UK NEQAS UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEMES FOR LEUCOCYTE IMMUNOPHENOTYPING

Sheffield Teaching Hospitals NHS
NHS Foundation Trust
Excellence as standard

JAK2 V617F False Negative Rate in the UK NEQAS LI Programme

JR Clark, AL Jack, L Whitby, D Barnett and JT Reilly
UK NEQAS for Leucocyte Immunophenotyping, Sheffield, UK

INTRODUCTION

Diagnosis of Philadelphia negative myeloproliferative neoplasms (MPNs) was revolutionised in 2005 with the discovery of the gain of function point mutation in the *Janus kinase 2* (*JAK2*) gene. Current draft recommendations for the molecular diagnostic testing in MPNs states that an allelic burden above 1% should be considered pathogenic, and that clinical assays should have a 1% level of sensitivity.

In 2006 UK NEQAS for Leucocyte Immunophenotyping (UK NEQAS LI) initiated an EQA programme for the detection of *JAK2* V617F mutation (from here on referred to as the *JAK2* programme). Currently, 150 laboratories from 30 countries participate in the programme. Over 16 samples covering a range of 1% to 37% *JAK2* V617F allelic burden have been issued.

METHODS

- Stable lyophilised samples were produced by mixing the *JAK2* V617F positive cell line HEL with the *JAK2* V617F negative cell line HL-60
- Using limiting dilution studies we were able to accurately produce samples of known *JAK2* V617F allelic burdens
- All samples were rigorously quality controlled for DNA quality and the presence/absence of the mutation prior to shipment
- Participating laboratories were asked to assay the samples using their local methodology for the presence or absence (and if available the quantitation) of the V617F mutation
- Results were submitted to UK NEQAS LI along with details of methodology
- The consensus allelic burden was calculated from all quantitative results submitted

RESULTS

- Qualitative results submitted to the programme have increased from 102 to 140. The number of quantitative results has increased from 26 to 52 (Table 1)
- Our data shows that up to 29% of participating laboratories fail to detect the *JAK2* V617F mutation at the pathogenic allelic burden of 1% (Figure 1)
- Up to 9% of laboratories fail to detect the *JAK2* V617F mutation at an allelic burden of 5% (Figure 1)
- No correlation was seen between PCR type or assay reference with the failure to detect the mutation

CONCLUSION

- This EQA programme has seen a steady increase in participant numbers, as well as an increase in those submitting quantitative results
- Our data suggests up to a fifth of laboratories do not use an assay with the required 1% *JAK2* V617F sensitivity, therefore resulting in false negatives
- Increased assay sensitivity could potentially reduce the need of further diagnostic tests, resulting in laboratory cost savings
- The ability to accurately diagnosis patients with low level *JAK2* V617F (<5%) MPNs would reduce false negatives and therefore facilitate better patient management

Table 1: Results of the *JAK2* V617F mutation EQA programme

Sample ID	No. of qualitative results	False negative rate	Consensus median %V617F	No. of Quantitative results
1	102	1.9	11%	26
2	108	0	32%	33
3	108	1.9	20%	33
4	117	0.9	37.2%	36
5	117	3.4	9.6%	36
6	123	0.8	26.2%	38
7	123	10	6.6%	38
8	130	0.8	21%	42
9	130	7.7	5%	42
10	137	0	22.4%	53
11	137	6.6	6.1%	53
12	128	8.7	5%	48
13	128	20.5	2%	48
14	140	3.6	7%	51
15	135	8.9	3%	51
16	135	29.6	1%	52

Figure 1: False negative rates compared to median % *JAK2* V617F. Graph shows the rapid rise in false negative rate at allelic burdens of less than 10% *JAK2* V617F