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Genetics Education Canada: Knowledge Organization
Centre d'éducation en génétique canadien: Connaissances organisées
DEEP DIVE

NON-INVASIVE PRENATAL TESTING BY CELL FREE DNA

Prenatal screening using cell-free DNA (cfDNA), also known as Non-Invasive Prenatal Testing (NIPT), is a test to prenatally detect Down syndrome and other common chromosome differences. This test assesses fragments of cfDNA derived from the placenta that are circulating in maternal blood to determine if there is an increased chance that the fetus has an aneuploidy. NIPT should be considered in pregnancies at increased risk of aneuploidy (e.g. positive conventional prenatal screen [such as enhanced First Trimester Screening (eFTS), Integrated Prenatal Screening (IPS)/ Maternal Serum Screening (MSS)], or an associated ultrasound finding). NIPT has **higher sensitivity and specificity** for trisomy 21 (Down syndrome) and trisomy 18 than conventional screening tests, however **it is not diagnostic.** Positive results should be confirmed by diagnostic testing (chorionic villus sampling or amniocentesis) prior to any irrevocable action. Negative results are reassuring, significantly reducing the likelihood of common chromosome differences. Additional follow-up testing and consultation may still be indicated as NIPT does not screen for congenital anomalies or many other genetic conditions. Some provinces fund NIPT as a second-tier screen for persons who meet certain high-risk criteria. Those who do not meet criteria can pay for NIPT themselves. Price varies by company and test selection.

What is Non-Invasive Prenatal Testing by cell-free DNA?

Non-**I**nvasive **P**renatal **T**esting (NIPT) by **c**ell-**f**ree DNA (cfDNA) is a **highly** sensitive **and specific** way to screen for aneuploidies (an extra or missing chromosome)) in pregnancy. Common chromosome aneuploidies are trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome), trisomy 13 (Patau syndrome). NIPT is a screening test providing a risk assessment, it is not a diagnostic test. NIPT is performed on a pregnant person's blood sample and poses no harm to the pregnancy.

A meta-analysis reviewing clinical validation and implementation studies found that, in singleton pregnancies, the detection rates (DR) and false positive rates (FPR) were:[1]

- DR: 99.7% and FPR: 0.04% for trisomy 21
- DR: 97.9% and FPR: 0.04% for trisomy 18
- DR: 99.0% and FPR: 0.04% for trisomy 13

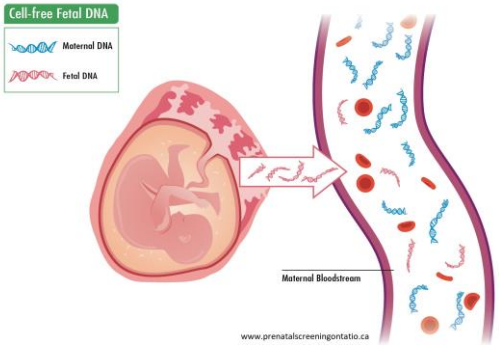
NIPT is not a diagnostic test. A positive/high risk NIPT result should be confirmed by diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) prior to any irrevocable action.[2]

NIPT can also be used for sex chromosome identification for the purpose of fetal sex determination. This can be clinically important in pregnancies with an increased risk for an X-linked disorder (e.g. haemophilia A, Duchenne muscular dystrophy).

How does NIPT work?

cfDNA refers to very small fragments of DNA not contained in a cell. They are always present in the blood stream. During pregnancy, some circulating cfDNA is derived from the placenta, and this is expected to represent the fetal genomic profile.

A blood sample is obtained from the pregnant person and the circulating cfDNA is analysed to assess the likelihood of extra or missing genomic information, as compared to the expected amount. Testing can be done as early as 9-10 weeks gestation (company specific) up to birth.



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cfDNA from the pregnancy comprises only a small percentage (<10%) of the total cfDNA in a pregnant person's blood. The quantity increases with gestational age. Various technologies and proprietary algorithms for analysis are used by each laboratory.[1]

A dating ultrasound is recommended prior to drawing the blood sample to: (1) ensure pregnancy viability, (2) obtain an accurate gestational age, and (3) exclude multiple pregnancies.

What are the Canadian recommendations on prenatal screening?

Current Canadian clinical practice guidelines by the Society of Obstetricians and Gynaecologists of Canada (SOGC) and the Canadian College of Medical Geneticists (CCMG) state that all pregnant persons in Canada, regardless of age, should be offered, through an informed counselling process, the option of a prenatal screening test for the most common fetal aneuploidies.[2]

All pregnant persons should be offered the options of:

1. No aneuploidy screening
2. Standard prenatal screening based on locally available programs e.g. enhanced First Trimester Screening (eFTS), Integrated Prenatal Screening (IPS)
3. Diagnostic testing (chorionic villus sampling (CVS) or amniocentesis) when appropriate indications are present e.g. a fetal nuchal translucency (NT) measurement of 3.5mm or greater or a congenital anomaly
4. NIPT screening where available, with the understanding that it may only be provincially funded when certain criteria are met

Additionally, where available, pregnant persons should be offered a first trimester ultrasound (at 11 to 14 weeks gestation) for accurate dating, determination of twin chorionicity, early anatomy assessment and NT measurement. A second trimester ultrasound (18 to 22 weeks gestation) should be offered for evaluation of structural anomalies. These ultrasounds should be performed at centres with expertise in fetal ultrasound.[2]

Note: The primary screening test for the detection of fetal structural abnormalities including open/closed neural tube defects (O/CNTDs) is the second trimester ultrasound. The primary use of maternal serum alpha fetoprotein for O/CNTD screening should be discontinued unless the pregnant person has a pre-pregnant BMI ≥ 35 kg/m2 or where access to timely and good quality ultrasound is limited.[3]

Red flags: When to consider offering NIPT and/or genetic consultation

SOGC and CCMG guidelines[2] recommend all pregnant persons be offered the option of a prenatal screening test for the most common fetal aneuploidies (trisomies 21, 18 and 13). While offering NIPT as a primary screening method is not funded in most provinces, NIPT is available in many provinces as a second-tier screen. See the online resource for links to provincial prenatal screening programs and NIPT funding criteria.

Evidence supports screening by NIPT in a pregnancy determined to be at increased risk for a common aneuploidy.

In general, a pregnancy is considered to have an increased chance of fetal aneuploidy when:

- The pregnant person is 40 years of age or older at the time of estimated date of birth
- There is an abnormal serum screen result i.e. eFTS/IPS/MSS
- The fetal nuchal translucency (NT) measurement is 3.5mm or greater
- There is a history of a previous pregnancy or child with trisomy 21, 18 or 13
- There are fetal congenital anomalies on ultrasound highly suggestive of trisomy 21, 18 or 13
- There are multiple soft markers[2,4] on ultrasound which are highly suggestive of a specific aneuploidy
- There is a risk of carrying a male fetus with an X-linked condition (NIPT screening could be used for sex determination)

As each prenatal genetics centre has its own referral criteria, abnormalities seen on ultrasound (e.g. congenital anomalies, NT ≥ 3.5mm or multiple soft markers) should be discussed with your local genetics centre or maternal

fetal medicine specialist to decide whether: (1) a referral is appropriate, (2) NIPT screening could be offered first, (3) additional testing could be considered e.g. microarray, additional imaging.

Private pay NIPT

NIPT may be obtained by paying out-of-pocket when public health care funding is not available. Cost and access vary by company, test selection and geographic location. A recent Canadian study found that between January 1, 2016, and December 31, 2017, 23,845 pregnant persons in Ontario had NIPT screening and, of those, one third (7,931) were self-paid.[5] GEC-KO does not endorse any particular company.

Before ordering NIPT for your patient, consider first talking to your local prenatal genetic counsellor about what is available near you. Genetics clinics across Canada often have a genetic counsellor available to answer questions from community providers and are happy to do so.[6] Genetic counsellors may also be available for questions through your provincial prenatal screening program. See program links in *Red flags: When to consider offering NIPT and/or genetic consultation*.

When ordering NIPT as a private pay option you may wish to discuss with a company representative the considerations below.

(1) Support:
   a. Is there direct support available by a board-certified genetic counsellor?
   b. Before and after testing?

(2) Process:
   a. How and where is blood drawn?
   b. Ideal gestational age for blood draw? *(*Some companies offer blood draw as early as 9 weeks but this may not be ideal, for example, if the patient has a high BMI the fetal fraction may be too low.*)*
   c. Is an ultrasound required?
      i. Note that ultrasound is recommended to confirm gestational age, confirm viability and to exclude multiples

(3) Cost:
   a. Are there added costs for blood draw, genetic counselling support?
   b. Are additional testing options available at extra cost e.g. fetal sex, opt in/opt out for sex chromosome differences, microdeletions/microduplications (see section on *Additional screening by NIPT*)

(4) Exclusion criteria:
   a. Is this test validated in all pregnancies? i.e. pregnancy achieved by in vitro fertilization, with egg donor, by surrogate, multiples, vanishing twin, consanguineous couple, pregnant person with high BMI

(5) Results:
   a. How are results reported/received?
   b. What happens in the event that no result is available **and** how often does this happen?

(6) Performance:
   a. What is the test performance (e.g. sensitivity, specificity, positive predictive value) ***for each*** condition screened and have these data been peer-reviewed and published?

(7) Privacy:
   a. Does the patient's sample leave the province and/or country? How are the patient's data protected? Data sharing policies?

What does the test result mean?

Depending on the company, results may be worded as: positive/negative; aneuploidy detected/no aneuploidy detected/aneuploidy suspected/borderline value; or high risk/low risk.

If the result is negative/low risk, this is reassuring.

Your patient should still be offered:

- First trimester ultrasound (at 11 to 14 weeks gestation) and nuchal translucency measurement where available
- Second trimester ultrasound (18 to 22 weeks gestation)
- Depending on the reason NIPT was ordered, a referral for genetic and/or maternal fetal medicine consultation for discussion of additional testing and/or follow up
    - e.g. for an ultrasound finding of an NT ≥ 3.5mm, a detailed ultrasound, fetal echocardiogram and additional genetic investigations would be offered

If the result is positive/high risk, follow-up genetic counselling is indicated and confirmation by diagnostic testing should be offered.

The SOGC/CCMG recommendations state that **no irrevocable obstetrical decisions should be made in pregnancies with abnormal NIPT results without confirmatory invasive testing**[2] (CVS or amniocentesis) as false positive results do occur.

While the detection rate of NIPT is quite high for most conditions, the positive predictive value (PPV) will vary based on the prevalence of (how common) the condition in a given population.

The PPV is not 100% in any population.

Consider the PPV of the result for <u>your</u> patient for the condition screened positive.

When NIPT is positive for trisomy 21, the PPV is expected to be higher if the *a priori* risk (expected prevalence) is higher (e.g. 40 years or older at the time of estimated date of birth, a previous positive serum screen, a suggestive finding on ultrasound)

When the NIPT is positive for a much rarer condition such as trisomy 13 or a microdeletion (see *Additional screening by NIPT*) the PPV is expected to be lower.

If there is no result, the laboratory will usually request that a second blood sample is drawn and the test repeated at no extra cost. Often (in 50-60%) a result will be available after a repeat blood draw.[2]

A request for repeat blood draw may occur in up to about 6% of cases, depending on the test methodology.[1] A recent study looking at Ontario's provincial data reported a 2.2% failure rate with no result after multiple blood draws.[7]

Possible reasons for test failure are (1) blood collection and/or transportation issues, (2) technical issues e.g. a poor sample or quality assessment failure, and, most often, (3) low fetal fraction (amount of fetal cfDNA relative to pregnant person's) which is influenced by a number of factors such as early gestational age, high BMI, or fetal aneuploidy.[8-10]

When contemplating repeating the blood test, the patient and provider should consider whether a delay in results/possibility of no results, and possible further delay to diagnostic testing is important to time sensitive decision making. If there is *a priori* low risk of aneuploidy and/or the pregnancy is at an early gestational age (e.g. 10 weeks), a delay in results may be acceptable. If the *a priori* risk of aneuploidy is increased, depending on the gestational age, moving ahead with diagnostic testing may be preferable for the patient.

If the NIPT result is not interpretable following a second attempt, test failure due to low fetal fraction is associated with an increased aneuploidy risk (~5%).[2] Consider genetic counselling where diagnostic testing and ultrasound may be offered.

What are the benefits and limitations of NIPT?

BENEFITS	LIMITATIONS
Increased accuracy: Higher detection rate, higher positive predictive value, lower false positive rate compared to conventional prenatal genetic screening. See *What does the test result mean?* for more	**NIPT is not a diagnostic test:** Although the accuracy is high, in the event of a positive/high risk result, no irrevocable obstetrical decisions should be made without confirmatory diagnostic testing for reasons mentioned above.
Fewer invasive procedures: With better performance compared to conventional screening, fewer pregnant individuals are expected to undergo invasive diagnostic testing associated with risk of miscarriage. Recent Ontario data reported a 2-fold reduction in invasive procedures since the introduction of NIPT in 2012.[7]	**No result:** This may occur in up to 6% of cases but may be resolved following a repeat blood test.[1] Ontario provincial data show a no result rate of 2.2%.[7] "No result" does not occur with conventional screening. Repeated attempts to obtain a result may delay a diagnosis.
Earlier timing: Screening by NIPT is a single blood test that is available as early as 9-10 weeks. Results are available within 1-2 weeks. Earlier screening results allow expectant parents more time for decision-making and potentially offer more options, e.g. CVS at 11-13 weeks versus amniocentesis after 15 weeks, or early reassurance.	**NIPT does not screen for all possible conditions:** NIPT cannot: — rule out all aneuploidies — detect single gene conditions — detect congenital anomalies — guarantee a healthy baby with a negative NIPT result
	Incidental findings: Although marketed to consumers as a screening test for Down syndrome, NIPT will screen for additional conditions. For example, the test could be ordered because of an increased fetal risk for trisomy 21 and the report could indicate a high risk for another chromosome abnormality, e.g. sex chromosome difference, like Turner syndrome (45,X) or Klinefelter syndrome (47,XXY). See *Additional screening by NIPT* for more on these and other conditions that might be uncovered. Additionally, NIPT analysis does not differentiate between maternal and fetal DNA and reports of maternal aneuploidy have occurred.[9] Pre-test counselling and appropriate follow up are important.

What about NIPT screening for sex chromosome differences?

NIPT for detection of sex chromosome differences (SCD) is also possible and offered by all companies. Even if fetal sex identification is not selected on a test requisition, some companies may still report a high risk of sex chromosome difference when detected.

SCD involve an extra or missing X or Y chromosome in whole or in part. Examples of SCD are Turner syndrome (45,X), and sex chromosome trisomies (e.g. Klinefelter syndrome (47, XXY), Triple X syndrome (47, XXX) and Jacobs syndrome (47, XYY)). The combined prevalence is about 1 in 500. Clinical presentation is highly variable among and within conditions with SCD. Counselling is difficult and complex. While Turner syndrome may be better described,[11,12] much of the literature to date reporting the clinical presentation of sex chromosome trisomies is flawed due to small numbers and ascertainment bias depicting the most severe presentations. Most individuals with sex chromosome trisomies (75-90%) are not diagnosed in childhood but may present in adulthood because of fertility problems or other issues,[13-17] while the diagnosis of Turner syndrome is at a median age of 15 years[11].

SOGC and CCMG state that prenatal determination of fetal sex is not clinically indicated, even when considering patient autonomy, except for assessing risk of an X-linked condition in a male fetus, or virilisation in a female fetus at risk of congenital adrenal hyperplasia.[2] Arguments for screening prenatally are that early diagnosis of SCD has shown better outcomes.[14-16,18] Additional large scale unbiased studies are needed to assess the screening performance of NIPT for detecting SCD.[1,18,19] Apart from Turner syndrome, there is not yet reliable data on the clinical sensitivity of NIPT for SCD.[18]

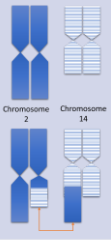
For more information on SCD, view the Deep Dive resource online for review articles and links.

What about additional screening by NIPT (e.g. microdeletions and microduplications)?

Most NIPT companies offer options for additional screening of conditions other than the common three trisomies (21, 18 and 13). Examples are microdeletion and microduplication syndromes, rare trisomies (e.g. trisomy 15 or 16), as well as genome-wide structural anomalies (i.e. to identify unbalanced translocations where a parent may be a carrier of a balanced reciprocal translocation – see below).

Microdeletion and microduplication syndromes are rare genetic conditions, occurring in about 1 in 5,000 to 1 in 50,000 pregnancies. They are caused by very tiny extra or missing pieces of a chromosome. A piece of a chromosome may contain tens or thousands of genes depending on the size. The most common example is 22q11.2 or DiGeorge syndrome.

A balanced reciprocal translocation is where two chromosomes have exchanged genomic information. All the necessary genomic information is present, but not in the usual location. Generally, this balanced rearrangement is of no consequence to the individual. During the formation of a mature egg or sperm (meiosis), the division of genetic material may result in a gamete with an unbalanced (extra or missing) amount of genetic information which can be passed on to a child.



Currently no national or international professional organizations support routine clinical implementation of additional screening by NIPT.[2,18] The main argument against routine use is that reliable published data are not available. While positive predictive value may look encouraging in proof-of-concept studies, the data are overestimated for the general obstetric population. The clinical utility and sensitivity have not been demonstrated.[18] The addition of these rare conditions to NIPT increases the false positive rate and decreases the positive predictive

value of the test. This would result in more pregnant persons having diagnostic testing with an associated risk of miscarriage.

What about NIPT for twins or higher order multiples (e.g. triplets)?

Because the risk for pregnancy loss or complication associated with diagnostic testing may be higher in twin pregnancies[20], a screening test more accurate than conventional screening is highly desirable. In 2017, the SOGC and CCMG recommended that NIPT in twins be undertaken with caution as additional validation studies and evaluation were needed before a recommendation could be made. Recommended screening modalities are (1) nuchal translucency (NT) with maternal age; (2) NT, maternal age and first trimester serum markers; (3) NT, maternal age plus first and second trimester serum markers, where available.[2]

In 2020, the International Society for Prenatal Diagnosis (ISPD) stated that there is now sufficient evidence to demonstrate high detection rate and positive predictive value and low false positive rate for twin pregnancies and that **NIPT as a first trimester screening is appropriate**.[21] Consult your local genetics centre as funding may be available.

A 2021 review in *Prenatal Diagnosis*, states that NIPT in twin pregnancies can complement, but not replace, first trimester ultrasound evaluation.[20] Overall, the observed performance of NIPT screening for trisomy 21 is comparable to singletons, and, while there are less data for trisomy 13 and 18, similar performance is expected.[20] Ultrasound between 11-14 weeks can provide information about chorionicity, fetal anomalies (e.g. increased nuchal translucency, major structural anomalies), and abnormal maternal anatomy (e.g. Mullerian differences, adnexal masses).

Zygosity: In twin pregnancies, the combined fetal fraction is higher than in singleton pregnancies, but the fetal fraction per fetus is lower. For monozygotic twin pregnancies (identical, derived from the splitting of a single egg fertilized by one sperm) this is not a concern as both twins are expected to have the same genetic profile. For dizygotic twin pregnancies (non-identical, derived from fertilization of two eggs by two sperm), fetal fraction of each twin is important for test accuracy.[20]

Sex chromosome differences**:** Testing for sex chromosome differences is generally not available for non-singleton pregnancies.

Test failure/ No-Call result: For twin pregnancies, the median failure rate is reported to be 5.6% at the first blood draw, with a revised rate of 3.1% upon a second blood draw. The main reported reason is low fetal fraction, which is associated with high maternal BMI, *in vitro* fertilization, aneuploidy and other factors.[21]

High order multiples: Triplet and higher order pregnancies are rare. Observed detection rates for trisomy 21 and other common aneuploidies are not available. Test failure rate is also expected to be higher than for singleton and twin pregnancies.[21]

What about NIPT and *in vitro* fertilization?

Pregnancies conceived through *in vitro* fertilization (IVF) are increasing in number.[22,23] Preimplantation genetic testing for aneuploidy (PGT-A) to screen embryos for chromosome differences prior to implantation is also being used more commonly. [22,23] The resulting pregnancies have a lower rate of aneuploidy than those conceived without PGT-A, although the precise sensitivity and specificity are not known due to limited available data.[22]

Conventional first trimester screening methods are less accurate in IVF pregnancies as there are differences in analyte levels (e.g. lower PAPP-A) associated with an increased potential for false positive results.[24] Additionally, these screening methods are not able to incorporate previous screening (e.g. by PGT-A which means a lower *a priori* risk than standard age-related risk) resulting in inaccurate assessments.[22,23]

NIPT for IVF pregnancies with or without PGT-A is the preferred method of prenatal screening. The sensitivity and specificity of NIPT has been validated for screening for common aneuploidies, more so than PGT-A. [22,23] **Diagnostic testing is not recommended unless NIPT screening result is high risk/positive, or ultrasound identifies a high risk**. [22,23]

Clinicians are advised to offer NIPT with caution as the performance in IVF pregnancies may be altered.[22-24] IVF pregnancies are reported to have lower fetal fraction[223,24], a suspected reason for the higher test failure rate among these pregnancies. [22-24] Successful results following a redraw and reanalysis are reported to be comparable to non-IVF pregnancies.[24]

For pregnancies where PGT-A was used and a euploid (typical chromosome number, not aneuploid) embryo was selected for implantation, the positive predictive value (PPV) of NIPT is lower and false positive rate is higher.[23] This is expected as the PPV is based on the prevalence of a condition in a population, and the prevalence of chromosome differences in embryos that have undergone screening by PGT-A would be lower than the general population.

Regardless of conception method, all pregnant persons should be offered a first trimester ultrasound (at 11 to 14 weeks gestation) for accurate dating, determination of twin chorionicity, early anatomy assessment and nuchal translucency (NT) measurement. A second trimester ultrasound (18 to 22 weeks gestation) should be offered for evaluation of structural anomalies. Ultrasounds should be performed at centres with expertise in fetal ultrasound.[2]

Check online for updates as prenatal genomics is a rapidly evolving area. References and specifics about provincial availability can also be found online.

See Public Resources for the updated A guide to understanding prenatal screening tests to help expectant parents and their healthcare providers choose the right testing option, including NIPT.

Authors: S Morrison MS CGC, JE Allanson MD FRCPC and JC Carroll MD CCFP

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