Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies

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Document management

Revision history

This document was initially issued for use during User Acceptance Testing (UAT) and Live Clinical Testing (LCT) phases for whole genome sequencing (WGS). The document was then updated for WGS testing as part of the genomic medicine service (GMS).

Version	Date	Author	Summary of changes
UAT 1.0	10/06/19	Shirley Henderson	Initial circulation for GLH Feedback
UAT 2.0	14/08/19	Shirley Henderson	Incorporation of GLH Feedback where appropriate
1.0	July 2021	Polly Talley	Incorporation of GLH and other relevant feedback where appropriate
2.0	Sept 2022	Polly Talley	Update of eligible samples, and incorporation of feedback where appropriate
3.0	May 2024	Polly Talley	Updated with changes to service including the introduction of tumour first / germline later

Reviewers

This document was reviewed by the following people:

Reviewer name	Title/responsibility	Date	Version
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Related documents

Title	Document number	Owner	Location
Sample Handling Guidance for Whole Genome Sequencing of Germline Samples	WGS-LAB-001	Rachael Mein	NHS Futures
Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples	WGS-LAB-002	Sandra Hing	NHS Futures
DNA extraction and Quality Control for Whole Genome Sequencing	WGS-LAB-005	Sandra Hing	NHS Futures

Document control

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Contents

1 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 genomic material for Whole Genome Sequencing (WGS) of patients with Haematological Malignancies (HaemOnc). This document is not a standard operating procedure (SOP) and it will be necessary for GLHs to produce detailed SOPs specifically relating to local practice.

2 Introduction

Multiple different genomic 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 if the germline is free of tumour, 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, skin biopsy, cultured fibroblasts and in some instances peripheral blood / bone marrow (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.

Modification to the HaemOnc WGS process now allows sequencing from a tumour sample only, with the option of sending a follow up germline sample at a later date. This has the benefit of allowing a faster, and therefore more clinically relevant turn-around time (TAT), but with initial results that do not include germline assessment, and therefore the results are more akin to the tumour in normal contamination (TINC) returns.

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.

4 Sample Requirements for WGS

6.1 Acute Myeloid Leukaemia

- **Tumour material**

Suitable tumour materials are bone marrow aspirate or peripheral blood samples containing ≥20% blasts morphologically or any blast percentage if there is an AML-defining genetic abnormality as per ICC or WHO 2022 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 in AML 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.

- **Germline DNA sample source**

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

- Cultured fibroblasts from a skin biopsy.
- Uncultured skin biopsy.
- Saliva - collected once sufficient treatment has been given to remove all circulating myeloid cells from the peripheral blood.
- Peripheral blood/bone marrow – when samples are shown molecularly to be clear of disease or have a measurable residual disease (MRD) marker (e.g. *NPM1*, *RUNX1::RUNX1T1*) detectable at a level of <0.1%.

Whenever possible germline sample types should be selected to minimise the delays in submitting paired tumour and germline samples for WGS. However, it is acknowledged that the ‘gold standard’ germline source is cultured fibroblasts which results in a short unavoidable delay in submitting samples. Recent data from the GMS WGS service has shown high levels of contamination associated with DNA directly extracted from skin biopsies and from saliva. Therefore, wherever possible DNA extracted from cultured fibroblasts should be utilised, if necessary using the tumour first, germline later submission pathway to minimise TAT delays.

6.2 Acute Lymphoid Leukaemia

- **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 proven 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.

- **Germline DNA sample source**

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

- Cultured fibroblasts from a skin biopsy.
- Uncultured skin biopsy.
- 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).
- Peripheral blood/bone marrow – when samples are shown molecularly to be clear of disease or have a measurable residual disease (MRD) marker (e.g. *IGH* or *TCR* gene rearrangement or *BCR::ABL1* transcript) detectable at a level of <0.1%.

Whenever possible germline sample types should be selected either to minimise the delays in submitting paired tumour and germline samples for WGS, or in the case of cultured fibroblasts, represent the 'gold standard' germline source.

6.3 Paediatric Patients with other Haematological Cancers

Children with acute leukaemia are considered paediatric patients up to the age of 25 years and the guidelines given in sections 4.1 & 4.2 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* and *Sample Handling Guidance for Whole Genome Sequencing of Germline Samples.*

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 neoplasms (MPN) 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%. 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

6.4 Proven or Suspected Haematological Tumours Exhausted all Standard of Care Testing (ESOC) and Treatment

This clinical indication has been added to ensure that there is a mechanism for clinicians to refer those patients where WGS is considered likely to have a real clinical impact but doesn't fit into the previously described eligible clinical indications.

In adults 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* and *Sample Handling Guidance for Whole Genome Sequencing of Germline Samples.*

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6.5 Patients who have relapsed following allogenic stem cell transplantation

Currently the WGS analytical pipeline is not able to process samples from transplanted patients with significant levels of donor chimerism. This is because the tumour:normal pipeline is only able to analyse data from one genome, meaning that chimeric samples which contain two different genomes would fail WGS contamination checks. Consequently, only cases where chimerism analysis shows 100% recipient DNA can be submitted for WGS.

It is not recommended that these patients are submitted as 'tumour only' samples until such a time as there are clear pathways and processes in place to manage the eventuality that some potential germline information of clinical significance which may pertain to the donor may be detected.

In the event a patient has relapsed post-allo HSCT and has 100% recipient chimerism, the appropriate germline sample would be cultured fibroblasts.

5 Sample Prioritisation

Until such a time as the AML/ALL/paediatric/other 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 (SOC) testing. 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.

6 Sample Processing

6.6 Peripheral blood & bone marrow aspirate samples

Samples should be collected into EDTA tubes. Currently samples collected into other tube 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 cancers will have elevated nucleated cell counts meaning the sample requires dilution prior to the use of automated DNA extraction protocols. Processing these samples without dilution risks suboptimal DNA extraction as these processes are usually optimised for a specific cell concentration range. This could result in poor quality and / or 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.

6.7 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.

6.8 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 or 15189:2022 accreditation for this process. DNA extraction from the cultured fibroblasts will be performed at the GLH WGS DNA extraction laboratory.

A video on how to take a skin punch biopsy can be found here https://youtu.be/Wk0edpXFevQ

6.9 Uncultured skin biopsy.

Skin biopsies should be processed in accordance with sample handling guidance produced for other fresh tissue samples collected for WGS (see *Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples*). 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

7 Storage and Transportation of Samples to the GLH WGS 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 / universal tube, sealed and wrapped in film. Alternatively, providing they are not going to be used for morphological assessment, it is acceptable to transport skin biopsies in saline, medium (provided lithium heparin is not an additive) or wrapped in damp gauze to prevent drying out of the sample.

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.

All samples should be sent to the GLH DNA extraction laboratory accompanied by a completed Cancer WGS test order form (TOF). For tumour samples the TOF will be completed by the SIHMDS once the diagnosis has been confirmed or the referring genomic laboratory. For germline samples the TOF will be completed by the clinical

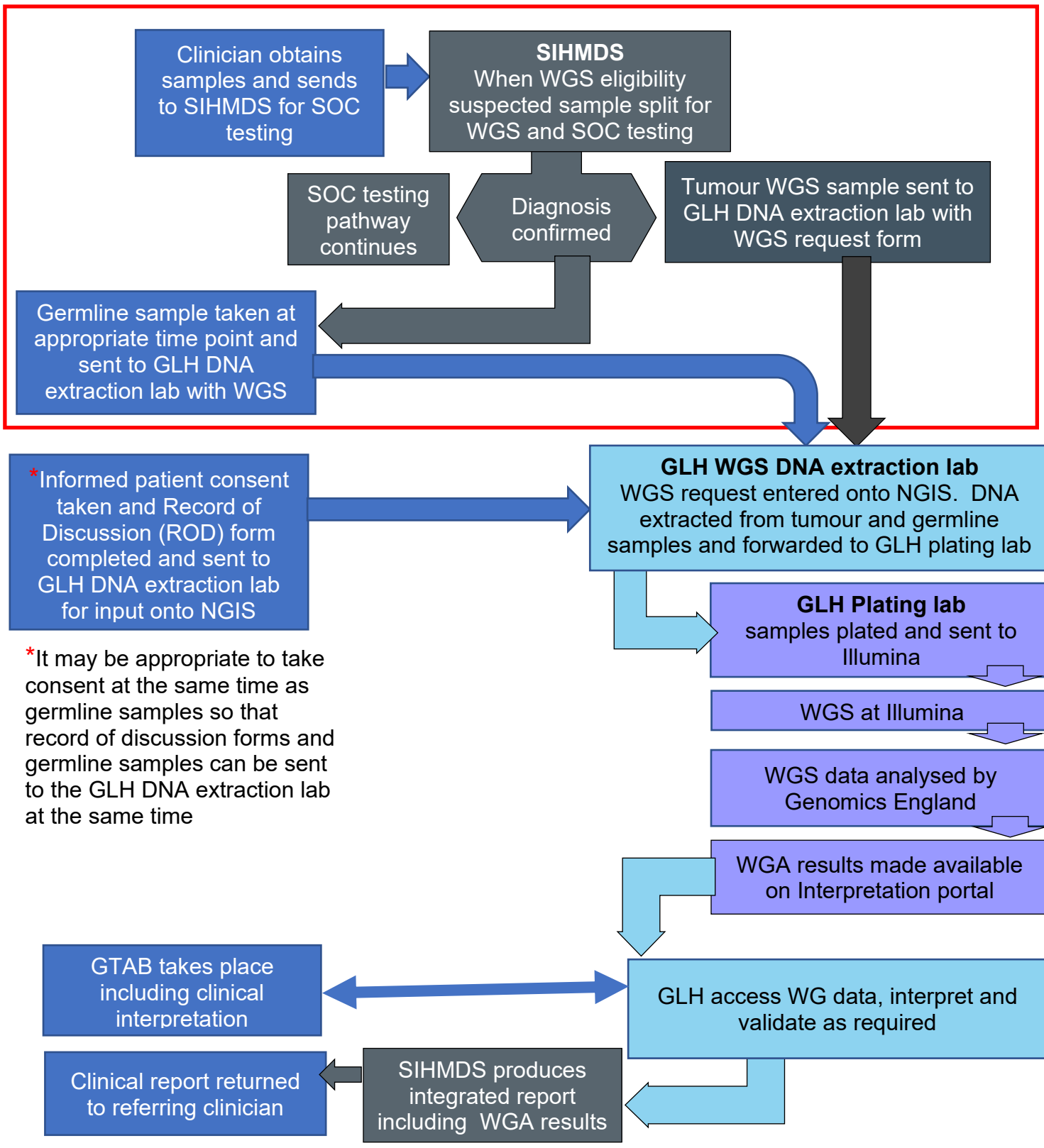
team responsible for obtaining and forwarding the germline samples or the referring genomic laboratory

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 prior to DNA extraction and to ensure the best quality DNA extraction is achieved.

However currently, matched germline and tumour DNA samples must be submitted together for plating and WGS, and therefore the GLH WGS DNA extraction laboratory must have provision to store DNA from tumour samples pending the arrival of the matched germline sample.

8 Sample Pathway

The flow chart below 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).



*It may be appropriate to take consent at the same time as germline samples so that record of discussion forms and germline samples can be sent to the GLH DNA extraction lab at the same time

9 Tumour First /Germline Later WGS Submissions

From May 2024, adjustments to the sample pathways and bioinformatic pipelines within GEL allow the submission of tumour only WGS. This allows the tumour sample to be sent for WGS without an accompanying germline.

These results will be processed using a pooled germline subtraction akin to the tumour in normal contamination (TINC) pathway, and will therefore have a larger number of variants for assessment and will contain limited information with respect to the germline.

The germline sample can be submitted at a later date if a sample becomes available: Once a germline sample has been successfully sequenced, the prior tumour-only sequencing will be subtracted against the patient's specific germline resulting in a more comprehensive Whole Genome Analysis which (assuming no tumour in normal contamination of the germline sample) includes all somatic domains as well as information on the relevant germline panels.

Test Directory codes will continue to be used for the standard submission of tumour and germline samples, but additional codes will be available for each clinical indication for both 'tumour first' and 'germline only'. These codes are detailed in the appendix.

10 Roles and responsibilities

It is the responsibility of the patient's managing clinicians and SIHMDS to ensure that whenever possible, appropriate 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 samples to the central GLH WGS DNA extraction laboratory.

It is the responsibility of the GLH senior management team and GLH HaemOnc 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.

11 References

1. Alaggio R, Amador C, Anagnostopoulos I, *et al.* The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia.* 2022 36(7):1720-1748. doi: 10.1038/s41375-022-01620-2
2. Khoury, J.D., Solary, E., Abla, O. *et al.* The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* **36**, 1703–1719 (2022). https://doi.org/10.1038/s41375-022-01613-

12 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
Proven or Suspected Haematological Tumours Exhausted all Standard of Care Testing and Treatment	Adults & Paediatrics	Malignant cell percentage

Table outlining the current NGTD codes for concurrent submission of the tumour and germline samples, as well as the codes aligned to the tumour first / germline later WGS service.

Clinical Indication	Current WGS Test code	Tumour First Code	Germline Later Code
Acute Myeloid Leukaemia	M80.1	M80.57	M80.58
Myelodysplasia	M82.6	M82.23	M82.24
Aplastic Anaemia	M83.4	M83.5	M83.6
Chronic Myeloid Leukaemia	M84.11	M84.24	M84.25
Myeloproliferative Neoplasm	M85.13	M85.37	M85.38
Systemic Mastocytosis	M86.3	M86.4	M86.5
Juvenile Myelomonocytic Leukaemia	M88.2	M88.11	M88.12
Acute Leukaemia Other	M89.1	M89.108	M89.109
Blastic Plasmacytoid Dendritic Cell Neoplasm	M90.1	M90.6	M90.7
Acute Lymphoblastic Leukaemia	M91.1	M91.82	M91.83
Lymphoma	M93.3	M93.4	M93.5
B Cell Non-Hodgkin Lymphoma	M95.7	M95.10	M95.11
Burkitt Lymphoma	M96.7	M96.9	M96.10
Burkitt Like Lymphoma with 11q Abnormalities	M97.2	M97.4	M97.5
Large B Cell Like Lymphoma with IRF4 Rearrangement	M98.2	M98.4	M98.5
High Grade Lymphoma	M99.8	M99.10	M99.11
Primary Mediastinal B Cell Lymphoma	M100.4	M100.7	M100.8
ALK Positive Large B Cell Lymphoma	M101.4	M101.6	M101.7
Malt-Lymphoma	M107.7	M107.9	M107.10
Paediatric Type Follicular Lymphoma	M110.2	M110.3	M110.4
T Cell Non-Hodgkin Lymphoma	M111.5	M111.7	M111.8
ALK Negative Anaplastic Large Cell Lymphoma (Including Primary Cutaneous Subtypes)	M112.5	M112.7	M112.8
ALK Positive Anaplastic Large Cell Lymphoma	M182.3	M182.5	M182.6
NK Cell/Gamma-Delta T Cell Lymphoma	M115.2	M115.3	M115.4
Hepatosplenic T Cell Lymphoma	M116.3	M116.5	M116.6
Histiocytosis	M117.16	M117.17	M117.18
MDS/MPN	M224.4	M224.41	M224.42
Proven or Suspected Haematological Tumours Exhausted all Standard of Care Testing and Treatment	M235.1	M235.2	M235.3