The Harmony™ Prenatal Test

A non-invasive approach to the detection of common fetal trisomies

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Most developed countries offer genetic screening and testing for aneuploidy as a standard part of prenatal care. Conventional screening requires a consideration of maternal age in the context of serum markers or ultrasonographic measurement of nuchal translucency or other fetal structures. Moreover, this conventional method of screening is associated with a false-positive rate of at least 2% to 3% and a false-negative rate of 5% or more.[1] Abnormal results necessitate the use of chorionic villus sampling (CVS) or amniocentesis to sample fetal cells and achieve a diagnosis. Even when these invasive diagnostic procedures are performed carefully by the most experienced practitioners, however, they carry a small risk of fetal injury and pregnancy loss.

What is needed is a non-invasive way to collect fetal cells. Although it has been known for some time that fetal cells enter the maternal circulation, it is still not feasible to sample them adequately in maternal plasma because they are proportionally scarce. However, when fragments of fetal DNA—known as cell-free DNA (cfDNA)—were identified in maternal plasma, the equation changed. cfDNA is far more plentiful than fetal cells, and researchers have shown that is specific to the current pregnancy.[2] By analyzing cfDNA from maternal plasma, it is now possible to screen for trisomies with high sensitivity and specificity. The Harmony™ Prenatal Test is an advanced blood test now available to screen for trisomies 13, 18, and 21 in high-risk pregnancies.

Disclosure
Dr Swarup reports that he is on the National Women's Health Advisory Board for Laboratory Corporation of America® Holdings.

A NEW GENERATION OF TESTING

The Harmony test is non-invasive, indicated for use in singleton pregnancies at 10 weeks of gestation or later, and it is simple, relying on a standard draw of whole maternal blood. Testing with cfDNA has been recommended as an option for patients at increased risk for aneuploidy by the American College of Obstetricians and Gynecologists (ACOG), the Society of

FIGURE 1 Harmony clinical performance[6-9]

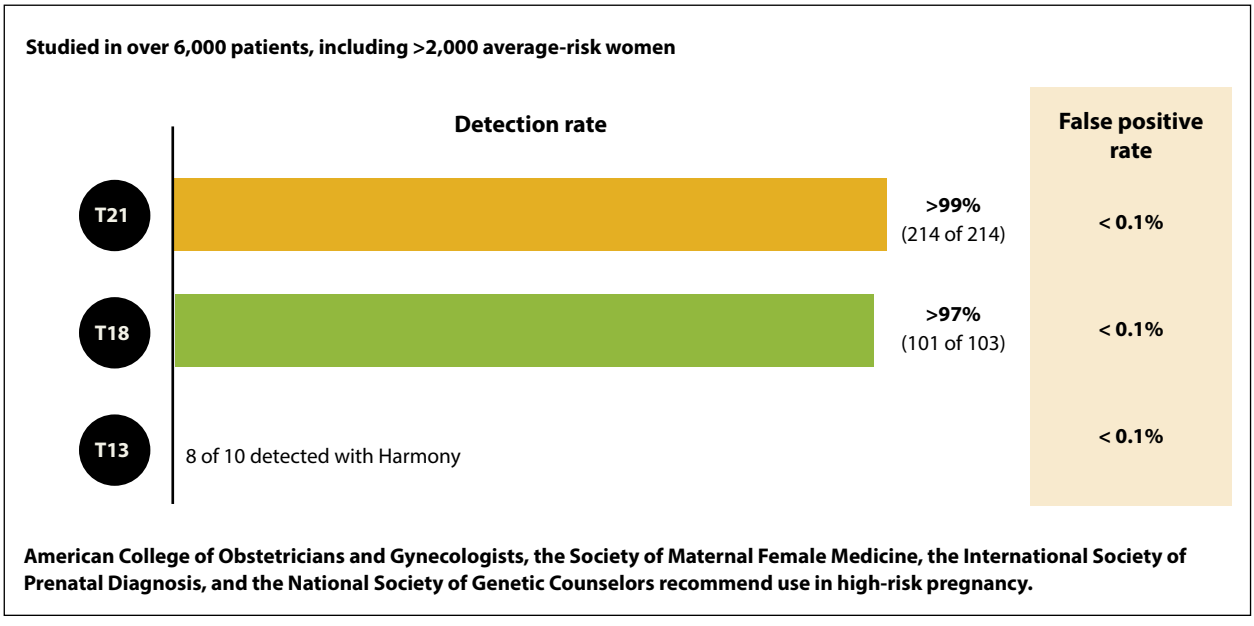


TABLE 1 Comparison of cell-free DNA (cfDNA) prenatal test methods[8]

Direct analysis	Massively parallel shotgun sequencing (MPSS)
• Direct analysis of cfDNA fragments	• Random analysis of cfDNA
• More efficient due to analysis of fewer cfDNA fragments than shotgun sequencing	• Requires millions of cfDNA fragments
• Simple binary results with individualized risk score for each trisomy	• No specific individual risk score

Maternal Fetal Medicine (SMFM)[3] as well as the International Society of Prenatal Diagnosis (ISPD) and the National Society of Genetic Counselors (NSGC).[4,5]

Overall, the Harmony test has been studied and validated in multiple published studies, in more than 6,000 patients, including over 2,000 average-risk women, identifying all 214 cases of trisomy 21, 101 of 103 cases of trisomy 18, and 8 of 10 trisomy 13 cases (**FIGURE 1**).[6-9] The performance of the Harmony test has been demonstrated to be consistent at any gestational age past 10 weeks. In addition, it is the only non-invasive prenatal test that has been evaluated in a population consisting exclusively of women in the first trimester of pregnancy.

TECHNOLOGY BEHIND THE HARMONY TEST

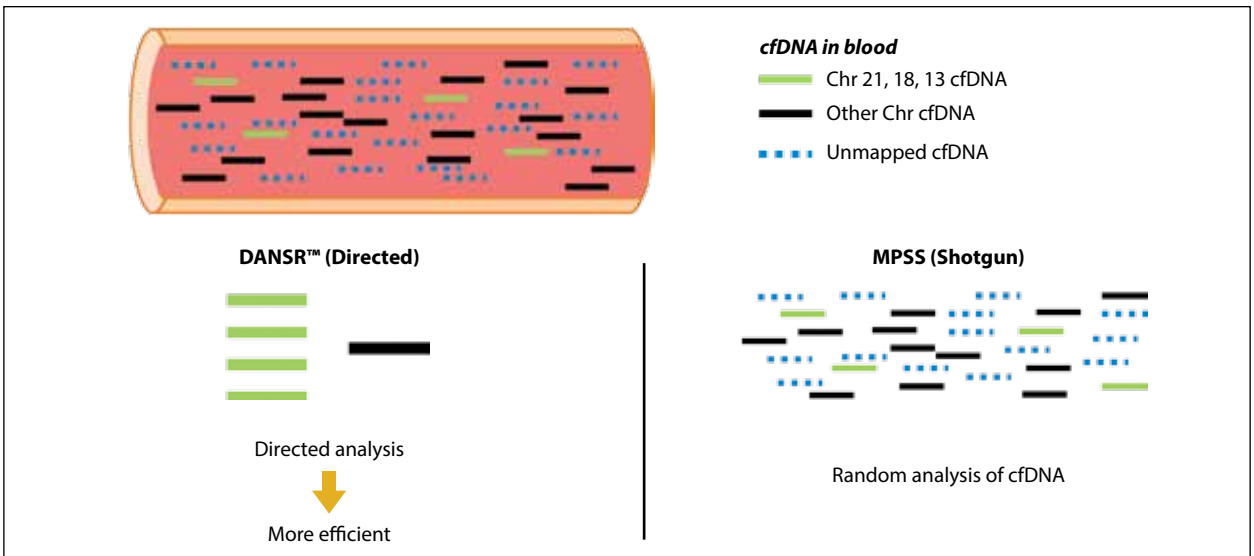
With cfDNA and special technology such as massively parallel shotgun sequencing (MPSS), which sequences large quantities of random fragments from each molecule of maternal cfDNA, it is possible to identify trisomies 13, 18, and 21 in maternal blood. There is a downside to MPSS, however: it is complex, generating enormous quantities of unnecessary data and requiring millions of fragments of cfDNA in the process, and it is expensive. One commercially available product that uses MPSS to detect trisomy 21 has a list price of approximately $2,700.[6]

Instead of relying on first-generation technology such as MPSS, the Harmony Prenatal Test uses Digital Analysis of Selected Regions (DANSR™) to *selectively* assess specific genome fragments from cfDNA to generate an individualized patient risk score for each trisomy (**TABLE 1, FIGURE 2**).[8] This approach is more efficient and is more accurate than MPSS.

In a separate study, Sparks and colleagues set out to develop an assay and a methodology for its use in detecting trisomy 21 and trisomy 18 using cfDNA from maternal plasma. They assessed cfDNA from a training set and from a blinded validation set of pregnant women that encompassed 250 disomy gestations, 72 trisomy 21 gestations, and 16 trisomy 18 gestations. They digitally analyzed selected regions of the DNA using a methodology known as "fetal-fraction optimized risk of trisomy evaluation" (FORTE™) to predict the risk of trisomy in each subject. The selective analysis in combination with the FORTE methodology correctly identified all trisomy 21 and trisomy 18 cases. There was a separation in the risk score between trisomic and disomic samples on a magnitude of 1,000 or greater.[6]

The ability to detect a small increase in the amount of an individual chromosome in a pregnancy, thereby determining whether it is trisomic or disomic, depends on the proportion of *fetal* cfDNA that is present in maternal plasma, compared with *maternal* cfDNA. The greater that fetal proportion—also known as the fetal fraction—the more easily detectable the trisomy. For example, Ashoor and colleagues conducted a nested case-control study in which cfDNA was extracted

FIGURE 2 Assay comparison—DANSR™ versus MPSS



cfDNA, cell-free DNA; DANSR, digital analysis of selected regions; MPSS, massively parallel shotgun sequencing.

TABLE 2 Findings from the Non-Invasive Chromosomal Evaluation (NICE) study[9]

- **50 participating clinical sites in United States and Europe**
- **Largest cohort study to date—all eligible subjects evaluated**
- **Study population was women undergoing invasive testing for any indication and thus included low risk women**

	Sensitivity	Specificity	False positive rate
Trisomy 21	100% (81/81)	99.97% (2887/2888)	0.03% (1/2888)
Trisomy 18	97% (37/38)	99.93% (2886/2888)	0.07% (2/2888)

from 400 pregnancies at 11 to 13 weeks' gestation, including 300 euploid gestations, 50 trisomy 21 pregnancies, and 50 cases of trisomy 18. Their goal was to explore possible influences on the fetal fraction. The fetal fraction was shown to increase with increasing pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotropin (beta-hCG), and to decrease with increasing maternal weight.[7]

DETECTION RATES ARE HIGH AND FALSE POSITIVES ARE LOW

The challenge with conventional screening for trisomies is the low specificity and sensitivity associated with serum markers and ultrasonographic measurement of nuchal translucency.[8]

Sensitivity and specificity are high using the chromosome selective sequencing approach. For example, Norton and colleagues utilized DANSR and FORTE on chromosomes 21 and 18 to report the risk of aneuploidy (high vs low). Of 81 cases of trisomy 21, they correctly classified all 81 as high-risk, with one false-positive finding among the 2,888 normal cases, for a sensitivity of 100% (95% confidence interval [CI], 95.5–100) and a false-positive rate of 0.03% (95% CI, 0.002–0.20).[9]

As for the 38 cases of trisomy 18, Norton and colleagues classified 37 as high-risk, with two false-positive results among the 2,888 normal cases, for a sensitivity of 97.4% (95% CI, 86.5–99.9) and a false-positive rate of 0.07% (95% CI, 0.02–0.25) (**TABLE 2**).[9]

And in an exploration of whether the mother's specific a priori risk factors for aneuploidy affected the percentage of fetal cfDNA, Brar and colleagues found that they did not. All cases of trisomy 21 were correctly identified in the study, regardless of the risk factors present. There was one false-negative case of trisomy 18, but no false-positive results in any of the groups analyzed.[10]

HARMONY OFFERS SEVERAL BENEFITS

The Harmony test offers features that benefit patient care:

- It has been studied specifically in the first trimester and proven to be safe and informative.*
- Results are reported as high- or low-risk, with an individualized risk score; other tests indicate only positive or negative based on a pre-set threshold.
- It is widely accessible, with in-network status in most insurance plans.
- The company offering the Harmony test has more than 150 genetic counselors on staff and an extensive phlebotomy network.

* The Harmony Prenatal Test has been developed and is performed as a laboratory test service by Ariosa Diagnostics, a CLIA-certified clinical laboratory.

FITTING THE HARMONY TEST INTO CLINICAL PRACTICE

According to ACOG, women at increased risk of aneuploidy may be offered testing using fetal cfDNA, with the expectation that about 98% of cases of Down syndrome will be identified, with a false-positive rate of less than 0.5%. That is, the Harmony test may be offered to women at high risk of aneuploidy by virtue of:

- maternal age of 35 years or older
- ultrasonographic findings that suggest an increased risk of aneuploidy
- history of trisomy in an earlier pregnancy
- parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21
- any positive test result for aneuploidy, including a first trimester, sequential, integrated, or quadruple screen
- presence of balanced Robertsonian translocation in either parent.[3]

Almost all patients undergo some prenatal screening, such as a nuchal translucency assessment or a quadruple screen, which yields a numerical value. It may be useful to offer the Harmony Prenatal Test to patients when their risk of aneuploidy reaches a specific numerical threshold, such as 1 in 1000 or greater.

Conversely, ACOG recommends non-invasive testing of fetal cfDNA should *not* be offered to women at low risk of aneuploidy, or women who have multiple gestations, because the test has not been sufficiently analyzed in these populations.[3] Nor should it be used in cases in which a structural abnormality has already been identified via ultrasound; in such cases, invasive diagnostic techniques should be offered.[3]

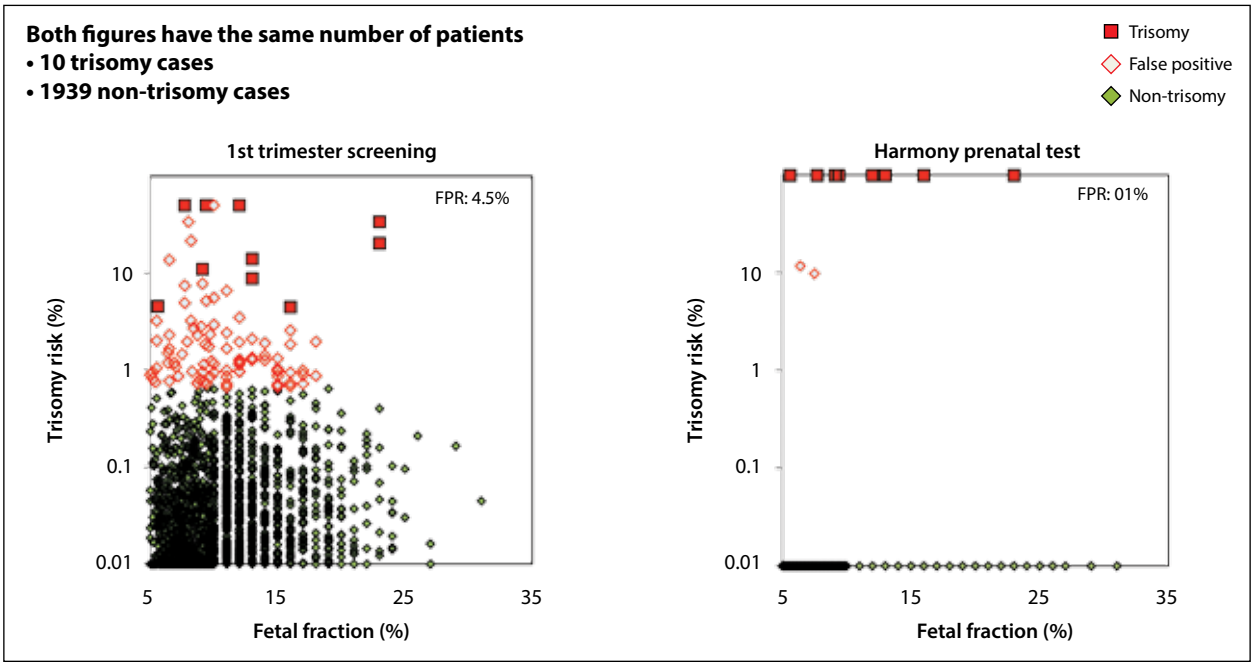
The Harmony test is indicated for use in the detection of common fetal trisomies in pregnancies of at least 10 weeks' gestation. It requires a specimen of whole blood from the mother, which should be stored in tubes (2 tubes, 8–10 mL each) provided by the manufacturer. Samples should be preserved at room temperature and transported at ambient temperature. The following clinical information must be supplied to the lab on the test requisition form: date of birth of the mother, if an IVF donor pregnancy, age of egg donor, gestational age and how it was determined, date of collection, and ordering physician.

A result is returned in approximately 8 to 10 days.

FUTURE APPLICATIONS

In a cohort study of 2049 women at average risk of aneuploidy, Nicolaides and colleagues used cfDNA analysis with selective chromosomal sequencing to test for trisomies 18 and 21. cfDNA

FIGURE 3 Average risk study—risk score comparison[11]



analysis correctly identified all 8 cases of trisomy 21, with a risk score exceeding 99% for trisomy 21 and a risk score of less than 0.01% for trisomy 18. Because the fetal fraction was below the minimum of 4%, the assay failed in 54 cases. These results of assay failure and low fetal fraction are similar to the 2.8% and 1.8%, respectively, reported in a previous cohort study. The problem of low fetal fraction may be impossible to overcome by currently available NIPT techniques.[11] For the 2 cases of trisomy 18 identified in this study, the risk score exceeded 99% for trisomy 18 and was less than 0.01% for trisomy 21. Nicolaides and colleagues concluded that non-invasive prenatal screening for trisomy 18 and 21 using selective sequencing of fetal cfDNA is effective in a routine population, with a detection rate exceeding 99% and a combined false-positive rate for trisomy 18 and 21 of 0.1% (**FIGURE 3**).[11]

OTHER BENEFITS

The Harmony Prenatal Test offers a non-invasive approach to screening for trisomies 13, 18, and 21, with quick results and an individualized risk score for each trisomy. At present, it is indicated for screening of women who have an elevated risk of aneuploidy, but its application may be broadened in the near future to encompass all women undergoing routine screening.

Although conventional screening methods have high detection rates, they may not be complete until the second trimester of pregnancy (eg, integrated screening), extending the wait for results—and the anxiety inherent in such waiting. In contrast, results from the Harmony test are available within the first trimester and are reported in approximately 8 to 10 days from the blood draw. The Harmony test features an individualized patient risk score for each trisomy evaluated—13, 18, and 21. Note that a low risk result does not ensure an unaffected pregnancy.[3]

For more information on the Harmony test, visit www.integratedgenetics.com or www.mytestingoptions.com.

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