The Royal Australian and New Zealand College of Obstetricians and Gynaecologists
Excellence in Women's Health

HUMAN GENETICS SOCIETY OF AUSTRALASIA

Prenatal screening and diagnostic testing for fetal chromosomal and genetic conditions

This statement was originally developed in March 2015 by the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening and approved by the Women's Health Committee and RANZCOG Council.

A list of members of the 2017-18 Review Group comprised of HGSA and RANZCOG representatives can be found in Appendix A and the current Women's Health Committee in Appendix B.

Disclosure statements have been received from all members of this committee and contributors.

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Objectives: This statement is intended to provide advice on the recommended screening and diagnostic tests for fetal chromosomal and other genetic conditions.

Outcomes: Informed decision-making and improved access to effective screening and diagnostic tests for chromosomal and genetic conditions.

Target audience: This statement is intended for use by health professionals providing antenatal care including:
- Clinicians: doctors (obstetricians, clinical geneticists, pathologists, radiologists and general practitioners), midwives, nurses and genetic counsellors;
- Scientists, laboratory staff and administrative staff delivering prenatal screening and diagnostic services.

Other audiences: This statement provides useful information for patients and carers, researchers, health policy makers, health regulators and those responsible for quality and safety of healthcare. This statement may also be a valuable resource to State and Federal Government bodies developing guidelines and other documents on prenatal screening and diagnosis.

Values: The evidence was reviewed by the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening, and applied to local factors relating to Australia and New Zealand. As part of the 2017 review of this statement, the evidence was updated.

Background: This statement was first developed by the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening (Prenatal Screening Tests for Trisomy 21, Trisomy 18 and Neural Tube Defects - C-Obs 4 in 1991 and Prenatal diagnosis policy - C-Obs 5 in 1990). C-Obs 4 and C-Obs 5 were significantly edited by the committee to create C-Obs 59 in 2014-15. That version was updated in 2017-18 to this current version.

Table of contents

1. Patient summary

Every baby has a small chance of having a chromosomal or genetic condition.* Prenatal screening for some chromosomal and genetic conditions is offered during pregnancy to provide the woman with more information about her unborn baby. All such testing should be **voluntary** and only undertaken when the woman has been informed about the nature of the screening test, the possible results, and the options available to her.

The basic principle of prenatal screening is to offer a safe, effective and accessible test in order to identify women with an increased chance of having a baby with a chromosomal or genetic condition. These women are then followed up with genetic counselling and offered diagnostic testing for a definite answer. Currently, only an invasive test (e.g. amniocentesis or chorionic villus sampling) can diagnose a genetic or chromosomal condition in an unborn baby. As all diagnostic tests carry a small risk of miscarriage, screening programs aim to minimise the need for invasive testing, while providing a high chance of identifying chromosomal or genetic conditions in the fetus.

While screening should be discussed and offered to all pregnant women, diagnostic testing for chromosome conditions may be preferable for some women and should be available as an alternative to screening, including on maternal request. This is because our current screening tests are designed to detect only the most common chromosome conditions. These make up about 75% of the total range of conditions detectable by modern prenatal diagnosis[1]. Furthermore, the risks of pregnancy loss following an invasive test are now considered lower than previously quoted, which may influence a woman's decision regarding a request for diagnostic testing.[2] However, it must be remembered that even a 'diagnostic test' is not a test for every disease and a normal result does not exclude the chance of the baby having a chromosomal or genetic disease.

The most common chromosomal cause of birth defects and intellectual disability in children and adults is **Down syndrome** (trisomy 21), which accounts for 52% of all major chromosomal conditions currently detected through prenatal testing.[3] This condition is caused when an individual is born with three copies of chromosome 21, instead of the usual two copies. Down syndrome is usually a sporadic (random) condition, meaning that there is usually no prior history of this condition in the family, and it is not inherited from a parent. Down syndrome has been the major focus of prenatal screening because it is common (occurs in about 1 in 400 pregnancies) and because it has effects on health and learning. Other chromosomal conditions that are commonly screened for include Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13). Trisomy 18 and trisomy 13 are more severe conditions than Down Syndrome and are associated with a high rate of pregnancy loss or death in infancy.

Women and their partners also have the option of being tested for changes in specific genes that can result in their baby inheriting a specific genetic condition. This is called **carrier screening**. The most common genetic conditions in this group include thalassemia, cystic fibrosis, spinal muscular atrophy and fragile X syndrome. If an unborn baby has a higher probability of having a condition based on the couple's results, then prenatal diagnostic testing with amniocentesis or chorionic villus sampling will be offered.

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* Our genetic material, or DNA, is organised into 46 packages called chromosomes. Large changes that cause gains or losses of whole chromosomes are referred to as chromosomal conditions. Smaller changes can also occur within individual genes on a chromosome, resulting in other types of genetic conditions. Sometimes the changes causing chromosomal or genetic conditions are present in the DNA of one or both parents and can be passed on to a baby via the egg or sperm. When a change is passed on to a baby by a parent, the condition is said to be **inherited**. Some conditions are caused by a change in the baby's DNA for the first time without being present in either parent. These are called **sporadic** conditions.

This statement summarises recommendations for prenatal screening for chromosomal and genetic conditions for pregnant women and their partners in the general population. Women with an increased chance of having a child with a chromosomal or genetic condition (e.g. having had a baby with a condition or due to a family history of a condition) should receive individualised counselling from a clinician with appropriate expertise, preferably prior to pregnancy.

2. Summary of recommendations

Prenatal tests for chromosome conditions	
Recommendation 1	**Grade and supporting references**
All pregnant women should be provided with information and have timely access to screening tests for fetal chromosome and genetic conditions. Prenatal screening options should be discussed and offered in the first trimester whenever possible.	Level III-3 Grade C [4]
Recommendation 2	**Grade and supporting references**
Screening or diagnostic testing for fetal chromosomal and genetic conditions is voluntary and should only be undertaken as an informed decision by the pregnant woman.	Consensus-based recommendation
Recommendation 3	**Grade and supporting references**
If a screening test result indicates an increased chance of a chromosome or genetic condition, the woman should have access to genetic counselling for further information and support. The available options for prenatal diagnosis should be discussed and offered.	Consensus-based recommendation
Recommendation 4	**Grade and supporting references**
Acceptable first-line screening tests for fetal chromosome abnormalities in the first trimester include either: a) combined first trimester screening with nuchal translucency and serum pregnancy-associated plasma protein A (PAPP-A) and beta human chorionic gonadotropin (βHCG) measurements OR b) cell-free DNA (cfDNA)-based screening. The choice of first line screening test will depend on local resources, patient demographics, and individual patient characteristics.	Consensus-based recommendation
Recommendation 5	**Grade and supporting references**
Pre-test counselling for cfDNA-based screening should include informed decision making regarding testing for fetal sex and sex chromosome aneuploidy. The potential for other unanticipated findings of relevance to maternal health (including maternal genomic imbalances), should be included in pre-test counselling.	Consensus-based recommendation

Recommendation 6	Grade and supporting references
Acceptable first-line screening tests for chromosome conditions in second trimester include: a) maternal serum screening (MA + AFP + βHCG +UE3 +/- Inhibin) and, b) cfDNA-based screening. The choice of first line screening test will depend on local resources, patient demographics, and individual patient characteristics.	Consensus-based recommendation
Recommendation 7	**Grade and supporting references**
The option of cfDNA-based screening as a second-tier test should be discussed with all women at increased probability of a chromosome condition after primary screening. The advantages and disadvantages of second tier cfDNA-based screening, compared with diagnostic testing, or no further assessment, should be discussed by a clinician with appropriate expertise.	Consensus-based recommendation
Recommendation 8	**Grade and supporting references**
Diagnostic testing with amniocentesis or chorionic villus sampling should be recommended prior to definitive management decisions (e.g. termination of pregnancy) in cases of "increased chance" screening results, including cfDNA-based screening.	Consensus-based recommendation
Recommendation 9	**Grade and supporting references**
Routine population-based screening for genome-wide chromosome abnormalities and microdeletion syndromes are not recommended due to the absence of well-performed clinical validation studies.	Consensus-based recommendation

Twin pregnancies and higher order multiple pregnancies	
Recommendation 10	**Grade and supporting references**
For all multiple pregnancies, first trimester ultrasound assessment of chorionicity and fetal morphology at 11-13 weeks is recommended for all women for interpretation of screening results and triaging to appropriate models of antenatal care, regardless of the choice of chromosome screening test.	Consensus-based recommendation
Recommendation 11	**Grade and supporting references**
In twin pregnancies, cfDNA-based screening may be offered with appropriate pre-test counselling regarding an increased test failure rate, and less available performance data compared with singletons.	Consensus-based recommendation
Recommendation 12	**Grade and supporting references**
In triplet and higher order pregnancies, screening for chromosome conditions should be performed with first trimester ultrasound markers (i.e. nuchal translucency thickness and nasal bone assessment +/- additional markers at 11-13 weeks).	Consensus-based recommendation
Recommendation 13	**Grade and supporting references**
Some women may prefer to directly access diagnostic testing, rather than undergo screening, in order to maximise genetic information about their fetus. This is an acceptable practice as long as the woman has had individualised counselling and is fully informed of the risks, benefits, costs and limitations of prenatal diagnosis.	Consensus-based recommendation

Carrier screening for inherited genetic disorders	
Good practice note	**Grade and supporting references**
Preconception screening is preferable to antenatal screening for heritable genetic conditions as this potentially allows more options for carrier couples, including pre-implantation genetic diagnosis.	Good practice notes (consensus-based)
Recommendation 14	**Grade and supporting references**
All couples intending to have children, or who are pregnant, should have a careful family history taken with a view to identifying relatives with heritable genetic disorders. Those identified with a family history of a specific inherited disorder should be offered referral to a genetic counselling service for information about carrier screening and prenatal diagnosis/ pre-implantation genetic diagnosis for the condition.	Consensus-based recommendation
Recommendation 15	**Grade and supporting references**
Carrier screening for the more common "monogenic" genetic conditions (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome, haemaglobinopathies) is available in Australia and in New Zealand (in the private sector). Information on carrier screening for the more common genetic conditions that affect children (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome) should be offered to all women planning a pregnancy or in the first trimester of pregnancy. Woman wanting more information about carrier screening should be given the opportunity to have a more detailed discussion about carrier screening with an informed clinician. The benefits and limitations of testing, and any associated costs should be discussed.	Consensus-based recommendation
Recommendation 16	**Grade and supporting references**
All pregnant women should be offered basic screening for thalassemia carrier status by a full blood examination at initial presentation. Screening with specific assays for haemoglobinopathies (such as HPLC or EPG and haemoglobinopathy gene DNA testing) should be considered in high risk ethnic or population groups.	Grade C [5]

3. Discussion and recommendations

The following section expands on each recommendation summarised earlier.

3.1 Prenatal tests for chromosome aneuploidies

Prenatal tests for chromosome aneuploidies	
Recommendation 1	**Grade and supporting references**
All pregnant women should be provided with information and have timely access to screening tests for fetal chromosome and genetic conditions. Prenatal screening options should be discussed and offered in the first trimester whenever possible.	Level III-3 Grade C [4]

General information on prenatal screening and diagnosis

3.1.1 All pregnant women should be advised of the availability of investigations for prenatal and diagnosis as early as possible in pregnancy to allow time to discuss the options available and facilitate an informed choice. An informed choice is "based on relevant knowledge, consistent with the decision maker's values".[4]

3.1.2 The offer of screening should be made to all people irrespective of the clinician's perception of what their likely choices might be. It is essential that the woman is not deprived of the opportunity to find out about the health of her fetus. It is not ethical to presuppose a course of action prior to this information being provided.

3.1.3 Some women may make an informed decision not to proceed with any testing. Counselling should follow a shared decision-making model, where health professionals discuss information based on their expertise and respect for the woman's values in arriving at an agreed course of action.

3.1.4 Information should be communicated using clear, simple and consistent language when discussing investigations, with confirmation that the information has been understood and with that understanding documented.

3.1.5 Information should be provided in a format that is easy to understand and accessible to pregnant women from culturally and linguistically diverse backgrounds (including Indigenous women) and women with additional needs (such as physical, sensory or learning difficulties). An interpreting service should be made available where it is required (see Appendix E).

3.1.6 Information should include the following:

a. A description of the conditions that can and cannot be detected through traditional screening processes and details of the testing process. This should include information about phenotypic variability of chromosomal conditions and the difficulties in being able to predict the extent of effect on a particular baby.
b. A discussion of the differences between screening and diagnostic tests.
c. Advantages and disadvantages of the different types of tests available (taking into account the gestation of the pregnancy).
d. Practical aspects of testing; including the timing of tests and the approximate costs involved.
e. The possibility that the screening and diagnostic pathway may reveal anomalies other than those expected.
f. Details of support groups and sources of further information (see How to decide on antenatal tests for chromosomal abnormalities and other conditions and Antenatal screening for Down syndrome and other conditions (New Zealand).

g. The understanding that, if a chromosome or genetic condition is diagnosed, a woman can choose whether to continue or terminate the pregnancy. Where a condition has been diagnosed, parents should be given sufficient information regarding the aetiology, associations, and implications of that diagnosis during pregnancy, the newborn period and beyond, in order to make informed decisions about their options.

3.1.7 There should be an assurance that regardless of their decision, women will be offered counselling and receive ongoing care and support. In the case of continuing the pregnancy, women and their partners should be provided with appropriate antenatal care with individualised preparations for birth and neonatal management. The option of neonatal palliative care should be discussed for conditions where the prognosis is poor and palliation is a realistic option. If they choose termination, they need to know that the mode of termination may be influenced by gestational age in line with State laws.[6]

3.2 Prenatal screening tests for fetal chromosome and genetic conditions

Recommendation 2	**Grade and supporting references**
Screening or diagnostic testing for fetal chromosomal and genetic conditions is voluntary and should only be undertaken as an informed decision by the pregnant woman.	Consensus-based recommendation
Recommendation 3	**Grade and supporting references**
If a screening test result indicates an increased chance of a chromosome or genetic condition, the woman should have access to genetic counselling for further information and support. The available options for prenatal diagnosis should be discussed and offered.	Consensus-based recommendation
Recommendation 4	**Grade and supporting references**
Acceptable first-line screening tests for fetal chromosome abnormalities in the first trimester include either: a) combined first trimester screening with nuchal translucency and serum pregnancy-associated plasma protein A (PAPP-A) and beta human chorionic gonadotropin (βHCG) measurements OR b) cell-free DNA (cfDNA)-based screening. The choice of first line screening test will depend on local resources, patient demographics, and individual patient characteristics.	Consensus-based recommendation
Recommendation 5	**Grade and supporting references**
Pre-test counselling for cfDNA-based screening should include informed decision making regarding testing for fetal sex and sex chromosome aneuploidy. The potential for other unanticipated findings of relevance to maternal health (including maternal genomic imbalances), should be included in pre-test counselling.	Consensus-based recommendation

Recommendation 6	Grade and supporting references
Acceptable first-line screening tests for chromosome conditions in second trimester include: a) maternal serum screening (MA + AFP + βHCG +UE3 +/- Inhibin) and, b) cfDNA-based screening. The choice of first line screening test will depend on local resources, patient demographics, and individual patient characteristics.	Consensus-based recommendation
Recommendation 7	**Grade and supporting references**
The option of cfDNA-based screening as a second-tier test should be discussed with all women at increased probability of a chromosome condition after primary screening. The advantages and disadvantages of second tier cfDNA-based screening, compared with diagnostic testing, or no further assessment, should be discussed by a clinician with appropriate expertise.	Consensus-based recommendation
Recommendation 8	**Grade and supporting references**
Diagnostic testing with amniocentesis or chorionic villus sampling should be recommended prior to definitive management decisions (e.g. termination of pregnancy) in cases of "increased chance" screening results, including cfDNA-based screening.	Consensus-based recommendation
Recommendation 9	**Grade and supporting references**
Routine population-based screening for genome-wide chromosome abnormalities and microdeletion syndromes are not recommended due to the absence of well-performed clinical validation studies.	Consensus-based recommendation

Prenatal screening programs for birth defects have traditionally focussed on the common autosomal aneuploidies (trisomy 21, trisomy 18 and trisomy 13) because they are major causes of perinatal morbidity and mortality and are amenable to definitive prenatal diagnosis via amniocentesis or CVS. Trisomy 21 (Down syndrome) is the most common aneuploidy seen in live born infants and is associated with intellectual disability and a range of other medical morbidities. The most important factor for having a child with trisomy 21 is maternal age. The chance of an affected newborn at term is approximately 1 in 300 for a woman aged 35 years, increasing to 1 in 100 by the maternal age of 40 years.[7] The overall prenatal prevalence of trisomy 21 has increased with the trend to later childbearing in many developed countries.

Trisomy 21 comprises approximately half of the chromosome abnormalities detected prenatally. The next most common autosomal trisomies are trisomy 18 and trisomy 13. Together, trisomies 21, 18 and 13 make up about 66% of major aneuploidies currently detected by prenatal diagnosis.[3]

A number of different screening methods for these common autosomal trisomies have been developed. The effectiveness of a screening test is defined in terms of the test parameters such as sensitivity, specificity, and positive and negative predictive value. The gestation at which a particular test is performed is also an important consideration in test choice, as women and clinicians usually prefer earlier diagnosis.[8]

All prenatal screening results should be communicated to the referring doctor and patient as soon as possible and in a manner that ensures clear understanding. The action to be taken on the basis of abnormal results is a decision for the couple concerned based on the information given with full counselling support.

Screening tests available in first trimester

i) Combined first trimester screening (CFTS) is performed at 11+0 to 13+6 weeks by incorporating maternal age, ultrasound measurement of fetal nuchal translucency, and maternal serum markers to generate an overall chance of trisomy 21. Calculations for trisomy 13 and 18 are also incorporated into the first trimester combined screening algorithm. CFTS is the standard of care in most developed countries, due to its dual advantages of high sensitivity and early detection. There are also the additional advantages of a routine ultrasound examination at 11+0 – 13+6 weeks, including confirmation of fetal number, gestation, viability and structural development.

ii) Cell-free DNA (cfDNA) -based screening using maternal plasma can be performed reliably from 10 weeks. This screening test became widely available in Australia in 2013 and has the highest sensitivity and specificity of all the screening tests for Down syndrome.[9] However, cfDNA testing is currently more expensive than CFTS and must be self-funded (currently no Medicare or private insurance rebate). Clinicians should inform women of the availability of this test as an alternative to CFTS as well as its associated costs. Women who choose to have cfDNA as a primary screening test should still be offered the opportunity to have an 11-13 week ultrasound for an early structural assessment, as 50% of major abnormalities can now be detected at this gestation. [10] cfDNA screening performs better than CFTS for aneuploidy detection and hence simultaneous screening with CFTS serum markers (PaPP-A and b-HCG) is not recommended as this increases the false positive rate but not the detection rate. [11]

Screening tests available in second trimester

Women in second trimester may be offered maternal serum screening at 15-20 weeks or cfDNA testing (available at any gestation from 10 weeks). Gestational age should have been confirmed by ultrasound dating prior to screening. The 18-20 week morphology ultrasound is not recommended as a primary screening test for trisomy 21 due to its relatively poor sensitivity and specificity.

The performance characteristics of current screening tests for trisomy 21 are contained in Table 1.

Table 1: Screening tests for trisomy 21 currently in use in Australia and New Zealand

Test	Gestation for screening	Sensitivity	Specificity	Positive predictive value[#]
Combined first trimester screening: MA + NT + βhCG + PAPP-A	11^{+0}- 13^{+6} weeks	85%	95%	~7-10% [12]
Second trimester serum screening: MA + AFP + βhCG + UE3 +/- Inhibin	15 – 20 weeks	70-75%[13, 14]	93%	~2-3%
cfDNA- based screening*	> 10^{+} weeks	99%	99% *	~45%[15]

*In a proportion (1-6%) of cases, cfDNA testing is unable to provide a result. These women should have follow up assessment including detailed ultrasound (if not already performed), and be offered the options of diagnostic testing, repeat cfDNA testing (successful in approximately 50%), or an alternative form of screening such as combined first trimester screening.
MA = maternal age; NT = nuchal translucency; βhCG = free B human chorionic gonadotrophin; PAPP-A = pregnancy associated plasma protein A; AFP = Alpha-fetoprotein; UE3 = oestriol.
# these positive predictive values are derived from test performance in the general pregnant population, but will vary according to the underlying prevalence of the condition.

Screening programs should ideally collate data to demonstrate the quality of assessment, including the collection of data demonstrating local performance (e.g. biochemical assays / the ultrasound marker nuchal translucency, cfDNA). Midwives, general practitioners and obstetricians ordering these tests should ensure that they use a quality assured product (see section 4.1 Governance for further details).

3.2.1 Additional first trimester markers of aneuploidy

The efficacy of combined first trimester screening can be enhanced by incorporating extra sonographic markers at the time of the nuchal translucency scan. These include assessment of the nasal bone[16], ductus venosus waveform[17] and tricuspid valve flow.[18] The addition of these markers to the first trimester combined test can improve detection rates to 96% and lower the false positive rate to 2.5%.[19] Extra biochemical markers, such as placental growth factor, have also been investigated in first trimester screening.[20] The incorporation of additional first trimester ultrasound markers depends on local availability and technical expertise, but is encouraged when adequately trained personnel are available.

Further information on technical aspects of nuchal translucency and nasal bone assessment can be obtained from the Australian Nuchal Translucency Online Learning Program (NTOLP) or the UK's FMF website.

3.2.2 Confounding maternal factors

Maternal factors such as maternal weight, smoking and conception by in-vitro fertilisation are recognised to affect the performance of screening tests, particularly the level of serum markers. Maternal weight is also a significant factor affecting the technical performance of cfDNA testing. It is important that referrers accurately report these elements of maternal history to test providers. It is also important that test providers include assessment of these features in the calculation of multiples of median (MoMs) for prediction algorithms. The presence of twins, or higher order multiples, also affects screening and needs to be flagged at the time of referral. The issue of screening in twin pregnancies is covered in more detail in section 3.2.4 of this statement.

3.2.3 Cell free DNA-based testing for fetal aneuploidy

Cell free DNA (cfDNA) based screening, commonly referred to as non-invasive prenatal testing (NIPT), uses DNA sequencing or array based technology to detect aneuploidy in placental tissues by measuring cfDNA in the maternal plasma. This test is highly sensitive and highly specific for trisomy 21 but does not have sufficient diagnostic accuracy to replace invasive testing (i.e. false positive and false negatives still occur). It was initially validated and clinically implemented as an "advanced" or secondary screening test for women at increased likelihood of having a child with aneuploidy based on maternal age, prior abnormal screening result, ultrasound irregularity or prior history of aneuploidy. Data are now available on its use in the general population, suggesting equal test performance characteristics (i.e. sensitivity and specificity) but a lower chance of an affected fetus given an abnormal screening result (approximately 45%)[15] as would be expected from its use in lower prevalence populations.[9, 15, 21] Diagnostic testing with amniocentesis or chorionic villus sampling should be recommended prior to definitive management decisions in cases of suspected aneuploidy on cfDNA-based screening.

Women should also be aware that between 1 to 6% of cfDNA tests are unreportable.[22] Women with such a "no call" result appear to have a higher rate of fetal abnormalities (e.g. triploidy),[23] and therefore should have follow up assessment including detailed ultrasound (if not already performed). They should be offered the available options of diagnostic testing, repeat cfDNA testing (successful in approximately 50%), or an alternative form of screening such as combined first trimester screening.

Most cfDNA screening tests offer fetal sex and sex chromosome aneuploidy detection in addition to trisomies 21, 18 and 13. There has, however, been no precedent for population screening for sex chromosome conditions due to their variable and usually mild phenotype. cfDNA based screening for sex chromosomes is also less accurate than for the autosomes, increases the false positive rate, and can be confounded by underlying maternal and placental factors (such as maternal age-related somatic mosaicism and confined placental mosaicism).[24] Pre-test counselling for cfDNA screening should include informed decision making regarding testing for fetal sex and sex chromosome aneuploidy. Women should be given the choice to opt out of receiving this information.[25] The potential for other unanticipated findings of relevance to maternal health (including maternal genomic imbalances), should be included in pre-test counselling.

cfDNA based screening tests for 22q11.2 deletion syndrome (DiGeorge syndrome), other microdeletion syndromes, and genome-wide chromosome abnormalities are commercially available.[26, 27] There is very limited clinical performance data for these assays compared with Down syndrome screening, partly due to their low prevalence, lack of population-based screening, and genetic variability.[28] It is not recommended to routinely offer screening for conditions other than the common autosomal aneuploidies and sex chromosomes with cfDNA.

cfDNA screening does not currently attract any Government or private health insurance rebates and therefore the test must be funded by the woman. cfDNA testing has only been widely available in Australia and New Zealand since 2013 with costs currently ranging from $385 to in excess of $1000. Women should be informed of the costs of cfDNA screening and its alternatives during the decision-making process.

In women with singleton pregnancies of 10 weeks gestation or greater, there is sufficient evidence to support the use of cfDNA as any of the following (i) a primary screening test for fetal aneuploidy, or as (ii) a secondary screen for women who have an increased probability result on a primary screening test, but does not wish to have diagnostic testing, or (iii) any woman with probability below the traditional threshold for offering diagnostic testing (i.e. less than 1 in 300), but who is insufficiently reassured by this and wishes to self-fund further screening. Further details on the clinical application of blood-based screening, ultrasound and diagnostic tests for the Australian context can be found in ANZJOG

(ref – Rieder W, McGillivray G, White S, Hui L. Contemporary prenatal aneuploidy screening practice in Australia: *Frequently asked questions in the cfDNA era*. Aust NZ JObstet Gynaecol 2018, DOI: 10.1111/ajo.12834 ePub ahead of print).

3.2.4 Screening for aneuploidy in multiple pregnancies

Twin pregnancies

Twin pregnancies and higher order multiple pregnancies	
Recommendation 10	**Grade and supporting references**
For all multiple pregnancies, first trimester ultrasound assessment of chorionicity and fetal morphology at 11-13 weeks is recommended for all women for interpretation of screening results and triaging to appropriate models of antenatal care, regardless of the choice of chromosome screening test.	Consensus-based recommendation
Recommendation 11	**Grade and supporting references**
In twin pregnancies, cfDNA-based screening may be offered with appropriate pre-test counselling regarding an increased test failure rate, and less available performance data compared with singletons.	Consensus-based recommendation
Recommendation 12	**Grade and supporting references**
In triplet and higher order pregnancies, screening for chromosome conditions should be performed with first trimester ultrasound markers (i.e. nuchal translucency thickness and nasal bone assessment +/- additional markers at 11-13 weeks).	Consensus-based recommendation

The performance of all screening tests that incorporate maternal blood biomarkers is reduced in twin pregnancies compared with singletons due to the inherent biological complexity of multiple gestation.

In twin pregnancies, the sensitivity of CFTS generally ranges from 72%-80%.[29] The use of nasal bone assessment can improve the sensitivity of CFTS in twin pregnancies to 89% for a fixed 5% false positive rate.[30]

Laboratories need specific clinical details to reliably calculate the likelihood of aneuploidy from biochemical data. These include:

- whether both twins are alive and, if not,
- the gestation of demise of the late twin
- the chorionicity (monochorionic /dichorionic), and
- the crown rump length (CRL) of both fetuses.

It is important to note that some screening algorithms require the CRL and NT of both twins to be done within a limited timeframe (1-2 days), otherwise screening results cannot be calculated.

cfDNA testing in twin pregnancies has not been as extensively evaluated as in singletons due to the limitations of smaller numbers. However, the most recent meta-analysis containing pooled data from 5 studies (24 cases of trisomy 21 and 1111 euploid twin pregnancies) estimated a sensitivity of 100% (95%CI 95.2-100%) for trisomy 21.[9] These studies noted a considerably higher "no call" rate for twin pregnancies of > 5%, which should be taken into consideration in pre-test counselling.

Women with a twin pregnancy who have missed the opportunity for first trimester screening may be offered second trimester maternal serum screening for Down syndrome (15-20 weeks) or cfDNA testing.

Triplets and higher order multiple pregnancies

In higher order multiples (triplets or more), aneuploidy screening should be performed with ultrasound markers at 11-13 weeks alone (e.g. nuchal translucency and nasal bone) as maternal serum screening and cfDNA testing cannot be used in higher order pregnancies.

3.3 Prenatal diagnostic procedures for suspected aneuploidy

Women at increased likelihood of having a child with aneuploidy on a screening test should be offered a prenatal diagnostic test for confirmation. All diagnostic procedures should be performed by trained operators or be closely supervised by a trained operator under direct ultrasound guidance. Commonly quoted estimates of total fetal loss rates following an invasive procedure range from 0.5 to 1.0% .[31] A recent meta-analysis suggests that fetal loss rates in the hands of experienced operators do not differ between CVS and amniocentesis and may be as low as 1 in 900.[32] However, there is also evidence that the fetal loss rates for invasive procedures are operator and experience-dependent, and hence actual complications rates may vary.[33]

Some women may prefer to directly access diagnostic testing, rather than undergo screening, in order to maximise genetic information about their fetus. This is particularly relevant now that chromosome analysis by microarray is widely available (see below), since this will detect disorders that are not detected with current blood-based screening. This is an acceptable practice as long as the woman has had individualised counselling and is fully informed of the risks, benefits, costs and limitations of prenatal diagnosis.

Recommendation 13	Grade and supporting references
Some women may prefer to directly access diagnostic testing, rather than undergo screening, in order to maximise genetic information about their fetus. This is an acceptable practice as long as the woman has had individualised counselling and is fully informed of the risks, benefits, costs and limitations of prenatal diagnosis.	Consensus-based recommendation

3.3.1 Amniocentesis

Amniocentesis is performed from 15 weeks gestation. This procedure should not be performed routinely before 14 weeks gestation because of the increased risk of adverse outcome such as talipes.[34]

3.3.2 Chorionic villus sampling (CVS)

CVS is performed from 11 weeks gestation. Before this gestation, CVS is associated with an increased risk of transverse limb reduction defects.

3.4 Assessment of fetal chromosomes following CVS or amniocentesis

There are a number of options for diagnostic tests on cells obtained from CVS or amniocentesis including:

- **Conventional (G-banded) Karyotyping** – uses cultured fetal cells to prepare stained metaphase chromosomes for microscopic inspection. Chromosome number, length, banding pattern and other physical characteristics are visually assessed by a cytogeneticist. It identifies changes in chromosome number as well as subchromosomal rearrangements down to 5-10 megabases in size.

- **Rapid aneuploidy tests - fluorescent in situ hybridisation (FISH), quantitative fluorescent polymerase chain reaction (QF-PCR), BACs on beads (BoBs)** - These technologies are usually employed as an adjunct to full karyotyping for a rapid assessment of the common autosomal trisomies (chromsomes 21, 18, 13) and sex chromosomes. FISH can also be used for the diagnosis of specific microdeletion syndromes such as 22q11 deletion (diGeorge syndrome).

- **Chromosomal microarray analysis** - Chromosome analysis by genome-wide oligonucleotide array (also called chromosomal microarray, molecular karyotype, and array CGH) identifies both large (5-10Mb) and sub-microscopic (< 5-10Mb) DNA variations across all chromosomes. Chromosomal microarrays (CMAs) assess the fetal genome in higher resolution than the conventional karyotype, but do not identify balanced chromosome rearrangements (e.g. balanced translocations) or the majority of mutations causing single gene disorders.

  Where structural fetal conditions are detected on ultrasound scan, CMA detects significantly more pathogenic aneuploidies than conventional karyotype.[35] [36] As a result, CMA is recommended as the "first tier" chromosome test in the presence of a structural fetal condition and replaces the need for banded karyotype.[37]

  In the setting of a normal fetal ultrasound scan (e.g. diagnostic testing after maternal serum screening), microarray identifies pathogenic chromosome changes not detected by conventional karyotype and can be used as a first tier test for women undergoing prenatal diagnosis with appropriate pre-test counselling.[35, 38]

  Single-nucleotide-polymorphism-based microarrays (SNP arrays) can also identify uniparental disomy (relevant for suspected imprinting disorders such as Angelman/Prader Willi syndromes), triploidy, and can be used to confirm zygosity in twin pregnancies. SNP based arrays can also identify parental relatedness (consanguinity).

  The diagnostic advantage of microarray is tempered by the fact that microarray detect variants of uncertain or unknown significance in about 5%[3], which may result in genetic counselling dilemmas and patient concern and distress.[39] The test therefore should only be offered in the context of pre-test and post-test counselling, especially when fetal ultrasound is normal. Patients who receive abnormal, uncertain or unknown microarray results should have access to a formal genetic counselling service staffed by genetic counsellors and/ or clinical geneticists.

  Laboratories offering a prenatal microarray service should be appropriately accredited with their regional authority. Reporting of microarrays is the responsibility of appropriately qualified medical laboratory professionals - i.e. FHGSA/FFSc medical scientists and/or FRCPA pathologists with scope of practice in genetic pathology.

3.5 Prenatal tests for other genetic disorders

3.5.1 Population-based reproductive carrier screening

Carrier screening for inherited genetic disorders	
Good practice note	**Grade and supporting references**
Preconception screening is preferable to antenatal screening for heritable genetic conditions as this potentially allows more options for carrier couples, including pre-implantation genetic diagnosis.	Good practice notes (consensus-based)
Recommendation 14	**Grade and supporting references**
All couples intending to have children, or who are pregnant, should have a careful family history taken with a view to identifying relatives with heritable genetic disorders. Those identified with a family history of a specific inherited disorder should be offered referral to a genetic counselling service for information about carrier screening and prenatal diagnosis/ pre-implantation genetic diagnosis for the condition.	Consensus-based recommendation
Recommendation 15	**Grade and supporting references**
Carrier screening for the more common "monogenic" genetic conditions (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome, haemaglobinopathies) is available in Australia and in New Zealand (in the private sector). Information on carrier screening for the more common genetic conditions that affect children (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome) should be offered to all women planning a pregnancy or in the first trimester of pregnancy. Woman wanting more information about carrier screening should be given the opportunity to have a more detailed discussion about carrier screening with an informed clinician. The benefits and limitations of testing, and any associated costs should be discussed.	Consensus-based recommendation
Recommendation 16	**Grade and supporting references**
All pregnant women should be offered basic screening for thalassemia carrier status by a full blood examination at initial presentation. Screening with specific assays for haemoglobinopathies (such as HPLC or EPG and haemoglobinopathy gene DNA testing) should be considered in high risk ethnic or population groups.	Grade C [5]

Preconception or prenatal genetic screening of couples will identify those with an increased likelihood of giving birth to a child with a specific heritable disorder. It does not refer to testing an individual with a strong family history of a known or possible genetic condition – these people should be offered direct referral to a specialist clinical genetics service.

It is estimated that all individuals are carriers for at least three clinically severe recessive childhood disorders.[40] Most of these are autosomal, meaning if both members of a couple are carriers of a mutation in one gene copy of a specific gene pair, and if both pass on the mutation, the offspring will develop a medically significant genetic condition. X-linked recessive conditions occur when a woman carries a mutation in a gene on the X-chromosome. If she passes this mutation on to her son, he will develop a medically significant genetic condition.

The carrier frequency of certain recessive conditions is higher in specific ethnic populations: e.g. cystic fibrosis in Northern Europeans and Ashkenazi Jews; thalassemia/haemoglobinopathies in South-East Asians; and Tay-Sachs disease in Ashkenazi Jews.

A number of carrier screening tests exist within Australasia (or are readily accessible from overseas), but currently these are generally not funded by the public health system (i.e. accessible only on a user pays basis).

Information on carrier screening for the more common genetic conditions that affect children (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome) should be offered to all women planning a pregnancy or in the first trimester of pregnancy. Woman wanting more information about carrier screening should be given the opportunity to have a more detailed discussion about carrier screening with an informed clinician. The benefits and limitations of testing, and any associated costs should be discussed.

One-step screening for carrier status, where both members of a couple are tested simultaneously and each given their result back individually is preferable as more carriers will be detected and the results will be available in a more timely fashion. However, it is recognised that it is more economical to undertake "two-step screening" – test the female first and then only test the male partner should she be found to be a carrier of the specific autosomal recessive condition(s) being screened for. The turn-around-time of screening tests and the anticipated gestational age at final diagnosis are important factors to consider when deciding between one-step or two-step carrier screening.

With the introduction of new genomic sequencing techniques, carrier screening for a multitude of recessive conditions is now available for couples who have no family history of a genetic disorder.[41] This so-called "expanded carrier screening" should only be offered in the context of well-defined clinical pathways for pre- and post-test genetic counselling, as up to 24% of adults will test positive for at least one recessive disorder.[42]

3.5.2 Prenatal diagnosis for genetic disorders on the basis of a family history of a known or suspected genetic disorder

If a woman and/or her partner have a family history of a known or suspected genetic disorder (e.g. fragile X syndrome, cystic fibrosis), refer the couple to specialist clinical genetics service (preferably prior to pregnancy) to assess reproductive risks and the availability of genetic testing to further refine reproductive risks. Options for pre-implantation genetic diagnosis and/or prenatal diagnosis should be discussed.

3.5.3 Prenatal diagnosis for genetic disorders suspected on the basis of fetal ultrasound abnormalities

Prenatal diagnosis for mutations in genes linked to specific disorders is currently available in Australasia for some genes using traditional sequencing methods. Examples include cystic fibrosis mutation panel screening and *CFTR* gene sequencing for fetal echogenic bowel, *FGFR* gene sequencing for possible fetal achondroplasia or craniosynostosis, and targeted testing for mutations in genes causing Noonan syndrome.

With the introduction of new genomic sequencing techniques, it is anticipated that prenatal diagnosis for mutations in multiple genes will be more readily available for a number of groups of conditions including skeletal dysplasias, Noonan syndrome, craniofacial disorders including craniosynostosis, arthrogryposis and others. In future, tests of this sort may also be available using maternal blood samples to analyse cell free DNA (noninvasive prenatal diagnosis, NIPD). These should currently only be offered through a specialist fetal medicine and genetics service.

3.6 Prenatal screening by fetal ultrasound in mid-trimester

It is recommended all women are offered a fetal morphology ultrasound scan at 18-22 weeks gestation, plus additional ultrasound scans depending on individual circumstances (Routine Antenatal Assessment in the Absence of Pregnancy Complications (C-Obs 03b) and HGSA/RANZCOG Prenatal Assessment of Fetal Structural Conditions (C-Obs 60)). Irregularities of fetal organ formation, growth or development may indicate an underlying chromosomal or single gene disorder.

3.7 Other issues

3.7.1 Assisted reproductive technologies (ART)

Pregnancies conceived using assisted reproductive technologies (ART) have been shown to have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to an increased likelihood of receiving false-positive results in first trimester screening for Down syndrome. Lower PAPP-A may reflect impairment of early implantation with some forms of ART. Some laboratories providing screening results incorporate this factor into their calculations, but not all. It is not certain that NT measurements are altered in pregnancies conceived by ART although some research has suggested this may be the case. [43-46] There is a higher risk of cfDNA test failure for IVF pregnancies compared with spontaneous conceptions (5.2% vs 2.2%).[47]

3.7.2 Pre-implantation genetic diagnosis (PGD)

Pre-implantation genetic diagnosis (PGD) is used to determine if genetic or chromosomal disorders are present in embryos produced through ART. PGD tests embryos before they are transferred to the uterus so couples can make informed decisions about their next steps in the IVF process. It was first used by couples with an increased chance of genetic conditions, to select embryos free of an inherited genetic disorder. They did not necessarily have infertility problems, but sought to have an embryo unaffected by the genetic disorder selected for transfer to the uterus. Pre-implantation genetic screening (PGS) for aneuploidy is now done in cases where there have been multiple miscarriages or lack of success with a large number of embryo transfer in couples seeking infertility treatment. PGD/PGS analysis is done on a small number of cells and hence is subject to error due to mosaicism. Couples are often offered confirmation of results with prenatal diagnosis.

4. Governance

4.1 Quality Assurance

4.1.1 Education for health professionals involved in prenatal screening

Health professionals caring for pregnant women should undertake continuing education regarding options available for prenatal screening and diagnosis, and should:

- Have up-to-date knowledge about the current screening modalities available and in what settings they can be implemented.
- Be able to provide pre-and post-test information, support and counselling, including written resources.
- Participate in continuing professional development (CPD) and courses that provide current evidence based information on prenatal screening and diagnosis.

Health professionals providing care to pregnant women will benefit from undertaking some modules of the Nuchal Translucency Online Learning Program (NTOLP) to gain an understanding of the complexities of prenatal screening and diagnosis in the first and second trimesters of pregnancy. See https://elearning.nuchaltrans.edu.au/

4.1.2 Performance quality standards and monitoring processes

What are the quality standards for prenatal screening programs?

i) Laboratory accreditation

All laboratories undertaking prenatal screening must be accredited by the National Association of Testing Authorities (NATA) in Australia, and International Accreditation New Zealand (IANZ) in New Zealand.

Those who undertake prenatal testing, whether laboratory or ultrasound units, should undertake overall audit and monitoring of their prenatal screening programs and participate in external quality assurance activities.

All pathology laboratories in Australia receiving funding via Medicare must be accredited by the National Association of Testing Authorities (NATA)/RCPA Laboratory Accreditation Program. The Standards are set by the National Pathology Accreditation Advisory Council (NPAAC). The Standards are based on the international standard ISO 15189 Standard for Medical Laboratories. In New Zealand laboratories are accredited via IANZ using the same ISO 15189 as their basis(https://www.rcpa.edu.au/Patients/Lab-Accreditation).

ii) Sonographer accreditation

Sonographers performing medical ultrasound examinations must be suitably qualified, involved in a relevant and appropriate Continuing Professional Development program and be registered on the Register of Accredited Sonographers held by Medicare Australia. For further information, please contact the Medicare Australia or the Australasian Sonographer Accreditation Registry. All operators should be certified to perform the NT scan in Australia and participate in regular audit. Operators performing nasal bone or ductus venosus assessments should be suitably trained and certified to perform this assessment (e.g. by participating in the Nuchal Translucency Online Learning Program (NTOLP) https://elearning.nuchaltrans.edu.au/).

iii) Internal and external performance audit

Ultrasound, operators (including obstetricians, radiologists, sonographers and midwives) should participate in audit to monitor their performance.

Ideally, the performance of the program of interest should be measured by routine monitoring of analyte medians, detection rate, screen positive rate, maternal age distribution of the screened population, uptake of screening and prenatal diagnostic tests and pregnancy outcome.

5. References

1. Lindquist A, Poulton A, Halliday J, Hui L. Prenatal diagnostic testing and atypical chromosome abnormalities following combined first-trimester screening: implications for contingent models of non-invasive prenatal testing. Ultrasound Obstet Gynecol. 2017.
2. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;45(1):16-26.
3. Hui L, Hutchinson B, Poulton A, Halliday J. Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy. Genet Med. 2017;19(12):1338-45.
4. Marteau TM, Dormandy E, Michie S. A measure of informed choice. Health expectations : an international journal of public participation in health care and health policy. 2001;4(2):99-108.
5. Ryan K, Bain BJ, Worthington D, James J, Plews D, Mason A, et al. Significant haemoglobinopathies: guidelines for screening and diagnosis. British Journal of Haematology. 2010;149(1):35-49.
6. de Costa C, Douglas H, Hamblin J, Ramsay P, Shircore M. Abortion law across Australia--A review of nine jurisdictions. The Australian & New Zealand journal of obstetrics & gynaecology. 2015;55(2):105-11.
7. Nicolaides KH. First trimester diagnosis of chromosomal defects. In: The 11-13^{+6} weeks scan. London: Fetal Medicine Foundation, 2004 Report No.: Contract No.: .
8. Lewis SM, Cullinane FM, Carlin JB, Halliday JL. Women's and health professionals' preferences for prenatal testing for Down syndrome in Australia. The Australian & New Zealand journal of obstetrics & gynaecology. 2006;46(3):205-11.
9. 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(3):302-14.
10. Kenkhuis MJ, Bakker M, Bardi F, Fontanella F, Bakker MK, Fleurke-Rozema H, et al. Yield of a 12-13 week scan for the early diagnosis of fetal congenital anomalies in the cell-free DNA era. Ultrasound Obstet Gynecol. 2017.
11. Salomon LJ AZ, Audibert F et al. ISUOG updated consensus statement on the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound practice. . Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2017;49:815-16.
12. Jaques AM, Halliday JL, Francis I, Bonacquisto L, Forbes R, Cronin A, et al. Follow up and evaluation of the Victorian first-trimester combined screening programme for Down syndrome and trisomy 18. BJOG. 2007;114(7):812-8.
13. Jaques AM, Collins VR, Muggli EE, Amor DJ, Francis I, Sheffield LJ, et al. Uptake of prenatal diagnostic testing and the effectiveness of prenatal screening for Down syndrome. Prenat Diagn. 2010;30(6):522-30.
14. Cocciolone R, Brameld K, O'Leary P, Haan E, Muller P, Shand K. Combining first and second trimester markers for Down syndrome screening: think twice. The Australian & New Zealand journal of obstetrics & gynaecology. 2008;48(5):492-500.
15. Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, et al. DNA sequencing versus standard prenatal aneuploidy screening. N Engl J Med. 2014;370(9):799-808.
16. Cicero S, Avgidou K, Rembouskos G, Kagan KO, Nicolaides KH. Nasal bone in first-trimester screening for trisomy 21. Am J Obstet Gynecol. 2006;195(1):109-14.
17. Maiz N, Valencia C, Emmanuel EE, Staboulidou I, Nicolaides KH. Screening for adverse pregnancy outcome by ductus venosus Doppler at 11-13+6 weeks of gestation. Obstet Gynecol. 2008;112(3):598-605.
18. Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0 to 13+6 weeks of gestation. Ultrasound Obstet Gynecol. 2009;33(1):18-22.
19. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011;31(1):7-15.
20. Maiz N, Wright D, Ferreira AF, Syngelaki A, Nicolaides KH. A mixture model of ductus venosus pulsatility index in screening for aneuploidies at 11-13 weeks' gestation. Fetal Diagn Ther. 2012;31(4):221-9.
21. Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, et al. Noninvasive Prenatal Testing for Trisomy 21, 18 and 13 - Clinical Experience from 146,958 Pregnancies. Ultrasound Obstet Gynecol [Internet]. 2015 Jan 19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25598039.
22. Yaron Y. The implications of non-invasive prenatal testing failures: a review of an under-discussed phenomenon. Prenat Diagn. 2016;36(5):391-6.
23. Palomaki GE, Kloza EM, Lambert-Messerlian GM, van den Boom D, Ehrich M, Deciu C, et al. Circulating cell free DNA testing: are some test failures informative? Prenat Diagn. 2015;35(3):289-93.
24. Bianchi DW, Parsa S, Bhatt S, Halks-Miller M, Kurtzman K, Sehnert AJ, et al. Fetal sex chromosome testing by maternal plasma DNA sequencing: clinical laboratory experience and biology. Obstet Gynecol. 2015;125(2):375-82.
25. Dondorp W, de Wert G, Bombard Y, Bianchi DW, Bergmann C, Borry P, et al. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. Summary and recommendations. European journal of human genetics : EJHG. 2015.

26. Gross SJ, Stosic M, McDonald-McGinn DM, Bassett AS, Norvez A, Dhamankar R, et al. Clinical experience with single-nucleotide polymorphism-based non-invasive prenatal screening for 22q11.2 deletion syndrome. Ultrasound Obstet Gynecol. 2016;47(2):177-83.
27. Pertile MD, Halks-Miller M, Flowers N, Barbacioru C, Kinnings SL, Vavrek D, et al. Rare autosomal trisomies, revealed by maternal plasma DNA sequencing, suggest increased risk of feto-placental disease. Sci Transl Med. 2017;9(405).
28. Hui L. Cell-free DNA testing for 22q11.2 deletion syndrome: appraising the viability, effectiveness and appropriateness of screening. Ultrasound Obstet Gynecol. 2016;47(2):137-41.
29. Gagnon A, Audibert F. Prenatal screening and diagnosis of aneuploidy in multiple pregnancies. Best practice & research Clinical obstetrics & gynaecology. 2014;28(2):285-94.
30. Cleary-Goldman J, Rebarber A, Krantz D, Hallahan T, Saltzman D. First-trimester screening with nasal bone in twins. Am J Obstet Gynecol. 2008;199(3):283 e1-3.
31. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther. 2010;27(1):1-7.
32. Ogilvie C, Akolekar R. Pregnancy Loss Following Amniocentesis or CVS Sampling—Time for a Reassessment of Risk. J Clin Med. 2014;3(3):741-6.
33. Halliday JL LJ, Sheffield LJ, Robinson HP, Renou P, Carlin JB. . Importance of complete follow-up of spontaneous fetal loss after amniocentesis and chorion villus sampling. Lancet. 1992;340:886-90.
34. Alfirevic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus sampling for prenatal diagnosis. The Cochrane database of systematic reviews. 2017;9:Cd003252.
35. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. The New England journal of medicine. 2012;367(23):2175-84.
36. Shaffer LG, Rosenfeld JA, Dabell MP, Coppinger J, Bandholz AM, Ellison JW, et al. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound. Prenat Diagn. 2012;32(10):986-95.
37. American College of Obstetricians and Gynecologists (ACOG). The use of chromosomal microarray analysis in prenatal diagnosis: Committee Opinion No. 581 Obstet Gynecol. 2013;122:1374-7.
38. Fiorentino F, Napoletano S, Caiazzo F, Sessa M, Bono S, Spizzichino L, et al. Chromosomal microarray analysis as a first-line test in pregnancies with a priori low risk for the detection of submicroscopic chromosomal abnormalities. European journal of human genetics : EJHG. 2013;21(7):725-30.
39. Wapner RJ, Driscoll DA, Simpson JL. Integration of microarray technology into prenatal diagnosis: counselling issues generated during the NICHD clinical trial. Prenatal diagnosis. 2012;32(4):396-400.
40. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, et al. Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med. 2011;3(65):65ra4.
41. Edwards JG, Feldman G, Goldberg J, Gregg AR, Norton ME, Rose NC, et al. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the american college of medical genetics and genomics, american college of obstetricians and gynecologists, national society of genetic counselors, perinatal quality foundation, and society for maternal-fetal medicine. Obstetrics and gynecology. 2015;125(3):653-62.
42. Lazarin GA, Haque IS, Nazareth S, Iori K, Patterson AS, Jacobson JL, et al. An empirical estimate of carrier frequencies for 400+ causal Mendelian variants: results from an ethnically diverse clinical sample of 23,453 individuals. Genet Med. 2013;15(3):178-86.
43. Maymon R, Shulman A. Serial first- and second-trimester Down's syndrome screening tests among IVF- versus naturally-conceived singletons. Human reproduction. 2002;17(4):1081-5.
44. Hui PW, Tang MH, Lam YH, Yeung WS, Ng EH, Ho PC. Nuchal translucency in pregnancies conceived after assisted reproduction technology. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2005;25(3):234-8.
45. Amor DJ, Xu JX, Halliday JL, Francis I, Healy DL, Breheny S, et al. Pregnancies conceived using assisted reproductive technologies (ART) have low levels of pregnancy-associated plasma protein-A (PAPP-A) leading to a high rate of false-positive results in first trimester screening for Down syndrome. Human reproduction. 2009;24(6):1330-8.
46. Gjerris AC, Loft A, Pinborg A, Tabor A, Christiansen M. First-trimester screening in pregnancies conceived by assisted reproductive technology: significance of gestational dating by oocyte retrieval or sonographic measurement of crown-rump length. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2008;32(5):612-7.
47. Lee TJ, Rolnik DL, Menezes MA, McLennan AC, da Silva Costa F. Cell-free fetal DNA testing in singleton IVF conceptions. Human reproduction (Oxford, England). 2018.

4. Other suggested reading

1. Royal College of Obstetricians and Gynaecologists. 2014. Non-invasive Prenatal Testing for Chromosomal Abnormality using Maternal Plasma DNA (Scientific Impact Paper No. 15).

2. Oepkes, D. Yaron, Y. Kozlowski, P. Rego de Sousa, M.J. Bartha, J.L. van den Akker, E.S. et al. Counseling for non-invasive prenatal testing (NIPT): what pregnant women may want to know. Ultrasound Obstet Gynecol. 2014; 44(1):1-5.

3. Edwards JG, Feldman G, Goldberg J, Gregg AR, Norton ME, Rose NC, et al. Expanded carrier screening in reproductive medicine-points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynaecologists, National Society of Genetic Counsellors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. Obstet Gynecol. 2015;125(3):653-62.

5. Links to other College statements

1. HGSA/RANZCOG Prenatal Assessment of Fetal Structural Conditions (C-Obs 60)
2. HGSA/RANZCOG Prenatal Screening for Adverse Pregnancy Outcomes (C-Obs 61)
3. RANZCOG Mid-trimester Fetal Morphology Ultrasound Screening (C-Obs 57)
4. RANZCOG Prenatal Screening for Fetal Conditions(C-Obs 35)
5. RANZCOG Pre-pregnancy Counselling (C-Obs 3(a))
6. RANZCOG Routine Antenatal Assessment in the Absence of Pregnancy Complications (C-Obs 3 (b))
7. RANZCOG Diagnostic Ultrasound, Position Statement on the Appropriate Use of (C-Gen 10)

6. Patient information pamphlets

A range of RANZCOG Patient Information Pamphlets can be ordered via:

https://www.ranzcog.edu.au/Womens-Health/Patient-Information-Guides/Patient-Information-Pamphlets

A decision aid has been developed by the Murdoch Children's Research Institute for women considering prenatal screening for chromosome conditions.
Your choice - Prenatal screening tests in pregnancy

7. Appendices

Appendix A – Updating Committee Membership (2017-2018)

Name	Expertise	Role
A/Professor Michael Gabbett – HGSA member	Clinical Genetics	Eminent Staff Specialist in Clinical Genetics, Genetic Health Queensland, Associate Professor, Griffith University, Senior Lecturer, The University of Queensland
Professor Jane Halliday – HGSA member	Epidemiology and Research	Head, Public Health Genetics Genetics Theme, Murdoch Children's Research Institute
Clinical Professor Jon Hyett – RANZCOG member	Obstetrics and Gynaecology	Head of High Risk Obstetrics, Royal Prince Alfred Women and Babies. Clinical Professor, Obstetrics and Gynaecology University of Sydney
Dr Scott White – RANZCOG member	Obstetrics and Gynaecology	Consultant to the Maternal Fetal Medicine Unit at King Edward Memorial Hospital Western Australia
A/Prof Lisa Hui– RANZCOG member	Obstetrics and Gynaecology Maternal Fetal Medicine	University of Melbourne Department of Perinatal Medicine Mercy Hospital for Women
Dr George McGillivray	Clinical Genetics	Genetics of the North East Victorian Clinical Genetics Service Royal Women's Hospital Melbourne, VIC

Appendix B Women's Health Committee Membership

Name	Position on Committee
Professor Yee Leung	Chair
Dr Joseph Sgroi	Deputy Chair, Gynaecology
Associate Professor Janet Vaughan	Deputy Chair, Obstetrics
Associate Professor Ian Pettigrew	EAC Representative
Dr Tal Jacobson	Member
Dr Ian Page	Member
Dr John Regan	Member
Dr Craig Skidmore	Member
Dr Lisa Hui	Member
Dr Bernadette White	Member
Dr Scott White	Member
Associate Professor Kirsten Black	Member
Dr Greg Fox	College Medical Officer
Dr Marilyn Clarke	Chair of the ATSI WHC
Dr Martin Byrne	GPOAC Representative
Ms Catherine Whitby	Community Representative
Ms Sherryn Elworthy	Midwifery Representative
Dr Amelia Ryan	Trainee Representative

Appendix C Overview of the development and review process for this statement

i. Steps in developing and updating this statement

This statement was originally developed in August 1991 (C-Obs 4), and subsequently updated in 1990 (C-Obs 5), and 2015 (C-Obs 59). During 2013-2015, the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening carried out the following steps in reviewing this statement:

- Declarations of interest were sought from all members prior to reviewing this statement.
- Structured clinical questions were developed and agreed upon.
- An updated literature search to answer the clinical questions was undertaken.
- At the March 2018 face-to-face committee meeting, the existing consensus-based recommendations were reviewed and updated (where appropriate) based on the available body of evidence and clinical expertise. Recommendations were graded as set out below in Appendix B part iii).

ii. Declaration of interest process and management

Declaring interests is essential in order to prevent any potential conflict between the private interests of members, and their duties as part of the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening.

A declaration of interest form specific to guidelines and statements was developed by RANZCOG and approved by the RANZCOG Board in September 2012. The HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening members were required to declare their relevant interests in writing on this form prior to participating in the review of this statement.

Members were required to update their information as soon as they become aware of any changes to their interests and there was also a standing agenda item at each meeting where declarations of interest were called for and recorded as part of the meeting minutes.

There were no significant real or perceived conflicts of interest that required management during the process of updating this statement.

iii. Grading of recommendations

Each recommendation in this College statement is given an overall grade as per the table below, based on the National Health and Medical Research Council (NHMRC) Levels of Evidence and Grades of Recommendations for Developers of Guidelines. Where no robust evidence was available but there was sufficient consensus within the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening Committee, consensus-based recommendations were developed or existing recommendations updated (and are identifiable as such). Consensus-based recommendations were agreed to by the entire Committee. Good Practice Notes are highlighted throughout and provide practical guidance to facilitate implementation. These were also developed through consensus of the entire Committee.

Recommendation category		*Description*
Evidence-based	*A*	*Body of evidence can be trusted to guide practice*
	B	*Body of evidence can be trusted to guide practice in most situations*
	C	*Body of evidence provides some support for recommendation(s) but care should be taken in its application*
	D	*The body of evidence is weak and the recommendation must be applied with caution*
Consensus-based		*Recommendation based on clinical opinion and expertise as insufficient evidence available*
Good Practice Note		*Practical advice and information based on clinical opinion and expertise*

Appendix D Full Disclaimer

This information is intended to provide general advice to practitioners, and should not be relied on as a substitute for proper assessment with respect to the particular circumstances of each case and the needs of any patient.

This information has been prepared having regard to general circumstances. It is the responsibility of each practitioner to have regard to the particular circumstances of each case. Clinical management should be responsive to the needs of the individual patient and the particular circumstances of each case.

This information has been prepared having regard to the information available at the time of its preparation, and each practitioner should have regard to relevant information, research or material which may have been published or become available subsequently.

Whilst the College endeavours to ensure that information is accurate and current at the time of preparation, it takes no responsibility for matters arising from changed circumstances or information or material that may have become subsequently available.

Appendix E Considerations for Indigenous and Culturally and Linguistically Diverse Populations

4.2.1 There should be appropriate communication with all women. Particular care should be taken to ensure that communication is clear and understood by women who are from culturally and linguistically diverse populations (including women from an Indigenous background).

4.2.2 In Australia, the Department of Immigration and Citizenship offers Free Interpreting Services through TIS National for private medical practitioners (defined as General Practitioners and Medical Specialists) providing Medicare rebate-able services and their reception staff to arrange appointments and provide results of medical tests. Free interpreters are also available in New Zealand.

4.2.3 A resource developed especially for Indigenous women by the Menzies School of Health Research is available on line at this link: - Fetal Anomaly Screening Resource "Take Home Booklet" Menzies School of Health Research

Appendix F Conditions with higher prevalence in people with particular ethnicity

Some places in Australia offer carrier screening for specific conditions before and/or during pregnancy. Examples of conditions screened for and the populations with the highest probability are shown in the Table below.

	Cystic fibrosis	Haemoglobinopathies/ thalassaemia	Common Ashkenazi mutations	Spinal muscular atrophy	Fragile X syndrome[1]
European	X			X	X
Ashkenazi Jewish	X		X	X	X
Asian		X		X	X
African		X		X	X
Mediterranean		X		X	X

[1]Only women need be offered FXS screening. FXS screening is particularly important if there is a family history of intellectual disability.

New genomic technologies are now facilitating carrier screening on a wider scale and in future these may well supersede existing programs.

Appendix G Definitions and Abbreviations

The following table details terms and abbreviations used throughout this statement. The definitions have been taken from the National Library of Medicines Medical Subject Headings (MeSH) database where available.

Term	Definition	Abbreviation
Alpha-fetoprotein	The first alpha-globulins to appear in mammalian sera during fetal development and are the dominant serum proteins in early embryonic life. AFP is measured in pregnant women through the analysis of maternal blood or amniotic fluid, as a screening test for a subset of developmental abnormalities.	AFP, α-fetoprotein
Amniocentesis	Percutaneous transabdominal puncture of the uterus during pregnancy to obtain amniotic fluid. It is commonly used for fetal karyotype determination in order to diagnose abnormal fetal conditions.	-
Assisted Reproductive Technology	Assisted reproductive technology (ART) is the application of laboratory or clinical technology to gametes (human egg or sperm) and/or embryos for the purposes of reproduction. Techniques include: embryo transfer; fertility preservation; in vitro fertilisation; gamete intrafallopian transfer; in vitro oocyte maturation; artificial insemination; in vitro oocyte maturation techniques; oocyte donation; oocyte retrieval; ovulation induction; posthumous conception; sperm retrieval; zygote intrafallopian transfer.	ART
Chorionic Villus Sampling	A method for diagnosis of fetal diseases by sampling the cells of the placental chorionic villi for DNA analysis, presence of bacteria, concentration of metabolites, etc. The advantage over amniocentesis is that the procedure can be carried out in the first trimester.	CVS
Cell free fetal DNA screening (or Noninvasive prenatal testing)	Cell-free fetal DNA of placental origin is detectable in maternal plasma from early first trimester. Cell-free fetal DNA screening is a screening test that indicates if a woman has a higher chance of having a fetus with Down syndrome (trisomy 21), Edward syndrome (trisomy 18) and Patau syndrome (trisomy 13). These cell-free fetal DNA fragments are released and comprise about 10% of the total cell-free DNA in maternal blood. CfDNA testing for fetal aneuploidy works by sequencing a portion of each DNA fragment in maternal plasma (both maternal and fetal), mapping each DNA sequence to a reference genome to determine its chromosome of origin, and counting the number of fragments arising from each chromosome.	cfDNA (or NIPT)

Combined First Trimester Screening	Combined first trimester screening test involves an ultrasound scan and a blood test at 11-13+6 weeks pregnancy.	cFTS
Cystic Fibrosis	An autosomal recessive genetic disease of the exocrine glands. Cystic fibrosis is characterised by epithelial secretory dysfunction associated with ductal obstruction resulting in airway obstruction; chronic respiratory infections; pancreatic insufficiency; maldigestion; salt depletion; and heat prostration.	CF
Diagnostic test	Any kind of medical test performed to aid in the diagnosis or detection of disease. In the context of this document, if a woman's result shows an increased chance of a particular condition or conditions, she is offered a diagnostic test.	-
Down syndrome	A chromosome disorder caused by either an extra chromosome 21 or an effective trisomy for chromosome 21. Clinical manifestations include hypotonia, short stature, brachycephaly, upslanting palpebral fissures, epicanthus, brushfield spots on the iris, protruding tongue, small ears, short, broad hands, fifth finger clinodactyly, Simian crease, and moderate to severe intellectual disability. Cardiac and gastrointestinal malformations, a marked increase in the incidence of leukemia, and the early onset of Alzheimer disease are also associated with this condition.	or Down's syndrome, also known as trisomy 21
Fragile X Syndrome	Fragile X syndrome (FXS) is a genetic condition causing intellectual disability, behavioural and learning challenges and various physical characteristics. Fragile X syndrome (FXS) is caused by the expansion or lengthening of the FMR1 gene on the X chromosome, known as a gene mutation. The X chromosome is one of two sex determining chromosomes. When the gene lengthens it switches off production of a protein that is involved in brain development and other functions. It is also the most common single gene cause of autism worldwide.	FXS
Free β human chorionic gonadotrophin	The beta subunit of human chorionic gonadotropin. Beta HCG is used as a diagnostic marker for early detection of pregnancy, Down syndrome, spontaneous abortion, ectopic pregnancy, hydatidiform mole or choriocarcinoma.	Beta HCG βhCG
Maternal Age	The age of the mother in pregnancy.	MA
Multiples of the Median	A multiple of the median (MoM) is a measure of how far an individual test result deviates from the median. MoM is commonly used to report the results of medical screening tests, particularly where the results of the individual tests	MoMs

	are highly variable.	
Negative Predictive Value	The negative predictive value is the proportion of negative results in tests that are true negative results. The NPV is not intrinsic to the test—it depends also on the prevalence.	NPV
Nuchal translucency	A prenatal ultrasonography measurement of the soft tissue behind the fetal neck.	NT
Oestriol	One of the three main estrogens produced by the human body. It is a hormone made during pregnancy that can be used to measure foetal health and predict when birth may happen.	UE3
Pregnancy associated plasma protein A	A product of the placenta, and decidua, secreted into the maternal circulation during pregnancy.	PAPP-A
Pre-implantation genetic diagnosis	Determination of the nature of a pathological condition or disease in the ovum; zygote; or blastocyst prior to implantation. It is used to test embryos for specific genetic or chromosomal abnormalities and enables the selection of unaffected embryos prior to implantation and pregnancy.	PGD
Positive Predictive Value	The positive predictive value is the proportion of positive results in tests that are true positive results. The PPV is not intrinsic to the test—it depends also on the prevalence.	PPV
Screening test	Screening is a strategy used to identify an unrecognised disease in individuals without signs or symptoms. This can include individuals with pre-symptomatic or unrecognised symptomatic disease. In the context of this document, if an individual in the general population is tested for a condition (e.g. with no known family history), the test is referred to as a screening test.	-
Turner Syndrome	A syndrome of defective gonadal development in phenotypic females associated with the karyotype 45,X (or 45,XO). Patients generally are of short stature with undifferentiated gonads (streak gonads), sexual infantilism, hypogonadism, webbing of the neck, cubitus valgus, elevated gonadotropins, decreased estradiol level in blood, and congenital heart defects.	-