

Development and Validation of a Synthetic External Control Panel to Confirm Linear Dynamic Range and Quantitative Detection of PML-RARA

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Introduction

Acute Promyelocytic Leukemia (APL), a subtype of Acute Myeloid Leukemia (AML), accounts for 10-15% of diagnosed adult AML cases per year¹. Almost all diagnosed APL patients express at least one isoform of the fusion gene PML-RARA [t(15;17)] which is formed by translocation between retinoic receptor alpha (RARA) gene on chromosome 16 and promyelocytic leukemia (PML) gene on chromosome 15^{1,2}. PML-RARA fusion isoforms depend on the breakpoint location in the PML gene and the three most common are bcr1 (L or long), bcr2 (V or variant), and bcr3 (S or short)².

Numerous quantitative assays measure levels of PML-RARA transcripts in peripheral blood by RT-PCR, a valuable tool to monitor response to drug treatment. Best practice and regulations require clinical laboratories to routinely monitor performance and periodically monitor the reportable dynamic range of quantitative assays. We developed and validate two control panels; one panel to ensure accurate linear quantification across the entire reportable dynamic range, and a second panel to confirm routine performance of accurate detection of the three PML-RARA isoforms when tested with a sample to answer RT-qPCR assay for the detection of PML-RARA.

Methods

The Xpert PML-RARA Linearity Panel C221 represents a range of PML-RARA (isoform bcr1) transcripts relative to a control (wild-type ABL1) transcript, to create five levels; bcr1 0.1%, bcr1 1.0%, bcr1 20%, bcr1 100%, and bcr1 450% within a proprietary stabilizing matrix formulation.

The Xpert PML-RARA External Control Panel C215 represents varying amounts of PML-RARA transcripts (isoforms bcr1, bcr2, and bcr3) relative to a control (wild-type ABL1) transcript to create five components: 0% (negative for PML-RARA transcript), bcr1 1.0%, bcr2 1.0%, bcr3 1.0%, and bcr1 20% within a proprietary stabilizing matrix formulation.

Three lots of each PML-RARA panel were tested on the Xpert PML-RARA assay using the GeneXpert® Dx system. Three cartridge reagent lots and different operators over multiple days were used to determine linearity, reproducibility, and repeatability.

Summary

The average % ratios were determined by testing 3 lots of Xpert PML-RARA External Control C215 and Xpert PML-RARA Linearity Panel C221 across three unique Xpert PML-RARA Assay cartridge lots on the Cepheid GeneXpert® Dx system.

Table 1a. Xpert PML-RARA External Control Panel Average % Ratio.

Xpert PML-RARA External Control Panel C215	Average % Values
Xpert PML-RARA bcr1 0%	0%
Xpert PML-RARA bcr1 1.0%	0.72%
Xpert PML-RARA bcr2 1.0%	0.86%
Xpert PML-RARA bcr3 1.0%	1.38%
Xpert PML-RARA bcr1 20%	17.7%

Table 1b. Xpert PML-RARA Linearity Panel Average % Ratio.

Xpert PML-RARA Linearity Panel C221	Average % Values
Xpert PML-RARA bcr1 0.1%	0.067%
Xpert PML-RARA bcr1 1.0%	0.72%
Xpert PML-RARA bcr1 20%	17.7%
Xpert PML-RARA bcr1 100%	93.8%
Xpert PML-RARA bcr1 450%	334.3%

Reproducibility

	% Level	Target	AVG Ct	95% CI, Ct		AVG % Ratio	95% CI, % Ratio	
				Lower	Upper		Lower	Upper
Xpert PML-RARA External Control Panel C215	0%	ABL	11.9	11.8	12.1			
		PML-RARA						
	1% bcr1	ABL	11.9	11.8	12.1	0.72	0.61	0.83
		PML-RARA	22.6	22.4	22.7			
	1% bcr2	ABL	11.9	11.7	12.0	0.86	0.75	0.97
		PML-RARA	22.1	22.0	22.3			
1% bcr3	ABL	12.0	11.9	12.2	1.38	1.15	1.61	
	PML-RARA	21.8	21.6	21.9				
Xpert PML-RARA Linearity Panel C221	0.1%*	ABL	11.9	11.8	12.0	0.067	0.054	0.08
		PML-RARA	25.9	25.7	26.1			
	1%	ABL	11.9	11.8	12.1	0.72	0.61	0.83
		PML-RARA	22.6	22.4	22.7			
	20%	ABL	12.0	11.8	12.1	17.70	15.10	20.29
		PML-RARA	18.0	17.8	18.1			
	100%	ABL	12.0	11.8	12.1	93.80	80.83	106.77
		PML-RARA	15.5	15.3	15.7			
	450%*	ABL	12.0	11.8	12.1	334.26	298.95	369.57
		PML-RARA	13.6	13.4	13.7			

Table 2. Reproducibility studies demonstrate high run-to-run precision of all PML-RARA levels. Three manufactured lots of the PML-RARA Control and Linearity Panels were tested across three lots of Xpert PML-RARA Assay cartridges (Cepheid). Average cycle threshold (Ct) values for each level of PML-RARA transcript to ABL1 transcript, with provided 95% confidence interval (CI) values showing less than a single cycle range for all levels. Average % PML-RARA to ABL1 transcript ratios, with 95% CI ranges. * 0.1% and 450% Levels had two outliers removed based on Grubb's outlier test³.

Repeatability

% Level	Lot	Avg. ABL Ct	ABL STDV	ABL %CV	Avg. PML-RARA Ct	PML-RARA STDV	PML-RARA %CV
0%	A	12.3	0.16	1.3	25.9	0.43	1.6
	B	11.7	0.17	1.5	26.2	0.55	2.1
1% bcr1	A	11.9	0.28	2.3	22.8	0.44	1.9
	B	12.1	0.27	2.2	22.7	0.56	2.5
1% bcr2	A	12.1	0.31	2.6	22.5	0.92	4.1
	B	11.9	0.34	2.9	22.8	0.51	2.2
1% bcr3	A	12.2	0.23	1.9	21.5	0.23	1.1
	B	11.8	0.30	2.4	21.7	0.70	3.3
20% bcr1	A	12.1	0.24	2.0	17.9	0.36	2.0
	B	11.8	0.28	2.4	17.8	0.41	2.3

% Level	Lot	Avg. %	Min %	Max %	AVG Fold Difference
0%	A	0.131	0.086	0.160	1.31
	B	0.083	0.036	0.140	1.21
1% bcr1	A	0.90	0.76	1.05	1.13
	B	1.06	0.56	1.54	1.25
1% bcr2	A	1.32	0.45	2.09	1.32
	B	0.85	0.49	1.15	1.18
1% bcr3	A	2.37	2.01	2.78	1.58
	B	2.31	1.26	3.28	1.54
20% bcr1	A	25.3	18.0	29.6	1.27
	B	22.1	15.5	31.9	1.11

Table 3a & 3b. Intra-run precision studies of the Xpert PML-RARA External Control and Linearity Panels. Two lots of 0.1% PML-RARA bcr1, 1% PML-RARA bcr1, 1% PML-RARA bcr2, 1% PML-RARA bcr3, and 20% PML-RARA bcr1 were tested on a single Xpert PML-RARA Assay cartridge lot (Cepheid). **a)** Average Ct values for each level. **b)** Average % ratio for each level.

Linearity

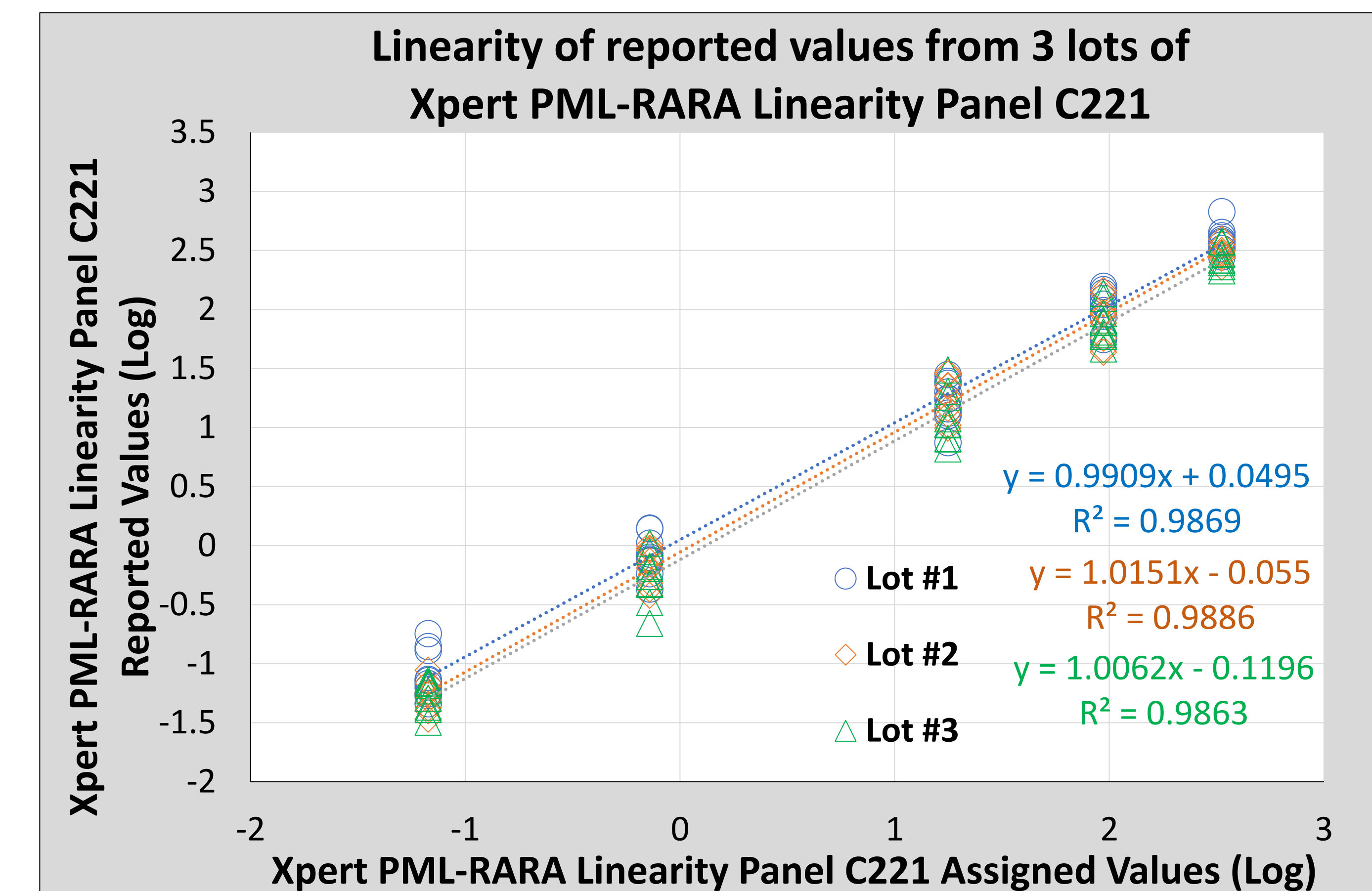


Figure 1. Linearity studies demonstrate high run-to-run precision of all PML-RARA bcr1 levels. Three manufactured lots of the Xpert PML-RARA Linearity Panel C221 were tested across three lots of Xpert PML-RARA Assay cartridges (Cepheid). Linear regression of Lots #1, #2 and #3, showing slope values between 0.9 and 1.1 and Pearson correlation coefficient, $R^2 > 0.98$.

Conclusion

Reproducibility: Reproducibility studies demonstrated high accuracy and precision of the Xpert PML-RARA External Control Panel C215 and Xpert PML-RARA Linearity Panel C221.

Linearity: Linear regression analysis of three lots of Xpert PML-RARA Linearity Panel C221 highlight the panel's strong linearity with R^2 values > 0.98 across the entire linear dynamic range of the Xpert PML-RARA Assay.

Repeatability: Repeatability studies demonstrated each of the Xpert PML-RARA levels tested performed with high precision with a %CV below 5%.

✎ The synthetic Xpert PML-RARA External Control Panel and Xpert PML-RARA Linearity Panel demonstrated high accuracy, precision and linearity with slope values between 0.9 and 1.1 and Pearson correlation coefficient, $R^2 > 0.98$ when tested across three Xpert PML-RARA Assay cartridge lots.

✎ Stability data for Xpert PML-RARA External Control Panel and Xpert PML-RARA Linearity Panel along with historical data of similar products containing MMQCI's proprietary matrix formulation supports stability for 2+ years when stored at -20°C.

✎ Reported % ratio values for the two Xpert PML-RARA Panels may vary among laboratories, test systems and reagent lots, however the use of the Xpert PML-RARA Linearity Panel C221 and Xpert PML-RARA External Control Panel C215 enables a laboratory to monitor and establish acceptable % ranges and confirm linearity across the reported dynamic range.

References:

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