

Evaluation of Monitoring Linearity of Quantitative Measurement of Oncology Assays with Synthetic Linearity Panels

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H045

Introduction

Clinical laboratory regulations require verification of an assay's reportable range to ensure reliable test results across the full range of target concentrations. Availability of linearity panels that provide quantification at the lowest and highest ranges for the assay's claimed limits is vital for laboratories to ensure accurate reporting for patient care.

This is especially critical for assays that monitor measurable residual disease (MRD), as they help to refine prognosis, inform and define treatment milestones and, in some cases, lead to the cessation of chemotherapy. Linearity panels for assays measuring transcript levels as prognostic markers for chronic and acute myeloid leukemia, including BCR-ABL1 (p190 or p210), PML-RARA fusions, and NPM1 mutations, have been validated to support accurate quantification across reportable dynamic ranges for multiple assays.

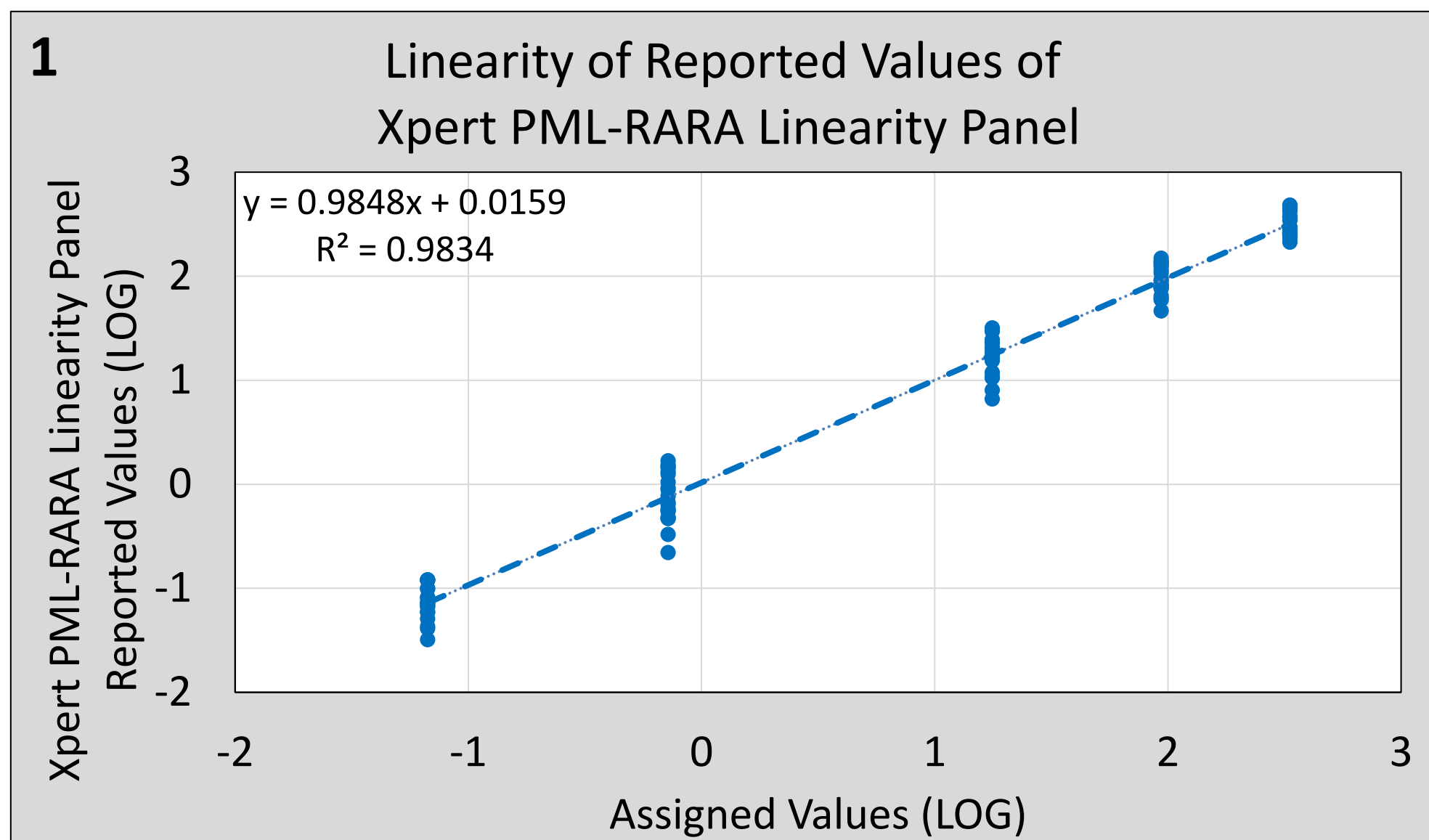
Methods

Five linearity panels were developed using synthetic transcripts mixed at precise ratios to represent a broad range of clinically relevant values. Four panels were designed to undergo the complete assay workflow, while the Birlinn™ BCR-ABL1 p210 IS Panel is formulated for direct addition to the amplification step.

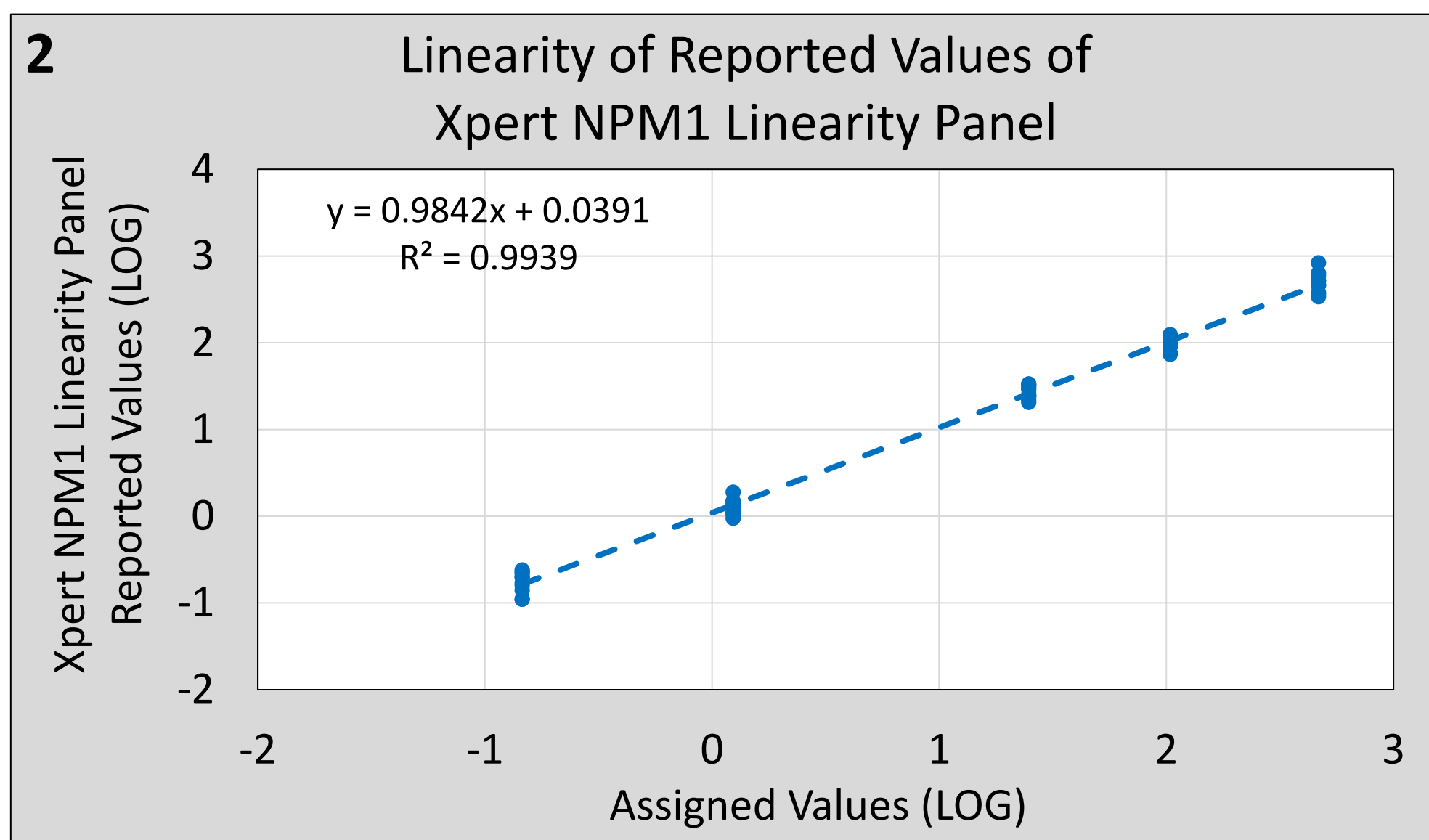
The Birlinn BCR-ABL1 p210 IS Panel and the Xpert BCR-ABL IS p210 Linearity Panel were tested alongside WHO standards to create lot-specific Correction Factors used to assign WHO-traceable %IS/MR values. The Birlinn BCR-ABL1 p210 IS Panel was used as a secondary standard across multiple assays using the assigned values and quantified by droplet digital PCR.

Three lots of the Xpert NPM1 (0.1% to 450% NPM1 MUT A), Xpert BCR-ABL1 p190 (0.008% to 20%) and Xpert PML-RARA (0.1% to 450% bcr1) panels were tested across 3 lots of their respective Xpert cartridges. Grubb's outlier test was applied to remove statistical outliers, and remaining data was used to assign % values to each level. Subsequent lots were manufactured and used to verify linearity across the entire reportable range of multiple cartridge lots using the assigned values.

Xpert PML-RARA and Xpert NPM1 Linearity Panels



Assigned %	Avg Reported %	N	2-fold range
0.067%	0.103%	19	0.034%-0.134%
0.72%	0.87%	20	0.36%-1.44%
17.7%	18.8%	20	8.8%-35.4%
93.8%	94.2%	19	46.9%-187.6%
334.3%	334.3%	19	167.1%-668.6%



Assigned %	Avg Reported %	N	2-fold range
0.146%	0.178%	10	0.073%-0.292%
1.24%	1.30%	10	0.62%-2.48%
24.8%	27.4%	10	12.4%-49.8%
104.3%	98.6%	10	52.1%-208.6%
468.8%	510.1%	10	234.4%-937.6%

Figure 1. Xpert PML-RARA Linearity Panel. 3 lots of the Xpert PML-RARA Linearity Panel were tested on multiple reagent lots of the Xpert PML-RARA assay to assign % values to each level. Linear regression analysis comparing reported ratios of subsequent lots to the assigned values resulted in strong linear relationship across the entire dynamic range of the assay ($R^2 > 0.98$).

Figure 2. Xpert NPM1 Linearity Panel. 3 lots of the Xpert NPM1 Linearity Panel were tested on multiple reagent lots of the Xpert NPM1 Mutation assay to assign % values to each level. Linear regression analysis comparing reported ratios of subsequent lots to the assigned values resulted in strong linear relationship across the entire dynamic range of the assay ($R^2 > 0.99$).

WHO-Traceability of Birlinn BCR-ABL1 IS p210 Linearity Panel

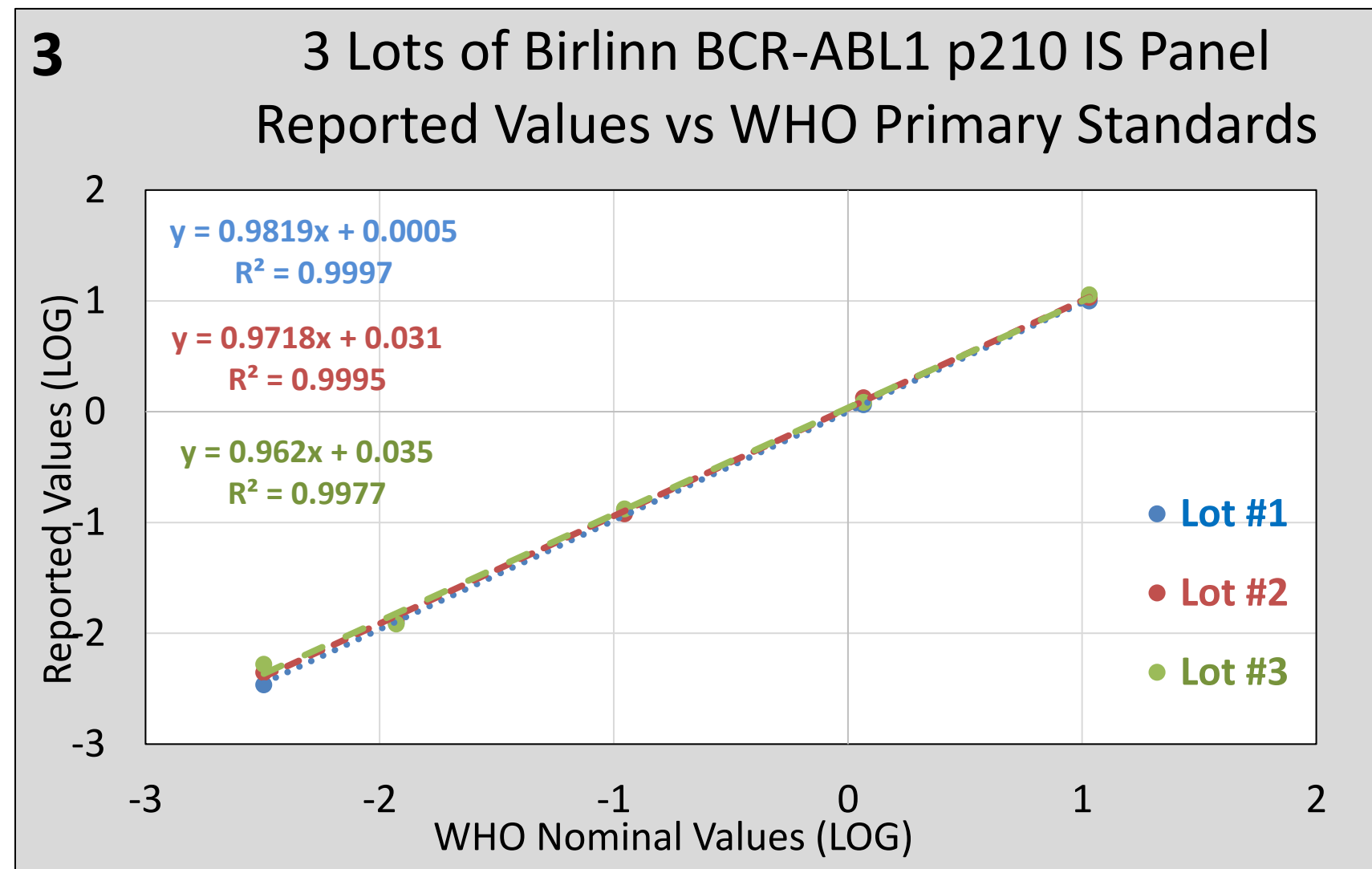
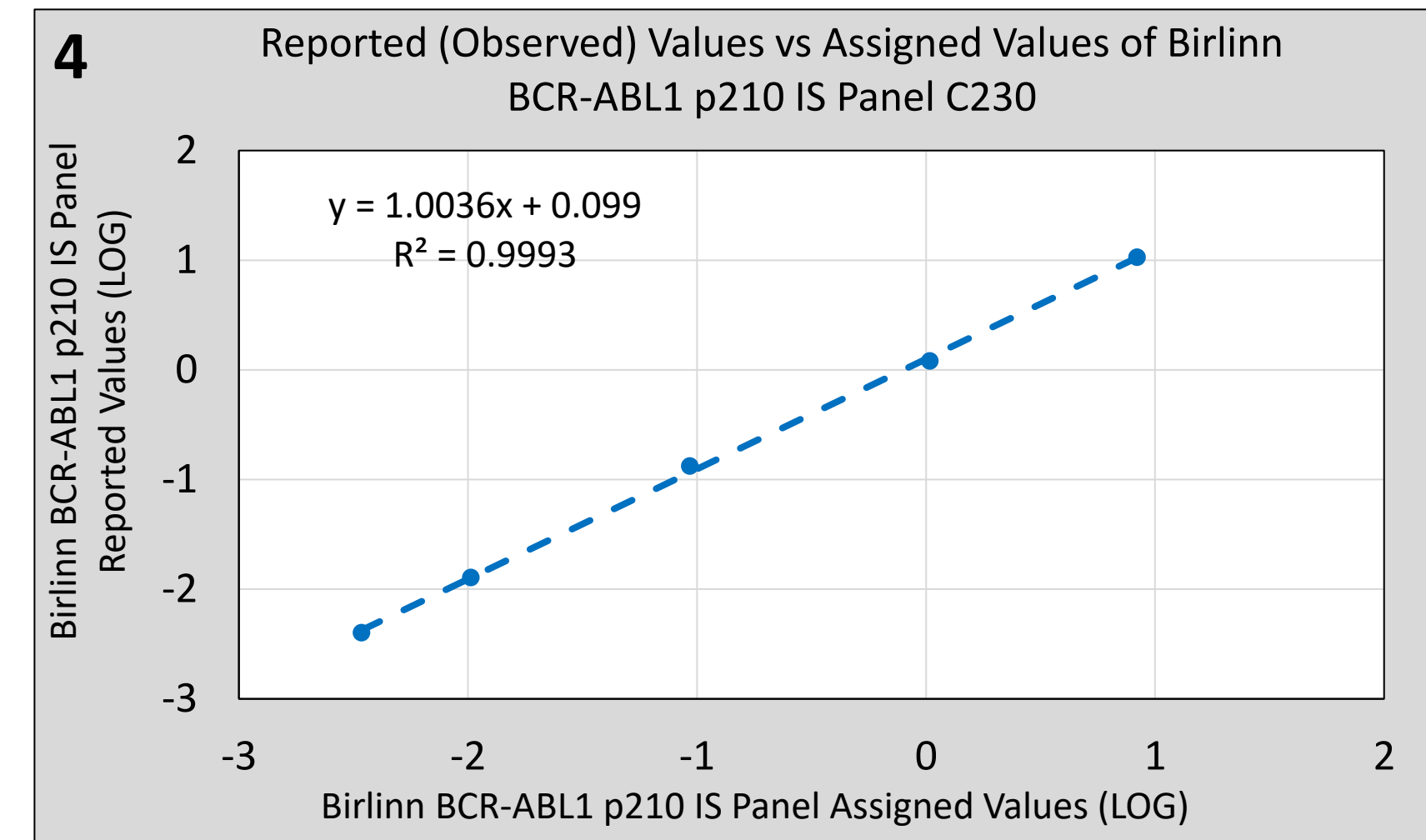


Figure 3. Linearity of Birlinn BCR-ABL1 p210 IS Panel vs WHO Primary Standards. 3 Lots of Birlinn BCR-ABL1 p210 IS Panel were tested on the REALQUALITY RQ-BCR-ABL p210 One-Step assay on the LC480II system alongside 5 WHO panels. Data was used to create lot-specific correction factors to assign values for use as secondary standards traceable to WHO Primary Standards.

Figure 4. Example of Calculating Correction Factor (CF). Reported %IS Values for Birlinn BCR-ABL1 p210 IS Panel are plotted against the assigned %IS values when tested with the REALQUALITY RQ-BCR-ABL p210 One-Step assay on the LC480II System. The Correction Factor can be calculated as the reciprocal of the anti-log10 of the intercept of the linear regression.



SUMMARY OUTPUT

Regression Statistics					
Multiple R	0.9996694				
R Square	0.9993389				
Adjusted R Square	0.9991185				
Standard Error	0.0416353				
Observations	5				

ANOVA					
	df	SS	MS	F	Significance F
Regression	1	7.861383	7.861383	4534.974	7.21546E-06
Residual	3	0.005201	0.001734		
Total	4	7.866584			

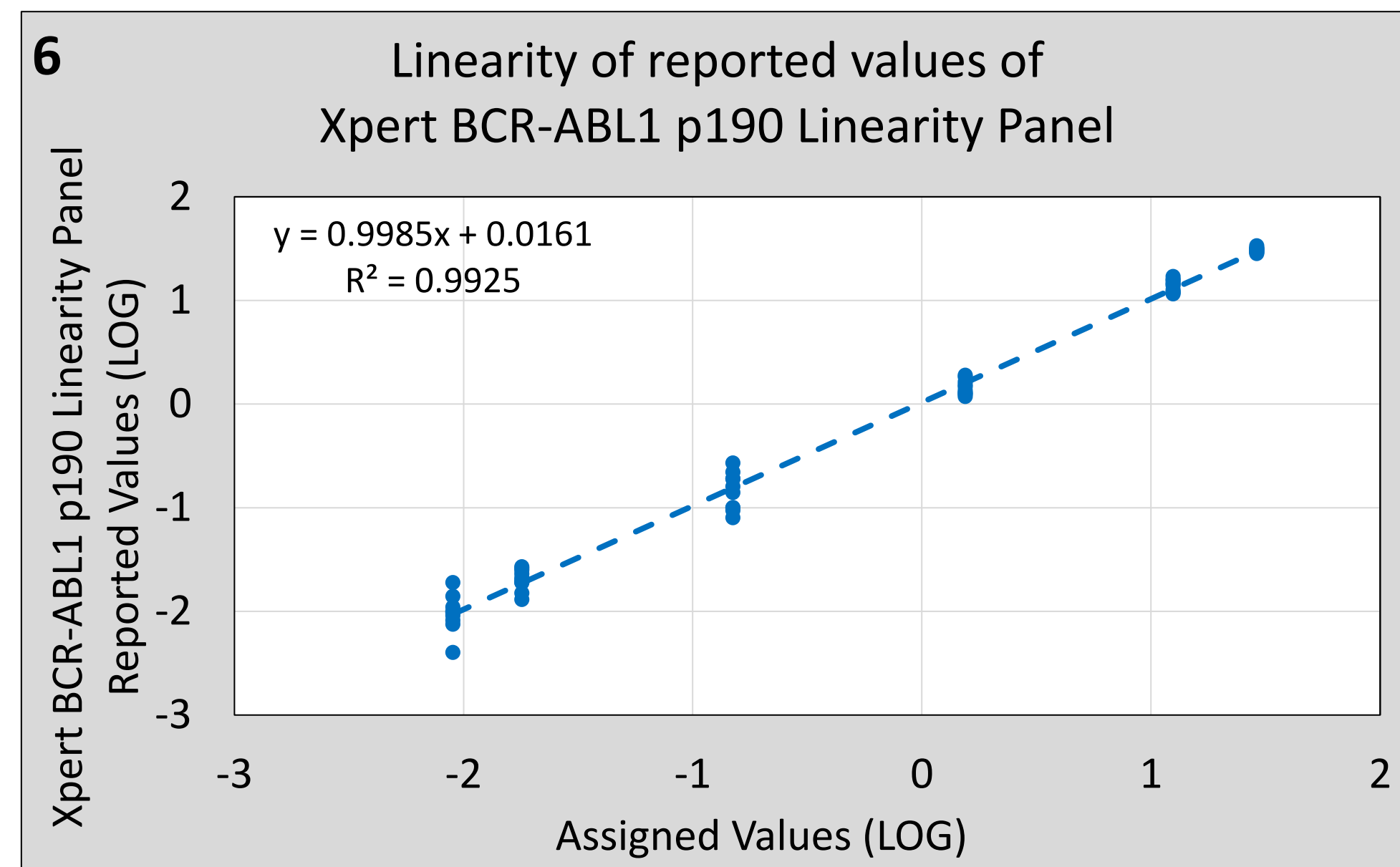
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	0.0990222	0.023022	4.301172	0.023123	0.02575547	0.172289
X Variable 1	1.0036385	0.014904	67.34222	7.22E-06	0.95620874	1.051068

y=1.0036x+ 0.099
R²= 0.9993
CF= 1/(10^intercept)
CF 0.80

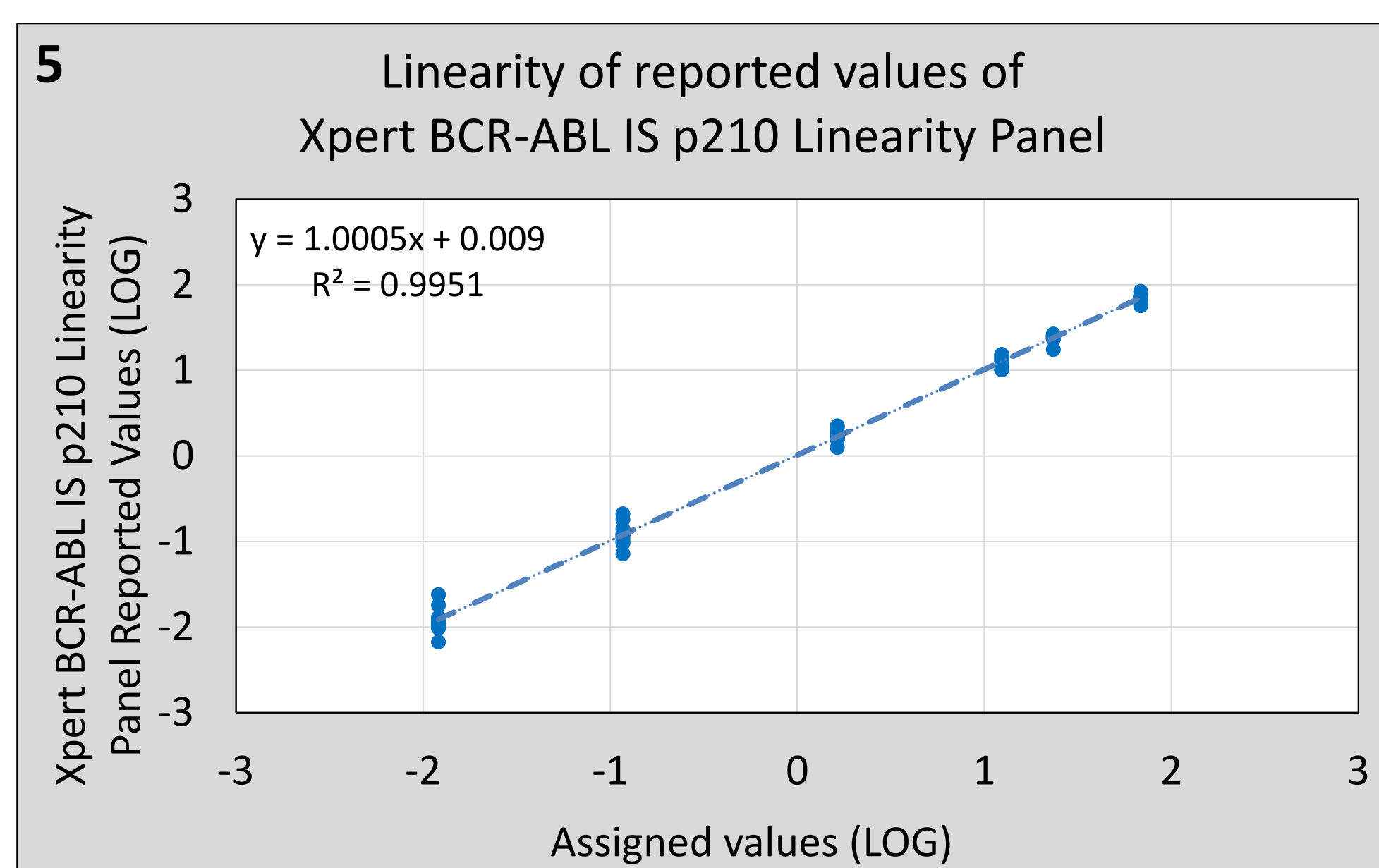
	Reported %	%IS
	10.6	8.5
	1.20	0.96
	0.13	0.10
	0.013	0.010
	0.0040	0.0032

xCF (0.80)

Xpert BCR-ABL Linearity Panels



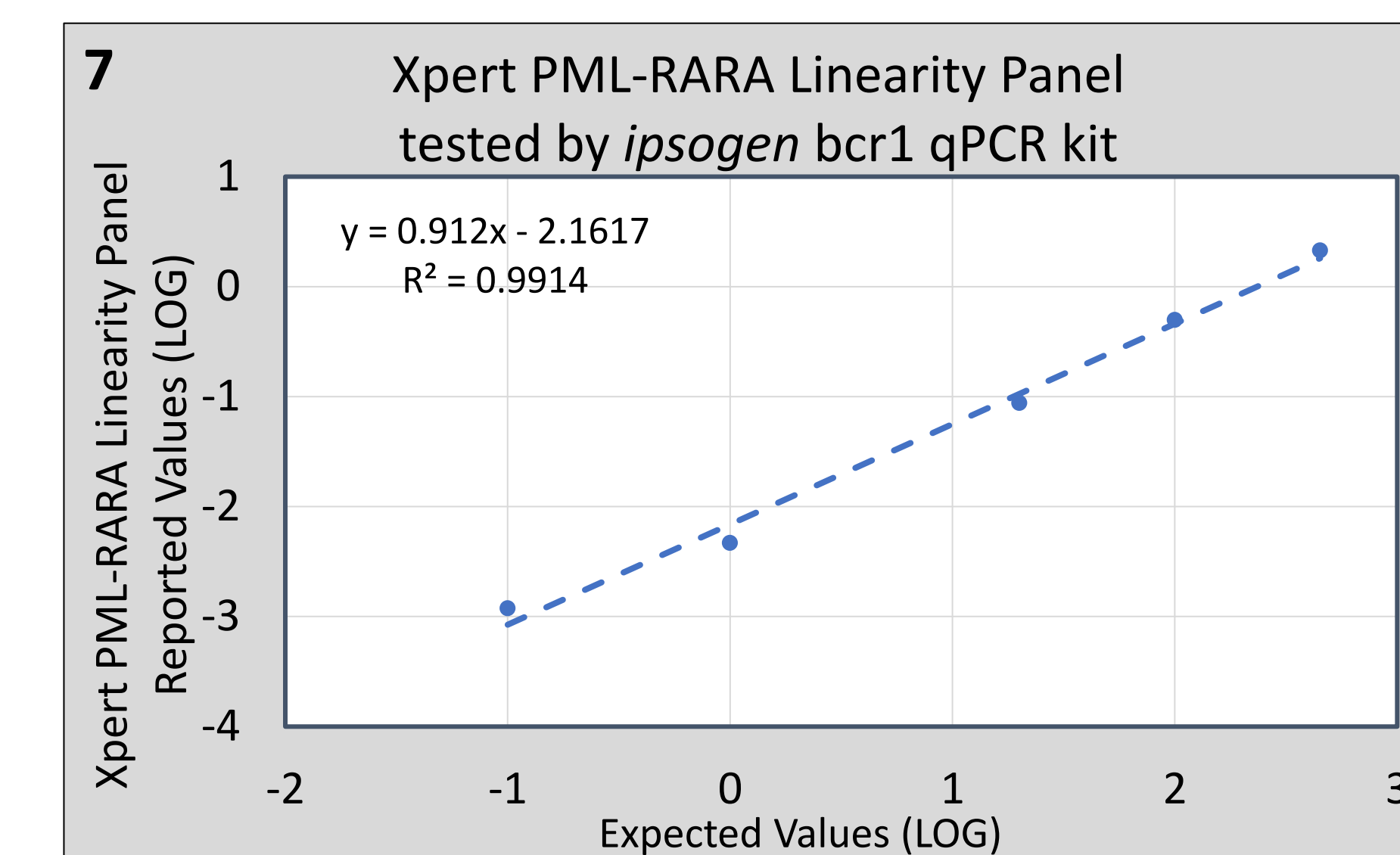
Assigned %	Avg Reported %	N	2-fold range
0.009%	0.0100%	10	0.0045%-0.02%
0.018%	0.021%	10	0.009%-0.036%
0.15%	0.15%	10	0.075%-0.30%
1.55%	1.47%	10	0.77%-3.10%
12.5%	14.0%	10	6.2%-25.0%
29.0%	30.7%	10	14.5%-58.0%



Assigned %	Avg Reported %	N	2-fold range
0.0121%	0.0128%	9	0.006%-0.0242%
0.117%	0.127%	9	0.058%-0.234%
1.64%	1.72%	9	0.82%-3.28%
12.4%	12.9%	9	6.4%-25.8%
23.4%	23.8%	9	11.7%-46.8%
68.6%	70.0%	9	34.3%-137.2%

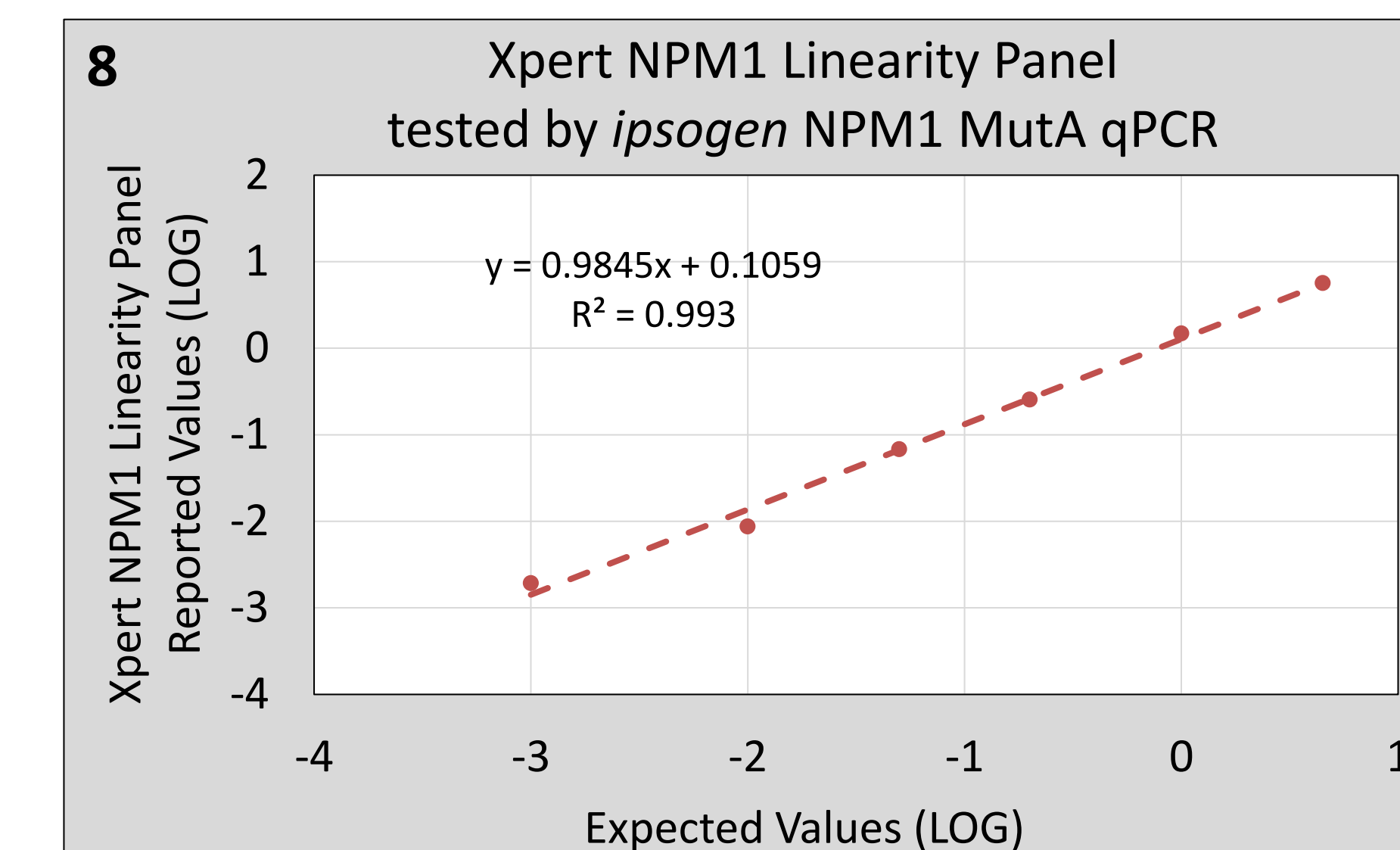
Figure 5. Xpert BCR-ABL1 p190 Linearity Panel. 3 lots of Xpert BCR-ABL1 p190 Linearity panel were tested on multiple reagent lots of Xpert BCR-ABL Ultra p190 assay to assign % values to each level. Linear regression analysis comparing reported ratios of subsequent lots to the assigned values resulted in strong linear relationship across the entire dynamic range of the assay ($R^2 > 0.99$). **Figure 6. Xpert BCR-ABL IS p210 Linearity Panel.** The Xpert BCR-ABL IS p210 Linearity panel was tested alongside WHO Panels to generate a CF to assign values on the Xpert BCR-ABL Ultra assay. Linear regression analysis comparing reported ratios of subsequent lots to the assigned values resulted in strong linear relationship across the entire dynamic range of the assay ($R^2 > 0.99$).

Linearity Panels tested by commercially available qPCR kits



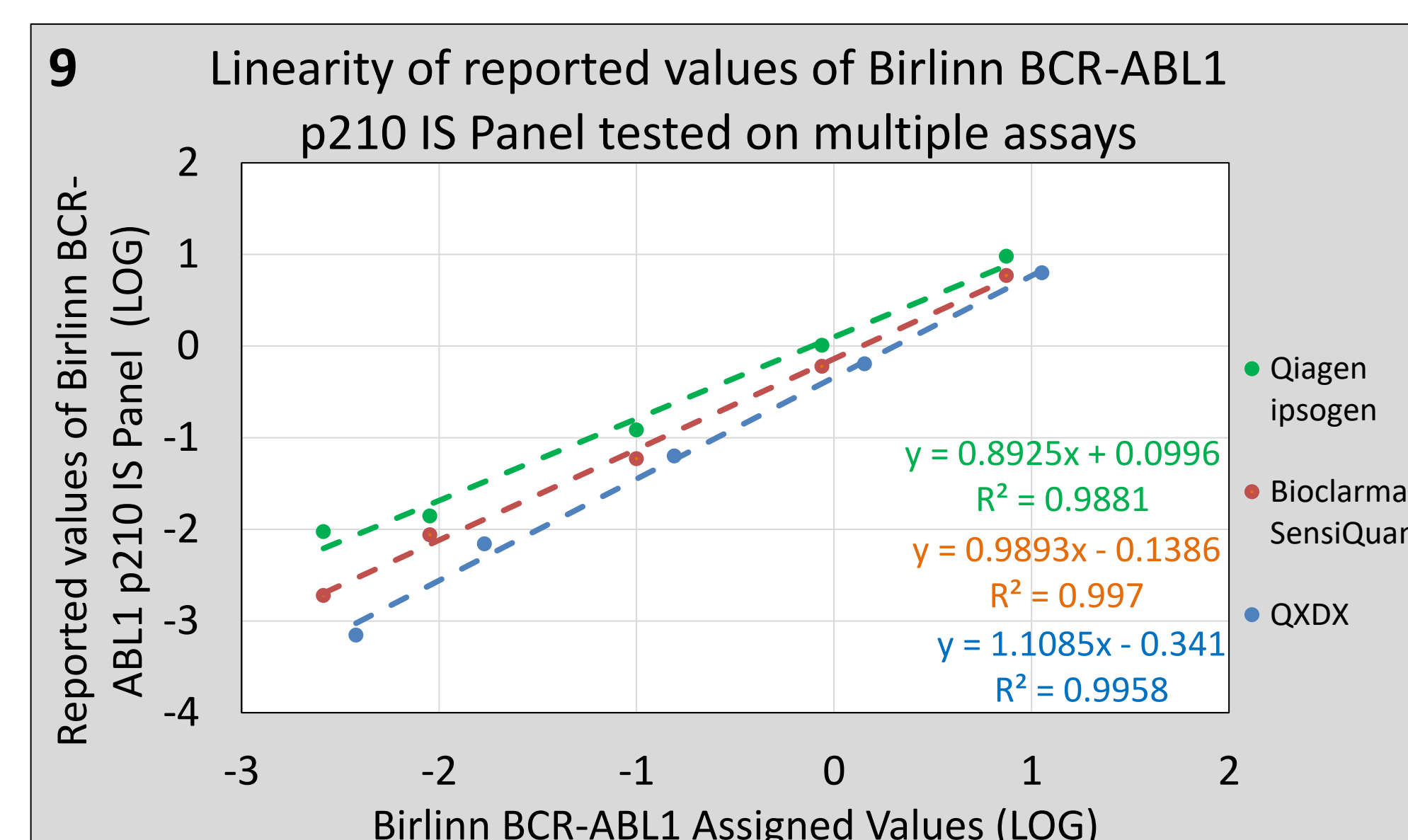
Expected %	<i>ipsogen</i> Reported %
0.1%	0.119%
1%	0.467%
20%	8.74%
100%	49.73%
450%	213.23%

Figure 7. Linear Regression of reported values of the Xpert PML-RARA Linearity panel tested on the *ipsogen* PML-RARA bcr1 kit. RNA was extracted (in duplicate) and tested using the *ipsogen*® PML-RARA bcr1 Kit. The Xpert PML-RARA Linearity Panel was able to confirm linearity across the entire range of the assay with a linear regression ($R^2 > 0.99$).



Expected %	<i>ipsogen</i> Reported %
0.10%	0.19%
1%	0.87%
5%	6.79%
20%	25.56%
100%	148.11%
450%	566.63%

Figure 8. Linear Regression of reported values of the Xpert NPM1 Linearity panel tested on the *ipsogen* NMP1 Mut A kit. RNA was extracted (in duplicate) and tested using the *ipsogen*® NPM1 Mut A Kit (QIAGEN). The Xpert NPM1 Linearity Panel was able to confirm linearity across the entire range of the assay with a linear regression ($R^2 > 0.99$).



Assigned Value	<i>ipsogen</i>	SensiQuant	QXDx
0.0026 (+/- 0.0012)	0.0076	0.0026	0.0007
0.009 (+/- 0.006)	0.011	0.012	0.007
0.10 (+/- 0.04)	0.10	0.08	0.063
0.87 (+/- 0.18)	0.82	0.83	0.64
7.46 (+/- 1.71)	7.65	8.15	6.31

Figure 9. Linear Regression of reported values of the Birlinn BCR-ABL IS p210 Linearity Panel tested on multiple assays. The Birlinn BCR-ABL1 p210 IS Panel was tested using *ipsogen*® BCR-ABL1 Mbcr IS-MMR DX kit (QIAGEN), SensiQuant p210 Master Mix (Bioclarma), and QXDx™ BCR-ABL %IS Kit (BIO-RAD). All three assays demonstrated linearity with R^2 values greater than 0.98. A linear relationship between the observed values and assigned values over the range of the secondary standards enables the calculation of a %IS correction factor (CF) for each assay.

Summary and Conclusions

All replicates across all panels reported % ratio values within 2-3-fold of assigned values and demonstrated linearity with $R^2 > 0.98$ across all reagent-lots.

The Birlinn BCR-ABL1 p210 IS Panel was accurately used as secondary standards across a laboratory developed ddPCR and qPCR assay, using the WHO-Traceable assigned values, and provided assurance of linearity ($R^2 > 0.99$) between the observed and assigned values

The synthetic linearity panels Xpert NPM1, Xpert PML-RARA, Xpert BCR-ABL1 p190, and Xpert BCR-ABL IS p210 demonstrated high accuracy and linearity across the entire reportable range of each assay and provide a standardized, reliable approach to verifying quantitative assay performance, supporting high-quality, actionable molecular results for precision monitoring in hematologic malignancies.