Society for Maternal-Fetal Medicine

Consult Series #74

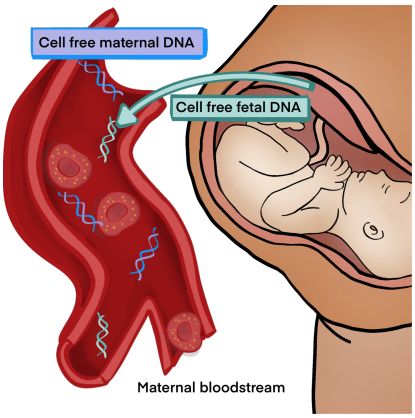
CELL-FREE DNA SCREENING FOR ANEUPLOIDIES

Updated guidance

Cell-free DNA (cfDNA) consists of short fragments of placental DNA found in the maternal circulation during pregnancy

Released into maternal bloodstream upon cell death of trophoblastic cells

Circulates within maternal serum with maternally derived cfDNA



- cfDNA is the most sensitive and specific SCREENING test for common aneuploidies
  - Not equivalent to a diagnostic test
- We recommend that cfDNA screening for common aneuploidies (trisomies 21, 13, and 18) be made routinely available to all patients
- After pretest counseling, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing
- All patients undergoing aneuploidy screening should be offered a 2nd-trimester assessment for open fetal defects (by ultrasonography, with or without serum AFP) and ultrasound screening for other abnormalities

Comparison of screening and diagnostic testing options for fetal aneuploidy

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.

Pretest counseling

- Explain that cfDNA is a screening test
- Provide explanation of cfDNA screening (conditions evaluated, limitations, potential for unanticipated results)
- Explain alternative testing options (screening & diagnostic)
- Clarify the option to pursue or decline tests
- Discuss performance in a singleton or twin pregnancy
- Highlight the possibility of false positive/ false negative results
- Explain possibility of unanticipated results due to maternal chromosomal variations or health conditions
- Offer resources if additional counseling is requested
- Document informed decision-making and plan for returning results

Patients who have undergone transplant or pregnancy achieved by donor oocytes should undergo genetic counseling

Cell-free DNA Test Performance Characteristics

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].

POSITIVE

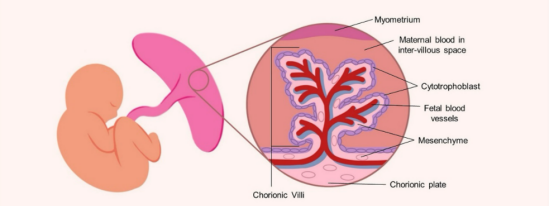
How should a positive test be interpreted?

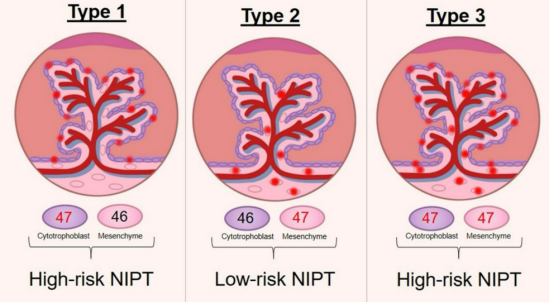
- Indicates an increased likelihood of a fetus having a specific genetic diagnosis
- Consider the PPV
- Recommend genetic counseling, detailed anatomical survey, and diagnostic testing with CVS or amniocentesis

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

- Maternal CNV
- Statistical chance
- Demised twin
- Previous transplant
- Fibroids
- Recent blood transfusion
- **Mosaicism in the fetus, placenta, or patient**
- Technical errors
- Maternal malignancy

Confined placental mosaicism (CPM)





Discuss options for evaluation, including:

- Detailed medical and family history
- Detailed physical exam (breast, head and neck, pelvic, rectal) and cervical cancer screening
- CBC, CMP, UA, fecal occult blood
- Chest X-ray and breast imaging
- Upper and lower GI tract endoscopy
- Targeted or whole-body MRI
- Referral to medical oncologist
- Explore clinical research

Suggested management of cfDNA

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].

NON-REPORTABLE

How should a non-reportable result be managed?

- Offer genetic counseling, comprehensive ultrasound evaluation, and diagnostic testing

  A repeat cfDNA screen will be successful in 75-80% of cases

- Depending on gestational age and ultrasound findings, a patient may choose to repeat cfDNA

What is the etiology of non-reportable cfDNA screening results?

Early gestational age	Low fetal fraction
Aneuploidy	Sample/lab error
CPM	Donor egg

Twin gestation (including vanishing twin)

Maternal conditions: Systemic lupus erythematosus, Antiphospholipid antibody syndrome, malignancy

"Fetal fraction" = The % of total cfDNA in maternal plasma of placental origin

Most common reason for a non-reportable result

Most labs require a minimum of 2-4%

Influenced by gestational age, BMI, use of low molecular weight heparin, autoimmune disorders, assisted reproductive technology

Observed in pregnancies affected with trisomy 13, trisomy 18, and maternal origin triploidy

SEX CHROMOSOME ANEUPLOIDY

What is a sex chromosome aneuploidy (SCA)?

Aneuploidy involving the X and Y chromosomes

Characteristics often not detectable on prenatal ultrasound

Collectively, the prevalence of SCA is greater than the prevalence of common aneuploidies

Individuals with SCA typically have normal intelligence with risk for learning issues or executive function diagnoses

We recommend that screening for SCAs be made available to obstetricians patients as an "opt-in" consideration with appropriate pretest counseling

Pretest counseling

Brief overview of SCA disorders

Prenatal detection of SCAs may result in improved outcomes

A positive result may lead to unexpected diagnosis of maternal SCA

Higher false positive rates and increased incidence of mosaicism

May result in the disclosure of fetal sex

SEX

The non-medical use of cfDNA solely for fetal sex determination is not recommended

Management of positive result

- Discuss results, diagnosis of concern, and PPV
- Refer for post-test counseling, diagnostic testing, and consideration of maternal karyotype in patients with 45,X or 47,XXX cfDNA results

MICRODELETION

Pathogenic chromosomal microdeletions are independent of maternal age and occur in 1.2% of fetuses

Some are associated with structural congenital anomalies

Others characterized by isolated neurodevelopmental disabilities not detectable by prenatal ultrasound

Validation data on the screening performance are limited

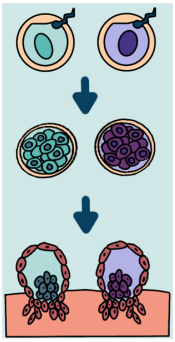
Differs by lab, but many offer

- 22q11.2 deletion syndrome (DiGeorge)
- 1p36 deletion syndrome
- 15q deletions associated with Prader-Willi and Angelman syndromes
- 5p deletion (Cri-du-Chat)
- 4p deletion (Wolf-Hirschorn)

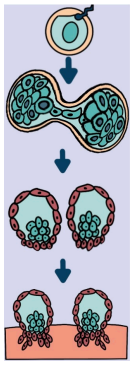
Patients desiring information regarding the risk for fetal CNVs should be offered diagnostic testing

We do not recommend routine general population screening for any microdeletion condition. Those who choose to undergo cfDNA screening for 22q11.2 deletion specifically should do so only after appropriate pretest counseling

TWINS



Dizygotic twins result from the fertilization of two separate ova by two separate sperm, which results in two genetically distinct fetuses



Monozygotic twins result from the splitting of a single fertilized ovum and share their genetic material

Timing of the splitting determines chorionicity

We recommend cfDNA as a first-line screening option for trisomies 21, 13, and 18 detection in twin gestations

Due to lack of data, cfDNA screening for SCA in twin gestations and cfDNA screening for higher-order multiples are not recommended

GENOME-WIDE

Detects subchromosomal imbalances of ≥ 7 Mb resolution for all chromosomes

Similar resolution to standard karyotype

We do not recommend the routine use of cfDNA testing for large genome-wide copy number deletions or duplications