BCR::ABL1 and AML Translocation Identification Programme

Distribution - 232401 Participant ID -

Date Issued - 27 April 2023 Closing Date - 26 May 2023

Trial Comments

This trial was issued to 198 participants, of which 187 (94.4%) returned results. Of the 11 non-returns, six participants pre-notified UKNEQAS LI of their intention not to return results for this trial.

Sample Comments

Two lyophilised cell line preparations were distributed for analysis: samples BCR 182 and AML 183. Subject to their laboratory repertoire, participants were requested to analyse sample BCR 182 for the presence of a BCR::ABL1 rearrangement and to analyse sample AML 183 for the presence of the t(8;21) RUNX1::RUNX1T1, t(15;17) PML::RARA and inv(16) CBFB::MYH11 rearrangements associated with AML. Sample BCR 182 was manufactured to be negative for a BCR::ABL1 rearrangement and sample AML 183 was manufactured to be positive for a t(8;21) RUNX1::RUNX1T1 rearrangement.

Results and Performance

Your Results

Identification	Your Results	Consensus Result
Sample 182		
BCR-ABL1 t(9;22)	No Rearrangement Detected	No Rearrangement Detected
Sample 183		
CBFB-MYH11 Inv(16)	No Rearrangement Detected	No Rearrangement Detected
RUNX1-RUNX1T1 t(8;21)	Rearrangement Detected	Rearrangement Detected
PML-RARA t(15;17)	No Rearrangement Detected	No Rearrangement Detected

All Participant Results

	Rearrangement Detected (Returns)	No Rearrangement Detected (Returns)
Sample 182		
BCR-ABL1 t(9;22)	2	183
Sample 183		
CBFB-MYH11 Inv(16)	0	159
RUNX1-RUNX1T1 t(8;21)	159	3
PML-RARA t(15;17)	1	169

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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Protocol Type

	Returns			
	BCR-ABL1 t(9;22)	*CBFB-MYH11* Inv(16)	*RUNX1-RUNX1T1* t(8;21)	*PML-RARA* t(15;17)
In-house Assay	63	28	30	33
Biomed 1	28	29	27	27
Hemavision Kit	10	23	25	25
EAC Protocol	12	16	17	16
Biotype Diagnostic GmbH Mentype AMLplex	8	13	13	12
Qiagen Ipsogen RUNX1-RUNX1T1 Kit	-	-	9	-
Modified EAC Protocol	11	9	8	10
Oncomine Myeloid Research Assay	5	8	8	8
Diatech Pharmacogenetics Easy Kit	8	6	6	7
Diatech EasyPGX ready BCR-ABL Fusion	9	4	4	4
Ampliseq for Illumina RNA Fusion	3	3	3	3
Biomed 3	-	2	2	2
Tib Molbiol LightMix kit	4	2	2	3
Archer FusionPlex Heme Kit	1	2	2	2
Entrogen Kit	-	1	1	1
Leukemia Fusion Gene (Q30) QuanDx kit	-	1	1	1
Archer FusionPlex Myeloid Kit	1	1	1	1
Illumina TruSeq Stranded Total RNA	1	1	1	1
Illumina TruSight RNA Fusion Panel	1	1	1	1
Qiagen Ipsogen BCR-ABL1 Mbcr RGQ RT-PCR Kit	1	-	-	-
Qiagen Ipsogen BCR-ABL1 Mbcr IS-MMR Kits CE	4	-	-	-
Qiagen Ipsogen PML-RARA bcr1 Kit CE	-	-	-	10
Invivoscribe PML RARA Kit	-	-	-	1
Qiagen Ipsogen BCR-ABL1 Mbcr Kit CE	5	-	-	-
Liferiver BCR-ABL Real Time RT-PCR Kit	1	-	-	-
Qiagen Ipsogen CBFB-MYH11 A Kit	-	8	-	-
Cepheid GeneXpert Ultra BCR-ABL assay	2	-	-	-
3B BlackBio TRUPCR BCR-ABL1 Kit	3	-	-	-
Genmark geneMAP Screening Kit	2	-	-	-
Bioclarma SensiQuant One-Step	1	-	-	-

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PCR Type

	Returns			
	BCR-ABL1 t(9;22)	*CBFB-MYH11* Inv(16)	*RUNX1-RUNX1T1* t(8;21)	*PML-RARA* t(15;17)
Real-Time PCR	71	62	67	72
Multiplex PCR	59	36	35	33
Single PCR	28	24	26	23
PCR for Next generation Sequencing	12	18	18	18
Nested PCR	15	19	16	24

Analysis Type

	Returns			
	BCR-ABL1 t(9;22)	*CBFB-MYH11* Inv(16)	*RUNX1-RUNX1T1* t(8;21)	*PML-RARA* t(15;17)
Real-Time PCR Fluorescent Detection	79	74	78	84
Agarose Gel Electrophoresis	63	41	39	40
Capillary Electrophoresis	24	19	20	21
NGS (Illumina)	8	10	10	10
NGS (ThermoFisher Ion Torrent)	5	9	9	9
Digital PCR	-	2	2	2
Acrylamide Gel Electrophoresis (PAGE)	2	1	1	2
Microfluidics Chip	1	1	-	-

Journal Reference for Assay

	Returns
van Dongen JJ et al. Leukemia. 1999 Dec;13(12):1901-28	66
Gabert J et al. Leukemia. 2003 Dec;17(12):2318-57	58
Cross NC et al Leukemia. 1994 Jan;8(1):186-9	26
Beillard et al. Leukemia 2003 Dec; 17 (12): 2474-86	14
Burmeister et al., Leuk Res. 2008 Apr;32(4):579-85.	9
Ruminy, P. et al. (2016) Leukemia, 30(3):757-60	7
Pallisgaard, N et al., (1998) Blood, 92(2):574-588	4
Emig M et al. Leukemia. 1999 Nov;13(11):1825-32	3
Evans et al., Leukemia 9: 1285-1286, 1995	3
Maurer et al., Lancet 337:1055-1058, 1991	3

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Sample BCR 182

In line with sample formulation, 183 of the 185 participants (98.9%) performing appropriate testing and returning a result for this sample correctly reported BCR 182 as being negative for a *BCR*::*ABL1* rearrangement.

The two participants that reported a false positive result for sample BCR 182 used either an in-house nested PCR approach with agarose gel electrophoresis, or the Diatech Pharmacogenetics Easy kit.

Sample AML 183

Sample AML 183 was formulated to be positive for a t(8:21) *RUNX1*::*RUNX1T1* rearrangement and negative for inv(16) *CBFB*::*MYH11* and t(15;17) *PML*::*RARA* rearrangements.

Of the 162 participants returning a result for t(8:21) *RUNX1*::*RUNX1T1,* 159 (98.1%) correctly identified sample AML 183 as being positive for this rearrangement. Three laboratories returned an out-of-consensus false negative result; of these, two used the EAC protocol (real-time PCR with fluorescent detection). The remaining laboratory (utilising multiplex PCR with capillary electrophoresis) also submitted a false positive result for t(15;17) *PML*::*RARA* analysis, potentially indicating a clerical error during data entry.

All participants returning results for inv(16) *CBFB*::*MYH11* (n = 159) correctly returned a negative result, and with the exception of the laboratory discussed above, all remaining laboratories performing t(15;17) *PML*::*RARA* testing (169/170, 99.4%), also correctly submitted a negative result.

Please note, for this programme samples are formulated to reflect the levels of fusion gene rearrangement typically identified at patient presentation.

Further Remarks

We remind participants to please test each sample for all the relevant assays available in their laboratory repertoire. For example, if a t(8;21) *RUNX1*::*RUNX1T1* transcript is detected in the AML sample, please do continue to analyse the sample for t(15;17) *PML*::*RARA* and inv(16) *CBFB*::*MYH11* rearrangements wherever possible. We acknowledge this approach may not reflect your strategy for a clinical case, however it is important all results are returned for trial scoring purposes.

If you have not already informed us of the relevant assays offered by your laboratory, please email this information to admin@uknegasli.co.uk, thus avoiding a future inappropriate non-consensus result designation, and the associated adverse impact on your laboratory performance status.

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 quality control (QC) thresholds (email: repeatsamples@uknegasli.co.uk). We recommend that participants

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

Final Comments

The persistent presence of *RUNX1*::*RUNX1T1*, *CBFB*::*MYH11* or *PML*::*RARA* fusion genes in patients with AML has demonstrated that these are stable markers enabling molecular assessment of measurable residual disease (MRD)[1]. For participants interested in EQA for MRD assessment using these AML rearrangements (as well as the canonical NM_002520.7(NPM1):c.860_863dup (type A) variant), UK NEQAS LI have recently developed a new pilot programme, 'Acute Myeloid Leukaemia Measurable Residual Disease by Molecular Methods'[2]. If participants require further information about this programme, please contact admin@ukneqasli.co.uk.

Finally, we would like to thank laboratories for their continued participation in the *BCR*::*ABL1* and AML Translocation Identification Programme.

References

1. Schuurhuis, G.J. et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 131(12), 1275-1291 (2018).

2. Scott, S. et al. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study. Blood Adv. (2023). doi: https://doi.org/10.1182/bloodadvances.2022009379

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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: 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 some 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