



Position Statement from the Aneuploidy Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis, April 2013

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BACKGROUND

Definitive prenatal diagnosis of chromosome abnormalities through the analysis of amniocytes or chorionic villus samples (CVS) is an accepted part of prenatal care. Chromosome numerical changes (aneuploidy, polyploidy), large deletions and duplications, and rearrangements can be detected through conventional chromosome analysis (karyotyping) and smaller copy number variations can be detected using microarrays (Wapner et al., 2012). However, amniocentesis and CVS procedures carry some degree of risk for miscarriage or other pregnancy complications (Tabor and Alfirevic, 2010). Therefore, in most developed countries it is routine practice to provide a woman's personal risk for fetal aneuploidy (screening) and to offer definitive diagnosis through amniocentesis or CVS if the risk is high. In the USA it has been recommended that amniocentesis and CVS should be available to all women whether or not they had aneuploidy screening (American College of Obstetricians, and Gynecologists, 2007a) although it is recognized that screening can be helpful to women before they decide whether to accept or reject invasive prenatal diagnostic testing (American College of Obstetricians, and Gynecologists, 2007b). Many countries, for example Canada, have national guidelines that recommend aneuploidy screening prior to invasive testing (Chitayat et al, 2011). In other countries there is no such national recommendation and in some countries and regions, a large proportion of women do receive invasive prenatal diagnosis regardless of screening results.

Fetal aneuploidy risk can be evaluated on the basis of a combination of maternal age, prior affected pregnancy or family history, maternal serum biochemical tests and fetal ultrasound markers (Cuckle and Benn, 2010). Recently, new non-invasive prenatal testing based on massively parallel sequencing of circulating free fetal DNA (cfDNA) in maternal plasma has been shown to be highly effective for aneuploidy detection. These analytic validation studies were performed on stored samples from women who were established as being high-risk on the basis of maternal age and/or maternal serum and

ultrasound markers (Chiu *et al.*, 2011; Ehrich *et al.*, 2011; Palomaki *et al.*, 2011; Palomaki *et al.*, 2012; Bianchi *et al.*, 2012; Sparks *et al.*, 2012; Ashoor *et al.*, 2012; Norton *et al.*, 2012). Based on these reports, this approach would appear to be the most effective method for screening for fetal trisomy 21 and trisomy 18 but is not fully diagnostic. Further clinical validation studies of maternal cfDNA screening are emerging, including studies on low risk women (Nicolaidis *et al.*, 2012).

All approaches to risk assessment appear to provide an opportunity to re-assure most women that their fetus is unlikely to be affected by a chromosomal disorder (and thereby reduce the number of unnecessary invasive procedures performed) while identifying those women at highest risk for an affected pregnancy. Potential follow up options for women who are identified as being at high risk based on any of these screening options can include further counseling, additional testing and appropriate follow-up obstetric care.

Because Down syndrome (trisomy 21) is the most common significant aneuploidy, prenatal screening has emphasized the detection of this disorder. However, it is recognized that many of the screening tests have a variable potential to detect other aneuploidies, some other genetic disorders, specific fetal anatomic abnormalities, and pregnancy complications such as preeclampsia.

GOAL OF FETAL ANEUPLOIDY RISK EVALUATION

Every pregnant woman should have the opportunity to receive the best possible estimate of her personal risk for fetal aneuploidy. Programs involved in risk evaluation aim to provide timely and accurate individual patient-specific estimates of risk for the most common and clinically significant fetal aneuploidies.

COUNSELING AND THE PROVISION OF PRENATAL SCREENING

Aneuploidy risk assessment is a component of a broad set of prenatal clinical services that should be offered from 9-13 weeks gestational age whenever possible. Services can include genetic counseling, screening for pregnancy complications and other fetal conditions, diagnostic testing (chromosome analysis, microarray analysis, other genetic testing), midwifery and obstetrical interventions. For women who only come into care after the first trimester, risk assessment testing should be made available as soon as possible.

Prior to undergoing prenatal screening, women should be given information on the screening process and be provided with an opportunity to discuss this with a health professional before making a personal decision to accept or decline screening. Whenever possible, the results from more than one screening approach in the same pregnancy should be combined into an accurate unified risk assessment. Following the screening, results should be explained in the context of the hazards and benefits of definitive diagnosis through amniocentesis and CVS.

Information must be provided through non-directive counseling. Each woman should make her own determination as to whether she wishes to receive screening and diagnostic services. Respect for ethical and cultural values, sensitivities and the decisions made by each patient are of key importance in the provision of prenatal testing services.

Prenatal aneuploidy risk assessment services often vary according to the healthcare systems that are present in different countries. Furthermore, service delivery may be modified to reflect individual women's clinical conditions such as infertility, past obstetrical history, co-existing risk for other genetic disorders, or their moral and ethical values. Use of risk cut-offs in recommendations for diagnostic testing, sequential versus concomitant offers of screening and diagnostic testing, and other programmatic differences exist. Providers may have differing opinions on these standards of care and differing access to the economic resources needed to provide risk assessment services. It is recognized that there are diverse approaches to these patient services that are compatible with beneficence to both individual women and to the populations served.

MEASURING EFFICACY OF PROTOCOLS

The efficacy of biochemical and ultrasound risk assessment protocols has traditionally been based on the detection rate (DR, or sensitivity), false-positive rate (FPR), and positive predictive value (PPV), or odds of being affected given a positive result (OAPR). These population-based screening performance indices are of considerable value in comparing different protocols. The relative efficacy of different protocols can be assessed by either fixing the FPR (between 1% and 5%) and comparing the DR, or fixing the DR (between 75% and 90%) and comparing the FPR. For a fixed risk cut-off, both the DR and FPR will vary between protocols. Statistical modeling using observational data is a reliable way of estimating the DR, FPR and OAPR of different screening protocols. Table 1 presents the modeled performance for various serum and ultrasound protocols for a fixed 3% FPR. Intervention studies can overestimate the screening performance but may provide important information on the practicality of a specific protocol.

Different criteria have been used in published studies to evaluate the efficacy of maternal cfDNA screening. Criteria include the DR and FPR defined by proportion of cases with a departure from the expected chromosome specific sequence counts (z-score) greater than 3 (Palomaki et al., 2011), or a normalized chromosome count greater than 4 or less than 2.5 (with intermediate values between 2.5 – 4.0 considered to be uninterpretable) (Bianchi et al., 2012). One algorithm includes maternal age, gestational age and the proportion of fetal DNA present with results presented as screen-positive or screen-negative based on a 1/100 cut-off (Sparks *et al.*, 2012). Direct comparison of the various clinical trials and approaches applied to studies of high-risk women (Table 2) is also confounded by the criteria used to select study cases, depth of sequencing, adjustments for GC content of the sequences, and number of acceptable mismatches in sequences and test failure criteria. None of the studies are sufficiently large to exclude occasional false-positive or false-negative results even when intermediate results are excluded (Benn *et al.*, 2012).

The full disclosure of the laboratory testing protocols, ultrasound markers, and computational methods used to obtain the results is essential. Individual test reports should contain the test numerical data with appropriate interpretative comments.

CHOICE OF PROTOCOL

(a) Maternal age alone

The use of maternal age as a sole criterion for aneuploidy risk assessment is not justifiable.

(b) Biochemical and serum markers

A range of maternal serum biochemical and fetal ultrasound markers have well-documented efficacy in distinguishing between affected and unaffected pregnancies. Each has validity within a specified time interval in pregnancy and should not be offered at earlier or later gestational ages. Combination of markers is valid, provided the correlation between them has been taken into consideration in the risk calculation.

Various first and second trimester approaches to aneuploidy screening as well as combinations of the two are listed in Table 1. Results are based on nuchal translucency (NT) at 12 weeks gestational age. This is generally preferred over 11 weeks in order to facilitate optimal patient scheduling, because fetal anatomy is more clearly visualized and is better than 13 weeks because the screening performance is superior.

A protocol based on first trimester measurement of NT for all women, with no additional tests, is insufficient for aneuploidy risk evaluation. However, NT is considered to be an extremely important marker because of the additional associations of large NT with cardiac defects and other serious fetal defects (Syngelaki *et al.*, 2011). It is common practice to regard chromosomally normal cases with increased NT as high risk for a broad range of fetal abnormalities. First trimester ultrasound examination with NT measurement should therefore be made available even when alternative screening and invasive diagnostic tests for fetal aneuploidy are being provided. NT measurement should be performed in centers with experience and demonstrated proficiency.

First trimester aneuploidy screening (the ‘combined’ test) is more advantageous than second trimester screening (the ‘quadruple’ test) not only because information is available earlier in pregnancy but also because the screening has greater efficacy (compare protocols 1a, b, c, d with 2a, b in Table 1). The combined test is the strategy that most European Public health agencies have chosen for their population based prenatal aneuploidy screening. The quadruple test can be provided from 14 to 21 weeks but 15-19 weeks is preferred because 15-19 weeks is optimal for open neural tube screening using AFP.

Many women who receive a first trimester risk estimate that is intermediate between very high or moderately low risk may benefit from the provision of additional screening tests in the second trimester (‘contingent’ screening) and this can be associated with highly effective screening (protocols 3a,b). Additional testing for those with low first trimester

risks ('step-wise' screening) can also be considered (protocols 3c,d) although this should not be needed for the majority of cases with very low first trimester risks (e.g. <1 in 1,500 at term). For both contingent and step-wise screening it is essential that the second trimester risk estimation incorporate both the first and second trimester tests that have been performed. The provision of separate risk assessments based on first trimester markers alone and second trimester markers alone ('independent' screening) should not be carried out as it is associated with a significantly higher overall false-positive rate and difficulties with second trimester counseling using two separate risk estimates. Protocols that include first and second trimester tests but only provide a risk figure after all screening tests are complete ('integrated' screening) are also associated with a high detection rate and low false-positive rate but will delay reassurance and/or restrict women's options in the first trimester (protocols 4a,b). When the same marker is tested in both trimesters ('repeat measures') there can be an additional benefit (protocols 4c, d).

The provision of additional first trimester sonographic markers can obviate the need for second trimester aneuploidy screening (Sonek and Nicolaides, 2010). The most widely used markers are absence of a fetal nasal bone (NB), tricuspid regurgitation (TR) determined by pulse wave Doppler ultrasound and abnormal blood flow in the ductus venosus (DV). The routine use of these markers can substantially increase detection (protocol 5a), but good results are also obtained when this is done contingently at specialist centers (protocols 5b, c, d). Use of ultrasound needs to be consistent with fetal safety recommendations; i.e. with an ultrasound exposure that is as low as reasonably achievable (AIUM Practice Guideline, 2007).

Aneuploidy screening can also be improved by additional second trimester ultrasound markers. One emerging approach is to measure three facial profile markers concurrently with the quadruple test (Miguelez *et al.*, 2010). These facial profile markers are nuchal fold thickness (NF), nasal bone length (NBL) and prenasal thickness (PT). The model predicted results are comparable with a first trimester combined test (protocol 6a, b). Currently, NF is more widely used than NBL and PT and additional clinical studies demonstrating the utility of the latter markers are expected.

In centers that routinely perform a 'genetic sonogram' or 'anomaly scan' at 18-23 weeks, presence or absence of a number of specific characteristics can be combined to assess risk (Aagaard-Tillery *et al.*, 2009). Findings that have been reported to be useful in modifying aneuploidy risk (abnormalities, anomalies and "markers") include major malformations (MM), increased nuchal fold thickness (NF), short femur or humerus length (FL or HL), echogenic intracardiac focus (EIF), pylectasis (P), echogenic bowel (EB), ventriculomegaly (V), and absent or hypoplastic nasal bone (NB). NF, FL, and HL should be expressed as continuous variables (e.g. with results expressed as MoMs) rather than categorical (i.e. on the basis of a value above or below a specific cut-off) because use of continuous variables maximizes the discriminatory power of the test and results in more specific information for each woman. Presence of EIF, P, and EB need to be based on objective criteria. Regional policies vary considerably with respect to the perceived value of the genetic sonogram and the individual markers that may be included (see for

example, policies adopted by the UK and Canada (UK National Screening Committee, 2002; Van der Hof et al., 2005)).

The genetic sonogram can be used for women who have received first trimester screening (protocols 7a, b), second trimester screening (protocols 8b, c) or both (protocols 9a, b). Although the second trimester anomaly scan can be used simply to modify the maternal age-specific aneuploidy risk alone, it is not a very effective screening test (protocol 8a). Using it to modify the risk following other aneuploidy screening can improve detection but when, as often happens, this is restricted to women with screen-positive results it can actually reduce detection. The genetic sonogram in combination with maternal age can be useful for women first receiving prenatal care at 21-23 weeks where rapid information about risk may be required.

Aneuploidy risks based on both NT and serum markers can be provided for twin pregnancies, despite poorer performance of the serum markers than in singletons. First trimester screening should take into consideration chorionicity; monochorionic twins are assumed to be monozygotic with an identical risk for each fetus while the majority of dichorionic twins are dizygotic and will be provided with separate risks for each fetus. First trimester serum markers require the use of gestation-specific and chorionicity-specific correction factors (Madsen *et al.*, 2011). Second trimester screening with serum markers alone is considerably less accurate than that in singleton pregnancies. For triplets and higher multiples, risks should be based on ultrasound markers alone. In the situation where there has been an early fetal loss (“vanishing twin”), the serum markers may be un-interpretable (Spencer et al., 2010).

(c) Maternal cfDNA screening

Various strategies have been proposed to develop screening or diagnostic tests for fetal aneuploidy by analyzing free fetal nucleic acids present in maternal plasma. However, at this time only those cfDNA analyses based on massively parallel sequencing with either “shotgun” counting of all free DNA sequences (Palomaki *et al.*, 2012a; Palomaki *et al.*, 2012b; Bianchi *et al.*, 2012) or “targeted” counting of specific DNA sequences (Sparks, *et al.*, 2012; Ashoor *et al.*, 2012; Norton *et al.*, 2012) have been sufficiently validated in trials to be considered analytically sound. Although rapid progress is being made in the development and validation of this technology, demonstration that in actual clinical practice the testing is sufficiently accurate, has low failure rates and can be provided in a timely fashion has not yet been provided. Therefore, at the present time the following caveats need to be considered by physicians, counselors and women considering this testing:

- Reliable non-invasive maternal cfDNA aneuploidy screening methods have only been reported for trisomy 21 and 18. cfDNA screening results have been reported for trisomy 13 but the numbers are not large and efficacy appears to be less than for trisomies 21 and 18. cfDNA screening results have also been reported for sex chromosome aneuploidy and the efficacy is unacceptably low

- There are insufficient data available to judge whether any specific cfDNA screening method is most effective
- The tests should not be considered to be fully diagnostic and therefore are not a replacement for amniocentesis and CVS. Some affected pregnancies may not be detected and there may be false-positive results.
- Analytic validity trials have been mostly focused on patients who are at high risk on the basis of maternal age or other screening tests. Efficacy in low risk populations has not yet been fully demonstrated. There are currently only limited data to suggest the test failure rate will not be appreciably higher for low-risk women (Brar et al., 2012) and the false-positive rate also appears to be comparable (Nicolaidis, et al., 2012)..
- There is insufficient information to know how well the test will perform in multiple gestation pregnancies that are discordant for trisomy but, theoretically, the detection of affected pregnancies could be lower than in singletons (Canick *et al.*, 2012). When there has been a known early demise of a co-twin (“vanishing twin”), results may be inaccurate.
- In cases where mosaicism is present (including confined placental mosaicism) results may be inaccurate.
- In a proportion of cases there is insufficient fetal cfDNA in the maternal plasma specimen or there is test failure for other reasons (Table 2). It is not known what proportion of women with insufficient fetal cfDNA or a failed or uninterpretable test would have an informative repeat test result. In addition, one of the cfDNA screening methods classifies a proportion of results as “unclassified” when they are in fact at somewhat increased risk of aneuploidy (Benn *et al.*, 2012)
- Specific independently developed laboratory minimum standards, quality control, proficiency testing and inspection requirements have not yet been developed for this testing. *It is expected that quality control standards will be developed and the ISPD strongly cautions providers to seek out laboratory services that meet national guidelines for quality control and proficiency testing that is the current standard for other molecular tests.*
- It has not been demonstrated that the test can be provided in a cost-effective, timely, and equitable manner to total populations.

Women interested in such testing should receive detailed counseling that explains the benefits and limitations of the test. cfDNA screening should only be provided after they have been informed that these tests are still under clinical development

Information that must be provided to the pregnant woman includes:

- (1) The testing currently available is mostly focused on the detection of fetal trisomies 21, 18, and 13.
- (2) Although detection rates are high, the test does not detect all cases of fetal trisomy 21, 18 and 13.

- (3) Although false-positive rates are low, there will be occasional false-positive results and therefore women with positive cfDNA screening results should be offered confirmatory fetal chromosome analysis either through an amniocentesis or CVS.
- (4) For some women the cfDNA screening test may not be informative and these patients may then need to consider invasive testing. In particular, women with an increased body mass index are at high risk of test failure or an inconclusive result. For late gestational age women, there may be insufficient time for a repeat screening test and/or invasive testing.

GENERAL CONSIDERATIONS FOR ALL ANEUPLOIDY SCREENING

When there is a known history of a previous pregnancy with trisomy 21, 13, or 18 or if a translocation involving these chromosomes is known to be segregating in the family, risks should be adjusted to allow for this additional information. Genetic counseling and prenatal diagnosis may be indicated. For those women who are at increased risk of a child with a prenatally diagnosable disorder with Mendelian pattern of inheritance, microdeletion syndrome, and some other conditions, amniocentesis or CVS would still be indicated.

There may also be limitations in the availability of reproductive genetic services, including but not limited to proficient sonographers, certified genetic counselors and physicians or requisite computer programs used to calculate risks. Early pregnancy referral patterns and economic considerations are also likely to result in geographic differences in the protocols used. The choice of protocol also must take into consideration the need to screen for open neural tube defects either through second trimester AFP or second trimester ultrasound.

No single combination of markers or screening cut-offs will therefore be appropriate for all situations.

SCREENING PROTOCOL RECOMMENDATIONS

Recommendations are based on our assessment of the current state-of-the-art of the various technologies, best practices for overall prenatal healthcare, and optimal use of resources. It is recognized that some areas of testing are rapidly changing with respect to the range of aneuploidies detectable, the demonstrated applicability to additional groups of women, and the costs of testing. As these developments evolve, new protocols or the inclusion of more women in contingent steps of some protocols may be appropriate.

Individual women perceive risk differently, may prefer particular approaches, or may choose to personally finance their testing. Our recommendations should not be the basis for the denial of testing.

The following protocol options are currently considered optimal:

1. Ultrasound nuchal translucency at 11-13 completed weeks¹ combined with serum markers at 10-13 weeks.
2. Extending option (1) to include other first trimester sonographic markers, provided ultrasound performance has been prospectively validated by the center where the screening is to be performed.
3. A 'contingent' test whereby women with borderline risks from option (1) have option (2) at a specialist center and risk is subsequently modified.
4. Four maternal serum markers (quadruple test) at 15-19 weeks, for women who first attend after 13 weeks 6 days.
5. Combining options (1) and (4) in either a stepwise or contingent protocol - provided that all screening test data is included in the final risk assessment. Integrated screening can be offered when CVS is not available. A serum integrated test when NT measurement is unavailable.
6. Contingent second trimester ultrasound to modify risks for aneuploidy for women having options (1), (4) or (5). Ultrasound performance must be prospectively validated by the center where the screening is performed.
7. cfDNA screening for women classified as high risk by any of the above options (1-6). cfDNA screening can also be considered for additional groups of women *who did not receive any other screening* (i.e. options 1-6) and who are considered to be high risk on the basis of: maternal age; presence of an ultrasound abnormality suggestive of trisomy 21, 18 or 13; family history of a chromosome abnormality that could result in *full* trisomy 21, 18 or 13; and history of a previous pregnancy/livebirth with trisomy 21, 18 or 13. Local economic considerations and access to sonography, invasive testing and counseling resources should be considered when deciding on the use of NIPT-MPS in additional groups of women.

QUALITY ASSURANCE

Laboratories providing maternal serum screening tests must participate in proficiency testing and monitor their performance through epidemiologic monitoring. Ultrasonographers performing NT ultrasound must participate in an on-going audit of performance. Computer programs used in calculating risk should be checked for design accuracy.

The current absence of specific guidelines for quality control and quality assurance for cfDNA screening is a serious concern. This methodology and computational analyses are highly complex, aspects are the subject of patents or are proprietary in nature, and the testing is the subject of intense commercial competitive pressures. Laboratories that have developed cfDNA screening must adhere to general guidelines for all clinical laboratories and participate in a proficiency testing program. Laboratory regulatory agencies should develop specific requirements for laboratory procedures, reporting, sample and data

¹ Completed weeks (e.g. 10=10 weeks 0 days to 10 weeks 6 days).

storage. Laboratory providers should also be prepared to provide ongoing specifics on accuracy, test failure rates and turn-around time.

Comprehensive registries of aneuploidy should be encouraged, provided confidentiality of individual patient data can be assured. These registries can provide validation of the risks and also have considerable research value.

SUMMARY

- I. Definitive diagnosis of Down syndrome and other fetal aneuploidies can only be achieved through amniocentesis or CVS.
- II. The use of maternal age alone to assess fetal Down syndrome risk in pregnant women is insufficient.
- III. A combination of ultrasound NT measurement and maternal serum markers in the first trimester should be made available to women who want an early risk assessment.
- IV. A four marker serum test should be available to women who first attend for their prenatal care after 13 weeks 6 days of pregnancy.
- V. Protocols that combine first trimester and second trimester markers are valid.
- VI. Second trimester ultrasound can be a useful adjunct to other aneuploidy screening protocols.
- VII. Maternal cfDNA screening is an emerging technology that can provide highly effective prenatal screening for Down syndrome, trisomy 18, and possibly trisomy 13 in high risk women. It is not a replacement for the analysis of amniotic fluid cells or CVS.

CONFLICTS OF INTEREST

The following have declared conflicts of interest. R Chiu: holds patents and patent applications on noninvasive prenatal diagnosis using fetal nucleic acids in maternal plasma with intellectual property partly licensed to Sequenom Inc. She also receives research support, is a consultant and equity holder in Sequenom. L Dugoff has received institutional research support from Perkin Elmer Inc. H Cuckle is a consultant to PerkinElmer Inc., Ariosa Diagnostics Inc., Natera Inc., and director of Genome Ltd. S Gross has received institutional research support from PerkinElmer Inc. M Norton is a principal investigator in clinical trial NCT0145167, sponsored by Ariosa Diagnostics Inc. K Spencer is a consultant for Brahms GmbH and his institution receives research funding from PerkinElmer Inc. D Wright is a consultant to the NHS Fetal Anomaly Screening Program and his employer, Plymouth University, has received research funding from PerkinElmer for his work.

ISPD MEMBERS COMMENTS

The Committee is grateful for the constructive suggestions from the ISPD membership.

There were diverse member opinions regarding who should be offered cfDNA screening. Several members suggested that cfDNA screening should be recommended to low risk women while others suggested an official ISPD position on cfDNA screening was

premature. It was also pointed out that some high risk women may be better served by the provision of invasive testing with microarray analysis.

As discussed above, the Committee had some concerns about test implementation and logistics (counseling, confirmation of positive tests, test failure rates, laboratory quality assurance) and the possible loss of the broader benefits associated with traditional screening approaches. However, it was concluded that this powerful new technology should be available to high risk women. Economic considerations were not formally evaluated by us but it was recognized that costs for cfDNA screening are currently high and that the expenditure does need to be justified for low risk women. We also acknowledge that, as with other prenatal screening protocols, there will be regional variations in the adoption and use of cfDNA screening. For example, in countries such as the Netherlands there is low utilization of existing serum and ultrasound screening protocols and this provides an additional impetus and greater advocacy for cfDNA screening as a new paradigm.

Finally, it is recognized that rapid progress is being made in cfDNA technology and we anticipate frequent revisions in our position.

REFERENCES

Aagaard-Tillery KM, Malone FD, Nyberg DA, Porter TF, Cuckle HS, Fuchs K, Sullivan L, Comstock CH, Sade GR, Eddleman K, Gross S, Dugoff L, Craigo S, Timor-Tritsch IE, Carr SR, Wolfe HM, Bianchi DW, D'Alton ME. 2009. Role of second-trimester genetic sonography after Down syndrome screening. *Obstet Gynecol* **114**:1189-1196.

American College of Obstetrics and Gynecologists. 2007a. ACOG Practice Bulletin 88. Invasive Prenatal Testing for Aneuploidy. *Obstet Gynecol.* **110**;1459-1467.

American College of Obstetrics and Gynecologists. 2007b. ACOG Practice Bulletin 77. Screening for Fetal Chromosomal Abnormalities. *Obstet Gynecol.* **109**;217-226.

AIUM Practice Guideline for the Performance of Obstetric Ultrasound Examinations. 2007. <http://www.aium.org/resources/guidelines/obstetric.pdf> Accessed September 10, 2012.

Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. 2012. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. *Am J Obstet Gynecol* **206**;322.e1-5.

Benn P, Cuckle H, Pergament E. 2012. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol.* **119**;1270.

Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. 2012. Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing. *Obstet Gynecol* **119**;890-901

Brar H, Wang E, Struble C, Musci T, Norton M. 2012. The fetal fraction of cell-free DNA in maternal plasma is not affected by *a priori* risk of fetal trisomy. *J Met Fetal Neonat Med*. Eprint.
<http://informahealthcare.com.online.uchc.edu/doi/pdfplus/10.3109/14767058.2012.722731>

Canick JA, Kloza, EM, Lambert-Messerlian GM, Haddow JE, Ehrich M, Boom D van der, Bombard AT, Deciu C, Palomaki GE. 2012. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. *Prenat Diagn.* **32**; 1-5.

Cicero S, Rembouskos G, Vandecruys H, Hogg M, Nicolaides KH. 2004. Likelihood ratio for trisomy 21 in fetuses with absent nasal bone at the 11-14-week scan. *Ultrasound Obstet Gynecol* **23**;218-223.

Chitayat D, Langlois S, Wilson RD; Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada; Prenatal Diagnosis Committee of the Canadian College of Medical Geneticists. 2011. Prenatal screening for fetal aneuploidy in singleton pregnancies. *J Obstet Gynaecol Can.* **33**:736-50.

Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, Lun FM, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. 2011. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validation study. *Brit Med J*; **342**: c7401.

Cuckle H, Aitken D, Goodburn S, Senior B, Spencer K, Standing S. 2004. Age-standardisation for monitoring performance in Down's syndrome screening programmes. *Prenat Diagn* **24**: 851-856.

Cuckle H, Benn P. 2010. Multianalyte Maternal Serum Screening for Chromosomal Defects. In: *Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment*. 6th edition. (Ed A Milunsky, JM Milunsky) Johns Hopkins University Press, Baltimore.

Ehrich M, Deciu C, Zweifellhofer T, Tynan JA, Cagasan L, Tim R, Lu V, McCullough R, McCarthy E, Nygren AO, Dean J, Tang L, Hutchison D, Lu T, Wang H, Angkachatchai V, Oeth P, Cantor CR, Bombard A, van den Boom D. 2011. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* **204**; 205.e201-205e211.

Masden HN, Ball S, Wright D, Topping N, Petersen OB, Nicolaides KH, Spencer K. 2011. A reassessment of biochemical marker distributions in trisomy 21 affected and unaffected twin pregnancies in the first trimester. *Ultrasound Obstet Gynecol* **37**: 38-47.

Miguel J, Moskovitch M, Cuckle H, Zugaib M, Bunduki V, Maymon R. 2010. Model-predicted performance of second-trimester Down syndrome screening with sonographic prenatal thickness. *J Ultrasound Med.* **29**:1741-7

Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. 2005. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies, results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* **25**:221-226.

Nicolaides KH, Syngelaki A, Ashoor G, Birdir C, Touzet G. 2012. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol.* 207:x.ex-x.ex.

Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, M. Rodriguez H, Williams J III, Mitchell ME, Adair CD, Lee H, Jacobsson B, Tomlinson MW, Oepkes D, Holleman D, Sparks AB, Oliphant A, Song K. 2012. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. *Am J Obstet Gynecol.* **207**; 137.e1-137.e8

Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Deciu C, Grody WW, Nelson SF, Canick JA. 2011. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* **13**:913-20.

Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, Nelson SF, Canick JA. 2012. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* **14**:296-305

Sonek J, Nicolaides K. 2010. Additional first-trimester markers. *Clin Lab Med.* **30**:573-592.

Sparks, AB, Struble CA, Wang ET, Song K, Oliphant A. 2012 Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. *Am J Obstet Gynecol* **206**; 319e1-319e9.

Spencer K, Staboulidou I and Nicolaides KH. 2010. First trimester aneuploidy screening in the presence of a vanishing twin: implications for maternal serum markers. *Prenat Diagn* **30**; 235-240

Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. 2011. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. *Prenat Diagn.* **31**;90-102.

Tabor A, Alfirevic Z. 2010. Update on procedure-related risks for prenatal diagnosis techniques. *Fetal Diagn Ther.* **27**:1-7.

UK National Screening Committee. 2002. Normal variant screening in pregnancy.

<http://fetalanomaly.screening.nhs.uk/programmestatements#fileid11216>

Accessed November 15, 2012.

Van den Hof MC, Wilson RD, Stephen Bly, S, Gagnon R, Lewthwaite B, Lim K, et al.

Fetal soft markers in obstetric ultrasound. 2005. *J Obstet Gynaecol Can* ;27;592–612

Wapner R et al., 2012. In Press.

Table 1. Model predicted Down syndrome detection rate for a 3% false-positive rate and positive predictive value for various screening protocols

Protocol (completed weeks*)	DR	OAPR 1:n
1a PAPPA+free β (10), NT (12)	82%	29
1b PAPPA+hCG (10), NT (12)	80%	29
1c PAPPA+free β (12), NT (12)	80%	29
1d PAPPA+hCG (12), NT (12)	79%	30
2a AFP+free β +uE ₃ +InhA (15-19)	64%	36
2b AFP+hCG+uE ₃ + InhA (15-19)	60%	39
3a PAPPA+free β (10), NT (12), contingent AFP+free β +uE ₃ +InhA (15-19)	90%	26
3b PAPPA+hCG (10), NT (12), contingent AFP+hCG+uE ₃ +InhA (15-21)	88%	27
3c PAPPA+free β (10), NT (12), stepwise AFP+free β +uE ₃ +InhA (15-21)	92%	25
3d PAPPA+hCG (10), NT (12), stepwise AFP+hCG+uE ₃ +InhA (15-21)	91%	26
4a PAPPA (10), NT (12), AFP+free β +uE ₃ +InhA (15-19)	91%	26
4b PAPPA (10), NT (12), AFP+hCG+uE ₃ +InhA (15-19)	89%	26
4c PAPPA+free β (10), NT (12), AFP+free β +uE ₃ +InhA (15-19)	93%	25
4d PAPPA+hCG (10), NT (12), AFP+hCG+uE ₃ +InhA (15-19)	91%	26
4e PAPPA+free β (10), AFP+free β +uE ₃ +InhA (15-19)	80%	29
4f PAPPA+hCG (10), AFP+hCG+uE ₃ +InhA (15-19)	75%	33
5a PAPPA+free β (10), NT+NB (12)	91%	26
5b PAPPA+free β (10), NT (12), contingent NB	89%	26
5c PAPPA+free β (10), NT (12), contingent TR	88%	27
5d PAPPA+free β (10), NT (12), contingent DV	88%	27
6a AFP+free β +uE ₃ +InhA+NF+NBL+PT (15-19)	90%	26
6b AFP+hCG+uE ₃ +InhA+NF+NBL+PT (15-19)	89%	27
7a PAPPA+free β (10), NT (12), ANOMALY (18+)	88%	27

7b	PAPPA+hCG (10), NT (12), ANOMALY (18+)	86%	27
8a	ANOMALY (18+)	56%	41
8b	AFP+free β +uE ₃ +InhA (15-19), ANOMALY (18+)	80%	29
8c	AFP+free β +uE ₃ +InhA (15-19), contingent ANOMALY (18+)	77%	30
9a	PAPPA+free β (10), NT (12), AFP+free β +uE ₃ +InhA (15-19), ANOMALY (18+)	96%	25
9b	PAPPA+hCG (10), NT (12), AFP+hCG+uE ₃ +InhA (15-19), ANOMALY (18+)	95%	25

The rates specified are for the purposes of comparison of protocols and do not necessarily indicate optimal cut-offs. NT=nuchal translucency, NB=nasal bone absence, TR=tricuspid regurgitation, DV=ductus venosus, NF=nuchal skinfold, NBL=nasal bone length, PT=prenasal thickness, contingent=1 in 50-1500 borderline risks (at term, equivalent to 1 in 38-1200 at mid-trimester), stepwise=borderline or lower risks, ANOMALY=major malformation, large NF, short femur, echogenic intracardiac focus, pyelectasis, echogenic bowel and ventriculomegaly, completed weeks, e.g. 10=10 weeks 0 days to 10 weeks 6 days (see recommendations for optimal times to provide tests). Predicted performance is based on published statistical parameters for NT and biochemical markers (Cuckle and Benn, 2010), NB (Cicero et al., 2004), TCR and DV (Sonek and Nicolaides, 2010), NF, NBL and PT (Migueluez *et al.*, 2010), ANOMALY (Aagaard-Tillery et al., 2009) and a standardized maternal age distribution (Cuckle et al., 2004).

Study	Method	Trisomy 21				Trisomy 18				Trisomy 13			
		DR (%)	FPR (%)	NR* (%)	Unclass** (%)	DR (%)	FPR (%)	NR* (%)	Unclass* (%)	DR (%)	FPR (%)	NR* (%)	Unclass** (%)
1. Chiu et al (2011)	Shotgun	86/86 (100)	3/146 (2.1)	11/764 (1.4)									
2. Ehrich et al (2011)	Shotgun	39/39 (100)	1/410 (0.2)	18/467 (3.9)									
3. Palomaki et al (2011, 2012)	Shotgun	209/212 (98.6)	3/1471 (0.2)	13/1686 (0.8)		59/59 (100)	5/1688 (0.3)	17/1988 (0.9)		11/12 (91.7)	16/1688 (0.9)	17/1988 (0.9)	
4. Bianchi et al (2012)	Shotgun	89/89 (100)	0/404 (0)	16/532 (3.0)	7/503 (1.4)	35/36 (97.2)	0/461 (0)	16/532 (3.0)	5/502 (1.0)	11/14 (78.6)	0/488 (0)	16/532 (3.0)	2/502 (0.4)
5. Sparks et al (2012)	Targeted	36/36 (100)	1/123 (0.8)	8/338 (2.4) ⁺		8/8 (100)	1/123 (0.8)	8/338 (2.4) ⁺					
6. Ashoor et al (2012)	Targeted	50/50 (100)	0/297 (0)	3/400 (0.8)		49/50 (98.0)	0/297 (0)	3/400 (0.8)					
7. Norton et al (2012)	Targeted	81/81 (100)	3/2888 (0.1)	148/3228 (4.6)		37/38 (97.4)	3/2888 (0.1)	148/3228 (4.6)					
TOTAL		590/593 (99.5)	11/5739 (0.2)	217/7415 (2.9)	7/503 (1.4)	188/191 (98.4)	9/5457 (0.2)	192/6486 (3.0)	5/502 (1.0)	22/26 (84.6)	16/2,176 (0.7)	33/2520 (1.3)	2/502 (0.4)

Table 2. Large clinical trials of Non-invasive Prenatal Testing by Massively Parallel Sequencing (NIPT-MPS) for fetal trisomy 21, 18 and 13.

* NR = no result due to low fetal DNA fraction or other causes of test failure. Excludes samples that were considered to be inadequate or ineligible prior to testing. Additional cases needed more than one sample to achieve a result.

** Unclass = Intermediate results which the laboratory interpreted as “unclassified”. Based on the proportion of affected pregnancies in the unclassified groups (trisomy 21 14% [1/7]; trisomy 18 40% [2/5]; trisomy 13 100% [2/2]), these women should be considered to be at high risk. Including them as positive changes the total discriminatory power: trisomy 21 DR 100% [90/90], FPR 1.5% [6/410]; trisomy 18 DR 97.3 [37/38], FPR 0.6% [3/464]; trisomy 13 DR 81.3% [13/16], FPR 0% [0/488].

⁺ No result rate based on training and validation samples combined.