Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: Non-invasive prenatal diagnosis (NIPT)

Model answers document

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About this document

This document has been designed to support the non-invasive prenatal testing (NIPT) virtual reality resource in training sessions. The questions and model answers below provide a reference point for discussions that will have happened during the session, and can be kept by learners for future reference, or used by trainers to aid in session planning.

Model answers

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 are blood collection containers that limit cellular degradation and prevent cross contamination of cell-free fetal DNA with maternal DNA. 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% of total cfDNA in maternal blood is placental. 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?

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

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?

- Streck tubes can only be stored at 4°C for five days.
- If they haven’t been processed by the fifth day, 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. The bottom layer is red blood cells and is red in colour. The thin middle layer is the buffy layer. The top layer is the 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 should be kept separate from the other layers.

As much fetal fraction (proportion of cfDNA vs maternal DNA) 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 to ensure traceability of reagents used in the process.
- Plate set-up, and reagents are checked for the correct barcode on the robot.
- Clear spacious workstation to reduce the likelihood of a laboratory incident and contamination.
- Pill box used to keep tubes and lids matched up, to reduce risk of sample contamination.
- A number of 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; if these are seen, the sample is invalidated.
- 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?

The fetal cfDNA fragment length is typically around 160bp–180bp in length and is different to maternal cfDNA. This is due to different methylation patterns between placental and maternal tissue.

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

- End repairing of the DNA fragments in the cfDNA, which converts the overhangs on the fragments into blunt ends.
- Addition of deoxyadenosine A-tailing, which creates a single base overhang.
- Ligation of DNA-indexed adapters, with a unique adapter for each patient.
- 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
Measures the intensity of the fluorescent dyes attached to the DNA with concentration determined by comparison to a DNA standard curve, thereby accurately quantifying the DNA. The workflow manager uses this information to automatically calculate how much of each sample to add to the pooled library. This ensures that it contains the same amount of DNA from each individual sample, reducing variation in sequencing coverage between samples.

Robot
Automates the processes to relieve the pressure on the technical teams. Ensures consistency throughout and increases the throughput of the number of samples.

Centrifuge
Spins at high speeds and collects the sample at the bottom of the plate or the tube. Removal of waste reagents is therefore easier and lowers the risk of discarding the cfDNA.

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?

DNA denatures from double-stranded into single-stranded at 95ºC. This is done 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?

Pooling libraries 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?

- The NextSeq performs massively parallel sequencing.
- Massively parallel sequencing is advantageous because it allows us to process numerous regions of the genome and patient samples at once. It is therefore 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 potentially cause a sharps injury.
- Know where the COSHH and risk assessments are.
- 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.