Concordance of assays designed for the quantification of *JAK2*V617F: a multicenter study

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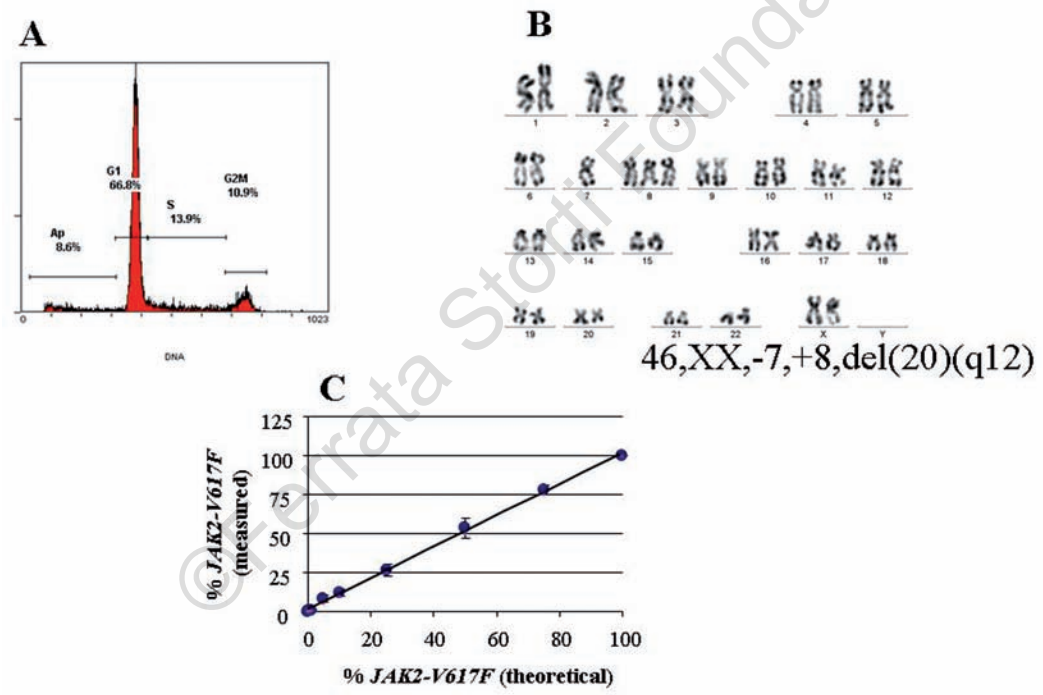
Citation: Lippert E, Girodon F, Hammond E, Jelinek J, Reading NS, Fehse B, Hanlon K, Hermans M, Richard C, Swierczek S, Ugo V, Carillo S, Harrivel V, Marzac C, Pietra D, Sobas M, Mounier M, Migeon M, Ellard S, Kröger N, Herrmann R, Prchal JT, Skoda RC, and Hermouet S. Concordance of assays designed for the quantification of JAK2V617F: a multi-center study. Haematologica 2009. doi: 10.3324/haematol.13486



Supplementary Figure S1. Characterization of the UKE-1 cell line. (A) DNA content of UKE-1 cells was measured after staining with propidium iodide and found to be similar to that of normal lymphocytes. (B) Karytotyping using R bands show monosomy 7, trisomy 8 and a deletion of the long arm of chromosome 20. (C) DNA from dilutions of UKE-1 cells in normal leukocytes was assessed for %JAK2V617F by comparison to plasmids A,B and C,D. Measured %JAK2-V617F values (y axis) were similar to theoretical values (x axis).

Supplementary Table S1. Sequences of primers and probes of the various techniques.

Center	Forward primers	Reverse primers	Probes
1	Under patent*	Under patent*	Under patent*
2	5'GGCAGAGAGAATTTTCTGAAC	5'GCTTTCCTTTTTCACAAGATA	Sensor mutant : 5'GTCTCCACAGAAACA TACTCCATAA (3'-FL) Anchor JAK2 5' Red640-AAAACCAAAT GCTTGTGAGAAAGCT-PH LNA probe: CCACAGACACATACTCCA-NH
3	5'GCAAGTATGATGAGCAAGCT	5'CTCTGAGAAAGGCATTAGAAAG	5'GGTTTTAAATTATGGAGTATGT**
4,5,6,7, 8	5'GCGCGGTTTTAAATTATGGAGTATGTG 5'GCGCGGTTTTAAATTATGGAGTATGTT	5'GCGGTGATCCTGAAACTGAATTTTC	5'TGGAGACGAGAGTAAGTAAAACTACAGGCT
9,10	5'TTATGGACAACAGTCAAACAACAAT	5'TTTACTTACTCTCGTCTCCACAGTC 5'TTTACTTACTCTCGTCTCCACAGTA	5'CTTGCTCATCATACTTGC
11	5'AAGCTTTCTCACAAGCATTTGGTTT	5'AGAAAGGCATTAGAAAGCCTGTAGTT	5'TCTCCACAGACACATAC 5'TCCACAGAAACATAC
12	5'GTTTCTTAGTGCATCTTTATTATGGCAGA	5'TTACTCTCGTCTCCACAGAC 5'AAATTACTCTCGTCTCCACAGAA	NA
13	5'GGGTTTCCTCAGAACGTTGA	5'TTTACTTACTCTCGTCTCCACATAC 5'TTTACTTACTCTCGTCTCCACATAA	NA
14	5'TTATGGACAACAGTCAAACAACAATTC	5'CTTACTCTCGTCTCCACAAAA	5'TTGTACTTTTTTTTTTCCTTAGTCTTTCTTTGAAGCAGCA
15	5'AGCATTTGGTTTTAAATTATGGAGTATATT	5'CAAAAACAGATGCTCTGAGAAAGG	5'CTCCACAGAAACATACTC
16	5'GGGTTTCCTCAGAACGTTGA	5'TCATTGCTTTCCTTTTTCACAA	NA

*NA: not applicable; *Ipsogen, Marseille, France. **sequencing primer.*

Supplementary Table S2. Analysis of genomic DNA standards from centers 1, 4, 7, and 11.

	%JAK2V617F				
	Part A (center 7)			Part B (center 4)	
Announced	Observed, with plasmids A,B	Observed, with plasmids C,D		Sample	Observed, with plasmids A,B
HEL, 100%	100	100		UKE-1, 50%	41 / 50*
HEL, 75%	97.8	98.1	1	BFU-E	100
HEL, 50%	88.6	89.5	2	BFU-E	45.3 / 47.3*
HEL, 30%	77.6	78.6	3	CFU-GM	47.3 / 47.0*
HEL, 20%	69.6	70.5	4	CFU-G	50.3
HEL, 10%	58.7	59.3	5	CFU-GM	45.9 / 49.0*
HEL, 5%	31.8	31.7			
HEL, 2.5%	36.2	36.3			
HEL, 1%	10.8	10.5			
HEL, 0.5%	4.0	3.7			
Patient 1, 97%	96.5	95.8			
UKE-1, 50%	52.1	51.33			

*Part A: %JAK2V617F of genomic DNA samples used as references in center 1 (HEL 0.5% to HEL100%: DNA from dilutions of cell line HEL in normal PBL), center 11 (patient 1) and UKE-1 (center 7), as determined by center 7 using dilutions of wild type (A, C) and mutant (B, D) plasmids. Part B: %JAK2V617F of DNA samples of UKE-1 standard with theoretical 50% JAK2V617F and DNA from single hematopoietic colonies, as determined by center 4 using dilutions of plasmids AB. For this experiment, blood or bone marrow mononuclear cells (600 cells/100 µL/well) of two JAK2V617F-positive patients with polycythemia vera were grown for 14 days in 96-well plates in collagen medium with stem cell factor (25 ng/mL) and interleukin-3 (10 ng/mL) without erythropoietin. Five wells with a single colony per well were identified by microscopic examination, individual colonies were picked and submitted to DNA extraction. *TaqMan AS-qPCR assay with specificity based on anti-sense reverse primers as described by Girodon et al.[25] Data shown are averages of duplicate or triplicate values.*