



Prenatal screening and diagnosis of chromosomal and genetic conditions in the fetus in pregnancy

This statement has been developed and reviewed by the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening and approved by the Women's Health Committee and RANZCOG Council. Dr Lisa Hui was the guest author of [Appendix D](#) on cell free fetal DNA screening.

A list of members of the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening can be found in [Appendix A](#) and Women's Health Committee in [Appendix B](#).

The committee acknowledges the contribution of Dr George McGillivray, Dr Lisa Hui and Professor Jane Halliday to this document.

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

Disclaimer This information is intended to provide general advice to practitioners. This information 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 document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The document has been prepared having regard to general circumstances.

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

Outcomes: 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, radiologists and general practitioners), midwives, nurses and genetic counsellors;
- Scientists, laboratory staff and administrative staff delivering services for prenatal screening and diagnosis.

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.

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). In 2014-15, C-Obs 4 and C-Obs 5 were significantly edited by the committee to create this current version (C-Obs 59).

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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 in maternity care to provide the pregnant woman with more information about her unborn baby. All such testing should be **voluntary** and only undertaken when the pregnant 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, accessible test to all pregnant women in order to identify those women with an increased likelihood of having a baby with a chromosomal or genetic condition. These women are then followed up with genetic counselling and offered diagnostic testing. Only an invasive test, either amniocentesis or chorionic villus sampling, can definitively diagnose a genetic or chromosomal condition in the baby. As all diagnostic tests carry a small risk of miscarriage, screening programs aim to minimise the need for invasive testing, while maximising the chance of identifying babies with chromosomal or genetic conditions.

The most common chromosomal cause of intellectual disability in children and adults is **Down syndrome** (trisomy 21). This condition is caused by the baby having three copies of chromosome 21, instead of the usual two copies. Down syndrome is usually a sporadic condition. Because of its frequency in the population (about 1 in 800) and its effects on health and learning, Down syndrome has been for many years the major focus of prenatal screening programs. Other chromosomal conditions (aneuploidies) that are screened for include Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13). These conditions are associated with disability, pregnancy loss or death in the newborn.

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

This statement summarises recommendations for prenatal screening for chromosomal and genetic conditions for pregnant women and their partners in the general population. However, women with an increased probability of having a child with a chromosomal or genetic condition due to past obstetric, medical or family history should receive individualised counselling from a specialist clinical genetics service, preferably prior to pregnancy.

* 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.

2. Summary of recommendations

Prenatal tests for chromosome aneuploidies	
Recommendation 1	Grade and supporting references
<p>All pregnant women* should be provided with information and offered the opportunity to have a discussion about the range of aneuploidies that can be detected and the characteristics of the available prenatal screening and diagnostic tests.</p> <p>* and their partners, or support person if appropriate</p>	<p>Level III-3 Grade C</p> <p>1</p>
Recommendation 2	Grade and supporting references
<p>Women should have timely access to tests for assessment of aneuploidies with adequate sensitivity and specificity (defined in table 1). Prenatal screening options should be discussed in the first trimester whenever possible in order to maximise screening options.</p>	<p>Consensus-based recommendation</p>
Good practice notes for maternal plasma cell free DNA (cfDNA) based testing for fetal aneuploidy	Grade and supporting references
<ul style="list-style-type: none"> • Accurate dating, confirmation of viability and determination of the number of embryos by ultrasound is recommended prior to cfDNA testing. • cfDNA based screening for fetal aneuploidy is not diagnostic. The chance of having an affected fetus following a cfDNA result reported as high risk (ie the positive predictive value, PPV) may be < 50%, depending on the specific chromosome involved and the background risk of the woman. Confirmatory diagnostic testing is strongly recommended after an abnormal cfDNA result. • If a woman has received a cfDNA reported as normal/low risk, an additional calculation for aneuploidy (e.g. by combined first trimester or second trimester serum screening) is not recommended as this will increase the false positive rate without substantially improving the detection rate. • The presence of a fetal structural anomaly remains an important indication for invasive prenatal testing, even in the presence of a prior cfDNA result reported as normal/low risk. • Pre-test counselling 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. 	<p>Consensus-based recommendation</p>
Recommendation 3	Grade and supporting references
<p>If a result is obtained indicating a greater probability of an aneuploidy, the woman should have access to genetic counselling services for support during decision-making and follow-up.</p> <p>The option of prenatal diagnosis should be discussed and offered.</p>	<p>Consensus-based recommendation</p>
Multiple pregnancies	

Recommendation 4	Grade and supporting references
In twin pregnancies, combined first trimester screening is the recommended modality for screening for aneuploidies. First trimester ultrasound assessment of chorionicity is recommended for interpretation of screening results and triaging to appropriate models of antenatal care.	Consensus-based recommendation
Good practice notes	Grade and supporting references
Aneuploidy screening for triplet and higher order pregnancies should be performed with first trimester ultrasound markers (ie. nuchal translucency thickness and nasal bone assessment +/- additional markers at 11-13 weeks).	Good practice notes (consensus-based)
Prenatal tests for other genetic disorders	
Recommendation 5	Grade and supporting references
All couples intending to have children, or who are pregnant, should have a careful family history taken regarding relatives with inherited disorders. Those identified with a family history of inherited disorders should be made aware of the availability of carrier screening for recessive conditions. (see Appendix F for particular population groups).	Consensus-based recommendation
Recommendation 6	Grade and supporting references
Where available, carrier status screening of women with a low probability for the more common genetic conditions (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome) may be offered. Women considering whether to have the test should be appropriately informed of the benefits and limitations of testing, and any associated costs.	Consensus-based recommendation
Good practice note	Grade and supporting references
Pre-pregnancy screening is preferable to antenatal screening for inheritable genetic conditions as this allows more options for carrier couples, including pre-implantation genetic diagnosis.	Good practice notes (consensus-based)
Recommendation 7	Grade and supporting references
All individuals with an increased likelihood of haemoglobinopathy based on their ethnic background should be offered basic screening for carrier status by a full blood examination at a minimum. Primary screening with specific assays for haemoglobinopathies (such as HPLC or EPG) can also be offered depending on local resources and population profile.	Grade C ²
Recommendation 8	Grade and supporting references
Where a fetal chromosome or genetic condition is suspected, diagnostic testing should be offered, depending on the nature of the condition found. Diagnostic tests that could be offered are discussed in section 3.4 .	Consensus-based recommendation

3. Discussion and recommendations

3.1 Prenatal tests for aneuploidies

General information on prenatal screening and diagnosis

3.1.1 All pregnant women* should be advised of the availability of prenatal screening 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”.¹

* and their partners, or support person if appropriate

3.1.2 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. Women electing not to have ultrasound screening in pregnancy should be aware of the other important benefits of routine scanning, including placental localisation, confirmation of gestational age, and excluding multiple pregnancy.

3.1.3 Information should be communicated using clear, simple and consistent language when discussing the tests, with confirmation that the information has been understood.

3.1.4 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](#)).

3.1.5 Information should include the following:

- 3.1.5.1 A description of the conditions that can be detected and the testing process. This should include information about phenotypic variability and the difficulties in being able to predict the extent of effect.
- 3.1.5.2 A discussion of the differences between screening and diagnostic tests
- 3.1.5.3 Advantages and disadvantages of the different types of tests available (taking into account the gestation of the pregnancy).
- 3.1.5.4 Practical aspects of testing; including the timing of tests and the approximate costs involved.
- 3.1.5.5 The possibility that the screening and diagnostic pathway may reveal anomalies other than those expected.
- 3.1.5.6 Details of support groups and sources of further information (see [Antenatal tests for child disability: what to consider](#), [Raising Children Network](#) and [Guidelines for maternity providers offering antenatal screening for Down syndrome and other conditions in New Zealand](#)).
- 3.1.5.7 The understanding that, if aneuploidy is diagnosed, women and their partners can choose whether to continue the pregnancy or have a termination. Where a genetic 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 an informed decision regarding pregnancy termination.
- 3.1.5.8 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 very poor. If they choose termination, they need to know that the mode of termination may be influenced by gestational age in line with local legal precedents.

- 3.1.5.9 The offer of screening should be made to all people irrespective of what may be their perceived likely choices if the result was an increased probability of a condition. 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 available.

Prenatal tests for aneuploidies	
Recommendation 1	Grade and supporting references
All pregnant women* should be provided with information and offered the opportunity to have a discussion about the range of aneuploidies that can be detected and the characteristics of the available prenatal screening and diagnostic tests.	Level III-3 Grade C 1
* and their partners, or support person if appropriate	

3.2 Prenatal screening tests for fetal aneuploidy

Prenatal screening programs for birth defects have traditionally focussed on aneuploidies 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 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 likelihood 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.³ The overall prevalence of trisomy 21 has increased with the trend to later childbearing in many developed countries.

Trisomy 21 comprises approximately half of the major aneuploidies detected prenatally. The next most common autosomal trisomies are trisomy 18 and trisomy 13. Together, trisomies 21, 18 and 13 make up about 80% of major aneuploidies detected by prenatal diagnosis.

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. Screening programs should be based on tests that perform to a minimum standard; that is with anticipated sensitivity >75% and specificity >95%. 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.⁴

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 the fetal nuchal translucency, and maternal serum markers levels to generate an overall figure for the likelihood of trisomy 21. Results for trisomy 13 and 18 can also be incorporated into the first trimester combined screening algorithm. This test is the standard of care in most developed countries, due to its dual advantages of high sensitivity and early detection.

ii) Cell-free DNA (cfDNA) 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. However, cfDNA testing is currently more expensive than CFTS and must be self-funded (currently no Medicare or private insurance rebate). This direct cost currently poses a significant barrier to accessibility and widespread clinical implementation. A detailed account of cfDNA testing is contained in [Appendix D](#).

Screening tests available in second trimester

Women in second trimester may be offered maternal serum screening with the quadruple test (15-20 weeks) or cfDNA testing (any gestation after 10 weeks). 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.

Integrated and sequential screening strategies that combine information from both first and second trimester serum screening results are not routinely used in Australia and New Zealand.

The performance characteristics of recommended screening tests for trisomy 21 are contained in table 1.

Table 1: Screening tests for trisomy 21 that meet the performance standard (i.e. have >75% sensitivity / >95% specificity).

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% ⁵
Quadruple test: MA + AFP + βhCG + UE3 + Inhibin	15 – 20 weeks	75%	95%	~2-3%
cell-free DNA screening*	> 10 ⁺ weeks	99%	99% [*]	~45% ⁶

*In a small proportion (<5%) of cases cfDNA testing is unable to provide a result

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.

Table 2: Screening tests for trisomy 21 that do not meet the performance criteria (i.e. have sensitivity <75% / specificity <95%)

Test	Gestation for screening	Sensitivity	Specificity
Maternal age alone:	Any stage	30-50% [*]	70% [*]
Double test: MA + AFP + βhCG	15 – 20 weeks	60%	95%
Triple test: MA +AFP + βhCG + UE3	15 – 20 weeks	70%	95%
Nuchal translucency alone (no biochemistry): MA + NT	11 ⁺¹ - 13 ⁺⁶ weeks	70%	95%

* varies according to maternal age distribution in the population

Screening programs should ideally collate data to demonstrate the quality of assessment, including the collection of data demonstrating local performance of measured characteristics (e.g. biochemical assays / the ultrasound marker nuchal translucency). 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 including assessment of the nasal bone⁷, ductus venosus waveform⁸ and tricuspid valve flow.⁹ 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%.¹⁰ Extra biochemical markers, such as placental growth factor, have also been investigated in first trimester screening.¹¹ 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 technology to detect an aneuploid pregnancy 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 emerging on its use in low probability or mixed probability populations, suggesting equal test performance characteristics (i.e. sensitivity and specificity) but a lower PPV as would be expected from its use in lower prevalence populations.^{6,12} Therefore, the uptake of this particular aspect of screening should be optional where possible.

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 and can be confounded by underlying maternal and fetal factors.¹³

This test does not attract any government or private insurance rebate and therefore 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 \$450-\$1000+. Clinicians should inform themselves about this new screening test and its potential place in screening and prenatal diagnosis pathways. Its use should be guided by local availability, patient preference, affordability, local institutional guidelines and individual clinician discretion.

Further detailed information is provided in [Appendix D](#).

Prenatal tests for aneuploidies	
Recommendation 2	Grade and supporting references
Women should have timely access to tests for assessment of aneuploidies with adequate sensitivity and specificity (defined in table 1). Prenatal screening options should be discussed in the first trimester whenever possible in order to maximise screening options.	Consensus-based recommendation
Good practice notes for cfDNA based screening for fetal aneuploidy	Grade and supporting references
<ul style="list-style-type: none"> • Accurate dating, confirmation of viability and determination of the number of embryos by ultrasound is recommended prior to cfDNA testing. • cfDNA based screening for fetal aneuploidy is not diagnostic. The chance of having an affected fetus following a cfDNA result reported as high risk (ie the positive predictive value, PPV) may be < 50%, depending on the specific chromosome involved and the background risk of the woman. Confirmatory diagnostic testing is strongly recommended after an abnormal cfDNA result. • If a woman has received a cfDNA reported as normal/low risk, an additional calculation for aneuploidy (e.g. by combined first trimester or second trimester serum screening) is not recommended as this will increase the false positive rate without substantially improving the detection rate. • The presence of a fetal structural anomaly remains an important indication for invasive prenatal testing, even in the presence of a prior cfDNA result reported as normal/low risk. <p>Pre-test counselling 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.</p>	Good practice notes (consensus-based)

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.

Recommendation 3	Grade and supporting references
If a result is obtained indicating a greater probability of an aneuploidy, the woman should have access to genetic counselling services for support during decision-making and follow-up. The option of prenatal diagnosis should be discussed and offered.	Consensus-based recommendation

3.2.4 Screening for aneuploidy in multiple pregnancies

Twin pregnancies

The performance of 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, CFTS is the recommended modality for screening. The sensitivity of CFTS in twins generally ranges from 72%-80%.¹⁴ The use of nasal bone assessment can improve the sensitivity of CFTS in twin pregnancies to 89% for a fixed 5% false positive rate.¹⁵

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.

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. cfDNA testing in twin pregnancies has not been as extensively evaluated as in singletons due to the limitations of smaller numbers. The two largest published studies have a combined total of 626 twin pregnancies. Their results suggest sensitivities of approximately 90% for trisomy 21, 50% for trisomy 13 and 83% for trisomy 18.^{16,17} These studies noted a considerably higher “no call” rate for twin pregnancies of > 5%, which should be taken into consideration in pretest counselling.

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 (e.g. nuchal translucency and nasal bone) as serum markers cannot be used effectively.

Maternal serum screening and cfDNA testing cannot be used in triplet or higher order pregnancies.

Multiple pregnancies	
Recommendation 4	Grade and supporting references
In twin pregnancies, combined first trimester screening is the recommended modality for screening for aneuploidies. First trimester ultrasound assessment of chorionicity is recommended for interpretation of screening results and triaging to appropriate models of antenatal care.	Consensus-based recommendation
Good practice notes	Grade and supporting references
Aneuploidy screening for triplet and higher order pregnancies should be performed with first trimester ultrasound markers (i.e. nuchal translucency thickness and nasal bone assessment +/- additional markers at 11-13 weeks).	Good practice notes (consensus-based)

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%.¹⁸ 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 900.¹⁹ There is also evidence that the fetal loss rates for invasive procedures are operator and experience dependent.²⁰ Prenatal diagnostic service providers or pregnancy care providers who do not perform sufficient procedures per year to maintain their skills should be encouraged to refer their cases to a specialised prenatal diagnostic service that does.

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.

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 (chromosomes 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.^{21 22} 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.²³

In the setting of a normal fetal ultrasound scan (e.g. for maternal age or maternal serum screening), microarray still identifies a greater number of pathogenic chromosome changes than banded karyotype.^{21, 24}

Single-nucleotide-polymorphism-based microarray (SNP array) can 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 can detect variants of uncertain significance that may cause genetic counselling dilemmas and patient concern and distress.²⁵ 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 or uncertain microarray results should have access to a formal genetic counselling service staffed by genetic counsellors and/ or clinical geneticists.

Laboratories providing prenatal microarray need staff appropriately trained in the analysis and reporting of microarray data. Laboratories offering a prenatal microarray service should be appropriately accredited with their regional authority and should have access to a clinical geneticist to aide in the interpretation and reporting of rare or complex microarray findings.

3.5 Prenatal tests for other genetic disorders

3.5.1 *Population-based preconceptional genetic screening*

Preconceptional genetic screening will identify couples at 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 referral to a specialist clinical genetics service.

It is estimated that all individuals are carriers for at least three clinically severe recessive childhood disorders.²⁶ 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; thalassemia/haemoglobinopathies in South-East Asians; and Tay-Sachs disease in Ashkenazi Jews.

A number of genetic carrier screening programs 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). Further information is available from your local genetics service.

“Expanded 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- or two-step carrier screening.

With the introduction of new genomic sequencing techniques, it is anticipated that carrier screening for a multitude of recessive conditions will be routinely available for couples, who have no family history of a genetic disorder, in the near future.²⁷

Prenatal tests for other genetic disorders	
Recommendation 5	Grade and supporting references
All couples intending to have children, or who are pregnant, should have a careful family history taken regarding relatives with inherited disorders. Those identified with a family history of inherited disorders should be made aware of the availability of carrier screening for recessive conditions. (see Appendix F for particular population groups).	Consensus-based recommendation
Recommendation 6	Grade and supporting references
Where available, carrier status screening of women with a low probability for the more common genetic conditions (e.g. cystic fibrosis, spinal muscular atrophy, fragile X syndrome) may be offered. Women considering whether to have the test should be appropriately informed of the benefits and limitations of testing, and any associated costs.	Consensus-based recommendation
Good practice note	Grade and supporting references
Pre-pregnancy screening is preferable to antenatal screening for inheritable genetic conditions as this allows more options for carrier couples, including pre-implantation genetic diagnosis.	Consensus-based good practice note
Recommendation 7	Grade and supporting references
All individuals with an increased likelihood of haemoglobinopathy based on their ethnic background should be offered basic screening for carrier status by a full blood examination at a minimum. Primary screening with specific assays for haemoglobinopathies (such as HPLC or EPG) can also be offered depending on local resources and population profile.	Level C ²

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), advice should be sought from a 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 offered when appropriate.

3.5.3 Prenatal screening for genetic disorders suspected on the basis of fetal conditions

Screening 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 screening 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, arthrogyposis and others. In future tests of this sort may also be available using maternal blood samples to analyse cell free DNA.

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.

Recommendation 8	Grade and supporting references
Where a structural fetal condition is suspected, diagnostic testing should be offered, depending on the nature of the condition found. Diagnostic tests that could be offered are discussed in section 3.4 .	Consensus-based recommendation

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.²⁸⁻³¹

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 <http://ntolp.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.

Laboratories that undertake prenatal testing for trisomy 21 and other chromosome anomalies during the first and second trimester should participate in an external quality assurance program e.g. United Kingdom National External Quality Assurance Service [UKNEQAS] provides assessment for T21 in first and second trimester and NTD in second trimester (but no other chromosomal anomalies). <http://www.ukneqas.org.uk/>

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.

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.

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6. Other suggested reading

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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):1-5.³³
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.

7. 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) Currently in development.
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 \(C-Gen 10\)](#)

8. Patient information

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

http://www.ranzcog.edu.au/publication/womens-health-publications/patient-information_pamphlets.html

Appendices

Appendix A

Human Genetics Society of Australia (HGSA) and the Royal Australian and New Zealand College of Obstetricians and Gynaecologists (RANZCOG) Joint Committee on Prenatal Diagnosis and Screening Membership

Name	Expertise	Role
Dr Agnes Wilson – RANZCOG member	RANZCOG Guideline developer Research Scientist	Committee Chair. RANZCOG Senior Coordinator, Guideline development and Women's Health
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 Childrens 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 Natalie Kiesey-Calding – RANZCOG member	Obstetrics and Gynaecology	Private Consultant, Cairns Obstetrics & Gynaecology
Ms Pauline McGrath – HGSA member	Genetic Counselling and Prenatal Screening and Diagnosis	HGSA Certified Genetic Counsellor at Queensland Health
Dr Andrew McLennan – RANZCOG member	Obstetrics and Gynaecology	Consultant to the Maternal Fetal Medicine Unit at Royal North Shore Hospital and a Partner at Sydney Ultrasound for Women
A/Professor Ricardo Palma-Dias – RANZCOG member	Obstetrics and Gynaecology	Clinical Director - Ultrasound Services, Royal Women's Hospital, Victoria. Clinical Associate Professor at University of Melbourne
Dr Jason Pinner – HGSA member	Medical Geneticist	University of Sydney (member to January 2014)
Professor Peter Stone – RANZCOG member	Obstetrics and Gynaecology Professor of Maternal Fetal Medicine	The University of Auckland
Dr Marleen Susman – HGSA member	Public Health Geneticist	Murdoch Childrens Research Institute (member to January 2014)
Professor Susan Walker – RANZCOG member	Obstetrics and Gynaecology Professor of Maternal Fetal Medicine	Shiela Handbury Chair of Maternal Fetal Medicine, Director Perinatal Medicine, Mercy Hospital for Women
Dr Dianne Webster – HGSA member	Laboratory Science	Lead Clinical Scientist, LabPlus, Auckland City Hospital, New Zealand

Appendix B Women's Health Committee Membership

Name	Position on Committee
Associate Professor Stephen Robson	Chair and Board Member
Dr James Harvey	Deputy Chair and Councillor
Associate Professor Anusch Yazdani	Member and Councillor
Associate Professor Ian Pettigrew	Member and Councillor
Dr Ian Page	Member and Councillor
Professor Yee Leung	Member of EAC Committee
Professor Sue Walker	General Member
Dr Lisa Hui	General Member
Dr Joseph Sgroi	General Member
Dr Marilyn Clarke	General Member
Dr Donald Clark	General Member
Associate Professor Janet Vaughan	General Member
Dr Benjamin Bopp	General Member
Associate Professor Kirsten Black	General Member
Dr Jacqui Boyle	Chair of the ATSIWHC
Dr Martin Byrne	GPOAC representative
Ms Catherine Whitby	Community representative
Ms Sherryn Elworthy	Midwifery representative
Dr Nicola Quirk	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 in 1990 (C-Obs 5) and was most recently reviewed in 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 February 2014 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). There was a teleconference held in August 2014 to further refine the recommendations. Further edits were made electronically by the committee from February 2014 to March 2015.

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 ones 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.

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

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 Cell-free DNA Screening for Fetal Aneuploidy

Introduction

Since 2011 multiple independent studies have demonstrated the clinical validity of maternal plasma DNA sequencing for the detection of fetal trisomy 21 in women with an increased probability. Cell-free (cf) DNA screening – also referred to as non-invasive prenatal testing (NIPT), and noninvasive prenatal screening (NIPS) - is a highly accurate screening method for trisomy 21. It has been commercially offered in Australia and New Zealand by overseas-based laboratories since late 2012. Provision of cfDNA testing by local Australasian laboratories is anticipated to become available in 2015. This field is changing rapidly and the contents of this appendix must be interpreted accordingly.

The College recommends that all pregnant women be offered the option of prenatal screening for fetal aneuploidies as early as possible in pregnancy (contained within this statement and in [C-Obs 35](#)). This appendix summarises the recent developments in cfDNA testing and highlights important issues for its use in Australia and New Zealand. The clinical implementation of cfDNA testing by Australian obstetric ultrasound specialists has been recently reported and summarises many of the issues encountered during the first year of local availability.^{34,35}

Background

Cell-free DNA of placental origin is detectable in maternal plasma from early first trimester.^{36,37} These cell-free DNA fragments are released from the trophoblast during apoptosis and comprise about 10% of the total cell-free DNA in maternal blood.³⁸ 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. If a woman is pregnant with a fetus affected by trisomy 21, her plasma will contain a greater than expected number of chromosome 21-derived DNA fragments because of the trisomic placenta. Millions of DNA fragments must be sequenced to achieve the statistical precision for accurate prenatal screening. Advances in next generation sequencing technologies have facilitated the rapid translation of this test to clinical practice.³⁹ While the sequencing and bioinformatics techniques vary among the published studies, they all employ the basic principle of counting DNA-fragments and comparing observed to expected numbers of sequences from chromosome 21.

Clinical validity studies in women with an increased probability of aneuploidy

There are now at least 24 published studies on cfDNA testing for the detection of trisomy 21 in women [with an increased probability of aneuploidy](#). These have been recently summarised in a meta-analysis by Gil *et al*.⁴⁰ The majority of these studies included women undergoing invasive testing for one or more of the following indications: advanced maternal age, high probability for trisomy 21 on serum or ultrasound screening tests, fetal structural condition on ultrasound, or personal or family history of affected pregnancy. The calculated sensitivity and specificity for trisomy 21 in singleton pregnancies in this meta-analysis were 99.2% and 99.91% respectively.

Several bodies have released statements on the use of cfDNA testing for the detection of fetal trisomy 21, approving its selective use as a screening test in high-probability women after appropriate pre-test counselling.⁴¹⁻⁴⁴ The International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) endorses the use of combined first trimester screening as their preferred primary screen, with the following options: 1. no further testing 2. cfDNA testing, or 3. invasive testing being offered to women depending on their

combined first trimester screening (cFTS) result.⁴⁵ The need for confirmatory diagnostic testing after an abnormal cfDNA testing result has been emphasised in all consensus statements to date.

The data on the other common autosomal trisomies suggest detection rates of 96.3% for trisomy 18 and 91.0% for trisomy 13.⁴⁰ Test specificity remains > 99% for assessment of these chromosomes but the false positive rates are cumulative for each additional chromosome tested. All commercial cfDNA assays in the local market now offer a combined test for chromosomes 21, 18 and 13. Sex chromosome testing is also an option that can be requested. The sensitivity of cfDNA testing for detection of monosomy X (Turner syndrome) is 90.3% and the specificity is 99.77%.⁴⁰ Test providers claim an overall accuracy of approximately 99% for fetal sex detection.

Studies in mixed or low probability populations

Data is still emerging on the use of cfDNA testing on unselected or mixed-probability screening populations.^{6, 12, 46-48} These data suggest that cfDNA testing performs with similar accuracy in these women, but with a lower positive predictive value (PPV) as would be expected due to the lower prevalence of aneuploidy. A US study that directly compared cfDNA testing with standard screening in a low probability population showed that cfDNA testing had a superior PPV of 45.5% compared with 4.2% for routine screening.⁶ These data underscore the continued need for confirmatory diagnostic testing with cfDNA based screening.

Factors that influence test performance

Test failures occur in 0.9-8.9% of samples, most commonly due to low fetal fraction.^{6, 48} The fetal fraction is the percentage of cell-free fetal DNA as a proportion of total cell-free DNA (maternal and fetal). Each commercial assay has its own quality assurance and fetal fraction thresholds. Biological factors that influence fetal fraction include gestational age, maternal weight and multiple pregnancy.^{38, 49} Fetal fraction appears to be inversely proportional to maternal weight.³⁸ This may affect the test performance in very large women.

Twin pregnancy has only been studied in relatively small numbers compared with singleton pregnancies. Although the results to date are promising, caution is advised during counselling as twin pregnancies appear to have a lower *per fetus* fetal fraction that may impact test performance.⁴⁹ Current limited experience does suggest that multiple pregnancies that are either discordant or concordant for aneuploidy are identifiable by cfDNA testing but at lower sensitivity than for singletons.^{12, 16, 17, 50-52} Several commercial providers currently offer testing for twins. The presence of a demised twin may impact on the accuracy of cfDNA testing and its use is not recommended in this situation. cfDNA testing is not available for higher order multiple pregnancies.

Advantages of cfDNA testing over current screening tests

- The improved sensitivity of cfDNA testing (>99%) offers better detection of affected pregnancies than any current screening method. Combined first trimester screening (cFTS), the current standard of care, has a sensitivity of approximately 90%.^{53, 54}
- The most immediate clinical utility of cfDNA testing stems from its very low false positive rate (< 0.5%). This is vastly superior to the screen positive rate of other methods such as cFTS (3-5%) or maternal age alone (up to 33% depending on the population). When used in women identified as

having an increased probability by a primary screening method, it has great potential to reduce invasive testing and thus procedure-related miscarriages.

- cfDNA testing also has a larger gestational age window for performance, being available from 10 weeks gestation onwards (no upper limit). Serum biochemistry screening and first trimester ultrasound screening all have narrow windows for testing.
- cfDNA testing does not require specially trained ultrasound personnel. The maternal plasma is collected by standard peripheral venepuncture and specimens can be transported at room temperature.
- Advances in cfDNA testing are expected to widen the scope of screening to include other abnormalities in addition to trisomies 21, 18 and 13 and sex chromosome aneuploidy. The clinical utility and cost-effectiveness of broadening prenatal screening to include other disorders such as microdeletion syndromes has not yet been examined in the Australian population.

Disadvantages of cfDNA testing

- The cost of cfDNA testing varies according to test provider and patient location and is currently not subsidised by the government or private health insurance. This financial barrier poses major ethical and economic challenges to the successful incorporation of cfDNA testing into prenatal care and precludes universal recommendations from RANZCOG. However, with industry competition and improvements in sequencing techniques, the costs of cfDNA testing are expected to drop below \$500 in the near future.
- cfDNA testing is currently provided to women in Australia and New Zealand from overseas laboratories via local distributors. The median turnaround time for a test result of 10 days may be unacceptable to some women. Future local provision of cfDNA testing is expected to reduce turnaround times substantially.
- cfDNA testing currently offers assessment of chromosomes 21, 18 and 13, X and Y. However, other significant chromosome abnormalities may go undetected if women identified as high risk by conventional first trimester screening decline invasive testing on the basis of a negative cfDNA result⁵⁵. The risk of these abnormalities varies according to various parameters within the combined test and may exceed 1% in some high risk groups.⁵⁶ Women should be aware that not all chromosomes are currently tested with cfDNA testing.
- cfDNA testing is not a diagnostic test. An abnormal cfDNA result still requires confirmation by amniocentesis or chorionic villus sampling. The PPV for trisomy 21 ranges from 45% in a low risk population⁵, to $\geq 90\%$ in a high risk population. Due to the possibility of false positive results and the potentially irreversible consequences of misdiagnosis, invasive testing for confirmation should always be recommended after an abnormal result.
- “False positive” cfDNA results may occur as a result of confined placental mosaicism⁵⁷, unsuspected maternal chromosome abnormalities⁵⁸, history of prior organ transplant, early twin demise, or rarely, maternal malignancy. The appropriate management of discordant cfDNA and fetal karyotype results is still evolving. Consultation with a clinical or laboratory geneticist should be considered if discordant results raise clinical concerns.
- Test failures occur in 1-9% of samples. When combined with 10-14 day turnaround time for results, a test failure may remove the opportunity for a woman to have another form of screening.

- Direct replacement of the first trimester nuchal translucency screening with cfDNA may increase the chance of delayed diagnosis of major structural abnormalities if the 11-13 week ultrasound is omitted.

Non-medical use of cfDNA testing

cfDNA will commonly lead to information which will identify fetal sex. There are some medical indications for which fetal gender identification is important. RANZCOG does not endorse sex selection using any prenatal testing modality.

Potential models for the implementation of cfDNA testing

There is currently insufficient data to prescribe a specific role for cfDNA testing in the prenatal care of women in Australia and New Zealand. Several models for its incorporation into existing Australian screening strategies have been published.^{59, 60} More research and consultation is required before general recommendations can be made for the Australian and New Zealand health care systems. In particular, the significant issues of availability, cost and patient affordability need to be addressed. The College does not endorse a particular model at this time. Below is a summary of some of the proposed models for the information of members.

cfDNA testing as a primary screening test

1. cfDNA testing as the primary screening test for women with an increased probability based on history or maternal age. A significant proportion of women included in the clinical trials of cfDNA testing were included for this indication. This has the potential to reduce the numbers of invasive tests performed for advanced maternal age alone.
2. cfDNA testing as a primary screening tool in the general population. This approach has the potential to increase the overall detection of trisomy 21 while reducing invasive testing rates, but would be costly.

cfDNA testing as a secondary “advanced” screening test

1. cfDNA testing as follow-up test in women with an increased probability after a first or second trimester screening test. Women with a high probability of aneuploidy who then go on to have a negative cfDNA test result would be expected to decline invasive testing. With this approach, the overall detection of Down syndrome would remain unchanged from that of the primary screening test, but invasive testing would be reduced.
2. cfDNA testing in combination with cFTS in a contingent manner. This involves using cFTS as the primary screening modality to stratify women into three groups. Those with the highest probability of aneuploidy would be offered invasive testing directly. Those with an intermediate probability of aneuploidy would be offered cfDNA testing, invasive testing, or no testing, and the those with the lowest probability of aneuploidy would have no further testing. The optimum thresholds for each group may vary according to local factors, but proposed reported risk thresholds include > 1 in 10-50 (to > 1 in 50) for the highest category and < 1 in 1000 for the lowest category. Approximately 15% of the total screened population would be expected fall into the intermediate category between these two cut-offs. This approach has the advantages of improving overall detection of trisomy 21 and reducing invasive testing at a lower cost than offering NIPT as a primary screening test. A UK study modelling various cut-off thresholds for this contingent approach has been published.⁶¹

Summary

- The current standard of care for prenatal screening in Australian and New Zealand is the use of cFTS as a primary screening test for pregnant women. Australia and New Zealand have a high utilisation of cFTS, which has a 90% detection rate for a 5% screen positive rate.^{53, 54} The incorporation of nasal bone assessment into cFTS can reduce the screen positive rate to \leq 3%.
- cfDNA testing is an option for those women who are able to self-fund their testing, after appropriate pre-test genetic counselling. The major benefits of cfDNA testing are the improved detection rate and the lower false positive rate compared with standard forms of screening.
- Women who do not have access to cFTS or who miss the gestational age window for cFTS can be offered the options of second trimester serum screening or cfDNA testing.
- cfDNA testing as a primary screening modality in the general pregnant population requires more clinical and economic evaluation. This situation may change in the future with the results from ongoing studies, changes in local laboratory capabilities and further decline in price.
- Pre-test counselling should include a discussion on the limitations of the test, including its positive predictive value and its inability to detect atypical aneuploidies. Women whose fetuses have an increased likelihood of aneuploidy, such as those with structural conditions, should be offered invasive diagnostic testing because cfDNA testing does not provide a genome-wide assessment of fetal karyotype.⁴⁵
- Pre-test counselling should also include informed decision making regarding the optional testing for fetal sex and sex chromosome aneuploidy. There has been no precedent for population screening for sex chromosome aneuploidies due to their variable and usually mild phenotype. The significant potential for an incidental diagnosis of maternal sex chromosome aneuploidy should be discussed in the pre-test counselling for X and Y chromosome assessment. Women should be given the opportunity to decline testing for fetal sex and sex chromosome aneuploidy.
- All women with an abnormal result on cfDNA testing should have genetic counselling and be offered invasive testing for confirmation of the diagnosis.
- Due to the public awareness of this technology, many of the consultations regarding cfDNA testing are likely to be initiated by pregnant women themselves. The principles outlined in [section 3.1](#) of this statement on pre-test counselling and information also apply to cfDNA testing, in particular the concept of informed choice and shared decision making.
- RANZCOG does not support direct-to-consumer marketing of prenatal tests for fetal conditions, including cfDNA testing. Prenatal screening tests are best implemented in the context of a therapeutic relationship and a comprehensive program that co-ordinates pre-test counselling, testing, post-test interpretation, support during decision-making, and where indicated, follow-up consultations and diagnostic testing.
- Practitioners who offer cfDNA testing to their patients are advised to prospectively collect data on uptake of the test and patient outcomes for audit and monitoring purposes. This is particularly important in cases where cfDNA testing results were discordant with the results of follow-up diagnostic testing. These collected data may also help inform future policy making.

- The cost of cfDNA testing is a major barrier to access for many women. Achieving support for public funding of cfDNA testing would require a cost-effectiveness analysis that includes local data on women's preferences and the impact of cfDNA testing on decision-making. This should be a high priority for research.

Appendix F 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 G Disorders Based on 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

¹Only 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 H 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)	<p>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 is at increased risk of having a fetus with Down syndrome (trisomy 21), Edward syndrome (trisomy 18) and Patau syndrome (trisomy 13).</p> <p>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.</p>	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 an individual is at increased risk, they are tested with 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 β hCG
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 are highly variable.	MoMs
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	<p>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.</p> <p>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.</p>	-
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.	-