

IgH/TCR Clonality Status

Performance Monitoring Cover Sheet

Participant No:

Trial No: IgH161701

Issue Date: 27th April 2016

Closing Date: 27th May 2016

Performance Monitoring:

	Final IgH Clonality Result IGH 127	Final TCR Clonality Result TCR 128
Your Result		
Consensus Clonality Result	Clonal	Clonal

Overall Classification for this Trial	Running Classification

The scoring system is a rolling scheme that will identify unsatisfactory performance or persistent unsatisfactory performance of any participant. This is in order that UK NEQAS LI can provide support and guidance where needed and ensure that the Genetics NQAAP are informed as appropriate.

Traffic light scoring system

Green = Satisfactory performance.

Amber = Unsatisfactory Performance: a single incidence of unsatisfactory performance in the last three trials. Support and guidance will be offered to the participant with the aim of improving performance prior to the next trial issue.

Red = Persistent Unsatisfactory Performance; two or three incidences of unsatisfactory performance in the last three trials. Immediate action required; support and guidance will continue to be offered but for UK laboratories, NQAAP Genetics will also be informed.

n/a not applicable

Visit www.ukneqasli.co.uk for further information on scoring systems.

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Trial Report

Participant No:

Trial No: IgH161701

Issue Date: 27th April 2016

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Two samples were issued for this trial: IGH 127, a lyophilised sample consisting of an immortalised hairy cell leukaemia cell line spiked into pooled buffy coat; and TCR 128, a lyophilised sample consisting of a T lymphocyte cell line spiked into pooled buffy coat. The samples were issued to 102 participants, who were asked to analyse the samples for IGH and TCR gene re-arrangements, respectively.

Clonality Results:

In this trial 99 (97.1%) participants returned results. Of these, 96 participants submitted results for IGH and 96 for TCR.

Please Note: Numbers in all tables may not equal the number of participants due to not all participants providing all information requested or to some participants performing combinations of techniques.

Table 1: Overall Results

	Final IgH 127 Clonality Result	Final TCR 128 Clonality Result
Your Result		
Not Clonal	1 (1.0%)	3 (3.1%)
Clonal	95 (99.0%)	93 (96.9%)

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NB: The loci and reporting nomenclature below has been standardised based on the Euroclonality/BIOMED 2 guidelines¹.

Table 2: IG Results by Locus	IGH VH-JH	IGH DH-JH	IGK VK-JK	IGK Kde	IGL
Your Result					
N° labs performing assay(s)	96	36	48	49	18
Polyclonal (not clonal)	1 (1.0%)	12 (33.3%)	1 (2.1%)	40 (81.6%)	14 (77.8%)
Clonal	95 (99.0%)	18 (50.0%)	43 (89.6%)	3 (6.1%)	-
Irregular polyclonal (not clonal)	-	2 (5.6%)	-	2 (4.1%)	2 (11.1%)
No (specific) product	-	-	-	1 (2.0%)	-
Multiple reproducible peaks	-	-	3 (6.3%)	-	-
Pseudo-clonal	-	-	-	-	-
Not evaluable	-	4 (11.1%)	1 (2.1%)	3 (6.1%)	2 (11.1%)

Table 3: TCR Results by Locus	TCR Beta V-J	TCR Beta D-J	TCR Gamma V-J	TCR Delta
Your Result				
N° labs performing assay(s)	58	55	93	23
Polyclonal (not clonal)	3 (5.2%)	2 (3.6%)	7 (7.5%)	17 (73.9%)
Clonal	53 (91.4%)	48 (87.3%)	84 (90.3%)	-
Irregular polyclonal (not clonal)	-	1 (1.8%)	1 (1.1%)	2 (8.7%)
No (specific) product	-	-	-	-
Multiple reproducible peaks	-	2 (3.6%)	-	-
Pseudo-clonal	-	-	-	-
Not evaluable	2 (3.4%)	2 (3.6%)	1 (1.1%)	4 (17.4%)

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Method Breakdown:

Table 4: Kit/Protocol	Total number of data points for all IG Loci (and as % of returned results)	Total number of data points for all TCR Loci (and as % of returned results)
Invivoscribe Identiclone (IVD)Kit	111 (43.9%)	118 (51.1%)
In-house method (BIOMED primers)	105 (41.5%)	63 (27.3%)
Invivoscribe (RUO)Kit	30 (11.9%)	29 (12.6%)
In-house (not BIOMED primers)	3 (1.2%)	14 (6.1%)
Master Diagnostica	3 (1.2%)	5 (2.2%)
Other	1 (0.4%)	2 (0.9%)

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Table 5: Analysis Method	Total (all IG Loci)	Total (all TCR Loci)
Capillary Electrophoresis	214 (78.1%)	198 (79.5%)
Acrylamide Gel Electrophoresis	28 (10.2%)	21 (8.4%)
Heteroduplex Analysis	19 (6.9%)	17 (6.8%)
Sequencing	4 (1.5%)	5 (2.0%)
Radioactive Labelling	4 (1.5%)	4 (1.6%)
Microfluidic Electrophoresis	3 (1.1%)	4 (1.6%)
Agarose Gel Electrophoresis	2 (0.7%)	-
Other	-	-

Table 6: PCR Type	Total (all IG Loci)	Total (all TCR Loci)
Multiplex	227 (90.8%)	219 (92.0%)
Single	22 (8.8%)	18 (7.6%)
Semi-Nested	1 (0.4%)	-
Other	-	1 (0.4%)

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Trial Summary IGH:

- In line with sample formulation, 95/96 (99.0%) participants who returned results reported the IgH clonality status of sample IGH 127 to be 'Clonal'.
- The one participant that reported the sample as 'Not clonal' used an in-house approach, not based on BIOMED, and acrylamide electrophoresis. They also only assayed a single target (IgH V-J) which is not a strategy recommended in the published guidelines¹.
- In this trial we compared the use of the 'The Euroclonality Uniform System for Molecular Conclusion' from the recent EuroClonality/BIOMED-2 guidelines¹, as an alternative way to classify clonality status, with our current system (Clonal/Not Clonal).
 - All participants who provided a 'Clonal' result using the current method classified the sample as Clonal using the EuroClonality system.
 - The participant who obtained a 'Not clonal' result for their final IgH clonality result classified the sample as 'Polyclonal (not clonal)' using recent guidelines.
 - We will be transitioning to the 'The Euroclonality Uniform System for Molecular Conclusion' as the primary method to classify clonality status from the next trial issue.
- There have been no significant changes in methodology since the last trial.

Trial Summary TCR:

- In line with sample formulation, 93/96 (96.9%) participants who returned results reported the TCR clonality status of sample TCR 128 to be 'Clonal'.
- The participants that reported the sample as 'Not clonal' used Invivoscribe Identclone(IVD) and Invivoscribe(RUO) kits.
- In this trial we compared the use of the 'The Euroclonality Uniform System for Molecular Conclusion' from the recent EuroClonality/BIOMED-2 guidelines¹, as an alternative way to classify clonality status, with our current system (Clonal/Not Clonal).
 - All participants who provided a 'Clonal' result using the current method classified the sample as 'Clonal' using the Euroclonality system, apart from two participants who did not provide an answer using the new guidelines.
 - Of the 3 participants who achieved a 'Not clonal' result for their final TCR clonality result, 2 classified the sample as 'Polyclonal (not clonal)' and 1 did not answer using the new guidelines.
 - We will be transitioning to the 'The Euroclonality Uniform System for Molecular Conclusion' as the primary method to classify clonality status from the next trial issue.
- There have been no significant changes in methodology since the last trial.

References:

1. Langerak, A. W. *et al.* EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. *Leukemia* **26**, 2159–71 (2012).

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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, Fax: +44 (0) 114 267 3601

e-mail: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:

Prof David Barnett, Director or Mr Liam Whitby, Operations Manager of UK NEQAS LI

4.8.2 d) No activities in relation to this EQA exercise were subcontracted.

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