Measurable Residual Disease for Lymphoid Neoplasms by Molecular Methods (Not Accredited)

TRIAL No: 252602 **Participant:** 40823 **ISSUED:** 26/03/2026 **CLOSING:** 08/05/2026

Please find enclosed three vials for MRD analysis of immunoglobulin (IG) gene rearrangements. Participant are expected to use their current in-house next generation sequencing (NGS) based approach for the identification and quantification of clonal IG gene rearrangement and will be expected to report qualitative and quantitative MRD results for each sample, alongside various methodological details.

Sample 017 is a lyophilised cell based sample provided to mimic a 'diagnostic' sample from a patient with a lymphoid neoplasm to facilitate the detection of a dominant clonal sequence(s), which is a suitable trackable marker, to be used as an MRD target in the other samples. Samples 018-019 are lyophilised cell-based samples which should be tested for the clonal IG gene rearrangement target(s) identified in sample 017. Please find further sample details below:

017: Lyophilised cells for IG Clonotype determination (12 x 10^6 cells)
018: Lyophilised cells for IG MRD detection and quantification (12 x 10^6 cells)
019: Lyophilised cells for IG MRD detection and quantification (12 x 10^6 cells)

Please note, sample 017 (ID sample for clonotype determination) and samples 018-019 (MRD samples) are designed to mimic the same patient at diagnosis and at multiple timepoints during treatment. As such please follow best practise to avoid contamination events e.g. the ID and MRD samples should not be on the same plate; however, MRD samples from the same patient can be on the same plate, but they should be at least 2 wells apart from each other.

SAMPLE STABILITY, STORAGE AND PROCESSING

The molecular haemato-oncology programmes provided by UK NEQAS LI are open to any appropriate DNA/RNA based approaches; UK NEQAS LI does not endorse any particular testing methodology.

Lyophilised material (for DNA preparation): Lyophilised (freeze dried) material has the advantage of improved stability. However, nucleic acid extraction from the material provided may benefit from minimal adaptations to your current methodology (see guidance below). However, wherever possible please treat the sample(s) as routine specimens adhering to standard operating procedures and local quality controls. There are no specific environmental conditions that need to be considered.

Always store lyophilised samples at 2-8°C in their stable lyophilised state until you are ready to proceed with nucleic acid extraction. **Do not reconstitute and store.**

The lyophilised material in each vial can differ slightly and the ease/difficulty when pipetting to breakdown the pellet may vary. Check the vials carefully when first reconstituting in order to not miss any remaining cell material. While pipetting, move the tip around over the bottom of the vial to ensure that the entire pellet is being disrupted. Look closely at vials in proper light conditions to check for any remaining cells sticking to the vial wall and bottom. Repeatedly and gently pipette when reconstituting but try not to create bubbles. Make sure the entire wall and bottom surface is being washed. The reconstituted sample(s) must be processed immediately. Lyophilised samples do not require a red cell lysis step. Even if they appear red, any red cells originally present in the specimen will have already lysed during the freeze-drying process.

It is important to always take account of the cell numbers in the sample(s) stated in the table above. Please note the approximate white cell counts are ascertained prior to lyophilisation, some cell loss is acknowledged during the freeze-drying process. If you are applying the sample to a column or cartridge it is important to take account of the maximum capacity of the column/cartridge; please read the manufacturer's information carefully. Reducing the number of cells can be facilitated by diluting the sample (after its initial reconstitution with 1ml DNase free water) using phosphate buffered saline (1x PBS). Centrifuge the determined volume of the cell suspension at 500-600 x g to pellet the leucocytes. Remove the supernatant and proceed with the nucleic acid extraction process as per your laboratory protocol, ensuring that the cell pellet is thoroughly homogenised in your chosen buffer. The DNA extracted should be subjected to local quality control procedures (e.g. spectrophotometry). If the extracted nucleic acid does not meet local quality control procedures a repeat sample should be requested as soon as possible.

DNA preparation, various methods: Subject to extraction technique it may be possible to add the calculated volume of lysis buffer directly to the lyophilised material in the glass vial in which the sample is provided. Alternatively, the lyophilised sample(s) may be reconstituted with 1mL RNase/DNase free water.

The sample now forms a suspension of leucocytes and can be processed from an appropriate point in your chosen extraction protocol. Proceed with the rest of your protocol. **Please do contact us (see contact details section) if you require any additional support optimising your standard nucleic acid extraction protocol for lyophilised material.**

Materials used in the production of samples for UK NEQAS LI EQA programmes are obtained from a variety of sources. In all cases these materials (patient samples, cell lines, blood products etc) are provided under the conditions that they be used only for the educational purpose of EQA. **Participants must only use the samples provided for the purpose intended**. UK NEQAS LI, Sheffield Teaching Hospitals NHS Foundation Trust and any of its employees will not be responsible for any misuse of samples issued in this programme.

COSHH (Control of Substances Hazardous to Health):
The cell line preparations utilised by this trial are human derived and judged as having a minimal likelihood that pathogens are present. They have been virologically tested at authentication and found negative for Hepatitis B (HBV), Hepatitis C (HCV), Human Immunodeficiency Virus (HIV), Human T cell Leukaemia virus I/II, Squirrel Monkey Retrovirus (SMRV) and Murine Leukaemia Virus (MLV). Samples may contain antibiotics (penicillin and streptomycin) and an antimycotic (amphotericin B).

The buffy coat units utilised by this study are human derived and judged as having a minimal likelihood that pathogens are present. They have been virologically tested and found negative for Hepatitis B, Hepatitis C, Hepatitis E, HIV-1, HIV-2, HTLV1 and Syphilis. No material is knowingly used that is positive for pathogens. However, it should be handled in accordance with local laboratory Health & Safety practices.

Packaging: UK NEQAS LI sample(s) are sent by first class post or courier accordingly. Packaging is guided by Package Instruction P650.

Disposal/Spillage: The sample(s) cannot be assumed to be free from infectious agents therefore the material should be assessed as potentially infectious (refer to COSHH). If found to be damaged the packaging and sample(s) should be disposed of in accordance with local Health & Safety and waste management practices. It is advised that any spillage of reconstituted material should be dealt with in line with the local protocol for small volume blood spills. If no specific protocol is available, UK NEQAS LI suggests liberally covering the area with a suitable disinfectant (allowing sufficient contact time for effective action), absorbing the treated spillage with a paper towel before rinsing the area with water and drying thoroughly. See the section below for guidance on requesting a repeat sample.

REPEAT SAMPLES

Requests for repeat samples should be made by email (repeatsamples@ukneqasli.co.uk). Should this not be possible please telephone our Administration team on the number provided below. Please make a repeat sample request as soon as possible. **If following repeat sample(s) processing, results obtained still do not pass local internal QC please contact UK NEQAS LI.**

RESULTS SUBMISSION

The data entry webpage for this trial can be accessed online at the UK NEQAS LI website via the Participant Hub (www.ukneqasli.co.uk). Participants are required to log into this area of the website using their Lab number (also known as PRN, participant reference number), Identity and Password.

Please only submit results applicable to the scope of this EQA programme.

Please note results for this trial distribution are to be submitted using an externally hosted data entry system (JotForm); the relevant link out to JotForm is provided on the UKNEQAS LI data entry webpage. Alternatively, please go to https://form.jotform.com/UKNEQASLI/MRDLNMM252602

Please only submit results for clonal sequences identified in the index case, DO NOT submit results for any emerging clones that may be present in the samples.

Participants are encouraged to carefully read and follow the instructions provided on the individual results submission pages. Please note, all numerical fields must be completed using only decimal points to separate numbers, and not commas (e.g. enter 6.3 not 6,3). If you experience any problems submitting your trial results please do contact us (see contact details section) for assistance. Failure to return your results will be recorded as a non-return and for an accredited programme impact upon your performance status. If you wish to return more than one set of results please contact UK NEQAS LI. If you have any queries with regards to online data entry, please do not hesitate to contact us. It is the responsibility of participants to ensure that their results have been received by UK NEQAS LI. Further information can be found in the associated trial issue email and on our website (www.ukneqasli.co.uk).

Participants can make changes to existing laboratory contact details, request a password reminder or add a new contact at any time via the Participant Hub. Alternatively please email (admin@ukneqasli.co.uk) or telephone the number provided below for assistance.

REPORT DISTRIBUTION

The trial report for this programme will be available online at the UK NEQAS LI website (www.ukneqasli.co.uk). Participants are required to log into the Participant Hub (using their web user details) to retrieve PDF report(s). Participants will be notified regarding the availability of an issued report by email. To ensure you receive such emails please check the contact details we hold for your laboratory are accurate and current at re-registration. Participants can make changes to existing laboratory contact details, request a password reminder or add a new contact at any time via the Participant Hub. Alternatively please email (admin@ukneqasli.co.uk) or telephone the number provided below for assistance.

CONTACT DETAILS

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Please state PRN (participant reference number) on all correspondence.