Noninvasive Prenatal Aneuploidy Screen by Cell-Free DNA Sequencing

Last Literature Review: April 2022 Last Update: December 2023

Cell-free DNA (cfDNA) screening, also referred to as noninvasive prenatal testing (NIPT) or noninvasive prenatal screening (NIPS), is the most sensitive and specific prenatal screening option for trisomy 13, trisomy 18, trisomy 21 (Down syndrome), and Turner syndrome.[1] The American College of Obstetricians and Gynecologists (ACOG), American College of Medical Genetics and Genomics (ACMG), and Society for Maternal-Fetal Medicine (SMFM) recommend that prenatal genetic screening, including NIPT, and prenatal diagnosis be offered to all pregnant women regardless of maternal age or fetal risk of chromosomal abnormality.[2,3,4,5] High-risk results merit prompt, appropriate follow-up; critical clinical decisions should be based on diagnostic rather than screening test results.[2,3,4,5] Guidelines recommend discussing the option of diagnostic testing (amniocentesis or chorionic villus sampling) instead of screening and after high-risk screening results. This is particularly important for women whose clinical and/or family history places them at high risk for genetic conditions not targeted by NIPT.[2,3,4] For more details about NIPT and a description of the spectrum of prenatal genetic testing, please refer to the ARUP Consult Prenatal Testing for Chromosomal Abnormalities and Neural Tube Defects topic. For more information about maternal serum screening, refer to ARUP's First and Second Trimester Screening Options.

Featured ARUP Testing

Non-Invasive Prenatal Aneuploidy Screen by cell-free DNA Sequencing 3003043

Method: Massively Parallel Sequencing

Testing may be offered to pregnant women with singleton pregnancies at high or low risk for aneuploidy from 10 weeks of gestation to term at the time of blood draw.

- Test may be ordered for women who have used an egg donor and for gestational carriers.
- Samples from twins and higher order multiple gestations will be sent out to Integrated Genetics (LabCorp) to perform the MaterniT21 PLUS Core test (test code 451927). ARUP only performs testing on singleton pregnancies.
- This test is contraindicated for pregnancies at less than 10 weeks of gestation, pregnancies with known twin demise, and in women with significant obesity.

Disease Overview

Aneuploidy is typically due to nondisjunction (uneven separation) of chromosome pairs/copies during gametogenesis (sperm and egg formation by meiosis). This leads to one or three copies of a chromosome in the zygote (fertilized egg), which is then replicated by mitosis into each cell of the body, causing disruption of the normal course of development and cellular function. The prevalence of aneuploidy is higher in early gestation pregnancies than in term pregnancies. The table below details specific disorders and their associated clinical findings and incidences.

Aneuploidies Tested, Incidence, and Associated Clinical Findings

Disorder	Clinical Features	Incidence at Birth
Trisomy 13 (Patau syndrome)	Clinical features include multiple congenital malformations and severe to profound intellectual disability Babies with trisomy 13 have high prenatal, neonatal, and infant mortality; approximately 10% survive past 5 years of age	1/5,000
Trisomy 18 (Edwards syndrome)	Clinical features include intrauterine growth restriction with low birth weight; multiple congenital anomalies involving the brain, spinal cord, heart, abdominal wall, and kidneys; hypotonia; and severe to profound intellectual disability Babies with trisomy 18 have high prenatal, neonatal, and infant mortality; approximately 12% survive past 5 years of age	1/3,330
Trisomy 21 (Down syndrome)	Clinical features include hypotonia, a characteristic facial appearance, developmental delays/intellectual disability, and short stature	1/660
Monosomy X (Turner syndrome)	Clinical features include fluid accumulations (cystic hygroma, lymphedema), short stature, low-set ears, broad chest, and cardiac and renal anomalies Infertility is typical Intelligence is generally within the normal range	1/2,500 female births
XXY syndrome (Klinefelter syndrome)	Males with Klinefelter syndrome, or 47,XXY, are typically taller than average, with delayed or incomplete puberty and infertility The effect on social skills and learning is widely variable and often mild	1/500 male births

Sources: Jones, 2013[6]; Meyer, 2016[7]; Otter, 2010[8]; Unique[9]; Bardsley, 2013[10]; Groth, 2013[11]

Disorder	Clinical Features	Incidence at Birth
XYY syndrome	Males with XYY syndrome and females with triple X syndrome (trisomy X) have tall stature and normal physical development They are fertile and cognition is typically within normal range	1/800 male births
XXX syndrome	They have some susceptibility to learning disabilities and other neurocognitive difficulties	1/1,000 female births

Sources: Jones, 2013[6]; Meyer, 2016[7]; Otter, 2010[8]; Unique[9]; Bardsley, 2013[10]; Groth, 2013[11]

Inheritance and Recurrence Risk

Most cases of trisomy and Turner syndrome are not inherited. Even in sporadic cases, however, the recurrence risk is usually increased over the background risk. Therefore, offering prenatal diagnosis in future pregnancies is warranted.[12,13]

Test Description

NIPT uses massively parallel whole genome sequencing of cfDNA fragments derived from maternal peripheral whole blood samples to generate paired-end sequencing data that are aligned to a reference genome. Regions of the genome that are over- or underrepresented are quantified. The probability (log likelihood ratio) of aneuploidy is calculated for targeted chromosomes, accounting for bias of GC-rich DNA fragments, region-specific genomic coverage, and fetal fraction. The results are reported as “high risk” or “low risk” for the aneuploidies of interest.[14,15,16]

Fetal fraction is estimated from the distribution of cfDNA fragment lengths and their genomic coordinates. A dynamic assessment of data quality and coverage profile determines whether data quality is sufficient for aneuploid risk interpretation.[14] Refer to the ARUP NIPT patient brochure for more information.

ARUP prohibits the use of Non-Invasive Prenatal Aneuploidy Screen by Cell-Free DNA Sequencing to further discrimination based on genetic characteristics, oppression of at-risk or minority populations, clinical utilization of human heritable genome editing, reproductive cloning of human beings, and/or bioterrorism.

Test Interpretation

Published Clinical Sensitivity and Specificity of NIPT

Clinical Sensitivity and Specificity[a,b]				
Condition	Clinical Sensitivity	Clinical Specificity	PPV[c]	NPV
Trisomy 21	98.9%	99.7%	20.9-95.1%	>99.9%
Trisomy 18	>99.9%	99.8%	6.2-94.1%	>99.9%
Trisomy 13	>99.9%	99.8%	1.6%-76.8%	>99.9%
Monosomy X	>99.9%	>99.8%	—	—

[a]Male/female concordance is >99%.

[b]Data are not sufficient to calculate sensitivity, specificity, PPV, and NPV for every condition.

[c]PPV ranges are calculated based on sensitivity and specificity in Borth[17] and the prevalence in a low-risk group (20 years of age, 30 weeks gestational age) through the prevalence in a high-risk group (44 years of age, 10 weeks gestational age). PPV is greatly affected by an individual’s pretest risk for each of the screened conditions, which varies based on gestational age and maternal age.

NPV, negative predictive value; PPV, positive predictive value

Sources: Borth, 2021[17]; Snidjers, 1999[18]; Snidjers, 1995[19]

Detailed information about PPV at increased pretest risk levels, various gestational ages, and maternal ages can be found in the Positive Predictive Value of a High-Risk Noninvasive Prenatal Screening Result for Various Increased Pretest Risk Levels and for Various Gestational Ages and Maternal Ages tables.

Analytic Validation Accuracy

Positive Percent Agreement (Sensitivity) and Negative Percent Agreement (Specificity)[a,b,c]					
Condition or Characteristic	**Mean PPA (Sensitivity)**	**5% to 95% Confidence Interval[d]**	**Number of Samples**	**PPV[e]**	**NPA (Specificity)**
Trisomy 21	94.9%	82.6%-99.3%	59	38.6%-97.5%	89.7%-99.9%
Trisomy 18	94.8%	78.6%-99.6%	39	8.6%-95.8%	94.8%->99.9%
Trisomy 13	86.7%	52.3%-98.9%	15	2.7%-85.0%	98.2%->99.9%
Monosomy X	>99.9%	64.1%-100%	10	—	—
XXX	>99.9%	34.4%-100%	4	—	—
XXY	80.0%	36.7%-98.4%	10	—	—
XYY	85.7%	34.5%-99.7%	7	—	—
Male	>99.9%	97.29%-100%	166	—	—
Female	99.3%	95.3%->99.9%	149	—	—
PPA (Sensitivity)					
Combined aneuploidy	93.1%	85.5%-97.4%	144	—	—
NPA (Specificity)					
Euploid/low risk	99.5%	96.2%-99.9%	186	—	—

[a]PPA and NPA are commonly referred to as sensitivity and specificity; in this validation the terms PPA and NPA are more appropriate.

[b]For samples with observed fetal fraction of ≤5.0%, the sensitivity to detect fetal aneuploidy and accuracy of the fetal fraction estimate are significantly lower.

[c]Data are not sufficient to calculate PPV and NPA for every condition.

[d]Jeffreys interval.

[e]PPV is greatly affected by an individual's pretest risk for each of the screened conditions, which varies based on gestational age and maternal age.

NPA, negative percent agreement; PPA, positive percent agreement

Sources: Snidjers, 1999[18]; Snidjers, 1995[19]

Reporting Information

- Autosomal aneuploidies
  - The following autosomal aneuploidies are reported:
    - Trisomy 13
    - Trisomy 18
    - Down syndrome (trisomy 21)
- Sex chromosome anomalies (SCA)
  - The following SCAs are reported in singleton pregnancies:
    - XO (Turner syndrome)
    - XXY (Klinefelter syndrome)
    - XXX
    - XYY
- Fetal sex
  - Fetal sex is reported; opting out of fetal sex reporting is available.
- Fetal fraction estimation (FFE)

- FFE is reported as a percentage (standard deviation, 1.2%).
- Risk results
  - The accuracy, or PPV, of NIPT varies depending on the pretest risk for the assessed condition. For more information, please refer to Positive Predictive Value (PPV) of a High-Risk Noninvasive Prenatal Screening Result for Various Increased Pretest Risk Levels and for Various Gestational Ages and Maternal Ages.
  - An over- or underrepresentation of targeted chromosomes is reported as a high-risk or low-risk result.
  - High risk for autosomal aneuploidy reports include a PPV statement.
  - High risk for SCA reports include data from a large, prospective study to aid in counseling patients.[20]
    - If the result is high risk for fetal SCA, this interpretation is reported even if the fetal sex reporting selection is "No." Sex of the fetus may be inferred.
  - Low-risk reports include negative likelihood ratios for trisomies 13, 18, and 21, calculated by Gil, 2017.[1]
  - Follow-up recommendations are provided for high-risk results and cases of "no-call" results.
- Enhanced reports
  - Enhanced reports for high-risk autosomal aneuploidy results include a PPV table.

Limitations

- Aneuploidy for higher-order multiple gestations (triplets) is not assessed.
- Fetal demise/miscarriage is not assessed.
- NIPT is a screening test; it is not a diagnostic test. Therefore, results should not be the sole basis for any pregnancy management decision.
- Results should be considered in the context of other clinical findings and test results.
- A low-risk screening result does not exclude a fetal diagnosis of aneuploidy.
- A high-risk screening result does not confirm a fetal diagnosis of aneuploidy.
- Analysis may fail due to a combination of insufficient fetal fraction and dynamic quality metrics including data quality and coverage profile.
- A result may be unreportable due to findings that are uninterpretable or outside the scope of the test.
  - Additional details about the category of the no-call result will be reported.
- Only the risk for fetal aneuploidy involving chromosomes 13, 18, 21, X, and Y is assessed.
- The VeriSeq NIPT Solution v2[14] is not intended to detect the following conditions:
  - Aneuploidies involving chromosomes not interrogated by this test
  - Polyploidy, such as triploidy
  - Deletions or duplications within any chromosome
  - Balanced chromosome rearrangements
  - Copy number variants of any chromosome
  - Other genetic disorders
  - Birth defects (eg, open neural tube defect)
- NIPT analysis may yield unexpected results that indicate abnormalities in maternal cfDNA and/or other fetoplacental conditions, such as mosaicism for the targeted chromosomes.
- The results of the test can be confounded by certain maternal and fetal factors including, but not limited to, the following:
  - Recent maternal blood transfusion
  - Maternal organ transplant
  - Maternal surgical procedure
  - Maternal immunotherapy or stem cell therapy
  - Maternal malignancy
  - Maternal aneuploidy or mosaicism
  - Fetoplacental mosaicism
  - Fetal demise or nonviable twin

References

1. Gil MM, Accurti V, Santacruz B, et al. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis [update in *Ultrasound Obstet Gynecol*. 2019;53(6):734-742]. *Ultrasound Obstet Gynecol*. 2017;50(3):302-314.

2. Screening for fetal chromosomal abnormalities: ACOG Practice Bulletin Summary, No. 226. *Obstet Gynecol*. 2020;136(4):859-867.

3. Practice Bulletin No. 162: Prenatal diagnostic testing for genetic disorders. *Obstet Gynecol*. 2016;127(5):e108-e122.

4. Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. *Genet Med*. 2016;18(10):1056-1065.

5. Driscoll DA, Gross SJ; Professional Practice and Guidelines Committee. First trimester diagnosis and screening for fetal aneuploidy. *Genet Med*. 2008;10(1):73-75.

6. Jones KL, Jones MC, del Campo M. *Smith's Recognizable Patterns of Human Malformations*. 7th ed. Elsevier Saunders; 2013:7-13.

7. Meyer RE, Liu G, Gilboa SM, et al. Survival of children with trisomy 13 and trisomy 18: a multi-state population-based study. *Am J Med Genet A*. 2016;170A(4):825-837.

8. Otter M, Schrander-Stumpel CT, Curfs LM. Triple X syndrome: a review of the literature. *Eur J Hum Genet*. 2010;18(3):265-271.

9. Unique. Understanding rare chromosome and gene disorders. Accessed Apr 2022.

10. Bardsley MZ, Kowal K, Levy C, et al. 47,XYY syndrome: clinical phenotype and timing of ascertainment. *J Pediatr*. 2013;163(4):1085-1094.

11. Groth KA, Skakkebæk A, Høst C, et al. Clinical review: Klinefelter syndrome--a clinical update. *J Clin Endocrinol Metab*. 2013;98(1):20-30.

12. McKinlay Gardner RJ, Amor DJ. *Gardner and Sutherland's Chromosome Abnormalities and Genetic Counseling*. 5th ed. Oxford; 2018.

13. Milunsky A, Milunsky JM. *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment*. 7th ed. John Wiley and Sons; 2016.

14. VeriSeq NIPT solution package insert. Updated Aug 2021; accessed Apr 2022.

15. Cirigliano V, Ordoñez E, Rueda L, et al. Performance of the neoBona test: a new paired-end massively parallel shotgun sequencing approach for cell-free DNA-based aneuploidy screening. *Ultrasound Obstet Gynecol*. 2017;49(4):460-464.

16. Pertile MD, Flowers N, Vavrek D, et al. Performance of a paired-end sequencing-based noninvasive prenatal screening test in the detection of genome-wide fetal chromosomal anomalies. *Clin Chem*. 2021;67(9):1210-1219.

17. Borth H, Teubert A, Glaubitz R, et al. Analysis of cell-free DNA in a consecutive series of 13,607 routine cases for the detection of fetal chromosomal aneuploidies in a single center in Germany. *Arch Gynecol Obstet*. 2021;303(6):1407-1414.

18. Snijders RJ, Sundberg K, Holzgreve W, et al. Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound Obstet Gynecol*. 1999;13(3):167-170.

19. Snijders RJ, Sebire NJ, Nicolaides KH. Maternal age and gestational age-specific risk for chromosomal defects. *Fetal Diagn Ther*. 1995;10(6):356-367.

20. Liang D, Cram DS, Tan H, et al. Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. *Genet Med*. 2019;21(9):1998-2006.

Related Information

Maternal Serum Screening
Prenatal Testing for Chromosomal Abnormalities and Neural Tube Defects

ARUP Laboratories is a nonprofit enterprise of the University of Utah and its Department of Pathology. 500 Chipeta Way, Salt Lake City, UT 84108
(800) 522-2787 | (801) 583-2787 | aruplab.com | arupconsult.com