# Non-Invasive Prenatal Testing In Maternal Plasma: Down Syndrome as a potential application

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## Aims and Methods

**Aims**

- To delve in non-invasive prenatal testing (NIPT) concretely with its applications and techniques.
- To compare between NIPT and the current prenatal screening.
- To consider the bioethical aspects related to prenatal testing.

**Methods**

Scientific literature search on PubMed database: recent papers and reviews, chosen depending on their data of publication and quality.

## 1. NIPT: an overview

NIPT is a potential emerging field in genetic prenatal diagnosis and screening. Thus, NIPT relies on the identification of fetal genetic material in the blood of pregnant women without no additional risk of miscarriage. The presence of fetal genetic material in the maternal bloodstream during the pregnancy is well-known and it is explained by the bidirectional traffic between the fetus and the mother through the placenta.

There are three possible fetal sources for NIPT (Figure 1): intact fetal cells, cell-free fetal DNA (cffDNA) and cell-free fetal RNA (cffRNA). Moreover, clinical applications of NIPT can be classified in four groups: sex-linked diseases, haemolytic disease of the fetus and newborn (HDFN), monogenic diseases and chromosomal aneuploidies.

The detection of universal fetal markers minimizes the interference between maternal and fetal DNA in maternal bloodstream. The different epigenetic pattern is an example of an useful method to detect fetal markers. In addition, PLAC4 mRNA is a placental transcript located on chromosome 21 so it is interesting for Down syndrome (DS) testing.

| Intact fetal cells | cffDNA | cffRNA |
|---|---|---|
| - Pregnancy specific<br>- Half-life: 25-35 days | - 3-6% of total cfDNA<br>- Pregnancy specific<br>- Half-life: 16.3 min | - Pregnancy specific<br>- Half-life: 14 min<br>- Stable and easy isolation |
| - Low concentration<br>- No identification of specific fetal markers | -Background of maternal DNA<br>-Specific fetal markers are necessary | - [cffRNA] is related to expression levels in placenta<br>- No need of specific fetal markers |
| Cell type: NRBC | 1st trimester | Depend on the marker (1st or 2nd trimester) |
| 2nd trimester | | |

Figure 1. Description of the different fetal sources for NIPT. cfDNA means cell-free DNA.

## 2. Techniques

**Single counting molecular techniques**

-The main advantage: analysis of total DNA (fetal plus maternal)

- The main inconvenient: cost

**Digital PCR**

Technical steps (Figure 2):

1. Dilution of the sample until <1 template DNA/well
2. Real-time PCR of each template DNA
3. Counting the number of positive reactions
4. Measure of the total DNA dosage

Advantages (comparing with Real-time PCR)

- Standards are no necessary
- Regulation of precision:

It depends on fetal DNA concentration

Application → Relative chromosome dosage (RCD)

Useful for Aneuploidy detection (Figure 3).

**Massively parallel sequencing (MPS)**

Technical steps (Figure 4):

1. Generation of millions of tags across the whole genome
2. Alignment and mapping of tags
3. Identification of chromosome origin
4. Counting of tags
5. Comparison of the number of tags from each chromosome to its reference value

Alternative approach:

**Digital analysis of selected regions (DANSR)**

Only it is analysed the chromosome of interest instead to whole genome

Application: → Aneuploidy detection

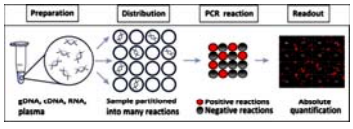


Figure 2. The main technical steps of Digital PCR.[1]

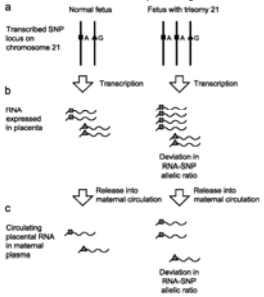


Figure 3. RCD scheme.[2]

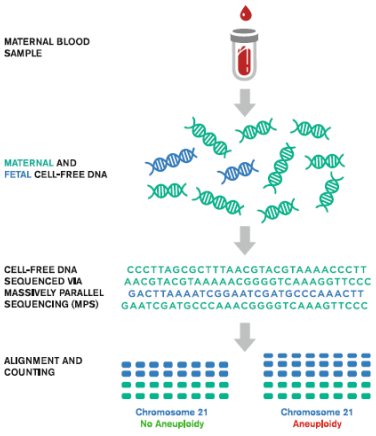


Figure 4. MPS scheme.[3]

## 5. Conclusions

- NIPT is performed with the aim to identify fetal genetic material in maternal bloodstream so as to detect genetic abnormalities. The absence of invasiveness allows to avoid additional risks caused by the procedure.
- NIPT can be applied for screening but not for definitive diagnosis.
- Economic, ethical, social and legal issues should be considered due to the ease and absence of risk performing NIPT.

## 3. Down Syndrome

Down syndrome (DS) or trisomy 21 is the most common aneuploidy (1/800 live births) and the most common cause of sever mental delay. DS is compatible with life but it involves some characteristic clinical features (Table 1). The high mortality is due to heart disease which is very frequent. In addition, maternal age and previous family history are the main risk factors (Figure 5).

**DS clinical features**

-Hypotonia
- Smaller nose and ears
- Slanted eyes
- Variable intellectual coefficient (45-70)
  - →intellectual impairment
  - →developmental delay
-Alzheimer susceptibility
- Leukaemia susceptibility (at earlier ages)

Table 1. It is summarised the main clinical features in DS.

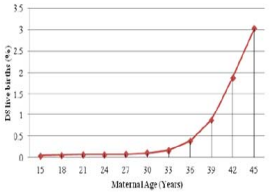


Figure 5. This graphic shows an exponential increase risk of carrying a DS baby as maternal age increases.

Owing to its high incidence, DS is the main reason why women undergo prenatal testing. The detection of DS prenatally involves screening and diagnostic testing. Currently, there are three screening algorithms designed with the aim to estimate a pregnancy-specific individual risk at carrying a DS fetus. These algorithms are based on the evaluation of three elements: maternal age, nuchal translucency (NT) and maternal serum biomarkers. Figure 6 only shows the first trimester combined test which is recommended by ISPD (International Society for Prenatal Diagnosis). Depending on the estimated risk level various strategies can be followed (Figure 7).

FIRST TRIMESTER SCREENING

| What is realized? | When? | Who? |
|---|---|---|
| 1. Echography<br>- Nuchal Translucency (NT)<br>2. Blood extraction:<br>- β-hCG and PAPP-A | 1. Week 11-13<br>2. Week 8-13 | All the women (age-independent) |

Combined test (NT + Biochemical markers): TP=75% and FP<3%

Figure 6. In this scheme is shown the essential information as regards first trimester screening. It allows to estimate risk level which can be low (<1:250) or high (>1:250).

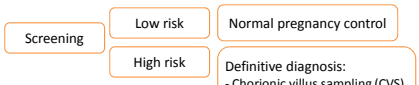


Figure 7. The risk level estimation is based on the screening results. A low risk pregnancy involves a normal pregnancy control with routine ecographies. Nevertheless, a high risk pregnancy requires to undergo a definitive diagnosis through invasive procedures (CVS and amniocentesis)

NIPT: screening or diagnosis?

Sensitivity and FPR are the variables that indicate if a test should be used for screening or diagnosis. NIPT presents high sensitivity and low false positive rate (FPR) (Table 2). Currently, NIPT cannot be considered diagnostic despite being a highly accurate screening test. Thus, invasive testing is also recommended in order to confirm a positive NIPT result (Figure 8). Improving sensitivity and specificity is crucial to reduce the number of invasive diagnostic tests offered to pregnant women and as a result reducing the number of miscarriages in affected and unaffected pregnancies.

| | Sensitivity (%) | FPR (%) |
|---|---|---|
| Current screening | 85-90 | 5 |
| NIPT | 79.1-100 | 0-2.1 |
| Definitive diagnosis | 100 | 0 |

Table 2. Comparison of sensitivity and FPR values among the different testing strategies.

**NIPT cannot be considered a diagnostic test**

**Two possible applications for NIPT:**
- First-line screening
- After a positive result from the current screening test

Figure 8. Currently NIPT is considered a screening test but it is not validated for definitive diagnosis.

## 4. Bioethical aspects

The development of NIPT has raised numerous ethical, social and legal issues so it is essential to evaluate the pros and cons (Figure 9). Some ethical concerns should be taken into account such as the right of "not to know" and the abortion.

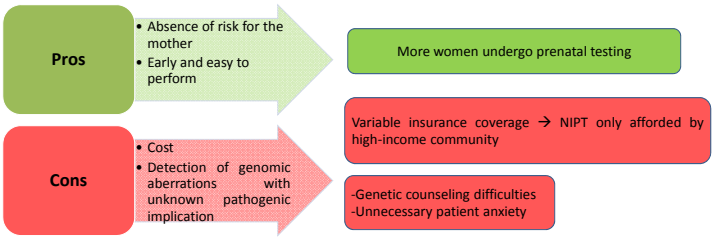


Figure 9. Summary of the pros and the cons of NIPT and their respective consequences.

## 6. References

1. http://www.lifetechnologies.com/es/en/home/life-science/pcr/digital-pcr.html
2. DENNIS LO YM, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nature Medicine* **(2007) (13):** 2
3. SWANSON A, et al. Non-invasive Prenatal Testing: Technologies, Clinical Assays and Implementation Strategies for Women's Healthcare Practitioners. *Genet Med Rep* **(2013) 1**:113–121