App Note

Countable Labs.

Consistent *BCR::ABL1* quantification across four transcripts in one reaction

Direct single-molecule counting of *e14a2*, *e13a2*, *e1a2*, *e19a2*, and *ABL1* without per-run standard curves

Introduction

Chronic myeloid leukemia (CML) is driven by the *BCR::ABL1* fusion gene, which produces a constitutively active tyrosine kinase that fuels uncontrolled cell growth. Quantitative monitoring of *BCR::ABL1* transcripts underpins measurable residual disease assessment in CML and Philadelphia chromosome-positive B-cell acute lymphoblastic leukemia and depends on comparing measurements across time. Laboratories need confidence that an observed change reflects biology rather than variation introduced by runs, operators, instruments, or analytical interpretation. That consistency is essential when evaluating molecular response, possible resistance, or remission.

Highlights

- **Trust every result.** Directly count molecules without per-run external curves or operator-set thresholds.
- **Get more biologically complete data from every sample.** Quantify four *BCR::ABL1* transcripts and *ABL1* in one reaction with practically no dead volume.
- **Expand capability without expanding workflow complexity.** Maintain four-transcript linearity from 10% to 0.0032%, with *ABL1* counting linear across six logs.

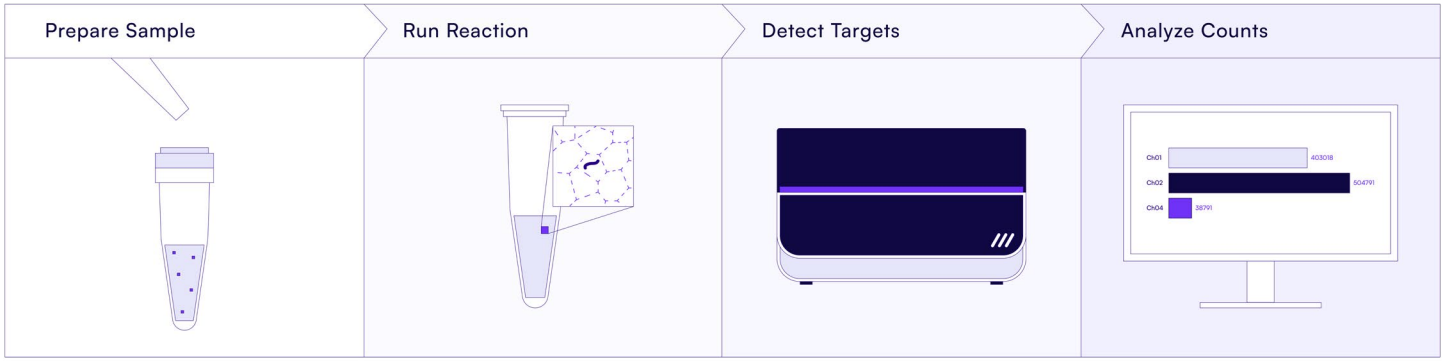

Figure 1. End-to-end workflow of the Ancora *BCR::ABL1* Assay. The workflow consolidates reverse transcription, amplification, and quantification into a closed, single-tube system.

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The Ancora *BCR::ABL1* Assay uses direct single-molecule counting to measure four *BCR::ABL1* transcripts and *ABL1* in one closed reaction. Multiplex optical signatures provide more biological information from each sample, while practically no dead volume preserves available input for deeper detection. Automated, fixed-rule analysis eliminates per-run external standard curves and operator-set amplification thresholds, expanding laboratory capability without adding separate assays or complex data analysis.

This study evaluates how those capabilities translate into analytical range, low background, transcript-specific sensitivity, between-day precision, and agreement between two Countable Systems.

Materials and Methods

Single-tube workflow preserves sample input for deeper detection

The Ancora *BCR::ABL1* Assay is a single-tube multiplex RNA assay that combines compartmentalization, reverse transcription, amplification, imaging, and automated analysis from a 50 µL reaction. Centrifugation divides the reaction into approximately 30 million compartments, allowing individual target molecules to be amplified and counted by three-dimensional light-sheet imaging. Because the workflow does not rely on fixed-volume metering, practically the entire reaction volume can be analyzed, preserving available RNA. For *BCR::ABL1* monitoring, the number of *ALB1* reference modules recovered and directly counted establishes the denominator used to calculate the ratio, and therefore the sensitivity achievable for each sample.

Isoform	Transcript	Assay Optical Signature
p210	*e14a2*	
	e13a2	
p190	*e1a2*	
p230	*e19a2*	
	ABL1 reference	

Figure 2. Distinct optical signatures enable transcript-level identification. Each *BCR::ABL1* fusion transcript and the *ABL1* reference are assigned a specific combination of fluorescent colors.

Multiplex optical signatures enable precise transcript level identification

Probes bind sequences associated with each fusion partner, and linkage analysis identifies amplified molecules carrying the expected optical signature. Each fusion call requires agreement between two independent signals on the same molecule, so a spurious event in one channel does not generate a count. The analysis software reports only molecules with the correct optical signature, supporting transcript-level discrimination (**Figure 2**).

Analytical materials

Analytical testing used RNA templates, cell-line materials containing each evaluated transcript, and a standardized reference panel consisting of K562 and HL60 cell mixtures (RNA sourced from Thermo Fisher Scientific) with assigned p210 *e14a2* %IS levels. Limits of detection and linearity for *e14a2*, *e13a2*, and *e1a2* were established with material purchased from Invivoscribe, and those for *e19a2* with contrived cell-line material (AR230 from ATCC) prepared at Countable Labs. Negative samples used to establish the limit of blank consisted of RNA carrying the *ABL1* reference transcript with no *BCR::ABL1* fusion present. RNA was extracted immediately before Countable PCR testing to maximize recovery of *ABL1* reference molecules and the sensitivity achievable per sample.

Automated analysis supports consistent, objective reporting

The Ancora *BCR::ABL1* Assay reports the number of molecules counted for each target and calculates each *BCR::ABL1* transcript as a percentage of the *ABL1* counted in the same reaction. Every step from image to reported result is carried out by the software using fixed rules. There is no baseline to set, no amplitude threshold to place, and no per-sample judgment by the operator.

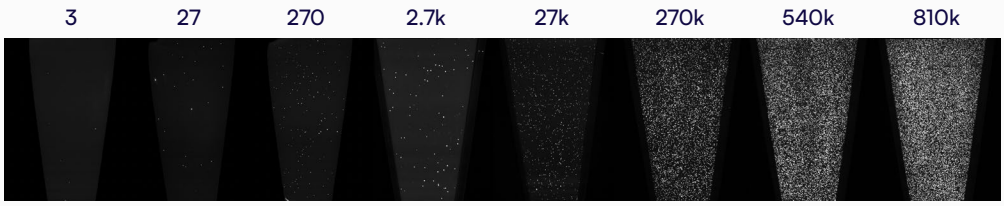
Calling a transcript detected. A transcript is reported as detected at three or more counts, and otherwise as NOT DETECTED. The threshold was derived from the limit of blank study reported below.

A three-count calling threshold corresponds to 95% expected detection at seven molecules per reaction. Under a Poisson model, a mean of approximately 6.3 molecules per reaction provides a 95% probability of observing three or more counts. This was rounded to seven molecules per 50 µL reaction for the theoretical sensitivity calculations. Expressed as *BCR::ABL/ABL1*, the theoretical detection limit is seven divided by the number of *ABL1* molecules counted in the same reaction. Achievable ratio sensitivity therefore depends on the *ABL1* recovery for the individual sample. Representative calculations are shown in **Table 2**.

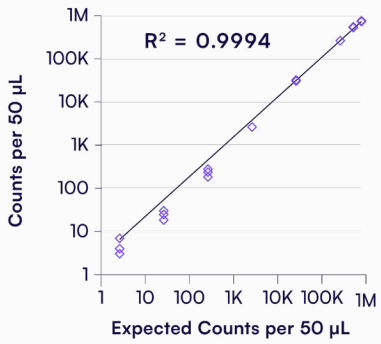
Fixed-rule reporting reduces operator-dependent interpretation and flags poor-quality samples. Detection calls are generated from molecular counts using a fixed three-count threshold, avoiding manual placement of amplification-curve thresholds. The ID score measures separation of assay signal from background; scores above 80 are generally considered acceptable. For p210, the software applies a lot-specific conversion factor supplied with each assay kit and entered during imaging setup to report %IS. The *e1a2* and *e19a2* transcripts are reported as *BCR::ABL1/ABL1* ratios, because no International Scale is defined for these transcripts. To allow comparison across transcript types, all results in this note are presented as *BCR::ABL1/ABL1* ratio (%).

Six-log *ABL1* counting expands the measurable range. To characterize the counting range, RNA template was serially diluted and quantified using a single-plex *ABL1* assay. Counts remained linear across six logs (R^2=0.9994), with coefficients of variation of 2.6% or lower above 1,000 expected counts (**Figure 3**). Below 1,000 counts, the %CV increased as expected from statistical sampling variation. Because quantification derives from a direct count rather than from interpolation against a standard curve, this range was measured without a per-run external standard curve or Poisson correction.

A.



B.



C.

Level	Expected counts	Average counts	% CV
1	804,692	759,084	1.5
2	536,461	535,545	1.8
3	268,231	268,231	1.0
4	26,823	31,756	2.6
5	2,682	2,629	1.4
6	268	233	17.3
7	27	25	20.1
8	3	5	38.5

Figure 3. *ABL1* counting remains linear across six logs. A single-plex assay for *ABL1* was tested with titration of HL60 RNA (N=4). (A) Light-sheet images at different RNA target levels. Every speckle in the light-sheet image represents a single detected molecule, so counts are read directly rather than inferred from an amplification curve. (B) Corresponding counts for each level. (C) Average counts and %CV.

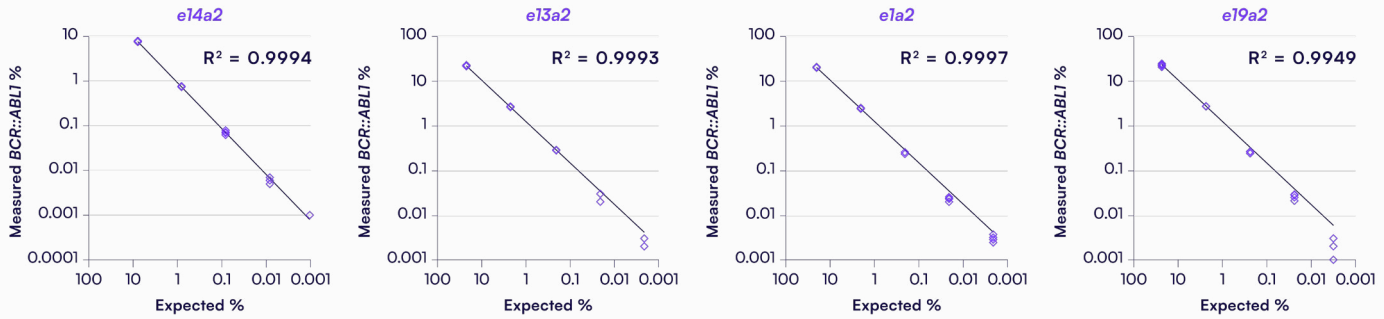


Figure 4. All four *BCR::ABL1* fusion transcripts remain linear from 10% to 0.0032% *BCR::ABL1/ABL1* in one reaction. RNA containing p210 *e14a2*, p210 *e13a2*, p190 *e1a2*, and p230 *e19a2* was diluted into wild-type *ABL1* RNA to generate varying *BCR::ABL1* to *ABL1* ratios. *ABL1* counts remained constant while *BCR::ABL1* counts decreased proportionally, demonstrating single-tube discrimination of all four transcripts (N=4).

One reaction maintains linear quantification across four fusion transcripts

Detecting a rare fusion event against an abundant reference transcript in the same reaction is the central requirement for MRD monitoring, so RNA containing each transcript was diluted into a wild-type *ABL1* background to generate a range of *BCR::ABL1* to *ABL1* ratios (**Figure 4**). *ABL1* counts remained constant while *BCR::ABL1* counts fell proportionally with dilution, and linearity was maintained from a 10% ratio down to 0.0032% for all four transcripts without per-run external standard curves or Poisson error correction. By maintaining linearity across all four targets in the same reaction, the assay provides broader transcript coverage without splitting the sample across separate tests.

Near-zero background supports confident low-level detection

To establish the limit of blank, 20 negative samples, each consisting of RNA carrying the *ABL1* reference transcript with no *BCR::ABL1* fusion present, were tested in the same experiment. Each measurement contributed approximately one million *ABL1* counts, so background was assessed against a reference burden comparable to that of a high-input specimen (**Table 1**).

No negative replicate produced more than one fusion count. The calling threshold was therefore set at three counts, providing additional margin above the highest observed background. The near-zero background is consistent with the panel's two-signal requirement: an event in a one channel does not generate a fusion count unless a coincident event is detected in the second channel assigned to that molecule. Together, the two-signal requirement and near-zero observed background support confident low-level detection at the three-count reporting threshold.

Table 1. Fusion transcript counts in negative samples. Negative samples carrying the *ABL1* reference transcript (HL60 cell line RNA) with no expected *BCR::ABL1* fusion present. Mean *ABL1* count was 1,045,864 per 50 µL reaction. No measurement returned more than one count using the automated software reporting feature.

Transcript	Samples with ≥1 count	Total counts across 20 samples	Mean counts per sample
e14a2	2 / 20	2	0.1
e13a2	2 / 20	2	0.1
e1a2	2 / 20	2	0.1
e19a2	0 / 20	0	0

Higher *ABL1* recovery enables deeper sample-specific sensitivity

Applying the 3-count calling threshold to the Poisson relationship described in the methods gives a theoretical detection limit of seven molecules per 50 µL reaction. Because quantification is a direct count and the reported value is a ratio, the corresponding sensitivity is set by how many *ABL1* molecules are counted in the same reaction: seven molecules against 100,000 *ABL1* copies is 0.007%,

and the same seven molecules against one million *ABL1* copies is 0.0007% (**Table 2**). The Countable System supports up to 1 µg of direct RNA input per reaction, but achievable ratio sensitivity depends on how many *ABL1* reference molecules are recovered and counted from that input. The values in **Table 2** are calculated from the calling threshold rather than measured at each input; transcript-specific limits of detection determined experimentally are given in **Table 3**.

Table 2. Theoretical limit of detection as a function of *ABL1* reference molecules. Limit of detection is defined as the input at which ≥95% of replicates are expected to yield three or more counts. Under Poisson statistics this corresponds to a mean of approximately 7 molecules per 50 µL reaction.

ABL1 copies per 50 µL reaction	Limit of detection (%*BCR::ABL1/ABL1*)	Estimated Molecular Response (MR)*
100,000	0.007%	MR4.2
500,000	0.0014%	MR4.9
1,000,000	0.0007%	MR5.2

* MR is defined on the International Scale for p210 and is reported only when the user enters the reagent lot conversion factor supplied with the assay kit.

Limits of detection were also established for each transcript individually using reference materials, and ranged from 0.0003% for *e14a2* to 0.0013% for *e1a2* and *e19a2* (**Table 3**). All four fall below 0.002%, and the rare transcripts are quantified at the same order of sensitivity as the common ones, so coverage of p190 and p230 is not a lower-performing addition to a p210 assay. Laboratories can therefore use the available high-quality RNA in each sample to pursue greater measurement depth without changing the assay workflow.

Table 3. Limit of detection by transcript. Values were derived from replicate data presented in Figure 4 using the reference materials indicated.

Transcript	Isoform	Limit of detection (%*BCR::ABL1/ABL1*)	Reference material
e14a2	p210	0.0003%	Invivoscribe
e13a2	p210	0.0008%	Invivoscribe
e1a2	p190	0.0013%	Invivoscribe
e19a2	p230	0.0013%	AR230 from ATCC

Between-day precision supports confidence in repeat measurements

Countable PCR is designed to produce comparable results without per-run standard curves, so five levels of *e14a2* reference material were tested with four replicates per level on two separate days (**Table 4**). The coefficient of variation was at or below 10% for every level above 100 counts and at or below 2.5% at the two highest levels, with no recalibration performed between the two days. At the lowest two levels the %CV rises as expected for a counting measurement approaching its limit of detection, and this variability is predictable from the count itself. This consistency helps laboratories distinguish changes in molecular burden from variability introduced by testing on different days.

Table 4. *e14a2* quantification remained within 10% CV above 100 counts across two days. Mean *BCR::ABL1/ABL1* % and %CV are shown for five levels of *e14a2* reference material tested with 4 replicates per level on two separate days.

Level	Day 1 (N=4)		Day 2 (N=4)		Combined (N=8)	
	e14a2/ABL1 ratio %		*e14a2/ABL1* ratio %		*e14a2/ABL1* ratio %	
	Mean	%CV	Mean	%CV	Mean	%CV
Level 1	0.001	56.0	0.001	91.7	0.001	69.0
Level 2	0.005	31.0	0.005	19.0	0.005	25.6
Level 3	0.044	**9.7**	0.048	**8.3**	0.046	**9.4**
Level 4	0.54	**2.3**	0.53	**1.6**	0.54	**2.0**
Level 5	6.00	**1.2**	5.74	**0.7**	5.87	**2.5**

Agreement across Countable Systems supports consistent quantification

The same analytical sample sets were run through two Countable Systems and analyzed using the production software (**Table 5**). Mean ratios differed by no more than 4.3% across the levels tested, with within-level coefficients of variation of 4.5% or lower. This agreement supports consistent qualification across Countable Systems. *e14a2* and *e13a2* were evaluated at three levels; *e1a2* and *e19a2* were evaluated at one level.

Table 5. Limit of detection by transcript. Values were derived from replicate data presented in Figure 4 using the reference materials indicated.

Transcript	Level	n	Mean counts	Mean % ratio	%CV	Difference between instruments
e14a2	1	8	103,820	17.02	2.0	−0.79%
e14a2	2	7	10,187	2.18	2.6	3.28%
e14a2	3	8	1,064	0.236	4.5	0.94%
e13a2	1	8	72,443	19.00	0.7	−0.85%
e13a2	2	7	7,472	2.34	0.8	1.12%
e13a2	3	7	724	0.238	4.3	−4.27%
e1a2	1	8	93,077	19.91	1.0	1.67%
e19a2	1	8	102,696	76.33	0.8	1.01%

Evaluation with patient samples and an FDA-approved RT-qPCR comparator

The analytical characterization presented here was performed on reference materials. In a separate study with the Molecular Pathology group at the University of Southern California, the Ancora *BCR::ABL1* Assay was evaluated alongside an FDA-approved RT-qPCR IVD using a %IS reference panel and patient samples. The study also evaluated transcript-specific quantification in samples from two patients with co-expressed *e14a2* and *e13a2* p210 transcripts and longitudinal samples from one patient carrying *e19a2* p230. Results were presented at the Association for Molecular Pathology Europe Congress in 2026 (Keum et al., 2026).

Conclusion

The Ancora *BCR::ABL1* Assay combines transcript identification and quantification in one closed reaction, resolving *e14a2* and *e13a2* p210 separately while also measuring *e1a2* p190, *e19a2* p230, and *ABL1*. Its centrifugation-based workflow leaves practically no dead volume, preserving available input and helping maximize the number of *ABL1* reference molecules counted, which determines the sensitivity achievable for each sample. Analytical characterization demonstrated linear *ABL1* counting across six logs and linear quantification of all four fusion transcripts across the shared range evaluated, from 10% to 0.0032% *BCR::ABL1/ABL1*. Background testing produced no more than one fusion count in any negative sample, supporting the three-count reporting threshold. Between-day precision was 10% CV or lower above 100 counts, and mean ratios differed by no more than 4.3% between two Countable Systems across levels tested.

Direct molecular counts and automated, fixed-rule analysis eliminate per-run external standard curves and operator-set amplification thresholds. Together, these capabilities provide broader transcript coverage from the same sample, support consistent measurement across days and instruments, and expand what laboratories can measure within a single PCR-based workflow.

Ordering

SKU	Kit	Contents
BK005-Ancora *BCR::ABL1* Assay Bundle	KT0012 Countable Matrix Consumables Kit	Matrix reagents, column strips, and tube strips for 48 reactions
	KT0013 Ancora *BCR::ABL1* Assay Kit	Enzymes, primer mixes, imaging control, and PCR reagents for 48 reactions

Required equipment: This assay is designed for use exclusively with the Countable System and its compatible equipment.

References

1. Keum JW, Ward PM, Campan M, Lai P, Hajjali I, Shum EY, Fan C. 2026. Single-tube multiplex quantification of *BCR::ABL1* fusion transcripts enables sensitive MRD monitoring across isoforms in CML/ALL. Presented at: Association for Molecular Pathology Europe Congress; May 2026. [Poster]

BEYOND *BCR::ABL1*

Bring your next assay challenge to Countable Labs

The Ancora *BCR::ABL1* Assay Kit is part of a growing family of heme-oncology assays for Countable PCR. Countable Labs also supports teams evaluating new targets, adapting existing assays, developing more complex panels, or exploring the platform before bringing it into their laboratory.

Expert-run assay services

Run a pre-designed Countable PCR assay on your samples. Your data package includes the protocol, assay characterization, and final results.

Custom assay development

Bring us an existing assay, new targets, or a workflow challenge. Countable can support assay conversion, optimization, performance characterization, and end-to-end development.

Instrument access and demos

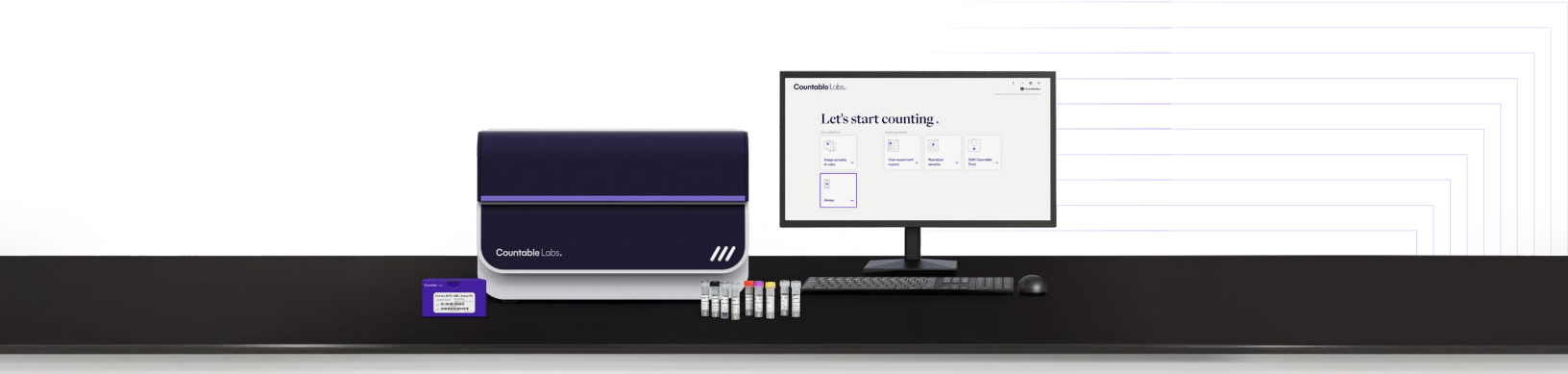
Explore Countable PCR through instrument-access and demonstration options aligned with your application and development needs.

Ancora Assay Kits

- ***BCR::ABL1***
  Quantify 4 *BCR::ABL1* transcripts plus *ABL1* beyond MR5.0
- ***KMT2A-r***
  Detect *KMT2A* rearrangements for MRD tracking
- ***NPM1* Mutation**
  Delivers the depth of RNA with the standardized precision of absolute counting of *NPM1* mutations
- ***KIT D816V***
  Simultaneously detect *KIT D816V* down to 0.015% VAF and *KIT* WT
- ***PML::RARA* RNA**
  Count all three canonical *PML::RARA* isoforms plus *ABL1*
- ***ABL1* TKI Resistance Mutation Phasing**
  Perform TKI resistance testing following a positive *BCR::ABL1* result

Have a target or workflow challenge?

Talk with a Countable Labs specialist at countablelabs.com/contact.



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