Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples

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This document was previously issued for use during User Acceptance Testing (UAT) and Live Clinical Testing (LCT) phases for whole genome sequencing (WGS). This current version is the first issue following these phases.

Version	Date	Author	Summary of changes
UAT 1.0	10/06/19	Jane Moorhead	Initial circulation for GLH Feedback
UAT 2.0	14/08/19	Jane Moorhead	Incorporation of GLH Feedback where appropriate
1.0	12/07/21	Sandra Hing	Incorporation of GLH and other relevant feedback where appropriate

Reviewers

This document was reviewed by the following people:

Reviewer name	Title/responsibility	Date	Version
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Prof Dame Sue Hill	SRO for Genomics, NHS England and NHS Improvement	July 2021	1.0
Prof Sandi Deans	Deputy Director (Laboratory & Scientific), Genomics Unit, NHS England and NHS Improvement	July 2021	1.0

Related documents

Title	Document number	Owner	Location
Sample Handling Guidance for Whole Genome Sequencing of Germline Samples	WGS-LAB-001	Rachael Mein	NHS Futures
DNA extraction and Quality Control for Whole Genome Sequencing	WGS-LAB-005	Sandra Hing	NHS Futures
Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies	WGS-LAB-003	Polly Talley	NHS Futures

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Contents

1 Summary

The purpose of this document is to provide guidance to NHS Genomic Laboratory Hubs (GLHs) and associated histopathology departments on the collection, processing and transportation of cellular materials for Whole Genome Sequencing (WGS) of solid tumours. The document covers the process from sample acquisition to transportation of samples to the GLH WGS DNA extraction laboratory. The document does not set out local operating procedures and it will be necessary for GLHs to produce detailed standard operating procedures (SOPs) specifically relating to local practice.

2 Overview of Solid Tumour Requirements

An array of molecular assays is often required to inform the diagnosis and clinical management of patients with sarcoma or paediatric cancer. WGS has the potential to detect all types of somatic and germline mutations including single nucleotide variants (SNVs), copy number variants (CNVs) and structural variants (SVs) and emerging biomarkers such as mutational signatures and burden in a single test without the need for multiple interrogations of the sample. To gain maximum information from WGS, tumour and germline DNA samples from the patient are both sequenced to allow germline variants to be subtracted from the tumour sequence, thus aiding the interpretation of somatic variants. Dual sequencing also allows clear differentiation between germline and somatic variants.

Peripheral blood is the usual choice for obtaining germline DNA for cancer patients and is suitable for all solid tumours. If it is thought that there is heavy infiltration of the bone marrow by metastatic spread, other suitable germline samples are discussed in the *Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies*

Handling of blood samples is covered in *Sample Handling Guidance for Germline DNA Extraction.*

3 Eligibility for WGS

The rare disease and cancer clinical indications eligible for whole genome sequencing are listed in the test directory published on the NHS England website https://www.england.nhs.uk/publication/national-genomic-test-directories/.

It is recognised that there will be instances when it may not be appropriate to submit samples for WGS on otherwise eligible cancer patients. This could include patients who die soon after diagnosis or patients whose management is purely palliative and will therefore not be able to benefit from testing. Given WGS should be considered a part of the normal diagnostic test repertoire it is recommended that Clinicians base decisions on whether to submit samples for a specific patient on similar criteria to those used for submission of other diagnostic tests. If a patient has been consented for WGS then samples should be submitted whenever possible regardless of plans for ongoing care.

4 Tumour Sampling Methods

4.1 Fresh tissue requirement

Formalin fixation causes a high sequencing failure rate and if WGS is achieved then the data quality is poor. Failure rates at pre-sequencing Quality Control (QC) measurement are substantially higher for formalin fixed paraffin embedded (FFPE), in the region of 20-30%, than for fresh tissue and even DNA extracted from FFPE tissue handled in a genomic optimised manner has a high false positive variant calling rate and false negative structural variant rate. Consequently, formalin fixed tumour tissue cannot be submitted for whole genome sequencing.

The amount of tissue required for DNA extraction should be determined by the local processing laboratory to meet the DNA output requirements.

The following are examples of usual sample quantities which will be adequate to achieve 2µg of DNA:

- 5mm x 5mm x 2mm of tumour tissue
- 15mm x 2mm needle core biopsy

It should be borne in mind that the quantity of extracted DNA is from solid tumour material is variable and will be dependent on the exact constituency/morphology of the tumour, extraction protocols and also other variables. Necrotic and haemorrhagic areas should be avoided.

4.2 Specimen macroscopic examination

- Specimen cut-up should take place in a clean environment.
- A clean cut-up board – either a disposable cardboard board or clean plastic board, with new sterile disposable blades and clean forceps should be used for each tumour sample to prevent cross contamination. Standard infection control precautions should be taken when handling fresh tissue.
- In order to minimise the risk of sample to sample contamination during processing it is essential that the following steps be considered standard practice:
- Disposable opening knives, scalpel blades, microtome blades and forceps should be single use only.
- Distel (or equivalent) should be used in conjunction with 70% ethanol for decontamination of workspaces. Please note: attempted decontamination of instruments and workspace with 70% ethanol is not sufficient to remove all traces of human tissue.
- Any instruments used for dissection which are not disposable, should be soaked in 10% Decon (or equivalent) and autoclaved before next use – this includes blades and dissection boards.
- To avoid bacterial contamination samples should not be left fresh at room temperature.

The specimen should be measured, weighed, painted and opened or sliced according to tumour site-specific Royal College of Pathology Guidelines[1]. On opening or slicing, the location of the tumour should be determined, and decision made regarding obtaining a fresh sample without compromising the histological diagnosis and standard of care testing (SoC) until WGS replaces SoC.

It is essential that all procedures are performed according to the ISO 15189:2012 standard and included within the UKAS accredited scope of the laboratory.

4.3 Selection of fresh sample

Areas of tumour that appear macroscopically necrotic or densely fibrotic should be avoided in order to maximise the number of tumour nuclei present for WGS.

4.4 Sampling large tumours

Sampling of the tumour can be made by either using a scalpel to select a tissue cube approximately 5 x 5 x 2mm, slice 10 x 3 x 2mm or punch biopsy (5mm Skin Punch Biopsy Utensil Meditech Systems Ltd. Dorset, UK – or equivalent) as appropriate to the lesion. If possible, more than one punch/slice should be taken to increase the chance of an appropriate area being selected. Whilst not mandatory, if the pathologist judges that the site(s) of sampling should be recorded, this may be captured with an image: different blocks can be indicated on a photograph or diagram.

4.5 Small tumour sampling

If the specimen comprises a small tumour, care should be taken to ensure sufficient material is available for both genomic sequencing and diagnosis. It is critical that the diagnostic pathway is not compromised in any way. Some alternative methods for sampling that can avoid impairing or compromising the diagnostic material have been identified.
A video demonstrating these techniques is available here:
https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/transforming-nhs-services/[2]

4.5.1 Core biopsy

A 20mm length 2mm diameter core biopsy contains a similar volume of tissue to a 4mm cube but removing it from the tumour can be much less disruptive. Opening the sample to take a core is not essential but it may help with directing where the core is taken from. Often palpation of the tumour is enough to direct where to take the core from. Clearly benign areas of the core can be removed prior to freezing or on assessment of the tissue prior to extraction.

Following agreement with the pathologist, it may be beneficial for the surgeon to take a post-operative core before fixing the specimen in the usual manner.

4.5.2 Mirror block of full face of tumour

Where a full face of tumour is taken for a formalin block, a mirror block from the facing half of the tumour can be taken and snap frozen. This sample can be kept until diagnosis is complete before DNA is extracted if there is a concern about diagnostic requirements.

4.5.3 Punch biopsies from fresh tissue blocks

A punch can be taken from well-preserved viable tumour in blocks that have been sampled fresh. By using a small punch e.g. 2 to 3mm the tumour can be sampled from more than one block to get material for whole genome sequencing while leaving sufficient residual tumour in each block for diagnosis. The remaining sample can then be fixed in formalin following usual practice.

4.6 Biopsy samples

Biopsy samples can be taken either during a diagnostic procedure or at surgical resection. Extra cores or forceps pieces may be taken at time of biopsy and stored for WGS if required. Where a decision is taken intraoperatively to not resect a tumour due to extensive metastases then a genomic specific biopsy can be taken during the procedure.

4.7 Biopsy sample requirements and handling

As a general recommendation, in cases where risk to patients is unlikely to be increased by taking more than one biopsy, then 2-3 needle cores or 1-2 standard endoscopic forceps biopsies, if feasible, are recommended.
The biopsy sample must be kept fresh in a way that preserves DNA as well as morphology and should be stored frozen (see Section 6), pending conventional histology on the remaining formalin fixed tissue. If required, such as when no tumour is seen in the formalin fixed samples, the fresh sample can be fixed and used for histology.
Sample handling should take place in a clean environment as for resections.

4.8 Frozen sections from samples

Frozen sections may be used to assess the tumour of the sample taken for WGS and also for submission to the DNA extracting laboratory.
The sample should be embedded within OCT (optimal cutting temperature compound). To ensure a flat cutting surface the following method is recommended:
a) The core is cut into shorter pieces.
b) These are placed adjacent to each other on a smooth flat clean surface (e.g. the inside foil from a scalpel blade).
c) OCT is placed over the pieces.
d) The foil is lowered into liquid nitrogen and frozen or sprayed with Cryospray to freeze.
e) The frozen block is inverted to reveal a flat surface for cutting.
f) It is placed in OCT on a chuck with the flat surface on top to allow usual cryotomy.




5 Tumour Content Assessment

Invasive malignant nuclei must account for at least 30% (with further guidance to be issued on certain tumour types) of the nuclei present in the tissue sample submitted for WGS. Additionally, the sample should have less than 20% necrosis by area. Macrodissection may be required to generate a suitable sample. Personnel involved in the assessment of sample tumour content should participate in the GenQA pilot on-line tumour assessment programme (Tissue-i) www.genqa.org. This is currently an educational tool with no formal assessment of competence.

Tumour content may be assessed on:

- A frozen section of the tissue to be submitted (see above, 4.3)
- A FFPE mirror block of the sample to be submitted
- An FFPE of area surrounding small punch biopsies frozen for submission

To enrich for tumour DNA, the biopsy may be macrodissected to remove any uninvolved part of the tissue. The whole (or remainder) of the biopsy/biopsies may then be homogenised for DNA extraction or multiple frozen sections cut according to usual protocol.
The laboratory may choose to evaluate further frozen sections for tumour assessment when cutting through the frozen block. This method has the advantage of estimating the tumour content throughout the sample; however, the DNA yield may be reduced as material can be left on the slide or instruments used.

Guidance on tumour assessment can be found at
https://www.genomicseducation.hee.nhs.uk/courses/[3]

6 Storage of Samples Prior to Freezing

6.1 Cold ischaemia effects

Postoperatively, biological processes continue at room temperature and these impact on protein expression and can affect morphology, immunohistochemistry, FISH results and possibly mitotic counts. To mitigate these effects of cold ischaemia, tissue specimens should be delivered to Pathology without any formalin fixation and sample selection should be undertaken within 2 hours of surgical excision unless refrigerated. Many samples can be maintained unfixed for up to 72 hours if *rapidly cooled* and kept at 4^{o}C. This can be done either by placing the dry specimen in a fridge, in a bag on wet ice, in a cold bag with ice blocks, or the sample can be placed in vacuum plastic bags, vacuum packed then placed in the fridge/ice/cold bag. Samples can be cooled in plastic pots, though rapid cooling is facilitated by placing the sample in plastic specimen bags purpose-designed for vacuum packing. These must have specimen and patient ID affixed in the usual manner.

The conventional practice of placing unopened samples into large buckets of formalin at room temperature for up to 72 hours does nothing to prevent damage due to cold ischaemia to samples except at the periphery once formalin has penetrated. It is therefore recommended that clinically actionable immunohistochemistry and FISH e.g. receptor status are performed on diagnostic biopsies which are fixed in formalin within an hour of removal from the patient if possible.

6.2 Biopsy samples

Biopsy samples can be kept refrigerated at 4^{o}C for up to 72 hours. To prevent drying out the biopsy can be kept in an Eppendorf, sealed and wrapped in cling film or kept in a larger tube adjacent to, but not touching, damp gauze. Biopsies should not be kept in saline for an extended period as it affects the morphology of the tissue.

7 Freezing

7.1 Snap Freezing in liquid nitrogen (LN2)

Immediately after sampling, the tissue is placed on a piece of foil, which is then immersed into LN2 for at least 60 seconds depending on the size of sample (samples can be kept longer in LN for convenience if required). The foil is removed using forceps and the sample transferred rapidly to a labelled Eppendorf which is then kept in LN2 or on dry ice prior to transfer to the freezer. Samples can be kept on dry ice for 4-6 hours if required. If preferable, the sample can be placed into OCT prior to freezing.

7.2 Isopentane on dry ice

Place a stainless steel, Pyrex or polypropylene container into a polystyrene container of dry ice. Add isopentane to this in a fume hood. When the isopentane settles the slurry is ready and the sample, on foil, can be immersed into the liquid to freeze. Freezing takes approximately 60 seconds; the sample can then be transferred to a labelled Eppendorf and stored on dry ice prior to transfer to a freezer.

7.3 Freezing with Cryospray

Samples are sprayed with Cryospray until solid (usually ~30 seconds). The sample is then transferred to a labelled Eppendorf on dry ice until transfer to a freezer. If desired the samples can be placed onto foil and loosely covered or orientated on a support such as cork with OCT before spraying.

7.4 Freezing on dry ice

Samples can be transferred directly into labelled Eppendorf tubes and placed into a polystyrene container of dry ice. The samples require at least 5 minutes for freezing but can be kept on dry ice for hours if necessary.

7.5 Other options

In exceptional circumstances submission of alternative sample types for WGS will be permitted. Where a sample has not been forwarded for DNA extraction because a diagnosis of a WGS eligible condition was not initially suspected and no further fresh material is available, it is permissible for stored locally extracted DNA to be transferred to the GLH DNA extraction laboratory once a diagnosis has been made. The submission of locally extracted DNA for WGS will be closely audited.

The method of freezing should take into account the manner in which the tumour assessment will be performed. If this is to be carried out using a frozen section from the sample itself, the first three methods only should be used as freezing artefact may be introduced by slower methods of freezing the tissue.

8 Storage and Transport of Frozen Material

Once frozen, samples should be stored in a freezer. A -80°C freezer is most appropriate however; samples have been kept in a -20°C freezer for months whilst in process with no discernible impact on whole genome sequencing.

Transport of the tissue to the GLH DNA extraction laboratory should be arranged accordingly, maintaining the appropriate temperature where the germline blood sample should be collated with the tissue sample.

For solid tumour referrals it is the responsibility of the patient's Home GLH to ensure that the germline sample has been taken, and then transported onto the Home GLH WGS DNA extraction laboratory. Alternatively, where the tumour sample has been sent to another GLH for histopathology processing then the fresh tissue must be transported and extracted at that GLH WGS DNA extraction laboratory. Obtaining consent should be the responsibility of the patient's clinical team.

All samples must be placed in standard specimen bags with a Genomic Medicine Service test order form and transported either to the GLH WGS DNA extraction Laboratory or transported to another GLH for DNA extraction. Blood samples for germline DNA extraction must be received in the WGS DNA extraction Laboratory and all processing completed within 72 hours.

Planning of working practices for sample collection and optimisation of transport logistics to the GLH WGS DNA extraction Laboratory from all sites of collection should be a priority with detailed process maps followed.

9 Sample Requirements for WGS

Both germline and tumour samples must be submitted for each case to allow interpretation of the whole genome sequencing. Sample handling and requirements for germline DNA are found in the Sample Handling Guidance for Germline samples[i]. The tumour DNA sample must be submitted for WGS at the same time as the germline DNA sample, however the tumour sample and the peripheral blood sample may be sent to the DNA extraction laboratory at different times with suitable tracking in place. 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 (see section 5 for details). Further guidance on the tumour content for certain cancer types will be issued in due course.

9.1 DNA requirements

- A minimum of 2µg of tumour DNA must be submitted for WGS at a preferred concentration of 45ng/µl in a minimum volume of 115µl.
- Reduced requirements under special circumstances are detailed in *DNA Extraction and Quality Control Guidance for Whole Genome Sequencing*[ii].

10 Roles and Responsibilities

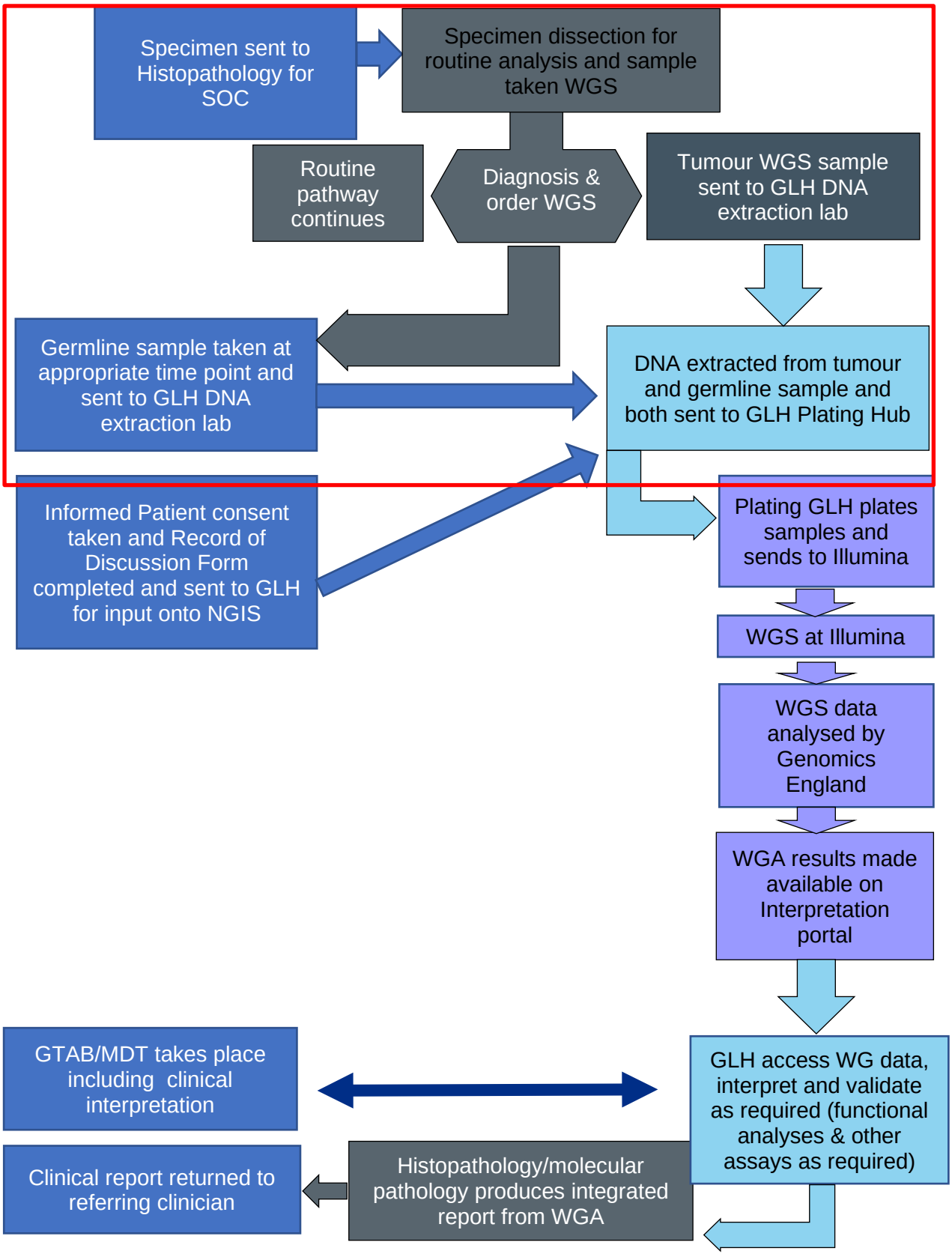
It is the responsibility of responsible clinician and reporting pathologist to ensure that whenever possible appropriate tissue samples are obtained and submitted for WGS from eligible patients.

It is the responsibility of the GLH to ensure that appropriate local pathways and SOPs are in place to facilitate the submission of cellular samples to the central GLH WGS DNA extraction laboratory.

It is the responsibility of the GLH Cancer Clinical and Scientific Leads to ensure that all appropriate clinical and laboratory teams across the geography of the GLH are fully aware of which patients are eligible for WGS and that the teams understand the local clinical pathways and processes that have been put into place to obtain and process samples from these patients for WGS.

11 Sample Pathway

The flow chart below provides a high-level overview of the process for WGS in eligible patients with solid tumours. DNA extraction processes are not covered by this guidance.



12 References

1. Royal College of Pathology Guidelines
2. https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/transforming-nhs-services/
3. https://www.genomicseducation.hee.nhs.uk/courses/[3]