Whole-Genome Sequencing Provides the Most Informative Noninvasive Prenatal Testing Results

A comparative analysis of commercially available methods for noninvasive prenatal tests.

Introduction

Noninvasive prenatal testing (NIPT) represents a major advance in prenatal screening, providing accurate information about fetal status as early as 10 weeks gestation using a single maternal blood draw. With NIPT, pregnant women can now screen for particular fetal chromosomal abnormalities, such as Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), Patau syndrome (trisomy 13), and sex chromosome–related syndromes such as Klinefelter and Turner syndromes.

Currently, next-generation sequencing (NGS) is the only proven method for performing NIPT. In fact, 99.8% of NIPT samples in published studies are run on Illumina systems with Illumina NGS technology (Table 1). In addition, the Illumina method for NIPT yields results faster than other tests. This white paper provides a comparison of the test failure rates and sample-to-answer times among NIPT methods currently available.

Table 1: 99.8% of NIPT Samples Run on Illumina NGS Systems

Test (Company)	Current Clinical NIPT Method	No. Published NIPT Samples
Bambni Assay (Berry Genomics)	Illumina NGS	2351
MaterniT21 PLUS Test (Sequenom)	Illumina NGS	108,665
NIFTY Test (BGI)	Illumina NGS	160,667
Panorama Prenatal Screen (Natera)	Illumina NGS	32,916
PrenaTest (LifeCodexx AG/GATC Biotech AG)	Illumina NGS	504
verifi Prenatal Test (Illumina)	Illumina NGS	113,367
Harmony Prenatal Test (Ariosa)[a]	Illumina NGS	37,206
Harmony Prenatal Test (Ariosa)[a]	Affymetrix Array	878

A PubMed search for "cell-free, DNA, prenatal", "noninvasive prenatal testing", and "noninvasive prenatal screening" was performed on April 30, 2015. All validation and clinical studies using unique samples were included, where a current clinical NIPT provider performed sample analysis. Case studies and studies published in a language other than English were excluded. Data from a 2015 ESHG conference abstract was also included. A total of 45 published studies were surveyed. Data calculations on file. Illumina, Inc. 2015. NGS = next-generation sequencing; either whole-genome or targeted.

a. In 2014, Ariosa switched from sequencing to arrays for clinical samples despite limited published data on this platform.

Background

Cell-Free Fetal DNA

Circulating cell-free DNA (cfDNA), from both the fetus and the mother, is found in maternal blood. On average, ~11% of the cfDNA circulating in maternal blood is from the fetus. Highly sensitive NGS, which uses millions of sequence reads per sample, can detect and measure aneuploidy within this mixed sample. Quantitative differences in cfDNA in maternal blood can be used to distinguish fetuses affected with trisomy 21 (and other fetal aneuploidies) from those that are unaffected.

In clinical trials in the general obstetrical population, prenatal screening by cfDNA has been demonstrated to have significantly lower false positive rates and higher positive predictive values for trisomies 21 and 18 than standard screening.[1]

NIPT Methods

Several options for NIPT are currently available (Table 2). Although all these tests enable screening for chromosomal abnormalities, not all provide the same level of accuracy (Figure 1). Studies have shown that NIPT performed using whole-genome sequencing (WGS) with next-generation sequencing (NGS), like the verifi® Prenatal Test from Illumina, offers an accurate, reliable, quick screen for chromosomal abnormalities.[2,3] High-sensitivity, high-specificity WGS-based NIPT results in low false-positive and false-negative rates and maintains a low test failure rate, minimizing the need for invasive testing procedures, such as amniocentesis and chorionic villus sampling (CVS).[1] Like WGS-based tests, NIPT using targeted sequencing, such as the Panorama Prenatal Screen from Natera, offers high sensitivity and specificity. However, these targeted sequencing tests have a higher failure rate than WGS tests. Sequenom, a provider of NIPT assays, recently evaluated the use of a targeted sequencing approach, only to find that acceptable performance comes at the price of a high "no call" rate.[4] Array-based NIPT, such as the Harmony Prenatal Test from Ariosa, is predicted to have reduced overall performance and higher failure rates than other methods. Both Natera and Ariosa have changed their workflows recently, with Ariosa switching from sequencing to arrays, resulting in a decline in test sensitivity and reduced performance for certain conditions.[5,6]

Table 2: NIPT Methods

Method	Description	Tests
Whole-Genome Sequencing (WGS)	Sequencing of the full fetal genome; provides comprehensive view of the chromosomes	• verifi Prenatal Test (Illumina) • PrenaTest (LifeCodexx) • NIFTY Test (BGI) • MaterniT21 PLUS Test (Sequenom) • Bambni Assay (Berry Genomics)
Targeted Sequencing	Sequencing of chromosome regions	• Harmony Prenatal Test (Ariosa, former version)
Targeted SNP Sequencing	Sequencing of a subset of SNPs	• Panorama Prenatal Screen (Natera)
SNP Arrays	Microarray analysis of preselected SNPs	• Harmony Prenatal Test (Ariosa, current version)

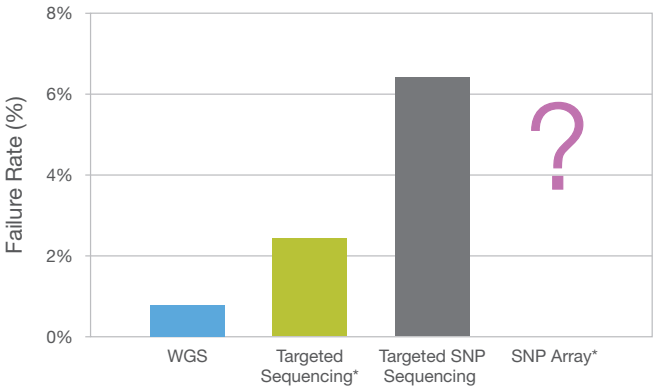

Figure 1: Lowest Test Failure Rates Observed Using WGS-Based NIPT—Data compiled from published clinical studies. WGS[3,10–15], targeted sequencing[16-22], targeted SNP sequencing[9,20].
* Published clinical studies for the Harmony test are based on targeted sequencing; however, this test is now run using SNP arrays. There are no publications of studies assessing failure rate for NIPT assays performed using SNP arrays

Test Failure Rates

Test failures, wherein no call for euploidy or aneuploidy can be made, are an important factor in the reliability and clinical utility of NIPT. NIPT test failure rates vary significantly based on the test used. Tests that use a targeted approach have demonstrated higher rates of test failure than WGS-based tests, in both validation and clinical experience studies (Figure 2). These "no call" results likely lead to invasive procedures that may have been avoided with a more reliable test.

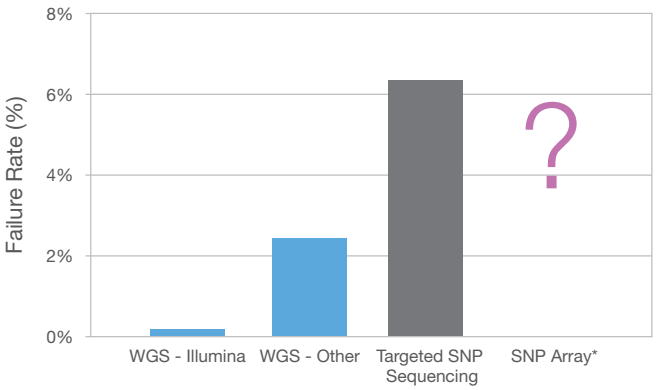

Figure 2: Technical Failure Rates in NIPT Clinical Experience Studies—Data compiled from published clinical studies. WGS - Illumina[3,13,14], WGS - other[14,15], targeted SNP sequencing[9,20].
* There are no publications of studies assessing failure rate for NIPT assays performed using SNP arrays.

This difference in test failure rates may be due to inherent biases in test design. Targeted sequencing and array-based methods tend to have longer laboratory protocols and employ more rounds of PCR than WGS methods, introducing potential sources of error and increasing bias. In addition, these targeted approaches focus on specific regions of the genome, but the design itself may not provide sufficient coverage to capture all variations. This lack of coverage may be further exacerbated when working with more challenging samples, such as those with a low percent of fetal fraction. In these cases, the information is not distinct enough for the test to provide a reliable result one way or the other, aneuploidy or euploidy. Results are deemed uninterpretable and the test is noted as failing.

WGS assays provide a comprehensive view of genomic material. This provides ample data across the entire genome, effectively removing any biases introduced by common molecular techniques, such as PCR. Having coverage data available across the entire diploid genome produces an analytical reference that current analytical techniques can use to reduce assay- and sample-specific biases. These normalization steps lead to high sensitivity when working with low fetal fraction samples, which means correct aneuploidy calls can be made in the range of fetal fractions that typically requires QC rejection when using targeted approaches.[7]

Higher Aneuploidy Rates in Failed Tests

An additional concern related to test failure rate is the high failure rate of aneuploid samples with non-WGS methods (Figure 3). According to the Society for Maternal-Fetal Medicine (SMFM), "women with failed cfDNA tests are at an increased risk for aneuploidy, and therefore need careful counseling about further testing, including the offer of diagnostic testing."[8] With a higher level of sensitivity, WGS-based assays are more likely to detect these aneuploidies in the first test. In addition to providing answers earlier in the pregnancy, this avoids unnecessary invasive procedures.

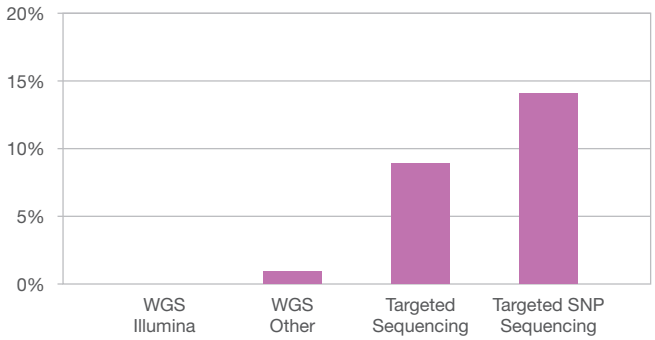

Figure 3: Percent of T21/T18/T13 (Combined) Aneuploidy Cases Missed Due to Technical Failure—Data compiled from published validation and clinical studies. References: Illumina[2], Sequenom[23,24], Ariosa[21,25,28], Natera[26,27].

Patient Impact of Test Failure

Test failure leads to increased anxiety on the part of the patient and the physician, and can potentially lead to increased unnecessary invasive procedures. As test failure is really an inconclusive result, the ordering physician has no information to share with the patient. "A failed test result causes a ripple effect, not only for the patient but also to the entire team helping take care of her and her pregnancy. It brings up anxiety and doubt for the patient and creates confusion as to what is the next step. More clinical evidence is building that a failed test should be considered a red flag and careful consideration given as to what is the best next option. We owe it to our patients and profession to consider how we can minimize these test failures and safely maximize the information we provide to our patients," states Dr. Martin Chavez, Chief of Maternal-Fetal Medicine at Winthrop-University Hospital.

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Although ordering a second blood draw to repeat NIPT is an option, there are no guarantees that repeated NIPT will provide a definitive answer. In fact, 65%* of patients with a first draw failure do not achieve resolution.[9] Another option is to perform a risky invasive procedure to obtain a definitive answer regarding chromosome number. In addition to the mental toll on patients and unnecessary inconvenience of additional testing, the patient is now further along in the pregnancy, reducing clinical management options.

Faster Results Using WGS

In addition to lower test failure rates, WGS-based NIPT is faster than other methods (Figure 4). The verifi Prenatal Test, a WGS test from Illumina, yields results in as little as 3 days. Targeted sequencing tests take 7-9 days, on average, to go from sample to answer.[9]

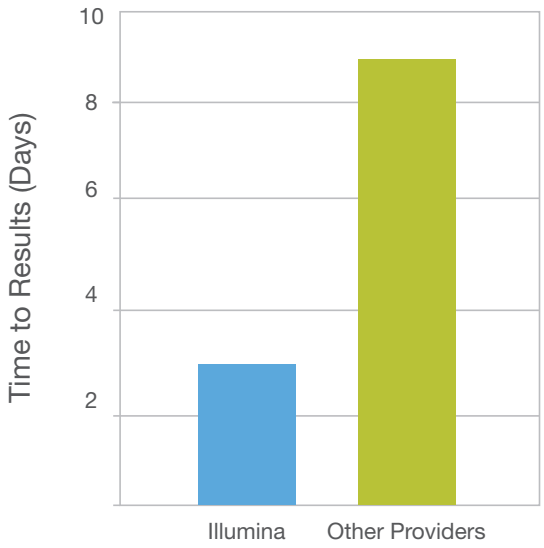

Figure 4: Sample-to-Answer Time for NIPT Assays—The verifi Prenatal Test, a WGS test from Illumina, yields results in as little as 3 days while NIPT assays from other providers can take up to 9 days.

Summary

WGS is a viable option for NIPT with distinct advantages over other methods, including significantly lower test failure rates, lower rates of missed aneuploidy, and a faster time to answer. To learn more about the WGS-based verifi Prenatal Test, visit www.verifitest.com.

* This 65% includes test failures from redraws and patients that either choose not to submit a second sample or are ineligible for a redraw due to specific features that prevent resolution with SNP-based NIPT (ie, large regions exhibiting loss of heterozygosity (LOH)).

References

1. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014;370(9):799-808.
2. Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol.* 2012;119(5):890-901.
3. Futch T, Spinosa J, Bhatt S, de Feo E, Rava RP, Sehnert AJ. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn*. 2013;33(6):569-574.
4. Jensen T. Noninvasive prenatal detection of selected fetal aneuploidies using targeted sequencing of homologs. Presented at: 2nd International Meeting on Cell-Free DNA;May 29, 2015;Copenhagen, Denmark.
5. Rosenman H. Natera, Inc. Form S-1. United States Securities and Exchange Commision web site. http://www.sec.gov/Archives/edgar/data/1604821/000104746915005154/a2224899zs-1.htm. Accessed June 4, 2015.
6. Musci T. Microarray-based cell-free DNA analysis for aneuploidy risk assessment. Presented at: 2nd International Meeting on Cell-Free DNA;May 29, 2015;Copenhagen, Denmark.
7. Rava RP, Srinivasan A, Sehnert AJ, Bianchi DW. Circulating fetal cell-free DNA fractions differ in autosomal aneuploidies and monosomy X. *Clin Chem.* 2014;60(1):243-250.
8. Cell-Free DNA Screening - Publications. Society for Maternal-Fetal Medicine. https://www.smfm.org/publications/193-cell-free-dna-screening. Accessed June 5, 2015.
9. Dar P, Curnow KJ, Gross SJ, et al. Clinical experience and follow-up with large scale single-nucleotide polymorphism-based non-invasive prenatal aneuploidy testing. *Am J Obstet Gynecol.* 2014;211(5):527.e521–517.
10. Dan S, Wang W, Ren J, et al. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. *Prenat Diagn*. 2012;32(13):1225-1232.
11. Lau TK, Cheung SW, Lo PS, et al. Non-invasive prenatal testing for fetal chromosomal abnormalities by low-coverage whole-genome sequencing of maternal plasma DNA: review of 1982 consecutive cases in a single center. *Ultrasound Obstet Gynecol*. 2014;43(3):254-264.
12. Zhang H, Gao Y, Jiang F, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies. *Ultrasound Obstet Gynecol*. 2015;45(5):530-538.
13. Taneja P, Snyder H, de Feo E, et al. Utilization of Noninvasive Prenatal Screening and its Relevance to Clinical Practice: Update on Clinical Outcome Metrics on Over 85,000 Cases. ESHG 2015;Glasgow, UK.
14. Beamon CJ, Hardisty EE, Harris SC, Vora NL. A single center's experience with noninvasive prenatal testing. *Genet Med.* 2014;16(9):681–687.
15. McCullough RM, Almasri EA, Guan X, et al. Non-invasive prenatal chromosomal aneuploidy testing--clinical experience: 100,000 clinical samples. *PLoS One.* 2014;9(10):e109173.
16. Bevilacqua E, Gil MM, Nicolaides KH, 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.
17. Fairbrother G, Johnson S, Musci TJ, Song K. Clinical experience of noninvasive prenatal testing with cell-free DNA for fetal trisomies 21, 18, and 13, in a general screening population. *Prenat Diagn.* 2013;33(6):580-583.
18. Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH. Implementation of maternal blood cell-free DNA testing in early screening for aneuploidies. *Ultrasound Obstet Gynecol*. 2013;42(1):34-40.
19. Willems PJ, Dierickx H, Vandenakker E. 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.
20. Comas C, Echevarria M, Rodriguez MA, Prats P, Rodriguez I, Serra B. Initial experience with non-invasive prenatal testing of cell-free DNA for major chromosomal anomalies in a clinical setting. *J Matern Fetal Neonatal Med.* 2014:1-6.

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21. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA Analysis for Noninvasive Examination of Trisomy. *N Engl J Med.* 2015;doi: 10.1056/NEJMoa1407349.
22. Quezada MS, Gil MM, Francisco C, Orosz G, Nicolaides KH. 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 weeks. *Ultrasound Obstet Gynecol*. 2015;45(1):36-41.
23. Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med.* 2011;13(11):913-920.
24. Palomaki GE, Deciu C, Kloza EM, et al. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med.* 2012;14(3):296-305.
25. Norton ME, Brar H, Weiss J, 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.e131-138.
26. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn.* 2013;33(6):575-579.
27. Pergament E, Cuckle H, Zimmermann B, et al. Single-Nucleotide Polymorphism-Based Noninvasive Prenatal Screening in a High-Risk and Low-Risk Cohort. *Obstet Gynecol.* 2014;124(2 Pt 1):210-218.
28. Ashoor G, Syngelaki A, Wang E, 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.

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