

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

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

Distribution - 222301
Date Issued - 08 August 2022

Participant -
Closing Date - 16 September 2022

Trial Comments

This trial was issued to 61 participants. Four participants did not return results. 49 participants returned results for t(8;21) *RUNX1::RUNX1T1*.

Sample Comments

Three vials of lyophilised cell line material, samples 025, 026 and 027 were issued to 61 participants for quantitative t(8;21) *RUNX1::RUNX1T1* analysis. Samples 025 and 027 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 026 was manufactured to be negative for the t(8;21) *RUNX1::RUNX1T1* transcript.

Results and Performance

Table 1: Your Results

| | Sample 025 | Sample 026 | Sample 027 |
|--|-------------------|-------------------|-------------------|
| Your qualitative result | Positive | Negative | Positive |
| Consensus qualitative result | Positive | Negative | Positive |
| Your % <i>RUNX1::RUNX1T1</i> / reference gene | 11.879 | 0 | 0.172 |
| Median % <i>RUNX1::RUNX1T1</i> / <i>ABL1</i> gene | 24.6 | n/a | 0.36 |
| Lower quartile | 16.9 | n/a | 0.25 |
| Upper quartile | 32.1 | n/a | 0.49 |
| Inter quartile range (IQR) | 15.2 | n/a | 0.24 |
| Your log change between sample 025 and 027 | -1.84 | n/a | -1.84 |
| Robust mean log change between sample 025 and 027 | -1.82 | n/a | -1.82 |
| Robust SD log change between sample 025 and 027 | 0.13 | n/a | 0.13 |

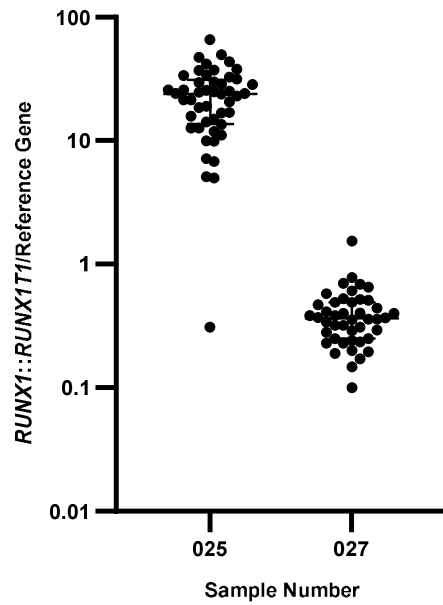


Figure 1: Scatter plot of % *RUNX1::RUNX1T1*/reference gene results for samples 025 and 027 submitted by all participants

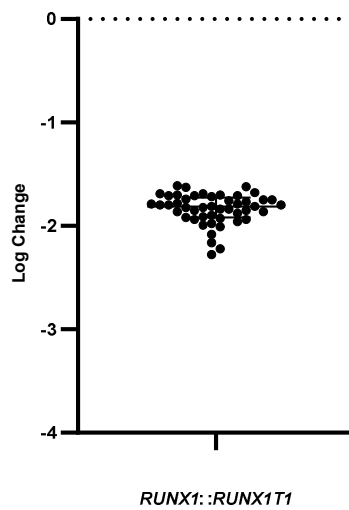


Figure 2: Plot to demonstrate calculated log change between samples 025 and 027 for each participant

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 |
|-------------|------------------------|
| <i>ABL1</i> | 43 |
| <i>GUSB</i> | 4 |
| <i>B2M</i> | 1 |
| <i>HMBS</i> | 1 |

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

Table 3: *ABL1* copy number

| | Sample 025 | Sample 026 | Sample 027 |
|----------------------------|------------|------------|------------|
| n | 42 | 43 | 43 |
| Median | 84,305 | 77,608 | 69,218 |
| Lower Quartile | 45,180 | 38,617 | 40,918 |
| Upper Quartile | 173,848 | 162,286 | 199,368 |
| Inter Quartile Range (IQR) | 128,668 | 123,669 | 158,451 |
| Min | 0 | 0 | 0 |
| Max | 863,281 | 767,463 | 1,177,271 |

Table 4: PCR Type

| | Number of Participants |
|---------------|------------------------|
| Real-Time PCR | 44 |
| Digital PCR | 4 |
| Nested | 1 |

Table 5: Assay protocol

| | Number of Participants |
|----------------------------------|------------------------|
| EAC Protocol | 22 |
| Qiagen Ipsogen RUNX1-RUNX1T1 Kit | 12 |
| In-house Assay | 9 |
| Modified EAC Protocol | 4 |
| Biomed | 2 |

Table 6: Analysis Type

| | Number of Participants |
|-------------------------------------|-------------------------------|
| Real-Time PCR Fluorescent Detection | 44 |
| Digital PCR | 4 |
| Agarose Gel Electrophoresis | 1 |

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

| | Number of Participants |
|---------------------------------------|-------------------------------|
| Qiagen/Ipsogen | 38 |
| In-house | 4 |
| None, dPCR | 2 |
| Delta Ct method, no standard curve | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |

Table 8: Source of Standard Curve – Reference Gene

| | Number of Participants |
|---------------------------------------|-------------------------------|
| Qiagen/Ipsogen | 31 |
| In-house | 6 |
| ERM-AD623 | 6 |
| None, as we are performing dPCR | 2 |
| Delta Ct method, no standard curve | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |

Trial Comments:

Sample 025

- Forty-seven laboratories classified the sample as suitable for analysis; two participants said the sample was suboptimal and one participant said the sample was not suitable for analysis.
- Sample 025 was manufactured to be positive for the t(8;21) *RUNX1::RUNX1T1* transcript. 49/49 (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 025 was 24.6% (n = 43).

Sample 026

- Forty-eight laboratories classified the sample as suitable for analysis; one participant said the sample was suboptimal and one participant said the sample was not suitable for analysis.
- Sample 026 was manufactured to be negative for the t(8;21) *RUNX1::RUNX1T1* transcript. 50/50 (100%) returning participants classified the samples as negative for the t(8;21) *RUNX1::RUNX1T1* transcript.

Sample 027

- Forty-eight laboratories classified the sample as suitable for analysis; one participant said the sample was sub optimal and one participant said the sample was not suitable for analysis.
- Sample 027 was manufactured to be positive for the t(8;21) *RUNX1::RUNX1T1* transcript. All participants returning results classified sample 027 as positive for the t(8;21) *RUNX1::RUNX1T1* transcript (n = 50).
- The median %*RUNX1::RUNX1T1/ABL1* calculated from participant returns for sample 027 was 0.36% (n=44).

Log Change

- The robust mean log change between sample 025 and 027, calculated from all participant returns was -1.82, with a robust SD = 0.13 (n = 48). One participant had a log change >3.5 SDs from the robust mean.

Reference Genes

- Median *ABL1* reference gene levels were 84,305 for sample 025, 77,608 for sample 026 and 69,218 for sample 027.

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

inv(16) *CBFB::MYH11*

Distribution - 222301
Date Issued - 08 August 2022

Participant -
Closing Date - 16 September 2022

Trial Comments

This trial was issued to 61 participants. Four participants did not return results. 50 participants returned results for inv(16) *CBFB::MYH11*.

Sample Comments

Three vials of lyophilised cell line material, samples 028, 029 and 030 were issued to 61 participants for quantitative *CBFB::MYH11* (Type A) analysis. Samples 028 and 030 were manufactured to be positive for the *CBFB::MYH11* (Type A) transcript, mimicking MRD levels seen following treatment in AML. Samples 029 was manufactured to be negative for the *CBFB::MYH11* (Type A) transcript.

Results and Performance

Table 9: Your Results

| | Sample 028 | Sample 029 | Sample 030 |
|--|-------------------|-------------------|-------------------|
| Your qualitative result | Positive | Negative | Positive |
| Consensus qualitative result | Positive | Negative | Positive |
| Your % <i>CBFB::MYH11</i> / reference gene | 31.54 | 0 | 0.004 |
| Median % <i>CBFB::MYH11</i> / <i>ABL1</i> | 37.2 | n/a | 0.015 |
| Lower quartile | 30.8 | n/a | 0.010 |
| Upper quartile | 53.5 | n/a | 0.020 |
| Inter quartile range (IQR) | 22.7 | n/a | 0.011 |
| Your log change between sample 028 and 030 | -3.90 | n/a | -3.90 |
| Robust mean log change between sample 028 and 030 | -3.42 | n/a | -3.42 |
| Robust SD log change between sample 028 and 030 | 0.26 | n/a | 0.26 |

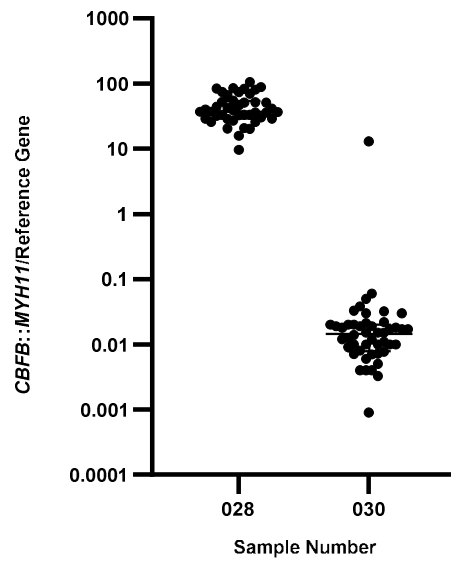


Figure 3: Scatter plot of % CFBF::MYH11 (Type A)/reference gene results for samples 028 and 030 submitted by all participants

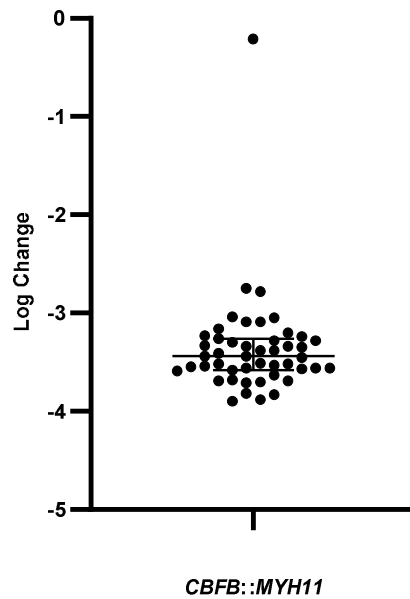


Figure 4: Plot to demonstrate the calculated log change between samples 028 and 030

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 |
|-------------|------------------------|
| <i>ABL1</i> | 43 |
| <i>GUSB</i> | 5 |
| <i>B2M</i> | 1 |
| <i>HMBS</i> | 1 |

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

Table 11: *ABL1* copy number

| | Sample 028 | Sample 029 | Sample 030 |
|----------------------------|------------|------------|------------|
| n= | 42 | 42 | 42 |
| Median | 81,354 | 64,792 | 59,000 |
| Lower Quartile | 44,941 | 22,815 | 36,500 |
| Upper Quartile | 171,943 | 159,387 | 139,270 |
| Inter Quartile Range (IQR) | 127,002 | 136,572 | 102,770 |
| Min | 0 | 0 | 0 |
| Max | 1,097,551 | 737,615 | 837,779 |

Table 12: PCR Type

| | Number of Participants |
|---------------|------------------------|
| Real-Time PCR | 45 |
| Other | 4 |
| Nested PCR | 1 |

Table 13: Assay protocol

| | Number of Participants |
|---------------------------------|-------------------------------|
| EAC Protocol | 23 |
| Qiagen Ipsogen CFBF-MYH11 A Kit | 11 |
| In-house Assay | 10 |
| Modified EAC Protocol | 4 |
| Biomed | 2 |

Table 14: Analysis Type

| | Number of Participants |
|-------------------------------------|-------------------------------|
| Real-Time PCR Fluorescent Detection | 45 |
| Digital PCR | 4 |
| Agarose Gel Electrophoresis | 1 |

Table 15: Source of Standard Curve – CFBF::MYH11

| | Number of Participants |
|---------------------------------------|-------------------------------|
| Qiagen/Ipsogen | 39 |
| In-house | 4 |
| Delta Ct method, no standard curve | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |
| None, digital PCR | 2 |

Table 16: Source of Standard Curve – Reference Gene

| | Number of Participants |
|---------------------------------------|-------------------------------|
| Qiagen/Ipsogen | 32 |
| In-house | 6 |
| ERM-AD623 | 6 |
| None, as we are performing dPCR | 2 |
| Delta Ct method, no standard curve | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |

Trial Comments:

Sample 028

- All participants classified the sample as suitable for analysis.
- Sample 028 was manufactured to be positive for the inv(16) *CBFB::MYH11* transcript. All returning participants classified the sample as positive for the inv(16) *CBFB::MYH11* transcript (n = 50).
- The median % inv(16) *CBFB::MYH11/ABL1* calculated from participant returns was 37.2 (n = 43).

Sample 029

- Forty-six participants classified the sample as suitable for analysis; one participant said the sample was sub optimal and one participant said the sample was not suitable for analysis.
- Sample 029 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 = 50).

Sample 030

- Forty-eight participants classified the sample as suitable for analysis; one participant said the sample was sub optimal and one participant said the sample was not suitable for analysis.
- Sample 030 was manufactured to be positive for the inv(16) *CBFB::MYH11* transcript. Forty-five participants classified the sample as positive for an inv(16) *CBFB::MYH11* transcript. Four participants classified the samples as negative. Of the four participants who missed the transcript, one used a qualitative Biomed 1, agarose gel-based approach with *HMBS* (HUGO gene nomenclature committee approved name (historical gene name: *PBGD*)) as a reference gene; one participant used Real-Time PCR and the Qiagen Ipsogen *CBFB-MYH11 A* kit with *GUSB* as a reference gene; one participant used a Real-Time PCR, Biomed 1 approach reporting a normalised ratio result of 0.005%; the final participant used Digital PCR and the EAC protocol and reported a normalised ratio result of 0.015%. All participants returning negative results classified the sample as suitable for MRD assessment.
- The median inv(16) *CBFB::MYH11* transcript/*ABL1* calculated from participant returns for sample 030 was 0.015 (n = 43).

Log Change

- The robust mean log change between sample 028 and 030, calculated from all participant returns was -3.42, with a robust SD = 0.26 (n = 47).
- No participants had a z-score >3.5 SDs from the robust mean log change.

Reference Genes

- Median *ABL1* reference gene levels were 81,354 for sample 028, 64,792 for sample 029 and 59,000 for sample 030.

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

t(15;17) PML::RARA

Distribution - 222301
Date Issued - 08 August 2022

Participant -
Closing Date - 16 September 2022

Trial Comments

This trial was issued to 61 participants. Four participants did not return results. 49 participants returned results for t(15;17) PML::RARA (BCR1, L form).

Sample Comments

Three vials of lyophilised cell line material, samples 031, 032 and 033 were issued to 61 participants for quantitative t(15;17) PML::RARA analysis. Samples 032 and 033 were manufactured to be positive for the t(15;17) PML::RARA transcript, mimicking MRD levels seen following treatment in AML. Samples 031 was manufactured to be negative for the t(15;17) PML::RARA transcript.

Results and Performance

Table 17: Your Results

| | Sample 031 | Sample 032 | Sample 033 |
|--|-------------------|-------------------|-------------------|
| Your qualitative result | Negative | Positive | Positive |
| Consensus qualitative result | Negative | Positive | Positive |
| Your % PML::RARA / reference gene | 0 | 0.0296 | 0.0633 |
| Median % PML::RARA / ABL1 | n/a | 0.019 | 0.048 |
| Lower quartile | n/a | 0.010 | 0.033 |
| Upper quartile | n/a | 0.030 | 0.070 |
| Inter quartile range (IQR) | n/a | 0.020 | 0.037 |
| Your log change between sample 032 and 033 | n/a | 0.33 | |
| Robust mean log change between sample 032 and 033 | n/a | 0.44 | |
| Robust SD log change between sample 032 and 033 | n/a | 0.16 | |

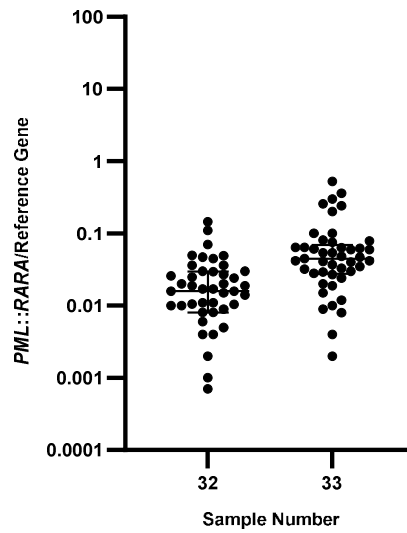


Figure 5: Scatter plot of % *PML::RARA*/reference gene results for samples 032 and 033 submitted by all participants

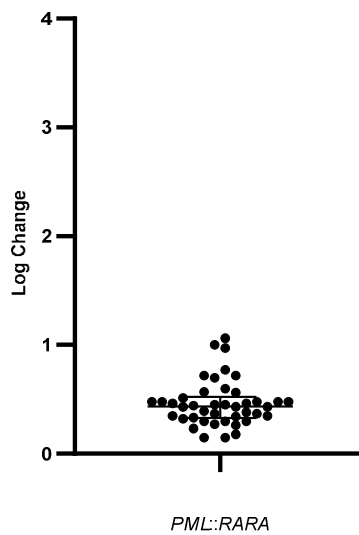


Figure 6: Plot to demonstrate the calculated log change between samples 032 and 033

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 |
|-------------|------------------------|
| <i>ABL1</i> | 41 |
| <i>GUSB</i> | 5 |
| <i>HMBS</i> | 1 |
| <i>B2M</i> | 1 |
| Other | 1 |

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

Table 19: *ABL1* copy number

| | Sample 031 | Sample 032 | Sample 033 |
|----------------------------|------------|------------|------------|
| N= | 39 | 39 | 39 |
| Median | 85,967 | 106,887 | 69,613 |
| Lower Quartile | 50,864 | 59,937 | 31,565 |
| Upper Quartile | 151,423 | 221,505 | 151,812 |
| Inter Quartile Range (IQR) | 100,559 | 161,567 | 120,247 |
| Min | 0 | 0 | 0 |
| Max | 524,912 | 926,354 | 538,333 |

Table 20: PCR Type

| | Number of Participants |
|---------------|------------------------|
| Real-Time PCR | 43 |
| Digital PCR | 3 |
| Nested PCR | 3 |

Table 21: Assay protocol

| | Number of Participants |
|--|-------------------------------|
| EAC Protocol | 20 |
| In-house Assay | 12 |
| Qiagen Ipsogen PML-RARA bcr1 Kit CE | 11 |
| Modified EAC Protocol | 2 |
| Biomed 1 | 2 |
| Diatech pharmacogenetics EasyPGX ready PML-RARA Fusion | 1 |
| Invivoscribe PML RARA Kit | 1 |

Table 22: Analysis Type

| | Number of Participants |
|-------------------------------------|-------------------------------|
| Real-Time PCR Fluorescent Detection | 44 |
| Digital PCR | 3 |
| Agarose Gel Electrophoresis | 1 |
| Capillary Electrophoresis | 1 |

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

| | Number of Participants |
|--|-------------------------------|
| Qiagen/Ipsogen | 35 |
| In-house | 5 |
| Digital PCR, no standard curve used | 3 |
| Capillary Electrophoresis, no standard curve | 1 |
| Delta Ct method, no standard curve | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |

Table 24: Source of Standard Curve – Reference Gene

| | Number of Participants |
|---------------------------------------|-------------------------------|
| Qiagen/Ipsogen | 28 |
| In-house | 7 |
| ERM-AD623 | 5 |
| None, as we are performing dPCR | 3 |
| In-house calibrated to Qiagen/Ipsogen | 1 |
| Delta Ct method, no standard curve | 1 |
| In-house and Qiagen/Ipsogen | 1 |

Trial Comments:

Sample 031

- Forty-seven participants classified the sample as suitable for analysis; two participants said the sample was suboptimal.
- Sample 031 was manufactured to be negative for the t(15;17) *PML::RARA* transcript. All participants classified the samples as negative for the t(15;17) *PML::RARA* transcript.

Sample 032

- Forty-seven participants classified the sample as suitable for analysis; two participants said the sample was suboptimal.
- Sample 032 was manufactured to be positive for the t(15;17) *PML::RARA* transcript. 44/49 (89.8%) of returning participants for this sample detected a t(15;17) *PML::RARA* transcript (n = 49). 5/49 (10.2%) participants reported the sample to be negative for the t(15;17) *PML::RARA* transcript.
- Of the five participants who classified the sample as negative, one used a nested PCR, Biomed 1, agarose gel electrophoresis approach using the *HMBS (PBGD)* reference gene; one used an EAC Protocol and real-time PCR approach using *ABL1*; one used the EAC protocol, real-time PCR and the *GUSB* reference gene and classified the sample as suboptimal; one used an in-house real-time PCR approach and the *B2M* reference gene; and one used the EAC protocol and digital PCR.
- The median % *PML::RARA/ABL1* calculated from participant returns was 0.019% (n = 39).

Sample 033

- Forty-six participants classified the sample as suitable for analysis; three participants said the sample was suboptimal.
- Sample 033 was manufactured to be positive for the t(15;17) *PML::RARA* transcript. 48/49 participants returning results classified the sample as positive for the t(15;17) *PML::RARA* transcript. One participant classified the sample as negative and used the EAC protocol and digital PCR.
- The participant who did not detect any transcript reported an *ABL1* level of 64,037.
- The median % *PML::RARA/ABL1* calculated from participant returns was 0.048 (n = 39).

Log Change

- The robust mean log change between sample 032 and 033, calculated from all participant returns was 0.44, with a robust SD = 0.16 (n = 42). Two participants had a result >3.5 SD from the robust mean.

Reference Genes

- Median *ABL1* reference gene levels were 85,967 for sample 031, 106,887 for sample 032 and 69,613 for sample 033.

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

***NPM1* (Type A)**

Distribution - 222301
Date Issued - 08 August 2022

Participant -
Closing Date - 16 September 2022

Trial Comments

This trial was issued to 61 participants. Four participants did not return results. 50 participants returned results for *NPM1* (Type A).

Sample Comments

Three vials of lyophilised cell line material, samples 034, 035 and 036 were issued to 61 participants for quantitative *NPM1* (Type A) analysis. Samples 034 and 035 were manufactured to be positive for the *NPM1* (Type A) transcript, mimicking MRD levels seen following treatment in AML. Samples 036 was manufactured to be negative for the *NPM1* (Type A) transcript.

Results and Performance

Table 25: Your Results

| | Sample 034 | Sample 035 | Sample 036 |
|--|-------------------|-------------------|-------------------|
| Your qualitative result | Positive | Positive | Negative |
| Consensus qualitative result | Positive | Positive | Negative |
| Your % <i>NPM1</i> (Type A) / reference gene | 1.081 | 152.437 | 0 |
| Median % <i>NPM1</i> (Type A) / <i>ABL1</i> | 0.44 | 43.0 | n/a |
| Lower quartile | 0.30 | 30.5 | n/a |
| Upper quartile | 0.59 | 66.7 | n/a |
| Inter quartile range (IQR) | 0.30 | 36.1 | n/a |
| Your log change between sample 034 and 035 | 2.15 | | n/a |
| Robust mean log change between sample 034 and 035 | 2.00 | | n/a |
| Robust SD Log change between sample 034 and 035 | 0.14 | | n/a |

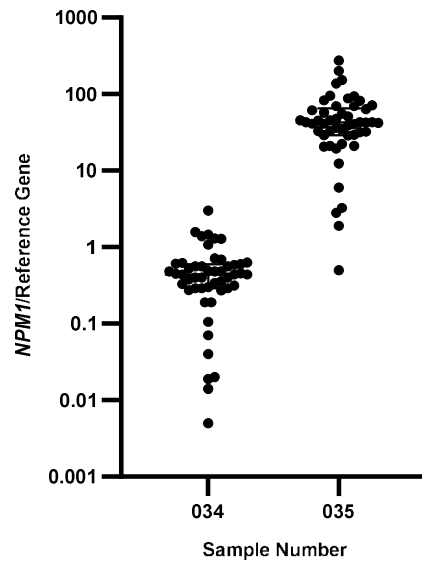


Figure 7: Scatter plot of *NPM1* (Type A)/reference gene results for samples 034 and 035 submitted by all participants

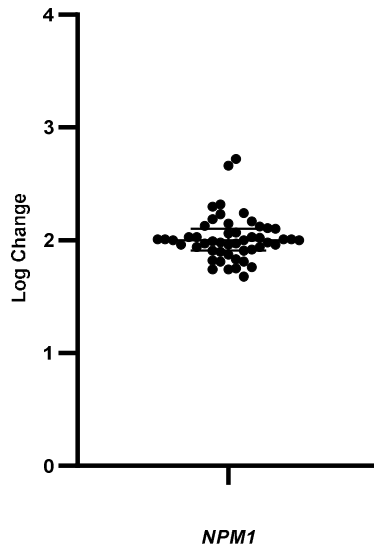


Figure 8: Plot to demonstrate the calculated log change between samples 034 and 035

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 |
|-------------|------------------------|
| <i>ABL1</i> | 46 |
| <i>GUSB</i> | 1 |
| <i>ALB</i> | 1 |
| Other | 1 |

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

Table 27: *ABL1* copy number

| | Sample 034 | Sample 035 | Sample 036 |
|----------------------------|------------|------------|------------|
| n= | 46 | 46 | 45 |
| Median | 64,726 | 101,042 | 72,800 |
| Lower Quartile | 34,746 | 43,866 | 27,033 |
| Upper Quartile | 160,013 | 206,655 | 128,129 |
| Inter Quartile Range (IQR) | 125,268 | 162,789 | 101,096 |
| Min | 0 | 0 | 0 |
| Max | 652,918 | 1,445,779 | 1,162,512 |

Table 28: PCR Type

| | Number of Participants |
|---------------|------------------------|
| Real-Time PCR | 45 |
| Digital PCR | 2 |
| Single PCR | 1 |
| Multiplex PCR | 1 |
| Other | 1 |

Table 29: Assay protocol

| | Number of Participants |
|---|-------------------------------|
| In-house Assay | 27 |
| Qiagen NPM1 mut A, B & D MutaQuant Kits | 14 |
| Qiagen NPM1 mut A MutaQuant Kits | 8 |
| Other | 1 |

Table 30: Analysis Type

| | Number of Participants |
|-------------------------------------|-------------------------------|
| Real-Time PCR Fluorescent Detection | 48 |
| Digital PCR | 2 |

Table 31: Source of Standard Curve – NPM1

| | Number of Participants |
|---|-------------------------------|
| Qiagen/Ipsogen | 40 |
| In-house | 4 |
| dCt method, no standard curve | 2 |
| In-house calibrated to ERM-AD623 (adjusted to single stranded copies) | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |
| Digital PCR, no standard curve | 1 |

Table 32: Source of Standard Curve – Reference Gene

| | Number of Participants |
|---------------------------------------|-------------------------------|
| Qiagen/Ipsogen | 34 |
| In-house | 6 |
| ERM-AD623 | 4 |
| Delta Ct method, no standard curve | 2 |
| None, as we are performing dPCR | 1 |
| In-house calibrated to Qiagen/Ipsogen | 1 |
| In-house calibrated to ERM-AD623 | 1 |

Trial Comments:

Sample 034

- Forty-seven participants classified the sample as suitable for analysis and three participants said the sample was suboptimal.
- Sample 034 was manufactured to be positive for the *NPM1* (Type A) transcript. All returning participants for this sample classified the sample as positive for the *NPM1* (Type A) transcript (n = 50).
- The median *NPM1* (Type A) transcript/*ABL1* calculated from participant returns for sample 034 was 0.44% (n = 47).

Sample 035

- Forty-eight participants classified the sample as suitable for analysis; one participant said the sample was not suitable for analysis.
- Sample 035 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 = 49).
- The median *NPM1* (Type A)/*ABL1* calculated from participant returns for sample 035 was 43.0% (n = 47).

Sample 036

- Forty-three participants classified the sample as suitable for analysis; five participants said the sample was suboptimal and two participants said the sample was not suitable for analysis.
- Sample 036 was manufactured to be negative for the *NPM1* (Type A) transcript.
- Forty-six participants classified the sample as negative; three participants reported sample 036 to be positive for the *NPM1* (Type A) transcript. Of the participants who reported a positive result, two used real-time PCR and an in-house assay and one used real-time PCR and the Qiagen *NPM1* mut A, B & D MutaQuant kit.

Log Change

- The robust mean log change between sample 034 and 035, calculated from all participant returns was 2.00, with a robust SD = 0.14 (n = 50).
- Two participant's log change results were > 3.5 SDs from the robust mean.

Reference Genes

- Median *ABL1* reference gene levels were 64,726 for sample 034, 101,042 for sample 035 and 72,800 for sample 036.

FLT3 ITD MRD testing

- Following several publications highlighting the importance of the detection of *FLT3* ITD MRD in AML (1), a survey was performed to identify the requirement for EQA.
- 15/58 participants who responded are currently performing *FLT3* MRD testing.
- A further 13 plan to implement it (four within six months; four within 12 months and five >12 months).
- As such, we plan to include educational samples for *FLT3* ITD MRD testing in trial MRD AML MM 222302 to be issued in March 2023.

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. 2022 Oct 31;JCO2200715.

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 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/>