Aneuploidy Screening Protocol

Aneuploidy screening or diagnostic testing should be discussed and offered to all pregnancy patients early in pregnancy, ideally at the first prenatal visit.

- **S**creening is designed to assess risk.
- 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.

There are a variety of screening test options, each offering varying levels of information and accuracy.

No one screening test is superior to other screening tests in all test characteristics.

Obstetric care providers are expected to discuss not only the risk of aneuploidy (see Table 1) but also the benefits, risks, and limitations of available screening tests (see Table 2).

Screening for aneuploidy should be an informed patient choice.

Recommendations for patients who desire aneuploidy screening:

- Regardless of the patient's baseline risk, all patients should be offered screening for chromosomal anomalies, including nuchal translucency (NT) measurement, serum screening and cell-free DNA (cfDNA), in addition to offering diagnostic testing.
- Test characteristics, advantages and disadvantages of screening options are listed in table 2.
- High risk patients based on ultrasound findings or screening test results should be counseled about advantages and disadvantages of advanced screening options vs diagnostic testing (see Table 4).

Aneuploidy screening in multiple gestations:

- Screens that include a serum sample will be less accurate in twin gestations as compared to singleton gestations.
    - No data available for higher order multiple gestations
- Consider obtaining genetic counseling to allow a more detailed discussion of the options listed below
- The decision regarding the appropriate screening option for patients with twin gestations is complex, and patients should be counseled prior to any screening or testing. Options for aneuploidy screening in twins include the following:
    - Combined first trimester screen with NT and serum analytes.
        - NT measurement directly evaluates each of the individual twins
        - Detection rate for Down syndrome 75-89% with 5% false positive.
        - Detection rate for T18 66.7%
    - Quad screen: If patients present after first trimester, serum Quad screening can be used in twin gestations and can identify

approximately 60% of fetuses affected with Down syndrome at a 5% positive screen rate.
    - CfDNA testing can be performed in twin gestations
        - Sensitivity for Trisomy 21 screening may be similar to singleton gestations (98-99%). Accurate detection rate for Trisomy 13 or Trisomy 18 is not definitely determined but appears to be >90% based on limited numbers.
        - CfDNA will give one test report for a twin pregnancy.
- Obtaining an ultrasound at 11-13 weeks for all twin gestations may be reasonable regardless of desires for genetic screening. This can allow determination of chorionicity and can serve as an early screen for certain twin-related complications.

Additional comments:

- Patients with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing.
- All patients should be offered second-trimester ultrasound to screen for fetal structural defects, ideally performed between 18-22 weeks, with or without second trimester MSAFP.
- CfDNA is the most sensitive and specific screening test for common aneuploidy, but still it has the potential for false-positive and false-negative test results. CfDNA should not be used as a substitute for diagnostic testing.
- CfDNA testing is only recommended for T21, T18, T13 and sex chromosome anomalies. Testing of other forms of aneuploidy, microdeletion syndromes or genome-wide copy number variants is not recommended in all-comers due to insufficient data, but these screens may be performed in a select population following appropriate counseling.
- All pregnant patients with a positive cfDNA test result should be recommended to have a diagnostic procedure before any irreversible action, such as pregnancy termination, is taken.
- Patients whose cfDNA 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.
- Patients with a low risk cfDNA screening test result for fetal aneuploidy should not undergo subsequent first trimester screening. (Society for Maternal-Fetal Medicine. Ultrasound and cell-free DNA screening. Am J Obstet Gynecol 2017)

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Table 1. Chromosomal Abnormalities in Second-Trimester Pregnancies Based on Maternal Age at Term

	Trisomy 21	Trisomy 18	Trisomy 13	Sex Chromosome Aneuploidy (XXX, XY, XYY, 45, X)	Microarray or Rare Chromosomal Abnormality	All Chromosomal Abnormalities
Age 20	8 per 10,000 1 in 1,250	2 per 10,000 1 in 5,000	1 per 10,000 1 in 10,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	82 per 10,000 1 in 122
Age 25	10 per 10,000 1 in 1,000	2 per 10,000 1 in 5,000	1 per 10,000 1 in 10,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	84 per 10,000 1 in 119
Age 30	14 per 10,000 1 in 714	4 per 10,000 1 in 2,500	2 per 10,000 1 in 5,000	34 per 10,000 1 in 294	37 per 10,000 1 in 270	91 per 10,000 1 in 110
Age 35	34 per 10,000 1 in 294	9 per 10,000 1 in 1,111	4 per 10,000 1 in 2,500	35 per 10,000 1 in 285	37 per 10,000 1 in 270	119 per 10,000 1 in 84
Age 40	116 per 10,000 1 in 86	30 per 10,000 1 in 333	14 per 10,000 1 in 714	51 per 10,000 1 in 196	37 per 10,000 1 in 270	248 per 10,000 1 in 40

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

Screening Approach	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate (DR) for Trisomy 21 (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
Cell-free DNA†	9–10 to term	99	2–4% Includes inability to obtain results, which is associated with increased risk†	1. Highest DR 2. Can be performed at any gestational age after 9–10 weeks 3. Lowest false-positive rate	Results may reflect underlying maternal aneuploidy or maternal disease	Several molecular methods
First trimester‡	10–13 6/7§	82–87‖	5	1. Early screening 2. Single time point test	Lower DR than tests with first and second trimester component NT required	NT+PAPP-A, free beta hCG, +/− AFP¶
Quad screen‡	15–22	81	5	1. Single time point test 2. No specialized US required	Lower DR than first trimester and first and second trimester combined tests	hCG, AFP, uE3, DIA
Integrated‡	10–13 6/7§, then 15–22	96	5	High DR	Two samples needed No first-trimester results NT required	NT+PAPP-A, then quad screen
Serum integrated‡	10–13 6/7§, then 15–22	88	5	1. DR compares favorably with first-trimester screening 2. No specialized US required	Two samples needed No first-trimester results	PAPP-A + quad screen
Sequential#: stepwise	10–13 6/7§, then 15–22	95	5	1. First-trimester results provided 2. Comparable performance to integrated, but FTS results provided	Two samples needed NT required	NT+ free beta hCG + PAPP-A, +/− AFP¶, then quad screen
Contingent screening**		88–94	5	First-trimester test result: Positive: diagnostic test or cell-free DNA offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed NT required	NT+hCG+ PAPP-A, +/− AFP¶, then quad screen

(*continued*)

Table 2. Characteristics, Advantages, and Disadvantages of Common Screening Tests for Chromosomal Abnormalities (*continued*)

Screening Approach	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate (DR) for Trisomy 21 (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
Nuchal translucency alone#	10–13 6/7§	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; NPV, negative predictive value; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein A; PPV, positive predictive value; uE3, unconjugated estriol; US, ultrasonography.

All patients should be offered second-trimester assessment for open fetal defects (by ultrasonography, with or without second-trimester serum AFP) and ultrasound screening for other fetal structural defects.

*A screen positive test result includes all positive test results: the true positives and false positives. For cell-free DNA, this includes the test failure rates given the association with increased risk of aneuploidy (see † below).

†Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2017;50:302–14.

‡First-trimester combined screening: 87%, 85%, and 82% for measurements performed at 11 weeks, 12 weeks, and 13 weeks, respectively (Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium. N Engl J Med 2005;353:2001–11.)

§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.

‖Use of free beta hCG in conjunction with nasal bone assessment increases the detection rate to 97% with a screen positive rate of 5% (Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. Prenat Diagn 2003;23:306–10.)

¶Testing of first trimester AFP depends on commercial lab used. First trimester AFP should not be used in lieu of second trimester AFP for open fetal defects screening.

#Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, et al. First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening. Cochrane Database of Systematic Reviews 2017, Issue 3. Art. No.: CD012600. DOI: 10.1002/14651858.CD012600.

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

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Table 3. The Effect of Maternal Age on the Positive Predictive Value of Cell-Free DNA Screening for Trisomy 21, 18, and 13 at 10 Weeks Gestation*

	Maternal Age	Age Related Risk†	Positive Predictive Value‡
Trisomy 21	20	1:804 or 12 per 10,000	38–80%
	35	1:187 or 53 per 10,000	73–95%
	40	1:51 or 196 per 10,000	91–99%
Trisomy 18	20	1:1,993 or 5 per 10,000	11–41%
	35	1:465 or 22 per 10,000	34–75%
	40	1:126 or 79 per 10,000	66–92%
Trisomy 13	20	1:6,347 or 1.6 per 10,000	5–13%
	35	1:1,481 or 7 per 10,000	17–40%
	40	1:401 or 24 per 10,000	43–71%

*Sensitivity and specificity approximately 99%

†Age related risk of aneuploidy per 10,000 pregnancies at 10 weeks gestation based on maternal age at term

‡Percent varies by laboratory

Adapted from University of North Carolina at Chapel Hill. Positive predictive value of cell free DNA calculator. Available at: https://www.med.unc.edu/mfm/nips-calc. Retrieved February 24, 2020.

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Table 4. Management of Ultrasonographic Markers for Aneuploidy

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
First trimester: enlarged nuchal translucency	Certified US measurement ≥3.0 mm or above the 99th percentile for the CRL	Aneuploidy risk increases with size of NT Also associated with other structural anomalies and genetic disorders	Genetic counseling. Offer diagnostic testing. Comprehensive US evaluation including a detailed US at 18–22 weeks. Fetal cardiac US may be considered if the NT is 3.0–3.4 and is recommended if the NT is 3.5 or greater.
First trimester: cystic hygroma	Large single or multilocular fluid-filled cavities, in the nuchal region and can extend the length of the fetus	About 50% are aneuploid	Genetic counseling. Offer diagnostic testing. Comprehensive US evaluation including a detailed US at 18–22 weeks and fetal cardiac US.
Second trimester: thickened nuchal fold	≥6 mm from outer edge of the occipital bone to outer skin in the midline at 15–20 weeks	Associated with Trisomy 21	Detailed anatomic survey. Genetic counseling. Aneuploidy testing should be offered if not previously performed.
Second trimester: absent or hypoplastic nasal bone	Nonvisualization of the nasal bone or nasal hypoplasia based on multiples of the median (MoM) or percentiles or the biparietal diameter/nasal bone length (BPD/NBL) ratio	Varies by race/ethnicity Absent in 30–40 percent fetuses with Trisomy 21 and 0.3 to 0.7 percent of euploid fetuses Hypoplastic in about 50–60 percent of fetuses with Trisomy 21 and 6 to 7 percent of euploid fetuses	Detailed anatomic survey. Genetic counseling. Aneuploidy testing should be offered if not previously performed.
Second trimester: pyelectasis	Renal pelvis measuring ≥4 mm in anteroposterior diameter up to 20 weeks of gestation	Associated with Trisomy 21	If isolated finding, aneuploidy testing should be offered if not previously performed. Repeat US in third trimester to assess need for postnatal imaging.
Second trimester: echogenic bowel	Fetal small bowel as echogenic as bone	Associated with Trisomy 21, intra-amniotic bleeding, CF, CMV, and FGR	Detailed anatomic survey. Genetic counseling. Offer CMV, CF, and aneuploidy testing. Consider follow up US for fetal growth because of the association with FGR.
Second trimester: mild to moderate ventriculomegaly*	Lateral ventricular atrial measurement measures between 10–15 mm	Associated with Trisomy 21, infection.	Detailed anatomic survey. Genetic counseling. Offer diagnostic testing for genetic conditions and CMV. Consider fetal MRI. Repeat US in third trimester.

(*continued*)

Table 4. Management of Ultrasonographic Markers for Aneuploidy (*continued*)

Soft Marker	Imaging Criteria	Aneuploidy Association	Management
Second trimester: short femur length	Measurement <2.5 percentile for gestational age	Can be associated with aneuploidy, FGR, skeletal dysplasia, or other genetic diagnosis	Aneuploidy testing should be offered if not previously performed. Consider repeat US in third trimester for fetal growth.
Second trimester: echogenic intracardiac foci	Echogenic tissue in one or both ventricles of the heart seen on standard four-chamber view	Seen in 15–30% of fetuses with trisomy 21 and 4–7% euploid fetuses	If isolated finding, aneuploidy testing should be offered if not done previously. Describe finding as not clinically significant or as a normal variant with normal screening.
Second trimester: choroid plexus cysts	Discrete small cyst(s) in one or both choroid plexus(es)	Seen as an isolated finding in 1–2% of the normal population. Associated with trisomy 18 when seen in combination with other anomalies.	If isolated finding, aneuploidy testing should be offered if not previously performed. Describe finding as not clinically significant or as a normal variant with normal screening.

Abbreviations: CF, cystic fibrosis; cfDNA, cell-free DNA; CMV, cytomegalovirus; CRL, crown–rump length; CVS, chorionic villus sampling; FGR, fetal growth restriction; MRI, magnetic resonance imaging; NT, nuchal translucency; US, ultrasound.

*Fox NS, Monteagudo A, Kuller JA, Craigo S, Norton ME. Mild fetal ventriculomegaly: diagnosis, evaluation, and management. Society for Maternal–Fetal Medicine (SMFM) Consult Series #45. Am J Obstet Gynecol 2018;219:B2–9.

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