QuantideX qPCR BCR-ABL IS Kit and ipsogen BCR-ABL1 Mbcr IS-MMR Kit Yield Highly Correlated Results

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INTRODUCTION

In this study, we characterized the correlation, bias, and agreement between two IS-harmonized, commercially available BCR-ABL1 quantification kits, both CE-marked to the European In-Vitro Diagnostic Devices Directive (98/79/EC).

METHODS

The QuantideX qPCR BCR-ABL IS Kit (Asuragen, CE IVD and US IVD) and ipsogen BCR-ABL1 Mbcr IS-MMR Kit (Qiagen, CE IVD and US RUO) were used according to each kit's CE IVD instructions, with both RT and qPCR performed on the ABI 7500 Fast Dx. Design of the primary arm (Fig. 1) was compliant with CLSI EP09 (3rd Ed.). Challenge panel sample order was randomized and then performed in the same sequence in each test (n=164 results across both arms and test methods). Per EUTOS scoring (Cross NCP, et al. *Leukemia* 29:999, 2015), copies were summed between duplicates for ipsogen. QuantideX is performed in singleton. A portion of positive detection events in ipsogen were indistinguishable from false positive measurements due to this method's LOB (Fig. 2).

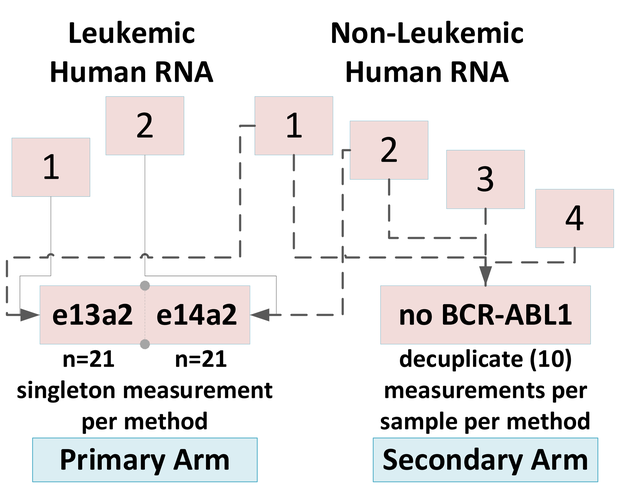

Figure 1. Challenge panel. Samples were formulated using 2 leukemic and 4 non-leukemic human RNAs. Both e13a2 and e14a2 were covered equally at levels targeting MR0.1 (80%IS) to MR5.0 (0.0010%IS), with heavier representation ≥MR4.0.

Table 1. Workflow comparison. The number of pipetting steps and reaction wells required for a batch run of 16 measurable results are shown.

Test	Pipetting Steps	Wells Used
QuantideX	118	27
ipsogen	238	96

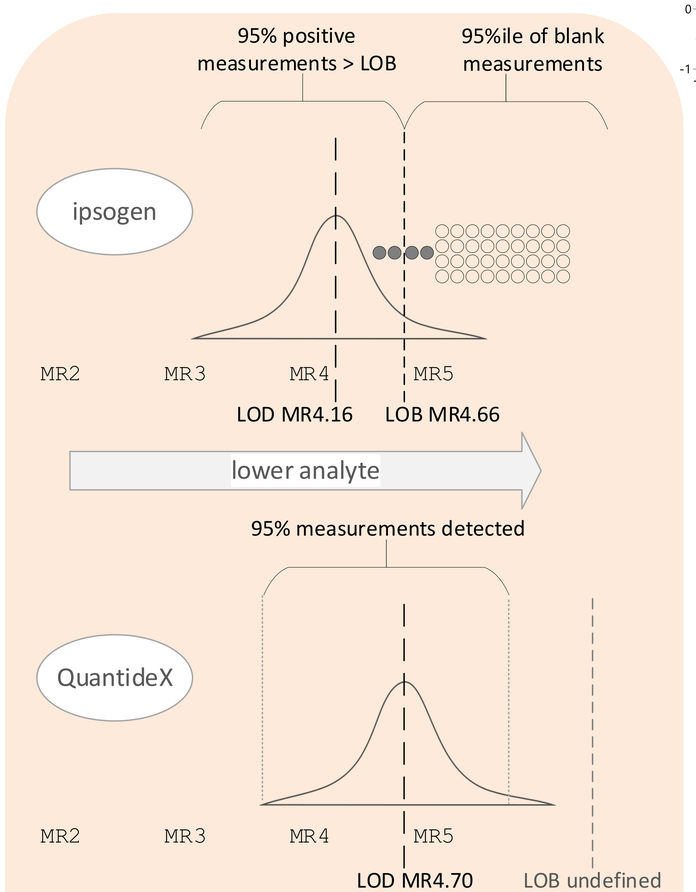

Figure 2. Comparison of performance limits. The limits of blank and detection from each kit's instructions for use are shown. Mock distributions are shown as Gaussian curves. Qualitative detection events are shown as positive (closed circles) and negative (open circles). Differences between these limits created challenges during analysis.

QUANTITATIVE RESULTS

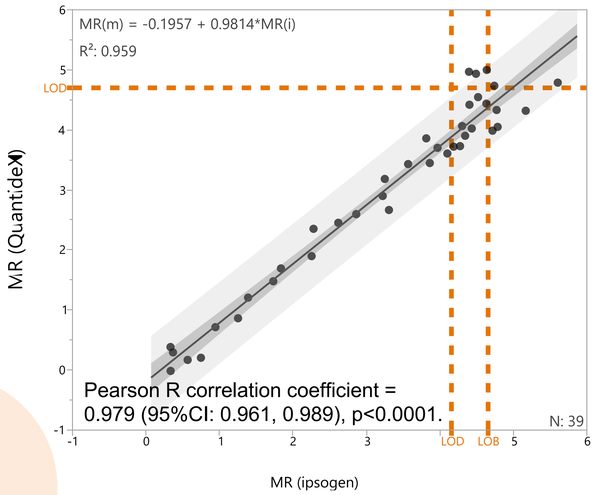

Figure 3. Correlation. All measured MR values included (n=39), regardless of performance limits (Fig. 2). Dotted lines represent QuantideX LOD (MR4.70 or 0.0020%IS), ipsogen LOD (MR4.16 or 0.0069%IS), and ipsogen LOB (MR4.66 or 0.0022%IS). QuantideX does not exhibit a numerical LOB.

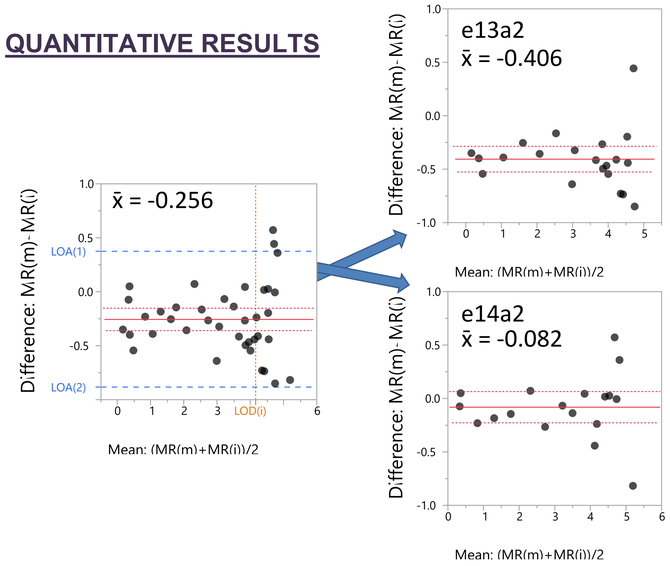

Figure 4. Bias. All measured MR values included (n=39), regardless of performance limits (Fig. 2). Dotted orange line represents ipsogen's LOD (MR4.16 or 0.0069%IS). Dotted red lines show 95%CI. Dotted blue lines show 95% LOA {-0.884, 0.371}, with 37/39 (94.9%) within these limits. Bias appeared uniform visually, and linear regression yielded slope of 0.0021 and r2<0.000. QuantideX MR values were on average 0.256 lower than ipsogen.

QUALITATIVE RESULTS

Table 2. Contingency analysis of qualitative detection, both study arms. This assessment included all samples in both study arms.

	ipsogen		
QuantideX	**Pos**	**Neg**	Total
Pos	**39***	**2**	41
Neg	**4****	**37**	41
Total	43	39	82

OPA = (39+37)/82 = 92.7% (95%CI: 84.9, 96.6%)

Cohen's Kappa Coefficient = 0.854 (95%CI: 0.741, 0.966)

* Three (3/39, 8%) duplicate-discordant BCR-ABL1 results (one positive, one negative) generated by the ipsogen test in the leukemic arm are included here as positive.

** These four discrepant results were false positives (4/40, 10%) generated by the ipsogen test in the non-leukemic study arm. All QuantideX test results (0/40, 0%) were negative in the non-leukemic arm.

CONCLUSIONS

The two methods were highly correlated over the full range of values obtained. Some bias was observed, arising from differences in quantifying e13a2. Both tests utilize the two-step RT-qPCR format, but showed differences in number of processing steps.

CONTACT

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