Myeloproliferative Neoplasms Diagnostic Testing Programme

Distribution - 242501

Participant ID -

Date Issued - 28 June 2024

Closing Date - 26 July 2024

Trial Comments

This report describes the first trial of the consolidated Myeloproliferative Neoplasms Diagnostic Testing (MPN DT) programme within which the UKAS accredited JAK2 p.Val617Phe (V617F) Mutation Status programme has been incorporated. From the outset, qualitative JAK2 p.(Val617Phe) testing will be subject to performance monitoring. UK NEQAS LI is working towards full accreditation of the MPN DT programme, and anticipates that similar performance monitoring for the remaining core MPN markers, as well as optional performance monitoring for JAK2 p.(Val617Phe) quantification, will be available by the end of 2025.

This trial was issued to 249 participants; 234 (94.0%) returned results. Of the 15 participants who did not submit results, three prenotified UK NEQAS LI of their intended non-return.

Sample Comments

Two vials of lyophilised cell line material were issued in this trial: MPN DT 112 and MPN DT 113. Participants were asked to consider both to be representative of diagnostic patient samples and (subject to their test repertoire) to perform analysis for all four core MPN variant types: 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 (v1.0) reference transcripts: NM_004972.4(JAK2), NM_004343.4(CALR), and NM_005373.3(MPL). MPN DT 112 was manufactured to be positive for JAK2 p.(Val617Phe) only, whilst MPN DT 113 was formulated to be negative for all four variant types.

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Sample MPN DT 112

Did you detect the clinically significant ***JAK2* p.(Val617Phe)** variant in sample MPN DT 112: **Yes**

Did you detect a clinically significant ***JAK2* exon 12** variant in sample MPN DT 112: **No**

Did you detect a clinically significant ***CALR* exon 9** variant in sample MPN DT 112: **No**

Did you detect a clinically significant ***MPL* exon 10** variant in sample MPN DT 112: **No**

Please note that results for *JAK2* exon 12, *CALR* exon 9 and *MPL* exon 10 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/Total number who tested the gene	Consensus DNA Sequence Variantᵠ	Consensus Protein Variantᵠ	Allele Burden (%)ᵟ	
				Robust Mean	Robust SD
JAK2 p.(Val617Phe)	232/233	c.1849G>T detected	p.(Val617Phe) detected	8.80	1.94
JAK2 exon 12	0/157	No variant detected	No variant detected		
CALR exon 9	1/179	No variant detected	No variant detected		
MPL exon 10	0/171	No variant detected	No variant detected		

ᵠResults returned by participants (at both the DNA and protein level) may have been harmonised to the equivalent Human Genome Variation Society (HGVS) approved nomenclature during the compilation of 'All Participant Results' tables . 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.
ᵟRobust 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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Sample MPN DT 113

Did you detect the clinically significant ***JAK2* p.(Val617Phe)** variant in sample MPN DT 113: **No**

Did you detect a clinically significant ***JAK2* exon 12** variant in sample MPN DT 113: **No**

Did you detect a clinically significant ***CALR* exon 9** variant in sample MPN DT 113: **No**

Did you detect a clinically significant ***MPL* exon 10** variant in sample MPN DT 113: **No**

Please note that results for *JAK2* exon 12, *CALR* exon 9 and *MPL* exon 10 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	No variant detected	No variant detected	
MPL exon 10	No variant detected	No variant detected	

All Participant Results

Gene/Region	Participants detecting a Variant/Total number who tested the gene	Consensus DNA Sequence Variantᵠ	Consensus Protein Variantᵠ	Allele Burden (%)ᵟ	
				Robust Mean	Robust SD
JAK2 p.(Val617Phe)	6/233	c.1849G>T not detected	p.(Val617Phe) not detected		
JAK2 exon 12	0/157	No variant detected	No variant detected		
CALR exon 9	1/179	No variant detected	No variant detected		
MPL exon 10	0/171	No variant detected	No variant detected		

ᵠResults returned by participants (at both the DNA and protein level) may have been harmonised to the equivalent Human Genome Variation Society (HGVS) approved nomenclature during the compilation of 'All Participant Results' tables . 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.
ᵟRobust 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	1	0

Please note: Performance monitoring is currently available for *JAK2* p.(Val617Phe) results only. We are working towards a qualitative performance monitoring system for all four MPN gene regions.
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 112	8.08	8.80	1.94	± 0.19
Sample MPN DT 113	N/A	N/A	N/A	N/A

Your *JAK2* p.(Val617Phe) Quantitative Performance

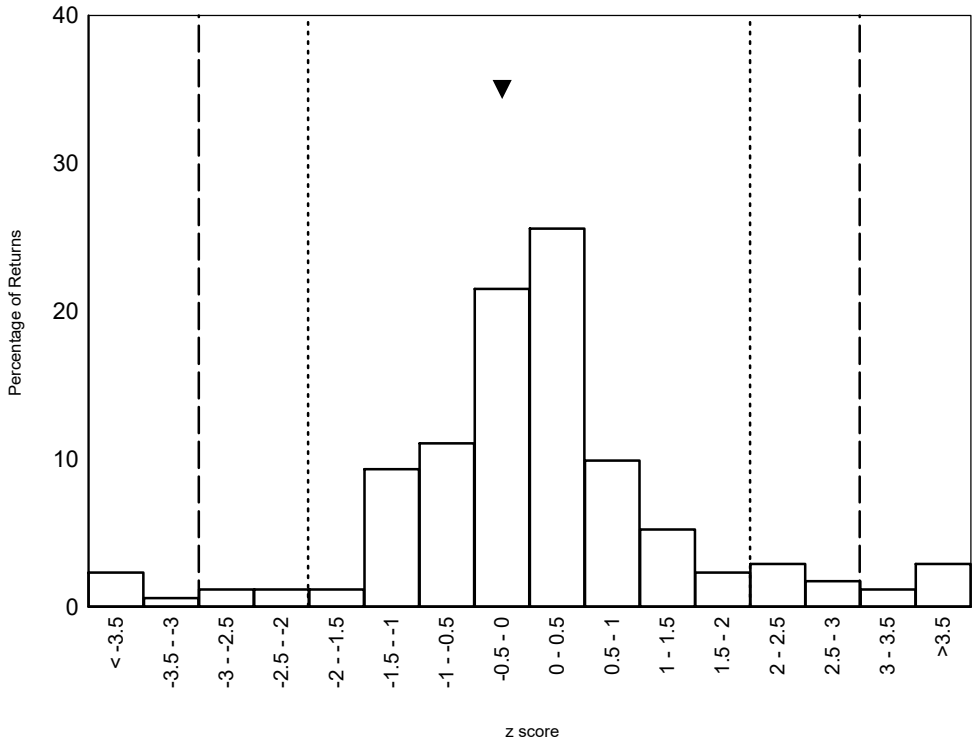
Your Quantitative Performance	z score	Performance Status for this Sample	Performance Status Classification Over Last 6 Positive Samples		
			Satisfactory	Action	Critical
Sample MPN DT 112	-0.37	N/A	N/A	N/A	N/A
Sample MPN DT 113	N/A	N/A	N/A	N/A	N/A

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

Myeloproliferative Neoplasms Diagnostic Testing Programme

Histograms of Participant z scores

JAK2 p.(Val617Phe) allele burden z score for sample MPNDT 112
Please note ▼ denotes your result



JAK2 p.(Val617Phe) allele burden z score for sample MPNDT 113
Please note ▼ denotes your result

No graph data: sample was formulated to be negative for *JAK2* p.(Val617Phe)

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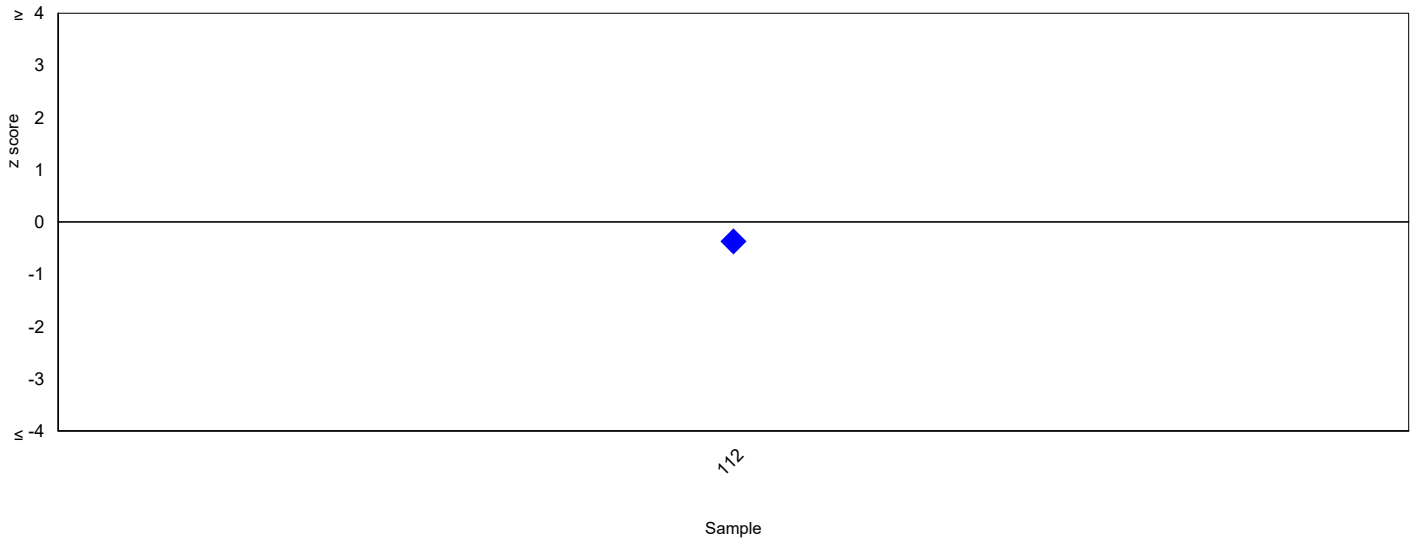
Shewhart Control Charts

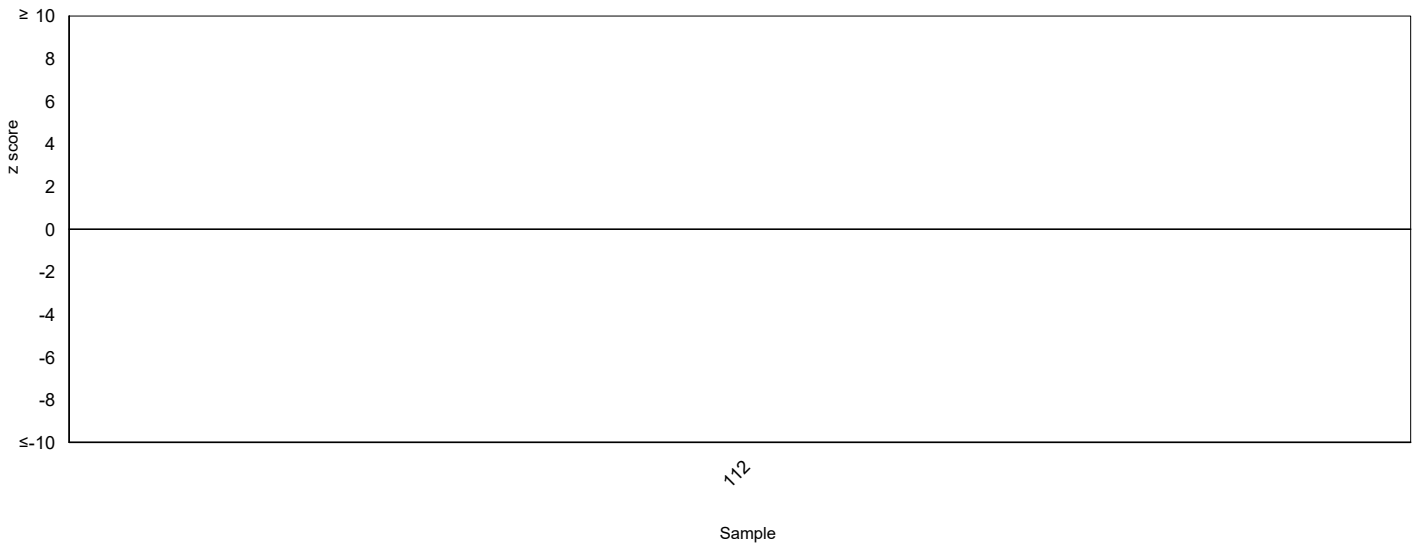
(Please note each data point represents a single sample)

JAK2 p.(Val617Phe) allele burden z score



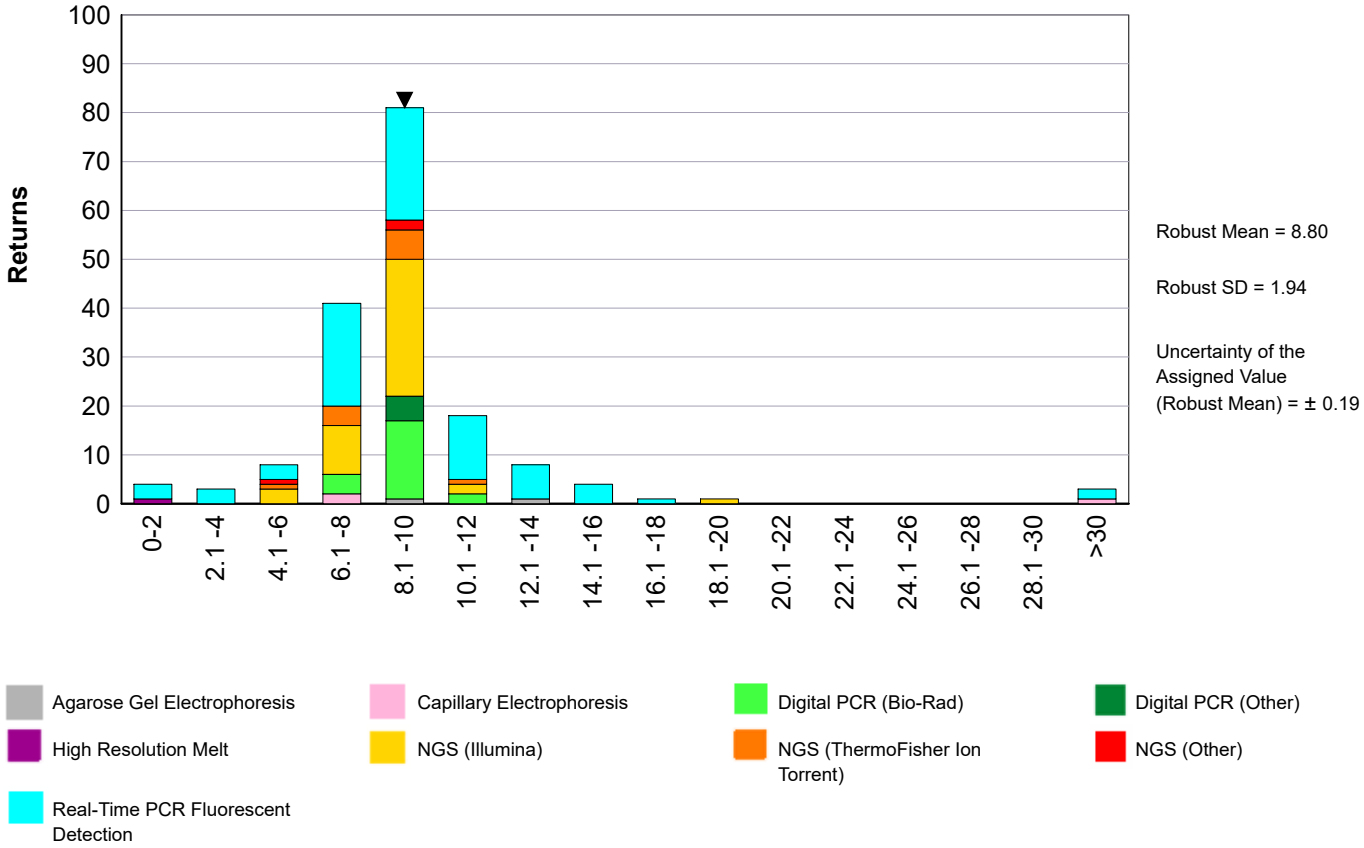
Cusum Control Charts

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



Myeloproliferative Neoplasms Diagnostic Testing Programme

Frequency distribution histogram showing % *JAK2* p.(Val617Phe) allele burden in sample MPNDT 112, classified by analysis method. Please note ▼ denotes your result



Frequency distribution histogram showing % *JAK2* p.(Val617Phe) allele burden in sample MPNDT 113, classified by analysis method. Please note ▼ denotes your result

No graph data: sample was formulated to be negative for *JAK2* p.(Val617Phe)

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PCR Type	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Allele specific PCR	50	6	8	18
Droplet digital PCR	27	-	1	3
Melting curve analysis	8	9	4	7
Multiplex PCR	3	7	5	6
PCR for NGS	59	84	75	78
Real-time PCR	83	7	19	27
Sanger sequencing	-	32	20	21
Single PCR	1	11	47	11

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

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Analysis Type	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Capillary Electrophoresis	8	16	57	15
Digital PCR (Other)	5	-	-	1
High Resolution Melt	5	10	4	9
NGS (Illumina)	45	59	53	58
NGS (Other)	3	4	5	3
Digital PCR (Bio-Rad)	23	-	1	3
Agarose Gel Electrophoresis	16	1	-	2
NGS (ThermoFisher Ion Torrent)	12	22	18	19
Real-Time PCR Fluorescent Detection	117	8	21	35
Sanger Sequencing	-	37	20	26

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

Protocol Type / Kit	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
Agilent SureSelect Custom QXT Panel	3	3	3	3
Archer DX VariantPlex Myeloid Panel	3	3	3	3
BioRad PrimePCR ddPCR Kit	19	-	1	1
Genesig JAK2 V617F QUASA Kit	3	-	-	-
Genmark geneMAP Somatic Mutation Detection Kit	2	5	2	2
Illumina AmpliSeq Panel	3	4	4	4
Illumina custom Panel	4	7	6	6
Illumina TruSight Myeloid Sequencing Panel	4	5	5	5
In-house Assay	97	84	98	76
Qiagen QiaSeq Custom Panel	8	9	8	9
Qiagen/Ipsogen MutaQuant Kit	39	-	-	2
Qiagen/Ipsogen MutaScreen Kit	11	-	1	24
Qiagen/Ipsogen MutaSearch Kit	4	-	-	1
Qiagen/Ipsogen RGQ PCR Kit	5	-	11	-
Sophia Genetics Myeloid Solution	4	4	4	4
ThermoFisher Ion Ampliseq Custom Panel	5	7	7	7
ThermoFisher JAK2 p.V617F TaqMan SNP Assay	3	-	-	-
ThermoFisher Oncomine Myeloid Gx v2 Panel	2	3	2	3
ThermoFisher Oncomine Myeloid Research Assay	4	10	8	8

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	6	5	6
Baxter et al (2005) Lancet 365:1054-61	26	4	2	2
Bench et al (2013) Br J Haematol 160:25-34	7	1	-	1
Boyd et al (2010) Br J Haematol 149:250-7	-	1	-	8
Cross et al (2021) Br J Haematol 195:338–51	4	3	3	5
Furtado et al (2013) J Mol Diagn 15:592-9	-	7	1	3
Furtado et al (2013) J Mol Diagn 15:810-8	-	-	-	7
James et al (2005) Nature 434:1144-8	8	2	2	-
Jones et al (2008) Haematologica 93:1560–1564	-	5	-	-
Jovanovic et al (2013) Leukemia 27:2032-9	7	-	-	-
Klampfl et al (2013) N Engl J Med 369:2379-90	2	-	38	1
Larsen et al (2007) Br J Haematol 136: 745-51	10	1	-	-
Levine et al (2005) Cancer Cell 7: 387-97	5	-	-	-
Nangalia et al (2013) N Engl J Med 369:2391-2405	-	-	11	1
Pardanani et al (2006) Blood 108:3472-6	-	-	-	5
Passamonti et al (2006) Blood 107:3676-82	6	-	-	-
Pietra et al (2008) Blood 111:1686-9	-	6	-	-
Pikman et al (2006) PLoS Med 3:e270	-	-	-	5
Scott et al (2007) N Engl J Med 356:459-68	-	10	-	-
Tefferi and Pardanani (2014) Nat Rev Clin Oncol 11: 125–6.	1	2	6	2
Other	28	28	30	32

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

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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 234 participants returning results for this trial, 233 (99.6%) indicated that they provide testing for the *JAK2* p.(Val617Phe) variant, and 157 (67.1%), 179 (76.5%) and 171 (73.1%) 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 (v1.0)[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) recommendations[2,3].

Qualitative results for *JAK2* p.(Val617Phe) testing have been subject to performance monitoring; all other results are currently appraised for information only.

Sample MPN DT 112

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

- In line with sample formulation, 232 participants (99.6% of those offering relevant testing) reported the presence of *JAK2* p.(Val617Phe) in sample MPN DT 112.
- A single participant submitted an out-of-consensus negative result, obtained using an in-house multiplex PCR assay with capillary electrophoresis.

***CALR* exon 9**

- In line with sample formulation, 178 participants (99.4% of those offering testing) submitted a negative result for *CALR* exon 9.
- The single participant submitting an out-of-consensus positive result utilised the Genmark geneMAP Somatic Mutation Detection kit (real-time quantitative PCR with fluorescent detection). This participant did not use HGVS nomenclature to describe their result but indicated the presence of "*CALR* Del I, Del II and/or Del III" variants.

***JAK2* exon 12 and *MPL* exon 10**

- In line with sample formulation, all 157 participants testing *JAK2* exon 12 and all 171 participants testing *MPL* exon 10 submitted negative results.

Sample MPN DT 113

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

- In line with sample formulation, 227 participants (97.4% of those offering testing) reported the absence of *JAK2* p.(Val617Phe) in sample MPN DT 113.
- Six participants submitted an out-of-consensus positive result. All six utilised the Qiagen/Ipsogen MutaQuant kit (real-time quantitative PCR) and provided a *JAK2* p.(Val617Phe) allele burden. These ranged from 0.009% to 0.53%. Given that quantitative PCR is associated with an inherent low level of background positivity, such low-level

results should be interpreted with caution and within context (in this case, participants were asked to consider the sample to have been taken at diagnosis).

***CALR* exon 9**

- In line with sample formulation, 178 participants (99.4% of those offering testing) submitted a negative result for *CALR* exon 9.
- The single participant submitting an out-of-consensus positive result also submitted the out-of-consensus positive result for sample MPN DT 112, and again they did not use HGVS nomenclature to describe their result but indicated the presence of "*CALR* Del I, Del II and/or Del III" variants.

***JAK2* exon 12 and *MPL* exon 10**

- In line with sample formulation, all 157 participants testing *JAK2* exon 12 and all 171 participants testing *MPL* exon 10 again submitted negative results.

General Methodology Comments

- Overall, the most commonly employed analysis methods were:
  - Real-Time PCR with fluorescent detection (n=117, 50.2%), followed by next generation sequencing (NGS, n=60, 25.8%) for *JAK2* p.(Val617Phe)
  - NGS (n=85, 54.1%), followed by Sanger sequencing (n=37, 23.6%) for *JAK2* exon 12 variants
  - NGS (n=76, 42.5%), followed by capillary electrophoresis (n=57, 31.8%) for *CALR* exon 9 variants
  - NGS (n=80, 46.8%), followed Real-Time PCR with fluorescent detection (n=35, 20.5%) for *MPL* exon 10 variants
- Thus, a wide range of methods were used to detect variants in the core MPN associated genes, the majority of which have an adequate theoretical limit of detection (LoD) in the context of MPNs.
- Good practice guidelines advocate 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[4]. Participants were asked to provide their LoD for each of their assays and the responses are summarised in the table below. This demonstrates that a subset of laboratories (highlighted red) use techniques with an inadequate LoD; transition to a technique with an appropriate LoD is therefore highly recommended for those centres.

Limits of Detection Reported by Participants

Limit of Detection (%)	*JAK2* p.(Val617Phe)	*JAK2* exon 12	*CALR* exon 9	*MPL* exon 10
<1	118	18	25	27
1-3	93	51	66	72
>3/<5	0	0	2	0
5	18	48	63	46
>5	2	39	22	25

The LoDs described in this table are those reported by participants, with no scrutiny as to how they were derived.

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Quantification Comments

- Of the 232 participants returning a positive *JAK2* p.(Val617Phe) result for sample MPN DT 112, 172 (74.1%) also submitted *JAK2* p.(Val617Phe) quantification data for this sample.
- Percentage variant allele burdens (defined as 100 x variant alleles/total alleles) ranged from 0.945% to 56.4%, with a robust mean of 8.8% and robust SD of 1.94%.

- Of those participants providing meaningful methodology information for quantification, the most commonly utilised methods were real-time qPCR (n=80), followed by NGS (n=59) and digital PCR (n=27).
- A minority of laboratories reported the use of capillary electrophoresis (n=3), agarose gel electrophoresis (n=2) or high-resolution melt analysis (n=1) for quantification.
- For the most commonly used methods, variant quantification information is shown in the table below.

Robust Statistics by Methodology

MPN DT 112	**qPCR** (n=80)	**NGS** (n=59)	**dPCR** (n=27)
Robust Mean (%)	9.08	8.58	8.90
Robust SD (%)	3.08	1.34	0.82
Range (%)	0.945 - 56	5.0 - 19.0	7.4 - 11.9

Robust mean and robust standard deviation of variant allele burdens in MPN DT 112 for the three most utilised quantification methods.

- As previously provided in reports from the retired JAK2 p.Val617Phe (V617F) Mutation Status programme, robust statistics and z scores have been calculated for submitted *JAK2* p.(Val617Phe) quantification results. Individualised longitudinal analysis in the form of Shewhart and Cusum control plots has commenced / restarted for all participants submitting quantification data; these plots will become more informative as further *JAK2* p.(Val617Phe) positive samples are issued within this programme.
- **Please remember that whilst the z score value for quantitative *JAK2* p.(Val617Phe) testing is 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.**
- As a guide, in our other 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, in this current trial nine laboratories' results would have been scored as 'Critical' for sample MPN DT 112.** These laboratories are represented beyond the outer dashed lines in the histogram of z scores on page 5. Six of these laboratories (including one using cDNA as the template material) employ a real-time qPCR-based technique, while one uses an in-house allele-specific PCR assay with capillary electrophoresis, one uses an in-house high resolution melt curve assay and one uses an in-house NGS assay (Illumina technology).
  We urge these nine laboratories to pay attention to these findings, particularly if they are using quantitative data in clinical reports.

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Final Comments

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

References

1. Morales J, Pujar S, Loveland JE, Astashyn A, Bennett R, Berry A, *et al*. (2022) A joint NCBI and EMBL-EBI transcript set for clinical genomics and research. *Nature* **604**:310–315.
2. den Dunnen JT, Dalgleish R, Maglott DR, Hart RK, Greenblatt MS, McGowan-Jordan J *et al*. (2016) HGVS recommendations for the description of sequence variants: 2016 Update. *Hum Mutat* **37**: 564–9.
3. den Dunnen JT Sequence Variant Nomenclature Version 21.0.4 Available at: https://hgvs-nomenclature.org/stable/ (accessed: Sept 2024).
4. Cross NCP, Godfrey AL, Cargo C, Garg M, and Mead AJ (2021) The use of genetic tests to diagnose and manage patients with myeloproliferative and myeloproliferative / myelodysplastic neoplasms, and related disorders. *Br J Haematol* **195**: 338-351.

Myeloproliferative Neoplasms Diagnostic Testing Programme

Information with respect to compliance with standards BS EN ISO/IEC 17043:2010

4.8.2 a) The proficiency testing provider for this programme is:
UK NEQAS for Leucocyte Immunophenotyping
Pegasus House, 4th Floor Suite
463A Glossop Road
Sheffield, S10 2QD
United Kingdom
Tel: +44 (0) 114 267 3600
e-mail: amanda.newbould@ukneqasli.co.uk

4.8.2 b) The 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 and post-closure testing of samples for this trial was 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/