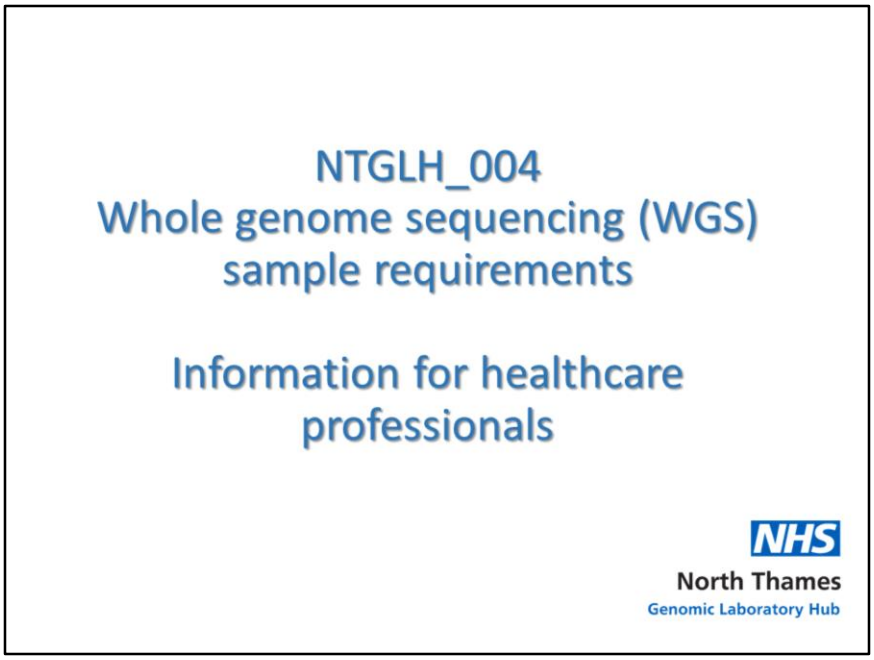
The genomics facilitator's toolkit

NTGLH Module ID	Module Title	Module Format
NTGHL_001	NHS Genomic Medicine Service	Handbook
NTGHL_002	Ordering from the National Test Directory	Handbook
NTGHL_003	Whole Genome Sequencing consent	Handbook
NTGHL_004	Whole Genome Sequencing sample requirements	Handbook
NTGHL_005	Clinical genetic testing methods	Powerpoint with narration
NTGHL_006	Clinical testing DNA sequence variant interpretation	Powerpoint with narration
NTGHL_007	Whole Genome Sequencing results	Powerpoint with narration
NTGHL_008	Introduction to genomics	Powerpoint with narration
NTGHL_009	Test cases in cancer	Handbook

NHS
North Thames
Genomic Laboratory Hub



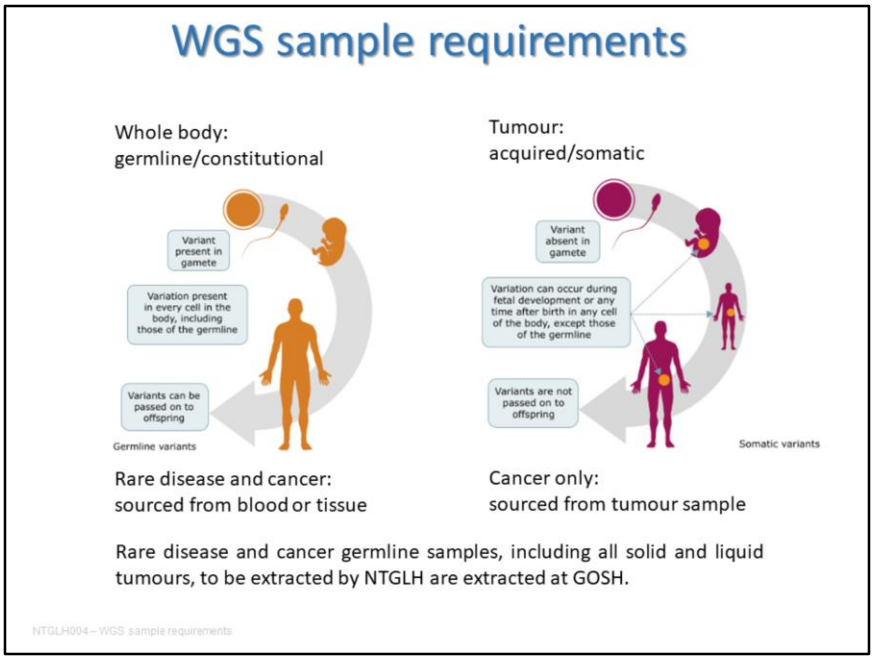
Published 14/10/2020. Content is current at time of publication. Contact your GLH test provider for current GMS test forms.

Contents

Guidance documents

Four whole genome sequencing sample guidance documents have been published by NHS England for the Genomic Medicine Service, please refer to and/or ask your local GLH test provider for:

1. Sample Handling Guidance for Whole Genome Sequencing for Germline Samples
2. Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples
3. Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies for Adults, Children and Young People
4. DNA Extraction and Quality Control Guidance for Whole Genome Sequencing.



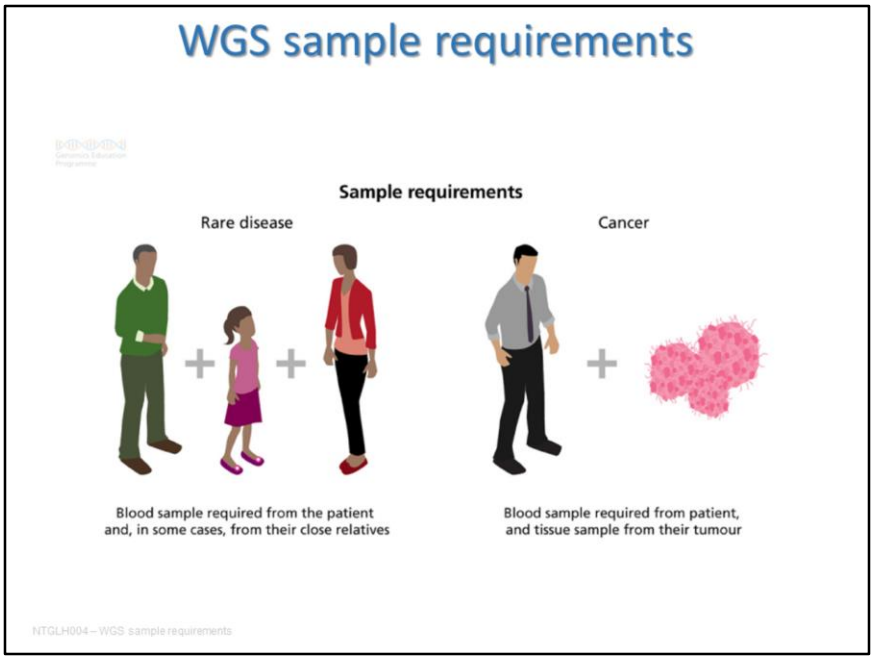
Genomics (and genetics) in rare disease or cancer affected patients can characterise DNA present in all cells of the body. Changes in the DNA here are termed as germline or constitutional variation. In cancer, DNA present in the cells of the tumour can also be characterised. Changes in the DNA here are termed as acquired or somatic variation.

Examples of source DNA:
1. Germline: Blood, saliva meeting certain criteria, fibroblast-derived DNA, uncultured skin biopsy or bone marrow aspirate meeting certain criteria
2. Somatic: tumour tissue sample including, fresh frozen tissue (not FFPE), bone marrow aspirate or peripheral blood meeting certain criteria.

Notes:
- Saliva DNA can yield low quality DNA and testing has a higher sample failure rate. Submit in exceptional circumstances.
- Participants who have had an allogenic bone marrow transplant (or in other atypical circumstances) should not have peripheral blood taken for DNA extraction. Instead use pre-bone marrow transplant stored DNA extracted from blood or DNA extracted from cultured fibroblasts.

- Stored samples can be used but must meet criteria set out by Genomics England [see guidance documents listed above]

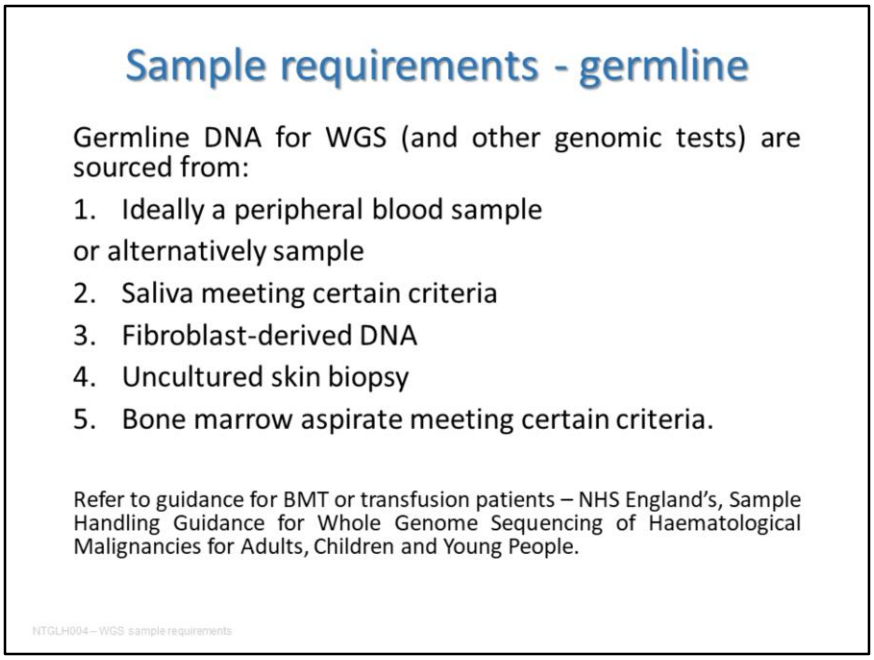
All North Thames GLH WGS test samples are to be sent for extraction to:
NORTH THAMES GENOMIC LABORATORY HUB
Great Ormond Street Hospital for Children NHS Foundation Trust
Specimen Reception, Level 5, Barclay House
37 Queen Square
London WC1N 3BH



Rare disease: Depending on the possible inheritance pattern and clinical indication, it is also important to include samples from other family members where possible. Please use the Test Selection Tool to determine the family structure that should be tested for each clinical indication - test-selection-private.beta.genomics.nhs.uk/test-selection/clinical-tests. Each family member submitting a sample for WGS will require consent see module, NTGLH003_Whole Genome Sequencing consent.

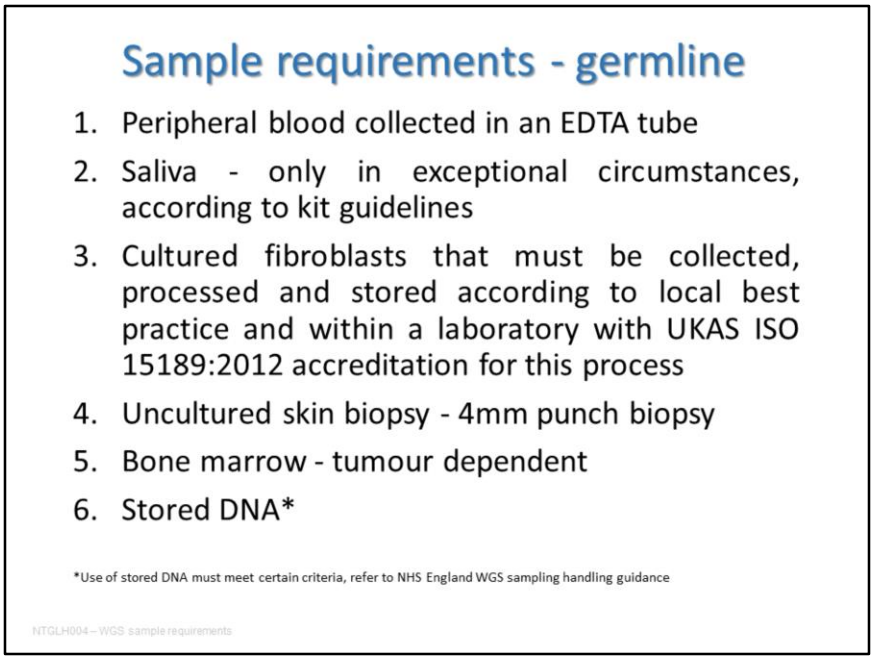
Cancer: In tumour sequencing, the sequence of the germline sample is subtracted from the sequence of the tumour sample. Dual sequencing allows clear differentiation between germline and somatic variants, aiding variant interpretation. Therefore, a tumour/normal matched sample pair is required for WGS to go ahead.

- Where a contemporaneous germline and tumour sample are available, please send them to the GLH WGS test provider paired with the required paperwork. Pack these samples according to standard guidelines and send them via recorded/tracked delivery.
- Where contemporaneous samples are not available then please send the tumour sample (and test order form). The tumour sample will be extracted and DNA will be stored for up to 40 days until the matched germline sample is available.

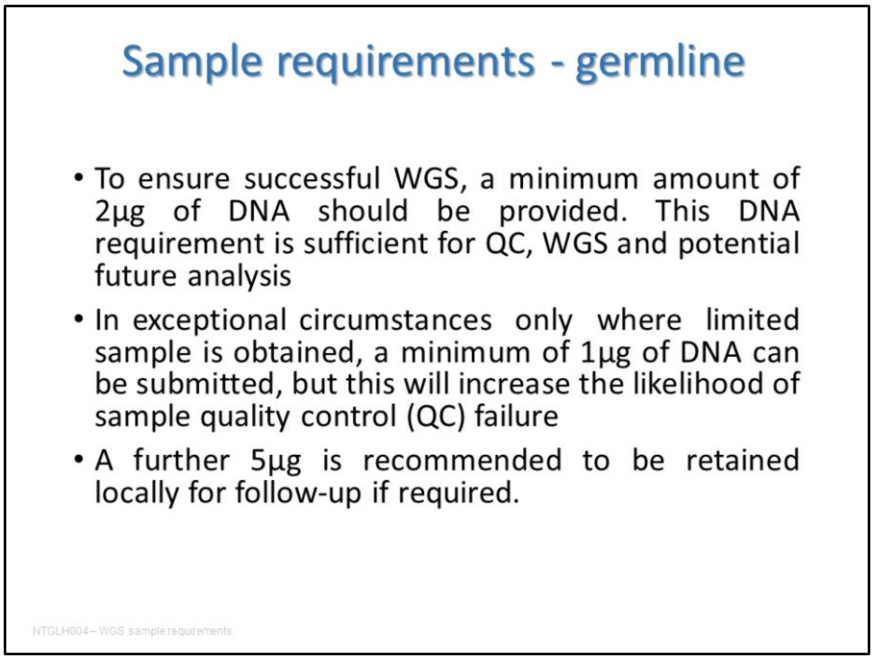


Foetal tissue will not be accepted for WGS testing in rare disease.

For haematological disease blood and saliva may be contaminated with tumour cells, so cannot be used for germline characterisation in certain circumstances. The most appropriate alternative germline source will vary depending on the haematological tumour type and the clinical circumstance. Guidance on selection of suitable germline material for haematological cancers is provided in NHS England document - Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies for Adults, Children and Young People. Contact your local GLH test provider for a copy.



See later slides for further specific WGS sample concentration input requirements.



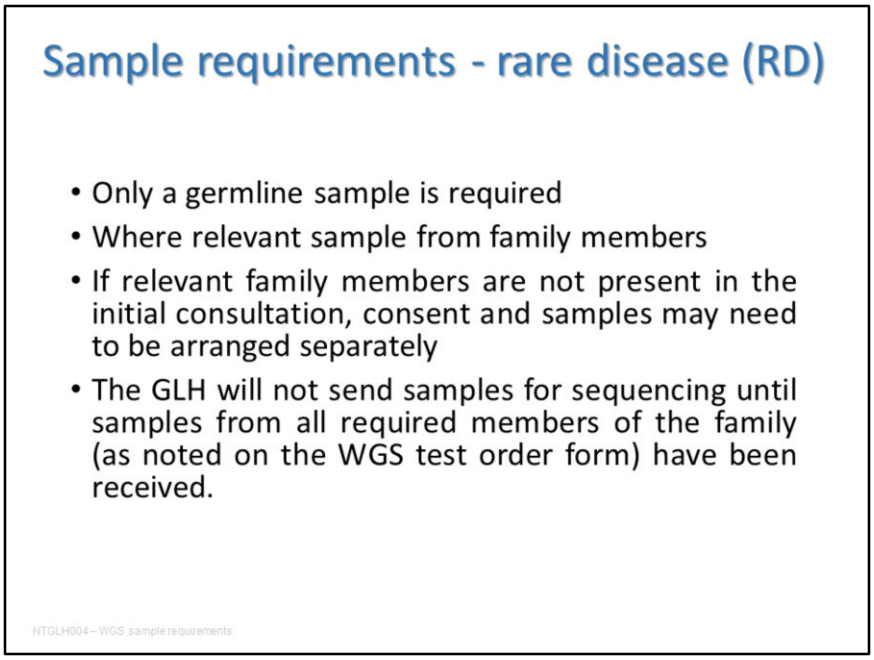
Sourced from NHS England, DNA Extraction and Quality Control Guidance for Whole Genome Sequencing.

Further information: Two tubes, each filled with 3-5ml of blood, should be sufficient to meet the above quantities in the majority of patients. This can be modified based on local laboratory evidence. Age appropriate quantity discretion can apply. See NHS England, Sample Handling Guidance for Whole Genome Sequencing for Germline Samples. Contact your local GLH test provider for a copy.

Minimum blood volume requirements for germline WGS

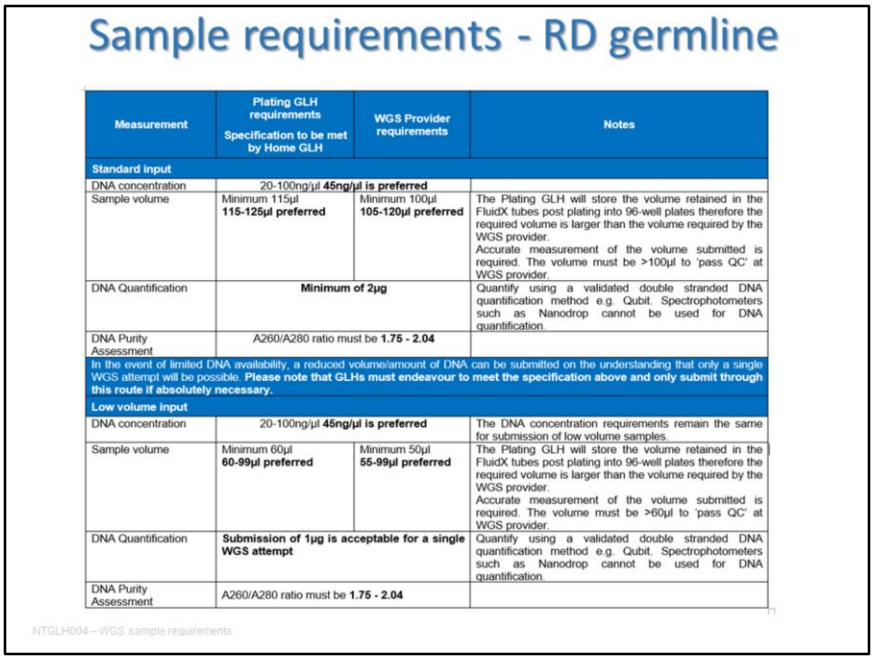
In neonates, acutely ill children and other patients where venepuncture is challenging, clinical discretion should be applied to the volume of blood drawn.

Age	EDTA DNA
14 years+	3-5 ml x2
3-14 years	> 3ml x 2
0-3 years	1-3 ml



In rare disease patients the sampling of family members may help with variant filtering and interpretation; a genome can contain up to five million variants when compared to a reference sequence. None, one or few variants identified by WGS may be disease causing in the context of the clinical indication tested for. For example, WGS may be requested to find the underlying cause of a condition in a child where neither parent has any of the same features. This means that variants that have arisen for the first time in the child (known as *de novo* variants) may be the cause of the condition. The genome of the average individual can contain up to 100 *de novo* single-nucleotide mutations. For more on variant interpretation see module, NTGLH006_Clinical testing DNA sequence variant interpretation. For more on inheritance models see module, NTGLH008_Introduction to genomics.

Therefore, WGS of a parent-proband trio maybe essential for certain gene-disease phenotypes. Please use the Test Selection Tool to determine the family structure that should be tested for each clinical indication - test-selection-private.beta.genomics.nhs.uk/test-selection/clinical-tests. To note, consenting family members may be carried out in a separate consultation, face-to-face or by telephone, depending on the clinical context.



Sample requirements - RD germline

Measurement	Plating GLH requirements Specification to be met by Home GLH	WGS Provider requirements	Notes
Standard input			
DNA concentration	20-100ng/µl **45ng/µl is preferred**		
Sample volume	Minimum 115µl **115-125µl preferred**	Minimum 100µl **105-120µl preferred**	The Plating GLH will store the volume retained in the FluidX tubes post plating into 96-well plates therefore the required volume is larger than the volume required by the WGS provider. Accurate measurement of the volume submitted is required. The volume must be >100µl to 'pass QC' at WGS provider.
DNA Quantification	**Minimum of 2µg**		Quantify using a validated double stranded DNA quantification method e.g. Qubit. Spectrophotometers such as Nanodrop cannot be used for DNA quantification.
DNA Purity Assessment	A260/A280 ratio must be **1.75 - 2.04**		
In the event of limited DNA availability, a reduced volume/amount of DNA can be submitted on the understanding that only a single WGS attempt will be possible. **Please note that GLHs must endeavour to meet the specification above and only submit through this route if absolutely necessary.**			
Low volume input			
DNA concentration	20-100ng/µl **45ng/µl is preferred**		The DNA concentration requirements remain the same for submission of low volume samples.
Sample volume	Minimum 60µl **60-99µl preferred**	Minimum 50µl **55-99µl preferred**	The Plating GLH will store the volume retained in the FluidX tubes post plating into 96-well plates therefore the required volume is larger than the volume required by the WGS provider. Accurate measurement of the volume submitted is required. The volume must be >60µl to 'pass QC' at WGS provider.
DNA Quantification	**Submission of 1µg is acceptable for a single WGS attempt**		Quantify using a validated double stranded DNA quantification method e.g. Qubit. Spectrophotometers such as Nanodrop cannot be used for DNA quantification.
DNA Purity Assessment	A260/A280 ratio must be **1.75 - 2.04**		

NTGLH004 – WGS sample requirements

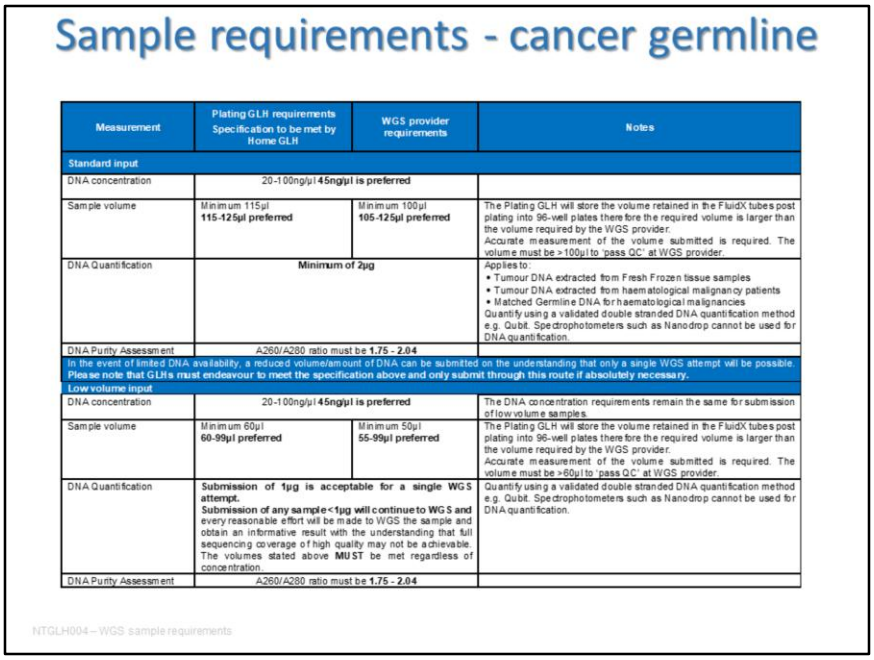
Slide sourced from, NHS England DNA Extraction and Quality Control Guidance for Whole Genome Sequencing v3.0

Germline DNA samples extracted for rare and inherited disease patients, and their family members, must meet the sample specification outlined above.

If the acquirement of sufficient DNA is not possible and a reduced amount of DNA in a reduced volume (see above) are submitted, this is on the understanding that there is an increased likelihood of no results being returned or partial WGS results are available due to the inability to repeat or rework the library preparation and/or WGS. Specific approval is not required for the submission of reduced DNA requirement samples, but this will be audited by NHS England.

Sample requirements - cancer

- A tumour/normal matched pair is required for WGS to go ahead
- For patients who have had a bone marrow transplant, a germline DNA sample would need to be acquired from fibroblasts, other unaffected tissue, or from a germline sample stored before the patient's transplant
- It can be more difficult to extract, sequence and obtain a high-quality result from DNA extracted from cancer cells of a solid tumour. Patients should be aware of the potential risk of sample failure and no results being obtained to provide information about their cancer
- Conversely, for haematological malignancies (such as leukaemias), it can be more challenging to obtain a high-quality and uncontaminated germline sample



Sample requirements - cancer germline

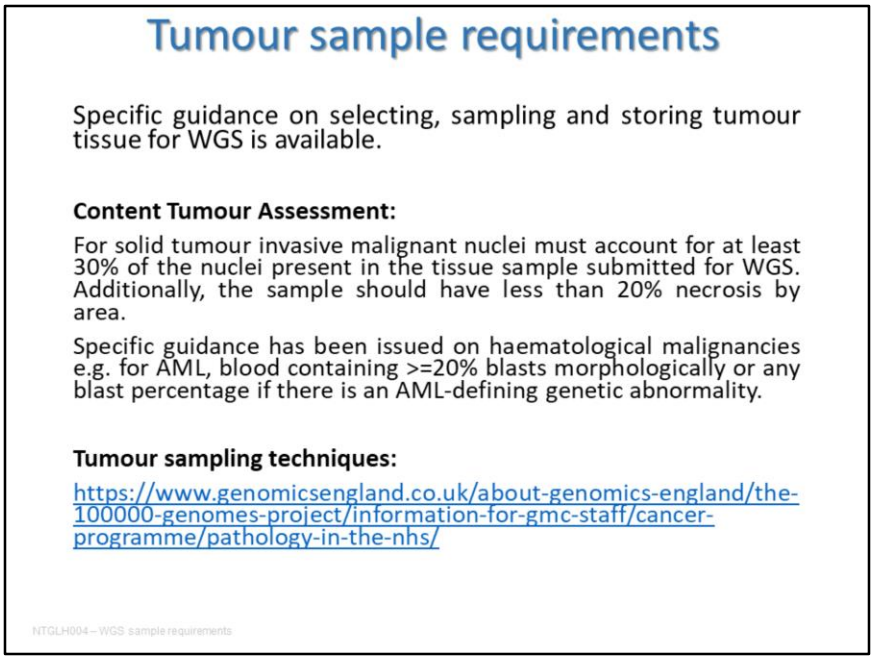
Measurement	Plating GLH requirements Specification to be met by Home GLH	WGS provider requirements	Notes
Standard input			
DNA concentration	20-100ng/µl **45ng/µl is preferred**		
Sample volume	Minimum 115µl **115-125µl preferred**	Minimum 100µl **105-125µl preferred**	The Plating GLH will store the volume retained in the FluidX tubes post plating into 96-well plates therefore the required volume is larger than the volume required by the WGS provider. Accurate measurement of the volume submitted is required. The volume must be >100µl to 'pass QC' at WGS provider.
DNA Quantification	**Minimum of 2µg**		Applies to: • Tumour DNA extracted from Fresh Frozen tissue samples • Tumour DNA extracted from haematological malignancy patients • Matched Germline DNA for haematological malignancies Quantify using a validated double stranded DNA quantification method e.g. Qubit. Spectrophotometers such as Nanodrop cannot be used for DNA quantification.
DNA Purity Assessment	A260/A280 ratio must be **1.75 - 2.04**		
In the event of limited DNA availability, a reduced volume/amount of DNA can be submitted on the understanding that only a single WGS attempt will be possible. Please note that GLHs must endeavour to meet the specification above and only submit through this route if absolutely necessary.			
Low volume input			
DNA concentration	20-100ng/µl **45ng/µl is preferred**		The DNA concentration requirements remain the same for submission of low volume samples.
Sample volume	Minimum 60µl **60-99µl preferred**	Minimum 50µl **55-99µl preferred**	The Plating GLH will store the volume retained in the FluidX tubes post plating into 96-well plates therefore the required volume is larger than the volume required by the WGS provider. Accurate measurement of the volume submitted is required. The volume must be >60µl to 'pass QC' at WGS provider.
DNA Quantification	**Submission of 1µg is acceptable for a single WGS attempt.** **Submission of any sample<1µg will continue to WGS and** every reasonable effort will be made to WGS the sample and obtain an informative result with the understanding that full sequencing coverage of high quality may not be achievable. The volumes stated above **MUST** be met regardless of concentration.		Quantify using a validated double stranded DNA quantification method e.g. Qubit. Spectrophotometers such as Nanodrop cannot be used for DNA quantification.
DNA Purity Assessment	A260/A280 ratio must be **1.75 - 2.04**		

NTGLH004 – WGS sample requirements

Slide sourced from, NHS England DNA Extraction and Quality Control Guidance for Whole Genome Sequencing v3.0. Germline DNA samples extracted for cancer patients must meet the sample specification outlined above.

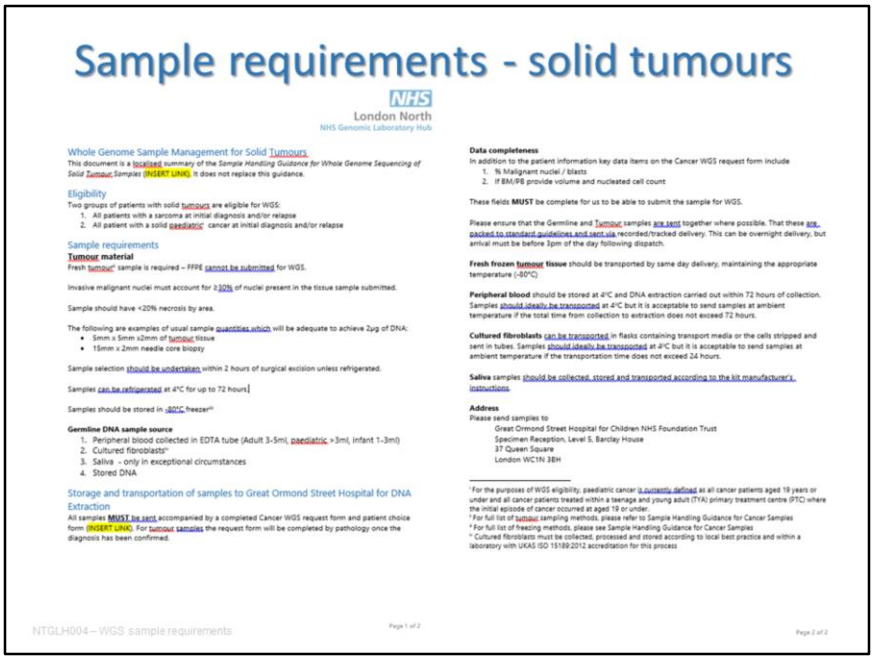
If the acquirement of sufficient DNA is not possible and a reduced amount of DNA in a reduced volume (see above) are submitted, this is on the understanding that there is an increased likelihood of no results being returned or partial WGS results are available due to the inability to repeat or rework the library preparation and/or WGS. Specific approval is not required for the submission of reduced DNA requirement samples, but this will be audited by NHS England.

For some patients the tumour samples available are small or for haematological patients, the germline sample is limited, and may not yield a sufficient amount of DNA to meet even the reduced sample specification outlined in the above table. In such cases then any amount of DNA can be submitted for WGS but the minimum volume of 50µl must be submitted to the local WGS test provider to enable processing and sub-optimal WGS data may be returned to the submitting GLH test provider.



For tumour requirements, use the appropriate guidance:

1. Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples
2. Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies for Adults, Children and Young People.



Sample requirements - solid tumours

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Whole Genome Sample Management for Solid Tumours

This document is a localised summary of the *Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples* (INSERT LINK). It does not replace this guidance.

Eligibility

Two groups of patients with solid tumours are eligible for WGS:

1. All patients with a sarcoma at initial diagnosis and/or relapse
2. All patient with a solid paediatric[i] cancer at initial diagnosis and/or relapse

Sample requirements

Tumour material

Fresh tumour[ii] sample is required – FFPE cannot be submitted for WGS.

Invasive malignant nuclei must account for ≥30% of nuclei present in the tissue sample submitted.

Sample should have <20% necrosis by area.

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

- 5mm x 5mm x2mm of tumour tissue
- 15mm x 2mm needle core biopsy

Sample selection should be undertaken within 2 hours of surgical excision unless refrigerated.

Samples can be refrigerated at 4°C for up to 72 hours.

Samples should be stored in ≤80°C freezer[iii]

Germline DNA sample source

1. Peripheral blood collected in EDTA tube (Adult 3-5ml, paediatric >3ml, infant 1-3ml)
2. Cultured fibroblasts[iv]
3. Saliva - only in exceptional circumstances
4. Stored DNA

Storage and transportation of samples to Great Ormond Street Hospital for DNA Extraction

All samples **MUST** be sent accompanied by a completed Cancer WGS request form and patient choice form (INSERT LINK). For tumour samples the request form will be completed by pathology once the diagnosis has been confirmed.

Data completeness

In addition to the patient information key data items on the Cancer WGS request form include

1. % Malignant nuclei / blasts
2. If BM/PB provide volume and nucleated cell count

These fields **MUST** be complete for us to be able to submit the sample for WGS.

Please ensure that the Germline and Tumour samples are sent together where possible. That these are packed to standard guidelines and sent via recorded/tracked delivery. This can be overnight delivery, but arrival must be before 3pm of the day following dispatch.

Fresh frozen tumour tissue should be transported by same day delivery, maintaining the appropriate temperature (-80°C).

Peripheral blood 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.

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.

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

Address

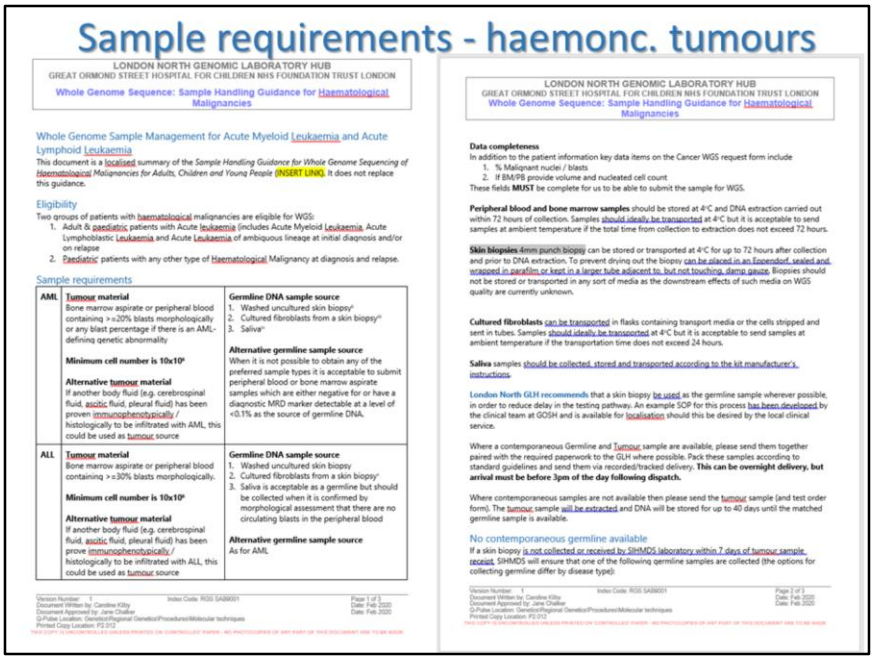
Please send samples to

Great Ormond Street Hospital for Children NHS Foundation Trust
Specimen Reception, Level 5, Barclay House
37 Queen Square
London WC1N 3BH

[i] 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.
[ii] For full list of tumour sampling methods, please refer to Sample Handling Guidance for Cancer Samples
[iii] For full list of freezing methods, please see Sample Handling Guidance for Cancer Samples
[iv] 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

Using knowledge at the time of publication of this handbook, The North Thames GLH has produced sample handling summary documents. Please contact the North Thames GLH for a copy.

Sample requirements - haemonc. tumours



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GREAT ORMOND STREET HOSPITAL FOR CHILDREN NHS FOUNDATION TRUST LONDON

Whole Genome Sequence: Sample Handling Guidance for Haematological Malignancies

Whole Genome Sample Management for Acute Myeloid Leukaemia and Acute Lymphoid Leukaemia

This document is a localised summary of the *Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies for Adults, Children and Young People* (INSERT LINK). It does not replace this guidance.

Eligibility

Two groups of patients with haematological malignancies are eligible for WGS:

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

Sample requirements

AML	**Tumour material** Bone marrow aspirate or peripheral blood containing >=20% blasts morphologically or any blast percentage if there is an AML-defining genetic abnormality **Minimum cell number is $10x10^6$** **Alternative tumour material** If another body fluid (e.g. cerebrospinal fluid, ascitic fluid, pleural fluid) has been proven immunophenotypically / histologically to be infiltrated with AML, this could be used as tumour source	**Germline DNA sample source** 1. Washed uncultured skin biopsy[c] 2. Cultured fibroblasts from a skin biopsy[d] 3. Saliva[e] **Alternative germline sample source** When it is not possible to obtain any of the preferred 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 detectable at a level of <0.1% as the source of germline DNA.
ALL	**Tumour material** Bone marrow aspirate or peripheral blood containing >=30% blasts morphologically. **Minimum cell number is $10x10^6$** **Alternative tumour material** If another body fluid (e.g. cerebrospinal fluid, ascitic fluid, pleural fluid) has been prove immunophenotypically / histologically to be infiltrated with ALL, this could be used as tumour source	**Germline DNA sample source** 1. Washed uncultured skin biopsy 2. Cultured fibroblasts from a skin biopsy[c] 3. Saliva is acceptable as a germline but should be collected when it is confirmed by morphological assessment that there are no circulating blasts in the peripheral blood **Alternative germline sample source** As for AML

Version Number: 1 Index Code: RGS SA86001 Page 1 of 3
Document Written by: Caroline Kilby Date: Feb 2020
Document Approved by: Jane Chalker Date: Feb 2020
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Whole Genome Sequence: Sample Handling Guidance for Haematological Malignancies

Data completeness
In addition to the patient information key data items on the Cancer WGS request form include

1. % Malignant nuclei / blasts
2. If BM/PB provide volume and nucleated cell count

These fields **MUST** be complete for us to be able to submit the sample for WGS.

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.

Skin biopsies 4mm punch biopsy 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 parafilm 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.

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.

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

London North GLH recommends that a skin biopsy be used as the germline sample wherever possible, in order to reduce delay in the testing pathway. An example SOP for this process has been developed by the clinical team at GOSH and is available for localisation should this be desired by the local clinical service.

Where a contemporaneous Germline and Tumour sample are available, please send them together paired with the required paperwork to the GLH where possible. Pack these samples according to standard guidelines and send them via recorded/tracked delivery. **This can be overnight delivery, but arrival must be before 3pm of the day following dispatch.**

Where contemporaneous samples are not available then please send the tumour sample (and test order form). The tumour sample will be extracted and DNA will be stored for up to 40 days until the matched germline sample is available.

No contemporaneous germline available

If a skin biopsy is not collected or received by SIHMDS laboratory within 7 days of tumour sample receipt, SIHMDS will ensure that one of the following germline samples are collected (the options for collecting germline differ by disease type):

Version Number: 1 Index Code: RGS SA86001 Page 2 of 3
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Document Approved by: Jane Chalker Date: Feb 2020
Q-Pulse Location: Genetics\Regional Genetics\Procedures\Molecular techniques
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Using knowledge at the time of publication of this handbook, The North Thames GLH has produced sample handling summary documents. Please contact the North Thames GLH for a copy.

Sample prioritisation

Until WGS bioinformatics pipelines are fully validated and results can be returned in an appropriate time frame, it will be necessary to run the assay in parallel with current standard of care testing (SOC).

Consequently, there will be occasions when 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.

Sample uses

Testing:

- The GLH WGS DNA extraction laboratory will carry out checks to ensure quality of the samples. The laboratory for the WGS test request that has been raised, will await samples from all family members, tumour/normal matched pair, prior to sending them for sequencing.
- A GLH WGS test provider will make contact if there are any issues with samples when they arrive at the laboratory, or if potential errors are identified at the time of sequencing or analysis.

For more information on sample uses see module, NTGLH007_Whole Genome Sequencing results.

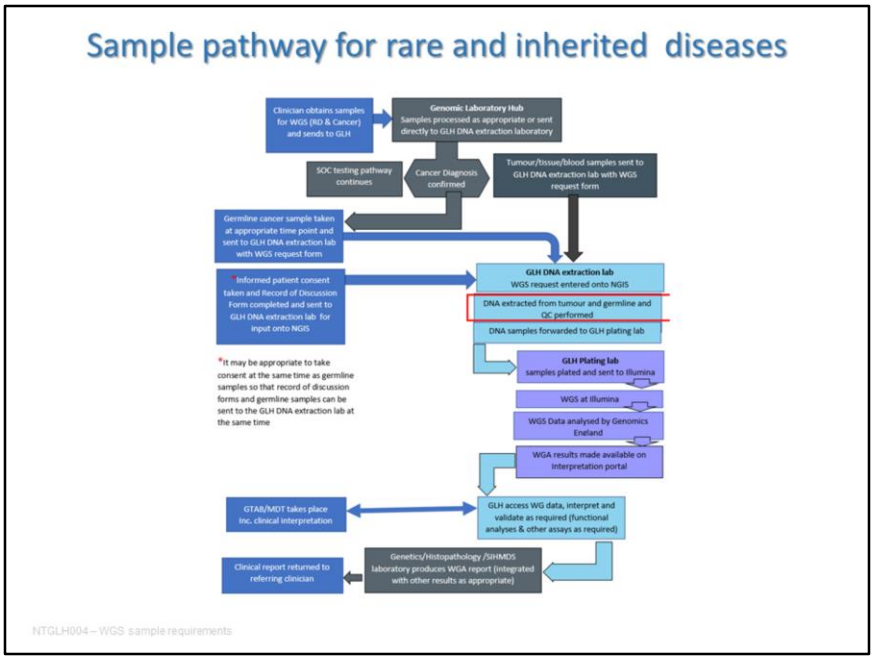
Sample uses

Storage:

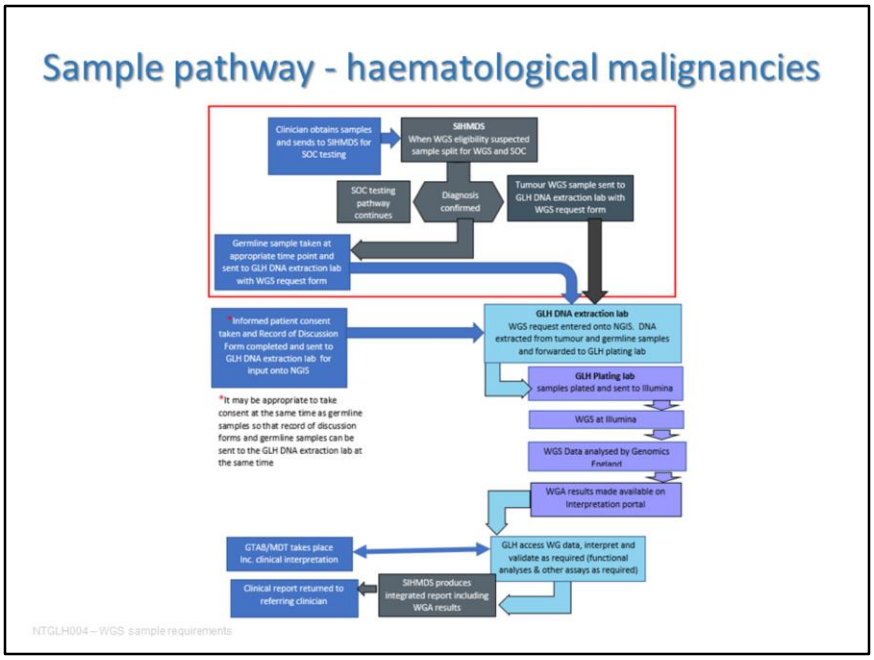
- DNA samples are stored in the local GLH and can be accessed by other laboratories within the GMS. Tumour samples may also be stored in the local hospital histopathology unit.
- Stored samples can be used:

1. In the future for further genomic tests provided appropriate consent has been obtained
2. As a control sample when testing family members of a proband
3. To help with laboratory test development and quality control procedures, although they are de-identified for this use.

- The laboratory will notify the clinician if there is a limited remaining amount of sample (for instance, if an individual is deceased) so a decision can be made on how it can be used.



Red box refers to steps considered in the NHS England DNA Extraction and Quality Control Guidance for Whole Genome Sequencing v3.0.



Red box refers to steps considered in the NHS England Sample Handling Guidance for Whole Genome Sequencing of Haematological Malignancies for Adults, Children and Young People v3.0.

Advice and educational resources

North Thames Genomic Laboratory Hub

- Follow us: @NorthThamesGLH
- Contact us at: gos-tr.norththamesglh@nhs.net

Further education

- https://www.genomicseducation.hee.nhs.uk/
  Free online course -5 weeks, 2 hours per week
- https://www.genome.gov/about-genomics/fact-sheets
- https://www.futurelearn.com/courses/the-genomics-era
- https://geneticsunzipped.com/blog/2019/3/4/008-getting-ready-for-genomic-medicine

Contacts and information

For any queries please contact:

North Thames GLH Education & Training Lead:
Corinne Trim
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Content of slides have been adapted from NHS England WGS sample handing guidance, see document list on slide 4.

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Published 14/10/2020. Content is current at time of publication. Contact your GLH test provider for current GMS test forms.