a guide for health professionals

nest

non-invasive early screening test

What is **nest**?

nest provides reliable, comprehensive answers about the health of a developing fetus

nest represents a major advance in prenatal screening, providing highly accurate answers about fetal chromosomal health — without the risks associated with invasive procedures, such as amniocentesis or chorionic villus sampling (CVS). Performed as early as 10 weeks gestation, the **nest** prenatal screening test demonstrates superb sensitivity and specificity for the most prevalent trisomies.

Medical societies agree that all pregnant women should be offered prenatal screening for fetal abnormalities and that NIPT is a major advance in screening methodologies.[1–5]

Test performance in most common chromosomal aneuploidies[6]

	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.)

nest can also screen for the most common sex chromosome aneuploidies as well as fetal sex

- **Monosomy X** (Turner syndrome)
- **XXX** (Triple X)
- **XXY** (Klinefelter syndrome)
- **XYY** (Jacobs syndrome)
- **Fetal sex** (XX or XY)—aids in risk stratification of X-linked disorders such as haemophilia

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.

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 test can screen twin pregnancies for T21, T18, T13 and the presence of Y chromosome (optional). The test can be used in both monozygotic and dizygotic pregnancies. Non-invasive prenatal testing (NIPT) is not recommended in the event of a vanishing or demised twin.

Committed to research

With its superior technology, **nest** provides clinical evidence showing across-the-genome analysis in a real-world population. The performance of the **nest** prenatal screening test was evaluated in a major scientific study that involved more than 60 leading US medical research and teaching institutions. The study findings were reviewed and published in the Journal of Obstetrics and Gynaecology in 2012.[8] A second study, published subsequently, presented the test's performance under regular clinical conditions and found similar results.[9] Monash IVF Group continues to expand the technology and innovate new solutions with its commitment to sponsor and support ongoing clinical studies to advance the effectiveness of NIPT.

Who should be offered **nest**?

nest screening is suitable for all women at 10 weeks or greater gestation with singleton and twin pregnancies. It may be of particular value for any women who meet the following criteria:

- Advanced maternal age (≥ 35 years at delivery)
- Positive serum screen
- Ultrasound softmarker
- History suggestive of increased risk for T21, T18, or T13, or sex chromosome aneuploidy

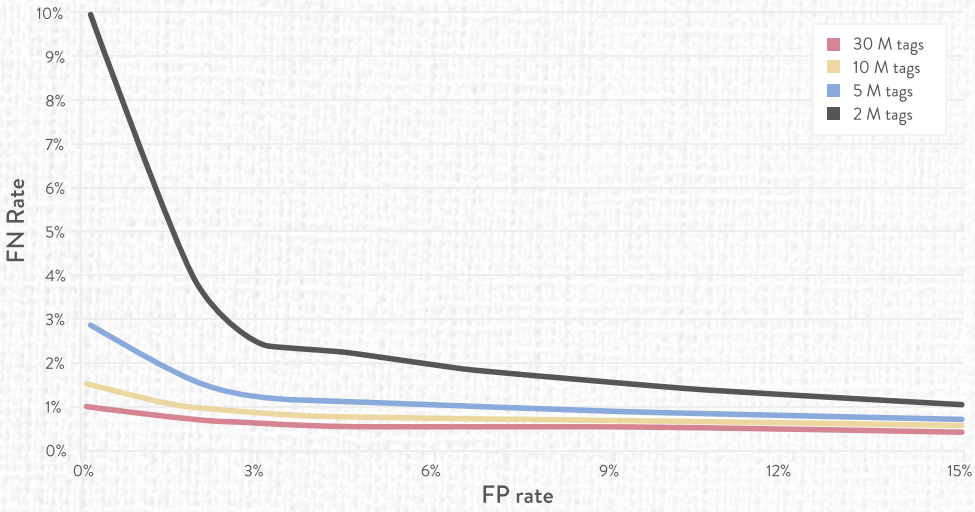
*Sex chromosome mosaicism cannot be distinguished by this method (the occurrence of which is < 0.3%). Patients with such mosaicism will have a sex chromosome result reported and will fall into one of the six categories (Monosomy X, XXX, XXY, XYY, XX, XY).

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.

The science of deeper sequencing

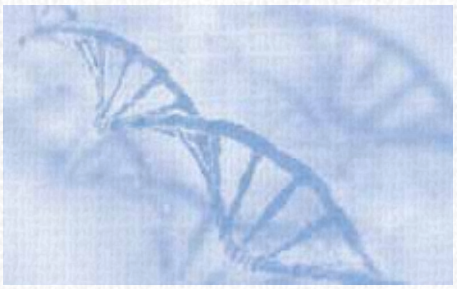


In this graph, shallower sequencing necessitates using fetal fraction (FF) estimates as compensation for weaker sequencing power. Without FF estimates, the incidence of false negatives would be clinically unacceptable and result in higher numbers of sample rejections and delayed result time.

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.

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 with our enhanced SAFeR™ algorithm 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 with SAFeR™	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

Patient referral and reporting

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 in 3-5 business days after sample receipt.*

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

Basic reports contain results for chromosomes 21, 18 and 13. Test reports include one of two possible results for chromosomes 21, 18 and 13: 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.

Know what a **nest** test case looks like

Scenario 1: High probability patient considering an invasive procedure.
38-year-old woman with history of infertility who conceived via in vitro fertilisation (IVF)

Counselling to discuss testing options	– Maternal serum screening – Invasive test (CVS/amniocentesis) – **nest** prenatal screening test – Ultrasound only
Patient elects the **nest** prenatal test	– Chromosome 21 — Low probability – Chromosome 18 — Low probability – Chromosome 13 — Low probability – Normal ultrasound

Patient is comfortable declining invasive testing as she has confidence in the high sensitivity of **nest** and normal ultrasound result. Procedural risks avoided.

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

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.

Limitations of 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.

References

1. ACOG Committee on Practice Bulletins. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol. 2007:109:217–227.

2. American College of Obstetricians and Gynecologists Committee on Genetics. Committee Opinion No. 545: noninvasive prenatal testing for fetal aneuploidy. Obstet Gynecol . 2012:120:1532–1534.

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4. Benn P, Borell A, Chiu R, et al. Position Statement from the Aneuploidy Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn . 2013;33:622–629.

5. Devers PL, Cronister A, Ormond KE, et al. Noninvasive prenatal testing/noninvasive prenatal diagnosis: the position of the National Society of Genetic Counselors. J Genet Couns. 2013:22:291–295.

6. Bhatt S, Parsa S, Snyder H, et al. Clinical Laboratory Experience with Noninvasive Prenatal Testing: Update on Clinically Relevant Metrics. ISPD 2014 poster.

7. Verinata Health, Inc. (2012) Analytical Validation of the verifi prenatal test: enhanced test performance for Detecting Trisomies 21, 18, and 13 and the Option for classification of Sex Chromosome Status. Redwood City, CA.

8. Bianchi DW, Platt LD, Goldberg JD, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol . 2012;119:890–901.

9. Futch T, Spinosa J, Bhatt S, et al. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. Prenat Diagn . 2013:33:569–574.

Additional studies

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Sehnert AJ, Rhees B, Comstock D, et al. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin Chem . 2011;57:1042–1049.

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