

## GENETICS

# Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18

Mary E. Norton, MD; Herb Brar, MD; Jonathan Weiss, MD; Ardeshir Karimi, MD; Louise C. Laurent, MD, PhD; Aaron B. Caughey, MD, PhD; M. Hellen Rodriguez, MD; John Williams III, MD; Michael E. Mitchell, MD; Charles D. Adair, MD; Hanmin Lee, MD; Bo Jacobsson, MD; Mark W. Tomlinson, MD; Dick Oepkes, MD, PhD; Desiree Hollemon, MSN, MPH; Andrew B. Sparks, PhD; Arnold Oliphant, PhD; Ken Song, MD

**OBJECTIVE:** We sought to evaluate performance of a noninvasive prenatal test for fetal trisomy 21 (T21) and trisomy 18 (T18).

**STUDY DESIGN:** A multicenter cohort study was performed whereby cell-free DNA from maternal plasma was analyzed. Chromosome-selective sequencing on chromosomes 21 and 18 was performed with reporting of an aneuploidy risk (High Risk or Low Risk) for each subject.

**RESULTS:** Of the 81 T21 cases, all were classified as High Risk for T21 and there was 1 false-positive result among the 2888 normal cases, for a sensitivity of 100% (95% confidence interval [CI], 95.5–100%) and a

false-positive rate of 0.03% (95% CI, 0.002–0.20%). Of the 38 T18 cases, 37 were classified as High Risk and there were 2 false-positive results among the 2888 normal cases, for a sensitivity of 97.4% (95% CI, 86.5–99.9%) and a false-positive rate of 0.07% (95% CI, 0.02–0.25%).

**CONCLUSION:** Chromosome-selective sequencing of cell-free DNA and application of an individualized risk algorithm is effective in the detection of fetal T21 and T18.

**Key words:** aneuploidy detection, cell-free fetal DNA, Down syndrome, noninvasive prenatal diagnosis, trisomy

Cite this article as: 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:x.ex-x.ex.

Currently, the most effective and commonly used prenatal screening tests for fetal aneuploidy use a combination of maternal age, sonographic measurement of the fetal nuchal translucency, and measurement of maternal serum

## ★ EDITORS' CHOICE ★

screening markers in the first and second trimesters.<sup>1-4</sup> Although prenatal screening tests have greatly improved in the past decade, the best performing screen-

ing tests have false-positive rates of 2-3% and false-negative rates of  $\geq 5\%$ .<sup>1-4</sup> Positive screening results require confirmation with diagnostic testing (eg, chorionic villus sampling [CVS] or amniocentesis); these tests carry fetal loss rates of approximately 1

From the Department of Obstetrics and Gynecology, Stanford University/Lucile Packard Children's Hospital, Stanford (Dr Norton); Riverside Perinatal Diagnostic Center, Riverside (Dr Brar); East Bay Perinatal Medical Associates, Oakland (Dr Weiss); Institute of Prenatal Diagnosis and Reproductive Genetics, San Gabriel (Dr Karimi); the Department of Obstetrics and Gynecology, University of California, San Diego, San Diego (Dr Laurent); San Gabriel Valley Perinatal Medical Group, West Covina (Dr Rodriguez); the Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles (Dr Williams); the Department of Surgery, University of California, San Francisco, San Francisco (Dr Lee); and Ariosa Diagnostics, San Jose (Ms Hollemon and Drs Sparks, Oliphant, and Song), CA; the Department of Obstetrics and Gynecology, Oregon Health Sciences University (Dr Caughey), and Northwest Perinatal Center (Dr Tomlinson), Portland, OR; the Department of Surgery, Medical College of Wisconsin, Milwaukee, WI (Dr Mitchell); the Department of Obstetrics and Gynecology, College of Medicine, University of Tennessee Health Science Center, Chattanooga, TN (Dr Adair); the Department of Obstetrics and Gynecology, Sahlgrenska University Hospital, Gothenburg, Sweden (Dr Jacobsson); and the Department of Obstetrics, Leiden University Medical Center, Leiden, The Netherlands (Dr Oepkes).

Received April 24, 2012; revised May 17, 2012; accepted May 24, 2012.

This study was financially supported by Ariosa Diagnostics.

A.B.C., M.E.M., and H.L. are consultants; M.E.M. and K.S. are board members; and D.H., A.B.S., A.O., and K.S. are paid employees of Ariosa Diagnostics. M.E.N. is co-principal investigator on NCT01511458, sponsored by Ariosa Diagnostics.

Reprints: Mary E. Norton, MD, Department of Obstetrics and Gynecology, Stanford University/Lucile Packard Children's Hospital, 300 Pasteur Dr., HH333, Stanford, CA 94305. [menorton@stanford.edu](mailto:menorton@stanford.edu).

0002-9378/free • © 2012 Mosby, Inc. All rights reserved. • <http://dx.doi.org/10.1016/j.ajog.2012.05.021>



For Editors' Commentary, see Contents

in 300 procedures.<sup>5,6</sup> Current screening paradigms are not uniform, with multiple algorithms available for use at various stages of pregnancy, and therefore can be confusing to incorporate into clinical practice.<sup>7</sup>

The presence of fetal and maternal cell-free DNA (cfDNA) circulating in maternal plasma is now widely appreciated, and several groups have demonstrated fetal trisomy 21 (T21) detection using massively parallel DNA shotgun sequencing (MPSS) in case-control studies.<sup>8-12</sup> This technique sequences cfDNA fragments to determine their specific chromosomal origin; a slightly higher than expected percentage of chromosome 21 fragments indicates that the fetus has a third chromosome 21.<sup>8,9</sup> In addition to detecting T21, several studies have reported on the use of MPSS in assaying trisomy 18 (T18) and trisomy 13 (T13).<sup>11-14</sup>

Despite these promising results, MPSS randomly analyzes DNA from the entire genome, resulting in higher cost and complexity than is practical for widespread clinical adoption. Recent studies have reported on an alternative assay, Digital ANalysis of Selected Regions (DANSR), that selectively evaluates specific genomic fragments from cfDNA, providing more efficient use of sequencing and potentially reduced costs when compared to MPSS.<sup>15-17</sup> This process of chromosome-selective sequencing has been extended to enable simultaneous determination of the fraction of fetal cfDNA in the maternal plasma as well as the chromosome proportion by assaying polymorphic and nonpolymorphic loci.<sup>16</sup> When combined with a novel analysis algorithm, the Fetal-fraction Optimized Risk of Trisomy Evaluation (FORTE), this information can provide an individualized assessment of trisomy risk.<sup>16</sup> In a recently published blinded independent study, the use of DANSR and FORTE was found to separate all cases of T21 and 98% of cases of T18 from euploid pregnancies in 400 singleton pregnancies at 11-13 weeks' gestation.<sup>17</sup>

This report describes the results of a multicenter study designed to evaluate the performance of this noninvasive prenatal assay and algorithm in a large cohort of women prior to invasive prenatal diagnostic testing.

## MATERIALS AND METHODS

### Study population

This was a prospective, cohort study comprising pregnant women aged  $\geq 18$  years, at gestational age  $\geq 10$  weeks, with a singleton pregnancy, who were planning to undergo invasive prenatal diagnosis for any indication. Subjects who were pregnant with  $>1$  fetus, or who themselves had a known aneuploidy, had active malignancy or a history of metastatic cancer, or had already undergone CVS or amniocentesis during the current pregnancy were excluded.

Subjects were prospectively enrolled after providing informed consent at selected prenatal care centers in the United States, The Netherlands, and Sweden. Institutional review board approval was obtained at all participating centers.

### Sample collection and preparation

Approximately 20 mL of blood was collected from each subject prior to any invasive procedure into a Cell-free BCT tube (Streck, Omaha, NE). Samples were sent directly to the laboratory without processing and needed to be received within 7 days of collection with no temperature excursions indicating freezing. Plasma was isolated from blood via a double centrifugation protocol. cfDNA was isolated from plasma using the Dynabeads Viral NA DNA purification kit (Dyna, Grand Island, NY) protocol, with minor modifications, and each sample was arrayed into individual wells of a 96-well microtiter plate.

### Test methods

Each subject's cfDNA sample was isolated and quantified using the DANSR assay, which has been described previously.<sup>15</sup> Briefly, this method uses ligation of locus-specific oligonucleotides to produce a sequencing template only from selected genomic loci, thus reducing the amount of DNA sequencing needed. The FORTE algorithm, also previously described in detail,<sup>16</sup> was used to estimate the risk of aneuploidy for chromosomes 21 and 18 in each sample. The FORTE risk score is determined by calculating the odds ratio for trisomy based on chromosome 21 and 18 cfDNA counts, and fraction of fetal cfDNA in the sample,

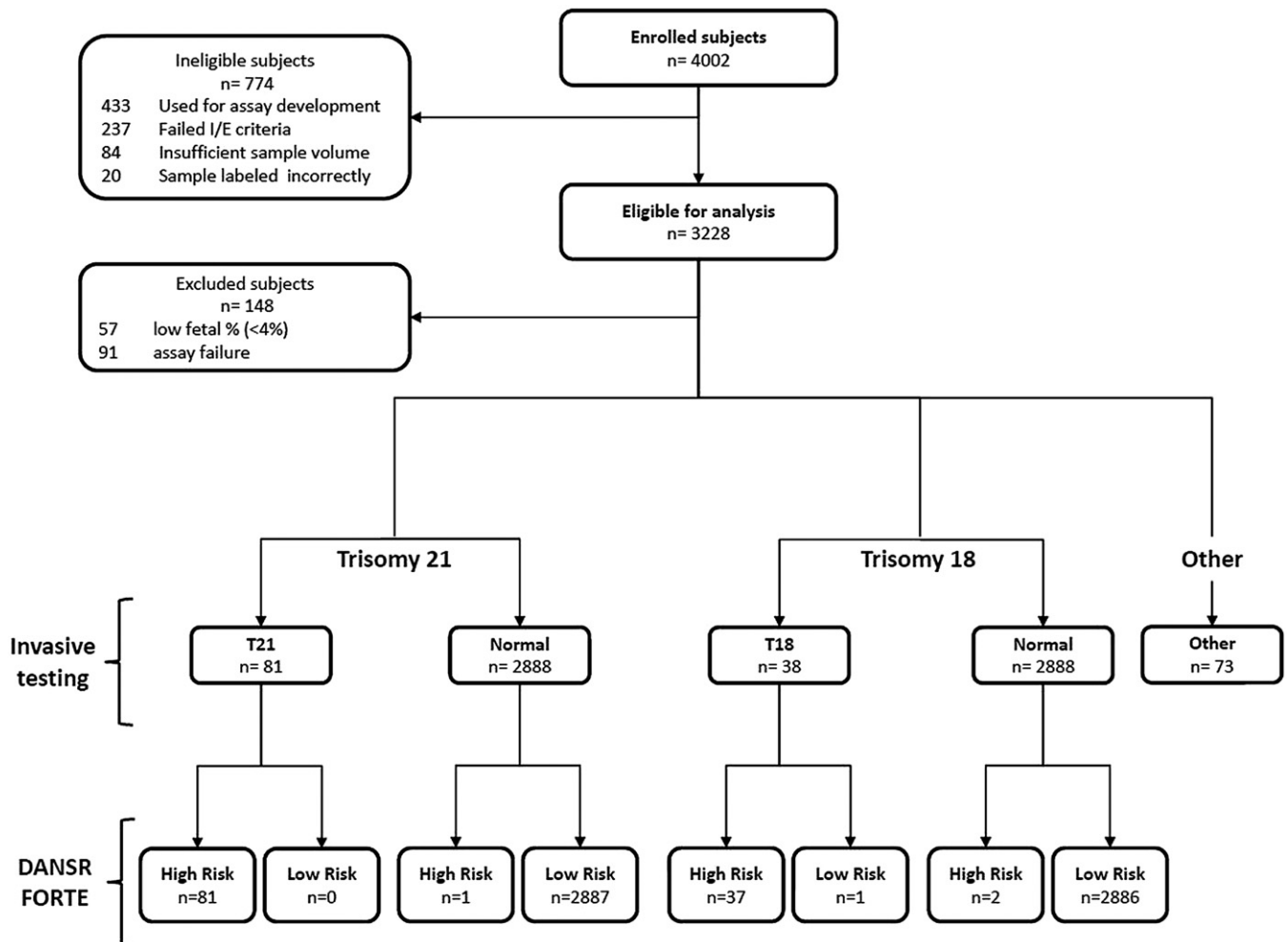
then applying this as a likelihood ratio to the a priori trisomy risk based on the maternal age and gestational age.<sup>16</sup> A predefined cutoff value of 1 in 100 (1%) was designated as the threshold for classifying a sample as High Risk vs Low Risk. The cutoff value was determined based on previous analyses that demonstrated an optimal separation between trisomy and euploid samples. Samples that did not generate a result were classified as low ( $<4\%$ ) fraction of fetal cfDNA, inability to measure fraction of fetal cfDNA, unusually high variation in cfDNA counts, and failed sequencing.

The laboratory personnel who performed the analyses were blinded to the clinical information associated with each sample. Finalized results were transferred to an independent data management center (Advance Research Associates, Mountain View, CA) for merging of assay and clinical data, and unblinding.

### Data analysis

Sample size was calculated based on obtaining sufficient cases of T21 to achieve lower bound 95% confidence intervals (CI) for sensitivity and specificity that were comparable or superior to current prenatal screening tests. The target performance for the DANSR and FORTE method was anticipated to be  $\geq 98\%$  for both sensitivity and specificity based on previous data.<sup>15-17</sup> Using this estimate, at least 60 cases of T21 would be required to provide a lower 95% CI of 90% for sensitivity. Assuming a T21 prevalence of 1 in 50 in the study cohort, based on a typical population of women undergoing invasive prenatal diagnosis, at least 3000 eligible subjects would be required. Categorical variables were summarized by the number and percentage of subjects in each category. Continuous variables were summarized as total number, mean, SD, minimum, median, and maximum values. We used  $\chi^2$  tests with Bonferroni correction when comparing categorical variables and proportions. Linear regression models were used to test the correlation between continuous variables (eg, percent fetal and gestational age) with the null hypothesis that the slope between 2 continuous variables is 0. Multivariate lo-

**FIGURE 1**  
**Flow diagram of subjects**



Flow diagram of subjects. Eligible subjects for analysis were classified into trisomy 21, trisomy 18, normal, and other based on invasive testing results. For purpose of this analysis, "normal" includes common chromosomal variants and balanced Robertsonian translocations.

DANSR, Digital ANALYSIS of Selected Regions; FORTE, Fetal-fraction Optimized Risk of Trisomy Evaluation; I/E, inclusion/exclusion.

Norton. Noninvasive chromosomal evaluation for fetal trisomy 21 and 18 detection. *Am J Obstet Gynecol* 2012.

gistic regression was used when the response variable was categorical. Standard analysis of variance models were used when the response variable was continuous and the explanatory variables were discrete. The 95% CI for sensitivity and specificity were computed using the method of Wilson.<sup>18</sup>

Analysis of samples using DANSR and FORTE included all evaluable subjects who had undergone invasive testing with fetal genotype analysis by karyotype, fluorescent in situ hybridization, or quantitative fluorescent polymerase chain reaction.

Prior to study unblinding, chromosomal abnormalities were categorized as T21, T18, T13, sex chromosome aneuploidy, triploidy, balanced translocation, unbalanced translocation, duplication, deletion, extra structurally abnormal chromosome, confined placental mosaic, mosaic (likely true), mosaic (likely artifact), and other. T21 cases resulting from unbalanced Robertsonian translocations were classified as "T21" and those with mosaicism for T21 or T18 were classified as "other." Subjects with commonly identified chromosomal rearrangements predicted to have a normal outcome, such as

inversions of chromosomes 1, 9, or 16, in addition to those with balanced Robertsonian translocations (inherited and de novo) were considered normal for the purpose of this analysis. Categorization was performed by a clinical geneticist (M.E.N.) based on review of each clinical karyotype report for all abnormal results.

For efficacy analysis, results from the DANSR assay and FORTE algorithm were compared against the reference standards of clinically adjudicated invasive testing results. Results from the DANSR assay and FORTE algorithm were provided as a trisomy risk score,

**TABLE 1**  
**Demographic and baseline characteristics of subjects evaluated**

| Demographic                            | Normal <sup>a</sup> (n = 3021) | Trisomy 21 (n = 84)    | Trisomy 18 (n = 42)    | Other (n = 81)                  | Total (n = 3228)     |
|--|--------------------------------|------------------------|------------------------|---------------------------------|----------------------|
| Maternal age, y, mean ± SD (range)     | 34.3 ± 6.3 (18–50)             | 35.4 ± 7.3 (18–47)     | 34.5 ± 6.1 (22–45)     | 32.0 ± 6.9 <sup>b</sup> (18–44) | 34.3 ± 6.4 (18–50)   |
| Gestational age, wk, mean ± SD (range) | 17 ± 4.1 (10–38.7)             | 16.4 ± 3.1 (11.6–25.7) | 16.2 ± 4.3 (10.9–29.4) | 16.6 ± 4.5 (10.0–34.1)          | 16.9 ± 4.1 (10–38.7) |
| Maternal ethnicity, n (%)              | 1504 (49.8)                    | 36 (42.9)              | 20 (47.6)              | 40 (49.4)                       | 1600 (49.6)          |
| Caucasian                              | 197 (6.5)                      | 3 (3.6)                | 2 (4.8)                | 5 (6.2)                         | 207 (6.4)            |
| African American                       | 406 (13.4)                     | 8 (9.5)                | 5 (11.9)               | 15 (18.5)                       | 434 (13.4)           |
| Asian                                  | 677 (22.4)                     | 27 (32.1)              | 10 (23.8)              | 18 (22.2)                       | 732 (22.7)           |
| Hispanic                               | 237 (7.8)                      | 10 (11.9)              | 5 (11.9)               | 3 (3.7)                         | 255 (7.9)            |
| Other                                  |                                |                        |                        |                                 |                      |
| Fetal DNA in sample                    |                                |                        |                        |                                 |                      |
| n <sup>c</sup>                         | 2888                           | 81                     | 38                     | 73                              | 3080                 |
| Percent fetal DNA, mean ± SD (range)   | 11 ± 4.5 (4.2–51.3)            | 11.6 ± 4.2 (5.1–23.3)  | 10 ± 3.8 (4.9–20.8)    | 11.6 ± 5.1 (4.5–32.0)           | 11 ± 4.5 (4.2–51.3)  |

<sup>a</sup> For purpose of this analysis, "normal" includes common chromosomal variants and balanced Robertsonian translocations; <sup>b</sup>  $P < .05$ ; <sup>c</sup> Based on 3080 samples successfully tested.

Norton. Noninvasive chromosomal evaluation for fetal trisomy 21 and 18 detection. *Am J Obstet Gynecol* 2012.

with the upper and lower risk value capped at 99% (99 in 100) and 0.01% (1 in 10,000), respectively. Primary calculations for sensitivity and specificity were based on a 1% (1 in 100) cutoff to designate results as High Risk or Low Risk for a given trisomy. Additional analyses on test performance were reported at different risk cutoff values.

## RESULTS

### Study participants

A total of 4002 pregnant women were enrolled in the study from Aug. 1, 2010, through Nov. 1, 2011, across 3 countries. Samples from all 3 countries were analyzed together as a single cohort given the use of specialized blood collection tubes that preserve cfDNA in blood for up to 14 days.<sup>19</sup> Of the 4002 plasma samples obtained, 433 samples were used for assay development with a subset of these reported on previously.<sup>16</sup> An additional 341 samples were ineligible prior to analysis for failing to meet inclusion/exclusion criteria (n = 237), insufficient sample volume (n = 84), and incorrect sample labeling (n = 20). Of the 3228 samples remaining, all underwent analysis. Of analyzed samples, 57/3228 cases (1.8%) were excluded due to low (<4%) fraction of fetal cfDNA and an additional 91/3228 (2.8%) samples were excluded due to assay failure. Assay failure modes

included inability to measure fraction of fetal cfDNA, unusually high variation in cfDNA counts, and failed sequencing. Figure 1 shows the flow of samples between enrollment and analysis.

The mean maternal age was 34.3 (range, 18–50) years for all subjects, and the cohort was racially and ethnically diverse, with 49.6% (1600/3228) of the population identified as Caucasian, 6.4% (207/3228) African American, 13.4% (434/3228) Asian, 22.7% (732/3228) Hispanic, and 7.9% (255/3228) other. For invasive testing, 818/3228 (25.3%) of subjects had karyotyping with CVS, and 2410/3228 (74.7%) by amniocentesis. There were no statistical differences as determined by  $\chi^2$  tests with Bonferroni correction between the normal and trisomy groups among these variables.

The mean gestational age of the cohort was 16.9 (range, 10.0–38.7) weeks with no statistical difference between normal and trisomy groups based on linear regression analysis (Table 1). In the entire cohort for analysis, there were 84 cases of T21, 42 cases of T18, and 81 other abnormal karyotypes including 4 cases of T13 and T21 sex chromosomal aneuploidies (Table 2).

### Fetal fraction

Overall, the fraction of fetal cfDNA expressed as a percentage was 11% in the

samples tested (Table 1). The fetal fraction did not vary with race/ethnicity, maternal age, or trisomy type using standard 1-way analysis of variance and linear regression analysis. The fraction of fetal cfDNA by gestational age week is depicted in Figure 2. For gestational ages between 10–22 weeks, there was no statistical difference in fraction of fetal cfDNA.

### Test performance

The classification of samples as High Risk or Low Risk at various cutoffs using the DANSR and FORTE method, and the correlation with invasive testing is shown in Table 3. Applying the predefined 1% cutoff to the 81 T21 cases in which a result was obtained, all yielded a High Risk result for a sensitivity of 100% (95% CI, 95.5–100%). Of the normal cases, 2887/2888 were classified as Low Risk for T21, yielding a specificity of 99.97% (95% CI, 99.8–99.99%) or false-positive rate of 0.03% (95% CI, 0.002–0.20%) (Figure 3 and Table 3). The 1 chromosomally normal case reported as High Risk for T21 had a risk score of 1.1% (1 in 92).

Applying the predefined 1% cutoff to the T18 cases in which a result was obtained, 37 of 38 yielded a High Risk result for a sensitivity of 97.4% (95% CI, 86.5–99.9%). Of the normal cases, 2886/2888

were classified as Low Risk for T18, yielding a specificity of 99.93% (95% CI, 99.75–99.98%) or false-positive rate of 0.07% (95% CI, 0.02–0.25%) (Figure 3 and Table 3). One T18 case by invasive testing was classified as Low Risk with a score of <0.01% (1 in 10,000). Two cases with a normal karyotype by invasive testing were classified as High Risk for T18 with risk scores of 73.8% and >99%.

Within this cohort, the positive predictive values for T21 and T18 were 98.8% and 94.9%, respectively. The negative predictive values for T21 and T18 were 100% and 99.96%, respectively.

The presence of other chromosomal abnormalities besides T21 or T18 did not impact the fetal fraction or the analysis of T21 or T18 risk. In other words, these cases did not have more false-positive results, or more cases of assay failure. Within this group, 1 case of confined placental mosaicism for T21 by CVS with a normal amniocentesis result was classified as Low Risk, and 1 case of confined placental mosaicism for T18 with a normal amniocentesis result was also classified as Low Risk. There was also 1 T21 mosaic on amniocentesis that was classified as Low Risk.

## COMMENT

This study demonstrates the efficacy of a chromosome-selective approach to cfDNA testing for fetal T21 and T18. Overall, this approach had 100% sensitivity and 99.97% specificity for T21 and 97.4% sensitivity and 99.93% specificity for T18 in this large cohort of primarily high-risk women. The improvement in sequencing efficiency achieved by the DANSR platform provides a more affordable, scalable approach to cfDNA analysis with high throughput and potential for widespread clinical utility.

In this study, there was generally at least a 100,000-fold magnitude separation between the risk score of trisomy vs disomy, with reported risks of >99% or  $\leq 1/10,000$  for trisomy. In a small number of cases (17, or 0.5% of the total cohort), the risk score fell between these dichotomous values. An advantage of the FORTE algorithm is that it allows an assessment of the magnitude of risk for

**TABLE 2**  
**Classification of chromosomal abnormalities**

| Chromosomal abnormality type           | n  | Percent of all abnormalities |
|--|----|------------------------------|
| Trisomy 21                             | 84 | 40.6                         |
| Trisomy 18                             | 42 | 20.3                         |
| Trisomy 13                             | 4  | 1.9                          |
| Sex chromosome aneuploidy <sup>a</sup> | 21 | 10.1                         |
| Triploidy                              | 5  | 2.4                          |
| Balanced translocation                 | 10 | 4.8                          |
| Unbalanced translocation               | 3  | 1.4                          |
| Duplication                            | 3  | 1.4                          |
| Deletion                               | 2  | 1.0                          |
| Extra structurally abnormal chromosome | 5  | 2.4                          |
| Confined placental mosaic              | 8  | 3.9                          |
| Mosaic—likely true                     | 11 | 5.3                          |
| Mosaic—likely artifact                 | 3  | 1.4                          |
| Other                                  | 6  | 2.9                          |

<sup>a</sup> Includes mosaics.

Norton. Noninvasive chromosomal evaluation for fetal trisomy 21 and 18 detection. *Am J Obstet Gynecol* 2012.

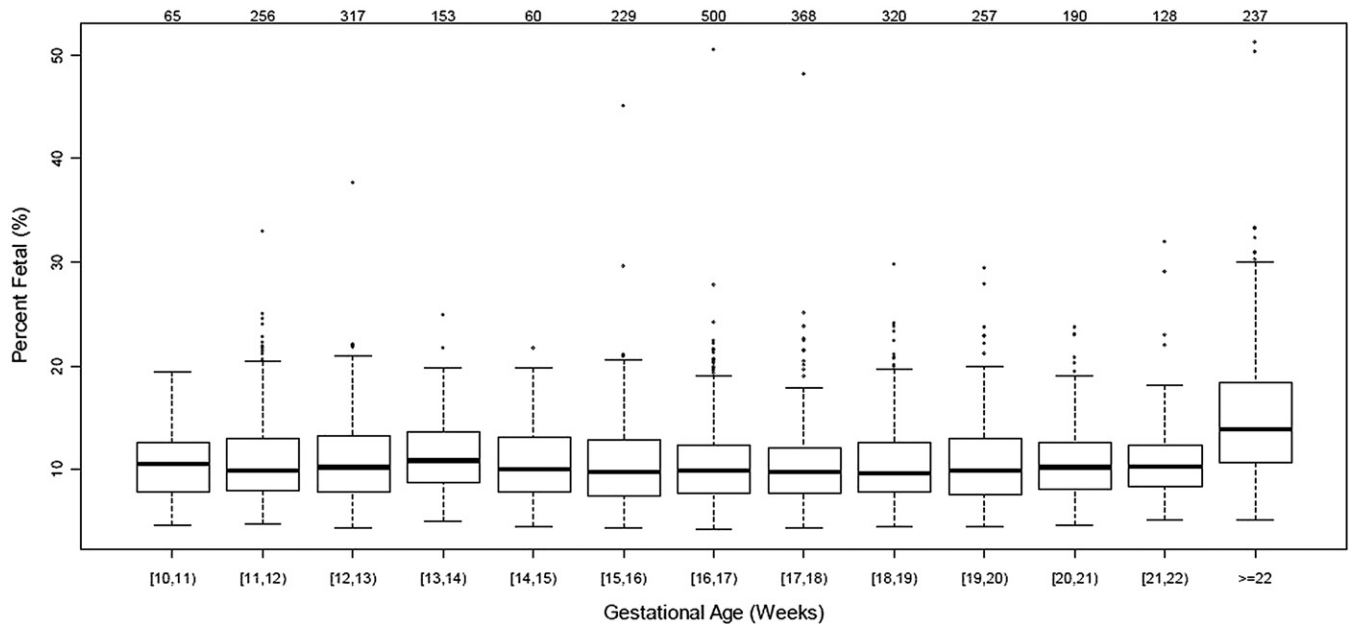
such cases. In the 1 false-positive T21 result, the risk was calculated at 1.1% (1 in 92), so was reported as High Risk, although clearly the most likely outcome with such a risk is a non-T21 fetus.

The fraction of cfDNA that is fetal is a key component of the FORTE algorithm, with trisomy becoming easier to detect at higher fetal fractions. Between 10–22 weeks of gestation, when the majority of patients would likely have this test performed, we did not find a significant difference in the fetal fraction. While earlier studies have shown a relationship between gestational age and fetal DNA amounts, the increase in fetal DNA was not seen until the third trimester.<sup>20</sup> More recent studies corroborate our findings that in the first and second trimester there is no relationship between gestational age and fetal DNA amounts.<sup>10,21</sup> Therefore, it appears that there is no benefit to waiting until later in the gestational age window to undergo testing. For cases in which the fetal fraction is too low to analyze, repeating the test at a later gestational age may be useful if fetal fraction varies within individuals. Further research to study this is warranted.

A limitation of this assay, as with MPSS, is that a percentage of cases do not provide a result. In this study, a total of 4.6% (148/3228) of cases had either low fetal DNA fraction or assay failure. Reassuringly, we found that the assay failure rate was comparable in normal vs trisomy cases. The reasons for assay failure, including low fetal fraction, are not known. In this study, we did not find assay failure to be associated with gestational age, maternal age, race/ethnicity, or fetal karyotype. Larger studies, with follow-up of cases in which results are not obtained, will be required to determine whether there is any clinical significance to this outcome. As with other published series<sup>11,14,17</sup> we also found that testing for T18 appears to be somewhat less robust than testing for T21. However, the overall numbers of abnormal cases were too small to identify statistically significant differences in fetal fraction, or in false-positive or false-negative rates.

In this cohort of women, it is notable that 39% of the abnormal karyotype results obtained from invasive testing and predicted to have phenotypic consequences were abnormalities other than

**FIGURE 2**  
**Fraction of cfDNA by gestational age**



Fraction of cell-free DNA (cfDNA) by gestational age. Fraction of cfDNA is plotted against each gestational week from weeks 10–22 and then grouped for gestational age >22 weeks. Median is represented by line within box plot, with box plot representing 25th and 75th percentile and outer hash marks representing lower and upper data within 1.5 interquartile ranges of box plot. Number of subjects within each gestational age bin are represented at top. Norton. *Noninvasive chromosomal evaluation for fetal trisomy 21 and 18 detection. Am J Obstet Gynecol* 2012.

T21 and T18. As with current screening, cfDNA at present is focused on the most common and clinically significant aneuploidies. Although the technology may improve and allow detection of an increasing number of chromosomal abnormalities, currently the avoidance of the risks of invasive testing come at the cost of more focused detection of only specific aneuploidies. With improvements in serum and ultrasound screening for T21 and T18, the rate of invasive testing has decreased in recent years.<sup>22–24</sup> In choosing screening over diagnostic

testing, women are making a choice to forego the additional information provided by a full karyotype to avoid the risk of loss associated with CVS or amniocentesis. They are also currently weighing the false-positive and false-negative rates of current testing, which are substantial.<sup>1–4</sup>

Prior studies of cfDNA have been case-control studies, comparing detection in subjects identified with fetal T21 or T18 to a selected group of those with normal karyotypes.<sup>8–17</sup> This current study included an entire large cohort of subjects

undergoing invasive prenatal diagnosis, and we were thus able to assess abnormalities such as Robertsonian translocations and the potential impact of other complex and unusual chromosomal abnormalities on cfDNA test results. Overall, the presence of chromosomal variants (eg, the common inversions of chromosomes 1, 9, and 16), as well as deletions, duplications, and other rare anomalies, did not interfere with detection of T21 or T18. In addition, while our study included primarily high-risk women, all women undergoing invasive

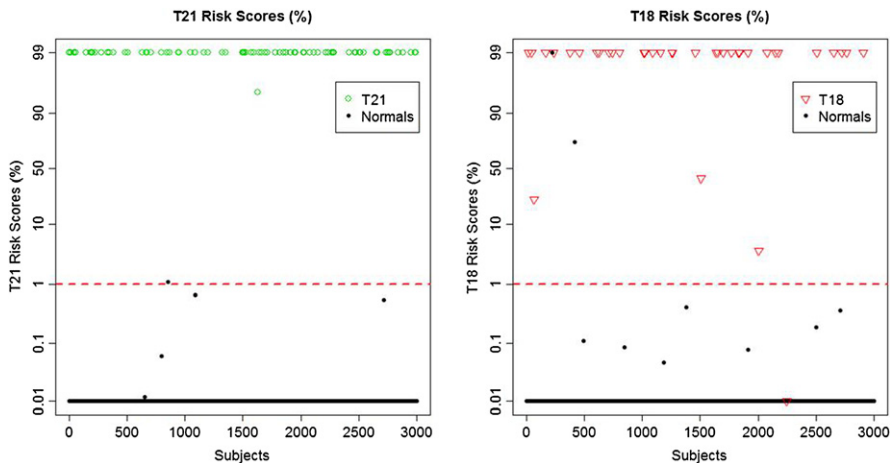
**TABLE 3**  
**Trisomy evaluation performance at various cutoff values**

| Cutoff           | Trisomy 21   |                    | Trisomy 18    |                    |
|------------------|--------------|--------------------|---------------|--------------------|
|                  | Sensitivity  | Specificity        | Sensitivity   | Specificity        |
| 1 in 1000 (0.1%) | 100% (81/81) | 99.90% (2885/2888) | 97.4% (37/38) | 99.79% (2882/2888) |
| 1 in 300 (0.33%) | 100% (81/81) | 99.90% (2885/2888) | 97.4% (37/38) | 99.86% (2884/2888) |
| 1 in 100 (1%)    | 100% (81/81) | 99.97% (2887/2888) | 97.4% (37/38) | 99.93% (2886/2888) |
| 1 in 10 (10%)    | 100% (81/81) | 100% (2888/2888)   | 94.7% (36/38) | 99.93% (2886/2888) |

Predetermined cutoff of 1 in 100 (1%) highlighted.

Norton. *Noninvasive chromosomal evaluation for fetal trisomy 21 and 18 detection. Am J Obstet Gynecol* 2012.

**FIGURE 3**  
**T21 and T18 detection with DANSR and FORTE**



Trisomy 21 (T21) and trisomy 18 (T18) detection with Digital Analysis of Selected Regions and Fetal-fraction Optimized Risk of Trisomy Evaluation. Risk scores are presented consecutively based on subject enrollment date with 1% cutoff designated by dashed line. T21 cases are *green*, T18 cases are *red*, and normal cases are *black*.

Norton. Noninvasive chromosomal evaluation for fetal trisomy 21 and 18 detection. *Am J Obstet Gynecol* 2012.

prenatal diagnosis for any indication were eligible, so our cohort represents a broader population than reported in previous studies.

There has been much discussion about how noninvasive prenatal testing via cfDNA might fit in with the current menu of prenatal testing options, including current screening and diagnostic testing.<sup>12</sup> The place of this technology remains uncertain, and there is concern that introduction of another test will potentially add to the confusion of an already complicated prenatal testing environment. Because of the high cost and relative inefficiency of MPSS, such testing has been suggested as a follow-up screening test to be offered to patients identified as high risk based on current multiple marker screening. While the use of cfDNA as an intermediate screening tool would likely reduce invasive testing rates, this approach would add complexity to patient decision-making and delay diagnosis for affected fetuses, without necessarily offering additional information for those women who do ultimately undergo invasive testing.

Conversely, cfDNA has also been discussed as an alternative to invasive diagnostic testing, although with apprecia-

tion that even the excellent detection rates achieved with cfDNA testing are not comparable to those obtained with invasive diagnosis. In addition, invasive testing allows analysis of a complete karyotype or application of microarray-based comparative genomic hybridization, providing substantial additional information beyond common fetal trisomy detection.

With the methods reported here, the higher throughput and lower cost<sup>16</sup> make this technique potentially scalable for population screening. cfDNA offers high accuracy with a single blood test, as opposed to the complex testing algorithms and multiple blood and ultrasound examinations required with current integrated screening. As such, this technology is potentially suitable as a replacement for current, relatively inefficient aneuploidy screening. If cfDNA were scalable as a population-based screening test, the type of abnormalities detected would not be different, but the test performance on both sensitivity and specificity would be far better. However, further experience in larger populations of average-risk women is needed to clarify the role and utility of cfDNA in clinical practice. ■

#### ACKNOWLEDGMENTS

We would like to thank the following institutions and individuals for recruiting subjects into the study: Stanford University, Stanford, CA: Amit Levi; Riverside Perinatal Diagnostic Center, Riverside, CA: Bobby Brar, Shelley Mueller; Institute of Prenatal Diagnosis and Reproductive Genetics, San Gabriel, CA: Yolanda Brown; University of California–San Diego, San Diego, CA: Susan Mayo, Aileen Fernando; San Gabriel Valley Perinatal Medical Group, West Covina, CA: Norma Sidhu; Cedars-Sinai Medical Center, Los Angeles, CA: Deebra Smith, Renatha Edwards; Medical College of Wisconsin, Milwaukee, WI: Aoy Tomita-Mitchell, PhD, Julie Deavers; University of Tennessee, Chattanooga, TN: Lorrie Mason, Mandy Schwartz; University of California–San Francisco, San Francisco, CA: Katherine Bianco, MD, Brian Shaffer, MD; Sahlgrenska University Hospital, Gothenburg, Sweden: Rose-Marie Holst, MD, PhD, Hans Bokström, MD, PhD; Northwest Perinatal Center, Portland, OR: Kelly Charland; Leiden University Medical Center, Leiden, The Netherlands: Phebe N. Adama van Scheltema, MD, PhD, Jennie Verdoes; NorthShore University HealthSystem, Evanston, IL: Mara Dinsmoor, MD, Megan Czarniecki; Oregon Health Sciences and University, Portland, OR: Leonardo Pereira, MD, Mary Ames, MD, Monica Rincon; Columbia University, New York, NY: Ronald Wapner, MD, Caroline Torres; Norton Healthcare, Louisville, KY: Helen How, MD, Christina Soto; Allina Health System, Minneapolis, MN: William A. Block Jr, MD, Lori Franelake; Center for Reproductive Medicine, Wichita, KS: David Grainger, MD, MPH, Bryan Baker; Prenatal Diagnosis of Northern California Medical Group Inc, Sacramento, CA: Douglas Hershey, MD, Dianna Allen; Center for Maternal and Fetal Medicine, Annapolis, MD: Jeffrey Spencer, MD, Georgette Saba; San Diego Perinatal Center, San Diego, CA: Holly Casele, MD, Larry Cousins, MD; Indiana University Women's Health Care, Indianapolis, IN: Men-Jean Lee, MD, Cornelia David-Moore; Fetal Diagnostic Center, Pasadena, CA: Gregory R. Devore, MD, Maria Meza; Northwestern Reproductive Genetics Inc, Chicago, IL: Eugene Pergament, MD, PhD, Candace Pennick; Virginia Women's Center, Richmond, VA: Peter Zedler, MD, Janet Phillips; Fetal Diagnostic Institute of the Pacific, Honolulu, HI: Greigh Hirata, MD, Tammy Lynn Stumbaugh; Rush University Medical Center, Chicago, IL: Jacques Abramowicz, MD, Amy Knight-Johnson; Atlanta Perinatal Consultants, Atlanta, GA: Lawrence Stone, MD, Erin Smith; Carolinas Medical Center, Charlotte, NC: Saiju Joy, MD, Susan Kullstam; White Memorial Medical Center, Obstetrics/Gynecology, Los Angeles, CA: Kathryn Shaw, MD, Evelyn Mata; Altus Research, Lake Worth, FL: Samuel Lederman, MD, Val Davis; Spectrum Women's Healthcare, Los Angeles, CA: Mark Dwight, MD, John Knight; Aurora Health Care, Milwaukee, WI: Tom Wigton, MD, Carol Davis; Houston Perina-

tal Associates, Houston, TX: Brian Kirson, MD, Becky Rodriguez; Complete Healthcare for Women, Columbus, OH: Milroy J. Samuel, MD, Sudha Suthanthira; Atlanta Perinatal Physicians, Atlanta, GA: Dexter Page, MD, Patrice Walker; Robert Wood Johnson University Hospital, New Brunswick, NJ: Todd J. Rosen, MD, Elena Ashkinadze; Bridgeport Hospital, Bridgeport, CT: Gary Kleinman, MD, Dallas Reed, MD; Genesis Health Central Women's Care, Dallas, TX: Natalie Chambliss Light, MD; Beth Israel Deaconess Medical Center, Boston, MA: Michele Hacker, PhD; Greenville Hospital System University Medical Center, Greenville, SC: Kenneth F. Trofatter Jr, MD, PhD, Barbara Strickland; Florida Obstetrics/Gynecology, Margate, FL: Ivonne Reynolds, DO, Octavia Reed; Pro-Health Care, Waukesha, WI: Randall Kuhlmann, MD, Lisa Baker; Medical University of South Carolina, Charleston, SC: Christopher Robinson, MD, Aimee King; Private practice, Los Angeles, CA: Janet Horenstein, MD; Texas Health Care, Dallas, TX: Delbert A. Johns, MD, Theresa Cheyne; Drexel Obstetrics and Gynecology, Philadelphia, PA: Lauren Plante, MD, Cheryl Tocci; Kentuckiana Perinatology, Louisville, KY: Marcello Pietrantoni, MD.

We would like to recognize the laboratory personnel at Ariosa Diagnostics for processing of the samples in this study.

## REFERENCES

1. American College of Obstetricians and Gynecologists. ACOG practice bulletin no. 77: screening for fetal chromosomal abnormalities. *Obstet Gynecol* 2007;109:217-27.
2. Malone FD, Canick JA, Ball RH, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med* 2005;353:2001-11.
3. Nicolaidis KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004;191:45-67.
4. Rozenberg P, Bussi eres L, Chevret S, et al. Screening for Down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population. *Am J Obstet Gynecol* 2006;195:1379-87.
5. American College of Obstetricians and Gynecologists. ACOG practice bulletin No. 88, December 2007: invasive prenatal testing for aneuploidy. *Obstet Gynecol* 2007;110:1459-67.
6. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. *Cochrane Database Syst Rev* 2003;3:CD003252.
7. Shulman LP. One small step and one giant leap for non-invasive prenatal screening: an editorial. *Am J Obstet Gynecol* 2011;204:183-5.
8. Chiu RWK, Akolekar R, Zheng YWL, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed plasma DNA sequencing: large scale validity study. *BMJ* 2011;342:1-9.
9. Ehrich M, Deciu C, Zwiefelhofer T, et al. Non-invasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011;204:205.e1-11.
10. 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;13:913-20.
11. Bianchi DW, Platt LK, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012;119:1-12.
12. Chitty LS, Hill M, White H, Wright D, Morris S. Noninvasive prenatal testing for aneuploidy—ready for prime time? *Am J Obstet Gynecol* 2012;206:269-75.
13. 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;14:296-305.
14. Chen EZ, Chiu RWK, Sun H, Akolekar R. Non-invasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. *PLoS One* 2011;6:e21791.
15. Sparks A, Wang E, Struble C, et al. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. *Prenat Diagn* 2012;32:1-7.
16. Sparks AB, Struble CA, Wang E, et al. Non-invasive 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;206:319.e1-9.
17. Ashoor G, Syngelaki A, Wagner M, 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;206:322.e1-5.
18. Wilson EB. Probable inference, the law of succession, and statistical inference. *J Am Stat Assoc* 1927;22:209-12.
19. Fernando MR, Chen K, Norton S, et al. A new methodology to preserve the original proportion and integrity of cell-free fetal DNA in maternal plasma during sample processing and storage. *Prenat Diagn* 2010;30:418-24.
20. Lun FM, Chiu RW, Chan KC, Leung TY, Lau TK, Lo YM. Microfluidics digital PCR reveals a higher than expected fraction of fetal DNA in maternal plasma. *Clin Chem* 2008;54:1664-72.
21. Ashoor G, Poon L, Syngelaki A, Mosimann B, Nicolaidis KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: effect of maternal and fetal factors. *Fetal Diagn Ther* 2012 [Epub ahead of print].
22. Fang YM, Benn P, Campbell W, Bolnick J, Prabalos AM, Egan JF. Down syndrome screening in the United States in 2001 and 2007: a survey of maternal-fetal medicine specialists. *Am J Obstet Gynecol* 2009;201:97.e1-5.
23. Norton ME, Norem C, Nakagawa S, Gregorich S, Kuppermann M. Effect of changes in prenatal screening and diagnostic testing policies on resources utilization in a large, integrated health care system. *Am J Obstet Gynecol* 2012;206:S317.
24. Goldman S, Currier R. Trends in prenatal diagnosis in CA over a decade, 1998-2009. *Am J Obstet Gynecol* 2012;206:S321.