harmony®
PRENATAL TEST
Testing service from Ariosa Diagnostics

Roche

Clinical Studies Abstract Booklet

The Harmony® prenatal 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 prenatal 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 prenatal 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 prenatal test.

MC-US-00844

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CLINICAL EVIDENCE

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).

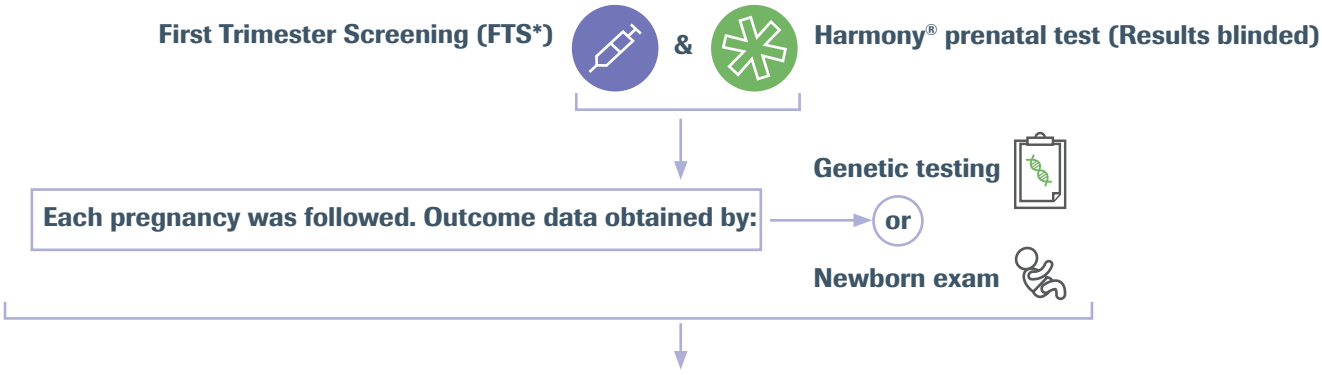
SUMMARY AND KEY POINTS

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

18,955 enrolled & each woman received both



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%** (6/11,975)	**0.05%** (8/14,949)
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: https://www.ncbi.nlm.nih.gov/pubmed/25830321

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

Results with microarray quantitation are entirely consistent with reported data on Harmony using sequencing quantitation, thus sensitivity data can be combined from nine previous studies with over 23,000 pregnancies.

	Sensititvity (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.

CLINICAL EVIDENCE

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

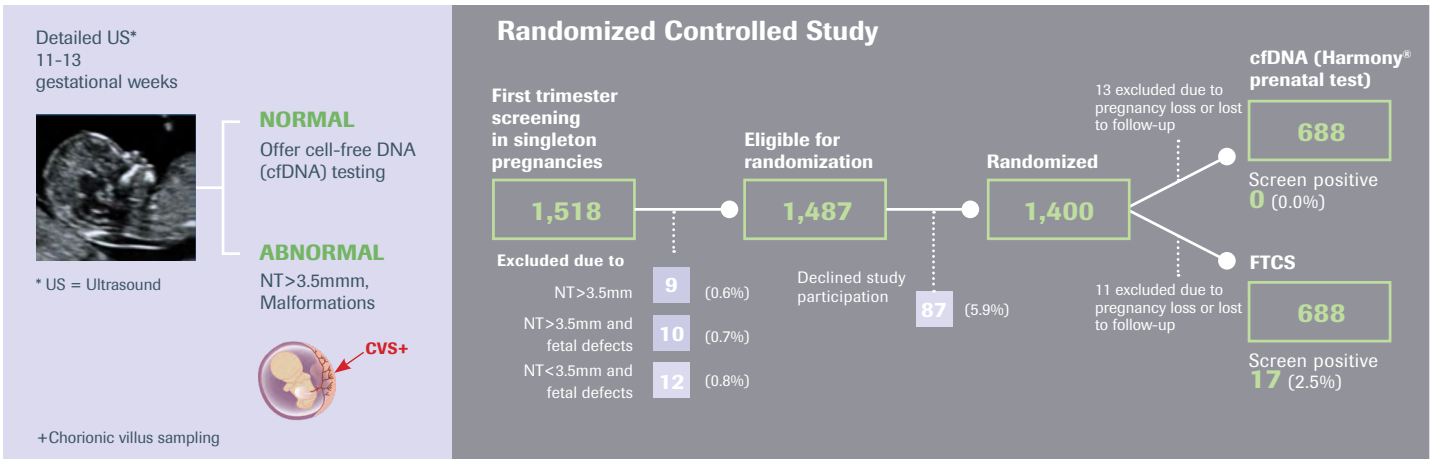
1,400 singleton pregnancies with normal first trimester ultrasound were randomized into two groups: FTCS or cfDNA screening.

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: https://www.ncbi.nlm.nih.gov/pubmed/28925570

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.

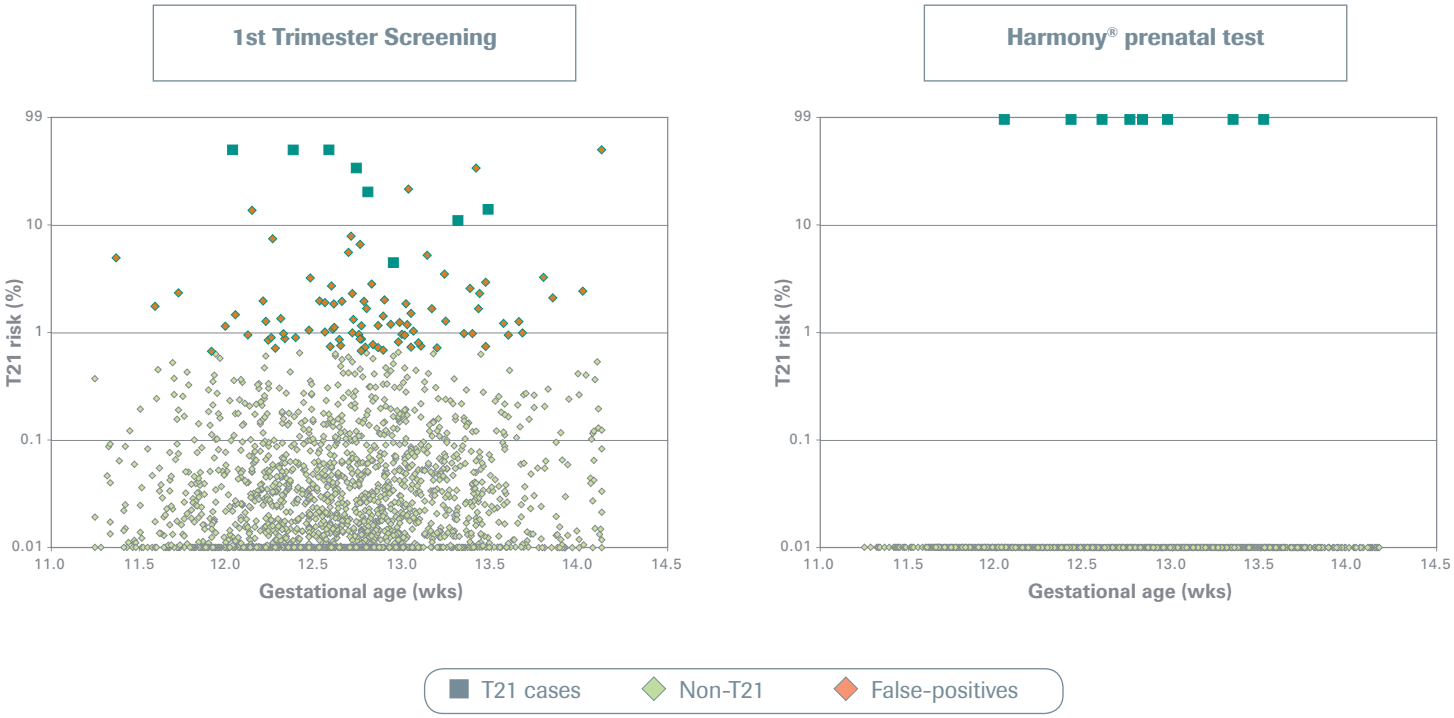
SUMMARY AND KEY POINTS

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® prenatal 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: https://www.ncbi.nlm.nih.gov/pubmed/23107079

Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma

Wang 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.

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%

Accuracy and reproducibility of fetal fraction using relative quantitation at polymorphic loci with microarray

Schmid M. et al. *Ultrasound Obstet Gynecol*. 2018 Jun;51(6):813-817.

SUMMARY AND KEY POINTS

Despite the increasing recognition of fetal fraction as an important quality metric in cfDNA testing, there has been little focus on the variety of methods employed and the need to ensure their reliability. This study evaluates the accuracy and reproducibility of fetal fraction measurement using polymorphic assays (aka SNPs) that are incorporated into test design as part the Harmony® Prenatal Test. This study confirms this DANSR SNP method is accurate and reproducible for fetal fraction estimation and is equally informative across global populations. This study provides a useful benchmark for ensuring the reliability and accuracy of fetal fraction measurement.

STUDY POPULATION

Fetal fraction measurement based on polymorphic assays were compared to those from Y-sequence quantitation in a consecutive series of 47,512 commercially tested maternal plasma samples.

STUDY DESIGN

Samples were assayed on custom microarrays designed to cover non-polymorphic targets on chromosomes of interest for aneuploidy assessment (21, 18, 13, X, and Y) and polymorphic targets for fetal fraction assessment. In a consecutive series of 47,512 clinical samples submitted to the Ariosa laboratory, fetal fraction measurements based on polymorphic assays were compared to those from Y-sequence quantitation. Fetal fraction reproducibility was also examined between the first and second tube measurements from 734 randomly chosen samples. To assess whether the assay was informative across different populations the fraction of informative loci was determined in 13,988 samples.

RESULTS

There was strong correlation between fetal fraction measured using the DANSR SNP assays and Y chromosome sequences ($r = 0.97$). Fetal fraction measurement between the first and second tubes was highly reproducible ($r = 0.98$). The fraction of informative loci observed in a clinical series was consistent with predictions based on assay design.

DISCUSSION

Despite the increasing recognition of fetal fraction as an important quality metric in cfDNA testing for fetal trisomy, there has been little focus on the variety of methods employed and the need to ensure their reliability. Underestimating fetal fraction may cause valid samples to be unnecessarily rejected. Overestimating fetal fraction may lead to samples with insufficient fetal cfDNA being tested and a risk of false negative results. Just as different methods for trisomy screening are well validated before clinical implementation, so should different assay designs for fetal fraction measurement.

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.

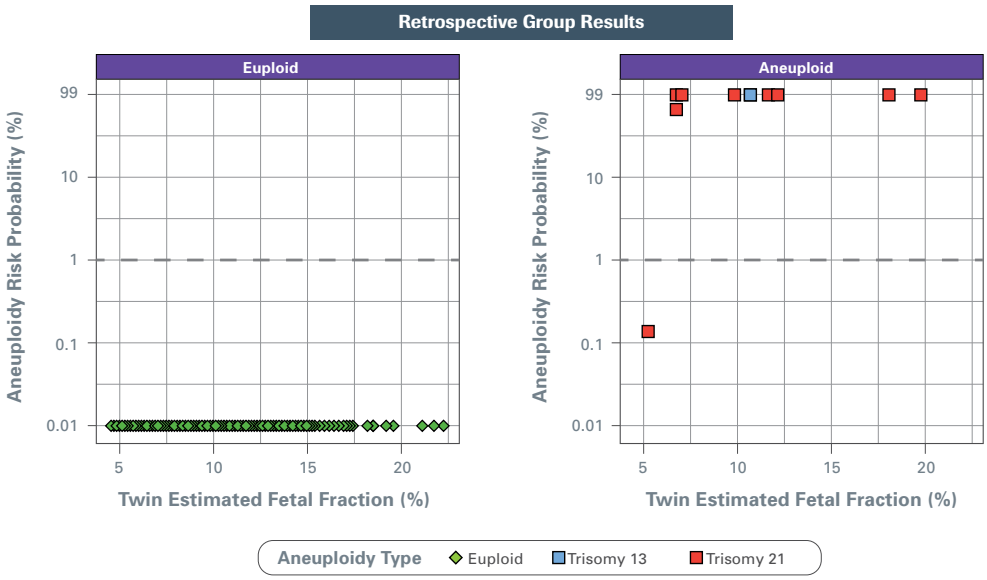
RESULTS

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%.



Open access: https://www.ncbi.nlm.nih.gov/pubmed/24247435

Performance of screening for aneuploidies by cell-free DNA analysis of maternal blood in twin pregnancies.

Bevilacqua et al *Ultrasound Obstet Gynecol* 2015:45(1):61-66

STUDY POPULATION

The study group was 515 twin pregnancies obtained at 10-28 weeks' gestation. The comparison group was comprised of 1847 singleton pregnancies. Median gestational age for the singleton comparison group was 13.6 weeks compared to the twin samples with a median gestational age of 13.0 weeks.

SUMMARY AND KEY POINTS

- Designed to report on the clinical implementation of a targeted cfDNA analysis screening for trisomies 21, 18, and 13 in twin pregnancies
- Prospective, multicenter study between May 2013 and September 2014
- Targeted cfDNA screening for trisomy 21, 18, and 13 is feasible in twin pregnancies
- Rate of reporting in twin pregnancies is lower than in singleton pregnancies due to lower fetal fractions in the twin cases

RESULTS

- The median fetal fraction in the singleton group was 11.7%. The median of the lower of the two fetal fractions in the twin group was 8.7%.
- The no result rate was higher in twin pregnancies at 5.6% as compared to the singleton comparison group with a no result rate of 1.7%. Second sampling was allowed in the study protocol and results were obtained in 50% of the twin pregnancies when a second blood sample was obtained.
- There were 323 euploid twin pregnancies and all were correctly identified as low risk for trisomy
  - No false positive results
- Correctly identified 11 of 12 trisomy 21 cases
  - There was 1 discordant low risk trisomy 21 case with a risk score of <1/10,000
  - Correctly identified 5 of 5 trisomy 18 cases

STUDY LIMITATIONS

Conclusive detection rates cannot be calculated due to the limited number of trisomy samples and the lack of complete outcome data.

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

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.

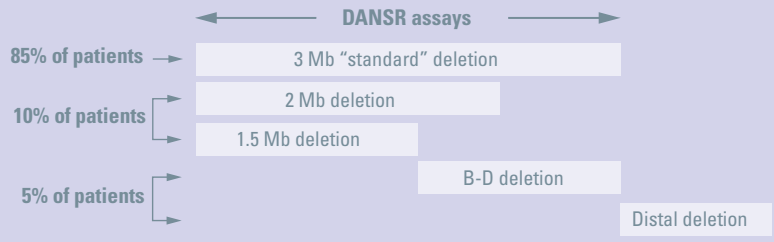
Fetal 22q11.2 deletions of 3 Mb and smaller were assessed.

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 verfication	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.*

Targeted cell-free DNA analysis with microarray quantitation for assessment of fetal sex and sex chromosome aneuploidy risk

Jones K. et al. *Ultrasound Obstet Gynecol* 2018; 51: 274–277.

SUMMARY AND KEY POINTS

This study expands upon the available published data by investigating the performance of targeted cfDNA analysis of the X and Y chromosomes using microarray quantitation for assessment of sex chromosome aneuploidy (SCA) probability in singleton pregnancy and fetal sex in twin and singleton pregnancies.

STUDY POPULATION

Samples of banked maternal plasma from 791 singleton and 51 twin pregnancies were obtained as part of ongoing multicenter clinical studies and from a sample bank at King's College London, UK. Gestational age and fetal fraction averaged 16.7 weeks and 13.4%, respectively

METHODS

Y-chromosome specific DANSR assays were used to evaluate fetal sex in twin and singleton pregnancies. Results were reported as male or female, depending on concluded presence or absence of Y-chromosome fragments. In twin pregnancies, a male result indicates the presence of at least one male fetus. Fetal SCA analysis was performed on samples from singleton pregnancies using X- and Y-specific DANSR assays followed by FORTE analysis adapted for this purpose. A probability cut-off of 1 in 100 was used for calculation of sensitivity and specificity.

RESULTS

Thirty-nine singleton and 12 twin pregnancy samples had insufficient fetal fraction or failed to pass quality control thresholds resulting in 752 and 39 samples undergoing testing for fetal sex in singleton and twin pregnancies.

Fetal sex results were yielded in 748/752 singleton and 39/39 twin samples, with 99.9% concordance. All 15 cases of SCAs were correctly identified (100% sensitivity; 95% CI, 79.6–100%). Out of 727 disomic pregnancies, 725 were correctly classified as low-risk for SCA (99.7% specificity; 95% CI, 99.0–99.9%) (see table below).

	*Fetal sex accuracy**		*Sex chromosome aneuploidy*						*Disomy accuracy**
			45,X†		*47,XXX †*		*47,XXY †*		
Study	*Singleton*	*Twin*	*DR*	*FPR*	*DR*	*FPR*	*DR*	*FPR*	
Current	747/748 (99.9 (99.3–100))	39/39 (100 (91.0–100))	13/13 (100 (77.2–100))	1/742 (0.1 (0–0.8))	1/1	1/742 (0.1 (0–0.8))	1/1	0/742 (0 (0–0.5))	725/727 (99.7 (99.0–99.9))
Hooks [2]	414/414 (100 (99.1–100))	—	26/27 (96.3 (81.7–99.3))	2/380 (0.5 (0.2–1.9))	1/1	2/380 (0.5 (0.2–1.9))	6/6	0/380 (0 (0–1))	378/380 (99.5 (98.1–99.9))
Nicolaides [3]	109/110 (99.1 (95–99.8))	—	43/47 (91.5 (80–96.6))	0/172 (0 (0–2.2))	5/5	1/172 (0.6 (0.1–3.2))	1/1	0/172 (0 (0–2.2))	115/116 (99.1 (95.3–99.9))

*Only first author of each study is given. Data are presented as n/N (% (95% CI)). *Accuracy defined as concordant cfDNA and karyotype test results. †Sensitivities for individual sex chromosome aneuploidies cannot be concluded due to small number of affected pregnancies. DR, detection rate; FPR, false-positive rate.*

DISCUSSION

Targeted cfDNA analysis performed with high accuracy for fetal sex assessment in twins and singletons, and correctly identified all SCAs with high specificity. This study provides a valuable supplement to the currently available data supporting the use of targeted cfDNA analysis for fetal sex and SCA assessment and substantiates previous conclusions that the performance of this methodology is robust across quantitation platforms.

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

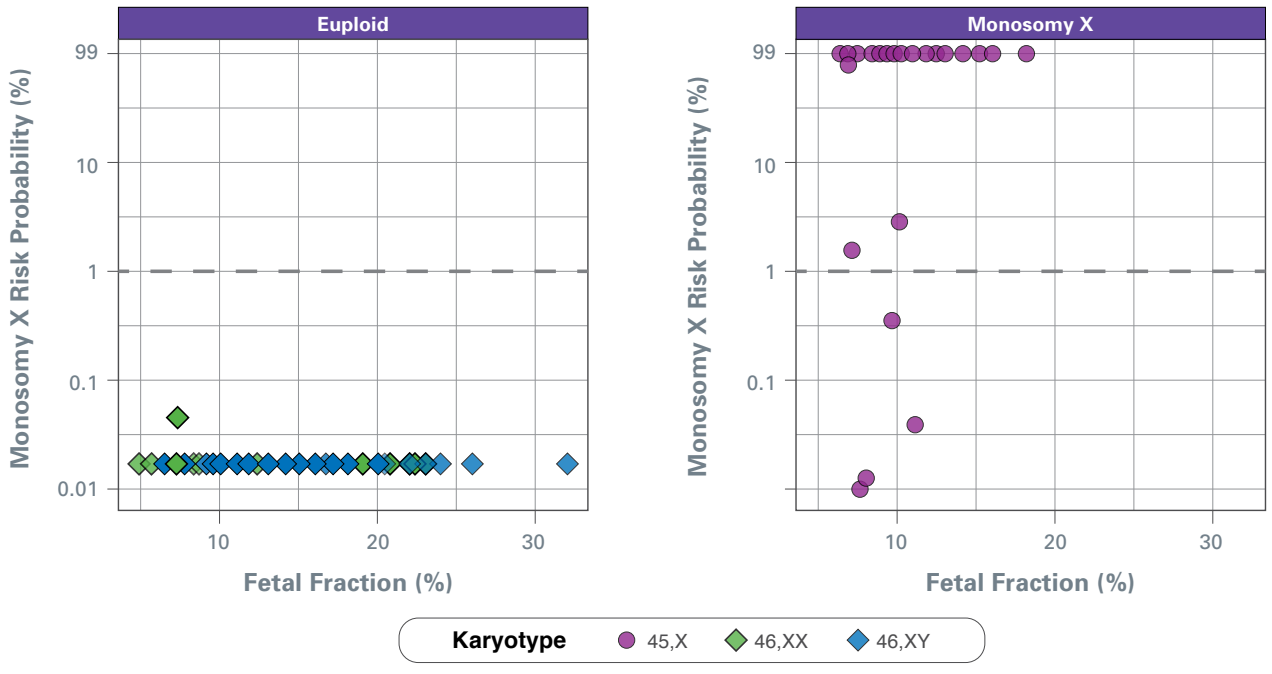
The objective of this study is to evaluate the performance of cfDNA analysis in the risk-assessment of fetal sex chromosome aneuploidies (SCAs).

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.



Open access: https://www.ncbi.nlm.nih.gov/pubmed/24335155

Maternal age trends support uptake of non-invasive prenatal testing (NIPT) in the low-risk population.

Chen et al. *Matern Neonatal Med*. June 2018:1-4.

KEY POINTS

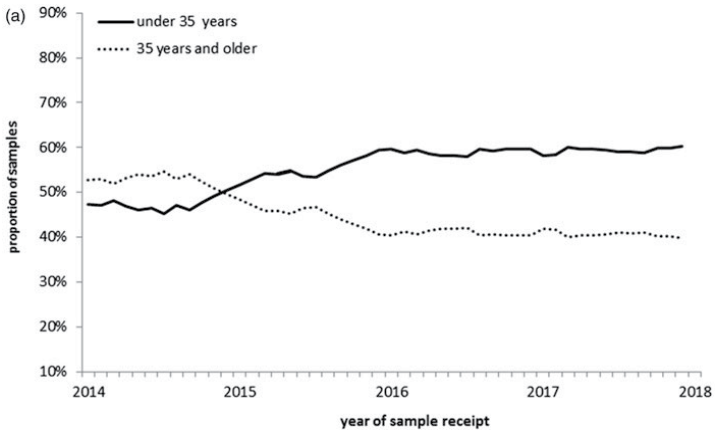
The proportion of samples submitted to the Ariosa laboratory by patients under 35 years has significantly increased in the 4-year subset, which represents the demographics of a diverse group of patients from across the globe. This suggests an increase in uptake of NIPT in the low-risk population.

METHODS

We performed a retrospective review of demographic information for all specimens submitted to the Ariosa Diagnostics clinical laboratory for the Harmony prenatal test between January 1, 2014 and December 30, 2017. Maternal age and country of origin were obtained from test requisition forms; given the limited clinical information provided to the laboratory, maternal age under 35 years was chosen as representative of a low-risk pregnancy.

RESULTS

Out of 903,789 specimens, the proportion of specimens submitted by patients under 35 years significantly increased across the study period, from 47.3% in 2014 to 60.3% in 2017. In December 2014, the proportion of specimens from patients under 35 reached 50%; this steadily increased to 60.3% by the end of 2017.



Proportion of samples by age group and time, US and outside of the US.

DISCUSSION

The group of samples in this study was received from over 65 countries and represents the demographics of a diverse group of patients. This study clearly demonstrates an international trend over a 4-year time period, with an increasing proportion of samples submitted by patients under 35 years of age. The data suggests an increase in use of NIPT in low-risk pregnancies.

These trends are likely due to many factors including the publication of clinical studies such as the NEXT study, the inclusion of NIPT in professional society guidelines, increased provider and public awareness across a broader segment of the pregnancy population, and a growing number of national and regional health plans supporting access to NIPT.

Screening for trisomies by cell-free DNA testing of maternal blood: consequences of failed result.

Revello et al. *Ultrasound Obstet Gynecol.* 2016 Jun;47(6):698-704. doi: 10.1002/uog.15851.

KEY POINTS

In trisomies 18 and 13, but not in trisomy 21, the fetal fraction is lower and the rate of failed cfDNA test is higher than in unaffected pregnancies. Consequently, pregnancies with a failed test can be considered as being at increased risk for trisomies 18 and 13, but not for trisomy 21.

Of the cases where a result was not obtained on first sample in the unaffected group, repeat testing provided a result for 62.8%. Management of pregnancies with failed cfDNA test should depend on any prior screening results and the results of a detailed ultrasound examination.

METHODS

This is a cohort study of 10,698 singleton pregnancies between 10-14 weeks gestation. Patients were offered the Harmony prenatal test either as an option following first trimester combined screening or as part of routine screening. If cfDNA testing did not provide a result, patients were offered repeat cfDNA testing, invasive testing or no further investigation. Similarly, the options for women with a second failed cfDNA test were invasive testing or no further investigation.

RESULTS

Results of cfDNA testing were provided after first sampling for 97.0% patients. The reasons for failure to provide a result were low fetal fraction in 219 (69.3%) cases and laboratory processing problems in 97 (30.7%) cases. There was a failed cfDNA result after first sampling in 2.9% (308/10 472) of cases in the unaffected group, in 1.9% (3/160) with trisomy 21, 8.0% (4/50) with trisomy 18 and 6.3% (1/16) with trisomy 13. Of the 308 cases where a result was not obtained on first sample in the unaffected group, 234 (76.0%) chose repeat cfDNA testing, which provided a result in 147 (62.8%) of the 234 cases. Of the 87 with failed second cfDNA, 7 (8.0%) had invasive testing and 80 (92.0%) opted for no further investigation. Decisions to have invasive testing for women with failed cfDNA results were largely based on results of the combined test and/or ultrasound findings.

DISCUSSION

The median fetal fraction was not significantly different in pregnancies with trisomy 21 than in unaffected pregnancies, but for trisomy 18 and trisomy 13, the fetal fraction was significantly reduced. The rate of failed result was similar in unaffected and trisomy 21 pregnancies, but was increased for trisomy 18 and trisomy 13 pregnancies. The main reason for test failure among those pregnancies with trisomy 18 and trisomy 13 was low fetal fraction. The risk of failed cfDNA test was affected by the same factors as those affecting fetal fraction: maternal age and body mass index, maternal serum level of free B-hCG and PAPP-A, racial origin (higher in women of South Asian racial origin than Caucasians), and method of conception. Options for management of pregnancies with failed first cfDNA test include repeat cfDNA testing, invasive testing, and no further investigation. Estimated risk for trisomies from the combined test and ultrasound findings should be taken into consideration when making management decisions.

PUBLICATIONS BY TOPIC

As of September 2018

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The Harmony Prenatal Test was developed by Ariosa Diagnostics, a CLIA-certified laboratory. As with other lab-developed tests, it has not been cleared or approved by the FDA and is not available for sales as an IVD in the U.S. Non-invasive prenatal testing (NIPT) based on cell-free DNA analysis is not diagnostic; results should be confirmed by diagnostic testing.

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Signature Page for MC-US-00844 v1.0

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Regulatory Approval	Beth Wolf Regulatory 09-Oct-2018 13:34:11 GMT+0000

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