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Single-molecule quantification of *BCR-ABL1* fusion transcripts in a single-tube workflow using PCR-based RNA counting

Jung won Keum, Emily Blair, Christina Fan, Eleen Shum
Countable Labs, Inc. 1810 Embarcadero Rd. Suite 200 Palo Alto, California 94303

Abstract

Accurate and streamlined methods for quantifying *BCR—ABL1* RNA transcripts are essential for monitoring treatment response in chronic myeloid leukemia (CML).

Here, we present a single-tube workflow for the detection of the three major *BCR-ABL1* isoforms (e14a2, e13a2, and e1a2) together with the reference ABL1 gene, integrating compartmentalization, reverse transcription (RT), PCR, and direct counting.

Using the one-step Countable RT-PCR platform, up to one million RNA molecules were accurately quantified with single-molecule resolution. Even in the presence of high background *ABL1*, different levels of *BCR-ABL1* variants were reliably detected. Our results closely matched expected %IS values from the reference panel, with successful detection down to 0.0032% *BCR-ABL1* and low %CV across all levels.

Our ability to directly count RNA molecules across a broad dynamic range makes the platform well-suited for minimal residual disease (MRD) monitoring. By combining sensitivity, specificity, and workflow efficiency in a single-tube format, our approach offers a scalable solution for RNA-based diagnostics.

Background

Importance of *BCR-ABL1* fusion transcripts in CML diagnosis and monitoring.

The *BCR-ABL1* fusion gene, generated by the Philadelphia chromosome translocation, is the genetic hallmark of CML. Quantifying *BCR-ABL1* RNA transcripts is critical for monitoring MRD and assessing molecular response to tyrosine kinase inhibitors.

There are several different *BCR-ABL1* fusion transcripts. The e14a2 and e13a2 transcripts, which encode the p210 protein, are most common in CML. The e1a2 transcript, which encodes p190, is uncommon in CML, but frequently observed in Acute Lymphoblastic Leukemia.

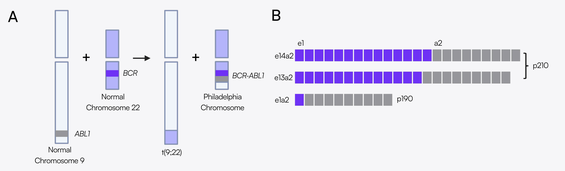


Figure 1. Schematic on the generation of Philadelphia chromosome and the resultant *BCR-ABL1* transcripts associated with CML. (A) The generation of Philadelphia chromosome results in *BCR-ABL1* oncogene. (B) Depending on the breakpoint in the *BCR* gene, three major *BCR-ABL1* isoforms are generated.

Methods

Direct quantification of single RNA molecules with one-step Countable RT-PCR.

The one-step Countable RT-PCR platform generates ~30 million compartments within a PCR tube through centrifugation, forming a clear gel-like matrix. These compartments can isolate up to 1 million RNA molecules, enabling independent amplification of each molecule without co-occupancy. Thus, unlike conventional digital PCRs (dPCR), which are limited in dynamic range and rely on Poisson correction to estimate target numbers, the Countable system provides true single-molecule counting.

Furthermore, the platform employs light sheet scanning to excite fluorophores, producing distinct high-resolution signals. It scans the entire PCR tube in less than one minute per channel, with counts reported simultaneously.

Importantly, the entire workflow—including compartmentalization, RT, PCR, and counting—occurs within a single tube, eliminating transfer steps and preventing sample loss or contamination risk.

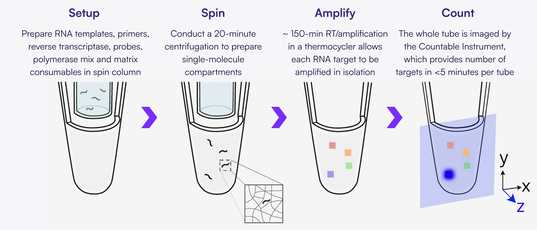


Figure 2. Schematic diagram of one-step Countable RT-PCR workflow for RNA counting. In Countable RT-PCR, target RNA molecules are partitioned into ~30 million compartments within an optically clear matrix. Subsequent RT, PCR amplification, and counting all occur within a single tube. Furthermore, the system employs high-resolution 3D light-sheet imaging to enable rapid and precise quantification.

Design of a 2-color assay for detection of *BCR—ABL1* variants.

In this study, we designed a 2-color assay to detect and quantify the three major *BCR-ABL1* isoforms (e14a2, e13a2, and e1a2), aiming to demonstrate accurate detection across a wide dynamic range of *BCR-ABL1* levels. The three *BCR-ABL1* isoforms are detected in channel 1, while the reference *ABL1* is detected in channel 2.

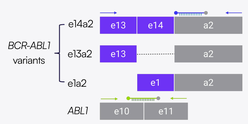


Figure 3. Assay design for detection of *BCR-ABL1* variants. The positions of each primer (arrows) and hydrolysis probe relative to *BCR* (purple box) and *ABL1* (gray box) are shown. *BCR-ABL1* variants are detected using a common *BCR-ABL1* probe in channel 1, while the reference *ABL1* is detected using a separate hydrolysis probe in channel 2.

Achieving comparable performance with the one-step Countable RT-PCR in a single tube, compared to the multiple transfers required for conventional dPCR.

The streamlined one-step RT-PCR workflow on the Countable system in a single tube provides a simplified process with minimal variation, while delivering comparable performance to dPCR, making it well suited for high-throughput testing.

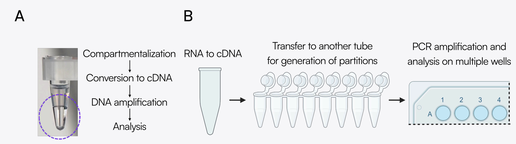
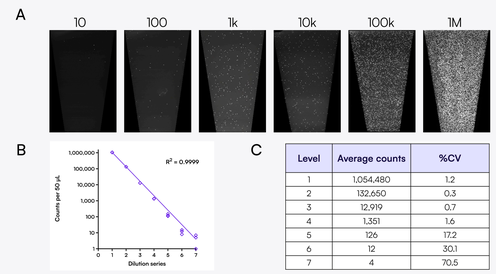


Figure 4. Workflow comparison to conventional dPCR. (A) In the Countable system, all steps required for detecting *BCR-ABL1* variants are performed within a single tube. (B) In contrast, conventional dPCR systems require multiple sample transfers between steps, and due to their limited dynamic range, results from multiple wells must be merged for analysis.

Results.

One-step Countable RT-PCR delivers direct RNA quantification over a 6-log linear dynamic range.

When a single-plex assay was tested with titrated RNA templates, Countable RT-PCR system produced linear counts across a 6-log dynamic range (R^2 = 0.9999), with %CV < 2% at inputs above 1,000 copies. Representative light sheet images at different RNA levels and their corresponding counts are shown below.



Level	Average counts	%CV
1	1,054,480	1.2
2	132,650	0.3
3	12,919	0.7
4	1,351	1.6
5	126	17.2
6	12	30.1
7	4	70.5

Figure 5. Linear dynamic range up to 1 million RNA copies. Single-plex assay was tested with titration of influenza B RNA templates (N=3). (A) Light sheet images at different RNA target levels are shown. The corresponding counts for each level are plotted in (B), with a summary provided in (C).

Specificity of *BCR-ABL1* assay is maintained in multiplex condition.

Each *BCR-ABL1* variant DNA template with expected counts of several thousands was incubated with its corresponding single-assay or multiplexed condition (e14a2/e13a2/e1a2/*ABL1* assays combined). The average counts from single-plex and multi-plex assays were comparable, demonstrating assay specificity in the multiplex condition.

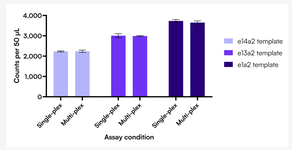


Figure 6. Multiplexing Capability of the *BCR-ABL1* Assay. Comparable counts were shown for each *BCR-ABL1* variant DNA template under both single-plex and multi-plex conditions (N=2).

The wide dynamic range of Countable RT-PCR enables detection of rare *BCR-ABL1* fusion events in the presence of abundant *ABL1* molecules in a single tube.

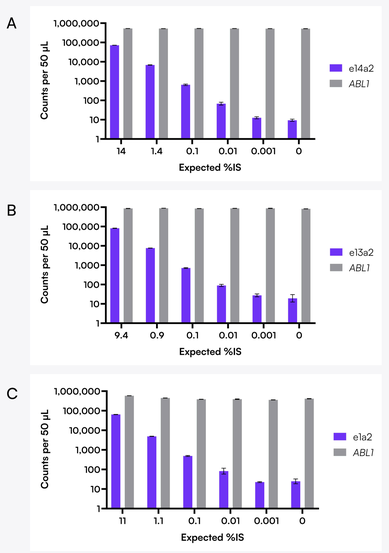


Figure 7. Quantification of different ratios of *BCR-ABL1* variants to wild-type (wt) *ABL1*. RNA containing the e14a2 (A), e13a2 (B), and e1a2 (C) isoforms was diluted into a background of wt *ABL1* RNA to generate varying *BCR-ABL1* to wt *ABL1* ratios (%IS). While wt *ABL1* counts remained constant (up to 900k), the counts of *BCR-ABL1* variants decreased proportionally with dilution, down to low 10s of molecules (N=4).

Results, cont.

RNA containing the e14a2, e13a2, and e1a2 isoforms was diluted into a background of wild-type *ABL1* RNA to generate varying *BCR-ABL1* to wt ABL1 ratios. While wt *ABL1* counts remained constant (up to 900k), the counts of *BCR-ABL1* variants decreased proportionally with dilution.

In other dPCR platforms, such high levels of wt *ABL1* cannot be loaded due to oversaturation of targets within partitions. The broad dynamic range of the Countable RT-PCR system enables simultaneous detection of both low- and high-abundance targets within a single tube, eliminating the need for merging multiple wells.

Detecting RNA fusion variants as low as 0.001% IS.

When measured %IS values were compared with the expected %IS (calculated based on the counts at the highest level for each target and 10-fold dilutions for subsequent levels), results from the highest level (~10%) down to 0.001% showed close agreement.

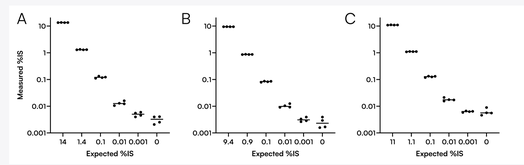
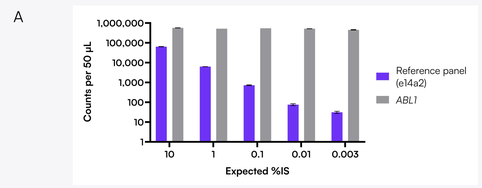


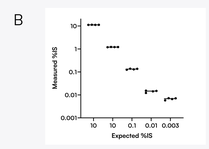
Figure 8. Comparison of Expected vs Measured %IS. Measured %IS was plotted against expected %IS for each *BCR-ABL1* variant: (A) e14a2, (B) e13a2, and (C) e1a2 (N=4). The measured %IS matched the expected values across tested levels.

Results of *BCR-ABL1* Countable RT-PCR assay match the %IS of reference panel.

The *BCR-ABL1* assay was evaluated using an AcroMetrix reference panel with e14a2 to wt *ABL1* ratios of 10%, 1%, 0.1%, 0.01%, and 0.0032%. The panel, consisting of a mixture of K-562 and HL-60 cells to mimic patient specimens, underwent RNA extraction prior to testing.

The assay results closely matched the expected values across all levels, with low %CV (Table C). We successfully detected *BCR-ABL1* levels as low as 0.0032%. Detecting at this level is particularly important, as it informs therapeutic decisions, including eligibility for tyrosine kinase inhibitor discontinuation in CML patients.





C

Expected %IS	Measured %IS		Counts of e14a2		Counts of ABL1	
	Value	%CV	Value	%CV	Counts	%CV
10	**11.3**	**0.7**	64,397	1.7	572,940	1.0
1	**1.2**	**1.2**	6,311	1.0	521,613	0.3
0.1	**0.1**	**5.0**	729	4.4	545,485	0.3
0.01	**0.01**	**9.7**	76	10.3	518,988	0.7
0.0032	**0.007**	**9.8**	32	11.7	456,483	3.1

Figure 9. Validation of *BCR-ABL1* assay in the reference panel. The assay was evaluated using a reference panel with known *BCR-ABL1* to wt *ABL1* ratios. Counts of e14a2 and *ABL1* (A), the corresponding %IS values (B), and a summary table (C) are shown (N=4).

Conclusion

We successfully detected the three major *BCR-ABL1* isoforms together with reference *ABL* using a streamlined single-tube workflow starting directly with RNA, eliminating the complexity of a conventional two-step workflow.

Our one-step Countable RT-PCR platform enabled direct quantification of RNA molecules with single-molecule precision across a 6-log dynamic range, making it ideal for MRD monitoring, as it allows detection of both abundant and rare molecules within a single tube.

Our results closely matched the reference panel values, successfully detecting 0.0032% *BCR-ABL1*, with low %CV across all tested levels.

Learn more about how Countable PCR transforms *BCR-ABL1* RNA fusion detection.