

PATIENT		SAMPLE		PROVIDER	
First Name	Jane	Sample Type	BLOOD	Name	Dr. Jane Smith
Last Name	Doe	Date Collected	05/03/2021	Address	1234 Street Name San Francisco, CA 94102
DOB	01/20/1982	Date Received	05/04/2021	Phone	555-555-5555
Ethnicity		Accession ID	123-45-6789	Fax	555-555-5555
Gender	Female	Requisition ID	987-65-4321		
Gestational Age	14w2d	Date Reported	05/11/2021		
Medical Record #	321-15-5321				

## UNITY™ Complete: Aneuploidy Screen

### Singleton Gestation

SUMMARY OF RESULTS			
<b>LOW RISK</b> FETUS	<b>FEMALE</b> FETAL SEX	<b>9.9%</b> FETAL FRACTION	
CONDITIONS SCREENED	FETAL RISK by NIPT	RISK Before NIPT	RISK After NIPT
Trisomy 21	Low Risk	1 in 86	<1 in 10,000
Trisomy 18	Low Risk	1 in 272	<1 in 10,000
Trisomy 13	Low Risk	1 in 760	<1 in 10,000
Monosomy X	Low Risk	1 in 250	<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 <a href="http://www.nsgc.org">www.nsgc.org</a> .			

## UNITY™ Complete: Rh(D)

### Singleton Gestation

SUMMARY OF RESULTS	
<b>NEGATIVE</b> FETAL Rh	
RECOMMENDED FOLLOW-UP	
<b>ANTI-D PROPHYLAXIS</b> is <b>NOT INDICATED</b> for this patient.	

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Patient Name Jane Doe

DOB 01/20/1982

Gestational Age 14w2d

Medical Record # 321-15-5321

## 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 9.9%**

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](http://www.nsgc.org).

### UNITY™ Complete: Rh(D) Singleton Gestation

**The fetus is NEGATIVE for RhD. The estimated fetal fraction was 9.9%**

NIPT was performed to evaluate for fetal Rh status. No copies of a functioning *RHD* gene were detected in the cell-free DNA. Therefore, the fetus is RhD negative.

This NIPT result is valid only for a singleton gestation.

Anti-D prophylaxis is not indicated for this patient.

## 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. Post-test residual risks ('Risk After NIPT') for high-risk results are calculated based on the test sensitivity, test specificity, and prior risks as determined by maternal age and are therefore personalized. Post-test residual risks for low-risk results are calculated based on the test sensitivity, test specificity, and prior risk in the general population, and are therefore not personalized.

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
<b>Trisomy 21</b>	99.8% [98.9% - 100%]	99.9% [99.8% - 99.9%]	84.2%	> 99.9%
<b>Trisomy 18</b>	99.9% [99.0% - 100%]	> 99.9% [99.9% - 100%]	94.2%	> 99.9%
<b>Trisomy 13</b>	99.1% [97.4% - 100%]	> 99.9% [99.9% - 100%]	52.5%	> 99.9%
<b>Monosomy X</b>	97.7% [93.7% - 100%]	> 99.9% [99.9% - 100%]	85.5%	> 99.9%
<b>Sex Chromosome Aneuploidy (XXX / XXY / XYY)</b>	Reported when identified	> 99.9%	n/a	> 99.9%
<b>Presence of Y Chromosome</b>	> 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 MiSeq. 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	CLINICAL ACTION
<b>Rh negative</b>	Rh negative	<i>RHD</i> gene deletion	negative	Anti-D prophylaxis is not indicated
	Rh negative	<i>RHDpsi</i>	negative	
	Rh negative	<i>RHD-CE-D</i> hybrid	negative	
	Rh negative	<i>RHD</i> rare mutation	likely positive	Anti-D prophylaxis is indicated
	Rh weak D	weak D type 1, 2, 3, etc.	likely positive	
Rh negative	<i>RHD</i> gene present	likely positive		
<b>Rh weak D</b>	Rh negative or positive	n/a	likely positive	If maternal RhD-genotyping is not performed, Anti-D prophylaxis is indicated
<b>Rh positive</b>	Rh negative or positive	n/a	likely positive	Anti-D prophylaxis is not indicated

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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