Method Comparison Between Two Commercially Available *BCR-ABL1* Quantitative Kits

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Summary

- QuantideX® qPCR BCR-ABL IS Kit (US IVD) and ipsogen BCR-ABL1 Mbcr IS-MMR Kit (RUO) were highly correlated.
- Some uniform bias was observed.
- Qualitative agreement of detection was high.
- Data analysis was complicated by replicate-discrepant *BCR-ABL1* results and false positive measurements in the ipsogen kit.

Introduction

Quantification of *BCR-ABL1* Major fusion transcripts of translocation t(9;22) assesses tumor burden in chronic myeloid leukemia (CML). This process has benefited from international harmonization efforts (International Scale, IS). Despite this, persistent differences between methods can change interpretations. Hence, characterizing variation between methods remains an important area of study, and, to our knowledge, this is the first such comparison for these two commercially available methods: QuantideX qPCR BCR-ABL IS Kit (US IVD) and ipsogen BCR-ABL1 Mbcr IS-MMR Kit (RUO).

Materials and Methods

Kits were used according to their instructions, with both RT and qPCR performed on the ABI 7500 Fast Dx. Design of the primary arm 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).

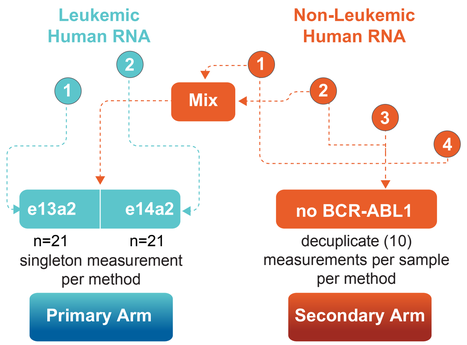

Figure 1. Challenge Panel. Samples were formulated using 2 leukemic and 4 non-leukemic human RNAs. The challenge panel of human RNA included both e13a2 and e14a2 and targeted MR0.1 (80%IS) to MR5.0 (0.0010%IS), with heavier representation ≥MR4.0.

Figure 2. Batch Run qPCR Plate Setup for Each Test. Following the instructions from the ipsogen kit, we were limited to 8 samples per run. For parity, the batch runs for the QuantideX kit were aligned with this (8 measurements each)–despite its ability to assay up to 49 samples per run.

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Results

Table 1. Results of Duplicate *BCR-ABL1* Wells in the ipsogen Test.* The number of observations for each category are shown. Note that positive detection events may be indistinguishable from false positive measurements for this kit in this study (see Table 4).

BCR-ABL1 Duplicates in ipsogen Test	*BCR-ABL1* Positive Samples	*BCR-ABL1* Negative Samples	Total
Negative / Negative	**3**	**36**	39
Positive / Negative	**3**	**4**	7
Positive / Positive	**36**	**0**	36
Total	42	40	82

*The protocol accompanying the ipsogen kit did not provide guidance on how to interpret discordant replicates. So, we followed the EUTOS scoring recommendations (Cross NCP, et al. Leukemia 29:999, 2015), where copies are summed between duplicates. This rendered all duplicate-discordant ipsogen results as positive, or detected. The QuantideX test is performed in singleton and analyzed with automated interpretive software.

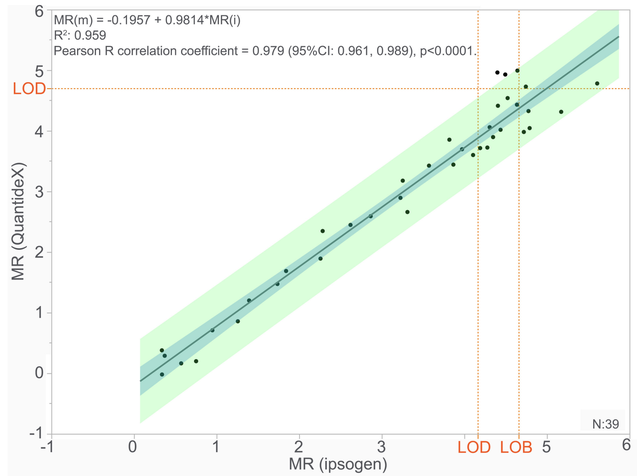

Figure 3. Correlation Plot. This analysis includes all MR values that were measured (n=39), regardless of each test's performance limits. Dotted lines represent QuantideX tests LOD (MR4.70 or 0.0020%IS), and ipsogen tests LOD (MR4.16 or 0.0069%IS) and LOB (MR4.66 or 0.0022%IS). The QuantideX test does not exhibit a numerical LOB. Confidence of fit is shown in darker color, with 95% limits of agreement (LOA) shown in light green.

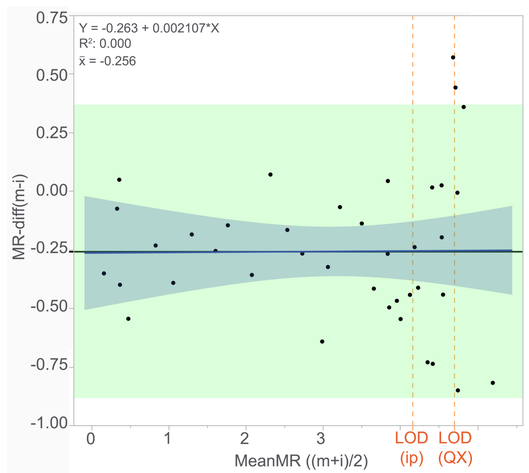

Figure 4. Bias Plot. This analysis includes all MR values that were measured (n=39), regardless of each test's performance limits. Dotted lines represent QuantideX test's LOD (MR4.70 or 0.0020%IS) and ipsogen test's LOD (MR4.16 or 0.0069%IS). X-axis represents the mean of both test's MR values; Y-axis, the difference as MR(QuantideX) – MR(ipsogen). The solid black line is drawn at the mean difference. Confidence of fit is shown in darker color. The 95% LOA {-0.884, 0.371} is shown in light green, with 37/39 (94.9%) within these limits. Bias appeared uniform by visual inspection, and linear regression showed a slope and R^2 near zero. QuantideX test's MR values were on average 0.256 lower than ipsogen test's MR values.

Table 2. Contingency Analysis at Deep Molecular Response (DMR). Since durable DMR is required for assessment of treatment discontinuation in CML to attempt treatment free remission (TFR), this assessment was limited to the primary arm of the study (CML positive). Results of Undetected and ≥MR4 (<0.01%IS) are coded as "DMR", while results <MR4 are coded as "Not DMR".

QuantideX Kit	ipsogen Kit		
	Not DMR	DMR	Total
Not DMR	21	5	26
DMR	0	16	16
Total	21	21	42

OPA = (21+16)/42 = 88.1% (95%CI: 75.0, 94.8%)
Cohen's Kappa Coefficient = 0.7627 (95%CI: 0.572, 0.952)

Table 3. Contingency Analysis of Qualitative Detection, Both Study Arms. All valid test results for samples presumed positive (leukemic) and negative (non-leukemic) in both study arms are included regardless of each test's test performance limits.

QuantideX Kit	ipsogen Kit		
	Positive	Negative	Total
Positive	**39**[a]	**2**	41
Negative	**4**[b]	**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)

[a]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. And 12/39 (31%) were positive but below LOD for the ipsogen test while being positive and within reportable range in the QuantideX test.
[b]These four discrepant results were examined further in Table 4.

Table 4. Unexpected Detection Events in the Non-Leukemic Secondary Arm. Total events are shown for each test. Such measurements are interpreted as false positive.

Test	Unexpected Detection Events
QuantideX Kit	0% (0/40)
ipsogen Kit	10% (4/40)

Conclusions

- 31% of leukemic sample results were positive but below LOD in the ipsogen test while being positive and above LOD in the QuantideX test.
- All dually positive values yielded a linear regression with slope of 0.981 and a Pearson R correlation coefficient of 0.979, as well as a mean bias of 0.26 MR units.
- The QuantideX test yielded no false positive results and the ipsogen test yielded 10%.
- Overall percent agreement of detection across both study arms was 92.7%, but a portion of results from the ipsogen test are indistinguishable from its false positives.

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