Performance characteristics of the first FDA-cleared droplet digital PCR (ddPCR) IVD assay for monitoring chronic myelogenous leukemia

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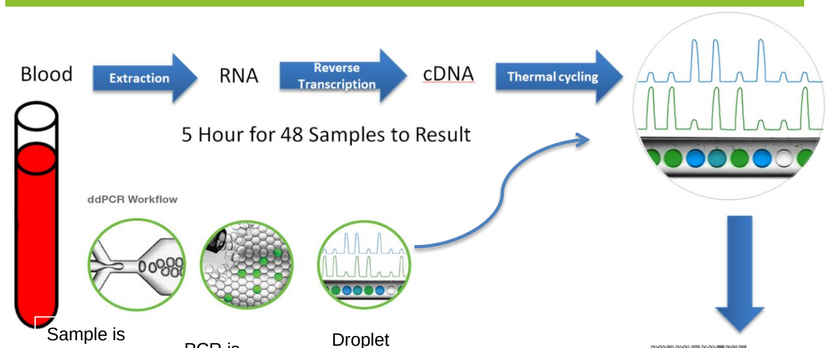
Introduction

Chronic myelogenous leukemia (CML) is a cancer of myeloid cells that accounts for 15-20% of all cases of leukemia. Most CML cases are caused by a translocation between chromosomes 9 and 22 that creates an abnormal fusion gene, BCR-ABL1. The BCR-ABL1 gene expresses an abnormal tyrosine kinase protein that causes unregulated growth and survival of granulocytes. CML is treated with TKIs (tyrosine kinase inhibitors). The concentration of BCR-ABL1 transcript RNAs is a marker of disease progression and the effectiveness of TKI treatment.
Bio-Rad's QXDx™ BCR-ABL %IS Kit quantifies the amount of BCR-ABL1 transcript as a ratio of BCR-ABL1/ABL1, then returns the value on the WHO International Scale(IS). The QXDx software performs automatic data analysis and reporting, results are reported in %IS, MR, and copies values.

Work Flow



Sample is partitioned into many thousand nanoliter droplets.

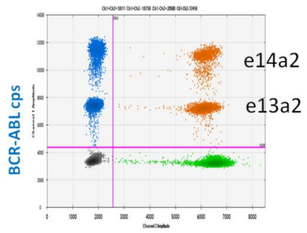
Each sample results in thousands of discrete measurements.

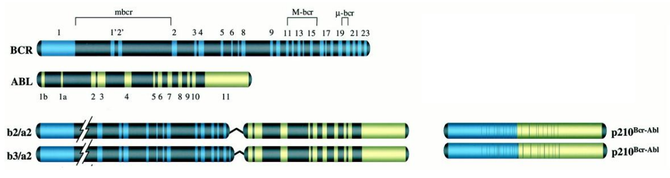
PCR is performed on the droplets.

1 or more DNA templates in the droplets amplify and generate fluorescence

Droplet fluorescence is read using 2 channels

Droplets are identified as positive or negative for each channel.





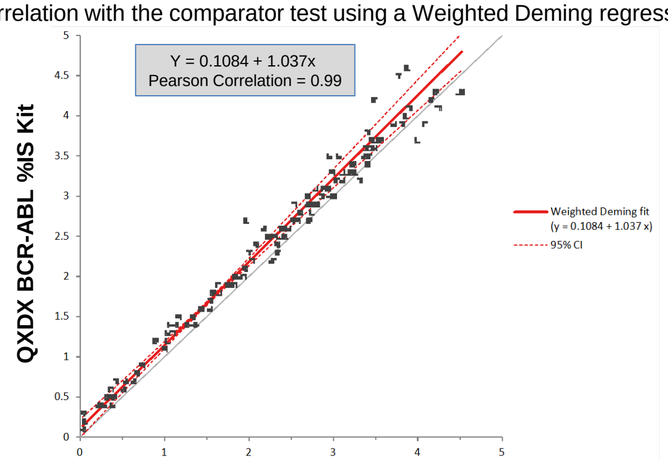
Bio-Rad's QXDx BCR-ABL System and %IS Kit

Key Performance Indicators

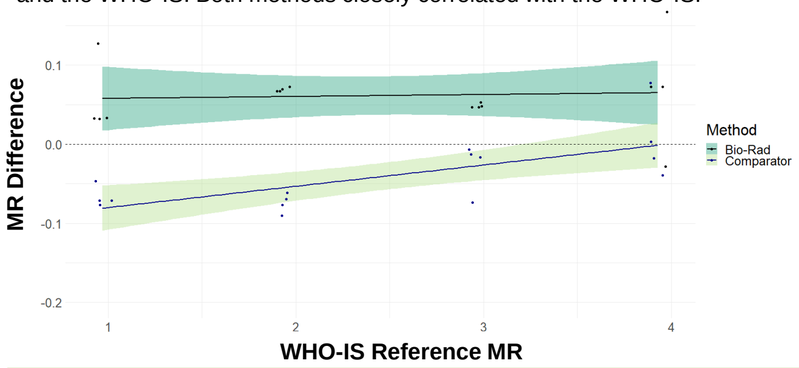
	Result	%IS	MR
LOB	95% Negative	0	∞
LLOQ	76%CV	0.003%	4.56
LOD	95% Positive	0.002%	4.7
Linear Range	y = 1.04x +0.058, R^2=0.996	50%-0.002%	0.3-4.7

Clinical Trial

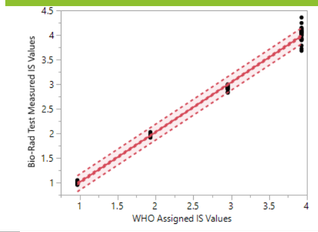
The clinical performance of the QXDx™ BCR-ABL %IS Kit was compared to the performance of an FDA-cleared BCR-ABL test kit on clinical samples from CML patients. The QXDx™ BCR-ABL %IS assay demonstrated excellent correlation with the comparator test using a Weighted Deming regression.



The WHO International Standard (WHO-IS) was tested by the QXDx™ BCR-ABL %IS Kit and comparator test to assess agreement between each method and the WHO-IS. Both methods closely correlated with the WHO-IS.



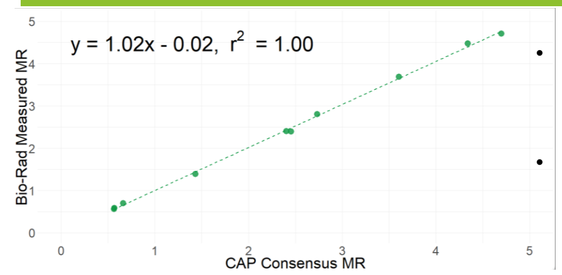
All Lots Aligned to the WHO International Scale



Linear regression on all seven assay kit lots.
BioRad IS = **-0.005794** + **1.014647***WHO IS,
R^2 =**0.995**

	Range for 7 Lots
Slope	0.97 to 1.03
Intercept	-0.045 to +0.09
R^2	0.99 to 1

College of American Pathologists Challenge



- Bio-Rad has participated in CAP Proficiency testing for BCR-ABL since 2015
- Bio-Rad's results track CAP mean results closely on n=10 studies.

Site/Day/Operator Reproducibility

MR Bin	Sample Name	Mean MR	N	Site/ Instrument	Day	Operator/ Run	Residual	Total Variance
1.0	C01	0.700	36	0.0000	0.0000	0.0000	0.0000	0.0000
	S07	0.975	36	0.0000	0.0000	0.0006	0.0014	0.0019
	C03	1.030	36	0.0001	0.0000	0.0007	0.0014	0.0022
	S01	1.347	36	0.0019	0.0000	0.0004	0.0014	0.0038
2.0	S08	1.994	36	0.0001	0.0000	0.0000	0.0005	0.0006
	S02	2.419	36	0.0000	0.0000	0.0008	0.0014	0.0022
	S09	2.503	36	0.0000	0.0000	0.0000	0.0003	0.0003
3.0	S03	2.778	36	0.0000	0.0004	0.0003	0.0011	0.0018
	S10	3.139	36	0.0008	0.0000	0.0008	0.0022	0.0039
	S04	3.286	36	0.0000	0.0000	0.0000	0.0041	0.0041
3.5	C04	3.444	36	0.0000	0.0000	0.0000	0.0054	0.0054
	S11	3.539	36	0.0000	0.0000	0.0015	0.0039	0.0053
	S05	3.622	36	0.0000	0.0000	0.0003	0.0083	0.0086
4.0	C02	3.700	36	0.0011	0.0015	0.0000	0.0070	0.0096
	S06	4.161	36	0.0000	0.0000	0.0000	0.0379	0.0379
	S12	4.225	36	0.0031	0.0000	0.0052	0.0336	0.0419

Samples were tested with the QXDx™ BCR-ABL %IS Kit with 2 replicates per run and 2 runs per day for 3 non-consecutive days, with one reagent lot for a total 36 replicate tests of each sample and 576 total measurements. Variance component analysis was estimated by Instrument, Day, Operator and Residual.

Lot-to-Lot Reproducibility

Sample ID	N	% Positive	Mean MR	Within Run SD	Operator SD	Day SD	Lot SD	Instrument SD
MR1	108	100	1.40	0.022	0.013	0.010	0.017	0.007
MR2	108	100	2.47	0.038	0.006	0.011	0.027	0.004
MR2.5	108	100	2.80	0.046	0.008	0.003	0.013	0.000
MR3	108	100	3.31	0.080	0.000	0.000	0.000	0.000
MR3.5	108	100	3.63	0.103	0.000	0.000	0.000	0.000
MR4	108	99	4.13	0.162	0.011	0.000	0.000	0.012
MR4-5	108	89	4.65	0.242	0.000	0.000	0.042	0.000
IHC 17%	108	100	0.73	0.068	0.000	0.000	0.018	0.057

Tested variance from 3 lots on n=108 samples across MR range, found no significant variance attributed to lot.

Additional Performance Indicators

- Remnant extraction reagents, triglycerides, conjugated and unconjugated bilirubin, cholesterol, hemoglobin, sodium heparin and EDTA were tested with QXDx™ BCR-ABL %IS Kit and no interference was observed
- QXDx™ BCR-ABL %IS Kit is stable for at least 1.5 years and at least 5 freeze thaw cycles
- QXDx™ BCR-ABL %IS Kit does not detect p190 and p230 variants
- All QXDx BCR-ABL %IS Kit lots are validated to the WHO-IS International Scale

Conclusions

- The QXDx™ BCR-ABL %IS Kit is a highly accurate, precise, and reproducible test for the monitoring of CML.

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