

Myeloproliferative Neoplasms Diagnostic Testing

TRIAL No: 262702 Participant: ISSUED: 25-Aug-26 CLOSING: 2-Oct-26

Important: 2 different sample types requiring differing testing strategies have been issued in this trial; please read the instructions below carefully.

SAMPLE INFORMATION

Please find enclosed one vial containing lyophilised cell line material for JAK2 p.(Val617Phe) measurable residual disease (MRD) analysis. Sample ID (approximate total white cell count) and relevant clinical details:

- **MPN DT 130** (7×10^6)

Clinical scenario: JAK2 p.(Val617Phe) positive myelofibrosis patient post haematopoietic stem cell transplant: ?MRD status

This sample represents a **follow-up sample**. Participants should **test sample MPN DT 130 for JAK2 p.(Val617Phe) only**. Participants will not be penalised for not testing this sample, or for failing to detect MRD, if we are aware that appropriate testing is not available at your centre.

At results submission, please ensure that all questions relating to MRD testing and limit of detection are answered accurately.

There will be no performance monitoring for MRD samples.

Please find enclosed one vial containing DNA for MPN diagnostic testing analysis. Sample ID (approximate volume and concentration):

- **MPN DT 131** (20µl of genomic DNA @ ~120ng/ µl in 1x TE)

This sample represents a **diagnostic** sample. Participants are requested to **test sample MPN DT 131 for all four core clinically significant MPN variant types** (exon numbering and variant nomenclature based on reference sequences: NM_004972.4(JAK2); NM_004343.4(CALR); NM_005373.3(MPL)):

- JAK2 p.(Val617Phe)
- JAK2 exon 12
- CALR exon 9
- MPL exon 10

Please complete all testing irrespective of test results. Please note that testing of all four markers is not mandatory for participation; if testing for one or more markers is not locally available, this will be accounted for at results submission. **Only JAK2 p.(Val617Phe) results will be subject to performance monitoring**; however please note that UK NEQAS LI is close to achieving full ISO/IEC 17043:2023 accreditation of the MPN Diagnostic Testing programme. Similar qualitative performance monitoring will be applied to the remaining core MPN markers later this year, and optional performance monitoring for JAK2 p.(Val617Phe) quantification in diagnostic samples will also then be available. We will keep participants informed via future trial reports and email notification(s).

This molecular haemato-oncology programme provided by UK NEQAS LI is open to any appropriate DNA 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. For this programme RNA based approaches are not advocated since they add additional unnecessary complexity and quantitative results cannot be directly compared to the wealth of published data from DNA evaluation.

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 (where applicable) 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 during testing of 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.

DNA samples provided in this trial should be subjected to local quality control and clean up procedures. The source sample has been subject to extraction using a magnetic (M-PVA) based method. Samples have been eluted in 1xTE. If the nucleic acid provided 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 store samples at 2-8°C and avoid freeze/thawing. **Spin the tube briefly to ensure content is at the bottom of the tube prior to opening the cap.**

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.**

Reconstitute the lyophilised sample with 1mL RNase/DNase free water or directly using an appropriate volume of initial buffer, as per your extraction protocol. 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.** 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 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). Centrifuge the determined volume of the cell suspension at 500-600xg to pellet the leucocytes. Remove the supernatant and proceed with the nucleic acid extraction process as per laboratory protocol, ensuring that the cell pellet is thoroughly homogenised in your chosen buffer.

The DNA/RNA extracted should be subjected to local quality control procedures (e.g. spectrophotometry, fluorometry, electrophoresis). 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.**

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, nucleic acids 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. The nucleic acid supplied is not known to contain any agents capable of harm; 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

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 choose 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.

This trial encompasses an MRD sample; we recognise that participants may use an alternative method (to their diagnostic approach) for JAK2 p. (Val617Phe) MRD testing. If relevant, details of an additional MRD assay should be provided on an additional submission page, hosted externally by the data entry system JotForm: https://form.jotform.com/UKNEQASLI/262702_MPNDT. Participants are encouraged to carefully read and follow the instructions provided on the individual JotForm submission pages, which can also be accessed from your UK NEQAS LI online data submission page.

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.

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.