Countable Labs.

Multiplexed *BCR::ABL1* panel on Countable RT-PCR enables ultrasensitive quantification of four *BCR::ABL1* fusion variants in a single tube.

Jung Won Keum[1], Janice Lai[1], Eleen Shum[1], Andras Saftics[1], Vladyslava Ratushna[1], Kelly Li[1], and Christina Fan[1]

Introduction

The *BCR::ABL1* fusion gene, generated by the Philadelphia chromosome translocation, is the genetic hallmark of chronic myeloid leukemia (CML). Sensitive and accurate quantification of *BCR::ABL1* fusion transcripts, together with differentiation of clinically relevant isoforms is important for CML research, including minimal residual disease (MRD) studies.

Conventional RT-qPCR requires a standard curve and extensive assay optimization, and its multiplexing capacity is limited when multiple fusion transcripts and reference targets are measured simultaneously.

Here, we describe a multiplex *BCR::ABL1* RNA fusion panel on the Countable system that uses a multi-probe labeling strategy to differentiate and quantify e14a2 and e13a2 (p210), e1a2 (p190), and e19a2 (p230) *BCR::ABL1* fusion variants, together with the reference gene *ABL1* in a single tube, achieving high sensitivity and specificity.

Materials and methods

The multiplex *BCR::ABL1* panel leverages Countable RT-PCR, a single-molecule counting platform that enables direct quantification of RNA molecules in a single-tube workflow.

A multi-probe labeling strategy detects *BCR::ABL1* fusion molecules by targeting each fusion partner with distinct probes. Linkage analysis identifies the expected optical signature from the same amplified molecule, enabling specific identification and quantification of individual *BCR::ABL1* fusion events.

The panel was evaluated using a range of RNA samples, including contrived materials prepared from RNA extracted from cell lines containing each fusion transcript, *BCR::ABL1* RNA dilution sets from Invivoscribe, and AcroMetrix reference materials with known International Scale (%IS) values. Samples containing *BCR::ABL1* fusion transcripts were also tested[2], and Countable PCR results were compared with those from orthogonal RT-qPCR method.

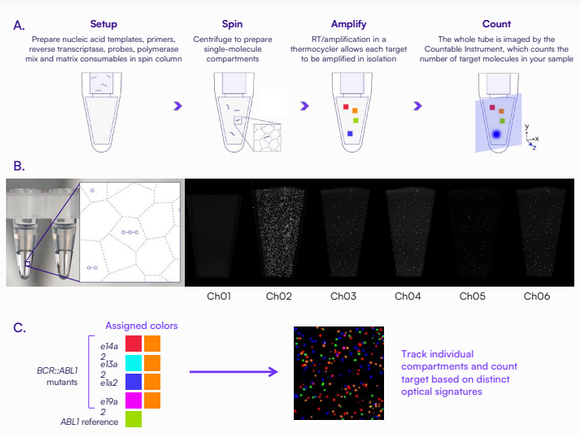


Figure 1. *BCR::ABL1* panel with a multi-probe labeling strategy on the Countable system. (A) Countable RT-PCR generates ~30 million compartments within a PCR tube through simple centrifugation, enabling reverse transcription, PCR amplification, imaging, and molecular counting in a single-tube workflow. (B) Representative image of a PCR tube showing the clear matrix after amplification, alongside light-sheet images of the amplified sample across six fluorescence channels. Positive compartments are counted directly, without relying on Poisson correction. (C) Each *BCR::ABL1* fusion target is labeled with multiple probes. This multi-probe design assigns each transcript a unique optical signature, enabling highly specific isoform identification while maintaining assay sensitivity.

Results

Low background across all four *BCR::ABL1* targets in the presence of 1 million *ABL1* counts.

- Twenty replicate negative samples were evaluated for nonspecific detection of four *BCR::ABL1* fusion targets (e14a2, e13a2, e1a2 and e19a2) in the presence of approximately 1 million *ABL1* counts per reaction.
- With automated count reporting, no fusion target generated more than one background count in any individual reaction, and 92.5% (74/80) of target measurements yielded zero counts.
- The limit of blank (LoB) was 1 count, corresponding to approximately 0.0001% *BCR::ABL1/ABL1* at 1 × 10⁶ *ABL1* counts.

Table 1. Analytical Specificity Using HL-60 Negative RNA.

Negative samples	e14a2 counts	e13a2 counts	e1a2 counts	e19a2 counts	ABL1 counts
Rep1	0	0	0	0	1,075,860
Rep2	0	0	0	0	1,171,030
Rep3	0	0	0	0	1,113,740
Rep4	0	0	0	0	1,189,220
Rep5	0	1	0	0	1,132,070
Rep6	0	0	0	0	1,147,620
Rep7	0	0	0	0	1,060,960
Rep8	0	0	1	0	1,094,660
Rep9	0	0	0	0	982,221
Rep10	0	0	0	0	969,554
Rep11	0	0	0	0	954,356
Rep12	0	0	0	0	1,057,740
Rep13	1	0	0	0	1,007,730
Rep14	0	0	1	0	979,934
Rep15	0	0	0	0	1,022,660
Rep16	0	0	0	0	994,610
Rep17	0	0	0	0	996,238
Rep18	1	0	0	0	1,005,840
Rep19	0	0	0	0	978,683
Rep20	0	1	0	0	982,560
Average	0	0	0	0	1,045,864

The wide dynamic range of the *BCR::ABL1* panel on Countable system enables simultaneous detection of both high- and low-abundance targets in a single tube without competitive interference.

- RNA containing each isoform was diluted into a background of wt *ABL1 RNA* to generate varying *BCR::ABL1/ABL1* ratios. Counts for all four targets remained linear across the dilution series, demonstrating broad transcript coverage without requiring the sample to be split across separate tests.
- In addition, the wide dynamic range supports accurate quantification of both abundant and rare target transcripts within a single tube.
- Based on the linearity data, the limit of detection (LoD) was estimated at approximately 0.001% for all *BCR::ABL1* isoforms.

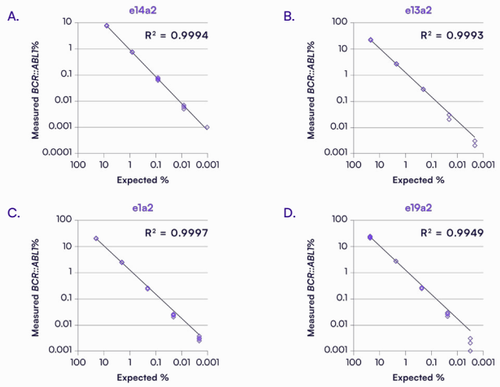


Figure 2. Linearity across four *BCR::ABL1* fusion transcripts (N=4). RNA containing e14a2 (A), e13a2 (B), e1a2 (C), and e19a2 (D) was serially diluted into wt *ABL1* RNA to generate *BCR::ABL1/ABL1* ratios ranging from 10% to 0.003%. All four fusion transcripts demonstrated strong linearity and high concordance between measured and expected ratios ($R^2 > 0.99$), demonstrating single-tube quantification across a broad dynamic range without assay interference.

The panel demonstrated reproducible performance across two independent testing days.

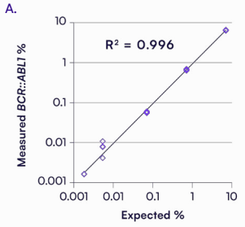
AcroMetrix reference materials containing different levels of e14a2 were tested with the panel across two independent days. Table 2 summarizes e14a2 counts and e14a2/*ABL1* percentage for Day 1, Day 2, and the combined two-day dataset. The Countable system demonstrated reproducible performance across both days, with an overall %CV of ≤10% for samples with more than 100 e14a2 counts.

Table 2. Reproducibility of e14a2 quantification across two days.

Level	Day1 (N=4)				Day2 (N=4)				Day1+Day2 (N=8)			
	e14a2 counts		e14a2 %		e14a2 counts		e14a2 %		e14a2 counts		e14a2 %	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
1	17,511	**1.1**	6.00	**1.2**	17,567	**1.3**	5.74	**0.7**	17,539	**1.1**	5.87	**2.5**
2	1,342	**1.2**	0.54	**2.3**	1,320	**3.8**	0.53	**1.6**	1,331	**2.8**	0.54	**2.0**
3	103	**9.0**	0.044	**9.7**	116	**8.1**	0.048	**8.3**	110	**10.0**	0.046	**9.4**
4	13	28.8	0.005	31.0	12	18.0	0.005	19.0	13	23.0	0.005	25.6
5	2	54.7	0.001	56.0	2	97.6	0.001	91.7	2	73.2	0.001	69.0

p210 targets are reported on the %IS using a lot-specific conversion factor.

To report p210 transcript levels on the %IS, the panel was calibrated against AcroMetrix reference material with assigned %IS values traceable to WHO standards. Measured *BCR::ABL1/ABL1* ratios closely matched expected %IS ($R^2 = 0.9960$), and the regression intercept yielded a conversion factor of 1.071.



Expected %IS	Measured BCR::ABL1/ABL1%	e14a2 counts	ABL1 counts
0.0019	0.0016	5	309,478
0.0055	0.0077	24	309,810
0.071	0.057	173	299,312
0.71	0.66	1,866	278,616
6.97	6.55	21,454	326,989

C.

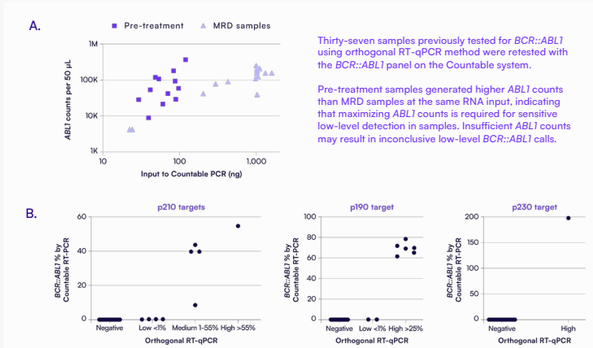
$$\%IS = \frac{BCR::ABL1\ counts}{ABL1\ counts} \times 100 \times \text{conversion factor}$$

$$\log_{10}(\text{Measured}) = \text{slope} \times \log_{10}(\text{Expected \%IS}) + \text{intercept}$$

$$\text{Conversion factor} = 10^{-\text{intercept}}$$

Figure 3. Assignment of %IS conversion factor using *BCR::ABL1* reference material (N=4). (A) Measured *BCR::ABL1/ABL1* (%) plotted against the expected %IS values for five AcroMetrix *BCR::ABL1* reference levels. (B) The corresponding expected %IS values, mean measured *BCR::ABL1/ABL1* ratios, and e14a2 and *ABL1* transcript counts are shown for each level. (C) The conversion factor is 10−intercept from the regression of $\log_{10}$ (Measured) against $\log_{10}$ (Expected %IS).

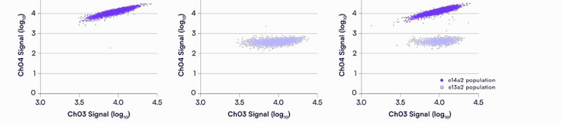
BCR::ABL1 panel showed good concordance with orthogonal RT-qPCR, while also detecting co-occurring fusion transcripts and rare a3 fusion variants in samples with *BCR::ABL1* mutations.



Thirty-seven samples previously tested for *BCR::ABL1* using orthogonal RT-qPCR method were retested with the *BCR::ABL1* panel on the Countable system.

Pre-treatment samples generated higher *ABL1* counts than MRD samples at the same RNA input, indicating that maximizing *ABL1* counts is required for sensitive low-level detection in samples. Insufficient *ABL1* counts may result in inconclusive low-level *BCR::ABL1* calls.

The Countable system identified the expected isoforms with good concordance across all targets. The orthogonal workflow tests p210, p190, and p230 separately, whereas the Countable panel distinguishes them in a single reaction.



Sample	Orthogonal RT-qPCR result	Countable PCR calling	BCR::ABL1/ABL1 %
Sample 1	p210 21.39 %IS	e14a2	42.48 %IS
Sample 2	p210 > 55 %IS	e13a2	58.39 %IS
Sample 3	p210 47.66 %IS	e14a2/e13a2 co-occurring	40.76 / 5.89 %IS
Sample 4	Outside assay design	e13a3	66.7%

The Compartment-level tracking resolved co-occurring e14a2/e13a2 transcripts without post-PCR steps such as electrophoresis. In an e13a3-positive sample, the standard a2 call was negative while the expected a3 signature was observed.

Figure 4. Evaluation of samples containing *BCR::ABL1* fusion transcripts on the Countable system (n = 37). (A) At the same RNA input, pre-treatment samples generated higher *ABL1* counts than MRD samples, which remained below 300K *ABL1* counts even with >1 µg RNA input. (B) Our results showed good correlation with orthogonal testing for all targets without splitting the sample per each assay. (C) Without prior knowledge or additional workflow steps, the panel distinguished e14a2, e13a2, and co-occurring e14a2/e13a2 transcripts, as well as a rare e13a3 variant.

Conclusion

- A single-tube multiplex *BCR::ABL1* panel on the Countable system enabled direct quantification of four fusion variants (e14a2, e13a2, e1a2, and e19a2) together with *ABL1*, without requiring a standard curve or Poisson correction.
- With automated count reporting, the panel showed minimal background (LoB of 1 count, ~0.0001% at 1 million *ABL1* counts) and an LoD of ~0.001%, supporting sensitive rare molecule detection.
- All four isoforms demonstrated linear quantification from 10% to 0.003% *BCR::ABL1/ABL1* ($R^2 > 0.99$), with ≤10% CV across two days for samples with >100 target counts.
- p210 results are reported on the %IS using a conversion factor calibrated with *BCR::ABL1* reference material traceable to WHO standards.
- In 37 samples containing *BCR::ABL1* fusion transcripts, isoform calls were concordant with previous RT-qPCR results. The panel also resolved co-occurring e14a2/e13a2 transcripts and detected a rare e13a3 variant.

[1] Countable Labs, Inc. 1810 Embarcadero Rd. Suite 200 Palo Alto, California 94303, USA

[2] We thank Dr. Pamela Ward of the University of Southern California for providing samples with *BCR::ABL1* mutation and performing RT-qPCR testing.

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Learn more about how Countable PCR enables multiplex molecular counting of *BCR::ABL1* fusion transcripts in a single tube.

Visit countablelabs.com/resources/bcr-abl1-fusion-variant-quantification-multiplex-pcr