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Prenatal screening for common aneuploidies using cell-free DNA

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INTRODUCTION

Prenatal screening for trisomy 21 (Down syndrome), trisomy 18 (Edwards syndrome), trisomy 13 (Patau syndrome), and selected sex chromosome aneuploidies can be performed using next-generation sequencing of cell-free DNA (cfDNA) in the maternal circulation. Circulating cfDNA is derived from both the mother and the fetal-placental unit [1-3] and cleared from the maternal circulation soon after delivery [4]. Although this approach is often called "noninvasive prenatal screening" (NIPS) or "noninvasive prenatal testing" (NIPT), these terms are nonspecific, as conventional serum screening tests, such as the second-trimester quadruple test or the first-trimester combined test, are also noninvasive.

The cfDNA test provides excellent performance (at least 99 percent of trisomy 21 pregnancies are detected with a screen-positive rate of approximately 1 per 1000, 0.1 percent) in patients who do not experience a test failure (ie, no call or no result). However, it is still considered a **screening** test due to infrequent false-positive and false-negative results. An invasive procedure (eg, amniocentesis or chorionic villus sampling) and subsequent karyotyping or microarray analysis are considered the gold standard **diagnostic** tests and should be offered to patients who are screen positive by cfDNA testing.

This topic will discuss prenatal aneuploidy screening via testing of cfDNA in maternal plasma. Other issues related to prenatal screening for trisomy 21 and other aneuploidies are reviewed separately:

- (See "Down syndrome: Overview of prenatal screening".)
- (See "First-trimester combined test and integrated tests for screening for Down syndrome and trisomy 18".)
- (See "Laboratory issues related to maternal serum screening for Down syndrome".)
- (See "Sonographic findings associated with fetal aneuploidy".)

CELL-FREE DNA

Origins — Both the mother and the fetal-placental unit produce cfDNA. The primary source of so-called "fetal" cfDNA in the maternal circulation is thought to be apoptosis of placental cells (syncytiotrophoblast), while maternal hematopoietic cells are the source of most maternal cfDNA [1-3]. A lesser source is apoptosis of fetal erythroblasts generating cfDNA in the fetal circulation; these fragments can cross the placenta and enter the maternal circulation [1,5,6]. Since the fetus and the placenta originate from a single fertilized egg, they are usually genetically identical, but differences between the placenta and fetus are important sources of discordant cfDNA test results (eg, confined placental mosaicism).

Circulating cfDNA, whatever its origin, is highly fragmented. Each fragment is between 50 and 200 base pairs [7]. There is a clear pattern to the fragmentation sizes relating to how the DNA is wrapped around histone proteins to form nucleosomes. These patterns differ between the maternal and fetal cfDNA, with longer fragments being

slightly more likely to be maternally derived. These differences can be used to both screen for specific disorders, such as aneuploidy, and to determine the fetal fraction.

Fetal fraction — The fetal fraction is the percentage of all cfDNA in maternal blood that is derived from the fetal-placental unit. Fetal-placental cfDNA can be detected in maternal blood as early as five weeks of gestation and almost always by nine weeks of gestation [8]. The relative concentration of fetal cfDNA increases modestly (0.1 percent per week) with gestational age from 10 to approximately 20 weeks and then increases rapidly (1 percent per week) until term [9].

An adequate amount of fetal-placental cfDNA must be present to obtain a reliable cfDNA screening result. In general, a minimum of 3 to 4 percent of the total circulating cfDNA should be derived from the fetal-placental unit for successful testing. Four factors can systematically reduce the fetal fraction, which can lead to an assay failure (a report of "no call" or "no result") or can result in a false-negative result. (See 'Test failures: Rates, reasons' below and 'False-negative cfDNA test results' below.)

A low fetal fraction may be due to:

- **Early gestational age** – The fetal fraction is substantially lower prior to 10 weeks of gestation, so most laboratories require that patients wait until at least 10 weeks of gestation to help ensure an adequate fetal fraction for testing. Fetal cfDNA comprises approximately 11 to 13 percent of the total cfDNA in the maternal circulation in the late first and early second trimesters when prenatal screening is typically performed [10-12]. It may comprise as much as 50 percent of the total cfDNA in the maternal circulation near term [13].

- **Suboptimal sample collection** – Appropriate sample collection and fragmented cfDNA stabilization are important to preserve the fetal fraction since a small number of degraded white blood cells from the mother's blood will greatly reduce the fetal fraction. To address this problem, the sample should be collected in a purple top (EDTA) tube and centrifuged within six hours; the resulting plasma is stable with -80°C freezer storage. Alternatively, a special cfDNA collection tube (eg, Cell-Free DNA BCT) that stabilizes the sample for up to five days at room temperature can be used. These tubes should not be refrigerated or frozen.

  An incomplete sample draw (eg, half-filled tubes) may be rejected by the laboratory or may result in a higher likelihood of test failure due to insufficient plasma volume for testing of fetal cfDNA.

- **Obesity** – As maternal weight (and to a lesser extent body mass index) increases, the fetal fraction systematically decreases. This inverse relationship has been attributed to the dilution of a relatively constant amount of fetal cfDNA in the larger maternal plasma volume of patients with obesity and also to an increase in the amount of maternally derived cfDNA as maternal weight increases [14]. In one study including almost 1500 patients with euploid pregnancies, a low fetal fraction (<3.5 percent) was noted in 1.1 percent of all samples but in only 0.2 percent of patients weighing <60 kg (132 pounds) versus 10.5 percent of patients weighing >110 kg (242 pounds) [14]. Others have observed that the risk of low fetal fraction increases for maternal weights as low as 81 kg (180 pounds) [14,15]. The increase in maternally derived cfDNA in patients who are obese may also be due to chronic inflammation and associated cell death [16].

  In contrast to serum marker screening, the laboratory is not able to mathematically adjust the result to correct for maternal weight. (See 'Implementation issues' below.)

- **Fetal karyotype** – The average fetal fraction at 10 to 20 weeks of gestation is lower in pregnancies with a trisomy 18 fetus (average fetal fraction 9 percent) than pregnancies with a euploid fetus (average fetal fraction 11 to 13 percent) and higher in pregnancies with a fetus with trisomy 21 (average fetal fraction 13 to 15 percent). This may partially explain why detection rates for trisomy 21 are higher than for trisomy 18, especially when test failures are considered. There are fewer data for other abnormalities, but it appears that the fetal fractions in both trisomy 13 and Turner syndrome are also lower than in euploid fetuses. Triploid fetuses have extremely low fetal fractions, usually below 4 percent [17,18].

- **Other less common factors** – A low fetal fraction has also been associated with:
  - Maternal use of low molecular weight heparin before 20 weeks of gestation [19-21].
  - Conception by in vitro fertilization [22].
  - Twin gestation, as the per fetus fetal fraction is lower in twins [23]. (See 'Twins' below.)

Clearance — After delivery, maternal clearance of fetal cfDNA occurs rapidly. In healthy pregnant people, the half-life is approximately one hour, with essentially all fetal cfDNA eliminated within two days of delivery [24,25]. Thus, future pregnancies are not affected by cfDNA in the circulation from prior pregnancies.

METHODOLOGY

The most common method of cfDNA screening counts cfDNA fragments for which the chromosome of origin has been identified (aligned). For example, in euploid nonpregnant females, approximately 1.3 percent of cfDNA fragments are derived from chromosome 21 (eg, chromosome 21 contains approximately 1.3 percent of the human genome). In pregnancy, the expected percentage of chromosome 21 fragments remains at 1.3 percent if both the fetus and mother are euploid. If the fetus has three copies of chromosome 21, the proportion of chromosome 21 fragments will be slightly higher, depending on the fetal fraction. For example, if the fetus has trisomy 21 and the fetal fraction is 10 percent, the expected proportion of chromosome 21 fragments will be 1.365 percent (1.30 x (1 + [0.10/2]). If the fetal fraction is lower, the increase in chromosome 21 fragments is smaller (eg, <1.365), and it becomes more difficult to detect trisomy 21.

In "shotgun sequencing," random cfDNA fragments are sequenced, which may require up to 10 million mapped fragments to obtain a reliable test result [4]. Alternatively, some laboratories use a preliminary step to enrich for fragments of interest (eg, chromosome 21, 18, 13, X, and Y); this "targeted sequencing" or "chromosome selective sequencing" approach allows the laboratory to map fewer fragments (approximately one or two million) to obtain a reliable test result.

A third method uses tens of thousands of highly polymorphic single nucleotide polymorphisms (SNPs) located only on chromosomes of interest (eg, 21, 18, 13, X, and Y). Maternal SNP genotypes are inputted and compared with the corresponding genotypes of the mixture of cfDNA from the mother and fetus in the plasma sample. An extra (or missing) chromosome in the plasma results in a shift in the pattern among informative SNPs. According to a commercial laboratory that introduced the SNP genotyping method, interpretations for common aneuploidies can be made for twin pregnancies, and the zygosity of each twin can be determined. SNP methods can also identify a vanished dizygotic twin [26]. Although this method originally required a paternal sample and sequenced the mother's DNA separately, these two steps are no longer required.

SNP testing cannot be used in the relatively uncommon instances of pregnancy achieved by egg donation, pregnancy in a bone marrow or organ transplant recipient, or a gestational carrier mother because additional confounding chromosomes are present in the maternal plasma. A sequencing method must be used for cfDNA screening in these pregnancies. Since a laboratory typically performs only one method of testing, the clinician needs to ensure that samples from these types of pregnancies are sent to a laboratory that performs sequencing-based testing.

SCREENING PERFORMANCE

Screening performance is described by the detection rate (DR) and the false-positive rate (FPR). It is important to note that a small proportion of cfDNA screening tests fails to provide a useable clinical result (ie, no result, test failure, or no call). (See 'Test failures: Rates, reasons' below.)

Trisomy 21, 18, and 13 — cfDNA is the most sensitive screening option for these aneuploidies. Performance varies by trisomy, but not by methodology, and is similar in both high- and low-risk pregnant patients [27]. Based on multiple meta-analyses [28-31], the consensus DRs and FPRs were as follows:

- **Trisomy 21** – DR 99.5 percent, FPR 0.05 percent
- **Trisomy 18** – DR 97.7 percent, FPR 0.04 percent
- **Trisomy 13** – DR 96.1 percent, FPR 0.06 percent

These data do not account for test failures in either aneuploid or normal samples, and many of the studies included in these meta-analyses did not have complete follow-up of all pregnancies. Thus, these DRs are likely an overestimate. Test failure rates are important to consider when reporting test performance since higher failure rates may decrease actual DRs, increase FPRs, and decrease positive predictive value (PPV) [32]. The direction and magnitude of these changes will depend on the actions taken when the test fails (eg, no further testing, repeat cfDNA testing, ultrasound, diagnostic testing).

Sex chromosome aneuploidies — The cfDNA DRs for these disorders are lower and FPR rates are higher than for the common autosomal trisomies (PPV for sex chromosome aneuploidy 37 percent in one study [33]). In the largest meta-analysis that evaluated cfDNA test performance for sex chromosome aneuploidies, the DR and FPR for monosomy X (177 cases and 9079 controls) were 90.3 and 0.23 percent, respectively [31]. For the sex chromosome trisomies 47,XXX; 47,XXY; and 47,XYY (56 cases and 6699 controls), the DR and FPR were 93 and 0.14 percent, respectively. Selected method-dependent results have been reported [34-36].

Test failures: Rates, reasons

- **Rate** – A wide range of cfDNA failure rates (ie, no result) has been reported, and these rates are likely dependent on population characteristics (eg, proportion of patients with obesity), test method, and whether there is routine testing of a second sample after an initial failure. No result rates generally fall between 1 and 3 percent [37,38].

- **Reasons for test failure** – The most common reasons for test failure include less than a specified absolute amount of total and/or fetal-placental DNA, fetal fraction below an acceptable level (eg, <3.5 or <4 percent), and insufficient numbers of fragments sequenced and/or aligned. Low fetal fraction may be responsible for up to 50 percent of all failures, depending on methodology. As discussed above, a low fetal fraction may be due to early gestational age, maternal obesity, maternal use of low molecular weight heparin, some fetal aneuploidies, suboptimal sample collection/processing, twin pregnancy (when fetal-specific fetal fractions are provided or estimated), and in vitro fertilization (IVF). For example, increasing data show that the fetal fraction is lower and the test failure rate is approximately two or three times higher for IVF pregnancies compared with naturally conceived pregnancies [22,39]. One laboratory with a 2 percent failure rate in naturally conceived pregnancies reported a 5 percent failure rate in IVF pregnancies [22]. However, there is no indication that a successful cfDNA test result in an IVF pregnancy is any less reliable. (See 'Fetal fraction' above.)

  Another reason for a test failure, depending on the laboratory method used, is long stretches of homozygosity (fragments in which identical gene sequences are discovered originating from the maternally and paternally derived chromosomes). Examples include uniparental disomy (inheritance of both chromosomes from one parent) or parental consanguinity. Some laboratories will identify results as "borderline" and will not make a screen-positive or screen-negative call even when all quality control parameters are met. In such instances, a "borderline" call should be considered screen positive and not a test failure or screen negative since follow-up action is needed.

  The laboratory's requirements for cfDNA test performance also affect its failure rate. Laboratories that prioritize minimizing false-positive and false-negative results may accept a higher rate of failures. This might be most appropriate if most of the samples are already considered to be at high risk. Other laboratories may consider false negatives and false positives to be an inevitable consequence of any screening test in a general pregnancy

population and may place more emphasis on a low failure rate. Reporting a test failure may not result in appropriate follow-up.

Lastly, it is important to consider whether the laboratory's reported failure rate includes only total assay failures (eg, the interpretation for trisomy 21 is present, but those for trisomy 18 and trisomy 13 are not); includes only failures related to chromosomes 21, 18, and 13; or includes failures to provide sex chromosome or other interpretations, such as microdeletion syndromes.

- **Management** – (See 'No call or no result' below.)

False-positive and false-negative results — Although the PPV for cfDNA screening for the common autosomal trisomies is approximately 90 percent in large studies or modeling exercises, this still means that 10 percent of patients with positive cfDNA results will not have an affected pregnancy [31,40]. There are several reasons why the result of a diagnostic test on amniocytes or fetal blood might not agree with the cfDNA test result. The cfDNA test result might be analytically correct (eg, correctly defines the placental genotype), while being clinically incorrect (eg, does not correctly define the fetal genotype). Although analytic test performance is important, the clinical test performance is the key component for patient care.

For the sex chromosomes, the genotype may be accurate but discordant with the phenotype. Genotype-phenotype discordance has been attributed to laboratory error, a vanishing twin, complex disorders of sexual differentiation, sex chromosome aneuploidy with/without mosaicism, and maternal chimerism [41,42].

False-positive cfDNA test results — Reasons for false-positive cfDNA (fetus is unaffected, but cfDNA testing indicates chromosomal abnormality) include [43]:

- **Confined placental mosaicism** – Since the primary source of "fetal" cfDNA in the maternal circulation is placental cells (syncytiotrophoblast), the cfDNA test will provide results relevant to the placenta, which may be discordant with fetal tissue. In these cases, the cfDNA test is analytically correct but clinically incorrect. Experience gained from chorionic villus sampling indicates that this may occur in up to 1 to 2 percent of pregnancies [44-47] and is more likely with monosomy X and trisomy 13 than for trisomy 21 or 18 [48]. (See "Chorionic villus sampling", section on 'Confined placental mosaicism'.)

- **Demised twin** – A demised twin can cause an FPR if, for example, the demised twin was aneuploid [49]. This is because the placenta from the demised twin (which is also more likely to be aneuploid) is still present at the time of testing and continues to shed DNA weeks after the demise. A twin rather than a singleton pregnancy may not have been recognized if the demise occurred very early in gestation, hence the term "vanishing twin."

- **Maternal mosaicism** – Most cfDNA testing methods assume that the mother has a normal karyotype, but this is not always true. For example, with advancing age, an increasing proportion of pregnant people have a small percentage of cells that have lost an X chromosome, and these can lead to a false-positive cfDNA result for laboratories reporting sex chromosome aneuploidies [50]. In such phenotypically normal females, the lower X chromosome signal on cfDNA testing would be attributed to the fetus and reported as fetal Turner syndrome. Follow-up fetal diagnostic testing would identify a euploid fetus.

  Although uncommon, some patients may have a nonmosaic sex chromosomal abnormality (eg, 47,XXX) and appear to have a normal phenotype [51]. Maternal mosaicism can be diagnosed by karyotyping peripheral blood lymphocytes [52]. Educational materials and counseling should include the possibility that a chromosomal abnormality may be identified in the mother.

- **Maternal cancer** – Cell-free circulating tumor DNA (ctDNA) may be shed into the maternal circulation in measurable levels in those with a malignancy [53-55]. In a pregnant patient with a tumor, cell-free fetal and maternal DNA as well as ctDNA contribute to total cfDNA.

In one study of 450,000 tested pregnancies, 55 potential cases of maternal cancer were identified: 40 of these were confirmed to be maternal neoplasms, 20 were benign uterine fibroids, 18 were malignant neoplasms (7 of which were already known), and 2 were unclassified [56]. Thus, among 450,000 tests, 11 new malignant maternal neoplasms were identified (1 in 41,000).

Not all cfDNA commercial tests are able to identify a pattern suggestive of maternal cancer. Programs that offer cfDNA testing that do identify the possibility of a maternal cancer should have referral plans in place to deal with these rare abnormal reports. The appropriate clinical evaluation of such patients is currently unclear. Various approaches have been suggested [57] but remain unvalidated. The most common malignancies in reproductive-age females are breast, cervical, ovarian, and colorectal cancers; leukemia; Hodgkin and non-Hodgkin lymphoma; thyroid cancer; and melanoma.

Educational materials and counseling of patients considering cfDNA for fetal aneuploidy screening should include the possibility that a maternal cancer may be identified. However, cfDNA testing should not be considered a screening test for maternal malignancy, given the paucity of data on this association, the potential for FPR, and the emotional and medical impact of such results on the patient's well-being.

- **Maternal copy number variants** – The methodology for cfDNA analysis assumes that every person carries the same proportion of genetic material on a given chromosome, but chromosomes vary slightly among individuals due to inherited or de novo copy number variants (ie, deletion or duplication of a genomic region[s]). In these individuals, cfDNA sequencing might yield a positive result when the size of the maternal duplication was relatively large and it occurred on a chromosome of interest (eg, chromosome 21) [58,59]. In two studies, maternal duplications on chromosome 18 were the likely cause of trisomy 18 FPR in six of seven cases examined [58,59]. Shallow sequencing (eg, a low number of fragments sequenced and limiting the number of referent chromosomes) makes this form of FPR more likely. Shotgun methods are less likely to be influenced by copy number variants on one chromosome if all autosomes are used to normalize counts from the chromosome of interest.

- **Transplant recipient** – If transplanted tissue (bone marrow or organ) was obtained from a male donor, cfDNA testing may incorrectly identify a female fetus as being male due to the release of male cfDNA from the donor organ into the maternal circulation [60].

- **Recent blood transfusion** – Maternal blood transfusion from a male donor performed <4 weeks prior to the blood draw for cfDNA may incorrectly identify a female fetus as being male [61].

- **Chance** – FPRs can also be the result of statistical chance, as the cutoff for a positive test is often set at +3 standard deviations. Therefore, 1 or 2 per 1000 euploid fetuses might have an FPR by chance alone, and if 100,000 tests were performed, an estimated 100 FPRs would be expected.

- **Technical issues** – As with all laboratory testing, rare sample mix-ups or other technical errors could lead to false-positive (or false-negative) test results. However, these would likely be identified as part of subsequent follow-up testing.

False-negative cfDNA test results — Reasons for false-negative cfDNA (fetus is affected, but cfDNA testing indicates no chromosomal abnormality) include:

- **Confined placental mosaicism** – As discussed above, the primary source of "fetal" cfDNA in the maternal circulation is placental cells (syncytiotrophoblast), which may be discordant with fetal tissue. It is possible that a fetus could be aneuploid even though the karyotype of the placenta does not reflect that finding. In these cases, the cfDNA test is analytically correct (ie, detecting those placental cells of the mosaicism that are euploid) but clinically incorrect (ie, the fetus itself is aneuploid). This is recognized to occur for trisomy 13 and 18 and rarely for trisomy 21 [62]. Isochromosome 21q rearrangements are overrepresented among false-negative cfDNA screening results involving trisomy 21 [63]. Postzygotic isochromosome formation leading to placental

mosaicism provides a biological cause for the increased prevalence of these rearrangements among false-negative cases. (See "Chorionic villus sampling", section on 'Confined placental mosaicism'.)

- **Borderline low fetal fraction** – A low but adequate fetal fraction (eg, between 3 and 5 percent) results in a very small difference in the expected (normal reference) versus observed percentage of chromosome fragments (eg, chromosome 21 fragments). If a sufficient number of fragments are not sequenced, this difference will not be identified, and the results will be incorrectly reported as screen negative. (See 'Methodology' above and 'Fetal fraction' above.)

- **Maternal copy number variants** – As described above, maternal duplications can cause an FPR. It is also theoretically possible for a maternal deletion to cause a false-negative result. However, this would be a much rarer event, as the fetus must be aneuploid and the maternal deletion would need to be on the same chromosome.

- **Technical issues** – Technical assay issues can make the identification of some aneuploidies more difficult. For example, the low guanine-cytosine content of chromosome 13 renders the polymerase chain reaction steps and subsequent sequencing counts less reliable. This results in lower DRs than for other aneuploidies. Laboratories attempt to correct for this in the bioinformatics analysis, but this is not always successful. There are also rare sample mix-ups or other laboratory-related issues that could cause a false-negative test result.

Predictive value — The DR and FPR for aneuploidy with cfDNA screening are unlikely to differ greatly between a low-risk (general population) and high-risk population. This conclusion is supported by findings in a meta-analysis [31]. However, the PPV and negative predictive value (NPV) will depend on the prevalence of each specific aneuploidy in the population. The PPV will also depend on the trimester in which the test is offered since the prevalence decreases as the pregnancy advances and all of the trisomies are associated with a higher than expected fetal loss rate [64].

The table (table 1) shows both PPV and NPV for trisomy 21, trisomy 18, and trisomy 13 in a low-risk (general population) setting and a higher risk setting (ie, ≥35 years of age). Some of the cfDNA tests may have slightly higher (or lower) predictive values due to minor differences in estimates of DR and FPR. This table is designed only to demonstrate reasonable expected rates. The performance of first-trimester combined screening is included as a reference point. First, all NPVs are quite high: ≥99.9 percent. This is due to the low a priori prevalence of these three disorders. These very high modeled NPVs for cfDNA screening were confirmed in a large study [65] where only two false-negative results were identified in over 100,000 pregnant patients screened. The first two rows of the table show the difference between combined serum marker/ultrasound-based screening and that based on cfDNA. The PPVs are higher for cfDNA compared with combined testing, and the PPVs are also higher in the higher risk setting. The DR, FPR, and PPV are all population statistics and are most useful for clinicians and patients when deciding on whether to test or which test to choose. After testing is completed, optimal laboratory practice would be to report a patient-specific risk based on both prior test risk (eg, age, abnormal ultrasound, prior history, screen-positive serum test) and the cfDNA test. However, most laboratories choose to report the average risk in screen-positive patients (the PPV). Some also account for the patient's age. PPVs are less commonly reported for other disorders such as sex chromosome aneuploidies and microdeletions.

The figure (figure 1) expands upon this analysis by creating a flowchart including all three common trisomies screened for by cfDNA in the first trimester. The prevalence and the DR are highest for trisomy 21, and both characteristics are lower for trisomies 18 and 13. In a first-trimester screened population of 100,000 pregnant people, 405 common trisomies would be detected (294 of which are trisomy 21), and only 6 would be missed (2 trisomy 21, 3 trisomy 18, and 1 trisomy 13). In general, a PPV of approximately 90 percent is reasonable for cfDNA screening for trisomy 21 in a general pregnancy population while lower PPVs of 70 and 30 percent are associated with trisomy 18 and 13, respectively.

Lastly, Turner syndrome (45X) is not associated with maternal age, and, therefore, the PPVs are expected to be the same in both the general pregnancy population and patients ≥35 years of age at delivery. However, specific ultrasound findings, such as increased nuchal translucency measurement, are associated with a much higher risk of Turner syndrome. A confounding factor is the increasing prevalence of 45X cells in females as they age [52].

Most of the observed PPVs are considerably lower than the individual risks reported by some laboratories (eg, >99:1 or 99 percent), even though this high individual risk is reported for nearly all positive calls. This discrepancy between the reported individual risks and reasonable PPVs may be the reason for the initial "surprise" at the presence of FPRs, especially among trisomy 18 positive results [66]. It is challenging to convey the difference between PPVs and individual patient risks on patient reports.

Similarly, commercial marketing of cfDNA screening for aneuploidy has not emphasized the difference between sensitivity and PPV. Patients should understand that, if they receive a positive result for trisomy 21, the likelihood that the fetus actually has trisomy 21 is less than 99 percent (ie, the PPV is less than the DR) and more likely to be approximately 90 percent. Likewise, patients who receive a negative result should understand that there is a high likelihood that the fetus does not have trisomy 21 (NPV) but that absence of trisomy 21 is not certain.

CLINICAL USE

Patients who choose to be screened for trisomy 21 by cfDNA will almost always also receive screening for trisomy 18 and trisomy 13, which are less common (figure 2). They may also choose to be screened for sex chromosome aneuploidies, or these may be included in the baseline set of conditions included in the screen. As discussed above, detection rates (DRs) for these aneuploidies are lower than for trisomy 21. (See 'Trisomy 21, 18, and 13' above and 'Sex chromosome aneuploidies' above.)

Secondary screening — cfDNA is commonly used as a secondary trisomy 21 screening test. It may also be used as a secondary screening test for trisomy 18 and trisomy 13.

By definition, secondary screening is a follow-up, nondiagnostic test offered to a population that has already been found to be screen positive (high risk) as a result of a previous screening test. For trisomy 21, these preliminary screening tests can include maternal age ≥35 years at delivery, abnormal ultrasound findings indicating increased risk (eg, increased nuchal translucency), an abnormal serum screening test (eg, first-trimester combined testing), a positive family history of aneuploidy (eg, previous aneuploid pregnancy), or a parent who carries a relevant Robertsonian translocation (eg, balanced translocation with risk for trisomy 13 or 21) [67].

The purpose of secondary screening in this setting is to take advantage of the high DR and low false-positive rate (FPR) of cfDNA screening. High specificity (low FPR) of cfDNA testing allows for a large reduction in the number of unnecessary invasive diagnostic procedures in initially screen-positive patients (high risk) (figure 2). The high sensitivity (DR) of cfDNA testing helps ensure that the few patients with an affected pregnancy who were initially screen positive will remain correctly classified as being screen positive. Since the majority of patients who undergo cfDNA screening prior to amniocentesis will receive a low-risk result and thus might want to avoid an invasive procedure, the cost of the cfDNA screening test may be justified by savings from averted diagnostic testing (amniocentesis and karyotype). Since 2012, cfDNA screening has resulted in a 40 to 76 percent reduction in the number of invasive procedures for prenatal genetic diagnosis [68,69]. Although cfDNA screening fails to give usable results for some patients (1 to 5 percent), those with test failures who are already classified as being at high risk can still be offered diagnostic testing.

There are two models (contingent and reflexive) that utilize serum and cfDNA screening in combination to screen the general pregnancy population. All patients begin the process with serum screening, but the risk cutoff and follow-up testing can differ. The aims of both models are to increase the DR above that usually found in serum screening and reduce the FPRs usually found with serum screening. These tests approach the screening performance of cfDNA testing in all patients while, at the same time, reducing the costs below that of offering cfDNA testing to all patients.

Contingent model — In this model [70], all patients from a general pregnancy population are offered first-trimester combined screening with two risk cutoffs. The "high risk" (eg, >1:150) identifies a group that could choose between going directly to invasive testing or to secondary cfDNA screening (or no further testing). Approximately 3 to 5 percent of patients would have a "high-risk" result. The low-risk group (eg, <1:1000) would receive routine prenatal care with no options for further testing. Approximately 80 to 85 percent of patients having the combined test would be low risk. The newly defined intermediate-risk group (eg, 1:151 to 1:1000) represents approximately 10 to 15 percent of the screened population. These patients are informed of their intermediate risk and offered cfDNA screening after counseling. If the cfDNA test is positive for patients in the high- or intermediate-risk group, those patients would also be offered invasive testing.

An important feature of contingent screening is that the patients receiving an intermediate-risk report must return for counseling and the offer of further testing. This contingent screening has the potential to increase detection because 10 to 15 percent of the population is offered secondary screening. However, the use of cfDNA as the secondary test results in very few "false-positive" findings (0.1 to 0.2 percent) that result in an unnecessary invasive test. The actual DRs and FPRs for a contingent model are dependent on the proportion of patients (and their risks) in the intermediate group that proceeds with further testing. It also requires additional resources to meet with and counsel these patients while collecting the second sample.

Reflexive model — This model [71] utilizes the same risk cutoff levels described above for contingent screening but collects a plasma sample suitable for cfDNA testing at the time the serum is collected. The "high-risk" patients are offered cfDNA or invasive testing, and the low-risk patients receive routine care. However, the intermediate-risk patients automatically (reflexively) have the plasma sample tested and the result returned, negating the need for a call-back or counseling session. This saves time and resources and provides a predictable increase in detection. However, there is an additional expense added by the upfront collection of plasma samples in 100 percent of patients when only 10 to 12 percent will have that sample reflexively tested.

Overall, the DRs and FPRs are more predictable for the reflexive model than the contingent model. In the contingent model, patients with an intermediate-risk result will need to return for a sample draw and cfDNA testing, and not all will choose to do so. In the reflexive model, a sample is already available for cfDNA testing for all patients in the intermediate-risk group. In the reflexive model, it is important that patients understand and consent to the possibility of the two tests and that the resulting individual risk may be quite high (similar to having cfDNA as a primary screen in the high-risk population).

Combined screening alone results in a DR and FPR of approximately 85 and 5 percent, respectively. Both the contingent and reflexive models will increase detection (by defining a new intermediate-risk group) and reduce the FPR as the highly specific cfDNA is the secondary screen. In a report summarizing the results of a reflexive model implementation in over 22,000 patients, the trisomy 21 DR was 95 percent (69 of 73) at an FPR of 0.02 percent (4 of 22,706) [72]. The DR for the contingent model will be slightly lower as not all patients with intermediate risks will chose secondary screening via cfDNA.

Although there are no authoritative data on the proportion of patients having primary screening for trisomy 21 via serum testing versus cfDNA testing, several points are clear. In the United States, the number of laboratories offering serum screening is decreasing as the number of laboratories offering cfDNA screening is increasing. Also, individual laboratories are experiencing an erosion in serum screening but a concomitant increase in alpha-fetoprotein (AFP)-only screening for open spina bifida [73]. This suggests that more patients have access to primary cfDNA testing now than in the recent past. In other countries [74], the test is available to all pregnant people at a low cost. In Belgium, for example, the cost for members of the Belgian service for public health insurance is 9 euros (approximately USD $9) [75].

Primary screening — Primary screening, by definition, is the first screening test for a given disorder or set of disorders. The use of cfDNA as a primary screening test in the United States is limited by some practical concerns but is an option for screening both singleton and twin pregnancies [76].

Important considerations include insurance coverage, concerns about the availability of appropriate pretest counseling, and concerns that some patients will terminate pregnancy after a positive screen without diagnostic testing even though the positive predictive value (PPV) of the test is lower in younger patients (eg, PPV trisomy 21 at age 20 = 48 percent versus 93 percent at age 40) [77]. In addition, there is a lack of consensus regarding the appropriate follow-up procedures for patients in the general population who have a cfDNA test failure (usually between 1 and 5 percent). Importantly, sufficient expertise and resources are not available in the United States to provide formal genetic counseling for any prenatal screening test in all low-risk patients. Although further study is needed to determine whether primary care practices can provide adequate counseling to allow for informed choice, at least one study in the United States reported that patient education about cfDNA screening can be conducted successfully through general obstetric providers [40]. (See 'Implementation issues' below.)

Screening for the most common sex chromosome aneuploidies is controversial because these individuals have fewer serious physical abnormalities than those with trisomy 21, 18, or 13 and the phenotypic features are much more variable. One rationale for screening is that, in the absence of prenatal or early childhood screening, these disorders are often diagnosed later in life after some options for treatment have passed, such as beginning low-dose testosterone therapy at age 13 for males with 47,XXY. (See "Down syndrome: Clinical features and diagnosis" and "Congenital cytogenetic abnormalities" and "Sex chromosome abnormalities".)

Implementation issues — Several factors need to be considered when offering noninvasive prenatal aneuploidy screening using cfDNA in either the high-risk or general population settings.

Patient education and counseling

- **Genetic counseling** – Ideally, genetic counseling would be available before testing; however, there are insufficient resources to make this practical for the general pregnancy population, and other options for informing patients in various settings and using multiple methodologies are being explored [78-80]. The key pretest points to discuss with patients who are considering cfDNA screening include the following: screening is optional, the difference between screening tests and diagnostic tests, basic principles of cfDNA technology, conditions that may be detected by screening and their clinical features and variability, the reporting format, test performance (eg, DR, FPR, test failure rate, PPV, and/or patient-specific risk), limitations of the test, incidental findings, timing, and need to confirm abnormal screening results before considering termination [81].

- **Screening is optional** – The decision to undergo screening depends on how each patient balances the benefits of obtaining information about aneuploidy with the potential emotional and physical risks of screening and testing.

- **Limitations of screening versus diagnostic testing** – Patients need to understand the difference between a screening test, which classifies patients as at higher or lower risk of specific fetal aneuploidies, and a diagnostic test, which can diagnose or eliminate the chance that a fetus has any chromosomal abnormality.

  Sequencing cfDNA is a screening test; it has false-positive and false-negative results and does not test for all genetic syndromes or all aneuploidies: it tests for trisomy 21, 18, and 13 and sometimes sex chromosome aneuploidy. Diagnostic procedures, such as amniocentesis, obtain fetal cells, and subsequent testing by karyotyping or microarray can diagnose all aneuploidies; distinguish between full trisomy and trisomy caused by an unbalanced chromosomal rearrangement; detect mosaicism and microdeletions/microduplications (only microarray); and, via amniotic fluid AFP and acetylcholinesterase, detect open neural tube defects. If there are one or more structural anomalies on ultrasound examination, a microarray on amniocytes is the preferred test [82]. (See "Prenatal diagnosis of chromosomal imbalance: Chromosomal microarray".)

  Even in the absence of any screening, or structural anomalies or soft markers on ultrasound, patients who want to maximize the amount of genetic information they can obtain about their fetus should consider an invasive diagnostic test (amniocentesis, chorionic villus sampling) with chromosomal microarray analysis. cfDNA screening, which is targeted at detecting trisomy 21, 18, or 13 and sex chromosome aneuploidies, is not

diagnostic and is not able to reliably identify the vast numbers of deleterious microdeletions/microduplications [83-86].

- **cfDNA screening versus serum marker screening** – Whether prenatal screening based on a combination of serum and ultrasound measurements will identify more chromosomal abnormalities than next-generation sequencing of circulating cfDNA is no longer controversial [87]. Although conventional serum marker screening for aneuploidy can lead to the serendipitous identification of chromosomal abnormalities not targeted by the screening test, it does so only by randomly identifying patients as being false positives. Prior to the widespread use of cfDNA as a secondary screening test, serum screen-positive patients generally chose an invasive procedure for conventional karyotype or microarray. However, many of the additional abnormalities identified are rare, with some having a very low chance of survival (eg, triploidy), while others were not phenotypically significant.

- **Cost** – Patients need to know whether their insurance covers the cfDNA screening costs completely and, if not, which charges will be their responsibility (eg, copayment, deductible). More insurance policies are now covering the cost of cfDNA testing for all pregnant people.

- **Turnaround time** – Overall turnaround time is slightly longer for cfDNA (five to seven days from sample collection to receipt of final report) than for serum screening (one to three days). In the high-risk setting, a shortened turnaround time may help in reducing the level of anxiety.

- **Incidental findings** – Patients should understand that prenatal screening may raise suspicion for conditions other than the fetal aneuploidies for which the test is being performed. These include maternal sex chromosome aneuploidy or mosaicism and maternal malignancy. (See 'False-positive cfDNA test results' above.)

- **Desire for fetal sex result** – In the general obstetric population, data suggest that one strong driver of interest in cfDNA screening is earlier identification of fetal sex (at 10 to 13 weeks via cfDNA rather than 17 to 20 weeks via ultrasound) [40]. Parents should understand that the test is intended to look for major fetal chromosomal anomalies and that they will receive this information along with the fetal sex. It is also possible that no result can be obtained for their test or that the fetal sex assignment will be incorrect.

Laboratory issues

- **Commercial marketing influences** – Nearly all testing is being performed by commercial companies (private or public) that have active sales and marketing forces. Individual clinicians, practices, and patients are targets for aggressive tactics to obtain market share. A systematic review concluded these companies' internet sites often do not provide supporting evidence for the information cited and may not provide adequate information about the need for an invasive test to definitively diagnose aneuploidy [88].

- **Choice of laboratory** – It is not possible to confidently conclude that any one laboratory has a superior combination of DR, FPR, and failure rates than another. It is most likely that decisions regarding choice of laboratory will be based on other factors, such as ease of ordering and receiving results, charges, turnaround time, special circumstances (eg, known twins), method of reporting results, identification of particular microdeletions, and customer service. This last item is crucial, as some report results are cryptic [89], and the availability of knowledgeable support personnel is invaluable in providing easy-to-understand results to patients.

- **Laboratory report** – Given that all tests are laboratory-developed, there is little consistency among laboratories in reporting negative or positive results or reporting test failures. Some laboratories report a risk based mainly on maternal age and cfDNA test results; others provide categorical results (consistent with extra chromosome 21 material). In some instances, the risks reported for a positive cfDNA test are unreasonably high (eg, >99 to 1), even though the laboratory has data that the PPV is far lower. None provide the ability to incorporate the a priori risk, even though an elevated a priori risk is the main reason for testing at this time.

Twins — The American College of Obstetricians and Gynecologists [76] and the International Society for Prenatal Diagnosis (ISPD) [23] allow for or recommend cfDNA screening for common trisomies in twin pregnancies. The amount of cfDNA for the pregnancy overall is approximately 35 percent higher in twin pregnancies than singleton pregnancies [90]. 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 [91]. For example, if the total fetal fraction for the twin pregnancy is 8 percent, one fetus may provide 6 percent and the other 2 percent. 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 comparing polymorphic loci that will differ in dizygotic twins and maternal loci [92]. A sample from a twin pregnancy with one low fetal fraction would be reported as a test failure (no call result), so the laboratory would not miss a trisomy in the fetus with only 2 percent fetal fraction.

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 (ie, the laboratory interpretation for a singleton and known or unknown twins are the same).

A 2020 International Society for Prenatal Diagnosis (ISPD) position statement included a summary analysis of screening for aneuploidy in twin pregnancies that included 83 trisomy 21, 27 trisomy 18, and 3 trisomy 13 cases [23]. The overall detection rates were 98.8, 93.1, and 75 percent, respectively. The associated total FPR was 0.29 percent, with estimated predictive values in a general pregnancy population of 75, 47, and 19 percent, respectively. The initial test failure rate ranged from 1.6 to 13.2 percent with a median of 3.6 percent, with insufficient fetal fraction accounting for most of these failures. Five studies provided revised failure rates from a combined population of 2938 twin pregnancies with 179 repeat tests. Between 83 and 100 percent of those offered repeat testing did so, and success rates ranged from 50 to 83 percent (overall 58 percent, 103 of 179). This reduced the median failure rate in these five studies from 5.6 to 3.1 percent, a 45 percent reduction.

A 2021 systematic review on the same topic included 137 trisomy 21, 50 trisomy 18, and 11 trisomy 13 pregnancies [93]. This review provided very similar estimates for trisomy 21 and 18 detection rates but a higher detection rate for trisomy 13 (94.7 percent), which was likely more accurate because of additional cases reported since the ISPD analysis.

Triplets — The data are even more limited for triplet pregnancies. One laboratory that used three times the needed fetal fraction as a minimum to interpret triplets reported test failures in 151 of 709 pregnancies (22 percent) [94]. No series with affected pregnancies have been reported. Given the rarity of triplet pregnancies and the reticence of providers to offer cfDNA screening in this group, it is unlikely that the type of dataset now available for twins will ever be available for triplet pregnancies. For this reason and the lack of other screening options, the ISPD position statement suggests that cfDNA may be a potential option in these pregnancies, but diagnostic testing should always be offered and limitations of screening tests stressed [23].

Maternal obesity — The concentration of fetal cfDNA falls with increasing maternal weight (or body mass index [BMI]) and is more often insufficient for prenatal screening in patients with obesity. Fetal cfDNA is still produced, but the fetal fraction in maternal blood is reduced because of an increased contribution of maternal cfDNA from apoptosis of adipose tissue and/or dilution of fetal (placental) DNA in the larger maternal blood volume.

Patients over 81 kg (180 pounds) can be informed that their chance of having a test failure or an inaccurate result is at least three or four times higher than in patients of lower weight (3.3 versus 0.5 percent in one study [14]) and that the chance of a false-negative result is also slightly higher [14,95]. A decision-analytic model found that serum-integrated screening was probably a better option (fewer unnecessary invasive tests) for patients over 136 kg (300 pounds) [96]. In patients with BMI ≥35 kg/m^2, postponing the test to an older gestational age would be unlikely to reduce test failures because the fetal fraction rises more slowly in patients with obesity [97].

Serum screening may be offered to patients with severe obesity as an alternative to cfDNA. If a no call cfDNA result is obtained late in the pregnancy and the patient is high risk, offering diagnostic testing rather than any screening test is an option. (See 'Fetal fraction' above.)

POSTTEST FOLLOW-UP

Screen positive — Even with the high performance of cfDNA screening, invasive diagnostic testing must be offered to patients in order to confirm the fetal karyotype. There is some controversy about whether an early gestation cfDNA positive screen should be confirmed by chorionic villus sampling (CVS) or postponed until ≥15 weeks when amniocentesis can be performed, as analysis of amniocytes is more definitive since it is representative of the fetal genotype rather than the analysis of placental cells [48]. For disorders where definitive diagnosis is unlikely to affect continuation of pregnancy or pregnancy management (eg, sex chromosome aneuploidy), the parental choice to delay diagnostic testing until after delivery, or even later, is also reasonable.

A conventional G-banded karyotype is critical to obtain in cfDNA screen-positive cases. Fluorescence in situ hybridization (FISH) and microarray can identify additional copies of aneuploid segments but not their orientation to the other chromosomes. In 5 to 10 percent of pregnancies with a common aneuploidy, one of the parents will be identified with a balanced translocation, and this information will alter reproductive counseling. Thus, if FISH or microarray is the initial diagnostic test and is positive, then a conventional karyotype should also be performed.

Screen negative — A screen-negative result means the fetus is at a reduced risk of having one of the aneuploidies in the test panel, but it does not eliminate the possibility of an affected fetus or the possibility of a fetus with a chromosomal abnormality not targeted by the screening test but detectable with diagnostic testing. Screen-negative patients are not usually offered invasive diagnostic testing.

However, screen-negative patients who go on to develop an indication for invasive diagnostic testing, such as a fetal anatomic anomaly on ultrasound examination, should be offered this testing. This recommendation does not apply to the fetus found to have an isolated soft marker. (See "Prenatal genetic evaluation of the fetus with anomalies or soft markers".)

No call or no result — As discussed above, 1 to 5 percent of cfDNA tests do not yield a result, and patients with obesity are at increased risk of receiving a test failure. (See 'Test failures: Rates, reasons' above and 'Maternal obesity' above.)

There is no standard approach. The patient has three options in this setting:

- Repeat the cfDNA test after seven days (or more), which is around the time that test failure is reported. Some failures (like large regions of homozygosity) will always cause the test to fail, so repeat testing is not an option. Repeat testing, when allowed, is successful in approximately 60 to 80 percent of cases [38,98]. Laboratory reports should indicate whether or not a repeat sample is recommended for each patient with a failed test.
- Standard serum marker or combined serum marker and ultrasound screening, if not already done.
- Invasive procedure (amniocentesis, CVS) and diagnostic testing (karyotyping/microarray).

A few studies suggest that there may be a higher than expected rate of aneuploidy in patients with cfDNA test failures [99-101]. Offering invasive diagnostic testing is appropriate, especially if the pregnancy with a failed test was already at high risk for aneuploidy prior to cfDNA testing (eg, advanced maternal age, abnormal ultrasound). However, one study found that the risk for aneuploidy is relegated to that group with very low fetal fractions (eg, <2 percent) [18]. When reviewing this literature, it is important to ensure that the testing was equivalent to that found in clinical practice. For example, the proportion of aneuploid pregnancies among initial test failures may not be representative of that found after the repeat cfDNA testing protocol is used, as is often the practice.

Standard serum/ultrasound screening is an option if cfDNA screening fails, with invasive diagnostic testing if that screen is positive. However, patients should understand that standard serum/ultrasound screening is a less sensitive test than cfDNA screening and typically screens for trisomies 21 and 18. Detection of trisomy 13 and sex chromosome aneuploidy are dependent on the test offered (eg, first-trimester screening will identify trisomy 13 as part of the trisomy 18 screen). In trisomy 18 and trisomy 13 pregnancies, in which test failures are more likely, there are most often clear ultrasound findings in the second trimester.

The American College of Obstetricians and Gynecologists recommends informing patients that test failure is associated with an increased risk of certain aneuploidies, providing additional genetic counseling, and offering comprehensive ultrasound evaluation and diagnostic testing [76].

Second-trimester screening for fetal structural anomalies — Patients who undergo first-trimester aneuploidy screening should be offered additional second-trimester ultrasound screening and/or maternal serum alpha-fetoprotein screening for fetal structural anomalies, including open neural tube defects [76].

SCREENING FOR GENETIC DISORDERS OTHER THAN THE COMMON ANEUPLOIDIES

Theoretically, cfDNA can be used to screen for genetic disorders other than the common aneuploidies discussed above (eg, other aneuploidies, microdeletions/microduplications, single gene disorders). Currently, none of the professional guidelines recommend routine expanded screening for any of these genetic disorders.

It is possible to detect aneuploidies of other chromosomes (eg, trisomy 16), especially when testing with sequencing-based methodologies. Although there are data that suggest the test may be highly predictive, the clinical utility of many of the findings has not been determined. Furthermore, many of these aneuploidies are lethal early in pregnancy, so miscarriage is likely to occur, or the aneuploidy may be confined to the placenta. In such cases, one option is to refer these cases to, or consult with, a genetics specialist who can provide additional information and guidance.

Several laboratories offer screening for select microdeletions. These include, for example, 22q11.2 (DiGeorge syndrome), 5P (Cri-du-chat syndrome), 1p36, and 15q (Prader-Willi and Angelman syndromes). When first introduced, the positive predictive value was relatively low, in the range of 15 to 30 percent. However, modification to the interpretation of results has resulted in higher predictive values [102]. One remaining issue with such testing is the penetrance associated with these findings. In some instances, the confirmed screen-positive rate is several times higher than the reported prevalence. This might indicate incomplete penetrance, under-ascertainment of the disorder after birth, or some other factor, such as a high spontaneous loss rate.

Although screening for single gene disorders using cfDNA is commercially available, routine clinical use remains largely investigational [103] and is not recommended.

SOCIETY GUIDELINE LINKS

Links to society and government-sponsored guidelines from selected countries and regions around the world are provided separately. (See "Society guideline links: Prenatal screening and diagnosis" and "Society guideline links: Down syndrome".)

SUMMARY AND RECOMMENDATIONS

- Prenatal screening for trisomy 21 (Down syndrome), trisomy 18, trisomy 13, and common sex chromosome aneuploidies can be performed using cell-free DNA (cfDNA) in the maternal circulation. Circulating cfDNA is derived from both the mother and the placenta and cleared from the maternal circulation soon after delivery. (See 'Origins' above and 'Clearance' above.)

- Maternal plasma must contain an adequate amount of fetal cfDNA to obtain a reliable test result. Several factors can reduce the fetal fraction, which can lead to an assay failure (a report of "no result"): screening before 10 weeks of gestation, suboptimal sample collection or processing, maternal obesity, select disorders (eg, trisomy 18, triploidy), as well as maternal use of low molecular weight heparin, conception by in vitro fertilization, or multiple gestation. (See 'Fetal fraction' above.)

- cfDNA is the most sensitive screening option (highest detection rate [DR]) for trisomy 21, 18, and 13 and the most specific. Performance varies by trisomy. Based on multiple meta-analyses, the consensus DRs and false-positive rates (FPRs) in successful tests are as follows:

  - **Trisomy 21** – DR 99.5 percent, FPR 0.05 percent
  - **Trisomy 18** – DR 97.7 percent, FPR 0.04 percent
  - **Trisomy 13** – DR 96.1 percent, FPR 0.06 percent

  The total FPR is the sum of the three rates, or 0.15 percent. However, these data do not account for test failures. (See 'Trisomy 21, 18, and 13' above.)

- In the largest meta-analysis that evaluated cfDNA test performance for sex chromosome aneuploidies, the DR and FPR for monosomy X (177 cases and 9079 controls) were 90.3 and 0.23 percent, respectively. For the sex chromosome trisomies, 47,XXX; 47,XXY; and 47,XYY (56 cases and 6699 controls), the DR and FPR were 93 and 0.14 percent, respectively. (See 'Sex chromosome aneuploidies' above.)

- A wide range of cfDNA failure rates has been reported. Although there is no standard approach to this situation, it is common practice to submit a repeat sample for cfDNA testing. Alternatively, one could offer a standard serum marker screen (if not already done) or a targeted ultrasound. An invasive procedure followed by diagnostic testing is an option if the pregnancy was already considered to be at "high risk."

  The most common reasons for test failure include less than a specified absolute amount of total and/or fetal-placental DNA, fetal fraction below an acceptable level (eg, <4 percent), and insufficient numbers of fragments sequenced and/or aligned. It is important to consider whether the laboratory's reported failure rate includes only total assay failures; includes only failures related to chromosomes 21, 18, and 13; or includes failures to provide sex chromosome interpretations. (See 'Test failures: Rates, reasons' above.)

- False-positive tests may be due to confined placental mosaicism, demised twin, maternal mosaicism, maternal cancer, maternal copy number variants, technical issues, or chance. (See 'False-positive cfDNA test results' above.)

- False-negative tests may be due to confined placental mosaicism, borderline low fetal fraction, maternal copy number variants, and technical issues. (See 'False-negative cfDNA test results' above.)

- Patients should understand that, if they receive a positive result for trisomy 21, the likelihood that the fetus actually has trisomy 21 (positive predictive value) is likely to be no higher than 90 percent. Patients ages 35 and older with a screen-positive result for trisomy 21 and trisomy 18 have risks on the order of 87 percent (odds 7:1) and 63 percent (odds 1:1), respectively (table 1). Likewise, patients who receive a negative result should understand that the likelihood of a normal fetus is high (>99.9 percent), but this does not rule out the possibility of trisomy 21. (See 'Predictive value' above.)

- cfDNA is commonly used as a secondary screening test in patients found to be screen positive (high risk) as a result of a previous screening test: maternal age ≥35 years at delivery, abnormal ultrasound findings indicating increased risk (eg, increased nuchal translucency), an abnormal serum screening test, a positive family history of aneuploidy, or a parent who carries a relevant Robertsonian translocation (figure 2). The majority of patients who undergo cfDNA secondary screening will receive a low-risk result and, thus, will avoid an invasive procedure. The high sensitivity of the test helps to ensure that very few patients with an affected pregnancy will be incorrectly reclassified as being at low risk. (See 'Secondary screening' above.)

- cfDNA screening is increasingly being offered as a primary screening test for fetal aneuploidy in the United States and elsewhere. However, in the United States, it is neither a universal nor routine test because of cost and implementation issues. This may change as more insurance companies provide coverage and/or the costs of testing decrease. (See 'Primary screening' above.)

- Issues that should be discussed with patients when offering noninvasive prenatal aneuploidy screening using cfDNA include the following (see 'Implementation issues' above):

  - Screening is optional and alternative screening and diagnostic options exist.

  - The conditions being tested for and what will not be identified.

  - Cost and insurance coverage.

  - Turnaround time (approximately five to seven days), how results are reported, incidental findings, and performance in twin pregnancy.

- Patients who want to maximize the amount of genetic information they can obtain about their fetus should consider an invasive procedure (amniocentesis, chorionic villus sampling) followed by diagnostic testing (karyotype or chromosomal microarray analysis). Screening using cfDNA has a high DR for trisomy 21, 18, or 13 but is not diagnostic. (See 'Patient education and counseling' above.)

- Patients weighing over 81 kg (180 pounds) can be informed that their chance of having an initial cfDNA test failure is three or four times higher than lower weight patients. Even if the test is successful, the chance of a false-negative result is also higher. Both of these limitations are due to lower fetal fraction. Patients in this weight range, at an advanced gestational age, at high risk of fetal aneuploidy, and with body habitus limitations on obtaining a complete fetal survey may want to consider diagnostic testing. (See 'Maternal obesity' above.)

- Use of cfDNA screening in twin pregnancies is an acceptable practice. The DRs and FPRs are similar to those in singleton pregnancies. Some commercial test offerings can identify twin pregnancies and provide zygosity, which may be helpful in the interpretation. Alternative options, such as ultrasound measurement of nuchal translucency or serum screening, have far lower DRs and higher FPRs. (See 'Twins' above.)

- An invasive procedure and diagnostic testing (eg, amniocentesis, karyotype, or microarray) should be offered to confirm all screen-positive test results. For disorders in which definitive diagnosis will not affect continuation of pregnancy or pregnancy management (eg, sex chromosome aneuploidies), the parents' choice to delay diagnostic testing until after delivery, or even later, is also reasonable.

  Screen-negative patients are not routinely offered invasive diagnostic testing. They are at a reduced risk of having a fetus affected by one of the aneuploidies in the test panel, although the possibility of an affected fetus cannot be eliminated. However, if fetal structural abnormalities are found on a later ultrasound, diagnostic testing with a chromosome microarray should be offered.

  Patients whose test does not yield a result can choose to undergo repeat cfDNA testing, an invasive diagnostic procedure (if already at high risk), or serum/ultrasound screening. (See 'Posttest follow-up' above.)

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Topic 458 Version 99.0

GRAPHICS

Positive and negative predictive values for combined first-trimester screening and cell-free DNA screening for the three common trisomies

Trisomy	Test	Test setting			
		General population		High-risk population	
		PPV (%)	NPV (%)	PPV (%)	NPV (%)
21	Combined	5 (1:20)	99.8 (600:1)	15 (1:6)	>99.9 (2200:1)
21	cfDNA	85 (6:1)	>99.9 (20,000:1)	95 (21:1)	>99.9 (>30,000:1)
18	cfDNA	69 (2:1)	>99.9 (12,000:1)	89 (8:1)	>99.9 (>30,000:1)
13	cfDNA	33 (1:2)	>99.9 (20,000:1)	68 (2:1)	>99.9 (>30,000:1)

The "general population" is defined as the maternal ages associated with live births in the United States in 2015 while the "high-risk population" is defined as all women ages 35 and older in that same population. The PPV is the proportion of true positives divided by the sum of true positive and false positives (TP/(TP+FP)). The NPV is the proportion of true negatives divided by the sum of true negative and false negatives (TN/(TN+FN)). The first-trimester "combined" test includes nuchal translucency measurements along with serum measurements, such as PAPP-A and hCG. The estimated detection and false-positive rates are 85% and 5%, respectively. The first-trimester prevalence of trisomies 21, 18, and 13 in the general population is 1:340, 1:1100, and 1:3500, respectively. The first-trimester prevalence of trisomies 21, 18, and 13 in the high-risk population (women ages 35 and older) is 1:100, 1:340, and 1:1200, respectively.

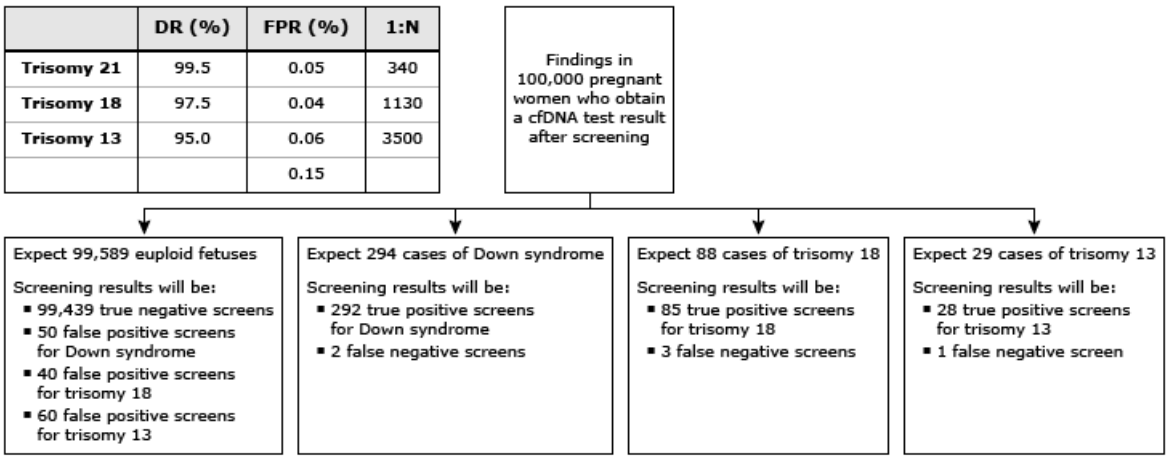
PPV: positive predictive value; NPV: negative predictive value; cfDNA: cell-free DNA; TP: true positive; FP: false positive; TN: true negative; FN: false negative; PAPP-A: pregnancy-associated plasma protein A; hCG: human chorionic gonadotropin.

Courtesy of Glenn E Palomaki, PhD, Geralyn M Messerlian, PhD, and Jacquelyn V Halliday, MS.

Graphic 104055 Version 6.0

Flowchart showing screening performance for the common trisomies in the general obstetric population

	DR (%)	FPR (%)	1:N
Trisomy 21	99.5	0.05	340
Trisomy 18	97.5	0.04	1130
Trisomy 13	95.0	0.06	3500
		0.15	



PPV	Detected cases	Missed	Number of FP	PPV (%)	Odds:1
Down syndrome	292	2	50	85	5.8:1.0
Trisomy 18	85	3	40	68	2.1:1.0
Trisomy 13	28	1	60	32	1.0:2.1
All	405	6	150	73	2.7:1.0

Overall DR (%)	Overall FPR (%)
98.5	**0.15**

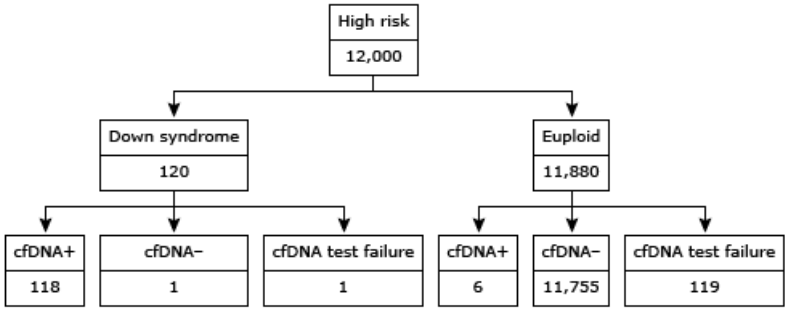
At the top left corner are the DRs, FPRs, and the expected prevalence of the three common trisomies. Overall, the FPR is expected to be approximately 0.15%. The flowchart shows the results of screening 100,000 women from the general population with singleton pregnancies (United States 2015 birth records). For example, among the 100,000 women with screening results, there will be 294 cases of Down syndrome. Among these, 292 will be screen positive, and 2 will be screen negative. Among the 99,589 euploid pregnancies, there will be 50 false-positive calls. Thus, the PPV for Down syndrome screening will be 85% (odds of 6:1). The table below the flowchart summarizes the results of Down syndrome screening as well as screening for trisomies 18 and 13. In summary, the overall DR is approximately 98.5% for the common trisomies with a 0.15% FPR. The PPV is highest for Down syndrome, with lower PPVs for trisomy 18, trisomy 13, and overall (approximately 3:1). The false-negative cases include 2 Down syndrome, 3 trisomy 18, and 1 trisomy 13 pregnancy. This figure does not account for the reported higher rate of test failure associated with trisomies 18 and 13.

DR: detection rate; FPR: false-positive rate; cfDNA: cell-free DNA; PPV: positive predictive value.

Courtesy of Glenn E Palomaki, PhD, Geralyn M Messerlian, PhD, and Jacquelyn V Halliday, MS.

Graphic 127261 Version 1.0

Use of cell-free DNA for secondary screening in women at high risk of Down syndrome



	Screen positive	Screen negative	Test failed
N	118+6	1+11,755	1+119
Offered follow-up	Diagnostic test	Routine	Options include repeat testing or a diagnostic test
Odds	20:1	1:11,755	1:120
PPV (%)	95.00	0.01	1.00

The flowchart assumes the prevalence of Down syndrome (trisomy 21) is 1:100 in the high-risk population. In addition, the cfDNA detection rate for Down syndrome is 99.5%, the associated false-positive rate is 0.05%, and the overall test failure rate is 1%. Among the 12,000 high-risk singleton pregnancies, 120 will have Down syndrome, and for simplicity, the remaining population will be euploid (unaffected). Among the 120 Down syndrome pregnancies, 1 will be a test failure, 1 will be screen negative, and the remaining 118 will be screen positive. Among the 11,880 euploid pregnancies, 6 will be false positive, 11,755 will be screen negative, and 119 will have a test failure. The associated table shows a summary of those pregnancies that screen positive (118 TP and 6 FP or approximately 1% of the 12,000), screen negative (1 FN and 11,755 TN or approximately 98%), as well as those in which the test failed to provide a result (1 TP and 119 TN or approximately 1%). Women who were screen positive would be offered diagnostic testing and would, as a group, have a PPV of 95% (odds 20:1). Among those women who screen negative, no further testing for Down syndrome would be warranted (routine care) as their risk has been reduced to approximately 1:12,000. Among those women with a failed test, there would be several options as their residual risk of 1:119 is quite similar to their group risk of 1:100 prior to testing. The test could be repeated and the subsequent result used, or, since the woman was a high risk to begin with and her risk has not changed, the offer of diagnostic testing is reasonable.

cfDNA: cell-free DNA; PPV: positive predictive value; TP: true positive; FP: false positive; FN: false negative; TN: true negative.

Courtesy of Glenn E Palomaki, PhD, Geralyn M Messerlian, PhD, and Jacquelyn V Halliday, MS.

Graphic 103823 Version 5.0

Contributor Disclosures

Glenn E Palomaki, PhD Grant/Research/Clinical Trial Support: PerkinElmer [Aneuploidies in cfDNA]; PerkinElmer [Preeclampsia]. Consultant/Advisory Boards: Ansh Labs [COVID-19 antibodies during pregnancy]; Abbott [COVID-19 antibodies during pregnancy]. **Geralyn M Messerlian, PhD** Grant/Research/Clinical Trial Support: PerkinElmer [Non-NGS methods to identify common aneuploidies in cfDNA, preeclampsia]; Ansh Labs [COVID testing]; Abbott Diagnostics [COVID testing]. **Jacquelyn V Halliday, MS** Nothing to disclose **Louise Wilkins-Haug, MD, PhD** Nothing to disclose **Vanessa A Barss, MD, FACOG** Nothing to disclose

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