Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: non-invasive prenatal testing (NIPT)

Trainer pack

V1 07/03/25

About the resource

The virtual reality resource has been developed to show the non-invasive prenatal testing (NIPT) procedure to learners without them having to go into a physical laboratory. This means that more learners can view a process at once than would otherwise be possible within limited laboratory space.

It is important to note that this resource is not designed to fully train someone to competency; learners who will ultimately be carrying out the procedure will need additional hands-on training within the laboratory. For some learners, however, observing and understanding the procedure is all that is required for their training pathway.

Note around laboratory processes

The general procedure for NIPT will be the same across all NHS genomic laboratories, however, there may be local variations depending on individual lab set-ups, including different types of equipment. In addition, some laboratories will have witnessed checks in specific areas. Please also refer to your local COSHH, risk assessments and SOPs for further information.

For trainer-led sessions

The virtual reality film is not designed to be used standalone. To support trainers and learners, there are two sets of supporting material that should be referenced and used during and after a training session.

- Trainer pack (this document) – for trainers leading a session with any number of learners.
- Model answers document – for learners who are attending a trainer-led session. We suggest that learners are directed to this material for their reference after their session, as it contains model answers to the formative questions that will be discussed.

For self-led sessions

- For learners working through the resource without a trainer present, please follow this link[1] to access the appropriate resource, which includes formative questions and model answers.

This material has been developed by practice educators and NHS England's Genomics Education Programme team to ensure that learners get the most out of the session.

[1] https://forms.office.com/e/1E9z07Jfzt

Learning objectives

By the end of the session, learners will be able to:

- Identify the principal components of NIPT.
- Outline the quality control procedures used during NIPT.
- Explain the key steps in the NIPT procedure.
- Describe how NIPT is used in a laboratory environment.

Assessment

There is a short assessment available for learners to complete after watching the VR film. We recommend that learners complete this assessment to assure themselves that they have met the learning objectives.

To get access to the assessment, learners should:

1. Register for a VLE account, using this form[2].
2. Wait for an email providing instructions on how to finish setting up their account (Note: applications are processed manually by the GTAC team and it may take time to receive this email).
3. Navigate to the VR assessment using this link[3]. Then click 'self-enrol' to access the assessment.

Pre-course reading

Understand the basic principles of NIPT by:

- reading about cell-free DNA (below);
- reading these articles: 'What is NIPT?' and 'Non-invasive prenatal testing (NIPT)';
- watching this animation: 'How fetal DNA revolutionized prenatal testing'; and
- watching this video about Illumina Veriseq (the workflow used in this VR resource): 'The Science behind the VeriSeq NIPT solution'.

[2] https://forms.office.com/e/tPaF1kLFcE
[3] https://pgvle.co.uk/course/view.php?id=666

An introduction to cell-free DNA

Cell-free DNA (cfDNA) are short fragments of DNA released into the bloodstream through a natural process of cell death. During pregnancy, the mother's blood contains cfDNA, both from her own tissue, and from the fetus (cell-free fetal DNA) via the placenta. Approximately 2%–20% of total cfDNA in maternal blood is placental.[1,2] cfDNA derived from the placenta can be detected after seven weeks' gestation and is undetectable within hours postpartum.[2] (Taken from Illumina: Cell-Free DNA Technology, Fetal Fraction, & NIPT).

References:

1. Barrett, A, Zimmerman BG, Wang D and others. 'Implementing prenatal diagnosis based on cell-free fetal DNA: Accurate identification of factors affecting fetal DNA yield'. PLoS One 2011: volume 6, issue 10. DOI: 10.1371/journal.pone.0025202
2. Nigam A, Saxena P, Prakash A and others. 'Detection of fetal nucleic acid in maternal plasma: A novel noninvasive prenatal diagnostic technique'. Journal International Medical Science Academy 2012: volume 25, issue 3, pages 119-120. DOI:

Trainer pack

There are a number of ‘bookmarks’ throughout the VR film – points that can be set to automatically pause the footage to allow for discussion to take place.

Important: when you first receive your VR kit, bookmarks will not be preloaded and will have to be added manually. We recommend that this is done as part of familiarising yourself with the equipment. After doing so once, they will be remain for future sessions. For help in adding bookmarks, please scan the QR code inside the kit box to be directed to supporting materials.

At each of these bookmarks, as well as at the end of the film, there are questions to ask and discuss, as outlined in this pack. Your role is to ensure that the group sufficiently covers each of the points. Each question is in **bold**. This is not a script – you may wish to discuss additional things, or additional questions may arise, however, these are the key points to cover.

Note: You may wish to look at the detailed information in the ‘model answers document’ in advance of leading a session; the answers provided here are intentionally brief and are designed to act as prompts rather than prescriptive responses.

After discussing the points below, finish the VR session with your group and direct them to the assessment so that they can test their knowledge.

Bookmark 1: Worksheet set-up and plasma isolation (10:48)

1. **What sample is required for NIPT testing? What type of collection tube is used and why?**

    - 7–10ml peripheral maternal whole blood.
    - Streck tubes.
    - For the Illumina VeriSeq workflow, samples must be less than five days old to be viable for processing, with the date of blood collection being Day 0.

2. **What is cell-free fetal DNA?**

    - Short fragments of DNA released into the bloodstream by the placenta during pregnancy.

3. **How much cell-free DNA in the bloodstream is cell-free fetal DNA?**

    - Approximately 2%–20%.
    - The amount of cell-free fetal DNA in the bloodstream increases with pregnancy and can be detected from seven weeks’ gestation.

4. **Why is a no-template control used?**

- It will identify any DNA contamination in the reagents used.
- It can be assumed that if there is not contamination in the no-template control that it is highly unlikely that the samples will be contaminated.
- Dulbecco's phosphate-buffered saline will be used for this control.
- It is also referred to as a negative or blank in some laboratories

5. **For this procedure, how long can Streck tubes be stored at 4°C? What do we do if the sample has not been tested within this time?**

- Five days.
- Plasma separation should be carried out to preserve the viability of the plasma and allow longer-term storage at -80°C.
- In case re-testing is required, post-processing samples may be recapped once plasma has been separated and stored at 4°C for an additional five days (up to a total of 10 days after blood collection).
- These storage requirements are specific for this procedure because this is what has been verified by Illumina. Storage requirements for other NIPT and non-invasive prenatal diagnosis (NIPD) testing usually follow the Streck tube manufacturer guidelines for storage at 18°C –25°C for up to seven days total.

6. **Why are the Streck tubes centrifuged at a low speed?**

- As the Streck tubes are glass, faster centrifugation speeds will break the tubes.

7. **What are the different layers observed after the sample has been centrifuged?**

- There are three layers:
- Bottom layer: red blood cells and red in colour.
- Thin middle layer: buffy layer.
- Top layer: plasma – it is straw coloured and there should be about 1.5ml of plasma above the buffy layer.

8. **Why do we need to isolate the sample within 15 minutes of centrifugation?**

- If it isn’t isolated, the plasma and maternal cells start to re-mix.
- The plasma is where the cfDNA is, and we do not want any of the other layers in the sample.

- As much fetal fraction as possible is needed to enable the changes in fetal DNA to be detected. If there is too much maternal DNA, the test is more likely to fail as there may not be enough fetal fraction to accurately give/call a result.

9. What quality processes have been undertaken so far in the procedure?

- Batch recording.
- Plate set-up, and reagents are checked for the correct barcode on the robot.
- Clear spacious workstation.
- Pill box used to keep tubes and lids matched up.
- Checks regarding the quantity of plasma and presence of the cell pellet.
- Small volumes of plasma are manually transferred rather than via the robot.
- Visual checks for consistent amounts of plasma in each well.
- Visual checks for the level of haemolysis and visible cell pellets.
- Checks by a supporting technician.

10. How long can the plasma plate be stored at 4°C?

- It can be stored for seven days if necessary.

Bookmark 2: cfDNA extraction (14:15)

11. What is the role of proteinase K?

- It breaks down any proteins that may be in the sample that could impact the extraction of the cfDNA.

12. What quality control processes are in place during cfDNA extraction?

- Expiration dates on cfDNA extraction kits are checked.
- Reagent locations on the robot are checked by a supporting technician.
- The plate is checked at the end of the process to confirm that all expected wells contain DNA samples.

13. Why are there checks in place? What would be the consequences of the following: incorrect sample transfer, reagent and robot set-up?

- If the wrong sample is transferred, the patient would receive an incorrect result.
- If the wrong reagent is used, the run would fail and would need to be repeated.
- If the robot is not set up correctly, it could crash, leading to damage, contamination and the likelihood that the run would need to be repeated. This could lead to a delay to patient results and there would be a cost associated with repeating the run. In addition, there could also be further delays if an engineer was required to attend to repair a damaged robot.

14. How does the DNA binding plate work?

- The binding plate has filters in it to which the nucleic acids from the plasma bind. The remainder of the liquid will pass through the filter and out of the bottom of the plate. Ethanol is then used to wash through the plate using a vacuum system on the Hamilton robot. Anything not bound to the filter is washed away.
- The bottom of the DNA binding plate is wiped with ethanol to ensure that there are no impurities left there.
- The DNA binding plate and plasma deep well plate are then centrifuged together, removing any remaining impurities and leaving only DNA in the filters.
- The DNA plate is then transferred to the top of the elution plate, and elution buffer is added by the robot, releasing the DNA off the filters. After centrifugation, there should be DNA in the elution buffer in each well of the plate.

Bookmark 3: Library preparation and quantification (18:48)

15. What size are fetal cfDNA fragments?

- Typically around 160bp–180bp
- This is different to maternal cfDNA due to different methylation patterns between placental and maternal tissue.

16. What are the main processes in the library preparation?

- End repair.
- A-tailing.
- Ligation of DNA-indexed adapters.
- Purification of ligated indexed DNA using magnetic sample purification beads.

17. What purpose does the unique sequence adaptor serve?

- Allows sample identification following sequencing when multiple samples have been pooled into one sequencing reaction.
- Contains sequences that allow library capture.

18. What pieces of laboratory equipment are used when quantifying the libraries and what do they do?

- Fluorometer: Quantifies DNA.
- Robot: Automates the processes.

- Centrifuge: Spins at high speeds and collects the sample at the bottom of the plate or tube.

Bookmark 4: Denaturing and pooling the libraries (20:40)

19. Why do the plates need to be sealed before we denature the libraries? What would happen if the plates were not sealed correctly? What is the knock-on effect for the patient?

- The plates are subjected to high temperatures so there is a risk that the sample would be subjected to evaporation**.**
- The water in the sample could potentially evaporate, which would alter the volume amount of sample available for testing and potentially result in the test failing if not enough DNA is transferred.
- The process would need to be restarted and could therefore result in a delay for the result reaching the patient.

20. What is the ideal temperature for DNA denaturation and how is this achieved?

- 95ºC.
- Using a thermocycler, often referred to as a PCR machine.

21. Why does the DNA need to be single-stranded for sequencing?

- So that each strand can separately attach to an oligonucleotide sequence anchored to the flow cell.

22. Why do we pool the libraries?

- It allows multiple samples to be sequenced in a single sequencing reaction.
- This reduces time and cost per sample.
- This is only possible with the addition of unique sequencing adaptors to each library.

End of film

23. What type of sequencing does the NextSeq perform? What are the advantages of this type of sequencing?

- Massively parallel sequencing.
- It is possible to process numerous regions of the genome and patients at once which makes it faster and more economical than other sequencing methods for looking at multiple genomic targets.

- To be able to confidently detect the small percentage change in the chromosome ratio that indicates that there is a high chance of a possible trisomy condition, a large amount of sequencing data is required. Massively parallel sequencing technologies are able to produce the high volumes of data required.

24. Briefly, how is the sequencing data analysed in NIPT?

- The NextSeq combined with the NIPT assay software automatically analyses the data generated and determines the aneuploidy score for each sample.
- It does this by analysing the raw sequence data for each sample multiplexed in the pool.
- It first aligns the reads against a reference sequence and performs analysis on reads that align to a unique location or site in the genome.
- Information on the fragment lengths from the paired end sequencing reads is obtained and it assesses sequencing coverage statistics on regions known to be enriched for either fetal or maternal cfDNA.
- Data generated from fragment length and coverage analysis are then used to estimate the fetal fraction for each sample. It is important to know the fetal fraction because the more fetal DNA there is, the greater confidence we have in the result.
- Sequencing reads are sorted by chromosome to look for deviations for the expected distribution. Analysis focuses on the shorter reads that are more likely to be the fetal DNA fragments.

25. What steps are taken to mitigate key health and safety risks throughout the NIPT procedure?

- Only using small quantities of hazardous reagents.
- Being aware that samples are received in Streck tubes which are made of glass, if not packaged correctly there is a risk they will get broken and therefore be a risk of a sharps injury.
- Know where the COSHH and risk assessments are for the process.
- Know where the spill kits are.
- Ensure correct disposal of waste.
- Minimise foot traffic by the robot, as its tracks will be extended out of the liquid handler into the laboratory space.
- Wear appropriate PPE.