UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

Paediatric Acute Leukaemia Translocations Programme

Distribution - 222303

Participant ID -

Date Issued - 14 February 2023

Closing Date - 17 March 2023

Trial Comments

This trial was issued to 58 participants; of which 54 (93.1%) returned results at the time of reporting. Of the non returns, one laboratory notified us of their intention not to return results and a further three participants requested an extension to results submission. Overall, 52 participants returned results for t(12;21) (ETV6::RUNX1), 51 participants returned results for t(4; 11) (KMT2A::AFF1) and 52 participants returned results for t(1;19) (TCF3::PBX1).

Sample Comments

Two vials of lyophilised cell line derived material (sample refs PALT 152 and PALT 153) were issued for t(12;21) (ETV6::RUNX1), t(4;11) (KMT2A::AFF1) and t(1;19) (TCF3::PBX1) analysis. PALT 152 was manufactured to be positive for the t(4;11) (KMT2A::AFF1) rearrangement. PALT 153 was formulated to be negative for all rearrangements.

Results and Performance

Your Results

PALT Identification	Your Results	Consensus Result
Sample PALT 152		
t(12;21) (*ETV6-RUNX1, TEL-AML1*)	No Rearrangement Detected	No Rearrangement Detected
t(4;11) (*KMT2A-AFF1, MLL-AF4*)	Rearrangement Detected	Rearrangement Detected
t(1;19) (*TCF3-PBX1, E2A-PBX1*)	No Rearrangement Detected	No Rearrangement Detected
Sample PALT 153		
t(12;21) (*ETV6-RUNX1, TEL-AML1*)	No Rearrangement Detected	No Rearrangement Detected
t(4;11) (*KMT2A-AFF1, MLL-AF4*)	No Rearrangement Detected	No Rearrangement Detected
t(1;19) (*TCF3-PBX1, E2A-PBX1*)	No Rearrangement Detected	No Rearrangement Detected

All Participant Results

	Rearrangement Detected (Returns)	No Rearrangement Detected (Returns)
Sample PALT 152		
t(12;21) (*ETV6-RUNX1, TEL-AML1*)	1	51
t(4;11) (*KMT2A-AFF1, MLL-AF4*)	49	2
t(1;19) (*TCF3-PBX1, E2A-PBX1*)	0	52
Sample PALT 153		
t(12;21) (*ETV6-RUNX1, TEL-AML1*)	1	51
t(4;11) (*KMT2A-AFF1, MLL-AF4*)	1	50
t(1;19) (*TCF3-PBX1, E2A-PBX1*)	0	52

Your Performance

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

N/A = Not Applicable

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PCR Type

	Returns		
	t(12;21) (*ETV6-RUNX1, TEL-AML1*)	t(4;11) (*KMT2A-AFF1, MLL-AF4*)	t(1;19) (*TCF3-PBX1, E2A-PBX1*)
Real-Time PCR	21	20	20
Single PCR	11	11	12
Nested PCR	7	7	6
Multiplex PCR	6	8	8
PCR for Next generation Sequencing	5	5	5
Droplet Digital PCR	1	-	1

Protocol Type

	Returns		
	t(12;21) (*ETV6-RUNX1, TEL-AML1*)	t(4;11) (*KMT2A-AFF1, MLL-AF4*)	t(1;19) (*TCF3-PBX1, E2A-PBX1*)
Biomed 1	12	11	12
Hemavision 28Q Kit	10	11	11
EAC Protocol	9	7	8
In-house Assay	8	11	7
Modified EAC Protocol	5	4	5
Hemavision Kit	2	2	4
Archer FusionPlex Heme Kit	2	2	2
Biomed 3	1	1	1
Oncomine Myeloid Research Assay	1	1	1
Archer Fusion Plex LLA Kit	1	1	1

Analysis Type

	Returns		
	t(12;21) (*ETV6-RUNX1, TEL-AML1*)	t(4;11) (*KMT2A-AFF1, MLL-AF4*)	t(1;19) (*TCF3-PBX1, E2A-PBX1*)
Real-Time PCR Fluorescent Detection	25	25	25
Agarose Gel Electrophoresis	18	18	19
NGS (Illumina)	4	4	4
Capillary Electrophoresis	2	2	2
NGS (ThermoFisher Ion Torrent)	1	1	1
Digital PCR (Biorad)	1	-	1
Qiaxcel	-	1	-

Journal Reference for Assay

	Returns
Gabert et al (2003). Leukemia, 17, 2318-2357	22
Van Dongen et al (1999). Leukemia, 13, 1901-1928	19
Pallisgaard et al (1998). Blood, 92, 574-588	3
In-house Designed	2

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Final Comments

PALT 152

- In total, 49 out of 51 (96.1%) participants testing for t(4;11) (*KMT2A*::*AFF1*) reported the presence of the rearrangement in PALT 152.
- Two participants reported a false negative result. One participant utilised the Biomed 1 protocol with agarose gel electrophoretic analysis. For the remaining participant, this result is likely due to a sample transposition between PALT 152 and 153 (see PALT 153 summary below).
- Fifty-two participants tested PALT 152 for the presence of t(12;21) (*ETV6*::*RUNX1*), of which 51 (98.1%) reported the sample to be negative for the rearrangement.
- The participant reporting a false positive result also reported an out of consensus false negative for the t(4;11) (*KMT2A*::*AFF1*) rearrangement. The participant utilised the Biomed 1 protocol with agarose gel electrophoretic analysis.
- All participants (n=52) testing for t(1;19) (*TCF3*::*PBX1)* found PALT 152 to be negative for the rearrangement.

PALT 153

- In line with sample formulation, 51/52 (98.1%) testing for t(12;21) (*ETV6*::*RUNX1*) reported the sample to be negative for the rearrangement.
- The participant reporting a false positive result also reported out of consensus results for the t(4;11) (*KMT2A*::*AFF1*) and t(12;21) (*ETV6*::*RUNX1*) rearrangements in PALT 152 (Biomed 1 protocol, agarose gel electrophoresis).
- Fifty-one participants tested PALT 153 for the presence of t(4;11) (*KMT2A*::*AFF1*), of which 50 (98.0%) reported the sample to be negative for the rearrangement.
- The participant reporting a false positive result also reported a false negative result for the t(4;11) (*KMT2A*::*AFF1*) rearrangement in PALT 152 (see above). This is suggestive of a sample transposition event.
- All participants (n=52) testing for t(1;19) (*TCF3*::*PBX1)* found PALT 153 to be negative for the rearrangement.

This represents the final trial issue and report of the 2022-2023 distribution period. We would like to thank all participants for their continued engagement in the Paediatric Acute Leukaemia Translocations programme. If participants have any suggestions/ improvement ideas for this programme, please contact admin@ukneqasli.co.uk.

Paediatric Acute Leukaemia Translocations 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
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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/