Evaluation of Equalis' international pilot round for EQA of *JAK2* V617F quantification using quantitative PCR or droplet digital PCR

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CONCLUSIONS

The number of participants (n=33) in this international EQA pilot round of *JAK2* V617F quantification using quantitative PCR or droplet digital PCR confirms an interest to obtain and/or maintain high quality and standardized quantification results of *JAK2* V617F. As a result of this evaluation, Equalis will continue to offer this as a regular EQA scheme starting in 2020. More information will be available on www.equalis.se later this year.

INTRODUCTION

Participating in an external quality assessment (EQA) scheme is important and valuable for a clinical laboratory to ensure and prove high quality of performance and analysis results. Equalis is a provider of over 100 EQA schemes within a variety of clinical laboratory investigations.

In the field of molecular diagnostics, we arranged an international pilot round for EQA of *JAK2* V617F quantification using quantitative PCR (qPCR) or droplet digital PCR (ddPCR). The pilot round was performed in collaboration with the MPN&MPNr-EuroNet network, who previously arranged a similar EQA scheme for quantification of *JAK2* V617F [1]. Quantifying the allelic burden, i.e. the ratio between mutant and total (mutant and wildtype) *JAK2* V617F is of importance in the diagnostics of myeloproliferative neoplasms and the allelic burden can also be monitored during treatment of *JAK2* V617F-positive patients to confirm a positive response to treatment.

METHOD

In this EQA pilot round, 33 international participants were asked to analyse and quantify *JAK2* V617F using qPCR or ddPCR. Two samples of extracted human DNA were distributed and results were reported via the web portal Equalis Online, where reports were also published after the round.

RESULTS

Twenty-seven out of 33 participants used qPCR to quantify *JAK2* V617F, and the remaining six participants used ddPCR (Table 1a-b). The most common assays for participants using qPCR were Ipsogen JAK2 MutaQuant kit (n=10) and Larsen in-house [2] (n=7), and the most common assay for ddPCR was Bio-Rad (n=3) (Table 1a-b).

As displayed in Figure 1, the quantification results displayed a larger variation (CV%) for results obtained using qPCR compared to ddPCR. However, the variation was within the expected range for a qPCR assay [1].

The variation was also slightly larger (27.2 CV% vs 19.7 CV%) for the sample with lower level of mutant *JAK2* (5.5% *JAK2* V617F) compared to the higher level (40.7% *JAK2* V617F), which is in line with previous observations by Asp et. al [1].

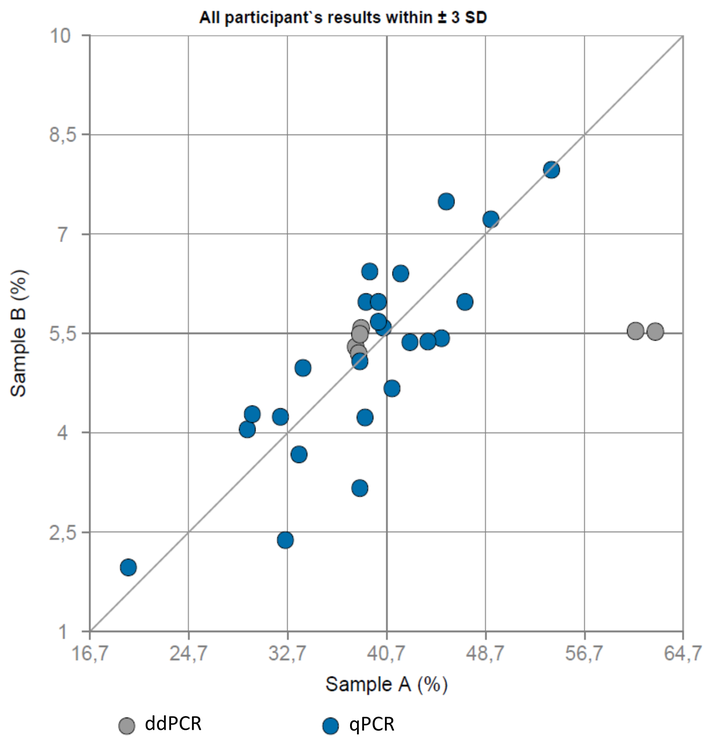

Figure 1. All participant's results (<±3SD) per output group for sample A and B

Table 1a. Distribution of *JAK2* V617F assays for participants using qPCR

qPCR (n=27)	No. of participants	Additional information
Ipsogen JAK2 MutaQuant Kit	10	-
Larsen in-house	7	Larsen *et al.*, 2011 [2]
Lippert in-house	1	Lippert *et al.*, 2006 [3]
Other	5	Denys *et al.*, 2010 [4]; Assay from Generi Biotech; Relative quantification using delta Ct method; In-house; Other assay not specified
Not available	4	-

Table 1b. Distribution of *JAK2* V617F assays for participants using ddPCR

ddPCR (n=6)	No. of participants	Additional information
Bio-Rad	3	-
In-house	1	-
Not available	2	-

REFERENCES

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