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Screening for Fetal Aneuploidy

Prenatal genetic screening is designed to assess whether a patient is at increased risk of having a fetus affected by a genetic disorder. In contrast, prenatal genetic diagnostic testing is intended to determine, with as much certainty as possible, whether a specific genetic disorder or condition is present in the fetus. The purpose of prenatal screening for aneuploidy is to provide an assessment of the woman's risk of carrying a fetus with one of the more common fetal aneuploidies. This is in contrast to prenatal diagnostic testing for genetic disorders, in which the fetal chromosomes are evaluated for the presence or absence of abnormalities in chromosome number, deletions, and duplications, or the fetal DNA is evaluated for specific genetic disorders. The wide variety of screening test options, each offering varying levels of information and accuracy, has resulted in the need for complex counseling by the health care provider and complex decision making by the patient. No one screening test is superior to other screening tests in all test characteristics. Each test has relative advantages and disadvantages. It is important that obstetrician–gynecologists and other obstetric care providers be prepared to discuss not only the risk of aneuploidy but also the benefits, risks, and limitations of available screening tests. Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.

The purpose of this Practice Bulletin is to provide current information regarding the available screening test options for fetal aneuploidy and to review their benefits, accuracy, and limitations. For information regarding prenatal diagnostic testing for genetic disorders, refer to Practice Bulletin No. 162, Prenatal Diagnostic Testing for Genetic Disorders.

Background

Aneuploidy is defined as having one or more extra or missing chromosomes, leading to an unbalanced chromosome number in a cell. Because each chromosome consists of hundreds of genes, the loss or gain of large chromosomal segments disrupts significant amounts of genetic material and often results in a nonviable pregnancy or offspring that may not survive after birth. In the case of a surviving newborn, congenital birth defects; failure to thrive; and functional abnormalities, including mild-to-severe intellectual disability, infertility, and shortened lifespan, may occur.

Although chromosomal abnormalities occur in approximately 1 in 150 live births (1), the prevalence is greater earlier in gestation because aneuploidy accounts for a large proportion of early pregnancy loss. The incidence of fetal aneuploidy increases as a woman ages (Table 1) but can affect any woman regardless of age and is not related to race or ethnicity. Other factors that increase the risk of fetal aneuploidy include a history of a prior aneuploid fetus and the presence of fetal anomalies. Autosomal trisomies are the most common aneuploidies that are not related to sex chromosome disorders. Down syndrome (trisomy 21) is the most common of these, with a prevalence of approximately 1 in 800 live births

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(1). The most common sex chromosome aneuploidy is Klinefelter syndrome (47,XXY) with a prevalence of 1 in 500 males. The only viable monosomy is Turner syndrome (45,X).

Down syndrome is the most common form of inherited intellectual disability, with approximately 6,000 affected infants born in the United States each year. It is estimated that 95% of cases of Down syndrome result from nondisjunction involving chromosome 21. The remaining cases result from translocations or somatic mosaicism (2). Although the clinical presentation of Down syndrome can vary, it is associated with characteristic facial features, learning disabilities, congenital heart defects (eg, atrioventricular canal defects), intestinal atresia, seizures, childhood leukemia, and early-onset Alzheimer disease. Fetuses affected with Down syndrome often do not survive pregnancy; between the first trimester and full term, an estimated 43% of pregnancies end in miscarriage or stillbirth (3). In economically developed countries, the median survival of individuals with Down syndrome is now almost 60 years (4). Factors associated with an increased risk of Down syndrome include higher maternal age, a parental translocation involving chromosome 21, a previous child with a trisomy, significant ultrasonographic findings, and a positive screening test result. After a prenatal diagnosis is made, prenatal assessment cannot predict the severity of the complications from Down syndrome.

In general, the process of aneuploidy screening identifies two groups of individuals: 1) those with a positive screening test result who have an increased risk of having a fetus with an aneuploidy and 2) those with a negative screening test result who have a lower posttest probability of the evaluated aneuploidies. Women with a positive screening test result should be counseled regarding their higher risk of aneuploidy and offered the option of diagnostic testing. Those who have a negative test result should be counseled regarding their lower adjusted risk and their lower residual risk. Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result. Even if a woman has a negative test result, she may choose diagnostic testing later in pregnancy, particularly if additional findings become evident (eg, fetal anomalies or markers of aneuploidy identified on follow-up ultrasonography).

Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit. The choice of whether to perform screening or diagnostic testing for aneuploidy depends on the woman's goals and values and her desire for informational accuracy. Although maternal age may

Table 1. Risk of Chromosomal Abnormalities Based on Maternal Age at Term

Age at Term	Risk of Trisomy 21*	Risk of Any Chromosome Abnormality†
15‡	1:1,578	1:454
16‡	1:1,572	1:475
17‡	1:1,565	1:499
18‡	1:1,556	1:525
19‡	1:1,544	1:555
20	1:1,480	1:525
21	1:1,460	1:525
22	1:1,440	1:499
23	1:1,420	1:499
24	1:1,380	1:475
25	1:1,340	1:475
26	1:1,290	1:475
27	1:1,220	1:454
28	1:1,140	1:434
29	1:1,050	1:416
30	1:940	1:384
31	1:820	1:384
32	1:700	1:322
33	1:570	1:285
34	1:456	1:243
35	1:353	1:178
36	1:267	1:148
37	1:199	1:122
38	1:148	1:104
39	1:111	1:80
40	1:85	1:62
41	1:67	1:48
42	1:54	1:38
43	1:45	1:30
44	1:39	1:23
45	1:35	1:18
46	1:31	1:14
47	1:29	1:10
48	1:27	1:8
49	1:26	1:6
50	1:25	§

*Data from Morris JK, Wald NJ, Mutton DE, Alberman E. Comparison of models of maternal age-specific risk for Down syndrome live births. Prenat Diagn 2003;23:252–8.

†Risk of any chromosomal abnormality includes the risk of trisomy 21 and trisomy 18 in addition to trisomy 13, 47,XXY, 47,XYY, Turner syndrome genotype, and other clinically significant abnormalities, 47,XXX not included. Data from Hook EB. Rates of chromosome abnormalities at different maternal ages. Obstet Gynecol 1981;58:282–5.

‡Data from Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. Br J Obstet Gynaecol 1987;94:387–402.

§Data not available.

be helpful in adjusting an individual woman's risk of having a fetus with aneuploidy, it should not be used as the sole determinant of whether aneuploidy screening or diagnostic testing is offered. Although the risk of aneuploidy increases with advancing maternal age, most children with Down syndrome are born to younger women because a larger proportion of all children are born to young women. An observational study of more than 38,000 women demonstrated that if all women aged 35 years and older had had diagnostic testing, the detection rate for Down syndrome would have been only 21.6% (5).

Screening tests for aneuploidy are now available for pregnant women in all trimesters of pregnancy. Among these are first-trimester, triple, quad, and penta screens; cell-free DNA; and ultrasonographic screening as single screening tests. Screening tests that are performed in the first and second trimesters include integrated, sequential, and contingent screening.

The intent of counseling for aneuploidy is to inform the pregnant woman about chromosomal disorders, provide information regarding her specific risk of carrying a fetus with aneuploidy, and review the available options so that she can make an informed choice regarding screening or diagnostic testing. After review and discussion, every patient has the right to pursue or decline screening or diagnostic testing. Pretest and posttest counseling are essential and must be a part of any screening program. When a positive or negative screening test result is obtained, the patient should be counseled regarding the adjusted likelihood of carrying a fetus with the evaluated aneuploidies. The potential for the fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should be reviewed. In the event that a prenatal diagnosis of fetal aneuploidy is made, the patient must be counseled appropriately so that she can make informed decisions regarding pregnancy management. Counseling should include family education and preparedness as well as options regarding adoption, pregnancy termination, referral to a tertiary care center for delivery of the newborn if needed, and perinatal hospice care as appropriate for a child with a condition that is incompatible with life. Patients found to have a fetus with a chromosomal abnormality often benefit from referral to a genetics professional for further detailed counseling.

Single Screening Tests

First-Trimester Screening

Typically performed when the crown–rump length measures between 38–45 mm and 84 mm (generally between 10 0/7 weeks and 13 6/7 weeks of gestation), first-trimester screening includes a nuchal translucency measurement, serum free β-hCG, or total human chorionic gonadotropin (hCG) along with pregnancy-associated plasma protein A analyte levels. A specific risk estimate for aneuploidy is calculated using these results as well as maternal factors such as maternal age, prior history of aneuploidy, weight, race, and number of fetuses.

The nuchal translucency refers to the fluid-filled space measured on the dorsal aspect of the fetal neck. An enlarged nuchal translucency (often defined as 3.0 mm or more or above the 99th percentile for the crown–rump length) is independently associated with fetal aneuploidy and structural malformations. The risk of adverse pregnancy outcome is proportional to the degree of nuchal translucency enlargement. Meticulous technique in nuchal translucency imaging is essential for accurate risk assessment because undermeasurement by even 0.5 mm can reduce the test sensitivity by 18% (6). Independent credentialing of ultrasonographers in appropriate technique is important to screening performance.

Quadruple Screen

The quadruple marker screen ("quad" screen) can be performed from approximately 15 0/7 weeks to 22 6/7 weeks of gestation; the range is dependent on the laboratory that the obstetrician–gynecologist or other obstetric care provider uses. This test does not require specialized ultrasonography for nuchal translucency measurement and gives information regarding the risk of open fetal defects in addition to aneuploidy risk assessment. The best time to perform a quad screen is from approximately 16 weeks to 18 weeks of gestation because this optimizes screening for neural tube defects. The quad screen involves the measurement of four maternal serum analytes: 1) hCG, 2) alpha fetoprotein (AFP), 3) dimeric inhibin A, and 4) unconjugated estriol, in combination with maternal factors such as age, weight, race, the presence of diabetes, and plurality to calculate a risk estimate. First-trimester and quad screening have similar detection rates for Down syndrome: more than 80% at a 5% positive result rate (Table 2) (5). Accurate gestational dating at the time of blood sampling is important because inaccurate gestational dating decreases the accuracy of the result. The later timing of this test leaves fewer options available for the patient if the results are positive.

Penta Screen

The penta screen includes hyperglycosylated hCG (also known as invasive trophoblast antigen) in addition to the quad screen markers and also is available for

Table 2. Characteristics, Advantages, and Disadvantages of Common Screening Tests for Aneuploidy

Screening Test	Gestational Age Range for Screening (Weeks)	Detection Rate for Down Syndrome (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
First trimester[†]	11–14[‖]	82–87	5	1. Early screening 2. Single test 3. Analyte assessment of other adverse outcome	Lower DR than combined tests NT required	NT+PAPP-A and hCG
Triple screen	15–22	69	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than with first-trimester or quad screening Lowest accuracy of the single lab tests	hCG, AFP, uE3
Quad screen[†]	15–22	81	5	1. Single test 2. No specialized US required 3. Also screens for open fetal defects 4. Analyte assessment for other adverse outcomes	Lower DR than combined tests	hCG, AFP, uE3, DIA
Integrated[†]	11–14, then 15–22	96	5	Highest DR of combined tests Also screens for open fetal defects	Two samples needed before results are known	NT+PAPP-A, then quad screen
Sequential[‡]: Stepwise	11–14, then 15–22	95	5	First-trimester results provided; Comparable performance to integrated, but FTS results provided; also screens for open fetal defects; analyte assessment for other adverse outcomes.	Two samples needed	NT+hCG+ PAPP-A then quad screen
Contingent screening[‡]		88–94	5	First-trimester test result: Positive: diagnostic test offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed	NT+hCG+ PAPP-A, then quad screen
Serum Integrated[†]	11–14; then 15–22	88	5	1. DR compares favorably with other tests. 2. No need for NT	Two samples needed; no first-trimester results	PAPP-A+quad
Cell-free DNA[§]	10 - term	99 (in patients who receive a result)	0.5	1. Highest DR for Down syndrome 2. Can be performed at any gestational age after 10 weeks 3. Low false-positive rate in high-risk women (or women at high risk of Down syndrome)	1. NPV and PPV not clearly reported 2. Higher false-positive rate in women at low risk of Down syndrome 3. Limited information about three trisomies and fetal sex 4. Results do not always represent a fetal DNA result	Three roughly equivalent molecular methods
Nuchal Translucency[†]	11–14[‖]	64–70	5	Allows individual fetus assessment in multifetal gestations Provides additional screening for fetal anomalies and possibly for twin–twin transfusion syndrome	1. Poor screen in isolation 2. Ultrasound certification necessary	US only

Abbreviations: AFP, alpha fetoprotein; DIA, dimeric inhibin-A; DR, detection rate; DS, Down syndrome; FTS, first-trimester screening; hCG, human chorionic gonadotropin; NPV, negative predictive value; NT, nuchal translucency; NTD, neural tube defect; PAPP-A, pregnancy-associated plasma protein A; PPV, positive predictive value; uE3, unconjugated estriol; US, ultrasonography.

*A screen positive test result includes all positive test results: the true positives and false positives.

[†]First-trimester combined screening: 87%, 85%, and 82% for measurements performed at 11 weeks, 12 weeks, and 13 weeks, respectively. Malone FASTER 2005.

[‡]Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.

[§]Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group [published erratum appears in Obstet Gynecol 2012;120:957]. Obstet Gynecol 2012;119:890–901 *and* Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med 2011;13:913–20.

[‖]Because of variations in growth and conception timing, some fetuses at the lower and upper gestational age limits may fall outside the required crown–rump length range.

Data from Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Semin Perinatol 2005;29:252–7.

second-trimester screening (7) by at least one national laboratory. Although there is some evidence from one limited retrospective trial that this test may improve second-trimester screening performance, its performance has not been evaluated rigorously in prospective studies and it is not widely used. Limited data are available to compare the accuracy of the penta screen with other second-trimester screening tests.

Triple Screen

The triple marker screen measures serum hCG, AFP, and unconjugated estriol to determine a risk estimate. This test provides a lower sensitivity for the detection of Down syndrome (sensitivity of 69% at a 5% positive screening test result rate) than quad screen and first-trimester screening (5).

Combined First- and Second-Trimester Screening

Combined first- and second-trimester screening with either integrated, sequential, or contingent screening protocol provides a higher detection rate than one-step screening. Depending on the test selected, results are not available until the second trimester or possibly in the first trimester under certain circumstances.

Integrated Screening and Serum Integrated Screening

With integrated screening, the patient undergoes a first-trimester nuchal translucency measurement and analyte screening followed by a second-trimester quad screen and receives a single test result in the second trimester. In locations where a nuchal translucency measurement by a certified ultrasonographer is unavailable, or if fetal position, maternal body habitus, or imaging properties preclude an accurate nuchal translucency measurement, serum integrated screening can be offered. Serum integrated screening has a similar but slightly lower detection rate than integrated screening (Table 2). Limitations of integrated screening include the withholding of first-trimester screening test results until the second trimester and nonadherence of the second blood draw; rates of nonadherence in practice have been reported to be as high as 25% without a written reminder to complete the test (8).

Sequential Screening: the Stepwise and Contingent Screening Models

Sequential screening was developed to maintain a high detection rate using the combined first- and second-trimester screening approach while also reporting the patient's first-trimester screening test risk, which allows for earlier management options. Using stepwise sequential screening, the patient is given a preliminary risk estimate after completion of the first-trimester analytes and nuchal translucency screening. If the first-trimester screening result indicates that the risk of aneuploidy is greater than the laboratory-derived positive screening cutoff, the patient is notified and offered a diagnostic test or cell-free DNA screening, and the screening protocol is discontinued. If the patient has a lower risk than the cutoff level, she is informed that she has received a negative screening test result and proceeds to quad screening in the second trimester. Sequential screening has a detection rate of 91–93% with a positive screening test result rate of 4–5% (9, 10).

The contingent model classifies aneuploidy risk as high, intermediate, or low on the basis of the first-trimester screening test results; women at high risk are offered cell-free DNA screening or diagnostic testing with chorionic villus sampling (CVS), and for those at low risk, no further screening or testing is recommended. Only those women at intermediate risk are offered second-trimester screening and, thus, fewer women go on to second-trimester screening.

In the stepwise and contingent models, the patients at highest risk identified by first-trimester screening are offered an early diagnostic procedure. First- and second-trimester results are used together to calculate a final risk of aneuploidy in patients at lower risk in the stepwise and sequential models. The sequential approach takes advantage of the higher detection rate achieved by incorporating the first- and second-trimester screening test results with only a marginal increase in the false-positive rate. Theoretically, the contingent approach should maintain high detection rates with low false-positive rates while reducing the number of second-trimester tests performed.

The use of multiple screening tests performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver confusing risk estimates to patients. In patients who undergo first-trimester screening, if later screening for risk of neural tube defects is to be done with maternal serum alpha-fetoprotein (MSAFP), the test should be performed as an isolated screening test and not as part of a quad screen.

Ultrasonographic Screening

Although fetuses with trisomy 13 (Patau syndrome, which occurs in 1 in 10,000 births) or trisomy 18 (Edwards syndrome, which occurs in 1 in 6,000 births)

usually have major structural anomalies that are evident on ultrasonography, the ultrasonographic identification of Down syndrome is more elusive. For several decades, the second-trimester "genetic ultrasonogram" has been used to screen for Down syndrome using specific ultrasonographic findings (11). This approach seeks to identify major structural abnormalities and minor ultrasonographic "soft markers" of aneuploidy. The major structural anomalies associated with fetal Down syndrome include cardiac anomalies (such as septal defects, tetralogy of Fallot, and atrioventricular canal defects) usually identified in the second trimester and duodenal atresia, which typically is identified in the third trimester. In contrast, second- and third-trimester soft ultrasonographic markers for aneuploidy are nonspecific physical characteristics that are more common among fetuses with Down syndrome and in some cases also can reflect or progress to an overt fetal abnormality (eg, thickened nuchal fold, renal pelvis dilation, or echogenic bowel). Because soft markers for aneuploidy also are common in unaffected fetuses, it is difficult to use these findings to distinguish between pregnancies affected or unaffected by aneuploidy. As an isolated finding, an increased nuchal skinfold thickness confers the highest risk of aneuploidy. In contrast, an isolated echogenic intracardiac focus carries one of the lowest risks of fetal aneuploidy (12, 13). If an isolated low-risk marker such as a choroid plexus cyst or intracardiac echogenic focus is identified on the fetal anatomic ultrasound survey, the patient's chart should be reviewed to determine if analyte screening has been performed previously; if not, it should be offered. Additional follow-up for isolated ultrasonographic markers generally is not indicated other than for isolated renal pelvis dilation, echogenic bowel, or shortened humerus or femur length (14). Patients with these markers may benefit from referral for detailed ultrasonography and follow-up. Major limitations of the use of second-trimester ultrasonographic markers include the lack of standardization in measurements and characteristics that define a positive test result, and the lack of understanding of how factors such as high maternal body mass index, multiple gestation, machine quality, and experience of the ultrasonographer and ultrasonologist affect screening performance.

Cell-free DNA Screening

Cell-free DNA screening evaluates short segments of DNA in maternal blood and can be used to screen for a variety of fetal conditions. The fetal component of cell-free DNA is released into the maternal circulation primarily from placental cells undergoing apoptosis or programmed cell death and comprises approximately 3–13% of the total cell-free DNA in maternal blood (15). This amount increases throughout gestation and is cleared from the maternal circulation within hours after childbirth (16). Several molecular methods have been developed to analyze cell-free DNA for the purpose of aneuploidy screening, and all appear to have similar detection and false-positive rates, although direct comparison trials have not been performed. Cell-free DNA screening also can be used to determine fetal sex, to identify the presence of a Rh-positive fetus in a Rh-negative mother, and to detect some paternally derived autosomal dominant genetic abnormalities (17–19). Screening can be performed from as early as 10 weeks of gestation until term and offers the highest reported detection rate for Down syndrome: more than 98% detection with positive screening rates of less than 0.5% among women with a reportable result (20). The detection rate is lower for trisomy 13 and trisomy 18 (21–27). Further, published studies have excluded those who have no reportable result, and these women are at increased risk of fetal aneuploidy (22, 23, 28). Inclusion of these women in the calculations would yield lower sensitivity for fetal aneuploidy. In addition, managing women with no reportable result as screen positive will decrease the specificity and increase the positive screening rate for this testing.

Clinical Considerations and Recommendations

▶ *Who should be offered screening for aneuploidy?*

All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age. The choice of screening test is affected by many factors, including a desire for information before delivery, prior obstetric history, family history, and the number of fetuses. Other factors to be considered include gestational age at presentation, the availability of a reliable nuchal translucency measurement, screening test sensitivity and limitations, the cost of screening, the constraints of long-term care of an affected child, and options for pregnancy care or termination for an abnormal diagnostic test result. No one test is superior for all test characteristics and not every test is available at all centers. Each test has advantages and disadvantages that should be discussed with each patient, with the appropriate test offered based on her concerns, needs, and values. Obstetrician–gynecologists and other obstetric care providers should become familiar with the available screening and diagnostic testing options for their patients within their practice and adopt a standard approach for counseling. Regardless of which screening tests are

offered, information about the detection (sensitivity) and positive screening and false-positive rates, advantages, disadvantages, and limitations should be communicated to the patient. At the time of counseling regarding aneuploidy screening, the benefits and risks of diagnostic testing (amniocentesis and chorionic villus sampling) also should be discussed (29). After counseling, patients may decline screening or diagnostic testing for any reason.

▶ *What is the role of ultrasonography in screening for fetal aneuploidy?*

In women of advanced maternal age, the absence of ultrasonographic markers has been used to decrease a woman's age-related risk of aneuploidy by greater than 80% (30, 31). However, with the exception of maternal age, second-trimester ultrasonography is the least effective primary screening test for Down syndrome, detecting only 50–60% of affected fetuses. As such, ultrasonography should not be used in isolation to diagnose or exclude Down syndrome. Ultrasonographic markers can identify other disorders, and the various soft markers have different degrees of association with Down syndrome. The risk of aneuploidy associated with each marker should be considered individually within the complete clinical context. The presence of soft ultrasonographic markers for aneuploidy warrants a targeted ultrasound to exclude other evident abnormalities and a review or offering of screening tests for fetal aneuploidy. Of the soft markers, third-trimester follow-up is only indicated for isolated renal pelvis dilation, echogenic bowel, or shortened humerus or femur (14). For women who have already undergone screening for aneuploidy and have received a negative screening test result, and for those who have had normal diagnostic testing, ultrasonography should not be used as an additional screening test for aneuploidy. If aneuploidy screening has been performed before ultrasonographic evaluation, no additional evaluation is indicated if an echogenic intracardiac feature or choroid plexus cysts is the sole identified marker (Table 3). However, further detailed counseling is recommended for fetuses with a hypoplastic or absent nasal bone, echogenic bowel, or nuchal skinfold thickening (14). If an isolated ultrasonographic marker for aneuploidy is detected, the patient should be offered aneuploidy screening if it was not offered previously.

With regard to first-trimester imaging, an increased nuchal translucency measurement increases the risk of genetic syndromes and isolated anomalies, such as congenital heart defects, abdominal wall defects, and diaphragmatic hernia, even with normal chromosomes on diagnostic testing (32). These patients should be offered a targeted ultrasound examination and fetal echocardiography in the second trimester.

The finding of an increased nuchal translucency extending along the length of the fetus in which septations are clearly visible is referred to as a cystic hygroma. This finding is associated with a 50% likelihood of fetal aneuploidy (most commonly Down syndrome, 45,X, and trisomy 18). Of the remaining euploid fetuses, one half will have a major structural malformation, such as congenital heart defects, diaphragmatic hernia, or skeletal dysplasia, or other genetic syndromes. Less than 20% of such pregnancies will result in a healthy live-born infant at term (33). A nuchal measurement for aneuploidy risk is not necessary at the time of cell-free DNA screening in the first trimester. However, ultrasound examination is useful to confirm viability, to confirm the number of fetuses and the presence of an empty gestational sac, to assign gestational age, and to identify some major fetal anomalies for patients who choose to have cell-free DNA screening (34). Patients who choose serum integrated screening may be offered first-trimester ultrasonography for gestational dating even if nuchal translucency measurement is unavailable or cannot be obtained. If an enlarged nuchal translucency, an obvious anomaly, or a cystic hygroma is identified on ultrasonography, the patient should be offered genetic counseling and diagnostic testing for aneuploidy as well as follow-up ultrasonography for fetal structural abnormalities. Given the high risk of congenital heart disease in these fetuses, referral for fetal cardiac ultrasonography should be considered. Patients with an enlarged nuchal translucency or cystic hygroma and normal fetal karyotype should be offered an anatomic evaluation in the second trimester, fetal cardiac ultrasonography, and further counseling regarding the potential for genetic syndromes not detected by aneuploidy screening.

▶ *What are the characteristics and limitations of the different screening tests?*

First-Trimester Screening

The first-trimester screening, or first-trimester combined screening, comprising nuchal translucency measurement and serum analyte measurements combined into a single test, is performed before 14 0/7 weeks of gestation (with the range determined by the laboratory accepting the sample, typically between 10 0/7 weeks and 13 6/7 weeks of gestation) and requires a crown–rump length between approximately 38–45 mm and 84 mm. Advantages of first-trimester screening are a slightly higher, but not significantly different, detection rate for Down syndrome compared with second-trimester screening. This test gives the potential for earlier diagnoses as well as the ability to screen for other fetal or placental disorders. However, first-trimester screening lacks the ability to assess the risk

Table 3. Management of Ultrasonographic Markers for Aneuploidy

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
First trimester: enlarged nuchal translucency	Certified ultrasonography measurement ≥ 3.0 mm or above the 99th percentile for the CRL	Aneuploidy risk increases with size of NT Also associated with Noonan syndrome, multiple pterygium syndrome, skeletal dysplasias, congenital heart disease, and other anomalies	1. Genetic counseling 2. Offer cfDNA or CVS 3. Second-trimester detailed anatomic survey and fetal cardiac ultrasonography
First trimester: cystic hygroma	Large single or multilocular fluid-filled cavities, in the nuchal region and can extend the length of the fetus	If septate, approximately 50% are aneuploid	1. Genetic counseling 2. Offer CVS 3. Second-trimester detailed fetal anatomic survey and fetal cardiac ultrasonography
Second trimester: echogenic intracardiac foci	Echogenic tissue in one or both ventricles of the heart seen on standard four-chamber view	LR 1.4–1.8 for Down syndrome Seen in 15–30% of Down syndrome and 4–7% euploid fetuses	1. If isolated finding, aneuploidy screening should be offered if not done previously 2. If aneuploidy screen result is negative, no further evaluation is required.
Second trimester: pyelectasis	Renal pelvis measuring ≥ 4 mm in anteroposterior diameter up to 20 weeks of gestation	LR 1.5–1.6 for Down syndrome	1. If isolated finding, aneuploidy screening should be offered if not performed previously 2. Repeat ultrasonography in third trimester for potential urinary tract obstruction
Second trimester: echogenic bowel	Fetal small bowel as echogenic as bone	LR 5.5–6.7 for Down syndrome Associated with aneuploidy, intra-amniotic bleeding, cystic fibrosis, CMV	1. Further counseling 2. Offer CMV, CF, and aneuploidy screening or diagnostic testing
Second trimester: thickened nuchal fold	≥ 6 mm from outer edge of the occipital bone to outer skin in the midline	LR 11–18.6 with 40–50% sensitivity and > 99% specificity for Down syndrome Most powerful second-trimester marker	1. Detailed anatomic survey 2. Further detailed genetic counseling and aneuploidy screening or diagnostic testing
Second trimester: mild ventriculomegaly	Lateral ventricular atrial measurement between 10–15 mm	Associated with aneuploidy LR 25 for Down syndrome	1. Genetic counseling 2. Second-trimester detailed anatomic ultrasound evaluation 3. Consider diagnostic testing for aneuploidy and CMV 4. Repeat ultrasound in third trimester
Second trimester: choroid plexus cysts	Discrete cyst(s) in one or both choroid plexus(es)	In isolation, no aneuploidy association	1. Second-trimester detailed anatomic survey and fetal cardiac ultrasound 2. No further follow-up if isolated 3. Consider aneuploidy screening or diagnostic testing if other markers are present
Second trimester: short femur length	Measurement < 2.5 percentile for gestational age	LR 1.2–2.2 for Down syndrome. Can be associated with aneuploidy, IUGR, short limb dysplasia	1. Second-trimester detailed fetal anatomic evaluation for short limb dysplasia 2. Further detailed counseling 3. Consider repeat ultrasonography in third trimester for fetal growth

Abbreviations: CF, cystic fibrosis; cfDNA, cell-free DNA; CMV, cytomegalovirus; CRL, crown–rump length; CVS, chorionic villus sampling; IUGR, intrauterine growth restriction; LR, likelihood ratio; NT, nuchal translucency.

Data from Reddy UM, Abuhamad AZ, Levine D, Saade GR. Fetal imaging: executive summary of a joint Eunice Kennedy Shriver National Institute of Child Health and Human Development, Society for Maternal-Fetal Medicine, American Institute of Ultrasound in Medicine, American College of Obstetricians and Gynecologists, American College of Radiology, Society for Pediatric Radiology, and Society of Radiologists in Ultrasound Fetal Imaging workshop. Fetal Imaging Workshop Invited Participants. Obstet Gynecol 2014;123:1070–82; Malone FD, Ball RH, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, et al. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. FASTER Trial Research Consortium. Obstet Gynecol 2005;106:288–94; Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, et al. Role of second-trimester genetic sonography after Down syndrome screening. First and Second Trimester Evaluation of Risk Research Consortium. Obstet Gynecol 2009;114:1189–96; *and* Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. BMJ 1992;304:867–9.

of open fetal defects and relies on the availability of a certified ultrasonographer. Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.

Second-Trimester Serum Screening Tests

Second-trimester serum screening, which typically is performed between approximately 15 0/7 weeks and 22 6/7 weeks of gestation, provides an adjusted risk assessment for Down syndrome, trisomy 18, and open fetal defects. The detection rate with quad screening is similar to first-trimester screening: more than 80% detection at a 5% positive screening test result rate for Down syndrome. Some laboratories offer additional screening for rare disorders such as Smith–Lemli–Opitz syndrome and placental sulfatase deficiency if indicated by an extremely low unconjugated estriol value. Also performed in the second trimester, the triple screen is less sensitive for Down syndrome (sensitivity of 69% at a 5% positive screening test result rate). The penta screen has no prospective validation trials to determine its efficacy; using modeling, it appears to perform well with the inclusion of invasive trophoblast antigen as an additional screening marker (7). None of these screening tests require specialized ultrasonographic measurements, although accurate gestational dating improves risk accuracy determinations.

Integrated Screening

Integrated screening combines first-trimester nuchal translucency and serum analyte screening with second-trimester quad screening to give one result for aneuploidy risk, with a detection rate for Down syndrome of approximately 96% at a 5% positive screening test result rate (Table 2). In addition to having a high sensitivity for Down syndrome, integrated screening provides information that is not available from nuchal translucency assessment regarding fetal abnormalities as well as a risk assessment for open fetal defects. However, integrated screening is complex, requiring first-trimester ultrasound assessment and two different blood tests, and the final result is not available until the second trimester.

Sequential Screening: Stepwise and Contingent Screening

Like integrated screening, both forms of sequential screening have the option of first- and second-trimester testing for a combined final test result. However, the first-trimester screening result is provided to the patient when it is available and, if the patient is found to be at high risk of aneuploidy after the first test, the patient can consider further evaluation with either cell-free DNA screening or with diagnostic testing. This allows the patient to receive an abnormal result in the first trimester when more diagnostic and management options are available.

▶ *What are the limitations of cell-free DNA screening?*

Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing. A large referral-based cytogenetics laboratory reported their experience with 109 consecutive fetal samples from pregnancies that had positive screening test results for cell-free DNA screening from four different laboratories that use varied cell-free DNA screening techniques. Based on cytogenetic confirmation, the positive predictive value, or chance that a positive screening test result was a true positive, using cell-free DNA screening was 93% for Down syndrome, 64% for trisomy 18, 44% for trisomy 13, and 39% for sex chromosome aneuploidy (35). Because the test usually cannot distinguish fetal DNA from maternal DNA, a positive screening test result could represent confined placental mosaicism, a resorbing twin or, in rare instances, a maternal malignancy or maternal aneuploidy (36).

The discrimination of euploid from aneuploid pregnancies with cell-free DNA screening is more effective with larger fetal fractions. At 11–13 weeks, the median fetal fraction of cell-free DNA in maternal plasma is approximately 10% (15). Factors contributing to low fetal fraction include sampling before 10 weeks of gestation, high maternal body mass index, and fetal aneuploidy. In some laboratories, cell-free DNA fractions less than 4% are considered too low to report a result, often referred to as a "no call" result. Recent studies have demonstrated that low fetal fractions indicate a high risk of aneuploidy (22, 23, 28). In one study of more than 1,000 analyzed samples, 8% failed to obtain a result, and 22% of those were aneuploid (28). Pregnancies that initially do not return a cell-free DNA test result because of low fetal fraction can be managed with repeat cell-free DNA screening or diagnostic testing. However, if repeat cell-free DNA screening is performed, this may delay diagnosis of fetal aneuploidy, which may affect reproductive options for an abnormal result.

To date, most published experience with cell-free DNA screening is based on studies conducted on high-risk populations. Data on the performance of cell-free DNA testing in the general obstetric population are now available (22, 37–40). The sensitivity and specificity in the general obstetric population are similar to the

levels previously published for the high-risk population. However, cell-free DNA screening cannot have the same accuracy in low-risk pregnancies (eg, in young women) because the positive predictive value is affected by the prevalence of the disorder in the population. The positive predictive value is lower in the general obstetric population because of the lower prevalence of aneuploidy in this population.

In low-risk populations, there is a larger proportion of false-positive test results among the patients who receive positive screening test results. This decrease in accuracy is especially concerning when pregnancy terminations have been reported in women who have positive screening test results for aneuploidy without a confirmatory cytogenetic result (38). All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken. Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy. Even if cell-free DNA test results are a true positive, cell-free DNA cannot distinguish aneuploidy derived from a translocation or nondisjunction, and this will affect counseling and understanding of the recurrence risk. Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy (28, 39).

Cell-free DNA screening currently gives information about the three most common aneuploidies and about fetal sex but does not typically provide information about other aneuploidies. Without published clinical validation trials, some laboratories have begun to offer cell-free DNA screening for additional disorders, including two forms of aneuploidy associated with nonviable pregnancies (trisomy 16 and trisomy 22) and five or more microdeletion syndromes. A microdeletion syndrome is caused by a chromosomal deletion encompassing contiguous genes that is too small to be detected by conventional cytogenetics. Given the rarity of these disorders, it is uncertain what a positive or negative screening test result means. Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time. For women who wish to know whether their fetus has a microdeletion, the best option is to undergo prenatal diagnostic testing with microarray of fetal cells from CVS or amniocentesis (34, 41).

Cell-free DNA screening tests do not provide information regarding the potential for open fetal defects. Therefore, women who undergo cell-free DNA screening should be offered assessment for open fetal defects with ultrasonography, MSAFP screening, or both.

▶ *How should aneuploidy screening test results be interpreted and communicated?*

Positive and negative screening cutoff levels usually are defined by the different laboratories that perform these analyses. Because of these differences, and because patients interpret information differently, laboratory results should be reported as either positive or negative, and the adjusted numerical risk of aneuploidy based on the test should be provided, regardless of the screening test performed. It also is useful to contrast this risk with the patient's prescreening age-related risk and the general population risk to put the test result in context. Graphical representations of results can be helpful to some patients. After all of this information is provided, the patient's understanding of the results should be confirmed and documented.

▶ *What additional screening or diagnostic tests should be offered after a positive screening test result?*

Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing. Women found to have a positive screening test result from a serum analyte or ultrasound screening test should be offered further detailed counseling and cell-free DNA screening or diagnostic testing by CVS or amniocentesis. Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed. However, use of cell-free DNA screening as a follow-up test for patients with a positive traditional screening test result is reasonable for patients who want to avoid a diagnostic test. However, this approach may delay definitive diagnosis and management. Given that the residual risk of a chromosomal abnormality with a normal cell-free DNA screening test result after an abnormal traditional screening test has been reported to be 2%, patients should be informed of this potential limitation (42). Women with an increased risk of aneuploidy based on cell-free DNA screening should be offered diagnostic testing and should undergo ultrasonography to evaluate for fetal structural anomalies. If MSAFP has not been obtained as part of aneuploidy screening, further screening for open fetal defects with MSAFP or ultrasonography should be offered. In addition, evaluation for fetal anomalies in the second trimester is appropriate for all patients. In the first trimester, maternal serum levels of

pregnancy-associated plasma protein A below the fifth percentile are independently associated with obstetric complications, such as spontaneous fetal and neonatal loss, fetal growth restriction, preeclampsia, placental abruption, and preterm delivery (43). In the second trimester, elevated hCG, AFP, and dimeric inhibin A levels in pregnancies without structural anomalies are associated with an increased risk of fetal death, intrauterine growth restriction, and preeclampsia (44, 45). The likelihood of an adverse pregnancy outcome increases with increasing number of abnormal markers in the same screening test and with more extreme analyte values (46). Although potential management strategies for women with abnormal serum markers have been proposed, they are not evidence based (46).

If a patient conceives and has undergone preimplantation genetic screening, prenatal screening for aneuploidy still should be offered because false-negative test results can occur with preimplantation genetic screening (47). Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during pregnancy.

▶ *How does screening for aneuploidy differ in multifetal gestations?*

In multifetal gestations, the risk of fetal aneuploidy is affected by the number of fetuses and the zygosity of the pregnancy; however, data regarding the risk of aneuploidy are more limited in multiple gestations compared with singleton pregnancies. In dizygous twin pregnancies, each fetus carries a risk of aneuploidy generally similar to the mother's age-adjusted risk, but the mother carries an increased risk of having a fetus with aneuploidy because there is more than one fetus. Typically, monozygous twins will have the same karyotype, with neither or both fetuses being affected; the risk of carrying aneuploid fetuses is similar to the mother's age-adjusted risk.

No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Analysis of the risks and benefits of screening or diagnostic testing in women carrying multiple fetuses is much more complicated, given the diminished effectiveness of screening and how the prenatal identification of a single aneuploid fetus might affect the pregnancy management. Diagnostic testing may be less acceptable to women with multiple gestations because of the increased difficulty and higher potential loss rates.

Nuchal translucency measurement allows each fetus in a multifetal pregnancy to be screened independently and, therefore, can be used in twin or high-order multifetal gestations. The distribution of nuchal translucency measurements does not differ significantly between singletons and multiples, and standard cutoffs can be used (48). One study reviewed individual first-trimester screening in twin gestations and generated individual risks for each fetus with nuchal translucencies and first-trimester screening. At a 1:300 cutoff, the detection rate was 75% with a 9% positive screening rate for trisomy 21 (49). However, the review concluded that a greater reliance should be placed on nuchal translucency to evaluate the fetuses for aneuploidy. A single enlarged nuchal translucency in monochorionic twins of discordant size could be an early sign of twin–twin transfusion syndrome rather than aneuploidy (50). These patients should be evaluated further for this possibility.

Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies. First-trimester, quad, and combined serum analyte screening are options available to screen twin gestations, although few data are available from prospective studies with regard to screening. Analyte screening test results typically are provided for the entire gestation and not each individual fetus. Second-trimester serum screening of twin gestations can identify approximately 50% of fetuses affected with Down syndrome at a 5% positive screening rate (51). Because of limited evidence regarding its efficacy, cell-free DNA testing is not recommended for aneuploidy screening in women with multiple gestations (34).

In multifetal gestations, if fetal demise or an anomaly is identified in one fetus, serum-based aneuploidy screening should be discouraged. There is a significant risk of an inaccurate test result in these circumstances. The patient should be offered counseling and consider diagnostic testing instead of a screening test. The accuracy of aneuploidy screening in a multiple gestation with a fetus that has an empty gestational sac is not known.

Summary of Recommendations and Conclusions

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- Women who have a negative screening test result should not be offered additional screening tests for aneuploidy because this will increase their potential for a false-positive test result.
- If an enlarged nuchal translucency, an obvious anomaly, or a cystic hygroma is identified on ultrasonography, the patient should be offered genetic counseling and diagnostic testing for aneuploidy as well as follow-up ultrasonography for fetal structural abnormalities.

- Patients with an enlarged nuchal translucency or cystic hygroma and normal fetal karyotype should be offered an anatomic evaluation in the second trimester, fetal cardiac ultrasonography, and further counseling regarding the potential for genetic syndromes not detected by aneuploidy screening.
- Women who undergo first-trimester screening should be offered second-trimester assessment for open fetal defects (by ultrasonography, MSAFP screening, or both) and ultrasound screening for other fetal structural defects.
- Because cell-free DNA is a screening test, it has the potential for false-positive and false-negative test results and should not be used as a substitute for diagnostic testing.
- All women with a positive cell-free DNA test result should have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken.
- Women whose cell-free DNA screening test results are not reported, are indeterminate, or are uninterpretable (a no call test result) should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.
- Women with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing.

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- Cell-free DNA screening tests for microdeletions have not been validated clinically and are not recommended at this time.
- Patients who conceive after preimplantation genetic screening for aneuploidy should be offered aneuploidy screening and diagnosis during their pregnancy.
- No method of aneuploidy screening is as accurate in twin gestations as it is in singleton pregnancies. Because data generally are unavailable for higher-order multifetal gestations, analyte screening for fetal aneuploidy should be limited to singleton and twin pregnancies.

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- Screening for aneuploidy should be an informed patient choice, with an underlying foundation of shared decision making that fits the patient's clinical circumstances, values, interests, and goals.
- Aneuploidy screening or diagnostic testing should be discussed and offered to all women early in pregnancy, ideally at the first prenatal visit.
- All women should be offered the option of aneuploidy screening or diagnostic testing for fetal genetic disorders, regardless of maternal age.
- If an isolated ultrasonographic marker for aneuploidy is detected, the patient should be offered aneuploidy screening if it was not offered previously.
- Some women who receive a positive test result from traditional screening may prefer to have cell-free DNA screening rather than undergo definitive testing. This approach may delay definitive diagnosis and management and may fail to identify some fetuses with aneuploidy.
- Parallel or simultaneous testing with multiple screening methodologies for aneuploidy is not cost-effective and should not be performed.
- In multifetal gestations, if fetal demise or an anomaly is identified in one fetus, serum-based aneuploidy screening should be discouraged. There is a significant risk of an inaccurate test result in these circumstances.

For More Information

The American College of Obstetricians and Gynecologists has identified additional resources on topics related to this document that may be helpful for obgyns, other health care providers, and patients. You may view these resources at www.acog.org/more-info/AneuploidyScreening.

These resources are for information only and are not meant to be comprehensive. Referral to these resources does not imply the American College of Obstetricians and Gynecologists' endorsement of the organization, the organization's web site, or the content of the resource. The resources may change without notice.

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51. Neveux LM, Palomaki GE, Knight GJ, Haddow JE. Multiple marker screening for Down syndrome in twin pregnancies. Prenat Diagn 1996;16:29–34. (Level III)

The MEDLINE database, the Cochrane Library, and the American College of Obstetricians and Gynecologists' own internal resources and documents were used to conduct a literature search to locate relevant articles published between January 1985–July 2014. The search was restricted to articles published in the English language. Priority was given to articles reporting results of original research, although review articles and commentaries also were consulted. Abstracts of research presented at symposia and scientific conferences were not considered adequate for inclusion in this document. Guidelines published by organizations or institutions such as the National Institutes of Health and the American College of Obstetricians and Gynecologists were reviewed, and additional studies were located by reviewing bibliographies of identified articles. When reliable research was not available, expert opinions from obstetrician–gynecologists were used.

Studies were reviewed and evaluated for quality according to the method outlined by the U.S. Preventive Services Task Force:

- I Evidence obtained from at least one properly designed randomized controlled trial.
- II-1 Evidence obtained from well-designed controlled trials without randomization.
- II-2 Evidence obtained from well-designed cohort or case–control analytic studies, preferably from more than one center or research group.
- II-3 Evidence obtained from multiple time series with or without the intervention. Dramatic results in uncontrolled experiments also could be regarded as this type of evidence.
- III Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

Based on the highest level of evidence found in the data, recommendations are provided and graded according to the following categories:

Level A—Recommendations are based on good and consistent scientific evidence.

Level B—Recommendations are based on limited or inconsistent scientific evidence.

Level C—Recommendations are based primarily on consensus and expert opinion.

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