UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

IG/TCR Clonality Status Programme

Distribution - 232403 Participant ID -

Date Issued - 13 December 2023 Closing Date - 19 January 2024

Trial Comments

This trial was issued to 112 participants, of which 110 (98.2%) returned results. One hundred and seven laboratories tested and returned results for IG and one hundred and seven tested and returned results for TCR .

Sample Comments

Two samples were issued for this trial: IG 174 and TCR 175. Sample IG 174 was formulated from cell line material and sample TCR 175 was manufactured from buffy coat material.

Results and Performance

Your Results

IG/TCR Clonality Status	Your Results	Consensus Result
Sample IG 174	Clonal	Clonal
Sample TCR 175	Polyclonal (Not Clonal)	Polyclonal (Not Clonal)

All Participant Results

	Clonal	Pseudoclonal	Multiple Reproducible Peaks (n>=3)	Polyclonal (Not Clonal)	No (specific) product	Not evaluable
Sample IG 174	105	0	0	2	0	0
Sample TCR 175	8	0	1	97	1	0

Your Performance

Performance	Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
		Satisfactory	Critical
	Satisfactory	2	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.

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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	Clonal	Polyclonal (Not Clonal)	Clonal	Clonal	
Returns	107	35	61	61	14
Clonal	105	31	59	54	0
Irregular Polyclonal (Not Clonal)	0	0	0	0	0
Multiple Reproducible Peaks (n>=3)	0	0	0	2	0
No (Specific) Product	0	1	0	0	5
Not Evaluable	0	0	1	2	2
Polyclonal (Not Clonal)	2	3	1	3	7
Pseudoclonal	0	0	0	0	0

TCR Results by Loci

TCR	TCRB Vβ-Jβ	TCRB Dβ-Jβ	TCRG Vγ-Jγ	TCRD
Your Result	Polyclonal (Not Clonal)	Polyclonal (Not Clonal)	Polyclonal (Not Clonal)	
Returns	71	65	105	11
Clonal	3	3	4	1
Irregular Polyclonal (Not Clonal)	4	5	1	1
Multiple Reproducible Peaks (n>=3)	1	1	1	0
No (Specific) Product	1	1	1	0
Not Evaluable	0	0	0	0
Polyclonal (Not Clonal)	62	55	98	9
Pseudoclonal	0	0	0	0

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Template Type

	IG Returns	TCR Returns
DNA	273	251

PCR Type

	IG Returns	TCR Returns
Multiplex PCR	236	211
PCR for Next generation Sequencing	28	29
Single PCR	13	12
Nested PCR	1	0

Protocol Type

	IG Returns	TCR Returns
Invivoscribe Identiclone (IVD) Kit	135	135
In-House Method (BIOMED Primers)	81	41
Invivoscribe (RUO) Kit	18	29
Invivoscribe LymphoTrack Dx TRB kit	0	18
LymphoTrack TRG Assay	0	12
Master Diagnostica	12	8
Euroclonality 2-Step NGS	9	6
In-House Method (Not BIOMED Primers)	6	5
LymphoTrack IGH FR1 FR2 FR3 IGK	22	0

Analysis Type

	IG Returns	TCR Returns
Capillary Electrophoresis	224	202
NGS (Other)	28	36
Acrylamide Gel Electrophoresis (PAGE)	7	5
Agarose Gel Electrophoresis	2	4
Heteroduplex Analysis	12	4
Microfluidic Electrophoresis	5	4
Radioactive Labelling	3	3
Sequencing	5	2

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Trial Summary IG 174

- The clinical scenario for sample IG 174 was: *A 63-year-old male presented with a 3cm left inguinal mass and loss of appetite. His haemoglobin was 9.1 g/dL and all other full blood counts were within normal ranges. The lymph node showed a diffuse large B cell lymphoma of germinal centre B subtype, and the neoplastic cells were CD10+, CD20+, CD19+, CD22+, CD79a+, sIgkappa+. A IGH::BCL2 fusion and a EZH2 somatic variant affecting codon Y641 were also demonstrated.*
- One hundred and five out of 107 participants (98.1%) who returned results for IG 174 reported a Final Molecular Conclusion of 'Clonal'.
- Two participants reported a Final Molecular Conclusion of 'Polyclonal (Not Clonal)'. Both participants utilised the Invivoscribe Identiclone (IVD) Kit with capillary electrophoresis.

Trial Summary TCR 175

- The clinical scenario for sample TCR 175 was: *A 64-year-old female had a severe noncyclic neutropenia of 0.24 x10^9/L. A blood smear indicated a large granular lymphocyte (LGL) expansion which accounted for 30% of the lymphocytes. The LGLs were CD3+, CD8+, TCR αβ+, CD57+ and CD4-.*
- Ninety-seven out of 107 participants (90.7%) who returned results for sample TCR 175 reported a Final Molecular Conclusion of 'Polyclonal (Not Clonal)'.
- Eight participants reported a Final Molecular Conclusion of 'Clonal'. Of the participants reporting an out of consensus 'Clonal' result, four utilised the Invivoscribe Identiclone (IVD) kit, one participant used the Invivoscribe (RUO) Kit, one an in-house (BIOMED) assay and on an in-house (Not BIOMED) assay. All utilised capillary electrophoretic analysis. A further participant utilised the Euroclonality 2-step NGS assay.
- Two of the eight participants reporting the final diagnostic conclusion for TCR 175 as 'Clonal' performed testing on one TCR locus (TCRG) only. Euroclonality guidelines state that when there is a clinical suspicion of T-cell clonality, it is best addressed by evaluating two TCR targets, usually TCRB and TCRG, either in parallel or consecutively[1]. The TCR Beta multiplex PCR has been shown to be slightly more informative than TCR Gamma amplification but both regions provide complementary information[1].
- A further participant reported a 'Clonal' result based on the findings from the TCR Delta locus. TCR Beta V-J, TCR Beta D-J and TCR Gamma V-J testing all indicated a 'Polyclonal (Not Clonal)' result. As outlined in the EuroClonality/BIOMED-2 guidelines, clonality testing using TCR Delta should only be used for well-defined clinical requests, suggestive of TCR Delta/Gamma proliferations or alternatively, immature T-cell proliferations[1]. Given the clinical scenario provided with TCR 175, indicating a possible mature T cell proliferation, TCR Delta testing would not routinely be undertaken.
- A breakdown for clonal peak sizes (bp) and molecular conclusions for each locus are outlined in Table 1, below.

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Participant	TCR Beta V-J		TCR Beta D-J		TCR Gamma V-J		TCR Delta		Assay utilised
	Molecular Conclusion	Clonal peak(s) (bp)	Molecular Conclusion	Clonal peak(s) (bp)	Molecular Conclusion	Clonal peak(s) (bp)	Molecular Conclusion	Clonal peak(s) (bp)	
1	Not Tested	-	Not Tested	-	Clonal	188 & 230	Not Tested	-	In-house (Not BIOMED primers)
2	Clonal	255 & 266	Clonal	311	Clonal	212, 167	Not Tested	-	Invivoscribe Identiclone (IVD)
3	Not Tested	-	Not Tested	-	Clonal	145, 179 & 230	Not Tested	-	In-house (BIOMED primers) assay
4	Polyclonal (Not Clonal)	n/a	Polyclonal (Not Clonal)	n/a	Polyclonal (Not Clonal)	n/a	Clonal	n/a	Euroclonality 2-step NGS
5	Polyclonal (Not Clonal)	-	Clonal	299	Polyclonal (Not Clonal)	-	Not Tested	-	Invivoscribe (RUO)
6	Polyclonal (Not Clonal)	-	Clonal	188, 298, 306	Polyclonal (Not Clonal)	-	Not Tested	-	Invivoscribe Identiclone (IVD)
7	Clonal	185,188, 240, 246, 267 & 268	Not Tested	-	Polyclonal (Not Clonal)	-	Not Tested	-	Invivoscribe Identiclone (IVD)
8	Polyclonal (Not Clonal)	-	Polyclonal (Not Clonal)	-	Clonal	No information provided	Not Tested	-	Invivoscribe Identiclone (IVD)

Table 1. Overview of clonal peak sizes (bp) and molecular conclusions for TCR loci tested by participants reporting a final molecular conclusion of 'Clonal'. Participant 4 clonal peak sizes marked as n/a as the methodology is Next Generation Sequencing and not capillary electrophoresis based.

- 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 cause of several 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 an out of consensus 'Multiple Reproducible Peaks (n>=3)' result for TCR 175.This participant utilised the Invivoscribe Identiclone (IVD) kit with capillary electrophoresis.
- A further participant reported an out of consensus 'No (specific) product' result. This participant also reported an out of consensus result for IG 174 and utilised the Invivoscribe Identiclone (IVD) kit with capillary electrophoresis.

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In this trial distribution, 13 participants utilised Next Generation Sequencing (NGS) for the testing of IG, TCR or both loci, consistent with data returns for IG 232402. Of the 13, one reported an out of consensus result (Euroclonality 2-step NGS assay). We continue to monitor the adoption of NGS among participants and unsatisfactory performance to ensure that the programme remains fit for purpose for these users. If NGS users have any feedback or comments on the reporting of results, please contact admin@uknegasli.co.uk.

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/