UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Post-Stem Cell Transplant Chimerism Monitoring Programme

All Participant Report

Distribution - 252603 Sample - 363 Participant ID:

Date Issued - 16 October 2025 Closing Date - 14 November 2025

Trial Comments

This trial was issued to 111 participants, of which 108 (97.3%) returned results by the trial deadline. Of those failing to return results, one informed UK NEQAS LI of extenuating circumstances.

Sample Comments

Four 1ml samples of peripheral blood representing Donor (361), Recipient (362) and two Post- Stem Cell Transplant samples (Post SCT 363 and 364) were distributed to participants. An additional (optional) educational Post- SCT sample (Edu H) was also included in this trial. Results of the educational sample were not subject to performance monitoring.

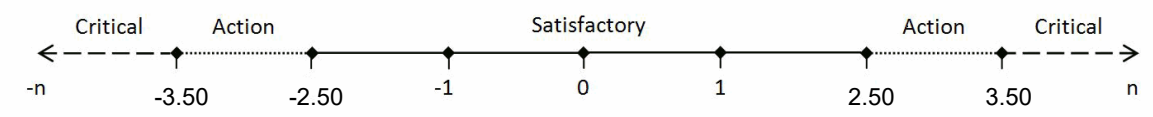
Results and Performance

Reported Percentage Donor	Your Results (%)	Robust Mean (%)	Robust SD (%)	Uncertainty of the Assigned Value (Robust Mean)
	23	22.0	2.1	± 0.25

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

*z Score Limits Definitions

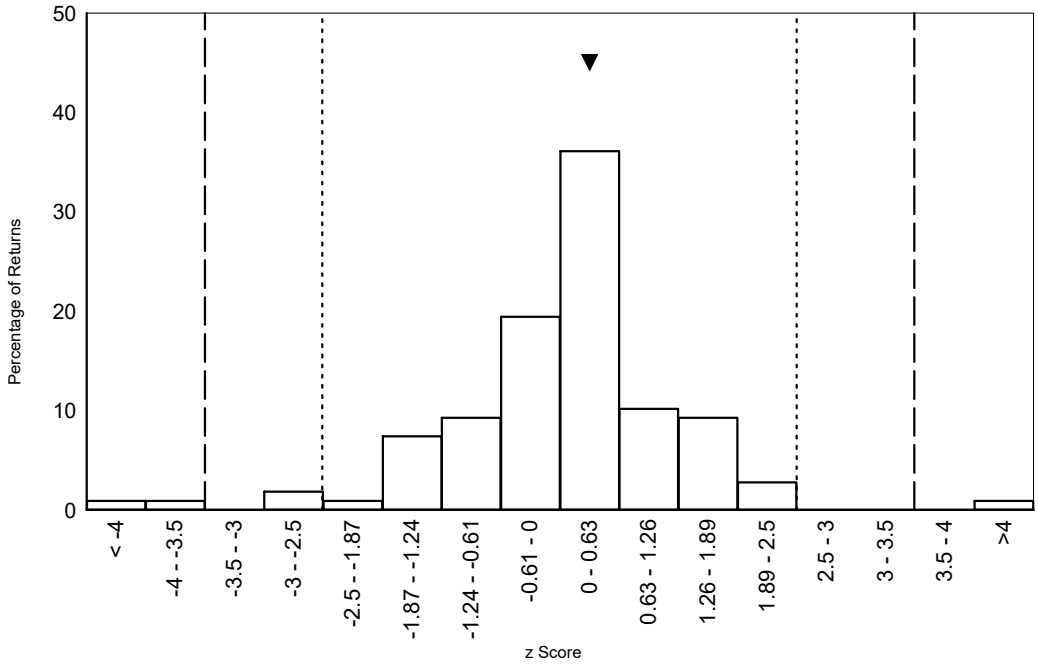
Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is not applicable to the Cusum control charts.



Histograms of Participant z Scores

Percentage donor chimerism result -

Please note ▼ denotes your result

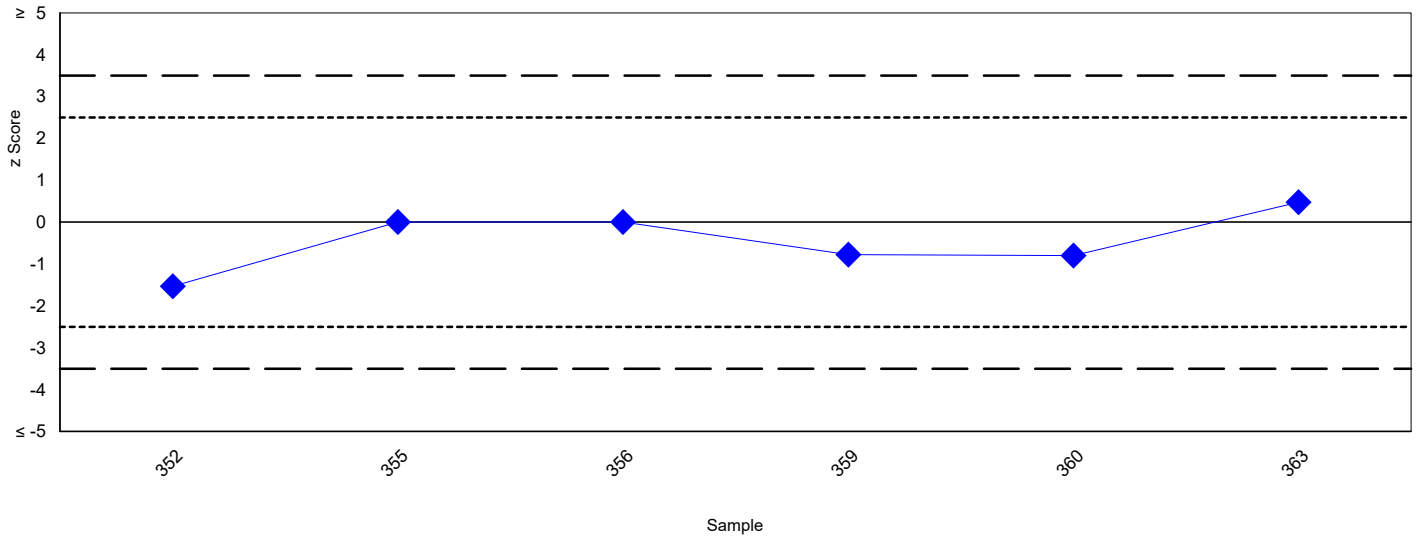


Post-Stem Cell Transplant Chimerism Monitoring Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

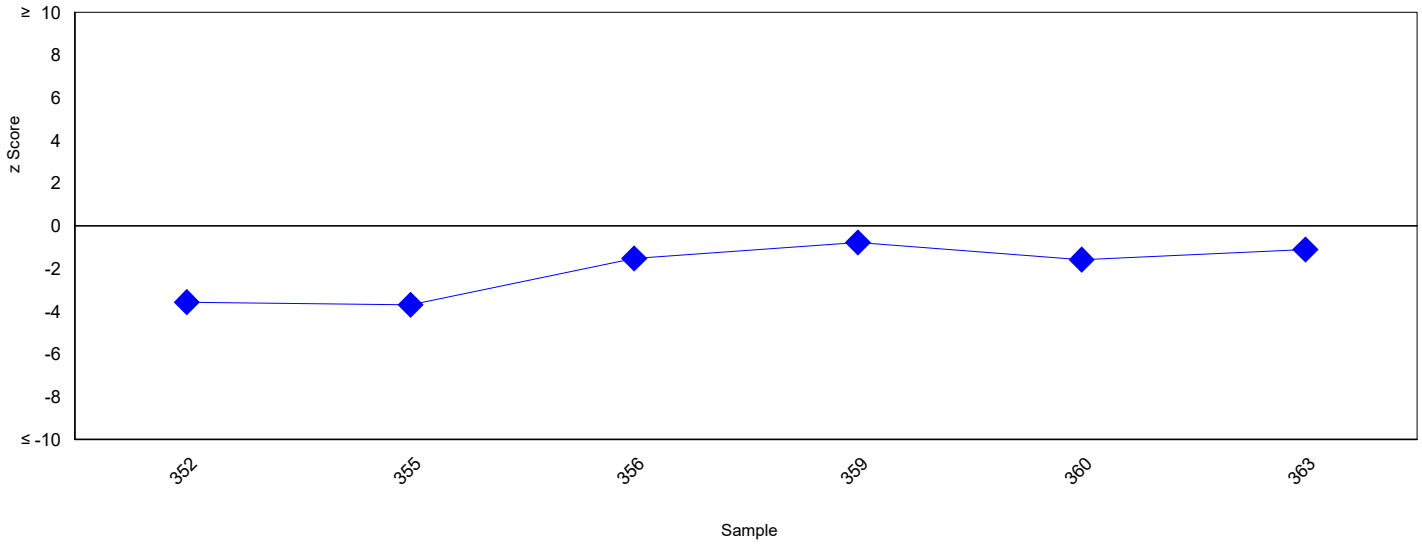
Values (Percentage (%) Donor)



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	30	21.9	1.9
Illumina MiSeq	15		
ABI SeqStudio	14		
ABI 3500xl	14		
ABI 3730	4		
ABI 3730xl	4		
ABI 3130xl	4		
Qiagen QIAcuity dPCR system	3		
ABI QuantStudio Dx	3		
ABI 7500 Real-Time PCR	3		
ABI QuantStudio 6	3		

PCR Type Specific Statistics

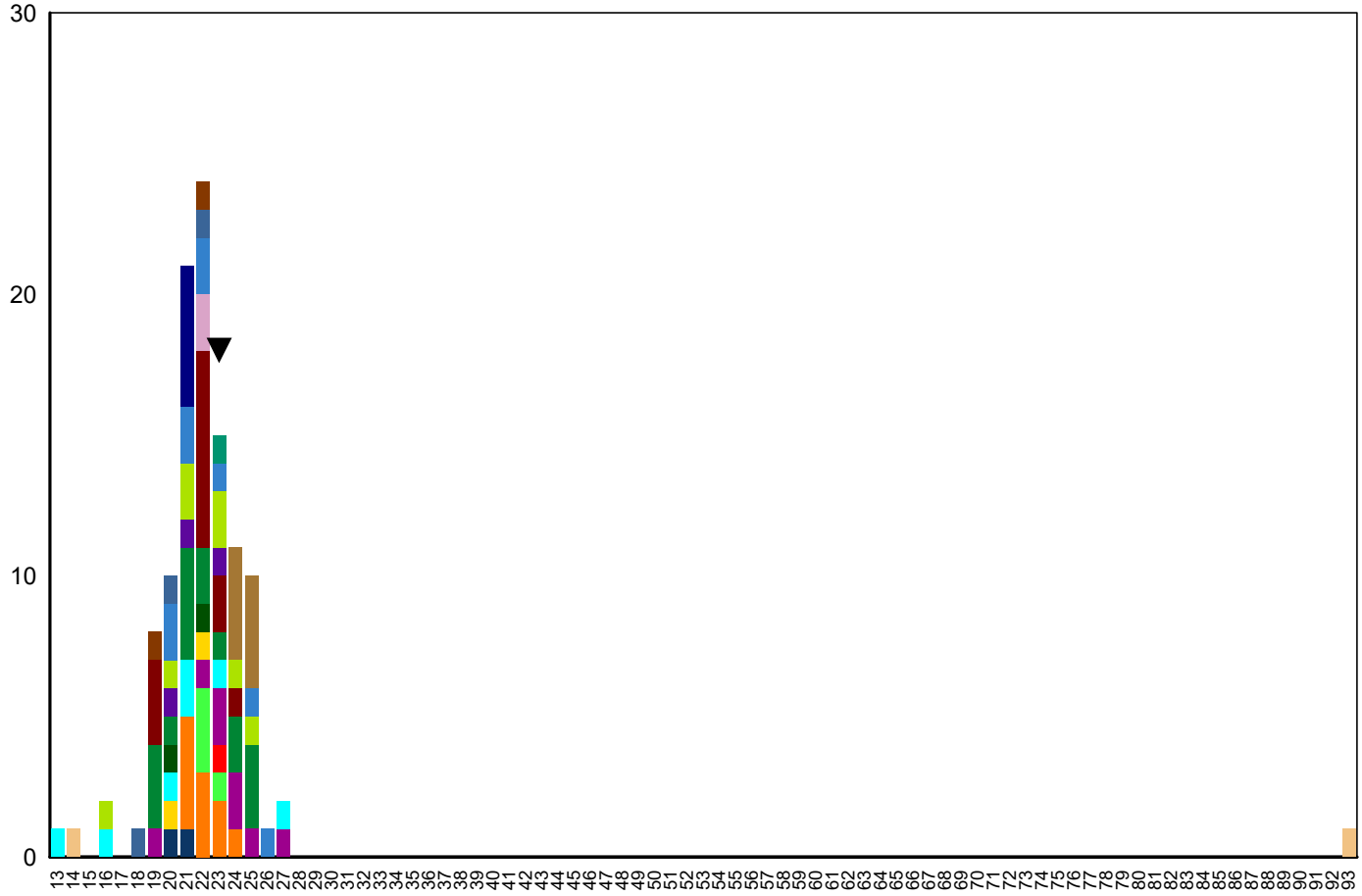
Method	Returns	Robust Mean	Robust SD
Multiplex	66	22.0	1.8
NGS	17		
Real - Time PCR	10		
Single	8		
Plate-based digital PCR	4		

Kit/Method Specific Statistics

Method	Returns	Robust Mean	Robust SD
In-house	16		
Promega Powerplex 16	13		
ABI AmpFISTR Identifiler Plus	10		
Promega PowerPlex Fusion	9		
Biotype Mentype Chimera	8		
Promega Powerplex 16 HS	8		
Devyser Chimerism Kit	8		
GenDx KMRtrack Monitoring Assay	7		
AlloSeq HCT CareDx	5		
ABI AmpFISTR NGM Select Kit	4		
Promega GenePrint 24	3		
Jeta Molecular QTRACE	3		

Post-Stem Cell Transplant Chimerism Monitoring Programme

Frequency distribution histogram showing percentage donor engraftment for sample Post-SCT 363



- ABI AmpFISTR Identifiler
- ABI AmpFISTR Identifiler Plus
- ABI AmpFISTR NGM Select Kit
- ABI AmpFISTR Profiler Plus
- Biotype Mentype Chimera
- Biotype Mentype DIPscreen
- GenDx KMRtrack Monitoring Assay
- GenDx NGStrack
- In-house
- Promega Powerplex 16
- Promega GenePrint 24
- Promega Powerplex 16 HS
- Promega Powerplex 21
- Promega PowerPlex Fusion
- Imegen Quimera dPCR Dry
- Devyser Chimerism Kit
- AlloSeq HCT CareDx
- Jeta Molecular QTRACE
- JETA Molecular DigitalTRACE
- Qiagen Plex Plus Kit (100) PCR Assay

Post-Stem Cell Transplant Chimerism Monitoring Programme

Distribution - 252603 Sample - 364 Participant ID:

Date Issued - 16 October 2025 Closing Date - 14 November 2025

Trial Comments

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Sample Comments

Four 1ml samples of peripheral blood representing Donor (361), Recipient (362) and two Post- Stem Cell Transplant samples (Post SCT 363 and 364) were distributed to participants. An additional (optional) educational Post- SCT sample (Edu H) was also included in this trial. Results of the educational sample were not subject to performance monitoring.

Results and Performance

Reported Percentage Donor	Your Results (%)	Robust Mean (%)	Robust SD (%)	Uncertainty of the Assigned Value (Robust Mean)
	72	73.3	2.2	± 0.26

Reported Percentage Donor	z Score*	Performance Status for this Sample	Performance Status Classification Over 6 Sample Period		
			Satisfactory	Action	Critical
	-0.59	Satisfactory	6	0	0

*z Score Limits Definitions

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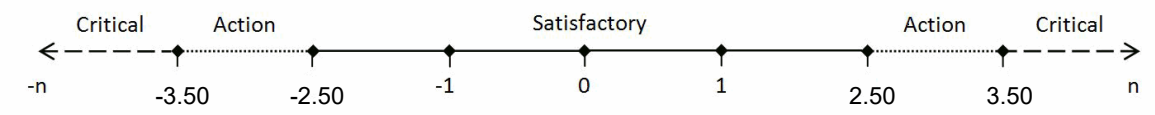

Histograms of Participant z Scores

Percentage donor chimerism result -

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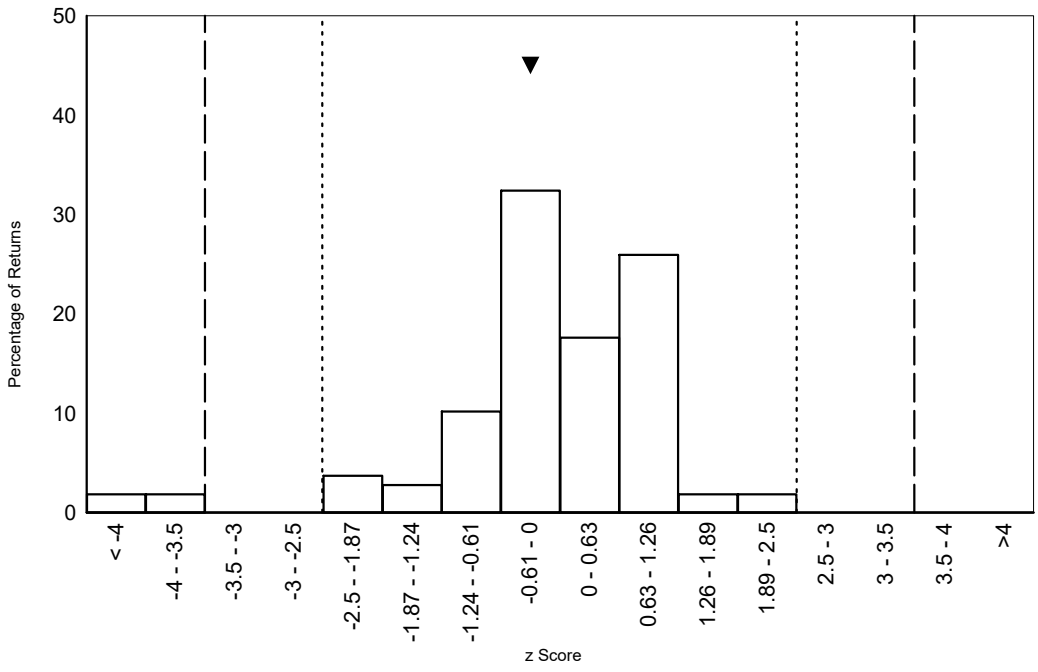
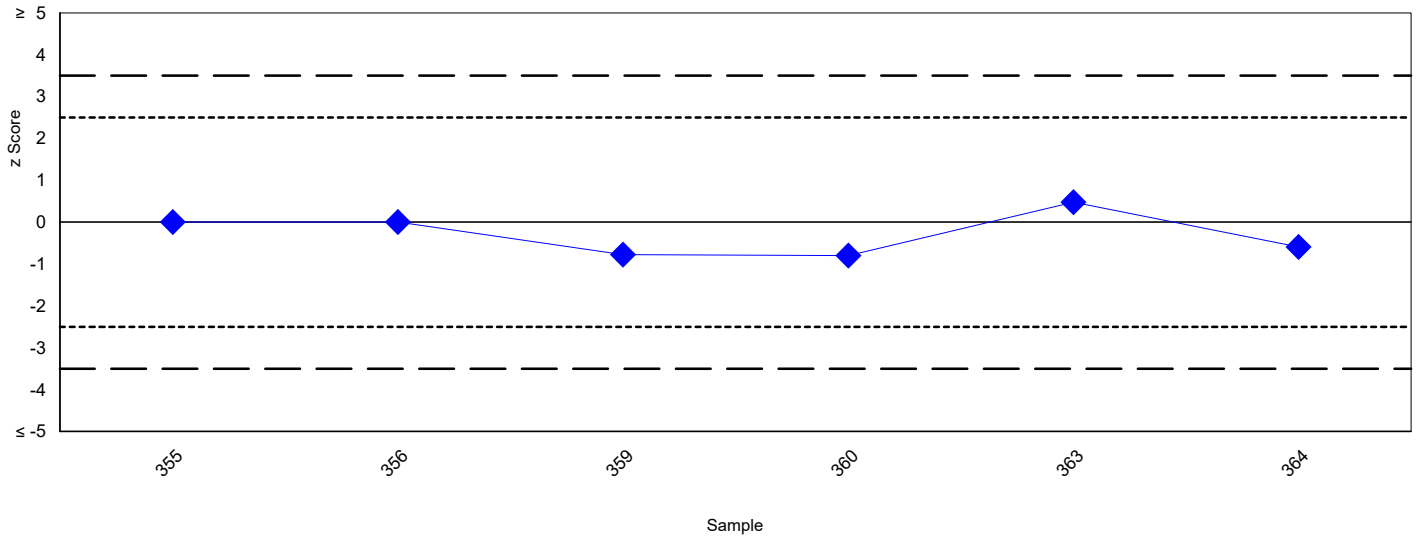


Post-Stem Cell Transplant Chimerism Monitoring Programme

Shewhart Control Charts

(Please note each data point represents a single sample)

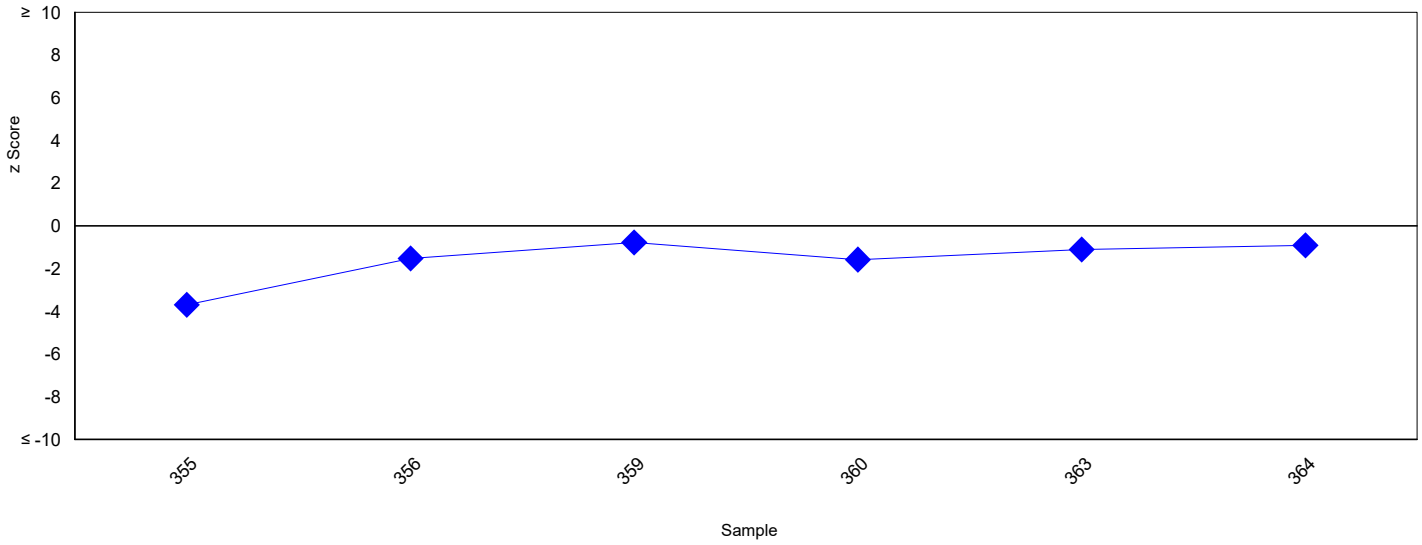
Values (Percentage (%) Donor)



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.

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ABI QuantStudio Dx	3		
ABI 7500 Real-Time PCR	3		
ABI QuantStudio 6	3		

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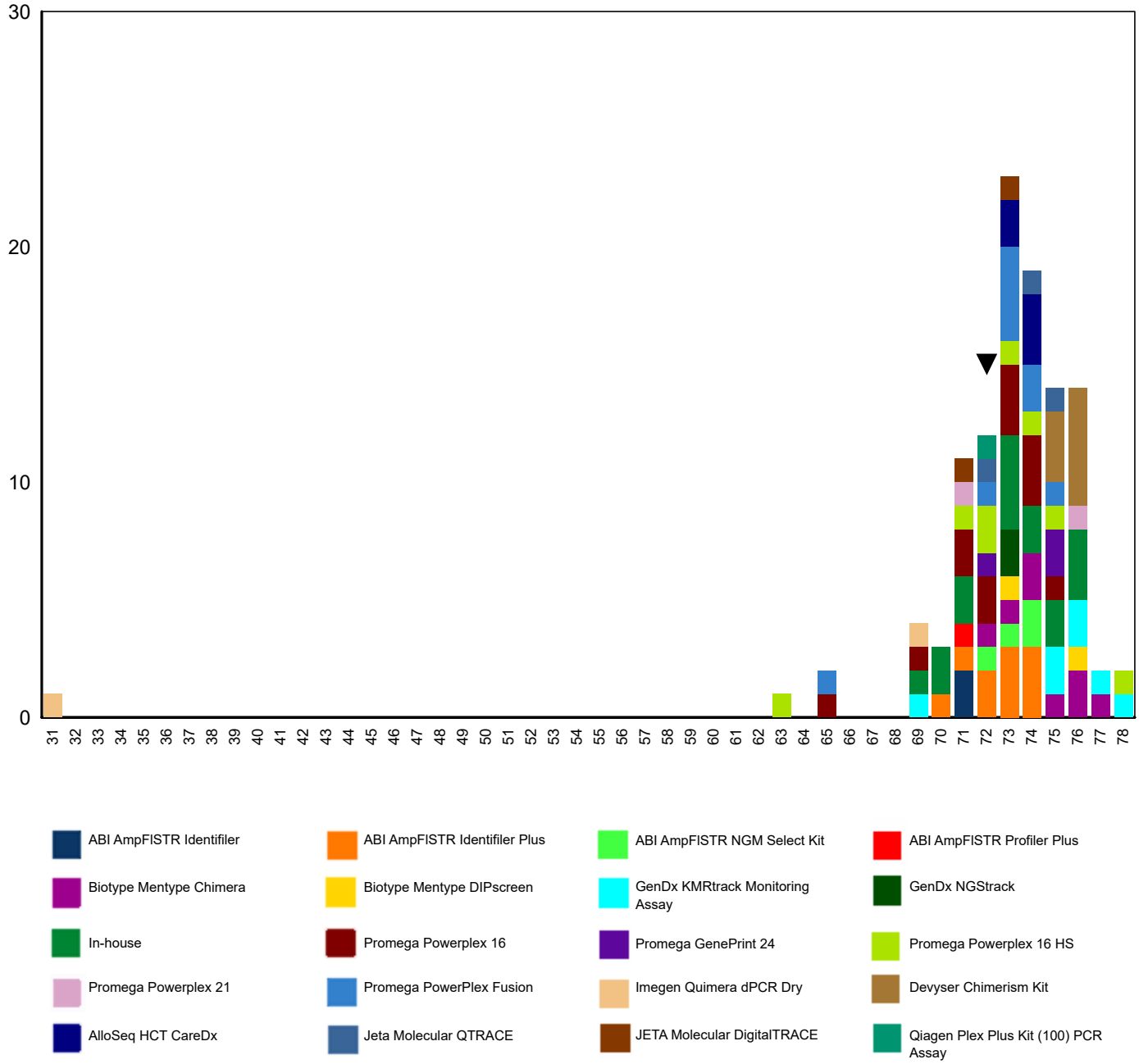
Post-Stem Cell Transplant Chimerism Monitoring Programme

Frequency distribution histogram showing percentage donor engraftment for sample Post-SCT 364



Post-Stem Cell Transplant Chimerism Monitoring Programme

Comments

Post SCT Sample 363

- The overall robust mean for sample Post SCT 363 was 22.0% donor chimerism with a robust standard deviation (SD) of 2.1%.
- Three participants (2.8%) received a critical z score for this sample, i.e. the submitted result was more than 3.5 SD from the robust mean.
- Of these, two utilised the Imegen Quimera dPCR Dry kit and calculated % donor chimerism using either one or two markers.
- The remaining participant utilised the GenDx KMRtrack Monitoring Assay and used two markers in their calculation.

Post SCT Sample 364

- The overall robust mean for sample Post SCT 364 was 73.3% donor chimerism, with a robust SD of 2.2%.
- Four participants (3.7%) received a critical z score for this sample, i.e. the submitted result was more than 3.5 SD from the robust mean.
- Of these, three used a Promega STR-based kit: one each of Powerplex 16, Promega Powerplex 16 HS and PowerPlex Fusion. These participants used 4, 6 and 17 markers respectively in their chimerism calculation.
- The final participant awarded a critical z score for sample Post SCT 364 used the Imegen Quimera dPCR Dry kit and also received a critical z score for sample Post SCT 363. This participant used a single marker in their chimerism calculation for both samples.

General Trial Comments

All 108 participants returned information on the number of markers used in their calculations.

- For both samples (363 and 364), 96 participants (88.9%) calculated percentage donor chimerism using a minimum of at least three informative markers, as recommended in the 2015 UK guidelines[1].
- The median number of markers used for sample 363 was six, whilst the median number used for 364 was five, however for each sample two participants used only a single locus to calculate percentage donor chimerism.

Educational Sample Edu H

Ninety-four participants (87.0% of participants returning results for this trial) submitted results for the educational sample Edu H. This is an increase from 79.2% of participants returning results for the previous educational sample in trial 242504.

Sample Edu H was formulated to exhibit approximately 99% donor chimerism.

- The robust mean for this sample was 99.0% donor chimerism, with a robust standard deviation of 0%.
- The submissions for Edu H are summarised in the table below:

Post-Stem Cell Transplant Chimerism Monitoring Programme

Edu H % Donor Chimerism	Number of Participants
100	11
99	56
98	16
97	4
96	4
89	1
1	2

Table summarising the % donor submissions for sample Edu H

- It is suspected that the two participants submitting the clearly erroneous result of 1% have submitted % recipient chimerism, rather than % donor chimerism. These participants have been excluded from further discussion.
- In line with sample formulation, 81/92 participants (88.0%) reported the presence of recipient DNA in sample Edu H.
- The remaining 11 participants (12.0%) reported 100% donor chimerism.
    - Eight utilised a commercially available short tandem repeat (STR) based assay: either Biotype Mentype Chimera (n=3), ABI AmpFlSTR Identifiler (n=1), ABI AmpFlSTR Identifiler Plus (n=1), ABI AmpFlSTR NGM Select Kit (n=1), Promega PowerPlex 16 HS (n=1) or Promega PowerPlex Fusion (n=1).
    - Two utilised the Biotype Mentype DIPscreen quantitative PCR kit.
    - One used an in-house NGS assay with Illumina technology.
- All 11 participants reporting 100% donor chimerism declared their assay's limit of detection (LoD). This is in the table below:

Stated LoD (%)	Number of Participants Submitting 100% Donor Chimerism
0.1	1
1	4
1.4	1
2	1
2.5	1
5	3

Table summarising the different LoDs stated by participants submitting a result of 100% donor chimerism for Edu H.

- The robust mean of % donor chimerism reported for sample Edu H (99.0%) indicates the contribution of recipient DNA to be ~1%. Therefore, at least the participant stating a LoD of 0.1% appears to have an assay sufficient to detect the recipient DNA present in sample Edu H, and could therefore be considered to have submitted a false negative qualitative result (i.e. no recipient DNA detected). This participant uses the in-house NGS assay mentioned above.
- Four further participants stated a LoD of 1%. These participants all utilise a commercially available STR based assay, either the Biotype Mentype Chimera kit (n=2), the ABI AmpFlSTR Identifiler kit, or the Promega Powerplex 16 HS.

- It is recommended that all five participants stating an LoD ≤1% consider their LoD in light of this result.

Increased discussion around microchimerism (defined as <1% recipient DNA[2]) also raises the issue of reporting results using a greater number of decimal places. Whilst participants are standardly requested to submit their results for this programme as integers (whole numbers), for Edu H we also requested that participants submit a further result to include the number of decimal places routinely included on their clinical reports (if appropriate).

- Forty-five participants (48.9% of the 92 submitting an integer result >1%) provided a further meaningful result for Edu H to one or more decimal places (1dp, n=28; 2dp, n = 16, 3dp, n=1).
- These results ranged from 96.0% to 100.00%, with a robust mean of 99.041% and RSD of 0.364%.
- Limits of detection submitted by these 45 participants ranged from <0.01% to 5%.
- Of the 11 previously discussed participants submitting a result of 100% donor chimerism for Edu H, one submitted a further result of 99.9% donor chimerism when asked to report using their standard number of decimal places. Therefore, once given the option of submitting a value with decimal places, this participant did not return a false negative qualitative result. This participant utilises the Biotype Mentype DIPscreen (stated LoD: 1.4%).

In summary, results for the educational sample Edu H were largely reassuring. There were two clearly erroneous results of 1% donor chimerism (likely resulting from transposition of donor and recipient values) and a further obvious outlier result of 89% donor chimerism (ABI AmpFlSTR Identifiler Plus, stated LoD: 0.1%). Additionally, it appears that only one participant (five, if including the participants with an LoD of 1%) could be considered to have submitted a result equivalent to a false negative qualitative result, based on their stated LoD.

The introduction of scored trial samples with a robust mean approaching full donor chimerism will likely require an alternative scoring system. Educational samples such as Edu H are therefore key to building experience and the development of this programme. UK NEQAS LI will discuss the results of this and future EQA rounds with the UK NEQAS LI Specialist Advisory Group to formulate a strategy, informed by the current literature, for the incorporation of microchimerism and increased decimal place reporting into the Chimerism programme.

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@ukneqasli.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, and particularly those that invested in the analysis of the educational sample and associated questionnaire.

- Data from a previous UK NEQAS LI chimerism survey features within the recently published UK best practice guidelines for chimerism testing and monitoring[3].

References

1. Clark, JR *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* **168**(1):26-37 (2015).

2. Blouin, AG *et al*. A practical guide to chimerism analysis: Review of the literature and testing practices worldwide. *Hum Immunol* **82**(11):838-849 (2021).

3. Clark, A *et al*. UK recommendations for chimerism testing and monitoring following allogeneic haematopoietic stem cell transplantation (HSCT): Best practice consensus guidelines from the British Society for Blood and Marrow Transplant and Cellular Therapies (BSBMTCT), NHS England Genomic Laboratory Hub (GLH) Haematological Malignancies Working Group, UK Cancer Genetics Group (UKCGG) and the UK National External Quality Assessment Service for Leucocyte Immunophenotyping (UK NEQAS LI). *Br J Haematol* **207**(5): 1802-1814 (2025).

Post-Stem Cell Transplant Chimerism Monitoring Programme

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 coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (Director).

4.8.2 c) Person(s) authorising this report: Mr Stuart Scott (Director) of UK NEQAS LI.

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited.
4.8.2 d) Post closure testing of samples for this programme is externally provided, although the final decision about sample suitability lies with the EQA provider; no other activities in relation to this EQA exercise were externally provided.
4.8.2 d) Where externally provided products or services are used in the delivery of EQA, a competent supplier is used, the EQA provider is responsible for this work and participants are informed accordingly.

4.8.2 g) The UK NEQAS LI Privacy Policy can be found at the following link: https://sheffield-ukneqas.ipassportqms.com/document_download/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. Participant details, their results and their performance data remain confidential unless we are required by law to share this information. Where required by law or authorised by contractual arrangements to release confidential information, UK NEQAS LI will notify those concerned of the information released, unless prohibited by law. For UK participants, the relevant National Quality Assessment Advisory Panel is informed when a UK participant is identified as having performance issues.

4.8.2 i) All EQA samples are prepared in accordance with strict standard operating 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 authorised 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/