



Performance of Pillar oncoReveal™ Essential MPN Panel: experiences of two clinical diagnostic laboratories



ACL - Advocate Clinical Laboratories

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Introduction

Standard of care for the clinical evaluation of myeloproliferative neoplasms (MPN) involves the molecular analysis of three driver genes, JAK2, CALR, and MPL using a single-gene testing cascading algorithm. Utilization of reflex PCR provides inadequate turn-around time (TAT), may introduce errors due to sample splitting, and expensive to logistically manage. In this study we evaluated the performance of the oncoReveal™ Essential MPN Panel from Pillar Bioscience.

Materials and Methods

Materials:

DNA extracted from blood/bone marrow clinical samples were used to evaluate the assay. DNA extraction was performed on a Promega Maxwell and a Roche Magna Pure 96. Identified reportable mutations were confirmed by a secondary orthogonal method such as (PCR, qPCR or PCR/CE).

Methods:

The oncoReveal™ Essential MPN Panel (Pillar Biosciences) utilizes proprietary SLIMamp® (stem-loop inhibition mediated amplification) technology, allowing amplification of regions of interest in a single tube, multiplex reaction. NGS libraries were prepared manually and sequenced on the Illumina MiSeq™ and Illumina NextSeq 550 Dx platform. Sample results and reports were generated by using PiVAT® (Pillar Biosciences' Variant Analysis Toolkit).

Assay was run in two ACL Laboratory sites Charlotte, NC and Rosemont, IL

Limit of Detection

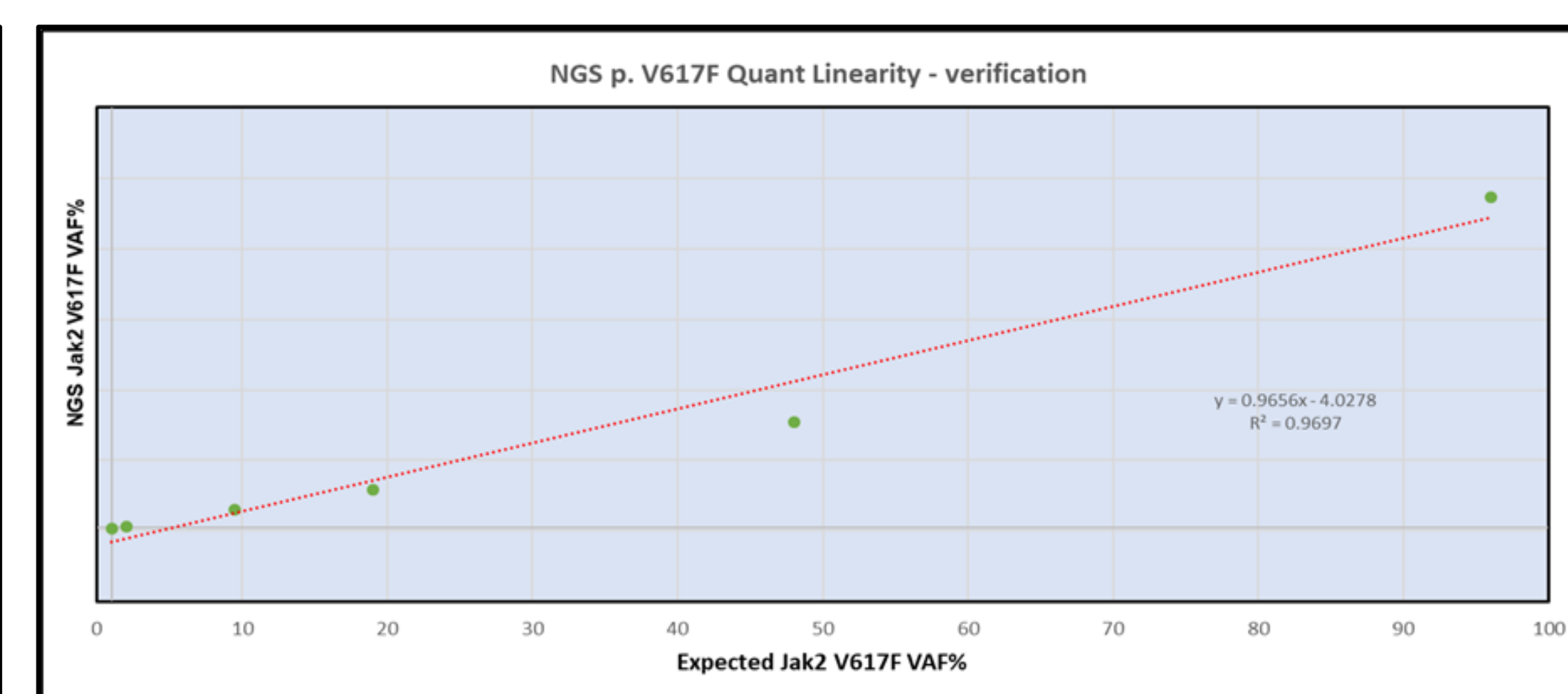
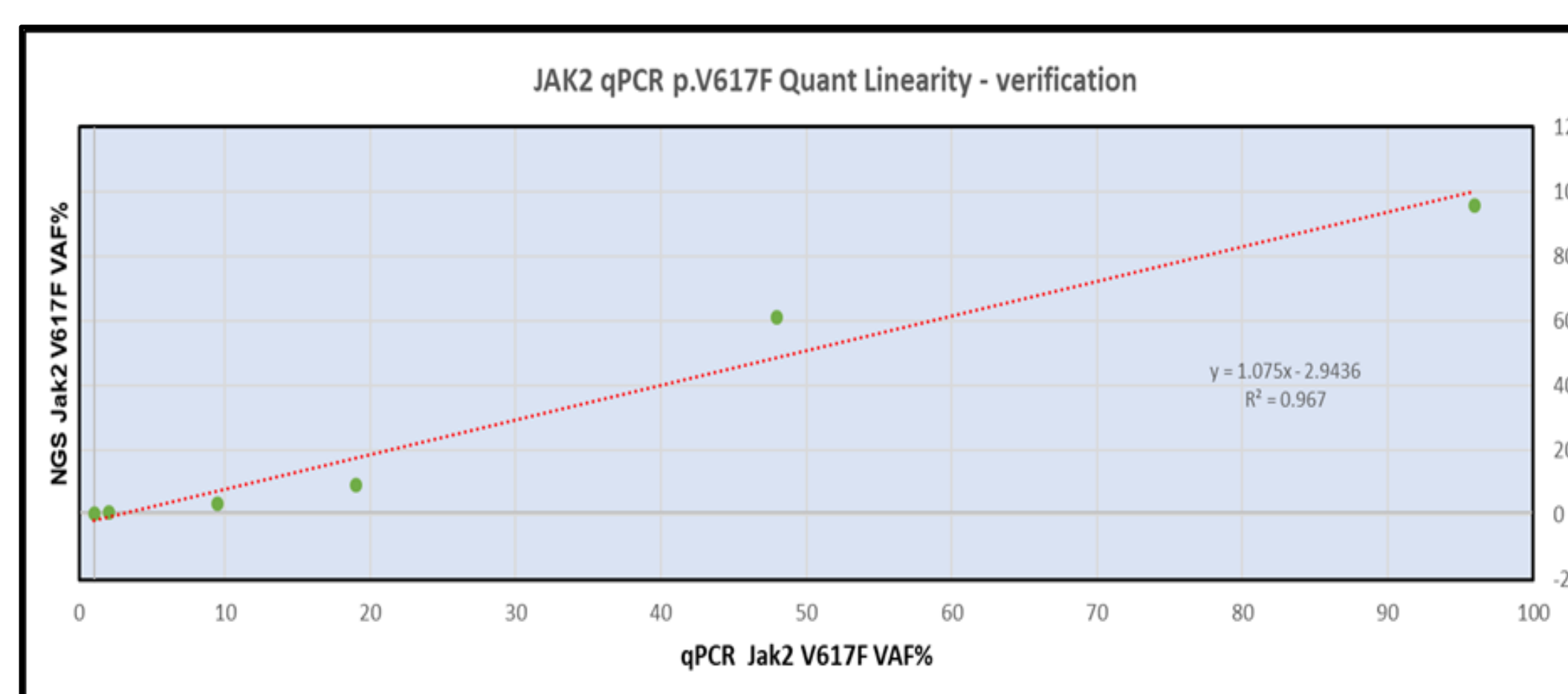
Dil.	Date	CALR (52 bp Del) p.L367T fsTer46			MPL (SNV) p.V515L			JAK2 (Del 1bp) + SNV NS42_E543del V617F					
		AF%	Var	Total	AF%	Var	Total	AF%	Var	Total	AF%	Var	Total
10ng/uL	RANGE	4.5	>10	>1000	5.5	>10	>1000	8.1	>10	>1000	7.5	>10	>1000
	5	773	16699	5	589	11319	7.4	954	12843	6.2	816	13069	
	AVE	4.7	791	16837	5.0	584	11666	7.8	1023	13038	6.5	862	13314
5ng/uL	5	758	16426	6	584	10366	9	1082	12176	6	738	11877	
	5	790	15998	5	484	9796	8	944	11909	6	766	12055	
	AVE	4.8	774	16212	5.3	534	10081	8.4	1013	12043	6.3	752	11966
2ng/uL	4	801	19760	5	663	12345	9	1196	13709	7	907	13559	
	5	1045	21758	6	860	13923	9	1402	16044	7	1125	15678	
	AVE	4.8	1045	21758	6.2	860	13923	8.7	1402	16044	7.2	1125	15678

Dil.	Date	CALR (52 bp Del) p.L367T fsTer46			MPL (SNV) p.V515L			JAK2 (Del 1bp) + SNV NS42_E543del V617F					
		AF%	Var	Total	AF%	Var	Total	AF%	Var	Total	AF%	Var	Total
1:3	RANGE	4.5	>10	>1000	5.5	>10	>1000	8.0	>10	>1000	7.0	>10	>1000
	1.5	120	7976	1.7	97	5728	2.2	144	6430	2.1	148	7033	
	1.3	151	11375	1.7	143	8271	2.7	258	9512	2.1	199	9601	
	1.3	110	8336	1.8	109	6197	2.8	181	6518	1.9	147	7720	
	1.5	122	8353	1.8	113	6319	2.6	176	6693	2.2	159	7151	
1.6	146	8975	1.9	118	6370	2.7	191	7189	2.1	165	8004		
AVE	1.5	1.5	100%	1.7	1.8	100%	2.6	3.0	100%	2.1	2.3	100%	
1:5	0.5	41	7800	0.6	37	6328	1.0	66	6924	0.7	53	7797	
	0.5	48	9040	0.5	35	6500	0.9	63	6873	0.6	48	8378	
	Failed	Failed	Failed	0.5	30	5703	1.0	63	6252	0.6	40	7123	
	Failed	Failed	Failed	0.6	42	7265	0.8	56	7239	0.5	44	8796	
	Failed	Failed	Failed	0.5	37	7258	0.6	49	7599	0.7	52	7716	
AVE	0.5	0.8	40%	0.6	1.1	100%	1.0	2.2	100%	0.70	1.5	100%	
1:10	0.6	35	5969	0.6	35	5969	0.6	35	5969	Failed	Failed	Failed	
	0.5	31	5778	0.6	37	5929	Failed	Failed	Failed	Failed	Failed	Failed	
	0.6	37	5929	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	
	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	
	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	

Results Comparison

ACL VALIDATION		CAP Cell Lines		Total
NGS MPN	+	+	-	
Illumina	-	132	0	132
		0	26	26
		Total		158
%				
100.0		PPA - Positive percent agreement (sensitivity)		
100.0		NPA - Negative percent agreement (specificity)		
100.0		Positive Predictive Value (PPV)		
100.0		Negative Predictive Value (NPV)		
100.0		Accuracy		

	ACC	DNA ng/ul	Spec type	Jak2 qPCR V617F	Pillar MPN Jak2	AVF%	EXON	COVR	Tot Cov	Agreed
Inhibition panel 1	EDTA Plasma	1.4	blood	21%	V617F	22.9	14	1663	7261	Y
Inhibition panel 2	Hemo. Low	1.4	blood	21%	V617F	22.6	14	1567	6924	Y
Inhibition panel 3	Hemo. Mid	0.95	blood	21%	V617F	23.6	14	1675	7113	Y
Inhibition panel 4	Hemo. High	0.7	blood	21%	V617F	22.4	14	1753	7839	Y
Inhibition panel 5	Hepar. Plasma	0.8	blood	21%	V617F	23.4	14	1703	7286	Y
Inhibition panel 6	Lipemic Plasma	0.3	blood	21%	V617F	22.9	14	1561	6823	Y
Inhibition panel 7	Icteric Plasma	2.23	blood	21%	V617F	21.6	14	1480	6847	Y
					AVE	22.8				
					STND	0.65				
					%CV	2.84				



NGS MPL exon 10 Illumina		+		-		Total
		+	-	+	-	
		11	0	0	108	11
		0	108	0	0	108
		Total		Total		119
%						
100.0		PPA - Positive percent agreement (sensitivity)				
100.0		NPA - Negative percent agreement (specificity)				
100.0		Positive Predictive Value (PPV)				
100.0		Negative Predictive Value (NPV)				
100.0		Accuracy				

NGS JAK2 V617F Illumina		+		-		Total
		+	-	+	-	
		25	0	0	94	25
		0	94	0	0	94
		Total		Total		119
%						
100.0		PPA - Positive percent agreement (sensitivity)				
100.0		NPA - Negative percent agreement (specificity)				
100.0		Positive Predictive Value (PPV)				
100.0		Negative Predictive Value (NPV)				
100.0		Accuracy				

NGS CALR exon 9 Illumina		+		-		Total
		+	-	+	-	
		18	0	0	101	18
		0	101	0	0	101
		Total		Total		119
%						
100.0		PPA - Positive percent agreement (sensitivity)				
100.0		NPA - Negative percent agreement (specificity)				
100.0		Positive Predictive Value (PPV)				
100.0		Negative Predictive Value (NPV)				
100.0		Accuracy				

NGS JAK2 exon 12-15 Illumina		+		-		Total
		+	-	+	-	
		7	0	0	112	7
		0	112	0	0	112
		Total		Total		119
%						
100.0		PPA - Positive percent agreement (sensitivity)				
100.0		NPA - Negative percent agreement (specificity)				
100.0		Positive Predictive Value (PPV)				
100.0		Negative Predictive Value (NPV)				
100.0		Accuracy				

Results

Of the 239 clinical specimens tested during validation; variants identified showed 100% concordance with the orthogonal methods. Out of 132 positive samples, 84 were SNVs and 48 were indels (including 14 CALR type 2,31 CALR Type 1, and 4 JAK2 ex12 indels). Repeatability and reproducibility demonstrated 100% concordance based on 28 repeats (SeraSeq QC reagent cat# 0710-0408). Limit of detection was established at; 1% for JAK2 p.V617F and JAK2 exons 12-15, 2% for MPL exon 10 – SNVs and 2% for CALR exon 9 InDels detected with 20-60ng input DNA. In analytical inclusivity study 132/132 samples were 100% concordant and in exclusivity study 26/26 samples were 100% concordant. The AcroMetrix™ Inhibition Panel (RUO) was used for an intrinsic inhibitors study and showed satisfactory performance

Workflow Comparison

The average laboratory TAT was shortened by 7-10 days, the cost was sufficiently reduced, and workflow efficiency increased with the oncoReveal™ Essential MPN Panel compared to the previous processes.

Conclusions

This intra-laboratory study demonstrates that the oncoReveal™ Essential MPN Panel performed very well against comparator methods. The performance characteristics, workflow benefits and TAT saving are suitable for clinical testing of MPN patient population to allow accurate clinical assessment.

References

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