Measurable Residual Disease for AML by Molecular Methods (Not Accredited)

t(8;21) *RUNX1*::*RUNX1T1*

Distribution - 222302
Date Issued - 01 March 2023

Participant - 43347
Closing Date - 31 March 2023

Trial Comments
This trial was issued to 74 participants. Six participants did not return results. A further four participants were pre-notified non-returns (PNNR). 54 participants returned results for t(8;21) *RUNX1*::*RUNX1T1*.

Sample Comments
Three vials of lyophilised cell line material, samples 037, 038 and 039 were issued to 74 participants for quantitative t(8;21) *RUNX1*::*RUNX1T1* analysis. Samples 038 and 039 were manufactured to be positive for the t(8;21) *RUNX1*::*RUNX1T1* transcript, mimicking measurable residual disease (MRD) levels seen following treatment in acute myeloid leukaemia (AML). Samples 037 was manufactured to be negative for the t(8;21) *RUNX1*::*RUNX1T1* transcript.

Results and Performance

Table 1: Your Results

	Sample 037	Sample 038	Sample 039
Your qualitative result	Negative	Positive	Positive
Consensus qualitative result	Negative	Positive	Positive
Your % *RUNX1*::*RUNX1T1* / reference gene	0	2.5126	4.7594
Median % *RUNX1*::*RUNX1T1* / *ABL1* gene*	n/a	2.3	4.2
Lower quartile*	n/a	1.8	3.4
Upper quartile*	n/a	2.9	5.8
Inter quartile range (IQR)*	n/a	1.2	2.4
Your log change between sample 038 and 039	0.28		
Robust mean log change between sample 038 and 039	0.28		
Robust SD log change between sample 038 and 039	0.09		
Your z score (for educational purposes only)**	0.00		

* Due to the differences in expression levels of the range of reference (control) genes used, results from different reference genes cannot be meaningfully compared. Therefore, we have only calculated median sample results and quartile values for participants using *ABL1* as the reference gene.

**The z score value (calculated from the $\log_{10}$ change between samples 038 and 039) is for educational purposes only and is not formally scored for this trial.

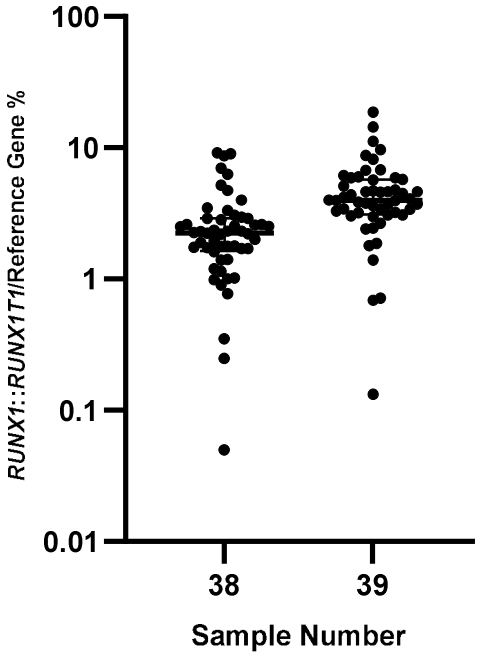

Figure 1: Scatter plot of % *RUNX1*::*RUNX1T1*/reference gene results for samples 038 and 039 submitted by all participants.

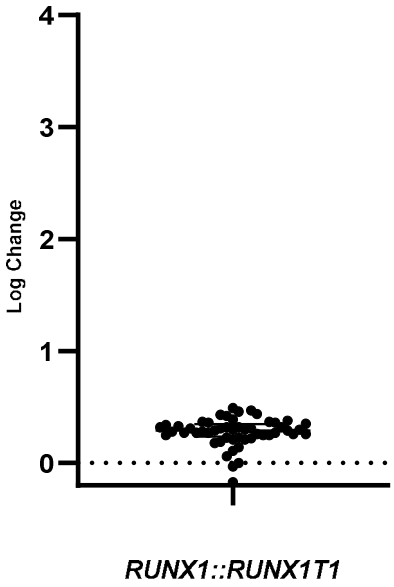

Figure 2: Plot to demonstrate calculated log change between samples 038 and 039 for each participant

Method Breakdown

The information in these tables is based on data provided from participants returning qualitative results at a minimum. Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Table 2: Reference gene* summary

	Number of Participants
ABL1	48
GUSB	4
B2M	1
HMBS	1

* HUGO Gene Nomenclature Committee (HGNC) approved gene names (www.genenames.org/)

Table 3: *ABL1* copy number

	Sample 037	Sample 038	Sample 039
n	44	44	44
Median	106,421	100,783	107,500
Lower Quartile	47,307	45,422	47,275
Upper Quartile	202,950	185,564	219,798
Inter Quartile Range (IQR)	155,643	140,142	172,523
Min	2	2	2
Max	1,029,832	1,174,927	1,327,180

Table 4: PCR Type

	Number of Participants
Real-Time PCR	49
Digital PCR	3
Nested PCR	2

Table 5: Assay protocol

	Number of Participants
EAC Protocol	19
Qiagen Ipsogen RUNX1-RUNX1T1 Kit	19
In-house Assay	10
Modified EAC Protocol	3
Biomed	1
Other	2

Table 6: Analysis Type

	Number of Participants
Real-Time PCR Fluorescent Detection	49
Digital PCR	3
Agarose Gel Electrophoresis	2

Table 7: Source of Standard Curve - *RUNX1*::*RUNX1T1*

	Number of Participants
Qiagen/Ipsogen	37
In-house calibrated to Qiagen/Ipsogen	5
No standard curve, dPCR	3
In-house	3
No standard curve, agarose gel	2
Delta Ct method, no standard curve	2

Table 8: Source of Standard Curve – Reference Gene

	Number of Participants
Qiagen/Ipsogen	31
ERM-AD623	6
In-house calibrated to Qiagen/Ipsogen	5
In-house	3
No standard curve, dPCR	3
No standard curve, agarose gel	2
No standard curve, Delta Ct method	2
Other	1

Trial Comments:

Sample 037

- Fifty laboratories classified the sample as suitable for analysis and four participants said the sample was suboptimal.
- Sample 037 was manufactured to be negative for the t(8;21) *RUNX1*::*RUNX1T1* transcript. 54/54 (100%) returning participants classified the samples as negative for the t(8;21) *RUNX1*::*RUNX1T1* transcript.

Sample 038

- Fifty-one laboratories classified the sample as suitable for analysis and three participants said the sample was suboptimal.
- Sample 038 was manufactured to be positive for the t(8;21) *RUNX1*::*RUNX1T1* transcript. 54/54 (100%) returning participants classified the sample as positive for the t(8;21) *RUNX1*::*RUNX1T1* transcript.
- The median %*RUNX1*::*RUNX1T1/ABL1* calculated from participant returns for sample 038 was 2.3% (n = 47).

Sample 039

- Fifty-one laboratories classified the sample as suitable for analysis and three participants said the sample was suboptimal.
- Sample 039 was manufactured to be positive for the t(8;21) *RUNX1*::*RUNX1T1* transcript. All participants returning results classified sample 039 as positive for the t(8;21) *RUNX1*::*RUNX1T1* transcript (n = 54).
- The median %*RUNX1*::*RUNX1T1/ABL1* calculated from participant returns for sample 039 was 4.2% (n=47).

Log Change

- The robust mean log change between sample 038 and 039, calculated from all participant returns was 0.28, with a robust SD = 0.09 (n = 52). Two participants had a log change >3.5 SDs from the robust mean. One reported both samples as suboptimal and the other used RTqPCR, the EAC protocol and Qiagen/Ipsogen standards.

Reference Genes

- Median *ABL1* reference gene levels were 106,421 for sample 037, 100,783 for sample 038 and 107,500 for sample 039.

Measurable Residual Disease for AML by Molecular Methods (Not Accredited)

inv(16) *CBFB*::*MYH11*

Distribution - 222302
Date Issued - 01 March 2023

Participant - 43347
Closing Date - 31 March 2023

Trial Comments
This trial was issued to 74 participants. Six participants did not return results. A further four participants were pre-notified non-return (PNNR). 56 participants returned results for inv(16) *CBFB::MYH11*.

Sample Comments
Three vials of lyophilised cell line material, samples 040, 041 and 042 were issued to 74 participants for quantitative *CBFB*::*MYH11* (Type A) analysis. Samples 040 and 042 were manufactured to be positive for the *CBFB*::*MYH11* (Type A) transcript, mimicking MRD levels seen following treatment in AML. Samples 041 was manufactured to be negative for the *CBFB*::*MYH11* (Type A) transcript.

Results and Performance

Table 9: Your Results

	Sample 040	Sample 041	Sample 042
Your qualitative result	Positive	Negative	Positive
Consensus qualitative result	Positive	Negative	Positive
Your % *CBFB*::*MYH11* / reference gene	0.0341	0	80.0992
Median % *CBFB*::*MYH11* / *ABL1**	0.020	n/a	65.0
Lower quartile*	0.013	n/a	41.5
Upper quartile*	0.031	n/a	82.0
Inter quartile range (IQR)*	0.019	n/a	40.5
Your log change between sample 040 and 042	3.37		
Robust mean log change between sample 040 and 042	3.46		
Robust SD log change between sample 040 and 042	0.19		
Your z score (for educational purposes only)**	-0.50		

* Due to the differences in expression levels of the range of reference (control) genes used, results from different reference genes cannot be meaningfully compared. Therefore, we have only calculated median sample results and quartile values for participants using *ABL1* as the reference gene.

**The z score value (calculated from the $\log_{10}$ change between samples 040 and 042) is for educational purposes only and is not formally scored for this trial.

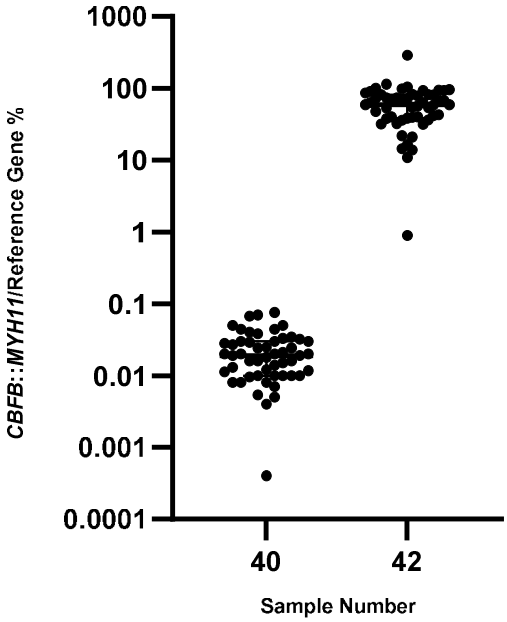

Figure 3: Scatter plot of % *CBFB*::*MYH11* (Type A)/reference gene results for samples 040 and 042 submitted by all participants.

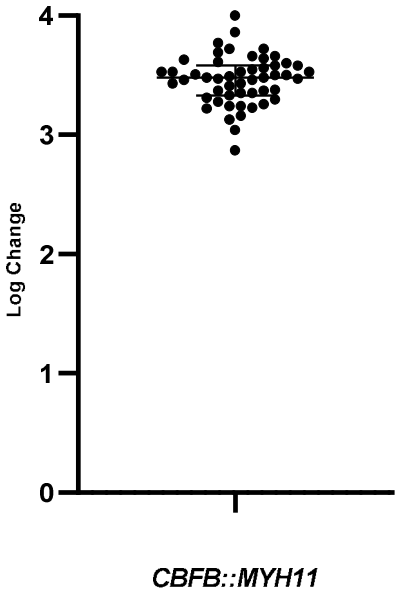

Figure 4: Plot to demonstrate the calculated log change between samples 040 and 042.

Method Breakdown

The information in these tables is based on data provided from participants returning qualitative results at a minimum. Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Table 10: Reference gene summary*

	Number of Participants
ABL1	50
GUSB	4
B2M	1
HMBS	1

* HUGO Gene Nomenclature Committee (HGNC) approved gene names (www.genenames.org/)

Table 11: *ABL1* copy number

	Sample 040	Sample 041	Sample 042
n=	46	46	45
Median	83,369	71,138	89,570
Lower Quartile	36,253	31,096	49,779
Upper Quartile	165,286	148,080	175,351
Inter Quartile Range (IQR)	129,033	116,985	125,572
Min	2	2	2
Max	1,150,938	1,125,544	766,306

Table 12: PCR Type

	Number of Participants
Real-Time PCR	52
Nested PCR	2
Digital PCR	2

Table 13: Assay protocol

	Number of Participants
EAC Protocol	20
Qiagen Ipsogen CBFB-MYH11 A Kit	19
In-house Assay	9
Modified EAC Protocol	4
Biomed 1	2
Other	2

Table 14: Analysis Type

	Number of Participants
Real-Time PCR Fluorescent Detection	51
Digital PCR	3
Agarose Gel Electrophoresis	2

Table 15: Source of Standard Curve – *CBFB*::*MYH11*

	Number of Participants
Qiagen/Ipsogen	40
In-house	4
In-house calibrated to Qiagen/Ipsogen	4
No standard curve, delta Ct method	2
No standard curve, agarose gel	2
No standard curve, digital PCR	2

Table 16: Source of Standard Curve – Reference Gene

	Number of Participants
Qiagen/Ipsogen	34
ERM-AD623	7
In-house calibrated to Qiagen/Ipsogen	4
No standard curve, dPCR	3
In-house	3
No standard curve, delta Ct method	2
No standard curve, agarose gel	2

Trial Comments:

Sample 040

- Fifty-four participants classified the sample as suitable for analysis and two participants said the sample was sub optimal for analysis.
- Sample 040 was manufactured to be positive for the inv(16) *CBFB*::*MYH11* transcript. Fifty-three participants (94.6%) classified the sample as positive for the inv(16) *CBFB*::*MYH11* transcript. Three participants identified the sample to be negative for the inv(16) *CBFB*::*MYH11* transcript. Two of the three participants who classified the sample as negative reported that the sample was suboptimal for analysis. The other participant used a RTqPCR, Qiagen Ipsogen CBFB-MYH11 A Kit and Qiagen/Ipsogen standards.
- The median % inv*(16) CBFB*::*MYH11/ABL1* calculated from participant returns was 0.020 (n = 47).

Sample 041

- Fifty-three participants classified the sample as suitable for analysis; three participants said the sample was sub optimal.
- Sample 041 was manufactured to be negative for the inv(16) *CBFB*::*MYH11* transcript.
- All participants returning results classified the samples as negative for the inv(16) *CBFB*::*MYH11* transcript (n = 56).

Sample 042

- Fifty-two participants classified the sample as suitable for analysis; three participants said the sample was sub optimal and one participant said the sample was not suitable for analysis.
- Sample 042 was manufactured to be positive for the inv(16) *CBFB*::*MYH11* transcript. All participants returning a result classified the sample as positive for an inv(16) *CBFB*::*MYH11* transcript (n=55).
- The median inv(16) *CBFB*::*MYH11* transcript/*ABL1* calculated from participant returns for sample 042 was 65.0 (n = 48).

Log Change

- The robust mean log change between sample 040 and 042, calculated from all participant returns was 3.46, with a robust SD = 0.19 (n = 51).
- No participants had a z-score >3.5.

Reference Genes

- Median *ABL1* reference gene levels were 83,369 for sample 040, 71,138 for sample 041 and 89,570 for sample 042.

Measurable Residual Disease for AML by Molecular Methods (Not Accredited)

t(15;17) *PML*::*RARA*

Distribution - 222302
Date Issued - 01 March 2023

Participant - 43347
Closing Date - 31 March 2023

Trial Comments
This trial was issued to 74 participants. Six participants did not return results. A further four participants were pre notified non-returns (PNNR). 55 participants returned results for t(15;17) *PML*::*RARA* (BCR1, L form).

Sample Comments
Three vials of lyophilised cell line material, samples 043, 044 and 045 were issued to 74 participants for quantitative t(15;17) *PML*::*RARA* analysis. Samples 043 and 044 were manufactured to be positive for the t(15;17) *PML*::*RARA* transcript, mimicking MRD levels seen following treatment in AML. Sample 045 was manufactured to be negative for the t(15;17) *PML*::*RARA* transcript.

Results and Performance

Table 17: Your Results

	Sample 043	Sample 044	Sample 045
Your qualitative result	Positive	Positive	Negative
Consensus qualitative result	Positive	Positive	Negative
Your % *PML*::*RARA* / reference gene	14.0224	0.0435	0
Median % *PML*::*RARA* / *ABL1**	7.0	0.024	n/a
Lower quartile*	4.5	0.015	n/a
Upper quartile*	10.6	0.033	n/a
Inter quartile range (IQR)*	6.1	0.018	n/a
Your log change between sample 043 and 044	-2.51		
Robust mean log change between sample 043 and 044	-2.51		
Robust SD log change between sample 043 and 044	0.15		
Your z score (for educational purposes only)**	0.02		

* Due to the differences in expression levels of the range of reference (control) genes used, results from different reference genes cannot be meaningfully compared. Therefore, we have only calculated median sample results and quartile values for participants using *ABL1* as the reference gene.

**The z score value (calculated from the $\log_{10}$ change between samples 043 and 044) is for educational purposes only and is not formally scored for this trial.

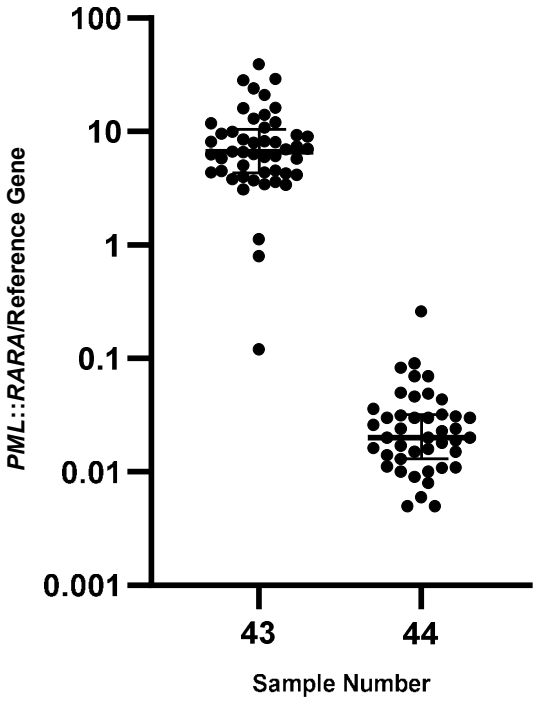

Figure 5: Scatter plot of % *PML::RARA*/reference gene results for samples 043 and 044 submitted by all participants

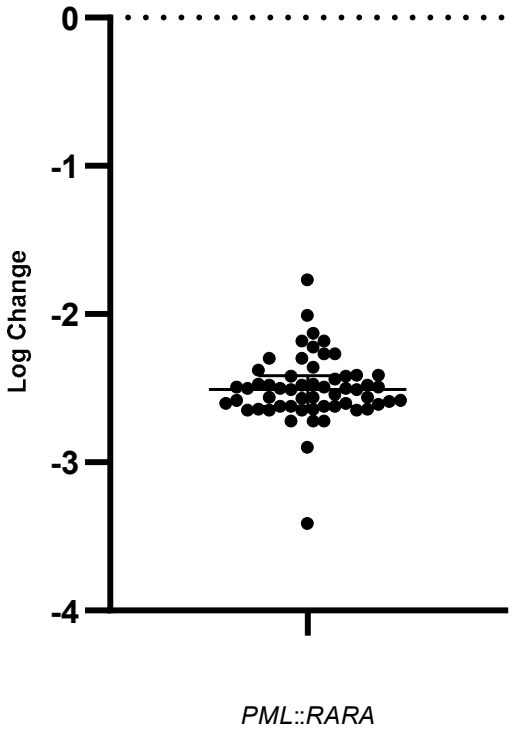

Figure 6: Plot to demonstrate the calculated log change between samples 043 and 044

Method Breakdown

The information in these tables is based on data provided from participants returning qualitative results at a minimum. Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Table 18: Reference gene summary*

	Number of Participants
ABL1	46
GUSB	4
HMBS	2
B2M	1

* HUGO Gene Nomenclature Committee (HGNC) approved gene names (www.genenames.org/)

Table 19: *ABL1* copy number

	Sample 043	Sample 044	Sample 045
N=	43	43	42
Median	68,500	95,903	70,181
Lower Quartile	26,967	27,131	34,486
Upper Quartile	200,798	204,007	191,270
Inter Quartile Range (IQR)	173,830	176,877	156,784
Min	2	2	2
Max	1,448,762	1,226,864	1,428,829

Table 20: PCR Type

	Number of Participants
Real-Time PCR	50
Nested PCR	4
Digital PCR	1

Table 21: Assay protocol

	Number of Participants
EAC Protocol	17
Qiagen Ipsogen PML-RARA bcr1 Kit CE	15
In-house Assay	13
Other	4
Modified EAC Protocol	3
Biomed 1	2
Invivoscribe PML RARA Kit	1

Table 22: Analysis Type

	Number of Participants
Real-Time PCR Fluorescent Detection	50
Digital PCR	2
Agarose Gel Electrophoresis	2
Capillary Electrophoresis	1

Table 23: Source of Standard Curve - *PML*::*RARA*

	Number of Participants
Qiagen/Ipsogen	33
In-house	6
In-house calibrated to Qiagen/Ipsogen	4
No standard curve, delta Ct method	3
No standard curve, other	3
No standard curve, dPCR	2
Bioclarma	1
SensiQuant PML/RARA Standard BIOCLARMA	1

Table 24: Source of Standard Curve – Reference Gene

	Number of Participants
Qiagen/Ipsogen	28
ERM AD623	6
In-house	5
In-house calibrated to Qiagen/Ipsogen	5
No standard curve, other	3
No standard curve, delta Ct method	2
No standard curve, dPCR	2
Bioclarma	1
SensiQuant PML/RARA Standard BIOCLARMA	1

Trial Comments:

Sample 043

- Fifty-one participants classified the sample as suitable for analysis; four participants said the sample was suboptimal.
- Sample 043 was manufactured to be positive for the t(15;17) *PML*::*RARA* transcript. Fifty-four participants classified the samples as positive for the t(15;17) *PML*::*RARA* transcript. One participant classified sample as negative. They classified the sample as suitable for analysis but provided an *ABL1* copy number of 2.
- The median % *PML*::*RARA*/*ABL1* calculated from participant returns was 7.0% (n=46).

Sample 044

- Fifty-one participants classified the sample as suitable for analysis; three participants said the sample was suboptimal; one participant said the sample was not suitable.
- Sample 044 was manufactured to be positive for the t(15;17) *PML*::*RARA* transcript. 46/54 (85.2%) of returning participants for this sample detected a t(15;17) *PML*::*RARA* transcript. 8/54 (14.8%) participants reported the sample to be negative for the t(15;17) *PML*::*RARA* transcript.
- Of the eight participants who classified the sample as negative, two classified the samples a suboptimal with one reporting ABL1 levels as 7800.
- Of the remaining six participants, five used RTqPCR with three participants utilising an in-house assay (each using different reference genes, ABL1, GUSB and B2M), two an Qiagen Ipsogen PML-RARA bcr1 Kit and one using the SensiQuant PML/RARA BIOCLARMA assay.
- The other participant used a nested PCR agarose gel approach.
- The median % *PML*::*RARA*/*ABL1* calculated from participant returns was 0.024% (n=40).

Sample 045

- Fifty-two participants classified he sample as suitable for analysis; three participants said the sample was suboptimal.
- Sample 045 was manufactured to be negative for the t(15;17) *PML*::*RARA* transcript. All participants returning results classified the sample as negative for the t(15;17) *PML*::*RARA* transcript (n=55).

Log Change

- The robust mean log change between sample 043 and 044, calculated from all participant returns was -2.51, with a robust SD = 0.15 (n = 43). Two participants had a result >3.5 SD from the robust mean. Both classified the positive samples as suitable for analysis, used RTqPCR and the EAC protocol.

Reference Genes

- Median *ABL1* reference gene levels were 68,500 for sample 043, 95,903 for sample 044 and 70,181 for sample 045.

Measurable Residual Disease for AML by Molecular Methods (Not Accredited)

NPM1 (Type A)

Distribution - 222302
Date Issued - 01 March 2023

Participant - 43347
Closing Date - 31 March 2023

Trial Comments
This trial was issued to 74 participants. Six participants did not return results. A further four participants were pre notified non-returns (PNNR). 56 participants returned results for *NPM1* (Type A).

Sample Comments
Three vials of lyophilised cell line material, samples 046, 047 and 048 were issued to 74 participants for quantitative *NPM1* (Type A) analysis. Samples 047 and 048 were manufactured to be positive for the *NPM1* (Type A) transcript, mimicking MRD levels seen following treatment in AML. Samples 046 was manufactured to be negative for the *NPM1* (Type A) transcript.

Results and Performance

Table 25: Your Results

	Sample 046	Sample 047	Sample 048
Your qualitative result	Positive	Positive	Positive
Consensus qualitative result	Negative	Positive	Positive
Your % *NPM1* (Type A) / reference gene	0.0142	9.622	2.7816
Median % *NPM1* (Type A) / *ABL1* *	n/a	5.1	1.1
Lower quartile*	n/a	3.2	0.6
Upper quartile*	n/a	6.8	1.3
Inter quartile range (IQR)*	n/a	3.6	0.7
Your log change between sample 047 and 048	-0.54		
Robust mean log change between sample 047 and 048	-0.70		
Robust SD Log change between sample 047 and 048	0.14		
Your z score (for educational purposes only)**	1.15		

* Due to the differences in expression levels of the range of reference (control) genes used, results from different reference genes cannot be meaningfully compared. Therefore, we have only calculated median sample results and quartile values for participants using *ABL1* as the reference gene.

**The z score value (calculated from the $\log_{10}$ change between samples 047 and 048) is for educational purposes only and is not formally scored for this trial.

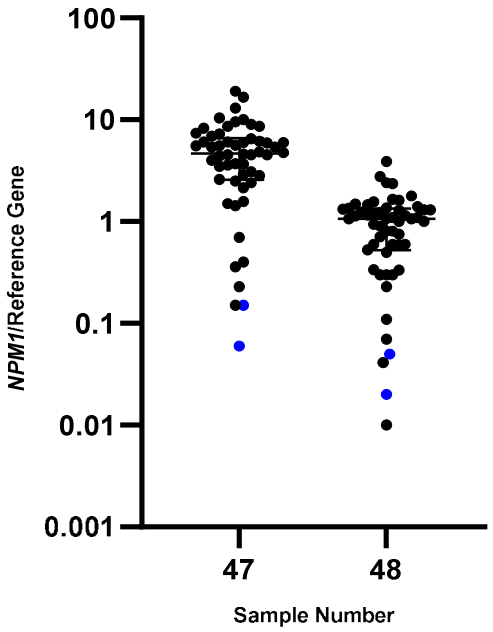

Figure 7: Scatter plot of *NPM1* (Type A)/reference gene results for samples 047 and 048 submitted by all participants. Participants normalising their results against NPM1 wildtype in blue.

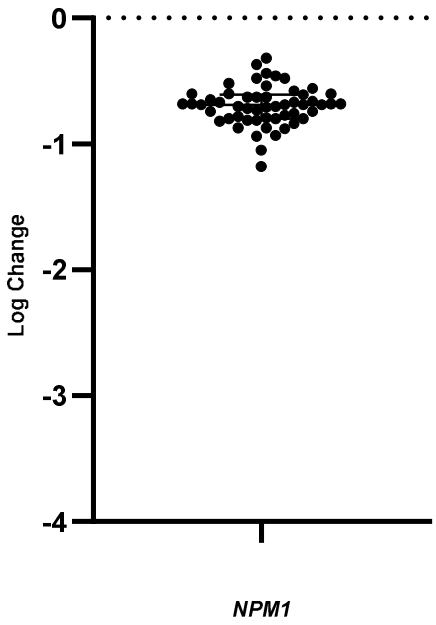

Figure 8: Plot to demonstrate the calculated log change between samples 047 and 048

Method Breakdown

The information in these tables is based on data provided from participants returning qualitative results at a minimum. Please note figures in the tables below may not tally with the total number of participants returning results due to some participants not returning all data requested or using multiple techniques.

Table 26: Reference gene summary*

	Number of Participants
ABL1	50
GUSB	1
NPM1 wildtype	4

* HUGO Gene Nomenclature Committee (HGNC) approved gene names (www.genenames.org/)

Table 27: *ABL1* copy number

	Sample 46	Sample 047	Sample 048
n=	48	48	48
Median	76,877	77,056	76,574
Lower Quartile	39,579	38,001	40,700
Upper Quartile	166,295	177,658	164,250
Inter Quartile Range (IQR)	126,716	139,657	123,550
Min	2	2	2
Max	733,027	622,227	695,442

Table 28: PCR Type

	Number of Participants
Real-Time PCR	50
Digital PCR	3
Single PCR	2
Other	1

Table 29: Assay protocol

	Number of Participants
In-house Assay	26
Qiagen NPM1 mut A, B & D MutaQuant Kits	15
Qiagen NPM1 mut A MutaQuant Kits	12
Other	3

Table 30: Analysis Type

	Number of Participants
Real-Time PCR Fluorescent Detection	52
Digital PCR	3
Next Generation Sequencing (Miseq)	1

Table 31: Source of Standard Curve – *NPM1*

	Number of Participants
Qiagen/Ipsogen	38
In-house calibrated to Qiagen/Ipsogen	5
In-house	3
No standard curve, dPCR	3
No standard curve, delta Ct method	2
No standard curve, other	2
In house calibrated to ERM AD623	1

Table 32: Source of Standard Curve – Reference Gene

	Number of Participants
Qiagen/Ipsogen	34
In-house calibrated to Qiagen/Ipsogen	5
ERM AD623	5
In-house	3
No standard curve, dPCR	3
No standard curve, delta Ct method	2
In-house calibrated to ERM AD623	1
In-house, NPM1 wt	1
No standard curve, other	1

Trial Comments:

Sample 046

- Fifty-four participants classified the sample as suitable for analysis and two participants said the sample was suboptimal.
- Sample 046 was manufactured to be negative for the *NPM1* (Type A) transcript. Fifty-one participants for this sample classified the sample as negative for the *NPM1* (Type A) transcript. Four participant's classified the sample as positive. All participants who classified the sample as positive said the sample was suitable for analysis. All used RTqPCR, utilising the Qiagen NPM1 mut A MutaQuant kit (n=2) or an in-house assay (n=2).

Sample 047

- Fifty-four participants classified the sample as suitable for analysis and two participants said the sample was suboptimal.
- Sample 047 was manufactured to be positive for the *NPM1* (Type A) transcript. All participants classified the sample as positive for the *NPM1* (Type A) transcript (n=56).
- The median *NPM1* (Type A)/*ABL1* calculated from participant returns for sample 047 was 5.1% (n = 50).

Sample 048

- Fifty-four participants classified the sample as suitable for analysis and two participants said the sample was suboptimal.
- Sample 048 was manufactured to be positive for the *NPM1* (Type A) transcript. All participants classified the sample as positive for the *NPM1* (Type A) transcript.
- The median *NPM1* (Type A)/*ABL1* calculated from participant returns for sample 047 was 1.1% (n = 50).

Log Change

- The robust mean log change between sample 047 and 048, calculated from all participant returns was -0.7, with a robust SD = 0.14 (n = 54).
- One participant's log change results were > 3.5 SDs from the robust mean using an in-house real-time PCR assay with in-house standards.

Reference Genes

- Median *ABL1* reference gene levels were 76,877 for sample 046, 77,056 for sample 047 and 76,574 for sample 048.

General comments

- Four participants tested the samples using NGS, dPCR or qPCR where they normalised the amount of *NPM1* Type A present against the *NPM1* wildtype, two of whom submitted quantitative data. This gave quantitative results that were an order of magnitude lower that those participants using RTqPCR as can be seen in

figure 7 (in blue). We expect the number of participants reporting *NPM1* MRD in this way to increase. Moving forward, we will optimise data entry for these participants and when there is sufficient data, we will calculate statistics bespoke to this group.

FLT3 ITD MRD testing

Table 33: Qualitative results for FLT3 ITD MRD

Analysis Type	n	Detection Rate		
		Edu A	Edu B	Edu C
Capillary Electrophoresis	16	8/16	2*/16	0/16
NGS (Illumina)	6	6/6	6/6	0/6
NGS (ThermoFisher Ion Torrent)	1	1/1	1/1	0/1
Agarose Gel Electrophoresis	1	1/1	1*/1	0/1
dPCR	1	1/1	1/1	0/1

*participants used cDNA as a template

Table 34: Quantitative results for FLT3 ITD MRD for participants using NGS

	Edu A	Edu B
n	7	7
Median VAF	0.14	0.028
25c VAF	0.13	0.018
75c VAF	0.23	0.036
IQR	0.11	0.019
Min VAF	0.07	0.011
Max VAF	0.35	0.050

Table 35: FLT3 ITD MRD log change between samples Edu A and Edu B MRD for participants using NGS and dPCR

	Log change
n	8
Median	0.98
25c	0.83
75c	1.00
IQR	0.17
Min	0.15
Max	1.10

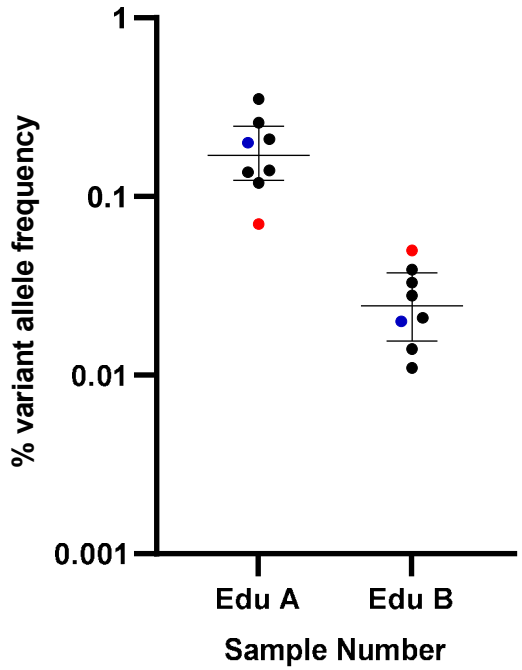

Figure 9: Scatter plot of *FLT3* ITD MRD VAF results for samples Edu A and Edu B submitted by all participants using NGS - Illumina (black marker), NGS - IonTorrent (red marker) and dPCR (blue marker)

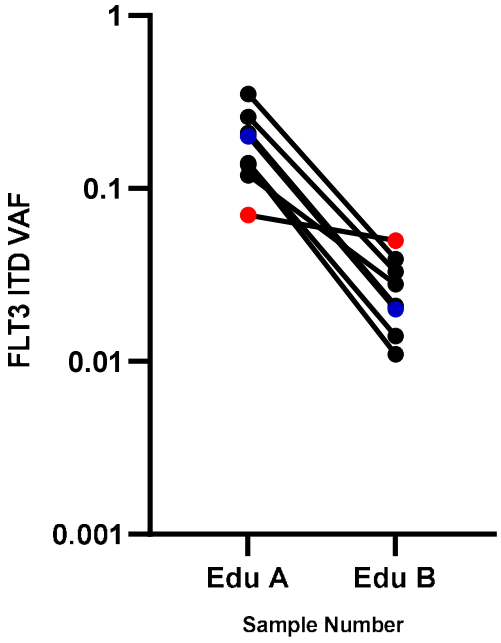

Figure 10: Scatter plot demonstrating the log change between participants *FLT3* ITD MRD VAF results for samples Edu A and Edu B submitted by all participants using NGS - Illumina (black marker), NGS - IonTorrent (red marker) and dPCR (blue marker)

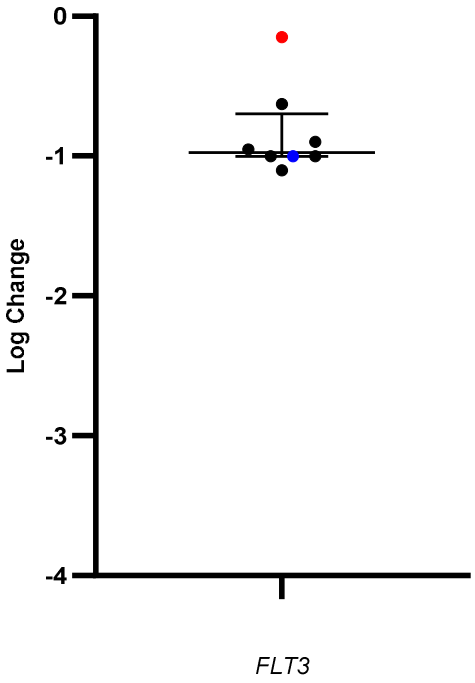

Figure 11: Plot to demonstrate the calculated log change between samples 047 and 048 submitted by all participants using NGS - Illumina (black marker), NGS - IonTorrent (red marker) and dPCR (blue marker)

Results Summary

- There have been several publications recently demonstrating the importance of *FLT3* ITD MRD testing in patients with AML (1–3). As such, in this round of EQA, UK NEQAS LI issued three samples for *FLT3* ITD MRD analysis.
- Of the 64 laboratories who returned results in the main trial, 25 participants returned results for the *FLT3* ITD MRD educational samples.
- Samples FLT3 Edu A and B were designed to be MRD positive, constituted of a *FLT3* ITD (30 bp) positive cell line at 0.2% and 0.02% in a *FLT3* ITD negative cell line background, respectively. Sample FLT3 ITD Edu C was designed to be MRD negative.
- The samples were designed to be tested by techniques sensitive enough to detect MRD. As such analysis has mainly been limited to results from participant using NGS and dPCR; capillary electrophoresis does not have an appropriate limit of detection for MRD analysis.
- All participants using these sensitive techniques correctly detected *FLT3* ITD MRD in the samples designed to be MRD positive and did not detect *FLT3* ITD MRD in the MRD negative samples.
- There was a generally good consensus in the *FLT3* ITD MRD VAF reported by participants; however, we acknowledge the limitations of this small data set.
- The majority of laboratories showed around a one log decrease in *FLT3* ITD MRD levels between samples Edu A and Edu B, in line with the expected difference from sample design. One laboratory showed very little difference between the *FLT3* ITD MRD levels in the two samples. This laboratory used a Thermofisher Ion Torrent Ion AmpliSeq Cancer Hotspot Panel v2 approach and did not provide a *FLT3* ITD MRD assay specific reference.

Reference(s)

1. Grob T, Sanders MA, Vonk CM, Kavelaars FG, Rijken M, Hanekamp DW, et al. Prognostic Value of FLT3 -Internal Tandem Duplication Residual Disease in Acute Myeloid Leukemia. J Clin Oncol [Internet]. 2022 Oct 31 [cited 2022 Nov 29];JCO2200715. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36315929
2. Loo S, Dillon R, Ivey A, Anstee NS, Othman J, Tiong IS, et al. Pretransplant FLT3-ITD MRD assessed by high-sensitivity PCR-NGS determines posttransplant clinical outcome. Blood [Internet]. 2022 Dec 1 [cited 2023 Jun 26];140(22):2407–11. Available from: https://dx.doi.org/10.1182/blood.2022016567
3. Dillon LW, Gui G, Page KM, Ravindra N, Wong ZC, Andrew G, et al. DNA Sequencing to Detect Residual Disease in Adults With Acute Myeloid Leukemia Prior to Hematopoietic Cell Transplant. JAMA [Internet]. 2023 Mar 7 [cited 2023 Jun 26];329(9):745–55. Available from: https://jamanetwork.com/journals/jama/fullarticle/2802059

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:
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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 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/

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