UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

# IG/TCR Clonality Status Programme

Distribution - 232401

Participant ID -

Date Issued - 25 April 2023

Closing Date - 26 May 2023

## Trial Comments

This trial was issued to 108 participants, of which one hundred (92.6%) returned results. Of the non returns, three participants informed us of their intention not to return results and two participants submitted a request for an extension to result submission. Ninety-nine laboratories returned results for IG and ninety- eight returned results for TCR. Please note, this report replaces v1.0.0 that showed incorrect running performance data.

## Sample Comments

Two samples were issued for this trial: IG 170 and TCR 171. Sample IG 170 was formulated from buffy coat material and sample TCR 171 was manufactured from a patient sample with a working diagnosis of T-ALL.

## Results and Performance

### Your Results

| IG/TCR Clonality Status | Your Results | Consensus Result |
|---|---|---|
| Sample IG 170 | Polyclonal (Not Clonal) | Polyclonal (Not Clonal) |
| Sample TCR 171 | Clonal | Clonal |

### All Participant Results

| | Clonal | Pseudoclonal | Multiple Reproducible Peaks (n>=3) | Polyclonal (Not Clonal) | No (specific) product | Not evaluble |
|---|---|---|---|---|---|---|
| Sample IG 170 | 7 | 0 | 0 | 91 | 0 | 1 |
| Sample TCR 171 | 97 | 0 | 1 | 0 | 0 | 0 |

### Your Performance

| Performance | Performance Status for this Trial | Performance Status Classification Over 3 Trial Period | |
|---|---|---|---|
| | | Satisfactory | Critical |
| | Satisfactory | 3 | 0 |

N/A = Not Applicable

The loci and reporting nomenclature in this report has been standardised to the Euroclonality/BIOMED 2 guidelines. Langerak, A. W. *et al.* (2012) EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia* 26, 2159-71.

# IG/TCR Clonality Status Programme

## IG Results by Loci

| IG | IGH $V_H$-$J_H$ | IGH $D_H$-$J_H$ | IGK $V_K$-$J_K$ | IGK Kde | IGL |
|---|---|---|---|---|---|
| Your Result | Polyclonal (Not Clonal) | Polyclonal (Not Clonal) | | | |
| Returns | 99 | 36 | 57 | 55 | 15 |
| Clonal | 6 | 0 | 1 | 2 | 0 |
| Irregular Polyclonal (Not Clonal) | 2 | 2 | 1 | 1 | 0 |
| Multiple Reproducible Peaks (n>=3) | 0 | 0 | 0 | 0 | 0 |
| No (Specific) Product | 0 | 0 | 1 | 1 | 0 |
| Not Evaluable | 1 | 0 | 0 | 0 | 0 |
| Polyclonal (Not Clonal) | 90 | 34 | 54 | 52 | 15 |
| Pseudoclonal | 0 | 0 | 0 | 0 | 0 |

## TCR Results by Loci

| TCR | TCRB Vβ-Jβ | TCRB Dβ-Jβ | TCRG Vγ-Jγ | TCRD |
|---|---|---|---|---|
| Your Result | | | Clonal | |
| Returns | 65 | 59 | 96 | 14 |
| Clonal | 53 | 55 | 93 | 13 |
| Irregular Polyclonal (Not Clonal) | 3 | 0 | 0 | 0 |
| Multiple Reproducible Peaks (n>=3) | 0 | 2 | 1 | 1 |
| No (Specific) Product | 1 | 1 | 0 | 0 |
| Not Evaluable | 0 | 0 | 0 | 0 |
| Polyclonal (Not Clonal) | 8 | 2 | 3 | 0 |
| Pseudoclonal | 0 | 0 | 0 | 0 |

# IG/TCR Clonality Status Programme

## Template Type

| | IG Returns | TCR Returns |
|---|---|---|
| DNA | 259 | 234 |

## PCR Type

| | IG Returns | TCR Returns |
|---|---|---|
| Multiplex PCR | 233 | 206 |
| PCR for Next generation Sequencing | 13 | 17 |
| Single PCR | 13 | 11 |
| Nested PCR | 1 | 0 |

## Protocol Type

| | IG Returns | TCR Returns |
|---|---|---|
| Invivoscribe Identiclone (IVD) Kit | 127 | 126 |
| In-House Method (BIOMED Primers) | 94 | 52 |
| Invivoscribe (RUO) Kit | 18 | 26 |
| Invivoscribe LymphoTrack Dx TRB kit | 0 | 15 |
| LymphoTrack TRG Assay | 0 | 10 |
| In-House Method (Not BIOMED Primers) | 3 | 6 |
| Master Diagnostica | 10 | 6 |
| LymphoTrack IGH FR1 FR2 FR3 IGK | 17 | 0 |

## Analysis Type

| | IG Returns | TCR Returns |
|---|---|---|
| Capillary Electrophoresis | 225 | 200 |
| NGS (Other) | 30 | 36 |
| Acrylamide Gel Electrophoresis (PAGE) | 8 | 5 |
| Heteroduplex Analysis | 12 | 5 |
| Microfluidic Electrophoresis | 5 | 4 |
| Agarose Gel Electrophoresis | 2 | 3 |
| Radioactive Labelling | 3 | 3 |

## IG/TCR Clonality Status Programme

### Trial Summary IG 170

- The clinical scenario for sample IG 170 was: *A 45-year-old female, a long term smoker, presented with a history of progressive fatigue and weight loss. Physical examination revealed splenomegaly, with no lymphadenopathy. Serum IgM was increased to 1200mg/dL and there was a lymphocytosis of 15 x 10^9/L. Lymphocytes expressed CD19, CD20, CD22, CD23, CD200 with polytypic light chains.*
- Ninety-one out of 99 participants (91.9%) who returned results for IG 170 reported a Final Molecular Conclusion of 'Polyclonal (Not Clonal)'.
- Seven (7.1%) participants reported a Final Molecular Conclusion of 'Clonal'. Of the participants reporting an out of consensus result, three utilised an in-house (BIOMED) assay with capillary electrophoresis, three reported use of the Invivoscribe Identiclone (IVD) Kit (two with capillary electrophoresis and one with acrylamide gel electrophoresis). A further participant utilised the Master Diagnostica kit with heteroduplex analysis.
- Of the seven participants reporting an out of consensus clonal result, three provided a detailed molecular interpretation of 'Clonality detected (with caution, plus advice for follow-up analysis/new sample).' One participant reported 'clonality detected (minor clonal product)' and one 'no further interpretation possible'. Two participants did not report a detailed molecular interpretation.
- Four participants reported clonality based on the results from the IGH VH-JH locus, two participants reported clonality based on IGH VH-JH and IGK Kde loci and one participant reported clonality in IGK VK-VJ. The breakdown for clonal peak sizes (bp) identified by these participants across these loci are outlined in Table 1, below.

| Participant | IGH VH-JH | | | IGK VK-VJ | IGK Kde | Comments |
|---|---|---|---|---|---|---|
| | FR1 | FR2 | FR3 | | | |
| 1 | - | 243.65 | 103.68 | - | - | |
| 2 | 366 | - | - | - | - | Reported clonal peak as out of range |
| 3 | Clonal at locus | | | - | Clonal | Reported clonality but provided no clonal peak information |
| 4 | - | - | - | 149 | - | |
| 5 | Clonal at locus | | | - | Clonal | Reported clonality but provided no clonal peak information |
| 6 | 212.5 and 212.7 | | | - | - | Did not specify the reaction tube that the clonal products were identified in |
| 7 | - | 274 & 133 | - | - | - | |

- Whilst it's difficult to be definitive in the absence of a review of the relevant Genescan profiles from these participants, the UK NEQAS LI molecular specialist advisory group commented that the most likely cause of a number of the out of consensus results was an overinterpretation of apparent dominant peaks within polyclonal profiles which may have presented with an irregular pattern, given the inconsistency in the size of peaks between participants. To minimise this, participants should always perform clonality testing using duplicates along a known polyclonal sample, with samples analysed within the context of the presenting clinical information. Analysis and reporting of clonality testing should be undertaken using the Euroclonality guidelines[1].

- One participant reported a Final Molecular Conclusion of 'Not evaluable', based on analysis of the IGH VH-JH locus only. This participant utilised the Invivoscribe Identiclone (IVD) Kit with Next Generation Sequencing.

**Trial Summary TCR 171**

- The clinical scenario for sample TCR 171 was: *A 30-year-old male presented with fatigue, lymphadenopathy, with a presenting white cell count of 60 x 10^9/L. The lymphoblasts accounted for 85% of the peripheral blood cells and on immunophenotyping the lymphoblasts were positive for CD2, CD5, CD7, cCD3, TdT, CD4, CD8 and CD10.*
- Ninety-seven out of 98 participants (99.0%) who returned results for sample TCR 171 reported a Final Molecular Conclusion of 'Clonal'.
- One participant reported a Final Molecular Conclusion of 'Multiple Reproducible Peaks (n>=3)' based on analysis of the TCR Gamma locus only. This participant utilised an in-house (BIOMED) assay with capillary electrophoresis.
- As previously reported, Euroclonality guidelines state that testing of TCR Gamma and
- Beta loci should be used in parallel for assessment of T-cell clonality. The TCR Beta multiplex PCR has been shown to be slightly more informative than TCR Gamma amplification but both regions provide complementary information[1].

**References**

1. Langerak, A. W., Groenen, P. J. T. A., Brüggemann, M., Beldjord, K., Bellan, C., Bonello, L., … van Dongen, J. J. M. (2012). EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia*, *26*(10), 2159–2171. https://doi.org/10.1038/leu.2012.246

## IG/TCR Clonality Status Programme
## Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600
e-mail: amanda.newbould@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Mr Liam Whitby (Director) and Mr Stuart Scott (Centre Manager).

4.8.2 c) Person(s) authorizing this report:
Mr Liam Whitby (Director) or Mr Stuart Scott (Centre Manager) of UK NEQAS LI.

4.8.2 d) Pre issue testing of samples for this programme is subcontracted, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were subcontracted. Where subcontracting occurs, it is placed with a competent subcontractor and the EQA provider is responsible for this work.

4.8.2 g) The UK NEQAS LI Confidentiality Policy can be found in the Quality Manual which is available by contacting the UK NEQAS LI office. Participant details, their results and their performance data remain confidential unless revealed to the relevant NQAAP when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict Standard Operational Procedures by trained personnel proven to ensure homogeneity and stability. Where appropriate/possible EQA samples are tested prior to issue. Where the sample(s) issued is stabilised blood or platelets, pre and post stability testing will have proved sample suitability prior to issue.

4.8.2 l), n), o), r) & s) Please refer to the UK NEQAS LI website at www.ukneqasli.co.uk for detailed information on each programme including the scoring systems applied to assess performance (for BS EN ISO/IEC 17043:2010 accredited programmes only). Where a scoring system refers to the 'consensus result' this means the result reported by the majority of participants for that trial issue. Advice on the interpretation of statistical analyses and the criteria on which performance is measured is also given. Please note that where different methods/procedures are used by different groups of participants these may be displayed within your report, but the same scoring system is applied to all participants irrespective of method/procedure used.

4.8.2 m) We do not assign values against reference materials or calibrants.

4.8.2 q) Details of the programme designs as authorized by The Steering Committee and Specialist Advisory Group can be found on our website at www.ukneqasli.co.uk. The proposed trial issue schedule for each programme is also available.

4.8.2 t) If you would like to discuss the outcomes of this trial issue, please contact UK NEQAS LI using the contact details provided. Alternatively, if you are unhappy with your performance classification for this trial, please find the appeals procedure at www.ukneqasli.co.uk/contact-us/appeals-and-complaints/

4.8.4) The UK NEQAS LI Policy for the Use of Reports by Individuals and Organisations states that all EQA reports are subject to copyright, and, as such, permission must be sought from UK NEQAS LI for the use of any data and/or reports in any media prior to use. See associated policy on the UK NEQAS LI website: http://www.ukneqasli.co.uk/eqa-pt-programmes/new-participant-information/