Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies for Adults, Children and Young People

Document management

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Reviewers

This document must be reviewed by the following people:

Reviewer name	Title/responsibility	Date	Version
Professor Dame Sue Hill	SRO for Genomics, NHS England		
Dr Sandi Deans	National Laboratory & Scientific Lead, Genomics Unit, NHS England		

Approved by

This document must be approved by the following people:

Name	Signature	Title	Date	Version
Professor Dame Sue Hill		SRO for Genomics, NHS England	14/08/2019	3.0
Dr Sandi Deans		National Laboratory & Scientific Lead, Genomics Unit, NHS England	14/08/2019	3.0

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Summary

The purpose of this document is to provide Guidance to NHS Genomic Laboratory Hubs (GLHs) and Specialised Integrated Haematological Malignancies Diagnostic Services (SIHMDS) on the collection, processing and transportation of cellular materials for Whole Genome Sequencing (WGS) of Haematological Malignancies. The document is not a standard operating procedure (SOP) and it will be necessary for GLHs to produce detailed SOPs specifically relating to local practice.

1. Introduction

Multiple different molecular tests are often required to inform the diagnosis and clinical management of patients with Acute Myeloid Leukaemia (AML) and Acute Lymphoblastic Leukaemia (ALL). WGS has the potential to detect all types of somatic and germline mutations (small, structural & copy number variants) and potential future 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 are sequenced simultaneously: This allows subtraction of germline variants from the somatic genome meaning there is clear differentiation between germline and somatic variants.

Peripheral blood is the usual sample choice for obtaining germline DNA to accompany tumour sequencing and is suitable for solid tumours. Obtaining an appropriate germline sample for bone marrow/blood based haematological cancers is more difficult and alternative sources such as saliva, cultured fibroblasts and in some instances peripheral blood (once it has been cleared of morphological disease) are required. The most appropriate source will vary depending on the haematological tumour type and the clinical circumstances therefore guidance on selection of suitable germline material for haematological cancers is provided in this document.

2. 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/.

At present the following two groups of patients with haematological malignancies are eligible for WGS:

- All patients (adult & paediatric) with Acute leukaemia (includes Acute Myeloid Leukaemia, Acute Lymphoblastic Leukaemia and Acute Leukaemia of ambiguous lineage) at initial diagnosis and/or on relapse.
- Paediatric patients with any other type of Haematological Malignancy at diagnosis and relapse.

For the purposes of WGS eligibility, paediatric cancer is currently defined as all cancer patients aged 19 years or under and all cancer patients treated within a teenage and young adult (TYA) primary treatment centre (PTC) where the initial episode of cancer occurred at aged 19 or under.

It is recognised that there will be instances when it may not be appropriate to submit samples for WGS on otherwise eligible 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.

3. Sample Requirements

3.1. Acute Myeloid Leukaemia

3.1.1 *Tumour material*:

Suitable tumour materials are bone marrow aspirate or peripheral blood containing >=20% blasts morphologically or any blast percentage if there is an AML-defining genetic abnormality as per WHO 2016 Guidelines[1]

If another body fluid (e.g. cerebrospinal fluid, ascitic fluid, pleural fluid) has been proven immunophenotypically / histologically to be infiltrated with AML, provided DNA quality and quantity QC metrics are met, this could be used as tumour source in the absence of an appropriate peripheral blood or bone marrow sample.

A lower blast cell percentage is acceptable compared to the required solid tumour neoplastic cell percentage (30%) as previous data suggests that many of the acquired variants will also be present in myeloid cells which do not have a morphological blast cell phenotype.

3.1.2 Germline DNA sample source

There are multiple sources of germline DNA and those listed below are acceptable for WGS.

- Saliva collected once sufficient treatment has been given to remove all circulating myeloid cells from the peripheral blood. e.g. on day 5 after administration of two doses of anthracycline chemotherapy (or equivalent) in patients receiving intensive induction chemotherapy.
- Cultured fibroblasts from a skin biopsy.
- Uncultured skin biopsy.

Whenever possible any of the above sample types should be used as the germline source as these will minimise the delays in submitting paired tumour and germline samples for WGS, or in the case of cultured fibroblasts, represent the traditionally acknowledged 'gold standard' germline source. However, when it is not possible to obtain any of the above sample types it is acceptable to submit peripheral blood or bone marrow aspirate samples which are either negative for or have a diagnostic minimal / measurable residual disease (MRD) marker (e.g. NPM1, RUNX1-RUNX1T1) detectable at a level of <0.1% as the source of germline DNA.

3.2. Acute Lymphoid Leukaemia

3.2.1 Tumour material

Suitable tumour materials are bone marrow aspirate or peripheral blood containing >=30% blasts morphologically.

If another body fluid (e.g. cerebrospinal fluid, ascitic fluid, pleural fluid) has been prove immunophenotypically / histologically to be infiltrated with ALL, provided DNA quality and quantity QC metrics are met, this could be used as tumour source in the absence of an appropriate peripheral blood or bone marrow sample.

3.2.2 Germline DNA sample source

Germline samples There are multiple sources of germline DNA and those listed below are acceptable for WGS.

- Saliva is acceptable as a germline in ALL but should be collected at a time when it is confirmed that there are no circulating blasts in the peripheral blood (by morphological assessment).
- Cultured fibroblasts from a skin biopsy.
- Uncultured skin biopsy.

Whenever possible any of the above sample types should be used as the germline source as these will minimise the delays in submitting paired tumour and germline samples for WGS, or in the case of cultured fibroblasts, represent the traditionally acknowledged 'gold standard' germline source. However, when it is not possible to obtain any of the above sample types it is acceptable to submit peripheral blood or bone marrow aspirate samples which are either negative for or have a diagnostic MRD marker (e.g. BCR or TCR gene rearrangement or BCR-ABL transcript) detectable at a level of <0.1% as the source of germline DNA.

3.3. Children with other Haematological Cancers

In children with acute leukaemia the guidelines given in sections 3.1 & 3.2 should be followed. In children with solid haematological malignancies such as lymphoma the normal sample types are fresh frozen tissue as the source of tumour DNA and peripheral blood as the source of germline DNA. Detailed guidance for the handling of these sample types can be found in "Sample Handling Guidance for Whole Genome Sequencing of Solid Tumours"[2]

In children with other 'liquid' non-acute leukaemia haematological malignancies (including lymphoid malignancies infiltrating blood and marrow as well as myeloid diseases such as myelodysplasia (MDS), myeloproliferative disorders (MPD) and chronic myeloid leukaemia (CML)), peripheral blood or bone marrow samples should be used as the tumour type.

In order to obtain informative WGS sequencing it is necessary for the 'malignant cell burden' of the sample to equal / exceed 30% (further guidance on handling of samples that do not meet this criterion will be issued). Whilst in some malignancies (e.g. lymphoid) this can be determined morphologically / immunophenotypically, it is acknowledged that in others (e.g. myelodysplastic syndrome /myeloproliferative neoplasms) there is no definitive malignant cell phenotype detected using standard diagnostic means. In these circumstances a surrogate marker of the malignant cell burden (e.g. myeloid cell percentage) can be used as outlined in the appendix.

4. Sample prioritisation

Until such a time as the AML, /ALL/paediatric haematological malignancies WGS bioinformatics pipeline has been fully validated and results can be returned in an appropriate time frame to inform the initial management of patients, it will be necessary to run the assay in parallel with current standard of care testing (SOC). Consequently, there will be occasions when, despite every effort being made to ensure efficient usage of all available material, there will not be sufficient material for all indicated tests. In this scenario priority should be given to those tests that will inform immediate management at the discretion of the treating clinician. However, it is envisaged that these cases will constitute a minority and in order to maximise potential future benefit to patients and to ensure equity of access to WGS samples should be submitted for WGS whenever possible.

5. Sample processing

5.1. Peripheral blood & bone marrow aspirate samples

Samples should be collected into EDTA tubes. Currently samples collected into other tubes types (e.g. Lithium Heparin) are not acceptable for WGS. A nucleated cell count should be performed in the SIHMDS prior to forwarding the sample to the GLH WGS DNA extraction laboratory. The rationale for performing a nucleated cell count prior to further processing of the sample is:

i) To ensure sufficient good quality DNA is obtained: A significant proportion of samples from patients with haematological cancer will have elevated nucleated cell counts meaning the sample requires dilution prior to the use of automated DNA extraction protocols. In the event these samples are processed without dilution there is a danger of suboptimal DNA extraction owing to these processes being optimised for a specific cell concentration range which is different to that of the sample being processed. This could result in in poor quality and / or paradoxically a low yield of DNA. The GLH DNA extraction laboratory will provide guidance on the degree of dilution required for different nucleated counts and the diluent that should be used.

ii) To ensure efficient usage of sample: This is particularly important for low volume samples e.g. paediatric bone marrow samples. Nucleated cell counts will allow the sample to be aliquoted appropriately at the SIHMDS to ensure optimal use of the available sample and that there is sufficient material for all the testing that is required. The GLH WGS DNA extraction laboratory will provide guidance on the number of cells required to ensure sufficient DNA can be extracted for WGS. The number of cells required will vary depending on the extraction system used therefore it will be necessary for GLHs to produce local guidance on this.

5.2. Saliva

A specialised saliva sample collection kit designed for downstream DNA extraction should be used. A variety of such kits are available commercially and any can be used providing local verification of performance has been performed and it has been shown to meet the sample requirements. DNA extraction from collected saliva samples will be performed at the GLH WGS DNA extraction laboratory.

5.3. Cultured Fibroblasts from a skin biopsy

Cultured fibroblasts must be collected, processed and stored according to local best practice and within a laboratory with UKAS ISO 15189:2012 accreditation for this process. DNA extraction from the cultured fibroblasts will be performed at the GLH WGS DNA extraction laboratory.

5.4. Uncultured skin biopsy.

Skin biopsies should be processed in accordance with sample handling guidance produced for other fresh tissue samples collected for WGS[2]. However, as the samples are collected as a source of germline DNA then no tumour content assessment is required. DNA extraction from uncultured skin biopsies will be performed at the GLH WGS DNA extraction laboratory.

6. Storage and transportation of samples to GLH DNA extraction laboratory

Peripheral blood and bone marrow samples should be stored at 4°C and DNA extraction carried out within 72 hours of collection. Samples should ideally be transported at 4°C but it is acceptable to send samples at ambient temperature if the total time from collection to extraction does not exceed 72 hours.

Saliva samples should be collected, stored and transported according to the kit manufacturer's instructions.

Cultured fibroblasts can be transported in flasks containing transport media or the cells stripped and sent in tubes. Samples should ideally be transported at 4°C but it is acceptable to send samples at ambient temperature if the transportation time does not exceed 24 hours.

Skin biopsies can be stored or transported at 4°C for up to 72 hours after collection and prior to DNA extraction. To prevent drying out the biopsy can be placed 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 stored or transported in any sort of media as the downstream effects of such media on WGS quality are currently unknown.

Other sample types: - 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 frozen cell pellets* from liquid tumours or locally extracted DNA to be transferred to the GLH DNA extraction laboratory once a diagnosis has been made. The submission of frozen pellets or locally extracted DNA for WGS will be closely audited.

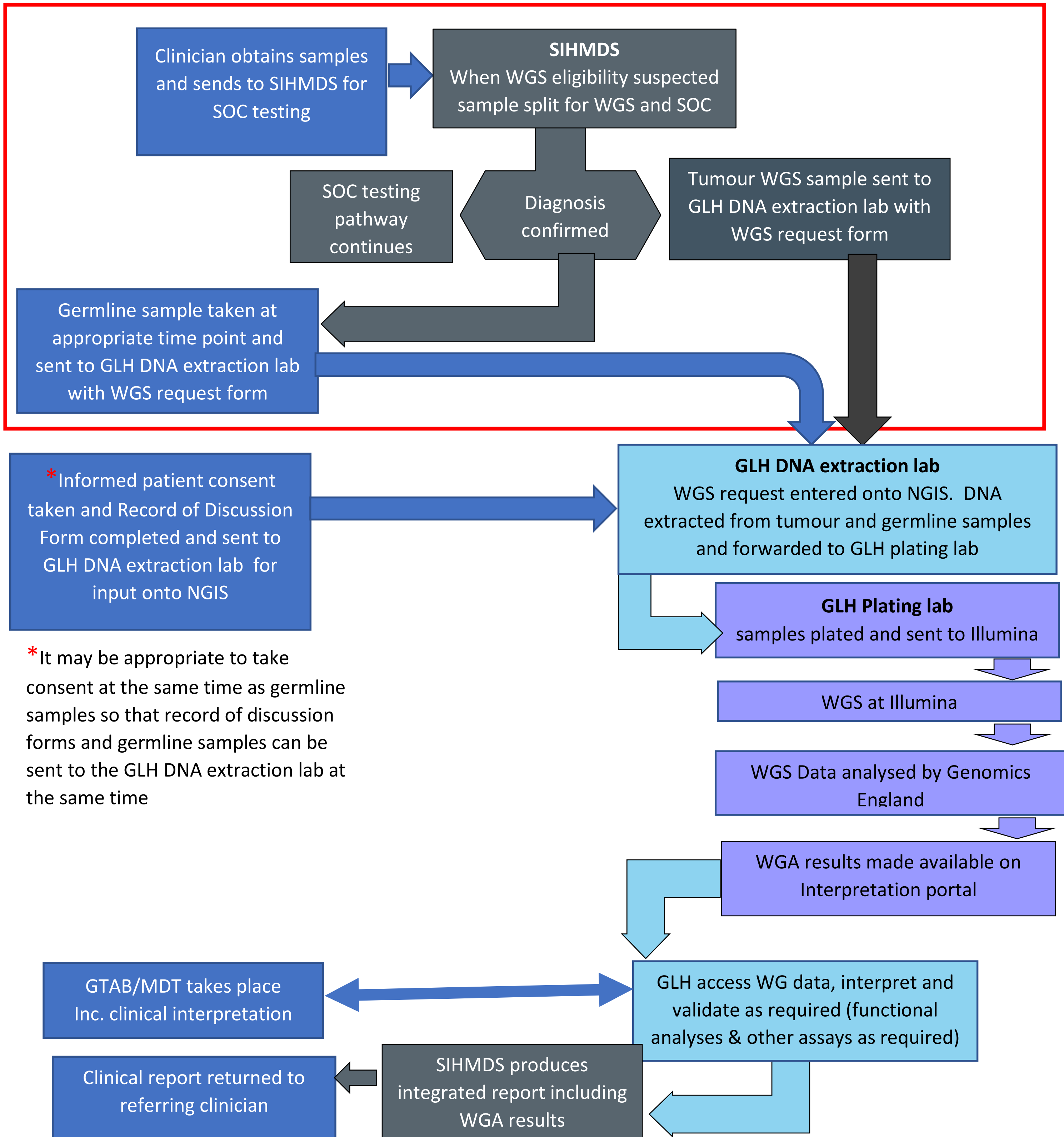
* Pellets may be produced from blood or bone marrow buffy coats or from other body fluids and must be transported frozen for DNA extraction.

All samples should be sent to the GLH DNA extraction laboratory accompanied by a completed Cancer WGS request form. For tumour samples the request form will be completed by the SIHMDS once the diagnosis has been confirmed. For germline samples the request form will be completed by the clinical team responsible for obtaining and forwarding the germline samples.

Although tumour samples will regularly be collected earlier than the matched germline sample it is recommended that all samples are transported for DNA extraction as soon as they are ready i.e. laboratories should not wait for the germline sample before transporting the tumour sample for DNA extraction. This is to avoid prolonged storage of samples in the cellular form and ensure the best quality DNA. However currently, matched germline and tumour DNA samples must be submitted together for plating and WGS therefore is will be necessary for the GLH DNA extraction laboratory to store DNA from tumour samples pending the arrival of the matched germline sample.

7.Sample Pathway

The below flow chart provides a high-level overview of the process for WGS in eligible patients with haematological malignancies. The area within the red box indicates the sample handling steps covered by this guidance document (DNA extraction is not included).



8. Roles and responsibilities

It is the responsibility of managing Clinicians and SIHMDS to ensure that whenever possible appropriate cellular 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 Haematological Malignancy 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 order, obtain and process samples from these patients for WGS.

9. References

1. Swerdlow, S. H. et al. (eds). WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th edition, (International Agency for Research on Cancer, Lyon, France, 2017).

2. GLH Sample Handling Guidance for Cancer Samples v2.0

10. Appendix

Table outlining markers of the malignant cell burden which should be used in different conditions.
*Malignant cell percentage as measured using morphology, immunohistochemistry or immunophenotyping

Clinical Indication Name	Eligibility	Parameter to use as malignant cell percentage*
Acute Myeloid Leukaemia	Adults & Paediatrics	Blast percentage
Myelodysplasia	Paediatrics only	Non-lymphoid nucleated cell percentage
Aplastic Anaemia	Paediatrics only	Non-lymphoid nucleated cell percentage
Chronic Myeloid Leukaemia	Paediatrics only	Myeloid cell percentage
Myeloproliferative Neoplasm	Paediatrics only	Non-lymphoid nucleated cell percentage
Systemic Mastocytosis	Paediatrics only	Non-lymphoid nucleated cell percentage
Juvenile Myelomonocytic Leukaemia	Paediatrics only	Non-lymphoid nucleated cell percentage
Acute Leukaemia Other	Adults & Paediatrics	Blast percentage
Blastic Plasmacytoid Dendritic Cell Neoplasm	Adults & Paediatrics	Malignant cell percentage
Acute Lymphoblastic Leukaemia	Adults & Paediatrics	Blast percentage
Lymphoma	Paediatrics only	Malignant cell percentage
B Cell Non-Hodgkin Lymphoma	Paediatrics only	Malignant cell percentage
Burkitt Lymphoma	Paediatrics only	Malignant cell percentage
Burkitt Like Lymphoma with 11q Abnormalities	Paediatrics only	Malignant cell percentage
Large B Cell Like Lymphoma with IRF4 Rearrangement	Paediatrics only	Malignant cell percentage
High Grade Lymphoma	Paediatrics only	Malignant cell percentage
Primary Mediastinal B Cell Lymphoma	Paediatrics only	Malignant cell percentage
ALK Positive Large B Cell Lymphoma	Paediatrics only	Malignant cell percentage
MALT Lymphoma	Paediatrics only	Malignant cell percentage
T Cell Non-Hodgkin Lymphoma	Paediatrics only	Malignant cell percentage
ALK Negative Anaplastic Large Cell Lymphoma (Including Primary Cutaneous Subtypes)	Paediatrics only	Malignant cell percentage
ALK Positive Anaplastic Large Cell Lymphoma	Paediatrics only	Malignant cell percentage
NK Cell/Gamma-Delta T Cell Lymphoma	Paediatrics only	Malignant cell percentage
Hepatosplenic T Cell Lymphoma	Paediatrics only	Malignant cell percentage
Histiocytosis	Paediatrics only	Histiocytic cell percentage
Paediatric Type Follicular Lymphoma	Paediatrics only	Malignant cell percentage