illumina®

verifi® Prenatal Test
Payer dossier

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Executive Summary

An estimated 2.6 million maternal serum screens for fetal aneuploidy are performed in the United States annually.[1] Before the introduction of cell-free DNA-based noninvasive prenatal testing (NIPT), prenatal screening options had variable performance and screen-positive rates of around 5%.[2] With a prenatal trisomy 21 incidence of approximately 0.45%[3] in the general pregnancy population, most screen positives were false positives. False positive results can lead to unnecessary patient anxiety and invasive diagnostic tests (amniocentesis and chorionic villus sampling [CVS]). These invasive tests carry risks, including procedure-related miscarriage.[4]

Because of the high false-positive rate with prenatal serum screening and the inherent risks associated with amniocentesis and CVS, there was a clear, unmet clinical need for a more accurate prenatal screen for fetal aneuploidy. Cell-free DNA-based NIPT has addressed this need. More accurate results should lead to fewer confirmatory invasive procedures and potentially less patient anxiety.

Growing evidence has demonstrated the benefit of NIPT as a first-tier fetal aneuploidy screen for all women,[5] not just high-risk women, dramatically reducing the number of costly, confirmatory invasive procedures. Further, economic models have demonstrated that NIPT as a first-tier screen would be cost-effective in the general US pregnancy population at a price of around $650.[6-8] Importantly, medical societies now support NIPT as a fetal aneuploidy screening option for all women.[9-11]

As demonstrated in this clinical dossier, the verifi Prenatal Test can significantly improve current prenatal screening and diagnostic strategies based on the following key points:

- The high sensitivity[12, 13] and specificity[12, 13] enable a reduction in confirmatory invasive procedures, their sequelae and costs.[14, 15]
- Commercial laboratory experience with the verifi Prenatal Test demonstrates a test failure rate of around 0.1% and an average turnaround time (TAT) of 3 business days.[12]

The verifi Prenatal Test is intended for use in women with a singleton or twin pregnancy, and can be performed at any time during pregnancy from 10 weeks' gestation to term.

The verifi® Prenatal Test was developed by, and its performance characteristics were determined by Verinata Health, Inc. (VHI) a wholly owned subsidiary of Illumina, Inc. The VHI laboratory is CAP-accredited and certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. It has not been cleared or approved by the U.S. Food and Drug Administration.

Issues with Prenatal Screening and Invasive Testing in the US

Practice guidelines by the American College of Obstetricians and Gynecologists (ACOG) recommend that pregnant women of all ages are offered aneuploidy screening before 20 weeks' gestation.[2]

Aneuploidy is a term used to describe a condition where there is an abnormal number of chromosomes. The clinical effects of aneuploidy are significant. Most pregnancies with aneuploid fetuses do not survive to term.[16] Infants with aneuploidies that survive to birth are generally affected by congenital birth defects and/or intellectual disability.[17] An estimated 30–60% of all miscarriages[16, 18, 19] and 1 in 300[16, 18] liveborns have aneuploidy. As such, aneuploidy is the leading known genetic cause of miscarriage and congenital birth defects.[18]

Current prenatal screening options are primarily used to identify trisomy 21 (Down syndrome, T21), trisomy 18 (Edwards syndrome, T18), and trisomy 13 (Patau syndrome, T13), which are the most common aneuploidies seen in live births. All three conditions lead to significant birth defects and intellectual disabilities.[17] The incidence of these trisomies increases with advancing maternal age.[20]

- Trisomy 21 occurs on average in about 1 in 660 live births.[17]
- Trisomy 18 occurs in about 1 in 3,333 live births.[17]
- Trisomy 13 occurs in approximately 1 in 5,000 live births.[17]

Older Prenatal Screening Modalities

Before the introduction of NIPT, prenatal aneuploidy screening options included the measurement of serum biomarkers and ultrasound examinations. In the first trimester, measurement of particular serum biomarkers (pregnancy-associated plasma protein A [PAPP-A] and human chorionic gonadotropin [hCG]) and ultrasound for nuchal translucency (NT) can be performed (commonly referred to as the "combined screen"). In the second trimester, a different set of serum biomarkers (hCG, Estriol, and alpha-fetoprotein [AFP] for the "triple" screen; add Inhibin-A for the "quadruple" screen) is measured. In some aneuploidy screening practices, both first trimester and second trimester measurements are performed in an attempt to increase the overall sensitivity (referred to as "sequential" or "integrated" screening).

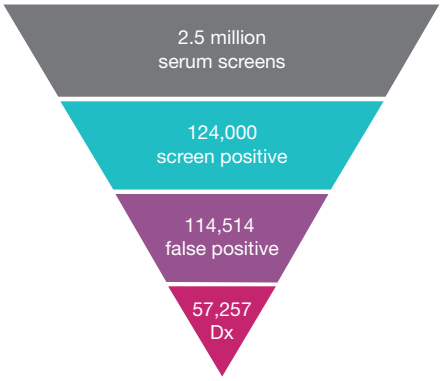
These screening options have suboptimal sensitivity and specificity (Table 1). Based on approximately 4M US births[21] and a screen rate of 62%,[1] around 2.5M annual prenatal serum screens are performed. Given a 5% screen-positive rate, there will be approximately 124,000 positive screen results. Assuming a prenatal trisomy 21 incidence of approximately 0.45%[3] and an 85% trisomy 21 detection rate,[22] this means that about 114,514 false positives are reported annually. With around 50% of women with a positive screening test electing invasive testing for confirmation,[23] this equates to around 57,257 invasive tests performed in women because of a false positive trisomy 21 screening result. Figure 1 illustrates the landscape of the prenatal screening for trisomy 21 in the United States before the introduction of NIPT.

Table 1: Performance characteristics of prenatal screening strategies in the first and second trimesters prior to NIPT

Trimester — Test	Sensitivity	Specificity
1st — Combined (serum plus NT) screen for T21	85%[22]	95%[22]
1st — Combined (serum plus NT) screen for T18	82%[24]	94%[24]
2nd — Quad screen for T21	81%[22]	95%[22]
2nd — Triple screen for T18*	77%[25]	99%[25]

* Using maternal age, β-hCG, and PAPP-A markers

Figure 1: Estimation of the US trisomy 21 annual prenatal screening landscape before the introduction of NIPT*



* Estimate based on published data for screening[1] and diagnostic testing[23] update rates, prenatal incidence of trisomy 21,[3] and performance of prenatal screening for trisomy 21.[22]

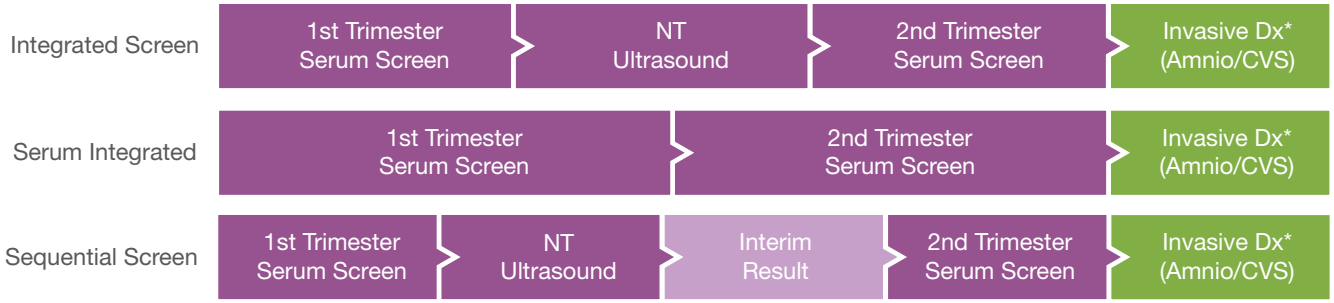
The number of diagnostic tests (Dx) shown indicates the number of trisomy 21 false positive cases undergoing invasive diagnostic procedures.

Clinicians have a range of screening and diagnostic tests available to offer to their patients. ACOG recommends that physicians provide the following information to their patients to enable them to make an informed decision about prenatal testing[4, 9]: the patient's risk for fetal aneuploidy and other genetic diseases; the difference between screening and diagnostic testing; detection rates, false-positive rates, and the advantages, disadvantages, and limitations of each screening test; the risks and benefits of invasive diagnostic testing.

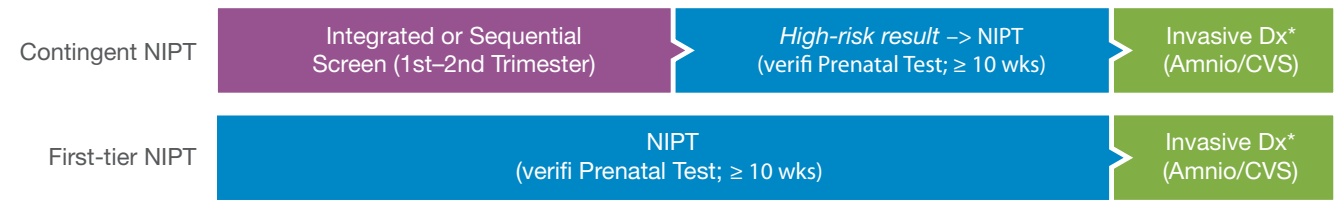
Figure 2 illustrates screening and diagnostic strategies for aneuploidy detection that clinicians currently provide to their patients, and how NIPT can, and is, being integrated into clinical care. Of note, integrated and sequential screens require multiple office visits and ultrasound measurement of nuchal translucency requires specialist training.

Figure 2. Prenatal screening strategies in the first and second trimesters

Screening options before the introduction of NIPT



New screening options after the introduction of NIPT



Invasive diagnostic (Dx) testing recommended for patients with a positive screening result.

Definitions: NT stands for nuchal translucency; amnio is short for amniocentesis; CVS stands for chorionic villus sampling; and wks is short for weeks.

Risks of Invasive Testing

High false-positive rates with prenatal screening can be a concern as many (≥ 50%) of these patients will go on to have invasive procedures.[23] These procedures carry inherent risks for adverse effects such as miscarriage, amniotic fluid leakage, infection, and infection transmission.[26, 27] The miscarriage rate for CVS and amniocentesis ranges from 1 in 500 to 1 in 1,000.[4]

verifi Prenatal Test – Overview

Intended Use

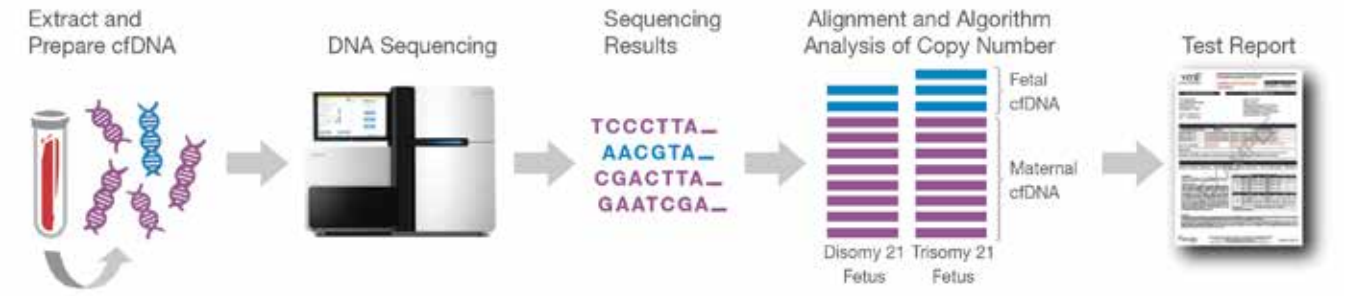
The verifi Prenatal Test is intended for use in women with a singleton or twin pregnancy who are electing to undergo prenatal screening for fetal aneuploidy. Testing can be performed from 10 weeks' gestation until term.

Test Technology

The verifi Prenatal Test is provided through the Illumina CLIA-certified, CAP-accredited clinical laboratory. The test utilizes next-generation sequencing (NGS) of circulating cell-free DNA (cfDNA) extracted from a maternal blood sample to screen for aneuploidy of chromosomes 21, 18, and 13, and the sex chromosomes; result reporting for the sex chromosomes is optional.

The circulating cfDNA found in maternal plasma is a combination of cfDNA from the mother and placenta (which is typically representative of the fetal DNA). Around 10-15% of the cfDNA in maternal blood is from the placenta,[28-30] this percentage is commonly referred to as the "fetal fraction". Millions of fragments from an individual patient's blood sample are sequenced, aligned to a reference human genome, and analyzed for any relative over- or under-representation of DNA from the chromosomes of interest (indicative of aneuploidy; *see Sections IV and V for additional information on the development of the verifi Prenatal Test*). Highly sensitive NGS combined with algorithmic analysis can be used to detect and measure aneuploidy within this mixed sample. Based on this analysis, the sample receives a classification of aneuploidy status for chromosomes 21, 18, and 13, as well as the sex chromosomes (if requested). An overview of the verifi Prenatal Test laboratory process is shown in Figure 3.

Figure 3: Cell-free DNA-based whole-genome NGS-based noninvasive prenatal testing: the verifi Prenatal Test



Several methods for NIPT are currently available. The verifi Prenatal Test harnesses the power of whole-genome sequencing (WGS) with a highly optimized algorithm,[31-34] which has been shown to be an accurate, reliable, and fast approach.[12, 32-35] Some NIPTs use a targeted approach, sequencing only a select number of chromosomes or select single-nucleotide polymorphisms (SNPs).[36, 37]

The verifi Prenatal Test is not dependent on maternal age, maternal weight, or gestational age (after 10 weeks). Further, unlike SNP-based tests,[38, 39] verifi can be used in twin pregnancies[34] and pregnancies conceived using an egg donor.[40] Table 2 highlights some of the important differences between the verifi Prenatal Test and targeted NIPT approaches.

Table 2: Comparison of verifi Prenatal Test and targeted NIPT approaches

verifi Prenatal Test (a WGS assay)[12, 32, 34]	Targeted NIPTs[37, 41-43]
Low failure rate (< 0.5%)	High failure rates (1–5% or greater)
Captures comprehensive genomic data	Analysis limited to a few chromosomes
Not constrained by patient factors	Can rely on patient factors
Available for singleton and twin gestations	May not offer testing for twin gestations

verifi Prenatal Test – Analytical Validity

Multiple clinical studies have established that sequencing of cfDNA from maternal blood can accurately detect fetal aneuploidy. Initial NIPT studies focused on the detection of the most common fetal aneuploidy, trisomy 21.[44, 45] As trisomy 18 is the next most common autosomal fetal aneuploidy in women undergoing prenatal screening, Verinata Health (a subsidiary of Illumina) developed a proprietary algorithm, SAFeR™ (Selective Algorithm for Fetal Results), to detect trisomy 21 and trisomy 18.[31] SAFeR calculates a Normalized Chromosome Value (NCV) for each chromosome of interest, which significantly reduces data variation caused by GC (guanine and cytosine) content, sample-to-sample and run-to-run variations, and other factors.

To validate the algorithm, Verinata Health conducted a blinded, prospective study in collaboration with 13 clinics throughout the United States.[31] Blood samples were collected from 1014 pregnant women who were at least 18 years of age and were undergoing a clinically indicated CVS or amniocentesis procedure. The blood samples were collected prior to the invasive procedures. Of the 1,014 eligible patient samples, 119 underwent cfDNA analysis; 53 of the 119 samples tested were from women with fetal aneuploidy. An optimized classification algorithm was developed from the sequencing data on 71 of the 119 samples (training set). The optimized classification algorithm was then evaluated on an independent test set of 48 samples. In this study, the optimized classification algorithm demonstrated 100% correct classification of T21 and T18.[31]

> *The study concluded that "…algorithms for quantification not only minimize random and systematic variation between sequencing runs but also allow for effective classification of aneuploidies across the entire genome…"*[31]

Analytical Performance

Analytical performance was assessed by testing samples with an unaffected karyotype and positive controls. The test process involves [1] collection of blood at clinics and shipment of the blood to the testing site, [2] isolation of plasma before cfDNA extraction from the plasma, [3] preparation of DNA libraries, [4] cluster generation and multiplexed sequencing on an Illumina NGS machine, [5] processing of sequencing results, which involves sequence alignment to the human genome and counting of unique tags, [6] sample classification.

The performance of the test was assessed using predefined acceptance criteria and comparison of the sequencing results with karyotype data. A set of unaffected samples (N=611) and controls (N=84 replicates of a contrived control) were used for accuracy and precision determinations.

From a total of 611 unaffected samples, 582 passed quality control metrics for DNA concentration (20–250 pg/µL) and library concentration (10 nM). The positive control was a contrived sample comprised of a mixture of sheared genomic DNA from three individual trisomic cell lines (representing trisomy 21, trisomy 18, and trisomy 13) sufficient to yield chromosome ratios representative of a trisomic fetal state for chromosomes 13, 18, and 21. The positive control was run in replicate in the analytical experiments described below.

Accuracy

The accuracy of the test is based on the comparison of the NIPT results to the karyotype results. Accuracy for chromosomes 13, 18, and 21 was determined for the positive controls and for the samples of unaffected karyotype. As a means to minimize experimental variance and account for experimental biases, chromosomal counts on test chromosomes were normalized by comparing to counts on a set of denominator chromosomes.

Accuracy for the positive control was determined from 84 replicates of a contrived control, sequenced across 13 flow cells. All 84 replicates were accurately classified for trisomy 13, 18, and 21 with mean NCVs of 8.07, 7.14, and 7.82, respectively (Table 3).

Table 3: Accuracy of positive control (n = 84 replicates)

	Ratio 13	Ratio 18	Ratio 21	NCV 13	NCV 18	NCV 21
Mean	0.27591	0.24410	0.08807	8.07	7.14	7.82
Min	0.27435	0.24289	0.08757	6.75	5.05	6.47
Max	0.27783	0.24588	0.08864	9.73	10.23	9.37

Of the 582 samples that went to sequencing, there were 15 individual sequencing failures, leaving 567 samples that passed all quality control metrics. Of the 567 analyzed samples, 555 were classified as “Unaffected” with an NCV of < 2.5. Two samples had a single chromosome with an NCV > 4 and were classified as “Affected” for that chromosome: 1 for chromosome 18 and 1 for chromosome 21. Ten samples were unclassified with NCVs between 2.5 and 4. Accuracy for the unaffected samples was calculated to be 99.6%.

Precision

Precision in unaffected samples was determined from the variability of 567 individual samples of unaffected karyotype that were sequenced across 13 flow cells. The mean, standard deviation, median, and CV (coefficient of variation) of chromosome 13, 18, and 21 ratios on each flow cell were calculated. The inter-flow cell CV was also determined.

A positive control was used to determine the precision for positive results. The control was tested in replicates (4–12 replicates within a flow cell) for a total of 84 measures across 13 flow cells. The variability (CV) of chromosome ratios for 13, 18, and 21 were determined; T21 ratios are shown in Table 4. The variability on multiple sequencing runs assessed with this positive control is an indication of the precision expected for chromosomes 13, 18, and 21 with affected samples.

Table 4: Intra- and inter-flow cell variation of ratio T21

Flow Cell ID	Mean	SD	Median	Intra-Flow Cell CV (%)	Inter-Flow Cell CV (%)
1	0.085204685	0.0003808	0.0852253	0.45	1.69
2	0.085313555	0.0003887	0.0853179	0.46	
3	0.085083745	0.0003458	0.0850902	0.41	
4	0.085176931	0.0003682	0.0851671	0.43	
5	0.085281972	0.0004553	0.0852451	0.53	
6	0.085095318	0.0004237	0.0851235	0.50	
7	0.085159962	0.0004214	0.0851081	0.49	
8	0.085135233	0.0004944	0.0851664	0.58	
9	0.085205245	0.0003955	0.085156	0.46	
10	0.085191251	0.0003174	0.0852005	0.37	
11	0.085126717	0.0004011	0.0850987	0.47	
12	0.085234313	0.000442	0.0852298	0.52	
13	0.085263435	0.0003756	0.085258	0.44	

verifi Prenatal Test – Clinical Validity

Performance Data in Singleton Pregnancies

Building on the findings of the Sehnert *et al* study,[31] the MELISSA clinical study was initiated to validate the diagnostic accuracy of the verifi Prenatal Test. Blood samples were collected in a prospective, blinded study from 2,882 women undergoing prenatal diagnostic procedures at 60 US clinical sites.[32] Chromosome classifications were made by verifi for each sample and compared with fetal karyotypes obtained by CVS or amniocentesis. This study demonstrated that the verifi Prenatal Test has high sensitivities and specificities for fetal trisomy 21, 18, and 13 (Table 5).[32]

Table 5: verifi Prenatal Test performance in the MELISSA study[32]

Condition	N	Sensitivity	95% CI	Specificity	95% CI
Trisomy 21, Down syndrome	493	>99.9% (89/89)	95.9–100	>99.9% (404/404)	99.1–100.0
Trisomy 18, Edwards syndrome	496	97.2% (35/36)	85.5–99.9	>99.9% (460/460)	99.2–100.0
Trisomy 13, Patau syndrome	499	78.6% (11/14)	49.2–99.9	>99.9% (485/485)	99.2–100.0
Monosomy X, Turner syndrome	433	93.8% (15/16)	69.8–99.8	99.8% (416/417)	98.7–100.0

Following the MELISSA study, updates to the DNA sequencing chemistry and algorithm used for verifi were made to improve test precision and performance. The updated test performance for the verifi Prenatal Test is shown below in Table 6.[12,13] Further, a new classification category "Aneuploidy Suspected" was introduced for samples with borderline results (NCVs above the cutoff for reporting as "No Aneuploidy Detected" but lower than the cutoff for reporting as "Aneuploidy Detected"). While some affected cases are expected to fall in the borderline zone, samples reported as "Aneuploidy Suspected" are more likely than samples reported as "Aneuploidy Detected" to be unaffected (false positive).

Table 6: verifi Prenatal Test performance in the MELISSA cohort after test updates[12,13]

Condition	N	Sensitivity	95% CI	Specificity	95% CI
Trisomy 21	500	>99.9% (90/90)	96.0–100.0	99.8% (409/410)	98.7–100.0
Trisomy 18	501	97.4% (37/38)	86.2–99.9	99.6% (461/463)	98.5–100.0
Trisomy 13	501	87.5% (14/16)	61.7–98.5	>99.9% (485/485)	99.2–100.0
Monosomy X	508	95.0% (19/20)	75.1–99.9	99.0% (483/488)	97.6–99.7

Performance of the verifi Prenatal Test has since been evaluated in the clinical population submitting samples to the Illumina clinical laboratory.[12, 35, 46, 47] Although clinical follow-up was limited, an evaluation of the first approximately 6,000 samples submitted to the Illumina laboratory suggested that clinical performance was in line with the performance parameters established in validation studies.[35] This is important because validation studies typically use later gestation samples, which can have a higher fetal cfDNA fraction, and exclude samples that can be more difficult to analyze (such as samples with a mosaic karyotype). In this study, the average turnaround time was 5 business days and the technical failure rate was 0.7%.[35]

A larger, subsequent study of around 85,000 clinical samples submitted for the verifi Prenatal Test has now been published.[12] During the timeframe of this study, several process improvements and analytic updates to the verifi Prenatal Test were implemented. These updates resulted in a reduction in the average turnaround time to 3 days, and a reduction in the test failure rate to 0.1%.[12] Importantly, this average turnaround time[12] is faster than with the targeted NIPT approaches that are offered by other laboratories.[48] Further, the 0.1% test failure rate determined in this study[12] is significantly lower than the failure rates observed with targeted NIPT approaches (1–5% or higher).[48] As in the

earlier clinical outcome study, outcome data was limited, particularly for cases reported as No Aneuploidy Detected. Observed positive predictive values (PPVs), indicating the proportion of cases reported as aneuploidy detected or suspected that were true affected fetuses, were calculated from cases with known clinical outcomes. The observed PPVs ranged from 50.0% to 92.8% for trisomies 13, 18, and 21.[12] These PPVs are consistent with those published by other NIPT clinical outcome studies.[35, 48]

Performance Data in Twin Pregnancies

In the US, the incidence of twin births is around 1 in 30, with the rate of twin births on the rise.[49] Traditional serum screening options have lower sensitivities and specificities in twin gestations.[50] Thus, the availability of an accurate and reliable fetal aneuploidy screen for use in twin pregnancies would be of significant value.

Improvements were made to the Illumina SAFeR algorithm to enable fetal aneuploidy screening in twin pregnancies with the verifi Prenatal Test.[34] An initial evaluation of test performance in twin pregnancies was made using maternal blood samples collected as part of two prospective clinical studies, MELISSA[32] and CARE[5]. A total of 115 twin pregnancy samples with known karyotypes were available for analysis. All samples were correctly classified, including 4 (three trisomy 21, one trisomy 18) affected pregnancies and 111 unaffected pregnancies.[34]

Test performance was next evaluated in clinical twin pregnancy samples submitted to the Illumina clinical laboratory for the verifi Prenatal Test.[34] A total of 487 samples were evaluated, of which 479 (98.4%) received a test result; all cancellations were for administrative, not technical, reasons. Of these, 9 cases were reported as aneuploidy detected or suspected, and 470 cases were reported as no aneuploidy detected. Of the 9 aneuploidy suspected/detected cases, 6 were true positives (at least one twin was affected), 1 was a false positive (both twins unaffected), and 2 were suspected to be true positives based on ultrasound findings but confirmatory karyotypes were unavailable. Within the 164 cases reported as no aneuploidy detected and with known outcomes, no false negatives were reported.

Overall, these two studies demonstrated that the verifi Prenatal Test performs well in twin pregnancies.

Applicability of NIPT for the General Pregnancy Population

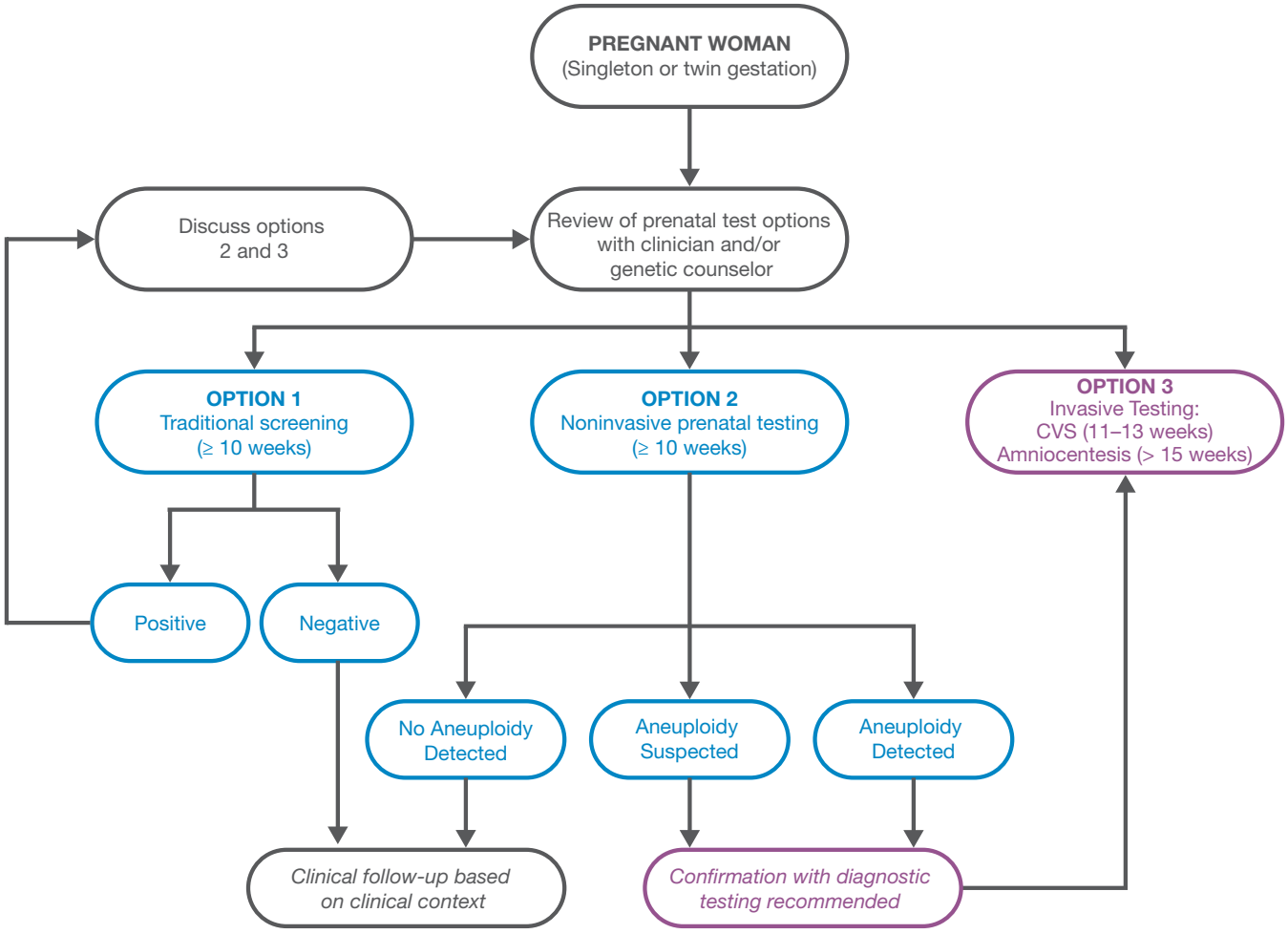
Initial NIPT validation studies were performed using women considered high risk for fetal aneuploidies. This was largely because this ensured a population enriched with affected samples, which powered the studies to determine sensitivity. However, questions quickly arose about the performance of NIPT in low- or general-risk pregnancy populations.

Performance of the verifi Prenatal Test in a general-risk population was determined in a prospective, blinded clinical study, CARE.[5] This study compared the results of the verifi Prenatal Test with results of conventional prenatal screening in a sample of 1,914 women recruited from the general obstetrical population. Verifi detected all cases of aneuploidy (five trisomy 21, two trisomy 18, and one trisomy 13) within this population. Importantly, the false-positive rate for the verifi Prenatal Test was significantly lower than standard screening for trisomy 21 (0.3% vs 3.6%) and trisomy 18 (0.2% vs 0.6%). Lower false-positive rates would result in far fewer women undergoing confirmatory invasive procedures. Further, the PPVs were markedly higher with verifi compared with standard screening: 45.5% versus 4.2% for trisomy 21; 40.0% versus 8.3% for trisomy 18. Thus, for women in this cohort with a positive screening result for trisomy 21 and undergoing a confirmatory invasive procedure, 1 in 2 will be truly affected with NIPT compared with only 1 in 25 with standard screening.

After the completion of the CARE study, updates were made to the analytics algorithm used for the verifi Prenatal Test that were anticipated to reduce technical causes of false positives.[51] Reanalysis of the CARE cohort with this updated algorithm showed that over 50% (6/11) of the previous false positives[5] were now correctly reported as "No Aneuploidy Detected".[51] This confirmed that the updated algorithm used in the Illumina laboratory for the verifi Prenatal Test prevents some technical causes of false positive results. A reduction in false-positive rates will result in higher PPVs, which is considered to be of significant value as NIPT is increasingly utilized within the general pregnancy population.

In summary, the high sensitivity and specificity for trisomies 21, 18, and 13[12, 13] (Table 6) and strong performance in the general pregnancy population[12] support that the verifi Prenatal Test can be successfully integrated into current prenatal screening strategies (see Figure 4). The screening strategy with the largest potential benefit to patients would be utilization of NIPT as a first-tier screen, as the test is availability from early pregnancy (≥10 weeks) and its use would result in fewer confirmatory invasive diagnostic procedures.

Figure 4: Potential prenatal testing strategies.



Screening options and potential results depicted in blue; diagnostic testing option depicted in purple. Patient can choose/decline any, and all, prenatal testing options.

verifi Prenatal Test – Clinical and Economic Utility

Clinical Utility and Economic Implications in the High-Risk Pregnancy Population

In the prospective, blinded MELISSA study, the verifi Prenatal Test demonstrated high sensitivity and specificity for the detection of trisomies 21, 18, and 13[32] suggesting that it could be incorporated into existing aneuploidy screening algorithms to reduce the number of confirmatory invasive procedures for high-risk women.

To evaluate the potential impact of the verifi Prenatal Test on current prenatal screening strategies and the associated rate of invasive diagnostic testing, a transition state probability model of current prenatal screening and diagnostic strategies was developed. This model used the performance data published in the MELISSA study. Bridgehead International developed this model to evaluate the impact of incorporating the verifi Prenatal Test into routine high-risk maternal screening practice.

The model took a theoretical cohort of 100,000 pregnant women at high risk for fetal aneuploidy (based on either first or second trimester screening) and assessed the expected clinical and cost impact of using the verifi Prenatal Test compared with current practice.[52] In the modeled population of five million covered lives with 100,000 pregnancies annually, invasive diagnostic induced miscarriages are reduced from 60 to 20, a 66% reduction (Table 7).

> *"The model demonstrates that inclusion of the verifi test into the prenatal testing paradigm for high-risk women will provide clear clinical benefits. The biggest benefit to women comes from a reduction in miscarriages due to invasive testing."*[52]

Often, new medical technologies add significant cost to the health system. A transition state probability model is a method of evaluating the clinical and economic impact of incorporating new technology into current standard of care. The transition state probability model for the verifi Prenatal Test illustrated that incorporating it into prenatal testing algorithms can greatly reduce the number of costly avoidable procedures and result in an overall cost savings to the health system (Table 7).

> *"The…savings are accompanied by an improved clinical experience by ruling out the need for clinically unnecessary invasive testing for many women."*[52]

Table 7: The clinical and cost impact of adopting the verifi Prenatal Test[52]

Measure	Traditional Prenatal Testing	verifi Prenatal Test	Impact	Benefit
Invasive diagnostic induced miscarriages	60	20	-40	66% reduction in invasive diagnostic-induced miscarriages
Unnecessary follow-up costs	$14.8M	$4.3M	-$10.5M	71% reduction in unnecessary follow-up costs
Use of CVS and amniocentesis	10,225	2,818	-7,407	72% fewer invasive procedures
No. invasive procedures to identify one aneuploidy	43	8	-35	81% fewer invasive procedures to identify one aneuploidy
T21 diagnoses	148	170	+22	15% increase in T21 diagnoses

Clinical Utility and Economic Implications in the General Pregnancy Population

While the clinical utility and economic value of NIPT in the high-risk pregnancy population are widely accepted by clinicians and payers, the benefit and cost implications in the general pregnancy population have been questioned.

Three studies have evaluated the cost savings of NIPT as a first-tier screen in the US using decision-analysis modeling.[6-8] These studies modeled the annual US pregnancy population that undergoes prenatal screening, and determined at what NIPT price point first-line screening by NIPT was cost effective compared with traditional screening options (measurement of serum markers with or without sonographic evaluation of the fetus).

These models factored in the primary cost drivers for prenatal screening: [1] detection rates and false-positive rates; [2] costs of traditional screening, diagnostic testing, and affected births; [3] current clinical practices in terms of screening uptake and termination rates. The per-patient cost-saving price of NIPT reflected the total costs incurred by payers for the screening population divided by the number of patients being screened.

These economic models demonstrated that NIPT as a first-tier screen in the general US pregnancy population is cost effective at a price of $619–744.[6-8]

> *"…universal application of NIPT would increase fetal aneuploidy detection rates and can be economically justified. Offering this testing to all pregnant women is associated with substantial prenatal healthcare benefits."*[6]

> *"Universal NIPT is less costly than MSS [maternal serum screening] as long as the cost of NIPT remains below $619."*[8]

> *"Based on our* cost-effectiveness *model looking at the U.S. general pregnancy population, NIPT can identify more fetal trisomy cases and at the same time reduce unnecessary invasive procedures and in turn fewer related normal fetus losses. These clinical benefits are realized in the setting of also achieving cost saving at the appropriate unit cost of NIPT."*[7]

One cost that was not considered in these models was the cost of NIPT test failures. As professional societies recommend that diagnostic testing be done following failed screening tests,[53, 54] high failure rates have the potential to reduce the value of NIPT. The verifi Prenatal Test offers the lowest reported technical failure rate at 0.1%.[12, 48] This failure rate is 10-fold less than that of other NIPTs on the market. Thus, the verifi Prenatal Test would substantially reduce the additional costs associated with test failures, making it the most cost saving NIPT option.[55]

Overall, these studies[6-8] demonstrate that first-tier screening with NIPT in the general pregnancy population has clinical utility and can result in an overall cost savings to the US health system at prices below $619–744.

Technology Assessments for Noninvasive Prenatal Testing

The Blue Cross Blue Shield Association's Technology Evaluation Center (TEC) has examined the analytic and clinical validity, and clinical utility of DNA sequencing-based prenatal screening (NIPT). Specifically, they assessed whether NIPT for trisomy 21, trisomy 18, and trisomy 13 improves net health outcomes compared with traditional combined screening approaches (maternal serum screening and ultrasound screening). These assessments determined that screening by NIPT would increase the number of affected pregnancies detected, and reduce the number of confirmatory diagnostic procedures.[56, 57]

Blue Cross Blue Shield Association's TEC Assessment

"…nucleic acid sequencing-based testing of maternal plasma for trisomy 21 with confirmatory testing of positive results (as is expected to be performed in a real-world clinical setting) in both high risk women and average-risk women being screened for trisomy 21 meets the TEC criteria"[56]

"Sequencing-based analysis of cell-free fetal DNA obtained from maternal plasma to screen for the presence of fetal T13 or T18—followed by diagnostic karyotype analysis of screen-positive results—in either high-risk or average-risk pregnant women being screened for fetal autosomal aneuploidies meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria."[57]

"In a decision model, sequencing-based maternal plasma fetal trisomy 21 testing reduced the number of invasive confirmatory procedures needed and consequent associated miscarriages, while improving the number of detected cases of trisomy 21, compared to standard screening procedures in either high- or average-risk populations of pregnant women."[56]

"Our findings indicate that for pregnant women undergoing aneuploidy screening, a strategy of using a cell-free fetal DNA–based screening test followed by confirmation of positive test results with an invasive procedure (amniocentesis or CVS) to determine fetal karyotype detected an equivalent or larger proportion of fetal T13 or T18 and missed fewer cases than a strategy employing the traditional integrated screen followed by amniocentesis or CVS diagnosis. Given that T13 and T18 cell-free fetal DNA–based tests will be performed along with T21 testing, the number of invasive procedures and miscarriages secondary to an invasive diagnostic procedure will be reduced with the cell-free fetal DNA–based strategy."[57]

Medical Society Opinions and Recommendations

The American College of Obstetricians and Gynecologists recommends that all women, regardless of maternal age, be offered prenatal assessment for aneuploidy either by screening or invasive prenatal diagnosis.[9]

Following the release of NIPT, medical societies initially endorsed its use in women at high risk for fetal aneuploidy.[58-60] Women are considered high risk for fetal aneuploidy when they have one or more of the following indications: Maternal age of 35 years or older at delivery; fetal ultrasonographic findings indicating an increased risk of aneuploidy; history of a prior pregnancy with a trisomy; positive serum screening test result for aneuploidy; parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or 21.[58]

However, following recent publications describing the performance of cell-free DNA-based NIPT in low-risk and all-risk populations,[5, 42, 51] medical societies have begun endorsing NIPT as a fetal aneuploidy screening option for all pregnant women.[9-11]

The American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal-Fetal Medicine (SMFM)

"Screening tests for aneuploidy are now available for pregnant women in all trimesters of pregnancy. Among these are first-trimester, triple, quad, and penta screens; cell-free DNA; and ultrasonographic screening as single screening tests."[9]

International Society for Prenatal Diagnosis (ISPD)

"The following protocol options are currently considered appropriate: cfDNA screening as a primary test offered to all pregnant women"[10]

American College of Medical Genetics and Genomics (ACMG)

"Noninvasive prenatal screening using cell-free DNA (NIPS) has been rapidly integrated into prenatal care since the initial American College of Medical Genetics and Genomics (ACMG) statement in 2013. New evidence strongly suggests that NIPS can replace conventional screening for Patau, Edwards, and Down syndromes across the maternal age spectrum, for a continuum of gestational age beginning at 9–10 weeks..."[11]

Noninvasive prenatal testing (NIPT) based on cell-free DNA analysis from maternal blood is a screening test; it is not diagnostic. Test results must not be used as the sole basis for diagnosis. Further confirmatory testing is necessary prior to making any irreversible pregnancy decision.

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The verifi® Prenatal Test was developed by, and its performance characteristics were determined by Verinata Health, Inc. (VHI) a wholly owned subsidiary of Illumina, Inc. The VHI laboratory is CAP-accredited and certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. It has not been cleared or approved by the U.S. Food and Drug Administration.

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