Myeloproliferative Neoplasms Diagnostic Testing Programme

Distribution - 252602

Participant ID

Date Issued - 27 August 2025

Closing Date - 03 October 2025

Trial Comments

The Myeloproliferative Neoplasms Diagnostic Testing (MPN DT) programme was developed to include the UKAS accredited JAK2 p.Val617Phe (V617F) Mutation Status programme last year. From the outset of this consolidated programme, qualitative JAK2 p.(Val617Phe) testing has been subject to performance monitoring. UK NEQAS LI is working towards full ISO/IEC 17043:2023 accreditation of the MPN DT programme during 2025. We anticipate that similar qualitative performance monitoring will be applied to the remaining core MPN markers, as well as optional performance monitoring for JAK2 p.(Val617Phe) quantification and measurable residual disease (MRD) testing. We will keep participants informed via future trial reports and email notification(s).

This trial was issued to 253 participants; 245 (96.8%) have successfully returned results for all MPN marker testing in line with their stated laboratory repertoire. Two further participants returned results which, due to IT constraints, are not included in the trial statistics. These participants utilise an RNA-based assay for some aspects of their MPN testing. The use of RNA/cDNA templates is not advocated for MPN testing since this adds additional unnecessary complexity and quantitative results cannot be directly compared to the wealth of published data from genomic DNA evaluation (Prof. N.C.P. Cross, personal communication).

Of the six participants who have not submitted results, one prenotified UK NEQAS LI of their intended non-return.

Sample Comments

Two samples were issued in this trial: MPN DT 122 and MPN DT 123. Participants were asked to consider both samples representative of patient samples at diagnosis and were requested (subject to their test repertoire) to perform qualitative analysis for all four core MPN markers: JAK2 p.(Val617Phe) and clinically significant variants within JAK2 exon 12, CALR exon 9 and MPL exon 10, with exon numbering according to the MANE Select (v1.4) reference transcripts: NM_004972.4(JAK2), NM_004343.4(CALR), and NM_005373.3(MPL). Participants were also given the option of submitting a variant allele burden, defined as (variant alleles/total alleles) x100, for any relevant variant. MPN DT 122 comprised genomic DNA extracted from peripheral blood donated by a patient known to harbour the NM_004343.4:c.1099_1150del p.(Leu367Thrfs*46) CALR variant; MPN DT 123 consisted of lyophilised cell line material formulated to be positive for the JAK2 p.(Val617Phe) variant and negative for the remaining three markers.

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Sample MPN DT 122

Did you detect the clinically significant ***JAK2* p.(Val617Phe)** variant in sample MPN DT 122: **No**

Did you detect a clinically significant ***JAK2* exon 12** variant in sample MPN DT 122: **No**

Did you detect a clinically significant ***CALR* exon 9** variant in sample MPN DT 122: **Yes**

Did you detect a clinically significant ***MPL* exon 10** variant in sample MPN DT 122: **No**

Please note that results for *JAK2 exon 12, CALR exon 9* and *MPL exon 10*, as well as MRD testing for *JAK2* p.(Val617Phe) are not yet subject to performance monitoring, however any out-of-consensus results should always be subject to appropriate investigation.

Your Qualitative Results

Gene/Region	Your DNA Sequence Variant	Your Protein Variant	Other Details
JAK2 p.(Val617Phe)	Variant not detected	Variant not detected	
JAK2 exon 12	No variant detected	No variant detected	
CALR exon 9	c.1099 1150del	p.(Leu367Thrfs*46)	
MPL exon 10	No variant detected	No variant detected	

All Participant Results

Gene/Region	Participants detecting a Variant[β]/Total number who tested the gene	Consensus DNA Sequence Variant[φ]	Consensus Protein Variant[φ]	Allele Burden (%)[δ]	
				Robust Mean	Robust SD
JAK2 p.(Val617Phe)	64/244	Marker Not Scored (see discussion)	Marker Not Scored (see discussion)	0.13	0.07
JAK2 exon 12	0/169	No variant detected	No variant detected		
CALR exon 9	191/194	c.1099_1150del	p.(Leu367Thrfs*46)	40.04	12.39
MPL exon 10	1/186	No variant detected	No variant detected		

[β]This includes results returned by participants unable to provide Human Genome Variation Society (HGVS) approved nomenclature due to assay limitations and those detecting a variant but submitting gross nomenclature errors.
[φ]Results returned by participants (at both the DNA and protein level) may have been harmonised to the equivalent HGVS approved nomenclature during the compilation of 'All Participant Results' tables. Note that HGVS recommendations allow the use of either Ter or * to indicate a translation termination (stop) codon. Nomenclature is based on the MANE Select reference transcript and genome build GRCh38. Protein nomenclature includes parentheses as it represents a prediction from analysis at the DNA level. Please see later discussion for up-to-date HGVS and MANE Select references.
[δ]Robust statistics calculated for any variant with ≥10 quantification data points. Percentage values quoted have been subjected to rounding up/down to 2 decimal places.
N/A = Not Applicable.

Your Nomenclature Guidance Comments

Gene/Region	DNA Description
JAK2 exon 12	
CALR exon 9	Consensus variant described, however nomenclature lacks full compliance with HGVS recommendations.
MPL exon 10	

Gene/Region	Protein Description
JAK2 exon 12	
CALR exon 9	Compliant with current HGVS recommendations.
MPL exon 10	

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Sample MPN DT 123

Did you detect the clinically significant ***JAK2* p.(Val617Phe)** variant in sample MPN DT 123: **Yes**

Did you detect a clinically significant ***JAK2* exon 12** variant in sample MPN DT 123: **No**

Did you detect a clinically significant ***CALR* exon 9** variant in sample MPN DT 123: **No**

Did you detect a clinically significant ***MPL* exon 10** variant in sample MPN DT 123: **No**

Please note that results for *JAK2 exon 12, CALR exon 9* and *MPL exon 10*, as well as MRD testing for *JAK2* p.(Val617Phe) are not yet subject to performance monitoring, however any out-of-consensus results should always be subject to appropriate investigation.

Your Qualitative Results

Gene/Region	Your DNA Sequence Variant	Your Protein Variant	Other Details
JAK2 p.(Val617Phe)	Variant detected	Variant detected	
JAK2 exon 12	No variant detected	No variant detected	
CALR exon 9	No variant detected	No variant detected	
MPL exon 10	No variant detected	No variant detected	

All Participant Results

Gene/Region	Participants detecting a Variant[β]/Total number who tested the gene	Consensus DNA Sequence Variant[φ]	Consensus Protein Variant[φ]	Allele Burden (%)[δ]	
				Robust Mean	Robust SD
JAK2 p.(Val617Phe)	243/244	c.1849G>T detected	p.(Val617Phe) detected	26.79	7.40
JAK2 exon 12	0/169	No variant detected	No variant detected		
CALR exon 9	1/194	No variant detected	No variant detected		
MPL exon 10	0/186	No variant detected	No variant detected		

[β]This includes results returned by participants unable to provide Human Genome Variation Society (HGVS) approved nomenclature due to assay limitations and those detecting a variant but submitting gross nomenclature errors.
[φ]Results returned by participants (at both the DNA and protein level) may have been harmonised to the equivalent HGVS approved nomenclature during the compilation of 'All Participant Results' tables. Note that HGVS recommendations allow the use of either Ter or * to indicate a translation termination (stop) codon. Nomenclature is based on the MANE Select reference transcript and genome build GRCh38. Protein nomenclature includes parentheses as it represents a prediction from analysis at the DNA level. Please see later discussion for up-to-date HGVS and MANE Select references.
[δ]Robust statistics calculated for any variant with ≥10 quantification data points. Percentage values quoted have been subjected to rounding up/down to 2 decimal places.
N/A = Not Applicable.

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Your *JAK2* p.(Val617Phe) Qualitative Performance

Performance Status for this Trial	Performance Status Classification Over 3 Trial Period	
	Satisfactory	Critical
Satisfactory	3	0

Please note: Performance monitoring is currently available for diagnostic *JAK2* p.(Val617Phe) results only. We are working towards a qualitative performance monitoring system for all four MPN markers, as well as *JAK2* p.(Val617Phe) MRD assessment.
N/A = Not Applicable

Your *JAK2* p.(Val617Phe) Quantitative Results

	Your Results	Robust Mean	Robust SD	Uncertainty of the Assigned Value (Robust Mean)
Sample MPN DT 122	Not Returned	0.13	0.07	± 0.01
Sample MPN DT 123	30.7	26.79	7.40	± 0.66

Your *JAK2* p.(Val617Phe) Quantitative Performance

Your Quantitative Performance	z score	Performance Status for this Sample	Performance Status Classification Over Last 6 Positive Samples		
			Satisfactory	Action	Critical
Sample MPN DT 122		N/A	N/A	N/A	N/A
Sample MPN DT 123	0.53	N/A	N/A	N/A	N/A

Please note: Performance monitoring is currently available for diagnostic *JAK2* p.(Val617Phe) qualitative results only. We are working towards a performance monitoring system for *JAK2* p.(Val617Phe) quantitative results.
N/A = Not Applicable

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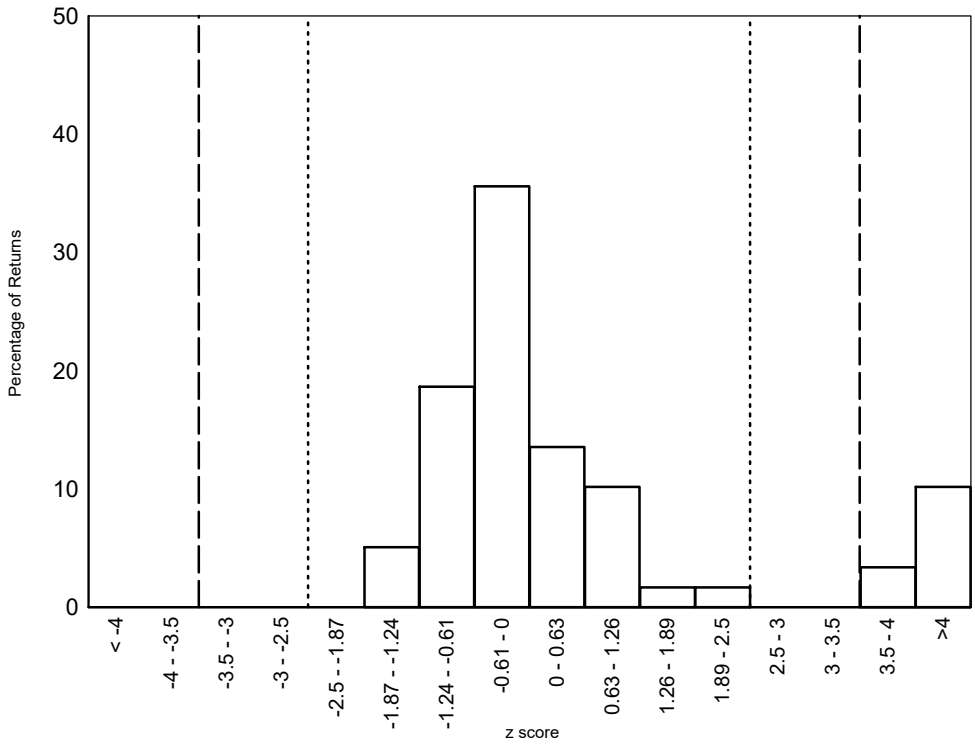
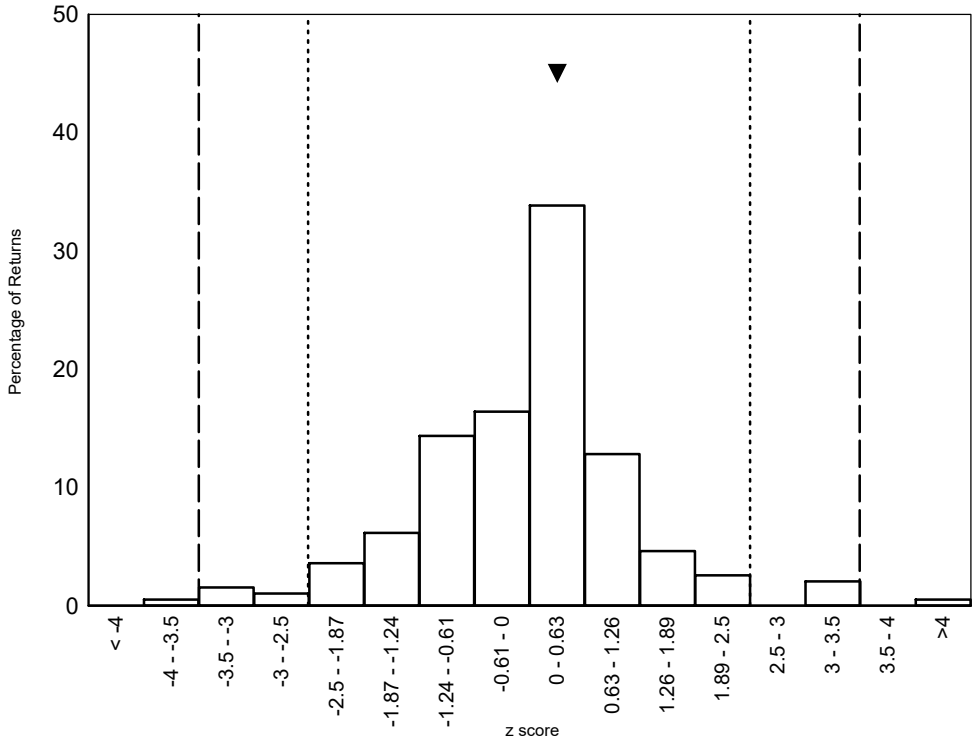
Histograms of Participant z scores for *JAK2* p.(Val617Phe) Quantification

JAK2 p.(Val617Phe) allele burden z score for sample MPNDT 122
Please note ▼ denotes your result



JAK2 p.(Val617Phe) allele burden z score for sample MPNDT 123
Please note ▼ denotes your result



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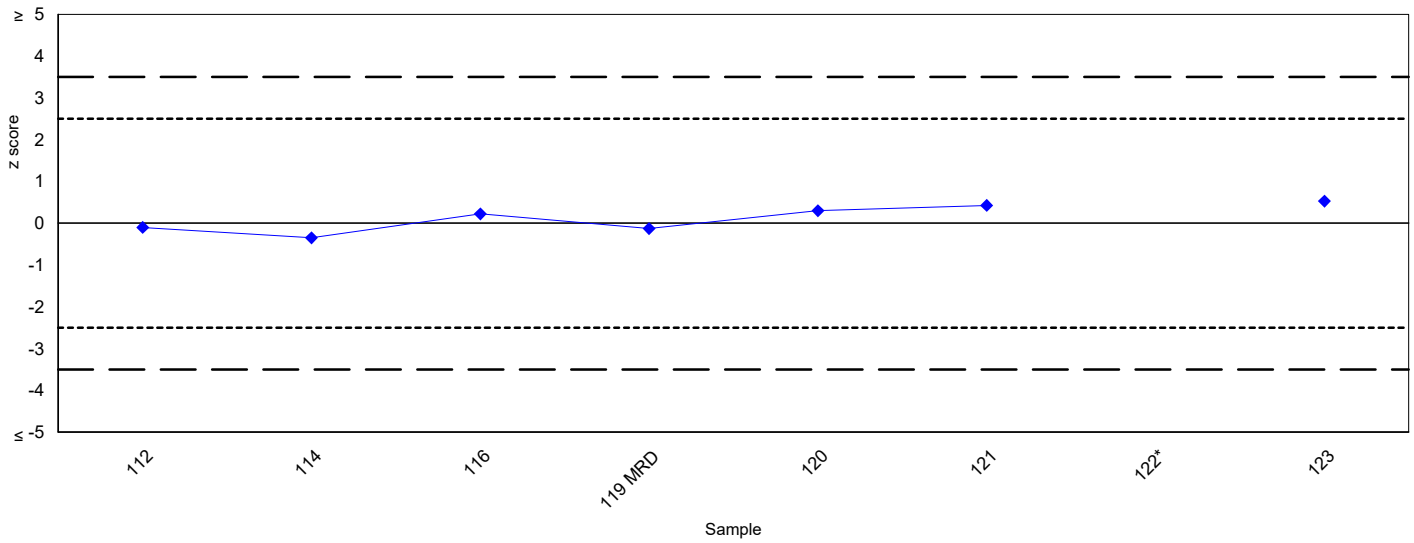
Shewhart Control Charts for *JAK2* p.(Val617Phe) Quantification

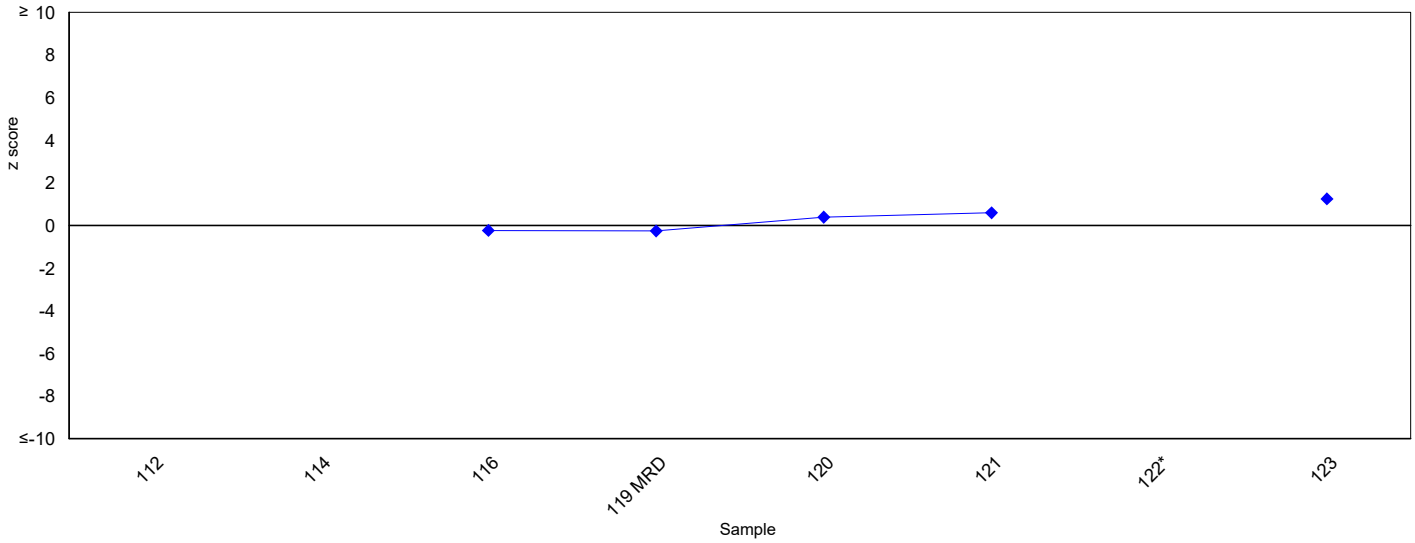
(Please note each data point represents a single sample)

JAK2 p.(Val617Phe) allele burden z score



Cusum Control Charts for *JAK2* p.(Val617Phe) Quantification

(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)



* very low allele burden, see MPN DT 252602 trial report for further details

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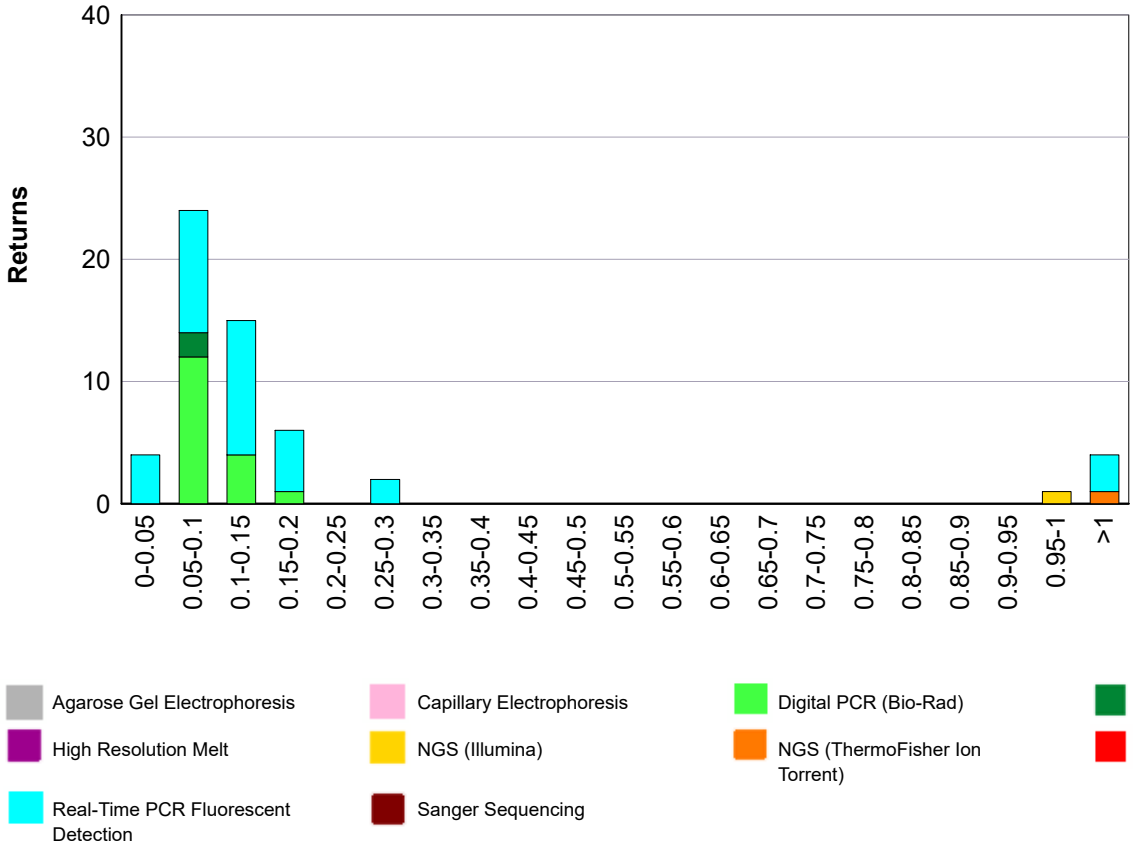
Frequency distribution histogram showing % *JAK2* p.(Val617Phe) allele burden in sample MPNDT 122, classified by analysis method.



Robust Mean = 0.13

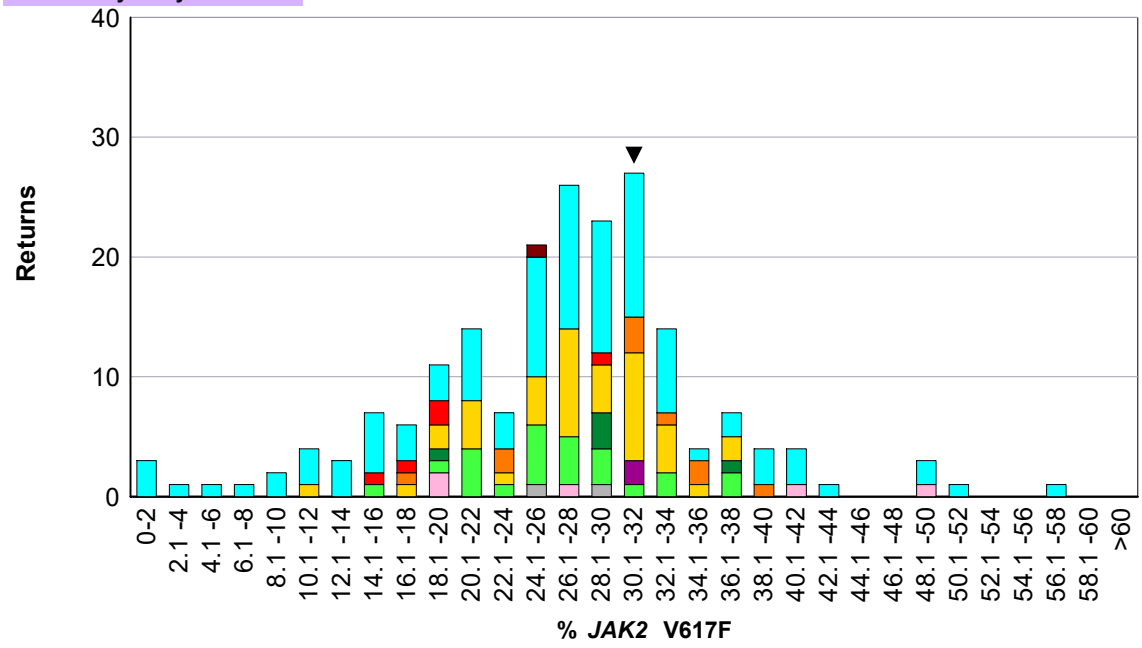
Robust SD = 0.07

Uncertainty of the Assigned Value (Robust Mean) = ± 0.01

Agarose Gel Electrophoresis
Capillary Electrophoresis
Digital PCR (Bio-Rad)
Digital PCR (Other)
High Resolution Melt
NGS (Illumina)
NGS (ThermoFisher Ion Torrent)
NGS (Other)
Real-Time PCR Fluorescent Detection
Sanger Sequencing

▼ denotes your result

Frequency distribution histogram showing % *JAK2* p.(Val617Phe) allele burden in sample MPNDT 123, classified by analysis method.



Robust Mean = 26.79

Robust SD = 7.40

Uncertainty of the Assigned Value (Robust Mean) = ± 0.66

Agarose Gel Electrophoresis
Capillary Electrophoresis
Digital PCR (Bio-Rad)
Digital PCR (Other)
High Resolution Melt
NGS (Illumina)
NGS (ThermoFisher Ion Torrent)
NGS (Other)
Real-Time PCR Fluorescent Detection
Sanger Sequencing

▼ denotes your result

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PCR Type	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Allele specific PCR	45	3	7	23
Droplet digital PCR	27	1	1	4
LNA PCR	4	-	-	-
Melting curve analysis	5	10	2	9
Multiplex PCR	1	5	5	4
PCR for NGS	56	87	80	83
Real-time PCR	101	4	23	33
Sanger sequencing	-	36	18	18
Single PCR	3	23	58	12

Please note: PCR Types cited by fewer than three participants are not represented in this table .

Analysis Type	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Digital PCR (Other)	5	-	-	1
NGS (Other)	5	5	4	4
NGS (Illumina)	42	65	61	63
High Resolution Melt	3	10	2	9
Digital PCR (Bio-Rad)	25	1	1	4
Real-Time PCR Fluorescent Detection	133	5	23	48
Agarose Gel Electrophoresis	10	-	1	1
Capillary Electrophoresis	10	19	66	16
NGS (ThermoFisher Ion Torrent)	10	19	16	17
Sanger Sequencing	1	45	20	23

Please note: Analysis Types cited by fewer than three participants are not represented in this table .

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Protocol Type / Kit	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Agilent SureSelect Custom QXT Panel	3	3	3	3
Agilent SureSelect Custom XT HS2 DNA Panel	2	4	4	4
Archer DX VariantPlex Myeloid Panel	3	3	3	3
Biomol CALR exon 9 Type I and II Mutation Kit	-	-	4	-
Biomol JAK2 V617F (qualitative)	5	-	-	-
Biomol MPL W515L/K Kit	-	-	-	3
BioRad PrimePCR ddPCR Kit	21	-	1	1
Generi Biotech gb ONCO JAK2 (V617F)	5	-	-	-
Genesig JAK2 V617F QUASA Kit	5	-	-	-
Genmark geneMAP Somatic Mutation Detection Kit	2	4	2	2
HealthInCode Imegen-CALR	-	-	4	-
HealthInCode Imegen-MPL	-	-	-	3
Illumina AmpliSeq Panel	3	6	5	5
Illumina custom Panel	4	6	6	6
Illumina TruSight Myeloid Sequencing Panel	2	3	3	3
In-house Assay	93	94	103	76
Qiagen QiaSeq Custom Panel	6	7	7	7
Qiagen/Ipsogen MutaQuant Kit	42	-	-	1
Qiagen/Ipsogen MutaScreen Kit	10	-	-	24
Qiagen/Ipsogen MutaSearch Kit	4	-	-	2
Qiagen/Ipsogen RGQ PCR Kit	7	-	9	-
Sophia Genetics Myeloid Solution	2	3	3	3
ThermoFisher Big Dye Terminator 3.1	-	3	3	2
ThermoFisher Ion Ampliseq Custom Panel	4	5	5	5
ThermoFisher Oncomine Myeloid Gx v2 Panel	3	5	5	5
ThermoFisher Oncomine Myeloid Research Assay	3	7	5	6
TRUPCR CALR Mutation Testing Kit	-	-	5	-
TRUPCR JAK2 Mutation Detection Kit	3	-	-	-
TRUPCR MPL Kit	-	-	-	6
Twist Custom Panel	3	4	4	3

Please note: Protocol Types / Kits cited by fewer than three participants are not represented in this table.

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Journal Reference for Assay	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Arber et al (2022) Blood 140:1200-28	7	5	6	8
Baxter et al (2005) Lancet 365:1054-61	27	4	2	4
Bench et al (2013) Br J Haematol 160:25-34	7	2	1	2
Boyd et al (2010) Br J Haematol 149:250-7	-	1	-	8
Chen et al (2007)J Mol Diagn 9:272-6	5	-	1	1
Cross et al (2021) Br J Haematol 195:338–51	3	3	4	6
Furtado et al (2013) J Mol Diagn 15:592-9	-	8	1	4
Furtado et al (2013) J Mol Diagn 15:810-8	-	-	-	7
James et al (2005) Nature 434:1144-8	8	2	1	-
Jovanovic et al (2013) Leukemia 27:2032-9	6	-	-	-
Klampfl et al (2013) N Engl J Med 369:2379-90	2	1	46	2
Kroger et al (2007) Blood 109:1316-21	5	-	-	-
Larsen et al (2007) Br J Haematol 136: 745-51	13	1	-	-
Levine et al (2005) Cancer Cell 7: 387-97	6	-	2	-
Nangalia et al (2013) N Engl J Med 369:2391-2405	-	1	15	1
Pardanani et al (2006) Blood 108:3472-6	-	1	-	6
Passamonti et al (2006) Blood 107:3676-82	6	-	-	1
Pietra et al (2008) Blood 111:1686-9	-	8	-	1
Pikman et al (2006) PLoS Med 3:e270	-	-	-	5
Scott et al (2007) N Engl J Med 356:459-68	-	14	-	-
Tefferi (2016) Am J Hematol 91:50–8	5	2	-	4
Tefferi and Pardanani (2014) Nat Rev Clin Oncol 11: 125–6.	1	2	6	1

Please note: assay references cited by fewer than five participants are not represented in this table.

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Sample MPN DT 122

Detailed breakdown of *CALR* exon 9 variant nomenclature

MANE Select transcript NM_004343.4

DNA sequence change	n	Nomenclature guidance comments (as applicable)
c.1099_1150del	106	Compliant with current HGVS recommendations.
c.1099_1150delCTTAAGGAGGAGGAAGAAGACAAGAAACGCAAAGAGGAGGAGGAGGCAGAGG	5	Consensus variant described, however providing the number of deleted nucleotides or listing the deleted nucleotide sequence is not endorsed.
c.1099_1150del with minor syntax error(s)	3	Consensus variant described, however nomenclature lacks full compliance with HGVS recommendations.
c.1099_1150del52	3	Consensus variant described, however providing the number of deleted nucleotides or listing the deleted nucleotide sequence is not endorsed.
NG_029662.1:g.10159_10210del	1	Consensus variant described using genomic reference sequence
c.1092_1143del	15	Nomenclature error at the DNA level: HGVS 3' rule not followed.
c.1092_1143del52	9	Nomenclature error at the DNA level: HGVS 3' rule not followed. Other nomenclature/syntax error(s) also present.
c.1099_1050del	2	Out of consensus description resulting from typographical error.
c.1099_1150del ref seq error	2	Consensus variant described, however reference sequence for an alternative gene was provided.
c.1099_1151del	1	Out of consensus description.
c.1099_150del	1	Out of consensus description resulting from typographical error.
c.1179_1230del	1	Out of consensus description.
c.1099_1150	1	Absent or inappropriate symbol.
C.1092-1143 del	1	Nomenclature error at the DNA level: HGVS 3' rule not followed. Other nomenclature/syntax error(s) also present.
Not Provided	40	HGVS nomenclature was not provided.

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Protein sequence change	n	Nomenclature guidance comments (as applicable)
p.(Leu367Thrfs*46)	92	Compliant with current HGVS recommendations.
p.(Leu367fs)	3	Compliant with HGVS recommendations (short format).
p.(L367Tfs*46)	2	Compliant with HGVS recommendations however the use of three letter amino acid codes is preferred.
p.Leu367Thrfs*46	12	Parentheses to indicate predicted status of the protein level description are absent and should be used when DNA is the input material.
p.L367fs*46	8	Consensus variant described, however a hybrid of standard and short format HGVS nomenclature is not advocated. Parentheses to indicate predicted status of the protein level description are absent and the use of three letter amino acid codes is preferred.
p.(Leu367Thrfs)	6	Consensus variant described, however a hybrid of standard and short format HGVS nomenclature is not advocated.
p.(Leu367fs*46)	4	Consensus variant described, however a hybrid of standard and short format HGVS nomenclature is not advocated.
p.L367Tfs*46	3	Parentheses to indicate predicted status of the protein level description are absent and should be used when DNA is the input material. Use of three letter amino acid codes is preferred.
p.(L367fs*46)	3	Consensus variant described, however a hybrid of standard and short format HGVS nomenclature is not advocated. The use of three letter amino acid codes is preferred.
p.Leu367fs*46	3	Consensus variant described, however a hybrid of standard and short format HGVS nomenclature is not advocated. Parentheses to indicate predicted status of the protein level description are absent.
p.Leu367Thrfs*46)	1	Incorrect use of parentheses.
p.(L367TfsTer46)	1	Consensus variant described, however nomenclature lacks full compliance with HGVS recommendations.
p.(Leu367)fs	1	Incorrect use of parentheses.
p.Leu367fs	1	Parentheses to indicate predicted status of the protein level description are absent and should be used when DNA is the input material.
(p.L367fs*46)	1	Consensus variant described, however a hybrid of standard and short format HGVS nomenclature is not advocated. Incorrect use of parentheses and the use of three letter amino acid codes is preferred.
L367fs*46	1	Nomenclature error at the protein level: absent letter prefix (HGVS recommendations state a prefix is mandatory to indicate type of reference sequence used). Parentheses to indicate predicted status of the protein level description are absent. Use of three letter amino acid codes is preferred.
p.(Leu367fs*Arg)	1	Nomenclature error at the protein level: incorrect amino acid code used.
p.(Leu367Thrfs*?)	1	Nomenclature error at the protein level. Current HGVS recommendations state 'fs*?' indicates a new reading frame which does not encounter a new termination codon.
p.L367Tfs*?	1	Nomenclature error at the protein level. Current HGVS recommendations state 'fs*?' indicates a new reading frame which does not encounter a new termination codon. Parentheses to indicate predicted status of the protein level description are absent and the use of three letter amino acid codes is preferred.
p.Leu367ThrfsTer?	1	Nomenclature error at the protein level. Current HGVS recommendations state 'fs*?' indicates a new reading frame which does not encounter a new termination codon. Parentheses to indicate predicted status of the protein level description are absent.
Not provided	45	HGVS nomenclature was not provided.

Colour coding reflects the level of compliance with current HGVS recommendations: green = fully compliant, amber = generally compliant with some omission(s) and red = nomenclature error(s)/ fails to comply with the recommendations. **See Trial Comments section for further discussion**.
Please note that whilst it is permissible to use either Ter or * to indicate a translation termination (stop) codon, due to space constraints submissions featuring Ter have been categorised with the equivalent submission featuring *.

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Trial Comments

Whilst participants in this trial were requested to investigate both samples for each of the four core MPN variant types, UK NEQAS LI understands that not all laboratories include assays for all four variant types in their test repertoire. Of the 245 participants returning results and contributing to this dataset, 244 (99.6%) indicated that they provide testing for the *JAK2* p.(Val617Phe) variant, and 169 (69.0%), 194 (79.2%) and 186 (75.9%) provide testing for variants in *JAK2* exon 12, *CALR* exon 9 and *MPL* exon 10, respectively.

Nomenclature used within this report is based on MANE Select (v1.4)[1] reference transcripts and genome build GRCh38. Where participants describe variants in *JAK2* exon 12, *CALR* exon 9 or *MPL* exon 10, these results (at both DNA and protein level) are considered in the context of Human Genome Variation Society (HGVS) Nomenclature recommendations[2-4].

Typically, qualitative results for *JAK2* p.(Val617Phe) testing are subject to performance monitoring; all other results are currently appraised for information only.

Sample MPN DT 122

Sample MPN DT 122 comprised genomic DNA extracted from an MPN patient known to harbour the NM_004343.4:c.1099_1150del p.(Leu367Thrfs*46) *CALR* somatic variant. This 52 bp deletion also known as the type 1 *CALR* variant, is well described in the literature[5-8], where historically it may be referred to as c.1092_1143del p.(Leu367Thrfs*46).

***CALR* exon 9**

- In line with expectation, 191 participants (98.5% of those offering testing) reported the presence of a clinically significant variant in exon 9 of the *CALR* gene.
- The three participants submitting an out-of-consensus negative result all used an NGS assay: one used an Illumina custom panel, one used the ThermoFisher Oncomine Myeloid Gx v2 panel and one used the ThermoFisher Oncomine Myeloid Research Assay. The latter two are both suspected of testing sample MPN DT 123, see below.
- One hundred and ten participants submitted an allele burden for the *CALR* variant; values ranged from 0.5% to 83.0%, with a robust mean of 40.04% and robust SD of 12.39%.

NM_004343.4: c.1099_1150del p.(Leu367Thrfs*46)

DNA sequence change

- In line with expectation, 106 participants (55.5% of those submitting a positive result) provided HGVS approved nomenclature[2-4] that correctly described the DNA sequence change (at the transcript level) as c.1099_1150del coded green in the table on pages 11 of this report).
- A further 11 participants (5.8%, coded amber) also described the consensus variant (at the transcript level) but used nomenclature not fully compliant with HGVS Nomenclature recommendations, for example unnecessarily naming the deleted nucleotides.
- Two participants (1.0%, coded red) described the consensus variant (at the transcript level) but provided a reference sequence that did not relate to the *CALR* gene.

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- One participant (coded amber) used gene (genomic region) coordinates to describe the consensus variant as NG_029662.1:g.10159_10210del. Although the use of genomic/gene coordinates does fulfil the HGVS Nomenclature requirement to describe the variant at the most basic level (DNA level), the application of this approach does not facilitate comparison with the published literature. The use of transcript based variant description(s) is the accepted standard in laboratory reports[9]. Although it can have limitations (e.g. for some intronic variants), standardised (MANE Select[1]) transcript based variant descriptions are typically more intuitive to the clinical team managing a patient's care.
- Thirty-one participants (16.2%, coded red) provided nomenclature with gross errors or described an alternative variant. This included 25 participants failing to follow the 3' rule and referring to the consensus variant using the historic nomenclature c.1092_1143del. For a unified approach, it is important to follow HGVS Nomenclature recommendations; however, we acknowledge that also including historic alternative legacy descriptions as an adjunct in clinical reports can be helpful.
- Forty participants (20.9%) did not provide any DNA nomenclature, including three who did provide protein nomenclature consistent with identification of the consensus variant.
- It is understood that some centres use methodologies that do not allow precise/complete variant identification but may afford some level of variant description. Of those 37 participants not providing any nomenclature in the appropriate HGVS nomenclature submission fields, 33 provided relevant information in the 'Other Details' field. As well as the 20 participants who correctly determined that a deletion of 52 nucleotides was present, one described a deletion of ~50bp, one indicated that a deletion was present and one stated that an insertion/deletion type mutation was detected. One participant incorrectly described a 51bp deletion, while another incorrectly described a 56bp deletion. Ten participants stated that a type 1 mutation had been detected (includes four that also indicated a deletion of 52bp) and three inappropriately added HGVS nomenclature to these fields (again includes one who also indicated a deletion of 52bp).

Protein sequence change

- In line with expectation, 92 participants (48.2% of those submitting a positive result) provided HGVS approved nomenclature[2-4] that correctly described the frameshift sequence change as either p.(Leu367Thrfs*46) or p.(Leu367ThrfsTer46). A further three participants used the appropriate short-format nomenclature, p.(Leu367fs), and two participants used single letter amino acid codes, p.(L367Tfs*46). All 97 descriptions (representing 50.8% of positive results) have been coded green in the table on page 12, however please note that three-letter codes are preferred by HGVS Nomenclature recommendations.
- When using single letter amino acid codes, * should be used to describe a translation termination (stop) codon. The single submission of p.(L367TfsTer46) has therefore been coded amber. Please note that when using three-letter amino acid codes the use of both * and Ter is acceptable.
- A further 43 participants (22.5%, coded amber) described the consensus variant protein change but used nomenclature not fully compliant with HGVS Nomenclature recommendations. Minor errors included the use of a hybrid of standard and short format nomenclature, and the omission/incorrect application of parentheses to indicate the

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predicted status of the description (inherent when genomic DNA is the assay input material).

- Five participants (2.6%, coded red) described an alternative variant or provided nomenclature with gross errors. Of these, three participants utilised '?' in their description, e.g. p.(Leu367Thrfs*?), which incorrectly indicates that the new reading frame does not encounter a termination codon and one omitted the p. prefix, described by HGVS Nomenclature as mandatory to indicate the type of reference sequence used.
- Forty-five participants (23.6%) did not provide a HGVS Nomenclature-based protein description, including eight who did provide a DNA description with varying degrees of compliance with HGVS Nomenclature specifications. The remaining 37 (referred to above) did not submit any (DNA or protein) HGVS Nomenclature-based descriptions.

Further comments

- The NM_004343.4: c.1099_1150del p.(Leu367Thrfs*46) type 1 *CALR* variant has been well described in MPN patients[5-7,10]. In line with other oncogenic gain of function *CALR* variants, this variant creates a +1 shift to an alternate reading frame, which ultimately alters the charge of the C-terminal protein domain with loss of the endoplasmic reticulum-retention motif[5,6]. The characteristic positive electrostatic charge has been shown to promote the association of aberrant calreticulin with the *MPL* encoded thrombopoietin receptor, resulting in activation of the JAK/STAT signalling pathway[11,12].

***JAK2* p.(Val617Phe) – not subject to PM, see below**

- Sixty-four of the 244 participants offering relevant testing (26.2%) reported the presence of the *JAK2* p.(Val617Phe) variant in sample MPN DT 122. Of these, 58 also submitted a variant allele burden: robust mean (RM) = 0.13%, robust standard deviation (RSD) = 0.07%.
- **Given that the *JAK2* p.(Val617Phe) allele burden in this sample is below the limit of detection (LoD) of many assays used in the context of MPN diagnosis, it is not surprising that it was not reported by the majority (180, 73.8%) of participants. For this reason, performance monitoring has not been applied to *JAK2* p.(Val617Phe) results for sample MPN DT 122.**

***JAK2* exon 12**

- In line with sample formulation, all 169 participants offering relevant testing submitted a negative result for *JAK2* exon 12.

***MPL* exon 10**

- In line with sample formulation, 185 participants offering relevant testing (99.5%) submitted a negative result for *MPL* exon 10.
- A single participant (0.5%) submitted an out of consensus positive result. This participant used the Qiagen/Ipsogen MutaScreen Kit and stated that the c.1544G>T p.(Trp515Leu) variant had been detected (with a quoted allele burden of 3%).

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Sample MPN DT 123

Sample MPN DT 123 comprised lyophilised cell line material formulated to be positive for the *JAK2* p.(Val617Phe) variant.

***JAK2* p.(Val617Phe)**

- In line with sample formulation, 243 participants (99.6%) offering relevant testing reported the presence of *JAK2* p.(Val617Phe) in sample MPN DT 123. One hundred and ninety-four participants, using a genomic DNA based assay, submitted a variant allele burden: RM = 26.79%, RSD = 7.40%.

***JAK2* exon 12**

- In line with sample formulation, all 169 participants offering relevant testing submitted a negative result for JAK2 exon 12.

***CALR* exon 9**

- In line with sample formulation, 193 participants (99.5%) offering relevant testing submitted a negative result for *CALR* exon 9.
- The single participant submitting an out-of-consensus positive result is suspected of a sample transposition error since they indicated that the *CALR* c.1099_1150del p.(Leu367Thrfs*46) variant had been detected and also submitted a false negative *CALR* result for sample MPN DT 122.

***MPL* exon 10**

- In line with sample formulation, all 186 participants offering relevant testing submitted a negative result for *MPL* exon 10.

General Methodology Comments

- Overall, the most commonly employed analysis methods were:
  - Real-time qPCR with fluorescent detection (n=133, 54.5%), followed by NGS (n=57, 23.4%) for *JAK2* p.(Val617Phe)
  - NGS (n=89, 52.7%), followed by Sanger sequencing (n=45, 26.6%) for *JAK2* exon 12 variants
  - NGS (n=81, 41.8%), followed by capillary electrophoresis (n=66, 34.0%) for *CALR* exon 9 variants
  - NGS (n=84, 45.2%), followed real-time qPCR with fluorescent detection (n=48, 25.8%) for *MPL* exon 10 variants
- Thus a range of methods, with differing theoretical LoDs, are used by participants to detect variants in the core MPN associated genes. UK good practice guidelines published in 2021 advocated the ability to detect 1-3% VAF or lower for *JAK2* p.(Val617Phe) and 5% VAF for *JAK2* exon 12, *CALR* exon 9 and *MPL* exon 10 variants[13]. In addition, the 'International Consensus Classification (ICC) of myeloid neoplasms and acute leukemias: myeloproliferative neoplasms' recommended the use of assays with enhanced sensitivities of ≤1% for *JAK2* p.(Val617Phe) and 1-3% for *JAK2* Exon 12, *CALR* exon 9 and *MPL* exon 10 variants[14,15].

- In this programme, participants were asked to provide the LoD for each of their assays and the responses are summarised in the table below. This demonstrates that a significant subset of laboratories (highlighted red or amber) use techniques with an LoD considered inadequate in recent guidelines; transition to a technique with an appropriate LoD is therefore highly recommended, particularly for those centres highlighted red in the table below.

Limits of Detection Reported by Participants

Limit of Detection (%)	*JAK2* p.(Val617Phe) (n)	*JAK2* exon 12 (n)	*CALR* exon 9 (n)	*MPL* exon 10 (n)
<1	136	14	20	22
1-3	90	54	78	82
>3-<5	1	1	4	1
5	14	51	66	50
>5	3	49	26	31

The LoDs described in this table are those reported by participants, with no scrutiny as to how they were derived. Participants with LoDs failing to meet UK good practice guidelines[13] are highlighted red. These participants, together with those highlighted amber, also fail to meet the more recent and more stringent ICC recommendations[14,15].

Please note, the UK good practice guidelines advocate the ability to detect 1-3% VAF or lower for *JAK2* p.(Val617Phe) and UK NEQAS LI are currently using the upper threshold of 3% for qualitative assessment of *JAK2* p.(Val617Phe). This means we currently expect all laboratories to be able to detect *JAK2* p.(Val617Phe) when the lower limit of the robust mean (LLRM) is ≥3% (LLRM = RM – Ux, where Ux is the uncertainty of the assigned value). Thus, laboratories are not currently penalised for a false negative result if the LLRM is less than 3%, and also less than the LoD of their assay. **However, we intend to move towards using the lower threshold of 1% which also better reflects the ICC guidance – further information will be provided when necessary, however we strongly encourage participants to continually consider their assays in the context of current guidelines.**

JAK2 p.(Val617Phe) Quantification Comments

Sample MPN DT 122

- Of the 64 participants using a genomic DNA based assay and returning a positive *JAK2* p.(Val617Phe) result for sample MPN DT 122, 58 (90.6%) also submitted *JAK2* p.(Val617Phe) quantification data for this sample.
- Percentage variant allele burdens (defined as 100 x variant alleles/total alleles) ranged from 0.02% to 43%, with a robust mean of 0.13% and robust SD of 0.07%.
- At least two of the three participants submitting outlier results with a strong positive bias (allele burden = 31% or 43%) are suspected of testing sample MPN DT 123, since they also submitted false negative *CALR* results for this sample. The third outlier result (allele burden = 30%) was submitted by a participant who does not provide *CALR* testing.

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Sample MPN DT 123

- Of the 243 participants using a genomic DNA based assay and returning a positive *JAK2* p.(Val617Phe) result for sample MPN DT 123, 194 (79.8%) also submitted *JAK2* p.(Val617Phe) quantification data for this sample.
- Percentage variant allele burdens (defined as 100 x variant alleles/total alleles) ranged from 0.2% to 57%, with a robust mean of 26.79% and robust SD of 7.40%.
- The participant submitting an outlier result with a negative bias (0.2%) also submitted a *JAK2* p.(Val617Phe) allele burden of 31% (along with a positive *CALR* result) for sample MPN DT 122. It is considered likely that this laboratory transposed the two samples provided in this trial.

- Of those participants providing meaningful methodology information for quantification in this trial, the most commonly utilised methods were real-time qPCR (n=99), followed by NGS (n=57) and digital PCR (n=29).
- A minority of laboratories reported the use of capillary electrophoresis (n=5), agarose gel electrophoresis (n=2), high-resolution melt analysis (n=2), or Sanger sequencing (n=1).
- For the most commonly used methods, variant quantification information, is shown in the tables below.

Robust Statistics by Methodology for *JAK2* p.(Val617Phe) quantification

MPN DT 122	**qPCR** (n=38)	**NGS** (n=2)	**dPCR** (n=18)
Robust Mean (%)	0.15	N/A	0.09
Robust SD (%)	0.10	N/A	0.03
Range (%)	0.02 – 31	1.0 - 43	0.054 – 0.2

Robust mean and robust standard deviation of variant allele burdens in MPN DT 122 for the three most utilised quantification methods. Note that outliers suspected of sample transposition events have not been excluded.

MPN DT 123	**qPCR** (n=99)	**NGS** (n=57)	**dPCR** (n=28)
Robust Mean (%)	25.90	27.53	26.73
Robust SD (%)	9.48	6.16	5.79
Range (%)	0.2 - 57	12.0 - 39.88	14.7 – 38

Robust mean and robust standard deviation of variant allele burdens in MPN DT 123 for the three most utilised quantification methods. Note that outliers suspected of sample transposition events have not been excluded.

- Robust statistics have been used to calculate z scores for all participants submitting *JAK2* p.(Val617Phe) quantification resulting from a genomic DNA-based assay. Individualised longitudinal analysis in the form of Shewhart and Cusum control plots is provided and is becoming more informative as further *JAK2* p.(Val617Phe) positive samples are issued within this programme. Z scores have not been calculated for participants utilising RNA or cDNA template in their assay, since such quantification is not directly comparable.
- **Please remember that whilst z score values for quantitative *JAK2* p.(Val617Phe) testing are currently provided for educational purposes only and official trial performance is based on qualitative results, we are working towards providing additional performance scoring for *JAK2* p.(Val617Phe) quantification data (Satisfactory / Action / Critical) for laboratories that require this information.**

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- As a guide, in other UK NEQAS LI quantitative programmes, a z score above 3.5 or below -3.5 is considered to be a 'Critical' result requiring immediate investigation by the laboratory.
- **Based on z scores <-3.5 or >3.5, two (1.0%) participants' results would have been classified as 'Critical' for sample MPN DT 123**. These participants are represented beyond the outer dashed lines in the MPN DT 123 histogram of z scores on page 5. Excluding the participant suspected of a sample transposition event, the remaining laboratory utilises the Biomol JAK2 V617F (qualitative) assay (real-time PCR with fluorescent detection). This participant has indicated that they would like performance monitoring for *JAK2* p.(Val617Phe) quantification once the programme achieves full accreditation, implying that quantitative data is included in their clinical reports. We particularly urge this laboratory react to these findings and instigate a root cause investigation.
- MPN DT 122 was a low-level positive sample with an allele burden RM of 0.13%. Eight participants (13.8% of those submitting a *JAK2* p.(Val617Phe) allele burden for this sample) were awarded z scores <-3.5 or >3.5. This included the three participants who submitted outlier results more consistent with sample MPN DT 123, and it should be noted that technically four of the remaining five participants stated an LoD that was above the LLRM for this sample (LLRM = RM – Ux = 0.12%).

Final Comments

- In addition to the *CALR* c.1099_1150del p.(Leu367Thrfs*46) type 1 variant, sample MPN DT 122 has been shown to harbour *JAK2* p.(Val617Phe) with an allele burden of ~0.13%. Whilst *JAK2* p.(Val617Phe) and *CALR* somatic variants were once thought to be mutually exclusive, there are now multiple reports of the coexistence of low level *JAK2* p.(Val617Phe) with other MPN driver variants[16-20]. This is almost certainly due to improved assay sensitivity[16].

  The clinical significance of detecting two MPN driver variants at diagnosis remains unclear and will likely depend upon the clonality status of the two variants. The consistently low *JAK2* p.(Val617Phe) allele burden in these cases raises the possibility that it may be explained by pre-existing clonal haematopoiesis of indeterminate potential (CHIP)[16,20,21] and that a subsequent *CALR* variant is associated with the MPN phenotype. Whilst it has been suggested that the two variants do mostly co-exist in separate clones[19,21], obtaining experimental proof is time-consuming and therefore rare[22,23]. Interestingly, Nishimura *et al*. (2021) provided the first report of *JAK2* p.(Val617Phe) being acquired in addition to an established *CALR* variant (in the same clone) during the progression from essential thrombocytosis to myelofibrosis[24]. Knock-in mouse studies of double-mutated *JAK2* and *CALR* support the negative impact of the co-existence of two driver mutations in the same clone[23].

  Longitudinal research is needed to further understand the long-term outcomes of patients with co-existing MPN driver mutations[20].

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- We would like to thank laboratories for their participation in the MPN Diagnostic Testing programme. If participants have any suggestions or ideas for this programme, please contact admin@ukneqasli.co.uk.

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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:
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Pegasus House, 4th Floor Suite
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4.8.2 b) The coordinator(s) of UK NEQAS LI programmes: Mr Stuart Scott (Director).

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

4.8.2 d) Administration and shipping for this programme is provided by EQA International Limited.

4.8.2 d) 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.

4.8.2 d) 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/NjRlNTgxYzctMTI4ZS00MTg4LWI2ZDMtZDdkYzJhMTFlZTg3. 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/