Non-Invasive Prenatal Testing

Information for medical practitioners

Harmony Prenatal Test evaluates the risk for trisomies 21, 18 and 13 in women of any age or risk category
Since non-invasive prenatal testing (NIPT) first became available in early 2013, there have been changes to test methodologies and the emergence of new evidence about performance from recent studies. After reviewing the currently available forms of NIPT, Sonic Genetics conclude that the Harmony™ Prenatal Test offers the best attributes of quality, reproducibility and consistency that will bring significant benefits to clinicians and the patients they care for.

NIPT referrals are accessible to any doctor caring for any pregnant woman anywhere across Australia via an extensive network of collection centres. If you require further information and copies of recent study findings, please visit Sonic Genetics NIPT website www.sonicgeneticsnipt.com.au

Harmony™ Prenatal Test is validated in women of all ages 2-8, 11-13, 15

- Harmony™ Prenatal Test is clinically validated for use in pregnant women, of any age or risk category, to assess the likelihood of fetal trisomies 21, 18 and 13.
- Harmony™ Prenatal Test is the most broadly studied cell-free DNA-based maternal blood test
  - Blinded clinical studies in more than 23,000 women of all ages
  - More than 500,000 pregnancies tested worldwide

The difference in performance is clear

- A study of blinded prospective 15,841 patients compares First Trimester Combined Screening (FTS*) with Harmony™ Prenatal Test in a general pregnancy population. 15
- The Harmony™ Prenatal Test test accurately identified all 38 trisomy 21 cases, versus 30 out of 38 with FTS*.
- FTS* produced 5.4% (n=854) false-positives, compared to Harmony which had 0.06% (n=9) false-positives for trisomy 21.

*Serum PAPP-A, total or free β-hCG & Nuchal Translucency

Unsurpassed accuracy

Harmony™ Prenatal Test delivers unsurpassed accuracy when compared to any other trisomy 21 blood test. In blinded studies of more than 22,000 pregnant women aged 18 to 50 for trisomy 21:

<table>
<thead>
<tr>
<th>Trisomy 21 PERFORMANCE</th>
<th>COMBINED PERFORMANCE (trisomies 21, 18, 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DETECTION RATE</td>
<td>&gt; 99%</td>
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<tr>
<td>FALSE-POSITIVE RATE</td>
<td>&lt; 0.1%</td>
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What is NIPT?

NIPT is an advanced screening test for common chromosomal abnormalities. It is more accurate than other screening tests, such as the combined first trimester screen (fetal ultrasound plus maternal serum screening) for Down syndrome (trisomy 21), Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13). However, NIPT is not 100% accurate and should not be regarded as a diagnostic test. All abnormal results should be confirmed by invasive testing before making significant clinical decisions, e.g. termination of pregnancy. An unexpected normal result, e.g. in a fetus with malformations, may also warrant invasive testing.

How does NIPT work?

NIPT involves testing millions of short fragments of DNA in maternal plasma. Some of these fragments will have come from the placenta, and most will be from the mother.

The number of fragments derived from each chromosome is determined by NIPT. In a mother with a chromosomally normal fetus, the proportion of fragments from each chromosome will be within a narrow normal range. But if the fetus has an abnormal number of chromosomes, the fetal contribution for that chromosome will be abnormal and will distort the overall proportion.

A number of different methods have been developed for NIPT, and others are under development. Sonic Genetics regularly reviews the various implementations of NIPT and assesses their performance, scope and cost.

Sonic Genetics recommends the Harmony™ Prenatal Test for NIPT.

What does NIPT screen for?

NIPT screens for Down syndrome (trisomy 21), Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13). These are the most common autosomal trisomies. They cause moderate to profound intellectual disability and are associated with major congenital malformations.

Sex chromosomal abnormalities, such as Turner syndrome (45,X) and Klinefelter syndrome (47,XXY), can also be detected, but with reduced accuracy. Sex chromosomal abnormalities are often clinically milder than other chromosomal abnormalities. Testing for sex chromosomal abnormalities will also reveal the fetal gender.

It is important to note that chromosomal abnormalities vary widely in the severity of the problems they cause. Some methods can detect trisomies of other chromosomes; however, these trisomies almost always result in spontaneous miscarriage. NIPT is also being extended to detect rare, small deletions in specific areas of certain chromosomes (microdeletions); these more recent developments also have reduced accuracy.

Can NIPT be used for twin pregnancies?

There is less experience with twin pregnancies than with singleton pregnancies. Nonetheless, the accuracy of the test for detecting trisomies 21, 18 and 13 appears to be very good, provided the laboratory is advised that it is a twin pregnancy.

It is also important to advise the laboratory of the presence of a non-viable fetus together with its viable twin, as the non-viable fetus may be releasing DNA into the maternal circulation, which can be detected by NIPT.

Abnormalities of sex chromosome number cannot be reliably detected in a twin pregnancy.

This test has not been validated for triplet or higher order multiple pregnancies for chromosomes 21, 18 and 13 and the sex chromosomes.
Should a woman have conventional first trimester screening and ultrasound as well as NIPT?

NIPT is more accurate than conventional first trimester screening but conventional screening still has a place in prenatal care. The two tests measure different things, that is, genetic code versus fetal and placental anatomy and biochemical function.

Two testing scenarios for FTS and NIPT exist:

**Option 1**  
Concurrent testing

When a woman has both conventional first trimester screening and NIPT, the conventional screening provides complementary information regarding risk of chromosomal abnormalities. It can also provide information not addressed by NIPT — such as an assessment of gestational age, detection of fetal structural abnormalities, and an assessment of risk of pre-eclampsia and fetal growth restriction.

**Option 2**  
Sequential testing

An alternative strategy is for a woman to have conventional first trimester screening initially, and then have NIPT only if this shows her to be at increased risk. This strategy is less costly overall (fewer women have NIPT), but the lower detection rate by conventional screening compared with NIPT means that some fetuses with trisomy 21, 18 or 13 will be missed. It would be a matter for the medical practitioner and patient to decide whether the risk of trisomy determined by conventional screening is sufficiently low that NIPT is not required.

The current position of RANZCOG is that NIPT “as a primary screening modality in the general pregnant population requires more clinical and economic evaluation”. Conventional first trimester screening and ultrasound still have important roles in prenatal care, and should be considered whether or not a woman has NIPT.

How accurate is NIPT?

NIPT is highly accurate, detecting more than 99% of fetuses with trisomy 21, and more than 95% of fetuses with trisomy 18, trisomy 13 or abnormalities of sex chromosomes. These are much better detection rates than we observe with conventional first trimester screening for these trisomies (85% for trisomy 21, 50% for trisomy 18 and 50% for trisomy 13). NIPT is also much better at identifying fetuses with normal chromosomes than conventional first trimester screening; more than 99.9% of normal fetuses are categorised correctly by NIPT, versus 95% by conventional first trimester screening.

NIPT is a very good screening test, but it is not a diagnostic test. There will occasionally be a difference between the result of the test and the actual chromosomal status of the fetus. This may be due to the statistical design of the test, or to biological factors, such as the fetus and placenta having different numbers of chromosomes.

What is NOT included in the NIPT test?

NIPT does not detect every genetic abnormality in the fetus, or every developmental problem that might occur during pregnancy. NIPT will not detect the following conditions:

1. Less common or ‘atypical’ chromosomal abnormalities. These make up approximately 20% of all chromosomal abnormalities, and occur more commonly in pregnancies with fetal malformations and/or with very high risk scores on combined first trimester screening. These abnormalities can often be detected by invasive genetic testing, that is, CVS or amniocentesis.
2. A specific mutation that might be known to run in the family, e.g. cystic fibrosis or Huntington disease. Please contact us on 1800 010 447 if you are concerned about this possibility.
3. Non-chromosomal disorders, such as neural tube defects, placental abnormalities and intra-uterine growth retardation.

This list of exclusions is not complete.

NIPT does not screen for all chromosomal abnormalities. It does not screen for other genetic disorders or birth defects.

Considerations before using NIPT

NIPT is a new and powerful investigation that carries major clinical implications for mother and fetus. Before proceeding with the test, it is vital that:

- clinicians understand the purpose, performance and limitations of the test
- patients are informed about these aspects of the investigation, and
- appropriate consent is provided before the test is initiated.

Sonic Genetics requires that patients provide written consent for this test to assure the medical practitioner and patient that these issues are understood. This leaflet provides a brief overview of these matters. Please contact us on either 1800 010 447 or (03) 9287 7700 or check our website, www.sonicgeneticsnipt.com.au, if you require further information.

References

14. Estimate based on an average-risk population with prevalence for T21, T18, and T13 of 1 in 700, 1 in 5000, and 1 in 16000 respectively.
How should the NIPT result be interpreted?

The accuracy of NIPT varies with the prior risk that the woman has an affected fetus. Three general scenarios are considered below.

Woman who is at low risk (1 in 1,000, or 0.1%) of her fetus having a chromosomal abnormality

This may apply to a young woman with normal fetal ultrasound, or a woman with a low-risk result from conventional first trimester screen.

**If the NIPT result is normal:**

It is highly likely that the result is correct, and that the fetus does not have any of the chromosomal abnormalities tested.

In such a low-risk setting, a normal result from NIPT is correct in more than 999 cases out of 1000. The power of NIPT to correctly identify a normal fetus is the main clinical benefit of this test.

**If the NIPT result is ABNORMAL:**

It is likely but not definite that the result is correct, and that the fetus has a chromosomal abnormality. The probability of the result being correct varies from 30-90% for different chromosomal abnormalities.

An abnormal NIPT result is more likely to be correct than an abnormal conventional first trimester screen. Nonetheless, an abnormal NIPT result must be confirmed by invasive testing by CVS or amniocentesis before making major medical decisions.

Woman who is at increased risk (1 in 100, or 1%) of her fetus having a chromosomal abnormality

This may apply to an older woman, or a woman with an increased risk result from conventional first trimester screen.

**If the NIPT result is normal:**

It is likely that the result is correct, and that the fetus does not have any of the chromosomal abnormalities tested.

In this setting, a normal result from NIPT is correct in more than 99.5 cases out of 100. The power of NIPT to correctly identify a normal fetus is the main clinical benefit of this test.

**If the NIPT result is ABNORMAL:**

It is highly likely but not definite that the result is correct, and that the fetus has a chromosomal abnormality. The probability of the result being correct varies from 80-95% for different chromosomal abnormalities.

In this setting, an abnormal NIPT result must be confirmed by invasive testing by CVS or amniocentesis before making major medical decisions.

Woman who is at high risk (1 in 10, or 10%) of her fetus having a chromosomal abnormality

This may apply to a woman whose fetus has malformations on ultrasound, or who has a very high risk from conventional first trimester screening.

NIPT may be less useful in this setting. A normal result for the tested chromosomes is still very likely to be correct, but the risk of a ‘false negative’ result is increased. It is also possible that the fetus has a chromosomal abnormality that is not tested by NIPT. Invasive testing by CVS or amniocentesis should be considered.

NiPT is a very good test, but it is not perfect. Abnormal results should be confirmed by invasive testing before acting on that result. Invasive tests may also be appropriate in the case of an unexpectedly normal result.
Arranging a test

When  The test can only be performed after 10 weeks’ gestation, and then at any time up to term. This test has not been validated at earlier gestations, as the concentration of fetal DNA in maternal plasma is too low prior to 10 weeks.

How  The blood sample is collected into specific tubes. The specific tube must be used to ensure that the DNA in the maternal plasma is not degraded.

Where  Testing is only available at specific collection centres and only on specific days (please call either 1800 010 447 or (03) 9287 7700 for details).

How long does the test take?

We aim to provide you with the test result within 5–10 business days. On rare occasions, the NIPT test is unable to provide results. This may be due to there being too little fetal DNA in the mother’s blood sample, or to problems in the shipping or analysis of the sample. The amount of fetal DNA in maternal blood increases during the pregnancy and it is usually possible to provide a result with a repeat blood sample. The need for such a repeat sample is usually not known until the final stages of the analysis.

What does the test cost?

NIPT is currently not rebated by Medicare nor covered by private health insurance.

Please visit www.sonicgeneticsnipt.com.au to find out the current price, or call us on either 1800 010 447 or (03) 9287 7700.

Payment is required in advance. In the rare circumstance that a repeat sample is required, the repeat test is performed at no additional cost.

NIPT request form

We require a specific request form that assists with the provision of the required clinical information. It must be signed by both requesting medical practitioner and patient to confirm that the patient has been adequately counselled and that the patient gives her written consent.

www.sonicgeneticsnipt.com.au