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Noninvasive Prenatal Testing for Fetal Aneuploidy

Powered by Natera's Constellation

Results to date as of November 2016

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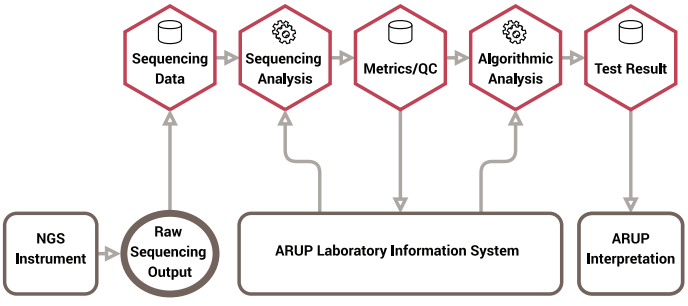
ARUP *is a nonprofit enterprise of the University of Utah and its Department of Pathology.*

EXECUTIVE SUMMARY

Since 2012, ARUP has offered non-invasive prenatal testing (NIPT) to its clients through a commercial agreement with Natera. Highly-multiplexed single-nucleotide polymorphism (SNP) analysis using massively parallel sequencing allowed for an NIPT with the highest clinical performance (1, 2, 3), the lowest false-negative rate (0.6%), one of the lowest false-positive rates (0.03%), complete accuracy in fetal gender prediction, and the ability to test as early as nine weeks of pregnancy. After scientific consultation with Natera, ARUP independently designed, developed, and validated NIPT at its own laboratory by integration with Natera's Constellation (a cloud-based general-purpose copy number analysis software) which facilitates the analysis and interpretation of data by ARUP medical directors and genetic counselors. ARUP's NIPT has a high degree of reproducibility, and excellent concordance with both Panorama (NIPT performed at Natera) and with known clinical outcomes. Over 200 samples were used to establish 100% concordance with Panorama (detected 55 out of 55 high risk, 146 of 146 low risk), and over 80 samples were used to establish 98% concordance with known clinical outcomes (detected 25 of 25 high risk, 58 of 59 low risk).

HOW ARUP'S NIPT WORKS

ARUP's NIPT assay is performed at ARUP's genomic laboratory in Salt Lake City, UT. Massively parallel sequencing data generated on targeted SNP regions are uploaded to the cloud where Natera's copy number calculator (CNC) algorithm within the Constellation platform de-multiplexes the data, calculates the QC metrics, and analyzes the SNP allele frequencies to provide Bayesian probabilities against copy number hypotheses. QC metrics are reviewed by ARUP staff against thresholds that were predetermined by ARUP, and copy number probabilities are interpreted to report the risk for chromosomal aneuploidies in placental cell-free DNA (against the background of maternal cell-free DNA). The current assay analyzes whole chromosome copy numbers in three autosomes (chromosomes 13, 18 and 21), and in the sex chromosomes, allowing the assessment of fetal sex in addition to the assessment of risk for aneuploidies.



The services performed by Constellation are shown in red hexagons.

Reportable elements in ARUP's NIPT are listed below:

What is reported in ARUP's NIPT
Risks for:
Trisomy 13, trisomy 18, trisomy 21 (Down syndrome), monosomy X (Turner syndrome), triploidy, XXX, XXY, and XYY.
Fetal fraction (%)
Fetal sex (unless opted out)

VALIDATION DATA

ARUP's validation strategy adheres to the guidelines for massively parallel sequencing from the College of American Pathologists (4), and the Clinical and Laboratory Standards Institute (5). The validation study included whole blood and plasma samples from pregnant and non-pregnant women, and whole genome amplified (WGA) samples from previously processed specimens. Plasma samples were obtained from patients with confirmed clinical outcome of the fetus by karyotyping (amniocentesis or chorionic villus sampling). The sample sets were enriched for aneuploidies to cover the entire spectrum of reportable results, and constituted a good representation of early to late gestational age, fetal fraction, and fetal sex distributions that will be encountered in NIPT.

STUDY 1: TECHNICAL PRECISION

Precision of ARUP's NIPT was assessed by duplicate or triplicate draws of fresh whole blood (four or six tubes per patient), as well as triplicate testing of WGA samples. Testing was performed using two sequencers and five operators. Interpretation was performed by three genetic counselors, and four medical directors who were blinded to the results of

Intra-assay Repeatability	
Triplicates (whole blood)	16 sets
Fetal fraction range	0.072~ 0.291
Mean fetal fraction SD	± 0.002
Mean fetal fraction CV	1.2%
Copy number assessment concordance (chromosomes 13, 18, 21, X, Y)	100% (112 out of 112 assessments)

Inter-assay Reproducibility	
Duplicates (whole blood)	24 sets
Fetal fraction range	<0.005~ 0.369
Fetal fraction SD	± 0.003
Fetal fraction CV	3.2%
Triplicates (WGA)	24 sets
Fetal fraction range	0.040 ~ 0.201
Mean fetal fraction SD	± 0.0008
Mean fetal fraction CV	0.8%
Copy number assessment concordance (chromosomes 13, 18, 21, X, Y)	100% (336 out of 336 assessments)

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other replicates. Intra-assay repeatability was assessed by 16 sets of triplicates (48 samples), and inter-assay reproducibility was assessed by 24 sets of duplicates and 24 sets of triplicates (120 samples). Precision was established using fetal fraction and concordance of copy number interpretations. Barring QC failures and fetal fractions below the performance limits of the algorithm (2.8%), the minimum confidence threshold was 0.98 for all chromosomal regions for a "High Risk" call and 0.85 for a "Low Risk" call. The majority of specimens had a confidence of >0.99 across all chromosomal regions tested. Overall, fetal fraction had a standard deviation (SD) of 0.003 or less, and a coefficient of variation (CV) of less than 4%. Fourteen specimens were from non-pregnant females in which the fetal fractions were correctly reported as being below the limit of detection for fetal DNA (0.01). Repeatability of copy number interpretation was compared for seven elements: i.e., likelihood of high risk for trisomy 13, trisomy 18, trisomy 21, monosomy X, sex chromosome trisomies, triploidy, and the assessment of fetal sex. Complete concordance was attained for a total of 448 assessments performed in duplicate or triplicate.

STUDY 2: AGREEMENT WITH PANORAMA

Agreement with Panorama (Natera) results were assessed by the use of WGA samples generated from specimens previously tested by Natera and whole blood samples that were tested at both laboratories. Similar to the precision study, multiple instruments, operators, genetic counselors and medical directors were used for testing and interpretation of blinded samples. Fetal fraction agreement with Natera's Panorama test was assessed using 111 samples with values ranging from 0.036 to 0.315. Variance from the Natera values ranged from -0.002 to +0.028 with a mean variance of +0.001, which was considered excellent agreement. For fetal sex assessment, 99 samples (male = 50; female = 49) were in complete concordance between ARUP and Natera. The remaining samples had no gender designations from Natera, either because the subjects did not wish to know the fetal sex, or the result voided the gender assignment (such as in the case of suspected twins). Agreement in copy number was assessed using 201 blinded specimens and complete concordance between ARUP and Natera was achieved. While outside of the performance claims of this assay, four confirmed egg donor cases were correctly identified as such. Additionally, one sample did not meet the minimum confidence threshold of 0.98 for trisomy 21 (0.96) resulting in a report of a "No call". In situations like this, provided all other parameters pass the quality thresholds, ARUP will add a "suspected T21" comment to the report recommending confirmatory testing rather than repeating the NIPT screen.

STUDY 3: CLINICAL PERFORMANCE

Blinded plasma specimens from 84 pregnant females were tested for copy number assessment. Each fetus had confirmatory diagnosis (karyotyping) for chromosomal aneuploidy. All 25 chromosomal aneuploidies were correctly called. Of the 59 normal samples, one sample, which was 46,XY (normal male) by karyotype on amniocytes, exhibited a distinct pattern of XXY, suggesting a possible difference between the placental DNA found in plasma and the actual fetal DNA. Independent review of the case by Natera supported the XXY call. Four egg donor cases from Study 2 were included in this

Agreement with Reference Laboratory (Natera, Inc., Panorama)

Sample type	Positive Percent Agreement n/N (%; 95% CI)	Negative Percent Agreement n/N (%; 95% CI)
Combined aneuploidy	55/55 (100%; 93.5–100)	146/146 (100%; 97.5–100)
Trisomy 13	5/5 (100%; 47.8–100)	196/196 (100%; 98.1–100)
Trisomy 18	9/9 (100%; 66.4–100)	192/192 (100%; 98.1–100)
Trisomy 21	18/18 (100%; 81.5–100)	183/183 (100%; 98.0–100)
Monosomy X	8/8 (100%; 63.1–100)	193/193 (100%; 98.1–100)
Sex chromosome trisomy (XXX, XXY, XYY)	11/11[a] (100%; 71.5–100)	190/190 (100%; 98.1–100)
Triploidy or twin [b]	4/4 (100%; 39.8–100)	197/197 (100%; 98.1–100)
Female	49/49 (100%; 92.8–100)	50/50 (100%; 92.9–100)
Male	50/50 (100%; 92.9–100)	49/49 (100%; 92.8–100)
Other [c]	4/4 (100%; 39.8–100)	201/201 (100%; 98.2–100)
No call [d]	1	NA

a. Three cases of XXX, four cases of XXY, and four cases of XYY

b. All four cases were confirmed twin or vanishing twin cases

c. Four confirmed egg donors were correctly flagged

d. Low confidence call on T21 due to borderline fetal fraction

NA: not applicable; n is the number of cases from this study; N is the number of cases from the reference laboratory (Natera Inc.)

Concordance with Clinical Outcome

Sample type	Sensitivity, n/N (%; 95% CI)	Specificity, n/N (%; 95% CI)
Combined aneuploidy	25/25 (100%; 86.3–100)	58/59 (98.3%; 90.9–100)
Trisomy 13	1/1 (100%; 2.5–100)	83/83 (100%; 95.7–100)
Trisomy 18	3/3 (100%; 29.2–100)	81/81 (100%; 95.6–100)
Trisomy 21	11/11 (100%; 71.5–100)	73/73 (100%; 95.1–100)
Monosomy X	1/1 (100%; 2.5–100)	83/83 (100%; 95.7–100)
Sex chromosome trisomy (XXX, XXY, XYY)	0/0 NA	83[a]/84 (98.8%; 93.5–100)
Triploidy or twin [b]	9/9 (100%; 66.4–100)	75/75 (100%; 95.2–100)
Female	41/41 (100%; 91.4–100)	32/32 (100%; 89.1–100)
Male	32/32 (100%; 89.1–100)	41/41 (100%; 91.4–100)
Other [c]	4/5 (80%; 28.4–99.5)	84/84 (100%; 95.7–100)
No call [d]	0	NA

a. A low risk male fetus (46,XY), confirmed by karyotype on amniocytes, exhibited a distinct pattern of XXY suggesting a possible difference between placental DNA found in plasma and the actual fetal DNA. Fetal fraction was 12%. Independent review of this case by reference laboratory (Natera) agreed with the XXY call.

b. Four confirmed egg donors were correctly flagged; one low level mosaic T21 (47,XY,+21[3]/46,XY[18]) was classified as low risk. Both situations are outside the performance claims of this assay.

NA: not applicable; n is the number of cases from this study; N is the number of cases with clinical outcome

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list as they all had confirmed clinical outcomes. One sample had fetal mosaicism for trisomy 21 (3 of 21 cells demonstrated trisomy 21). This was not detected and reported as a normal low risk sample for trisomy 21. The detection of mosaicism is outside the scope of this assay.

CONCLUSION

This blinded validation study of over 430 samples demonstrated that ARUP's NIPT is highly precise, and concordant with Natera's Panorama test. For clinical performance to detect fetal aneuploidy, ARUP's NIPT was 100% sensitive (95% confidence interval 86.3% – 100%) and 98% specific (95% confidence interval 90.9% - 100%) based on 84 samples enriched for aneuploidy. For fetal sex, ARUP's NIPT was 100% accurate based on 73 samples that had known fetal genders. While outside of the scope of the test, ARUP's NIPT was able to accurately determine subjects that carried no fetus (not pregnant). It was also able to detect pregnancies from an egg donor (mismatch between maternal and fetal alleles). In conclusion, the performance of ARUP's NIPT is substantially equivalent to that of Natera's Panorama NIPT and has the clinical performance that satisfies the needs for this important screening test.

This study was conducted under University of Utah IRBs #7740 and #30110 and Intermountain Healthcare IRBs #1013818 and #1010437.

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