

Trial by Dutch Laboratories for Evaluation of Non-Invasive Prenatal Testing.

Part I - Clinical Impact

Running head: TRIDENT: Dutch trial of implementing NIPT

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What's already known about this topic?

- Non-Invasive prenatal Testing (NIPT) for trisomies 21, 18, and 13 is mainly offered in a commercial setting.
- Offering NIPT to pregnant women at increased risk for fetal trisomy leads to a reduction in invasive testing.

What does this study add?

- NIPT implemented as a contingent test under the umbrella of a national health authority-supported fetal trisomy screening program facilitates optimal counseling with close to 100% follow-up, results in 86% uptake and confirms a major reduction of invasive tests.

Abstract

Objective. To evaluate the clinical impact of nationwide implementation of genome-wide Non-Invasive Prenatal Testing (NIPT) in pregnancies at increased risk for fetal trisomies 21, 18 and 13.

Method. Women with elevated risk based on first trimester combined testing ($FCT \geq 1:200$) or medical history, not advanced maternal age alone, were offered NIPT as contingent screening test, performed by Dutch University Medical laboratories. We analyzed uptake, test performance, redraw/failure rate, turn-around time and pregnancy outcome.

Results. Between April 1st and September 1st, 2014, 1413/23232 (6%) women received a high-risk FCT result. Of these, 1211 (85.7%) chose NIPT. 179 women had NIPT based on medical history. 1386/1390 (99.7%) women received a result, 6 (0.4%) after redraw. Mean turn-around time was 14 days. Follow-up was available in 1376 (99.0%) pregnancies. NIPT correctly predicted 37/38 (97.4%) trisomies 21, 18 or 13 (29/30, 4/4 and 4/4 respectively). 5/1376 (0.4%) cases proved to be false positives: trisomies 21 (n=2), 18 (n=1) and 13 (n=2). Estimated reduction in invasive testing was 62%.

Conclusion. Introduction of NIPT in the Dutch National healthcare-funded Prenatal Screening Program resulted in high uptake and a vast reduction of invasive testing. Our study supports offering NIPT to pregnant women at increased risk for fetal trisomy.

Keywords: fetal trisomy, prenatal screening, non-invasive prenatal testing, cfDNA, implementation study.

Introduction

Non-invasive prenatal testing (NIPT) for fetal trisomy using cell-free DNA analysis from maternal plasma is increasingly offered to pregnant women worldwide, reducing the number of invasive diagnostic tests.¹ Of the women eligible for invasive testing by maternal age and first trimester combined test (FCT), more than 90% carry a healthy child. Fear for invasive testing makes many of these women refrain from further testing,²⁻⁴ and some even refrain from screening.^{5;6}

In March 2011, a multidisciplinary Dutch NIPT Consortium was founded as a platform for collaboration between obstetric caregivers, clinical geneticists and laboratory specialists, the national prenatal screening organization, the Dutch Genetic Alliance (VSOP), ethicists, insurance companies and policy makers. In an increasing number of countries, NIPT has been introduced commercially without governmental guidance. In the Netherlands such an introduction is subject to a governmental license under the Population Screening Act because screening is offered for untreatable disorders. The NIPT Consortium received this license as well as funding for the nationwide TRIDENT study (Trial by Dutch laboratories for Evaluation of Non-Invasive Prenatal Testing), starting April 1st, 2014. The license approved NIPT as an alternative option to invasive testing for women with a $\geq 1:200$ FCT result and for women with an increased risk for trisomy 21, 18 or 13 because of medical history. Direct access to NIPT based on maternal age alone was not allowed, and the direct access to invasive testing based on maternal age alone was halted.

The TRIDENT license was initially granted for two years. We expected to perform 1000-2000 NIPTs in the first year. The aim of this study was to evaluate uptake, test results, test performance and pregnancy outcomes of all pregnancies after the first 1000 participating women had delivered. Parallel to this study, we performed a questionnaire study to analyze

women's preferences and decision-making. Results will be reported separately (Van Schendel *et al.* Paper Part II).

Methods

Organization of prenatal screening in the Netherlands

In the Netherlands, FCT has been available for all pregnant women since 2007, upon payment of €165. Uptake is between 25 and 30%.⁷ All follow-up testing including NIPT is currently reimbursed by the compulsory health insurance after subtraction of a deductible of 360 euros. National screening programs are coordinated and monitored by the Centre for Population Screening of the National Institute for Public Health and the Environment (RIVM/CvB), that resorts under the Ministry of Health, Welfare and Sport. This organization provides uniform patient information in different languages, guidance documents, and e-learning for professionals.

Participating women and inclusion procedure

Our study group consisted of all pregnant women who chose NIPT because of an increased risk for trisomy 21, 18 or 13 based on FCT result or based on medical history (previous child with such a trisomy or a balanced translocation in one of the parents). FCT results were reported as risks for trisomy 21, 18 and 13. FCT is monitored and audited per region and reported to the RIVM/CvB. A program committee (with representatives of the participating professions and organizations) advises the CvB about the program. Eight Regional Centers (affiliated to the University Medical Centers) have been licensed for the FCT screening and are monitoring and auditing the quality of Nuchal Translucency (NT) measurements as well as the biochemical part of the testing as the final metrics of the FCT. FCT includes

measurement of: PAPP-A (Pregnancy-Associated Plasma Protein A) and β -hCG (beta subunit of human Chorionic Gonadotrophin). NT measurements are performed by trained ultrasonographers according to guidelines and with requirements for a minimal number of measurements annually. Exclusion criteria were: multiple pregnancies, vanishing twins, fetal nuchal translucency ≥ 3.5 mm or other structural anomalies, chromosome anomaly or history of maternal malignancy, gestational age $<10+0$ weeks, women <18 years old, and inability to give informed consent.

As required in the National Prenatal Screening Protocol all pregnant women were asked whether they wanted to be informed on options for screening for fetal anomalies at the first prenatal care visit. If so, they were counseled by a certified midwife or obstetrician on the FCT and the 20-week anomaly scan, and about NIPT as an alternative for invasive testing. As part of the TRIDENT study, counselors had received additional training on relevant aspects of NIPT. Written information and a website (www.meerovernipt.nl) were available for further reading.

Women with an increased risk based on FCT or medical history were referred for further counseling to one of the eight Centers for Prenatal Diagnosis, or one of their satellite centers. Women were given the choice between NIPT, invasive testing or no (follow-up) test. Extensive oral and written information on all tests was provided. For counseling on NIPT, performance data from a review by Mersy *et al.*⁸ were used: sensitivity and specificity around 99.0% for trisomy 21. We predicted a turn-around time (TAT) of 14-21 days. Women were informed that NIPT examines DNA fragments from the placenta cells rather than from the fetus itself, providing a biological explanation for some false positive and very rare false negative results. The study was a nationwide research study on all aspects of introducing NIPT in the prenatal screening program for trisomy 21, 13 and 18. A genome-wide approach was used allowing the detection of other chromosomal abnormalities. Women were informed

that other abnormal results besides trisomy 21, 18 or 13 are occasionally found, and that any potentially clinically relevant abnormal NIPT result would be discussed with them, potentially leading to an offer of detailed ultrasound, invasive testing and/or parental testing. Women were informed that fetal sex and sex chromosomal aneuploidies would not be communicated, as the ministerial license did not allow analysis of sex chromosomes.

For counseling on invasive testing the following information was given: how the procedures are performed, risk-figures for procedure-related miscarriage (0.3-0.5%), TAT, and genetic tests done by chorionic villi sampling (CVS) or amniocentesis (AS). The latter differed according to the indication and patient history and local policy and could be QF-PCR for trisomy 13, 18 and 21 only, karyotyping, or array.

All women signed an informed consent form. Permission for the study was granted by the Minister of Health (11016-118701-PG). The study was also approved by local University Medical Center Ethics Committees.

Sample collection

All eight University Hospitals and 13 satellite centers participated. Women were given an unique TRIDENT study number. A custom-made TRIDENT page was integrated in the Astraia fetal medicine database (version 1.23, astraia software GmbH, Munich, Germany) and contained fields for entry of maternal characteristics, FCT results, invasive prenatal diagnosis and pregnancy outcome data.

Two to four EDTA anticoagulated or Cell-Free DNA BCT (Streck, Omaha NE, USA) tubes of 6-10 ml blood were taken and transported the same day (EDTA tubes) or within 7 days (Streck tubes) to a Clinical Genetics laboratory for analyses. The laboratories kept a dataset for cross-checking. The RIVM/CvB database Peridos, to which data on prenatal

screening are uploaded from the electronic patient files of obstetric caregivers, was used to calculate uptake of NIPT in the group of women with an increased risk based on the FCT.

Massively parallel shotgun sequencing

In the years preceding the TRIDENT study, all eight University Hospital Genetic Laboratories had validated massively parallel shotgun sequencing for the prediction of fetal trisomies, and shared validation samples for quality control. During the project, six laboratories performed the testing, and all were involved in analysis, interpretation and reporting. Genome-wide sequencing was performed with the Illumina HiSeq 2500 or the Life Technologies 5500 W SOLiD sequencer. Bioinformatic analysis was performed either by z-score calculation, with z-scores above 3.0 as a cut-off value between indicative or not indicative for trisomy, or by the WISECONDOR algorithm under standard settings to call trisomies.⁹ As an extra check, several labs combined the two methods. WISECONDOR detects trisomies and monosomies of all autosomes, as well as deletions and duplications >20Mb. Fetal fraction was not routinely measured. One laboratory estimated fetal fraction in male fetuses, and requested a redraw when fetal fraction was lower than 4%.

Outcome variables

The primary clinical outcome data for this part of the TRIDENT study were uptake, defined as the percentage of women eligible for NIPT based on FCT result that actually chose to have NIPT, test characteristics of NIPT performed by the Dutch laboratories, failure rate, and TAT (days between blood draw and reporting to the patient). As a secondary outcome, we made an inventory of abnormal findings other than full trisomy 21, 18 and 13 and their outcome. The standard follow-up procedure was a return form filled out and returned by the woman after pregnancy/birth. In case of abnormal findings, follow-up data were gathered as needed:

invasive testing results, ultrasound data, genetic testing in products of conception (cord blood, placenta), birth data and data of postnatal examination.

Statistical analysis

Descriptive statistics, with mean, SD and range, were used to describe uptake, failure rate and TAT. Predictive performance was analyzed using two-by-two tables, and calculation of sensitivity, specificity, and positive and negative predictive values.

Results

Five months into the TRIDENT study, we already had included over 1000 pregnancies. We decided to report on the results between the start of the study, April 1, 2014, and September 1, 2014, as soon as clinical follow-up was complete. The last woman of this group delivered in March 2015. Characteristics of the study group are given in Table 1.

During the first five months of the study, 1413/23232 women (6.1%) who underwent FCT received a trisomy risk $\geq 1:200$. Of these, 1211 underwent NIPT, uptake in the screening group was 1211/1413 (85.7%). An additional 179 NIPT tests were done because of medical history. The uptake in this subgroup could not reliably be assessed due to an unknown denominator. In total, 1390 (1211+179) pregnant women had a blood sample taken.

A result was issued after a single blood draw in 1380/1390 (99.3%) cases. Of the ten remaining cases, one woman elected to directly undergo amniocentesis, showing a normal karyotype. In the other nine cases a redraw was done and three samples failed again (overall failure rate 0.3% (4/1390)). The failures were due to fetal fractions $< 4\%$ ($n=3$), or the results of the WISECONDOR algorithm being inconclusive, most likely due to bad DNA quality ($n=1$). One woman was morbidly obese (BMI 52), the other three had a normal weight/BMI. Four children without anomalies were born.

Results were issued after a mean of 14 days (range 5-32); 95% of results were available within 21 days. TAT improved in later phases of the study due to faster equipment.

Accuracy

Of the 1386 successful NIPT samples, 1325 (95.6%) were reported as normal, and 42 (3.0%) as either trisomy 21 (n=31), 18 (n=5) or 13 (n=6). Of these 42 cases, a full trisomy was confirmed in the fetus in 34 cases, by invasive testing (n=33) or karyotyping of postnatal tissues (n=1) (Table 2). 19/1386 cases had an abnormal result involving other trisomies or subchromosomal aberrations (see following paragraph for details). Five false positive cases were found (0.4%, n=2 trisomy 21, n=1 trisomy 18 and n=2 trisomy 13). In two pregnancies, NIPT showed unclear results, which were reported as very unlikely to be full T21 but mosaic could not be ruled out. In both cases the parents opted for amniocentesis which revealed a normal karyotype; both children are alive and well. As these two events were not reported as T21 they are included in the 'normal result' group for this paper. No pregnancies with an abnormal NIPT result were terminated without confirmation by invasive testing.

We report one false negative case: trisomy 21 (46,XX,i(21)(q10)) was detected in amniotic fluid after invasive testing because of ultrasound anomalies at routine scanning. The parents chose to continue the pregnancy. Analysis of the placenta after birth showed absence of this isochromosome from the cytotrophoblast layer, whereas it was present as a mosaic (66%) in the mesenchyme layer.

Details of the discordant NIPT results are given in table 3. Two-by-two tables and test characteristics are given in tables 4a,b and c for each of the three trisomies.

Additional findings

In 19/1386 (1.4%) cases, which is 31% of all abnormal cases, other full trisomies (1.1%, n=15) (T22 n=1, T20+T2 n=1, T16 n=6, T9 n=1, T8 n=2, T7 n=4) or subchromosomal aberrations (0.3%, n=4) (deletions n=3 (11p, 12q, 18p), duplications n=1 (7q)) were reported (table 2). As 1076/1386 cases underwent genome-wide analysis by WISECONDOR, the frequency of additional findings is actually somewhat higher (1.7%). No sex chromosomal aneuploidies were found as the license did not allow analysis of sex chromosomes. All cases were counseled by the clinical geneticist on possible consequences and options for further testing. In two cases the pregnancy was terminated after confirmation by invasive testing (12q and 18p deletion, both without clear ultrasound abnormalities). The 11p deletion was confirmed to originate from the healthy mother. In two of the 17 ongoing pregnancies a child with multiple structural anomalies was born, one case of T16 NIPT result but normal genotype (array) in the child, and one case of T9 NIPT result which was mosaic in the child. In both cases parents refrained from invasive testing. In ten additional children a growth-restricted infant was born. A detailed report of an extended cohort with additional findings, including follow-up testing, clinical relevance and pregnancy outcomes will be published separately.

Outcome

Complete follow-up of pregnancy outcomes was obtained for 1376/1390 (99.0%) pregnancies. Three children died in the neonatal period, two related to severe prematurity without evidence of structural or chromosomal anomalies, and one due to severe cardiac malformations (all three normal array). One live-born child was found to have a 22q11.2 deletion, associated with diGeorge syndrome (too small to detect with the method used). Its heart defect was not detected during pregnancy. Three women with a prenatal diagnosis of

trisomy 21 elected to continue the pregnancy, all three children were born alive. No other live-born child was reported to have chromosomal abnormalities.

Invasive testing

Fifty-three invasive tests were done following NIPT results (5 CVS, 46 AS, 1 CVS and AS): 37 for suspected trisomy 21, 18 or 13, two for possible mosaics, an additional 12 due to other NIPT findings, and one after a failed result. In addition, invasive testing was done in 21 women with a normal NIPT result, mostly indicated by ultrasound anomalies. Of those, five showed an abnormal karyotype: the already described missed trisomy 21 isochromosome, a triploidy, a small (0.1Mb) 9p deletion, a 1.7Mb deletion on Xp and one mosaic trisomy 8.

A precise estimation of the reduction of the overall number of invasive procedures attributable to the availability of NIPT in the TRIDENT study is hampered by several factors. NIPT performed by foreign commercial laboratories has been available to Dutch women in neighboring countries, paid out-of-pocket, since 2012. Of the 202 women who in the study period received a high-risk result from the FCT and declined NIPT, some may have chosen to have their blood sample analyzed in a foreign laboratory. Numbers and outcome data are unavailable to us.

What we do know however is that in The Netherlands consistently around 50% of women with an increased FCT risk result elect to undergo invasive testing.^{7;10} In our study, 1413 women undergoing FCT received a risk $\geq 1:200$. Theoretically, had NIPT not been available, 50% or 706 of them would have opted for invasive testing. Adding the 21 who had invasive testing due to ultrasound abnormalities, results in a total of 727 invasive tests. The actual number of invasive tests in our study group was 53 plus 21 is 74, plus an unknown part of the 202 women who declined our NIPT. The most conservative calculation, if all 202 women would have chosen invasive testing, results in a reduction from 727 to 276, or 62%.

Discussion

Our first evaluation of the TRIDENT study confirms the expected high accuracy of NIPT in a real-world setting. The uptake of NIPT in this high-risk cohort was 86%. This led to a major reduction in the number of invasive diagnostic procedures, at the expense of one trisomy missed. No unexpected live birth of a child with a trisomy occurred in this cohort. No pregnancies were terminated after abnormal NIPT without confirmation in chorionic villi or amniotic fluid cells. Detection rates (DR) for the common trisomies (96% for trisomy 21, 100% for trisomies 13 and 18) and false positive rates (0.14% for trisomies 21 and 13, 0.07% for trisomy 18) were comparable to previous studies.¹¹ Our data support introduction of NIPT as a safe second-tier screening test to accurately select the small proportion of women truly at high risk for fetal trisomy, and to reliably reassure all others.

The uptake of NIPT of 86% was similar to the hypothetical interest of 82% in a previous questionnaire study,¹² and the 88% found in an UK survey.¹³ Smaller prospective studies in the US have reported uptakes of NIPT by women at elevated risk ranging from 43-69%.^{3;14;15} However, data are difficult to interpret and compare due to ample opportunities to obtain NIPT as a first-tier screening test across the border. Also costs and reimbursement policies, and the extent of invasive testing, are likely to have a considerable impact on choices. The latter may vary from QF-PCR testing for chromosomes 21, 18, 13, X and Y only, to traditional karyotyping or high-resolution microarray. Preferences may change again when more extended cfDNA testing becomes available.

The true clinical value of our study is that it confirms the expected major reduction in the number of invasive procedures, which was the primary goal of NIPT development.¹⁶ It is well known that for biological reasons NIPT will never be as accurate as the analysis of fetal cells taken from amniotic fluid.¹⁷ However, according to our data, there appears to be only a small price to pay for trading some accuracy for safety. The single fetal trisomy missed by

NIPT was detected at routine prenatal ultrasound. The underlying isochromosome was absent in the cytotrophoblast, which is the source of the cfDNA tested by NIPT. Although rare, false negative trisomy 21 cases have been reported.^{18;19} Adequate counseling should ensure that women understand the high but not 100% accuracy of NIPT.

Although there is still controversy on the magnitude of the fetal loss rate caused by invasive testing, the reduction of invasive test procedures is certainly perceived as better care both by health care providers and pregnant women. Moreover, costs of invasive testing, a sum of laboratory work and procedure by maternal-fetal medicine specialists, are higher than the cost of NIPT, and this gap is rapidly widening. Health economic consequences of our study will be subject of reviewing.²⁰ Others found similar reductions in invasive testing after introduction of NIPT.^{1;21-23} A recent decision-analysis modelling study concluded that offering NIPT to the high-risk population would reduce the number of invasive procedures by 72%, and NIPT for the general population a reduction of 60%.²⁴ An important consequence is the rapidly declining experience of clinicians in performing invasive procedures.^{1;25} Quality assurance and training programs need to be adapted, and more centralized care seems unavoidable.

In the Netherlands only University Genetic Laboratories are licensed to do genetic testing. Some of these laboratories already had several years of experience in fetal cfDNA analysis while others started this service especially for the TRIDENT study.²⁶⁻²⁸ Although the numbers are still limited, we found no evidence for an inferior performance of NIPT by our laboratories compared to other providers.⁸

A possible limitation of the method used in our study was our inability to measure fetal fraction in all cases. Only recently algorithms became available to calculate fetal fraction based on the single read NGS data that we have used.²⁹

Our choice for whole genome sequencing entailed that other trisomies, as well as large duplications and deletions also became detectable. In such cases decisions on informing and advising couples were taken after multidisciplinary discussions between lab specialists, clinical geneticists, and maternal-fetal medicine specialists. NIPT data on the occurrence of these anomalies in a population with an increased risk based on the FCT are slowly emerging.^{30;31} In our study, 31% of the chromosomal abnormalities found were other than trisomy 21, 13 or 18. This is higher than the number calculated by Norton *et al.*,³² which might be due to the fact that the study populations were different due to different screening protocols. Using our protocol, many of the non-detectable anomalies as described by Norton *et al.*³² such as other trisomies, unbalanced rearrangements and insertions/deletions would have been detectable.

Biologically NIPT resembles direct analysis of chorion villus cytotrophoblasts, and additional findings are likely to be comparable.^{17;26;33} As expected therefore, a number of cases of confined placental mosaicisms such as trisomy 7, 8 and 16 and one case of true fetal mosaicism leading to respectively false positive and a false negative result were found. Our series confirmed the previously described association between a mosaic placenta and fetal growth restriction.^{34;35} As with CVS, advance knowledge of such a risk may be beneficial to clinical management. The need to perform invasive testing in these cases, and if so either with CVS or AS, will be discussed between laboratory specialists and clinicians on a case to case base. The advice to the parents will depend on gestational age, type of chromosome anomaly, presence or absence of ultrasound findings and parent preferences.^{33;36} This is a new area of research into possible additional clinical value of NIPT beyond trisomy 21, 13 and 18. In our study, couples had no option to reject information on secondary findings, but a choice will be offered in our future studies as not all couples may want this information.

The use of NIPT as a secondary screening test does not diminish the false negative rate of the initial screening test (FCT) but mainly lowers the false positive rate. As the DR of NIPT for common aneuploidies is higher than the DR of FCT (~80-95%),³⁷⁻³⁹ replacement of FCT by NIPT as a first-tier test will lower the false negative rate. We expect that the indication for NIPT will broaden to a larger proportion of pregnant women in the coming years, in line with international trends. This will enable us to further invest in equipment and improve workflow, leading to higher efficiency, lower cost per test and faster turn-around times, an issue our pregnant women highly value. With improving technology also the scope of testing may become wider. The overall impact of the introduction of NIPT into our national screening program will therefore need continuous monitoring with emphasis not only on technical performance but also on patient preferences and outcome data of all pregnancies.

In conclusion, the first evaluation of our TRIDENT study with full clinical follow-up confirms the clinical usefulness of NIPT as a safe and reliable alternative to invasive diagnostic testing for high-risk pregnant women. Our trial license was granted for two years, and has been extended for another two years. We are continuing to follow-up all cases of NIPT, we will separately publish the questionnaire results on women's views and preferences (Van Schendel *et al.*, Paper Part II) and re-analyze the health economic consequences. With these combined data, we will discuss with the government and all involved stakeholders a plan of action for formal implementation of NIPT in the Dutch national prenatal screening program.

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Table 1 Characteristics of 1390 pregnant women at increased risk for fetal trisomy who underwent NIPT

| Characteristics | n (%) |
|-----------------------------------|--------------|
| Maternal age (y) | |
| <36 | 603 (43) |
| ≥36 | 703 (51) |
| Unknown | 84 (6) |
| Parity | |
| 0 | 300 (22) |
| 1 or more | 486 (35) |
| unknown | 604 (43) |
| Indication for NIPT | |
| FCT risk ≥ 1:200 | 1211 (87) |
| Medical history | 179 (13) |
| FCT risk for fetal trisomy | |
| ≥ 1:10 | 48 (3) |
| 1:11 – 1:50 | 239 (17) |
| 1:51 – 1:100 | 282 (20) |
| 1:101 – 1:200 | 482 (35) |
| unknown | 160 (25) |

FCT, first trimester combined test

Table 2 Outcome of NIPT in pregnancies at increased risk for trisomy 21, 18 or 13

| NIPT result | All | Invasive tests | | | Live-born |
|------------------------------|------|---------------------|----------------|------|------------------------|
| | | (abnormal genotype) | TOP | IUFD | (abnormal genotype) |
| Normal | 1311 | 21 (5) | 8 ^a | 16 | 1287 (2 ^b) |
| Trisomy 21 | 31 | 29 (27) | 25 | 2 | 4 (2) |
| Trisomy 18 | 5 | 5 (4) | 3 | 1 | 1 (0) |
| Trisomy 13 | 6 | 3 (2) | 2 | 2 | 2 (0) |
| Other Trisomies | 15 | 9 (1) | 0 | 0 | 15 (2) |
| Subchromosomal abnormalities | 4 | 3 (2) | 2 | 0 | 2 (0) |
| Failed | 4 | 1 (0) | 0 | 0 | 4 (0) |

Data are n. Data from 1376 pregnancies with complete outcome data.

TOP, termination of pregnancy; IUFD, intrauterine fetal demise.

^aOne for triploidy, seven for ultrasound anomalies.

^bOne trisomy 21, one 22q11.2 del.

Table 3. Outcome of pregnancies with false positive and false negative NIPT results

| NIPT | MA | | GA | | FCT risk | z-score | Ultrasound | Invasive test | Child phenotype | Cord blood |
|------|-----|-----|------|-----------------------------|----------|--------------------|--------------------------------------|-----------------------------------|------------------|------------|
| | (y) | BMI | (wk) | | | | | | | |
| T21 | 41 | 26 | 12+3 | 1:147 (T21) | 4.96 | NI | Amnio QF-PCR and FISH normal | NI female | | |
| T21 | 29 | 22 | 13+5 | 1:80 (T21) | 3.29 | NI | None | NI female | | |
| T18 | 39 | 29 | 15+0 | 1:85 (T18) | 4.40 | NI | Amnio QF-PCR and karyotype normal | NI female | | |
| T13 | 39 | 22 | 15+4 | 1:5000 (T13) 1:35 (T21) | 5.11 | NI | None | NI male | QF-PCR normal | |
| T13 | 37 | 22 | 13+5 | 1:6134 (T13) 1:121 (T21) | 4.4 | NI | None | NI male | Karyotype normal | |
| NI | 34 | 23 | 13+5 | 1:140 (T21) | <3 | Markers for T21 | Amnio T21, 46,XX,i(21)(q10) | Live-born Down syndrome female | | |

MA, maternal age; BMI, body mass index; GA, gestational age; FCT, first trimester combined test; NI, normal.

Table 4a Test characteristics of NIPT for fetal trisomy 21

| | Trisomy 21 | Non-trisomy 21 | |
|--------------------|------------|----------------|------|
| NIPT high risk T21 | 29 | 2 | 31 |
| NIPT low risk T21 | 1 | 1354 | 1355 |
| | 30 | 1356 | 1386 |

Sensitivity $29/30 = 96.7\%$

Specificity $1354/1356 = 99.9\%$

Positive Predictive Value $29/31 = 93.5\%$

Negative Predictive Value $1354/1355 = 99.9\%$

Table 4b Test characteristics of NIPT for fetal trisomy 18

| | Trisomy 18 | Non-trisomy 18 | |
|--------------------|------------|----------------|------|
| NIPT high risk T18 | 4 | 1 | 5 |
| NIPT low risk T18 | 0 | 1381 | 1381 |
| | 4 | 1382 | 1386 |

Sensitivity $4/4 = 100\%$

Specificity $1381/1382 = 99.9\%$

Positive Predictive Value $4/5 = 80.0\%$

Negative Predictive Value $1381/1381 = 100\%$

Table 4c **Test characteristics of NIPT for fetal trisomy 13**

| | Trisomy 13 | Non-trisomy 13 | |
|--------------------|------------|----------------|------|
| NIPT high risk T13 | 4 | 2 | 6 |
| NIPT low risk T13 | 0 | 1380 | 1380 |
| | 4 | 1382 | 1386 |

Sensitivity $4/4 = 100\%$

Specificity $1380/1382 = 99.9\%$

Positive Predictive Value $4/6 = 66.7\%$

Negative Predictive Value $1380/1380 = 100\%$