Harmony™
PRENATAL TEST

Clinical Studies Abstract Booklet

The Harmony™ Prenatal Test is a non-invasive prenatal test (NIPT) that assesses the risk of trisomies by analyzing cell-free DNA (cfDNA) in maternal blood. Since January 2012, there have been over a dozen articles accepted for publication in peer-reviewed medical journals regarding the Harmony test. The purpose of this booklet is to highlight data from some of these publications, including the following:

- Clinical performance and validation of the Harmony test in:
  - Women at high risk for fetal aneuploidy
  - General screening population
- Importance of fetal fraction in cfDNA testing
- Clinical utility of NIPT
- Implementation of NIPT into clinical practice

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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 study to date regarding performance of non-invasive prenatal testing

Summary and Key Points

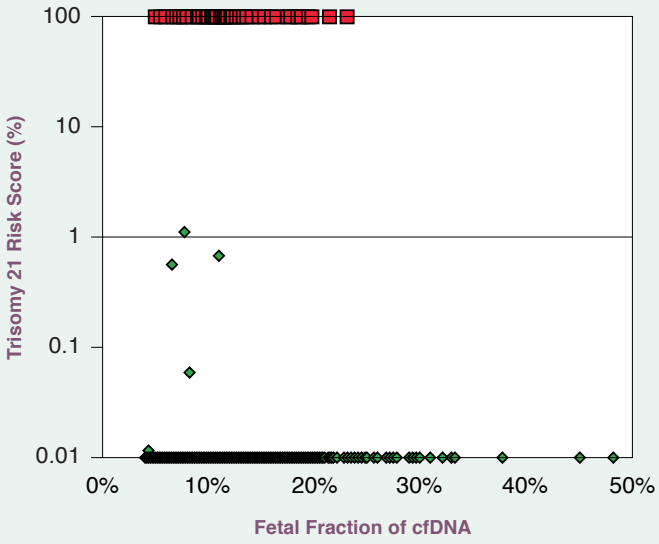
The NICE Study is an international, multicenter cohort study of pregnant women at gestational age 10-weeks or later from 50 clinical sites in which the Harmony test’s performance in assessing the risk for fetal trisomies 21 (T21) and 18 (T18) was evaluated.

- 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 is <0.1%.
- To date, this is the largest validation study of non-invasive prenatal testing.

Results

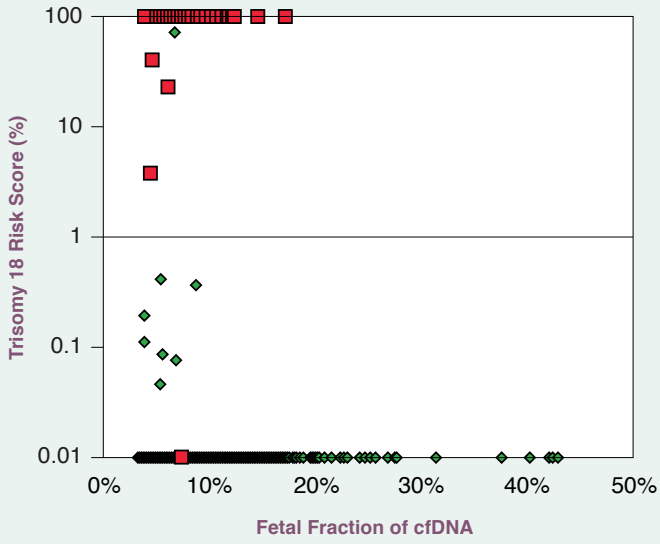
TRISOMY 21

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)



Trisomy Non-trisomy

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

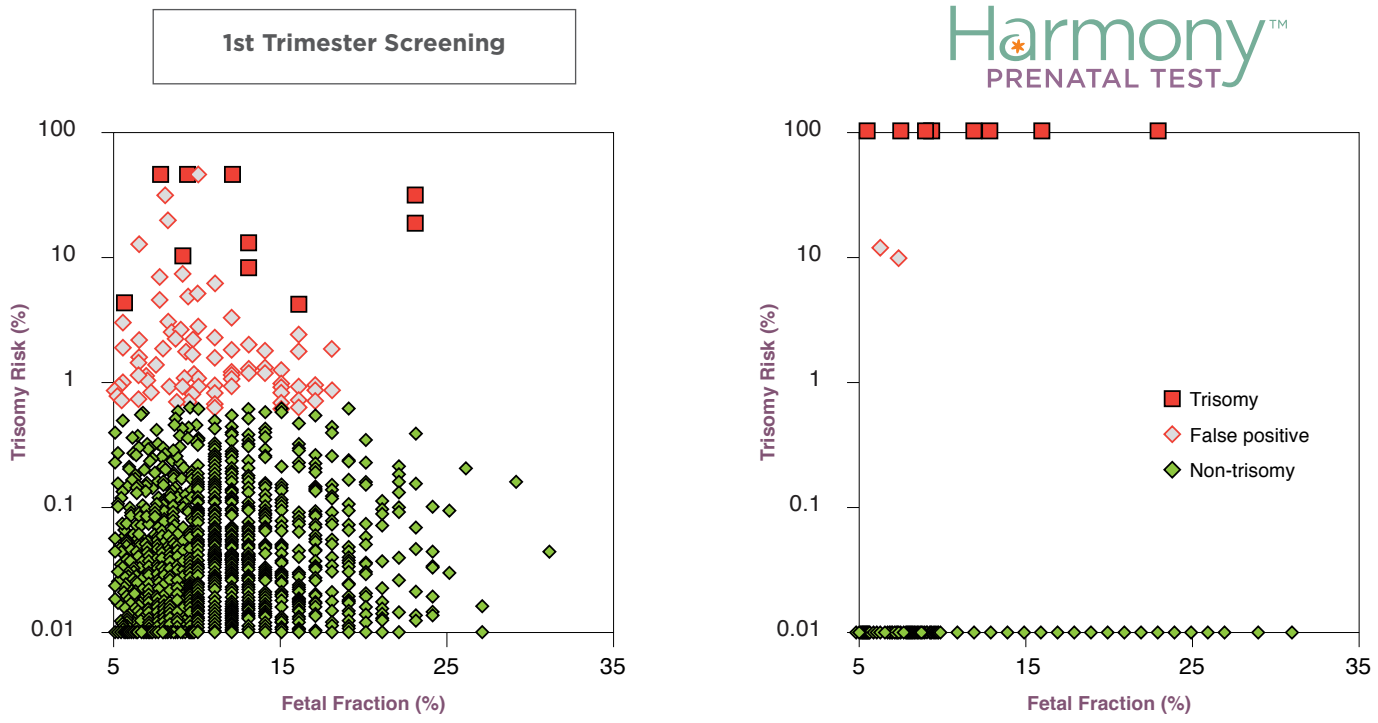
Summary and Key Points

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 trisome 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



Logarithmic distribution of trisomy risk scores (as a percentage, 1 in 100 = 1%) with the different prenatal testing modalities plotted by the fetal fraction. Red squares represent the trisomy 21 and trisomy 18 cases and green diamonds represent the non-trisomy cases. Gray diamonds represent the false positive cases at a risk cut-off of 1 in 150.

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

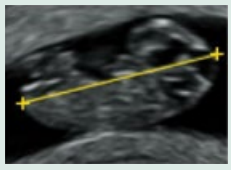
Summary and Key Points

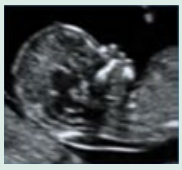
This is a prospective study to explore the feasibility of maternal blood cell-free (cf) DNA testing in screening for trisomies 21, 18 and 13 (T13) at 10-weeks' gestation. Women with singleton pregnancies were tested at 10-weeks by cfDNA and combined test/first-trimester combined screening at 12 weeks. The cfDNA test was performed with the Harmony Prenatal Test. The median maternal age was 36.7 with maternal age range from 20.4-48.8.

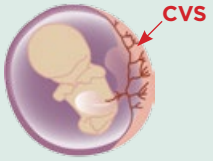
Results from this study demonstrated the feasibility of implementing routine testing for trisomies 21, 18 and 13 by cfDNA testing in singleton pregnancies at 10 weeks' gestation.

Results

- 98% of women tested with Harmony test received a result.
- 15 Harmony "High Risk" results for T21, T18, and T13 were confirmed by invasive testing.
- There was no false positives (FP) for T21 using the Harmony cfDNA test;
- For all trisomies combined, Harmony's false-positive rate (FPR) was 0.1% vs. 3.4%FPR with the combined test/first trimester combined screening test.







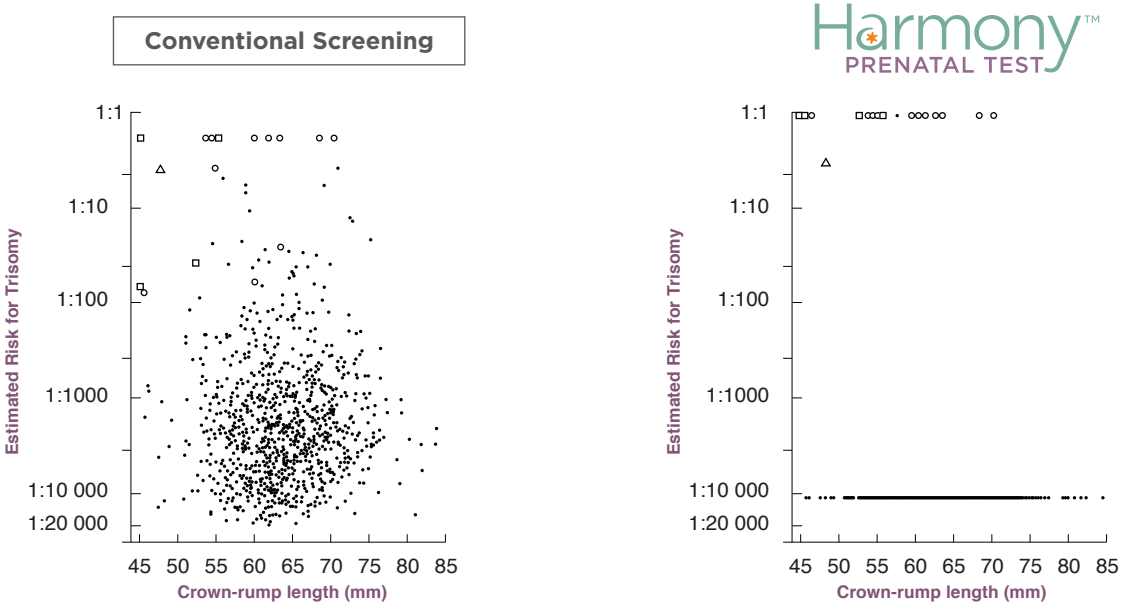
Harmony™ PRENATAL TEST

- Scan to measure the fetus
- Blood for cfDNA and combined test

- Detailed scan
- Discuss screening results and determine if CVS is necessary

- High-risk cfDNA test result
- Fetal defects
- NT ≥ 3.5mm

Estimated risk for trisomy in the pregnancy with trisomy 21 (o), trisomy 18, or trisomy 13. (a) combined test (b) cfDNA



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

The Fetal Fraction of Cell-Free DNA in Maternal Plasma is Not Affected by A Priori Risk of Fetal Trisomy

Brar H, Wang E, Struble C, Musci TJ, Norton ME.

Study Population

A comparative analysis on fetal cfDNA amounts was performed in pregnant women stratified into a priori risk groups based on maternal age, prenatal screening results or nuchal translucency measurement. The women were 18 years and older with a singleton pregnancy of at least 10 weeks gestational age who were undergoing an invasive procedure for any reason.

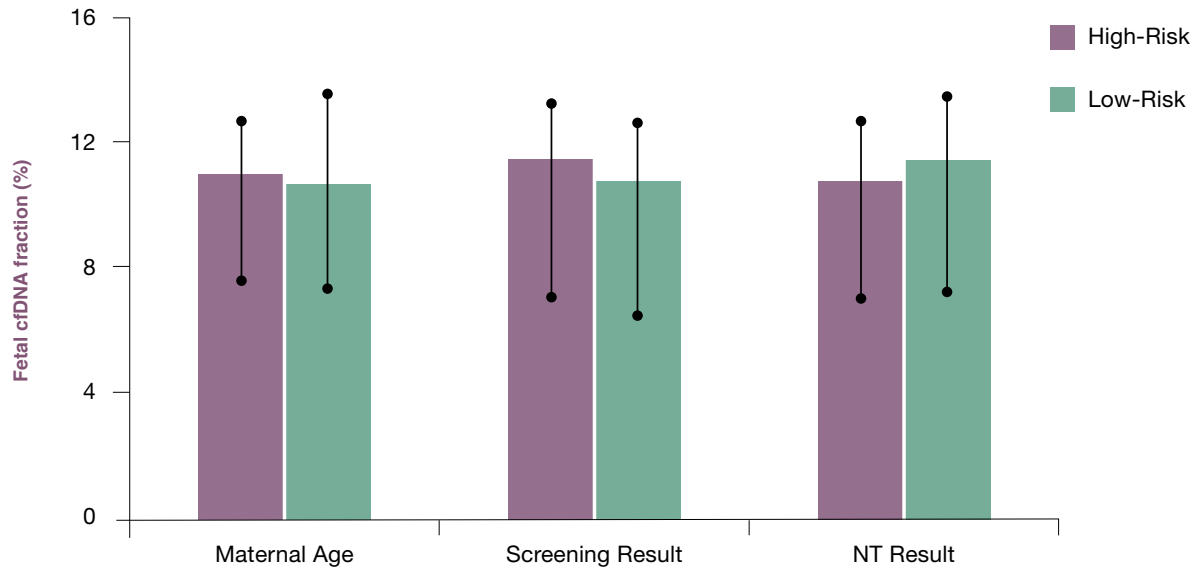
Summary and Key Points

Across the highest and lowest deciles within each group, there were no significant differences in the fetal cfDNA fraction.

- Maternal age, serum screening and NT measurements show no significant effect of the fetal fraction of cfDNA in maternal plasma.
- No false positive results were found in any of the low-risk or high-risk groups.
- All T21 cases correctly identified in low- and high-risk groups regardless of clinical risk factors.

Results

Fetal Fraction Constant Across Risk Groups



May support that NIPT performance is consistent between “high-risk” and “low-risk” pregnancies

Full manuscript available at: http://informahealthcare.com/doi/abs/10.3109/14767058.2012.722731

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 cfDNA does not materially change between 10 to 21 weeks of 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.
  - Minimum percent fetal cfDNA is approximately 4%.
- The vast majority of samples greater than 10 weeks gestation contain an adequate fetal cfDNA proportion to allow for useful clinical results.
  - Findings did indicate a correlation between higher maternal weight and lower percent fetal cfDNA.
- 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)	Pregnancies with ≥4% fetal cell-free DNA (%)
<50	>99
≥50 & <60	>99
≥60 & <70	>99
≥70 & <80	>99
≥80 & <90	98
≥90 & <100	96
≥100 & <110	95
≥110 & <120	90
≥120 & <130	88
≥130 & <140	81
≥140	71

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/pd.4119/full

Clinical Utility and Cost of Non-Invasive Prenatal Testing with cfDNA Analysis in High-Risk Women Based on a US Population

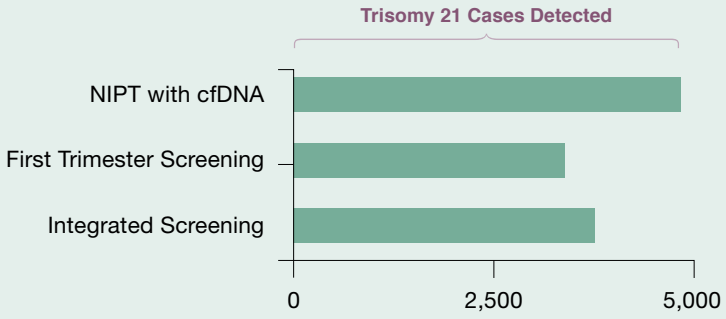
Song K, Musci TJ, Caughey AB.

Summary and Key Points

NIPT in high-risk pregnancies, at a price of **$795**, was a cost savings when compared to the current standard of care with First Trimester Combined Screening (FTS) or Integrated Screening (INT).

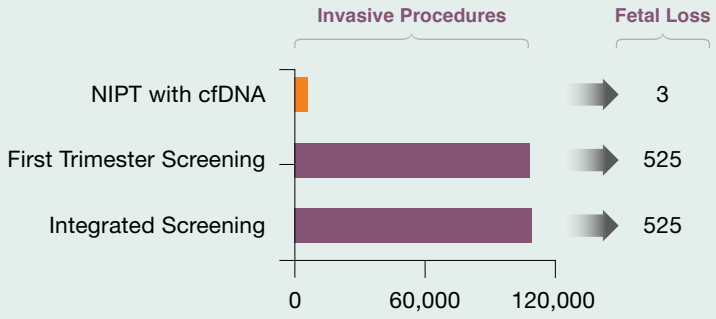
- NIPT detected 28% and 43% more trisomy 21 cases compared to INT and FTS, respectively.
- NIPT reduced invasive procedures by >95%.
- NIPT reduced normal fetal losses by >99%.
- NIPT reduced healthcare costs by >10%.

BETTER DETECTION



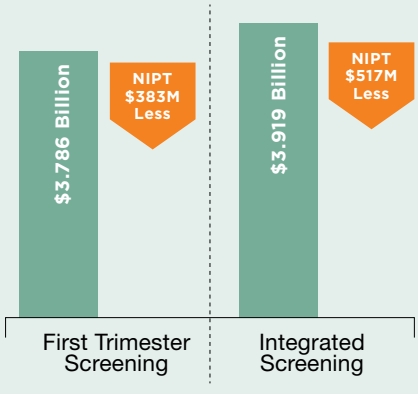
NIPT detects 28-43% more T21 cases

FEWER COMPLICATIONS



NIPT reduces invasive procedures by >95% and saves >99% of normal fetuses

NIPT has better clinical outcomes compared to conventional screening



Ariosa DIAGNOSTICS

NIPT at a Price of $795 is Cost Saving over Conventional Prenatal Screening

Full manuscript available at: http://informahealthcare.com/doi/abs/10.3109/14767058.2013.770464

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.

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.

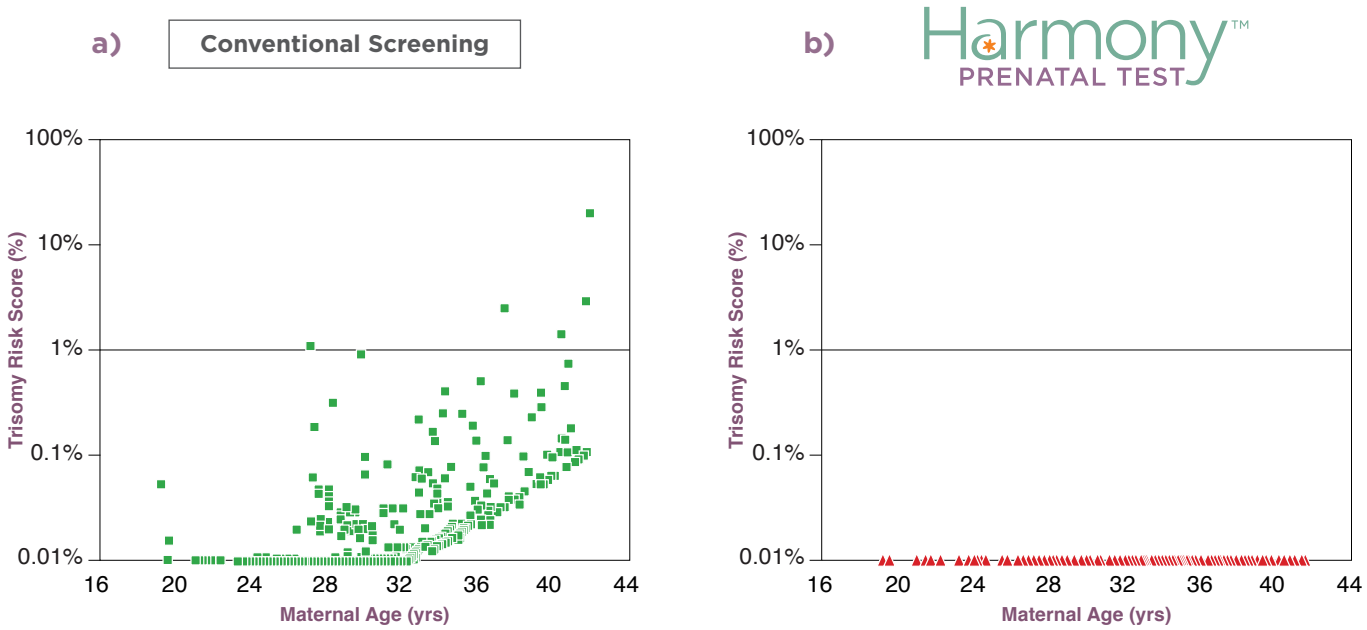


Figure 2 (a) Risk scores (n = 267) with first trimester combined screening (serum PAPP-A, serum ß-hCG, and nuchal translucency) based on maternal age. (b) Risk scores (n = 287) with NIPT based on maternal age. Risk scores are plotted on a logarithmic scale

Full manuscript available at: http://onlinelibrary.wiley.com/doi/10.1002/pd.4092/full

Non-Invasive Prenatal Testing with Cell-Free DNA: US Physician Attitudes Toward Implementation in Clinical Practice

Musci TJ, Fairbrother G, Batey A, Bruursema J, Struble C, Song K. Prenat Diagn. 2013 May;33(5):424-8. doi: 10.1002/pd.4091. Epub 2013 Mar 22.

Study Population

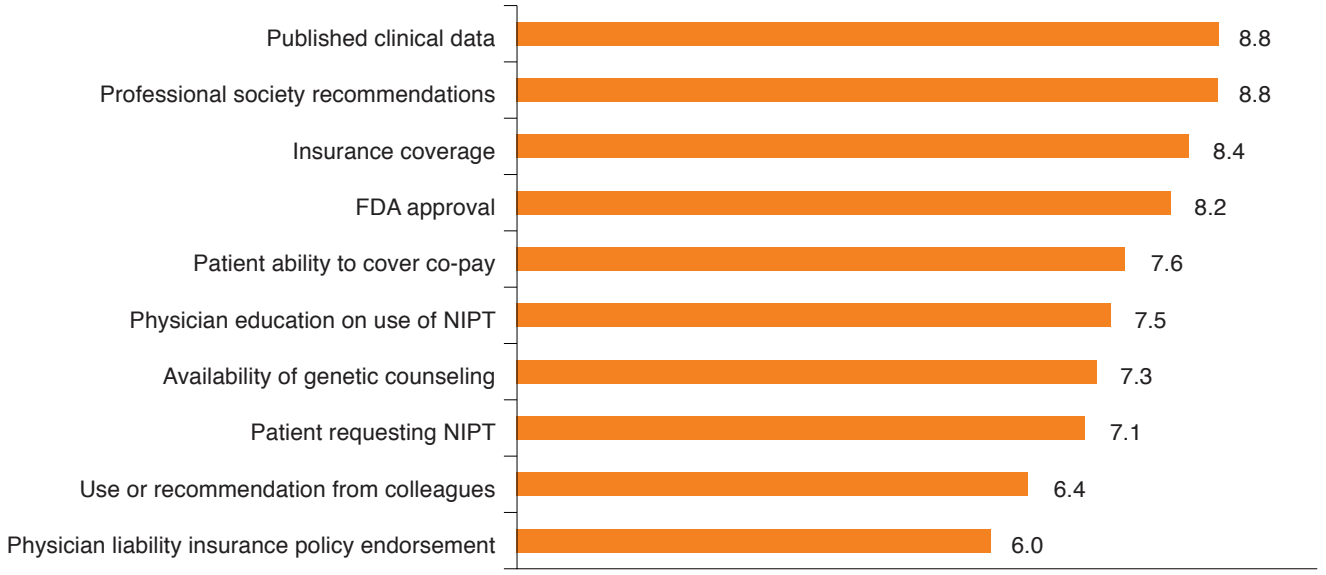
The purpose of this study was to assess awareness, potential adoption and current utilization of NIPT for common fetal aneuploidies among U.S.-based obstetricians.

Summary and Key Points

A 36-item web-based survey was designed to assess the current practice of fetal aneuploidy screening and knowledge and utilization of NIPT for fetal trisomy. Practicing obstetricians in the United States were invited via email to participate in the survey.

- Of the 101 obstetricians who completed the survey (27% academic-based, 73% private practice), 97% offer screening to high-risk patients and 91% offer screening to average-risk patients.
- With regard to current screening tests,the top three limitations were as follows: patient anxiety, risks of follow-up invasive testing, and high false positives.
- NIPT had been used by 32% of respondents and 22% were familiar with NIPT and the associated clinical data. The majority of physicians predicted that they would offer NIPT to high-risk women (86.1%) and average-risk women (76.2%) within 12 months.

Importance of factors for NIPT adoption (mean values, 1=not at all important, 10=very important)



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

E.J. Verweij1, B. Jacobsson[2], P.N. Adama van Scheltema1, 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.

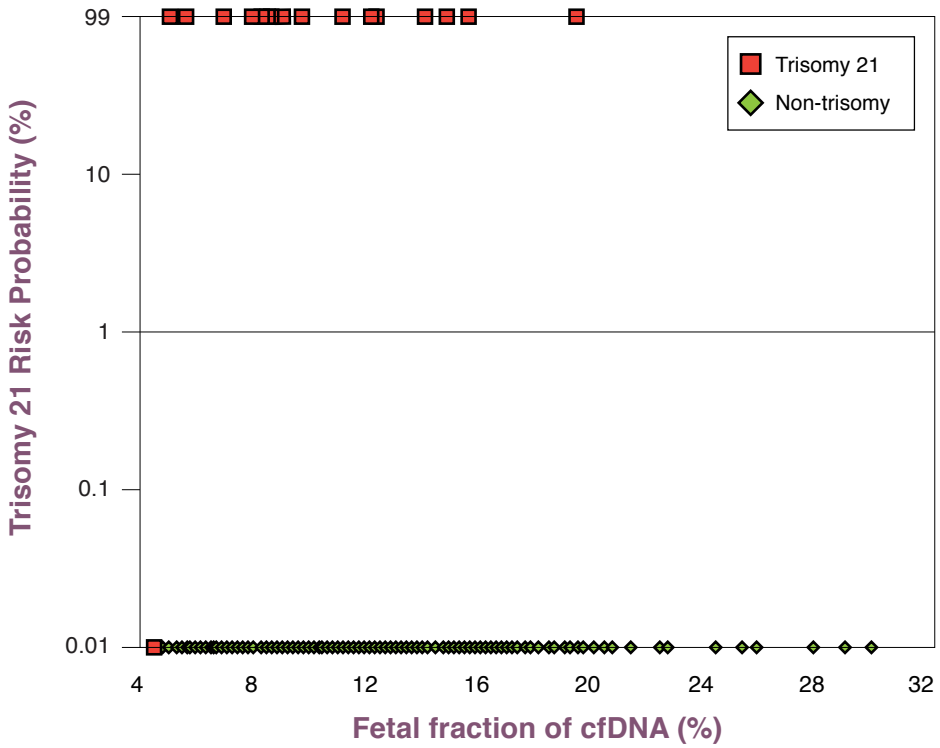
Summary and Key Points

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 Unites States (US).

- This is the first prospective European multicenter study showing that noninvasive prenatal testing using directed sequencing of cfDNA, applied to blood samples shipped across the Atlantic Ocean, 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

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