UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

4th Floor Suite, Pegasus House, 463A Glossop Road, Sheffield, S10 2QD, United Kingdom

Office Main Number: +44 (0) 114 267 3600 **Mr L Whitby (Director): +44 (0) 114 267 3607**
Main Fax: +44 (0) 114 267 3601 **Mr S Scott (Centre Manager): +44 (0) 114 267 3603**
Website: www.ukneqasli.co.uk

Dear Participant:

As part of our ongoing efforts to improve the JAK2 p.Val617Phe (V617F) Mutation Status programme and to ensure it reflects current laboratory practise, you will note several changes to the trial report.

With longitudinal monitoring of JAK2 p.Val617Phe mutation load/allelic burden increasingly being used to assess the efficacy of treatments for myeloproliferative neoplasms (Haslam & Langabeer, 2016; Stasik *et al*, 2018; Hjelmgren *et al*, 2020), the report has been redesigned to provide robust statistics and z-score (ISO, 2015) -in line with already accredited UK NEQAS LI programmes- for quantitative results provided by participants.

The robust statistics are derived from participant data using Algorithm A (ISO 5725-5) that ensures that all data is included in the generation of the robust mean, but also minimises the effect of outliers upon the final statistics.

Due to the nature of how z-scores are generated a positive z-score highlights a positive bias in a laboratory's results whereas a negative z-score shows a negative bias. As such, this adds value to the performance monitoring information provided to laboratories because the z-score immediately highlights to the participating centre if their result is above or below the expected consensus value.

Furthermore, histograms, Shewhart and Cusum control plots have been added to the trial report to facilitate longitudinal analysis of your quantitative results.

At this time, quantitative data 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.

Please find an annotated report that explains the changes here:

http://www.ukneqasli.co.uk/eqa-pt-programmes/molecular-haemato-oncology-programmes/jak2-p-val617phe-v617f-mutation-status-accredited/

If you have any questions, please contact us using the details below.

4th Floor Suite, Pegasus House, 463A Glossop Road, Sheffield, S10 2QD, United Kingdom

Office Main Number: +44 (0) 114 267 3600 **Mr L Whitby (Director): +44 (0) 114 267 3607**
Main Fax: +44 (0) 114 267 3601 **Mr S Scott (Centre Manager): +44 (0) 114 267 3603**
Website: www.ukneqasli.co.uk

UK NEQAS for Leucocyte Immunophenotyping
Telephone: (+44) 0114 267 3600
Fax: (+44) 0114 267 3601
Email: admin@ukneqasli.co.uk

Reference(s)

Haslam, K. & Langabeer, S.E. (2016) Monitoring minimal residual disease in the myeloproliferative neoplasms: Current applications and emerging approaches. *BioMed Research International*, **2016,**.

Hjelmgren, J., Nilsson, K. & Birgegård, G. (2020) JAK2 V617F as a Marker for Long-Term Disease Progression and Mortality in Polycythemia Vera and its Role in Economic Modeling. *Journal of Health Economics and Outcomes Research*, **7,** 61–70.

ISO (2015)ISO 13528:2015. Statistical methods for use in proficiency testing by interlaboratory comparison.

Stasik, S., Schuster, C., Ortlepp, C., Platzbecker, U., Bornhäuser, M., Schetelig, J., Ehninger, G., Folprecht, G. & Thiede, C. (2018) An optimized targeted Next-Generation Sequencing approach for sensitive detection of single nucleotide variants. *Biomolecular Detection and Quantification*, **15,** 6–12.

UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

JAK2 p.Val617Phe (V617F) Mutation Status Programme

Distribution - 202103 Participant ID -

Date Issued - 10 February 2021 Closing Date - 19 March 2021

Trial Comments

FINAL REPORT: In this trial, there were 240 participating laboratories; 228 (95.0 %) participants returned results. Of the 12 participants who failed to return results, two pre-notified us of their non-return and one requested an extension to the submission deadline.

Sample Comments

Two lyophilised samples (JAK2 169 and JAK2 170) were distributed to participants for JAK2 p.Val617Phe variant analysis in this trial. JAK2 169 and JAK2 170 were duplicate samples formulated to be positive for the JAK2 V617F variant. For educational purposes, a further lyophilised sample (Edu I) was also distributed. Edu I was formulated to be positive for the JAK2 p.Val617Phe (V617F) variant around the 1-3% level specified to be significant at diagnosis in myeloproliferative neoplasms (Bench et al., 2013).

Results and Performance

Your Qualitative Results

JAK2 Mutation Status	Your Results	Consensus Result
Sample JAK2 169	Mutation Detected	Mutation Detected
Sample JAK2 170	Mutation Detected	Mutation Detected

All Participant Qualitative Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample JAK2 169	221	7
Sample JAK2 170	221	7

Your Qualitative Performance

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 169		4.92	1.40
Sample JAK2 170		5.12	1.78

Your Quantitative Performance

Your Quantitative Performance	z-score	Performance Status for this Sample	Performance Status Classification Over 6 Sample Period		
			Satisfactory	Action	Critical
Sample JAK2 169	0.34	N/A	N/A	N/A	N/A
Sample JAK2 170	-0.35	N/A	N/A	N/A	N/A

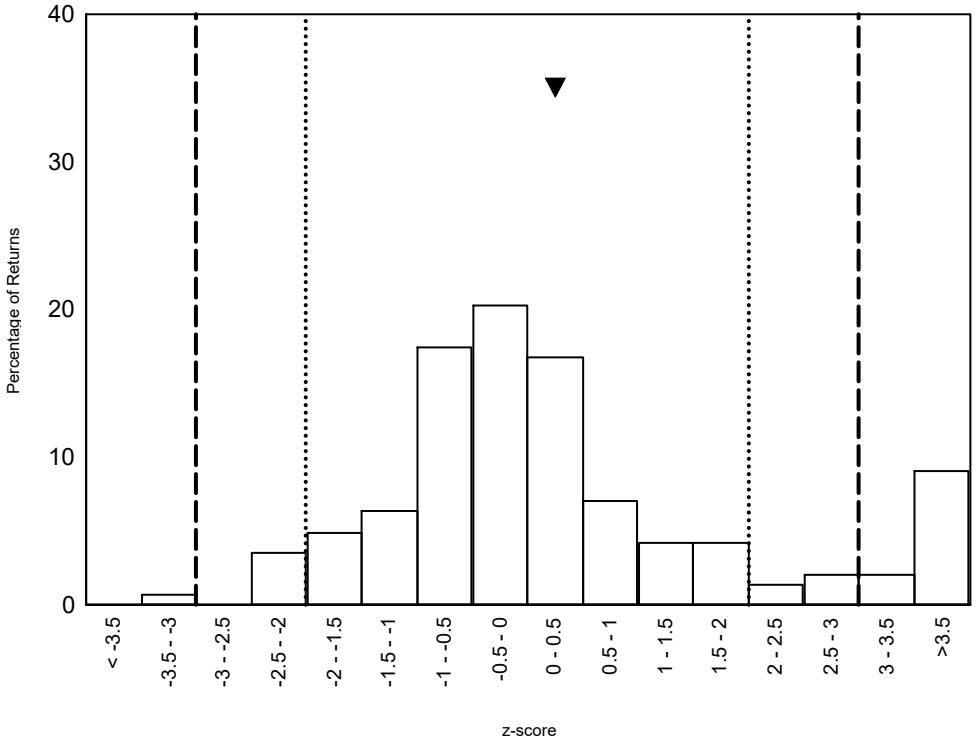
N/A = Not Applicable

JAK2 p.Val617Phe (V617F) Mutation Status Programme

Histograms of Participant z-scores

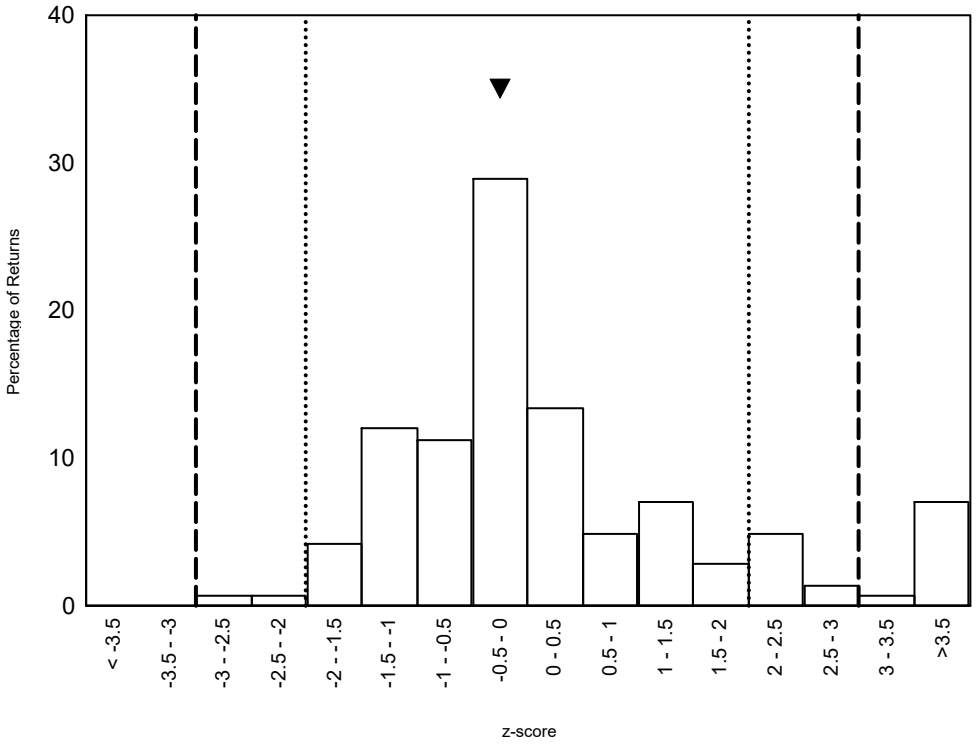
JAK2 p.V617F % mutation load z-score

Please note ▼ denotes your result



JAK2 p.V617F % mutation load z-score

Please note ▼ denotes your result

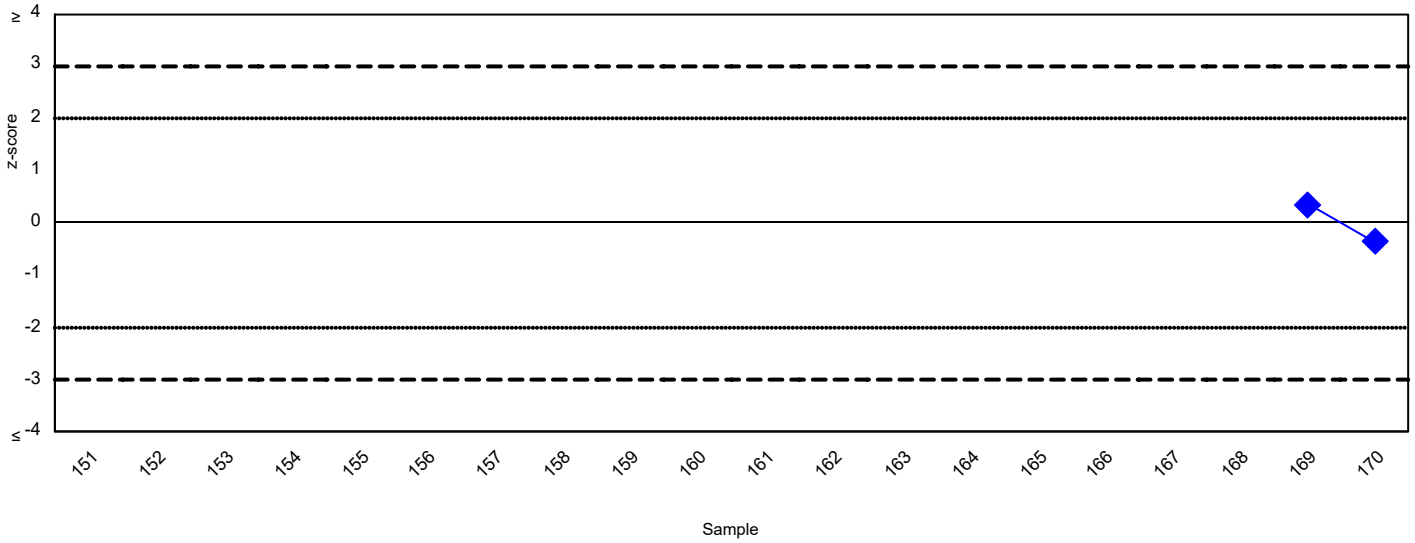


JAK2 p.Val617Phe (V617F) Mutation Status Programme

Shewhart Control Charts

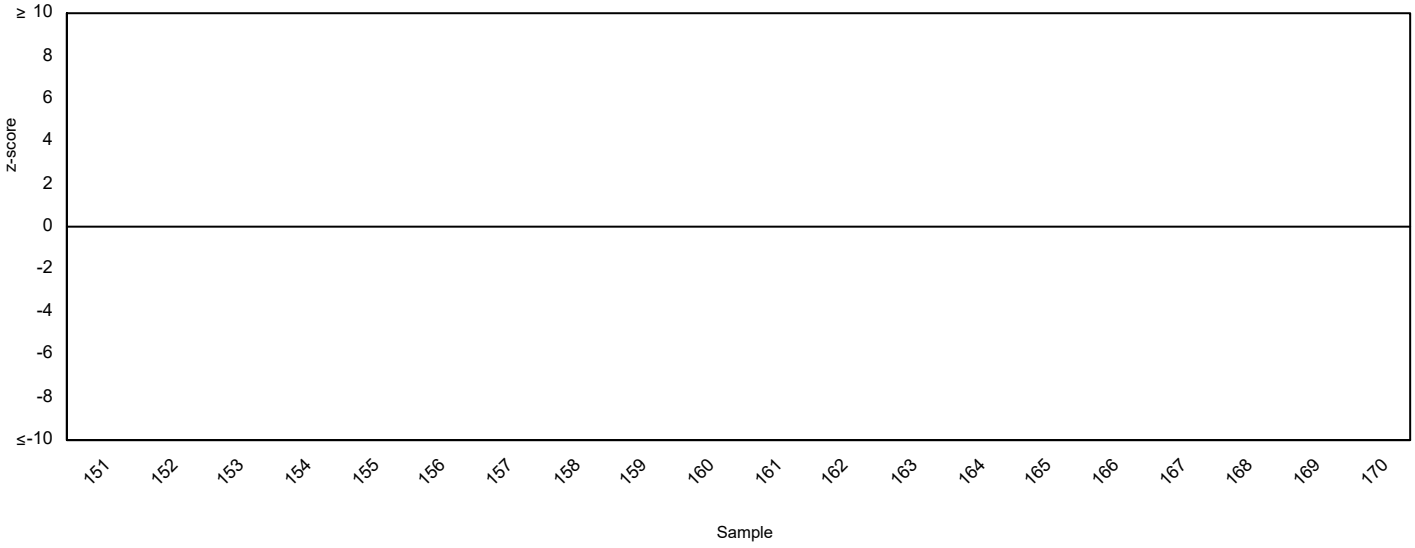
(Please note each data point represents a single sample)

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)



JAK2 p.Val617Phe (V617F) Mutation Status Programme

Template Type

	Returns
DNA	225
cDNA	4

PCR Type

	Returns
Real-Time PCR	88
Allele Specific PCR	68
Droplet Digital PCR	24
Sequencing	15
Melting Curve Analysis	11
Single PCR	11
Multiplex PCR	6
LNA PCR	3
Allele Specific Competitive Blocker PCR	1
COLD PCR	1

Protocol Type

	Returns
In-house Assay	137
Ipsogen JAK2 MutaQuant Kit CE	36
BioRad PrimePCR ddPCR kit	20
Ipsogen JAK2 MutaScreen Kit CE	16
Ipsogen JAK2 RGQ PCR Kit CE	6
Ipsogen JAK2 MutaSearch Kit	5
ThermoFisher JAK2 p.V617F TaqMan SNP assay	4
Genesig JAK2 V617F QUASA kit	2
Ion Torrent Oncomine Myeloid Panel	2
Rotor-Gene Q MDx	1

JAK2 p.Val617Phe (V617F) Mutation Status Programme

Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	127
Agarose Gel Electrophoresis	36
Digital PCR (Biorad)	22
Capillary Electrophoresis	13
NGS (Illumina)	8
NGS (Other)	6
High Resolution Melt	5
NGS (ThermoFisher Ion Torrent)	5
Mass Spectometry	2
Digital PCR (Other)	1
Digital PCR (Raindance)	1
Microchip Electrophoresis System	1
Restriction Enzyme Digest	1

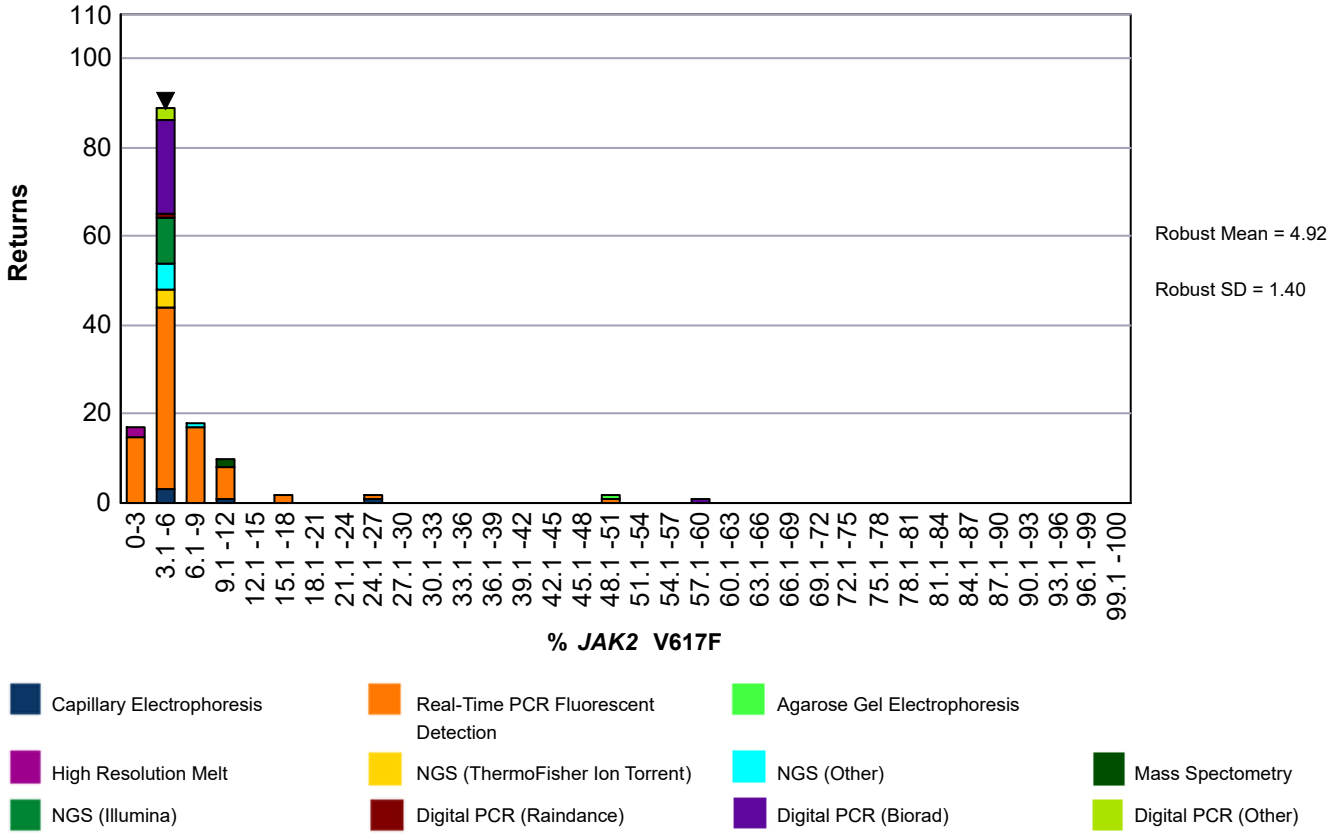
JAK2 p.Val617Phe (V617F) Mutation Status Programme

Journal Reference for Assay

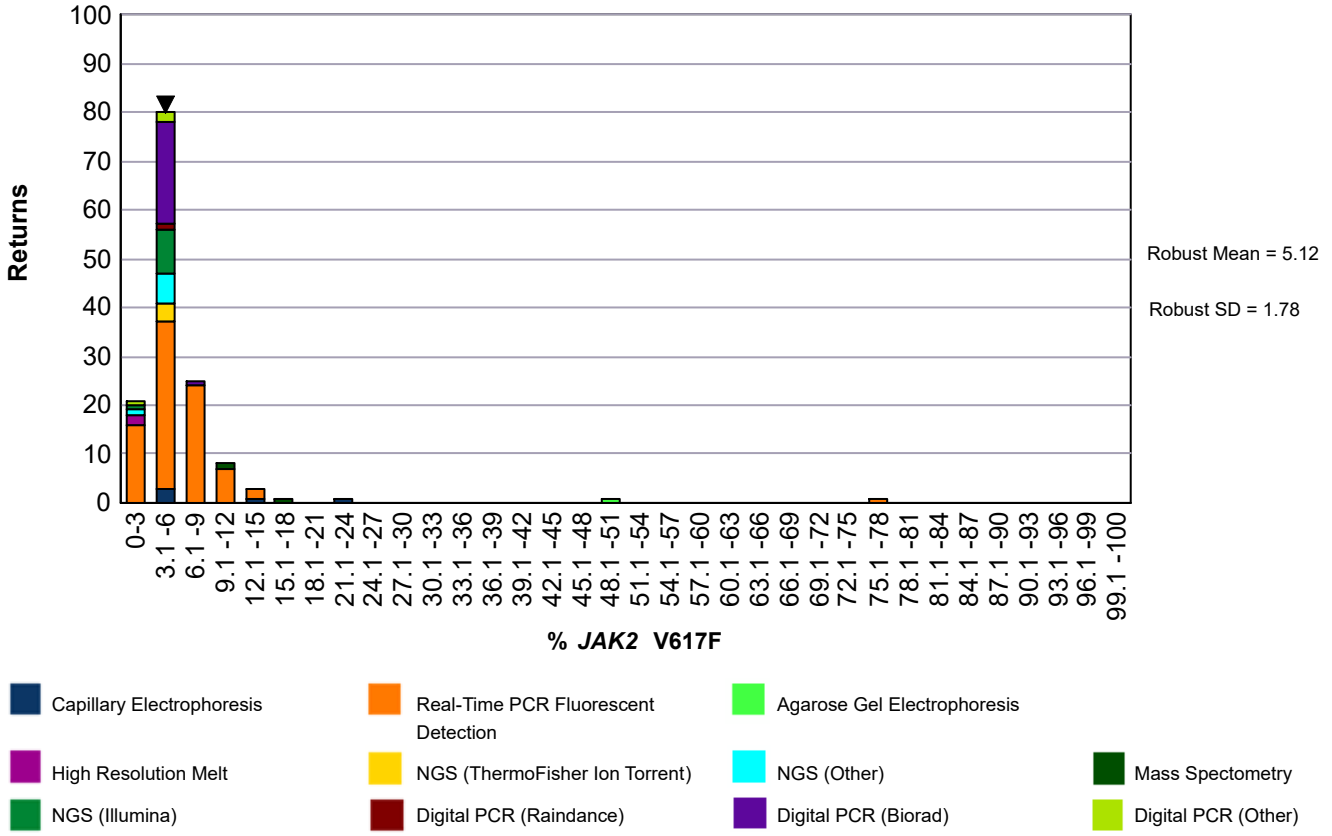
	Returns
Baxter et al (2005) Lancet; 365 (9464):1054-61	74
Levine et al (2005) Cancer Cell 7(4): 387-97	24
Larsen et al (2007)BJH, 136, 745-751	19
Tefferi et al Leukemia 22 (1): 14-22	17
Lippert et al (2006), Blood 108(6):1865-7	14
Denys B et al (2010)J Mol Diagn 12(4):512-9	9
Jones et al (2005) Blood 106(6):2162-21681	9
Passamonti et al (2006) Blood 107 (9):3676-3682	7
Jovanovic et al (2013) Leukemia 27, 2032–2039	6
McClure et al (2006) Leukemia 20 (1) 168-71	6
Chen et al (2007) J Mol Diagn (9):272-276	5
Sidon et al (2006) Clin Chem :52(7):1436-8	5
Vannucchi et al (2009) 33(12):1581-3	5
Cankovic et al (2009) Am J Clin Pathol 132 (5): 713-21	4
Kroger et al (2007) Blood 109(3):1316-21	4
Lay et al (2006) J Mol Diagn; 8(3):330-4	4
James et al (2006) Leukemia (2)350-353.	3
Murugesan et al (2006) AM J Clin Pathol :125(4):625-33	3
Wolstencroft EC (2007) J Mol Diagn 9(1):42-6	3
Horn et al (2006) J Mol Diagn 8(3): 299-304.	2
Olsen et al (2006) Arch Pathol Lab Med;130(7):997-1003	2

JAK2 p.Val617Phe (V617F) Mutation Status Programme

Frequency distribution histogram showing % JAK2 mutation load in sample JAK2 169, classified by analysis method



Frequency distribution histogram showing % JAK2 mutation load in sample JAK2 170, classified by analysis method



JAK2 p.Val617Phe (V617F) Variant Status Programme

Trial comments:

- In line with sample formulation, 221 laboratories (96.9% of returning participants) detected the JAK2 p.Val617Phe (V617F) variant in samples JAK2 169 and JAK2 170
- Five laboratories did not detect the JAK2 p.Val617Phe (V617F) variant in either sample. Three of these laboratories employed an in-house method (two utilised allele specific PCR with agarose gel electrophoresis and one used melting curve analysis with Real-Time PCR fluorescent detection), one used the BioRad PrimePCR ddPCR kit and one used the Ipsogen JAK2 MutaScreen Kit.
- For sample JAK2 169, the remaining two participants returning a false negative result employed an Ipsogen kit: one used JAK2 MutaScreen, the other used JAK2 MutaSearch.
- For sample JAK2 170, the remaining two participants returning a false negative result both employed in-house methodology: one used melting curve analysis with Real-Time PCR fluorescent detection, the other performed next generation sequencing using the ThermoFisher Ion Torrent platform.

Quantification comments:

- One hundred and forty-two laboratories (62.3% of returning participants) submitted quantification data for sample JAK2 169, and 141 laboratories (61.8%) submitted quantification data for JAK2 170.
- For sample JAK2 169, variant levels ranged from 1.6% to 60.0% (robust mean 4.9%, with a robust SD 1.4%).
- For sample JAK2 170, variant levels ranged from 1.5% to 78.0% (robust mean 5.1%, with a robust SD 1.8%).
- Regarding methodology, the most commonly utilised quantitative methods were real time PCR (n=85), followed by digital PCR (n=26), next generation sequencing (n=21), and capillary electrophoresis (n=5).
- For methods utilised by more than 20 participants, variant level information is shown in the table below.

JAK2 169	**RT-PCR** (n=84)	**ddPCR** (n=25)	**NGS** (n=21)
Robust Mean (%)	5.3	4.6	4.8
Robust SD (%)	2.5	0.5	0.5
Range (%)	1.6-51.0	3.9-60.0	4.0-6.3
JAK2 170	**RT-PCR** (n=84)	**ddPCR** (n=24)	**NGS** (n=21)
Robust Mean (%)	5.6	4.7	5.0
Robust SD (%)	2.8	0.4	0.8
Range (%)	1.5-78.0	3.7-6.0	3.0-6.0

Table: Robust mean and robust SD of variant levels in JAK2 169 and JAK2 170 for the three most commonly utilised quantification methods. Please note that two laboratories did not return quantification data, despite stating the method they employ.

JAK2 p.Val617Phe (V617F) Variant Status Programme

- Samples JAK2 169 and JAK2 170 were manufactured as duplicate samples. For participants providing quantification data for both samples, the absolute difference between the variant levels ranged from 0% to 55.4% (median 0.4%, IQR 0.9%). The following table shows this data for the three most commonly employed methodologies.

Difference between JAK2 169 and JAK2 170	**RT-PCR** (n=84)	**ddPCR** (n=24)	**NGS** (n=21)
Median (%)	0.5	0.2	0.8
IQR (%)	1.1	0.2	0.8
Range (%)	0.0-27.0	0.0-55.4	0.0-3.0

Table: Median, range and interquartile range (IQR) of participants' absolute differences between JAK2 169 and JAK2 170 for the three most commonly utilised quantification methods.

Educational Sample Comments:

- One hundred and fifty-one participants returned data for the educational sample Edu I. The submissions for this sample were not scored.
- In line with formulation, 143 participants (94.7%) detected the JAK2 p.Val617Phe (V617F) variant in sample Edu I, with a further two laboratories (1.3%) returning a "suspicious" or "equivocal" result. Five participants (3.3%) did not detect the JAK2 p.Val617Phe (V617F) variant and one (0.7%) indicated that their testing returned an invalid result.
- Four of the five laboratories that did not detect the JAK2 p.Val617Phe (V617F) variant in Edu I also failed to detect the variant in JAK2 169 and / or JAK2 170. The fifth laboratory used the BioRad PrimePCR ddPCR kit.
- Edu I was formulated to reflect a sample with a variant level around the 1-3% level specified to be significant at diagnosis in myeloproliferative neoplasms (Bench *et al.,* 2013). One hundred and one participants returned quantification data (66.9% of returning participants) and variant levels ranged from 0.9% to 96.0% (robust mean 3.5%, robust SD 1.3%). The table below resolves this data for the three most commonly employed quantification methodologies.

Edu I	**RT-PCR** (n=57)	**ddPCR** (n=18)	**NGS** (n=16)
Robust Mean (%)	3.8	3.2	3.6
Robust SD (%)	2.1	0.4	0.6
Range (%)	0.9-96.0	2.6-4.7	2.0-4.6

Table: Robust mean and robust SD of variant levels in Edu I for the three most commonly utilised quantification methods.

- Forty-one participants provided additional report comments as they would in a clinical scenario. Of these, 10 centres indicated that they would suggest repeat sampling given the low variant level and 14 stated that the result should be interpreted with caution and/or within the clinical context.

JAK2 p.Val617Phe (V617F) Variant Status Programme

Participant Satisfaction Survey:

- One hundred and forty-six participants (99.3%) stated that they were happy with the service provided by UKNEQAS LI. A single laboratory (0.7%) indicated that they would prefer increased cellular composition of trial samples.

References

- Bench, A. J., White, H. E., Foroni, L., Godfrey, A. L., Gerrard, G., Akiki, S., … Cross, N. C. P. (2013). Molecular diagnosis of the myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and other relevant mutations. *British Journal of Haematology*, 160(1), 25–34. https://doi.org/10.1111/bjh.12075

JAK2 p.Val617Phe (V617F) Variant 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, 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) No activities in relation to this EQA exercise were subcontracted.

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/