

# the partumpost

## Why NIPT is not a diagnostic test

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### High sensitivity for T21, T18, T13

Non-invasive prenatal testing (NIPT) using cell-free DNA (cfDNA) from maternal plasma has unparalleled sensitivity and specificity when screening for trisomies 21, 18 and 13.

In a large meta-analysis, the sensitivity (detection rate) for these trisomies was 99.7%, 97.9% and 99.0% respectively, while a false-positive rate (FPR) of just 0.04% was reported for each trisomy tested (1). The cumulative FPR of 0.12% means only about 1 in 830 pregnancies tested will receive a false-positive call for any one of these conditions.

In contrast, the screening performance of monosomy X was lower, with a sensitivity of 95.8% and a FPR of 0.14%.

These performance statistics confirm NIPT as a highly accurate screening test. However, the test cannot be considered diagnostic.

### Biological causes for inaccurate NIPT results

- **Fetal cfDNA is derived from the placenta**

Placental cfDNA is released into the maternal bloodstream from cytotrophoblast cells that undergo apoptosis. Thus, NIPT primarily analyses cfDNA from the outer cell layers of the chorionic villi; essentially it is a 'liquid biopsy' of the placenta (2).

It is well known that the chromosome constitution of placental cells can differ from the fetus. This is called confined placental mosaicism (CPM) and occurs when the placenta harbours a chromosome abnormality but the fetus is chromosomally normal.

CPM is the most common cause of false-positive NIPT results and occurs more frequently for trisomy 13 and monosomy X than it does for either trisomy 18 or trisomy 21 (3).

To help address this problem, VCGS calculates a mosaicism score on every trisomic sample called. This is then used to modify the positive predictive value (PPV) of the NIPT result.

The chance of confirming a chromosome condition in the fetus is significantly reduced when there is evidence of placental mosaicism. This knowledge can then be used to help patients with the most appropriate choice of follow-up testing. For example, performing amniocentesis instead of CVS.

- **Placental mosaicism as a cause of false-negative results**

Rarely, placental mosaicism can lead to false-negative NIPT results (4). Here, the placental cytotrophoblast cells are predominantly normal while the fetus has a chromosome abnormality.

We have investigated several false-negative trisomy 18 results and one false-negative trisomy 21 result caused by a high proportion of normal cells in the placenta. This type of placental mosaicism can lead to false-negative results despite the presence of average, or even high, fetal fractions in these samples.

- **CPM & fully discordant NIPT results**

Very rarely, CPM can cause a fully discordant NIPT result. We have seen one remarkable high risk monosomy X result where the fetus had a 47,XYY karyotype.

This unusual finding can be explained by a 46,XY zygote having misdivision of the Y chromosome during the first cell division after conception. This leads to 45,X and 47,XYY cell lines. The 45,X cells contributed to the majority of the placental trophoblast, while the inner cell mass and fetal precursor cells were comprised of 47,XYY cells. This error caused a false-positive result for monosomy X and a false-negative result for 47,XYY in the same NIPT sample. It clearly highlights the complexities of mosaicism in the early embryo.

- **Co-twin demise**

The presence of a demised co-twin (vanishing twin) earlier in pregnancy can cause a false-positive NIPT result when the demised twin is trisomic. This is because placental cells from the demised twin continue to release cfDNA into the maternal bloodstream (5). This can occur for many weeks after the initial demise and is also gestation dependent. The later the gestation at demise, the longer the cfDNA persists.

VCGS uses a sophisticated analysis algorithm to estimate the degree of trisomy mosaicism in the cfDNA sample. This algorithm can be used to aid pregnancy management, as the demised twin contributes less trisomic cfDNA over time.

Contact the *percept* NIPT service for advice on the timing of blood collection when caring for a patient with known co-twin demise.

- **Maternal chromosomal mosaicism**

On average, NIPT samples collected at 10-12 weeks of gestational age comprise 90% maternal cfDNA and 10% placental cfDNA. A low grade chromosome mosaicism carried by the mother can sometimes confound results.

This is particularly true when the mother carries a low grade monosomy X mosaicism (6). Again, more sophisticated analysis algorithms can minimise false-positive calls, thereby avoiding unnecessary prenatal diagnostic procedures.

The algorithm used by VCGS has recently been upgraded to help identify maternal mosaicism. Re-analysis of approximately 100 VCGS plasma samples previously called at increased risk for monosomy X returned significantly fewer false-positive calls.

This improvement is achieved by using additional statistics that interrogate the length of the cfDNA molecules. Maternal cfDNA fragments are, on average, slightly longer than placental cfDNA fragments. This difference is then used to help differentiate maternal cfDNA from the fetal cfDNA, thereby reducing false-positive calls associated with maternal mosaicism.

Besides maternal sex chromosome mosaicism, we have also seen discordant NIPT results caused by maternal trisomy mosaicism, copy number variant mosaicism and sex-mismatched transplants of donor tissues. Maternal mosaicism is often, but not always suspected as the cause of these discordant findings.

Lastly, maternal malignancy can be a rare cause of unusual NIPT results that do not reflect the fetal karyotype (7,8).

## Summary

While NIPT is often described as >99% accurate, placental and maternal mosaicism can contribute to false-positive screening results. Technical considerations can also lead to incorrect calls.

**Consequently, NIPT results should never be considered in isolation. Diagnostic testing is recommended to confirm high risk results.**

See our website for more information about *percept* NIPT: [vcgs.org.au/perceptNIPT](http://vcgs.org.au/perceptNIPT)

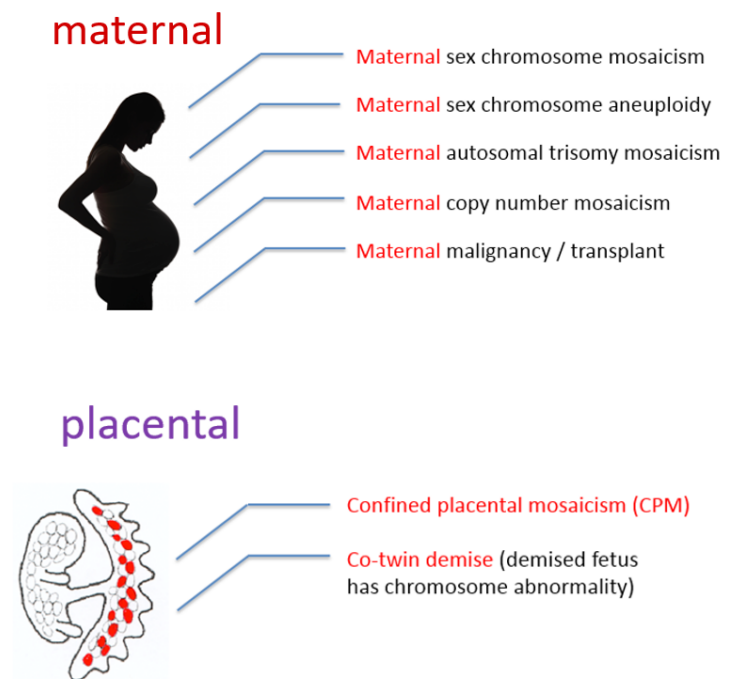


Figure 1. Maternal & fetal causes of false NIPT results

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