BCR::ABL1 and AML Translocation Identification Programme

Distribution - 232403

Date Issued - 07 December 2023 Closing Date - 12 January 2024

Trial Comments

This trial was issued to 199 participants, of which 194 (97.5%) returned results. Of the five non-returns, two laboratories requested an extension to the results submission period.

Sample Comments

Two lyophilised cell line preparations were distributed for analysis: samples BCR 186 and AML 187. Subject to their laboratory repertoire, participants were requested to analyse sample BCR 186 for the presence of a BCR::ABL1 rearrangement and to analyse sample AML 187 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 186 was manufactured to express the e13a2 major BCR::ABL1 transcript and sample AML 187 was manufactured to contain a t(15;17) PML::RARA rearrangement. Please note that it is the participant's responsibility to ensure we have details of their current test repertoire†. A centre will not be penalised for failure to detect a transcript when we are aware that the laboratory does not perform the relevant investigation. A further sample, Edu C, was also included in this trial. Participants were invited to test Edu C for KMT2A rearrangement, however participation was optional and not subject to performance monitoring. Edu C was manufactured to be positive for the KMT2A::MLLT3 fusion transcript.

Results and Performance

Your Results

Identification	Your Results	Consensus Result
Sample 186		
BCR-ABL1 t(9;22)	Rearrangement Detected	Rearrangement Detected
Sample 187		
CBFB-MYH11 Inv(16)	No Rearrangement Detected	No Rearrangement Detected
RUNX1-RUNX1T1 t(8;21)	No Rearrangement Detected	No Rearrangement Detected
PML-RARA t(15;17)	Rearrangement Detected	Rearrangement Detected

All Participant Results

	Rearrangement Detected (Returns)	No Rearrangement Detected (Returns)
Sample 186		
BCR-ABL1 t(9;22)	191	1
Sample 187		
CBFB-MYH11 Inv(16)	0	160
RUNX1-RUNX1T1 t(8;21)	0	163
PML-RARA t(15;17)	171	4

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	65	28	28	32
Hemavision Kit	11	25	26	25
Biomed 1	26	27	25	26
EAC Protocol	12	16	18	19
Biotype Diagnostic GmbH Mentype AMLplex	8	14	14	13
Diatech EasyPGX ready AML1-ETO Fusion	-	-	10	-
Modified EAC Protocol	12	8	9	9
Qiagen Ipsogen RUNX1-RUNX1T1 Kit	-	-	9	-
Oncomine Myeloid Research Assay	5	6	6	7
Diatech Pharmacogenetics Easy Kit	7	3	3	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	2	2	2	2
Leukemia Fusion Gene (Q30) QuanDx kit	-	1	1	1
Archer FusionPlex Myeloid Kit	1	1	1	1
Genmark geneMAP Screening Kit	3	1	1	2
Illumina TruSeq Stranded Total RNA	1	1	1	1
Illumina TruSight RNA Fusion Panel	1	1	1	1
Qiagen QiaSeq Targeted RNAScan	1	1	1	1
Entrogen Kit	-	-	-	1
Qiagen Ipsogen BCR-ABL1 Mbcr RGQ RT-PCR Kit	2	-	-	-
Qiagen Ipsogen BCR-ABL1 Mbcr IS-MMR Kits CE	5	-	-	-
Qiagen Ipsogen PML-RARA bcr1 Kit CE	-	-	-	10
Invivoscribe PML RARA Kit	-	-	-	1
Qiagen Ipsogen BCR-ABL1 Mbcr Kit CE	3	-	-	-
Qiagen Ipsogen CBFB-MYH11 A Kit	-	8	-	-
Cepheid GeneXpert Ultra BCR-ABL assay	1	-	-	-
Diatech EasyPGX ready BCR-ABL Fusion	13	-	-	-
Bioclarma SensiQuant One-Step	1	-	-	-
AB Analitica Real Quality One-Step	-	-	-	1
Generi Biotech gb ONCO BCR-ABL DETECT	2	-	-	-
Diatech EasyPGX ready CBFB-MYH11 Fusion	-	10	-	-
Diatech EasyPGX ready PML-RARA Fusion	-	-	-	10
3B BlackBio TRUPCR BCR-ABL1 Qualitative Kit	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	75	66	71	76
Multiplex PCR	59	36	35	35
Single PCR	27	24	25	24
PCR for Next generation Sequencing	14	17	17	18
Nested PCR	15	17	15	22

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	87	80	84	92
Agarose Gel Electrophoresis	63	39	36	40
Capillary Electrophoresis	18	17	19	19
NGS (Illumina)	10	11	11	11
NGS (ThermoFisher Ion Torrent)	5	7	7	8
Acrylamide Gel Electrophoresis (PAGE)	3	2	2	2
Digital PCR	-	2	2	2
Microfluidics Chip	2	1	-	-

Journal Reference for Assay

	Returns
van Dongen JJ et al. Leukemia. 1999 Dec;13(12):1901-28	62
Gabert J et al. Leukemia. 2003 Dec;17(12):2318-57	57
Cross NC et al Leukemia. 1994 Jan;8(1):186-9	22
Beillard et al. Leukemia 2003 Dec; 17 (12): 2474-86	16
Burmeister et al., Leuk Res. 2008 Apr;32(4):579-85.	7
Pallisgaard, N et al., (1998) Blood, 92(2):574-588	5
Emig M et al. Leukemia. 1999 Nov;13(11):1825-32	4
Ruminy, P. et al. (2016) Leukemia, 30(3):757-60	4
Chen Z et al. PLoS One. 2015 Mar;10(3): e0122530	2
Evans et al., Leukemia 9: 1285-1286, 1995	2
Maurer et al., Lancet 337:1055-1058, 1991	2

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Sample BCR 186

In line with sample formulation, 191 of the 192 participants (99.5%) performing appropriate testing and returning a result for this sample correctly reported BCR 186 as being positive for *BCR*::*ABL1* rearrangement.

Of the 191 laboratories that detected a *BCR*::*ABL1* transcript in sample BCR 186, 180 provided interpretable details of the transcript type detected: 162 participants (90.0%) participants detected a major (p210) *BCR*::*ABL1* transcript, three (1.7%) detected a minor (p190) *BCR*::*ABL1* transcript, ten (5.6%) detected both the major (p210) and minor (p190) *BCR*::*ABL1* transcripts, and five (2.8%) detected >2 *BCR*::*ABL1* transcript types. Of those 162 participants reporting a major transcript, 118 further specified the transcript type: 115 participants reported that an e13a2 (b2a2) major transcript had been detected; conversely three reported the presence of the e14a2 (b3a2) major transcript.

A single participant reported an out-of-consensus negative result for sample BCR 186. This centre used the EAC protocol (real time PCR with fluorescent detection).

Sample AML 187

Sample AML 187 was formulated to be positive for the t(15;17) *PML*::*RARA* rearrangement, and negative for the inv(16) *CBFB*::*MYH11* and t(8:21) *RUNX1*::*RUNX1T1* rearrangements.

One hundred and seventy-one of the 175 participants (97.7%) returning a result for t(15;17) *PML*::*RARA* correctly reported a positive result. One of the four laboratories reporting an out-of-consensus negative result used the EAC protocol and also reported the out-of-consensus negative result for sample BCR 186, potentially indicating a sample transposition error. A further two out-of-consensus results were reported by participants using the Biomed 1 protocol (one employing a single PCR approach followed by agarose gel electrophoresis, the other employing a nested PCR approach followed by capillary electrophoresis), and the final participant used the Hemavision kit (real time PCR with fluorescent detection).

All participants returning results for inv(16) *CBFB*::*MYH11* (n=160) and t(8:21) *RUNX1*::*RUNX1T1* (n=163) correctly returned a negative result.

Further Remarks

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

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 now offer a pilot programme, 'Acute Myeloid Leukaemia Measurable Residual Disease by Molecular Methods'[2]. If participants require further information about this programme, please contact admin@uknegasli.co.uk.

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Educational Sample and *KMT2A* survey

In December 2021 a participant survey was included in the 212203 trial of this programme (see report issued 7 Mar 2022). This identified several gene rearrangements associated with myeloid neoplasms for which it would be desirable to have EQA provision. To this end we have recently sourced a number of additional cell lines, and as part of their validation, these cell lines will initially be featured in optional, educational (non-scored) samples. This trial included an educational sample with a *KMT2A*::*MLLT3* fusion (Edu C), as well as a short survey regarding laboratories' testing of *KMT2A* rearrangements.

Eighty-one participants responded to the current survey, with 62 indicating that their laboratory standardly offers *KMT2A* rearrangement testing. The table below reveals the context in which *KMT2A* rearrangement testing is offered by these laboratories:

Context of *KMT2A* testing	Number of participants (%)
Diagnosis only	31 (50.0)
Diagnosis, follow-up and MRD	16 (25.8)
Diagnosis and follow-up	10 (16.1)
Diagnosis and MRD	3 (4.8)
Diagnosis and relapse	1 (1.6)
Diagnosis (MRD in validation)	1 (1.6)

Fifty-six participants (90.3%) indicated that their assay allowed them to determine the partner gene rearranged with *KMT2A*. Participants were also asked which of the common *KMT2A* fusions their assay is able to detect and these results are summarised in the table below. Please note that two participants indicated that their assay does not allow identification of the partner gene, but also provided details of the rearrangements their assay will detect. Nine participants stated that their assay is able to detect all/multiple (e.g. >50) *KMT2A* fusions.

KMT2A Fusion	Number of participants
KMT2A::*AFF1*	47
KMT2A::*AFDN* (*MLLT4*)	45
KMT2A::*ELL*	51
KMT2A::*MLLT1*	40
KMT2A::*MLLT3*	57
KMT2A::*MLLT10*	40

Of the 62 centres standardly offering *KMT2A* rearrangement testing, 15 (24.2%) quantify the fusion transcript, and of these, 13 include quantification in their clinical reports.

Educational sample Edu C

Sixty-three participants tested sample Edu C. In line with formulation, 61/63 (96.8%) detected a *KMT2A* rearrangement. All 61 were able to determine the partner gene, and in line with sample formulation, all correctly reported the presence of a *KMT2A*::*MLLT3* rearrangement. Eight participants indicated that two isoforms were detected. Please note that one participant used the alternative alias *MLL-AF9* to refer to *KMT2A*::*MLLT3*. We recommend that participants utilise the official gene symbols approved by the HUGO Gene Nomenclature Committee (HGNC, https://www.genenames.org/).

Two laboratories submitted an out-of-consensus negative result: one participant used an in-house single PCR assay followed by agarose gel electrophoresis but stated that they do not standardly offer this testing and the other participant only provides testing for *KMT2A* partial tandem duplications and therefore would not necessarily be expected to detect a *KMT2A*::*MLLT3* rearrangement.

Quantification of Edu C

Seven laboratories submitted a % normalised ratio for sample Edu C (copies of *KMT2A* fusion/copies of reference gene x100). There was considerable variation in the results, with values ranging from 7-100%. In addition, some participants indicated methodologies inconsistent with reporting a % normalised ratio. These issues, together with the small size of the data set, preclude the publication of descriptive statistics for quantification of the *KMT2A* fusion transcript in sample Edu C.

Edu C final remarks

In agreement with the 212203 survey, there appears to be sufficient demand for *KMT2A* diagnostic testing EQA provision. Qualitative results for the educational sample Edu C demonstrated good consensus, confirming the cell line used is suitable for this purpose. UK NEQAS LI will now look towards incorporating *KMT2A*::*MLLT3* positive samples into our programme repertoire. Further details will be provided when appropriate.

Finally, we would like to thank laboratories for their continued participation in the *BCR*::*ABL1* and AML Translocation Identification Programme, and particularly those participants who have engaged with the optional educational sample, Edu C.

References

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

2. Scott, S. *et al*. Assessment of acute myeloid leukemia molecular measurable residual disease testing in an interlaboratory study (2023) *Blood Adv* **7**(14): 3686-3694.

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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