H010

KIT(D816V) Digital PCR Testing

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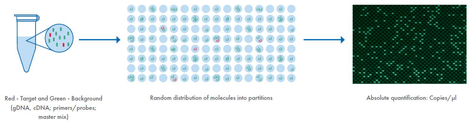
Background

Systemic mastocytosis (SM) is a hematopoietic neoplasm characterized by an abnormal growth of clonal mast cells in bone marrow and other extracutaneous organs. KIT is a receptor tyrosine kinase involved in proliferation of mast cells, melanocytes, germ cells, and hematopoietic stem cells. The vast majority (>90%) of SM cases have a somatic missense mutation c.2447A>T (p.D816V) of the KIT gene in exon 17. This change results in ligand-independent constitutive activation of KIT and leads to increased cell proliferation and accumulation in various organs, and a reduction in cell death. The detection of KIT D816V is one of the minor diagnostic criteria for SM per the WHO system. Quantitative detection using digital PCR may aid physicians in diagnosis and therapeutic monitoring of patients with SM. In this study, we have evaluated the clinical and analytical performance features of the assay.

Methods

Total genomic DNA is extracted and amplified using a multiplex digital PCR with wild-type and mutant-specific probes on QIAcuity One 5plex Digital PCR system. DNA from SM specimens were used to evaluate accuracy, repeatability, reproducibility, analytical detection sensitivity and stability of the assay.



Red - Target and Green - Background (gDNA, cDNA, primers/probes; master mix)

Random distribution of molecules into partitions

Absolute quantification: Copies/µl

Results

Of the 21 specimens (blood, bone marrow and cell pellet) tested during validation, results from 11 specimens with p.D816V mutation and 10 specimens without the mutation were 90.4% concordant with results obtained by next-generation sequencing (NGS). Repeatability was 100% and reproducibility was 95% concordant using 10 specimens with p.D816V mutation and 10 specimens without the mutation. This assay has a sensitivity to detect approximately 0.03% KIT p.D816V somatic mutations. The DNA stored at 2-8ºC was stable for at least one year. The EDTA, heparin blood and bone marrow specimens were stable for at least 30 days stored at 15-30ºC.

Accuracy Validation Data

10 wild type and 11 samples of known p.D816V mutation of the KIT gene were assayed and compared to the sample results obtained by next-generation sequencing (NGS). The results were 90.4% concordant.

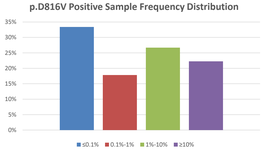
Sample#	Sample Type	MUT Concentration (copies/ul)	WT Concentration (copies/ul)	Result	Expected %	Difference	Variance	Concordant call
NTC	n/a	0	0	#DIV/0!	n/a	n/a	n/a	n/a
Sample 01	LT BM	84	460	15.46%	21.70%	-6.24%	28.74%	Variance>10%
Sample 02	GT BM	63	699	8.26%	8.30%	-0.04%	-0.49%	yes
Sample 03	GT BM	125	360	25.82%	26.80%	-0.98%	-3.66%	yes
Sample 04	LT BM	191	354	34.99%	33.50%	1.49%	4.45%	yes
Sample 05	GT BL	229	381	37.55%	39.90%	-2.35%	-5.89%	yes
Sample 06	GT BM	87	295	22.68%	24.70%	-2.02%	-8.18%	yes
Sample 07	GT BM	345	443	43.82%	42.90%	0.92%	2.14%	yes
Sample 08	GT BM	129	307	29.64%	27.60%	2.04%	7.39%	yes
Sample 09	LT BM	108	141	43.24%	45.99%	-2.75%	-5.98%	yes
Sample 10	LT BM	89	965	8.40%	9.75%	-1.35%	13.87%	Variance>10%
Sample 11	GT BM	0	621	0.01%	0.00%	0.01%		yes
Sample 12	LT BL	0	729	0.02%	0.00%	0.02%		yes
Sample 13	LT BM	0	741	0.02%	0.00%	0.02%		yes
Sample 14	GT BM	0	848	0.01%	0.00%	0.01%		yes
Sample 15	GT BM	0	681	0.01%	0.00%	0.01%		yes
Sample 16	GT BM	0	629	0.03%	0.00%	0.03%		below NGS detection level
Sample 17	GT BM	0	572	0.02%	0.00%	0.02%		yes
Sample 18	GT BM	0	769	0.02%	0.00%	0.02%		yes
Sample 19	GT BM	0	628	0.00%	0.00%	0.00%		yes
Sample 20	LT BL	0	822	0.00%	0.00%	0.00%		yes
Sample 21	Cell Pellet	419	684	46.39%	47.80%	-1.41%		yes

Precision

Repeatability (intra-assay precision) was 100% concordant for KIT p.D816V results using 10 positive and 10 negative specimens.

Reproducibility (inter-assay precision) was 95% concordant for KIT p.D816V results using 10 positive and 10 negative specimens. One negative specimen showed borderline positive 0.03% and 0.04% in 2 of 3 runs.

The KIT (D816V) Test has been offered as a clinical test at LabCorp. Of the 258 specimens tested, 80.23% (207) were negative, 17.44% (45) were positive. The age distribution for patients with a positive result is 30-79 in this set of data. Results could not be obtained in 2.33% (6) specimens due to low wild type copy number (specimen degradation and limited amount of DNA).



p.D816V Variant Allele Frequency (VAF)	Percentage in Positive Samples
≤0.1%	33%
0.1%-1%	18%
1%-10%	27%
≥10%	22%

-Our data showed that 33% positive patient samples had <0.1% KIT p.D816V variant. A High sensitivity PCR detection method is needed for the variant detection.
-KIT p.D816V mutation with variant allele frequency (VAF) ≥10% in bone marrow cells or peripheral blood leukocytes qualifies as a B-finding (burden of disease). 22% of positive patient samples had a VAF ≥10%

Conclusions

The KIT (D816V) Test is a robust, reproducible, and sensitive assay using blood, bone marrow and cell pellet specimens for systemic mastocytosis assessment and therapeutic monitoring.

References

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