Wessex Genomics Laboratory Service (WGLS)
Oncology - Sample Requirements

Sample Types

- **Bone Marrow (BM)** is the tissue of choice to investigate patients suspected of having leukaemia or related haematological neoplasms. BM aspirates are routinely received although a BM trephine crush is an option if the marrow is fibrotic or otherwise difficult to aspirate. DNA can be extracted from dried, unstained BM aspirate slides if liquid samples are not available.

    - **Conventional cytogenetics/FISH**: **1-2mls BM** in transport medium or in lithium heparin (please try to avoid excess haemodilution)
        - For paediatric acute leukaemias also add three drops of BM to KCH solution, when available; this will then be fixed at the referring laboratory
        - For **FISH** analysis, we can also accept unstained BM smears or trephine rolls; these may be useful for many months after being made. EDTA is also acceptable if lithium heparin sample is not available. Please note that FISH results on trephine biopsies are difficult to obtain; however, we will attempt FISH investigations if required.

    - **Molecular investigations**: **2-3mls BM in EDTA** (**5mls** for AML molecular monitoring).
        - Lithium Heparin is also acceptable if EDTA sample is not available for WGLS Salisbury tests only – requestor must refer to WGLS Oncology Test List

- **Peripheral blood (PB)** is perfectly adequate for FISH studies in lymphoproliferative disorders if there is PB lymphocytosis and for most molecular studies. PB can be used for conventional cytogenetics provided that immature cells capable of division are present (i.e. frequently in acute leukaemias and diagnostic CML, but rarely in MDS or MPD). If the BM is a dry tap or you are doubtful about its quality, consider sending a blood sample as well. DNA can be extracted from dried, unstained PB smears slides if liquid samples are not available.

    - **Conventional cytogenetics/FISH**: at least **5mls** in lithium heparin; if fresh material is not available, FISH can be performed on unfixed, unstained blood smears (at least 4 slides). EDTA is also acceptable if lithium heparin sample is not available

    - **Molecular studies:** Preferably, at least **5mls** in EDTA (**12mls** for *BCR::ABL1*/*ABL1* monitoring, **20mls** for AML molecular monitoring).
        - Lithium Heparin is also acceptable if EDTA sample is not available for WGLS Salisbury tests only – requestor must refer to WGLS Oncology Test List

*For BM/PB samples intended for **RNA extraction**, please note the transport and mailing instructions below.*

- **Unfixed air-dried tissue imprints or 2-4µm thick paraffin embedded tissue sections** (mounted on positively charged slides) are used for interphase FISH analysis for NHL and solid tumours. Both sample preparations are acceptable for rearrangement testing, while

imprints are the only suitable tissue for numerical abnormalities. Samples should only be forwarded to the laboratory once the pathologist has determined the need for a specific genetic result. Imprints should be stored at room temperature prior to sending and they are likely to be successful even when several weeks old. For those samples, where there is limited infiltration of the tissue, the involved areas must be clearly marked on an additional H&E slide. In the context of B cell NHL, fresh biopsy material for cell culture will only be accepted in specific cases after discussion with laboratory personnel. **Please note that slides should have two patients' identifiers, including the patient's name and the histology ID number**.

- **Fresh biopsy samples** for paediatric solid tumours; these should be placed in sterile culture medium and sent to the laboratory as quickly as possible. Where material is extremely limited and a FISH marker could be used, touch preparations of biopsy specimens may be adequate. These should be placed on slides in a suitable way for the FISH to be carried out under 22x22mm coverslips. For tumours with classic chromosomal rearrangements that help in diagnosis or prognosis, we suggest that imprints are always made in the referring laboratory and sent with the fresh tissue.

- **Other fresh tissues** like lymph nodes, spleen, ascitic fluid, CSF, buccal swabs and non-paediatric solid tumours can also be analysed following discussion with the laboratory. Pleural fluid cannot be processed.

- **Formalin Fixed Paraffin Embedded (FFPE)** material can be tested for most DNA applications, however please note that the processing of such tissue can fragment DNA and impair testing. The % neoplastic nuclei must be recorded on the form.

    - **FFPE blocks** can be sent for scroll cutting and DNA extraction. The % neoplastic nuclei must be recorded on the form. FFPE material will be cut in its entirety. If the tumour content is low or localized, please consider instructions below to macrodissect the specimen.

    - **Tissue scrolls** for solid organ testing should be prepared on a clean microtome, ideally using a fresh blade per case, to avoid the risk of cross contamination. Please send 3x20µm scrolls in a clean laboratory container. If the tumour content is low or localized, please consider instructions below to macrodissect the specimen.

    - **Unstained slides for macrodissection** are preferred when the tumour content is low (<30%), or for MSI testing, *MLH1* promoter methylation or *MGMT* promoter methylation. Please send 6x unstained slides (8µm thick) together with a H&E stained section with area identified for macrodissection. The tissue on unstained slides should be orientated as per the reference.

Specimen Containers

For samples which require **cytogenetic analysis**, the laboratory will provide containers to regular referrers for sample collection (transport medium and KCH with full instructions are sent out by the WGLS Salisbury on the FIRST Tuesday of every month). These bottles contain heparinised tissue culture medium with antibiotics, to facilitate the transport of the small amount of BM and avoid desiccation. More bottles can be sent upon request, at any time, by hospital transport or by post. However, please note that i**n emergency, a tube containing lithium heparin (green top) can be used** for both BM and PB.

EDTA samples are preferred for molecular investigations.

Fresh solid tissues should be placed in one of our transport containers or other a sterile liquid such as culture medium, Hank's balanced salt solution or saline. The department is pleased to advise on the use of alternative specimen containers.

Fresh liquids, such as CSF, can be transported in universal containers.

Samples for molecular investigations should be sperate samples, not previously used on a laboratory automated analyser.

Identification of the Tube

Specimen containers must have **at least** three points of legible ID which match the accompanying request form to be accepted by the laboratory. Label the tube with surname and first name, date of birth as a minimum, as well as date of withdrawal. The laboratory reserves the right to refuse to accept and/or process samples with incomplete, illegible or ambiguous patient information.

Completing the Order Form

Please send a COMPLETED referral form with all samples. The reason for referral is essential to determine which culture types need to be set up, which tests to perform, numbers of cells to analyse and sample prioritisation. All relevant clinical and haematological information and likely diagnosis should be included. If the patient is a participant of a research trial, it is important to give details as certain trials can have specific requirements.

For samples from patients who underwent BM transplant, the sex of the donor should be stated.

Please ensure direct contact details for consultant and medic collecting the samples is recorded

Transport and Mailing

Sample and referral card should be sent **together** in a secure leakproof package (hard cardboard box not a padded envelope) according to UN P650 packaging instructions, to arrive as soon as possible after collection. Please note that the Salisbury laboratory no longer provides an emergency weekend rota; however, for **urgent** referrals (?APL, ?Burkitt, paediatric acute leukaemia), samples can still be sent during the weekend. For these cases, arrange special transport and phone the lab on Friday afternoon or the Hospital switch board during the weekend (the hospital switchboard will contact WGLS Salisbury staff). **It is advisable to telephone about any samples that could arrive at the laboratory late in the day or out of hours.**

All samples should be sent directly to the Wessex Genomics Laboratory Services – Southampton or Salisbury by guaranteed next day delivery, courier service, hospital transport, etc.

While samples from the majority of chronic myeloid conditions will survive comfortably for 48 hours, ALL and Burkitt lymphoma samples die very rapidly, so they should reach the laboratory within a few hours of being taken, and myeloma samples cannot be processed effectively if they are more than 24 hours old. It is advisable that all myeloma samples sent on Friday arrive before 2pm to allow time for cell separation; all myeloma samples arriving on Friday after this time will be refrigerated over the weekend and processed on Monday morning, in such cases the success of the investigation is likely to be compromised.

Samples for **RNA** based analysis should ideally reach the laboratory within 24 hours and must reach the laboratory within 48 hours of collection as degradation of RNA may compromise the

assay. Please avoid colleting samples on a Friday where avoidable (for example, routine BCR::ABL1 RT-qPCR monitoring).

Please protect PB/BM/DNA samples from extremes of temperatures and consider refrigeration (2-8ºC) when transportation/delivery is delayed. DNA samples can be frozen.

Testing for disease pathways can be performed at both the WGLS Southampton and WGLS Salisbury sites (please refer to the HaemOnc Test Request form and the Solid Tumour test request form for specific information). When concurrent testing is known to be required, independent samples can be sent to both sites upfront. Alternatively, samples (or extracted nucleic acids from samples) may be transported between sites as required.

Laboratory testing is coordinated within the Genomic Laboratory Hubs (GLH) with some services being provided within the Central and South (C&S) GLH. On occasion, testing is sent external to the C&S GLH.

Further advice

Please do not hesitate to contact the laboratories for any advice regarding sample types.

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