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Evaluation of a deep-sampling PCR assay for KIT D816V detection at ultra-low variant allele frequencies

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Introduction

Rare variant detection in liquid biopsy and heme malignancies is limited by the partition count and DNA input capacity of conventional digital PCR (dPCR).

Countable PCR is a deep sampling approach that partitions large DNA inputs (up to >1 µg) into over 30 million single-molecule compartments per reaction.

Here, the KIT D816V assay on Countable PCR is analytically and clinically evaluated against a reference dPCR platform, also uncovering samples that were undetected by dPCR.

Objectives

To evaluate Countable PCR for KIT D816V detection against a commercial dPCR comparator, with focus on:

1. Analytical sensitivity at ultra-low VAF;
2. Site-to-site reproducibility on patient samples previously characterized on commercial dPCR; and
3. Performance across a wide DNA input range without upfront sample normalization, or splitting wells.

Methods

Assay. A multiplexed Countable PCR assay targeting KIT D816V, a key biomarker in systemic mastocytosis, was evaluated on the Countable platform. VAF was calculated directly from variant-positive vs total partition counts. Poisson correction was not applied.

Analytical testing. Contrived samples spanning expected KIT D816V VAF of 0.015%, 0.03%, and 0.06% were used to evaluate ultra-low VAF detection. A 3-fold synthetic dilution series (n=4) was used to establish sensitivity limits, performed at Labcorp.

Inter-site reproducibility. 15 patient samples were analyzed at two independent Countable PCR sites at (Countable Labs and Labcorp) and compared to results from a commercial dPCR platform (Labcorp).

Expanded clinical evaluation. 23 patient samples previously analyzed on commercial dPCR were re-tested on Countable PCR. Total DNA input ranged from 290 to 2,116 ng, loaded without normalization to reflect production workflow, performed at Labcorp.

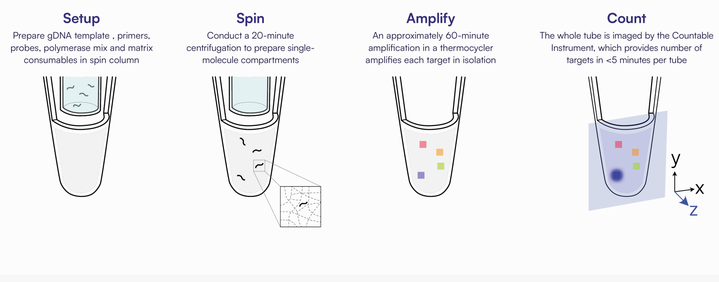

Figure 1. Countable PCR workflow. A non-microfluidic, centrifugation-based method partitions a 50 µL PCR reaction into over 30 million single-molecule compartments for true digital counting.

Results

Analytical performance. Signal distribution showed clear separation between variant (KIT D816V), wild-type (KIT WT), and background, with Intensity Distribution (ID) scores of 97.6 and 98.5, respectively (Figure 2). Sensitivity was evaluated using contrived samples spanning expected VAF of 0.015%, 0.03%, and 0.06%. Consistent variant detection was observed at all input levels, including 0.015% VAF, below the 0.03% limit of detection of the commercial dPCR comparator (Figure 3).

Figure 2. Fluorescence intensity histograms for the Countable KIT D816V assay. Target signal (KIT D816V, blue; KIT WT, green) is cleanly separated from background (grey) without manual thresholding. An Intensity Distribution (ID) score is reported per sample to quantify automated call confidence (ID 97.6 and 98.5 shown).

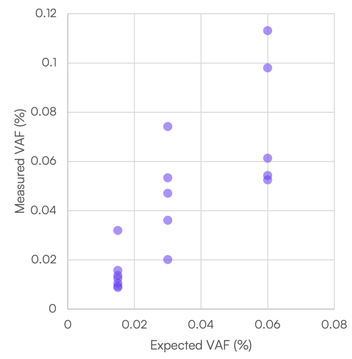

Figure 3. Ultra-low VAF detection. Contrived samples at expected KIT D816V VAF of 0.015%, 0.03%, and 0.06% (replicates per level). Measured VAF tracked expected across the range; consistent detection was observed at 0.015% VAF, below the 0.03% LoD of the commercial dPCR comparator. Variability is consistent with stochastic sampling at low copy number.

Clinical application. Fifteen patient samples were re-analyzed on Countable PCR at two independent sites and compared to results from a commercial dPCR platform (Table 1). Inter-site agreement was high across the full VAF range (0.03% to >40%), with each Countable site reporting from a single well versus three wells required for commercial dPCR.

Sample ID	dPCR VAF	Countable PCR Site 1 VAF	Countable PCR Site 2 VAF
Patient 1	0.10%	0.11%	0.12%
Patient 2	0.06%	0.05%	N/A
Patient 3	0.03%	0.04%	0.03%
Patient 4	0.06%	0.09%	0.06%
Patient 5	39.41%	39.24%	N/A
Patient 6	0.06%	0.09%	N/A
Patient 7	0.06%	0.10%	N/A
Patient 8	0.03%	0.06%	0.03%
Patient 9	0.03%	0.08%	N/A
Patient 10	0.05%	0.14%	N/A
Patient 11	0.19%	0.13%	0.17%
Patient 12	0.14%	0.39%	0.18%
Patient 13	19.00%	18.91%	18.93%
Patient 14	44.00%	45.80%	45.60%
Patient 15	5.49%	5.86%	N/A

Table 1. KIT D816V VAF measured on 15 patient samples by commercial dPCR and by Countable PCR at two independent sites. Inter-site agreement was maintained across the full VAF range (0.03% to >40%). Each Countable PCR result was generated from a single well; the commercial dPCR result required three wells per sample. N/A means sample not tested.

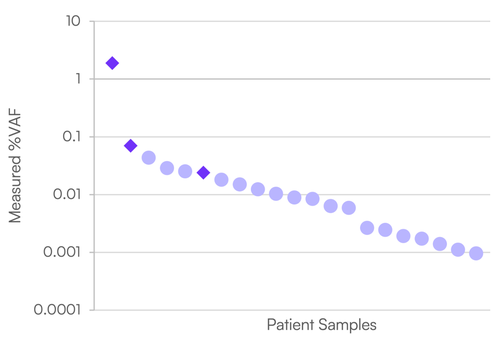

Figure 4. Expanded patient cohort (n=23). Per-sample Countable PCR VAF, ranked. Diamonds (◆) denote samples previously called KIT D816V positive by commercial dPCR; circles (●) denote previously called wild-type. Several samples called wild-type by commercial dPCR returned detectable variant signal on Countable PCR, consistent with the analytical sensitivity below the 0.03% commercial dPCR LoD.

An expanded cohort of 23 deidentified patient samples (290 to 2,116 ng DNA input in a single reaction, no normalization) was then evaluated on Countable PCR (Figure 4). Several samples previously called wild-type by commercial dPCR showed detectable variant signal on Countable, consistent with the analytical sensitivity demonstrated above.

Conclusion

- Countable PCR enabled detection of KIT D816V at 0.015% VAF in a single reaction, extending sensitivity below the limit of detection of the commercial dPCR comparator and removing the need for multi-well pooling.
- Inter-site agreement was high across the full VAF range (0.03% to >40%) in 15 patient samples re-analyzed at two independent sites.
- An expanded cohort of 23 patient samples was reproducibly analyzed across a 7x range of DNA input (290 to 2,116 ng) without upfront normalization, and detected mutation in patient samples previously undetected in dPCR.
- The wide input tolerance of Countable PCR simplifies workflow by removing the upstream normalization step required in conventional dPCR, reducing hands-on time and sample re-runs due to oversaturation.
- These results support deep-sampling, single-molecule digital PCR as a viable approach for rare-variant quantification in translational and clinical research.

Learn more about how Countable PCR redefines sensitivity in genomics.

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