Non-invasive prenatal testing
Information for Doctors

Now including 22q11.2 deletion
What is NIPT and cfDNA?

Non-invasive prenatal testing (NIPT) is intended to screen a pregnant woman’s blood to determine the chance that the developing baby has specific chromosome abnormalities. During pregnancy, DNA from the mother and placenta circulate in the mother’s plasma. This DNA is called cell-free DNA (cfDNA) and results from the natural breakdown of maternal and placental cells. cfDNA is the basis for the most accurate screening test for common chromosome abnormalities in the fetus. A number of different methods have been developed for NIPT, and Sonic Genetics recommends the Harmony Prenatal Test.

As early as 10 weeks’ completed gestation, the Harmony Prenatal Test evaluates the risk of trisomy 21, 18 and 13, plus 22q11.2 deletion, if requested. It is validated for use in pregnant women of any risk category.
How does Harmony work?

Harmony requires a single blood draw from a pregnant woman, and can be performed as early as 10 weeks’ completed gestation. Harmony involves testing millions of short fragments of cfDNA in maternal plasma. Most of the fragments will be from the mother, but as the pregnancy progresses, an increasing proportion (the ‘fetal fraction’) will be derived from the placenta. If the fetal fraction is sufficient for accurate analysis, the test determines whether the amount of cfDNA from specific chromosomes is consistent with the fetus having the normal number of those chromosomes. It can also screen for presence of a microdeletion at 22q11.2, if requested. The analysis does not proceed if the fetal fraction is too low for accurate analysis.

The measurement of fetal fraction is the key differentiator of the Harmony NIPT. Many other NIPTs do not assess fetal fraction, and therefore cannot reveal if there is sufficient fetal DNA to generate an accurate result.

Clinicians in more than 100 countries have trusted Harmony to screen more than 1,000,000\(^1\) pregnancies.
What does Harmony screen for?

Harmony screens for the three most common autosomal chromosome trisomies, as well as aneuploidies of the sex chromosomes and deletion, upon request.

- **Trisomy 21 (Down syndrome)** is the most common trisomy identified at birth. Down syndrome is associated with moderate intellectual disability and may also cause congenital heart defects and other malformations.

- **Trisomy 18 (Edwards syndrome) and Trisomy 13 (Patau syndrome)** are associated with a high rate of miscarriage. These babies are born with severe brain abnormalities and often have congenital heart defects as well as other birth defects. Most affected babies die before or soon after birth, and very few survive beyond the first year of life.

- **22q11.2 deletion (DiGeorge syndrome)** is the most common microdeletion syndrome. It is often associated with cleft palate and congenital heart disease.

- **Sex chromosome abnormalities (SCA)**, i.e. abnormalities of the X and Y sex chromosomes, are less common and often less severe than trisomy 21, 18 and 13. Monosomy X can have effects including heart and kidney disease and could be as severe as 22q11.2 deletion (or more so). Some sex chromosome abnormalities are associated with infertility, for example, Turner syndrome (45,X) and Klinefelter syndrome (47,XXY), and may be associated with other malformations or developmental issues. Other sex chromosome abnormalities, such as triple X (XXX) and Jacobs syndrome (XYY), are of less medical consequence. These sex chromosome abnormalities can be detected with Harmony.

Some NIPT methods can detect trisomies of other chromosomes; however, these trisomies almost always result in early spontaneous miscarriage.
Why choose Harmony for your patients?

- Test earlier than conventional first trimester screening (FTS)
- Superior accuracy to other NIPTs and conventional FTS
  - Fetal fraction assessment
- Clinically proven results
  - Exceptionally accurate results
  - Reduction in false-positive rate by 90-fold
  - Higher detection rate
- Significantly reduces invasive procedures, such as CVS and amniocentesis
- Free genetic counselling for eligible high-risk patients
- Quality assurance
  - Performed in Australia at our NATA-accredited laboratory

Test earlier than conventional first trimester screening (FTS)

Conventional FTS can be performed after 12–14 weeks’ gestation. Harmony can be performed from 10 weeks’ completed gestation – giving your patients peace of mind sooner.

Superior accuracy to other NIPTs and conventional FTS

Fetal fraction assessment

All forms of NIPT rely on the presence of sufficient fetal DNA for analysis. Unlike many other types of NIPT, Harmony includes a precise and accurate measure of the fetal fraction (the proportion of cfDNA in maternal circulation derived from the fetus and placenta). This important step identifies samples that do not have sufficient fetal DNA to draw an accurate conclusion, minimising the risk of a false-negative result. NIPT methods that do not have this step could potentially provide a normal result from an abnormal pregnancy (because the fetal fraction is so low, the result simply reflects the mother’s chromosomes rather than those of the fetus).

Clinically proven results

Most of the published experience with NIPT has involved small cohorts of women at relatively high risk of having an abnormal fetus. A publication in the New England Journal of Medicine (23 April 2015) involved a prospective blinded cohort of more than 15,000 pregnant women of various ages and risk, across multiple international sites, comparing the performance of Harmony versus first trimester screening. Harmony was far more accurate in identifying both normal and affected fetuses. Note that 22q11.2 was not part of this study and the performance data shown below is from a separate study, published in Fetal Diagnosis and Therapy (8 November 2017).

<table>
<thead>
<tr>
<th></th>
<th>EFFECTIVENESS</th>
<th>EFFECTIVENESS</th>
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<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>False-positive</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>75-92%</td>
<td>3-5%</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>86-96%</td>
<td>3-5%</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>73-90%</td>
<td>3-6%</td>
</tr>
<tr>
<td>22q11.2 deletion</td>
<td>Not detectable</td>
<td></td>
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</tbody>
</table>
Exceptionally accurate results

<table>
<thead>
<tr>
<th>T21: Harmony (38 of 38)</th>
<th>&gt;99%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T21: First trimester screening (30 of 38)</td>
<td>79%</td>
</tr>
</tbody>
</table>

Higher detection rate

Harmony 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 the sex chromosomes, plus more than 75% of fetuses with 22q11.2 deletion. These are higher detection rates than observed with conventional first trimester screening for these conditions. More than 1 out of 5 affected pregnancies may go undetected with FTS.

Reduction in T21 false-positive rate by over 90-fold

<table>
<thead>
<tr>
<th>9 of 15,803</th>
<th>False-positives with Harmony</th>
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<tbody>
<tr>
<td>854 of 15,803</td>
<td>False-positives with FTS</td>
</tr>
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</table>

Significantly reduces invasive procedures, such as CVS and amniocentesis

Following a high probability result with FTS or NIPT, the recommended course of action is further diagnostic testing, including CVS or amniocentesis. Aside from the stress and anxiety likely caused by a high probability result to the pregnant woman, these invasive procedures also carry a risk of miscarriage. Since the implementation of NIPT, there has been a significant reduction in the requirement for invasive procedures, due to the reduction in the rate of false-positives.

Quality assurance

The quality and stability of Harmony has been documented in more than 1,000,000¹ patient samples worldwide. In Australia, the test provided by Sonic Genetics is listed with the TGA (ARTG) and performed in a laboratory accredited by local regulatory authorities (NATA).
Why choose Sonic Genetics as your Harmony provider?

We support our referring clinicians

Counselling a patient with a high-risk result can be challenging. If you receive a high-risk result for a patient, you can speak directly with one of our genetic pathologists or clinical geneticists to discuss your patient’s result. If that patient has paid Sonic Genetics directly for the test, full instructions on how to access a free and independent genetic counselling service will accompany the result. We have extensive educational resources at www.sonicgenetics.com.au/nipt to help you use the test and its outcome confidently. Brochures and forms can be downloaded, and there are links to further summaries about the various disorders being screened.

Ultimate convenience for you and your patients

Sonic Genetics has developed a simple Harmony ordering process for you and your patients, including the availability of Harmony Non-invasive Prenatal Test Request Forms on most Practice Management Software platforms. Following counselling and completion of a request form between doctor and patient, the patient can go online and pay for the test and book a collection appointment. An SMS and email are sent directly to the patient confirming sample collection booking. The patient presents at one of our collection centres for a blood collection.

We have the largest number of collection centres across Australia, giving convenience and ease of access for your patients. Our extensive nationwide logistics network ensures rapid sample transport and delivery to our testing laboratory in Australia.

Experience you can trust

Our parent company, Sonic Healthcare, is a global leader in diagnostics, and Australia’s largest provider of diagnostic services. We have a national network of pathology laboratories, as shown on this map, with almost 300 pathologists, hundreds of collection centres, and extensive experience in the delivery of genetic testing, including Harmony. We were the third laboratory in the world to implement the Harmony Prenatal Test.

Access to Genetic Pathologists

Sonic Genetics employs genetic pathologists who oversee the laboratory performing the testing and who are available for consultation with you.

Quality assurance for reliable results

Sullivan Nicolaides Pathology (SNP) is NATA-accredited to perform the Harmony Prenatal Test. SNP provides Harmony testing for Sonic Genetics and the Sonic Healthcare group. You can be confident we have been audited and perform testing to Australian standards.

Access to your patients’ results, anywhere, anytime

Sonic Dx, our secure online results portal, is accessible from your mobile phone, iPad, tablet or desktop. Simply log in via the internet, and you can view all your patients’ results for any tests performed through Sonic Genetics and your local Sonic laboratory.
Where does Harmony fit in the prenatal pathway?

Should a woman have conventional first trimester screening and ultrasound as well as Harmony?

While Harmony is more accurate than conventional first trimester screening, conventional screening still has a place in prenatal care. The two tests measure different things: Harmony evaluates cfDNA from specific chromosomes, while first trimester screening assesses anatomy and biochemical function. The two tests provide complementary information about the fetus. This recommendation is consistent with a policy statement from the RANZCOG and the Human Genetics Society of Australasia (www.ranzcog.edu.au).
The Harmony report and interpretation of results

What does Harmony report?

- The percentage fetal fraction in the maternal sample – it must be above 4% to generate an accurate result
- The probability the tested cfDNA has trisomy 13, 18 and 21, along with other parameters requested
- A recommendation to the clinician as to what is required following Harmony testing

Harmony® Prenatal Test Report

<table>
<thead>
<tr>
<th>CHROMOSOME</th>
<th>RESULT</th>
<th>RECOMMENDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21 (T21)</td>
<td>High Risk</td>
<td>Genetic counselling and additional testing</td>
</tr>
<tr>
<td>Trisomy 18 (T18)</td>
<td>Low Risk</td>
<td>Review results with patient</td>
</tr>
<tr>
<td>Trisomy 13 (T13)</td>
<td>Low Risk</td>
<td>Review results with patient</td>
</tr>
<tr>
<td>Fetal Sex</td>
<td>Male</td>
<td>Review results with patient</td>
</tr>
<tr>
<td>Sex Chromosome Aneuploidy (SCA)</td>
<td>Low Risk</td>
<td>Review results with patient</td>
</tr>
<tr>
<td>22q11.2</td>
<td>Low probability of a deletion</td>
<td>Review results with patient</td>
</tr>
</tbody>
</table>

EXAMPLE

Will my patient always get a result for Harmony?

In rare cases (less than 3% of initial blood collections), Harmony will not give a result. The most common reason is biological, i.e. there is insufficient fetal DNA in the mother’s sample for analysis. Increasing maternal body weight is associated with reduced fetal fraction. Less commonly, a test failure may be due to the DNA in the mother’s sample being degraded and not suitable for analysis.

Patients who do not yield a result after the first collection can have their samples re-collected and re-analysed at no additional charge. Approximately 1% of re-collected blood samples will not yield a result with Harmony. Fetal aneuploidy can result in low fetal fraction in a small percentage of patients. If analysis of the re-collected sample does not yield a result, the risk of fetal aneuploidy may be 3-5% (N Engl J Med 2015, 372:1589-97).

What if my patient’s result is abnormal?

A Harmony result should always be confirmed by CVS or amniocentesis before any major clinical decisions are made regarding the pregnancy.

Harmony is a screening test; it is not a diagnostic test. False-positives can occur, usually due to the placenta having an area of aneuploidy and the remainder of the placenta and the fetus being normal, i.e. confined placental mosaicism (CPM). Other biological causes of false-positive results may include placental DNA released from an abnormal demised twin, or a maternal chromosomal abnormality. For these reasons, further intervention in a pregnancy should not be based solely on an abnormal Harmony result.
When

We recommend that the test be performed between 10 and 14 weeks’ gestation. The superior accuracy of Harmony relies on the presence of sufficient fetal DNA in the maternal circulation for analysis. The concentration of fetal DNA increases during pregnancy and more than 95% of women will have sufficient fetal DNA for analysis after 10 weeks’ completed gestation. We do not test samples collected at less than 10 weeks’ completed gestation, as the chances are very high that there will be insufficient fetal fraction to provide an accurate result.

Where

Collection can take place at any one of hundreds of our collection centres and is couriered to our testing laboratory in Brisbane. The blood sample is collected into two specific tubes, designed to preserve the fetal fraction. Sample transport typically takes 24 hours and results are available 5–8 business days from date of collection. Results are available electronically via Sonic Dx or downloaded to Practice Management Software. Alternatively, fax or hard-copy reports may be requested.

On the rare occasions where further analysis of the sample is required, a repeat blood collection may be recommended.

How

The clinician managing a woman’s pregnancy is required to complete a Harmony Non-invasive Prenatal Test Request Form (available online or via your Practice Management Software). Clinical information, such as number of fetuses, gestational age, IVF status, maternal age and weight, must be provided. These data are essential for the test algorithm.

The requesting clinician must ensure that the patient has given informed consent before the test is requested.

Cost and Additional Testing

Medicare does not currently cover any form of NIPT. Current pricing for Harmony is found on our website. Pre-payment by the patient is required, and can be done via our website.

In the event that your patient returns a high-risk result, free genetic counselling will be provided. Sonic laboratories can also offer the following testing services with no out-of-pocket costs for patients that fulfil the Medicare criteria:

<table>
<thead>
<tr>
<th>Service</th>
<th>Description</th>
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<tr>
<td>Rapid FISH</td>
<td>Confirmatory diagnostic test for aneuploidy of chromosomes 13, 18, 21, X, Y. This can be extended for other chromosomes, e.g. 22q11.2 deletion.</td>
</tr>
<tr>
<td>Karyotype</td>
<td>Confirmatory diagnostic test performed on amniotic fluid and CVS.</td>
</tr>
</tbody>
</table>

The above additional testing services are offered at the Medicare rebate with no out-of-pocket fee to patients who have had the Harmony Prenatal Test with Sonic Genetics.
Any further questions?

Does Harmony test for fetal sex?
Yes. The Harmony Prenatal Test can report the sex chromosomes of the fetus if requested, however this is not the primary purpose of the test.

Can Harmony be used for twins?
Yes. The Harmony Prenatal Test can be used in twin pregnancies to test for trisomies 21, 18 and 13 and fetal sex chromosomes. The test can tell whether both twins are girls or whether at least one of the twins is a boy. It cannot tell if both twins are boys, or if there is one boy and one girl. It isn’t possible to test for sex abnormalities in twin pregnancies. The Harmony Prenatal Test cannot be used to test for 22q11.2 deletions in twin pregnancies.

The Harmony Prenatal Test is not available for triplet or higher order multiple pregnancies.

If there is no result, does the patient get a refund?
If the patient has pre-paid Sonic Genetics directly for their Harmony test and no result is given for trisomy 13, 18 and 21, then a free-of-charge repeat test may be offered. Should the repeat test also yield no result (approx. 1% of cases), then a full refund will be given upon request.

The Harmony result included the trisomies but failed to provide the fetal sex or sex chromosome abnormalities. Why? And can the patient get a refund?
The Harmony Prenatal Test is primarily intended to determine the absence of certain chromosomal abnormalities. Due to the biological nature of the sex chromosomes, X and Y being physically small and similar in genetic code, a determination of sex or sex chromosome abnormalities cannot be guaranteed. No refund is given in the unlikely event of fetal sex or sex chromosome abnormalities being indeterminable by Harmony.

Can Harmony be performed on IVF pregnancies?
Yes. The Harmony Prenatal Test can be used for both self-conceived and egg donor pregnancies.
References

- Palomaki G et al., Genet Med. 2011 Nov;13(11):913-20
- Nicolaides K et al., Fetal Diagn Ther. 2014;35(1):1-6
- Gil M et al., Fetal Diagn Ther. 2014;35:204-11
- 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
- Norton M et al., N Engl J Med. 2015; Published online https://doi.org/10.1056/NEJMoia407349
- Schmid, M et al., Fetal Diagn. Ther. 2017; Published online https://doi.org/10.1159/000484317
Non-invasive prenatal testing based on cell-free DNA analysis is not diagnostic: results should be confirmed by diagnostic testing. Before making any treatment decisions, all women should discuss their results with their healthcare provider, who can recommend confirmatory, diagnostic testing where appropriate. The Harmony Prenatal Test was developed by Ariosa Diagnostics. Sonic Genetics performs the Harmony Prenatal Test in Australia at our NATA-accredited Sullivan Nicolaides Pathology (SNP) laboratory. The Harmony Prenatal Test is included on the Australian Register of Therapeutic Goods.

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