NEXT BIOSCIENCES

TriScreen Non-Invasive Prenatal Testing (NIPT)

Cumulative prenatal testing statistics - 2024

Contents

Introduction

Dear Colleague,

Next Biosciences has collated summary statistics on TriScreen NIPT performed in-house by our laboratory from January 2020 to December 2023. We are pleased to report that our data continues to correlate very closely to international statistics. We are hoping that you find the data that we present informative and interesting. Non-Invasive Prenatal Testing (NIPT) is becoming widely recognised as a significant and important screening test during pregnancy with improved sensitivity and specificity compared to traditional screening options. The American College of Obstetricians and Gynaecologists (ACOG) and the Society of Maternal-Fetal Medicine (SMFM) recommend that NIPT be offered as a first-tier screening test for all pregnant women, regardless of risk.[1]

Please feel free to contact us if you have any comments or queries.

Kind regards,

Dr Yvonne Holt
Next Biosciences Chief Medical Officer

Overview

It is estimated that chromosomal anomalies occur in 1 in 154 live births, with a higher prevalence earlier in gestation as aneuploidy accounts for a large proportion of early pregnancy loss.[2] Factors increasing a patient's risk of a chromosomal anomaly include; advanced maternal age (AMA), a parental balanced translocation, ultrasound anomalies or an elevated risk indication from a maternal serum screening test.

TriScreen NIPT is a highly accurate screening test used to determine the risk of fetal chromosomal anomalies. In principle, cell-free fetal DNA (cffDNA) in maternal plasma, which is primarily derived from the placenta, is detectable from as early as 10 weeks' gestation. **TriScreen** offers various NIPT options to cater for multiple patient scenarios. The first of which is the standard panel NIPT option (TriScreen), which includes testing of **chromosomes 13, 18, and 21** for both singleton and higher order pregnancies, with the addition of **sex chromosome anomalies** for singleton pregnancies. The next option offered is **TriSceen+**, which offers an extended panel for **singleton pregnancies, including all 23 pairs of chromosomes** and allows for the detection of **rare autosomal anomalies (RAAs) and copy number variations (CNVs)**. The third option is the **Next T21,** designed to be an affordable testing option if Trisomy 21 is the only concern. This option does not report on sex chromosomes. All samples are processed in-house at our laboratory in Midrand with the Illumina CE-IVD VeriSeq NIPT platform, utilising high-throughput whole-genome next generation sequencing on a NextSeq 550Dx instrument.

Key differentiators for TriScreen NIPT:

- Expanded panel for all chromosomes available for singleton pregnancies.
- Testing of higher order pregnancies (standard panel only).
- Testing of IVF pregnancies, including surrogate and egg donor pregnancies.
- Sex chromosome testing.
- Reporting of segmental duplications or deletions larger than seven mega base pairs (Mbp).
- Reporting on lower fetal fractions (FF).
- Low test failure rates.
- Commitment to making NIPT more accessible through assistance with medical aid authorisation and convenient payment plan options for patients.
- Free genetic counselling offered to patients with a high risk NIPT result.
- Ten years' experience in offering NIPT with clinical advice which is readily accessible.
- Flexible blood draw options, including the option of a travelling nurse in larger centres.
- Personalised client services assistance throughout the NIPT process.

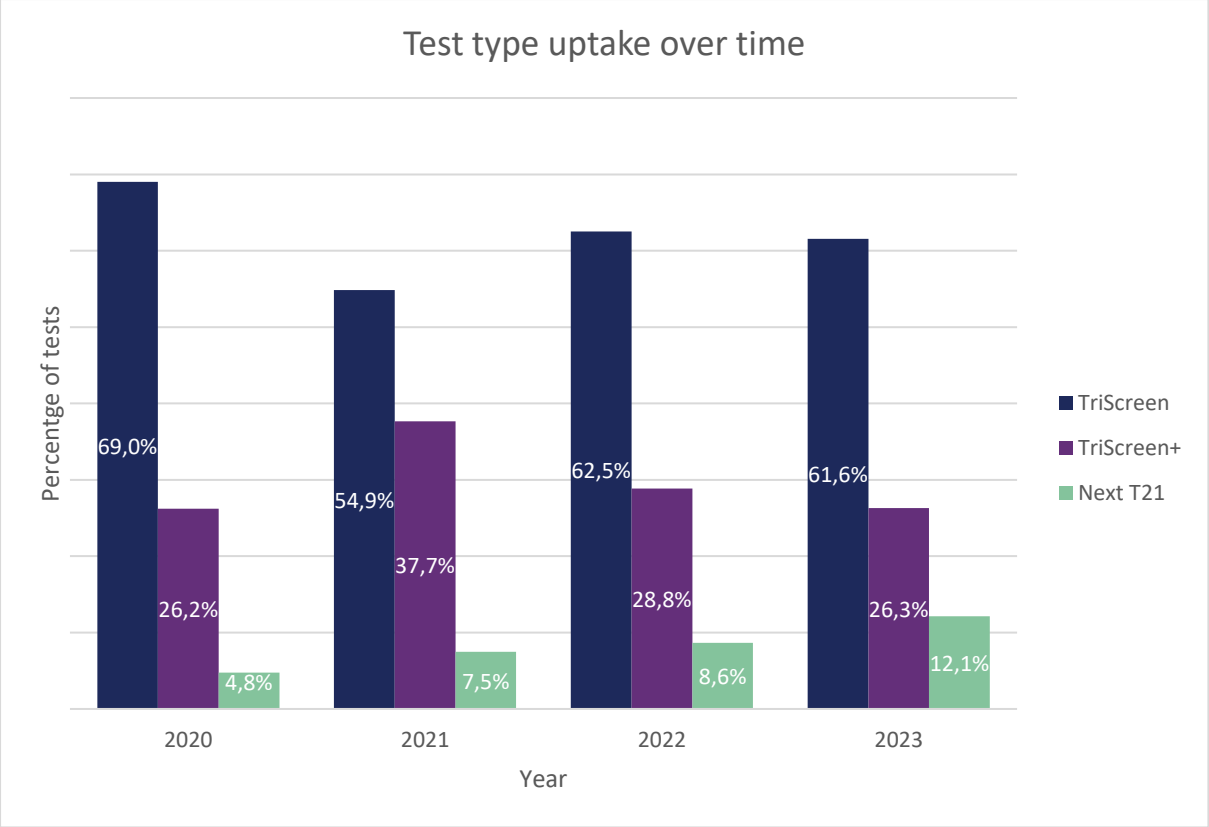

Figure 1: Uptake of the three testing options: TriScreen (standard panel), TriScreen+ (all chromosomes), and Next T21 (chromosome 21 only) since 2020.

Overall test performance

For the period January 2020 to December 2023, results were obtained for 99.9% of pregnancies, of which 2.64% represented twins. We are, therefore, proud to report a no-result rate of only 0.09%, which is lower than what has been published for the Illumina VeriSeq NIPT clinical validation study.[3] This is important because 'no call' results (uninterpretable and therefore not reported) can lead to increased anxiety for both the patient and the healthcare provider and may lead to an increased number of follow-up invasive procedures to obtain information about the pregnancy. Low risk results were reported for 97.4% of pregnancies tested.

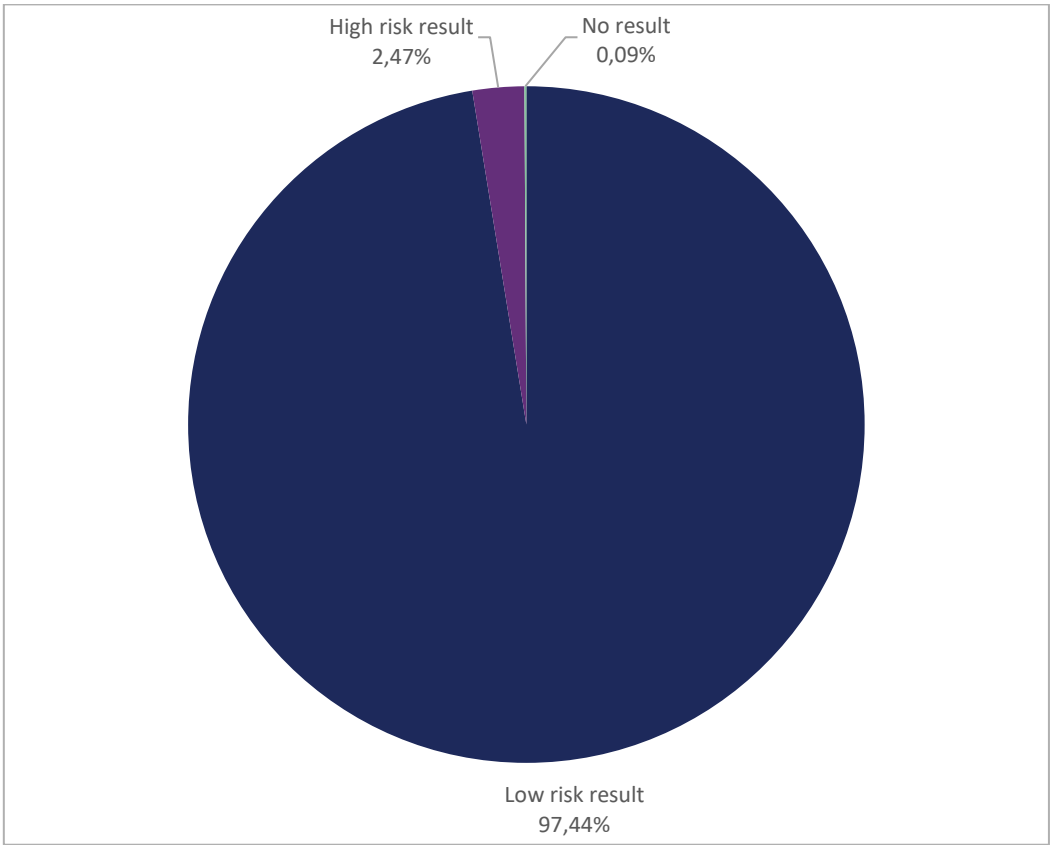

Figure 2: Overall performance of the TriScreen NIPT test.

Results obtained

We reported a high-risk result for 2.5% of pregnancies tested. The anomalies detected are shown in Figure 3. As expected, Trisomy 21 is observed most frequently.[4] Sex chromosome anomalies (SCAs) include Turners (45,X0), Jacobs (47,XYY), Klinefelter (47,XXY), and Triple X (47,XXX) syndrome. TriScreen+ represented 29.8% of requests, which enabled us to report on RAAs and CNVs in this subset of patients. Together these groups account for 24.5% of anomalies detected, therefore making them the second most common type of anomaly in this data set. This is in line with international data studies of large cohorts.[4]

Although TriScreen has a very high sensitivity and specificity (more than 99.9% for trisomy 21, 18 and 13[3]), it is considered a screening test. Next Biosciences strongly advise that high risk test results are followed up by a diagnostic test (chorionic villus sampling or amniocentesis). A high risk NIPT result should not be considered in isolation and irreversible clinical decisions not made without confirmation or additional support for such a result. **A free genetic counselling session is available to all patients who received a high risk NIPT result.**

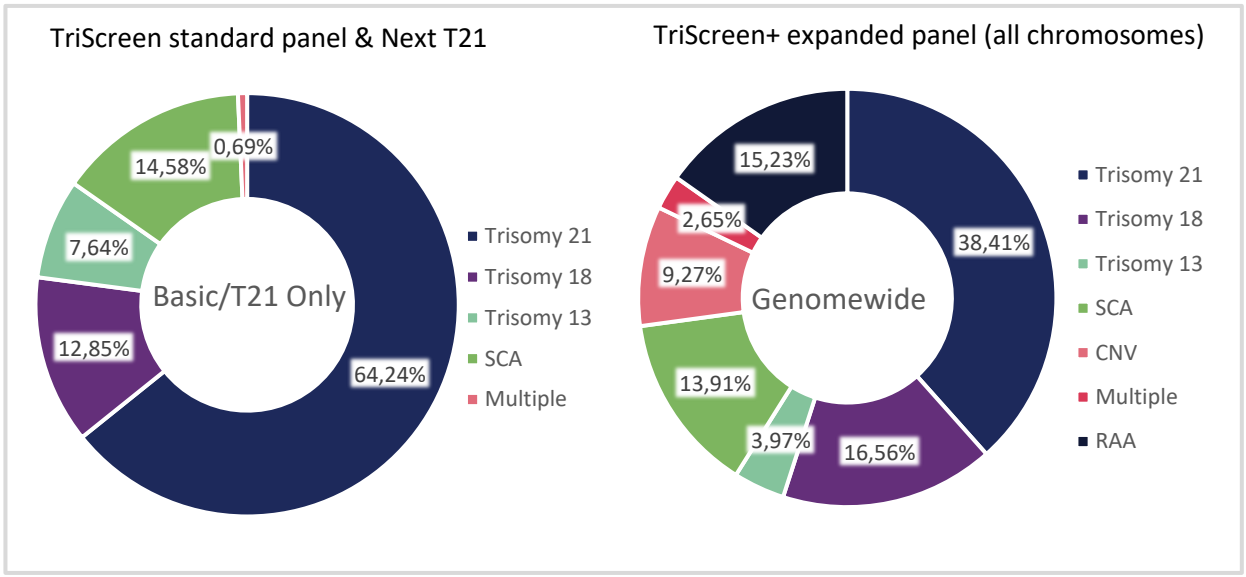

Figure 3: Distribution of anomalies obtained from TriScreen, Next T21 and TriScreen+ NIPT. RAA – rare autosomal anomalies, SCA – sex chromosome anomalies, CNVs – copy number variants. Multiple refers to aneuploidy which involved more than one chromosome.

TriScreen NIPT is a highly accurate screening test for aneuploidy, but it is important to be aware of the limitations of the technology. The test is not designed to detect mosaicism and cannot detect triploidy or tetraploidy. Unbalanced structural changes (segmental duplications/deletions) larger than 7Mbp can be detected with TriScreen+ but balanced structural changes cannot.

Referrals per gestational age

Our results show that the largest proportion of NIPT referrals are from early pregnancies (before 14 weeks' gestation) (Figure 4), comprising 29.7% of tests. The majority of trisomy's detected with a gestational age of >24 weeks was Trisomy 21.

We would encourage doctors to refer patients for NIPT in the first trimester of their pregnancy, as this will allow more time to consider and plan for a diagnostic test in the event of a high risk NIPT result.

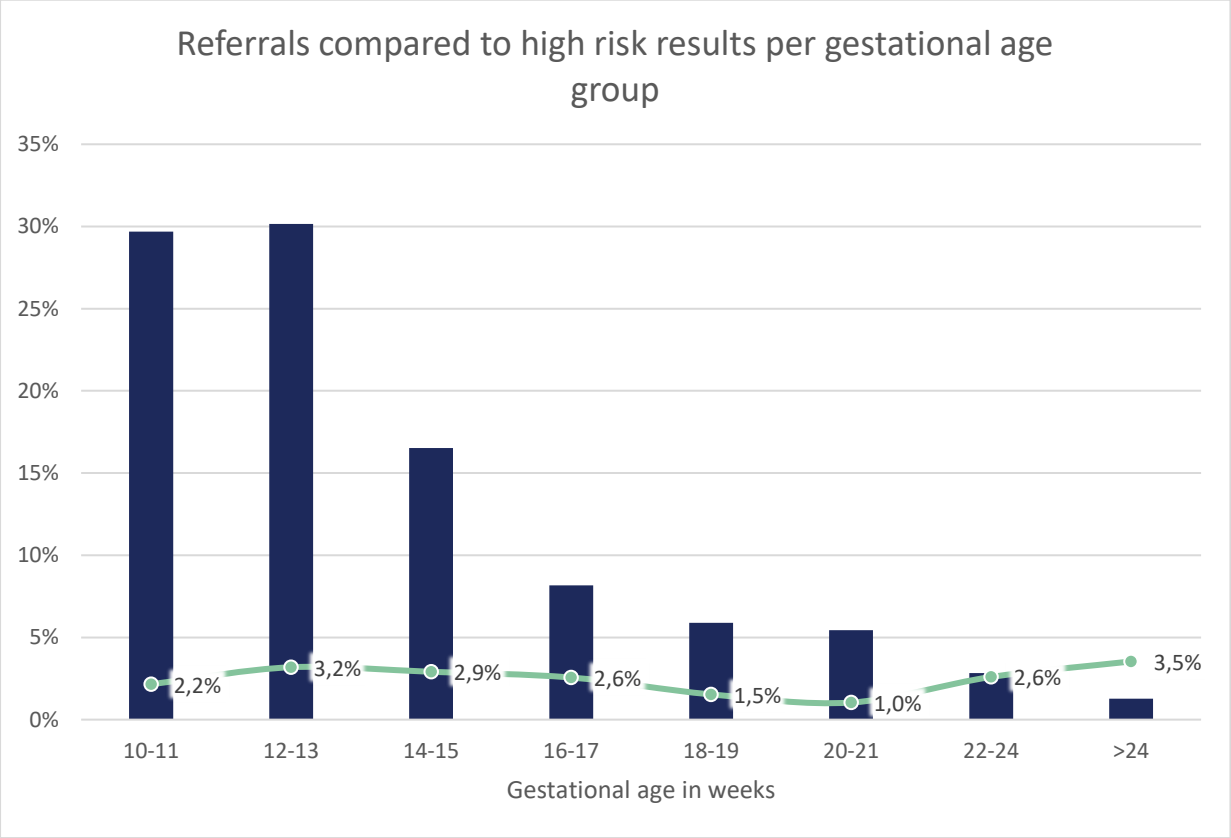

Figure 4: The referrals received per gestational age are represented by the solid bars and the percentage of referrals at each gestational age group that resulted in a high risk NIPT result indicated for each.

Referrals per maternal age group

Results obtained per maternal age group is indicated in Figure 5. As expected, we saw an increase of anomalies as maternal age advanced, with the highest incidence of aneuploidy detected within the maternal age groups of >35 years. It is well documented that advanced maternal age (AMA) is an indication for aneuploidy risk that applies to pregnant women over the age of 35 years[5]. However, the aneuploidy risk in younger women is not negligible. Please note that the lower aneuploidy rate reported of the 40-45 year age group may misrepresent the actual risk due to the low number of pregnancies in this group and the potential inclusion of *in vitro* fertilisation pregnancies using egg donors.

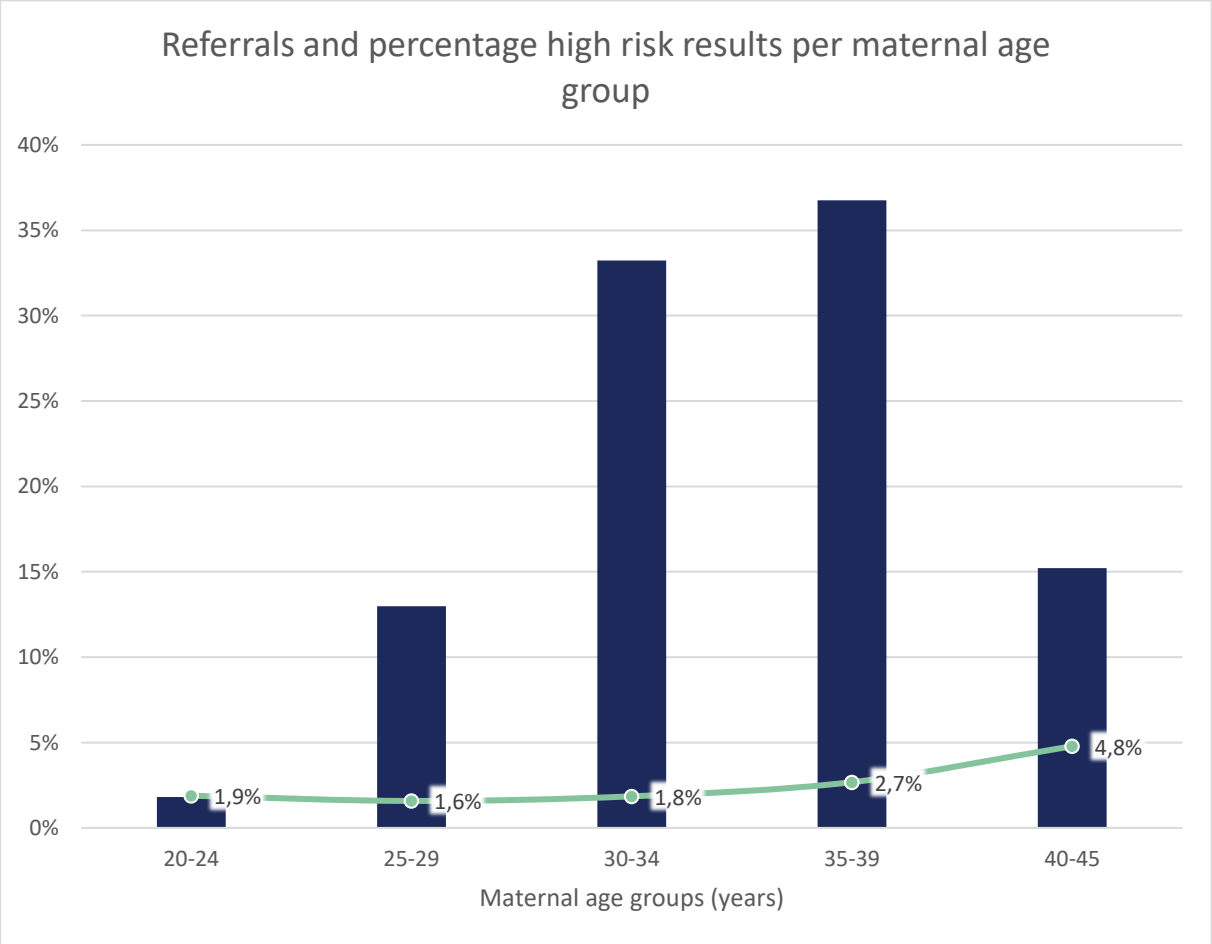

Figure 5: TriScreen NIPT referrals per maternal age group (five-year intervals) are represented by the solid bars and the percentage of referrals that resulted in a high risk for an anomaly indicated for each age group.

Fetal Fraction (FF)

The importance of FF as a test statistic is often emphasised in the literature, with mention that a FF of 2-4% may be considered minimal and too low for an accurate result, however, the threshold of detection depends on the NIPT technology.[6] TriScreen utilises whole-genome sequencing, which has a lower detection limit as it does not rely on FF alone, but considers parameters, such as sequencing depth, to generate a dynamic quality control threshold metric referred to as the iFACT (individualised fetal aneuploidy confidence test). Our data indicates that FF does not increase significantly during the first two trimesters (Figure 6).

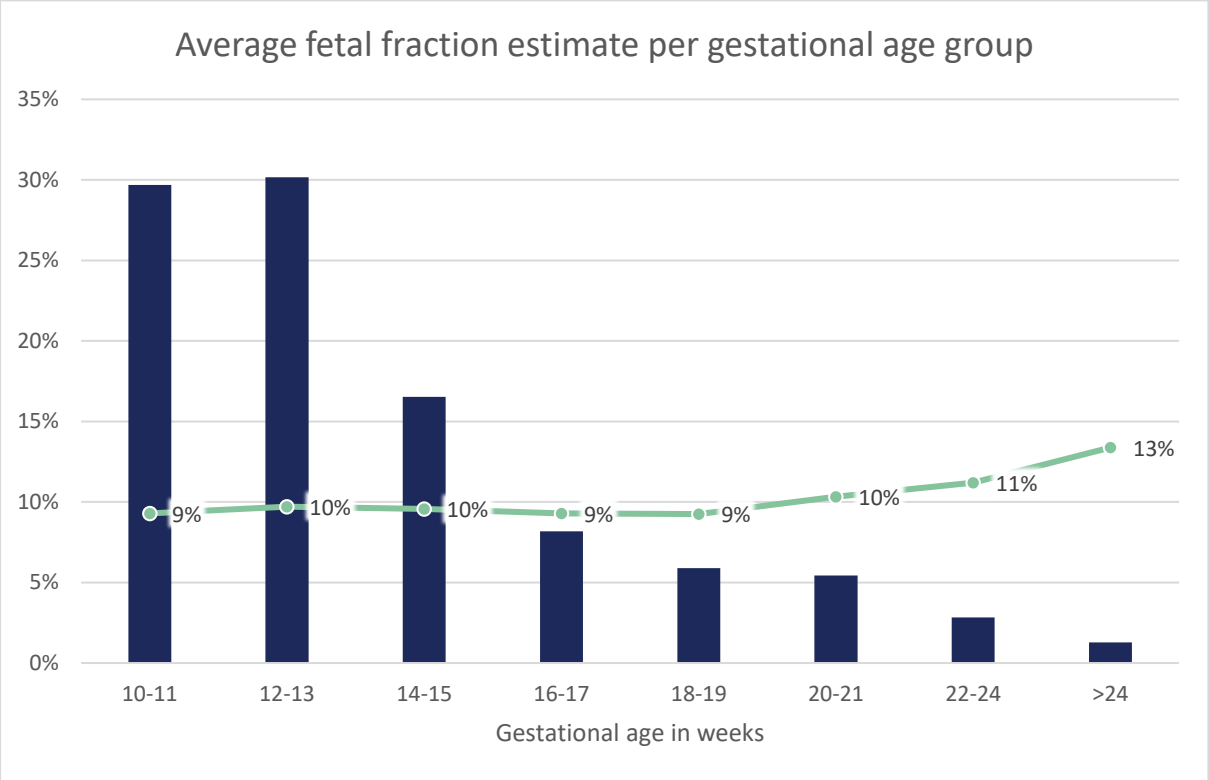

Figure 6: TriScreen NIPT referrals received for the various gestational ages are represented by solid bars, with the average fetal fraction percentage for each group indicated.

Test failures are usually FF-related. Besides gestational age, the FF is affected by many biological factors, including maternal body mass index (BMI), maternal inflammatory and autoimmune conditions, as well as medication exposure.[7] Literature suggests that trisomy 13, trisomy 18, and triploidy have all been associated with a lower FF, which could contribute to a 'no call' test result. Similarly, BMI has also been shown to have a significant effect, and can be used to explain a large majority of test failures.[8] In an attempt to lower our no result rate, we have implemented a testing policy for no result samples. Our policy is to repeat all tests that fail to produce a result after the first run. After a second test failure, a redraw is requested and the test is repeated a third time at no additional cost to the patient.

Funder reimbursement

It is important to note that we rely on the accuracy of the information provided on the test request form for patient referral reasons. Figure 7 shows how the average medical aid reimbursement rate for NIPT can vary between different test indications. The overall reimbursement rate has remained close to 60% for all pregnancies tested over the last four-year period.

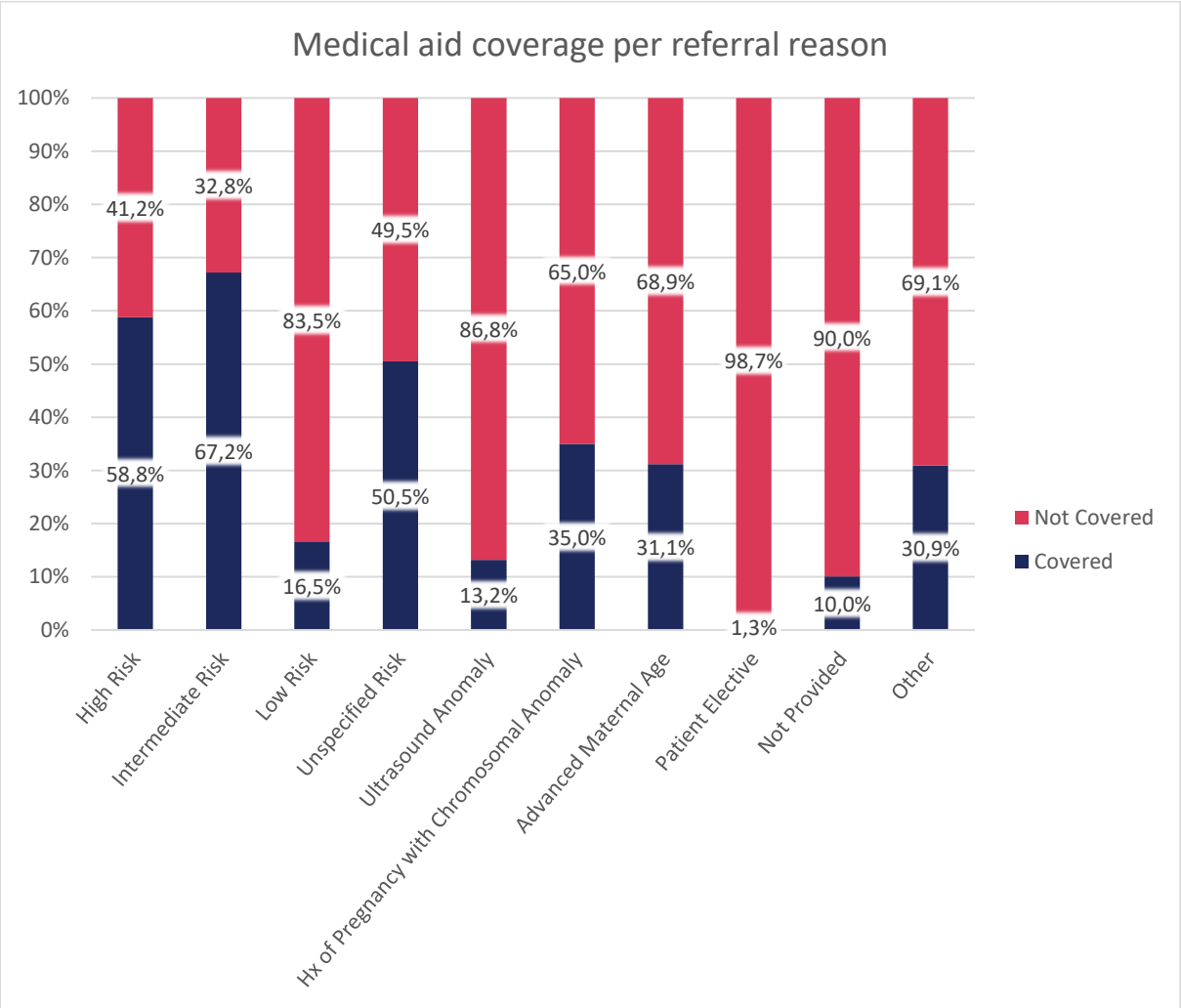

Figure 7: Percentage of referrals for each test indication and the associated percentage of referrals that were authorised or declined by the patients' medical aids.

A higher anomaly detection rate was obtained for patients referred for patient concern or anxiety only (patient elective), compared to patients with an intermediate serum screen result (1.2% vs. 1.0% respectively) (Figure 8). This is an important finding considering that medical aid reimbursement was only 1.3% for the low risk patient group (Figure 7). This lends itself to the significant value NIPT has for all pregnancies. In addition, pregnancies that had an ultrasound anomaly detected were the largest high risk cohort at 10.1%, however, many medical aids do not provide coverage for NIPT based on these criteria.

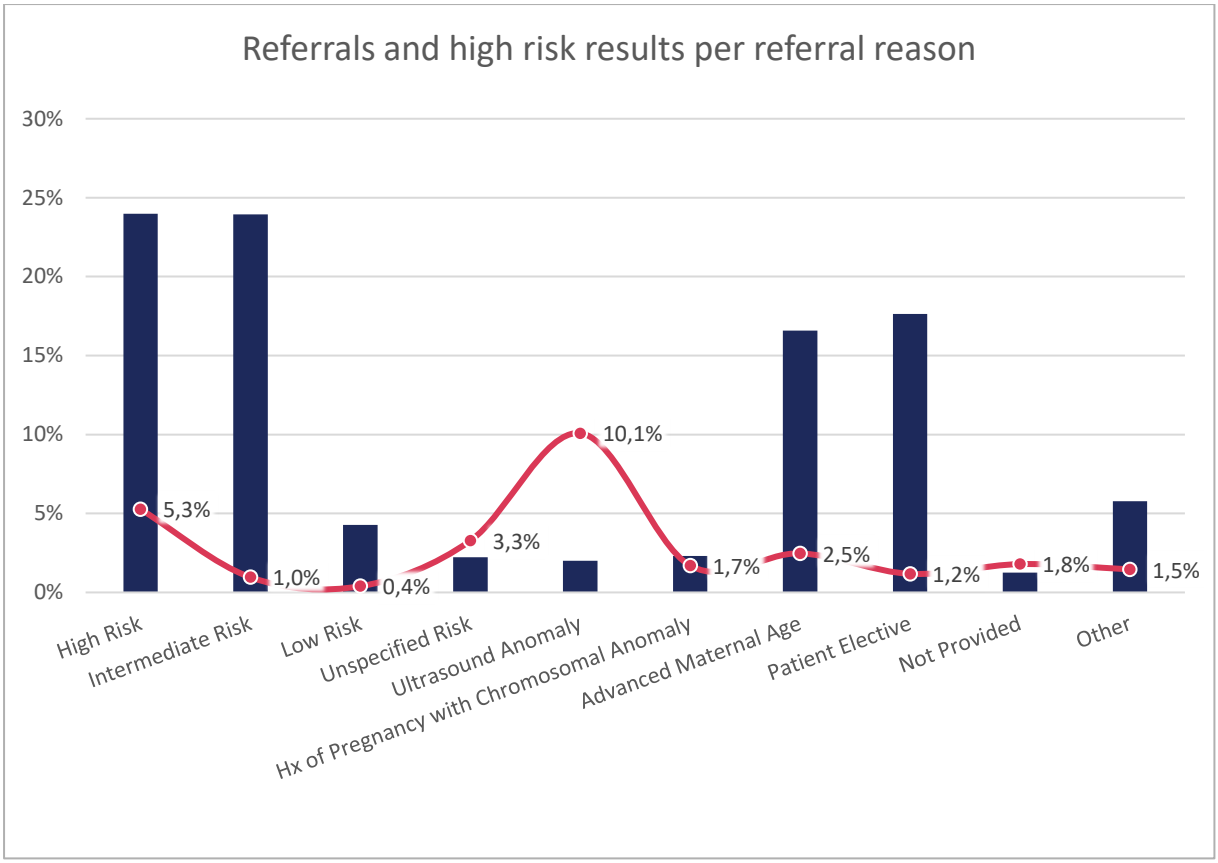

Figure 8: The tests performed for the various referral reasons are indicated by solid bars, with the percentage of high risk results (showing aneuploidy) reported indicated for each category by the red line.

Nine percent of test requests were cancelled by patients before their sample was processed. This is largely due to medical aid rejection and financial constraints not allowing the patient to pay out of pocket for the test. Next Biosciences is committed to increasing access to care. As a result, we will continue to engage with medical funders and motivate for more inclusive coverage criteria for better access to NIPT for South African patients.

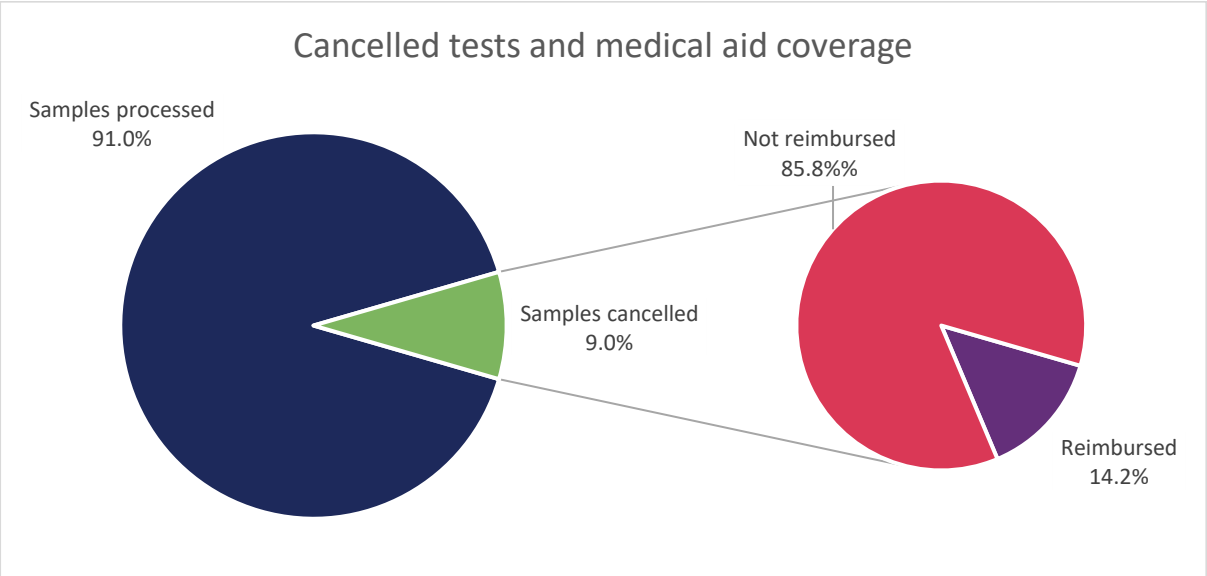

Figure 9: Proportion of cancelled test requests and the medical aid reimbursement.

Follow up studies

Although TriScreen is considered a highly sensitive screening test with equally high specificity, false positive and negative results are a reality of all NIPT technologies. We have embarked on a follow-up study to assist us with internal quality control and to improve on the accuracy of our reports. To facilitate this, we contacted healthcare professionals for information on the outcomes of 302 pregnancies with high risk NIPT results. We were able to obtain feedback for 75% of cases and assign an outcome to 67% of these cases (Figure 10). Cases where we were not able to assign an outcome include cases with no data obtained as well as the undetermined category which includes cases where we do not have enough information to assign an outcome. The undetermined category includes cases where a termination of pregnancy was done without invasive testing. No additional clinical information was provided to us. We acknowledge that multiple factors including patient choice may play a role in the decision to proceed to a termination. To be able to produce accurate follow-up data having all relevant information regarding the pregnancy is of utmost importance.

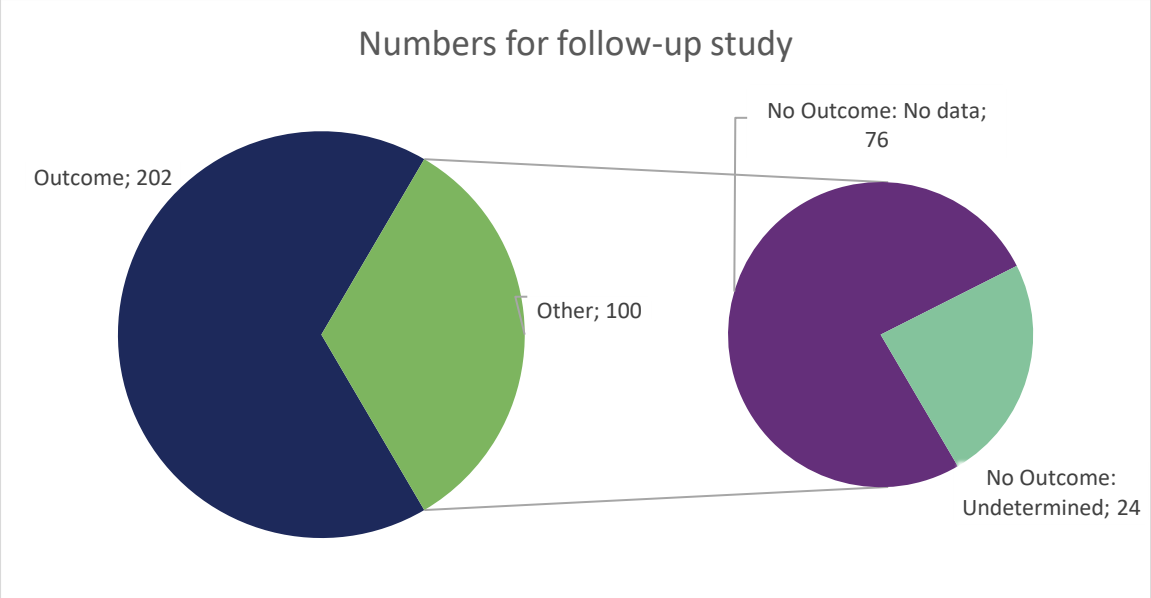

Figure 10: Summary of cases included in follow-up study, showing the number of cases for which outcomes could be obtained. The no outcomes category includes cases for which limited information was available (undetermined) and cases for which no information was available (no data).

The follow-up outcome categories have been defined as follows:

- **True positives** are cases where diagnostic testing or postnatal testing confirmed the NIPT result.
- **Likely true positive** are cases where no diagnostic testing was done but scan or postnatal examinations support the NIPT result.
- **False positives** are cases where a diagnostic test result during pregnancy or testing done postnatally did not detect the presence of a chromosomal anomaly in the fetus.
- **Likely false positive** cases had no diagnostic testing done but no clinical features were noted following delivery.

Overall, our data show that 83.7% of high risk TriScreen NIPT results were true positives or likely true positives. As expected, the highest specificity was seen for detecting Trisomy 21 (94.9% true and likely

true positive results), followed by Trisomy 18 (89.3%), Trisomy 13 (71.4%) and SCAs (83.3%). Although RAAs and CNVs showed the largest number of false positives (77.8% and 66.7% respectively), the numbers were also much lower which will affect the accuracy of the data. It does, however, demonstrate the value of TriScreen+ and the testing of all chromosomes as a number of cases were confirmed with diagnostic testing.

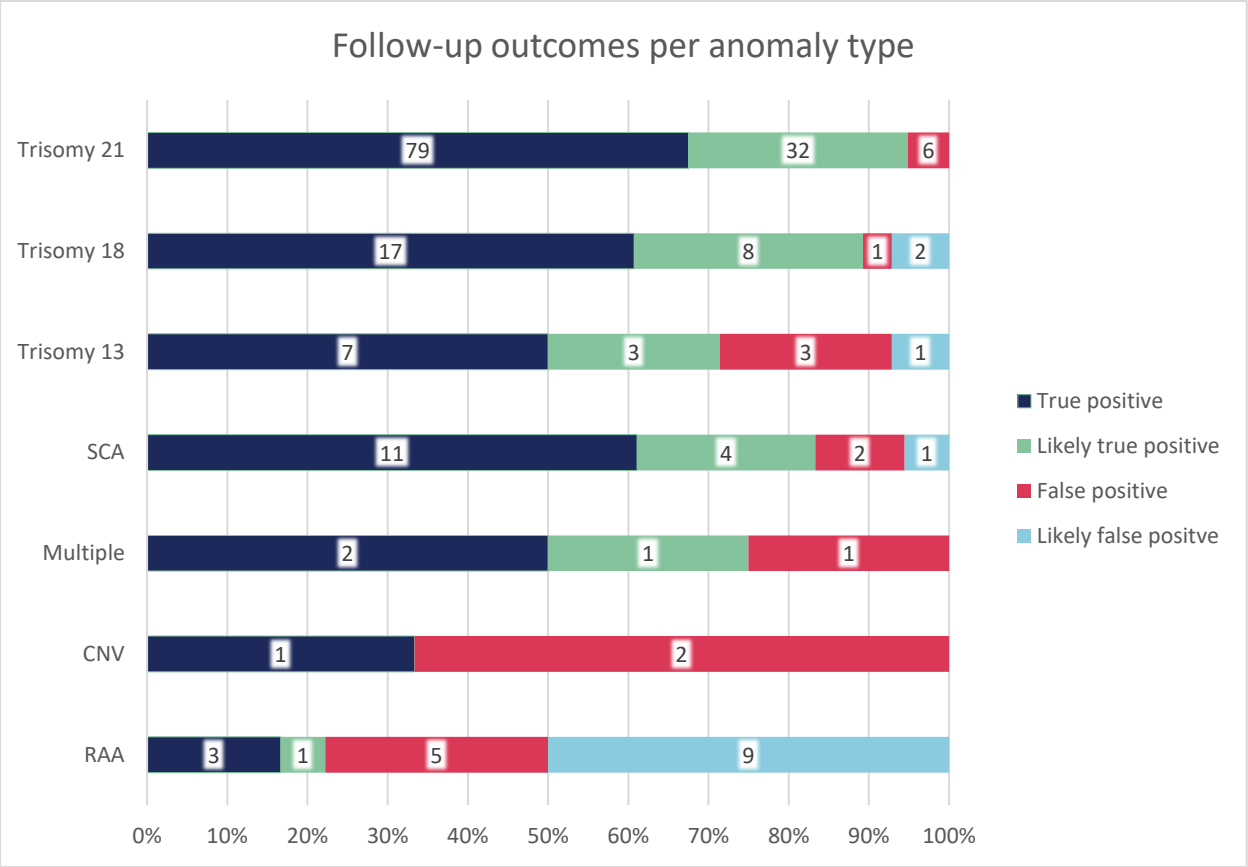

Figure 11: Outcome data for high risk TriScreen NIPT results based on the type of anomaly detected and the outcome category. The figure shows both actual numbers and percentages. SCA – sex chromosome anomalies; CNV – copy number variations; RAA – rare autosomal anomalies; Multiple refers to aneuploidy which involved more than one chromosome.

Most false positive results are due to underlying biological factors, such as the persistence of biological material from a vanishing twin, maternal malignancy or maternal chromosomal mosaicism, confined placental mosaicism (CPM), or true fetal mosaicism.[9,10] CPM is the most common reason for discordant results. CPM involving aneuploidy has the potential to adversely affect the pregnancy, it has been associated with low birth weight, fetal growth restriction, preterm birth and structural fetal anomalies.[11] It is, therefore, important to be aware of CPM and manage the pregnancy accordingly.

NIPT technologies are designed to have the highest sensitivity for detecting chromosomal anomalies and therefore to limit false negative results. We have not received any reports of false negative results for TriScreen NIPT during this period, providing assurance of the accuracy of this test.

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