

FETAL ANEUPLOIDY DETECTION BY MATERNAL PLASMA DNA SEQUENCING
PART 2
A Technology Assessment

INTRODUCTION

The California Technology Assessment Forum is requested to review the scientific evidence for the use of maternal plasma DNA sequencing for fetal aneuploidy detection. This topic is being addressed because of recent publications of studies of diagnostic accuracy of this technology in high risk women. This is the second time that CTAF has addressed this topic. The focus of this evaluation will be its use in women at high risk for fetal aneuploidy vs those at average risk and its use as a primary vs an advanced screening test.

BACKGROUND

Chromosomal Aneuploidy

Prenatal testing is widely recommended and widely used to screen for chromosomal abnormalities. Down syndrome (trisomy 21), Edwards syndrome (Trisomy 18) and Patau syndrome (Trisomy 13) are three aneuploid disorders with significant consequences which can be detected by prenatal evaluation.

Down syndrome (Trisomy 21) is the most common chromosome abnormality in live births. Down syndrome accounts for the commonest form of intellectual disability caused by a known chromosome problem. Individuals with Down syndrome have a learning disability that is moderate to severe (average IQ of 50), characteristic facial features, short stature, cardiac and intestinal defects, problems with vision hearing and increased infection risk. There is significant morbidity and mortality in affected individuals as well as adding a psychosocial and financial burden to the family.

The prevalence of Down syndrome is overall about 1 in 629 births.¹ However the prevalence increases with increasing maternal age, so that the risk is about one in 1,340 at age 25; one in 353 at age 35 and in 35 at age 45.^{2,3}

Trisomy 18 (Edwards syndrome) is the second most common autosomal trisomy. The incidence is about 1 in 5,500 live births, but the risk increases with increasing maternal age. About half of babies born with Trisomy 18 die within the first week of life and only five to ten percent survive to one year. Those who do survive have intellectual disability.⁴

Trisomy 13 or Patau syndrome is another chromosomal trisomy where 80% of infants die within the first month. Those who survive have intellectual disability, seizures and failure to thrive.⁴

Prenatal diagnostic testing

The American College of Obstetricians and Gynecologists (ACOG) recommends that pregnant women be offered screening for chromosomal abnormalities, regardless of maternal age.⁵ There are multiple options for noninvasive prenatal screening for fetal

chromosomal abnormalities. The noninvasive tests typically include measurement of maternal serum markers which are interpreted in the context of maternal age and many also include ultrasound findings (nuchal translucency- ultrasound measurement of the back of the neck).

The first trimester combined test includes nuchal translucency (NT), crown-rump length as well as pregnancy associated plasma protein-A (PAPP-A) and free or total beta human chorionic gonadotropin (beta-HCG). This screening test can be performed in the first trimester (before 13 weeks).

A second category of noninvasive tests are the “integrated tests” which require maternal plasma analytes during both the first and the second trimesters. These include additional markers such as alpha fetoprotein (AFP), unconjugated estriol (uE3) and inhibin A. The full integrated test has the highest detection rate for Down syndrome and the lowest screen positive rate, but the disadvantage is that the testing cannot be completed until the second trimester.

The results of the noninvasive tests can either be “screen negative” or “screen positive.” A screen negative result means that the patient’s risk of having a baby with Down syndrome is less than some predetermined level. The patient is provided with the actual risk (eg risk for Down Syndrome is 1/500). A screen positive result means that the risk for Down syndrome is at or above the cut off point. A patient who has a “screen positive” result is offered a diagnostic test, either chorionic villus sampling (CVS) or amniocentesis. ACOG recommends that all patients should be provided with an individualized risk acknowledging that different patients have different concepts of what would be considered a personal positive result.

In summary, for the currently available noninvasive tests, despite the various proposed combinations of noninvasive tests, existing screening methods have detection rates of 90-95% and false positive rates of 3-5%, but all offer a combination of noninvasive testing followed by an offer of diagnostic testing if the screening test is positive.⁶⁻⁹

Diagnostic Tests

The currently available diagnostic tests are amniocentesis and CVS. Both have the ability to definitely show fetal karyotyping, but are associated with the risk of pregnancy loss. CVS has the advantage of being able to be performed earlier in pregnancy (during the first trimester), but is associated with a small risk of pregnancy loss. Amniocentesis cannot be performed until later in the pregnancy, (often after a woman is already obviously pregnant) and is also associated with a small risk of miscarriage. Both of these tests provide actual chromosomal analyses and can either confirm or disconfirm an abnormal screening test.

Cell Free Fetal DNA (cfDNA)

Prenatal diagnosis can use cell-free fetal nucleic acids in maternal blood to detect fetal chromosomal abnormalities. Intact fetal cells can be found in the maternal circulation, leading to the possibility that maternal blood could be used to diagnose fetal disease. Because the absolute amount of fetal cells relative to maternal blood is very small, they can be difficult to detect. In addition, cells from prior pregnancies can persist in the maternal circulation, which further limits the use of actual fetal cells.

However, cell free nucleic acids (DNA and RNA) are more plentiful in the maternal circulation. In addition, fetal DNA has a very short half life; thus any fetal DNA that is found is due to the current pregnancy. Fetal DNA can often be detected by the fifth postmenstrual week and can almost always be detected by the 9th postmenstrual week.¹⁰ Fetal DNA is approximately three to six percent of total DNA in the maternal circulation. The percentage of fetal DNA increases with increasing gestational age.¹¹

One of the main challenges of the use of cfDNA for diagnosis is differentiating the fetal DNA from the maternal DNA. One of the best methods is to identify DNA sequences only found on the Y chromosome, since normal women do not have Y chromosomes. Another way is to identify DNA sequences which are associated with paternally inherited fetal conditions, since normal women would not be expected to have them. Finding maternally linked alleles is potentially more difficult, since it would be harder to differentiate maternal and fetal DNA. Strategies that have been used include finding fetal DNA that is methylated differently than maternal DNA or looking at differences in DNA length.^{12,13} Fetal DNA fragments are shorter than maternal DNA fragments; however this difference has not proven useful in differentiating the two types of DNA.¹⁴

There are at least two techniques for isolating fetal DNA from maternal DNA. Massively parallel signature sequencing (MPSS) is a random analysis of millions of cfDNA fragments. This technique sequences short segments of cfDNA from the mother and the fetus and assigns them to specific chromosomes. The number of chromosome counts are then compared to a control value of other chromosomes and if there is an excess of a particular chromosome (e.g. 21), trisomy is suspected. This technique requires analysis of a very large number of DNA fragments per sample – estimates are about 25 million, which could potentially limit its clinical utility.

The other technique is directed DNA analysis. The goal of directed DNA analysis is to selectively sequence relevant chromosomes. Digital analysis of selected regions (DANSR) is a recently developed process of analyzing counts from assays targeted against selected genomic regions on particular chromosomes.¹⁵ This technique is touted as being potentially more efficient than MPSS because it uses fewer genetic fragments. Both of these techniques are currently being evaluated for use in noninvasive prenatal testing.

The potential role of cfDNA in prenatal diagnosis would be either as a primary screening test (replacing the currently available non-invasive tests) or as an “advanced screening test.” As an advanced test, it would potentially be used in women who have a “screen positive” result on a noninvasive test before proceeding to invasive diagnostic testing. Since it is not a diagnostic test, confirmation of positive results by a diagnostic test would still be required. However, the potential advantage of using cfDNA as an advanced screening test would be to reduce the number of invasive procedures and the resulting loss of normal fetuses. Thus CTAF is evaluating the role of cfDNA as a primary or advanced screening test for fetal aneuploidy. In addition, CTAF is evaluating the role of cfDNA in high risk vs average risk women.

TECHNOLOGY ASSESSMENT (TA)

TA Criterion 1: The technology must have final approval from the appropriate government regulatory bodies.

All laboratory tests (except those for research) performed on humans in the United States are regulated by the Centers for Medicaid and Medicare Services (CMS) through the Clinical Laboratory Improvement Amendments (CLIA). The Division of Laboratory Services under the Office of Clinical Standards and Quality (OCSQ) is responsible for

implementing the CLIA Program. Laboratories performing laboratory developed tests LDT(s) are required to have CLIA certification to ensure the quality and validity of the LDT(s). At this time, LDTs are not subject to U.S. FDA regulations.

<http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/index.html?redirect=/clia/>

Currently, there are three companies with LDT products for fetal aneuploidy detection via maternal plasma is performed in CLIA certified laboratories.

Company	LDT name	Trisomy identified			Testing approach	
		Trisomy 21 (Down Syndrome)	Trisomy 18 (Edwards Syndrome)	Trisomy 13 (Patau Syndrome)	Directed Analysis	Random Sequencing (MPSS*)
Ariosa Diagnostics	Harmony™ Prenatal Test	X	X		X	
Sequenom	MaterniT21™	X	X	X		X
Verinata Health	verifi™ prenatal test	X	X	X		X

*MPSS: Massively Parallel Signature Sequencing

These tests are recommended for singleton pregnancies only, and so CTAF is only evaluating the use of these tests in singleton pregnancies.

TA Criterion 1 is met.

TA Criterion 2: The scientific evidence must permit conclusions concerning the effectiveness of the technology regarding health outcomes.

For the first assessment of cfDNA, the Medline database, Cochrane clinical trials database, Cochrane reviews database and the Database of Abstracts of Reviews of

Effects (DARE) were searched using the key words : “aneuploid” or “down syndrome” or “chromosome disorders” or “trisomy and prenatal diagnosis” or “fetal diseases” or “fetus” AND “cell free system” or “cell free” or “DNA” or “RNA” or “maternal plasma DNA” or “maternal blood DNA’. The search was performed from database inception to May, 2012. The bibliographies of systematic reviews and key articles were manually searched for additional references. The abstracts of citations were reviewed for relevance and all potentially relevant articles were reviewed in full.

- Study had to evaluate cfDNA as a prenatal screening test in pregnant women
- Study had to compare cfDNA with a gold standard
- Included only humans
- Published in English as a peer reviewed article

Our initial search revealed 265 potentially relevant articles. We reviewed all the titles and identified 16 potentially relevant abstracts. Abstracts were reviewed in full and we identified seven studies as potentially relevant for inclusion. Reasons for exclusion included a focus on cfDNA technique rather than test performance, use of a novel test other than cfDNA or being a duplicate of an earlier publication.

We updated our search to include articles published through August 31, 2012. As a result of the Pub Med search we reviewed 24 abstracts. After excluding duplicates and studies that focused on technique rather than test performance, or use of a novel test other than cfDNA, we identified one additional relevant study.¹⁶ A repeat search of the Cochrane database through August 31, 2012 did not reveal any additional publications.

A total of eight studies evaluated the use of cfDNA for screening for fetal aneuploidy. Two studies were validation studies where part of the sample was used as the training set and the other part of the sample was used as the validation set.^{15,17} Although some of the studies were done prospectively, in all but two studies, not all

samples in a cohort were analyzed for trisomy status;^{16,18}, the majority of studies selected trisomy cases and additional controls for analysis from the overall cohort.

We did not identify any studies that compared an aneuploidy screening strategy incorporating cfDNA to the standard of care aneuploidy screening strategy that starts with noninvasive tests and proceeds to diagnostic tests without use of cfDNA nor did we identify any studies that compared cfDNA with standard of care prenatal screening.

After this assessment was complete, one “in press” article was sent to CTAF that evaluated the accuracy of T13 detection but that was not identified in the literature search¹⁹.

Level of Evidence: 3

TA Criterion 2 is met

DRAFT

Table 1 : Studies using cell free fetal DNA to screen for fetal aneuploidy

Author, year	Study Type	N total	N with abnormal karyotype	Trisomy targeted	Test	Inclusion Criteria
Palomaki, 2011 ²⁰ Palomaki, 2012 ²¹	Multicenter nested case control	1971	283 212 (T21) 59 (T18) 12 (T13)	21 18 13	MPSS	High risk for Down syndrome: maternal age, family history or positive screening test
Sehnert, 2011 ¹⁷	Multicenter cross sectional validation study	119	53	21 18	MPSS	18 years and older and pregnant
Ehrich, 2011 ²²	Blinded prospective	480	39	21	MPSS	Advanced maternal age, positive screening test, personal or family history of Down Syndrome
Bianchi, 2012 ²³	Blinded prospective; multicenter with nested case control	532	221	21 18 13	MPSS	Women undergoing an invasive prenatal procedure; aged 38 and over; positive screening test for aneuploidy or prior aneuploidy fetus
Chiu, 2011 ¹⁸	Diagnostic accuracy with prospectively collected or archived serum	753	86	21	MPSS	Women with “clinical indications for CVS or amniocentesis”
Ashoor, 2012 ²⁴	Nested case control	400	100	21 18	DANSR	Stored plasma

Author, year	Study Type	N total	N with abnormal karyotype	Trisomy targeted	Test	Inclusion Criteria
						High risk women where risk for aneuploidy was >1/300
Sparks, 2012 ¹⁵	Nested case control with training and validation sets	338	88	21 18	DANSR	At least 18 years of age; at least 10 weeks pregnant; singleton pregnancy (subset of larger prospective group chosen based on ploidy status)
Norton, 2012 ¹⁶	Prospective Cohort	3228	119 81 (T21) 38 (T18)	21 18	DANSR	At least 18 years of age; at least 10 weeks pregnant; singleton pregnancy; planning to undergo invasive procedure for any reason

MPPS: Massively Parallel Signature Sequencing
DANSR: Digital Analysis of Selected Regions

Table 2 Results of studies using cell free fetal DNA to screen for fetal aneuploidy

Author, year	N Total	N with abnormal karyotype	Main results: T21	Main results: T18	Main results: T13	Comments
Palomaki, 2011 ²⁰ Palomaki, 2012 ²¹	1,971	212 (T21) 59 (T18) 12 (T13)	98.6% detection rate 99.8% specificity	100% detection rate 99.72% specificity	91.7% detection rate 99.03% specificity	Overall detection rate 98.9% for common aneuploidies 17 samples could not be interpreted: three were T18 Very few T13 cases
Sehnert, 2011 ¹⁷	119	53	100% sensitivity	100% sensitivity	N/A	Training and validation set: Training set - 71 samples (26 abnormal karyotypes) and validation set - 48 samples (27 abnormal karyotypes) 13 T21 and 8 T18 samples
Ehrlich, 2011 ²²	480	39	100% sensitivity 99.7% specificity	N/A	N/A	38 T21 samples
Bianchi, 2012 ²³	532	221 Total 89 (T21) 36 (T18) 14 (T13)	100 sensitivity	97.2% sensitivity	78.6% sensitivity	No false positives for autosomal euploidies

Author, year	N Total	N with abnormal karyotype	Main results: T21	Main results: T18	Main results: T13	Comments
Chiu, 2011 ¹⁸	753	86	100% sensitivity 97.9% specificity	N/A	N/A	Two different methods tested (2-plex and 8-plex). 2-plex method had better sensitivity
Ashoor, 2012 ²⁴	400	100 50 (T21) 50 (T18)	100% sensitivity 99%	100% sensitivity 99%	N/A	Specificity calculated on total of 300 euploid fetuses although three samples were not available for analysis
Sparks, 2012 ¹⁵	338	88	100% sensitivity	100% sensitivity	N/A	36 T21 cases in validation set 7 T18 cases in validation set
Norton, 2012 ¹⁶	3,228	119 81 (T21) 38 (T18)	100% sensitivity (95% C.I. 95.5%-100%) False positive rate 0.03% (95% C.I. 0.002%-0.20%)	97% sensitivity (95% C.I. 86.5%-99.9%) False positive rate 0.07% (95% C.I. 0.02%-0.25%)	NA	4.6% of samples were not able to be analyzed either due to low fetal DNA fraction or assay failure

TA Criterion 3: The technology must improve the net health outcomes.

A total of eight studies evaluated the use of cfDNA for screening for fetal aneuploidy. Five used the MPSS technology^{17,18,20-23} and three used the DANSR technology.^{15,24} All of the eight studies evaluated cfDNA as a screening test for T21;^{15,17,18,20-24} six studies evaluated it as a screening test for trisomy 18^{15,17,20,21,23,24} and two studies evaluated it as a screening test for T13.²³ In all the studies, the true chromosomal state of the fetus was known from either amniocentesis or CVS. Two studies were validation studies where part of the sample was used as the training set and the other part of the sample was used as the validation set.^{15,17} Although some of the studies were done prospectively, in many cases, all samples in a cohort were not analyzed for trisomy status. These studies selected trisomy cases and additional controls for analysis from the overall cohort. In one study¹⁸ which compared two protocols for MPSS, all samples (regardless of ploidy status) were analyzed with MPSS with one of the MPSS protocols, but only about 146 of the 571 euploid fetuses were evaluated with one of the MPSS protocols. One large prospective multicenter cohort study did test all subjects for aneuploidy status¹⁶.

The number of fetuses with abnormal karyotypes varied in the studies but ranged from 39 to 283 in the largest study. In the two studies that had the most aneuploidy fetuses,^{21,23} they were assessing for more than one type of aneuploidy so the number of fetuses with each type of aneuploidy was somewhat less. For example, in the Bianchi study, there were 89 cases of T21, 36 cases of T18 and 14 cases of T13. In the multicenter prospective cohort study by Norton and colleagues there were 81 T21 fetuses and 38 T18 fetuses.¹⁶

All seven of the studies evaluated for T21 status. The number of T21 cases in each study ranged from 39 to 212; however in the validation studies, where they used a

training set and a validation set, the total number of aneuploidy fetuses on whom the cfDNA test was validated was smaller.^{15,17} The sensitivity rate for the detection of T21 ranged from 98.6% to 100%. Specificity ranged from 97.9% to 99.97%.

Six of the studies evaluated cfDNA for the detection of Trisomy 18. Two of those studies were validation studies and so the total number of T18 fetuses in the validation set were only eight and seven respectively.^{15,17} Although both of these two validation studies reported a 100% sensitivity for the detection of T18, the total number of cases on which this estimate is based is only 15. In one study which had 59 T18 cases, the reported sensitivity of cfDNA was 100%,²⁰ and in another study that included 36 T18 cases, the sensitivity was 97.2%.²³ Finally in a multicenter prospective study that included 38 evaluable cases of T18, the sensitivity was 97%.¹⁶

Only two studies evaluated the use of cfDNA in the detection of T13.^{21,23} Between them these studies included a total of 26 T13 cases. The detection rate was 78.65% in one study²³ and 91.7% in the other study²¹; however, the ability to draw conclusions is limited by the small number of cases.

A recent case-control study, identified, after the literature search was completed, showed that the sensitivity for detecting Trisomy 13 could be accurately determined, but was based on only 11 cases of T13.¹⁹

In the MELISSA study (Maternal Blood IS source to Accurately diagnose fetal aneuploidy), Bianchi and colleagues collected blood samples in a multicenter blinded study from 2,882 women that were undergoing prenatal diagnostic procedures at 60 different U.S. sites²³. In this nested case control study, they selected all singleton pregnancies with any abnormal karyotype (not just T21) as well as a balanced number of randomly selected euploid pregnancies. MPSS was performed on all samples and

the results were compared with the karyotypes as determined by amniocentesis or CVS. They had a total of 532 samples, 221 of which had abnormal karyotypes. Among 89 fetuses with T21, 89 were correctly classified with MPSS (100% sensitivity). Of the 36 cases of T18, 35 were appropriately classified as T18 with MPSS (sensitivity 97.2%). Among 14 fetuses with T13, 11 were correctly classified as T13 (sensitivity 78.6%). The controls for each analysis were a mixture of aneuploidy and euploid samples. For example, for T18, the controls included all euploid fetuses as well as all other “non-T18” fetuses, including T21 and T13. This resulted in control group sizes of 493 for T21, 496 for T18 and 499 for T13. In this study, there were no false positive results in screening for chromosomal aneuploidies, resulting in specificities for T21, T18 and T13 of 100% each. Thus, the specificity is calculated based on less than 500 fetuses for each aneuploidy. Because this was a nested case control study, and therefore it did not reflect true population prevalence of the fetal aneuploidies, positive and negative predictive values cannot be calculated.

Chiu and colleagues used prospectively collected or archived serum from 753 women at high risk for T21.¹⁸ High risk was defined as women with “clinical indications for either CVS or amniocentesis” defined by the obstetric practice at each recruiting site. All women were undergoing or had undergone a diagnostic procedure. A total of 86 had a fetus with T21. MPSS, according to two different protocols, was performed on all samples. The protocols evaluated two levels of multiplexing. Multiplexing involves having more than one plasma sample mixed and sequenced jointly and increased the number of samples that can be analyzed in each sequencing run. They evaluated two levels of multiplexing where either two or eight maternal samples were co-sequenced in each slide segment. The two plex protocol was performed on samples from 314 pregnancies and the eight plex protocol was performed on samples from 753 pregnancies. The two plex protocol detected T21 fetuses with 100% sensitivity and 97.9% specificity, resulting in a positive predictive value (PPV) of 96.6% and a negative

predictive value (NPV) of 100%. The eight plex protocol detected 79.1% of the T21 cases and had a specificity of 98.9%, resulting in a PPV of 91.9% and a NPV of 96.9%. Of note, the PPV calculated for the two plex protocol includes the same total number of T21 cases and fewer euploid cases (not all members of the cohort), which may artificially elevate true disease prevalence and result in a higher PPV.

Recently, the NICE Study (Non-Invasive Chromosomal Evaluation), a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18 was published¹⁶. This study did not evaluate T13. [In this study, conducted in three countries, a total of 4,002 women were eligible for inclusion. Women who were at least 18 years old, with a gestational age of ≥ 10 weeks, with a singleton pregnancy, who were undergoing invasive prenatal diagnosis for any reason, were included. Blood was collected from participants prior to the invasive procedure. Of the 4,002 potential participants, 774 were ineligible due to either being used for the assay development, not meeting inclusion criteria, insufficient sample volume or incorrect labeling of the sample. This left 3,228 participants as eligible for analysis. Of the 3,228 participants, 2,888 of them had normal karyotypes, 84 had T21 karyotype (results obtained in 81), 42 had T18 karyotype (results obtained in 38) and 73 had other chromosomal abnormalities. Calculations for sensitivity and specificity were based on a 1% cut off (1/100) to designate results as “high risk” or “low risk.” Based on this risk classification, the sensitivity for T21 in the 81 samples in which a result was obtained was 100% (95% C.I. 95.5-100%). Among the normal cases, 2,887 out of 2,888 cases were classified as normal, for a specificity of 99.97% (95% C.I. 99.8-99.99%), resulting in a false positive rate of 0.03% (95% C.I. .002-0.20%). The one chromosomally normal case that was classified as “high risk” had a risk score of 1.1%, very close to the 1% cutoff. For Trisomy 18, 37 out of 38 cases resulted in a high risk result, for a sensitivity of 97.4% (95% C.I. 86.5-99.9%). Among the normal cases, 2,886 out of 2,888 cases were classified as normal, resulting in a specificity of 99.93% (95% C.I. 99.75-99.98%).

Overall, the PPVs for T21 and T18 were 98.8% and 94.9% respectively. The NPVs were 100% and 99.96% for T21 and T18 respectively. One potential limitation is that overall 148 out of 3,228 (4.6%) of cases could not be analyzed either due to assay failure or low fetal DNA fraction. The authors report that the assay failure rate was similar in trisomy and normal cases and the cause of assay failure is not currently known. However, it is important to recognize that the published sensitivity, specificity and predictive values are reported only for those samples that could be analyzed. Nevertheless, this large prospective study does provide important information about test performance in a population of high risk women undergoing invasive procedures.

Potential Benefits

1. Can cfDNA testing replace invasive prenatal diagnostic testing for aneuploidies?

Despite its reported high sensitivity and specificity, its accuracy is not that of the gold standard of fetal karyotyping; thus cell free DNA cannot currently replace either CVS or amniocentesis, both of which are invasive procedures with associated risks. In addition, karyotyping can provide information about other conditions besides fetal aneuploidy status, whereas cfDNA cannot.

2. Can cfDNA testing replace current noninvasive screening strategies for fetal aneuploidy?

Existing screening strategies have an accuracy ranging from 90-95% for the detection of fetal aneuploidy, with a false positive rate of 3-5%.⁶⁻⁹ Cell free fetal DNA screening has a higher reported accuracy than the currently available screening tests, and at some point in the future, could potentially replace some of the current tests that are standard of care but less accurate tests. However, to date, cfDNA has not been evaluated as a primary screening test. It has only been evaluated in women who are already considered high risk (e.g. after having a “positive” screening test) or those who

are already planning to undergo a diagnostic test, either amniocentesis or CVS.

3. Does cfDNA testing have a role as an “advanced” screening test for women at high risk for fetal aneuploidy?

Another potential role for cfDNA testing is as a “secondary” screening test, which could be performed on women deemed at high risk after undergoing conventional noninvasive screening tests for fetal aneuploidy. These women could then undergo cfDNA testing for further risk refinement and some could potentially avoid many invasive procedures, and the potential loss of normal fetuses. Since all the currently published studies have evaluated cfDNA for use in women who have already had a “positive” screening test, or who have “clinical indications for a diagnostic test” or who are “planning to undergo an invasive test for any reason,” cfDNA does have a potential role as a secondary screening test.

The potential impact of incorporating a cfDNA test into routine prenatal care was recently calculated using a theoretical model. It was estimated that including the veriFi™ prenatal test as a screening test for fetal trisomies *in high risk women* would result in a 66% reduction in invasive diagnostic induced miscarriages and would lead to 38% more women receiving a T21 diagnosis.²⁵ They also estimated that total costs for prenatal screening and diagnosis would be decreased by 1% annually.²⁵

Potential Harms

The potential harms are mostly related to the possibility of incorrectly classifying a fetus. Using this test has four possible outcomes:

- 1) correctly identifying fetuses with aneuploidy;
- 2) correctly identifying fetuses without aneuploidy;

- 3) incorrectly identifying a normal fetus as being aneuploidy (false positive); and
- 4) incorrectly identifying an aneuploidy fetus as being normal (false negative).

The main potential harms are the false positive tests and the false negative tests. The false positive tests could lead to unnecessary diagnostic procedures, with their related risks. The false negative tests could lead to a fetus with Down syndrome being carried to term, when the mother would have either preferred to prepare herself for this or may have chosen to terminate the pregnancy had she known the true ploidy status of the fetus. In the one large prospective cohort study which allows an estimate of population prevalence, in a population of high risk women, the false positive rate for T21 was 0.03% and the false positive rate for T18 was 0.07%, both of which are relatively low rates. The corresponding false negative rates for T21 were 0% and for T18 were 3.6%. Since the estimates for T18 are based on only 38 cases, they are probably less reliable than the T21 estimates¹⁶.

With respect to the procedure itself, since it is a noninvasive blood test, the procedure related harms are minimal.

Summary

Overall cfDNA has the potential benefits of improved diagnostic accuracy for Trisomy 21 and T18 over existing screening tests, although to date, its utility has only been evaluated in high risk women. It has the potential to have a role as a secondary screening test, and may ultimately lead to fewer unnecessary diagnostic procedures. The potential harms are primarily related to the downstream effects of false positives and false negatives. Overall, the potential benefits of testing for T21 and T18 outweigh the potential harms in high risk women. Less evidence is available for

testing for T13. Since the test has not been evaluated in women at average risk, the potential benefits and harms cannot current be weighed.

TA Criterion 3 is met for cfDNA as a prenatal screening test for T21 and T18 in high risk women

TA Criterion 3 is not met for cfDNA as a prenatal screening test for T 13 in high risk women

TA Criterion 3 is not met for average risk women.

TA Criterion 4: The technology must be as beneficial as any established alternatives.

There are currently two main levels of prenatal tests - screening tests and diagnostic tests. There are several noninvasive screening tests for fetal abnormalities. Each has its own advantages and disadvantages. In order to maximize their utility, they are frequently used in combination, sometimes during the first trimester and sometimes during the second trimester. The main goals of the noninvasive screening tests are for risk stratification. A screening test can refine a woman's likelihood of carrying a fetus with fetal aneuploidy. Her baseline risk is age dependent and a screening test can either increase or decrease the likelihood of aneuploidy. A "positive" test is defined as a test that meets a certain cut-off point (eg risk greater than 1/120). Women with positive tests are given the option of proceeding to a diagnostic test (either CVS or

amniocentesis). A diagnostic test that actually samples fetal chromosomes, is the gold standard for detecting fetal aneuploidy.

Despite the various proposed combinations of noninvasive tests, existing screening methods have detection rates of 90-95% and false positive rates of 3-5%.⁶⁻⁹ Thus in theory, a goal for newer noninvasive tests would be to maximize detection rates while minimizing the rate of false positives, thus leading to fewer unnecessary diagnostic tests.

High risk Women

Studies of cfDNA to date have evaluated it in women already identified as high risk by age personal or family history of Down's syndrome and/or with initial positive screening tests. Given its significantly greater sensitivity and specificity than currently available noninvasive screening tests and combinations of noninvasive screening tests, one of the proposed uses of cfDNA has been as an "advanced" screening test. Individuals who have a positive test on one of the currently available screening tests could potentially have cfDNA as the next step. However, a positive cfDNA test would still require progression onto a definitive diagnostic test. To date, no studies have directly compared incorporating cfDNA into the screening strategy with the current strategy of starting with established noninvasive screening tests and then offering definitive tests to high risk individuals.

The potential impact of incorporating a cfDNA test into routine prenatal care for high risk women was recently calculated using a theoretical model. This model was developed using the clinical results from the MELISSA study described above²³. The MELISSA study included women at high risk for fetal aneuploidy based on either first or second trimester screening. The model evaluated the addition of cfDNA testing using

verifi™ test for Trisomy 21, 18 and 13 into routine clinical practice for high risk pregnancies. Using this model, it was estimated that including the verifi™ prenatal test as a screening test for fetal trisomies in high risk women would result in a 66% reduction in invasive diagnostic induced miscarriages and would lead to 38% more women receiving a T21 diagnosis.²⁵ They also estimated that total costs for prenatal screening and diagnosis would be decreased by 1% annually.²⁵

Average Risk Women

Despite the significantly higher sensitivity and specificity reported for the cfDNA for the detection of trisomies, the sensitivity and specificity are not 100% and so it can never be used as a diagnostic test. If it were used in an average risk population (population with a relatively low disease prevalence), the impact of false positive test results could potentially be significant.

Currently no studies have evaluated incorporating cfDNA into prenatal clinical practice in average risk women compared with the current standard of care. Similarly, no studies have compared using cfDNA as a primary screening test vs the current standard of care.

In summary, for high risk women, although there are no studies directly comparing cfDNA to current prenatal screening strategies, cfDNA has excellent sensitivity and specificity, and using a cost model analysis, when incorporated into routine prenatal care has the potential to achieve accurate trisomy diagnosis with a reduction in invasive diagnostic-induced miscarriages.

For average risk women, although the use of cfDNA for detection of fetal aneuploidy is promising, there is currently no evidence about how it compares to the established standard of care as a primary screening test, nor of how it might fit in as an “advanced” screening test into the current algorithm of prenatal testing for aneuploidy.

TA Criterion 4 is met for the detection of T21 and T 18 for women at high risk for fetal aneuploidy when cfDNA is used as an advanced screening test.

TA Criterion 4 is not met for the detection of T13 for women at high risk for fetal aneuploidy when cfDNA is used as an advanced screening test.

TA Criterion 4 is not met for women at high risk for fetal aneuploidy when cfDNA is used as a primary screening test.

TA Criterion 4 is not met for women at average risk for fetal aneuploidy.

TA Criterion 5: The improvement must be attainable outside the investigational settings.

High Risk Women

To date, the use of cfDNA has only been evaluated in women at high risk for chromosomal abnormalities. A potential improvement when compared with the current standard of care of prenatal testing or when incorporated into existing noninvasive screening strategies has been shown based on the above economic model based on

the MELISSA study²⁵. In addition, the recent large cohort study by Norton et al was performed in multiple settings in three different countries, reflective of real world settings. In addition, it is a simple blood test sent for analysis at centralized laboratories and so can easily be performed in either investigational or non-investigational settings.

Average Risk Women

For average risk women, since an improvement has not been shown in the investigational setting, an improvement cannot be attainable outside the investigational setting.

TA Criterion 5 is met for women at high risk for fetal aneuploidy T21 and T 18 when cfDNA is used as an advanced screening test.

TA Criterion 5 is not met for women at high risk for fetal aneuploidy T13 when cfDNA is used as an advanced screening test.

TA Criterion 5 is not met for women at high risk for fetal aneuploidy when cfDNA is used as a primary screening test.

TA Criterion 5 is not met for women at average risk for fetal aneuploidy.

CONCLUSION

High risk Women

In conclusion, cfDNA is a promising new technology with high sensitivity and specificity for the prediction of fetal aneuploidy, in particular T 21 and T18, when evaluated as an advanced screening test in high risk women. In high risk women, when used as an advanced screening test, it has the potential to reduce the number of invasive diagnostic procedures, with their associated risks of fetal loss.

Average Risk Women

To date, no studies have evaluated cfDNA for use in women at average risk for fetal aneuploidy. Cell free fetal DNA for the diagnosis of fetal aneuploidy is a promising technology, but it is currently not ready for routine use in average risk women.

RECOMMENDATIONS

The following recommendations are proposed:

It is recommended that the use of cell free fetal DNA as a prenatal **advanced** screening test for fetal aneuploidy for Trisomy 21 and Trisomy 18 in high risk women meets CTAF criteria 1-5 for safety and efficacy and improvement in health outcomes.

It is recommended that the use of cell free fetal DNA as a prenatal screening test for fetal aneuploidy for Trisomy 13 in high risk women does not CTAF criteria 4-5 for safety and efficacy and improvement in health outcomes.

It is recommended that the use of cell free fetal DNA as a prenatal **primary** screening test for fetal aneuploidy for Trisomy 21, Trisomy 18, and Trisomy 13 in

average and high risk women does not meet CTAF criteria 3 through 5 for safety and efficacy and improvement in health outcomes.

DRAFT

RECOMMENDATION(S) OF OTHERS

Blue Cross Blue Shield Association (BCBSA)

No assessments on this topic were found on the BCBSA TEC website.

Canadian Agency for Drugs and Technologies in Health (CADTH)

No assessments or guidelines on this topic were found on the CADTH website.

National Institute for Health and Clinical Excellence (NICE)

No assessments or guidelines on this topic were found on the NICE website.

Centers for Medicare and Medicaid Services (CMS)

No information on this topic was found on the CMS website.

Agency for Healthcare Research and Quality

No evidence reports or technology assessments were found on this topic on the AHRQ Genetic Testing section of the AHRQ website.

National Society of Genetic Counselors (NGSC)

NGSC was invited to send an opinion on this technology and to send a representative to the meeting. NGSC forwarded their position statement dated February 18, 2012 and sent a representative to the meeting via teleconference.

The position statement of the NGSC as of February 18, 2012 states "... that NGSC currently supports Noninvasive Prenatal Testing/Noninvasive Prenatal Diagnosis (NIPT/NIPD) as an option for patients whose pregnancies are considered to be at an increased risk for certain chromosome abnormalities. NSGC urges that NIPT/NIPD only be offered in the context of informed consent, education, and counseling by a qualified

provider, such as a certified genetic counselor. Patients whose NIPT/NIPD results are abnormal, or who have other factors suggestive of a chromosome abnormality, should receive genetic counseling and be given the option of standard confirmatory diagnostic testing.”

American College of Obstetricians and Gynecologists (ACOG), District IX (CA)

ACOG was invited to send an opinion on this technology and to send a representative to the meeting. ACOG did not send in an opinion nor send a representative to the meeting.

In its 2007 clinical management guidelines ACOG recommended offering prenatal screening and invasive diagnostic testing for fetal aneuploidy to all pregnant women before 20 weeks of gestation, regardless of maternal age.⁵ The ACOG guideline algorithm for prenatal aneuploidy screening and diagnostic testing is summarized below.

Test used to detect fetal aneuploidy	First trimester	Second trimester
<ul style="list-style-type: none"> • Screening Test 	<ul style="list-style-type: none"> • Integrated Screen • First Trimester Screen 	<ul style="list-style-type: none"> • Integrated Screen • Triple Screen • Quad Screen
<ul style="list-style-type: none"> • Invasive Diagnostic Test 	<ul style="list-style-type: none"> • Chorionic Villi Sampling 	<ul style="list-style-type: none"> • Amniocentesis

International Society for Prenatal Diagnosis (ISPD)

ISPD was invited to send an opinion on this technology and to send a representative to the meeting. ISPD did not send an opinion nor send a representative to the meeting

Society for Maternal-Fetal Medicine (SMFM)

SMFM was invited to send an opinion on this technology and to send a representative to the meeting. SMFM sent an opinion but did not send a representative to the meeting

DRAFT

ABBREVIATIONS USED IN THIS ASSESSMENT:

AFP:	Alpha FetoProtein
cfDNA:	Cell-Free Fetal DNA
CLIA:	Clinical Laboratory Improvement Amendments
CMS:	Centers for Medicaid and Medicare Services
CVS:	Chorionic Villus Sampling
DANSR:	Digital Analysis of Selected Regions
DARE:	Database of Abstracts of Reviews of Effects
HCG:	Human Chorionic Gonadotropin
LDT:	Laboratory Developed Tests
NT:	Nuchal Translucency
MPSS:	Massively Parallel Signature Sequencing
OCSQ:	Office of Clinical Standards and Quality
PAPP-A:	Pregnancy Associated Plasma Protein-A
PPV:	Positive Predictive Value
NPV:	Negative Predictive Value
uE3:	Unconjugated Estriol
MELISSA:	MatErnal BLood IS Source to Accurately detect fetal aneuploidy

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