CM-Path SOP for Fresh Tissue Sampling

## Clinical Relevance

Molecular testing will become routine for many if not all pathology specimens in the future, as further genomic, transcriptomic, and proteomic changes present in disease can be harnessed for prognostication, therapeutic decision making, family screening, and prevention strategies. Accurate molecular testing is predicated upon well sampled, handled and preserved tissues, preferably fresh frozen. It is vital that fresh tissue sampling does not compromise the histological diagnosis and staging.

## Principle of procedure

There is a move to all specimens arriving in lab fresh, following removal of formalin from theatres. This coincides with a drive to sample fresh tissue from all cancer resection specimens for retrospective molecular testing as required. Samples can be taken from tissues treated preoperatively with chemo/radiotherapy, from metastatic and nodal deposits and from background normal tissue. Sampling will primarily involve fresh frozen (FF) tissue, although formalin-fixed paraffin-embedded (FFPE) samples can be taken as long as the conditions of the sample handling, fixation and processing described below are adhered to.

## Personnel / training requirements

Band 6 BMS and pathologists with relevant training in fresh tissue handling.

## Specimen requirements

Tumour biopsies and resections.

Unfixed tumour specimens delivered to the lab within 2 hours of removal from patient or stored at 4oC until the delivery is possible within 24-72 hours

FFPE samples may be taken when it is not possible to sample the fresh tissue, but this must be tightly controlled and recorded. The tissue should be placed in a standard size cassette and processed on a routine overnight schedule. The tissue fixation needs to be done in 10% neutral buffered formalin for 12-24 hours optimally, with a maximum of 72 hours in certain situations. The times referred include the fixation time on the tissue processor. The tissue should not be left in wax for an extended time once processed. If these times are exceeded then the exact time should be documented, with a note stating that this may adversely affect any subsequent molecular testing.

Tissue decalcified in acid or EDTA should not be routinely used for molecular testing. If unavoidable, EDTA is prefered, and any specimens subject to decalcification should be documented clearly.

## Equipment

Ventilated bench or safety hood.

Standard specimen dissection equipment.

-80oC freezer

Cryostat

## Health and Safety

All fresh specimens must be treated as potentially infectious.

Full PPE should be worn at all times.

High-risk specimens must be handled in a Class I cabinet.

|  |  |  |  |
| --- | --- | --- | --- |
| Reagent | Risk | Precaution | COSHH Ref |
| Clarke’s Fluid | Low | Wear gloves, lab coat, and eye protection | \* |
| Cryospray | Low | Wear gloves, lab coat, and eye protection | \* |
| Eosin | Low | Wear gloves, lab coat, and eye protection | \* |
| Industrial methylated spirits | Low | Wear gloves, lab coat, and eye protection | \* |
| Isopentane | Medium | Wear gloves, lab coat, and eye protection | \* |
| Haematoxylin | Low | Wear gloves, lab coat, and eye protection | \* |
| OCT | Low | Wear gloves, lab coat, and eye protection | \* |
| Paraplast | Low | Wear gloves, lab coat, and eye protection | \* |
| Scotts Tap Water Solution | Low | Wear gloves, lab coat, and eye protection | \* |
| Xylene | Medium | Wear gloves, lab coat, and eye protection. Use fume extraction | \* |

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## Biological hazards:

All fresh specimens must be treated as potentially infectious.

Full PPE should be worn at all times.

High-risk specimens must be handled in a Class I cabinet.

### Reagents

Clarke Fluid

Cryospray

Eosin

Haematoxylin

Industrial Methylated Spirits

Isopentane

Molten paraffin wax (Paraplast)

OCT

Scotts Tap Water Substitute

Xylene

## Quality control

Specimen must meet handling requirements.

Cryostat/microtome must be cleaned before sectioning.

## Computer codes

Various specimen specific codes as per FFPE specimens.

*Any additional as per local biobanking/100K genomes protocols.*

## Methodology

All specimens should be received fresh from theatre and sampled within 2 hours if held at room temperature and within 24 hours if held at 4oC. The specimen should be opened and inked as per Royal College of Pathologists (RCPath) / Local Guidelines, including weighing and measuring in three dimensions as appropriate, and noting any measurements or structural relations of tumour to margins etc as required by the tissue specific dataset.

If the tumour can be identified a full face should be left intact for diagnostic histological assessment, with fresh samples taken from the adjoining face / parallel slice. Areas of frank necrosis should be avoided where possible to improve the quality of the sample obtained. Samples should be taken with a fresh scalpel blade and clean forceps to avoid contamination with non-tumour DNA.

If the tumour cannot be identified, a sample of possible tumour may be taken fresh if it does not interfere with diagnostic reporting. For example, if no tumour is present in the diagnostic block the fresh tissue block may have to be used for diagnostics instead. Otherwise tissue must be processed as for FFPE by fixation in Neutral Buffered Formalin (NBF) followed by cut up to produce genomic blocks with total fixation time not exceeding 24-36 hrs (including processor time). The tumour can be identified on diagnostic slides with the block showing greatest tumour proportion used for molecular testing, and clearly identified in the integrated report.

Where frozen sections are taken tissue can be sampled from the frozen section specimen if there is enough or from the completion surgery if tumour remains.

Tissue can be sampled from primary tumour, metastases or nodal involvement depending on the surgery and indications for molecular testing. For example, it may be that prognosis or therapeutics may alter based on molecular discrepancies between primaries and metastases. Exhaustive sampling may not be required in extensive disease and decisions should be made on a case by case basis where possible.

For minimally invasive procedures, such as bladder tumour resections via cystoscopy, tissue for molecular testing should be separated out at time of surgery and transported to the lab in a separate pot to that destined for FFPE.

Tissue taken should be immediately frozen and stored at -80oC.

Details of fresh tissue collection should be documented in the integrated report, describing site and number taken.

The tumour content, cellularity and necrosis assessment should be performed by trained individuals according to agreed protocols - guidance for this can be found on the CMPath website.

## Limitations

Communications between theatre, porters, lab, and pathologist need to be optimal to ensure fresh specimens are delivered and processed in a timely manner. If any delay is expected refrigeration should be a priority. The lab should be informed ahead of time as to the expected arrival of a fresh specimen to avoid delays that would impact specimen quality.

Fresh specimens may not contain any tumour.

FFPE samples must be fixed in NBF, not formal saline.

Fixed sample processing often exceeds the desired time limits.

## Reference range / Action limits

Fresh samples should not be taken if they would risk the primary diagnosis of the patient.

Up to three fresh specimens should be taken from each tumour specimen as a minimum (more if multiple tumours or metastases or nodal involvement tissue required).

If fresh tissue cannot be sample in the time frame required for quality samples it should not be performed and the specimen should be processed in NBF to ensure adequate fixation for histological assessment.