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SMFM CONSULT SERIES

Society for Maternal-Fetal Medicine Consult Series #74: Cell-free DNA screening for aneuploidies: Updated guidance

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Replaces Society for Maternal-Fetal Medicine Consult Series #36: Prenatal aneuploidy screening using cell-free DNA

The American College of Obstetricians and Gynecologists endorses this document.

Abstract

In this Consult, we provide updated guidance on the evolving applications of cell-free DNA (cfDNA) screening, including its use in detecting sex chromosome aneuploidies and microdeletions and its application in multifetal gestations. We compare cfDNA test performance with conventional screening methods and offer recommendations for managing inconclusive results and unexpected secondary findings. The following are the Society for Maternal-Fetal Medicine's (SMFM's) recommendations: (1) we recommend that cfDNA screening for common aneuploidies (trisomies 21, 18, and 13) be made routinely available to all obstetrical patients (GRADE 1B); (2) we recommend cfDNA as the most sensitive and specific screening test for common fetal aneuploidies (trisomies 21, 18, and 13) in any patient population. After pretest counseling, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing (GRADE 1B); (3) we recommend that screening for sex chromosome aneuploidies be made available to obstetrical patients as an "opt-in" consideration with appropriate pretest counseling (GRADE 1C); (4) we do not recommend routine general population screening for any microdeletion condition. Patients who choose to undergo cfDNA screening for 22q11.2 deletion specifically should do so only after appropriate pretest counseling. Pregnant people who are interested in obtaining information regarding the risk for fetal copy number variants should be offered diagnostic testing as opposed to cfDNA screening for microdeletion syndromes (GRADE 1C); (5) we recommend cfDNA as a first-line screening option for trisomy 21 detection in twin gestations (GRADE 1B); (6) although the numbers of affected pregnancies are limited, the detection rates associated with trisomy 18 and 13 appear to be consistently high in twin gestations, and cfDNA screening for these conditions is recommended (GRADE 1B); (7) because of a lack of data, cfDNA screening for sex chromosome aneuploidy in twin gestations and cfDNA screening for higher-order multiples are not recommended (GRADE 1C); (8) we do not recommend the routine use of cfDNA testing for large

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genome-wide copy number deletions or duplications (GRADE 1C). This document replaces SMFM Consult Series #36: Prenatal aneuploidy screening using cfDNA.

KEYWORDS
confined placental mosaicism, fetal aneuploidy, genetic counseling, microdeletion, noninvasive, screening, sex chromosome aneuploidy, twin gestation

1 | INTRODUCTION

Screening for fetal aneuploidy has significantly changed since noninvasive cell-free DNA (cfDNA) screening became commercially available in 2011. Numerous studies have demonstrated improved test performance, including higher detection rates for the common aneuploidies, with cfDNA screening compared with traditional ultrasound and serum-based screening strategies. However, the complexity of this technology, combined with commercial interest in rapidly incorporating these tests into clinical practice despite inadequate provider education, has resulted in misinformation and unintended consequences for patients [1–5]. Some of these challenges include a lack of recognition of reduced positive predictive value (PPV) in low-prevalence populations, unexpected clinical consequences associated with test failures (e.g., confined placental mosaicism [CPM], vanishing twins), unanticipated results related to maternal health conditions (e.g., maternal malignancy), and expanded use beyond common aneuploidies despite limited clinical utility and minimal validation data.

This Consult aims to enhance clinician knowledge regarding these evolving genomic technologies with updated guidance for sex chromosome aneuploidy (SCA) and microdeletion screening, as well as application in multifetal gestations. We also discuss cfDNA test performance compared with conventional screening options; managing nonreportable test results (also described as "no result," "no call," or "low fetal fraction"); and unanticipated secondary findings. The importance of pretest counseling is detailed, along with specific points to consider when discussing cfDNA screening with patients.

2 | CLINICAL QUESTIONS

2.1 | What is cfDNA screening?

cfDNA consists of short fragments of placental DNA (~142 base pairs) found in the maternal circulation during pregnancy. These cfDNA segments are released into the maternal bloodstream upon cell death or apoptosis of trophoblastic cells. Placental cfDNA circulates within maternal serum in a background of maternally derived cfDNA differentiated by DNA fragment size, placental-specific methylation signatures, and single-nucleotide polymorphic alleles [6]. The amount of placental cfDNA found within the background of maternal cfDNA increases as gestation advances; generally, it represents 10% to 20% of the total cfDNA in maternal plasma at its peak between 10 and 21 weeks of gestational age [7]. This total percentage is termed the "fetal fraction." Certain pregnancy conditions or maternal health factors are associated with higher or lower rates of cellular apoptosis (and, therefore, aberrant fetal fractions), which may influence the test performance or sensitivity of the cfDNA analysis.

Prenatal cfDNA screening (previously termed noninvasive prenatal testing, or NIPT) is available as early as 9–10 weeks of gestation [8, 9]. Screening for common fetal aneuploidies (trisomies 21, 18, and 13) by cfDNA began in 2011 and has become the standard for most obstetrical patients. However, cfDNA technology has also been marketed to screen for a variety of additional genetic conditions, including SCAs, targeted microdeletion syndromes, rare autosomal trisomies (RATs), and genome-wide imbalances, despite minimal validation or clinical utility data.

2.2 | How is cfDNA screening performed?

Methods for cfDNA aneuploidy screening vary between laboratories. Proprietary differences in bioinformatic algorithms and a lack of standardized reporting policies further complicate screening methodology. Currently, clinical laboratories use three primary cfDNA testing techniques, each with benefits and limitations. Despite platform differences, screening performance for the common autosomal trisomies is remarkably similar among the available methodologies reported in clinical trials [10]. Each platform has specific maternal or fetal criteria that must be met for optimal test performance. Based on peer-reviewed and published performance data, no one technology is superior to another [10–12].

All three techniques begin with the amplification of circulating cfDNA. Next-generation sequencing is the most frequently used technology to evaluate the amplified

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cfDNA through massively parallel sequencing random fragments throughout the genome (sometimes called "shotgun sequencing") or chromosome-selective targeted sequencing. The short DNA sequences are mapped to specific chromosomes with subsequent dosage analysis to determine whether excess DNA is present from the chromosome(s) of interest [13, 14]. A second technique is single-nucleotide polymorphism (SNP) analysis, which compares thousands of single DNA base pairs from the maternal genotype to circulating fetal DNA. Using a bioinformatic algorithm, the laboratory can identify aneuploidy by skewed ratios of expected allele frequency [15]. Unique aspects of SNP technology include the ability to screen for both triploidy and zygosity in twins, characteristics not currently possible by other cfDNA modalities. A third, less-used technique is targeted microarray of the amplified cfDNA, which measures copy number variation (CNV) in the fetal genome by comparing it to a known reference DNA sample on an array platform. Overrepresentation of a targeted chromosome region is suggestive of aneuploidy compared with the reference sample [16]. Providers should understand the cfDNA screening technology used by their chosen laboratory, including its limitations and benefits, to provide comprehensive pre- and posttest counseling. (See guidance in Section 2.5.)

2.3 | Who should consider cfDNA screening?

Screening for fetal aneuploidy using cfDNA has increased dramatically since its inception [10, 17, 18]. In 2020, guidance from the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine (SMFM) [19] recognized cfDNA as the most sensitive and specific screening test for common fetal aneuploidy in all obstetrical patients [10, 19–23]. The organizations further acknowledged that no single screening test performed optimally in all clinical scenarios and highlighted the importance of pretest counseling to enable informed decisions about all testing options, including diagnostic testing. Similarly, in 2022, the American College of Medical Genetics and Genomics (ACMG) issued an updated statement [24] acknowledging the superior performance of cfDNA screening compared with traditional screening methods for common aneuploidies [17, 25–28]. These guidelines state that cfDNA screening is not equivalent to a diagnostic test and stress the importance of pretest counseling and informed consent [19, 24]. **We recommend that cfDNA screening for common aneuploidies (trisomies 21, 18, and 13) be made routinely available to all obstetrical patients (GRADE 1B)**. Routine use implies that the screening is offered to all patients in the context of general obstetrical care.

2.4 | How does cfDNA screening compare to conventional screening?

Multiple studies have identified cfDNA as a superior screening test for trisomies 21, 18, and 13 compared with traditional serum and ultrasound screening tests (Table 1) [29–32]. Test performance for first- and second-trimester combined screening has a slightly lower detection rate for trisomy 21 than cfDNA screening [33–36]. Conventional combined serum or first-trimester ultrasound screening or both can lead to a range of findings that cfDNA may not detect, including fetal structural abnormalities, unanticipated genetic diagnoses, or risk for adverse perinatal outcomes [19]. When evaluating test options, it is crucial to consider factors such as test performance, availability, cost, personalized risk profiles, and patient information preferences. Although less frequently used since the uptake of cfDNA screening, laboratories remain available for first- and second-trimester serum analyses, and the Fetal Medicine Foundation offers nuchal translucency certification [37]. An anatomic survey is recommended for all patients to detect structural abnormalities regardless of aneuploidy screening method [19].

Patient risk and priorities for information guide the choice of prenatal genetic testing strategies: no testing, screening and screening modality, or diagnostic testing [19, 38–41]. **We recommend cfDNA as the most sensitive and specific screening test for common fetal aneuploidies (trisomies 21, 18, and 13) in any patient population. After pretest counseling, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing (GRADE 1B)**. Patients may consider alternate screening modalities or diagnostic testing based on personalized risk assessment, availability of testing options, cost, or individual desire for information.

2.5 | What are important points for pretest counseling for cfDNA screening?

The healthcare provider offering cfDNA screening should comprehensively understand cfDNA screening methodologies and test performance. This understanding includes basic knowledge of the specific laboratory testing technique, the benefits and limitations of the test, what conditions are available for screening, how the available options align with each patient's desire for information, and the test's performance. Additionally, providers should have a reliable system to communicate relevant

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TABLE 1 Comparison of prenatal screening and diagnostic testing options for fetal aneuploidy [19].

Screening approach	Approximate gestational age range for screening (weeks)	DR for trisomy 21 (%)	Screen-positive rate[a] (%)	Advantages	Disadvantages	Method
Cell-free DNA [10]	9–10 to term	99	2–4 (includes inability to obtain results, which is associated with increased risk) [10]	• Highest DR • Can be performed at any gestational age after 9–10 weeks • Lowest false-positive rate	• Results may reflect underlying maternal aneuploidy or maternal disease	Several molecular methods
First trimester[b]	10–13 6/7[c]	82–97[d]	5	• Early screening • Single time point test • Nasal bone assessment increases DR to 97% with screen-positive rate of 5%[d]	• Lower DR than tests with first- and second-trimester components • NT required	NT ± nasal bone + PAPP-A, free beta hCG, ±AFP[e]
Quad screen[b]	15–22	81	5	• Single time point test • No specialized US required	• Lower DR than first-trimester and first- and second-trimester combined tests	hCG, AFP, uE3, DIA
Integrated[b]	10–13 6/7,[c] then 15–22	96	5	• High DR	• Two samples needed • No first-trimester results • NT required	NT + PAPP-A, then quad screen
Serum integrated[b]	10–13 6/7,[c] then 15–22	88	5	• DR compares favorably with FTS • No specialized US required	• Two samples needed • No first-trimester results	PAPP-A + quad screen
Sequential: stepwise [29]	10–13 6/7,[c] then 15–22	95	5	• First-trimester results provided only to patients with high-risk results • Comparable performance to integrated screening but FTS results provided	• Patients without high-risk first-trimester results only receive results after the second-trimester sample • NT required	NT + free beta hCG + PAPP-A, ±AFP,[e] then quad screen
Sequential: contingent [35]	10–13 6/7,[c] then 15–22	88–94	5	• Classified aneuploidy risk based on FTS results as high, intermediate, or low	• Second serum sample required for combined risk assessment of intermediate-risk patients • NT required	NT + hCG + PAPP-A, ±AFP,[e] then quad screen
NT alone [36]	10–13 6/7[c]	70	5	• Allows individual fetus assessment in multifetal gestations • Provides additional screening for fetal anomalies	• Poor sensitivity and specificity in isolation • NT required	US only

Abbreviations: AFP, alpha-fetoprotein; DIA, dimeric inhibin-A; DR, detection rate; FTS, first-trimester screening; hCG, human chorionic gonadotropin; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; uE3, unconjugated estriol; US, ultrasonography.

[a]A screen-positive test result includes true positives and false positives. For cell-free DNA, this includes the test failure rates given the association with increased risk of aneuploidy (see Gil et al. [10]).

[b]First-trimester combined screening: 87%, 85%, and 82% for measurements performed at 11, 12, and 13 weeks, respectively [29].

[c]Because of variations in growth and pregnancy dating, some fetuses at the lower and upper gestational age limits may fall outside the required crown–rump length range. Also, different laboratories use slightly different gestational age windows for their testing protocol.

[d]Use of free beta hCG in conjunction with nasal bone assessment increases the DR to 97% with a screen-positive rate of 5% [34].

[e]Testing of first-trimester AFP depends on the commercial laboratory used. First-trimester AFP should not be used in lieu of second-trimester AFP for open fetal defects screening.

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information to patients, including pretest education and posttest disclosure of results. Open communication between the provider and the patient is essential to facilitate informed decision-making.

Patients may be more familiar with the term NIPT despite efforts to phase out that nomenclature in favor of the more accurate "prenatal screening with cfDNA." Calling prenatal screening with cfDNA "noninvasive prenatal testing" suggests that cfDNA screening is diagnostic, minimizes other noninvasive screening options (e.g., quad screen), and implies that diagnostic testing is not preferred because of its invasive nature [42].

Pretest counseling should incorporate the potential outcomes of the screening test, which may include a positive result, an inconclusive result, or an unexpected result, any of which may necessitate further testing for the fetus or the patient. Abnormalities in cfDNA that are not consistent with fetal aneuploidy may indicate benign or malignant maternal disease (i.e., an unexpected maternal result) and require further evaluation [43]. Pretest counseling should also address practical aspects of genetic testing, including cost, insurance coverage, and return of results. Individual patient scenarios may require additional counseling and expertise. Patients who have undergone transplant or pregnancy achieved by donor oocytes should undergo genetic counseling to discuss the nuances of cfDNA screening.

Providers can use a range of resources to ensure comprehensive and accurate pretest counseling. These may include decision aids that use narrative or interactive elements to facilitate a decision-making process aligned with the patient's values and preferences. Other valuable tools to assist in pretest counseling include brochures, accessible online materials, multimedia presentations, and educational resources in multiple languages. Incorporating such resources can enhance and support the counseling process, ensuring that patients receive the information they need to make informed decisions about testing. Patients can proceed with, decline entirely, or decline certain aspects of testing once these issues are addressed.

Pretest counseling for cfDNA screening should include the following components (see Box 1 for an example):

- Explain that cfDNA is a screening test, results are not considered diagnostic, and pregnancy decision-making solely on the basis of cfDNA results is not recommended.
- Provide a clear explanation of cfDNA screening, including conditions evaluated, limitations, and potential for uninformative or unanticipated results. Emphasize that cfDNA does not screen for all possible genetic conditions.
- Explain alternative testing options (both screening and diagnostic tests) based on personalized risk assessment, cost, and individual desire for information.
- Clarify that every patient has the option to pursue or decline diagnostic or screening tests.
- Discuss the test performance of cfDNA in a singleton or twin pregnancy (PPV/negative predictive value and sensitivity/specificity). Reinforce that confirmatory diagnostic testing is recommended if a positive result is obtained.
- Highlight the possibility of false-positive and false-negative results with cfDNA screening and explain the implications of each.
- Explain the possibility of receiving unanticipated results due to maternal chromosomal variations or maternal health conditions not known before the test. Discuss the need for follow-up testing in such cases.
- Offer support and resources for individuals and couples who request additional counseling or guidance.
- Document the informed decision-making process and the plan for returning results.

2.6 | How should a positive cfDNA test be interpreted?

A positive cfDNA test result indicates an increased likelihood of a fetus having a specific genetic diagnosis. Interpreting a positive cfDNA test requires careful consideration of multiple factors and a thorough laboratory report review. The PPV is a helpful calculation to assess the probability that a positive cfDNA screen result accurately indicates the presence of a specific condition or disease in a fetus. Calculating a PPV is based on the prevalence of the condition in the population being tested and the test's sensitivity and specificity. As the prevalence of autosomal trisomies (trisomies 21, 18, and 13) increases with maternal age, so does the PPV of a positive test result [44, 45]. For example, the prevalence of trisomy 21 is much lower in a 20-year-old patient than a 40-year-old patient, so the PPV of a positive or "high-risk" cfDNA screening result is much lower in a 20-year-old than a 40-year-old individual (Table 2) [19]. In contrast, the prevalence of 45,X does not significantly differ by maternal age. Unlike common autosomal aneuploidies, the PPV of cfDNA screening for 45,X remains constant irrespective of maternal age [46, 47]. Several online tools are available to assist providers during posttest counseling to calculate the PPV based on maternal age prevalence for high-risk cfDNA screening test results [46, 47]. Laboratories often report risk estimates, including PPV, in the test report. As some laboratories use their own internal data or proprietary metrics in these calculations, clinicians may consider using independent PPV resources to verify the laboratory report. The presence of other risk

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BOX 1: Example of pretest counseling before ordering cell-free DNA screening

Cell-free DNA (cfDNA) screening is a test offered to all pregnant people to take a closer look at the baby's genetics before birth. This test helps us check for certain conditions, like Down syndrome (trisomy 21), by looking at genetic information in your blood from the pregnancy. It is important to know that this test only includes a limited number of genetic conditions. You can decide if you want this test or not based on your own risk and how much information you want. cfDNA screening doesn't give a "yes" or "no" result. It tells us whether the chance of having a baby with these genetic conditions is higher or lower. When the cfDNA result says there is a higher chance for a genetic condition, we recommend meeting with specialists who can talk with you more about the result and offer a confirmatory test that can give a "yes" or "no" answer. cfDNA screening can sometimes give us uncertain information or suggest a health concern for you. It might say there is a problem when there isn't one or miss a problem that's actually there. Occasionally, the test is unable to provide a result. In these situations, your doctor will discuss the information and make a plan for additional testing if you desire.

Some patients choose to have testing of the sex chromosomes. We don't do this test just to predict the sex of the baby, but to see if there is a higher chance that the baby has an abnormal number of sex chromosomes. The usual sex chromosome combinations are XX for biologically female sex and XY for biologically male sex. This screening checks for variations in these chromosomes, because a child can be born with extra or too few sex chromosomes. Children with sex chromosome variations generally don't have major birth defects. These conditions are most often associated with infertility, developmental disabilities, learning disabilities, or social-emotional difficulties, for which treatment in early childhood may be beneficial. When we screen for variations of the sex chromosomes, there is a higher chance of getting a false-positive result. This may be caused by a variation of sex chromosomes in the pregnant person that was not known before the test. Before taking this test, it is important to consider how you might feel should you get information about yourself or your health you didn't expect. For this reason, we ask you to choose if you want information about the sex chromosomes from this test.

You have the right to decide if you want any genetic testing or not, based on what information you want about your pregnancy. You should also understand the cost of the test before you take it. If you ever feel confused or want to talk more about it, many specialists can help to answer questions and offer support.

TABLE 2 Cell-free DNA test performance characteristics [1, 48, 49].

Condition	Sensitivity (%)	Specificity (%)	PPV (%) Age 20 years	PPV (%) Age 25 years	PPV (%) Age 35 years	PPV (%) Age 40 years
Trisomy 21[a]	99.2	99.91	48	51	79	93
Trisomy 18[a]	96.3	99.87	14	15	39	69
Trisomy 13[a]	91	99.87	6	7	21	50
45,X[b]	90.3	99.77	32	32	32	32
47,XXX[a]	93.1	99.86	27	27	28	45
47,XXY[a]	93	99.86	29	29	30	52
47,XYY[c]	93	99.86	71	71	71	71

Abbreviation: PPV, positive predictive value.

[a]Calculated based on National Society of Genetic Counselors and Genetic Support Foundation noninvasive prenatal testing/cfDNA calculator [47].

[b]95% confidence interval (CI), 27.0%–37.3% [50].

[c]95% CI, 63.9%–77.1% [50].

factors for aneuploidy, such as fetal structural abnormalities or ultrasound "soft" markers, also influences the PPV and should be taken into consideration when counseling a patient. There is no available resource to individualize PPV based on various combinations of structural abnormalities, ultrasound soft markers, and cfDNA results. A positive cfDNA result should be followed by genetic counseling, a detailed anatomic survey, and a recommendation for diagnostic testing with chorionic villus sampling (CVS) or amniocentesis.

2.7 | What is the etiology of false-positive cfDNA screening results?

The marketing of cfDNA tests and the language used to report results can cause confusion [2]. Lack of consistency in reporting language can make interpreting the test results challenging for patients and providers. For example, a report for a screen-positive test result for trisomy 21 may read "positive," "high risk," "aneuploidy detected," or "aneuploidy suspected," depending on the laboratory. The

language used in reports may also incorrectly imply that screening is diagnostic [2, 3, 51]. In a 2014 study describing outcomes of commercial cfDNA testing in more than 30,000 women, approximately 6% of those with a positive cfDNA test result had an abortion without confirmatory diagnostic testing [52]. Approximately 20% of patients underwent abortion for suspected autosomal trisomy without karyotype confirmation in a 2016 retrospective cohort study by Dobson et al. [4]. Although neither study provides insight into the decision for abortion or any potential relationship to abnormal screening, the US Food and Drug Administration issued a warning to the public on April 19, 2022, regarding the risk of false-positive results, inappropriate use of screening, and inappropriate interpretation of results [53]. Obstetrical providers must understand and appropriately interpret the results of cfDNA screening and accurately convey this information to their patients as part of posttest counseling.

Although false-positive results can occur with cfDNA, they are less common when compared with traditional serum or ultrasound aneuploidy screening methods. In some cases, false positives stem from biological factors or laboratory methods. Potential causes of false-positive results include (1) mosaicism in the fetus, placenta, or patient; (2) maternal CNVs; (3) demised twin; (4) maternal malignancy; (5) previous transplant; (6) fibroids; (7) recent blood transfusion; (8) statistical chance; and (9) technical errors [54–57]. By directly testing the fetal chromosomal complement, a diagnostic test (CVS or amniocentesis) can determine whether a cfDNA test result is indicative of a true fetal abnormality [19].

2.7.1 | Confined placental mosaicism

CPM is defined as genetic variation detected in chorionic villi but not in fetal tissue. There are three types of CPM (as shown in Figure 1): type 1 (abnormality only in cytotrophoblasts), type 2 (abnormality only in the mesenchyme), and type 3 (abnormality in both cytotrophoblasts and mesenchyme). Because cfDNA is derived from placental trophoblasts, CPM identified by a high-risk cfDNA result is most likely to be type 1 or type 3. Overall, chromosomal mosaicism occurs in approximately 1% to 2% of placental tissues from CVS specimens and varies by chromosome of interest [58]. For example, the incidence of CPM associated with trisomy 21 is 2%, but for 45,X it is as high as 59% [59–62]. Available data indicate that true fetal mosaicism occurs in approximately 10% to 15% of cases of CPM and varies depending on the chromosome of origin [63–66]. Type 1 CPM is least likely to be associated with true fetal mosaicism [67]. Providers should recognize the two types of CVS preparations that evaluate different tissue sources when considering CPM and CVS results: direct preparation evaluates cytotrophoblasts, while cultured preparations evaluate the mesenchymal cells. For some common aneuploidy conditions, including trisomy 13 and monosomy X, the high rate of CPM is such that CVS may not be the preferred test for fetal diagnosis (Table 3). Ultrasonography may help guide the preferred modality of diagnostic testing based on the presence or absence of ultrasound findings that would increase the likelihood of an affected fetus. If there are no abnormalities identified by ultrasonography in the first trimester, CVS can be offered with the caveat that there is a high rate of CPM that may require amniocentesis for confirmation. If the CVS cultured preparation reveals monosomy X (45,X), trisomy 13 (without mosaicism), or a normal fetal karyotype, the result can be considered diagnostic, and follow-up amniocentesis is not recommended. Counseling should be individualized based on the aneuploidy suspected by cfDNA and the risk for CPM in the context of the patient's desire for information and reproductive autonomy [19, 62]. (For more information, see Section 2.15.) Further evaluation of suspected CPM when the fetus is euploid is beyond the scope of this document.

2.7.2 | Maternal malignancy

Nonreportable and false-positive cfDNA screening results can be caused by maternal malignancy, which occurs in approximately one in 1000–1500 pregnancies [68, 69]. The presence of multiple aneuploidies, a single autosomal monosomy, or copy number gains and losses across multiple (three or more) chromosomes raises the likelihood of an underlying maternal malignancy [70–72]. This unanticipated finding is identified in approximately 0.03% to 0.12% of patients undergoing cfDNA screening for fetal aneuploidy [70, 71]. Generally, full fetal autosomal monosomies or multiple autosomal trisomies are not compatible with a viable pregnancy or live birth [71, 73]. When these patterns are identified on cfDNA testing, there is a 5% to 50% risk of an underlying maternal malignancy [70, 72]. A 2024 National Institutes of Health study examined the potential of cfDNA screening to detect occult cancer in pregnant or postpartum individuals with unusual or nonreportable prenatal screening results from 12 different commercial laboratories. Among 107 participants with unusual or nonreportable results, 48.6% were diagnosed with cancer [72].

Offering fetal diagnostic testing is the first step in investigating any abnormal or inconclusive cfDNA screening result. If the fetal testing aligns with the cfDNA screening, it likely explains the results and rules out concerns of maternal malignancy. When there is a discrepancy between the cfDNA screening and fetal diagnostic testing

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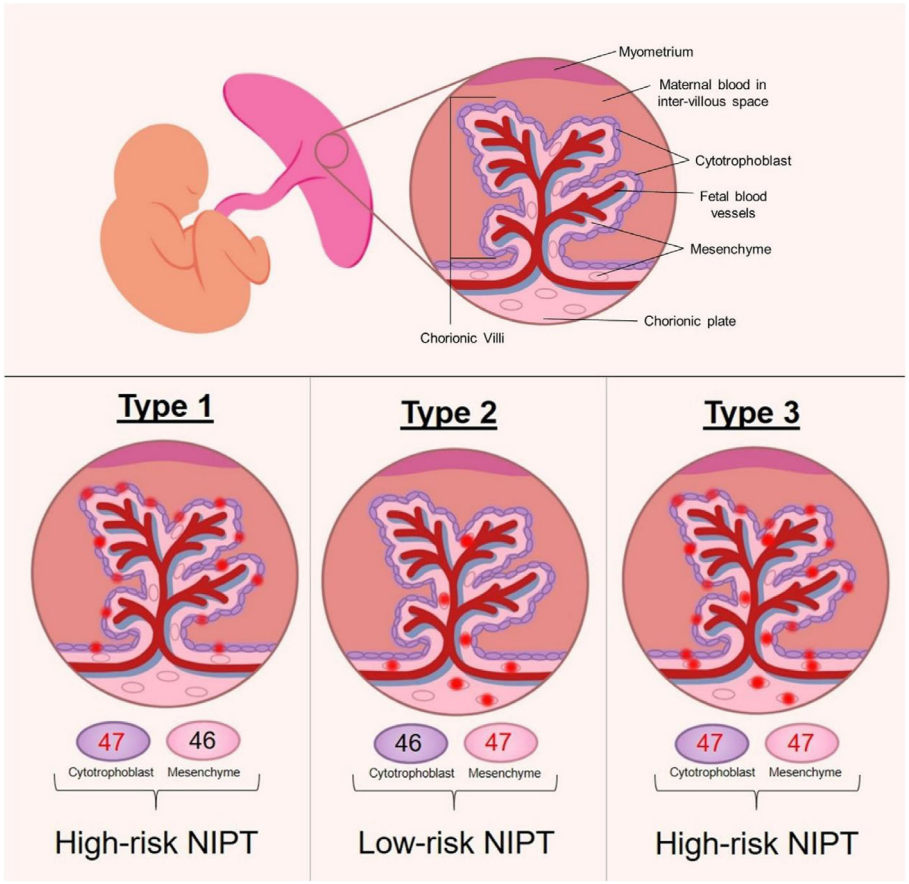


FIGURE 1 Confined placental mosaicism types involving trisomy and expected screening result [55]. Noninvasive prenatal testing (NIPT) analyzes DNA arising only from the cytotrophoblast. Type 1 confined placental mosaicism (CPM) describes aneuploidy exclusively in the cytotrophoblast. Type 2 CPM involves aneuploidy exclusively in the mesenchymal layer. Type 3 involves aneuploidy in both the cytotrophoblast and mesenchyme. Note that the term "prenatal cell-free DNA (cfDNA) screening" is preferable to "noninvasive prenatal testing," or NIPT. Reprinted with permission from [55].

results or the patient declines diagnostic testing, the counseling and management approach depends on the patient's desire for information, family history, and personal medical history. The cost of additional evaluation should also be considered. Currently, there is no consensus guideline for counseling and evaluating patients with suspected malignancy based on cfDNA testing, and therefore, recommendations are based on expert opinion [74–76]. Providers should counsel patients about the potential for malignancy and discuss options for additional evaluation, which may include:

- Detailed medical and family history
- Detailed physical examination, including breast, head and neck, pelvic, and rectal examination, as well as cervical cancer screening
- Laboratory tests: complete blood count with differential, complete metabolic profile, urine analysis, and fecal occult blood testing
- Chest radiography and breast imaging
- Upper and lower gastrointestinal tract endoscopy
- Targeted or whole-body magnetic resonance imaging
- Referral to a medical oncologist
- Exploration of ongoing clinical research available to patients with potential for malignancy as a result of cfDNA screening.

2.8 | What is the etiology of nonreportable cfDNA screening results?

Nonreportable cfDNA test results (sometimes described as "no call," "no result," "low fetal fraction," or "test failure") have been reported in 0.03% to 11.1% of studies [18]. Despite technological advancements leading to fewer nonreportable rates in recent years, variations persist based on the laboratory platform and other laboratory-specific approaches, including bioinformatic algorithms. A recent ACMG systematic evidence review reported an estimated nonreportable cfDNA test rate of 0.85% in 31 studies [17]. Test failures can result from technical causes, including sample handling or processing, difficulties interpreting

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TABLE 3 Suggested management of cell-free DNA results in pregnancy [62].

Chromosome	Category	Suggested invasive procedure	Other considerations
Trisomy 1, 2, 3, 4, 5, 7, 8, 9, 10, 12, 16, 17, 19, 22	Rare autosomal trisomy	Normal first-trimester ultrasound: amniocentesis Abnormal first-trimester ultrasound: CVS	Third-trimester growth scan (especially in trisomy 16)
Trisomy 6, 7, 11, 14, 15, 20	Rare autosomal trisomy, imprinted genes	CVS, followed by amniocentesis if abnormal	If unaffected fetus with normal karyotype, consider methylation studies or testing for uniparental disomy
Trisomy 13	Common aneuploidy, high rate of CPM	Normal first-trimester ultrasound: amniocentesis[a] Abnormal first-trimester ultrasound: CVS	Amniocentesis if mosaicism on CVS
Trisomy 18, 21	Common aneuploidy, low rate of CPM	CVS	Amniocentesis if mosaicism on CVS
Monosomy X	Common aneuploidy, high rate of CPM	Normal first-trimester ultrasound: amniocentesis[a] Abnormal first-trimester ultrasound: CVS	If unaffected fetus, consider maternal karyotype for mosaic Turner syndrome
Multiple aneuploidies	Risk of maternal malignancy	Normal first-trimester ultrasound: amniocentesis Abnormal first-trimester ultrasound: CVS	If unaffected fetus, consider work up for maternal malignancy

Abbreviations: CPM, confined placental mosaicism; CVS, chorionic villus sampling.

[a]If there are no abnormalities identified by ultrasonography in the first trimester, CVS can be offered with the caveat that there is a high rate of CPM that may require amniocentesis. If the CVS cultured preparation reveals monosomy X (45,X), trisomy 13 (without mosaicism), or a normal fetal karyotype, the result can be considered diagnostic, and follow-up amniocentesis is not recommended.

Source: Adapted with permission from [62].

sequencing data, inconclusive data, or low fetal fraction. The potential etiologies associated with a nonreportable cfDNA result are summarized in Box 2. Some studies have demonstrated an association between nonreportable cfDNA results and adverse obstetrical outcomes, including preeclampsia and preterm birth [77, 78]. Alternative strategies of repeating cfDNA screening with a different commercial laboratory or serum screening after a nonreportable result have not been investigated. Additional large studies are warranted to identify best practices to inform management.

Various factors influence the fetal fraction, including gestational age, maternal body mass index, and technical aspects of the cfDNA testing method [54, 80–82]. Generally, the fetal fraction increases as pregnancy progresses, and it is typically higher in the second and third trimesters than in the first trimester. An insufficient fetal fraction is the most common reason for a nonreportable cfDNA test. Although the minimum fetal fraction threshold required to generate a cfDNA result varies by assay, most laboratories require a minimum fetal fraction of 2% to 4%.

Low fetal fraction is associated with less accurate test results and is more frequently observed in pregnancies affected with trisomy 13, trisomy 18, and digynic (maternal origin) triploidy [21, 22, 39, 77, 83, 84]. Low fetal fraction has also been associated with several maternal conditions and agents that can cause decreased trophoblast apoptosis, inhibit placental growth, or increase maternal cell degradation, including autoimmune disorders, conception via assisted reproductive technology, and the use of low-molecular-weight heparin [85–87]. Obesity increases the maternally derived cfDNA fraction relative to the fetal contribution due to increased necrosis and apoptosis of

BOX 2: Etiology of nonreportable cell-free DNA test [78, 79]

- Obesity
- Early gestational age
- Aneuploidy
- Low fetal fraction
- Maternal low-molecular-weight heparin use
- Sample/laboratory error
- Maternal medical conditions: systemic lupus erythematosus, antiphospholipid antibody syndrome, malignancy
- Assisted reproductive technologies
- Donor egg
- Confined placental mosaicism
- Twin gestation (including vanishing twin)

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adipose tissue. As such, increasing maternal weight is negatively associated with fetal fraction [88–90]. Despite this recognized association, offering cfDNA regardless of maternal weight with a specific discussion of increased risk for test failure during pretest counseling is recommended. Over 80% of women weighing more than 400 lb will receive a result when screened between 9 and 12 weeks of gestation [82].

2.9 | How should a nonreportable cfDNA screening result be managed?

Patients with nonreportable cfDNA results should be offered genetic counseling, comprehensive ultrasound evaluation, and diagnostic testing because of the increased risk for fetal aneuploidy. A population-based retrospective cohort study in 2023 evaluated singleton pregnancies and reported a relative risk of 130.3 (95% confidence interval [CI], 64.7–262.6) for trisomy 21, trisomy 18, or trisomy 13 in pregnancies with a nonreportable cfDNA screen on the first attempt [91]. Genetic counseling and offering diagnostic testing after a nonreportable cfDNA screening test result are critical to informed decision-making and reproductive autonomy. Whether the patient chooses to forgo diagnostic testing and instead reattempts cfDNA screening may depend on several factors, including the presence of abnormal ultrasound findings and gestational age. A repeat cfDNA screen will be successful in 75% to 80% of cases [77, 81, 82, 92]. Importantly, a patient may not wish to delay obtaining definitive information, given the increased risk for aneuploidy associated with a nonreportable result. If a patient elects to have repeat cfDNA screening between 10 weeks and 0/7 days and 13 weeks and 6/7 days, storing a serum sample at the time of the second cfDNA blood draw can be considered to ensure that an opportunity for first-trimester or combined screening will not be missed should the second attempt fail. Future studies are needed to determine optimal management in the setting of an initial nonreportable result. There are no data on the performance of conventional screening, antenatal testing regimen, or repeat cfDNA testing by a laboratory with a different testing methodology in patients with an initial failed cfDNA screening test because of a low fetal fraction [93].

2.10 | What are SCAs?

SCAs involving the X and Y chromosomes are among the most common aneuploidies compatible with live birth. The most common aberrations of sex chromosomes are 47,XXX (triple X syndrome, live birth prevalence: 1/700), 45,X (Turner syndrome: 1/2000), 47,XXY (Klinefelter syndrome: 1/660), and 47,XYY (Jacob syndrome: 1/1000) [94–96]. Collectively, the prevalence of SCA (1/350–500) is greater than the prevalence of common aneuploidies for many obstetrical patients [97–99]. Approximately half of 47,XXY cases and the majority of 47,XXX cases result from maternal nondisjunction associated with advanced maternal age [100–102]. Most individuals with nonmosaic 45,X have loss or abnormality of the second X chromosome due to paternal meiotic errors [103, 104].

A 45,X karyotype occurs in 1% to 2% of all pregnancies, with more than 99% of cases resulting in spontaneous fetal loss [105]. Many prenatally identified pregnancies with 45,X are diagnosed by amniocentesis or CVS performed for other indications [106, 107]. Ultrasound findings associated with 45,X include increased nuchal translucency or cystic hygroma in the first trimester [108]. Structural anomalies observed in the second trimester include renal abnormalities or congenital heart disease (e.g., hypoplastic left heart, abnormalities of the aortic valve, coarctation of the aorta) [109]. More than half of all patients with 45,X have a mosaic cell line (e.g., 45,X/46,XX) as a result of a mitotic error. Most mosaic fetuses with a 45,X cell line and a second cell line of a structurally normal sex chromosome are born phenotypically normal [105]. The highly variable phenotype of individuals with SCA and the potential for placental mosaicism or an unexpected maternal diagnosis complicate prenatal counseling and obstetrical decision-making. The clinical characteristics of SCAs are often not detectable during a prenatal ultrasound examination [110]. Further, most individuals with SCA, particularly when mosaic, have no outward phenotype after birth [108, 110, 111]. Although a significant ascertainment bias exists relative to the clinical characteristics of SCAs [108], commonly reported postnatal features include growth differences and infertility with 45,X and 47,XXY karyotypes [94, 95]. Individuals with SCA typically have normal intelligence with increased risk for learning issues (e.g., reduced visuospatial organization, math abilities) or executive function diagnoses (e.g., attention deficit disorders) [112].

2.11 | How accurate is cfDNA screening for SCA?

Currently, cfDNA is the only available method to screen for SCA prenatally. The unanticipated expansion of cfDNA to routinely include SCA has resulted in a dramatic increase in the prenatal detection of SCA [106, 113]. Approximately 50% of confirmed cases of SCA are suspected because of a positive cfDNA screening test [106]. For most patients, a positive result for SCA is an unforeseen finding [106]. Clinical performance data of cfDNA screening for SCA

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have become increasingly available for both the general population and people at high risk for aneuploidy. A 2023 systematic review and meta-analysis evaluated 94 studies that included 1,531,240 women screened with cfDNA, all with confirmatory diagnostic testing [50]. Out of 94 studies on cfDNA screening, 41 predominantly focused on high-risk populations and the rest on the general obstetrical population. Amniocentesis was identified as the ideal standard for diagnostic testing and used by 71% of providers. The pooled sensitivity and specificity for all cfDNA-identified SCAs were 94.1% (95% CI, 90.8%–96.3%) and 99.5% (95% CI, 99%–99.7%), respectively. The PPV was not significantly different between the high-risk population and the general risk group (52% vs. 48%, $p = 0.232$). Prior studies found that the pooled PPV varied significantly by specific SCA diagnosis. For 45,X, the PPV was 32% (95% CI, 27%–37.3%); for 47,XXX, the PPV was 57.5% (95% CI, 51.7%–63.1%); for 47,XXY, the PPV was 67.6% (95% CI, 62.5%–72.5%); and for 47,XYY, the PPV was 70.9% (95% CI, 63.9%–77.1%) [50]. Shear et al. evaluated the performance of cfDNA screening for SCA in a systematic review and meta-analysis, including 28 studies [114]. The calculated PPV was 14.5% (95% CI, 7%–43.8%) for 45,X and 97.7% (95% CI, 78.6%–100%) for 47,XXY.

Screening with cfDNA has a higher sensitivity and specificity for disorders involving the Y chromosome than those involving the X chromosome only. As part of the evidence-based clinical guideline by ACMG for noninvasive prenatal screening for fetal chromosome abnormalities in a general-risk population, a systematic evidence review identified a notable performance difference for SCA screening compared with test performance for trisomy 21. In that review, 45,X had the lowest PPV (29.5%; 95% CI, 22.7%–37.4%) of the various SCA conditions in this analysis [17]. The unique biological characteristics of these disorders contribute to higher rates of false-positive and false-negative results. Despite these limitations, no alternative prenatal screening method currently exists for SCAs, underscoring the importance of patient education before testing and shared decision-making in the screening process.

2.12 | What are the benefits of prenatal diagnosis of SCA?

Prenatal diagnosis of SCA provides prospective parents with valuable information about the genetic condition and its implications, allowing them to make informed decisions about the pregnancy. Diagnosis may also enable parents and healthcare providers to anticipate and prepare early intervention or management strategies. For example, individuals prenatally diagnosed with 45,X karyotypes, including those with a mosaic karyotype, should follow a lifelong schedule of screening and treatment, including early echocardiography [111]. Early diagnosis is also beneficial to access early developmental evaluation and indicated therapies. Both early intervention services (e.g., physical therapy, audiological evaluation, occupational therapy, developmental assessments) and early hormonal therapy have been shown to improve outcomes, particularly for individuals with 45,X syndrome [115] or 47,XXY syndrome [82–84, 111, 116, 117]. The benefits of early hormonal therapies on quality of life and improved neurodevelopmental outcomes, in addition to pubertal development and fertility, are important components of ongoing research [117].

2.13 | What are the benefits and limitations of cfDNA screening for SCA?

The use of cfDNA has become widespread because of multiple advantages. It provides a noninvasive method of aneuploidy detection, avoiding the risk of miscarriage from invasive testing methods. The screening test has high sensitivity and specificity. The accuracy of cfDNA screening for SCA is lower than for the common trisomies, particularly trisomies 21 and 18 (see Table 2) [1, 48, 49]. The higher false-positive rate for SCA is associated with unique biological mechanisms that result in this group of disorders. These factors include maternal mosaicism for SCA, structural abnormalities of the X chromosome, fetal mosaicism, and placental mosaicism. A small study by Wang et al. reported that 8.6% of positive cfDNA results for SCA resulted from maternal sex chromosome mosaicism [118]. This may be a true germline SCA mosaic condition or a somatic change with variable clinical significance. The role of increasing age-related loss of a single X chromosome on cfDNA screening is unclear. This established phenomenon is reported to occur at frequencies ranging from 0.07% in those younger than 16 years of age to more than 7.3% at 65 years of age [119]. The low frequency is likely below the ability of cfDNA technologies to detect routinely. Maternal solid organ or bone marrow transplant from a male donor or donor with SCA can also complicate cfDNA screening results for fetal sex determination and SCA [120]. Other factors that confound all aneuploidy screening, including SCA, include low fetal fraction or the presence of a vanishing twin [121, 122].

2.14 | What are the important components of pretest counseling for SCA screening with cfDNA?

Pretest counseling is essential for patients undergoing SCA screening, as a positive result for SCA presents unique

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clinical challenges compared with screening for common autosomal trisomy. Challenges include the high rates of CPM (up to 50% for monosomy X) [59, 60] and false-positive results, lack of prenatal ultrasound findings, variable postnatal phenotype, and unexpected maternal SCA diagnosis with subsequent medical and psychosocial consequences.

Available research highlights the unpreparedness that patients experience when receiving a positive cfDNA result for SCA, attributed to a lack of sufficient pretest counseling and education, the variability of SCA diagnoses, and the complexity of the information presentation [123]. Many patients experience cfDNA screening as a "routine" blood test, do not undergo any pretest counseling, and are not informed that SCA may be included in the screening. In a survey of 323 parents, of whom 122 received a diagnosis of SCA, many respondents described feeling angry or unprepared for a positive result. They could not recall whether any information was given about SCA screening [124]. Many national health systems and organizations recognize the inherent technical, economic, cultural, and ethical factors uniquely associated with SCA and recommend population-based SCA screening as an optional test with pretest counseling to disclose the variations in test performance and the potential for unanticipated maternal findings [125–133]. **We recommend that screening for SCAs be made available to obstetrical patients as an "opt-in" consideration with appropriate pretest counseling (GRADE 1C)** [25]. Opting in for SCA screening implies the patient provides informed consent for testing after pretest counseling.

Pretest counseling for SCA screening should include the following information:

- A brief overview of SCA disorders, highlighting their variable and typically milder features when compared with autosomal trisomies.
- Prenatal detection of SCAs may result in improved outcomes, especially for individuals with 45,X and 47,XXY, as they may otherwise go undiagnosed for years because of lack of symptoms.
- A positive cfDNA result for SCA may result in the unexpected diagnosis of a maternal SCA.
- Screening for SCAs is associated with higher false-positive rates and an increased incidence of mosaicism, which may be fetal, placental, or maternal in origin.
- cfDNA screening for SCAs may result in disclosure of the fetal sex.
- Following pretest counseling, patients should decide whether to undergo cfDNA screening for SCAs.

2.15 | What is the appropriate evaluation to conduct after a positive cfDNA result for SCA?

Positive cfDNA results should be presented clearly and objectively, followed by an explanation of the diagnosis of concern and phenotype. The PPV of the test, which may be found in the laboratory report or by using online resources, should be communicated to the patient [46, 47]. The patient should be referred for posttest counseling to a provider with genetics expertise (e.g., a genetic counselor, geneticist, or maternal-fetal medicine subspecialist) to discuss the test result and further explore the specific condition. Next steps include fetal diagnostic testing and consideration for maternal karyotype assessment in patients with 45,X or 47,XXX cfDNA results. Posttest counseling should also include a discussion of plausible alternative contributions to a false-positive result associated with cfDNA for SCA.

Choosing a diagnostic test to further evaluate a positive cfDNA result for SCA requires consideration of various factors (see Table 3 and Section 2.7.1). Confirmatory testing for an abnormal cfDNA screening result involving chromosomes highly associated with CPM, such as the X and Y chromosomes, either by CVS or amniocentesis, is a matter of ongoing debate. The fetal component of circulating cfDNA originates primarily from cytotrophoblasts, raising the question of whether the direct preparation from CVS may evaluate the same DNA source and leading some content experts to advocate for amniocentesis [59, 60]. Grati et al. analyzed 52,673 CVS samples, of which 1073 had at least one abnormal cell line [59]. The proportion of abnormal CVS samples with a mosaic cell line for 45,X was 59%. In these patients, a follow-up amniocentesis confirmed either mosaicism or aneuploidy in only 26% of cases. The high frequency of placental mosaicism for 45,X warrants consideration of prenatal diagnostic testing by amniocentesis rather than CVS, particularly in the absence of sonographic findings [62]. False-positive results are less likely in cases with ultrasound features of 45,X in the first trimester (enlarged nuchal translucency or cystic hygroma), in which case CVS may be a reasonable consideration [134].

Informed consent for diagnostic testing should include recognition that information will be delayed by waiting for amniocentesis results in the second trimester and balancing that against the potential for inaccurate information about the true fetal karyotype with the use of CVS. If, after informed consent, a patient with a positive result for SCA (especially 45,X) elects CVS, both direct and cultured karyotype analysis should be performed to

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provide the most information about the fetus. Aneuploidy found in the cytotrophoblast layer, detected by cfDNA screening, is also detected by direct preparation of CVS tissue. Aneuploidy found in the mesenchymal core, which can only be detected by cultured CVS, is more likely to reflect the actual fetal karyotype. Amniocentesis is recommended if mosaicism is identified on CVS from either the direct preparation or cultured tissue, given the potential for discordance between the placenta and fetus, as previously described (see Figure 1).

2.16 | What is the role of cfDNA in fetal sex determination?

Data on cfDNA performance for sex determination demonstrate a remarkably accurate detection rate for fetal sex [135, 136]. A meta-analysis reported the test performance for Y chromosome sequences to have a sensitivity of 95.4% (95% CI, 94.7%–96.1%), specificity of 98.6% (95% CI, 98.1%–99.0%), PPV of 98.8%, and negative predictive value of 94.8%. Identification of fetal sex has medical benefits for families at risk for X-linked disorders in male offspring. However, most patients and families desire information on fetal sex for nonmedical reasons. Ongoing research is necessary to evaluate motivation, ethical issues, and outcomes of cfDNA for sex determination. The nonmedical use of cfDNA solely for fetal sex determination is not recommended [25].

2.17 | What is the role of cfDNA in screening for microdeletions?

Pathogenic chromosomal microdeletions or microduplications (CNVs) throughout the genome are independent of maternal age [137]. Although many of these diagnoses are associated with structural congenital anomalies, a significant portion are uniquely characterized by isolated neurodevelopmental disabilities that would not be ascertained by prenatal ultrasonography [138]. Most laboratories have a limited, targeted screening panel using cfDNA for specific microdeletion conditions, including 22q11.2 deletion (DiGeorge) syndrome, 1p36 deletion syndrome, 5p deletion (Cri-du-Chat) syndrome, 4p deletion (Wolf-Hirschhorn syndrome), and 15q deletions associated with Prader-Willi and Angelman syndromes. Laboratories differ in the specific microdeletion conditions available for screening and may require the ordering provider to opt out if microdeletion testing is not desired.

The performance of microdeletion cfDNA screening can vary among different laboratories and testing platforms. Differences in sensitivity, specificity, and accuracy exist, leading to inconsistent results and interpretations. The low prevalence of these conditions impacts the PPV. Furthermore, microdeletion cfDNA screening panels may not include all known molecular mechanisms of a specific condition. For example, Prader-Willi syndrome is caused by a paternal deletion of 15q11.2-q13 in 65% to 75% of affected individuals, which may be identified by cfDNA screening. However, the remaining patients with Prader-Willi syndrome have this diagnosis because of maternal uniparental disomy (20%–30%) or an imprinting defect (1%–3%) that would not be identified by cfDNA microdeletion testing [139]. Screening with cfDNA does not comprehensively assess a patient's risk for all microdeletion syndromes. Most studies on microdeletion screening with cfDNA have not included genetic confirmation of all enrolled participants, limiting the ability to determine test performance characteristics.

Validation data on the screening performance of tests for microdeletion syndromes are limited, primarily because of the scarcity of these conditions. The most common pathogenic microdeletion syndrome is 22q11.2 deletion syndrome, with a reported prevalence of one in 3000 to 6000 live births [140]. A 2021 systematic review of seven studies that included 474,189 pregnancies and 210 cases of microdeletion and microduplication syndromes reported a weighted, pooled, false-positive rate of 0.07% (95% CI, 0.02–0.15) and a PPV of 44.1% (95% CI, 31.49–63.07) [141]. Sensitivity and specificity could not be calculated, as genetic confirmation was not ascertained in most cases.

Dar and colleagues performed a prospective study on an international cohort of 18,289 pregnancies to assess the performance of cfDNA screening for 22q11.2 deletion syndrome using an SNP-based approach (the SNP-based Microdeletion and Aneuploidy Registry [SMART] study) [142]. This study was the first to include comprehensive genetic confirmation for all cfDNA samples. Of the 12 infants affected by 22q11.2 deletion syndrome, 10 were detected by cfDNA, yielding a sensitivity of 83.3% (95% CI, 51.6–97.9) and a PPV of 52.6% (95% CI, 28.9–75.6) using an updated algorithm developed after enrollment was completed. The false-positive rate was 0.05%. Of note, four of the 12 cases had cardiac anomalies associated with 22q11.2 deletion syndrome before cfDNA screening; by including these four cases, the authors calculated a prevalence of 22q11.2 deletion syndrome of one in 1524 but acknowledged that doing so may have enriched the 22q11.2 deletion syndrome population. This resulted in an increased PPV compared with the PPV calculated by the prevalence of one in 2312 if these cases were excluded.

Thus, while cfDNA screening for 22q11.2 deletion syndrome appears promising, the data are limited. The prevalence rates and PPVs reported in the SMART study may

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not reflect an average-risk population, and the 95% CIs are wide because of the small number of affected cases. In addition, it is not clear whether the performance characteristics are generalizable to other cfDNA platforms used to screen for microdeletion syndromes. Despite the incentive to include 22q11.2 deletion screening for the general population with the goal of improving neonatal outcomes, a secondary analysis from the SMART study found that more than 50% of infants at high risk for 22q11.2 deletion syndrome, suspected by fetal cfDNA screening, were discharged from the hospital without genetic testing. These data highlight areas of necessary research and process improvement before implementing population-wide screening.

A study using a targeted cfDNA approach reported a sensitivity of 69.6% (95% CI, 55.2%–80.9%) with a specificity of 100% (95% CI, 99.5%–100%) in a cohort that included 34 patients with 22q11.2 deletion syndrome and fetal cardiac anomalies identified prospectively and 12 patients diagnosed based on banked samples from pregnant patients with affected fetuses [143]. While the reported performance was promising in this specific population, these data do not apply to general population screening for 22q11.2 deletion syndrome. Furthermore, cfDNA screening for 22q11.2 deletion syndrome in a fetus with congenital heart disease does not replace diagnostic testing with a chromosomal microarray, which remains the recommended evaluation approach for a fetus with a structural birth defect [129, 144]. Chromosomal microarray is used to identify submicroscopic deletions and duplications (CNVs) throughout the genome and includes testing for 22q11.2 deletion [144]. Diagnostic testing to include microarray is important in the setting of a fetus with congenital anomalies, as many other CNVs beyond 22q11.2 deletion are associated with congenital heart disease.

Based on the available data, routine population screening for microdeletion conditions is not recommended. Patients who are interested in obtaining information to determine whether their fetus is affected with a pathogenic CNV should be offered CVS or amniocentesis with a chromosomal microarray as opposed to cfDNA screening for microdeletion syndromes [129]. **We do not recommend routine general population screening for any microdeletion condition. Patients who choose to undergo cfDNA screening for 22q11.2 deletion specifically should do so only after appropriate pretest counseling** [24]**. Pregnant people who are interested in obtaining information regarding the risk for fetal CNVs should be offered diagnostic testing as opposed to cfDNA screening for microdeletion syndromes (GRADE 1C)**.

2.18 | What is the accuracy of cfDNA in twin gestations?

Screening with cfDNA is more complex in twin pregnancies than in singleton pregnancies. Aneuploidy rates in twins are relatively low compared with singleton pregnancies, making it difficult to perform an adequately powered prospective trial [145, 146]. Zygosity determines the degree of risk for chromosomal abnormalities and whether the fetuses are concordant or discordant for these risks. Approximately two-thirds of twins are dizygotic, and one-third are monozygotic, with this distribution influenced by the use of fertility treatments, maternal age, and ancestry of the population [122]. Monozygotic twins result from the splitting of a single fertilized ovum and share their genetic material. The timing of the splitting of the cells determines chorionicity. Very early separation at the morula stage at 1 to 3 days results in dichorionic diamniotic placentation. Separation at the hatching stage (4–8 days after fertilization) results in monochorionic diamniotic placentation, and later separation (8–13 days after fertilization) results in monochorionic monoamniotic placentation. Approximately 75% of monozygotic twins are monochorionic, and 25% are dichorionic [122]. Dizygotic twins result from the fertilization of two separate ova by two separate sperm, which results in two genetically distinct fetuses. Dizygotic twins generally have dichorionic placentation. An SNP-based cfDNA approach can be used to assess twin zygosity. Although ultrasonography remains the gold standard for determining chorionicity, cfDNA-based zygosity determination may prove to be of clinical value when ultrasonography is not available or yields uncertain results. The SNP pattern from a monozygotic pregnancy will be identical for the twins and the same as for a singleton pregnancy; for a dizygotic pregnancy, the nonmaternal SNP alleles are derived from one or both dizygotic fetuses. A validation study involving 95 samples reported 100% sensitivity (95% CI, 96.1%–100%) in the determination of zygosity with a nonreportable result rate of 2.1% [147, 148]. In a retrospective cohort study of cfDNA screening tests from 59,471 twin gestations using an SNP-based approach, zygosity assignment was not possible in 6.9% of cases.

The reporting of cfDNA screening results and fetal fraction in twin pregnancies varies among different analysis techniques. Factors known to be associated with a low fetal fraction and cfDNA test failure in singleton pregnancies, including conception by in vitro fertilization and higher maternal weight, are more prevalent in twin pregnancies [82, 149, 150]. A fetal-specific assessment for aneuploidy (none, one fetus, or both fetuses) using SNP technology can be provided based on minor allele frequency rather

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than the genome-wide counting method, which provides a global aneuploidy risk assessment for the pregnancy [151]. A combined estimation of the fetal fraction is reported in monozygotic twins, as they are genetically identical, and can be reported separately for each dizygotic twin using SNP analysis. In a dizygotic twin pregnancy, one aneuploid twin may provide a significantly lower fetal fraction amount compared with the euploid co-twin, which may provide a higher-than-expected contribution. The combined fetal fraction from the aneuploid and euploid twins can be adequate for analysis for laboratories that do not use SNP analysis [152, 153]. This approach could, however, lead to a false-negative low-risk result for the pregnancy with one aneuploid twin.

In a monozygotic twin pregnancy, the estimated fetal fraction is greater than the mean reported in a singleton pregnancy. This results in comparable test performance for aneuploidy screening as a singleton with a lower nonreportable result rate from the higher fetal fraction level [151]. Nonreportable result rates are higher in dizygotic than monozygotic twin pregnancies and depend on the cfDNA method used. A prospective study of 928 twin and 23,495 singleton pregnancies undergoing targeted cfDNA screening for fetal trisomy at 10 weeks and 0/7 days to 14 weeks and 1/7 days of gestation reported higher nonreportable result rates in twin pregnancies than singleton pregnancies because of low fetal fraction [150]. The nonreportable result rates were 4.9% in monochorionic twins and 11.3% in dichorionic twins, compared with a 3.4% nonreportable result rate in singletons. While nonreportable result rates in dichorionic twins are anticipated to remain higher compared with monochorionic twins, ongoing research is necessary to determine optimal methodologies for aneuploidy risk assessment in twin pregnancies.

Although the data are limited, cfDNA screening for trisomy 21 in twins is effective and comparable in performance to singleton pregnancies [154]. Screening performance in twins appears to be equivalent among the available cfDNA screening methodologies [155]. The majority of initial studies included small numbers of fetuses with trisomy 21, with cfDNA screening performed largely in the second trimester. An updated cohort study on first-trimester cfDNA screening from the Fetal Medicine Foundation on 1272 twin pregnancies, including 20 pregnancies with trisomy 21, reported a 95% detection rate for trisomy 21 (95% CI, 75.1–100) with a 0.08% false-positive rate (95% CI, 0–0.44) [156]. The authors included data from their study in a systematic review of 13 studies. However, there were notable differences among the studies with respect to sample size, nonreportable result rates, and gestational age at the time of cfDNA screening, which ranged from 8 weeks and 0/7 days to 36 weeks and 6/7 days of gestation. Detection rates were not provided based on chorionicity. The pooled weighted detection rate for trisomy 21 was 99% (95% CI, 92–99.9), and the false-positive rate was 0.02% (95% CI, 0.001–0.43) in the combined total of 137 cases of trisomy 21 and 7507 non-trisomy-21 twin pregnancies. The pooled weighted detection rate and false-positive rate were 92.8% (95% CI, 77.6–98.0) and 0.01% (95% CI, 0–0.44), respectively, in the combined total of 50 cases of trisomy 18 and 6840 non-trisomy-18 pregnancies. The pooled weighted detection rate for trisomy 13 was 94.7% (95% CI, 9.14–99.97), and the false-positive rate was 0.10% (95%, CI, 0.03–0.39) in the combined total of 11 cases of trisomy 13 and 6290 non-trisomy-13 cases. The small number of total cases is reflected by the wide CIs. A recent multicenter retrospective cohort study of 1764 samples sent for twin cfDNA screening at a median gestational age of 12 weeks and 3/7 days from 17 US centers included 42 pregnancies with trisomy 21 [157]. The overall detection rate for trisomy 21 was 97.6% (95% CI, 83.8–99.7). Of the 42 cases of trisomy 21, 38 were from dichorionic pregnancies, including the one false-negative case, resulting in a 97.4% sensitivity (95% CI, 82.6–99.7) for the dichorionic cases and a 100% sensitivity (95% CI, 43.9–100) for the three monochorionic cases. There were no false-positive cases. Although the performance characteristics for screening for trisomy 18 and 13 were favorable, the numbers of cases of trisomy 18 (9/10 cases dichorionic) and trisomy 13 (4/4 cases dichorionic) were too small to make definitive conclusions. **We recommend cfDNA as a first-line screening option for trisomy 21 detection in twin gestations (GRADE 1B). Although the numbers of affected pregnancies are limited, the detection rates associated with trisomy 18 and 13 appear to be consistently high in twin gestations, and cfDNA screening for these conditions is recommended (GRADE 1B). Because of a lack of data, cfDNA screening for SCA in twin gestations and cfDNA screening for higher-order multiples are not recommended (GRADE 1C) [**17, 158].

2.19 | What is genome-wide cfDNA screening?

Genome-wide cfDNA screening detects subchromosomal imbalances of 7 or more megabase (Mb) resolution for all chromosomes. The resolution of genome-wide cfDNA screening is similar to the 5–10-Mb resolution of a standard karyotype, enabling the detection of RATs in addition to the three common autosomal trisomies. The PPVs for RATs are significantly lower than the PPVs for the common trisomies because of the low prevalence and high incidence of CPM. The clinical utility of screening for these rarer conditions has not been established [17]. Of note, genome-wide cfDNA screening will not detect

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the majority of microdeletion and microduplication syndromes, which are typically less than 5 Mb. Genome-wide cfDNA screening may be useful in cases in which a parent has a known balanced translocation. While diagnostic testing is the gold standard to identify a fetus with an unbalanced chromosomal rearrangement, cfDNA testing may be an alternative for patients who wish to avoid diagnostic testing. These patients should meet with a genetic counselor who should review the parental karyotype and confirm that the laboratory is potentially able to identify the specific unbalanced chromosomal rearrangements pertinent to the case. Diagnostic testing should be recommended to confirm abnormalities detected on cfDNA screening. **We do not recommend the routine use of cfDNA testing for large genome-wide copy number deletions or duplications (GRADE 1C)**.

2.20 | What is cfDNA testing for single-gene disorders?

Advances in cfDNA testing for the identification of pathogenic variants in single-gene diseases are a promising technology for the future of prenatal diagnosis. Although not recommended for routine use, laboratories currently offer such testing, particularly to detect dominant disorders with a high de novo rate or for paternally inherited autosomal dominant pathogenic variants. The assay and analysis necessary to identify single-gene disorders from cfDNA are not the same as those used for aneuploidy screening. Additional research and findings from peer-reviewed publications are necessary to establish best clinical practices, including data on clinical utility and validity, as well as an enhanced understanding of the complex counseling and ethical issues that this testing presents.

3 | CONCLUSION

The availability of cfDNA screening for all patient populations as a first-line screening test for common aneuploidy (trisomies 21, 18, and 13) in singleton and twin pregnancies is a significant advancement in reproductive medicine. Pretest counseling and patient education are imperative to its effective implementation. Further research is necessary to evaluate clinical utility, validity, impact on the healthcare system, and psychosocial outcomes before cfDNA is implemented for genetic disorders beyond common aneuploidy.

Summary of recommendations.[a]

Number	Recommendation	GRADE
1	We recommend that cell-free DNA (cfDNA) screening for common aneuploidies (trisomies 21, 18, and 13) be made routinely available to all obstetrical patients.	1B
2	We recommend cfDNA as the most sensitive and specific screening test for common fetal aneuploidies (trisomies 21, 18, and 13) in any patient population. After pretest counseling, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.	1B
3	We recommend that screening for sex chromosome aneuploidies be made available to obstetrical patients as an "opt-in" consideration with appropriate pretest counseling.	1C
4	We do not recommend routine general population screening for any microdeletion condition. Patients who choose to undergo cfDNA screening for 22q11.2 deletion specifically should do so only after appropriate pretest counseling. Pregnant people who are interested in obtaining information regarding the risk for fetal copy number variants should be offered diagnostic testing as opposed to cfDNA screening for microdeletion syndromes.	1C
5	We recommend cell-free DNA as a first-line screening option for trisomy 21 detection in twin gestations.	1B
6	Although the numbers of affected pregnancies are limited, the detection rates associated with trisomy 18 and 13 appear to be consistently high in twin gestations, and cfDNA screening for these conditions is recommended.	1B
7	Because of a lack of data, cfDNA screening for sex chromosome aneuploidy in twin gestations and cfDNA screening for higher-order multiples are not recommended.	1C
8	We do not recommend the routine use of cfDNA testing for large genome-wide copy number deletions or duplications.	1C

[a]See the Supporting Information for evidence summary table.

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Society for Maternal-Fetal Medicine grading of recommendations assessment, development, and evaluation (GRADE) system [159, 160].

Grade of recommendation	Clarity of risk and benefit	Quality of supporting evidence	Implications
1A. Strong recommendation, high-quality evidence	Benefits clearly outweigh risks and burdens, or vice versa.	Consistent evidence from well-performed, randomized controlled trials, or overwhelming evidence of some other form. Further research is unlikely to change confidence in the estimate of benefit and risk.	Strong recommendation that can apply to most patients in most circumstances without reservation. Clinicians should follow a strong recommendation unless a clear and compelling rationale for an alternative approach is present.
1B. Strong recommendation, moderate-quality evidence	Benefits clearly outweigh risks and burdens, or vice versa.	Evidence from randomized controlled trials with important limitations (inconsistent results, methodologic flaws, indirect or imprecise), or very strong evidence of some other research design. Further research (if performed) is likely to have an impact on confidence in the estimate of benefit and risk and may change the estimate.	Strong recommendation that applies to most patients. Clinicians should follow a strong recommendation unless a clear and compelling rationale for an alternative approach is present.
1C. Strong recommendation, low-quality evidence	Benefits appear to outweigh risks and burdens, or vice versa.	Evidence from observational studies, unsystematic clinical experience, or randomized controlled trials with serious flaws. Any estimate of effect is uncertain.	Strong recommendation that applies to most patients. Some of the evidence base supporting the recommendation is, however, of low quality.
2A. Weak recommendation, high-quality evidence	Benefits closely balanced with risks and burdens.	Consistent evidence from well-performed randomized controlled trials or overwhelming evidence of some other form. Further research is unlikely to change confidence in the estimate of benefit and risk.	Weak recommendation; best action may differ depending on circumstances or patients or societal values.
2B. Weak recommendation, moderate-quality evidence	Benefits closely balanced with risks and burdens; some uncertainty in the estimates of benefits, risks, and burdens.	Evidence from randomized controlled trials with important limitations (inconsistent results, methodologic flaws, indirect or imprecise), or very strong evidence of some other research design. Further research (if performed) is likely to influence confidence in the estimate of benefit and risk and may change the estimate.	Weak recommendation; alternative approaches likely to be better for some patients under some circumstances.
2C. Weak recommendation, low-quality evidence	Uncertainty in the estimates of benefits, risks, and burdens; benefits may be closely balanced with risks and burdens.	Evidence from observational studies, unsystematic clinical experience, or randomized controlled trials with serious flaws. Any estimate of effect is uncertain.	Very weak recommendation, other alternatives may be equally reasonable.
Best practice	Recommendation in which either (i) there is an enormous amount of indirect evidence that clearly justifies strong recommendation (direct evidence would be challenging, and inefficient use of time and resources, to bring together and carefully summarize) or (ii) recommendation to the contrary would be unethical.		

Source: Adapted from [160].

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Guidelines referenced.

Organization	Title	Year of publication
American College of Medical Genetics and Genomics	Noninvasive Prenatal Screening for Fetal Aneuploidy, 2016 Update: A Position Statement of the American College of Medical Genetics and Genomics [25]	2016
American College of Medical Genetics and Genomics	Noninvasive Prenatal Screening (NIPS) for Fetal Chromosome Abnormalities in a General-Risk Population [24]	2023
American College of Obstetricians and Gynecologists	Practice Bulletin No. 162 Summary: Prenatal Diagnostic Testing for Genetic Disorders [41]	2016
American College of Obstetricians and Gynecologists	Screening for Fetal Chromosomal Abnormalities: ACOG Practice Bulletin No. 226[19]	2020
International Society for Prenatal Diagnosis	International Society for Prenatal Diagnosis Position Statement: Cell Free (cf)DNA Screening for Down Syndrome in Multiple Pregnancies [155]	2020
International Society for Prenatal Diagnosis	Position Statement from the International Society for Prenatal Diagnosis on the Use of Noninvasive Prenatal Testing for the Detection of Fetal Chromosomal Conditions in Singleton Pregnancies [129]	2023
Society of Obstetricians and Gynaecologists of Canada and the Canadian College of Medical Geneticists	No. 348-Joint SOGC-CCMG Guideline: Update on Prenatal Screening for Fetal Aneuploidy, Fetal Anomalies, and Adverse Pregnancy Outcomes [127]	2017

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