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What is NIPT and cfDNA?

Non-invasive prenatal testing (NIPT) is a test of a pregnant woman’s blood to determine the chance that the developing baby has a serious chromosome abnormality. During pregnancy, DNA from the mother and fetus circulate in the mother’s plasma. This DNA is called cell-free DNA (cfDNA) and results from the natural breakdown of maternal and fetal 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, the Harmony™ Prenatal Test evaluates the risk of trisomy 21, 18 and 13, and is validated for use in pregnant women of any age or risk category.

A higher standard in trisomy testing
How does Harmony™ work?

Harmony™ requires a single blood test from a pregnant woman, and can be done as early as 10 weeks’ 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 fetus. 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. 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 insufficient fetal DNA to generate an accurate result.

Clinicians in more than 100 countries have trusted Harmony™ to screen more than 500,000 pregnancies.
The targeted approach of Harmony™ yields a deeper analysis versus sequencing.

In contrast to tests that randomly sequence all cell-free DNA (cfDNA), the Harmony™ test focuses on cfDNA from only the chromosomes of interest. This unique, directed approach allows deeper analysis and ultimately yields more accurate results.

What does Harmony™ screen for?

Harmony™ tests for the three most common autosomal chromosome trisomies (21, 18 and 13), as well as aneuploidies of the sex chromosomes.

- **Trisomy 21 (Down syndrome)** is the most common trisomy identified at birth. Down syndrome is associated with moderate to severe 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.

- **Sex chromosome abnormalities (SCA)**, i.e. abnormalities of the X and Y sex chromosomes, are less common and often less severe than trisomies 21, 18 and 13. 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 and the result is of little clinical significance.
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’ 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 there being 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). This important step identifies samples that do not have sufficient fetal DNA to draw an accurate conclusion. This minimises 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, i.e. a false-negative result).

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 recent 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 Combined Screening. Harmony™ was far more accurate in identifying both normal and affected fetuses.

<table>
<thead>
<tr>
<th>FTS Performance</th>
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<tbody>
<tr>
<td></td>
<td>EFFECTIVENESS</td>
<td>EFFECTIVENESS</td>
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<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>
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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
- Quality assurance
Exceptionally accurate results

<table>
<thead>
<tr>
<th></th>
<th>FTS Performance</th>
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<tbody>
<tr>
<td><strong>COST/BURDEN</strong></td>
<td><strong>COST/BURDEN</strong></td>
</tr>
<tr>
<td><strong>amnios per case</strong></td>
<td><strong>amnios per case</strong></td>
</tr>
</tbody>
</table>
| Trisomy 21           | ![Graph](image1)
| Trisomy 13           | ![Graph](image2)
| Trisomy 18           | ![Graph](image3)
| 45,X                 | Not tested      |
| 47,XXY               | Not tested      |

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. These are higher detection rates than observed with conventional First Trimester Screening for these trisomies. 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></th>
<th>False positives with Harmony™</th>
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<tbody>
<tr>
<td>9 of 15,803</td>
<td>854 of 15,803</td>
</tr>
</tbody>
</table>

Significantly reduces invasive procedures such as CVS and amniocentesis

Following a positive result with FTS or NIPT, the recommended course of action is further diagnostic testing, including amniocentesis or CVS. Aside from the obvious stress and anxiety caused by a positive result to the pregnant woman, these invasive procedures carry with them 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 500,000 patient samples through the Ariosa laboratory in the USA. In Australia, the test provided by Sonic Genetics has been approved by Ariosa and is 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. 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. 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 an 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 the map below, 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.

Quality assurance for reliable results
Sullivan Nicolaides Pathology (SNP) is the only Australian provider that is NATA-accredited to perform the Harmony™ non-invasive prenatal test. SNP provides Harmony™ testing for Sonic Genetics and the Sonic Healthcare group. You can be confident we have been audited and test to Australian standards.

Access to your patients’ results, anywhere, anytime
Have you used our online app – Sonic Dx? It’s 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 the genetic code, 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 (http://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 risk assessment (low to high risk)
- The probability of the presence of each of the three autosomal trisomies (T21, T18 and T13)
- A recommendation to the clinician as to what is required following Harmony™ testing

<table>
<thead>
<tr>
<th>Harmony™ Prenatal Test Report</th>
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<tbody>
<tr>
<td><strong>CHROMOSOME RESULT PROBABILITY</strong></td>
</tr>
<tr>
<td><strong>RECOMMENDATION</strong></td>
</tr>
<tr>
<td>Trisomy 21 (T21) Low Risk Less than 1/10,000 (0.01%)</td>
</tr>
<tr>
<td>Trisomy 18 (T18) Low Risk Less than 1/10,000 (0.01%)</td>
</tr>
<tr>
<td>Trisomy 13 (T13) High Risk Greater than 99/100 (99%)</td>
</tr>
<tr>
<td>Fetal Sex Female Greater than 99/100 (99%)</td>
</tr>
<tr>
<td>Sex Chromosome Aneuploidy (SCA) Low Risk</td>
</tr>
<tr>
<td>Fetal Fraction 12.0% Fetal fraction refers to the percentage of fetal cell-free DNA in the mother’s blood. The test is reported only if the fetal fraction is ≥ 4%.</td>
</tr>
</tbody>
</table>

Will my patient always get a result for Harmony™?

In rare cases (less than 4%), 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 technical 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 recollected and re-analysed at no additional charge. Approximately half of the recollected samples yield results. Fetal aneuploidy can result in low fetal fraction in a small percentage of patients. If analysis of the recollected sample does not yield a result, the risk of fetal aneuploidy may be approximately 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 amniocentesis or CVS 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. This phenomenon is called ‘confined placental mosaicism’. Other biological causes of false-positive results may include placental DNA released from an abnormal demise 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’ gestation. We do not test samples collected at <10 weeks’ gestation.

WHERE

Collection can take place at any one of hundreds of our collection centres and is shipped to our testing laboratory in Brisbane. The blood sample is collected into two specific tubes. Sample transport takes 24 hours and results are available 5–8 business days from date of collection. You can access the results via our web based app – Sonic Dx – or request results to be faxed directly to you.

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™ Request Form (available at www.sonicgenetics.com.au/nipt). 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 for the test 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, Sonic laboratories can also offer the following testing services with no out-of-pocket costs:

RAPID FISH
Confirmatory diagnostic test for aneuploidy of chromosomes 13, 18, 21, X, Y. This can be extended for other chromosomes, e.g. 22q deletion.

Karyotype Confirmatory diagnostic test performed on amniotic fluid and CVS.

The above additional testing services are offered at the Medicare rebate with no out-of-pocket fee to patients who have had Harmony™ with Sonic Genetics.
Does Harmony™ test for fetal sex?
Yes. The Harmony™ Prenatal Test can report the fetal sex chromosomes of the fetus if requested.

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 is not available for triplet or higher order multiple pregnancies.

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

- Data on file at Ariosa Diagnostics, Inc.
- 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, NEJM DOI: 10.1056/NEJMoA1407349 (published online April 1, 2015)
The published evidence that is the basis for this leaflet is detailed in other leaflets about Harmony™ available on the Sonic Genetics website.

Non-invasive prenatal testing services (NIPT) based on cell-free DNA analyses are not diagnostic; positive results should be confirmed by a diagnostic test. The Harmony™ Prenatal Test is developed by Ariosa Diagnostics. Testing is performed in Australia by Sonic Genetics in our Sullivan Nicolaides Pathology Laboratory, which is NATA accredited. As with other laboratory-developed tests, this test is pending inclusion on the Australian Register of Therapeutic Goods.

For further information, including scientific and peer-reviewed publications, please refer to our website, www.sonicgenetics.com.au/nipt or call us on 1800 010 447