Development of full-process quality control material *BCR-ABL* panel traceable to WHO international standard

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ABSTRACT

Introduction: Chronic myeloid leukemia (CML) is driven by the *BCR-ABL* fusion gene, formed by the t(9;22). Sequential monitoring of the expression of *BCR-ABL* mRNA in CML patients is critical for optimal disease management. Expression of *BCR-ABL* is measured by RT-qPCR or digital PCR relative to a reference gene (*ABL, GUSB* or *BCR*), and results are expressed on the International Scale (IS). These methods require full-process control that captures any variability during RNA extraction, cDNA synthesis and PCR. However, there is no such full process control material available today, and the World Health Organization (WHO) international primary *BCR-ABL* reference material is only available for the calibration of secondary reference reagents or kits. In this study, we developed a cell-line based *BCR-ABL* panel that is traceable to the WHO primary *BCR-ABL* panel, and has an additional panel member for 0.0032%IS (Molecular Response MR4.5).

Methods: The *BCR-ABL* and *ABL* gene copy numbers of HL60 (*BCR-ABL* negative) cells and K562 (*BCR-ABL* positive; e14a2 fusion transcript) cells were monitored in cell culture for three passages before mixing to approximate to the target values of 10%IS, 1%IS, 0.1%IS, 0.01%IS and 0.0032%IS. The cell mixture were lyophilized and stored at -20ºC. The RNA was extracted using the Qiagen™ RNeasy™ mini kit, and cDNA synthesized using two-step Applied Biosystems™ High Capacity cDNA Reverse Transcription Kit. The copy numbers of *BCR-ABL* and *ABL* were then determined using custom assays on the Bio-Rad™ QX200™ Droplet Digital™ PCR (ddPCR™) system. Precise IS values were assigned by reference to the WHO international primary panel. The product performance was evaluated in an external testing site using Bio-Rad QXDx™ *BCR-ABL* %IS kit (CE-IVD). The stability of the control panel has been monitored in accelerated and real-time studies.

Results: Five panel members of *BCR-ABL* control material were created at approximately 10%, 1%, 0.1%, 0.01% and 0.0032% target %IS values by mixing the HL60 and K562 cells at different ratios. The panel is traceable to the WHO international primary *BCR-ABL* panel value and performed well in external testing. The accelerated stability study supports the control shelf-life for 2 years stored at -20ºC, and the real-time stability study is on-going.

Summary: A set of full-process *BCR-ABL* Panel have been produced in the development phase that are traceable to the WHO international standard and including an additional member at 0.0032%IS. This panel could help to support analytical validation of high sensitivity assays, enable results to be expressed on the IS, provide improved calibration for deep molecular response and enable assay variation over time to be monitored. The control is for research use only, not for clinical use.

INTRODUCTION

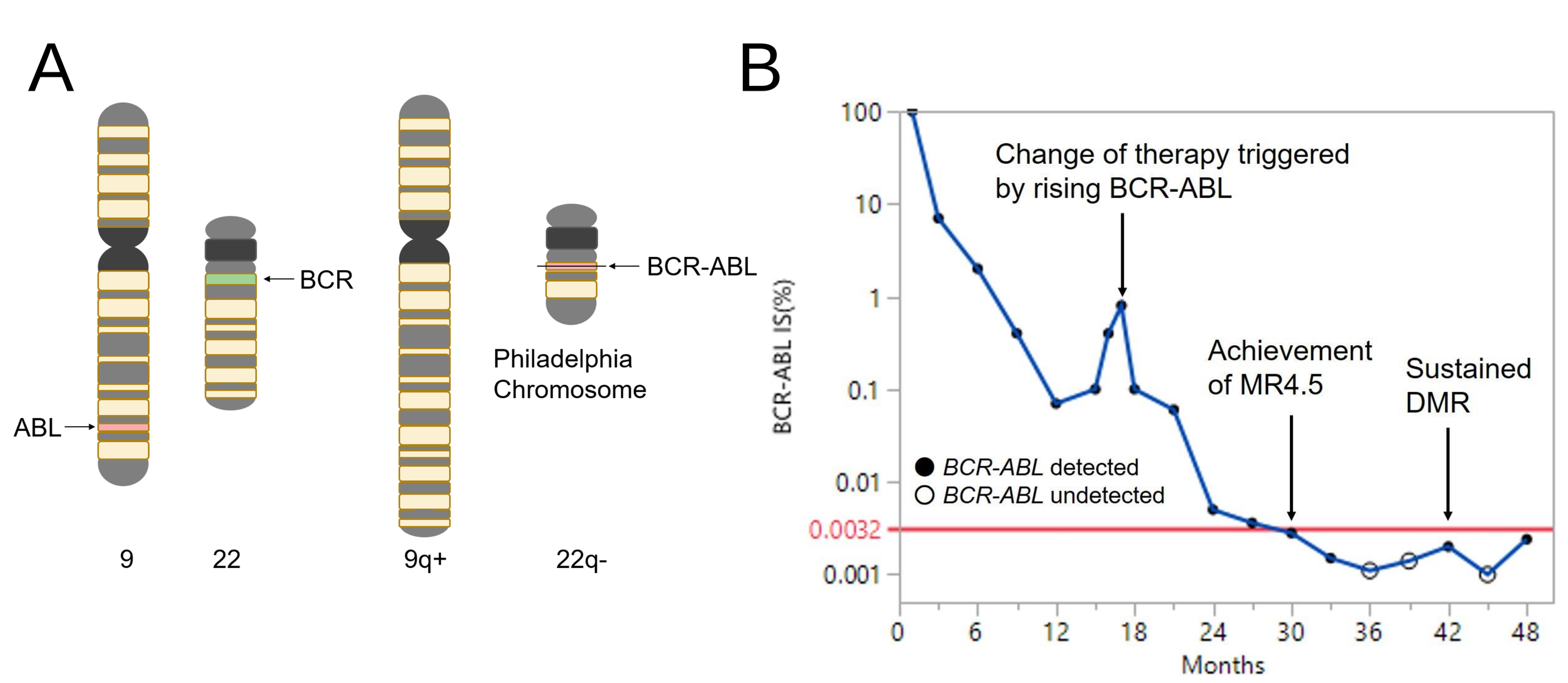

Figure 1. (A) Formation of the *BCR-ABL* gene. The *BCR-ABL* gene is formed by translocation of the *ABL* gene on chromosome 9 and the *BCR* gene on chromosome 22, which results in the Philadelphia chromosome that carries the *BCR-ABL* fusion gene. (B) An example clinical course for a CML patient. Before imatinib therapy, the BCR-ABL^{IS} was close to 100% but decreased on therapy resulting in the achievement of MR4.5 (BCR-ABL^{IS} <0.0032%^{IS}).

	WHO Standard	AMX *BCR-ABL* Panel
Format	Lyophilized cells	Lyophilized cells
Storage condition	-20ºC or below	-20ºC or below
Content	K-562 & HL-60 cells	K-562 & HL-60 cells
Total cell number/vial	~1.5 x 10^6	~1.0 x 10^6
MR 1 (10%^{IS})	Yes	Yes
MR 2 (1%^{IS})	Yes	Yes
MR 3 (0.1%^{IS})	Yes	Yes
MR 4 (0.01%^{IS})	Yes	Yes
MR 4.5 (0.0032%^{IS})	No	Yes

Table 1. Comparison between WHO *BCR-ABL* International Standard vs. the AcroMetrix™ (AMX) *BCR-ABL* Panel.

METHODS

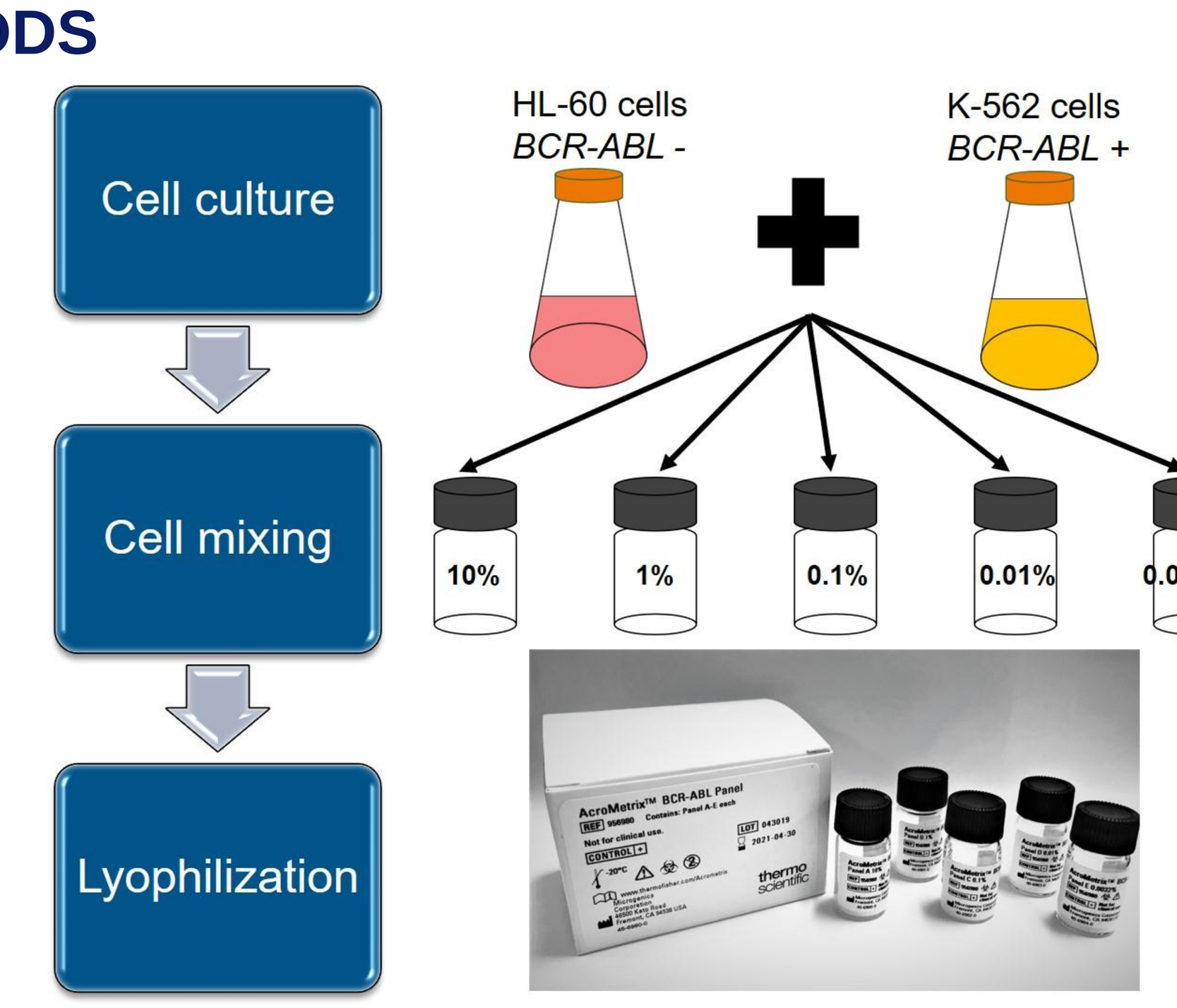

Figure 2. Manufacturing workflow of the AcroMetrix™ *BCR-ABL* Panel. HL-60 (*BCR-ABL* negative cell-line) cells and K-562 (*BCR-ABL* positive cell-line; e14a2 *BCR-ABL*) cells were cultured and expended in IMDM and RPMI media, respectively, over several days. Cells were then spun down and resuspended using 1X PBS before mixing at the target 10%^{IS}, 1%^{IS}, 0.1%^{IS}, 0.01%^{IS} and 0.0032%^{IS} values. After cell mixing and filling, the vials were freeze dried and stored at -20ºC.

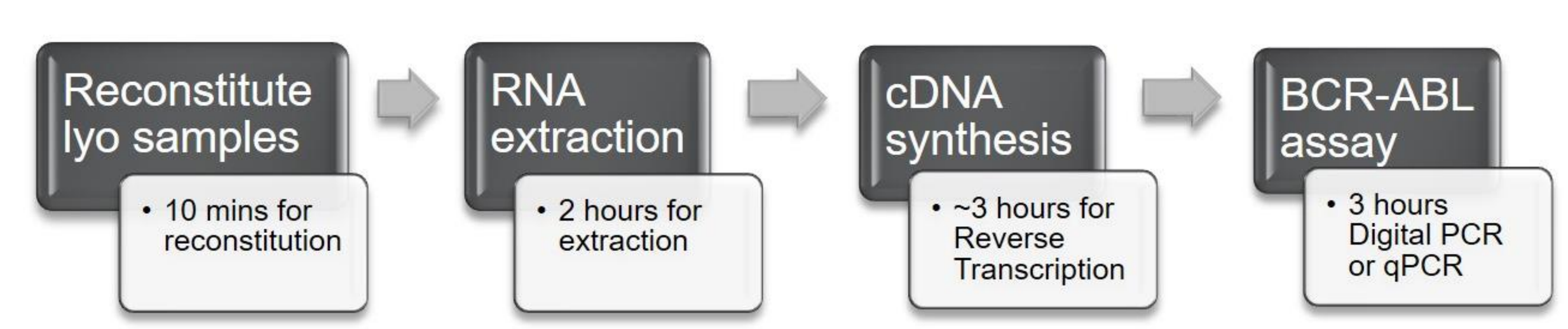

Figure 3. Workflow of using the full process AcroMetrix™ BCR-ABL Panel. Each vial is reconstituted using desired cell lysis buffer before RNA extraction. Extracted RNA was then converted into cDNA using reverse transcription kits and further tested on digital PCR or qPCR platform.

Gene	Primer/Probe	Sequence (5'→3')
BCR-ABL	Forward primer	CCGCTGACCATCAATAAGGAA
	FAM MGB probe	AAGCCCTTCAGCGGC
	Reverse primer	CTGAGGCTCAAAGTCAGATGCTACT
ABL	Forward primer	ACCACTGACGTGCCTGAGATG
	FAM MGB probe	AGAGAGCGATCCTCTGG
	Reverse primer	GAGACACGGCAGGCTCATG

Table 2. *BCR-ABL* and *ABL* primers and probes sequences[1].

	Hold 1	40 Cycle		Hold 2	Hold 3
Temperature	95ºC	94ºC	56ºC	98ºC	4ºC
Time	10 mins	30 sec	60 Sec	10 mins	∞

Table 3. Thermal cycling program for *BCR-ABL* assays[1].

RESULTS

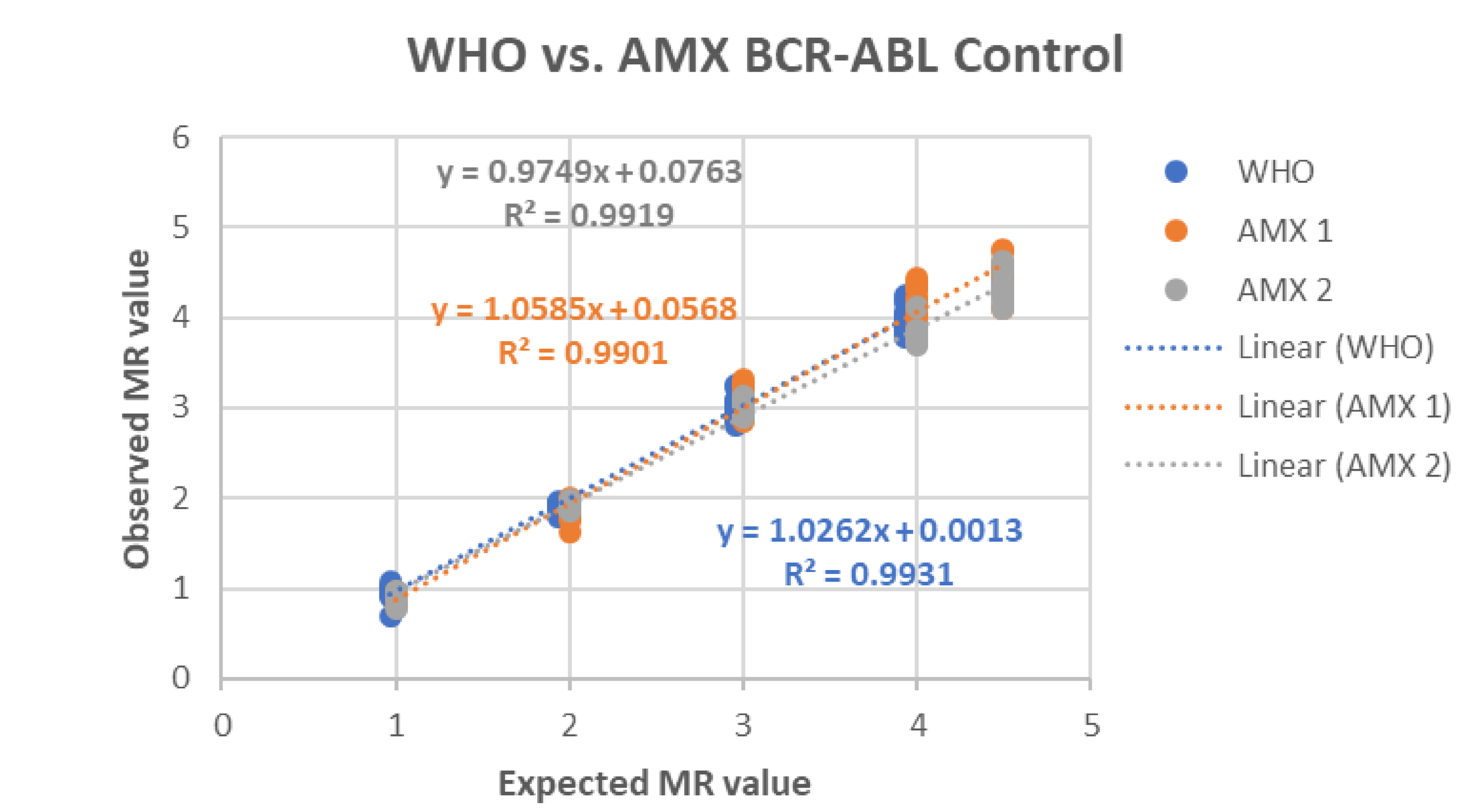

Figure 4. Linearity comparison between WHO and 2 different lots of AcroMetrix™ *BCR-ABL* Panel (AMX 1 and AMX 2). The AMX 1 and AMX 2 showed similar linearity as the WHO international *BCR-ABL* standard, with the additional MR4.5 panel member.

	BCR-ABL^{IS}		
Panel	AMX 1	AMX 2	AMX 3
A	11.8	12.3	9.3
B	1.23	1.31	0.96
C	0.095	0.093	0.083
D	0.016	0.0074	0.0071
E	0.0049	0.0035	0.0030

Table 4. Three different development lots of AcroMetrix™ BCR-ABL panel. BCR-ABL^{IS} values were assigned by reference to the WHO primary standard.

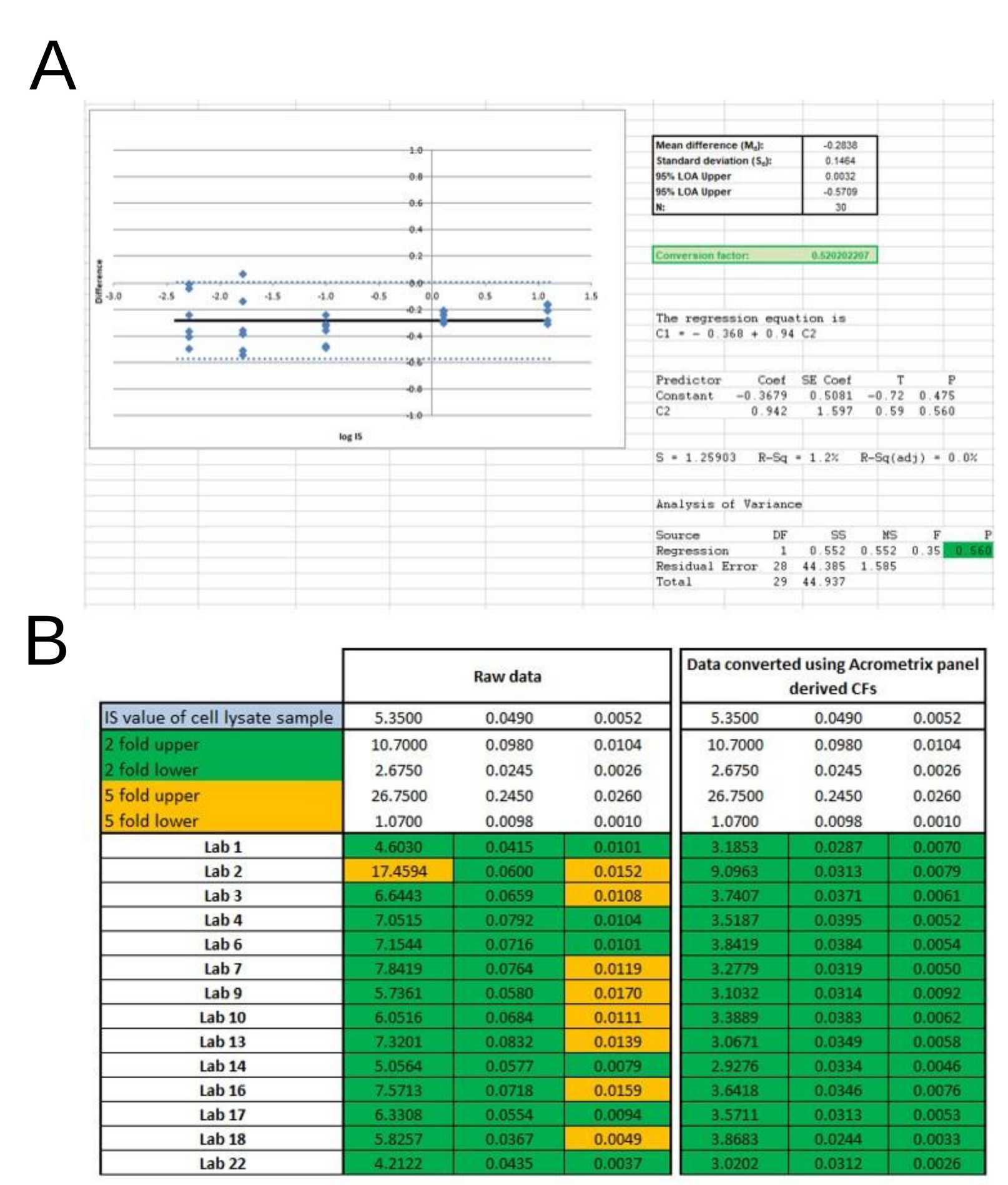

Figure 5. (A) Example of replicate RT-qPCR analysis of the AcroMetrix panel to derive a laboratory-specific conversion factor (CF) by Bland-Altman analysis. (B) Validation of the AcroMetrix panel-derived CFs in 14 UK testing laboratories using different RT-qPCR tests to measure BCR-ABL/ABL.

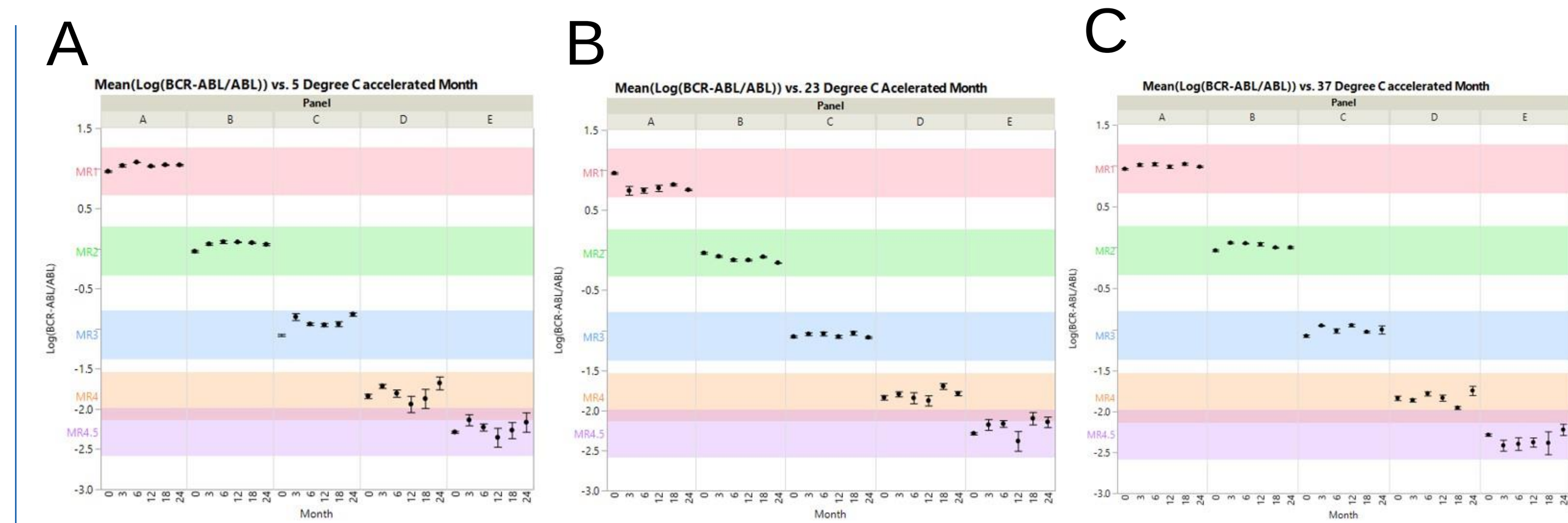

Figure 5. Accelerated stability results at 4ºC (A), 25ºC (B) and 37ºC (C). The acceptance range for MR1, MR2, MR3, MR4 and MR4.5 is based on ±0.3 Log of T_0 value. Samples were stored at respective temperature for 16, 32, 64, 95 and 127 days, representing the stability for 3, 6, 12, 18, 24 months. At each time points, four replicates from each panel were extracted and tested. The error bar represents the standard error of each time points. All of the stability time points pass the acceptance criteria (±0.3Log of T_0 value, shaded area), which supports the product stability at -20ºC for 24 months.

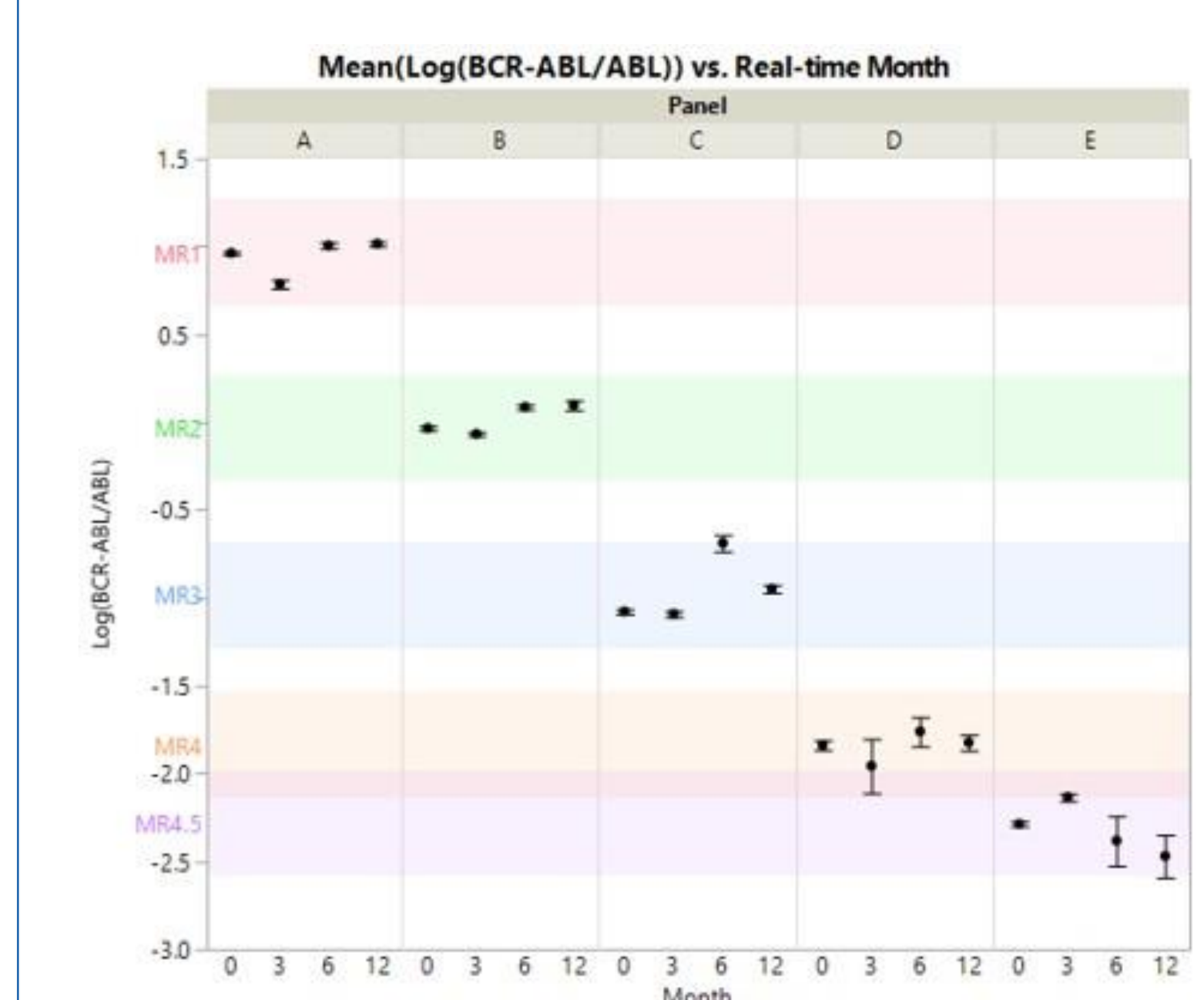

Figure 6. Real-time Stability of the AcroMetrix™ *BCR-ABL* Panel stored at -20ºC. The acceptance range for MR1, MR2, MR3, MR4 and MR4.5 is based on ±0.3Log of T_0 value. The study is still on-going.

SUMMARY

- A full process BCR-ABL external molecular control panel for the analytical evaluation of BCR-ABL test methods is developed. This panel is not intended for clinical use.
- The %IS value of the BCR-ABL control is traceability to WHO international standard.
- This research use only panel has all the members as the WHO International Standard (10%, 1%, 0.1%, and 0.01%), and the additional 0.0032%.
- Accelerated stability study supports the product stable for 24 months at -20ºC; the real-time stability is still on-going.

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TRADEMARKS

The control is For Research Use Only. Not For Clinical Use.