Paediatric Acute Leukaemia Translocations

TRIAL No:262702 **Participant:** **ISSUED:** 06/10/2026 **CLOSING:**06/11/2026

Please find enclosed 2 vials containing lyophilised cell line derived material for Paediatric Acute Leukaemia Translocations Identification. Sample ID (approximate total white cell count):

PALT 174 (9 x10^6)
PALT 175 (9 x10^6)

Please assay both samples for **t(12;21)(*TEL*::*AML1* or *ETV6*::*RUNX1*), t(4;11)(*MLL*::*AF4 or KMT2A*::*AFF1*) and t(1;19) (*E2A*::*PBX1* or *TCF3*::*PBX1*).** Please note samples are representative of diagnostic samples and therefore currently not suitable for Minimal Residual Disease (MRD) analysis. This may be subject to change dependent on participant feedback.

Participants are requested to inform UK NEQAS LI of their relevant assay repertoire when joining the programme and to notify us of any subsequent change in scope. Please telephone or email UK NEQAS LI using the contact details overleaf to supply/update this information for our records.

This molecular haemato-oncology programme provided by UK NEQAS LI is open to any appropriate RNA based approaches. UK NEQAS LI does not endorse any particular testing platform/methodology. However, where a particular input material or approach may increase the chances of an erroneous result, we will communicate this in the trial report.

Wherever possible, please ensure that you treat the samples as routine specimens, adhering to standard operating procedures and local quality control (QC). Details of methodologies used should be included in your results submission. Please note that results from all methods are performance monitored collectively, and not by individual methodological approaches, however, results may be considered by method in tables and figures for information only. **Nucleic acid extraction from the External Quality Assessment (EQA) material provided may benefit from minimal adaptations to your current methodology (see guidance below).** There are no specific environmental conditions that need to be considered when testing this EQA material.

SAMPLE STABILITY, STORAGE AND PROCESSING

Whilst the EQA sample(s) provided should ideally be stored at 2-8°C, they are expected to remain stable at typical ambient temperatures for the duration of transit, even for extended transit periods. However, if samples appear to have visibly deteriorated or do not pass local QC, please contact UK NEQAS LI to arrange repeat samples - see below for details.
Lyophilised (freeze dried) material has the advantage of improved stability. However, inherent differences to fresh patient samples means that making slight modifications to your extraction protocol may further improve resultant nucleic acid quality and yield. **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.** General guidance is provided below for the most widely used nucleic acid extraction methodology principles. UK NEQAS LI are in no way endorsing the use of any particular manufacturer's reagent(s)/kit in the advice given.

Phenol-chloroform based techniques (e.g. TRIzol), Silica binding columns (e.g. Qiagen RNeasy) and Magnetic bead-based extraction methods (e.g. Roche MagNA Pure):
Add the calculated volume of phenol reagent or lysis buffer directly to the lyophilised material in the glass vial in which the sample is provided. Initially it may appear difficult to homogenise and may require up to 5 minutes incubation at room temperature with gentle pipetting (there is no requirement for vortexing). 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. Proceed with the rest of your protocol (also see additional advice below). **The reconstituted sample(s) must be processed immediately.**
Additional advice - applicable to many methods:

- 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.
- Prior to nucleic acid extraction the lyophilised sample(s) may alternatively be reconstituted with 1ml RNase/DNase free water. The sample will reconstitute with gentle pipetting (there is no requirement for vortexing). The sample now forms a suspension of leucocytes and can be processed from an appropriate point in your chosen extraction protocol. **The reconstituted sample(s) must be processed immediately.**
- It is important to always take account of the cell numbers in the sample(s) stated above. Please note the approximate white cell counts are ascertained prior to lyophilisation, some cell loss is acknowledged during the freeze-drying process. **You may need a larger volume of reagent(s) or smaller amount of sample than routinely used for patient samples**; cell line samples are often highly expressing and/or polyploid. If you are applying the sample to a column or cartridge it is important 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 RNase/DNase free water) using phosphate buffered saline (1x PBS).
- The RNA extracted should be subjected to local quality control procedures (e.g. spectrophotometry). **It is likely that a high yield of RNA will be achieved. We therefore advise that extracted RNA is quantified in order to optimise the amount inputted into cDNA synthesis reaction.** If the extracted nucleic acid does not meet local quality control procedures a repeat sample should be requested as soon as possible (see the section below for guidance on requesting a repeat sample).

Please do contact us (see contact details section) if you require any additional support optimising your standard nucleic acid extraction protocol for lyophilised material.

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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 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 Epstein-Barr virus (EBV), Hepatitis B (HBV), Hepatitis C (HCV), Human Herpesvirus-8 (HHV-8), Human Immunodeficiency Virus (HIV), Human T-cell Leukaemia virus I/II, Murine Leukaemia Virus (MLV) and Squirrel Monkey retrovirus (SMRV). Samples may contain antibiotics (penicillin and streptomycin) and an antimycotic (amphotericin B). 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.

UK NEQAS LI website hosted results submission pages have been designed to facilitate local independent checking of trial results prior to final submission. Once data has been entered into the relevant fields a participant can chose to click the [Save] button to permit independent checking by a second operative before final submission to UK NEQAS LI. The date and time will appear in the corresponding field to indicate data has been successfully saved. Subsequent editing of the data fields is still possible at this stage. **The [Submit] button must be clicked for data to be locked (preventing further editing) and transferred to UK NEQAS LI.** The date and time will appear in the corresponding field to indicate the trial data has been successfully submitted. Additionally, the date of successful results submission will also appear in the 'completed' column of the relevant programme trial list. Please note, all data **saved** or submitted in the UK NEQAS data entry system will be downloaded and analysed at trial closure.

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. 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.

If you wish to return more than one set of results, please contact UK NEQAS LI. **Failure to return your results will be recorded as a non-return and for an accredited programme impact upon your performance status.** 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).

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

UK NEQAS LI, Pegasus House, 4th Floor, 463A Glossop Road, Sheffield, S10 2QD, UK.
Tel: +44 (0) 114 267 3600, e-mail: admin@ukneqasli.co.uk
Please state PRN (participant reference number) on all correspondence.

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