


a guide for health professionals



Non- invasive prenatal screening tests



RANZCOG Prenatal screening recommendations



Non-invasive prenatal testing has had a profound influence on the management of pregnant women worldwide.

The Australian based nest non-invasive prenatal screening tests provides reliable and accurate answers about fetal chromosomal health, with a low test failure rate of 0.1%¹⁰

The following information is sourced from the Royal Australian and New Zealand College of Obstetricians and Gynaecologists: Prenatal screening and diagnostic testing for fetal chromosomal and genetic conditions, July 2018. The statement was first developed by the HGSA/RANZCOG Joint Committee on Prenatal Diagnosis and Screening and is intended to provide advice on the recommended screening and diagnostic tests for fetal chromosomal and other genetic conditions.

Cell free DNA- based testing aneuploidy (3.2.3)

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. 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 a primary screening test for fetal aneuploidy, a secondary screen for women who have an increased probability result on a primary screening test, but does not wish to have diagnostic testing, 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.



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What is nest?

nest is a comprehensive non-invasive prenatal screening solution that provides important genetic information about a developing baby.

nest provides highly accurate genetic information about pregnancy chromosomes without the risks associated with invasive procedures such as amniocentesis or chorionic villus sampling (CVS).

Performed as early as 10 weeks gestation, a **nest** screening test only requires a single 7 mL sample of blood and results are received within 5 business days from the receipt of the sample.

Personalised prenatal screening options

nest can be offered to all pregnant women and may be of particular value for any pregnant woman who meets one or more of the following criteria:

Advanced maternal age (>35 years at delivery)

Positive maternal serum screen*

History suggestive of increased risk for T13, T18, T21, RAT (rare autosomal trisomy), or sex chromosome aneuploidy

Seek information about their baby's gender. There is no upper gestational time limit for a **nest** prenatal screening test.

*Diagnostic testing should be considered if serum screening results indicate a high risk (e.g. > 1:10) or if ultrasound abnormalities are present

Clinicians have the option to elect to request:



The standard nest screening test

Screen for aneuploidies in the most common trisomies (T13, T18, T21) and sex chromosome aneuploidies.



The new nest+ screening test

Screen for aneuploidies in all 22 autosomes (includes T13, T18, T21) and sex chromosome aneuploidies.

Note: Non-invasive prenatal testing (NIPT) is not recommended in the event of a vanishing or demised twin.

What is nest (cont).



Autosomal aneuploidies screened	13, 18, 21 Patau, Edwards and Down Syndrome	1-22 Patau, Edwards and Down syndrome & rare autosomal trisomies
Sex Chromosome aneuploidy screening	Yes (singleton gestation only) Monosomy X (Turner syndrome) XXX (Triple X) XXY (Klinefelter syndrome) XYY (Jacobs syndrome)	Yes (singleton gestation only) Monosomy X (Turner syndrome) XXX (Triple X) XXY (Klinefelter syndrome) XYY (Jacobs syndrome)
Reports fetal fraction	Yes	Yes
Reports on fetal gender	Yes	Yes
What else is routinely reported	No further reporting parameters	Deletions and Duplications >7Mb
Microdeletion reporting	No	No
When can the test be done	10 weeks gestation onwards	10 weeks gestation onwards
Suitable for twin pregnancies	Yes	No
Turnaround time	5 business days from receipt in lab	5 business days from receipt in lab
Genetic counselling	Available at no cost	Available at no cost
Cost	\$470.00 (PAPP-A and PLGF can be tested at no additional cost) (Effective August 2021)	\$470.00 (PAPP-A and PLGF can be tested at no additional cost) (Effective August 2021)

The science behind nest

The nest advantage—a more stringent and optimised approach to genetic sequencing

nest leverages the power of massively parallel sequencing (MPS) across the whole genome. The industry’s deepest sequencing approach combined with a highly optimised algorithm provides a clearer, more reliable answer than other methods.

Utilising the power of deeper sequencing, **nest** gives reassurance by:

- Providing an accurate result with a lower FF threshold
- Eliminating unnecessary sample rejections
- Reducing the need for redraws
- Only requires maternal blood sample
- Providing fast report time to requesting doctor or clinic: 3–5 business days after sample receipt.

nest prenatal screening tests	Other targeted sequencing tests
Definitive cut-off values provide clear screening results	Provides ambiguous risk scores similar to serum screens
Lowest test failure rate (0.1%) ⁶	High failure rates (4%–10% or greater)
Not constrained by BMI or ethnicity and only requires maternal blood sample	May rely on BMI, ethnicity, or paternal sample to improve accuracy
Accepts egg donors	May exclude egg donors

The importance of the inclusion of fetal fraction

nest screening is able to detect fetal fraction. This is the amount cell-free DNA in the maternal blood that is of fetal origin. During the first trimester, an average 2-10% of the DNA in the maternal blood is of fetal origin. Placental cells break down as part of their natural life cycle, releasing fragmented DNA particles into the maternal circulation. The inclusion of fetal fraction in NIPT screening is essential for accurate test results.

The nest prenatal screening test increases the specific signal of aneuploid chromosomes and hence improves the overall accuracy of classifying affected samples. The test output provides unambiguous results, not a risk score, and it is not dependent on maternal age, maternal weight, gestational age (after 10 weeks), donor egg conception or ethnicity.

nest laboratories elects to report fetal fraction as low as 2% in their nest screening tests.

The cut-off value has been shown to reduce the chance of a false positive result due to low fetal fraction.

Test performance

The standard **nest** and more comprehensive **nest+** non-invasive prenatal screening tests provides one of the lowest NIPT test failure rate available on the Australian market of just 0.1%⁶.

Test performance in most common chromosomal aneuploidies⁶

	N	Observed sensitivity	95% CI	Observed specificity	95% CI
T21 Down syndrome	577	99.14%	98.0–99.7	99.94%	99.90–99.97
T18 Edwards syndrome	175	98.31%	95.0–99.6	99.90%	99.86–99.93
T13 Patau syndrome	53	98.15%	90.0–99.9	99.95%	99.91–99.97

(For test metrics from the MELISSA validation study, please see Bianchi DW, Platt LD, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. 2012;119:890-901. In accordance with medical societies' requests, the observed metrics shown above are provided to reflect more recent clinical experience.)

Test performance for sex chromosomes^{*7}

	Sensitivity	95% CI	Specificity	95% CI	Accuracy	95% CI
MX	95.0% (19/20)	75.1–99.9	99.0% (483/488)	97.6–99.7	-	-
XX	97.6% (243/249)	94.8–99.1	99.2% (257/259)	97.2–99.9	98.4%	96.9–99.3
XY	99.1% (227/229)	96.9–99.9	98.9% (276/279)	96.9–99.8	99.0%	97.7–99.7

XXX, XXY, XYY: Limited data of these more rare aneuploidies preclude performance calculations.

Test performance^a for rare autosomal trisomies (RATs)¹² and copy number variation (CNV)¹²

	Sensitivity ^b	95% CI	Specificity	95% CI
RATs^d	96.4% (27/28)	82.3–99.4%	99.8% (2001/2005)	99.49–99.92%
CNV	74.1% (20/27)	55.3–86.8%	99.8% (2000/2004)	99.49–99.92%

a. Seven twin pregnancies reported correctly as T21 not shown on table b. Genome-wide screen performance is reported for RATs and CNVs c. CI based on Wilson's score methods d. RAT excludes chromosomes 21, 18 and 13

Test performance of Rare Autosomal Trisomies (RATs)

International studies have reported similar sensitivity and specificity for RATs using whole genome sequencing based non-invasive prenatal screening.¹¹ Confirmation of results is complicated by the diagnostic method used for follow-up of abnormal results (amniocentesis) which may not reflect placental cfDNA.

Screening twin pregnancies

Screening for fetal aneuploidy in twin gestations poses unique challenges such as lower levels of DNA available for analysis from each fetus. By expanding the sensitivity and overall capability of the assay, the standard nest test can screen twin pregnancies for T21, T18, T13 and the presence of Y chromosome (optional). The nest screening test can be used in both monozygotic and dizygotic pregnancies.

Exceptions:

Non-invasive prenatal testing (NIPT) is not recommended in the event of a vanishing or demised twin.

nest+ is not available as a screening option for twin pregnancies.

^{*}Sex chromosome mosaicism cannot be distinguished by this method (the occurrence of which is < 0.3%). Patients with such mosaicism will have a sex chromosome result reported and will fall into one of the six categories (Monosomy X, XXX, XXY, XYY, XX, XY).

Patient referral and reporting

How do I arrange a nest prenatal screening test for my patient?

- Discuss the suitability of either the **nest** or **nest+** prenatal screening test with your patient.
- Prenatal testing request forms may be downloaded from the **nest** website, go to the **arrange-a-test** page or delivered to your clinic by request (please phone or email nest laboratories with your resource request).
- Specialist collection centres for **nest** can be located on the **locate a collection centre** page.

This is the 'nest' Prenatal Testing Request Form. It includes fields for patient details (name, date of birth, sex, client ref no), pregnancy details (gestational age, ultrasound results), and test selection (chromosomes 1-22, sex chromosomes, fetal fraction). It also has sections for essential information, comments, and a declaration by the practitioner.

OR

This is the 'nest+' Prenatal Testing Request Form. It includes the same fields as the 'nest' form but also includes a section for 'Comprehensive Chromosome Screening' (chromosomes 1-22, sex chromosomes, and fetal fraction) and a declaration by the practitioner.

Note: Comprehensive chromosome screening can only be reported if the **nest+ option is selected and will not be tested for the standard **nest** option. Please carefully consider your choice of test as any changes will require redraw, reprocessing and the application of an additional test fee.**

Visit nestscreens.com.au

nest is an easy, non-invasive blood test that delivers the answers you seek in just days

nest is easy to order and needs only 1 tube of blood (just a 7mL sample).

Our reports are available 5 business days after sample receipt.*

The **nest** screening test report is well organised and easy to read.

nest+ reports contain results for chromosomes 1-22 : low probability or high probability with an accompanying interpretation. Test reports include one of two possible results for chromosomes 13, 18 and 21: low probability or high probability. The report will contain fetal fraction result.

For singleton pregnancies, sex chromosome results are reported where requested. If there are no sex chromosome aneuploidies, then the report will indicate XX or XY status.

It is recommended that no irreversible clinical decisions be made based on these screening results alone. If a definitive diagnosis is desired, chorionic villus sampling or amniocentesis should be undertaken.

This is a sample of a 'nest' Prenatal Anomaly Test Report. It shows the patient's details, the test results for chromosomes 1-22, sex chromosomes, and fetal fraction. The report is organized into sections for 'Test Results' and 'Interpretation'.

*Time to report may vary based on the location of collection centre. Please refer to our website for blood collection locations and opening hours.

Notes

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

Limitations of the nest prenatal screening test:

The **nest** prenatal test is a highly accurate, advanced screening test that is non-invasive. This test is designed to screen for chromosome aneuploidies and is validated for chromosomes 21, 18, and 13, X and Y. The test is validated for singleton and twin pregnancies with gestational age of at least 10 weeks. Genetic counselling before and after testing is recommended. These results do not eliminate the possibility that this pregnancy may be associated with other chromosomal abnormalities, birth defects, or other complications. A

low probability result does not preclude the presence of trisomy 21, trisomy 18, or trisomy 13, monosomy X, XXX, XXY, and XYY. When an aneuploidy detected result is reported in a twin pregnancy, the status of each individual fetus cannot be determined. The presence or absence of Y chromosome material can be reported in a twin pregnancy; however, the occurrence of sex chromosome aneuploidies such as MX, XXX, XXY, and XYY, cannot be evaluated in twin pregnancies. There is a small possibility that the

test results might not reflect the chromosomes of the fetus, but may reflect the chromosomal changes of the placenta (confined placental mosaicism), or of the mother (chromosomal mosaicism). Results of 'High probability' are considered to be screened positive. Monash IVF Group recommends that no irreversible clinical decisions should be made based on these screening results alone. If definitive diagnosis is desired, chorionic villus sampling or amniocentesis would be necessary. This test is accredited by RCPA/NATA; accreditation number 2774.

Limitations of the nest+ prenatal screening test:

This test is designed to screen for chromosome aneuploidies via analysis of cell-free DNA from maternal plasma and is a screening test only, it is not diagnostic. If the basic test is requested it is validated for chromosomes 21,18,13, X and Y in both singleton and twin pregnancies. This test is validated for all chromosomal aneuploidies and gains/losses greater than 7MB in singleton pregnancies only. This test can only be performed from a gestational age of at least 10

weeks and 0 days as estimated by LMP, crown rump length or other appropriate method. This test does not eliminate the possibility that this pregnancy is affected by other chromosome abnormalities, small subchromosomal abnormalities, haploidy, triploidy or tetraploidy, birth defects or other conditions. Aneuploidies can also be influenced by the following clinical conditions: abnormal maternal karyotype, single fetal demise of a twin pregnancy, placental

mosaicism, cell, tissue or organ transplant or metastasis. The specificity for this testing is outlined in table below. This test was developed by Verinata health, Inc a wholly owned subsidiary of Illumina, Inc.

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Disclaimer

The manner in which this information is used to guide patient care is the responsibility of the health care provider, including advising for the need for genetic counselling or additional diagnostic testing. Any diagnostic testing should

be interpreted in the context of all available clinical findings. This test was developed by, and its performance characteristics were determined by, Verinata Health, Inc., a wholly-owned subsidiary of Illumina, Inc. It is not registered

with the Therapeutic Goods Association as an In Vitro Device.

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