Expanding Access to the QuantideX® qPCR BCR-ABL IS Kit Through Validation on Multiple Quantitative Real-Time Instruments

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Summary

- In chronic myeloid leukemia (CML), regular patient monitoring via quantification of BCR-ABL1 fusion transcripts normalized to the International Scale (IS) is critical for assessing treatment efficacy
- While validated assays currently exist, currently supported platforms like the 7500 Fast Dx will only be supported through at least the end of 2027, necessitating the need for modern instrumentation
- Here, we show preliminary results from analytical validation of a commercially available BCR-ABL1 monitoring assay on two modern qPCR platforms (QuantStudio™ 5 Dx and QuantStudio 7 Pro Dx), including limits of detection, quantification, and blank (LoD/LoQ/LoB), linearity, and precision
- Performance reported herein indicates that critical performance metrics on modern platforms are consistent with the 7500 Fast Dx, demonstrating that newer technologies may adequately address critical performance needs while offering modernized workflow improvements

Introduction

Quantification of BCR-ABL1 Major fusion transcripts of translocation t(9;22) is critical for monitoring the tumor burden in chronic myeloid leukemia (CML). While this process has benefited from international harmonization efforts (International Scale, IS), differences between methods can impact measurements. A key component of performance is the qPCR instrument used to amplify, detect, and quantify both the target of interest and endogenous normalizer. The QuantideX qPCR BCR-ABL IS Kit* (IVD) was cleared for use on the ABI 7500 Fast Dx (Thermo Fisher Scientific, Inc.), which is no longer manufactured and only has guaranteed support through December 31, 2027. Herein, we have assessed performance of these RT-qPCR materials with data from our ongoing analytical validation on two new diagnostic-labeled instruments.

Methods

We evaluated the QuantideX qPCR BCR-ABL IS Kit (Asuragen, Inc.) on the QuantStudio® 5 Dx (QS5) and QuantStudio 7 Pro Dx (QS7) from Applied Biosystems™ and compared results to validation data on the 7500 Fast Dx. The kit was used according to the kit's instructions, with updated instrument-specific settings to accommodate new platforms. Identical thermal cycling parameters were used on the new instruments for both the RT and PCR steps. Results were analyzed using the QuantStudio 5 Instrument Software (QS5 only) or Diomni Software (QS5 and QS7) using auto threshold and baseline for both ABL1 and BCR-ABL1 targets. Blends of RNA isolated from whole blood specimens collected from BCR-ABL1-positive and non-leukemic donors were generated to create challenge panels for analytical validation studies, which included within-lab precision, limits of blank/detection/ quantitation (LoB/LoD/LoQ), and linearity. Data herein represents a preliminary analysis of completed analytical validation testing to date. Testing with additional lots and operators in our within-lab precision and LoD/LoQ studies and other studies not shown here (multi-site reproducibility, method comparison) are currently underway.

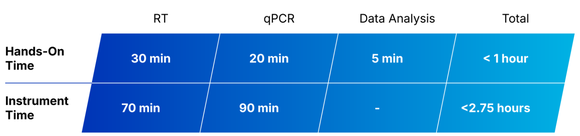


Figure 1. Assay Time of a Typical QuantideX qPCR BCR-ABL IS Kit Batch Run. Timing was estimated from two runs with 60 reactions each on the 7500 Fast Dx, which has identical cycling parameters and similar run times to the QuantStudio 5 Dx and QuantStudio 7 Pro Dx. Including both hands-on and Instrument time, total turnaround time is under four hours.

Results

Table 1. Preliminary Limit of Detection and Quantitation Data Indicates LoD/LoQ of MR4.7 range on the QuantStudio 5 Dx (1A) and QuantStudio 7 Pro Dx (1B). CML-positive human RNA with either e13a2 or e14a2 fusions was diluted into a background of CML-negative human RNA to a targeted MR of 4.5-4.8 to create 7 unique specimens (3 e13a2, 4 e14a2), each tested at the minimum RNA input of the assay (1000 ng/RT) with 24 replicates per sample. Testing consisted of 1 reagent lot, 3 batch runs, 3 days, 2 operators, and 2 instruments for each instrument model, generating 336 measurements total. Samples with the highest mean MR meeting acceptance criteria for each breakpoint are indicated in bold. The LOD of the assay was similar between breakpoints and was consistent with the previously validated BCR-ABL1 monitoring assay on the 7500 Fast Dx platform (MR4.7). All samples met assay precision criteria (SD ≤ 0.36), indicating the limit of quantitation is identical to the limit of detection. Validation testing with additional lots is ongoing.

1A. QuantStudio 5 Dx

Sample	Breakpoint	N Detected	% Detected	MR Mean	MR SD
1	e13a2	24	100%	4.56	0.19
2	**e13a2**	**24**	**100%**	**4.71**	**0.29**
3	e13a2	23	95.8%	4.70	0.22
4	e14a2	23	95.8%	4.48	0.29
5	e14a2	23	95.8%	4.60	0.28
6	**e14a2**	**24**	**100%**	**4.69**	**0.34**
7	e14a2	23	95.8%	4.67	0.30

1B. QuantStudio 7 Pro Dx

Sample	Breakpoint	N Detected	% Detected	MR Mean	MR SD
1	e13a2	24	100%	4.56	0.30
2	e13a2	23	95.8%	4.60	0.20
3	**e13a2**	**23**	**95.8%**	**4.76**	**0.29**
4	e14a2	24	100%	4.38	0.30
5	e14a2	24	100%	4.45	0.24
6	e14a2	23	95.8%	4.55	0.27
7	**e14a2**	**23**	**95.8%**	**4.66**	**0.31**

Table 2. Preliminary Limit of Blank Data Indicates LoB of Undetected (sufficient ABL1) on the QuantStudio 5 Dx (1A) and QuantStudio 7 Pro Dx (1B). 30 unique CML-negative human RNA specimens were tested with RNA inputs spanning the full input range of the assay (1,000-5,000 ng). Testing consisted of 3 reagent lots with one replicate per sample per lot for each instrument model, generating 360 measurements total. The average percent undetected was 99.4% across all lots and samples, meeting criteria of >95%.

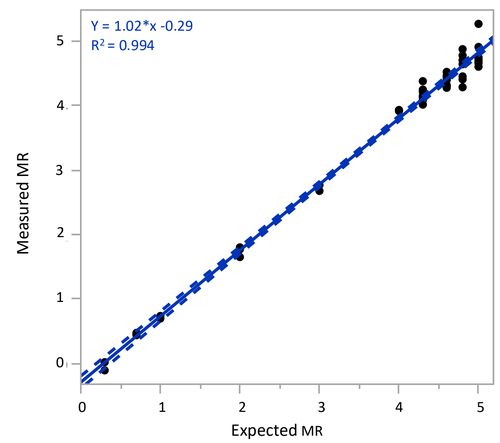
2A. QuantStudio 5 Dx

Lot	N Undetected (Percent)
1	59/60 (98.3%)
2	60/60 (100%)
3	60/60 (100%)
Overall	**179/180 (99.4%)**

2B. QuantStudio 7 Pro Dx

Lot	N Undetected (Percent)
1	60/60 (100%)
2	59/60 (98.3%)
3	59/59 (100%)
Overall	**178/179 (99.4%)**

2A. QuantStudio 5 Dx



2B. QuantStudio 7 Pro Dx

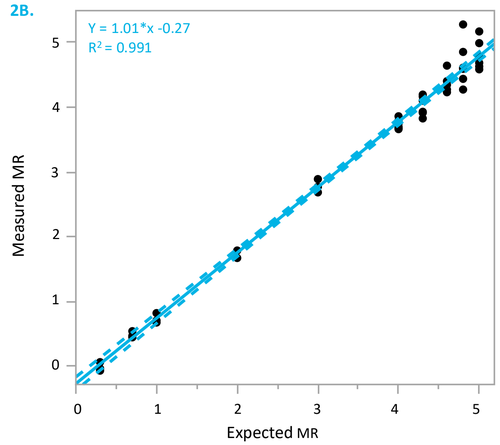


Figure 2. Linearity shows ~4.5-log linear range on the QuantStudio 5 Dx (2A) and QuantStudio 7 Pro Dx (2B). CML-positive human RNA with either e13a2 or e14a2 fusions was diluted into a background of CML-negative human RNA to a targeted MR of 0.3-5.0 to create 20 unique specimens (10 e13a2, 10 e14a2) with 2-4 replicates per sample per instrument model. Both instrument models demonstrated linearity from MR 0.3-5.0.

Table 3. Within-Lab Precision Data Indicates Acceptable Precision Spanning the Full Linear Range on the QuantStudio 5 Dx (3A) and QuantStudio 7 Pro Dx (3B). CML-positive human RNA with either e13a2 or e14a2 fusions was diluted into a background of CML-negative human RNA to a targeted MR of 1-4.5 to create 20 unique specimens (10 e13a2, 10 e14a2) tested with 1000 ng or 3000 ng mass input (5 at each input per breakpoint, one for each MR target) with 24 replicates per sample per instrument. For each instrument model, testing consisted of 2 reagent lots, 8 batch runs, 8 days, 2 operators, and 2 instruments, generating 960 measurements total. All samples met precision acceptance criteria. Validation testing with additional lots is ongoing.

3A. QuantStudio 5 Dx

Target MR	Mean MR Range	SD MR Range
1	1.10 – 1.16	0.05 – 0.14
2	1.92 – 2.10	0.05 – 0.13
3	2.96 – 3.23	0.09 – 0.17
4	4.02 – 4.29	0.15 – 0.23
4.5	4.43 – 4.78	0.22 – 0.29

3B. QuantStudio 7 Pro Dx

Target MR	Mean MR Range	SD MR Range
1	1.09 – 1.20	0.04 – 0.12
2	1.92 – 2.06	0.04 – 0.11
3	2.97 – 3.25	0.08 – 0.13
4	4.01 – 4.26	0.17 – 0.30
4.5	4.44 – 4.78	0.22 – 0.33

Conclusions

- Overall, analytical validation data generated to date demonstrates that the QuantideX qPCR BCR-ABL IS Kit has strong performance on the QuantStudio 5 Dx and QuantStudio 7 Pro Dx, similar to the originally validated platform (7500 Fast Dx) to meet the stringent needs of clinical BCR-ABL1 monitoring
- Limits of detection and quantification on both platforms were approximately MR 4.7 for both e13a2 and e14a2 and the limit of blank was Undetected (sufficient ABL1), meeting stringent needs of LOD ≥ MR4.5 for clinical utility
- The assay was linear from MR 0.3 to 5.0 with both e13a2 and e14a2 on both platforms, demonstrating a 4.5-log linear range
- The precision of the assay was consistent across multiple reagent lots, runs, operators, and instrument models, demonstrating strong within-lab precision
- Combined with multisite reproducibility and method comparison studies underway, these analytical validation data indicate that modern qPCR platforms will provide excellent performance with workflow and analysis improvements, ensuring continuity as the legacy instrument is phased out

*FDA cleared IVD medical device in the US and Class C IVDR certified in the EU. Intended for use only with the 7500 Fast Dx instrument platform (US and EU) and the Roche cobas™ z480 (EU only). The performance study product described herein on the QuantStudio 5 Dx and QuantStudio 7 Pro Dx instruments is under development; performance characteristics and final product features to be determined. The performance study product is not authorized as an IVD medical device in any region.