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PRENATAL TEST

performed in Sweden

Clinical Studies Abstract Booklet

The Harmony™ Prenatal Test is a non-invasive prenatal test (NIPT) that evaluates the risk of trisomies by analyzing cell-free DNA (cfDNA) in maternal blood. Since January 2012, there have been over 17 Harmony test studies accepted for publication in peer-reviewed medical journals.

This booklet highlights some of these studies and covers the following:

- Clinical performance and validation of the Harmony test in:
  - Women at high risk for fetal aneuploidy
  - Women in the general screening population
  - Twin Pregnancies
- Fetal fraction and impact on cfDNA testing
- Clinical utility of NIPT
- Implementation of Harmony Prenatal Test
- Clinical performance of sex chromosome aneuploidies

Clear ANSWERS
to Questions that Matter

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

Norton M, Jacobsson B, Swamy G, Laurent L, Ranzini A, Brar H, Tomlinson M, Pereira L, Spitz J, Hollemon D, Cuckle H, Musci T, Wapner R.

Study Population

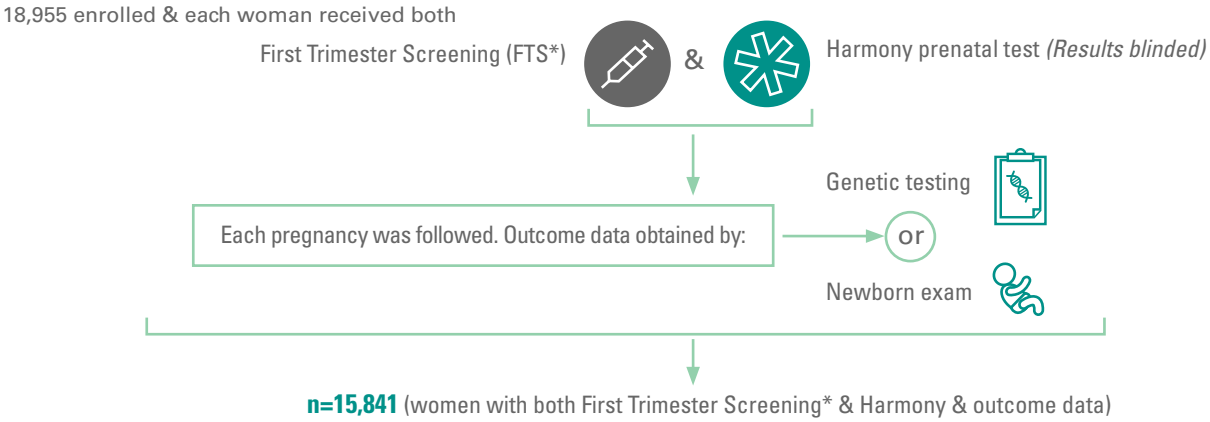
15,841 singleton pregnancies from a general prenatal screening population. The mean maternal age was 30.7 (range 18-48). The mean gestational age was 12.5 weeks (range 10.0-14.3).

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 and specificity (detection rate)

Study Design



Results

Study Results (n=15,841)	FTS*	Harmony Prenatal Test	p-value
Detection Rate (affected pregnancies correctly identified as high risk)	79% (30/38)	100% (38/38)	0.008
False-Positive Rate (unaffected pregnancies incorrectly identified as high risk)	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 performance is consistent in all risk categories

Full manuscript: Norton M, et al, NEJM DOI: 10.1056/NEJMoa1407349 (published online April 1, 2015)

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

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

Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, Rodriguez MH, Williams J 3rd, Mitchell ME, Adair CD, Lee H, Jacobsson B, Tomlinson MW, Oepkes D, Hollemon D, Sparks AB, Oliphant A, Song K.

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

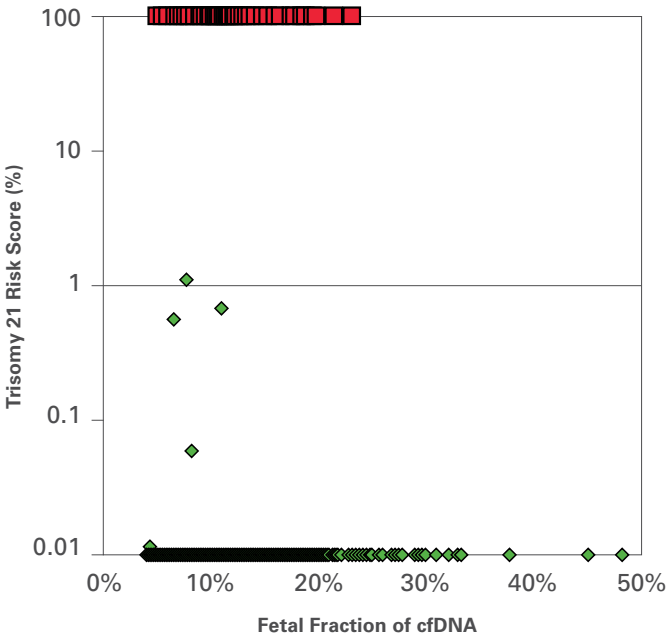
The NICE Study is an international, multicenter cohort study of pregnant women at gestational age 10-weeks or later from 50 clinical sites in which the Harmony test's performance in assessing the risk for fetal trisomies 21 (T21) and 18 (T18) was evaluated.

- 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

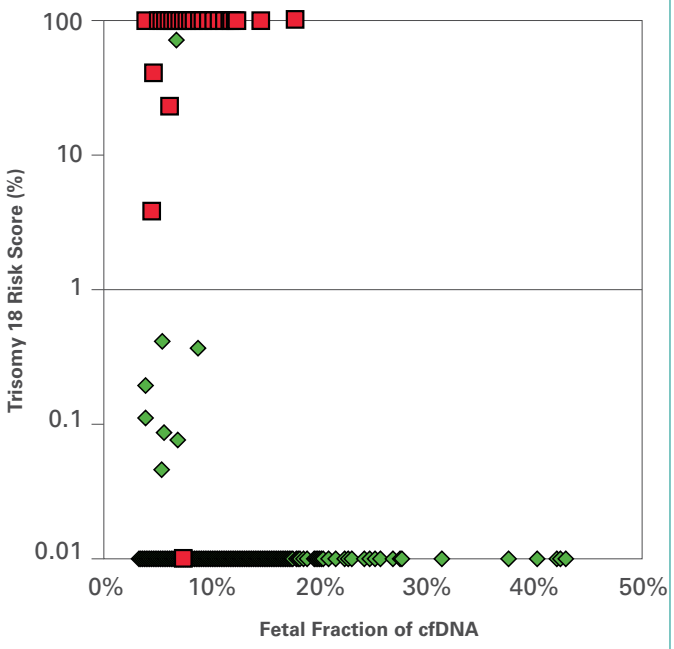
TRISOMY 21

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)



Full manuscript available at: http://ajog.org/article/S0002-9378%2812%2900584-4/fulltext

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

Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G.

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.

Full manuscript available at: http://ajog.org/article/S0002-9378%2812%2900913-1/fulltext

Implementation of Maternal Blood Cell-free DNA Testing in Early Screening for Aneuploidies

Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH.

Study Population

1,005 singleton pregnancies in the first trimester from the Fetal Medicine Centre. The median maternal age was 36.7 years [range: 20.4-48.8].

Summary and Key Points

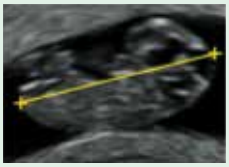
This study explored the feasibility of routine clinical use of cell-free DNA (cfDNA) testing as a primary screening tool for trisomies 21, 18, and 13 at least 10 weeks' gestation. Pregnant women who presented at The Fetal Medicine Centre in London between October 2012 and April 2013 were screened for trisomies 21, 18, and 13 by both NIPT and first trimester combined screening (the combined test). The cfDNA test used in this study was the Harmony Prenatal Test.

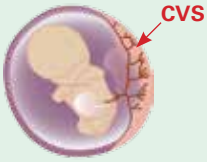
Results

- 98% of participants received a Harmony Prenatal Test result.
- 15 High Risk Harmony results (ten trisomy 21, four trisomy 18, and one trisomy 13) were confirmed by invasive diagnostic testing.
- There were no "false positive" Harmony results for trisomy 21.
- For all trisomies combined, Harmony's false positive rate (FPR) was 0.1% vs. 3.4% with the combined test (nuchal translucency measurement and first trimester biochemistry).

Conclusion

Results from this study demonstrate the feasibility of routine testing for trisomies 21, 18 and 13 by cfDNA testing in singleton pregnancies at least 10 weeks' gestation.





10 weeks:

harmony™ PRENATAL TEST

- Ultrasound to confirm viability and gestational age
- Blood drawn for Harmony Prenatal Test and combined test

12 weeks:

- Nuchal translucency ultrasound
- Review cfDNA results and combined test results with patient.

Offered in the case of:

- High-risk NIPT test result
- NT > 3.5mm
- Other ultrasound findings

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

European Non-Invasive Trisomy Evaluation (EU-NITE) Study: A Multicenter Prospective Cohort Study for Noninvasive Fetal Trisomy 21 Testing

E.J. Verweij[1], B. Jacobsson[2], P.N. Adama van Scheltema[1], M.A. de Boer1, M.J.V. Hoffer[3], D. Hollemon[4], M. Westgren[5], Ken Song[4], D. Oepkes[1]

Study Population

520 women with singleton pregnancies were enrolled in this study. Enrollment criteria included those with an increased risk on first trimester combined screening or detection of fetal abnormalities with ultrasound evaluation. Women requesting invasive testing without these findings were also included. Maternal age ranged from 20 to 47.

Summary and Key Points

The objective of this study was to evaluate the performance of the Harmony Prenatal Test (non-invasive prenatal test using cfDNA) for fetal trisomy 21 (T21) by shipping whole blood samples from Europe to Ariosa Diagnostics's laboratory in the United States (US.

- This is the first prospective European multicenter study showing that non-invasive prenatal testing using directed sequencing of cfDNA, is highly accurate for assessing risk of fetal T21.

Results

- T21 test results were obtained in 504/520 (96.9%) of patients. Risk assessment was accurate in 503/504 subjects (99.8%).
- There were no false positive results and one false negative result for T21 (sensitivity 17/18, 94.4%, specificity 100%).

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/pd.4182/full

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

Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A.

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)	(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)	(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, Quezada M, Bregant B, Syngelaki A, and Nicolaides K

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 T21, 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. The Harmony™ Prenatal Test algorithm, FORTE, incorporated the lower fetal fraction contribution of the 2 fetuses in the twin pregnancy[1].

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.

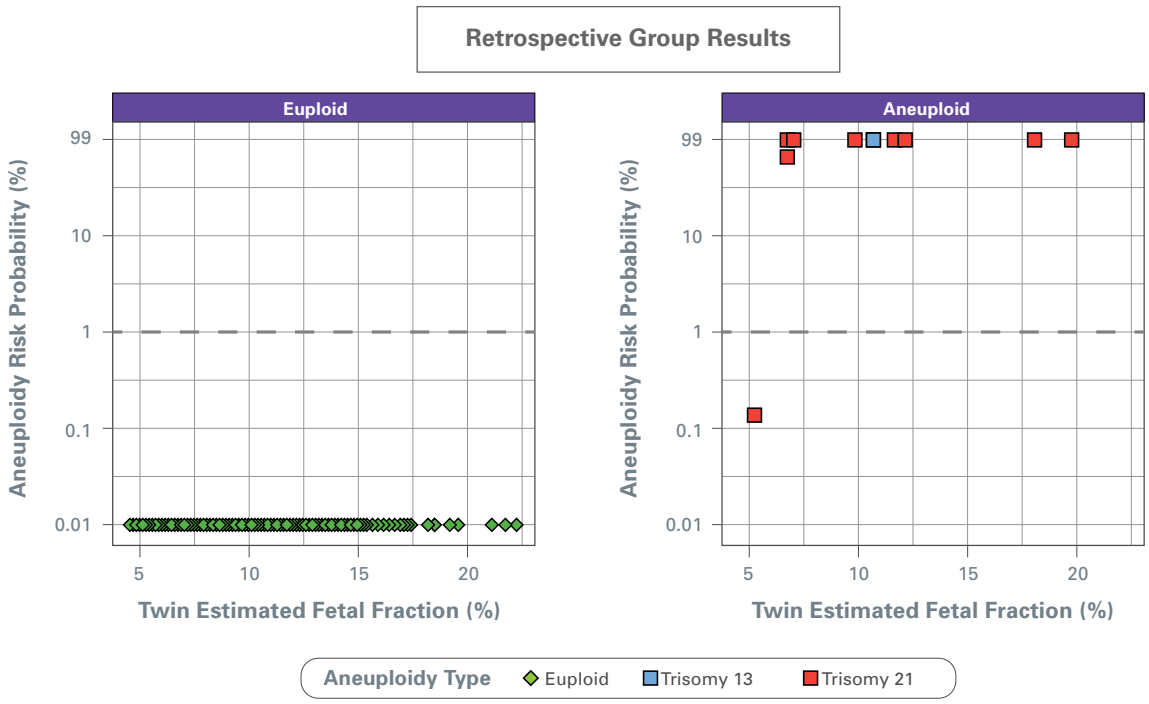
Results

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 T13, 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 T21, T18 and T13 was <0.01%.
  - In 2/63 cases, risk score for T21 was >99%.
  - In 1/63 cases, risk score for T18 was 59%.



1. Fetal Fraction Estimate in Twin Pregnancies Using Directed Cell-Free DNA Analysis. Struble C, Syngelaki A, Oliphant, Song A, Nicolaides KH, Fetal Diagn Ther DOI: 10.1159/000355653

Open access: http://www.karger.com/Article/Pdf/356495

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

Nicolaides KH, Musci T, Struble C, Syngelaki A, Gil M. Fetal Diag Ther. 2013 Dec;DOI:10.1159/000357198

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

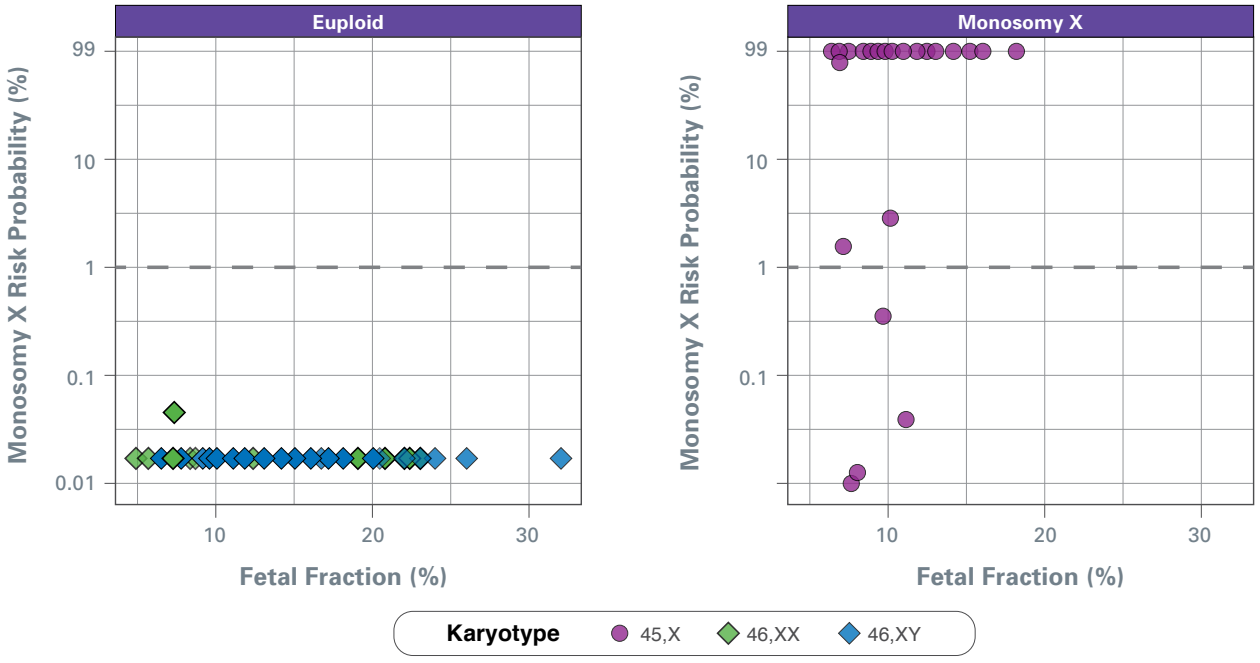
The objective of this study is to evaluate the performance of cfDNA analysis in the risk-assessment of fetal sex chromosome aneuploidies (SCAs).

The results of this study show that evaluation of cfDNA by directed analysis (DANSR) 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.



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

Hooks J, Wolfberg AJ, Wang ET, Struble CA, Zahn J, Juneau K, Mohseni M, Huang S, Bogard P, Song K, Oliphant A, Musci TJ

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 376/380 euploid pregnancies. Fetal sex was correctly identified in 414/414 samples

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/pd.4338/pdf

Clinical Experience of Noninvasive Prenatal Testing with Cell-Free DNA for Fetal Trisomies 21, 18, and 13, in a General Screening Population

Fairbrother G, Johnson S, Musci TJ, Song K.

Study Population

The purpose of this study is to evaluate NIPT with cfDNA as a primary screening method for trisomy 21, 18, and 13 in an obstetrical clinical practice setting. The cohort included 289 women with mean age of 32.3 years (range: 17.8–42.0) who underwent testing at 13.0 gestational age weeks (range: 10.1–20.7).

Summary and Key Points

NIPT has the potential to be a highly effective screening method as a standard test for risk assessment of fetal trisomies 21, 18, and 13 in general pregnant populations.

- NIPT results were provided for 98.6% of patients at a mean reporting time of 9.3 calendar days.
- With NIPT, all patients had a risk less than 1:10000 for trisomy 21, 18, or 13.
- With FTS, 4.5% of patients had screening results indicating an increased risk for trisomy 21. One patient who had an elevated trisomy 21 risk with FTS elected to have an amniocentesis, which revealed a euploid fetus. NIPT on this same patient provided a low-risk result for trisomy 21.

Full manuscript: http://onlinelibrary.wiley.com/doi/10.1002/pd.4092/pdf

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LG-H-AB-Rev1.0

The Harmony® Prenatal Test was developed by Ariosa Diagnostics (San Jose, California, USA). The Harmony® reagents and Ariosa cell-free DNA System (AcfS) software used as part of the Harmony Prenatal Test are CE Marked under the IVD Directive 98/79/EC. Harmony is a non-invasive prenatal test (NIPT) based on cell-free DNA analysis. The results are intended for prenatal screening and are not intended to be the sole basis for diagnosis. Harmony does not screen for potential chromosomal or genetic conditions other than those expressly identified in this document. Before making any treatment decisions, all women should discuss their results with their healthcare provider, who can recommend confirmatory, diagnostic testing where appropriate.