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VeriSeq™ NIPT Solution v2

Revolutionizing NIPT, the VeriSeq NIPT Solution v2 is an end-to-end, accessible, whole-genome sequencing assay that generates accurate results for up to 96 samples in ~ 26 hours.

Highlights

- **Most comprehensive view of the fetal genome**
  IVD in-lab NIPT solution offering the broadest test menu validated in a clinical accuracy study of > 2000 samples
- **Superior test performance**[1]
  Unparalleled accuracy, fastest results, low failure rates
- **Simple, scalable IVD solution**
  Easy-to-implement solution analyzes 24, 48, or 96 samples per run

Introduction

Noninvasive prenatal testing (NIPT) performed with next-generation sequencing (NGS) provides reliable screening results for fetal chromosomal aneuploidies as early as 10 weeks gestation—from a single tube of maternal blood.[2,3] VeriSeq NIPT Solution v2 takes advantage of powerful Illumina NGS technology to bring a whole-genome sequencing (WGS) approach to NIPT, expanding test menu options to include common aneuploidies (chromosomes 21, 18, and 13), all rare autosomal aneuploidies (RAAs), sex chromosome aneuploidies (SCAs), and partial deletions and duplications, referred to as copy number variations (CNVs), ≥ 7 Mb in size. Combining a broad test menu, accurate results, and low failure rates, the VeriSeq NIPT Solution offers the most comprehensive screening of the fetal genome, enabling informed, timely pregnancy management decisions.[4,5]

The VeriSeq NIPT Solution v2 incorporates workflow, instrument, and software innovations that make NGS-based NIPT accessible to any lab performing clinical prenatal aneuploidy screening. Providing reagents, instruments, software, installation, and training, VeriSeq NIPT is an automated, reliable solution for in-house NIPT (Figure 1 and Table 1).

Table 1: VeriSeq NIPT Solution v2, at a glance

Parameter	Description
Method	Whole-genome sequencing
Library preparation	PCR-free
Chemistry	Paired-end sequencing
No. samples	24, 48, or 96 per batch
Time to report	~ 26 hours
No. technicians	1
Specimen	7-10 ml of a single tube of maternal blood
Analysis offered	Aneuploidy status of all autosomes and sex chromosomes Certain subchromosomal copy number variations ≥ 7 Mb

Comprehensive view of the fetal genome

Many in-lab NIPT solutions focus on trisomies in chromosomes 21, 18, and 13, but these chromosomes represent only a portion of the genome. These tests will miss CNVs ≥ 7 Mb that can occur at an incidence rate of 0.10% and have been associated with fetal anomalies and developmental delay. These tests will also miss pregnancies that screen positive for RAAs, which may be associated with adverse outcomes, including miscarriage, intrauterine growth restriction (IUGR), uniparental disomy (UPD), preterm labor, and fetal anomalies, among others.[6] With RAAs occurring at a combined rate of 0.34%,[6] compared to 0.30% for trisomy 21,[7] it's possible that basic NIPT screening alone may miss significant anomalies.

Superior test performance

Based on accuracy of results, time to answer, and failure rates, the VeriSeq NIPT Solution v2 demonstrates excellent performance.

Figure 1: Full IVD NIPT workflow—The VeriSeq NIPT Solution v2 provides everything needed for NIPT using NGS, including reagents for DNA extraction, library preparation, and sequencing; instrumentation for automated library preparation and sequencing with workflow manager software; an onsite server for secure data storage and analysis; and data analysis software capable of generating clinical results.

Table 2: Clinical performance of VeriSeq NIPT Solution v2[a]

	Trisomy 21	Trisomy 18	Trisomy 13	RAA[d]	CNV ≥ 7 Mb	Any anomaly[e]
Sensitivity[b]	> 99.9% (130/130)	> 99.9% (41/41)	> 99.9% (26/26)	96.4% (27/28)	74.1% (20/27)	95.5% (318/333)
2-sided 95% CI[c]	97.1%, 100%	91.4%, 100%	87.1%, 100%	82.3%, 99.4%	55.3%, 86.8%	92.7%, 97.3%
Specificity	99.90% (1982/1984)	99.90% (1995/1997)	99.90 (2000/2002)	99.80% (2001/2005)	99.80% (2000/2004)	99.34% (1954/1967)
2-sided 95% CI[c]	99.63%, 99.97%	99.64%, 99.97%	99.64%, 99.97%	99.49%, 99.92%	99.49%, 99.92%	98.87%, 99.61%

a. Seven twin pregnancies reported correctly as T21 not shown in table

b. Basic screen performance is reported for T21, T18, and T13 and excludes 16 samples with known mosaics and an additional 49 samples affected with anomalies for the genome-wide screen only; genome-wide screen performance is reported for RAAs and CNVs

c. CI based on Wilson's score method

d. RAA excludes chromosomes 21, 18, and 13

e. Any anomaly includes samples from SCA basic and genome-wide screens

Table 3: Concordance of VeriSeq NIPT Solution v2 results for fetal sex classification with clinical reference standard outcome

VeriSeq NIPT Solution v2 results	Newborn physical exam outcome[a]		Cytogenetic results					
	Female	Male	XX	XY	XO	XXX	XXY	XYY
Percent concordant	100%	100%	100%	100%	90.5%	100%	100%	91.7%

a. No cytogenetic results

Unparalleled accuracy

VeriSeq NIPT Solution v2 has undergone extensive testing to determine clinical accuracy and reliability. Samples from affected pregnancies were eligible for testing if clinical outcomes were available and met sample inclusion criteria. The cohort comprised gestational ages distributed around 11 weeks, samples with low fetal fractions, and twin pregnancies. The study screened > 2300 maternal samples with known outcomes for trisomy 21, trisomy 18, trisomy 13, all rare autosomal trisomies, CNVs ≥ 7 Mb, and SCAs using the VeriSeq NIPT Solution v2 and compared the results to clinical reference data. Results demonstrated exceptionally high sensitivity and specificity for the target aneuploidies and CNVs ≥ 7 Mb (Table 2 and Table 3) and an overall low sample failure rate of 1.2%.[8]

Fastest results

The VeriSeq NIPT Solution v2 offers a fast three-step workflow for NIPT that generates accurate results in just over 1 day (Table 4). Following the simple, automated workflow, one technician can analyze 24-96 samples in < 8 hours with minimal hands-on time. Targeted sequencing and array-based methods tend to have longer laboratory protocols, requiring more hands-on time.

Table 4: VeriSeq NIPT complete in ~ 1 day

Step	Hands-on time	Total time
Sample preparation (plasma isolation, DNA extraction, library preparation)	~ 2 hours	~ 8 hours
Sequencing (library loading and run set up)	~ 15 min	~ 14 hours
Data analysis and report generation[a]	N/A	~ 4 hours
Total time	~ 2.25 hours	~ 26 hours

a. Actual time depends on individual lab processes and may vary from site to site. N/A: not applicable

Low test failure rates

Test failures, where no call for disomy 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 demonstrate higher rates of test failure than tests that sequence the whole genome in both validation and clinical experience studies.[9] The VeriSeq NIPT Solution v2 uses WGS to provide ample data across the entire diploid genome, without impacting accuracy or increasing failure or false positive rates. In the clinical performance study, ~ 99% of samples yielded a result on the first run.[8] In lab practice, sufficient material may be available from the initial blood draw to repeat the VeriSeq NIPT workflow, if needed.

Simple, scalable IVD solution

The integrated VeriSeq NIPT Solution v2 provides everything needed to run the assay. The automated workflow easily scales to analyze 24, 48, or 96 samples per run to allow for efficiency and flexibility in managing sample volumes.

Automated library preparation

The fully automated VeriSeq NIPT process provides a simple workflow that minimizes technician time and the potential for error. The protocol starts with 7-10 ml of maternal peripheral whole blood collected in the provided Streck Blood Collect Tube (BCT). Optimized VeriSeq NIPT sample prep kits contain reagents and consumables for preparing sequencing libraries from cfDNA. Plasma isolation, cfDNA extraction, and PCR-free library preparation, including quantification plate creation, library quantification, and library pooling, are automated on the VeriSeq NIPT Microlab STAR, a Hamilton Microlab STAR system custom configured specifically for use in the VeriSeq NIPT workflow. The

user-friendly VeriSeq NIPT Workflow Manager controls all aspects of sample preparation, including sample tracking.

Sequencing

The VeriSeq NIPT Solution v2 is powered by proven Illumina sequencing by synthesis (SBS) chemistry, the chemistry that generates over 90% of the sequencing data in the world.[10]

Improved efficiency with paired-end sequencing

A maternal blood sample contains different lengths of cfDNA; longer lengths tend to be maternal while shorter lengths have stronger fetal correlation (Figure 2).[11] Using paired-end sequencing, which produces twice the amount of data with the same amount of time and effort as single-read sequencing, the VeriSeq NIPT Solution v2 quickly and efficiently identifies the lengths of all cfDNA fragment within a single sample. The algorithm in the VeriSeq NIPT Assay Software uses this data to focus analysis on the shorter fragments to increase the signal from fetal cfDNA.

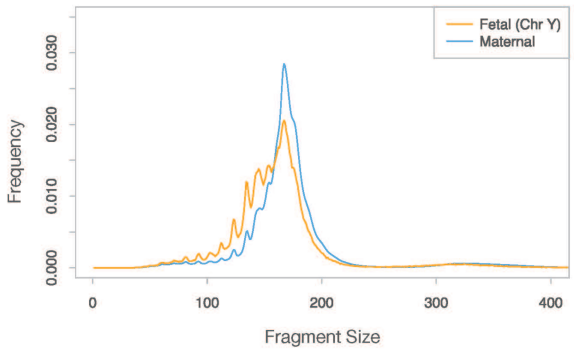

Figure 2: Size comparison of maternal and fetal cfDNA fragments—Paired-end sequencing differentiates cfDNA fragments based on size. Longer fragments tend to be maternal in origin while shorter fragments are fetal.

Diagnostic power in a desktop sequencer

Sequencing in the VeriSeq NIPT Solution v2 is performed on the Illumina NextSeq™ 550Dx System, an FDA-regulated and CE-IVD marked platform that delivers the power of high-throughput NGS with the affordability of a benchtop system (Table 5). The NextSeq 550Dx System features a load-and-go workflow that requires roughly 30 minutes of hands-on time. An intuitive user interface allows users to perform sequencing applications with minimal training or instrument set-up time.

Table 5: NGS instrument performance requirements

Parameter	Specification
Read length	2 × 36 bp
Sequencing file type	.BCL file
Sequencing output	400M reads
Run time	~ 14 hours
Multiplexing	24 or 48 samples per run

Onsite analysis

Data analysis is performed on a dedicated VeriSeq Onsite Server v2 with the IVD-marked VeriSeq NIPT Assay Software v2. The server automatically processes sequencing data. Multiple sample batches can be queued for analysis on a single server. There is no need to send out data for analysis, saving time and protecting sample identity.

VeriSeq NIPT Assay Software

VeriSeq NIPT Assay Software filters and aligns the reads to a reference genome. An advanced algorithm counts the reads per chromosome. Scores reflect normalized coverage of the test chromosome, or subchromosomal region, and aid in the detection and differentiation of aneuploidy and CNVs. The software also generates and reports a fetal fraction estimate for each sample. Fetal fraction data are combined with coverage and other statistical inputs generated during sequencing to assess aneuploidy status.

To assure low test failure rates, VeriSeq NIPT Assay Software includes the individualized fetal aneuploidy confidence test (iFACT) sample quality scoring metric. iFACT indicates if the instrument has generated sufficient sequencing coverage and data quality, given the fetal fraction estimate for each sample, to enable an aneuploidy or CNV call, even for samples with low fetal fraction.[12-14] This dynamic cutoff enables VeriSeq NIPT Assay Software to report on low fetal fraction samples, resulting in low test failures.[14-16]

Report generation

After data analysis, the VeriSeq NIPT Assay Software generates an "Aneuploidy Detected" or "No Aneuploidy Detected" call for chromosome tested in each sample. If a CNV is detected, the exact coordinates in the genome are displayed in the output report for that sample. Information is output in a .CSV file that can be integrated with an existing LIMS. Data can be used to create a custom clinical report according to laboratory needs.

Fully supported implementation

For smooth laboratory integration, the VeriSeq NIPT Solution v2 includes complete system installation by a skilled Illumina Field Service Engineer and hands-on training. Knowledgeable Illumina scientists lead laboratory personnel step by step through sample extraction, library preparation, sequencing, and analysis (Table 6). When laboratories are up and running, continued support is provided by the Illumina Technical Support team.

Table 6: VeriSeq NIPT Solution v2 training

Topic	Details
Introduction to the VeriSeq NIPT Solution v2	Seminar overview of workflow and analysis • Ancillary equipment guide • Consumables guide • Blood draw protocol • Plasma isolation protocol
Instrument operation training	Onsite training • Requires installed instrument
Site inspection	Onsite confirmation • Ancillary equipment installation • Needed reagents • Connectivity of system components
Onsite training	Assay performed by Illumina scientist • Pretested plasma samples with known performance characteristics (provided by Illumina) • Walkthrough of assay workflow from plasma isolation to instrument operation and data analysis • Data analysis training
Onsite competency testing	Assay performed by customer • Pretested plasma samples with known performance characteristics (provided by Illumina)

Summary

VeriSeq NIPT Solution v2 revolutionizes the accessibility, reliability, and power of NIPT. Now any laboratory can harness NGS for fast, reliable, highly accurate NIPT results with low failure rates.

Learn more

To learn more about the VeriSeq NIPT Solution v2, visit www.illumina.com/VeriSeqNIPT.

Ordering Information

Product	Catalog no.
VeriSeq NIPT Sample Prep Kit (24 samples)	20025895
VeriSeq NIPT Sample Prep Kit (48 samples)	15066801
VeriSeq NIPT Sample Prep Kit (96 samples)	15066802
VeriSeq Onsite Server v2	20028403
Veriseq NIPT Solution Enablement	15076162
Streck cell-free DNA BCT (CE)	15073345
NextSeq 550Dx Instrument	20005715

Intended use statement

The VeriSeq NIPT Solution v2 is an *in vitro* diagnostic test intended for use as a screening test for the detection of genome-wide fetal genetic anomalies from maternal peripheral whole blood specimens in pregnant women of at least 10 weeks gestation. VeriSeq NIPT uses whole-genome sequencing to detect CNVs for all autosomes and aneuploidy status for all chromosomes. The test offers an option to request the reporting of sex chromosome aneuploidy (SCA). This product must not be used as the sole basis for diagnosis or other pregnancy management decision.

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