Fetal Aneuploidy: Screening and Diagnostic Testing

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Aneuploidy is the presence of one or more extra chromosomes or the absence of one or more chromosomes. The risk of fetal aneuploidy rises with increasing maternal age. Because fetal aneuploidy can affect any pregnancy, all pregnant women should be offered screening. First-trimester combined screening performed between 10 and 13 weeks' gestation detects 82% to 87% of trisomy 21 (Down syndrome) cases. Second-trimester serum quadruple screening performed between 15 and 22 weeks' gestation detects 81% of trisomy 21 cases. Combinations of these tests include integrated or serum integrated, stepwise sequential, and contingent sequential screenings, all of which improve detection rates compared with each test alone. Fetal cell-free DNA testing (noninvasive prenatal testing) performed at or after 10 weeks' gestation detects more than 99% of trisomy 21 cases, with a lower false-positive rate than traditional first- or second-trimester screening methods. Fetal cell-free DNA testing has similar detection rates in high- and low-risk populations but has lower positive predictive values in younger women. It may be performed as primary screening or as a follow-up test to abnormal findings on first- or second-trimester screenings. Second-trimester ultrasonography has limited utility in aneuploidy screening in women who have already been screened with a first- or second-trimester serum test. Diagnostic tests following a positive screening result include chorionic villus sampling performed between 10 and 13 weeks' gestation or amniocentesis performed after 15 weeks' gestation. (*Am Fam Physician*. 2020;101(8):481-488.

Chromosomal abnormalities affect approximately one in 150 pregnancies[1] and are responsible for 50% of early pregnancy losses.[2] Aneuploidy is the presence of one or more extra chromosomes or the absence of one or more chromosomes.[3] The consequences of fetal aneuploidy vary from incompatibility with life to intellectual and physical disability. Prenatal screening aims to detect the most common forms of aneuploidy compatible with survival beyond early embryologic development into viability. The risk of fetal aneuploidy rises with increasing maternal age. For example, the risk of a woman giving birth to a live newborn with trisomy 21 (Down syndrome) increases from one in 1,480 at 20 years of age to one in 85 at 40 years of age.[1] Although the overall birth rate in the United States has declined, the portion of first births to women older than 30 years increased from 23.9% in 2000 to 30.2% in 2014.[4,5] Because fetal aneuploidy can affect any pregnancy, all pregnant women should be counseled and offered aneuploidy screening regardless of age.[1,6,7]

CME **This clinical content** conforms to AAFP criteria for continuing medical education (CME). See CME Quiz on page 461.

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Counseling and Delivering Results

Information from prenatal aneuploidy screening facilitates anticipatory planning and may affect the decision to continue an established pregnancy. Physicians should counsel pregnant women on available screening and diagnostic tests for aneuploidy.[8] Counseling should be nondirective, with

WHAT'S NEW ON THIS TOPIC

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Although the overall birth rate in the United States has declined, the portion of first births to women older than 30 years increased from 23.9% in 2000 to 30.2% in 2014.

Fetal cell-free DNA testing (noninvasive prenatal testing), which is generally performed at or after 10 weeks' gestation, can be used to determine the likelihood of trisomies 21, 18, and 13, as well as fetal sex and sex chromosome aneuploidy.

the physician supporting the autonomy of the woman and her partner in choosing whether to be screened.

Pretest counseling should include a discussion of baseline age-dependent risk, the potential for false-negative and false-positive results, the difference between screening and diagnostic tests, and what types of follow-up testing to expect.[9] The use of decision aids (examples are available at https://www.psychosocialresearchgroupunsw.org/decision-aids.html) may improve a woman's ability to make an informed choice.[10] All prenatal aneuploidy screening tests optimize detection rates (high sensitivity) and test for relatively uncommon conditions, resulting in high negative predictive values but low positive predictive values. Physicians should communicate test results in a timely manner and discuss the likelihood that a positive result is a true positive. *Table 1* defines common terms related to aneuploidy screening.[1,9,11]

Preimplantation Genetic Screening

Only preimplantation genetic screening performed during the in-vitro fertilization process provides information on aneuploidy before an embryo's implantation in the uterus. Because this type of screening biopsies the portion of an embryo that becomes the placenta, it is susceptible to false-positive and false-negative results attributable to mosaicism (aneuploidy in the placenta that is not present in the fetus).[12] Therefore, women who have conceived via in-vitro fertilization and undergone preimplantation genetic screening should still be offered aneuploidy screening during pregnancy.[1]

Prenatal Screening Tests

A summary of available aneuploidy screening tests is provided in *Table 2*.[1,11,13-17] The optimal test may depend on patient risk, preference, gestational age, availability, and cost. There is no standard algorithm recommended by professional organizations.

FIRST-TRIMESTER SCREENING

First-trimester combined screening consists of ultrasound testing of fetal nuchal translucency, maternal serum pregnancy-associated plasma protein A (PAPP-A) levels,

TABLE 1

Common Terms Related to Aneuploidy Screening

Term	Definition
Noninvasive prenatal testing	Amplification of the placental cell-free DNA circulating in the maternal bloodstream to determine the likelihood of fetal aneuploidy
First-trimester combined screening	Combination of nuchal translucency testing and maternal serum measurement of PAPP-A and free or total hCG levels
Second-trimester quadruple (quad) screening	Combination of alpha fetoprotein, unconjugated estriol, hCG, and inhibin A levels from maternal serum to produce a single risk estimate
Integrated screening	First-trimester nuchal translucency and PAPP-A testing are integrated with second-trimester quad screening to produce a single risk estimate; results are withheld until after second-trimester quad screening; serum integrated screening is an alternative method that omits first-trimester nuchal translucency testing
Stepwise sequential screening	First-trimester combined screening (nuchal translucency, PAPP-A, and hCG) is used to determine risk; patients at high risk are offered invasive diagnostic testing (chorionic villus sampling or amniocentesis), and patients at low risk receive second-trimester quad screening to refine the risk estimate
Contingent sequential screening	First-trimester combined screening (nuchal translucency, PAPP-A, and hCG) classifies patients as low, intermediate, or high risk; low-risk patients need no further testing, intermediate-risk patients may have second-trimester quad screening to refine the risk estimate, and high-risk patients are offered invasive diagnostic testing (chorionic villus sampling or amniocentesis)
Sensitivity (detection rate)	The percentage of individuals with a condition correctly identified as positive for that condition; depends on the characteristics of the test
Specificity	The percentage of individuals without a condition correctly identified as negative for that condition; depends on the characteristics of the test
Negative predictive value	The likelihood that a negative test result reflects a true negative (the condition is not present); depends on the test and the prevalence of the condition in the population screened
Positive predictive value	The likelihood that a positive test result reflects a true positive (the condition is present); depends on the test and the prevalence of the condition in the population screened

hCG = human chorionic gonadotropin; PAPP-A = pregnancy-associated plasma protein A.

Adapted with permission from Anderson CL, Brown CE. Fetal chromosomal abnormalities: antenatal screening and diagnosis. Am Fam Physician. *2009;79(2):118, with additional information from references 1 and 9.*

TABLE 2

Characteristics of Aneuploidy Screening Tests

Screening test	Timing (weeks' gestation)	Sensitivity for trisomy 21	Advantages and disadvantages
First-trimester combined	10 0/7 to 13 6/7	82% to 87%[1,13]	Results available early; nuchal translucency measurement requires a sonographer with special certification
Second-trimester quadruple (quad)	15 0/7 to 22 6/7	81%[1]	Screens for aneuploidy and neural tube defects; abnormal results may also predict adverse pregnancy outcomes
Integrated	10 0/7 to 13 6/7 *and* 15 0/7 to 22 6/7	96%[13,14]	Improved detection rates compared with first-trimester or second-trimester quad screening, but abnormal first-trimester results are withheld until after quad screening
Serum integrated	10 0/7 to 13 6/7 *and* 15 0/7 to 22 6/7	88%[1]	Improved sensitivity over second-trimester quad screening alone without a need for a sonographer with special certification
Stepwise sequential	10 0/7 to 13 6/7 *and* 15 0/7 to 22 6/7	95%[15]	Women who are high risk based on first-trimester tests are offered invasive diagnostic testing early; the remainder of patients must remember to have a second blood draw for quad screening
Contingent sequential	10 0/7 to 13 6/7 *and* 15 0/7 to 22 6/7	85% to 88%[1,16]	Avoidance of second-trimester quad screening in low-risk women
Cell-free DNA (NIPT)	After 10	> 99%[17]	Generally done at or after 10 weeks' gestation; high sensitivity and specificity and fewer false positives than other tests; more costly

NIPT = noninvasive prenatal testing.

Information from references 1, 11, and 13-17.

and free or total human chorionic gonadotropin (hCG) levels obtained between 10 0/7 and 13 6/7 weeks' gestation.[1,18,19] Nuchal translucency alone should not be used to screen for trisomy 21 in singleton pregnancies. First-trimester combined screening is designed to report 5% of all results as positive, most of which will be false positives. A randomized controlled trial reported a detection rate for trisomy 21 of 87% at 11 weeks' gestation, 85% at 12 weeks, and 82% at 13 weeks.[13]

Abnormal nuchal translucency is also a predictor of subsequent structural anomalies, and all women with abnormal nuchal translucency should receive detailed ultrasonography at 18 to 22 weeks' gestation.[7] The American College of Obstetricians and Gynecologists (ACOG) recommends fetal echocardiography in these cases.[1] Women who choose first-trimester combined screening may still be offered maternal serum alpha fetoprotein measurement between 15 and 22 weeks' gestation (ideally between 16 and 18 weeks) as a screen for open neural tube defects and anencephaly. However, Canadian guidelines suggest that this measurement is unnecessary when high-quality second-trimester ultrasonography is available.[7]

SECOND-TRIMESTER SCREENING

Second-trimester quadruple (quad) screening includes alpha fetoprotein, unconjugated estriol, hCG, and inhibin A levels from maternal serum. The test is performed between 15 0/7 and 22 6/7 weeks' gestation, although this range may vary slightly by reference laboratory; accurate pregnancy dating is imperative.[1,20] Reports will include a baseline risk of trisomies 21 and 18 based on maternal age and the current pregnancy's risk of those trisomies, as well as open spina bifida. As with first-trimester combined screening, laboratories report 5% of all second-trimester quad screening tests as positive, most of which will be false positives. Second-trimester quad screening detects 81% of trisomy 21 cases[1] *(Table 3[1,21])*.

A retrospective analysis demonstrated associations between abnormal quad screening markers and adverse pregnancy outcomes.[13,22] Women with abnormal quad screening results without subsequent evidence of aneuploidy or neural tube defect may have increased risk of adverse pregnancy outcomes, including preterm birth, fetal growth restriction, preeclampsia, and fetal loss. Increased monitoring for these complications is suggested but has not been shown to improve outcomes.[22]

COMBINATION FIRST- AND SECOND-TRIMESTER SCREENING

Combinations of first- and second-trimester screening are available to increase the detection rate of trisomy 21.[1,13] Integrated screening combines first-trimester maternal serum PAPP-A and fetal nuchal translucency with second-trimester quad screening and detects 96% of trisomy 21 cases.[13,14] When performed without first-trimester nuchal translucency (the "serum" integrated screening), the trisomy 21 detection rate is 88%.[1] First-trimester results are withheld from the patient until the second-trimester screening is performed.

In stepwise sequential screening, first-trimester combined screening (PAPP-A, hCG, and nuchal translucency) results are given to the patient if positive so that she may be offered early invasive diagnostic testing. When results are negative, quad screening is added in the second trimester to refine risk, resulting in an overall trisomy 21 detection rate of 95%.[15]

In the contingent sequential screening approach, the results of first-trimester combined screening are classified into three risk categories: high (1% of results), intermediate (18% of results), or low (81% of results).[18] Patients at high risk are offered invasive diagnostic testing, and patients at low risk receive no further testing. Patients with intermediate risk are offered second-trimester quad screening to refine risk estimates. Detection rates of 85% to 88% have been reported for this approach.[1,16]

CELL-FREE DNA TESTING (NIPT)

Placental DNA fragments circulating in the maternal bloodstream are known as fetal cell-free DNA. Cell-free DNA testing, or noninvasive prenatal testing (NIPT), amplifies this DNA to determine if equal amounts are present from each chromosome.[23] NIPT, which is generally performed at or after 10 weeks' gestation, can be used to determine the likelihood of trisomies 21, 18, and 13, as well as fetal sex and sex chromosome aneuploidy. It is superior to first- or second-trimester serum screenings with fewer false positives and higher positive predictive values for trisomies 18 and 21.

In a 2015 randomized controlled trial comparing NIPT with first-trimester combined screening, NIPT detected

TABLE 3

Test Performance of Second-Trimester Serum Quadruple Screening for Trisomy 21

Maternal age (years)	Prevalence of trisomy 21 at 16 weeks' gestation	Sensitivity	False-positive rate	Negative predictive value	Positive predictive value*
20	1 per 1,177	81%	4.9%	> 99%	1.3%
25	1 per 1,040	81%	4.9%	> 99%	1.6%
30	1 per 700	81%	4.9%	> 99%	2.3%
35	1 per 296	81%	4.6%	> 99%	5.5%
40	1 per 86	81%	3.8%	> 99%	19.1%

Note: Statistics in this table were calculated by the author using reported prevalence data from the California Department of Public Health Prenatal Screening Program,[21] with a reported sensitivity of 81% and a fixed screen-positive rate of 5%.[1]

*—The portion of patients who screen positive for trisomy 21 who have fetuses affected by trisomy 21 (true positive).

Information from references 1 and 21.

TABLE 4

Test Performance of Cell-Free DNA (Noninvasive Prenatal Testing) for Trisomy 21

Maternal age (years)	Prevalence of trisomy 21 at 16 weeks' gestation	Sensitivity	False-positive rate	Negative predictive value	Positive predictive value*
20	1 per 1,177	99.7%	0.04%	> 99%	68%
25	1 per 1,040	99.7%	0.04%	> 99%	71%
30	1 per 700	99.7%	0.04%	> 99%	78%
35	1 per 296	99.7%	0.04%	> 99%	89%
40	1 per 86	99.7%	0.04%	> 99%	97%

Note: Statistics in this table were calculated by the author using reported prevalence data from the California Department of Public Health Prenatal Screening Program[21] and reported sensitivity and specificity data from reference 17.

*—The portion of patients who screen positive for trisomy 21 who have fetuses affected by trisomy 21 (true positive).

Information from references 17 and 21.

BEST PRACTICES IN GENETIC MEDICINE

Recommendations from the Choosing Wisely Campaign

Recommendation	Sponsoring organization
Do not order serum aneuploidy screening after noninvasive prenatal testing has already been performed.	Society for Maternal-Fetal Medicine

Source: For more information on the Choosing Wisely Campaign, see https://www.choosingwisely.org. For supporting citations and to search Choosing Wisely recommendations relevant to primary care, see https://www.aafp.org/afp/recommendations/search.htm.

100% of trisomy 21 cases (false-positive rate of 0.06%) and 78.9% of trisomy 18 cases (false-positive rate of 0.01%).[24] A 2017 meta-analysis reported that NIPT had a detection rate of 99.7% for trisomy 21 and 97.9% for trisomy 18, with a false-positive rate of 0.04% for both[17] *(Table 4*[17,21]*)*. Multiple studies have since reported similar or better test performance across low- and high-risk populations.[25-28]

NIPT can be performed as primary screening or as a follow-up test when first- or second-trimester serum screening results are abnormal. First- or second-trimester screening should not be performed after NIPT.[1] Using NIPT only as a contingent follow-up test avoids invasive testing and its associated risks in most women,[29] although some models suggest that as many as one in 50 pregnancies with positive first- or second-trimester screening and normal NIPT results may have an undetected chromosomal abnormality.[30] The contingent approach is supported by the Society of Obstetricians and Gynaecologists of Canada.[7] ACOG and the Society for Maternal-Fetal Medicine note that NIPT can be used in low-risk populations,[1] although positive predictive values are lower. Universal NIPT adoption is not yet cost-effective.[31] The Society for Maternal-Fetal Medicine designates some high-risk women as ideal candidates for NIPT screening (risk factors include maternal age of 35 years or older at the time of delivery; ultrasound findings indicating higher risk of aneuploidy; a previous pregnancy affected by trisomy 13, 18, or 21; or positive results from first- or second-trimester serum screenings).[32] Positive NIPT results should be confirmed with invasive diagnostic testing, particularly if pregnancy termination is being considered.

Before 10 weeks' gestation, the percentage of fetal vs. maternal cell-free DNA circulating in maternal serum (the fetal fraction) may be too low to create a result. These "no-call" results may indicate an increased risk of aneuploidy.[33] Of those women with no-call results, 50% to 80% will receive a reportable result on a repeat test.[7,34] Low fetal fraction is more common in pregnant women who are obese, with 7% of women weighing more than 100 kg (220 lb, 7 oz) and 51.1% of women weighing more than 160 kg (352 lb, 12 oz) receiving fetal fractions too low to report at 11 to 13 weeks' gestation.[35]

Any NIPT test may have a false-positive, false-negative, or no-call result. When abnormal NIPT screening is discordant with (normal) invasive diagnostic testing, it may be attributable to placental mosaicism, maternal aneuploidy, or sometimes occult maternal malignancy. Discordant results, particularly when more than one aneuploidy is seen on NIPT and not confirmed by invasive diagnostic testing, may require a discussion with the patient regarding the risks and benefits of an occult malignancy workup.[36,37]

TWIN PREGNANCIES

First- and second-trimester serum screening or first-trimester nuchal translucency alone can be used to screen women with twin pregnancies for aneuploidy, although detection rates are lower.[1] In higher order pregnancies (triplets or more), serum screening is unvalidated, and only nuchal translucency alone can differentiate which fetus is potentially affected. The Society of Obstetricians and Gynaecologists of Canada notes that NIPT is less validated in twin pregnancies and should be used with caution, and ACOG recommends against it.[1,7] However, a meta-analysis of NIPT in twin pregnancies reported a sensitivity of 99% for trisomy 21 and 85% for trisomy 18.[38]

SECOND-TRIMESTER ULTRASONOGRAPHY

As a stand-alone test, second-trimester ultrasonography has a reported sensitivity of 50% to 60% for trisomy 21.[1] A series of "soft markers" for aneuploidy, none of which are considered congenital anomalies, may suggest a higher likelihood of trisomy 21 or 18 when seen on second-trimester ultrasonography.[1,39] Many fetuses with aneuploidy will not have these soft markers on ultrasonography, and these soft markers are common in normal fetuses. A meta-analysis found that a thickened nuchal fold is the only soft marker associated with increased risk of trisomy 21.[40] When soft markers are isolated, reassurance can be offered to most women after negative quad screening or NIPT testing. The interpretation of isolated soft markers is summarized in *Table 5*.[1,7,41,42] When multiple soft markers are found, referrals to maternal fetal medicine and genetic counseling are warranted.[42]

Invasive Diagnostic Testing

Women with positive aneuploidy screening results should be offered referral to maternal fetal medicine and genetic

SORT: KEY RECOMMENDATIONS FOR PRACTICE

Clinical recommendation	Evidence rating	Comments
All pregnant women should be counseled and offered aneuploidy screening regardless of maternal age.[1,6,7]	C	Expert consensus guidelines
Fetal cell-free DNA testing (NIPT), which is generally performed at or after 10 weeks' gestation, is superior to first- or second-trimester serum screenings with fewer false positives and higher positive predictive values for trisomies 18 and 21.[1,7,17,23-32]	A	Systematic reviews and meta-analyses of high-quality diagnostic accuracy studies; NIPT performs similarly in high- and low-risk populations, although positive predictive values are lower in low-risk populations
First-trimester nuchal translucency, NIPT, and first- or second-trimester serum testing can be performed in twin pregnancies.[1,7,38]	B	Meta-analysis of diagnostic accuracy studies with limitations; detection rates are lower in twin pregnancies
Women with positive results on aneuploidy screening should be offered referral for invasive diagnostic testing.[1,7]	C	Expert consensus guidelines; no screening test, including cell-free DNA, is considered diagnostic

NIPT = noninvasive prenatal testing.

A = consistent, good-quality patient-oriented evidence; **B** = inconsistent or limited-quality patient-oriented evidence; **C** = consensus, disease-oriented evidence, usual practice, expert opinion, or case series. For information about the SORT evidence rating system, go to https://www.aafp.org/afpsort.

TABLE 5

Interpretation of Isolated Soft Markers of Aneuploidy on Second-Trimester Ultrasonography

Marker	Additional screening	Counseling and follow-up
Choroid plexus cyst Echogenic intracardiac focus	Offer second-trimester quadruple (quad) screening* or cell-free DNA testing (NIPT) if not yet obtained	If results are negative (low risk) on serum screening or NIPT, these findings are considered a normal variant and not a marker of aneuploidy risk
Clinodactyly Sandal gap toe	Offer NIPT if not yet obtained	If results are negative (low risk) on NIPT, these findings are considered a normal variant and not a marker of aneuploidy risk
Echogenic bowel Hypoplastic nasal bone Pyelectasis Shortened humerus or femur Single umbilical artery Thickened nuchal fold Ventriculomegaly	Offer NIPT if not yet obtained	If results are negative (low risk) on NIPT, these findings are not considered a marker of increased aneuploidy risk; however, patients should be referred to maternal fetal medicine for further workup and follow-up

NIPT = noninvasive prenatal testing.

*—Alpha fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin A levels from maternal serum.

Information from references 1, 7, 41, and 42.

counseling to discuss invasive diagnostic testing with chorionic villus sampling or amniocentesis.[1,7] Chorionic villus sampling is performed between 10 and 13 weeks' gestation and tests placental tissue obtained transcervically or transabdominally.[43] Amniocentesis tests fetal cells grown in a culture from an amniotic fluid sample obtained transabdominally. It is performed any time after 15 weeks' gestation; earlier amniocentesis has higher complication rates.[44] Both tests carry a risk of pregnancy loss, with an estimated risk of one in 455 for chorionic villus sampling and one in 900 for amniocentesis.[1,45] The laboratory tests performed depend on the indication for the diagnostic procedure but may include karyotyping, chromosomal microarray, or fluorescent in situ hybridization.

This article updates a previous article on this topic by Anderson and Brown.[11]

Data Sources: The authors searched PubMed for systematic reviews, meta-analyses, and randomized controlled trials involving aneuploidy screening and diagnosis in pregnancy. The TRIP database was queried with similar terms. Relevant guidelines from the Society for Maternal-Fetal Medicine, American College of Obstetricians and Gynecologists, Society of Obstetricians and Gynaecologists of Canada, and Royal College of Obstetricians and Gynaecologists were reviewed. The Cochrane database was also searched. An Essential Evidence Plus summary of patient-oriented evidence that matters was reviewed. Individual references were reviewed from the bibliographies of other specialty guidelines with relevant articles reviewed in full text. Search dates: March 2019 and January 2020.

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