UNITY

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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
DOB	01/20/1982	**Date Received**	05/04/2021		San Francisco, CA 94102
Ethnicity		**Accession ID**	123-45-6789	**Phone**	555-555-5555
Gender	Female	**Requisition ID**	987-65-4321	**Fax**	555-555-5555
Gestational Age	14w2d	**Date Reported**	05/11/2021		
Medical Record #	321-15-5321				

UNITY™ Complete: **Aneuploidy Screen**

Singleton Gestation

SUMMARY OF RESULTS

H **HIGH RISK** Trisomy 21 | **FEMALE** FETAL SEX | **9.9%** FETAL FRACTION

CONDITIONS SCREENED	FETAL RISK *by* NIPT	RISK *Before* NIPT	RISK *After* NIPT
Trisomy 21	**HIGH RISK**	**1 in 86**	**9 in 10**
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

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

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.
A genetic counselor can also be found at www.nsgc.org.

UNITY™ Complete: **Rh(D)**

Singleton Gestation

SUMMARY OF RESULTS

Rh+ **LIKELY POSITIVE** FETAL Rh

RECOMMENDED FOLLOW-UP

ANTI-D PROPHYLAXIS is **INDICATED** if the patient is Rh negative and not alloimmunized.

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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 HIGH RISK to be affected with TRISOMY 21. 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.

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. 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 be found at www.nsgc.org.

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

This fetus is LIKELY POSITIVE for RhD. The estimated fetal fraction was 9.9%

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 if the patient is Rh negative and not alloimmunized.

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
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 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
Rh negative	Rh negative	*RHD* gene deletion	negative	Anti-D prophylaxis is not indicated
	Rh negative	*RHD* psi	negative	
	Rh negative	*RHD-CE-D* hybrid	negative	
	Rh negative	*RHD* rare mutation	likely positive	Anti-D prophylaxis is indicated
	Rh weak D	weak D type 1, 2, 3, etc.	likely positive	
	Rh negative	*RHD* gene present	likely positive	
Rh weak D	Rh negative or positive	n/a	likely positive	If maternal RhD-genotyping is not performed, Anti-D prophylaxis is indicated
Rh positive	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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Trisomy 21 - Patient Information Sheet

YOUR BABY'S RISK: HIGH CHANCE TO BE AFFECTED WITH TRISOMY 21

The testing performed by UNITY™ shows your baby's chance of being affected with trisomy 21 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 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.

WHAT IS TRISOMY 21?

Individuals with trisomy 21, or Down syndrome, have three copies of chromosome 21. Children with trisomy 21 typically look like their parents, but also have some unique features such as a flat facial profile, small nose, and upward slanted eyes. In addition, they are at risk for various medical health issues. Approximately 50% of children have heart defects. Other issues may include hearing or vision impairment, feeding and digestion issues, and leukemia. Children with Down syndrome typically learn to walk and talk but are slower to achieve language and motor milestones than their siblings or peers. Almost all individuals with Down syndrome have some degree of intellectual disability.

Pregnancies affected with trisomy 21 may have anomalies identified on first or second trimester ultrasound. These pregnancies may have a higher risk for miscarriage or stillbirth.

Most health issues are treatable but may require surgery after birth. The average life expectancy is approximately 60 years. Most adults require support; however, some individuals may work or live in a supervised setting. The type and severity of symptoms varies from one person to another. Genetic testing cannot predict the severity or ultimate level of functioning.

WHAT CAUSES TRISOMY 21?

The average person has 46 chromosomes; males typically have an X and a Y chromosome, while females have two X chromosomes. An individual inherits one of each chromosome from each parent. An individual with trisomy 21 has an extra 21st chromosome. Trisomy 21 is not usually inherited. This is a mistake that happens at conception. There is nothing you could have done to cause or prevent trisomy 21.

CONFIRMATORY TESTING

Although UNITY™ is a highly accurate screening test, diagnostic testing is strongly recommended to determine whether your baby is affected with trisomy 21. UNITY™ uses an advanced technology to determine whether your baby's risk to have trisomy 21 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.

After Birth

Infants with Down syndrome typically have physical features of the condition. However, if confirmatory testing during pregnancy is declined, the baby's pediatrician should be informed of this result. An individual inherits one of each chromosome from each parent. An individual with trisomy 21 has an extra 21st chromosome. Trisomy 21 is not usually inherited. This is a mistake that happens at conception. There is nothing you could have done to cause or prevent trisomy 21.

RESOURCES

- National Down Syndrome Society - https://www.ndss.org/
- Down Syndrome Pregnancy - https://www.downsyndromepregnancy.org
- Global Down Syndrome Foundation - https://www.globaldownsyndrome.org/
- Lettercase, Understanding Down Syndrome - https://understandingdownsyndrome.org/
- National Society of Genetic Counselors - https://www.nsgc.org
- American College of Obstetricians and Gynecologists Guide to Prenatal Diagnosis - https://www.acog.org/Patients/FAQs/Prenatal-Genetic-Diagnostic-Tests