

# Single-tube Multiplex Quantification of *BCR::ABL1* Fusion Transcripts enables Sensitive MRD Monitoring across Isoforms in CML/ALL

J W Keum<sup>1</sup>, PM Ward<sup>2</sup>, M Campan<sup>2</sup>, P Lai<sup>2</sup>, I Hajjali<sup>2</sup>, EY Shum<sup>1</sup>, and C Fan<sup>1</sup>

<sup>1</sup>Countable Labs, Inc. 1810 Embarcadero Rd. Suite 200 Palo Alto, California 94303, USA

<sup>2</sup> University of Southern California, Molecular Pathology, 2250 Alcazar St, Los Angeles, CA 90033

## Introduction

Quantitative monitoring of *BCR::ABL1* transcripts is central to MRD assessment in CML and B-ALL. Current clinical workflows rely on RT-qPCR or digital PCR (dPCR), which require multi-step processing and, in the case of qPCR, calibration to external standards for quantification. A simplified approach that enables direct, absolute quantification of *BCR::ABL1* transcripts without reliance on standard curves could offer an alternative paradigm for MRD monitoring.

## Objectives

To demonstrate the high-level precision of Countable PCR and the clinical utility of a single-tube multiplex assay.

## Methods

A single-step multiplex RNA assay was developed for the detection and absolute counting of e14a2, e13a2, e1a2, and e19a2 *BCR::ABL1* fusion transcripts. Reverse transcription, amplification, and molecular counting occur in a closed 50  $\mu$ L reaction without separate cDNA conversion, sample transfer, or external standard curves. Individual molecules are directly counted by 3D light-sheet microscopy imaging of the complete reaction volume, eliminating dead volume and partitioning statistics.

## Results

Figure 1: How Countable PCR works.

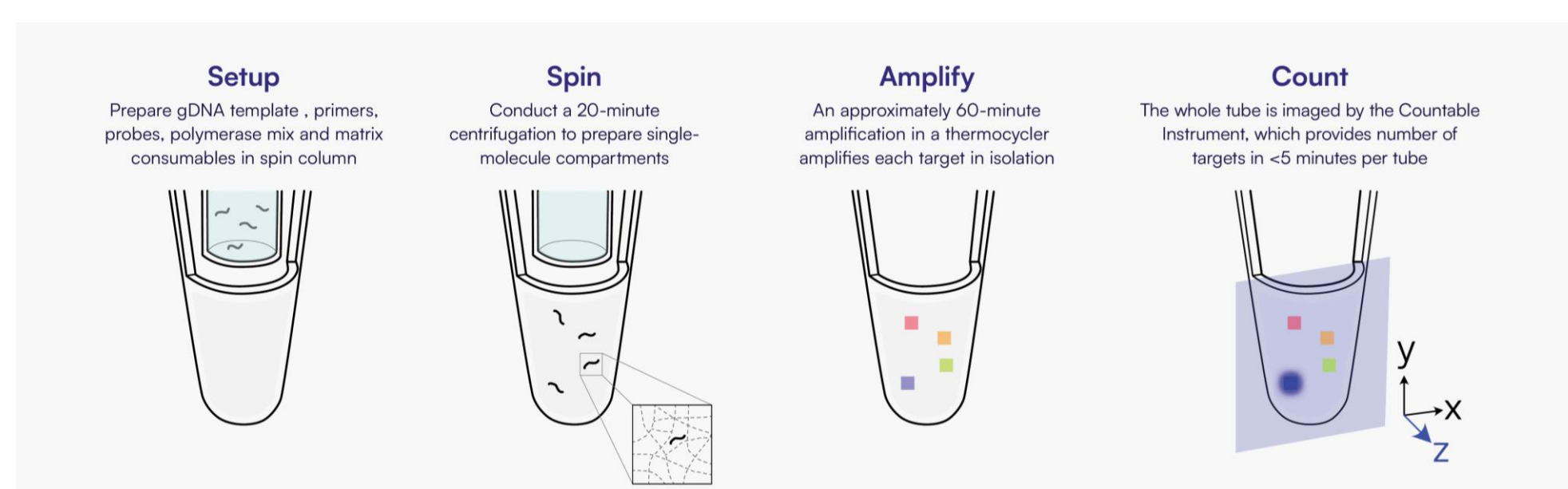


Figure 2: 3D light sheet microscopy: absolute counts from RNA of different inputs.



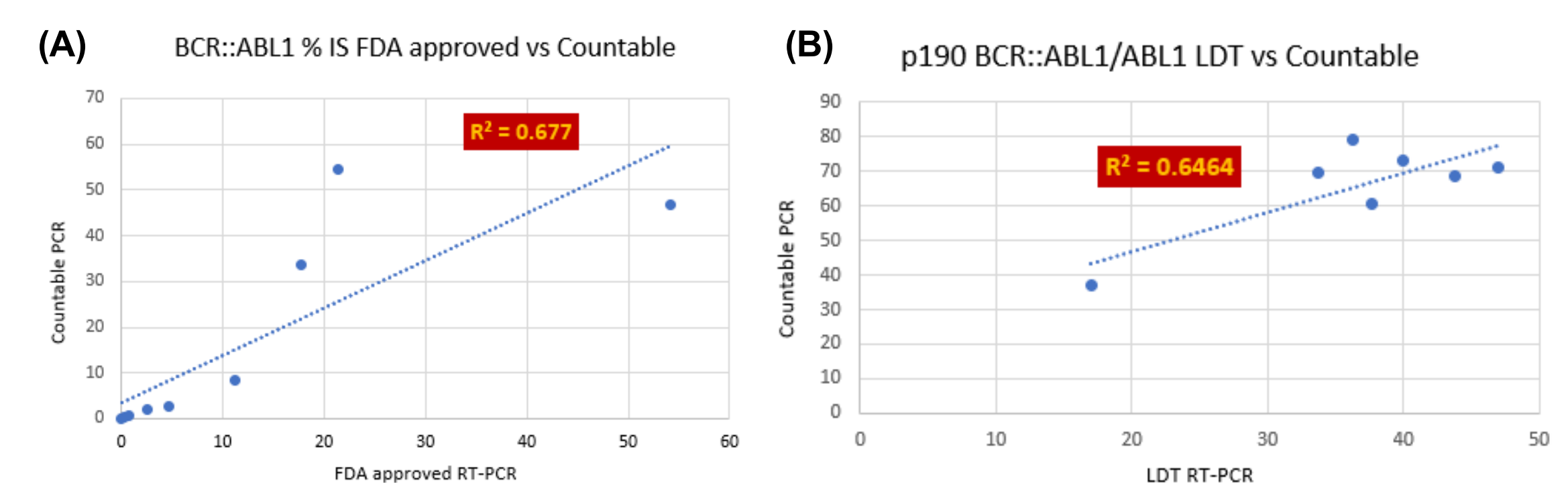
## Results

The precision of Countable PCR was evaluated against RT-PCR using a vendor-provided %IS reference panel. Figure 1 illustrates Countable PCR processing workflow. Representative 3D light-sheet images are presented in Figure 2, with corresponding quantitative results shown in Figure 3. Countable PCR demonstrated superior precision, with coefficients of variation ranging from 0.7% to 22%.

Figure 3: Comparison of replicate p210 % IS RT-PCR results versus Countable PCR.

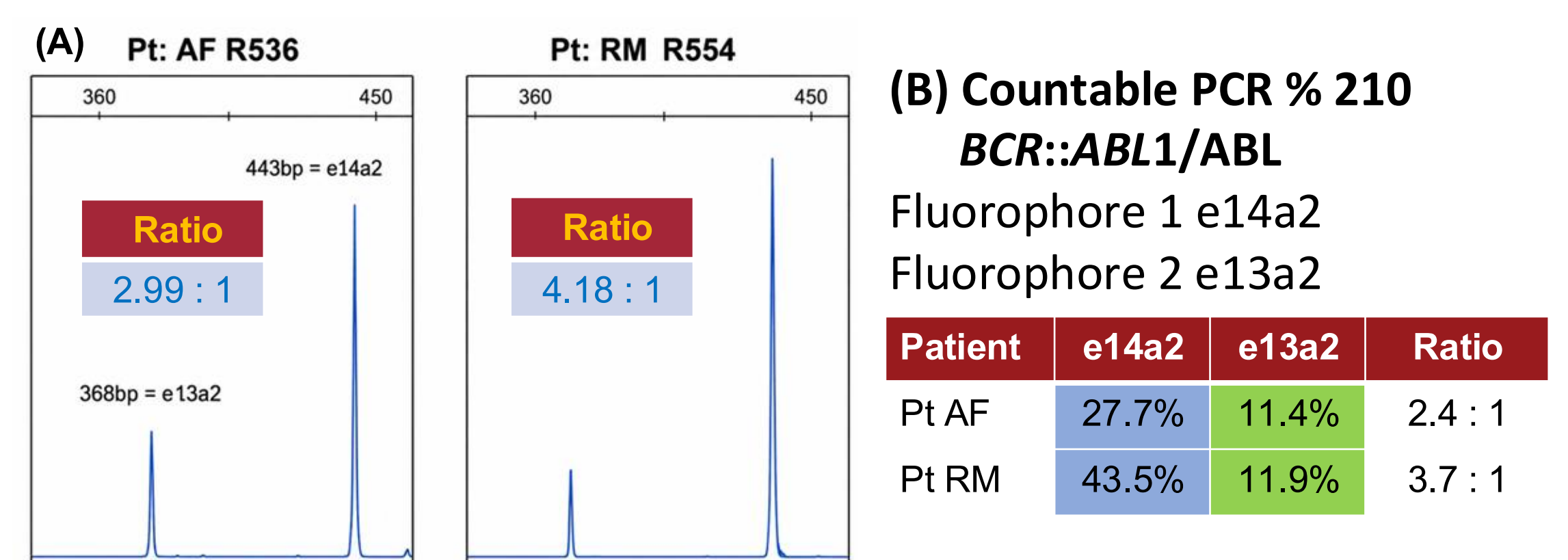
Vendor controls % IS	FDA RT-PCR %IS Mean (n=7) (%CV)	Countable PCR % <i>BCR::ABL1/ABL1</i> Mean (n=2-4) (%CV)
10%	14.77 (22.84)	9.947 (2.03)
1%	1.63 (23.69)	1.105 (0.7)
0.1%	0.07 (53.79)	0.116 (4.04)
0.01%	0.009 (46.67)	0.034 (14.2)
0.0032%	0.0038 (20.27)	0.019 (21.9)

Fig. 4: Orthogonal testing of clinical samples (A) p210 and (B) p190.



*BCR::ABL1* p210 and p190 samples were orthogonal evaluated using Countable PCR. There was moderate correlation demonstrated with  $R^2$  values of 0.67 and 0.64 respectively (Figure 4), likely due to the nature of relative quantitation of qPCR.

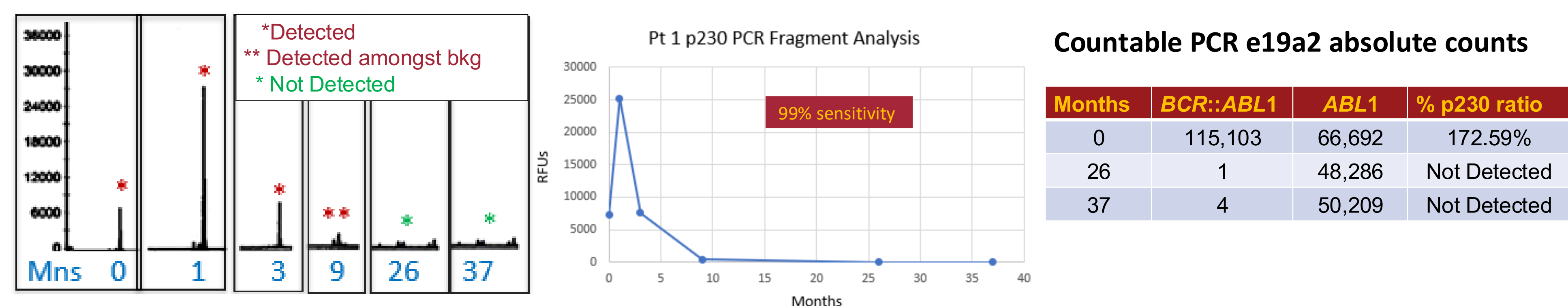
Figure 5: Patients with double p210 transcripts identified by PCR fragment analysis (A) were quantitated using Countable PCR (B)



Individual e13a2 and e14a2 Countable probes enabled the detection (and monitoring) of patients harboring double transcript variants (Figure 5).

Inclusion of Countable PCR e19a2 primers and probes allowed monitoring of patients with p230 transcripts (Figure 6).

Fig 6: Pt with p230 transcripts monitored by diagnostic PCR fragment analysis assay (A and B) quantitated using Countable PCR.



## Conclusions

- Countable PCR p210 absolute counts align well to %IS.
- Countable PCR demonstrated higher precision when evaluated using %IS reference panel.
- Enhanced clinical utility of the Countable PCR assay enables detection of p230 and dual transcript variants in addition to the common p210 and p190 transcripts

