harmony®

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Clinical Studies Abstract Booklet

The Harmony® test is a non-invasive prenatal test (NIPT) that evaluates the probability of trisomies (trisomy 21, 18 and 13) and additional menu options, including sex chromosome aneuploidies and 22q11.2 microdeletion by analyzing cell-free DNA (cfDNA) in maternal blood. Using DANSR assay, a proprietary targeted DNA-based technology that focuses on cfDNA from chromosomes of interest, and FORTE, a powerful algorithm that calculates probability by incorporating fetal fraction, the Harmony test provides accurate and reliable NIPT results. To date, over 1.4 million tests have been run, and 48 peer-reviewed publications have been reported, providing clinical evidence for the Harmony test performance across any age or risk category.[1-48]

This booklet provides performance data from selected publications and a list of peer-reviewed publications evaluating the Harmony test.

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** This reference also includes test performance in twin pregnancies.*

Non-Invasive Examination of Trisomy (NEXT) Using Cell Free DNA Analysis

Norton M. et. al. N Engl J Med. 2015 Apr 23;372(17):1589-9

Study Population

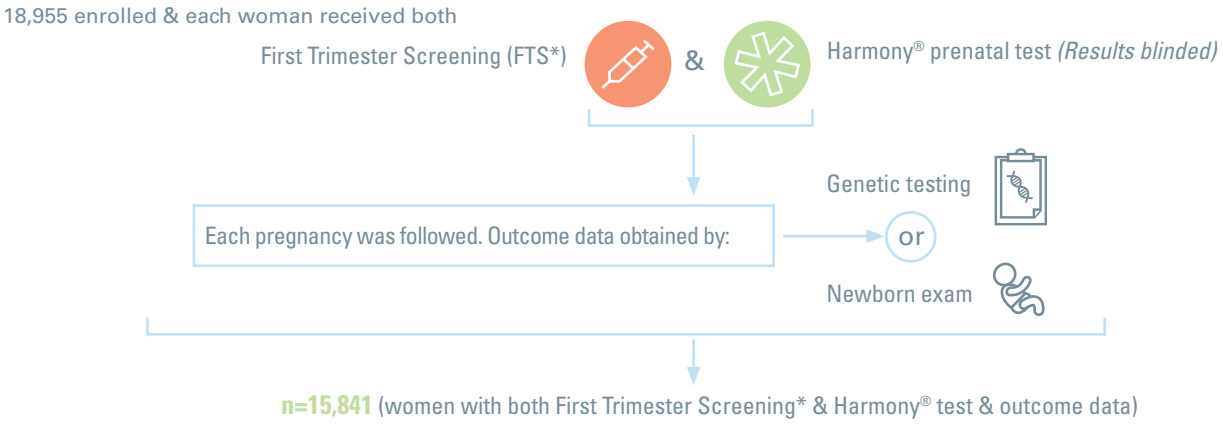
15,841 singleton pregnancies from a general prenatal screening population. The mean maternal age was 31 (range 18-48). The mean gestational age was 12.5 weeks (range 10.0-14.3).

Summary and Key Points

The study is the largest direct comparison of cfDNA screening (Harmony® prenatal test) to standard screening (first trimester screening*) for aneuploidy detection and shows superior test performance of cfDNA screening regardless of prior risk.

- Prospective, international, multi-center, blinded study of pregnant women undergoing standard aneuploidy screening. Pregnancy outcome was obtained on all patients.
- Powered for sensitivity (detection rate) and specificity for trisomy 21.

Study Design



Results

Study Results (n=15,841)	FTS*	Harmony® prenatal test	p-value
Detection Rate (affected pregnancies correctly identified as high probability)	79% (30/38)	100% (38/38)	0.008
False-Positive Rate (unaffected pregnancies incorrectly identified as high probability)	5.4% (854/15,803)	0.06% (9/15,803)	<0.001
Positive Predictive Value (PPV) (likelihood that a positive result is confirmed on diagnostic testing, based on false-positive rate and population frequency)	3.4%	81%	<0.001

Sub-group analysis – Harmony® in "Low Risk" Patients	Less than 35 years old (n= 11,994)	Screen negative on FTS (n= 14,957)
Sensitivity	100% (19/19)	100% (8/8)
False Positive Rate	0.05% (6 of 11,975)	0.05% (8 of 14,949)
Positive Predictive Value	76%	50%

PPV of FTS in general study population: **3.4%**

Harmony® test performance is consistent in all risk categories

Full manuscript available at: http://www.nejm.org/doi/full/10.1056/NEJMoa1407349#t=article

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

Clinical Performance of Non-Invasive Prenatal Test (NIPT) Using Targeted Cell-Free DNA Analysis in Maternal Plasma with Microarrays or Next Generation Sequencing (NGS) is Consistent Across Multiple Controlled Clinical Studies

Stokowski et. al. Prenat Diagn. 2015;35(12):1243-1246

Key Points

- Demonstrates the consistently high sensitivity and specificity of the Harmony® prenatal test across quantitation platforms.
- Combines data from this study with all published Harmony® clinical performance studies using the targeted DANSR assay with FORTE analysis software to calculate specificity and sensitivity for trisomy 21, 18, and 13 screening in more than 23,000 pregnancies.

Study Population

799 blinded maternal plasma samples from an intentionally diverse group of pregnancies (759 singleton, 40 twin, and 5 IVF) were evaluated for trisomy 21, trisomy 18, and trisomy 13 risk using the Harmony® prenatal test with microarray quantitation. Mean maternal age was 36 years; mean gestational age was 16 weeks (interquartile range 13-19 weeks). All subjects had prenatal diagnosis or were followed to birth with evaluation for fetal aneuploidies performed using newborn exam and subsequent karyotype confirmation of any suspected aneuploidies.

Results

All 641 euploid pregnancies which produced a risk score were correctly classified as low risk (specificity: 100%). Harmony® test identified 107/108 trisomy 21 cases (sensitivity 99.1%), 29/30 trisomy 18 cases (sensitivity 96.7%), and 12/12 trisomy 13 cases (sensitivity 100%). This high sensitivity and specificity using microarray-based quantitation is consistent with previously published test performance using next generation sequencing quantitation.

The single trisomy 18 case that was identified as low risk was retested with a modified FORTE analysis on fetal tissue. This yielded male results with no evidence for trisomy 18, suggesting that there may have been incorrect clinical annotation of the case.

The paper combines the current dataset with all data from nine previous studies and provides a comprehensive view of the Harmony® test clinical performance in over 23,000 pregnancies. The included studies are either blinded cohort studies with known pregnancy outcome or prospective studies with complete follow-up. There are no registry reports or other studies with incomplete follow-up.

	Sensitity (n)	Specificity (n)
Trisomy 21	**99.3%** (421)	**99.96%** (22,734)
Trisomy 18	**97.4%** (151)	**99.98%** (22,248)
Trisomy 13	**93.8%** (32)	**99.98%** (14,211)

n = number of pregnancies studied (with trisomy for sensitivity or non-trisomy for specificity)

Conclusion

The conclusion is demonstration of high sensitivity for trisomy 21, trisomy 18 and trisomy 13 in well-controlled studies. The specificity for each of the three trisomies is greater than 99.9% with many thousands of pregnancies studied. The extremely high specificity provides for a high positive predictive value.

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/pd.4686/full

Non-Invasive Chromosomal Evaluation (NICE) Study: Results of a Multicenter, Prospective, Cohort Study for Detection of Fetal Trisomy 21 and Trisomy 18

Norton M. et. al. Am J Obstet Gynecol. 2012 Aug;207(2):137.e1-8

Study Population

3,228 singleton pregnancies undergoing invasive testing for any indication (includes both "high" and "low" risk women). Largest blinded study to date regarding performance of non-invasive prenatal testing.

Summary and Key Points

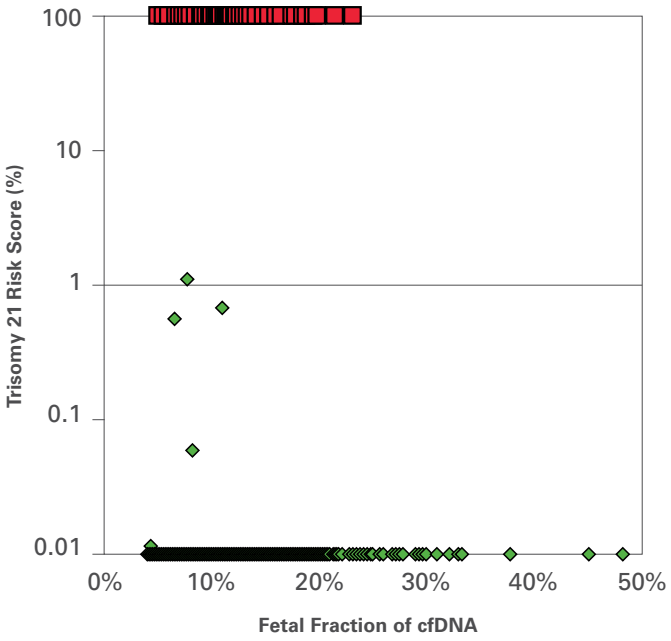
The NICE Study is an international, multicenter cohort study of pregnant women at gestational age 10-weeks or later from 50 clinical sites in which the Harmony® test's performance in assessing the risk for fetal trisomies 21 (T21) and 18 (T18) was evaluated.

- Chromosome-selective sequencing of cfDNA and application of an individualized risk algorithm is effective in the risk assessment of fetal T21 and T18.
- The FORTE risk algorithm provides an individualized risk assessment for T21 and T18. In this study, 99.5% of patients received a risk of either >99% or <1/10,000 for these trisomies.
- False positive rates for trisomy 21 and 18 are <0.1%.
- To date, this is the largest validation study of non-invasive prenatal testing.

Results

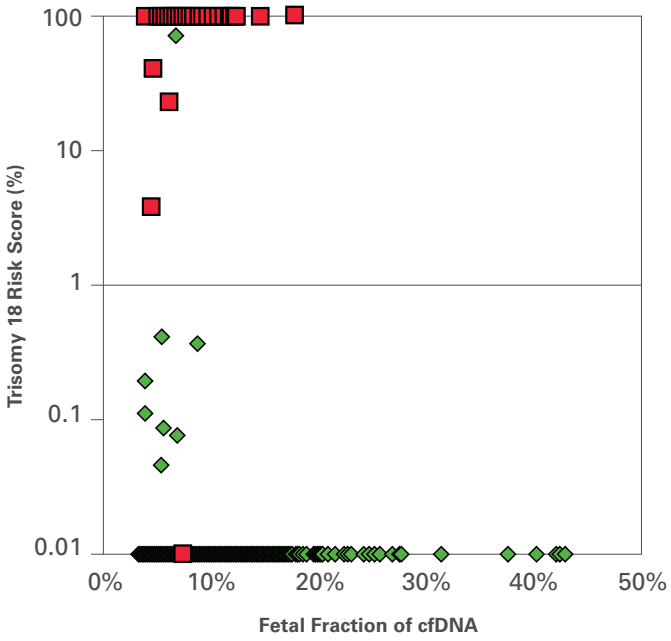
TRISOMY 21

Detection Rate: 100% (81/81)
False positive rate: 0.03% (1/2888)



TRISOMY 18

Detection Rate: 97% (37/38)
False positive rate: 0.07% (2/2888)



First trimester screening based on ultrasound and cfDNA vs. first-trimester combined screening - a randomized controlled study

Kagan KO et. al. Ultrasound Obstet Gynecol. 2017 Sep 19

Study Population

1,400 singleton pregnancies with normal first trimester ultrasound were randomized into two groups: FTCS or cfDNA screening.

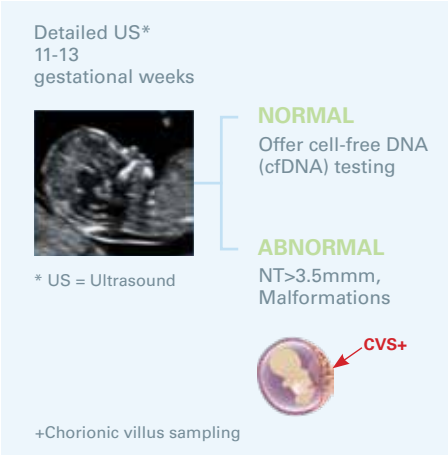
FTCS includes: maternal and gestational age, fetal nuchal transluscency (NT), maternal serum pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotropin (hCG). First trimester ultrasound protocol followed ISUOG recommendations. Pregnancies with fetal defects and/or increased nuchal translucency noted during ultrasound were excluded from randomization and counseled regarding follow-up testing options. Only pregnancies with complete outcome information were included in the study results.

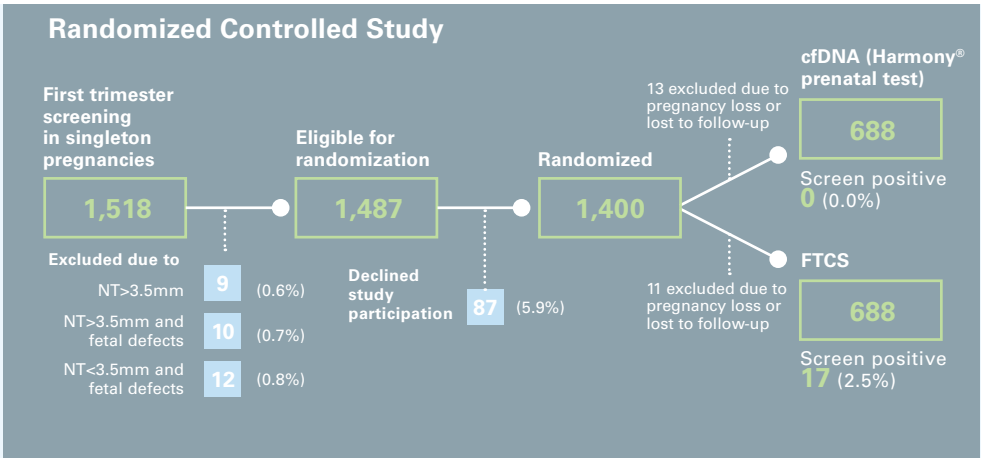
Median maternal age: 33.9 years; Median gestational age: 12.7 weeks.

Summary and Key Points

Purpose: To compare the false positive rates of First Trimester Combined Screening (FTCS) against a combination of ultrasound examination with cfDNA (Harmony® prenatal test) analysis

Results: cfDNA analysis using the Harmony® prenatal test in combination with first trimester ultrasound examination led to significantly lower false positive rates for trisomy 21 as compared to FTCS.





Conclusion/Discussion:

No false positives were seen in the group receiving cfDNA screening; 2.5% of cases in the FTCS group were false positives.

Authors discuss the superior detection of cfDNA screening for Down syndrome as compared to FTCS and contingent screening models.

Authors suggest implementation of a primary screening approach using cfDNA analysis and first trimester ultrasound. Benefits of this approach:

- Excellent detection rate of rare and common trisomies as well as fetal structural abnormalities
- Low false positives leading to reduction in unnecessary anxiety and follow-up testing
- Less complicated protocol than the contingent screening model, with almost all patients getting clear results from the first blood draw

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/uog.18905/pdf

Non-Invasive Prenatal Testing for Fetal Trisomies in a Routinely Screened First-Trimester Population

Nicolaides KH et. al. Am J Obstet Gynecol. 2012 Nov;207(5):374.e1-6

Study Population

2,049 singleton pregnancies in the first trimester from a general screening population.

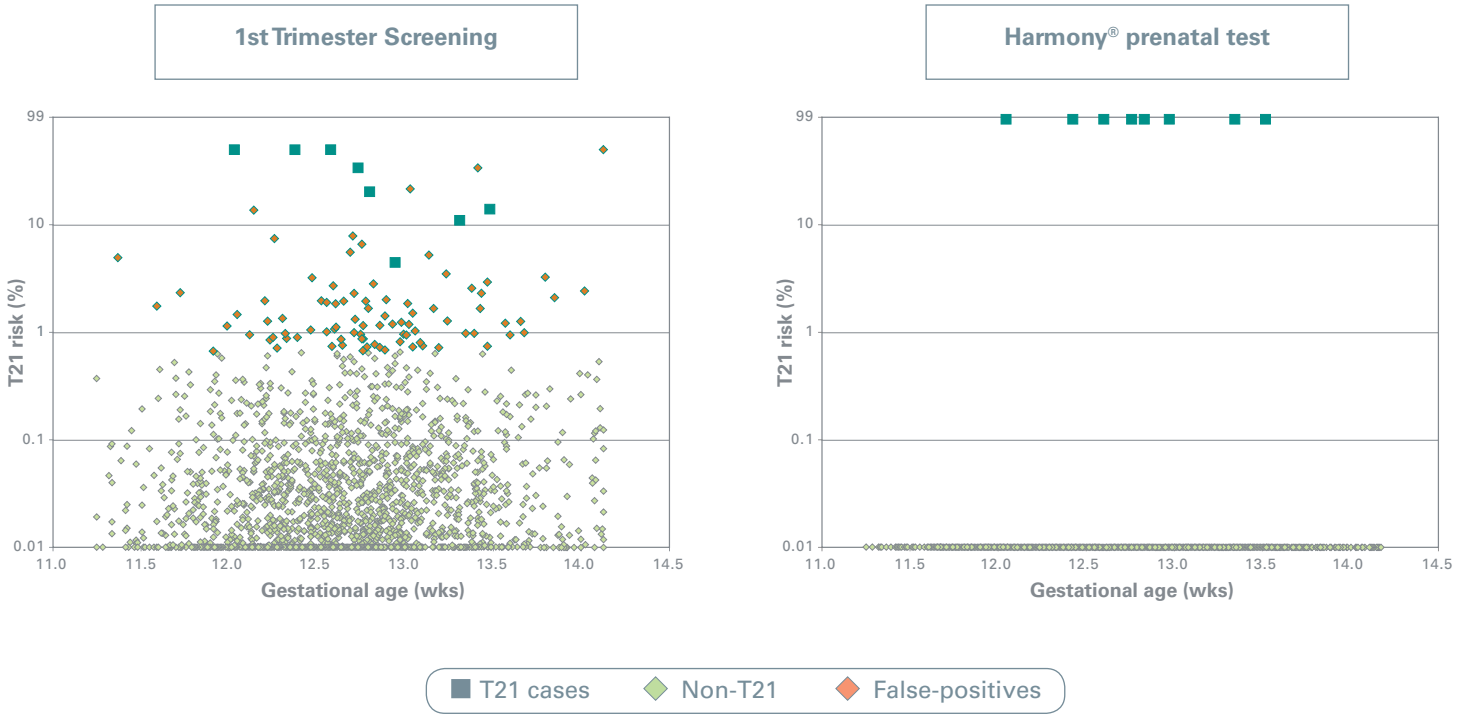
Summary and Key Points

This study is an external, independent and blinded study exclusively conducted during the 1st trimester to assess the prenatal detection rate and false positive rate of trisomies 21 and 18 by chromosome-selective sequencing of cfDNA. This study compared the Harmony® test to first trimester combined screening in an average-risk population.

- NIPT using chromosome-selective sequencing in a routinely screened population identified trisomies 21 and 18 with a false positive rate of 0.1%.
- The Harmony® test accurately identified all trisomy cases among the tested samples.
- False positive rate for first trimester combined screening was 4.5% compared to 0.1% in the Harmony® test analysis.

Results

Clinical Performance Comparison of the Harmony® prenatal test and First-Trimester Combined Screening.



Gestational Age and Maternal Weight Effects on Fetal Cell-Free DNA in Maternal Plasma

Wang E et. al. Prenat Diagn. 2013 Jul;33(7):662-6

Study Population

22,384 singleton pregnancies of at least 10 weeks' gestational age.

Summary and Key Points

- This is the largest sample set to date to report on the relationship between fetal fraction and both maternal weight and gestational age.
- Fetal cell-free DNA (cfDNA) increases by an average of 0.1% per week between 10 to 21 weeks gestation.
- Regardless of NIPT approach, the ability to report out a reliable result is related to the proportion of fetal to maternal cfDNA in maternal plasma.
  — The minimum percent fetal cfDNA required for reliable analysis is 4%.
- The vast majority of samples greater than 10 weeks gestation contain an adequate fetal cfDNA proportion to allow for reliable clinical results.
- Accurate gestational age determination is critical to the likelihood of receiving a result and in determining when to schedule a redraw.

Results

- 1.9% of pregnant women had insufficient fetal cfDNA amounts (<4% cfDNA fraction) for testing on the first blood draw.
- Increasing maternal weight is associated with lower fetal fraction of cfDNA.
- On the second blood draw, 56% of women had more than 4% fetal fraction of cfDNA.
- Fetal fraction increased 0.1% per week between 10 to 21 weeks and 1% per week after 21 weeks.

Maternal Weight (kg)	Maternal Weight (lb)	Pregnancies with ≥4% fetal cfDNA (%)
<50	<110	>99%
≥50 - <60	≥110 - <132	>99%
≥60 - <70	≥132 - <154	>99%
≥70 - <80	≥154 - <176	>99%
≥80 - <90	≥176 - <198	98%
≥90 - <100	≥198 - <220	96%
≥100 - <110	≥220 - <243	95%
≥110 - <120	≥243 - <265	90%
≥120 - <130	≥265 - <287	88%
≥130 - <140	≥287 - <309	81%
≥140	>309	71%

Maternal Weight (kg)	Maternal Weight (lb)	Pregnancies with ≥4% fetal cfDNA (when second draw was required)
<90	<198	71%
≥90 - <100	≥198 - <220	61%
≥100 - <110	≥220 - <243	59%
≥110 - <120	≥243 - <265	59%
≥120 - <130	≥265 - <287	29%
≥130 - <140	≥287 - <309	39%
≥140	>309	18%

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/uog.12504/pdf

Cell-Free DNA Analysis for Trisomy Risk Assessment in First-Trimester Twin Pregnancies

Gil M. et. al. Fetal Diagn Ther. 2014;34(5):496-499

Study Population

Two groups of twin pregnancies were evaluated in this study:

- Retrospective group: 207 stored plasma samples with known karyotype obtained at 11-13 weeks gestation.
- Prospective group: 68 twin pregnancies underwent prospective screening for trisomies 21, 18, and 13 by cfDNA testing between 10-13 weeks gestation. Karyotype only known for those with invasive procedures.

Summary and Key Points

This study evaluates the test performance of cfDNA testing for trisomies 21, 18, and 13 in twin pregnancies. The cfDNA test used in this study was the Harmony® prenatal test.

cfDNA testing in twins with the Harmony® test is feasible, with a higher detection rate and lower false positive rate compared to combined (serum) screening. The reporting rate of results is lower than in singleton pregnancies due to lower fetal fraction in the twin study population.

Results

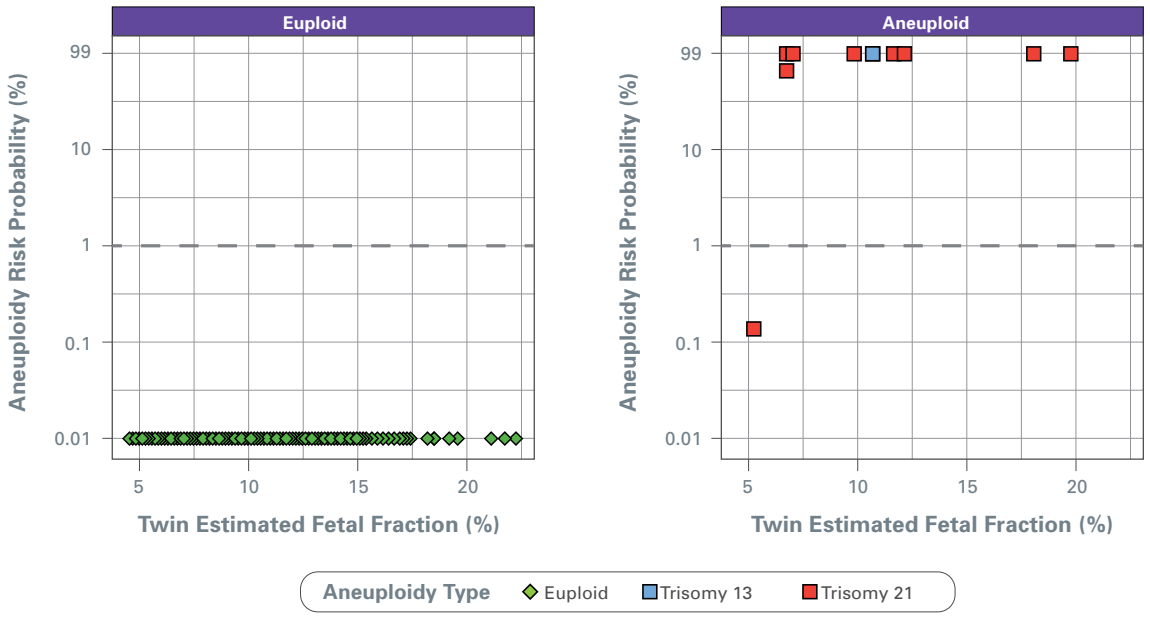
Retrospective Group

- Results were correctly classified in 191/192 cases with known karyotype
  - No false positive results.
- Correctly classified 9 of 10 trisomy 21 cases, with risk scores of >99% in 8 cases and a 72% risk in 1 case
  - There was one false negative trisomy 21 case with a risk of 1:714 (0.14%).
  - Correctly classified 1 case of trisomy 13, with a risk score of >99%
  - All euploid cases were correctly classified and had a risk score for each trisomy of <0.01%.
  - 11/207 samples (5.3%) failed due to low fetal fraction

Prospective Group

- Risk scores provided for 63/68 samples (92.6%); risk scores not provided in 5/68 samples (7.3%) due to low fetal fraction.
  - In 60/63 cases with a result, risk score for trisomies 21, 18 and 13 was <0.01%.
  - In 2/63 cases, risk score for trisomy 21 was >99%.
  - In 1/63 cases, risk score for trisomy 18 was 59%.

Retrospective Group Results



Prenatal Screening for 22q11.2 Deletion Using a Targeted Microarray-based Cell-free DNA (cfDNA) Test

Maximilian Schmid et. al. Fetal Diagnosis and Therapy 2017 Nov 8

Study Population

Two part-study (analytical validation and clinical verification) of 1953 plasma samples, 122 of which had confirmed deletions.

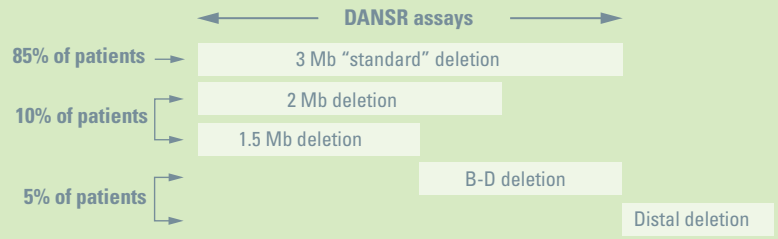
Fetal 22q11.2 deletions of 3 Mb and smaller were assessed.

Summary and Key Points

Purpose: To evaluate the performance of the Harmony® prenatal test, a targeted micro-array based cfDNA test, in identifying pregnancies at increased risk for a 22q11.2 deletion.

Result: The Harmony® prenatal test is able to identify pregnancies at increased risk for 22q11.2 deletions of 3Mb and smaller while maintaining a low false positive rate.

Harmony® prenatal test DANSR assays coverage



Results

Analytical validation: 92 out of 122 samples with confirmed deletions were identified as having a high probability of 22q11.2 deletion. 1606 out of 1614 presumed unaffected pregnancies were reported as having no evidence of deletion. Specificity of 99.5%.

Smallest size deletion detected: 1.96 Mb. No correlation observed between sensitivity and deletion size.

Clinical verification: 5 out of 7 samples with deletions were reported as having a high probability of deletion. No false positives in the 210 unaffected samples.

Conclusions

The Harmony® prenatal test identifies pregnancies at increased risk for 22q11.2 deletions of 3Mb and smaller with high specificity.

	Analytical validation	Clinical verification	Combined
Total samples (N)	1736	217	1953
22q11.2 (n/N)	92/122	5/7	97/129
No evidence of a deletion (n/N)	1606/1614*	210/210	1816/1824
Sensitivity %, (95% CI)	75.4 (67.1-82.2)	71.4 (35.9-91.8)	75.2 (67.1 - 81.8)
Specificity %, (95% CI)	99.5 (99.0-99.7)	100 (98.2- 100)	99.6 (99.1-99.8)

*Estimations were made using samples with no known 22q11.2 deletion and were presumed to be unaffected. Actual specificity could be higher.

Non-invasive risk assessment of fetal sex chromosome aneuploidy through directed analysis and incorporation of fetal fraction

Hooks J. et. al. Prenat Diagn. 2014;34(5):496-499

Study Population

Study of 432 stored maternal plasma samples taken >10 weeks gestation from singleton pregnancies. 398 were from euploid pregnancies. 34 were from pregnancies affected with Sex Chromosome Aneuploidies (27 cases 45,X;1 case 47,XXX; 6 cases 47,XXY; no cases 47,XYY). All fetuses had a karyotype by invasive testing. Karyotype was blinded at time of cfDNA analysis.

Total group population characteristics:

- Mean: maternal age 35.6 yrs, gestational age 15.4 weeks

Summary and Key Points

The purpose of this study was to evaluate the test performance of the Harmony® prenatal test in the assessment of risk for SCAs.

- 414/432 (96%) samples passed quality control metrics and generated an SCA result.
- Detection rate for 45, X was 96.3% (26/27) in this study with a false positive rate of 0.5% (2/380).
- Detection rate for all other SCAs was 100% with a false positive rate of 0.5% (2/380).

Results

The cohort included 34 cases of sex chromosome aneuploidy. The Harmony® prenatal test correctly identified the following SCA cases as high-risk:

- 26/27 (96.3%) cases of 45,X
- 1/1 (100%) cases of 47,XXX
- 6/6 (100%) case of 47,XXY

The overall false positive rate for all SCAs was 1% in 4/380 euploid pregnancies. Fetal sex was correctly identified in 414/414 samples.

Assessment of Fetal Sex Chromosome Aneuploidy Using Directed Cell-Free DNA Analysis

Nicolaides KH et. al. Fetal Diag Ther. 2014;35(1):1-6

Study Population

Case control study of 177 maternal plasma samples taken at 11-13 weeks gestation. All fetuses had a confirmatory karyotype by invasive testing. Karyotype was blinded at time of cfDNA test. The cfDNA test used in this study was the Harmony® prenatal test.

Summary and Key Points

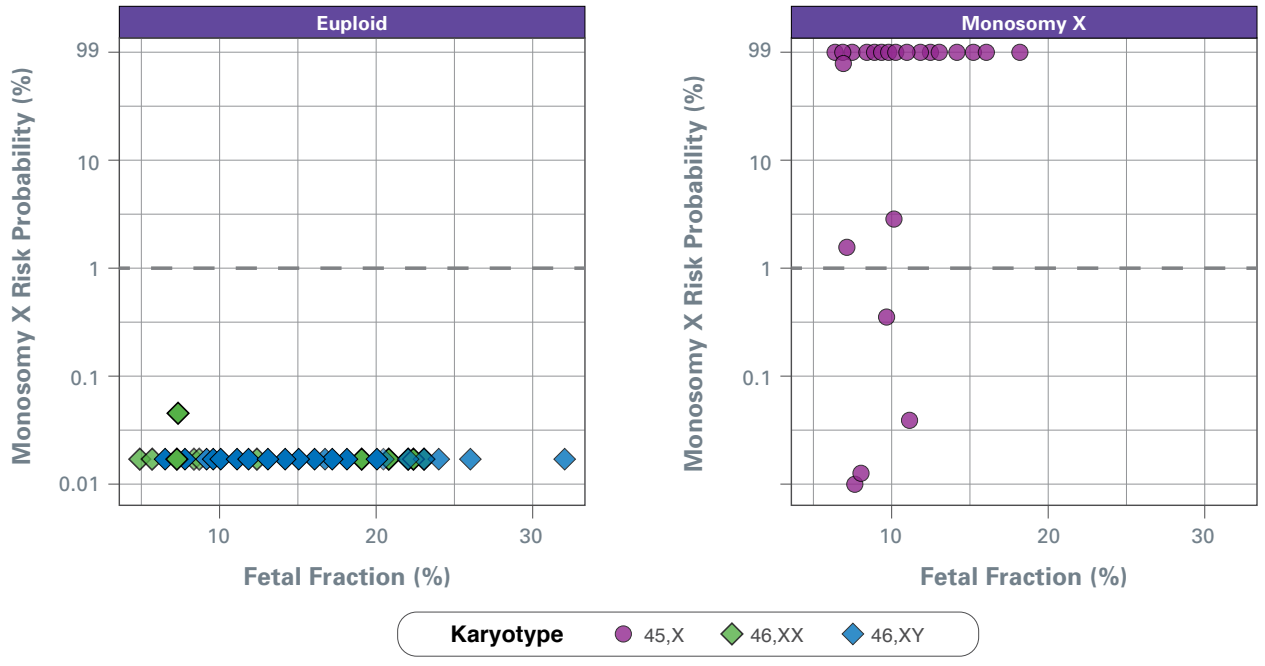
The objective of this study is to evaluate the performance of cfDNA analysis in the risk-assessment of fetal sex chromosome aneuploidies (SCAs).

The results of this study show that evaluation of cfDNA by directed analysis (DANSR assay) can correctly classify fetal sex chromosome aneuploidy with reasonably high sensitivity.

- Detection rate for 45,X was 91.5% in this study with NO false positives.
- Detection rate for all other SCAs was 100% with a false positive rate of <1%.

Results

- Risk results were obtained for 172/177 (97.2%) of samples; median fetal fraction was 12.0%.
- Of fetuses affected with SCA, the following were appropriately identified as "High Risk":
  - 43/47 (91.5%) cases of 45,X
  - 5/5 (100%) cases of 47,XXX
  - 1/1 (100%) case of 47,XXY
  - 3/3 (100%) cases of 47,XYY
- In 115/116 euploid pregnancies, correct classifications were made.
  - 1 False Positive: 47,XXX with a risk of 55/100 that was actually a 46,XX euploid.



LIST OF PEER-REVIEWED PUBLICATIONS USING THE HARMONY® TEST AS OF 2017

1. Ashoor G et al. Fetal Diagn Ther. 2012;31(4):237-43.
2. Ashoor G et al. Am J Obstet Gynecol. 2012;206(4):322.e1-5.
3. Nicolaides KH et al. Am J Obstet Gynecol. 2012;207(5):374 e1-6.
4. Norton ME et al. Am J Obstet Gynecol. 2012;207(2):137.e1--8.
5. Sparks et al. Am J Obstet Gynecol. 2012;206(4):319e1-9.
6. Sparks et al. Prenat Diagn. 2012;32(1):3-9.
7. Ashoor G et al. Ultrasound Obstet Gynecol. 2013;41(1):21-25.
8. Ashoor et al. Ultrasound Obstet Gynecol. 2013;41(1):26-32.
9. Brar et al. J Matern Fetal Neonatal Med. 2013;26(2)-143-45.
10. Fairbrother et al. Prenat Diagn. 2013: March 1-5.
11. Gil MM et al. Ultrasound Obstet Gynecol. 2013;42(1):34-40.
12. Verweij et al. Prenat Diagn. 2013;33(10):996-1001.
13. Wang et al. Prenat Diagn. 2013;33(7):662-6.
14. Gil MM et al. Fetal Diagn Ther. 2014;35(3):204-211.
15. Feenstra et al. Prenat Diagn. 2014;34(2):195-8.
16. Juneau et al. Fetal Diagn Ther. 2014;36(4):282-6.
17. Hooks et al. Prenat Diagn. 2014;34(5):496-499.
18. Nicolaides KH et al. Fetal Diagn Ther. 2014;35(1):1-6.
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26. Quezada et al. Ultrasound Obstet Gynecol. 2015;45(1):101-105.
27. Quezada et al. Ultrasound Obstet Gynecol. 2015;45(1):36-41.
28. Stokowski et al. Prenat Diagn. 2015;35(12):1243-1246.
29. Chen et al. Prenat Diagn. 2016;36(13):1217-1224.
30. Gil MM et al. Ultrasound Obstet Gynecol. 2016;47(1):45-52.
31. Gil MM et al. J Matern Fetal Neonatal Med. 2016: November 1-7.
32. Wallerstein et al. J Pregnancy 2014
33. Kagan et al. Arch Gynecol Obstet. 2016;294(2):219-224.
34. McLennan et al. Aust NZ J Obstet Gynaecol. 2016;56(1):22-28.
35. Revello et al. Ultrasound Obstet Gynecol. 2016;47(6):698-704.
36. Sarno et al. Ultrasound Obstet Gynecol. 2016;47(6):705-711.
37. Bevilacqua et al. Fetal Diagn Ther. 2017 Aug 23.
38. Bjerregaard et al. Dan Med J. 2017 Apr;64(4).pii: A5359.
39. Chan et al. BJOG An Int J Obstet Gynaecol 2017
40. Jones et al. Ultrasound Obstet Gynecol. 2018 51:274-277.
41. Kagan et al. Ultrasound Obstet Gynecol. 2017 Sep 19
42. Kornman et al. Fetal Diagn Ther. 2017 Sep 6
43. Langlois et al. Prenat Diagn. 2017 37(12) 1238-1244.
44. Miltoft et al. Ultrasound Obstet Gynecol. 2017 Jun 22.
45. Richardson et al. Prenat Diagn. 2017 Dec 37(13) 1298-1304.
46. Schmid et al. Fetal Diagn Ther. 2017 Nov 8
47. Scott et al. J Matern Neonatal Med. June 8 2017:1-8.
48. Rolnik et al. Ultrasound Obstet Gynecol. 2018 Jan 10

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