystic Fibrosis

Analysis of the cell free fetal DNA was performed to evaluate for fetal *CFTR* variants and no paternally inherited *CFTR* variants were detected in the cell free fetal DNA.

The fetus is LOW RISK to be affected with cystic fibrosis. The estimated fetal fraction was 8%.

Prenatal diagnosis via chorionic villus sampling or amniocentesis can be considered if the patient desires additional information. 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.

METHODS AND LIMITATIONS

UNITY™ Five Gene Carrier Screen

DNA was extracted and purified from leukocyte enriched peripheral blood. The resulting DNA was subjected to two separate Laboratory Developed Procedures, digital Multiplex Ligation Probe Amplification (digitalMLPA) and a Custom Amplicon Panel PCR, that was sequenced by synthesis on an Illumina MiSeq. Results were aligned and examined on a custom bioinformatics pipeline and compared to the published human genome build GRCh37/hg19 reference sequence. Small nucleotide variants were confirmed using Sanger Sequencing and pathogenic and likely pathogenic variants were reported.

Test limitations: No test is perfect. While results of BillionToOne's Five Gene Carrier Screen test are highly accurate, a negative result significantly reduces but does not eliminate the chance of being a carrier. This test result assumes that the patient is not Ashkenazi Jewish, French Canadian or Cajun as these patients are at higher risk of diseases that we do not test in our panel.

Test sensitivity and mutation spectrum: The carrier screen panel is designed to detect the majority of pathogenic alleles for cystic fibrosis, sickle cell disease, alpha-thalassemia, beta-thalassemia, and spinal muscular atrophy. The test has sensitivity of >99% for cystic fibrosis, >99% for sickle cell disease and beta-thalassemia, >95% for alpha thalassemia and >90% for spinal muscular atrophy. All exons, exon-intron junctions and select intronic regions of *CFTR, HBB, HBA1* and *HBA2* are sequenced. Copy number analysis is performed on *SMN1, HBA1, HBA2, HBB* and *CFTR*. The carrier screen covers all 23 variants recommended by ACMG, all common *HBB* pathogenic 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 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 a maternal variant, 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.

Test Limitations: Single gene NIPT may not be reported when the fetal fraction is <5% or 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).

Test sensitivity and mutation spectrum: The *HBB* NIPT detects inheritance of >590 pathogenic alleles encompassing >99% of pathogenic alleles. The cystic fibrosis NIPT detects inheritance of >110 pathogenic alleles encompassing >90% of pathogenic alleles. The *HBA* NIPT detects paternal inheritance of the double gene deletion in *cis* (SEA, SA, BRIT, FIL or THAI) through a combination of detection of common SNVs in the *HBA1-HBA2* locus and breakpoint PCR. The *HBA* NIPT additionally performs Relative Mutation Dosage analysis to detect inheritance of the Constant Spring allele. The SMA NIPT detects inheritance of *SMN1* copy number.

Carrier screen genotypes excluded from NIPT analysis: The *HBA* NIPT is designed to detect Hb Barts and HbH Constant Spring disease. The *HBA* NIPT is not designed to detect non-Constant Spring HbH disease. Therefore, the *HBA* NIPT is only performed when the mother is found to be a carrier of an *HBA1, HBA2* two-gene deletion in *cis* (e.g. SEA, MED-I, SA, 20.5, BRIT, FIL or THAI) or a carrier for HbH Constant Spring. The *HBA* NIPT is not performed for "silent carriers" who only have alpha3.7 or alpha4.2 single-gene deletions. The *SMN1* NIPT is not performed for two copy, SNP positive silent carriers.

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.

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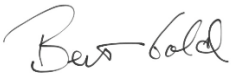

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UNITY™ CARRIER SCREEN: CARRIER FREQUENCIES

Disease	Gene	Ethnicity	Carrier Frequency *Before* Testing	Detection Rate	Carrier Risk *After* Negative Testing
Alpha-Thalassemia	*HBA1, HBA2*	African American	1 in 30	>95%	<1 in 600
		Asian	1 in 20	>95%	<1 in 400
		Northern European	1 in 500	>95%	<1 in 1000
		General Population	1 in 25	>95%	<1 in 500
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)