

BCR::ABL1 and AML Translocation Identification Programme

Distribution - 252601

Participant ID -

Date Issued - 23 April 2025

Closing Date - 23 May 2025

Trial Comments

This trial was issued to 203 participants, of which 198 (97.5%) returned results. Of the five non-returns, one participant prenotified UK NEQAS LI of their intention not to return results.

Sample Comments

Two lyophilised cell line preparations were distributed for analysis: samples BCR 194 and AML 195. Subject to their laboratory repertoire, participants were requested to analyse sample BCR 194 for the presence of a BCR::ABL1 rearrangement and to analyse sample AML 195 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 194 was manufactured to express the e1a2 minor BCR::ABL1 transcript and sample AML 195 was manufactured to be negative for all three AML-associated rearrangements. 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.

Results and Performance

Your Results

Identification	Your Results	Consensus Result
Sample 194		
BCR-ABL1 t(9;22)	Rearrangement Detected	Rearrangement Detected
Sample 195		
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)	No Rearrangement Detected	No Rearrangement Detected

All Participant Results

	Rearrangement Detected (Returns)	No Rearrangement Detected (Returns)
Sample 194		
BCR-ABL1 t(9;22)	188	7
Sample 195		
CBFB-MYH11 Inv(16)	0	167
RUNX1-RUNX1T1 t(8;21)	0	169
PML-RARA t(15;17)	0	179

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	66	36	38	36
Hemavision Kit	9	23	23	22
Biomed 1	19	22	20	21
EAC Protocol	12	15	17	17
Biotype Diagnostic GmbH Mentype AMLplex	12	17	17	17
Oncomine Myeloid Research Assay	8	10	10	10
Diatech EasyPGX ready AML1-ETO Fusion	-	-	9	-
Qiagen Ipsogen RUNX1-RUNX1T1 Kit	-	-	7	-
Modified EAC Protocol	10	6	6	7
Diatech Pharmacogenetics Easy Kit	8	5	4	7
Entrogen Kit	1	3	3	3
Ampliseq for Illumina RNA Fusion	2	3	3	3
Tib Molbiol LightMix kit	3	2	2	3
Bioclarma SensiQuant One-Step	3	2	2	2
Biomed 3	-	1	1	1
BioRad PrimePCR ddPCR kit	2	1	1	3
Leukemia Fusion Gene (Q30) QuanDx kit	-	1	1	1
Archer FusionPlex Myeloid Kit	1	1	1	2
Archer FusionPlex Heme Kit	-	1	1	-
Qiagen QiaSeq Targeted RNAScan	1	1	1	1
Genmark geneMAP Detection Kit	2	1	1	3
Roche LightCycler BCR-ABL1 Quantification Kit	1	-	-	-
Qiagen Ipsogen BCR-ABL1 Mbcr RGQ RT-PCR Kit	1	-	-	-
Qiagen Ipsogen BCR-ABL1 mbcr Kit CE	2	-	-	-
Qiagen Ipsogen PML-RARA bcr1 Kit CE	-	-	-	6
Invivoscribe PML RARA Kit	-	-	-	1
Qiagen Ipsogen BCR-ABL1 Mbcr Kit CE	2	-	-	-
Qiagen Ipsogen CBFB-MYH11 A Kit	-	6	-	-
Cepheid GeneXpert Ultra BCR-ABL assay	3	-	-	-
3B BlackBio TRUPCR BCR-ABL1 Kit	2	-	-	-
Genmark geneMAP Screening Kit	1	-	-	-
Diatech EasyPGX ready BCR-ABL Fusion	19	-	-	-
Generi Biotech gb ONCO BCR-ABL DETECT	4	-	-	-
Diatech EasyPGX ready CBFB-MYH11 Fusion	-	9	-	-
Diatech EasyPGX ready PML-RARA Fusion	-	-	-	12
TRUPCR BCR ABL Qualitative qPCR Kit	1	-	-	-

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PCR Type

	Returns			
	<i>BCR-ABL1</i> t(9;22)	<i>CBFB-MYH11</i> Inv(16)	<i>RUNX1-RUNX1T1</i> t(8;21)	<i>PML-RARA</i> t(15;17)
Real-Time PCR	82	71	75	82
Multiplex PCR	64	40	38	38
Single PCR	21	20	21	22
PCR for Next generation Sequencing	15	21	21	20
Nested PCR	14	15	14	17

Analysis Type

	Returns			
	<i>BCR-ABL1</i> t(9;22)	<i>CBFB-MYH11</i> Inv(16)	<i>RUNX1-RUNX1T1</i> t(8;21)	<i>PML-RARA</i> t(15;17)
Real-Time PCR Fluorescent Detection	93	82	88	98
Agarose Gel Electrophoresis	52	36	32	33
Capillary Electrophoresis	30	20	22	20
NGS (ThermoFisher Ion Torrent)	8	12	12	12
NGS (Illumina)	8	11	11	10
Digital PCR	2	2	2	4
Acrylamide Gel Electrophoresis (PAGE)	1	1	1	1
Microfluidics Chip	1	1	-	-

Journal Reference for Assay

	Returns
van Dongen JJ et al. Leukemia. 1999 Dec;13(12):1901-28	60
Gabert J et al. Leukemia. 2003 Dec;17(12):2318-57	58
Cross NC et al Leukemia. 1994 Jan;8(1):186-9	28
Beillard et al. Leukemia 2003 Dec; 17 (12): 2474-86	18
Burmeister et al., Leuk Res. 2008 Apr;32(4):579-85.	9
Ruminy, P. et al. (2016) Leukemia, 30(3):757-60	6
Pallisaard, N et al., (1998) Blood, 92(2):574-588	4
Chen Z et al. PLoS One. 2015 Mar;10(3): e0122530	3
Emig M et al. Leukemia. 1999 Nov;13(11):1825-32	3
Tong et al. (2018) Leuk Res 69:47-53	2

***BCR::ABL1* and AML Translocation Identification Programme**

Sample BCR 194

In line with sample formulation, 188 (96.4%) of the 195 participants performing appropriate testing and returning a result for this sample correctly reported BCR 194 as being positive for *BCR::ABL1* rearrangement.

Of the 188 laboratories that detected a *BCR::ABL1* transcript in sample BCR 194, 178 provided interpretable details of the transcript type detected: 169 participants (94.9%) detected a minor (p190) *BCR::ABL1* transcript, three (1.7%) detected both the major (p210) and minor (p190) *BCR::ABL1* transcripts and six participants (3.4%) appeared to detect >2 *BCR::ABL1* transcript types, however it is possible that these laboratories were describing the scope of their assay, rather than which transcript was detected. Of the 169 participants detecting a p190 minor transcript, 125 provided further details: 121 specified e1a2, two specified b1a2 and two specified e1a2/e1a3 had been detected.

Eight participants reported an out-of-consensus negative result for sample BCR 194, however one of the eight has previously informed UK NEQAS LI that that testing for the minor (p190) *BCR::ABL1* transcript is not included in their assay repertoire[†]. This centre was designated as a prenotified non-return for this sample. Therefore, seven laboratories (3.6%) returned a false negative result for sample BCR 194. Four participants used a real-time PCR assay with fluorescent detection: either the Diatech EasyPGX ready BCR-ABL Fusion kit (n=2), the Genmark geneMAP Detection kit (n=1) or the Roche LightCycler BCR-ABL1 Quantification kit (n=1). Two participants utilised an in-house nested PCR assay with agarose gel electrophoresis, and one employed the EAC protocol (multiplex PCR followed by capillary electrophoresis).

Sample AML 195

Sample AML 195 was formulated to be negative for each of the inv(16) *CBFB::MYH11*, t(8;21) *RUNX1::RUNX1T1* and t(15;17) *PML::RARA* rearrangements associated with AML.

In line with sample formulation, all participants returning a result for inv(16) *CBFB::MYH11* (n=167), t(8;21) *RUNX1::RUNX1T1* (n=169) and t(15;17) *PML::RARA* (n=179) correctly returned a negative result.

***BCR::ABL1* and AML Translocation Identification Programme**

Further Remarks

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

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) and *FLT3* internal tandem duplication variants), UK NEQAS LI 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.

Finally, we thank laboratories for their continued participation in the *BCR::ABL1* and AML Translocation Identification Programme and welcome any suggestions for programme improvement or development.

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* **7**(14): 3686-3694 (2023).

BCR::ABL1 and AML Translocation Identification 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 coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (acting Director).

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

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited. Pre issue and 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. 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/NjRINTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFIZTg3. 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/>