UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Post-Stem Cell Transplant Chimerism Monitoring Programme

All Participant Report

Distribution - 232403 Sample - 323 Participant ID -

Date Issued - 19 October 2023 Closing Date - 17 November 2023

Trial Comments

This trial was issued to 112 participants, of which 108 (96.4%) returned results. Of the four non returns, one laboratory prenotified us of their intention not to return results and two laboratories requested an extension to the results submission period.

Sample Comments

Four 1ml samples of peripheral blood representing Donor (321), Recipient (322) and two Post- Stem Cell Transplant samples (Post-SCT 323 and 324) were distributed to participants.

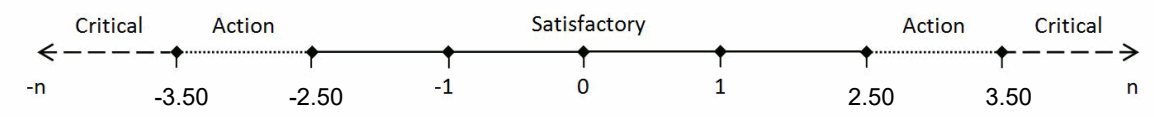
Results and Performance

Reported Percentage Donor	Your Results (%)	Robust Mean (%)	Robust SD (%)
	51	56.0	3.0

Reported Percentage Donor	z Score*	Performance Status for this Sample	Performance Status Classification Over 6 Sample Period		
			Satisfactory	Action	Critical
	-1.33	Satisfactory	6	0	0

*z Score Limits Definitions

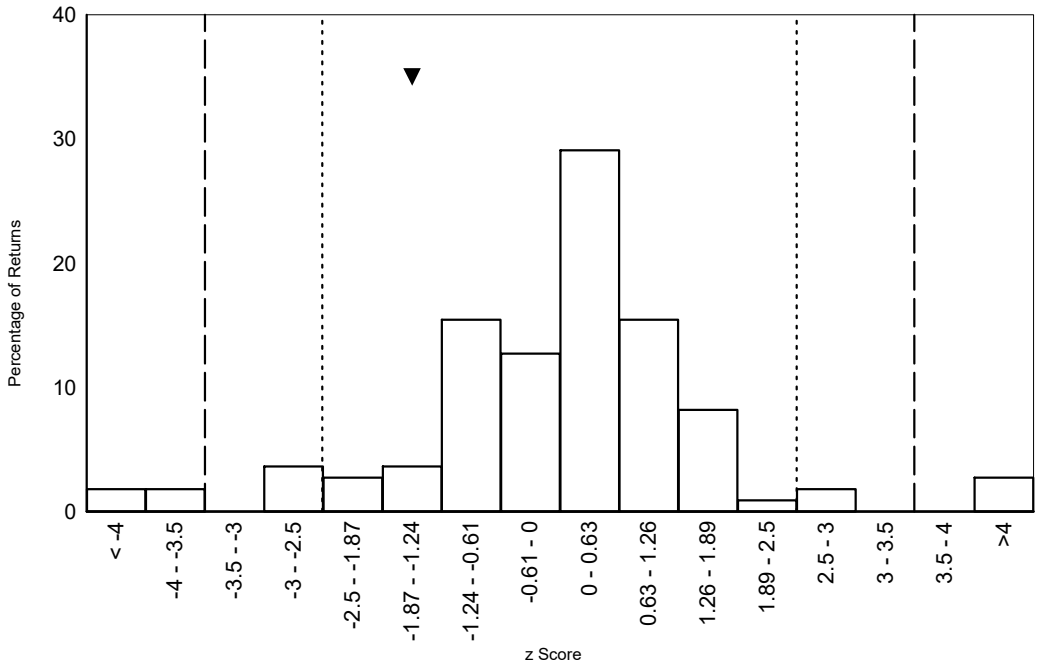
Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.



Histograms of Participant z Scores

Percentage donor chimerism result -

Please note ▼ denotes your result

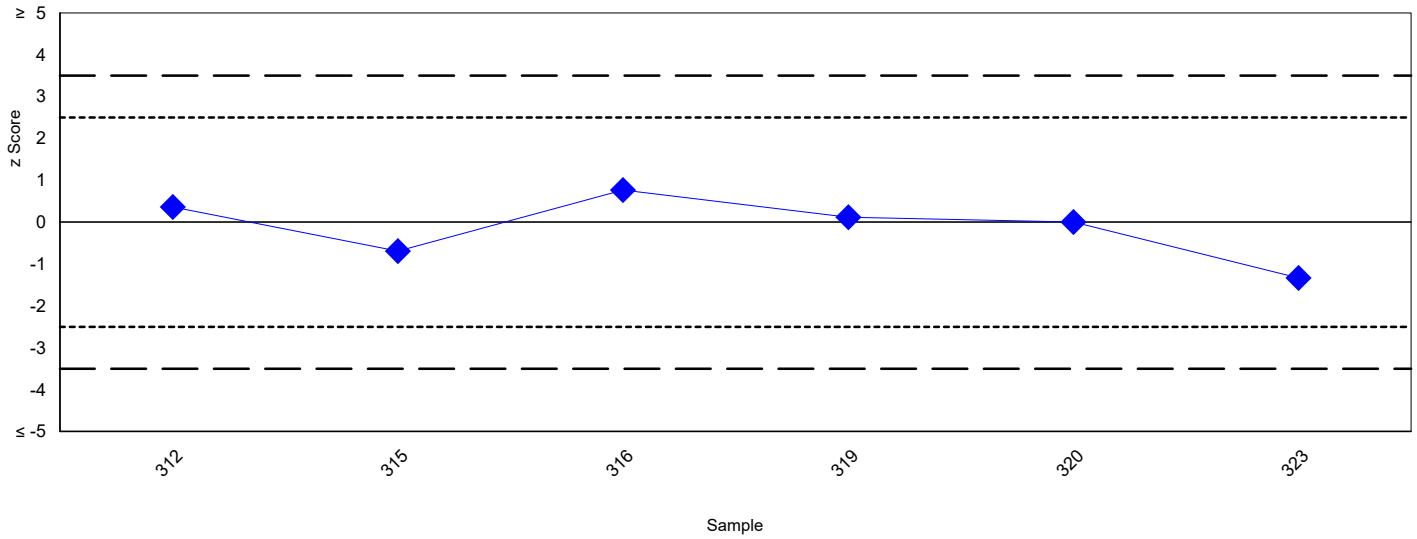


Post-Stem Cell Transplant Chimerism Monitoring Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

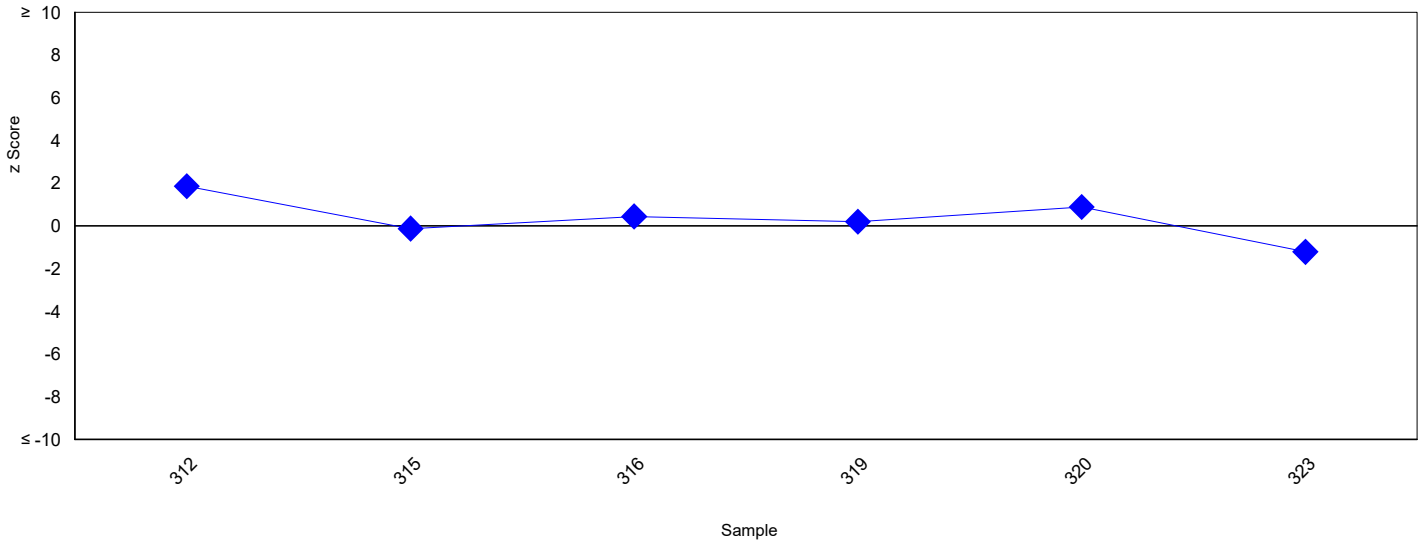
Values (Percentage (%) Donor)



Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Values (Percentage (%) Donor)



Post-Stem Cell Transplant Chimerism Monitoring Programme

Please note, only methods/instruments used by >2 participants are included in the tables. Robust statistics can only be calculated where we have ≥ 20 returns.

Instrument Specific Statistics

Method	Returns	Robust Mean	Robust SD
ABI 3500	32	56.2	3.2
ABI 3500xl	16		
Illumina MiSeq	11		
ABI SeqStudio	10		
ABI 3130xl	8		
ABI 3130	5		
ABI 7500 Real-Time PCR	4		
ABI 3730xl	3		
ABI QuantStudio Absolute Q dPCR Sys	3		

PCR Type Specific Statistics

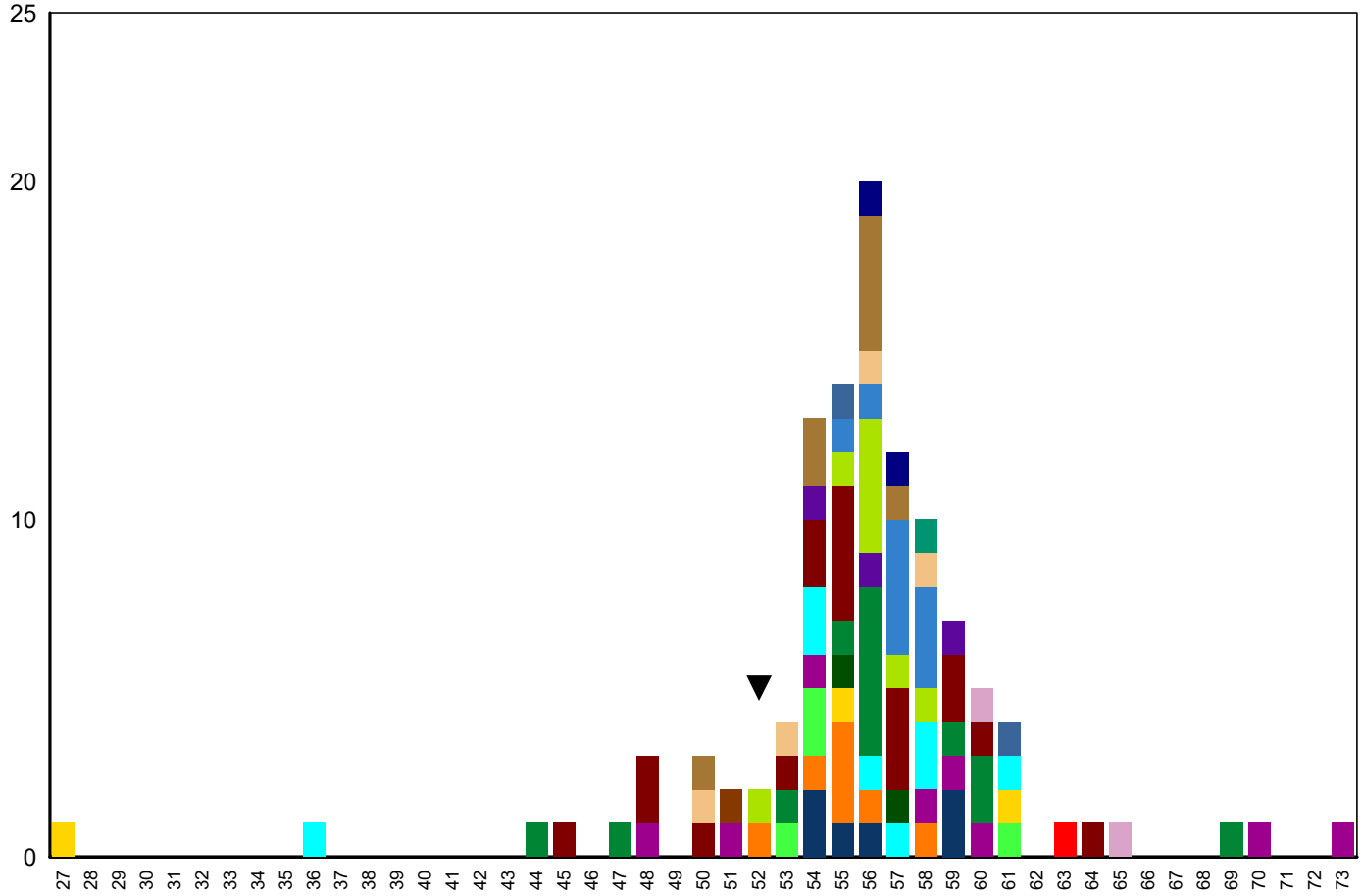
Method	Returns	Robust Mean	Robust SD
Multiplex	72	56.1	3.1
NGS	12		
Real - Time PCR	10		
Single	8		
Droplet Digital PCR	3		
Plate-based digital PCR	3		

Kit/Method Specific Statistics

Method	Returns	Robust Mean	Robust SD
Promega Powerplex 16	17		
In-house	12		
Promega PowerPlex Fusion	9		
GenDx KMRtrack Monitoring Assay	8		
Biotype Mentype Chimera	8		
Devyser Chimerism Kit	8		
Promega Powerplex 16 HS	8		
ABI AmpFISTR Identifiler Plus	7		
ABI AmpFISTR Identifiler	6		
ABI AmpFISTR NGM Select Kit	4		
Imegen Quimera dPCR Dry	4		
Promega GenePrint 24	3		
Biotype Mentype DIPscreen	3		

Post-Stem Cell Transplant Chimerism Monitoring Programme

Frequency distribution histogram showing percentage donor engraftment for sample Post-SCT 323



Post-Stem Cell Transplant Chimerism Monitoring Programme

Distribution - 232403 Sample - 324 Participant ID -

Date Issued - 19 October 2023 Closing Date - 17 November 2023

Trial Comments

This trial was issued to 112 participants, of which 108 (96.4%) returned results. Of the four non returns, one laboratory prenotified us of their intention not to return results and two laboratories requested an extension to the results submission period.

Sample Comments

Four 1ml samples of peripheral blood representing Donor (321), Recipient (322) and two Post- Stem Cell Transplant samples (Post-SCT 323 and 324) were distributed to participants.

Results and Performance

Reported Percentage Donor	Your Results (%)	Robust Mean (%)	Robust SD (%)
	94	94.7	0.9

Reported Percentage Donor	z Score*	Performance Status for this Sample	Performance Status Classification Over 6 Sample Period		
			Satisfactory	Action	Critical
	-0.78	Satisfactory	6	0	0

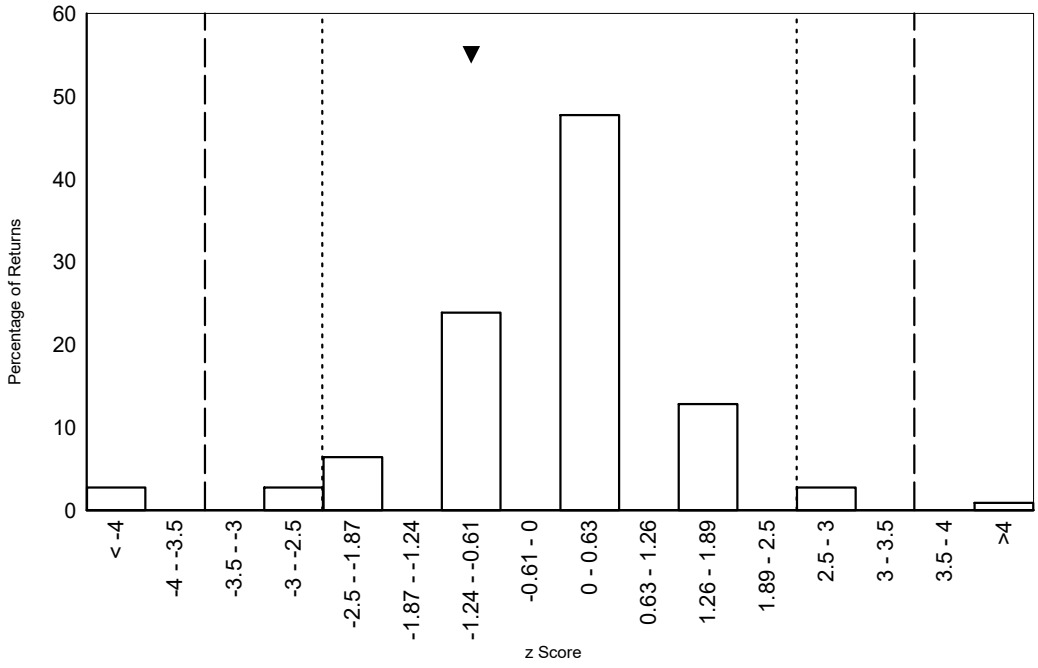
*z Score Limits Definitions

Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.

Histograms of Participant z Scores

Percentage donor chimerism result -

Please note ▼ denotes your result

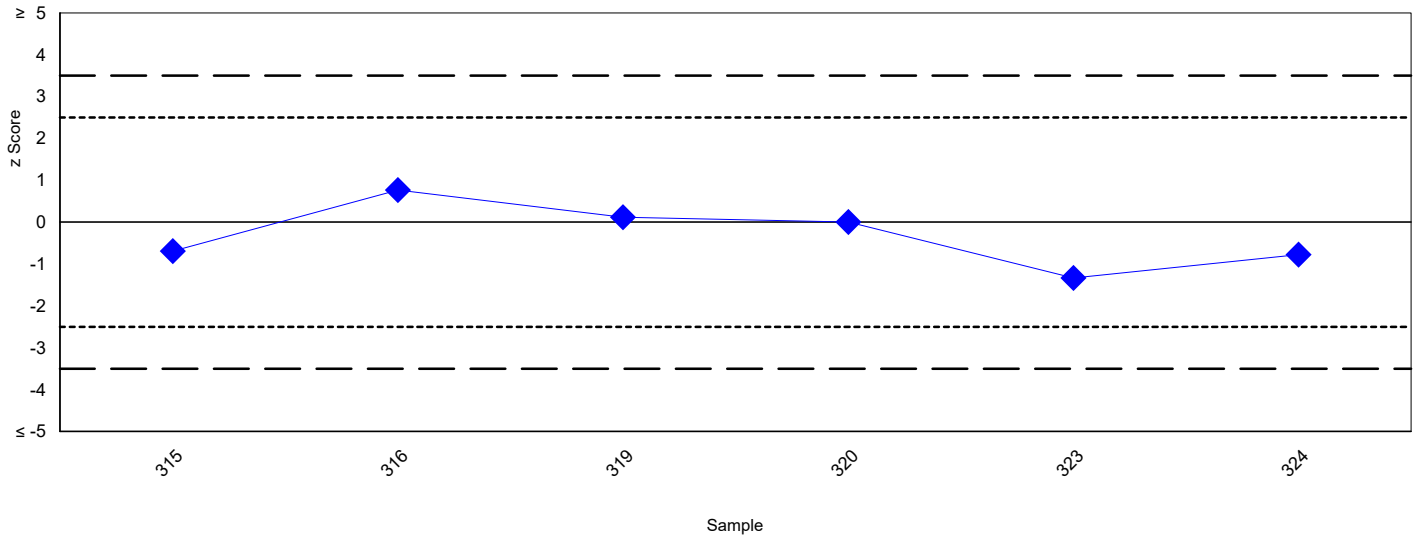


Post-Stem Cell Transplant Chimerism Monitoring Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

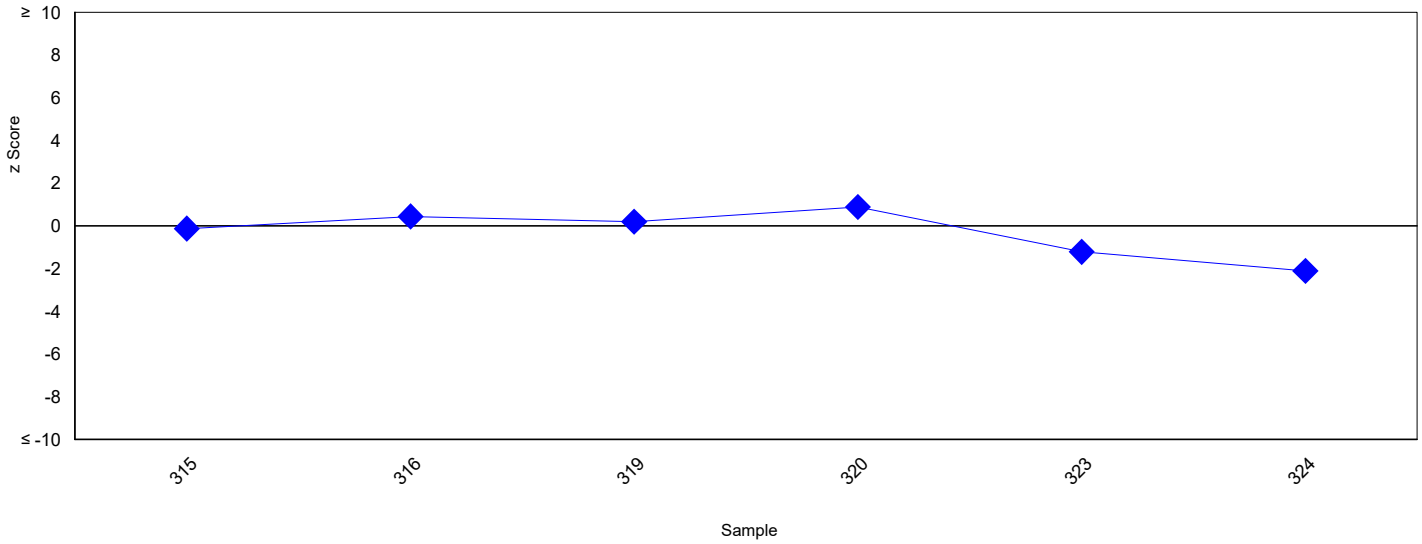
Values (Percentage (%) Donor)



Cusum Control Charts

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Values (Percentage (%) Donor)



Post-Stem Cell Transplant Chimerism Monitoring Programme

Please note, only methods/instruments used by >2 participants are included in the tables. Robust statistics can only be calculated where we have ≥ 20 returns.

Instrument Specific Statistics

Method	Returns	Robust Mean	Robust SD
ABI 3500	32	94.6	0.7
ABI 3500xl	16		
Illumina MiSeq	11		
ABI SeqStudio	10		
ABI 3130xl	8		
ABI 3130	5		
ABI 7500 Real-Time PCR	4		
ABI QuantStudio Absolute Q dPCR Sys	3		

PCR Type Specific Statistics

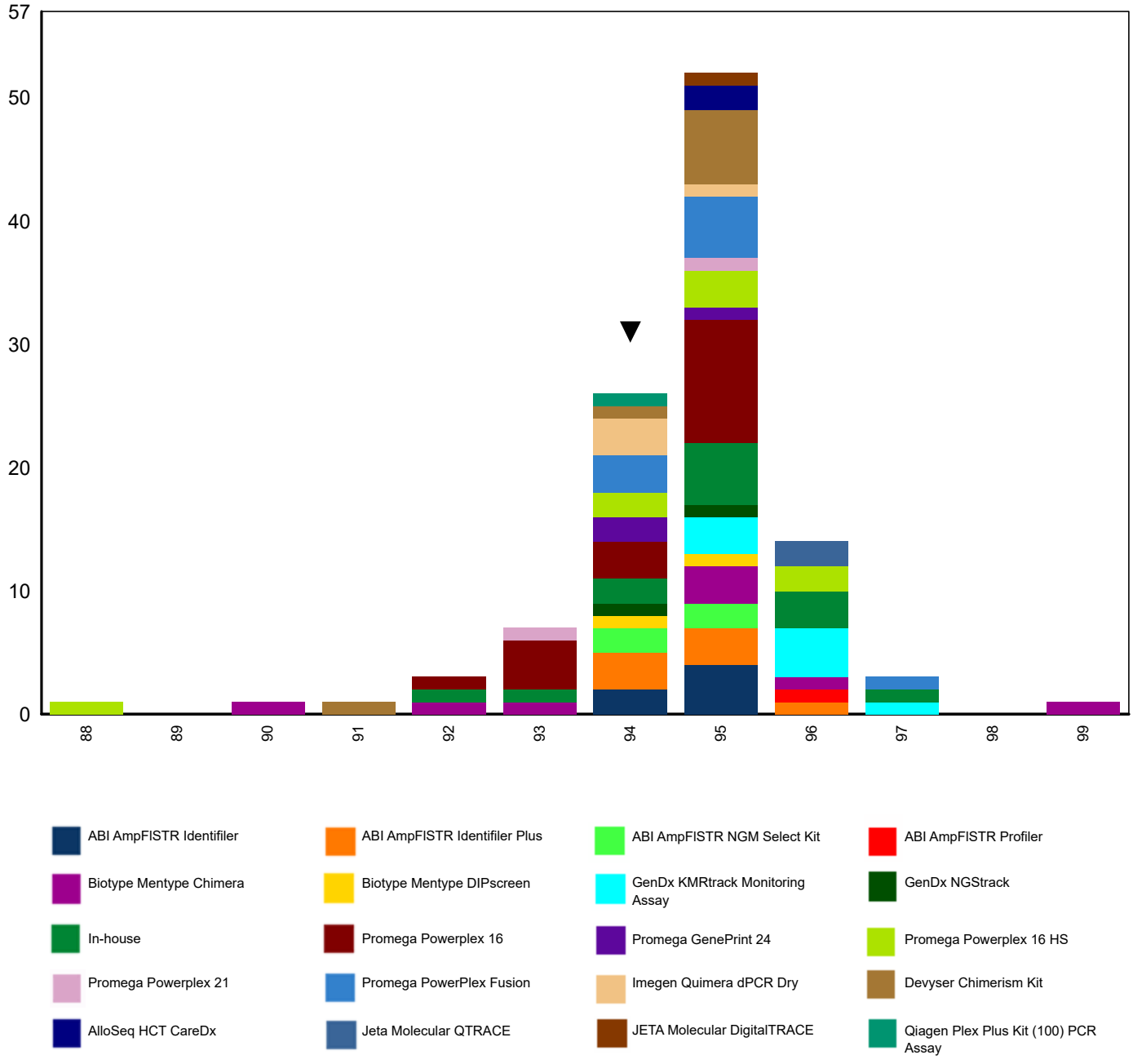
Method	Returns	Robust Mean	Robust SD
Multiplex	71	94.6	1.0
NGS	12		
Real - Time PCR	10		
Single	8		
Plate-based digital PCR	3		
Droplet Digital PCR	3		

Kit/Method Specific Statistics

Method	Returns	Robust Mean	Robust SD
Promega Powerplex 16	17		
In-house	12		
Promega PowerPlex Fusion	9		
Promega Powerplex 16 HS	8		
Biotype Mentype Chimera	8		
Devyser Chimerism Kit	8		
GenDx KMRtrack Monitoring Assay	8		
ABI AmpFISTR Identifiler Plus	7		
ABI AmpFISTR Identifiler	6		
Imegen Quimera dPCR Dry	4		
ABI AmpFISTR NGM Select Kit	4		
Promega GenePrint 24	3		

Post-Stem Cell Transplant Chimerism Monitoring Programme

Frequency distribution histogram showing percentage donor engraftment for sample Post-SCT 324



Post-Stem Cell Transplant Chimerism Monitoring Programme

Comments

Post-SCT Sample 323

- The overall robust mean for sample Post-SCT 323 was 56.0% donor chimerism with a robust standard deviation (SD) of 3.0%.
- Seven participants (6.5%) received a critical z score for this sample, i.e. the submitted result was more than 3.5 SD from the robust mean. Two participants used a real- time quantitative PCR approach, either the GenDx KMRtrack Monitoring Assay or an in-house developed assay. Four participants used a multiplex PCR approach, either Promega Powerplex 16 (n=1), Biotype Mentype Chimera (n=2) or Biotype Mentype DIPscreen (n=1), though it is suspected that the latter may have used the quantitative PCR-based assay Biotype Mentype DIPquant for their assessment of percentage donor chimerism. The final participant used an in-house single PCR approach. All used at least three markers in their chimerism calculation, with the exception of the GenDx KMRtrack user (two markers used).

Post-SCT Sample 324

- The overall robust mean for sample Post-SCT 324 was 94.7% donor chimerism, with a robust SD of 0.9%. Please note that one participant was unable to return a result for this sample since testing did not meet their internal quality standards.
- Four participants (3.7%) received a critical z score for this sample. Two participants used the Biotype Mentype Chimera assay (including one of the participants awarded a critical z score for sample Post-SCT 323). The remaining two participants used either the Promega Powerplex 16 assay, or the NGS-based Devyser Chimerism kit. All four participants used at least seven markers in their calculation.

General Trial Comments

- All 108 participants returned information on the number of markers used in their calculations.
- For sample Post-SCT 323, 97/108 participants (89.8%) calculated percentage donor chimerism using a minimum of at least three informative markers, as recommended in the 2015 UK guidelines[1]. Whilst the median number of markers used was seven, three participants used a single locus to calculate percentage donor chimerism.
- For sample Post-SCT 324, 95/107 participants (88.8%) calculated percentage donor chimerism using a minimum of at least three informative markers. Whilst the median number of markers used was eight, the same three participants used a single locus to calculate percentage donor chimerism.

Final Comments

- Participants are reminded to request repeat samples if the original samples have not arrived within two weeks of trial distribution, or if initial testing does not meet internal QC thresholds (email: repeatsamples@uknegasli.co.uk). We recommend that

participants contact us prior to the trial deadline if this delay prevents timely submission. Please do not submit results from testing that has not met internal quality standards.

- We thank participants for their continued engagement with the Post-Stem Cell Transplant Chimerism Monitoring Programme.

Reference

1. Clark, J. R. *et al.* Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): Technical recommendations for the use of Short Tandem Repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *Br. J. Haematol.* (2015). 168(1):26-37 doi:10.1111/bjh.13073

Post-Stem Cell Transplant Chimerism Monitoring 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) 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/

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/