VCGS Leaders in Genetic Health

the partumpost

fetal fraction facts

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What is cfDNA?

Cell-free (cf)DNA is the name given to short, fragmented molecules of DNA that are released into the plasma following apoptosis (programmed cell death). The blood plasma from a pregnant woman will contain cfDNA that is both maternal and fetal in origin (1). Both components are analysed during non-invasive prenatal testing (NIPT).

What is fetal fraction?

Fetal fraction describes the proportion of the total cfDNA that is fetal in origin. The 'fetal' component is actually derived from placental trophoblast (2, 3). Thus, cfDNA analysis (NIPT) for fetal trisomy can be considered a 'liquid biopsy' of the placenta.

Why is fetal fraction important?

All methods of NIPT require a minimum fetal fraction for accurate trisomy screening, commonly estimated at 4% (4).

Several NIPT providers will fail NIPT samples at fetal fractions below this level. However, high analytical sensitivity can be achieved at lower fetal fractions by using protocols that combine statistical methods based on normalised chromosome values in combination with higher sequencing read counts (5, 6).

High read counts (counting more cfDNA molecules) is known to improve analytical sensitivity at lower fetal fractions. *percept* NIPT employs these methods to accurately detect trisomy at fetal fractions as low as 2.0-2.5% (VCGS validation data on file).

The *percept* NIPT assay has recently achieved NATA/RCPA accreditation for compliance with NPACC Standards and ISO 15189 for screening at lower fetal fractions.

What biological factors influence fetal fraction?

The average fetal fraction in samples taken between 10 and 14 weeks of pregnancy has been described as around 10% (4). The actual value is influenced by a number of factors. These include the woman's gestation, her weight, placental size, whether the pregnancy is singleton or twin and also whether a trisomy is present (4, 7-10).

Pregnancies with Down syndrome will have the highest average fetal fraction for gestation. Pregnancies with trisomy 18 or 13 have lower average fetal fractions. The average in chromosomally normal pregnancies lies somewhere between these two (6).

The mean gestational age on NIPT samples received by VCGS is 11 weeks and 3 days, with an average fetal fraction of 8% (VCGS data n=25,000). Blood collected at earlier gestations (10-12 weeks) results in many women having a fetal fraction measurement below 4% (Fig. 1).

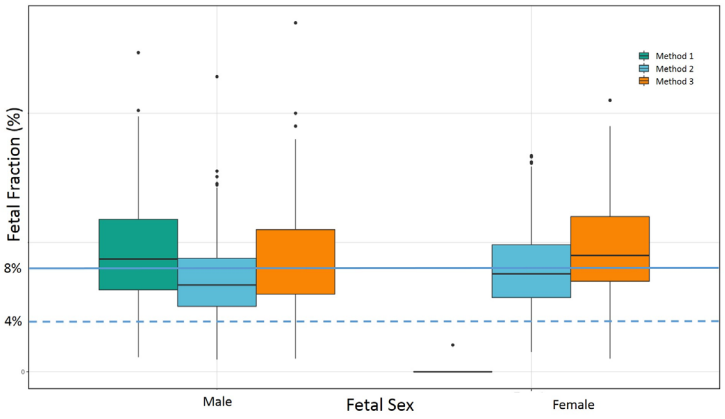

Figure 1. Fetal fraction measurement for a set of 423 male and 383 female pregnancies using multiple fetal fraction measurement tools.

In a continuous set of 3,500 *percept* NIPT referrals, 180 samples (5.1%) had a fetal fraction below 4%. This frequency is similar to the 6.1% of NIPT samples that were cancelled for fetal fractions below 4% when VCGS used an external NIPT provider during 2013-2014 (n=994).

By lowering the limit of detection for fetal trisomy using percept's improved methodology, the failure rate from low fetal fraction is now only 0.2%.

How does VCGS measure fetal fraction?

VCGS is the only NIPT provider in Australia that measures fetal fraction on every sample using more than one method.

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There is no gold standard for measurement, although most consider Y chromosome sequence count comes closest. Unfortunately this method is only suitable for male pregnancies. VCGS estimates the fetal fraction in male pregnancies using four methods, all of which are incorporated into its analytical bioinformatics pipeline. This includes Y chromosome sequence count.

In female pregnancies, two bioinformatics methods are used. In trisomic pregnancies, an additional method is applied to estimate the relative proportion of trisomic cfDNA in the sample (we call this the 'trisomic fraction'). This measurement is used to help guide clinicians with the choice of follow-up diagnostic testing.

What are the benefits of sensitive screening at low fetal fractions?

- Highly sensitive screening for trisomy at low fetal fractions reduces initial NIPT test failure rates to well below 1%. Women are not inconvenienced by repeat blood collection and will increase their probability of successful screening (9).
- High sensitivity is achieved on every pregnancy, not just on those with a fetal fraction above 4%.
- *percept* NIPT is ideal for screening at earlier gestations, for women with high BMI and in dichorionic twin pregnancies where the average fetal fraction per fetus is generally lower. *percept* is also available for screening triplet pregnancies with prior approval.
- Observed sensitivity and specificity for trisomy 21 using *percept* NIPT is >99.5% and 99.98% respectively, based on 25,000 pregnancies screened.
- The positive predictive value (PPV) for increased risk trisomy 21 results is 99.4% based on available outcome data.

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