NHS
South East
Genomic Laboratory Hub

Department of Cellular Pathology

Title: Tissue Pathway Standard Operating Procedure

Subject: Solid tumour for genomic testing

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Tissue Pathway Standard Operating Procedure

1. Introduction

NHS England are currently reconfiguring the way genomic and genetic laboratory services are commissioned and delivered. They have established 7 Genomic Laboratory Hubs to lead this reconfiguration. The South East Genomics Laboratory Hub (SEGLH), a network of leading foundation trusts and pathology providers, has been commissioned to deliver the genomics laboratory service across South London, Kent, Surrey and Sussex. More information about the new NHSE national Genomics Medicine Service can be found here: https://www.england.nhs.uk/genomics/genomic-laboratory-hubs/

A key requirement of the reconfiguration is to consolidate cancer genomic testing activity across our region; to standardise provision of services with a new national genomics test directory and improve equity of access to these new genomics tests. As a result, the SEGLH is currently implementing new commissioned pathways for adult solid cancers including large tumour agnostic gene panels with high-throughput next generation sequencing technology. This will be a phased approach, initially providing access to a 57 gene panel which meets the current diagnostic targets while a 500 gene panel is validated. A key part of this plan is to work with pathology colleagues to embed tumour pathways, work towards integrated or composite reporting models and standardise tissue processing methods to optimise DNA/RNA quality.

This document provides guidance for submitting samples for genomic testing.

2. Objective

This standard operating procedure is for all hospital trusts and cellular pathology laboratories submitting samples to the South East Genomics Laboratory Hub (SE GLH) based at Guy's Hospital site, for genomic Next Generation Sequencing (NGS) and Whole Genome Sequencing (WGS) testing according to the tests prescribed in the NHSE National Test Directory: https://www.england.nhs.uk/publication/national-genomic-test-directories/.

The aim is to ensure that nucleic acids extracted from material are of a suitable quality for NGS and that the correct information is provided to direct analysis and reporting of results.

3. Scope

The activities covered in this document are:

- The handling of formalin fixed paraffin embedded (FFPE) samples for submission to the SE GLH for genomic testing.
- The assessment and recording of tumour cellularity for the above samples.
- A summary of the sample requirements for WGS.

For full details on WGS preparation of tissue samples, tumour assessment, freezing and transport for WGS please refer to the SOPs below:

i. GLH Sample Handling Guidance for Cancer Samples

ii. GLH Sample Handling Guidance for Germline Samples

4. Responsibility

The clinical leads or molecular pathology leads, together with the laboratory managers of the pathology laboratories will be responsible for implementing this SOP within the department. This includes training of staff and documentation and auditing of the entire process.

The reporting and referring cellular pathologist will be responsible for ordering the tests, selecting the appropriate blocks and sections, assessing the tumour cellularity of the sample and ensuring the samples are handled and submitted by the laboratory to the GLH.

The pathology laboratory technical staff members are responsible for the tissue preparation of the sample for dispatch and transport to the GLH.

5. Sample preparation at local pathology laboratory

Pathology samples should be fixed, process and embedded as per routine laboratory protocols. For optimal DNA and RNA preservation, the sample must be fixed in 10% neutral buffered formalin within 1 hour after obtaining the sample. For large resection specimens, the sample must be sliced or opened to enable adequate penetration of formalin into the tissue. The tissue should be fixed for <48 hours, and not be fixed over the weekend where possible. Rapid processing may also have a detrimental effect on the quality of extracted nucleic acids and suitable alternative material sent, where possible. Sampling of the tissue for blocks prior to embedding should be done as per local protocols for diagnostic purposes. There is no requirement to sample extra material for genomic testing.

Cytology samples are also accepted by the GLH. Cytology samples with diagnostic material should be made into a cell block according to local laboratory protocols and embedded in paraffin blocks as per histology specimens.

To prevent wastage or loss of tissue for analysis, it is recommended that once the pathologist has made an initial assessment of the specimen and the sample is appropriate for genomic analysis, sections for submission to the GLH may be made, but without compromise to other tests (such as

deeper levels or immunohistochemistry), which are required for making a histological diagnosis. This will be at the discretion of the reporting pathologist, but may include: preparation of sections immediately after cutting sections for other tests without trimming, or cutting unstained sections between levels of material. Please avoid excessive trimming or thick sections in the routine diagnostic setting.

For preparation of the material for genomic analysis, the microtome blade should be cleaned with 70% IMS prior to cutting sections of each separate sample. Alternative methods of preventing contamination may also be used, including changing the microtome blade between sectioning each sample or the use of commercial agents (eg. DNA-away). This is to prevent cross-contamination of DNA/ RNA. It is not necessary to change the water in the water-bath, but the bath surface should be wiped with tissue paper. The uppermost section should be discarded at any new sectioning time-point and only subsequent sections collected.

In order to minimise the turnaround times for obtaining the results of the genomic testing, the GLH has proposed reflex testing pathways for common tumour types which are recommended for use in the local laboratories (Appendix A).

6. Tumour block selection and tumour cellularity assessment at local pathology laboratory

All samples for submission for genomic testing must be assessed for tumour content. For NGS genomic analysis, a minimum tumour cellularity required is >20%.

Assessment of tumour cellularity is performed by the reporting pathologist, who will have the histopathological expertise for the particular tumour specialty. To ensure the pathologist is competent in the assessment, the pathologist should:

- Attend the online course module on assessing tumour cellularity by Health Education England at: https://www.genomicseducation.hee.nhs.uk/courses
- Participate in the GenQA pilot online tumour assessment EQA, found at: https://www.genqa.org

The pathologist should select the most appropriate block from the specimen, which provides the highest tumour cellularity, as well as the most representative morphology of the tumour. Where there is tumour heterogeneity on morphological assessment, the tumour with the most invasive component or that which confers the worst prognosis should be selected. Based on above online guidance, the H&E slide for the block is assessed for:

- The overall tumour cellularity (estimated to nearest 100 cells if <1000, nearest 1000 if <10000, or nearest 10000).

- The percentage of numbers of tumour cells, as a total of all nucleated cells (including admixed inflammatory and stromal cells) rather than relative surface area of tumour on slide. This should be estimated to the nearest 10%.

- This percentage should be adjusted for the 3-dimensional arrangement of cells, based on nuclear size/volume, such that a small cluster of lymphocytes will yield more DNA than the equivalent sized nest of tumour cells, in which each cell will be larger and deeper.

- The presence of tumour necrosis and melanin pigmentation (in melanoma specimens).

If the tumour cellularity on the whole section is >20%, the entire section may be used for processing at the GLH (see below for the options). If the cellularity is <20%, another more appropriate block should be selected if available. Alternatively, the pathologist can mark on the H&E slide an area of tumour which will yield a tumour cellularity of >20%, a clear statement of the tumour cellularity in the marked area should be given on the referral form. This will allow the marked area of the sample to be selected for testing and macrodissected at the GLH.

The tumour cellularity assessment of either the whole slide or the marked area selected for genomic analysis must be recorded in the test request form (Appendix B). Please ensure that the area of tumour cellularity referred to on the referral form is clear (either in marker area or as a whole section assessment).

If it is not possible to identify any area on all the blocks which will yield tumour cellularity of >20%, this sample will not be suitable for genomic (NGS) analysis. However, for specific tumours, the sample can be tested using salvage analysis techniques. In this case, the pathologist should perform tumour cellularity assessment with/without marking a chosen area, recording the tumour cellularity as <20%.

7. Tissue preparation

The GLH does not have microtome sectioning capabilities, and therefore whole paraffin blocks are not accepted.

The GLH can receive samples in the following formats:

1. Curls/scrolls

- If the tumour cellularity of the whole section is >20% curls/scrolls of paraffin sections are acceptable. Please send in a sealed single-use microtube, either 1.5ml or 2ml. Ideally 5 sections of 10micron thickness should be submitted.
- Please do not send curls with an overall tumour cellularity of <20%; see section 2.

2. Slides

- 5 sections of 10 micron thickness on uncharged unstained glass slides are required.
- The slides must be clearly labelled with the sample histology number and patient surname.
- An H&E section at 4micron should be submitted with the unstained sections – this can be the diagnostic H&E or an additional slide cut at the same time as the unstained sections. This slide should be submitted with the USS and the request form
- The area of the tumour must be outlined on the H&E section with a permanent marker pen: this will allow microdissection if necessary.
- The marked H&E slide and FFPE unstained slides must have the histology number and patient surname clearly marked on the slides and must correspond to that on the request form.
- Sections should be dried prior to transfer to the hub lab
- Sections must NOT be baked onto slides.
- Please note that spare unstained slides sectioned previously should not be used for molecular analyses.

All slides or microtubes should be labelled with the laboratory histology number and patient surname. Unstained slides should be air-dried before packaging for dispatch. As RNA integrity from FFPE sections has been observed to decrease over time when exposed to light and air, air-dried sections intended for RNA analysis may benefit from storage in a fridge if not dispatched promptly.

Samples that are referred for MLH1 promoter hypermethlyation as part of the Lynch pathway require a matched normal control sample to run alongside the tumour sample; please send 5ml blood in EDTA. If it is not possible to obtain a blood sample from the patient, please send material from one normal tissue block in addition to the material from the tumour block as either curls or slides as described above.

The sample should be submitted together with the GLH request form fully completed, and a copy of the pathology report if authorised. The transport of the sample and documentation should be via either tracked Royal Mail or other recognised courier services.

8. WGS Sample Requirements

Both germline and tumour samples must be submitted for each case to allow interpretation for WGS. The tumour samples and germline sample may be sent to the DNA extraction laboratory at different times with suitable tracking in place.

Formalin fixation causes a high sequencing failure rate and therefore formalin fixed tumour tissue cannot be submitted for WGS. **For optimal, high quality sequencing a fresh tumour sample is required**. Tumour cells must account for at least 30% of the nucleated cells present in the tissue used for DNA extraction.

For details of the preparation of tissue samples, tumour assessment, freezing and transport please refer to:

i. GLH Sample Handling Guidance for Cancer Samples
ii. GLH Sample Handling Guidance for Germline Samples