Serum and Urine Marker Screening for Fetal Aneuploidy

Number: 0464

Policy

Note on Temporary Policy Liberalization in NIPT Testing During the COVID-19 Pandemic:

In order to reduce viral transmission during the COVID-19 pandemic, the Society for Maternal Fetal Medicine (SMFM) has recommended limiting obstetric ultrasound, including fetal nuchal translucency screening, where possible. In response to this unique situation, noninvasive prenatal testing (NIPT) (CPT codes 81420, 81507) will be covered as a substitute for fetal nuchal translucency screening in all pregnant women, including women at average risk for fetal aneuploidy. Note that this temporary coverage liberalization is not due to a change in the underlying evidence base for NIPT use in average risk women. Rather, it is a response to the lack of availability of other aneuploidy screening technologies in average risk women during this time. This policy will remain in effect until June 4, 2020.

Policy History

Last Review 03/27/2020
Effective: 06/12/2001
Next Review: 05/08/2020

Definitions
I. Aetna considers multiple serum marker testing (dimeric inhibin A, human chorionic gonadotropin (hCG) with maternal serum alpha-fetoprotein (MSAFP), and unconjugated estriol) medically necessary for pregnant women who have been adequately counseled and who desire information on their risk of having a Down syndrome fetus.

According to recommendations of the U.S. Preventive Services Task Force and the American College of Obstetricians and Gynecologists, women aged 35 and older who desire information of their risk of having a Down syndrome fetus should have chorionic villus sampling (CVS) or amniocentesis for detection. Multiple serum marker testing is considered medically necessary for women who decline these more invasive procedures.

II. Aetna considers the use of the following serum markers experimental and investigational for second trimester serum marker screening for Down syndrome because the clinical use of these markers is under investigation:

- Beta subunit of hCG
- Human placental lactogen
- Pregnancy-associated plasma protein A (PAPP-A)
- Urinary beta-core.

III. Aetna considers the use of serum markers A Disintegrin And Metalloprotease 12 (ADAM 12) and placental protein 13 (PP13) experimental and investigational for first trimester screening for Down syndrome because their clinical use is under investigation.

IV. Aetna considers measurement of cell-free fetal nucleic acids in maternal blood (e.g., MaterniT21, MaterniT21 PLUS, Verifi Prenatal Test, Harmony Prenatal Test, Panorama Prenatal Test, QNatal Advanced) medically necessary for testing for fetal aneuploidy (trisomy 13, 18 and 21) in pregnant women with single gestations who meet any of the following indications:
A. Fetal ultrasonographic findings predicting an increased risk of fetal aneuploidy (absent or hypoplastic nasal bone, choroid plexus cyst, echogenic bowel, echogenic intracardiac focus, fetal pyelectasis, nuchal translucency, nuchal fold, ventriculomegaly, and shortened femur or humerus); or
B. History of a prior pregnancy with an aneuploidy; or
C. Parental balanced robertsonian translocation with increased risk for fetal trisomy 13 or trisomy 21; or
D. Positive screening test for an aneuploidy, including first trimester, sequential, or integrated screen, or a positive quadruple screen; or
E. Pregnant women age 35 years and older at expected time of delivery.

V. Aetna considers measurement of cell-free DNA experimental and investigational for fetal genotyping for RHD (Sensigene), screening of micro-deletion syndrome, micro-duplication syndrome, and rare autosomal trisomies (e.g., trisomy 2, 5, 7, 8 (Warkany syndrome 2), 9, 12, 14, 15, 16, 17 and 22), and for other indications not listed above (e.g., low-risk women, women with multiple gestations) because its effectiveness has not been established for other indications. Cystic hygroma is not considered a medically necessary indication for cell-free DNA screening; given the increased likelihood of fetal aneuploidy, identification of a cystic hygroma is an indication for definitive testing of the fetus with amniocentesis.

VI. Aetna considers the use of maternal serum anti-Mullerian hormone level for first or second trimester screening for Down syndrome experimental and investigational because its effectiveness has not been established.

VII. Aetna considers the use of urinary markers (measurement of cell-free DNA and metabolomic profiling) for testing for fetal aneuploidy (trisomy 13, 18 and 21) in pregnant women experimental and investigational because the effectiveness of this approach has not been established.

VIII. Aetna considers evaluation of DSCR4 gene methylation in plasma for non-invasive prenatal diagnosis of fetal aneuploidy experimental and investigational because the effectiveness of this approach has not been established.
IX. Aetna considers measurement of circulating fetal nucleated red blood cells and extra-villous trophoblastsis for non-invasive prenatal diagnosis of fetal aneuploidy experimental and investigational because the effectiveness of this approach has not been established.

X. Aetna considers PreSeek (a prenatal single gene cell-free fetal DNA screening panel for detecting multiple Mendelian monogenic disorders) experimental and investigational.

See also CPB 0140 - Genetic Testing (./100_199/0140.html), and CPB 0282 - Noninvasive Down Syndrome Screening (./200_299/0282.html).

Background

Maternal Serum Screening for Fetal Aneuploidy

Levels of human chorionic gonadotropin (hCG), maternal serum alpha-fetoprotein (MSAFP), and unconjugated estriol have been associated with maternal risk of Down syndrome, a chromosomal abnormality associated with mental retardation, congenital heart defects, and physical anomalies. Measurement of these serum markers has been proposed as a means of identifying pregnant women of all ages who are likely to have a Down syndrome fetus.

All pregnant women should be counseled about the risk of having a Down syndrome fetus. Multiple serum marker testing, in conjunction with adequate counseling, should be offered to pregnant women under age 35 who desire information on their risk of having a Down syndrome fetus. Women found to be at high-risk would be candidates for amniocentesis or chorionic villus sampling (CVS), with karyotyping of the tissue obtained to confirm the diagnosis. Multiple serum markers testing and counseling should also be offered to women age 35 or older who wish to avoid the risks of amniocentesis or CVS but desire information on their risk of having a Down syndrome fetus.
High maternal serum levels of hCG with low levels of MSAFP and/or unconjugated estriol in pregnant women has been associated with an increased risk of carrying a Down syndrome fetus. Measurement of multiple serum markers offers a means of identifying young women who are at high-risk of having a Down syndrome fetus; women found to be at high-risk would be offered confirmatory testing by karyotyping tissue obtained by amniocentesis or CVS. Multiple marker testing may also allow pregnant women at high-risk (such as pregnant women age 35 and older) an alternative means of determining the likelihood of having a Down syndrome fetus if they wish to avoid the risk of fetal harm and death associated with amniocentesis and CVS.

Dimeric inhibin A is now used by some commercial laboratories in combination with the 3 traditional analytes. With a screen-positive rate of 5 % or less, this new 4-analyte combination appears to detect 67 % to 76 % of Down syndrome cases in women younger than 35 years (ACOG, 2001).

Efforts to improve biochemical screening have centered on the investigation of screening in the first trimester and on the search for better markers. Two potential serum markers that can be measured during the first trimester are the free beta subunit of hCG and pregnancy-associated protein A. Serum concentrations of the free beta subunit of hCG are higher than average, and pregnancy-associated protein A concentrations are lower, in the presence of a fetus with Down syndrome. The combination of free beta-hCG, PAPP-A, and maternal age appears to yield detection and false-positive rates comparable to second-trimester serum screening (63 % and 5.5 %, respectively) (ACOG, 2001). Unfortunately, free beta-hCG may not be higher in Down syndrome pregnancies until 12 weeks of gestation, and PAPP-A seems to lose its discrimination value after 13 weeks of gestation, making accurate assessment of gestational age and careful timing of the screening test essential. The American College of Obstetricians and Gynecologists (2001) states, however, that “preliminary data [regarding these analytes] remains controversial and testing is not yet standard of care.” Many other screening analytes, including urinary beta-core and human placental lactogen, are currently being investigated for use in the first and second trimesters to determine whether they alone or in combination, will increase detection to a rate greater than the current 60 %.
Maternal serum screening for Down syndrome in the first trimester, rather than second, is not widespread because: (i) CVS and early amniocentesis are not as widely available as amniocentesis during the second trimester, and they may be less safe; (ii) maternal serum screening for Down syndrome in the first trimester, followed by screening for open neural-tube defects during the second, is likely to be less cost effective than performing all the screening at the same time; (iii) because serum concentrations of pregnancy-associated protein A change rapidly during the first trimester, gestational age needs to be established by ultrasonography in order for the sensitivity of first-trimester screening to be equivalent to second-trimester screening; and (iv) assays for serum pregnancy-associated protein A and the free beta subunit of hCG are not licensed for clinical use in the United States.

The National Institutes of Health sponsored a multi-center prospective study (the First and Second Trimester Evaluation of Aneuploidy Risk or 'FASTER' trial) that compared first- and second-trimester non-invasive methods of screening for fetal aneuploidies with second trimester multiple marker maternal serum screening that is the current standard of care (NICHD, 2001). The results of the FASTER trial are described in CPB 282 - Noninvasive Down Syndrome Screening. First-trimester screening, taken together with maternal age, involves an ultrasound measurement of fetal nuchal translucency thickness at 10 to 14 gestational weeks, as well as serum levels of pregnancy-associated protein A and free beta-hCG. Second-trimester screening is based on the serum “triple screen,” which consists of measurement of levels of AFP, unconjugated estriol (uE3), and hCG, performed at 15 to 18 gestational weeks, taken together with maternal age and serum inhibin-A levels (so called “quad test”).

In October 1999, the ACOG issued a position statement that first trimester screening is investigational and should not be used in routine clinical practice. The ACOG statement concluded that “[f]irst-trimester screening for fetal chromosome, cardiac, and other abnormalities using the nuchal translucency marker alone or in combination with serum markers appears promising but remains investigational.”
Based on the results of the FASTER trial, which found that first-trimester screening is as good as or better than second-trimester screening, ACOG (2004) stated that first-trimester screening using nuchal translucency, free beta-hCG, and pregnancy-associated plasma protein-A has comparable detection rates and positive screening rates for Down syndrome as second-trimester screening using 4 serum markers (AFP, beta-hCG, uE3, and inhibin-A). The American College of Obstetricians and Gynecologists stated that, although first-trimester screening for Down syndrome and trisomy 18 is an option, it should be offered only if certain criteria can be met.

Wright et al (2010) provided estimates and confidence intervals for the performance (detection and false-positive rates) of screening for Down's syndrome using repeated measures of biochemical markers from first and second trimester maternal serum samples taken from the same woman. Stored serum on Down's syndrome cases and controls was used to provide independent test data for the assessment of screening performance of published risk algorithms and for the development and testing of new risk assessment algorithms. A total of 78 women with pregnancy affected by Down's syndrome and 390 matched unaffected controls, with maternal blood samples obtained at 11 to 13 and 15 to 18 weeks' gestation, and women who received integrated prenatal screening at North York General Hospital at 2 time intervals: between December 1, 1999 and October 31, 2003, and between October 1, 2006 and November 23, 2007 were include in this analysis. Repeated measurements (first and second trimester) of maternal serum levels of hCG, uE3 and PAPP-A together with AFP in the second trimester were carried out. Main outcome measures were detection and false-positive rates for screening with a threshold risk of 1 in 200 at term, and the detection rate achieved for a false-positive rate of 2 %. Published distributional models for Down's syndrome were inconsistent with the test data. When these test data were classified using these models, screening performance deteriorated substantially through the addition of repeated measures. This contradicts the very optimistic results obtained from predictive modeling of performance. Simplified distributional assumptions showed some evidence of benefit from the use of repeated measures of PAPP-A but not for repeated measures of uE3 or hCG. Each of the 2 test data sets was used to create new parameter estimates against which screening test performance was assessed using the other data set. The results were equivocal but there was evidence suggesting improvement in screening performance through the use of
repeated measures of PAPP-A when the first trimester sample was collected before 13 weeks’ gestation. A Bayesian analysis of the combined data from the 2 test data sets showed that adding a second trimester repeated measurement of PAPP-A to the base test increased detection rates and reduced false-positive rates. The benefit decreased with increasing gestational age at the time of the first sample. There was no evidence of any benefit from repeated measures of hCG or uE3. The authors concluded that if realized, a reduction of 1% in false-positive rate with no loss in detection rate would give important benefits in terms of health service provision and the large number of invasive tests avoided. The Bayesian analysis, which shows evidence of benefit, is based on strong distributional assumptions and should not be regarded as confirmatory. The evidence of potential benefit suggests the need for a prospective study of repeated measurements of PAPP-A with samples from early in the first trimester. A formal clinical effectiveness and cost-effectiveness analysis should be undertaken. This study has shown that the established modeling methodology for assessing screening performance may be optimistically biased and should be interpreted with caution.

Cell-Free DNA

Wright and Burton (2009) stated that cell-free fetal nucleic acids (cfNA) can be detected in the maternal circulation during pregnancy, potentially offering an excellent method for early non-invasive prenatal diagnosis (NIPD) of the genetic status of a fetus. Using molecular techniques, fetal DNA and RNA can be detected from 5 weeks gestation and are rapidly cleared from the circulation following birth. These investigators searched PubMed systematically using keywords free fetal DNA and NIPD. Reference lists from relevant papers were also searched to ensure comprehensive coverage of the area. Cell-free fetal DNA comprises only 3% to 6% of the total circulating cell-free DNA, thus diagnoses are primarily limited to those caused by paternally inherited sequences as well as conditions that can be inferred by the unique gene expression patterns in the fetus and placenta. Broadly, the potential applications of this technology fall into 2 categories: (i) high genetic risk families with inheritable monogenic diseases, including sex determination in cases at risk of X-linked diseases and detection of specific paternally inherited single gene disorders; and (ii) routine antenatal care offered to all pregnant women, including prenatal screening/diagnosis for aneuploidy, particularly Down syndrome (DS), and diagnosis of Rhesus factor status in RhD negative women. Already sex determination
and Rhesus factor diagnosis are nearing translation into clinical practice for high-risk individuals. The authors concluded that the analysis of cfDNA may allow NIPT for a variety of genetic conditions and may in future form part of national antenatal screening programs for DS and other common genetic disorders.

The American College of Obstetricians and Gynecologists (2012) stated that non-invasive prenatal testing that uses cell-free fetal DNA from the plasma of pregnant women offers tremendous potential as a screening tool for fetal aneuploidy. The ACOG Committee Opinion concluded that measurement of cell-free DNA may be considered for the following indications: maternal age 35 years or older at delivery; fetal ultrasonographic findings predicting an increased risk of fetal aneuploidy; history of a prior pregnancy with an aneuploidy; positive screening test for an aneuploidy, including first trimester, sequential, or integrated screen, or a positive quadruple screen; parental balanced robertsonian translocation with increased risk for fetal trisomy 13 or trisomy 21. The ACOG Committee Opinion stated that cell-free fetal DNA testing should be an informed patient choice after pre-test counseling and should not be part of routine prenatal laboratory assessment. The ACOG Committee Opinion stated that cell-free fetal DNA testing should not be offered to low-risk women or women with multiple gestations because it has not been sufficiently evaluated in these groups. A negative cell-free fetal DNA test result does not ensure an unaffected pregnancy. The Committee Opinion stated that a patient with a positive test result should be referred for genetic counseling and should be offered invasive prenatal diagnosis for confirmation of test results.

Currently available cell-free DNA tests are laboratory developed tests, and there is no requirement for premarket approval by the U.S. Food and Drug Administration. Such laboratory developed tests are regulated by the Centers for Medicare & Medicaid Services as part of the Clinical Laboratory Improvement Amendments of 1988 (CLIA). However, CLIA regulations are restricted to certifying internal procedures and qualifications of laboratories rather than the safety and efficacy of laboratory developed tests specifically. CLIA regulations of genetic tests are designed to ensure procedural compliance at laboratory level and do not extend to validation of specific tests.
Ehrich and colleagues (2011) evaluated a multi-plexed massively parallel shotgun sequencing assay for non-invasive trisomy 21 detection using circulating cell-free fetal DNA. Sample multi-plexing and cost-optimized reagents were evaluated as improvements to a non-invasive fetal trisomy 21 detection assay. A total of 480 plasma samples from high-risk pregnant women were employed. In all, 480 prospectively collected samples were obtained from third-party storage site; 13 of these were removed due to insufficient quantity or quality. Eighteen samples failed pre-specified assay quality control parameters. In all, 449 samples remained: 39 trisomy 21 samples were correctly classified; 1 sample was misclassified as trisomy 21. The overall classification showed 100 % sensitivity (95 % confidence interval [CI]: 89 to 100 %) and 99.7 % specificity (95 % CI: 98.5 to 99.9 %). The authors concluded that extending the scope of previous reports, this study demonstrated that plasma DNA sequencing is a viable method for non-invasive detection of fetal trisomy 21 and warrants clinical validation in a larger multi-center study.

Sehnert et al (2011) reported on a cross sectional study of the use of cell-free DNA to detect fetal aneuploidy. Blood samples from 119 adult pregnant women underwent massively parallel DNA sequencing. Fifty-three sequenced samples came from women with an abnormal fetal karyotype. To minimize the intra- and inter-run sequencing variation, the investigators developed an optimized algorithm by using normalized chromosome values (NCVs) from the sequencing data on a training set of 71 samples with 26 abnormal karyotypes. The classification process was then evaluated on an independent test set of 48 samples with 27 abnormal karyotypes. The authors reported that sequencing of the independent test set led to 100% correct classification of T21 (13 of 13) and T18 (8 of 8) samples. The authors noted that other chromosomal abnormalities were also identified.

Palomaki et al (2011) reported on a nested case-control study of the use of massively parallel DNA sequencing to detect fetal aneuploidy. The investigators used blood samples that were collected in a prospective, blinded study from 4,664 pregnancies at high risk for Down syndrome by maternal age, family history, or positive screening test. Fetal karyotyping results from amniocentesis or chorionic villus sampling were compared to cell-free DNA sequencing in 212 Down syndrome and 1,484 matched euploid pregnancies. Down syndrome detection rate was 98.6 % (209/212), the false-positive rate was 0.20 % (3/1,471), and the testing failed in 13 pregnancies (0.8 %); all were euploid.
In a subsequent report, Palomaki et al (2012) selected 62 pregnancies with trisomy 18 and
12 with trisomy 13 from the cohort of 4,664 pregnancies along with matched euploid
controls (including 212 additional Down syndrome and matched controls already reported in
Palomaki, et al., 2011), and their samples tested by massively parallel DNA sequencing.
Among the 99.1% of samples interpreted (1,971/1,988), observed trisomy 18 detection
rates was 100% (59/59), with a false positive rate of 0.28%. Observed trisomy 13 detection
rate was 91.7% (11/12) with a false-positive rate of 0.97% however, this estimate was
based upon only 12 cases. Among the 17 samples without an interpretation, three were
trisomy 18. The authors stated that, if z-score cutoffs for trisomy 18 and 13 were raised
slightly, the overall false-positive rates for the three aneuploidies could be as low as 0.1%
(2/1,688) at an overall detection rate of 98.9% (280/283) for common aneuploidies.

Bianchi et al (2012) reported on a nested case-control study of the use of massively
parallel DNA sequencing to detect fetal aneuploidy. The investigators used blood samples
that were collected in a prospective, blinded study from 2,882 high-risk women scheduled to
undergo amniocentesis or chorionic villus sampling procedures at 60 U.S. sites. An
independent biostatistician selected from these blood samples all singleton pregnancies
with any abnormal karyotype, and for comparison, selected a balanced number of randomly
selected pregnancies with euploid karyotypes. Chromosome classifications were made for
each sample by massively parallel sequencing and compared with fetal karyotype
determined by amniocentesis or chorionic villus sampling. The authors had 532 samples,
221 of which had abnormal karyotypes. The authors reported that 89 of 89 trisomy 21 cases
were classified correctly (sensitivity 100%, 95% confidence interval [CI] 95.9 to 100), 35 of
36 trisomy 18 cases were classified correctly (sensitivity 97.2%, 95% CI 85.5 to -99.9), 11 of
14 trisomy 13 cases (sensitivity 78.6%, 95% CI 49.2 to 99.9), and 15 of 16 monosomy X
cases (sensitivity 93.8%, 95% CI 69.8 to 99.8). The authors reported that there were no
false-positive results for autosomal aneuploidies (100% specificity, 95% CI more than 98.5
to 100). In addition, fetuses with mosaicism for trisomy 21 (3/3), trisomy 18 (1/1), and
monosomy X (2/7), three cases of translocation trisomy, two cases of other autosomal
trisomies (20 and 16), and other sex chromosome aneuploidies (XXX, XXY, and XYY) were
classified correctly. 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. Specificities were estimated based upon a relatively small number of controls; further studies involving a larger number of unaffected controls would estimate the specificity with greater precision.

Regarding the evidence for cell-free DNA tests for fetal aneuploidy, Allyse et al (2012) has commented that companies offering such tests have limited themselves to publishing the results of clinical and analytic validation studies. However, more contextual, but no less important, issues of consistent and systematic validation, timing, risk and scope of cell-free DNA testing still need to be resolved. The authors stated that, at present, however, cell-free DNA tests have not achieved sufficient specificity and sensitivity to replace existing invasive tests as a diagnostic tool. The authors stated that demonstrated detection rates with cell-free DNA show a significant improvement over existing noninvasive integrated screening regimes. Nevertheless, they do not match the near-perfect diagnostic capabilities of invasive tests. Furthermore, existing clinical validation trials have taken place only in high-risk populations. The authors stated that it is unclear whether acceptable positive and negative predictive values can be attained in lower risk populations.

In addition to its noninvasive nature, another commonly espoused feature of non-invasive tests using cell-free DNA is its potential to detect fetal DNA beginning at 10 weeks of gestation (Allyse et al, 2012). Some women may want cell-free DNA testing to enable them to terminate a non-viable pregnancy as early as possible to avoid physical and emotional discomfort. However, a majority of pregnancies with trisomy 13, 18 and 21 spontaneously abort during the first trimester. Conducting early testing to recognize a trisomic pregnancy could require women to make wrenching decisions about termination and generate considerable guilt and stress that might have been avoided had the fetus spontaneously aborted. In addition to the psychosocial effects, this process would also entail spending considerable medical resources on prenatal care for non-viable pregnancies (Allyse, et al., 2012).

The Society for Maternal-Fetal Medicine (2015a) has stated that cell-free DNA (cfDNA) screening has been largely recommended in patients at higher risk for aneuploidy, given more limited evidence regarding effectiveness in lower risk populations. A subsequent statement by SMFM (2015b) indicated that SMFM does not recommend that cfDNA
aneuploidy screening be offered to all pregnant women. SMFM stated that "limited data at
the present time on the effectiveness and clinical utility for improving patient outcomes
preclude a recommendation that cfDNA be actively offered to all pregnant women...SMFM
recognizes the value of cfDNA screening for women at higher risk for aneuploidy but
considers that cfDNA screening is not the appropriate choice for first-line screening for the
low-risk obstetric population at the present time. For this population, conventional screening
methods remain the preferred approach. Given the misconceptions regarding interpretation
of cfDNA screening results and the serious consequences that have been documented,
there are significant concerns about the consequences of broad utilization of this test in low-
risk women, the vast majority of whom do not undergo genetic counseling or detailed pre-
test counseling with a provider."

The UK National Health Service Fetal Anomaly Screening Programme has stated that cell-
free DNA testing "is very much in the early stages of development" and more research is
needed to make sure cell-free DNA is a better test than those currently offered to women
wanting information on the health of their baby (Delbarre, 2012). As such, non-invasive
prenatal diagnosis using cell-free DNA to test for genetic conditions is unlikely to be
available on the UK National Health Service for at least five years. The UK National Health
Service would not currently consider using these tests to replace any of the tests currently
offered as part of the Fetal Anomaly Ultrasound and Down’s syndrome Screening
Programme.

Mersy and associates (2013) noted that research on non-invasive prenatal testing (NIPT) of
fetal trisomy 21 is developing fast. Commercial tests have become available. These
investigators provided an up-to-date overview of NIPT of trisomy 21 by evaluating
methodological quality and outcomes of diagnostic accuracy studies. These researchers
undertook a systematic review of the literature published between 1997 and 2012 after
searching PubMed, using MeSH terms “RNA”, “DNA” and “Down Syndrome” in combination
with “cell-free fetal (cf) RNA”, “cfDNA”, “trisomy 21” and “noninvasive prenatal diagnosis”
and searching reference lists of reported literature. From 79 abstracts, 16 studies were
included as they evaluated the diagnostic accuracy of a molecular technique for NIPT of
trisomy 21, and the test sensitivity and specificity were reported. Meta-analysis could not
be performed due to the use of 6 different molecular techniques and different cut-off points.
Diagnostic parameters were derived or calculated, and possible bias and applicability were evaluated utilizing the revised tool for Quality Assessment of Diagnostic Accuracy (QUADAS-2). Seven of the included studies were recently published in large cohort studies that examined massively parallel sequencing (MPS), with or without pre-selection of chromosomes, and reported sensitivities between 98.58 % [95 % CI: 95.9 to 99.5 %] and 100 % (95 % CI: 96 to 100 %) and specificities between 97.95 % (95 % CI: 94.1 to 99.3 %) and 100 % (95 % CI: 99.1 to 100 %). None of these 7 large studies had an overall low-risk of bias and low concerns regarding applicability. Massively parallel sequencing with or without pre-selection of chromosomes exhibits an excellent negative predictive value (100 %) in conditions with disease odds from 1:1,500 to 1:200. However, positive predictive values were lower, even in high-risk pregnancies (19.7 to 100 %). The other 9 cohort studies were too small to give precise estimates (number of trisomy 21 cases: less than or equal to 25) and were not included in the discussion. The authors concluded that NIPT of trisomy 21 by MPS with or without pre-selection of chromosomes is promising and likely to replace the prenatal serum screening test that is currently combined with NT measurement in the first trimester of pregnancy. Moreover, they stated that before NIPT can be introduced as a screening test in a social insurance health-care system, more evidence is needed from large prospective diagnostic accuracy studies in first trimester pregnancies. Furthermore, they believed further assessment, of whether NIPT can be provided in a cost-effective, timely and equitable manner for every pregnant woman, is needed.

Norton and colleagues (2013) stated that the recent introduction of clinical tests to detect fetal aneuploidy by analysis of cell-free DNA in maternal plasma represents a tremendous advance in prenatal diagnosis and the culmination of many years of effort by researchers in the field. The development of NIPT for clinical application by commercial industry has allowed much faster introduction into clinical care, yet also presents some challenges regarding education of patients and health care providers struggling to keep up with developments in this rapidly evolving area. It is important that health care providers recognize that the test is not diagnostic; rather, it represents a highly sensitive and specific screening test that should be expected to result in some false-positive and false-negative diagnoses. Although currently being integrated in some settings as a primary screening test for women at high-risk of fetal aneuploidy, from a population perspective, a better option for NIPT may be as a second-tier test for those patients who screen positive by conventional
aneuploidy screening. The authors concluded that how NIPT will ultimately fit with the
current prenatal testing algorithms remains to be determined. They stated that true cost-
utility analyses are needed to determine the actual clinical effectiveness of this approach in
the general prenatal population.

Lutgendorf et al (2014) stated that the clinical use of NIPT to screen high-risk patients for
fetal aneuploidy is becoming increasingly common. Initial studies have demonstrated high
sensitivity and specificity, and there is hope that these tests will result in a reduction of
invasive diagnostic procedures as well as their associated risks. Guidelines on the use of
this testing in clinical practice have been published; however, data on actual test
performance in a clinical setting are lacking, and there are no guidelines on quality control
and assurance. The different NIPT employ complex methodologies, which may be
challenging for health-care providers to understand and utilize in counseling patients,
particularly as the field continues to evolve. The authors concluded that how these new
tests should be integrated into current screening programs and their effect on health-care
costs remain uncertain.

Moise (2012) reviewed the evidence for the use of cell-free fetal DNA to determine the fetal
RHD gene. The feasibility of using cell-free fetal DNA circulating in maternal serum to
determine fetal RHD gene and guide administration of prophylaxis has been shown in
several studies (Rouillac-Le Sciellour et al, 2004; Finning et al, 2008; Muller et al, 2008; Van
der School et a., 2006; Clausen et al, 2012). In the largest of these studies (n = 2,312
Rh(D)-negative women), fetal RHD gene detection sensitivity was 99.9 % at 25 weeks of
gestation using an automated system that targeted 2 RHD exons (Clausen et al, 2012). Six
fetuses were falsely identified as RHD-positive and 74 results were inconclusive due to
methodologic issues or variant D types; all of these women received antenatal Rh(D)
prophylaxis. Prophylaxis was unnecessary and avoided in 862 true-negative cases (37.3
%), unnecessary antenatal prophylaxis was administered to 39 women who had a positive
or inconclusive result antenatally but delivered a Rh(D)-negative newborn (1.7 %). In 2
pregnancies (0.087 %), a Rh(D)-positive fetus was not detected antenatally so antenatal
prophylaxis was not given; however, the women received post-natal prophylaxis.
An assessment by the Swedish Council on Technology Assessment in Health Care (SBU, 2011) found that there is moderately strong scientific evidence that fetal RHD determination by non-invasive fetal diagnostic tests has a sensitivity and specificity of nearly 99%. The assessment stated that these results are largely based on studies of RhD-negative pregnant women who are not RhD-immunized. The report stated that those studies that also included pregnant women who have been immunized against RhD showed similar results. The report concluded that screening for fetal blood group using non-invasive fetal diagnostic tests, in combination with specific prenatal preventive measures (targeted RhD prophylaxis), could result in fewer RhD-negative pregnant women developing antibodies to RhD. The report concluded that the organizational and health economic consequences of introducing this type of screening have not been established.

In some European countries, fetal RHD gene determination is performed clinically in Rh(D)-negative women and administration of antenatal anti-D is avoided in the case of a RhD-negative fetus. An opinion by the Royal College of Obstetricians and Gynaecologists (Chitty and Crolla, 2009) stated that obstetricians have used non-invasive prenatal diagnosis to guide management of women who are RhD-negative and at risk of hemolytic disease of the newborn for years and guidelines should be revised to reflect this change in practice.

The American College of Obstetricians and Gynecologists (2012) has no recommendation for use of fetal cell-free DNA in preventing RHD alloimmunization.

Moise and Argoti (2012) evaluated the application of new technologies to the management of the red cell alloimmunized pregnancy. These investigators searched 3 computerized databases for studies that described treatment or prevention of alloimmunization in pregnancy (MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials [1990 to July 2012]). The text words and MeSH included Rhesus alloimmunization, Rhesus isoimmunization, Rhesus prophylaxis, Rhesus disease, red cell alloimmunization, red cell isoimmunization, and intrauterine transfusion. Of the 2,264 studies initially identified, 246 were chosen after limiting the review to those articles published in English and cross-referencing to eliminate duplication. Both authors independently reviewed the articles to eliminate publications involving less than 6 patients. Special emphasis was given to
publications that have appeared since 2008. Quantitative polymerase chain reaction can be used instead of serology to more accurately determine the paternal RHD zygosity. In the case of unknown or a heterozygous paternal RHD genotype, new DNA techniques now make it possible to diagnose the fetal blood type through cell-free fetal DNA in maternal plasma. Serial Doppler assessment of the peak systolic velocity in the middle cerebral artery is now the standard to detect fetal anemia and determine the need for the first intrauterine transfusion. Assessment of the peak systolic velocity in the middle cerebral artery can be used to time the second transfusion, but its use to decide when to perform subsequent procedures awaits further study. New data suggested normal neurologic outcome in 94% of cases after intrauterine transfusion, although severe hydrops fetalis may be associated with a higher risk of impairment. Recombinant Rh immune globulin is on the horizon. The authors stated that cell-free fetal DNA for fetal RHD genotyping may be used in the future to decide which patients should receive antenatal Rh immune globulin.

An ACOG guideline on “Cell-Free DNA Testing for Fetal Aneuploidy” (2015) stated that “given the performance of conventional screening methods, the limitations of cfDNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population. Routine cfDNA screening for micro-deletion syndromes should not be performed and cfDNA screening is not recommended for women with multiple gestations”.

Bianchi et al (2014) noted that in high-risk pregnant women, non-invasive prenatal testing with the use of massively parallel sequencing of maternal plasma cell-free DNA (cfDNA testing) accurately detects fetal autosomal aneuploidy. Its performance in low-risk women is unclear. At 21 centers in the U.S., these researchers collected blood samples from women with singleton pregnancies who were undergoing standard aneuploidy screening (serum biochemical assays with or without nuchal translucency measurement). They performed massively parallel sequencing in a blinded fashion to determine the chromosome dosage for each sample. The primary end-point was a comparison of the false positive rates of detection of fetal trisomies 21 and 18 with the use of standard screening and cfDNA testing. Birth outcomes or karyotypes were the reference standard. The primary series included 1,914 women (mean age of 29.6 years) with an eligible sample, a singleton fetus
without aneuploidy, results from cfDNA testing, and a risk classification based on standard screening. For trisomies 21 and 18, the false positive rates with cfDNA testing were significantly lower than those with standard screening (0.3 % versus 3.6 % for trisomy 21, p < 0.001; and 0.2 % versus 0.6 % for trisomy 18, p = 0.03). The use of cfDNA testing detected all cases of aneuploidy (5 for trisomy 21, 2 for trisomy 18, and 1 for trisomy 13; NPV, 100 % [95 % CI: 99.8 to 100]). The PPV for cfDNA testing versus standard screening were 45.5 % versus 4.2 % for trisomy 21 and 40.0 % versus 8.3 % for trisomy 18. The authors concluded that in a general obstetrical population, prenatal testing with the use of cfDNA had significantly lower false positive rates and higher PPVs for detection of trisomies 21 and 18 than standard screening. The drawbacks of this study included a relatively small number of true positive results for determining test sensitivity and the need to base the outcome data mainly on clinical examinations. Furthermore, 28.5 % of the results of cfDNA testing were obtained in the third trimester. Since the fetal fraction increases with gestational age, this factor may have contributed to the improved performance of cfDNA testing. Also, 0.9 % of cfDNA tests did not provide results. Although this rate of failure was lower than rates in other studies of DNA testing, the possibility of test failure should be discussed during pretest counseling. The authors stated that these findings suggested that cfDNA testing merits serious consideration as a primary screening method for fetal autosomal aneuploidy.

Norton et al (2015) noted that cfDNA testing for fetal trisomy is highly effective among high-risk women. However, there have been few direct, well-powered studies comparing cfDNA testing with standard screening during the first trimester in routine prenatal populations. In this prospective, multi-center, blinded study conducted at 35 international centers, these investigators assigned pregnant women presenting for aneuploidy screening at 10 to 14 weeks of gestation to undergo both standard screening (with measurement of nuchal translucency and biochemical analytes) and cfDNA testing. Participants received the results of standard screening; the results of cfDNA testing were blinded. Determination of the birth outcome was based on diagnostic genetic testing or newborn examination. The primary outcome was the area under the receiver-operating-characteristic curve (AUC) for trisomy 21 (Down’s syndrome) with cfDNA testing versus standard screening. These researchers also evaluated cfDNA testing and standard screening to evaluate the risk of trisomies 18 and 13. Of 18,955 women who were enrolled, results from 15,841 were
available for analysis. The mean maternal age was 30.7 years, and the mean gestational age at testing was 12.5 weeks. The AUC for trisomy 21 was 0.999 for cfDNA testing and 0.958 for standard screening (p = 0.001). Trisomy 21 was detected in 38 of 38 women (100%; 95% CI: 90.7 to 100) in the cfDNA-testing group, as compared with 30 of 38 women (78.9%; 95% CI: 62.7 to 90.4) in the standard-screening group (p = 0.008). False positive rates were 0.06% (95% CI: 0.03 to 0.11) in the cfDNA group and 5.4% (95% CI: 5.1 to 5.8) in the standard-screening group (p < 0.001). The PPV for cfDNA testing was 80.9% (95% CI: 66.7 to 90.9), as compared with 3.4% (95% CI: 2.3 to 4.8) for standard screening (p < 0.001). The authors concluded that in this large, routine prenatal-screening population, cfDNA testing for trisomy 21 had higher sensitivity, a lower false positive rate, and higher PPV than did standard screening with the measurement of nuchal translucency and biochemical analytes. The authors stated that “Before cfDNA testing can be widely implemented for general prenatal aneuploidy screening, careful consideration of the screening method and costs is needed. Although the sensitivity and specificity of cfDNA testing are higher than those of standard screening, these benefits are lower when cases with no results on cfDNA are considered …. Although these data support the use of cfDNA testing in women regardless of age or risk status, further cost utility studies are warranted”.

Dondorf et al (2015) presented a joint European Society of Human Genetics (ESHG) and the American Society of Human Genetics (ASHG) position document with recommendations regarding responsible innovation in prenatal screening with NIPT. By virtue of its greater accuracy and safety with respect to prenatal screening for common autosomal aneuploidies, NIPT has the potential of helping the practice better achieve its aim of facilitating autonomous reproductive choices, provided that balanced pretest information and non-directive counseling are available as part of the screening offer. Depending on the health-care setting, different scenarios for NIPT-based screening for common autosomal aneuploidies are possible. The trade-offs involved in these scenarios should be assessed in light of the aim of screening, the balance of benefits and burdens for pregnant women and their partners and considerations of cost-effectiveness and justice. With improving screening technologies and decreasing costs of sequencing and analysis, it will become possible in the near future to significantly expand the scope of prenatal screening beyond common autosomal aneuploidies. Commercial providers have already begun expanding their tests to include sex-chromosomal abnormalities and micro-
deletions. However, multiple false positives may undermine the main achievement of NIPT in the context of prenatal screening: the significant reduction of the invasive testing rate. This document argued for a cautious expansion of the scope of prenatal screening to serious congenital and childhood disorders, only following sound validation studies and a comprehensive evaluation of all relevant aspects. A further core message of this document is that in countries where prenatal screening is offered as a public health program, governments and public health authorities should adopt an active role to ensure the responsible innovation of prenatal screening on the basis of ethical principles. Crucial elements are the quality of the screening process as a whole (including non-laboratory aspects such as information and counseling), education of professionals, systematic evaluation of all aspects of prenatal screening, development of better evaluation tools in the light of the aim of the practice, accountability to all stake-holders including children born from screened pregnancies and persons living with the conditions targeted in prenatal screening and promotion of equity of access.

The position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis (Benn et al, 2015) stated that "High sensitivities and specificities are potentially achievable with cfDNA screening for some fetal aneuploidies, notably trisomy 21 …. When cfDNA screening is extended to micro-deletion and micro-duplication syndromes or rare trisomies, the testing should be limited to clinically significant disorders or well-defined severe conditions. There should be defined estimates for the detection rates, false positive rates, and information about the clinical significance of a positive test for each disorder being screened”.

Yaron et al (2015) stated that NIPT using cfDNA in maternal blood for trisomy 21 was introduced in 2011. This technology has continuously evolved with the addition of screening for trisomy 18 and trisomy 13 followed by the inclusion of sex chromosome aneuploidies. Expanded non-invasive prenatal test panels have recently become available, which enable screening for micro-deletion syndromes such as the 22q11.2 deletion (associated with the velocardiofacial syndrome) and others. However, the performance data for these micro-deletion syndromes are derived from a small number of samples, mostly generated in-vitro. Rigorous performance evaluation, as was done at least for trisomy 21 testing using cfDNA analysis, is difficult to perform given the rarity of each condition. In addition, detection rates
may vary considerably depending on deletion size. More importantly, PPVs strongly influenced by the low prevalence, are expected to be significantly lower than 10% for most conditions. Thus, screening in an average-risk population is likely to have many more false-positives than affected cases detected. Conversely, testing in a high-risk population such as fetuses with cardiac anomalies may have higher PPVs, but a negative result needs to be considered carefully as a result of uncertain information about detection rates and a significant residual risk for other copy number variants and single gene disorders. The authors concluded that cfDNA testing for micro-deletion syndromes and rare autosomal trisomies (e.g., trisomy 2, 5, 7, 8 [Warkany syndrome 2], 9, 12, 14, 15, 16, 17, and 22) is currently unsupported by sufficient clinical evidence. Routine testing for these conditions should await comprehensive clinical validation studies and a demonstration of PPV and clinical utility in the population to be tested.

The ACOG/SMFM practice bulletin on “Screening for Fetal Aneuploidy” (2016) provided the following recommendations:

**Level A Recommendations:**

- Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing.
- All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken.
- Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.

**Level B Recommendations:**

- Cell-free DNA screening tests for micro-deletions have not been validated clinically and are not recommended at this time.
Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy.

In a systematic review and meta-analysis, Taylor-Phillips et al (2016) measure test accuracy of NIPT for Down (trisomy 21), Edwards (trisomy 18) and Patau (trisomy 13) syndromes using fetal cfDNA and identified factors affecting accuracy. PubMed, Ovid Medline, Ovid Embase and the Cochrane Library published from 1997 to February 9, 2015, followed by weekly auto-alerts until April 1, 2015. English language journal articles describing case-control studies with greater than or equal to 15 trisomy cases or cohort studies with greater than or equal to 50 pregnant women who had been given NIPT and a reference standard.

A total of 41, 37 and 30 studies of 2012 publications retrieved were included in the review for Down, Edwards and Patau syndromes. Quality appraisal identified high risk of bias in included studies, funnel plots showed evidence of publication bias. Pooled sensitivity was 99.3 % (95 % CI: 98.9 % to 99.6 %) for Down, 97.4 % (95.8 % to 98.4 %) for Edwards, and 97.4 % (86.1 % to 99.6 %) for Patau syndrome. The pooled specificity was 99.9 % (99.9 % to 100 %) for all 3 trisomies. In 100,000 pregnancies in the general obstetric population these researchers would expect 417, 89 and 40 cases of Downs, Edwards and Patau syndromes to be detected by NIPT, with 94, 154 and 42 false positive results. Sensitivity was lower in twin than singleton pregnancies, reduced by 9 % for Down, 28 % for Edwards and 22 % for Patau syndrome. Pooled sensitivity was also lower in the first trimester of pregnancy, in studies in the general obstetric population, and in cohort studies with consecutive enrolment. The authors concluded that NIPT using cell-free fetal DNA has very high sensitivity and specificity for Down syndrome, with slightly lower sensitivity for Edwards and Patau syndrome. However, it is not 100 % accurate and should not be used as a final diagnosis for positive cases.

In a review on “Aneuploidy screening: Newer noninvasive test gains traction”, Nickolich and colleagues (2016) stated that the overall cost-effectiveness of cfDNA for aneuploidy screening in low-risk women is unknown.
Natera's Panorama® non-invasive prenatal test (NIPT) is a cell-free DNA test and is the first NIPT that can determine whether twins are monozygotic (identical) or dizygotic (non-identical or fraternal), determine the gender of each twin, and detect chromosomal abnormalities as early as nine weeks' gestation. It also helps identify risk for more genetic conditions in twin pregnancies than other NIPTs, including monosomy X, sex chromosome trisomies, and 22q11.2 deletion syndrome.

UpToDate states the use of cfDNA for screening twin pregnancies is not yet endorsed by ACOG, ACMG, or other professional societies. The amount of cfDNA for the pregnancy overall is about 35 percent higher in twin pregnancies than singleton pregnancies. In turn, the amount of cfDNA contributed by each twin is lower than in a singleton pregnancy and may be quite different for the two fetuses in dizygotic twins. One approach, therefore, is to modify the algorithm used for singleton pregnancies to estimate the smallest fetal fraction contribution of the two fetuses, which involves identifying nonpolymorphic and polymorphic loci where fetal alleles differ from maternal alleles. However, this type of analysis is not performed by the majority of laboratories providing cfDNA testing. Because it is impossible to determine which twin is abnormal based on cfDNA analysis alone, results are reported for the entire pregnancy, and invasive testing is required to distinguish which twin, if either one, is affected. Some laboratories that offer cfDNA screening in twin pregnancies use methods that are "blind" to the number of fetuses.

Based on preliminary data from 758 twin pregnancies with known outcomes, the detection rates for trisomy 21, 18, and 13 were 95 (37/39), 85 (6/7), and 100 percent (2/2), respectively. An important ancillary finding was a higher test failure rate, especially if the methodology was able to determine the fetal-specific fetal fraction and the lower of the two was used for inclusion. One methodology using single nucleotide polymorphisms can identify dizygotic twins but cannot provide an interpretation. This may be seen as a disadvantage, but such a test can also identify a vanished twin in the same way, which may have clinical advantages. Based on two meta-analyses, the consensus detection rate and false positive rate for trisomy 21 in twin pregnancies were 98.7 and 0.11 percent, respectively. In addition, 13 of 14 twins with trisomy 18 were detected, along with two of three trisomy 13 pregnancies.
Sarno et al (2016) aimed to examine in twin pregnancies the performance of first-trimester screening for fetal trisomies 21, 18 and 13 by cell-free (cf) DNA testing of maternal blood and, second, to compare twin and singleton pregnancies regarding the distribution of fetal fraction of cfDNA and rate of failure to obtain a result. This was a prospective study in 438 twin and 10 698 singleton pregnancies undergoing screening for fetal trisomies by cfDNA testing at 10 + 0 to 13 + 6 weeks’ gestation. Chromosome-selective sequencing of cfDNA was used and, in twin pregnancies, an algorithm was applied that relies on the lower fetal fraction contributed by the two fetuses. Multivariate regression analysis was used to determine significant predictors of fetal fraction and a failed result. In twin pregnancies, the median fetal fraction was lower (8.0% (interquartile range (IQR), 6.0-10.4%) vs 11.0% (IQR, 8.3-14.4%); P < 0.0001) and failure rate after first sampling was higher (9.4% vs 2.9%; P < 0.0001) compared to in singletons. Multivariate logistic regression analysis demonstrated that the risk of test failure increased with increasing maternal age and body mass index and decreased with fetal crown-rump length. The risk was increased in women of South Asian racial origin and in pregnancies conceived by in-vitro fertilization (IVF). The main contributor to the higher rate of failure in twins was conception by IVF which was observed in 9.5% of singletons and 56.2% of twins. In the 417 twin pregnancies with a cfDNA result after first or second sampling, the detection rate was 100% (8/8) for trisomy 21 and 60% (3/5) for trisomies 18 or 13, at a false-positive rate (FPR) of 0.25% (1/404). In the 10 530 singleton pregnancies with a cfDNA result after first or second sampling, the detection rate was 98.7% (156/158) for trisomy 21 and 80.3% (49/61) for trisomies 18 or 13, at a FPR of 0.22% (23/10 311). The authors concluded that in twin pregnancies undergoing first-trimester screening for trisomies by cfDNA testing, the fetal fraction is lower and failure rate higher compared to in singletons. The number of trisomic twin pregnancies examined was too small for an accurate assessment of performance of screening, but it may be similar to that in singleton pregnancies.

Milan et al (2018) sought to develop an accurate sex classification method in twin pregnancies using data obtained from a standard commercial non-invasive prenatal test. A total of 706 twin pregnancies were included in this retrospective analytical data study. Normalized chromosome values for chromosomes X and Y were used and adapted into a sex-score to predict fetal sex in each fetus, and results were compared with the clinical outcome at birth. Outcome information at birth for sex chromosomes was available for 232
twin pregnancies. From these, a total of 173 twin pregnancies with a Y chromosome identified in non-invasive pregnancy testing were used for the development of a predictive model. Global accuracy for sex classification in the testing set with 51 samples was 0.98 (95% confidence interval [0.90,0.99]), with a specificity and sensitivity of 1 (95% confidence interval [0.82,1.00]) and 0.97 (95% confidence interval [0.84,0.99]), respectively. The authors concluded that while non-invasive prenatal testing is a screening method and confirmatory results must be obtained by ultrasound or genetic diagnosis, the sex-score determination presented herein offers an accurate and useful approach to characterizing fetus sex in twin pregnancies in a non-invasive manner early on in pregnancy.

Quibel and Rozenberg (2018) noted that in France, the recommended method for Down syndrome screening is the 1st trimester combined test, the risk assessment, based on maternal age, ultrasound (US) measurement of fetal nuchal translucency and maternal serum markers (free β-hCG and PAPP-A). The Down syndrome detection rate is 78.7 % at a screen positive rate of 5 %. However, the best screening test is the integrated test using a combination of 1st trimester combined test and 2nd trimester quadruple test (serum AFP, hCG, unconjugated E3, and dimeric inhibin-A) and being able to achieve a detection rate for Down syndrome of approximately 96 % at a screen-positive rate of 5 %. In recent years, the isolation of small fragments of "fetal" cfDNA in the maternal blood dramatically changed the screening strategy paradigm allowing a Down syndrome detection rate and false positive rate of 99.2 % and 0.09 %, respectively. However, aneuploidy screening based on cfDNA presents 2 major limitations, which must be taken into account because they considerably limit its benefit. First, not every woman will receive an interpretable result and that those who fail to receive a result are at increased risk for fetal aneuploidy: whether an inconclusive result is treated as screen positive or screen negative affects the overall detection rate (sensitivity) and false-positive rate (specificity) of the test. Secondly, the limited number of targeted aneuploidies (trisomies 21, 18, 13 and common sex chromosome aneuploidies) in contrast to conventional non-invasive screening, which is also able to detect rare aneuploidies, duplications, deletions, and other structural re-arrangements. Of course, genetic counseling has to include a discussion about benefits and limitations of aneuploidy screening based on cfDNA. However, it should not be considered as a new screening test to substitute for conventional non-invasive screening. Moreover, if the ultimate goal is to deliver the most information regarding potential risk of
various chromosomal abnormalities associated with adverse perinatal outcomes, then
current cfDNA screening strategies may not be the best approach. These data highlighted
the limitations of cfDNA screening and the importance of a clear and fair information during
pre-test genetic counseling regarding benefits and limitations of any prenatal non-invasive
screening (whether conventional or by cfDNA), but also about risks and benefits of invasive
diagnostic procedures (in 1st- or 2nd-line), especially since the cytogenetic analysis with
chromosomal microarray analysis has improved the detection of genome microdeletions
and microduplications (variants of the copy number) that cannot be detected by standard
cytogenetic analysis.

Hayward and Chitty (2018) noted that emerging genomic technologies, largely based
around NGS, are offering new promise for safer prenatal genetic diagnosis (PGD). These
innovative approaches will improve screening for fetal aneuploidy, allow definitive NIPD of
single gene disorders at an early gestational stage without the need for invasive testing,
and improve the ability to detect monogenic disorders as the etiology of fetal abnormalities.
In addition, the transformation of prenatal genetic testing arising from the introduction of
whole genome, exome and targeted NGS produces unprecedented volumes of data
requiring complex analysis and interpretation. Now translating these technologies to the
clinic has become the goal of clinical genomics, transforming modern healthcare and
personalized medicine. The achievement of this goal requires the most progressive
technological tools for rapid high-throughput data generation at an affordable cost.
Furthermore, as larger proportions of patients with genetic disease are identified clinicians
must be ready to offer appropriate genetic counselling to families and potential parents. In
addition, the identification of novel treatment targets will continue to be explored, which is
likely to introduce ethical considerations, especially if genome editing techniques are
included in these targeted treatments and transferred into mainstream personalized
healthcare. The authors reviewed the impact of NGS technology to analyze cfDNA in
maternal plasma to deliver NIPD for monogenic disorders and allow more comprehensive
investigation of the abnormal fetus through the use of exome sequencing.

Zhang and colleagues (2019) stated that current non-invasive prenatal screening is targeted
toward the detection of chromosomal abnormalities in the fetus. However, screening for
many dominant monogenic disorders associated with de-novo mutations is not available,
despite their relatively high incidence. These investigators reported on the development and validation of, and early clinical experience with, a new approach for non-invasive prenatal sequencing for a panel of causative genes for frequent dominant monogenic diseases; cfDNA extracted from maternal plasma was bar-coded, enriched, and then analyzed by NGS for targeted regions. Low-level fetal variants were identified by a statistical analysis adjusted for NGS read count and fetal fraction. Pathogenic or likely pathogenic variants were confirmed by a secondary amplicon-based test on cfDNA. Clinical tests were performed on 422 pregnancies with or without abnormal US findings or family history. Follow-up studies on cases with available outcome results confirmed 20 true-positive, 127 true-negative, 0 false-positive, and 0 false-negative results. The authors concluded that the initial clinical study showed that this non-invasive test could provide valuable molecular information for the detection of a wide spectrum of dominant monogenic diseases, complementing current screening for aneuploidies or carrier screening for recessive disorders.

Sonographic Markers of Fetal Aneuploidy

Raniga et al (2006) stated that chromosomal abnormalities occur in 0.1 % to 0.2 % of live births, and the most common clinically significant aneuploidy among live-born infants is DS (trisomy 21). Other sonographically detectable aneuploidies include trisomy 13, 18, monosomy X, and triploidy. Second-trimester ultrasound scan detects 2 types of sonographic markers suggestive of aneuploidy. Markers for major fetal structural abnormalities comprise the first type; the second type of markers are known as "soft markers" of aneuploidy. These latter markers are non-specific, often transient, and can be readily detected during the 2nd-trimester ultrasound. The most commonly studied soft markers of aneuploidy include absent or hypoplastic nasal bone, choroid plexus cyst, echogenic bowel, and echogenic intracardiac focus, mild fetal pyelectasis, and rhabdomyel limb shortening. There is a great deal of interest in the ultrasound detection of aneuploidy, as evidenced by the large number of publications in the literature on this topic. Unfortunately, studies evaluating the significance of the soft markers of aneuploidy varied widely and showed contradictory results. These investigators reviewed the most common ultrasonographic soft markers used to screen aneuploidy and discussed ultrasonographic technique and measurement criteria for the detection of soft markers. They also reviewed the clinical relevance of soft markers to aneuploidy risk assessment and evidence-based
strategies for the management of affected pregnancies with each of these markers in light of current literature. The authors concluded that the detection of any abnormal finding on ultrasound should prompt an immediate detailed ultrasound evaluation of the fetus by an experienced sonographer. If there is more than 1 abnormal finding on ultrasound, if the patient is older than 35 years of age, or if the multiple marker screen is abnormal, an amniocentesis should be recommended to rule out aneuploidy.

Coco and Jeanty (2005) examined if isolated pyelecasis is a risk factor for trisomy 21. A total of 12,672 unselected singleton fetuses were examined by prenatal ultrasound during the 2nd trimester at a single institution. The sensitivity, specificity, positive-predictive value (PPV), negative-predictive value (NPV), and likelihood ratio of pyelecasis (either isolated or in association with other soft markers/structural anomalies) to detect trisomy 21 were calculated. Pyelecasis (antero-posterior pelvic diameter greater than or equal to 4 mm) was detected in 2.9 % (366/12,672) of the fetuses. Among these, 83.3 % (305/366) were isolated, and 16.7 % (61/366) were associated with other markers/structural anomalies. The prevalence of trisomy 21 was 0.087 % (11/12,672) and, among these fetuses, 2 (18.1 %) had pyelecasis, 1 isolated, and 1 associated with other markers/structural anomalies. The presence of isolated pyelecasis had 9.09 % sensitivity, 97.6 % specificity, 0.33 % PPV, and 99.9 % NPV to detect fetuses with trisomy 21. The likelihood ratio of trisomy 21 in this group of fetuses was 3.79 (95 % CI: 0.582 to 24.616). Among fetuses with pyelecasis and other associated markers/structural anomalies, the sensitivity, specificity, PPV, NPV, and likelihood ratio for trisomy 21 were 9.09 %, 99.5 %, 1.64 %, 99.9 %, and 19.2 (95 % CI: 2.91 to 126.44). The authors concluded that in the absence of other findings, isolated pyelecasis is not a justification for the performance of an amniocentesis.

Smith-Bindman et al (2007) examined the association between 2nd trimester ultrasound findings (genetic sonogram) and the risk of DS. This was a prospective population-based cohort study of women who were at increased risk of chromosome abnormality based on serum screening. Overall, 9,244 women with singleton pregnancies were included, including 245 whose fetuses had DS. Overall, 15.3 % of the women had an abnormal genetic sonogram, including 14.2 % of pregnancies with normal fetuses and 53.1 % of those with DS. If the genetic sonogram were normal, the risk that a woman had a fetus with DS was reduced (likelihood ratio 0.55 [95 % CI: 0.49, 0.62]). However, if the normal genetic
sonogram were used to counsel these high-risk women that they could avoid amniocentesis, approximately 50% of the cases of DS (115 of 245) would have been missed. The isolated ultrasound soft markers were the most commonly observed abnormality. These were seen in a high proportion of DS fetuses (13.9%) and normal fetuses (9.3%). In the absence of a structural anomaly, the isolated ultrasound soft markers of choroid plexus cyst, echogenic bowel, clenched hands, clinodactyly, renal pyelectasis, short femur, short humerus, and 2-vessel umbilical cord were not associated with DS. Nuchal fold thickening was a notable exception, as a thick nuchal fold raised the risk of DS even when it was seen without an associated structural anomaly. The authors concluded that the accuracy of the genetic sonogram is less than previously reported. The genetic sonogram should not be used as a sequential test following serum biochemistry, as this would substantially reduce the prenatal diagnosis of DS cases. Moreover, they stated that in contrast to prior reports, most isolated soft markers were not associated with DS.

Cho and associates (2009) described ultrasound findings in fetuses with trisomy 18. These investigators performed a prospective population-based cohort study of 2nd trimester ultrasound among Californian women who were at increased risk of chromosome abnormality based on serum screening between November 1999 and April 2001. Structural anomalies plus the following soft markers were assessed: choroid plexus cyst (CPC), clenched hands, clinodactyly, echogenic bowel, echogenic intracardiac focus, nuchal fold thickening, renal pyelectasis, short femur, short humerus and a single umbilical artery (SUA). Overall, 8,763 women underwent ultrasound evaluation, including 56 whose fetuses had trisomy 18. Ultrasound anomalies were seen in 89% of trisomy 18 fetuses, as compared with 14% of normal fetuses. If the genetic sonogram was normal (no structural anomaly and no soft marker), the risk was reduced by approximately 90%. The ultrasound soft markers were typically seen in conjunction with structural anomalies in affected fetuses and in the absence of a structural anomaly, most isolated ultrasound soft markers were not associated with trisomy 18. The only exception was an isolated CPC, seen as the only finding in 11% of fetuses with trisomy 18. The authors concluded that if the genetic sonogram is used as a sequential test following serum biochemistry, a normal ultrasound study reduces the likelihood of trisomy 18 substantially even if a woman has abnormal serum biochemistry. The presence of an isolated CPC raised the risk, but not high enough to prompt invasive testing.
Ting and colleagues (2011) examined the significance of isolated absent or hypoplastic nasal bone in the 2nd trimester ultrasound scan. All cases of absent or hypoplastic nasal bone (length less than 5th percentile) encountered during 2007 to 2009 were retrieved from database and all the ultrasound findings including structural abnormalities and soft markers for DS and fetal karyotype were reviewed. The cases were categorized into a study group with isolated absent or hypoplastic nasal bone and a comparison group with additional ultrasound findings. The incidence of DS confirmed by karyotyping was compared between the 2 groups. Among 14 fetuses with absent or hypoplastic nasal bone identified, 6 (42.9 %) had DS and 8 (57.1 %) were normal. All (100 %) of the 6 fetuses with isolated absent or hypoplastic nasal bone (Study Group) had normal karyotype, while 6 (75 %) of the other 8 fetuses with additional ultrasound findings (Comparison Group) had DS (p=0.010). The authors concluded that the use of isolated absent or hypoplastic nasal bone in the 2nd trimester ultrasound scan for DS screening may not be effective. Amniocentesis, however, is indicated for fetuses with structural abnormality or additional soft markers, which should be carefully searched by an experienced ultrasonographer.

Ameratunga et al (2012) described the association between fetal echogenic bowel (FEB) diagnosed during the 2nd trimester and adverse perinatal outcomes in an Australian antenatal population. A retrospective analysis of ultrasound scans was performed between March 1, 2004 and March 1, 2009 at The Royal Women's Hospital, Melbourne, Vic., Australia. Cases reported as having FEB on 2nd trimester ultrasound were included. Medical records of each case were reviewed and information concerning additional investigations and perinatal outcomes were extracted. A total of 66 cases were identified in the database. Three patients (5 %) were excluded from further analysis as they were lost to follow-up, leaving 63 (95 %) cases in this series. Thirty-two fetuses (52 %) underwent karyotyping via amniocentesis, 5 (16 %) of which were found to have chromosomal defects. Maternal serology for cytomegalovirus (CMV) was performed in 49 (78 %) cases. Investigations indicated a total of 5 women who had CMV infection during their pregnancy. Thirty-three pregnancies (53 %) were tested for cystic fibrosis (CF) and 1 baby was confirmed to have CF post-natally. Among the 50 live-born infants, 3 cases of fetal growth restriction were apparent. Overall, 42 of the 50 live-born infants (84 %) and 67 % of the entire cohort of 63 patients with a mid-trimester diagnosis of FEB had a normal short-term neonatal outcome. The authors concluded that the findings of this study reiterated the
increased prevalence of aneuploidy, CMV, CF and fetal growth restriction in pregnancies complicated by the mid-trimester sonographic finding of FEB. However, reassuringly, 67 % of cases with ultrasound-detected echogenic bowel in the 2nd trimester had a normal short-term neonatal outcome in this multi-ethnic Australian population.

Butler et al (2013) determined the outcome of infants who presented with FEB and identified additional sonographic findings that might have clinical relevance for the prognosis. These investigators reviewed all pregnancies in which the diagnosis FEB was made in the authors’ Fetal Medicine Unit during 2009 to 2010 (n = 121). They divided all cases into 5 groups according to additional sonographic findings. Group 1 consisted of cases of isolated FEB, group 2 of FEB associated with dilated bowels, group 3 of FEB with 1 or 2 other soft markers, group 4 of FEB with major congenital anomalies or 3 or more other soft markers, and group 5 consisted of FEB with isolated intra-uterine growth restriction (IUGR). Of 121 cases, 5 were lost to follow-up. Of the remaining 116 cases, 48 (41.4 %) were assigned to group 1, 15 (12.9 %) to group 2, 15 (12.9 %) to group 3, 27 (23.2 %) to group 4, and 11 (9.5 %) to group 5. The outcome for group 1 was uneventful. In group 2 and 3, 2 anomalies, anorectal malformation and cystic fibrosis, were detected post-natally (6.7 %). In group 4, mortality and morbidity were high (78 % and 22 %, respectively). Group 5 also had high mortality (82 %) and major morbidity (18 %). The authors concluded that if FEB occurs in isolation, it is a benign condition carrying a favorable prognosis. If multiple additional anomalies or early IUGR are observed, the prognosis tends to be less favorable to extremely poor.

ADAM12

Laigaard and colleagues (2006) stated that maternal serum A Disintegrin And Metalloprotease 12 (ADAM 12) is reduced, on average, in early first trimester Down and Edwards’ syndrome pregnancies; however the extent of reduction declines with gestation. These investigators examined the levels of ADAM 12 at 9 to 12 weeks when the marker might be used concurrently with other established markers. Samples from 16 Down and 2 Edwards’ syndrome cases were retrieved from storage and tested together with 313 unaffected singleton pregnancies using a semi-automated time-resolved immuno-fluorometric assay. Results were expressed in multiples of the gestation-specific median (MoM) based on regression. The median in Down syndrome was 0.94 MoM with a 10th to
90th percentile range of 0.22 to 1.63 MoM compared with 1.00 and 0.33 to 2.24 MoM in unaffected controls (p = 0.21, one-side Wilcoxon Rank Sum Test). The 2 Edwards' syndrome cases had values 0.31 and 2.17 MoM. The authors concluded that ADAM12 can not be used concurrently with other markers in the late first trimester. However, it does have the potential to be used earlier in pregnancy either concurrently with other early markers or in a sequential or contingent protocol. The authors stated that more research is needed to reliably predict the performance of either approach. Furthermore, the ACOG practice bulletin on screening for fetal chromosomal abnormalities (2007) does not mention ADAM 12 as a serum marker for screening Down syndrome.

Christiansen et al (2007) examined the potential of ADAM 12 as a second-trimester maternal serum marker of Down syndrome (DS). The concentration of ADAM 12 was determined in gestational week 14 to 19 in 88 DS pregnancies and 341 matched control pregnancies. Medians of normal pregnancies were established by polynomial regression and the distribution of log(10) MoM ADAM 12 values in DS pregnancies and controls determined. Correlations with alpha-fetoprotein (AFP) and free beta-hCG were established and used to model the performance of maternal serum screening with ADAM 12 in combination with second-trimester serum markers. The ADAM 12 maternal serum concentration was significantly increased with a median MoM of 1.85 and a mean log(10) MoM (SD) of 0.268 (0.2678) compared to a mean log(10) MoM (SD) of 0.013 (0.4318) in controls. ADAM 12 correlated with maternal weight and ethnicity (with the serum concentration increased in Afro-Caribbeans), but neither with maternal age nor gestational age, and only marginally with AFP (r(DS) = 0.078, r(controls) = 0.093) and free beta-hCG (r(DS) = 0.073, r(controls) = 0.144. The increase in detection rate – for a false positive rate of 5 % – by adding ADAM 12 to the double test (AFP + free beta-hCG) was 4 %, similar to that of adding unconjugated estriol to the double test. The authors concluded that ADAM 12 is an efficient second-trimester marker for DS. Moreover, they stated that further studies should be conducted to determine whether it may be a useful additional or alternative marker to those currently used in the second-trimester.

Koster and co-workers (2010) ascertained the distributions of pregnancy-associated plasma protein A (PAPP-A), fbeta-hCG, ADAM12 and PP13 in first trimester twin pregnancies. Serum marker concentrations were measured in monochorionic and dichorionic twin
pregnancies and singleton controls to study differences in MoMs. Median PAPP-A and 
ftbeta-hCG MoMs were 2.03 and 1.87 for monochorionic twins (n = 116) and 2.18 and 1.89 
for dichorionic twins (n = 650). Furthermore, ADAM12 and PP13 MoMs were 1.66 and 1.56 
for monochorionic twins (n = 51) and 1.64 and 1.53 for dichorionic twins (n = 249). No 
statistically significant differences between monochorionic and dichorionic twin pregnancies 
were found. Correlations between markers in these pregnancies did not differ from 
singletons. The authors concluded that for first-trimester screening, different parameters for 
monochorionic and dichorionic twin pregnancies is not necessary. Furthermore, if ADAM12 
and PP13 will be adopted as screening markers, the presented median MoM values, 
standard deviations and correlation coefficients for twin pregnancies may contribute to a 
proper twin risk estimation.

In a case control study, Torring and colleagues (2010) examined if ADAM12-S is a useful 
serum marker for fetal trisomy 21 using the mixture model. These researchers measured 
ADAM12-S by KRYPTOR ADAM12-S immunoassay in maternal serum from gestational 
weeks 8 to 11 in 46 samples of fetal trisomy 21 and in 645 controls. Comparison of 
sensitivity and specificity of first trimester screening for fetal trisomy 21 with or without 
ADAM12-S was included in the risk assessment using the mixture model. The 
concentration of ADAM12-S increased from week 8 to 11 and was negatively correlated 
with maternal weight. Log MoM ADAM12-S was positively correlated with log MoM PAPP-A 
(r = 0.39, p < 0.001), and with log MoM free beta hCG (r = 0.21, p < 0.001). The median 
ADAM12-S MoM in cases of fetal trisomy 21 in gestational week 8 was 0.66 increasing 
to about 0.9 MoM in weeks 9 and 10. The use of ADAM12-S along with biochemical 
markers from the combined test (PAPP-A, free beta-hCG) with or without nuchal 
translucency measurement did not affect the detection rate or false positive rate of fetal 
aneuploidy as compared to routine screening using PAPP-A and free beta-hCG with or 
without nuchal translucency. The authors concluded that these findings showed moderately 
decreased levels of ADAM12-S in cases of fetal aneuploidy in gestational weeks 8 to 11. 
However, including ADAM12-S in the routine risk does not improve the performance of first 
trimester screening for fetal trisomy 21.
Cowans et al (2010) examined the stability of ADAM-12 with time and at different temperatures. Maternal serum and whole blood pools were stored at 30 degrees C, room temperature and refrigerator temperature or subjected to repeated freeze-thaw cycles. ADAM-12 was measured at set time points using an automated DELFIA research assay. Using a 10% change in concentration as a limit of stability, ADAM-12 is stable in serum for less than 15 hrs at 30 degrees C, less than 20 hrs at room temperature and for 51 hrs at refrigerator temperature. ADAM-12 levels are not altered following 3-20 degrees C to room temperature freeze-thaw cycles. The stability of ADAM-12 in whole blood appears similar to that in serum. The authors concluded that these findings suggested that ADAM-12 may be unstable under many routine laboratory conditions, and the marker's instability may also be partly responsible for the discrepancies in the literature.

Other Markers of Fetal Aneuploidy

Koster and colleagues (2009) examined if placental protein 13 (PP13) could be an additional marker in first trimester screening for aneuploidies. These researchers assessed differences in multiples of the gestation-specific normal median (MoMs), PP13 concentrations were measured in serum samples from DS, trisomy 18 and 13 affected pregnancies and euploid singleton pregnancies (4 for each case matched for duration of storage, maternal weight and age). The PP13 MoM in DS cases (n = 153) was 0.91 [not statistically significant from controls (n = 853); p = 0.06; Wilcoxon rank sum test, 2-tail]. Placental protein 13 MoMs were decreased in trisomy 18 (n = 38- median MoM 0.64; p < 0.0001) and trisomy 13 cases (n = 23-median MoM 0.46; p < 0.0001). There was a slight upward trend in MoM values of the DS cases with gestational weeks. The PP13 MoM was significantly correlated with the pregnancy associated plasma protein-A MoM and the free beta-subunit of hCG (fbeta-hCG) MoM. The authors concluded that PP13 does not seem to be a good marker for DS.

Li et al (2010) compared the difference in maternal serum anti-Mullerian hormone (AMH) level between DS pregnancies and unaffected pregnancies, and evaluated its performance as a screening marker for DS pregnancy. A total of 145 pregnancies affected by fetal DS and 290 unaffected controls matched with maternal age and gestational age were selected, and their archived first or second trimester serum retrieved for AMH assay. There was no significant difference in maternal serum AMH level between pregnancies affected and
unaffected by fetal DS. First trimester serum samples had higher AMH level compared to second trimester samples. The authors concluded that maternal serum AMH level, as a marker of ovarian age, is not superior to chronological age in predicting DS pregnancies. They stated that despite the cross-sectional nature of the study, the variation of maternal serum AMH concentration with gestational age warrants further investigation.

Maternal Urinary Markers

Iles and colleagues (2015) noted that the established methods of antenatal screening for Down syndrome are based on immunoassay for a panel of maternal serum biomarkers together with ultrasound measures. Recently, genetic analysis of maternal plasma cfDNA has begun to be used but has a number of limitations including excessive turn-around time and cost. These researchers developed an alternative method based on urinalysis that is simple, affordable and accurate. A total of 101 maternal urine samples (12 to 17 weeks gestation) were taken from an archival collection of 2,567 spot urines collected from women attending a prenatal screening clinic; 18 pregnancies in this set subsequently proved to be Down pregnancies. Samples were either neat urine or diluted between 10- to 1,00- fold in distilled H2O and subjected to matrix assisted laser desorption ionization (MALDI), time of flight (ToF) mass spectrometry (MS). Data profiles were examined in the region 6,000 to 14,000 m/z. Spectral data was normalized and quantitative characteristics of the profile were compared between Down and controls. In Down cases there were additional spectral profile peaks at 11,000 to 12,000 m/z and a corresponding reduction in intensity at 6,000 to 8,000 m/z. The ratio of the normalized values at these 2 ranges completely separated the 8 Down syndrome from the 39 controls at 12 to 14 weeks. Discrimination was poorer at 15 to 17 weeks where 3 of the 10 Down syndrome cases had values within the normal range. The authors concluded that direct MALDI ToF mass spectral profiling of maternal urinary has the potential for an affordable, simple, accurate and rapid alternative to current Down syndrome screening protocols.

Trivedi and Iles (2015) stated that in DS the precise cellular mechanisms linking genotype to phenotype is not straightforward despite a clear mapping of the genetic cause. Metabolomic profiling might be more revealing in understanding molecular-cellular mechanisms of inborn errors of metabolism/syndromes than genomics alone and also result in new prenatal screening approaches. The urinary metabolome of 122 maternal urine from
women with and without an aneuploid pregnancy (predominantly DS) were compared by
both zwitterionic hydrophilic interaction chromatography (ZIC-HILIC) and reversed-phase
liquid chromatography (RPLC) coupled to hybrid ion trap time of flight mass spectral
analysis. ZIC-HILIC mass spectrometry resolved 10-fold more unique molecular ions than
RPLC mass spectrometry, of which molecules corresponding to ions of m/z 114.07 and m/z
314.20 showed maternal urinary level changes that significantly coincided with the presence
of a DS fetus. The ion of m/z 314.20 was identified as progesterone and m/z 114.07 as
dihydrouracil. A metabolomics profiling-based maternal urinary screening test modeled
from this separation data would detect approximately 87 and 60.87 % (using HILIC-MS and
RPLC-MS), respectively of all DS pregnancies between 9 and 23 weeks of gestation with no
false positives.

Furthermore, UpToDate reviews on “Down syndrome: Overview of prenatal screening”
(Messerlian and Palomaki, 2016) and “Prenatal screening for Down syndrome using cell-
free DNA” (Palomaki et al, 2016) do not mention the use of urinary markers for screening of
Down syndrome.

Evaluation of DSCR4 Gene Methylation in Plasma

Hu and Zhou (2018) noted that DS results in patients suffering from delayed body growth,
special facies, mild-to-moderate mental retardation and other symptoms, seriously affecting
the life of patients. These researchers examined the association between Down’s
syndrome critical region 4 (DSCR4) gene methylation in plasma in high-risk pregnant
women with DS in early pregnancy (referred to as pregnant women in early pregnancy) and
DS, in order to screen new epigenetic markers for the clinical diagnosis of DS. DNA in
peripheral blood cells and plasma in pregnant women in early pregnancy were treated with
hydrosulphit; DSCR4 genes with different methylation levels were amplified by methylation-
specific polymerase chain reaction (PCR), and the methylation difference of the CpG site of
the DSCR4 amplification product in peripheral blood DNA was verified via restriction
endonuclease analysis. The expression of DSCR4 with different methylation levels in
peripheral blood of pregnant women in early pregnancy were detected via reverse
transcriptase-quantitative PCR (RT-qPCR), and the DSCR4 gene functions were studied via
the intervention in DSCR4 expression with small interfering RNA (siRNA). Methylation-
specific PCR and restriction endonuclease analysis revealed that DSCR4 genes were
differentially methylated in peripheral blood DNA in pregnant women in early pregnancy. Additionally, DSCR4 showed a low methylation status in plasma but a high methylation status in peripheral blood cells. RT-qPCR revealed that non-methylated DSCR4 was highly expressed in the peripheral blood of pregnant women in early pregnancy, and thus was an epigenetic marker of fetal DS. siRNA results showed that the down-regulation of DSCR4 inhibited cell migration and invasion, but had no effect on cell proliferation. The authors stated that these results suggested that the DSCR4 gene was differentially methylated in peripheral blood DNA in pregnant women in early pregnancy. Furthermore, DSCR4 exists in a non-methylated state in plasma and in a hyper-methylated state in blood cells. They noted that DSCR4 can therefore promote the migration and invasion of trophocytes and serve as an epigenetic marker of non-invasive clinical diagnosis of DS. The authors concluded that this study provided a theoretical basis for the non-invasive prenatal diagnosis of DS and screened new biomarkers for maternal-fetal epigenetic differences; it also provided a new perspective for studying the role of DSCR4 in pathological process of DS and placental development.

Measurement of Circulating Fetal Nucleated Red Blood Cells and Extra-Villous Trophoblastsis for Non-Invasive Prenatal Diagnosis of Fetal Aneuploidy

In a proof-of-principle, pilot study, Huang and colleagues (2017) presented a novel silicon-based nano-structured microfluidics platform named as "Cell Reveal" to demonstrate the feasibility of capturing circulating fetal nucleated red blood cells (fNRC) and extra-villous cytotrophoblasts (EVT) for cell-based non-invasive prenatal diagnosis (cbNIPD). The "Cell Reveal" system is a silicon-based, nano-structured microfluidics using immuno-affinity to capture the trophoblasts and the nucleated RBC (nRBC) with specific antibodies. The automated computer analysis software was used to identify the targeted cells through additional immunostaining of the corresponding antigens. The identified cells were retrieved for whole genome amplification for subsequent investigations by micro-manipulation in 1 microchip, and left in-situ for subsequent fluorescence in-situ hybridization (FISH) in another microchip. When validation, bloods from pregnant women (n = 24) at gestational age 11 to 13 weeks were enrolled. When verification, bloods from pregnant women (n = 5) receiving CVS or amniocentesis at gestation age 11 to 21 weeks with an aneuploid or euploid fetus were enrolled, followed by genetic analyses using FISH, short tandem repeat (STR) analyses, array comparative genomic hybridization (aCGH), and next
generation sequencing (NGS), in which the laboratory was blind to the fetal genetic complement. The numbers of captured targeted cells were 1 to 44 nRBC/2 ml and 1 to 32 EVT/2 ml in the validation group. The genetic investigations performed in the verification group confirmed the captured cells to be fetal origin. In every 8 ml of the maternal blood being blindly tested, both fnRBC and EVT were always captured. The numbers of captured fetal cells were 14 to 22 fnRBC/4 ml and 1 to 44 EVT/4 ml of maternal blood. The authors concluded that this report was one of the first few to verify the capture of fnRBC in addition to EVT; and the scalability of their automated system made them one step closer toward the goal of in-vitro diagnostics.

Hou and associates (2017) noted that circulating fetal nucleated cells (CFNCs) in maternal blood offer an ideal source of fetal genomic DNA for NIPD. These researchers developed a class of nano-Velcro microchips to effectively enrich a subcategory of CFNCs, i.e., circulating trophoblasts (cTBs) from maternal blood, which can then be isolated with single-cell resolution by a laser capture microdissection (LCM) technique for down-stream genetic testing. These investigators first established a nano-imprinting fabrication process to prepare the LCM-compatible nano-Velcro substrates. Using an optimized cTB-capture condition and an immunocytochemistry protocol, these researchers were able to identify and isolate single cTBs (Hoechst+/CK7+/HLA-G+/CD45−, 20 μm > sizes > 12 μm) on the imprinted nano-Velcro microchips; 3 cTBs were polled to ensure reproducible whole genome amplification on the cTB-derived DNA, paving the way for cTB-based aCGH and STR analysis. Using maternal blood samples collected from expectant mothers carrying a single fetus, the cTB-derived aCGH data were able to detect fetal genders and chromosomal aberrations, which had been confirmed by standard clinical practice. The authors concluded that these findings supported the use of nano-Velcro microchips for cTB-based non-invasive prenatal genetic testing, which holds potential for further development toward future NIPD solution.

PreSeek

PreSeek is a cell-free fetal DNA non-invasive prenatal multi-gene sequencing screen for multiple Mendelian monogenic disorders using maternal blood. Pre-Seek evaluates fetal DNA for pathogenic and likely pathogenic variants in 30 genes (BRAF, CBL, CDKL5, CHD7, COL1A1, COL1A2, FGFR2, FGFR3, HDAC8, HRAS, JAG1, KRAS, MAP2K1, MAP2K2,
MECP2, NIPBL, NRAS, NSD1, PTPN11, RAD21, RAF1, RIT1, SHOC2, SMC1A, SMC3, SOS1, SOS2, SYNGAP1, TSC1, TSC2). PreSeek does not screen for fetal chromosome, or other copy number, abnormalities commonly detected by traditional (aneuploidy) NIPT. Positive screening results should always be followed-up with an invasive, diagnostic test before any medical decisions are made. Currently, there are no published studies or guidelines regarding this test.

### CPT Codes / HCPCS Codes / ICD-10 Codes

Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "#".

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
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<tbody>
<tr>
<td><strong>CPT codes covered if selection criteria are met</strong></td>
<td></td>
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<tr>
<td>81507</td>
<td>Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy</td>
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<tr>
<td>81508 - 81509</td>
<td>Fetal congenital abnormalities, biochemical assays two or three proteins (PAPP-A, hCG [any form], or DIA), utilizing maternal serum, algorithm reported as a risk score</td>
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<td>81510 - 81512</td>
<td>Fetal congenital abnormalities, biochemical assays analytes (AFP, uE3, hCG [any form], DIA), utilizing maternal serum, algorithm reported as a risk score</td>
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<tr>
<td>82105</td>
<td>Alpha-fetoprotein (AFP); serum</td>
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<td>82106</td>
<td>amniotic fluid</td>
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<tr>
<td>82677</td>
<td>Estriol</td>
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<tr>
<td>84702</td>
<td>Gonadotropin, chorionic (hCG); quantitative</td>
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<td>84703</td>
<td>qualitative</td>
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<td>84704</td>
<td>free beta chain</td>
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<td>86336</td>
<td>Inhibin A</td>
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**CPT codes not covered for indications listed in the CPB:**

No specific codes
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<tr>
<th>Code</th>
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<tr>
<td>81422</td>
<td>Fetal chromosomal microdeletion(s) genomic sequence analysis (eg, DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood</td>
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<tr>
<td>83516</td>
<td>Immunoassay for analyte other than infectious agent antibody or infections agent antigen; qualitative or semiquantitative, multiple step method [not covered for anti-Mullerian hormone level for first or second trimester screening for Down syndrome]</td>
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<tr>
<td>83520</td>
<td>Immunoassay, analyte, quantitative; not otherwise specified [not covered for anti-Mullerian hormone level for first or second trimester screening for Down syndrome]</td>
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<td>83632</td>
<td>Lactogen, human placental (HPL) human chorionic somatomammotropin</td>
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<td>84163</td>
<td>Pregnancy-associated plasma protein-A (PAPPA-A)</td>
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**CELL-FREE DNA testing of maternal blood:**

CPT codes covered if selection criteria are met

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<th>Code</th>
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<tr>
<td>0009M</td>
<td>Fetal aneuploidy (trisomy 21, and 18) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy</td>
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<tr>
<td>0060U</td>
<td>Twin zyosity, genomic targeted sequence analysis of chromosome 2, using circulating cell-free fetal DNA in maternal blood</td>
</tr>
<tr>
<td>81420</td>
<td>Fetal chromosomal aneuploidy (eg, trisomy 21, monosomy X) genomic sequence analysis panel, circulating cell-free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18, and 21</td>
</tr>
<tr>
<td>81507</td>
<td>Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy</td>
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</table>

**CPT codes not covered for indications listed in the CPB:**
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<tr>
<th>Code</th>
<th>Code Description</th>
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<tr>
<td>0060U</td>
<td>Twin zygosity, genomic targeted sequence analysis of chromosome 2, using</td>
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<td></td>
<td>circulating cell-free fetal DNA in maternal blood</td>
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</table>

**ICD-10 codes covered if selection criteria are met:**

- **G91.2** (Idiopathic) normal pressure hydrocephalus [Ventriculomegaly]
- **O09.291 - O09.299** Supervision of pregnancy with other poor reproductive or obstetric history [prior pregnancy with an aneuploidy]
- **O09.511 - O09.529** Supervision of elderly primigravida and multigravida [high-risk]
- **O09.521 - O09.529** Supervision of elderly multigravida
- **O28.1** Abnormal biochemical finding on antenatal screening of mother [fetal ultrasonographic findings predicting an increased risk of fetal aneuploidy or positive screening test for an aneuploidy]
- **O28.5** Abnormal chromosomal and genetic finding on antenatal screening of mother [fetal ultrasonographic findings predicting an increased risk of fetal aneuploidy or positive screening test for an aneuploidy]
- **O35.0XX0 - O35.0XX9** Maternal care for (suspected) central nervous system malformation in fetus
- **O35.1XX0 - O35.1XX9** Maternal care for (suspected) chromosomal abnormality in fetus [aneuploidy in mother, fetal aneuploidy]
- **Q90.0 - Q91.7** Down syndrome, Patau's syndrome [Trisomy 13], Edward's syndrome [Trisomy 18]
- **Q92.0 - Q92.9** Other trisomies and partial trisomies of the autosomes, not elsewhere classified [aneuploidy in mother, fetal aneuploidy] [not covered for micro-duplication syndrome] [not covered for Warkany syndrome 2 or trisomies other than 13, 18, and 21]
- **Q95.0 - Q95.9** Balanced translocation and insertion in normal individual [Robertsonian translocation]
- **Z34.80 - Z34.93** Encounter for supervision of normal pregnancy [low risk women]
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<tbody>
<tr>
<td>ICD-10 codes not covered for Indications listed in the CPB:</td>
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<td>O30.001 - O30.93</td>
<td>Multiple gestations</td>
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<tr>
<td>Q93.88</td>
<td>Other microdeletions [micro-deletion syndrome]</td>
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<tr>
<td>Z13.228</td>
<td>Encounter for screening for other metabolic disorders [not covered for use of urinary markers (measurement of cell free DNA and metabolomic profiling) for testing for fetal aneuploidy (trisomy 13, 18 and 21) in pregnant women]</td>
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<td>Z13.71 - Z13.79</td>
<td>Encounter for screening for genetic and chromosomal anomalies</td>
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<tr>
<td>Z31.438</td>
<td>Encounter for other genetic testing of female for procreative management</td>
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</tbody>
</table>

The above policy is based on the following references:

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