

# Clinical Studies Abstract Booklet

The Harmony<sup>®</sup> 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.<sup>1-48</sup>

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



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# 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).

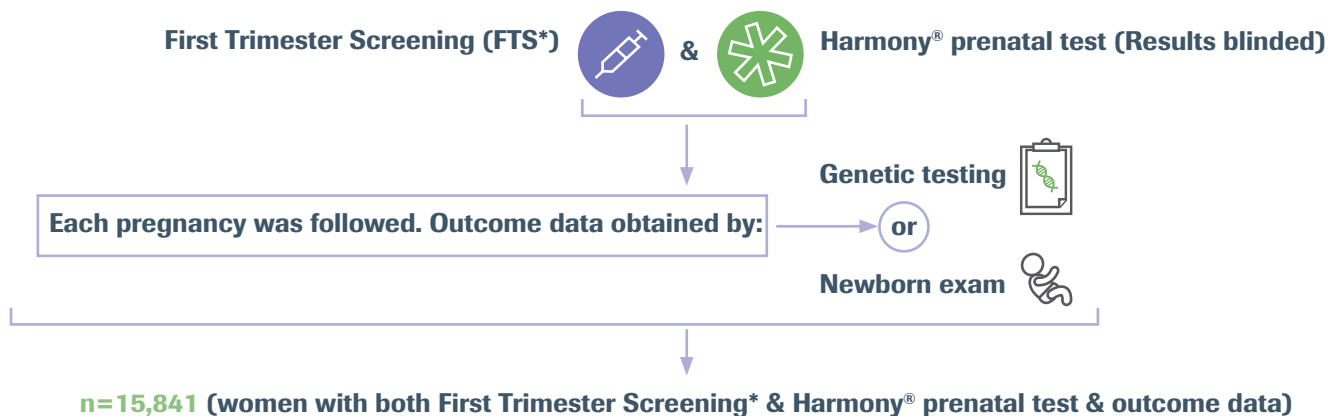
## SUMMARY AND KEY POINTS

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
<b>Detection Rate</b> (affected pregnancies correctly identified as high probability)	<b>79%</b> (30/38)	<b>100%</b> (38/38)	<b>0.008</b>
<b>False-Positive Rate</b> (unaffected pregnancies incorrectly identified as high probability)	<b>5.4%</b> (854/15,803)	<b>0.06%</b> (9/15,803)	<b>&lt;0.001</b>
<b>Positive Predictive Value (PPV)</b> (likelihood that a positive result is confirmed on diagnostic testing, based on false-positive rate and population frequency)	<b>3.4%</b>	<b>81%</b>	<b>&lt;0.001</b>

Sub-group analysis Harmony® in "low risk" patients	Less than 35 years old (n=11,994)	Screen negative on FTS (n=14,957)
<b>Sensitivity</b>	<b>100%</b> (19/19)	<b>100%</b> (8/8)
<b>False-Positive Rate</b>	<b>0.05%</b> (6/11,975)	<b>0.05%</b> (8/14,949)
<b>Positive Predictive Value</b>	<b>76%</b>	<b>50%</b>

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

Harmony® test performance is consistent in all risk categories

# 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.

	Sensitivity (n)	Specificity (n)
<b>Trisomy 21</b>	<b>99.3%</b> (421)	<b>99.96%</b> (22,734)
<b>Trisomy 18</b>	<b>97.4%</b> (151)	<b>99.98%</b> (22,248)
<b>Trisomy 13</b>	<b>93.8%</b> (32)	<b>99.98%</b> (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.

# 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

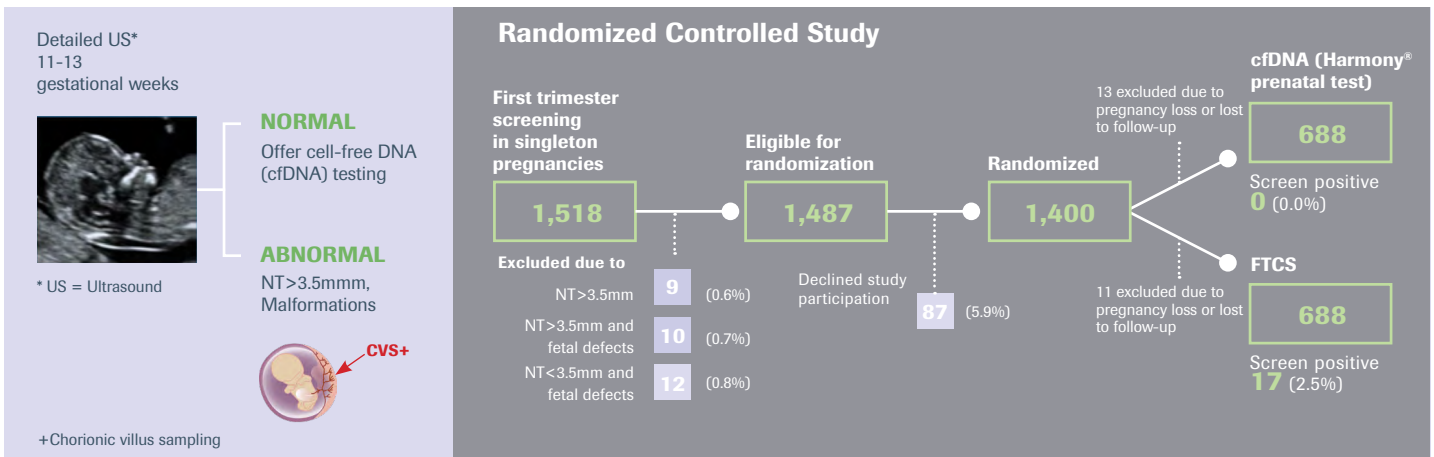
1,400 singleton pregnancies with normal first trimester ultrasound were randomized into two groups: FTCS or cfDNA screening.

FTCS includes: maternal and gestational age, fetal nuchal translucency (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

# 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.

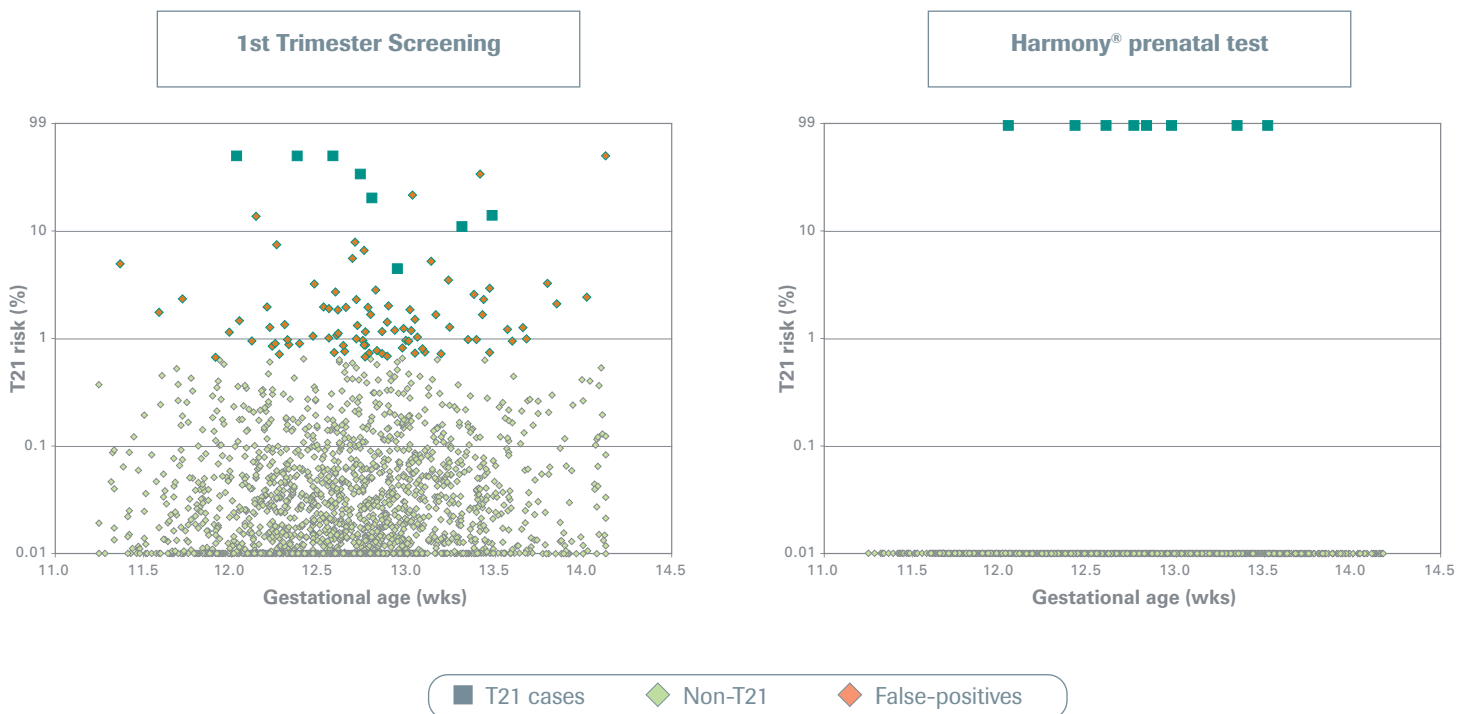
## SUMMARY AND KEY POINTS

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.



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

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

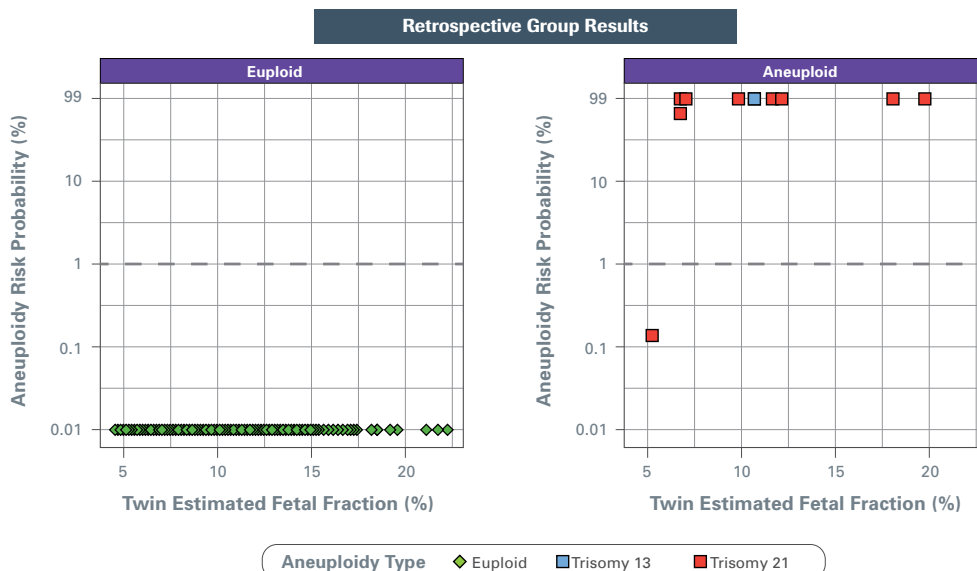
## RESULTS

### 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

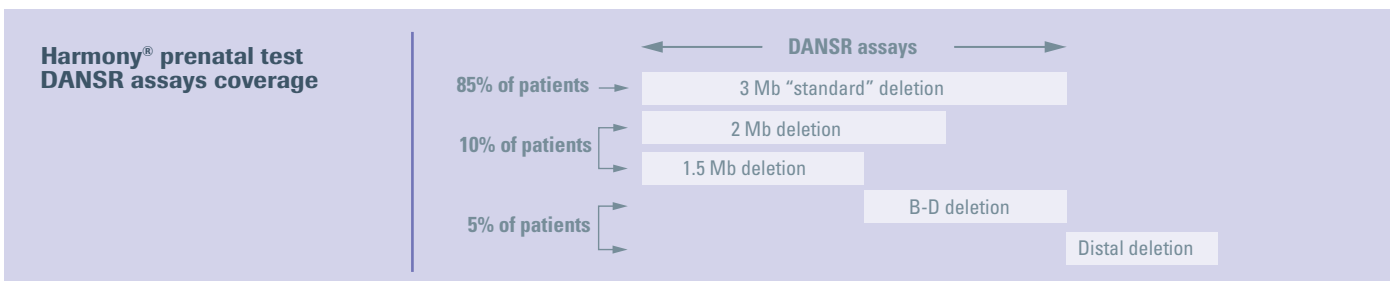
Two part-study (analytical validation and clinical verification) of 1953 plasma samples, 122 of which had confirmed deletions.

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

## SUMMARY AND KEY POINTS

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

**Result:** The Harmony<sup>®</sup> 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.



## 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<sup>®</sup> 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.

# 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).

Study	Fetal sex accuracy*		Sex chromosome aneuploidy						Disomy accuracy*
			45,X †		47,XXX †		47,XXY †		
	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 <sup>2</sup>	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 <sup>3</sup>	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

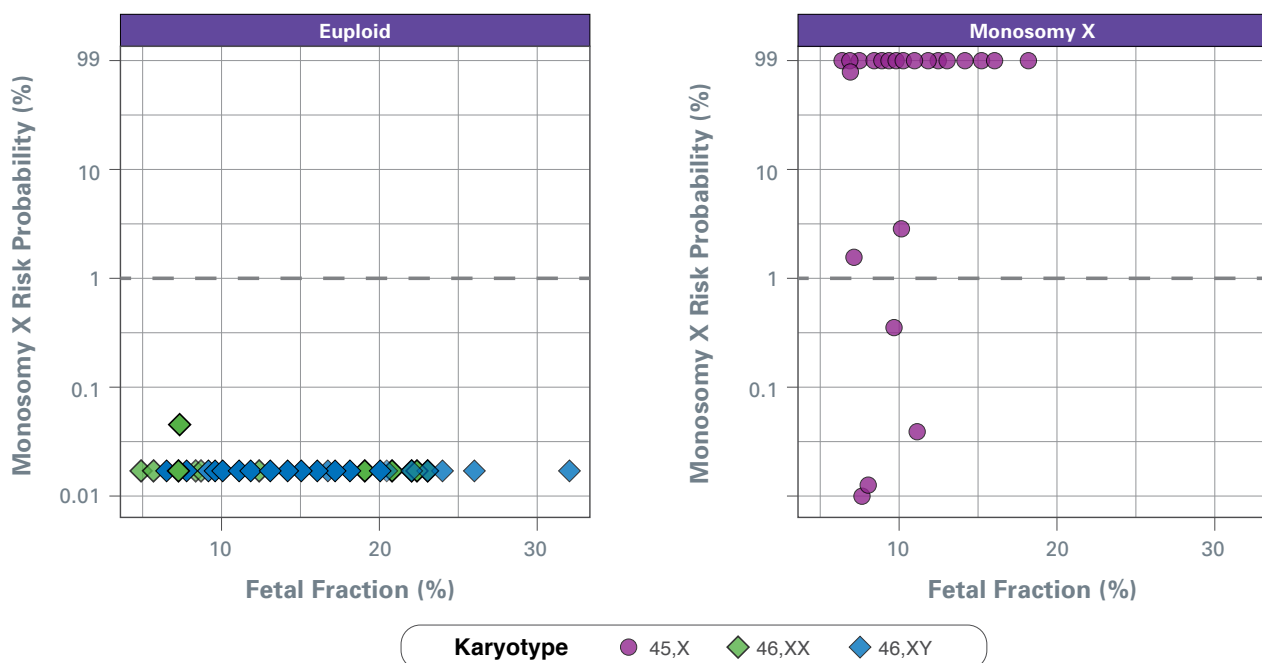
The objective of this study is to evaluate the performance of cfDNA analysis in the risk-assessment of fetal sex chromosome aneuploidies (SCAs).

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

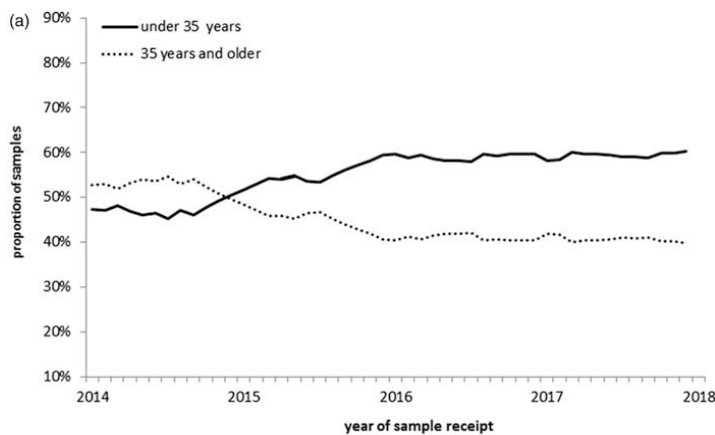
The proportion of samples submitted to the Ariosa laboratory by patients under 35 years has significantly increased in the 4-year subset, which represents the demographics of a diverse group of patients from across the globe. This suggests an increase in uptake of NIPT in the low-risk population.

## 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 December 2019

## CLINICAL EVIDENCE

- Ashoor et al. Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: effect of maternal and fetal factors. *Fetal Diagn Ther.* 2012;31(4):237–243.
- Ashoor et al. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* 2012;206(4):322.e1–5.
- Norton et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* 2012;207(2):137.e1–8.
- Sparks et al. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* 2012;206(4):319.e1–9.
- Sparks et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. *Prenat Diagn.* 2012;32(1):3–9.
- Ashoor et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method. *Ultrasound Obstet Gynecol.* 2013;41(1):21–25.
- Verweij et al. European non-invasive trisomy evaluation (EU-NITE) study: a multicenter prospective cohort study for non-invasive fetal trisomy 21 testing. *Prenat Diagn.* 2013;33(10):996–1001.
- Feenstra et al. Complexity of noninvasive prenatal screening and diagnostic testing for an unbalanced translocation involving chromosomes 5 and 18. *Prenatal Diagnosis* 34.2(2014):195–198.
- Juneau et al. Microarray-Based Cell-Free DNA Analysis Improves Noninvasive Prenatal Testing. *Fetal Diagn Ther.* 2014;36(4):282–286.
- Stokowski et al. Clinical performance of non-invasive prenatal testing (NIPT) using targeted cell-free DNA analysis in maternal plasma with microarrays or next generation sequencing (NGS) is consistent across multiple controlled clinical studies. *Prenat Diagn.* 2015 Dec;35(12):1243–6.
- Schmid et al. Prenatal Screening for 22q11.2 Deletion Using a Targeted Microarray-Based Cell-Free DNA Test. *Fetal Diagn Ther.* 2018;44(4):299–304.

## FETAL FRACTION

- Rolnik et al. Associated between fetal fraction on cell free DNA testing and first trimester markers for preeclampsia. *Ultrasound Obstet Gynecol* 2018 Jan 10.
- Ashoor G et al. Fetal fraction in maternal plasma cell-free DNA at 11–13 weeks' gestation: relation to maternal and fetal characteristics. *Ultrasound Obstet Gynecol* 2013;41(1):26–32.
- Brar H et al. The fetal fraction of cell-free DNA in maternal plasma is not affected by a priori risk of fetal trisomy. *J Matern Fetal Neonatal Med* 2013;26(2):143–45.
- Wang E et al. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma *Prenat Diagn.* 2013;33(7):662–6.
- Quezada MS et al. Fetal fraction of cell-free DNA in maternal plasma in the prediction of spontaneous preterm delivery. *Ultrasound Obstet Gynecol* 2015 Jan;45(1):101–5.
- Revello R et al. Screening for trisomies by cell-free DNA testing of maternal blood: consequences of failed result. *Ultrasound Obstet Gynecol.* 2016 Jun;47(6):698–704.
- Scott FP et al. Factors affecting cell free DNA fetal fraction and the consequences for test accuracy *J Matern Neonatal Med.* 2018 Jul;31(14):1865–1872.
- Schmid et al. Accuracy and reproducibility of fetal fraction measurement using relative quantitation at polymorphic loci with microarray. *Ultrasound Obstet Gynecol.* 2018 Jun;51(6):813–817.
- White et al. Factors associated with obtaining results on repeat cell-free DNA testing in samples redrawn due to insufficient fetal fraction *J Matern Neonatal Med.* March 2019:1–6
- Miltoft et al. Cell-Free Fetal DNA in the Early and Late First Trimester. *Fetal Diagn Ther.* 2019 Sep 18:1–9.

## TWIN PREGNANCIES

- Gil et al. Cell-Free DNA Analysis for Trisomy Risk Assessment in First-Trimester Twin Pregnancies. *Fetal Diagn Ther.* 2014;35(3):204-211
- Struble et al. Fetal Fraction Estimate in Twin Pregnancies Using Directed Cell-Free DNA Analysis. *Fetal Diagn Ther.* 2014;35(3):199-203.
- Bevilacqua et al. Performance of screening for aneuploidies by cell-free DNA analysis of maternal blood in twin pregnancies. *Ultrasound Obstet Gynecol.* 2015;45(1):61-66.
- Sarno et al. Prospective screening for trisomies by cell-free DNA testing of maternal blood in first trimester twin pregnancies. *Ultrasound Obstet Gynecol.* 2016;47(6):705-711.
- Galeva et al. Routine first-trimester screening for fetal trisomies in twin pregnancies: cell-free DNA test contingent on results from the combined test. *Ultrasound Obstet Gynecol.* 2019 Feb;53(2):208-213.
- Gil et al. Screening for trisomies by cfDNA testing of maternal blood in twin pregnancy: update of The Fetal Medicine Foundation results and meta-analysis. *Ultrasound Obstet Gynecol.* 2019 Jun;53(6):734-742.

## AVERAGE RISK

- Chen et al. Maternal age trends support uptake of non-invasive prenatal testing (NIPT) in the low-risk population. *J Matern Fetal Neonatal Med.* 2019 Dec;32(23):4039-4042.
- Nicolaidis et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol.* 2012;207(5):374 e1-6.
- Fairbrother et al. Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. *Prenat Diagn.* March 2013:1-5
- Gil et al. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. *Ultrasound Obstet Gynecol.* 2013;42(1):34-40.
- Norton et al. Cell-free DNA Analysis for Noninvasive Examination of Trisomy (NEXT). *N Engl J Med.* 2015;372(17):1589-1597.
- Comas et al. Initial Experience with Non-Invasive Prenatal Testing of Cell-Free DNA for Major Chromosome Anomalies in a Clinical Setting. *J Matern Fetal Neonatal Med.* 2015;28(10):1196-1201.
- Kagan et al. First trimester screening based on ultrasound and cfDNA vs. first-trimester combined screening - a randomized controlled study. *Ultrasound Obstet Gynecol.* 2018 Apr;51(4):437-444.
- Langlois et al. Comparison of first tier cell-free DNA screening for common aneuploidies with conventional publicly funded screening. *Prenat Diagn.* 2017 37(12):1238-1244.

## CONTINGENT SCREENING

- Gil et al. UK NHS pilot study on cell-free DNA testing in screening for fetal trisomies: factors affecting uptake *Ultrasound Obstet Gynecol.* 2015;45(1):67-73.
- Quezada et al. Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks gestation and the combined test at 11-13 week. *Ultrasound Obstet Gynecol.* 2015;45(1):36-41.
- Gil et al. Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test. *Ultrasound Obstet Gynecol.* 2016;47(1):45-52.
- Gil et al. Screening for trisomies 21 and 18 in a Spanish public hospital: from the combined test to the cell-free DNA test. *J Matern Neonatal Med.* November 2016:1-7.
- McLennan et al. Noninvasive prenatal testing in routine clinical practice - An audit of NIPT and combined first-trimester screening in an unselected Australian population. *Aust N Z J Obstet Gynaecol.* 2016;56(1):22-28.
- Miltoft et al. Contingent first-trimester screening for aneuploidies with cell-free DNA in a Danish clinical setting. *Ultrasound Obstet Gynecol.* 2018 Apr;51(4):470-479..

## SEX CHROMOSOME ANEUPLOIDY

- Richardson et al. Sex discordance identification following non-invasive prenatal testing. *Prenat Diagn.* 2017 Dec;37(13):1298-1304..
- Hooks et al. Non-invasive risk assessment of fetal sex chromosome aneuploidy through directed analysis and incorporation of fetal fraction. *Prenat Diagn.* 2014;34(5):496-499.
- Nicolaidis et al. Assessment of Fetal Sex Chromosome Aneuploidy Using Directed Cell-Free DNA Analysis. *Fetal Diagn Ther.* 2014;35(1):1-6.
- Kagan et al. Discordance between ultrasound and cell free DNA screening for monosomy X Arch. *Gynecol Obstet.* 2016;294(2):219-224.
- Bevilacqua et al. Screening for Sex Chromosome Aneuploidy by Cell-Free DNA Testing: Patient Choice and Performance. *Fetal Diagn Ther.* 2018;44(2):98-104.
- Korrmann et al. Non-invasive Prenatal Testing for Sex Chromosome Aneuploidy in Routine Clinical Practice. *Fetal Diagn Ther.* 2018;44:(2):85-90.
- Jones et al. Performance of targeted cell free DNA (cfDNA) analysis with microarray quantitation for assessment of fetal sex and sex chromosome aneuploidy risk. *Ultrasound Obstet Gynecol.* 2018 51:274-277.

## IMPLEMENTATION & UTILIZATION

- Musci et al. Non-invasive prenatal testing with cell-free DNA: US physician attitudes toward implementation in clinical practice. *Prenat Diagn.* 2013 May;33(5):424-8.
- Song et al. Clinical utility and cost of non-invasive prenatal testing with cfDNA analysis in high-risk women based on a US population. *J Matern Fetal Neonatal Med.* 2013 Aug;26(12):1180-5.
- Willems et al. The first 3,000 Non-Invasive Prenatal Tests (NIPT) with the Harmony test in Belgium and the Netherlands. *Facts Views Vis Obgyn.* 2014;6(1):7-12.
- Wallerstein et al. A New Model for providing cfDNA and risk assessment in a public hospital setting. *Journal of Pregnancy* Volume 2014, Article ID 962720, 7 pages, 2014.
- Hernández-Gómez et al. Non invasive prenatal test (NIPT) in maternal blood by parallel massive sequencing. Initial experience in Mexican women and literature review. *Ginecol Obstet Mex.* 2015;83(5):277-288.
- Brewer et al. Survey of US Obstetrician Opinions Regarding NIPT Use in General Practice: Implementation and Barriers. *J Matern Neonatal Med.* 2016;7058(August):1-13.
- Chen et al. Women's choices for invasive or non-invasive testing: Influence of gestational age and service delivery. *Prenat Diagn.* 2016 Dec;36(13):1217-1224.
- Fairbrother et al. Prenatal screening for fetal aneuploidies with cell-free DNA in the general pregnancy population: a cost-effectiveness analysis. *J Matern Fetal Neonatal Med.* 2016;29(7):1160-1164.
- Bjerregaard et al. The rate of invasive testing for trisomy 21 is reduced after implementation of NIPT. *Dan Med J.* 2017 Apr;64(4):pii: A5359.
- Kagan et al. False-Positive Rate in First-Trimester Screening Based on Ultrasound and Cell-Free DNA versus First-Trimester Combined Screening with Additional Ultrasound Markers *Fetal Diagn Ther.* 2019;45(5):317-324.
- Bevilacqua et al. Cell-Free DNA Analysis in Maternal Blood: Differences in Estimates between Laboratories with Different Methodologies Using a Propensity Score Approach. *Fetal Diagn Ther.* 2018 J2019;45(5)302-311.
- Chan et al. Implications of failure to achieve a result from prenatal maternal serum cell-free DNA testing: a historical cohort study. *BJOG.* 2018 Jun;125(7):848-855.
- Lee et al. Cell-free fetal DNA testing in singleton IVF conceptions. *Hum Reprod.* 2019;45(5):302-311.
- Bevilacqua et al. Profile of women choosing the Harmony Prenatal Test. *Expert Rev Mol Diagn.* 2018 Jul;18(7):591-599.
- Kostento et al. Clinical and Economic Impact of Adopting Noninvasive Prenatal Testing as a Primary Screening Method for Fetal Aneuploidies in the General Pregnancy Population. *Fetal Diagn Ther.* 2019;45(6):413-423.
- Grati et al. Noninvasive screening by cell-free DNA for 22q11.2 deletion: Benefits, limitations, and challenges. *Prenat Diagn.* 2019 Jan;39(2):70-80.
- Bevilacqua et al. Cell-free DNA analysis after reduction in multifetal pregnancies. *Ultrasound Obstet Gynecol.* 2019 Jun 10. doi: 10.1002/uog.20366.
- Galeva et al. First-trimester screening for trisomies by cfDNA testing of maternal blood in singleton and twin pregnancies: factors affecting test failure. *Ultrasound Obstet Gynecol.* 2019 Jun;53(6):804-809.
- de Wergifosse et al. Cell-free DNA analysis in maternal blood: comparing genome-wide versus targeted approach as a first-line screening test. *J Matern Fetal Neonatal Med.* 2019 Nov 13:1-10.
- Kagen et al. False-positive rates in screening for trisomies 18 and 13: a comparison between first-trimester combined screening and a cfDNA-based approach. *Arch Gynecol Obstet.* 2019 Feb;299(2):431-437.

