Development of synthetic secondary standards for BCR-ABL1 quantification on GeneXpert® BCR-ABL Monitor V2 and Xpert® BCR-ABL Ultra assays.

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Introduction

Chronic myeloid leukemia (CML) is characterized by the presence of Philadelphia chromosome (Ph) arising from translocation of ABL1 gene on chromosome 9 and BCR gene on chromosome 22, resulting in BCR-ABL1 fusion, an oncogenic tyrosine kinase protein. Quantitative monitoring of BCR-ABL1 transcripts by RQ-PCR in patient blood is an important tool for measuring molecular response to therapy. Current recommendations for monitoring CML patients call for measuring the levels of BCR-ABL1 standardized to the International Scale (IS) to ensure harmonized reporting across laboratories[1]. To this end, the 1st WHO International Genetic Reference Panel can be used. However, due to the limited supply of the WHO primary panel, development of reliable secondary reference standards is essential for monitoring of BCR-ABL1 quantitative assays.

In this study, we describe the development and performance of MMQCI's Xpert BCR-ABL IS Panel C130 as secondary standards calibrated and traceable to WHO primary panel for BCR-ABL1 quantification using GeneXpert® Instrument Systems with the GeneXpert® BCR-ABL Monitor V2 or Xpert® BCR-ABL Ultra assay.

Materials and Methods

Xpert BCR-ABL IS Panel C130 is composed of six standards. Each standard contains an increasing concentration of BCR-ABL1 (e14a2/b3a2) RNA transcript mixed with a fixed concentration of ABL1 RNA transcript to produce six levels, 0.0%IS, 0.0032%IS, 0.01%IS, 0.1%IS, 1%IS and 10%IS when analyzed, on the GeneXpert® Instrument Systems with the GeneXpert® BCR-ABL Monitor V2 or Xpert® BCR-ABL Ultra assay. Three lots of Xpert BCR-ABL IS Panel C130 were manufactured and calibrated against primary WHO BCR-ABL1 reference panel using one cartridge lot of the GeneXpert® BCR-ABL Monitor V2 assay. The following protocol was used as per White et al.[2] and NIBSC code: 09/138 Instructions for use.[3]:

1. 5 panels of WHO (4 levels) primary panel were reconstituted and tested alongside 3 lots of C130 over 8 days per NIBSC protocol. An additional two levels of C130 (Xpert BCR-ABL1 0% IS Level C131 and Xpert BCR-ABL1 0.0032% IS Level C132) were tested for the remaining two days for a total of 10 days of testing.
2. Assay lot specific efficiency "$E_{\Delta CT}$" was generated by performing linear regression analyses of ΔCt of the WHO tested samples against the log values of the theoretical assigned %IS. The efficiency was calculated using the formula: $E_{\Delta CT} = 10^{(1/slope)}$.
3. Grubbs' outlier test was used to detect potential outliers using z statistic for each sample. Two outliers were removed from the final analysis.
4. Bland Altman test was used to calculate a Correction Factor (CF) for assignment of IS values to Xpert BCR-ABL IS Panel C130. CF was calculated using the formula $CF = 10^{M_d}$ where M_d is the mean of the differences between log10 WHO assigned IS values and %IS values generated using GeneXpert® BCR-ABL Monitor V2 assay.
5. The BCR-ABL1/ABL1 ratios for all six levels of Xpert BCR-ABL IS Panel C130 were converted to IS scale by multiplying the observed ratios for each level by CF.
6. Long term stability at -20°C for the panel is being established by testing 4 lots of the panel in duplicate with GeneXpert® BCR-ABL Monitor V2 assay.

Results

Figure 1. Determination of Assay Lot-Specific Efficiency for WHO Calibration. Each cartridge lot of GeneXpert BCR-ABL Monitor V2 and Xpert BCR-ABL Ultra has a lot-specific Scaling Factor (SF) and Efficiency value ($E_{\Delta CT}$) embedded within the test cartridge barcode. The lot-specific SF and $E_{\Delta CT}$ values are determined by the assay manufacturer (Cepheid) and used to calibrate the assay to the International Standard (IS). In order for MMQCI to determine a Correction Factor to calibrate Xpert BCR-ABL IS Panel C130 as secondary standards, the cartridge lot-specific SF and $E_{\Delta CT}$ were removed and a linear regression was performed of the ΔCt of the WHO tested samples against the log values of the theoretical assigned %IS. A new Efficiency value of the assay was calculated using the formula: $E_{\Delta CT} = 10^{(1/slope)} = 10^{1/3.64} = 1.88$

Delta Ct Plot for Assay Efficiency Generation

y = 3.6393x - 7.3343
R² = 0.9908

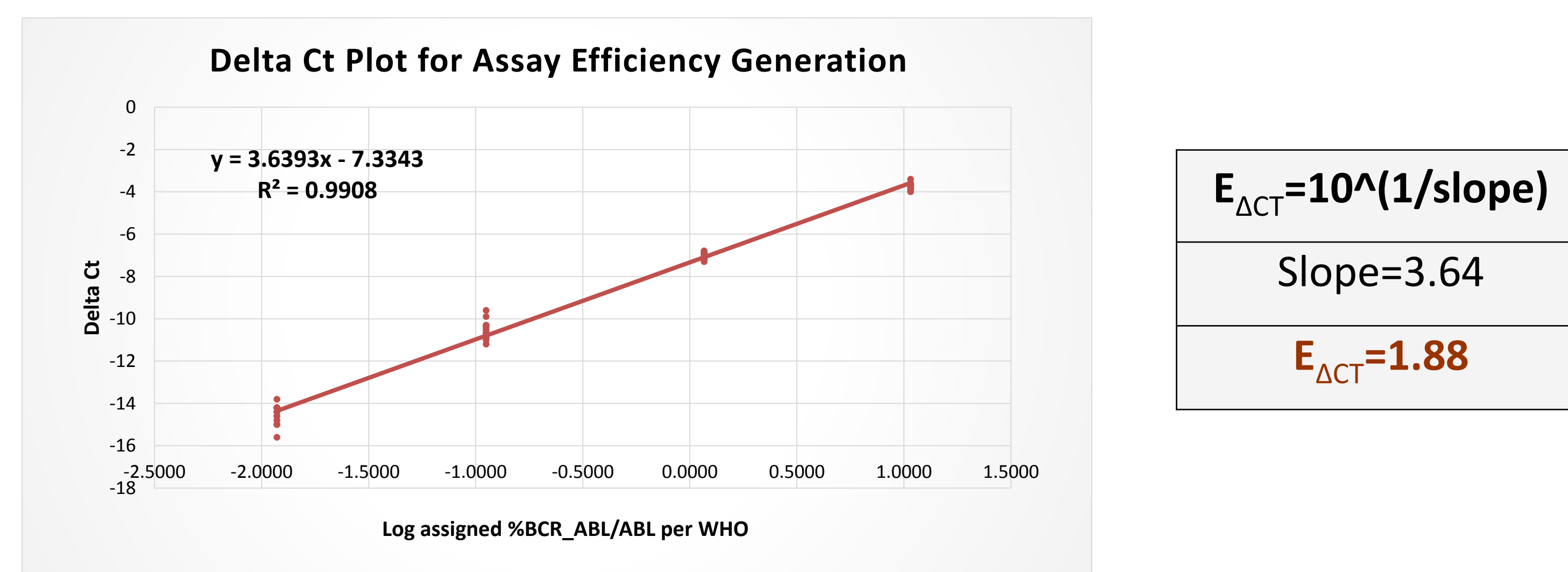$E_{\Delta CT} = 10^{(1/slope)}$

Slope = 3.64

$E_{\Delta CT} = 1.88$

Figure 2. Calculation of Correction Factor (CF) for Assignment of IS values. Since the Bland Altman requirement of p>0.05 was met, IS values can be assigned by applying a CF factor, calculated as the anti-log of the mean of differences between the log10 WHO assigned and WHO actual values generated for each lot of Xpert BCR-ABL IS Panel C130.
$CF = 10^{M_d} = 10^{0.0095} = 1.02$. **A. Bland Altman Bias plot for WHO primary standards on GeneXpert® BCR-ABL Monitor V2 assay.** No significant trend in bias was seen in the quantification across all 4 levels of WHO primary standards on GeneXpert® BCR-ABL Monitor V2 assay. (p=0.626911). **B. Correlation between WHO primary standards nominal values and test values.** Linear regression analysis between nominal values of WHO primary standards and test values obtained when tested on one cartridge lot of GeneXpert® BCR-ABL Monitor V2 assay demonstrated correlation between the two values (r²=0.9943).

A. Bias Plot of WHO primary Standards on Xpert BCR-ABL Monitor V2 Assay

B. LOG WHO Actual vs LOG WHO Nominal

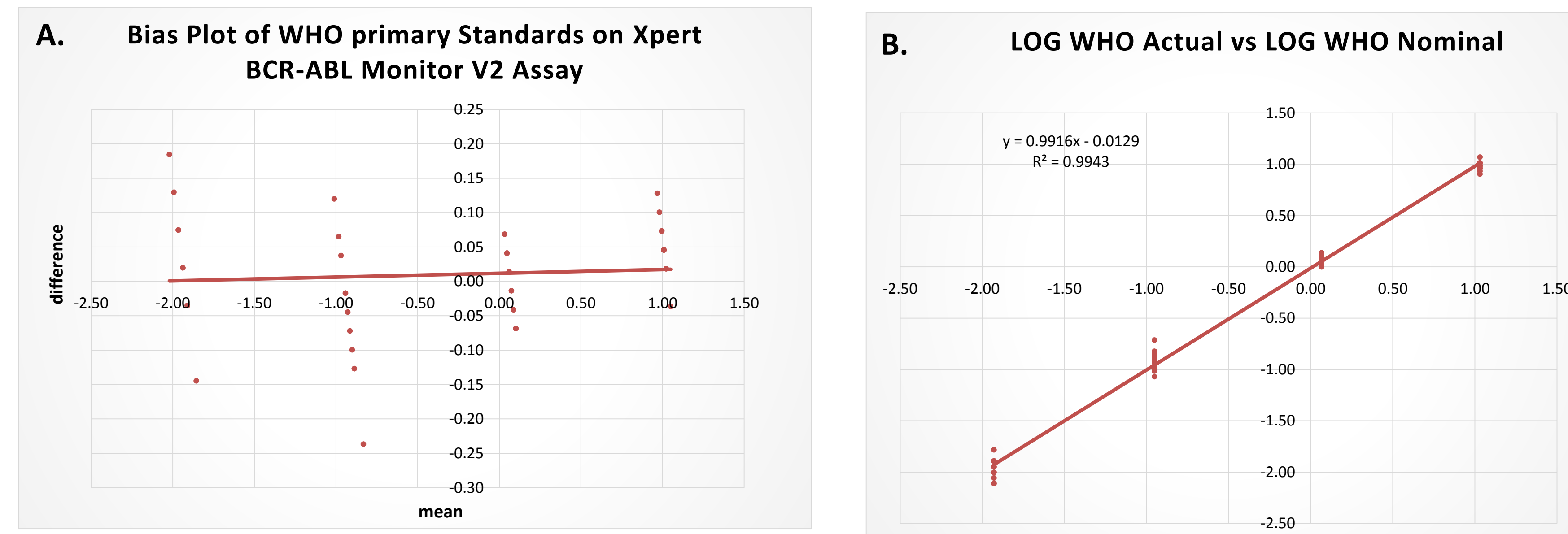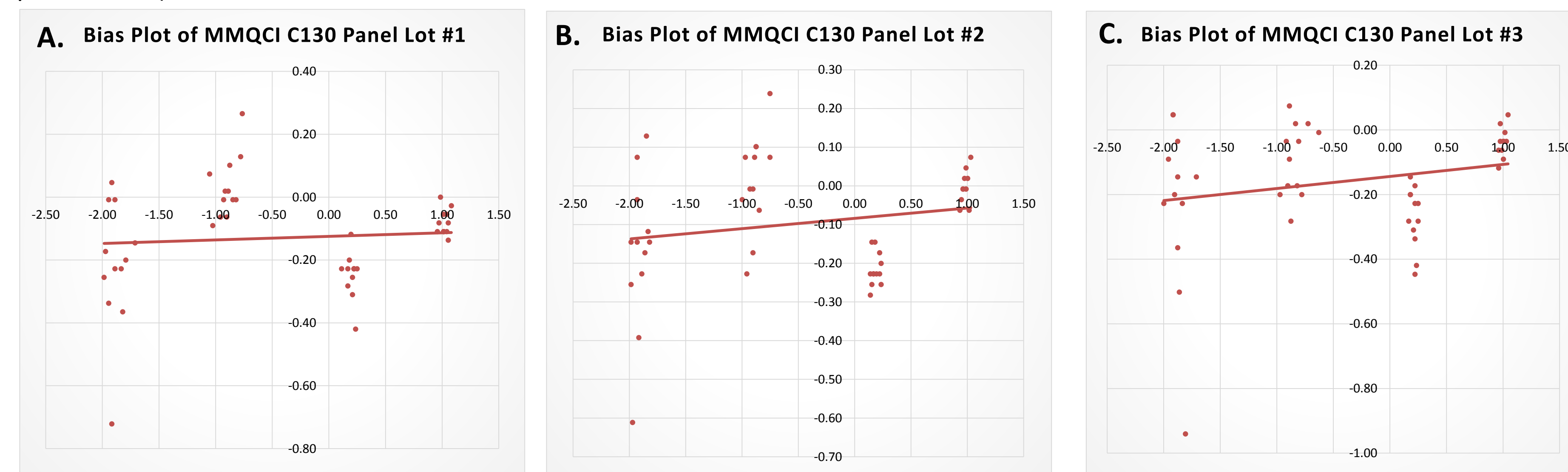y = 0.9916x - 0.0129
R² = 0.9943

Figure 3. Bland Altman test to detect bias across four levels of Xpert BCR-ABL IS Panel C130 on GeneXpert® BCR-ABL Monitor V2 assay. Bland Altman test shows no significant trend in bias across all four levels of Xpert BCR-ABL IS Panel C130 assigned values against WHO panel nominal values on GeneXpert® BCR-ABL V2 assay. **A.** Bias plot for Xpert BCR-ABL IS Panel Lot 1 (p=0.598267). **B.** Bias plot for Xpert BCR-ABL IS Panel Lot 2 (p=0.197907). **C.** Bias plot for Xpert BCR-ABL IS Panel Lot 3 p=0.121397).

A. Bias Plot of MMQCI C130 Panel Lot #1

B. Bias Plot of MMQCI C130 Panel Lot #2

C. Bias Plot of MMQCI C130 Panel Lot #3

Table 1. Xpert BCR-ABL IS Panel C130 %IS values. The BCR-ABL %IS actual values were generated across three lots of MMQCI standards using one Xpert BCR-ABL cartridge lot. The assigned %IS values traceable to WHO primary standards were calculated by multiplying the observed ratios with the CF (1.02).

C130 Levels	WHO Actual	Lot #1		Lot #2		Lot #3	
		Actual	WHO-Traceable	Actual	WHO-Traceable	Actual	WHO-Traceable
C132 0.0032%IS	-	0.0047%	0.0048%	0.0057%	0.0058%	0.0051%	0.0052%
C133 0.01%IS	0.0110%	0.0177%	0.0180%	0.0151%	0.0154%	0.0185%	0.0189%
C134 0.1%IS	0.128%	0.122%	0.124%	0.127%	0.129%	0.162%	0.166%
C135 1.0%IS	1.2%	2.08%	2.12%	1.93%	1.97%	2.20%	2.25%
C136 10.0%IS	9.6%	11.6%	11.8%	9.4%	9.6%	10.2%	10.4%

Figure 4. Correlation between Xpert BCR-ABL IS Panel C130 and WHO nominal values. Linear regression analysis between %IS assigned values for all four levels of Xpert BCR-ABL IS Panel C130 vs WHO actual values demonstrated equality between the two values for all three lots of C130.
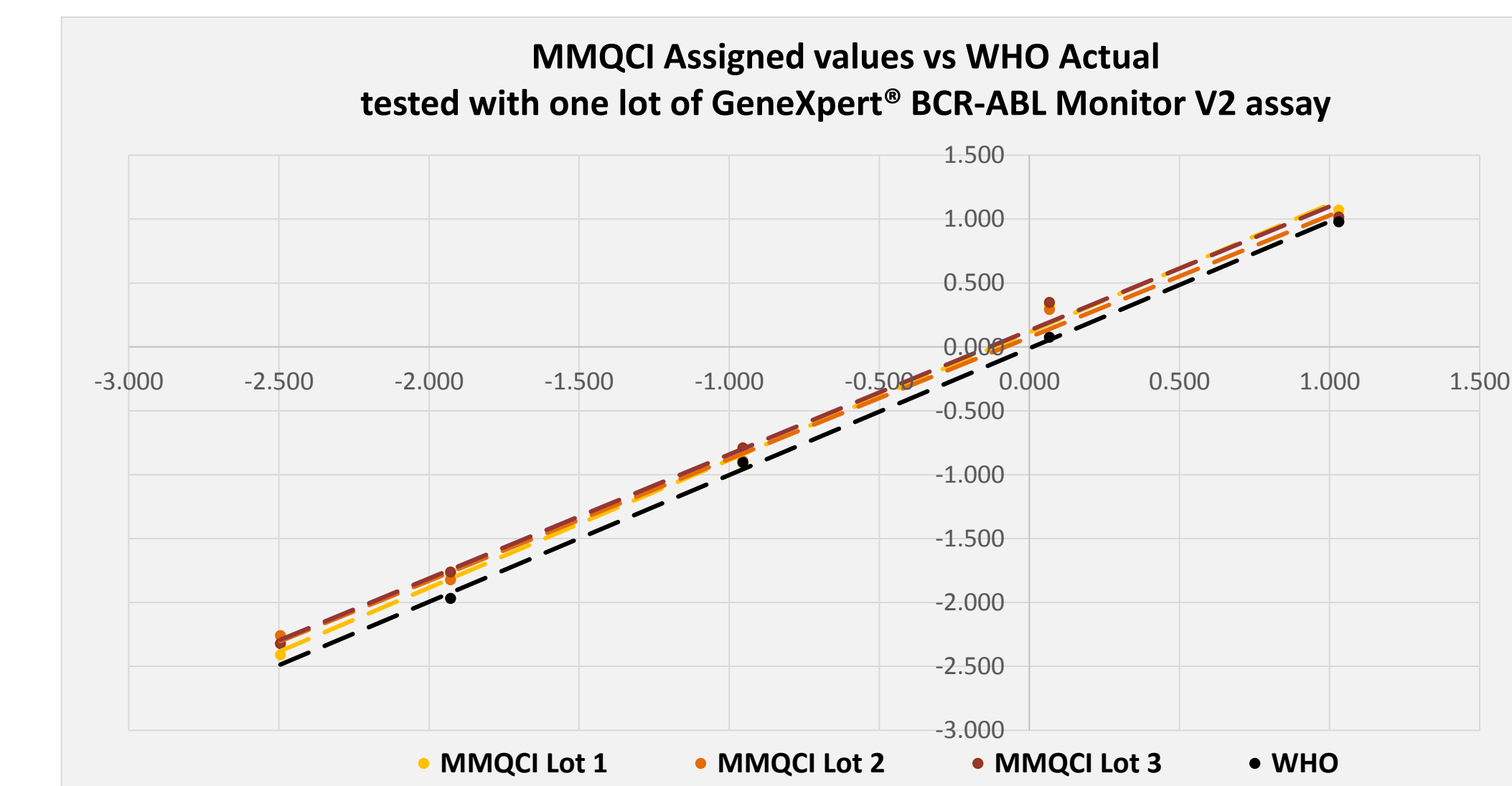

MMQCI Assigned values vs WHO Actual tested with one lot of GeneXpert BCR-ABL Monitor V2 assay

● MMQCI Lot 1　● MMQCI Lot 2　● MMQCI Lot 3　● WHO

Figure 5. Stability of Xpert BCR-ABL IS Panel C130 when stored at -20°C. All levels of 4 manufactured lots of C130 were tested for stability using the GeneXpert BCR-ABL Monitor V2 assay over 18 months. **A. Stability of ABL transcript.** No trending was observed of Ct values of the ABL transcripts across the four lots of Xpert BCR-ABL IS Panel C130. **B. Stability of BCR-ABL transcript.** No trending was observed for Ct values of BCR-ABL transcripts across the four lots of Xpert BCR-ABL IS Panel C130.

A.

B.
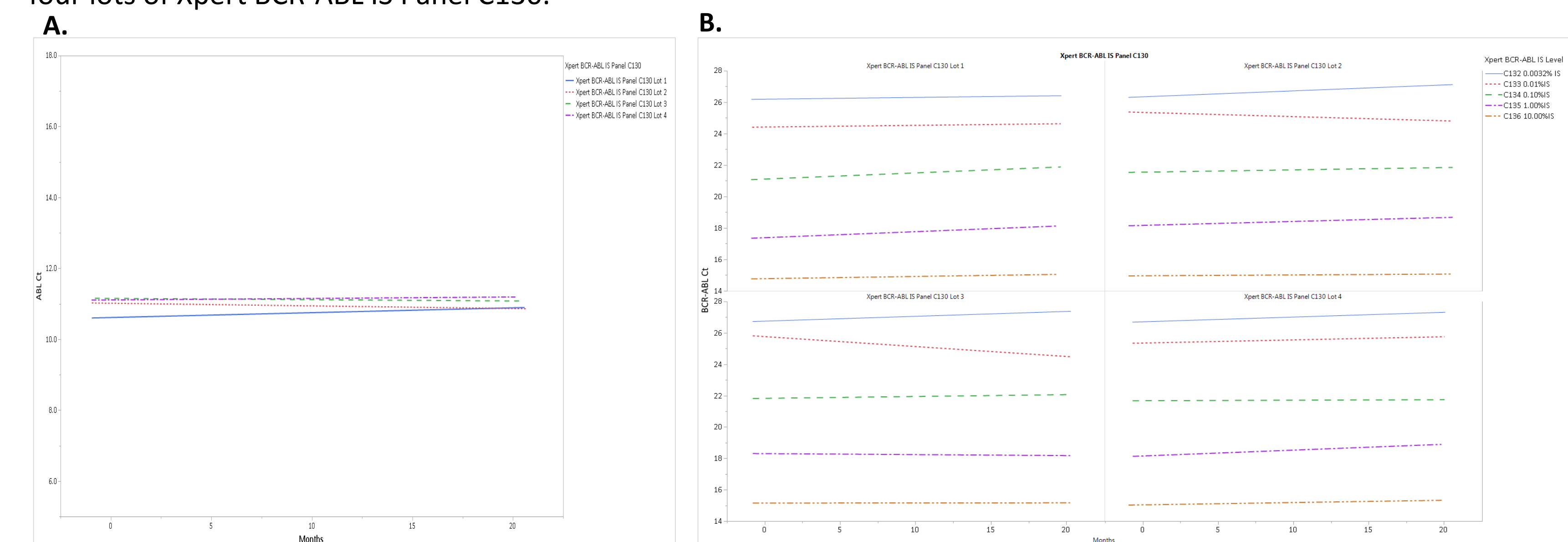
Conclusions

○ MMQCI has reproducibly manufactured BCR-ABL1 secondary standards traceable to WHO BCR-ABL1 primary panel.
○ Xpert BCR-ABL IS Panel C130 performs reliably on the GeneXpert® BCR-ABL Monitor V2 assay over time and is stable for at least 18 months when stored at -20°C.
○ Xpert BCR-ABL IS Panel C130 can be used to monitor the performance of GeneXpert® BCR-ABL Monitor V2 and Xpert® BCR-ABL Ultra assays which report on the International Scale.

References
[1]Branford S et al. Desirable performance characteristics for BCR-ABL measurement on an international reporting scale to allow consistent interpretation of individual patient response and comparison of response rates between clinical trials. Blood 2008, 112:3330-38
[2]White HE et al. Establishment of the first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood 2010, 116:e111-117
[3]1st WHO International Genetic Reference Panel for quantitation of BCR-ABL translocation by RQ-PCR NIBSC code: 09/138 Instructions for use (Version 4.0, Dated 13/12/2012)