

PATIENT		SAMPLE		PROVIDER	
First Name	Jane	Sample Type	Blood	Provider	John Smith
Last Name	Doe	Date Collected	08/27/2022	Clinic Address	1035 O'Brien Drive Menlo Park, CA 94025
DOB	01/01/2004	Date Received	09/01/2022	Phone Number	650.460.2551
Ethnicity	African or African American	Accession ID	1234567K1234	Fax Number	833.915.0146
Gender	Female	Requisition ID	1234567K1234-5		
Gestational Age	18w3d	Date Reported	09/06/2022		
Medical Record #	N/A				

UNITY™ Complete: Aneuploidy Screen

Singleton Gestation

SUMMARY OF RESULTS			
LOW RISK FETUS	FEMALE FETAL SEX	12.2% FETAL FRACTION	
CONDITIONS SCREENED	FETAL RISK by NIPT	RISK Before NIPT	RISK After NIPT
Trisomy 21	Low Risk	1 in 1025	<1 in 10,000
Trisomy 18	Low Risk	1 in 4576	<1 in 10,000
Trisomy 13	Low Risk	1 in 15656	<1 in 10,000
Monosomy X	Low Risk	1 in 560	<1 in 10,000
Sex Chromosome Aneuploidy (XXX / XXY / XYY)	Not Detected		
RECOMMENDED FOLLOW-UP			
Genetic counseling is available 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 .			

UNITY™ Complete: Rh(D)

Singleton Gestation

SUMMARY OF RESULTS	
LIKELY POSITIVE FETAL Rh	Anti-D prophylaxis is indicated for RhD negative patients who are not alloimmunized and carrying an RhD positive fetus.

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Patient Name Jane Doe	DOB 01/01/2004	Gestational Age 18w3d	Medical Record # N/A
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INTERPRETATION

UNITY™ Complete: Aneuploidy Screen Singleton Gestation

The fetus is LOW RISK to be affected with aneuploidy of chromosomes 13, 18, 21, X & Y. The estimated fetal fraction was 12.2%

NIPT was performed to evaluate for fetal copy number of chromosomes 13, 18, 21, X & Y.

This NIPT result is valid only for a singleton gestation.

A low risk NIPT result significantly reduces the risk of the screened aneuploidies; it does not eliminate the risk. NIPT does not exclude abnormalities of other chromosomes not evaluated by the screen, microdeletions, triploidy, other genetic syndromes, or birth defects. A low risk NIPT result does not guarantee a normal pregnancy outcome.

Genetic counseling is available 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. Comprehensive genetic counseling is recommended for a patient with a family history of a chromosome abnormality or other genetic disorder so that risks can be accurately discussed, as well as additional testing options that may be available. A genetic counselor can also be found at www.nsgc.org.

UNITY™ Complete: Rh(D) Singleton Gestation

This fetus is LIKELY POSITIVE for RhD. The estimated fetal fraction was 12.2%

NIPT was performed to evaluate for fetal Rh status. The cell-free DNA tested positive for the presence of the *RHD* gene. Therefore, this fetus is likely positive for RhD.

This NIPT result is valid only for a singleton gestation.

In rare cases, a fetus with a likely positive result may have a RhD negative phenotype as the result of a mutation in the *RHD* gene this test is unable to identify. Additionally, fetuses with a weak D genotype will have a likely positive NIPT result, however, they may present with a RhD negative phenotype at birth.

Anti-D prophylaxis is indicated for RhD negative patients who are not alloimmunized and carrying an RhD positive fetus.

METHODS AND LIMITATIONS

UNITY™ Complete: Aneuploidy Screen

Cell-free DNA (cfDNA) was isolated from 2-4 mL 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. A modified fetal fraction calculation is performed for twin pregnancies and pregnancies conceived with a gestational carrier. The fetal risk for aneuploidy of chromosomes 13, 18, 21, X, & Y for singleton pregnancies or chromosomes 13, 18, & 21 for twin pregnancies, was determined by a separate multiplex PCR on cfDNA using no more than 99 amplicons per chromosome to perform relative chromosomal dosage analysis. When multiple blood tubes are analyzed for NIPT (e.g. for redraws), we report the combined reported fetal fraction by taking the arithmetic mean of fetal fractions across different tubes. 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. Pre-test risks for aneuploidy ('Risk Before NIPT') are based on maternal age and gestational age. Post-test residual risks ('Risk After NIPT') for high-risk results are personalized and calculated based on the test sensitivity, test specificity, and prior risks, as determined by maternal age and gestational age. Post-test residual risks for low-risk results are not personalized and calculated based on the test sensitivity, test specificity, and prior-risk in the general population.

Test Limitations: Results may not be reported when the amount of cell-free fetal DNA in the blood sample is too low. Results from this test are highly accurate; however, discordant results may occur. Potential causes of discordant results include: maternal, fetal, or placental mosaicism, low fetal fraction, vanishing twin, maternal malignancy, maternal organ or bone marrow transplant, or other reasons. Findings of unknown significance will not be reported. UNITY™ does not screen for neural tube defects or abdominal wall defects. This test does not screen for microdeletions. UNITY™ NIPT is not diagnostic. No irreversible decisions regarding the pregnancy should be made without confirmatory invasive prenatal testing.

CONDITIONS SCREENED	SENSITIVITY	SPECIFICITY	POSITIVE PREDICTIVE VALUE	NEGATIVE PREDICTIVE VALUE
Trisomy 21	99.8% [98.9% - 100%]	99.9% [99.8% - 99.9%]	84.2%	> 99.9%
Trisomy 18	99.9% [99.0% - 100%]	> 99.9% [99.9% - 100%]	94.2%	> 99.9%
Trisomy 13	99.1% [97.4% - 100%]	> 99.9% [99.9% - 100%]	52.5%	> 99.9%
Monosomy X	97.7% [93.7% - 100%]	> 99.9% [99.9% - 100%]	85.5%	> 99.9%
Sex Chromosome Aneuploidy (XXX / XXY / XYY)	Reported when identified	> 99.9%	n/a	> 99.9%
Presence of Y Chromosome	> 99.9%	> 99.9%	n/a	> 99.9%

* Internal analytical validation data for singleton pregnancies. Performance metrics are estimated based on analytical data from contrived aneuploidy samples at 9% fetal fraction. Representative coefficients of variance from human plasma samples were applied to this analytical data to perform Monte Carlo simulations to calculate these metrics, assuming prior risks for a patient population of advanced maternal age. Mosaicism is not reflected in the validation.

METHODS AND LIMITATIONS

UNITY™ Complete: Rh(D)

Cell-free DNA (cfDNA) was isolated from 2-4mL of plasma from whole blood collected in a Streck cell-free DNA tube. The resulting DNA was subjected to a Custom Amplicon Panel PCR with proprietary Quantitative Counting Template molecules. The amplified DNA was sequenced by synthesis on an Illumina sequencer. Results were aligned and examined on a custom bioinformatics pipeline and compared to the published human genome build GRCh37/hg19 reference sequence along with the sequence of *RHDpsi*. A modified fetal fraction calculation is performed for twin pregnancies and pregnancies conceived with a gestational carrier.

Paternal alleles present in common single nucleotide variants (SNVs) were used to measure the fraction of cell-free DNA of fetal origin. Quantitative Counting Templates were used to determine the presence or absence of molecules of the *RHD* gene and the *RHDpsi* variant in the cell-free DNA.

Test Limitations: The RhD NIPT may not be reported when the amount of cell-free fetal DNA in the blood sample is too low. RhD pseudogenes not mentioned above may not be able to be differentiated from RhD and may be reported as RhD positive. Positive RhD results are returned for RhD positive mothers, due to the presence of maternal RhD in the cell-free DNA.

MATERNAL PHENOTYPE	FETAL PHENOTYPE	FETAL GENOTYPE	UNITY™ FETAL Rh(D) NIPT RESULT
Rh negative	Rh negative	<i>RHD</i> gene deletion	negative
	Rh negative	<i>RHD psi</i>	negative
	Rh negative	<i>RHD-CE-D</i> hybrid	negative
	Rh negative	<i>RHD</i> rare mutation	likely positive
	Rh weak D	weak D type 1, 2, 3, etc.	likely positive
	Rh positive	<i>RHD</i> gene present	likely positive
Rh weak D	Rh negative or positive	n/a	likely positive
Rh positive	Rh negative or positive	n/a	likely positive

This 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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