JAK2 p.Val617Phe (V617F) Mutation Status Programme

Distribution - 232401 Participant ID -

Date Issued - 14 June 2023 Closing Date - 14 July 2023

Trial Comments

This trial was issued to 226 particpants; 217 participants (96.0%) returned results. Of the nine participants who failed to return results, three pre-notified us of their intended non-return and one requested an extension to the submission deadline.

Sample Comments

Two lyophilised samples (JAK2 183 and JAK2 184) were distributed to participants for JAK2 p.Val617Phe (V617F) variant analysis in this trial. These duplicate samples were formulated to be positive for JAK2 p.Val617Phe.

Results and Performance

Your Qualitative Results

JAK2 Mutation Status	Your Results	Consensus Result
Sample JAK2 183	Mutation Detected	Mutation Detected
Sample JAK2 184	Mutation Detected	Mutation Detected

All Participant Qualitative Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample JAK2 183	215	2
Sample JAK2 184	215	2

Your Qualitative Performance

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

N/A = Not Applicable

Your Quantitative Results

JAK2 Mutation Load	Your Results	Robust Mean	Robust SD
Sample JAK2 183	41.5	45.19	6.35
Sample JAK2 184	44.7	47.47	6.58

Your Quantitative Performance

Your Quantitative Performance	z-score	Performance Status for this Sample	Performance Status Classification Over Last 6 Positive Samples		
			Satisfactory	Action	Critical
Sample JAK2 183	-0.58	N/A	N/A	N/A	N/A
Sample JAK2 184	-0.42	N/A	N/A	N/A	N/A

N/A = Not Applicable

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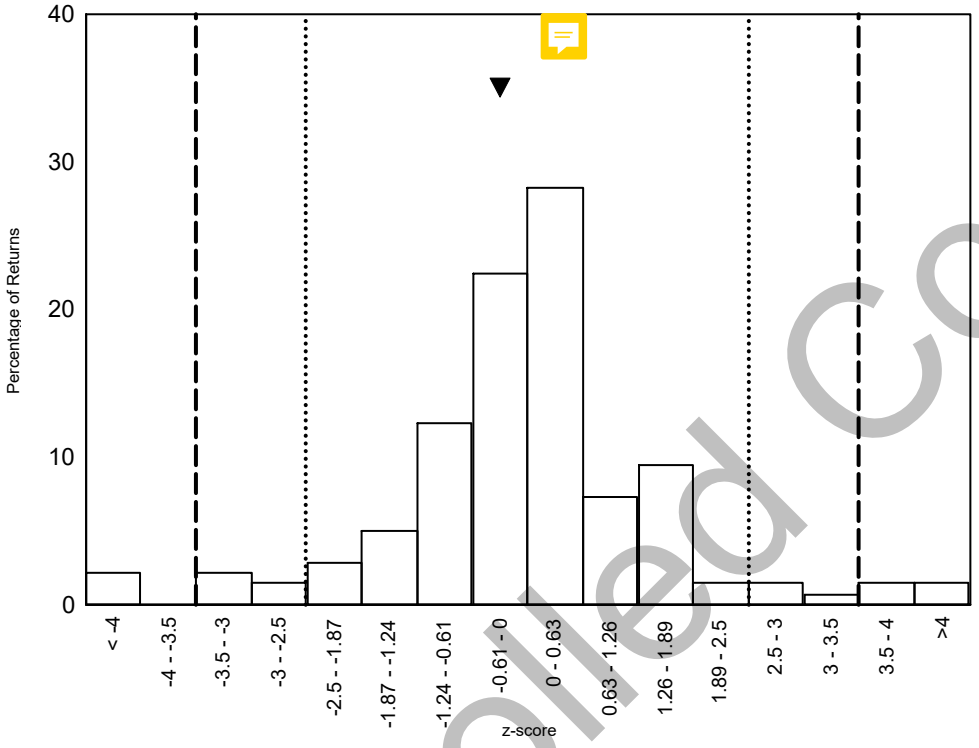
Histograms of Participant z-scores

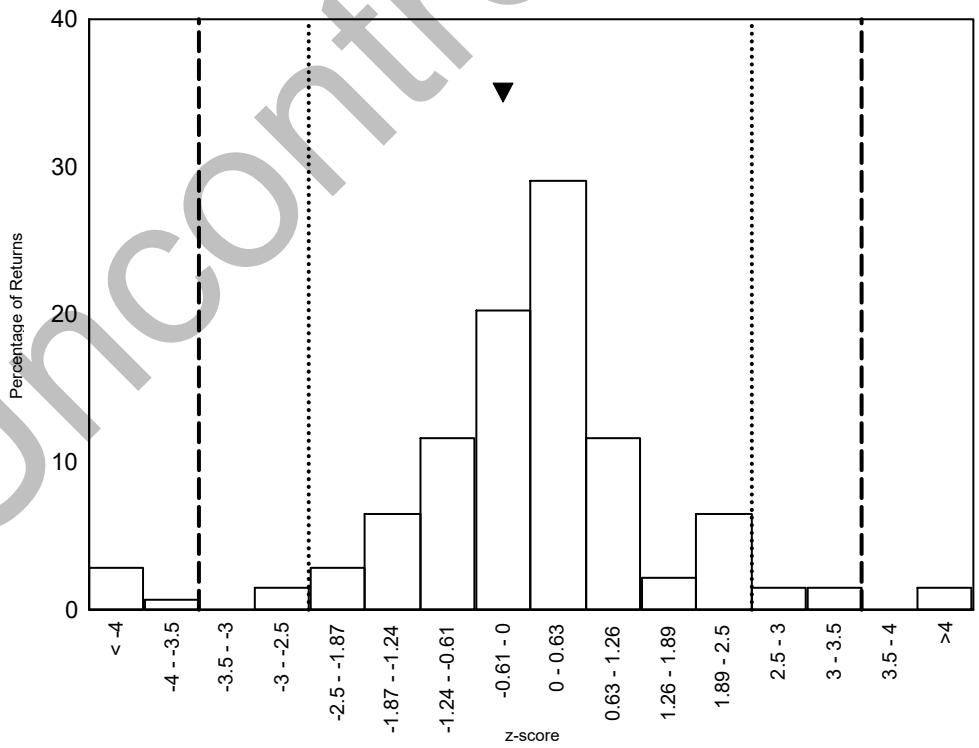
JAK2 p.V617F % mutation load z-score for sample JAK2 183

Please note ▼ denotes your result



JAK2 p.V617F % mutation load z-score for sample JAK2 184

Please note ▼ denotes your result



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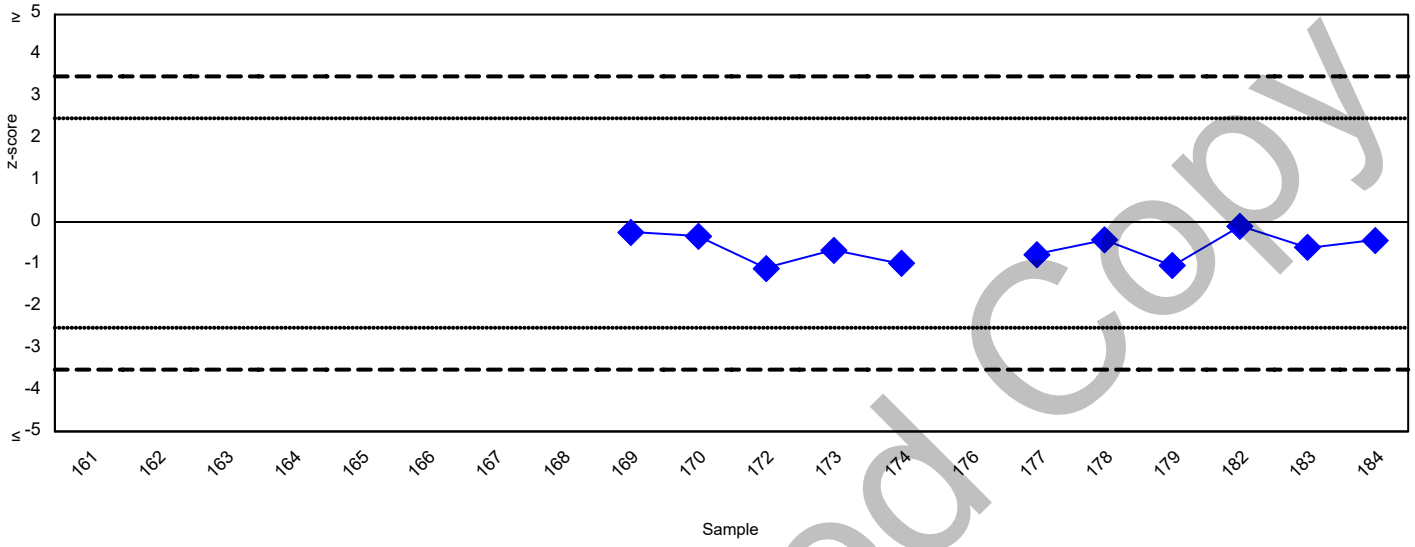
Shewhart Control Charts

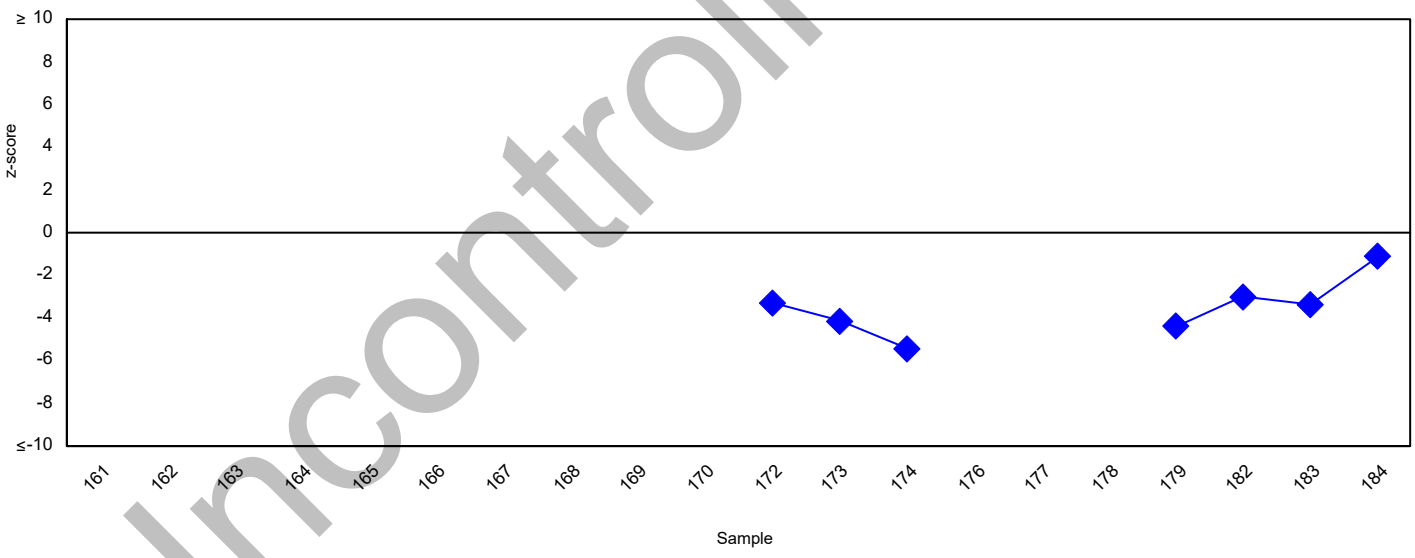
(Please note each data point represents a single sample)

JAK2 p.V617F % mutation load 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)



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Template Type

	Returns
DNA	215
cDNA	2

PCR Type

	Returns
Real-Time PCR	103
Allele Specific PCR	54
Droplet Digital PCR	26
PCR for Next generation Sequencing	13
Melting Curve Analysis	7
Single PCR	6
Sequencing	5
LNA PCR	1
Multiplex PCR	1

Protocol Type

	Returns
In-house Assay	114
Ipsogen JAK2 MutaQuant Kit CE	38
BioRad PrimePCR ddPCR kit	21
Ipsogen JAK2 MutaScreen Kit CE	14
Ipsogen JAK2 MutaSearch Kit	6
Ipsogen JAK2 RGQ PCR Kit CE	5
Oncomine Myeloid Research Assay	5
3B BlackBio TRUPCR JAK2 Kit	3
Genesig JAK2 V617F QUASA kit	3
ThermoFisher JAK2 p.V617F TaqMan SNP assay	3
AmoyDx JAK2 Mutation Detection Kit	2
Genmark geneMAP Somatic Mutation Detection Kit	1
Illumina TruSight Myeloid Sequencing Panel	1
Rotor-Gene Q MDx	1

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Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	131
Agarose Gel Electrophoresis	24
Digital PCR (Biorad)	24
Capillary Electrophoresis	9
NGS (Illumina)	9
NGS (ThermoFisher Ion Torrent)	7
NGS (Other)	4
Digital PCR (Other)	3
High Resolution Melt	3
Mass Spectometry	2
Microchip Electrophoresis System	1

Journal Reference for Assay

	Returns
Baxter et al (2005) Lancet; 365 (9464):1054-61	64
Levine et al (2005) Cancer Cell 7(4): 387-97	23
Larsen et al (2007)BJH, 136, 745-751	21
Tefferi et al Leukemia 22 (1): 14-22	17
Lippert et al (2006), Blood 108(6):1865-7	10
Passamonti et al (2006) Blood 107 (9):3676-3682	10
Denys B et al (2010)J Mol Diagn 12(4):512-9	8
Jones et al (2005) Blood 106(6):2162-21681	8
Jovanovic et al (2013) Leukemia 27, 2032–2039	7
Cankovic et al (2009) Am J Clin Pathol 132 (5): 713-21	6
Chen et al (2007) J Mol Diagn (9):272-276	6
Vannucchi et al (2009) 33(12):1581-3	5
Kroger et al (2007) Blood 109(3):1316-21	4
James et al (2006) Leukemia (2)350-353.	3
Lay et al (2006) J Mol Diagn; 8(3):330-4	3
McClure et al (2006) Leukemia 20 (1) 168-71	3
Murugesan et al (2006) AM J Clin Pathol :125(4):625-33	2
Sidon et al (2006) Clin Chem :52(7):1436-8	2

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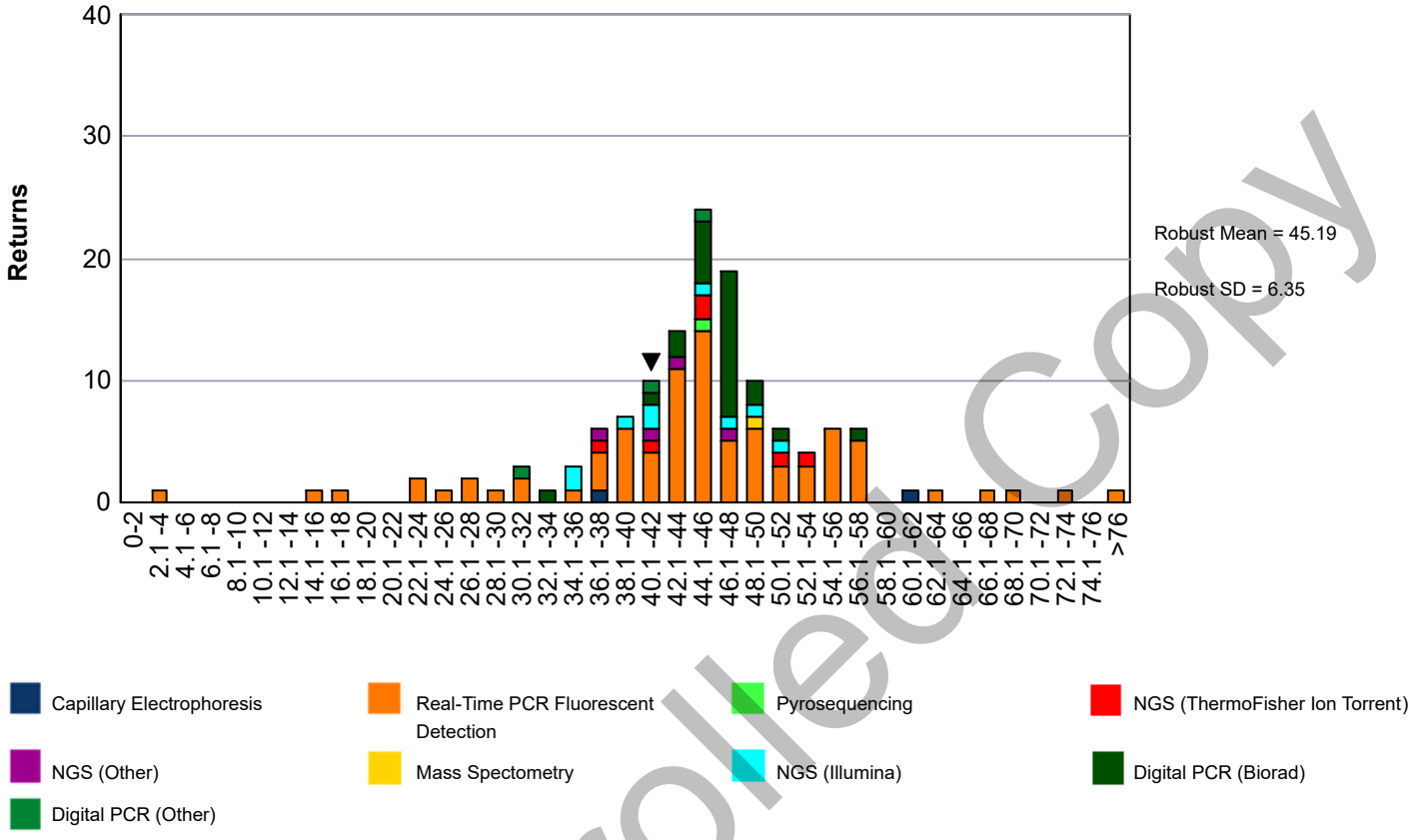
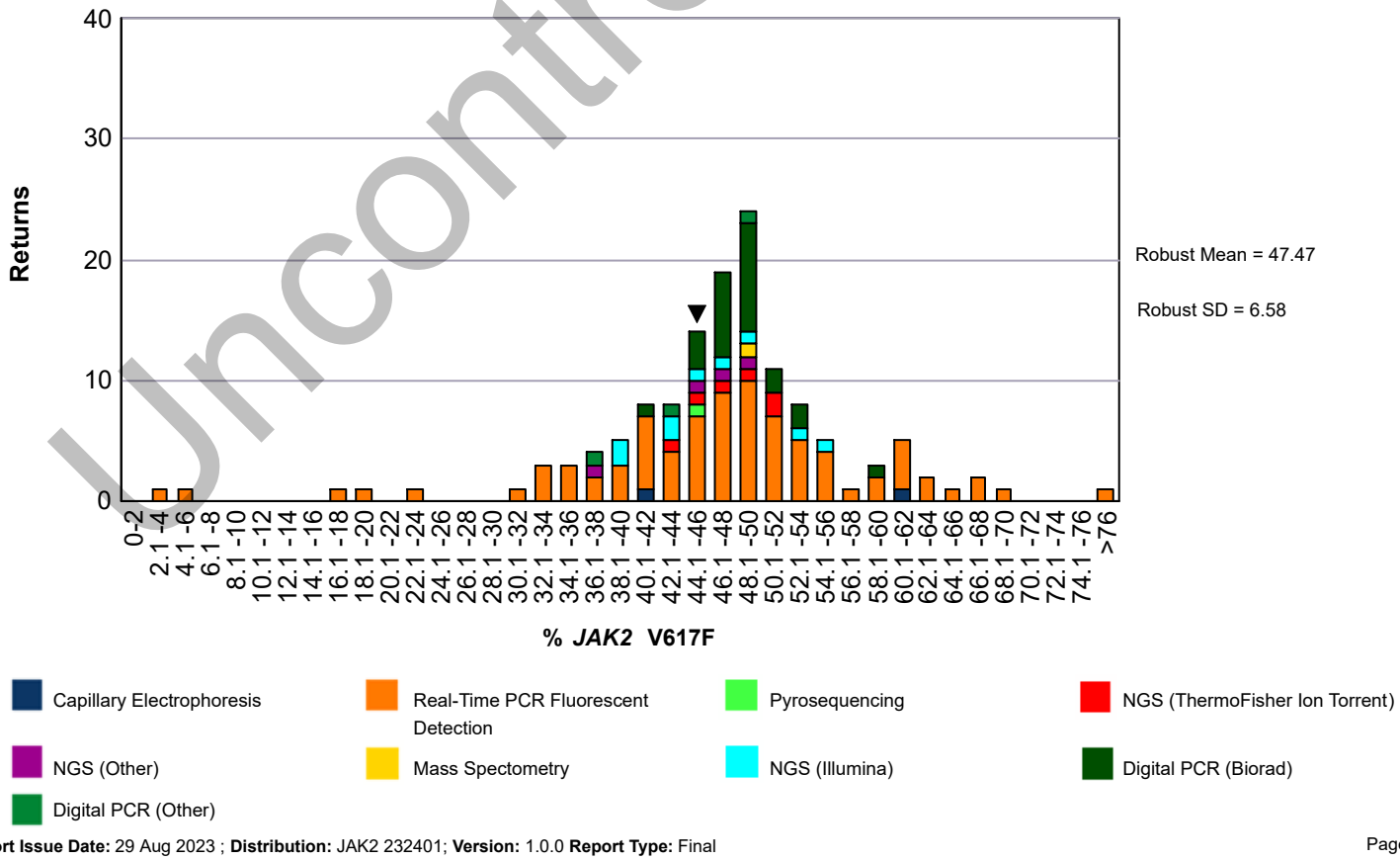
Frequency distribution histogram showing % JAK2 mutation load in sample JAK2 183, classified by analysis method



Frequency distribution histogram showing % JAK2 mutation load in sample JAK2 184, classified by analysis method



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Trial comments:

Sample JAK2 183

- In line with sample formulation, 215 laboratories (99.1% of returning participants) detected the JAK2 p.Val617Phe variant in sample JAK2 183.
- Two participants returned an out-of-consensus negative result for sample JAK2 183, both of whom utilised a real-time quantitative PCR approach: one used an in-house assay, and one employed the AmoyDx JAK2 Mutation Detection Kit.

Sample JAK2 184

- In line with sample formulation, 215 laboratories (99.1% of returning participants) detected the JAK2 p.Val617Phe variant in sample JAK2 184.
- Of the two laboratories returning an out-of-consensus negative result for sample JAK2 184, one also returned an out of consensus result for JAK2 183 (in-house real-time qPCR assay), the other employed the Ipsogen JAK2 MutaSearch Kit.

Quantification comments:

- One hundred and thirty-eight laboratories (64.2% of laboratories returning positive results) submitted quantification data for the samples in this trial.

- For sample JAK2 183, submitted variant allele burdens ranged from 3.3% to 136.1% (robust mean 45.19%, robust SD 6.35%).
- For sample JAK2 184, submitted variant allele burdens ranged from 3.6% to 129.8% (robust mean 47.47%, robust SD 6.58%).
- Please note that % allele burdens are requested in the following format, and therefore values >100% are mathematically impossible:

  [V617F alleles / (V617F alleles + wildtype alleles)] x 100
- The same participants provided the minimum and maximum outlier results for each sample, using an in-house real-time qPCR based assay and the Genmark geneMAP Somatic Mutation Detection Kit respectively.

- Of those participants providing meaningful methodology information for quantification, the most commonly utilised methods were real-time qPCR (n=87), followed by digital PCR (n=28), and next generation sequencing (n=19).
- A minority of laboratories reported the use of capillary electrophoresis (n=2), mass spectrometry (n=1) or pyrosequencing (n=1) for quantification.
- For the most commonly used methods, variant quantification information is shown in the tables below.

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JAK2 183	**qPCR** (n=87)	**dPCR** (n=28)	**NGS** (n=19)
Robust Mean (%)	45.13	46.20	43.64
Robust SD (%)	9.05	1.89	5.11
Range (%)	3.3-136.1	32.0-56.2	34.1-52.7

Table: Robust mean and robust SD of variant allele burdens in JAK2 183 for the three most utilised quantification methods.

JAK2 184	**qPCR** (n=87)	**dPCR** (n=28)	**NGS** (n=19)
Robust Mean (%)	47.6	48.04	46.28
Robust SD (%)	9.58	2.28	4.69
Range (%)	3.6-129.8	38.0-58.9	38.0-55.4

Table: Robust mean and robust SD of variant allele burdens in JAK2 184 for the three most utilised quantification methods.

- As introduced in the 202103 JAK2 p.Val617Phe (V617F) trial, robust statistics and z-scores have been calculated for the submitted quantification results and individualised longitudinal analysis in the form of Shewhart and Cusum control plots is continuing.
- **Please note that currently this quantitative analysis is provided for information only and official trial performance is based on the qualitative result; however, we are working towards providing performance scoring for quantitative data (Satisfactory / Action / Critical) for laboratories that require this information.** Importantly, based on z-scores <-3.5 or >3.5, in this current trial seven laboratories would have been scored as critical for sample JAK2 183 and seven would have been scored as critical for JAK2 184 (nine laboratories in total). Five laboratories would have been scored as critical for both samples; all employ real-time qPCR-based techniques. We urge all nine laboratories to pay attention to these findings, particularly if they are using quantitative data in clinical reports.

Final Comments

- UK NEQAS LI is working towards the consolidation of the JAK2 p.Val617Phe (V617F) Variant Status Programme and the MPN Diagnostic Programme for the 2024/2025 registration period. Further details will be provided as this development progresses.
- We would like to thank laboratories for their continued participation in the JAK2 p.Val617Phe (V617F) Variant Status Programme.

JAK2 p.Val617Phe (V617F) Mutation Status 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 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/