Supplementary Appendix

Methods

Table 1: PrimerDesign Quasa® kit PCR preparation

Reagent	Volume per reaction (µL)
2x Precision Quasa Mastermix	10
Primer/probe mix	1
RNAse/DNAse free water	4
Total volume	15
Added DNA template	5
Final Volume	20

Table 2: PrimerDesign Quasa® kit amplification protocol

	Step	Time (sec)	Temp (ºC)
	qPCR Enzyme Activation	120	95
5 Cycles	Denaturation	10	95
	Annealing	15	50
	Extension	15	72
40 Cycles	Denaturation	10	95
	Annealing*	30	60
	Extension	15	72

* Detection of fluorogenic data through the FAM channel.

Results

Table 3: Accuracy of the PrimerDesign assay for detection of the JAK2V617F mutation compared to that of the reference method (Melting Curve Analysis)

#	PrimerDesign Quasa® assay	Reference method
A01	Positive	Positive
A02	Negative	Negative
A03	Positive	Positive
A04	Negative	Negative
A05	Positive	Positive
A06	Negative	Negative
A07	Negative	Negative
A08	Positive	Positive
A09	Negative	Negative
A10	Positive	Positive
D01	Positive	Positive
D02	Negative	Negative
D03	Negative	Negative
D04	Negative	Negative
D05	Positive	Positive
D06	Negative	Negative
D07	Positive	Positive
D08	Positive	Positive
D09	Positive	Positive
D10	Positive	Positive
D11	Positive	Positive
D12	Positive	Positive
E01	Positive	Positive
E02	Positive	Positive
E03	Negative	Negative
E04	Negative	Negative
E05	Negative	Negative
E06	Negative	Negative
E07	Negative	Negative
E08	Negative	Negative

E09	Negative	Negative
E10	Negative	Negative
E11	Negative	Negative
E12	Negative	Negative

Table 4: WHO comparative results. The PrimerDesign assay was run in duplicate, and a mean value was established.

	15/172	15/170	15/168	15/166	15/244	15/246	15/164
WHO JAK2V617F %	0	0.03	1.0	10.8	29.6	89.5	100
PrimerDesign	0	0	2.5	29	47	95	99.8

Figure 1: *Linear Regression between WHO percentages and the PrimerDesign mean values. All values were plotted within the accepted limits of agreement.*

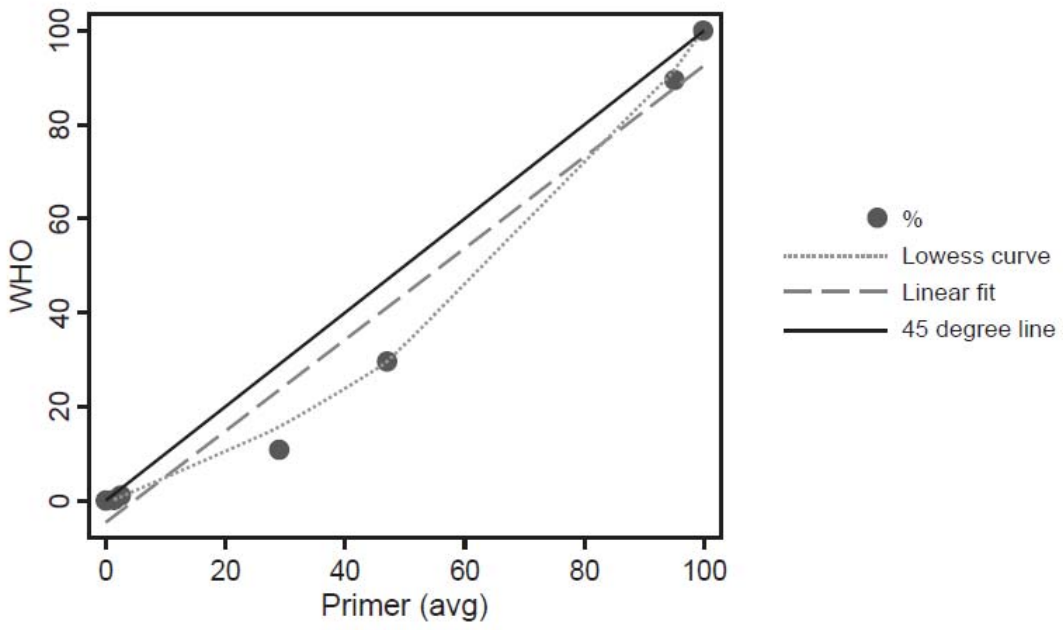

Table 5: Summary of the performance of the Primerdesign Quasa assay in the UK NEQAS JAK2V617F mutation status program – qualitative results.

	TAD Haematology result	Consensus result
Sample #		
159	mutation detected	mutation detected
160	mutation detected	mutation detected
161	no mutation detected	no mutation detected
162	no mutation detected	no mutation detected
163	mutation detected	mutation detected
164	mutation detected	mutation detected
165	no mutation detected	no mutation detected
166	mutation detected	mutation detected
167	mutation detected	mutation detected
168	no mutation detected	no mutation detected
169	mutation detected	mutation detected
170	mutation detected	mutation detected
Edu1	mutation detected	mutation detected
172	mutation detected	mutation detected
176	mutation detected	mutation detected
177	mutation detected	mutation detected
179	mutation detected	mutation detected
184	mutation detected	mutation detected
186	mutation detected	mutation detected
Eduk	mutation detected	mutation detected

Table 6: Intra-assay precision results

	TEST 1		TEST 2	
Specimen #	Quantitative (%)	Qualitative	Quantitative (%)	Qualitative
A01	93	Positive	93	Positive
A02	0	Negative	0	Negative
A03	5	Positive	5	Positive
A05	44	Positive	47	Positive
A06	0	Negative	0	Negative
A08	88	Positive	87	Positive
D03	0	Negative	0	Negative
D04	0	Negative	0	Negative
D06	0	Negative	0	Negative

Table 7: Inter-assay precision results

Specimen #	A01	A03	A05	A08	D03	D04	D06	D08
Day 1 Quantitative (%)	93	5	47	87	0	0	0	79
Day 1 Qualitative	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Day 2 Quantitative (%)	98	2	50	96	0	0	0	80
Day 2 Qualitative	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Positive
Day 3 Quantitative (%)	96	3	45	92	0	0	0	76
Day 3 Qualitative	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Positive

Table 8: Results of the JAK2-M tested samples based on inclusion criteria defined by the JAK2-Tree Predictive Model.

	True positive (n)	False positive (n)	Total (n)
JAK2-tree indicated testing	47	130	177
	True negative (n)	False negative (n)	Total (n)
JAK2-tree not indicated to test	26	4	30