Genomics Training Academy (GTAC)

NHS England

Virtual reality resource supporting material: Nucleic acid extraction from FFPE tissue

Model answers document

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About this document

This document has been designed to support the ‘Nucleic acid extraction from FFPE tissue’ 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: Samples (05:48)

1. What is the difference between DNA and RNA?

- DNA – double-stranded, using adenine, thymine, cytosine and guanine; it makes up the genetic sequence.
- RNA – generally single-stranded, using adenine, uracil, cytosine and guanine; it is used to build proteins.

2. Why might some tests use DNA and some RNA?

Changes to DNA can result in changes to the amino acid or protein it produces. Many genomic tests look for these DNA variants. However, not all genes are expressed all the time. RNA is transcribed from DNA and then into amino acids and proteins. By looking at the RNA, we can establish which genes are actively producing proteins and which are not. This can be useful in identifying aberrant gene activity and targeting subsequent treatments, improving outcomes for our patients.

3. What are slides and curls (may be referred to as scrolls in some laboratories)?

Slides: Thin sections of FFPE tissue, 2–5 microns thick, are cut from the tumour block and mounted on slides. Some of these are stained to visualise the cells, usually with hematoxylin and eosin (H&E) stain. The hematoxylin stains the nuclei on the section a purple/blue colour, and the eosin stains the cytoplasm a pink colour. This enables the histopathologist to look at the cell shapes and structures and identify different cell types, including any abnormal cells.

The histopathologist who is assessing the original blocks/specimens for tumour content will mark up the H&E slide to ensure that the correct abnormal tissue is marked for extraction.

Slides adjacent to the stained slide (chosen because they have very similar abnormal areas) are sent for DNA or RNA extraction. The abnormal areas are scraped off the slides to be processed (as demonstrated).

Curls: Curls are thicker sections (10 microns) of FFPE tissue which are taken off the block and placed into an Eppendorf. These sections are only generally used for DNA or RNA extraction and will not have areas of abnormal cells marked.

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4. **What is the clinical reason for receiving slides instead of curls?**

Slides may be received where there are significant levels of normal tissue in the section. By highlighting the area where abnormal cells have been identified and on the matching H&E slide, it is possible to remove this region for DNA extraction. Therefore, you would expect there to be a concentration of tumour cells. Without removing the normal tissue, there is a risk that a false negative result may be reported due to the dominance of normal tissue. For those samples received on slides, it is normal practice to give a percentage of tumour compared to normal tissue.

Curls can be used when there is a significant proportion of tumour cell line present and there are not concerns that the normal cell line will mask the abnormal. Tissue adherence to slides can also be an issue and therefore curls may be an option in these situations.

5. **Why can FFPE samples be stored at room temperature?**

The tissue blocks are stable, however exposure to high temperature will result in degradation over time. Many labs store these samples at 4ºC to ensure stability. Any unused sections are usually sent back to the appropriate histopathology laboratory for long term storage.

6. **Why is it important that the worksheet contains several identifiers including name, laboratory number and histology number?**

Worksheet identifiers ensure that the correct patient samples are being processed at all times and reduce the risk of a sample mix-up. A sample mix-up could result in incorrect or no treatment being given to a patient which is catastrophic. Also, it would result in issues when following up the patient during and after treatment where we may find different clonal changes.

7. **What is the histology number?**

This is the laboratory number for the histopathology teams. It means that they can track the exact block and section for that patient and will identify which tumour it is. This is essential for all patients, but particularly when a patient has more than one tumour at a time or over periods of time.

8. **In this laboratory, the laboratory information management system (LIMS) is called StarLIMS. The LIMS in your laboratory may have a different name. What are the key functions of a LIMS in the laboratory?**

The LIMS will enable tracking of the sample from when it is booked into a system. It should enable every part of the processing to be recorded, including the operators involved. Some LIMS also allow communications between clinical teams and the laboratory to be recorded, as well as batch numbers of reagents and analysis of results. Systems are also being developed where reports are automatically uploaded to the EPR (electronic patient record)

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so clinicians have direct access. This is vital within the oncology networks for MDT discussions and also treatment decisions.

Bookmark 2: Scraping slides/transferring curls (09:48)

9. During this procedure, we use a slide warmer. What purpose does it serve?

The slide warmer dewaxes the slides and enables the material to be taken off them more easily. The optimal temperature to do this is 56ºC.

10. Considering the slide warmer's optimal temperature, did you notice anything unusual in the film?

Ideally, we put the slides onto the slide warmer when it has reached temperature but putting them on earlier does not adversely affect the process and is sometimes done by technicians – as shown in the film – because it is more convenient to do so.

11. Why is it so important to extract only tumour tissue? How can this affect the result?

Extracting tissue other than the tumour tissue will potentially lead to a false negative result. This is due to the abnormal cell line being potentially lower level and not detected. Reporting a normal result because tumour tissue has not been assessed could lead to incorrect treatments or no treatments. It could also affect any follow up for the patient.

12. A scalpel is used to scrape the tissue from the slides – what are the safety implications of this process? How should the scalpel be discarded?

Scalpels are treated as sharps in the laboratory, therefore additional care is required when using them. As with all processing, the appropriate PPE should be worn including gloves, and care and concentration is required. Scraping should be done as much as possible away from the body and fingers as this will limit the chances of the blade slipping. Once used, the blade will be contaminated with patient material and chemicals and should be disposed of into a sharps bin. Local procedures for sharps injury should be followed in case of any accidents.

13. What happened during the first day of processing?

- Worksheets were created to include all patient samples requiring DNA and RNA extractions – these will be laboratory dependent.
    - Including patient details, laboratory number, volume of master mixes.
- Tubes were all prepared for the process.
- Checks were performed ensuring the correct samples will go into the correct tubes.

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- Curls were transferred to the extraction tube
- A slide warmer was used to soften the wax to enable the section to be removed from the slides. Up to 10 slides were processed for each patient.
- Checks were undertaken to confirm that all numbers match on the slides, H and E slides match and the extraction tubes were labelled correctly.

Note that in this laboratory, the process is paused between Day 1 and Day 2, however this may not be the same in all laboratories.

Bookmark 3: Pre-treatment and incubation (12:03)

14. What does the mineral oil do?

Mineral oil is used for deparaffinisation. It removes the paraffin wax that is used to preserve the tissue so that the cells can be lysed and the nucleic acids can be purified. Depending on the protocol, the method of deparaffinisation may differ. Other methods include xylene and QIAGEN deparaffinisation solution.

15. Why do we heat the sample to 80 ºC, then to 56 ºC and finally to 80 ºC again? Describe what is happening at each stage.

80ºC (following mineral oil addition) – this step is to heat the sample in the mineral oil to remove the paraffin.

56ºC – this step is to lyse the cells. Proteinase K is an enzyme that degrades proteins in the cell membrane, as well as proteins and nucleases that could degrade the DNA/RNA. It operates best at this temperature. Alongside the Proteinase K, the lysis buffer disrupts the cell and nuclear membranes to release the cell components, including DNA and RNA, into solution.

80ºC – this step is to reverse the cross-linking of molecules that is introduced to the DNA during the formalin fixation process. Removal of the cross-linking allows the DNA and RNA to be used in downstream processes such as PCR.

16. Why is it important to record the lot numbers of the reagents and the dates they were opened?

The lot number and opening dates of all reagents used in the procedure are recorded to provide an audit trail of what happened to each sample. Quality assurance is a vital part of the department’s quality management system and assures service users that all samples are tested to highest quality standards and is required for compliance with UKAS accreditation to ISO15189.

17. What other records should be kept about the process?

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- Equipment – the equipment used in the procedure is recorded, particularly if there is more than one instrument that could be used. All equipment will be logged as part of the department's quality management system and this record will include details of regular maintenance and calibration as well as a log of any repairs that have been made.
- Operator – the name of the operator will be recorded.
- Checks – any checks made by a second person will be recorded.
- Temperature monitoring – the temperature of fridges, freezers and ambient locations where reagents and equipment are located will be recorded to ensure that they are within the temperature limits defined by the manufacturer.

Bookmark 4: Preparing for extraction on the Maxwell instrument (16:54)

18. Why might different sample types affect the colour when the lysate is added to the cartridge?

FFPE samples may come from a range of tissue types. Some of these tissue types will contain molecules, for example melanin in skin, that may not be degraded during lysis and will therefore colour the sample.

19. Why is it important that the liquid is at the bottom of the elution tube?

During the extraction process on the instrument, the DNA/RNA will be attached to magnetic beads. These beads are placed into the elution tube at the end of the instrument protocol to allow the DNA/RNA to be eluted into the liquid. If the liquid is not at the bottom of the tube, the DNA/RNA will remain on the beads and will be lost.

End of film

20. Why does the RNA lysate need to be stored in the freezer if not being extracted the same day?

RNA, as a single stranded molecule, is more sensitive to degradation than DNA. Therefore, it should not be kept at room temperature for longer than necessary for the process and should be stored at -80ºC when not in use. Cool blocks are usually used for RNA processes to keep the RNA cool and reduce degradation when not in a freezer.

21. Why is RNA processing carried out in a different laboratory?

As above, RNA is more sensitive to degradation than DNA. Handling RNA in a separate laboratory or workspace reduces the exposure to RNases that may degrade the RNA.

22. What is the purpose of adding DNase or RNase to the lysate?

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For DNA extraction, RNase is used to remove any RNA; for RNA extraction, DNase is used to remove any DNA. Unwanted nucleic acids need to be removed as they would interfere with downstream procedures.

23. Why are the cartridges discarded into a sharps bin? What type of waste disposal will this sharps bin go to?

The cartridge is discarded into a sharps bin as it contains sharp edges that would not be suitable to go into a waste bag. The sharps bin will be disposed of via the clinical waste (orange) waste stream as it contains biological material.

24. What types of nucleic acid quantification are there, what does each method tell us about the quantity and quality of the DNA or RNA, and when would you use them?

- Spectrophotometer – measures light absorbance. Different types of molecule have different absorbances: nucleic acids (260nm absorbance), proteins (280nm absorbance) and salt contamination (230nm absorbance). This method is quick and cheap (after purchase of instrument), provides quantity of nucleic acids and an indication of the purity and presence of contaminants.

- Fluorescence – makes use of a fluorescent molecule that binds to double-stranded DNA. This method is time consuming and expensive due to reagent use (plus instrument purchase), however, it gives an accurate measure of double-stranded DNA which is more accurate than a spectrophotometer.

- TapeStation (electrophoresis) – measures the DNA fragment size and integrity. This method is time consuming and expensive due to reagent/consumables use (plus instrument purchase), however it indicates fragment length, a measure of DNA quality, which is important for some downstream processes.

- Real-time PCR – quantifies the amount of DNA. Very time-consuming and expensive due to reagent/consumables use, however, it gives an accurate assessment of amplifiable DNA.

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