Clinical Validation of the Bio-Rad ddPCR MSI Test for the Detection of Microsatellite Instability in Tumor and Plasma cfDNA Samples

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Microsatellite Instability Introduction

Microsatellite Instability is a tumor phenotype that arises from defects in the Mismatch Repair (MMR) pathway[1]. Tumors exhibiting microsatellite instability are called MSI-H and have distinct prognostic as well as therapeutic options[2]. MSI-H tumors are more likely to respond to immunotherapy compared to Microsatellite Stable (MSS) tumors[3]. Therefore, a non-invasive test that can detect MSI status with high sensitivity is urgently needed in the clinic. Additionally, given the evolving clinical relevance of liquid biopsies, tests that can be used in both tissue and blood are highly desired. We have developed a ddPCR-based test for determining the MSI status in tissue and cfDNA (liquid biopsy) samples by analyzing 5 common mononucleotide MSI markers[4]. The test can be applied to tumor samples (either fixed or fresh tumor samples) as well as to liquid biopsy (plasma cell-free DNA) samples.

Clinical Performance of Bio-Rad ddPCR MSI Test

Clinical samples were obtained from commercial vendors. Samples were either tissue (FFPE or fresh-frozen) or plasma, and the tumor MSI status was determined by the vendors. Demographic information on the samples is shown in Table 1.

	Male	Female
All samples, number (%)	49 (0.48)	54 (0.52)
Age range, median	22-90, 64	24-86, 71
MSI-H, number (%)	39 (0.38)	41 (0.40)

Table 1. Demographic characteristics of samples.

MSI Detection Rate in Tissue Samples by Tumor Stage

DNA was extracted from 69 paraffin-embedded and micro dissected colorectal cancer tissues and tested with the Bio-Rad ddPCR MSI test. Table 3 and Figure 2 show the concordance between ddPCR and vendor-tested MSI status by tumor stage.

Tissue Concordance in Colorectal Cancer by Tumor Stage		Bio-Rad	
		MSI-H	MSS
Stage I	MSI-H	1	0
	MSS	0	4
Stage II	MSI-H	27	1
	MSS	0	9
Stage III	MSI-H	15	1
	MSS	0	9
Stage IV	MSI-H	1	0
	MSS	0	1

Table 3. MSI status concordance between various MSI by tumor stage.

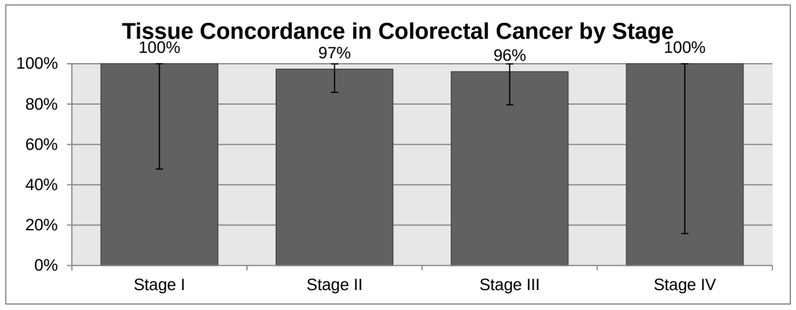

Figure 2. Percent concordance of MSI results by tumor stage.

MSI Detection in Plasma cfDNA Samples

Plasma samples from patients with MSI-H CRC tumors as confirmed by tissue testing were tested with the Bio-Rad ddPCR MSI test. The test demonstrated 100% PPV and 53% sensitivity. Incomplete concordance may be attributed to low tumor shedding and plasma volume (average 1.9mL, range 1.0-4.75mL). Table 4 and Figure 3 show the performance of the test by tumor stage in plasma.

Plasma Concordance in Colorectal Cancer by Tumor Stage*		Bio-Rad	
		MSI-H	MSS
Stage II	MSI-H	6	10
	MSS	0	0
Stage III	MSI-H	8	3
	MSS	0	0
Stage IV	MSI-H	3	2
	MSS	0	0

Table 4. MSI status concordance between tissue and matched plasma by tumor stage. *Two samples omitted; one failed post-droplet generation (stage II sample depleted), and one suspected of having a germline polymorphism (stage III sample indeterminate).

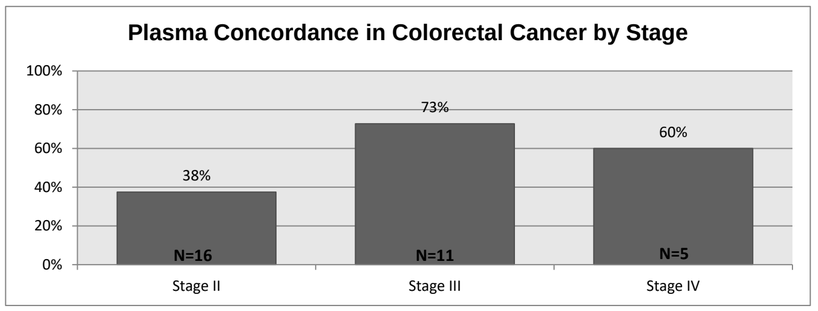

Figure 3. Percent concordance between tissue and matched plasma samples by tumor stage.

Concordance of Bio-Rad ddPCR MSI Test with Vendor-Tested MSI Tests

Tissue samples from colorectal cancer (CRC) tumors were purchased from 4 commercial vendors. The samples were tested with the Bio-Rad ddPCR MSI test, and the results were compared with the MSI status information provided by the vendors. Table 2 and Figure 1 show the concordance between the Bio-Rad ddPCR MSI test and other common MSI testing modalities.

Tissue Concordance in CRC Stages I-IV by Vendor and Test Method		Bio-Rad	
		MSI-H	MSS
Vendor 1 (IHC)	MSI-H	16	1
	MSS	0	0
Vendor 2 (PCR-CE)	MSI-H	7	0
	MSS	0	1
Vendor 3 (PCR-CE)	MSI-H	12	0
	MSS	0	12
Vendor 4 (PCR-Melt Curve)	MSI-H	9	1
	MSS	0	10

Table 2. MSI status concordance for the Bio-Rad ddPCR MSI test against other MSI tests. Vendor 3 (one sample set, IHC and PCR-CE tested) was IHC-neg and PCR-pos for 2 samples, or 10/12 (83%) IHC concordant with ddPCR. The cohort of 69 tissue samples tested with ddPCR was 67/69 (97%) concordant with vendor-tested PCR.

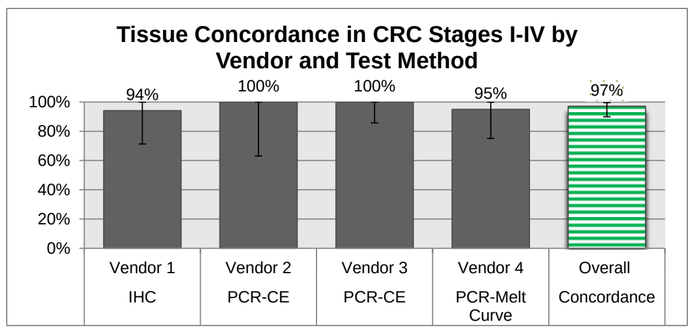

Figure 1. Percent agreement between various MSI tests and the Bio-Rad ddPCR MSI test.

Conclusions

We have developed a digital PCR–based multiplex assay for determining the microsatellite instability (MSI) status in solid tumor and plasma cell-free DNA samples. In tissue samples, the Bio-Rad ddPCR MSI test demonstrated high (>97%) concordance with all reference MSI test methods, and it performed consistently well in samples from all tumor stages. Compared with IHC and conventional PCR methods, ddPCR and tissue DNA had a specificity of 97.1%, PPV of 100%, and NPV of 92%. In cfDNA samples the test had an overall sensitivity of 53% (69% in late stage samples) with 100% PPV.

References

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See corresponding poster # ST063/Abstract # 16906 “Analytical Performance of a Novel Microsatellite Instability (MSI) Droplet Digital PCR Test”

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