

JAK2 V617F Mutation Status

Participant:	Trial No:
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Batches of JAK2 positive and negative lyophilised cell-lines samples were produced and subsequently labelled as sample JAK2 118 and JAK2 119, respectively.

Samples were distributed to 160 participating laboratories; 154 (96.3%) of participants returned results.

62 (40%) and 60 (39%) participants submitted quantitative data for samples JAK2 118 and JAK2 119, respectively. This data is represented graphically in frequency histograms below.

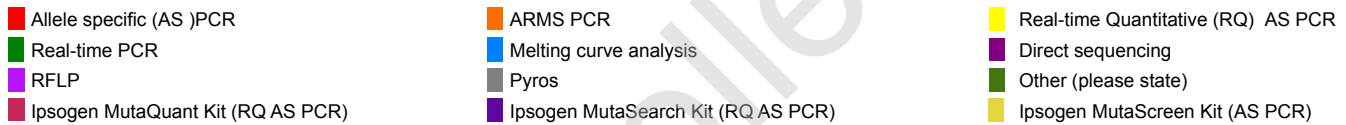
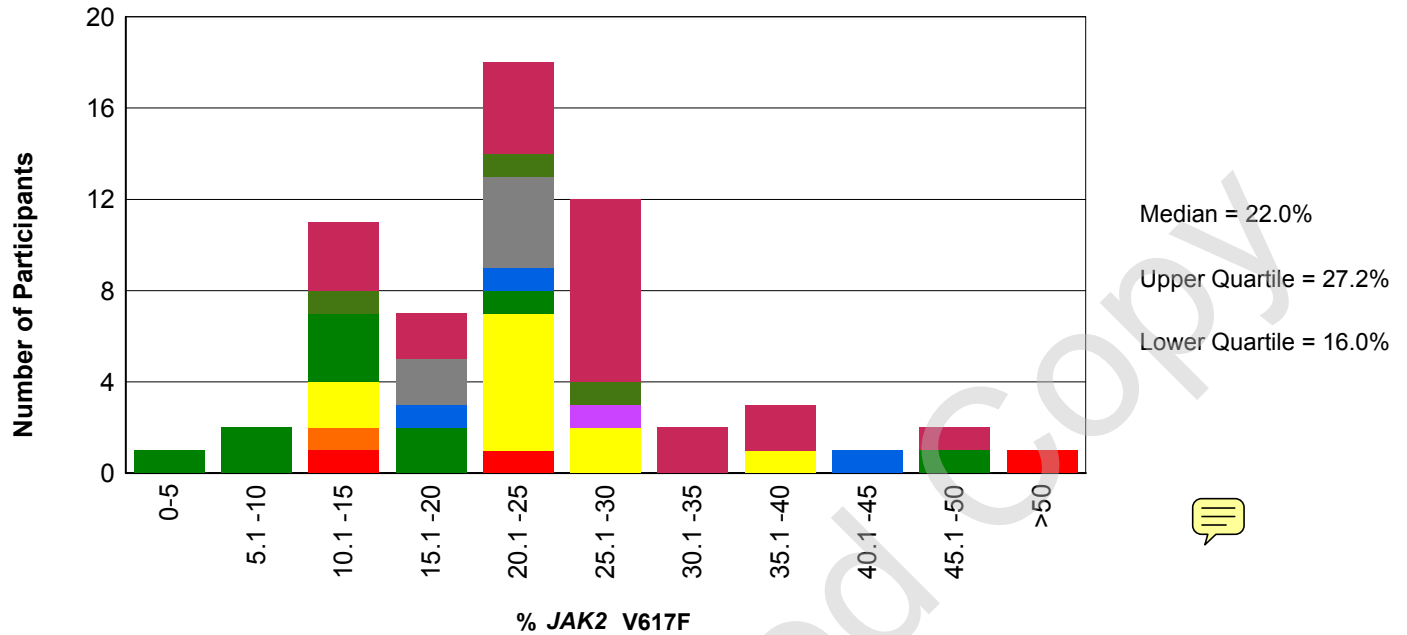
Your Results

Your result	118		119	
	V617F detected	Wild type allele detected	V617F detected	Wild type allele detected
	No Result	No Result	No result	No Result
Overall result	Y = 154 N = 0 ND = 0 Fail = 0	Y = 120 N = 5 ND = 29 Fail = 0	Y = 2 N = 152 ND = 0 Fail = 0	Y = 126 N = 2 ND = 26 Fail = 0

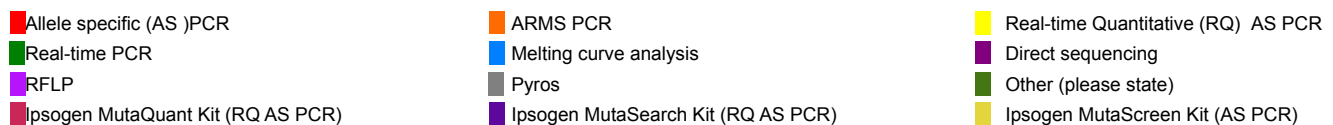
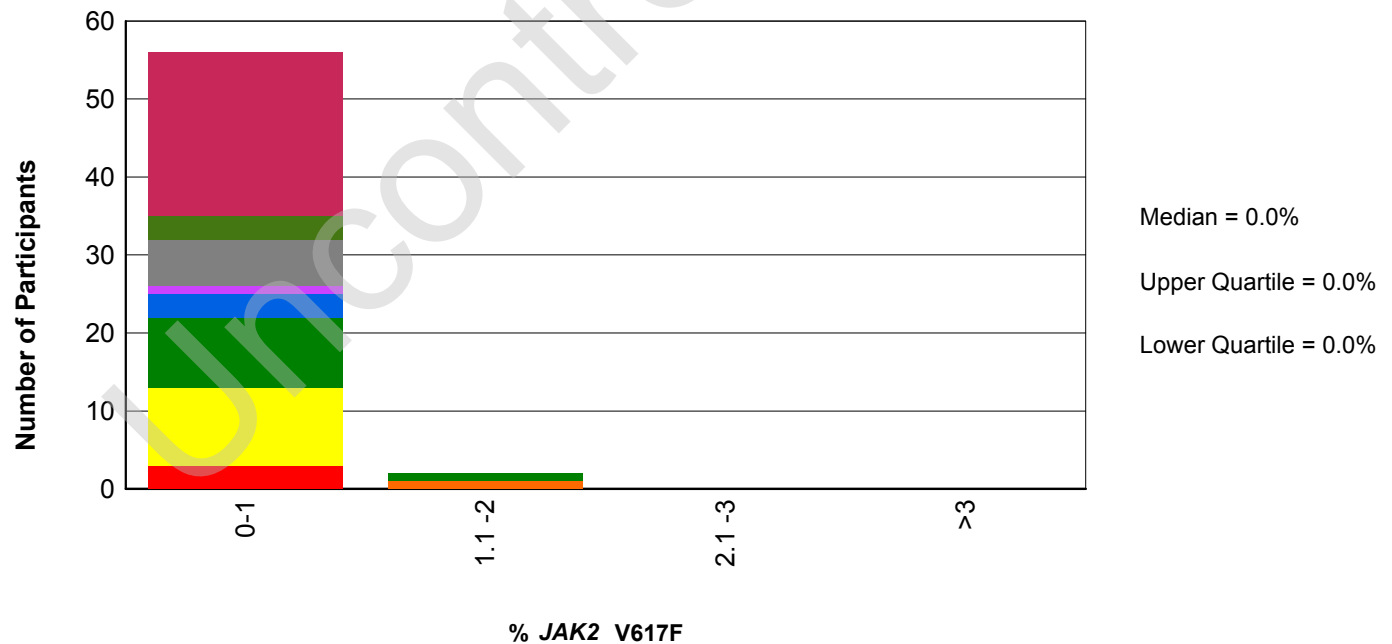
Key: Y=Yes, N=No, ND=Not Determined

Quantitative Results

V617F Quantitative Result for 118



V617F Quantitative Result for 119





Method Breakdown

Method	Number of Participants
Qualitative Method:	
Allele specific (AS)PCR	50
ARMS PCR	18
Real-time Quantitative (RQ) AS PCR	21
Real-time PCR	19
Melting curve analysis	10
Direct sequencing	2
RFLP	1
Pyrosequencing	3
Other	5
Ipsogen MutaQuant Kit (RQ AS PCR)	13
Ipsogen MutaSearch Kit (RQ AS PCR)	7
Ipsogen MutaScreen Kit (AS PCR)	5
Quantitative Method:	
Allele specific (AS)PCR	3
ARMS PCR	1
Real-time Quantitative (RQ) AS PCR	12
Real-time PCR	11
Melting curve analysis	3
RFLP	1
Pyrosequencing	6
Other	4
Ipsogen MutaQuant Kit (RQ AS PCR)	22



Summary Sample 118

- All participants correctly detected the presence of the JAK2 V617F mutation.
- The median %JAK2 V617F was 22%. A large spread of quantitative data was submitted with results ranging from 5% to 72.7%.
- When the quantitative data is analysed by methodology, the median for Ipsogen Mutaquant Kit (n=23) was 27% with an interquartile range of 9.01%. The median for pyrosequencing (n=6) was 21.50% with an interquartile range of 2.50%. The median for real-time PCR (n=11) was 13.35% with an interquartile range of 7.90%. The median for real-time quantitative allele specific PCR (n=12) was 23.60% with an interquartile range of 4.90%.

Summary Sample 119

- Two participants detected a JAK2 V617F mutation; the remaining participants (n=152) did not detect the mutation.
- The same two participants quantified the mutation as 0.16% and 0.006%. Care must be taken when reporting low burden JAK2 V617F mutations. Clinical, biological and technical considerations must be should be taken into account. (Bench *et al* 2012).
- Two participants quantified JAK2 V617F mutation burden at 1.3% and 2%, however they submitted results of no mutation detected.
- To highlight the issues surrounding the reporting of low burden JAK2 V617F mutations we are planning to ask clinical scenario questions with the next JAK2 trial issue.

Reference

Bench AJ, White, HE, Foroni L, Godfrey AL, Gerrard G, Akiki S, Awan A, Carter I, Goday-Fernandez A, Langabeer SE, Clench T, Clark J, Evans PA, Grimwade D, Schuh A, McMullin MF, Green AR, Harrison CN, Cross NC. Molecular diagnosis of myeloproliferative neoplasms: UK guidelines for the detection of JAK2 V617F and relevant mutations. BJH. 2012 Epub.