JAK2 p.Val617Phe (V617F) Mutation Status

Distribution - 161702

Participant ID -

Date Issued - 19 October 2016

Closing Date - 18 November 2016

Trial Comments

In this trial, there were 215 participating laboratories; 211 (98.1%) of participants returned results.

Sample Comments

Two lyophilised samples (JAK2 143 and JAK2 144) were distributed to participants for JAK2 p.Val617 Phe mutation analysis. JAK2 143 and 144 were duplicate samples formulated to be positive for the JAK2 V617F mutation.

Results and Performance

Your Results

JAK2 Mutation Status	Your Results	Consensus Result
Sample JAK2 143		Mutation Detected
Sample JAK2 144		Mutation Detected

All Participant Results

	Mutation Detected (Returns)	No Mutation Detected (Returns)
Sample JAK2 143	207	4
Sample JAK2 144	208	3

Your Performance

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

N/A = Not Applicable

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Template Type

	Returns
DNA	208
cDNA	3

PCR Type

	Returns
Allele Specific PCR	89
Real-Time PCR	83
Single PCR	15
Melting Curve Analysis	11
Multiplex PCR	4
Droplet Digital PCR	3
Allele Specific Competitive Blocker PCR	2
LNA PCR	2
COLD PCR	1
Nested PCR	1

Protocol Type

	Returns
In-house Assay	145
Ipsogen JAK2 MutaQuant Kit CE	32
Ipsogen JAK2 MutaScreen Kit CE	12
Ipsogen JAK2 MutaSearch Kit	11
Ipsogen JAK2 RGQ PCR Kit CE	5
AB Analitica REALQUALITY RS-JAK-2 V617F Q	3
AmoyDx JAK2 Mutation Detection Kit	1
Rotor-Gene Q MDx	1
Tib Molbiol LightMix kit	1

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Analysis Type

	Returns
Real-Time PCR Fluorescent Detection	130
Agarose Gel Electrophoresis	50
Capillary Electrophoresis	16
High Resolution Melt	4
Droplet Digital PCR	3
Next Generation Sequencing (Ion Torrent)	3
Pyrosequencing	2
Mass Spectometry	1
Restriction Enzyme Digest	1
Sanger Sequencing	1

Journal Reference for Assay

	Returns
Baxter et al (2005) Lancet; 365 (9464):1054-61	67
Levine et al (2005) Cancer Cell 7(4): 387-97	22
Tefferi et al Leukemia 22 (1): 14-22	17
Larsen et al (2007)BJH, 136, 745-751	16
Jones et al (2005) Blood 106(6):2162-21681	15
Lippert et al (2006), Blood 108(6):1865-7	13
Denys B et al (2010)J Mol Diagn 12(4):512-9	11
Passamonti et al (2006) Blood 107 (9):3676-3682	9
Chen et al (2007) J Mol Diagn (9):272-276	6
Vannucchi et al (2009) 33(12):1581-3	6
Kroger et al (2007) Blood 109(3):1316-21	5
James et al (2006) Leukemia (2)350-353.	4
Sidon et al (2006) Clin Chem :52(7):1436-8	3
Goerttler et al Blood (2005) 106(8):2862-4	2
McClure et al (2006) Leukemia 20 (1) 168-71	2
Merker et al (2010) J Mol Diagn 12(1):58-64	2
Murugesan et al (2006) AM J Clin Pathol :125(4):625-33	2
Olsen et al (2006) Arch Pathol Lab Med;130(7):997-1003	2
Schnittger et al (2006) Leukemia (12):2195-2197	2
Zhou et al (2004) Clin Chem 50(8):1328-35	2

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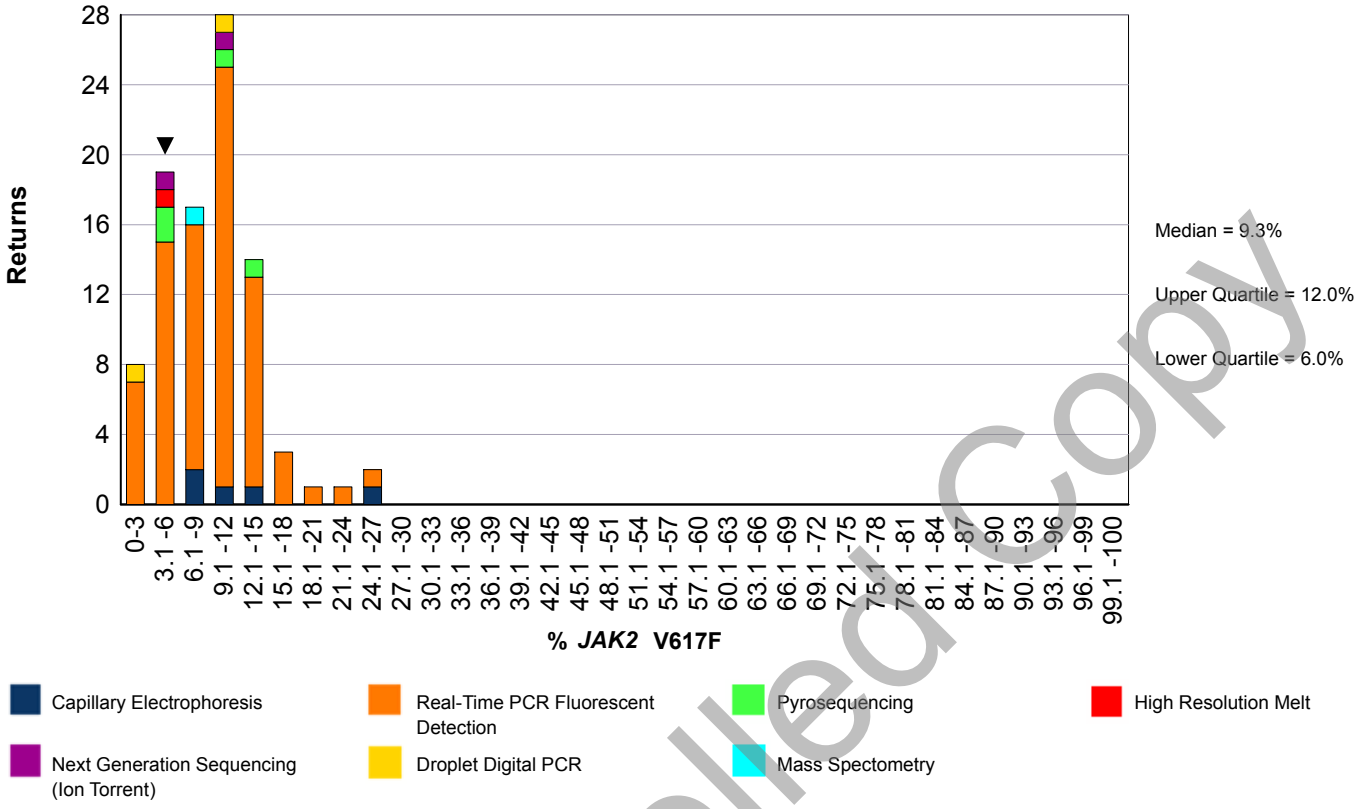
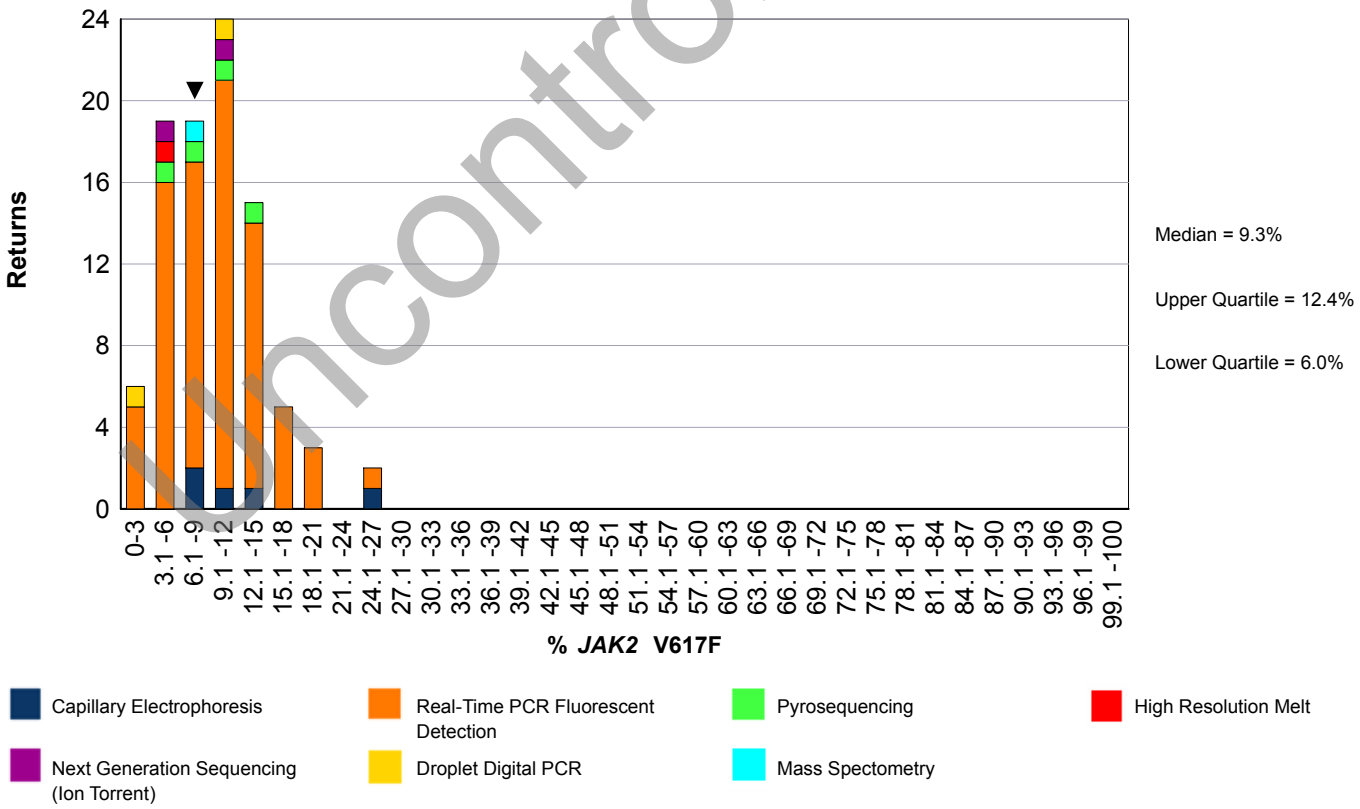
Frequency distribution histogram showing % JAK2 mutation load in sample JAK2 143, classified by analysis method



Frequency distribution histogram showing % JAK2 mutation load in sample JAK2 144, classified by analysis method



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Trial comments

- In line with sample formulation, 207 laboratories (98.1% of returning participants) detected the *JAK2* p.Val617Phe (V617F) mutation in sample JAK2 143.
- In line with sample formulation, 208 laboratories (98.6% of returning participants) detected the *JAK2* p.Val617Phe (V617F) mutation in sample JAK2 144. All three participants who didn't detect the mutation in JAK2 144 also failed to detect it in JAK2 143.
- Two of the four participants who didn't detect the mutation used Real-Time PCR Fluorescent Detection as their analysis method with the other two participants utilising Agarose Gel Electrophoresis.

Quantification comments:

- Ninety-five laboratories (45.0% of returning participants) submitted quantification data for JAK2 143, mutation levels ranged from 1.1%-26.9% (median 9.3%, inter quartile range (IQR) 6%).
- Ninety-six participants (45.5% of returning participants) submitted quantitative results for sample JAK2 144 with levels ranging from 1.1%-25.4% (median 9.3%, IQR 6.4%).
- The median difference in mutation load reported by participants between the duplicate samples 142 and 143 was 0.7% (min 0.0%; max 9.9%).
- Overall the most commonly utilised quantitative methods were real time PCR (n=73), followed by agarose gel electrophoresis (n=7), capillary electrophoreses (n=5), droplet digital PCR, high resolution melt, NGS (n=3), pyrosequencing (n=2) and mass spectometry (n=1).

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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:
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: nicola.rose@ukneqasli.co.uk

4.8.2 b) The coordinators of UK NEQAS LI programmes are Prof David Barnett and Mr Liam Whitby.

4.8.2 c) Person(s) authorizing this report:
Prof David Barnett, Director or Mr Liam Whitby, Operations 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