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# Noninvasive prenatal testing (NIPT) advanced-uses and limitations-a review article

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**Abstract**
Noninvasive prenatal testing (NIPT), or noninvasive prenatal screening (NIPS), determines the risk of fetal chromosomal aneuploidies. When the chromosome number is more or less than standard (46), the fetus is called aneuploidic and will show abnormality, both structural and functional. The pregnant mother's bloodstream contains a mix of cell-free DNA (cfDNA) made up of both maternal and placental cells. The DNA of placental cells and fetal cells is usually identical. Analyzing placental cfDNA allows early screening of the fetus for chromosomal anomalies.

**Keywords:** Gestational thrombocytopenia, pregnancy, asymptomatic thrombocytopenia

## Introduction

Noninvasive prenatal testing (NIPT) is a screening test which indicates whether the risk of chromosomal abnormalities in the fetus is high or low, using maternal blood. NIPT is rapidly becoming a first-tier aneuploidy screening test.

This test analyzes small DNA fragments circulating in maternal blood. These fragments are free-floating, cell-free DNA (cfDNA), containing < 200 DNA base pairs. During pregnancy, the mother's bloodstream harbours both maternal and placental cfDNA. Placental cfDNA is usually identical to the fetal DNA. Thus, it is an indirect method to analyse fetal chromosomes, without actually taking blood from the fetus, (Fig 1).

## Technologies used in NIPT

Targeted Approaches for NIPT, employing counting methods

- **SNP Analysis:** As the amount of cfDNA is very small, it must be amplified before analysis. PCR method is ued to amplify cfDNA, using specific SNP targets. After amplification, the next step is to sequence the targeted SNPs, to find the chromosomal pattern of specific chromosomes, comparing them between mother and fetus.
- **Microarray Analysis:** cfDNA fragments, amplified by PCR, are tagged with a fluorescent probe, then the fluorescent probe is attached (hybridised) to complimentary sequences on the NIPT microarray slide, and counted. If the fetal cfDNA is greater or lesser than the maternal cfDNA, it is inferred that the fetus has chromosomal aneuploidy.
- **Rolling Circle Amplification:** Rolling circle amplification targets specific cfDNA fragments which bind to a circular template and replicate by a rolling mechanism, thus getting amplified. The amplified products are fluorescently labeled and counted. Deviations in expected fluorescent counts indicate fetal aneuploidy.

With targeted approaches, only selected regions of specific chromosomes are being assessed. The whole chromosome is not being analysed, and there may be regions of deletion /duplication/structural rearrangements in chromosomes, which are escaping attention. These targeted approaches have additional steps and require more rounds of amplification than whole-genome sequencing methods.

Nowadays, NIPT uses next-generation sequencing (NGS) to sequence short cffDNA fragments to identify the genetic variants that represent chromosomal abnormalities [1]. It reduces the time and costs needed to analyse nucleic acids as compared to conventional methods.

**NIPT Is Reliable at Detecting $T_{13}$, $T_{18}$, and $T_{21}$, klinefelter syndrome (47XXY), Turner syndrome (45X):** NIPT should be mainly used for detecting trisomy 21, trisomy 18 and trisomy 13 and extra or missing copies of the sex chromosomes (Fig 2). The detection rate for chromosome 21 is very high, using NIPT (99.2% for singleton pregnancies and 93.7% for twin pregnancies). The detection rates for T18 and T13 are also quite high - 96.3% and 91%, respectively. Thus NIPT detection rates for the common aneuploidies are far more reliable, compared to traditional (dual and quadruple marker) techniques, (80-85% reliable detection rates).

The last decade has seen a large number of studies confirming the high accuracy of NIPT for screening common chromosome aneuploidies in singleton pregnancy. The overall sensitivity of NIPT was 99.17, 98.24, and 100% for T21, T18, and T13, respectively, and the specificity was 99.95, 99.95, and 99.96% for T21, T18, and T13, respectively [2].

**NIPT to detect chromosomal microdeletions and microduplications:** Chromosomes can have very small deletion/duplication areas in the p and q arms, which cannot be detected by karyotype. These anomalies are called submicroscopic copy number variation (CNV); they will not cause death of fetus, but they are responsible for major structural malformations and functional deficits like speech and developmental delay, autism, mental retardation, etc of the fetus. With the sequencing and bioinformatics analysis, NIPT can detect some microdeletion/microduplication syndromes. Factors that influence the performance of CNVs detection by NIPT include CNV size, sequencing depth, Fetal Fraction (FF), and GC content of the DNA fragment

The NIPT sequencing depth is 0.15×, and the data volume is 3 million reads; the NIPT-plus sequencing depth is 0.4×, and the data volume is 8 million reads [3].

Fetal CNVs ≥ 10 Mb length can be reliably captured by NIPT at conventional sequencing depths. However, NIPT cannot be relied upon to detect smaller CNVs [4, 5]. In case of an NIPT report indicating small CNV detection in different chromosomes, the report needs to be validated by further testing with either microarray or whole exome sequencing.

**Fetal fraction**

As the placenta reflects the fetus (trophoblast and inner cell mass both originating from the blastmere), fetal fraction (FF) is the percentage of cell-free DNA (cfDNA) that the placenta contributes. It is calculated by dividing the fetal cfDNA by the total amount of fetal and maternal cfDNA. FF, usually measured between 10 and 20 weeks of pregnancy, is 10-15%. Adequate FF improves the accuracy of NIPT.

Usually, a FF of 4% is set as the minimum threshold to obtain accurate NIPS results. A low FF should ring alarm bells for aneuploid fetus, hypertensive disorders of pregnancy and preeclampsia whereas significantly high FF may be early warning for a possible preterm birth. If a sample's FF is < 4% or >40%, it is considered a “no-call” result, meaning the NIPT report is invalid.

FF increases with gestational age and possibly female fetuses. The prevalence of twin pregnancies has increased greatly due to the advanced maternal and paternal age across society as well as the increased use of ART In twin pregnancies, FF ranges between 8-36%, with a mean of 12.2%. The total FF of DZ twins and monozygotic twins is 35% and 26% higher than that of singleton fetuses, respectively, since in dizygotic twins, there are two separate placentae, while monozygotic twins share a single placenta. Thus, large FF in twin pregnancies should be interpreted differently, and different zygosity should be considered. In case of miscarriage of one twin, the “vanishing twin’s” necrotic cytotrophoblasts flood the maternal plasma, causing a rapid nflux of cffDNA, but it stops by 12 - 14weeks. Therefore, if early USG indicates that previously there were 2 gestation sacs, but later, one fetus is found in late first trimester scan, then the blood sampling for NIPS for the alive fetus should be done after 14 weeks of gestation for NIPS so as to reduce the impact of a vanishing twin.

FF is higher in the trisomy 21 group, lower in the trisomy 18 and trisomy 13 groups than in the euploid group, which may complicate the efficacy of NIPS in detecting trisomy 18 and trisomy 13.

**What the NIPT Test Does Not Detect**

NIPT test is highly accurate screening test for detecting selected chromosomal abnormalities, but not diagnostic for trisomy 21, 13, 18. If NIPT indicates a higher risk, diagnostic testing, such as amniocentesis or CVS, may be recommended.

NIPT cannot detect conditions that involve single-gene disorders (e.g., cystic fibrosis or sickle cell anemia), neural tube defects (like spina bifida), or other non-chromosomal conditions.

**Limitations of NIPT**

The false positives present in NIPT is typically <1%. Confined Placental Mosaicism for aneuploidy is the most common cause. Certain chromosomal aneuploidies are confined to the trophoblast cells and may not be found in the fetal cells. ‘Vanishing twins’, maternal copy variants and maternal tumours are also possible causes. Tumour-derived cell-free DNA can add to the amount of maternal cfDNA in the maternal serum, thereby masking the cffDNA and its chromosomal profile.

One study detected a false negative rate of 0.09% with NIPT in determining T21 [6].The false negatives in NIPT is possibly due to a low FF, advanced maternal age, maternal obesity, sampling in early gestation. If shorter cffDNA fragments can be created during DNA extraction and library sequencing, the cffDNA fraction could be increased. Thus, repeating NIPT (using shorter cffDNA fragments) for false negative cases can identify cases overlooked due to low cffDNA fractions, e.g., CPM or twin pregnancy.

**Conclusion**

NIPT is a highly accurate screening test for detecting trisomy 21, 13, 18 but the accuracy is not as high in detecting aneuploidies in other chromosomes. CNV detection by NIPT is not widely accepted, and when detected, must be validated by follow up diagnostic tests, like microarray or sequencing. Due to false positives and false negatives reports, NIPT will remain a highly efficient screening test for common chromosomal aneuploidies, but not a diagnostic test.

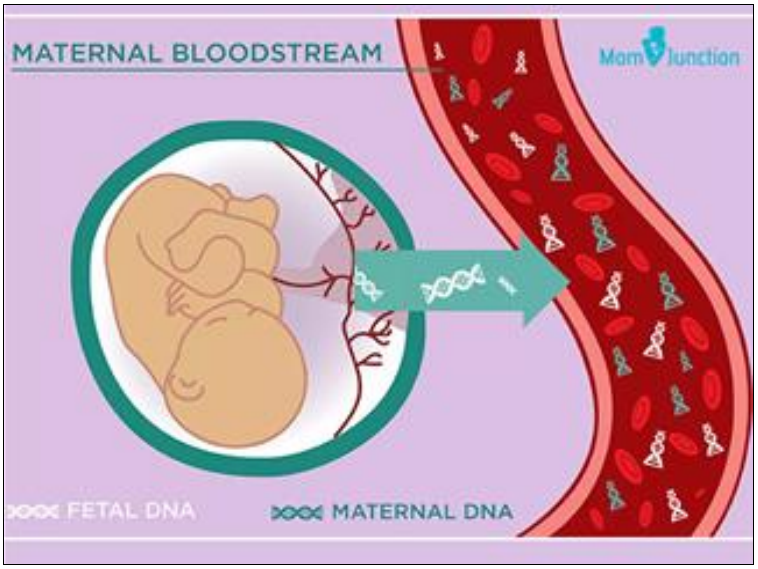


**Fig 1:** Maternal blood stream with maternal and fetal cfDNA

The detection efficiency of T21, T18, and T13 in NIPT

| NIPT | TP | FP | PPV (%) | Sensitivity (%) | TN | FN | NPV (%) | Specificity (%) |
|---|---|---|---|---|---|---|---|---|
| T21 | 88 | 11 | 88.89 | 98.88 | 38,874 | 1 | 99.99 | 99.97 |
| T18 | 16 | 14 | 53.33 | 100 | 38,944 | 0 | 100 | 99.96 |
| T13 | 5 | 20 | 20 | 100 | 38,949 | 0 | 100 | 99.95 |

**Fig 2:** Accuracy of NIPT in detecting trisomy 21, trisomy 13 and trisomy 18

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