Evaluation of a Fully Automated Idylla Test System for Microsatellite Instability in Colorectal Cancer

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Abstract

A fast and accurate test for microsatellite instability (MSI) is crucial in colorectal cancer prognosis and treatment. In this manuscript, we validated an automated Idylla MSI detection platform using 42 patient samples. The new Idylla MSI system provided accurate results in 2.5 hours. This automated tool greatly simplified the MSI detection process.

Background: Microsatellite instability (MSI) is a phenotype commonly observed in colorectal cancer, and is caused by a deficient mismatch repair system. Determining MSI status greatly aids tumor prognosis and treatment plans in colorectal cancer, and plays a critical role in recent United States Food and Drug Administration-approved immunotherapies. As recognition of its importance grows, MSI has been identified in more types of cancers, underscoring the importance of accurate assays for determining MSI status in tumor cells. Currently, tumor MSI status is detected via polymerase chain reaction-based methods or immunohistochemistry. **Materials and Methods:** In this study, we tested a new, fully automated MSI detection system (Idylla MSI detection kit) released by Biocartis. We evaluated 42 formalin-fixed paraffin-embedded tumor tissues, which were clinically tested for MSI status using the polymerase chain reaction or immunohistochemistry method, with the Idylla MSI detection system. **Results:** The Idylla MSI detection system showed an overall 97.62% concordance rate with previously used methods. Moreover, this fully automated system requires less than 5 minutes "hands on" preparation time and 150 minutes total run time per sample. **Conclusion:** The Biocartis Idylla MSI kit proves a powerful tool to accurately detect MSI status in tumor cells in a rapid and almost labor-free manner.

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Introduction

Mutations, like insertions and deletions, are easily introduced into the genome during DNA replication and cause genomic instability. During DNA replication, mismatches are corrected by the mismatch repair (MMR) system to ensure accurate duplication of genetic information.[1] A proficient MMR system recognizes mismatches during DNA replication and corrects errors via digesting the mismatch sequences. The proteins that form the MMR system are encoded by 4 genes: MLH1, MSH2, MSH6, and PMS2. A mutation within any of these 4 genes results in a deficient MMR (dMMR) system.[2] With a dMMR system, DNA replication errors are not fixed efficiently.

Microsatellites are short DNA sequences with repetitive motifs that naturally occur in the human genome.[3] Owing to their repetitive structure, microsatellites are especially prone to DNA replication errors. When combined with a dMMR, DNA

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replication errors in microsatellite regions are not fixed efficiently, causing microsatellite instability (MSI). Therefore, MSI is an excellent marker for a dMMR system.

A dMMR system is a common cause of cancer. Hence, MSI is also a hallmark of dMMR-related cancer. Clinical data suggests 15% of colorectal cancer (CRC) results from a dMMR system, characterized by MSI.[4] In CRC, one-third of MSI is caused by germline mutations in 1 or more of the 4 MMR genes (MLH1, MSH2, MSH6, and PMS2) (Lynch Syndrome).[5] Approximately two-thirds of MSI are owing to the loss of MLH1 caused by hypermethylation of the MLH1gene promoter region.[6,7]

MSI status of CRC plays an important role in disease prognosis and treatment. Data from multiple clinical studies revealed patients with CRC with microsatellite instability status (MSI-H) had a better prognosis when treated or untreated with 5-fluorouracil (5-FU)-based adjuvant chemotherapy compared with patients with microsatellite stable status (MSS or MSI-L).[8-12] The relationships between MSI status, tumor prognosis, and treatment responses in patients with CRC are stage-dependent. Results from a series of clinical studies indicated that, in patients with stage II CRC, MSI-H/dMMR tumors did not benefit from 5-FU-based adjuvant therapy.[13-19] Surgery alone provided better survival rates for those patients.[20,21] In stage III CRC, the association between MSI status and tumor prognosis and treatment is more complicated. MSI-H tumor cell lines were sensitive to oxaliplatin in preclinical studies.[22] However, clinical findings were contradictory, revealing MSI status did not correlate with oxaliplatin efficacy.[23] Results from other clinical trials suggested FOLFOX (5-FU plus folic acid plus oxaliplatin) therapy may be more effective for certain subgroups of MSI-H/dMMR tumors at stage III with proximal location.[24-26]

MSI status has become a decisive factor when electing an immunotherapy treatment. MSI-H tumors present a strong inflammatory response, triggering an immune escape mechanism in tumor cells, often via production of checkpoint proteins programmed cell death protein 1 and programmed death-ligand 1. Therefore, MSI-H tumors are highly sensitive to immunotherapy.[27-29] Clinical trials of anti-programmed cell death protein 1 drugs pembrolizumab and nivolumab showed promising results in patients with MSI-H cancer.[30,31] The United States Food and Drug Administration granted accelerated approval for these drugs in patients with MSI-H or dMMR solid tumors. MSI-H tumors are common and not limited to CRC. They were also found in non—small-cell lung cancer, genitourinary cancer, breast cancer, and many other type of cancers. This underscores the importance of accurate testing of MSI status in these cancers, enabling patients to get the best treatment.

Traditionally, MSI status is detected via polymerase chain reaction (PCR) of a panel of microsatellite markers.[32] The Bethesda panel, a 5-marker panel, was established for MSI testing in a 1997 meeting. This panel includes 2 mononucleotide (BAT-25 and BAT-26) and 3 dinucleotide (D5S345, D2S123, and D17S250) repeats.[33] MSI status is categorized based on the number of mutated markers: A MSI-H classification is given when 2 or more of the 5 markers showed instability, MSI-L when only 1 marker showed instability, and MSS when no markers showed instability. In 2002, a second consensus meeting recommended substituting dinucleotide repeats with mononucleotide repeats.[34]

Currently, the Bethesda panel and a commercial panel developed by Promega are widely used by most labs for MSI status testing. In 2004, Buhard and colleagues demonstrated a panel of 5 quasimonomorpic mononuleotide makers (BAT-25, BAT-26, NR-27, NR-21, and NR-24) were able to accurately evaluate tumor MSI status without normal tissue for comparison.[35] An alternative method is using MLH1, MSH2, PMS2, and MSH6 antibodies to perform immunohistochemistry (IHC) analysis to determine MSI status (MMR status).[36] With the development of next generation sequencing technology, multigene panel next generation sequencing is also used to test tumor MSI status.[37]

Recently, Biocartis released a fully automated Idylla MSI testing assay. This assay utilizes a new set of 7 biomarkers to evaluate tumor MSI status without the need for reference tissue DNA. In this study, we tested 42 CRC samples with known MSI status as detected by previously described methods[35,36] on the Idylla MSI testing system. In addition to a high concordance rate between the Idylla MSI test and traditional methods, the automated Idylla MSI assay required minimal labor and significantly less run time to get results.

Materials and Methods

Sample Collection and Experiment Design

We collected 42 formalin-fixed paraffin-embedded (FFPE) CRC tissues with known MSI status from 2 major hospitals in China (Shengjing Hospital, Shenyang, Liaoning Province and Qingdao Hospital, Qingdao, Shandong Province). These samples' MSI status was previously tested using a PCR-based MSI assay or IHC. Of the 42 samples, there were 20 MSI-H, 20 MSS, and 2 MSI-L. Based on Idylla MSI assay reporting, we grouped these 42 samples into a positive group (20 MSI-H) and a negative group (22 MSS and MSI-L), respectively.

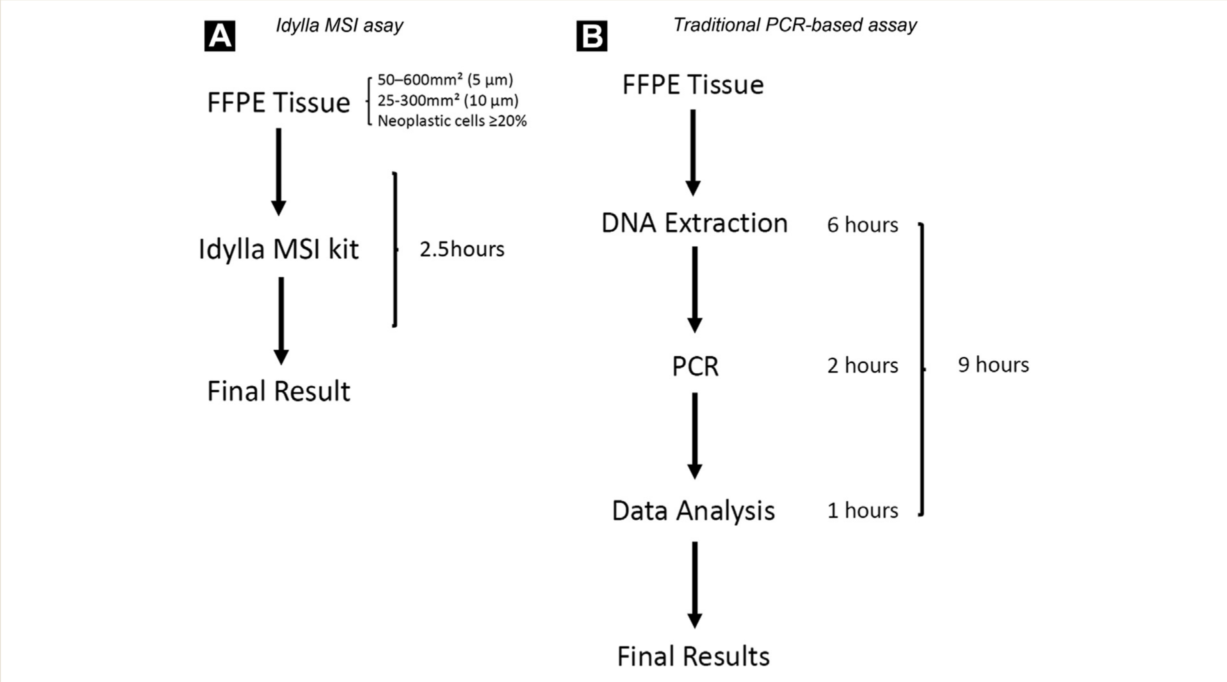
Idylla MSI Assay

Following manufacturer's instructions, 100 to 200 mm^2 of each sample's 10-μm thick FFPE tissue was placed between 2 wet filter papers (GE Healthcare Life Sciences Whatman, Cat# 1001-6508) soaked with nuclease-free water (Thermo Scientific, REF# R0581). Each sample-paper sandwich was transferred into an Idylla MSI cartridge. After scanning the MSI cartridge barcode, the assay type and designated protocol were automatically registered, and the sample ID was manually entered in the console's software. The MSI cartridge was then loaded into the Idylla console, and the assay started automatically. Each sample's run took 150 minutes total. After each run, a detailed report with biomarker scores and quality control status was generated by the built-in software. The run information was simultaneously deposited to an online web application, Idylla Explore (https://idyllaexplore.biocartis.com/), for remote access.

Results

The Idylla MSI test workflow is user-friendly and straightforward. As shown in Figure 1A, a minimum of 50 mm^2 of 5-μm FFPE tissue with greater than 20% neoplastic cell content is required for the assay. Within the reaction cartridge, DNA is directly liberated from FFPE tissue without requiring a long deparaffinization process. Next, 7 biomarkers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2) are amplified via PCR for a downstream melting curve analysis. The analysis

Figure 1 Comparison of the Idylla MSI Assay and Conventional PCR-based Method Workflow. A, Idylla MSI Assay Workflow. B, Traditional PCR-based Assay Workflow



Abbreviations: FFPE = formalin-fixed paraffin-embedded; MSI = microsatellite instability; PCR = polymerase chain reaction.

software, built on a neural network-based algorithm, then detects the mutation status of each marker by evaluating a qualitative score derived from the melting curve analysis (Figure 2A). A report with test results, marker scores, and quality control status is then automatically generated. Consistent with previously established criteria, tumors with 2 or more mutated markers are reported as MSI-H by the software. However, the Idylla MSI assay does not have a MSI-L category. One or no mutated markers will both be considered as microsatellite stable (MSS).

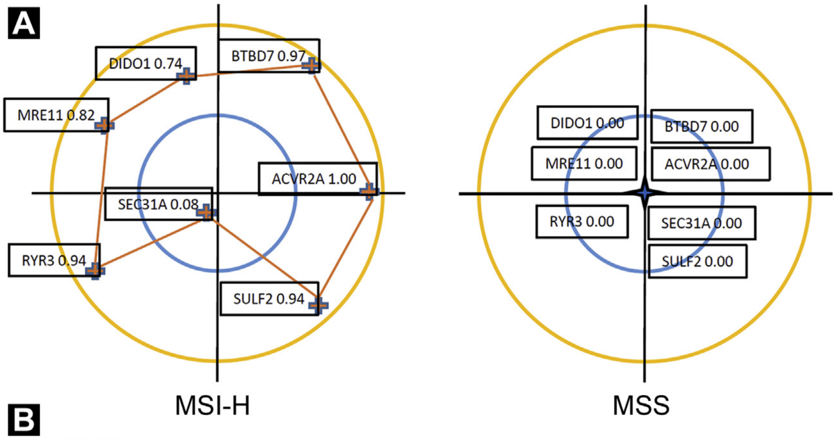
We obtained 42 FFPE tumor tissues from 2 medical centers. These samples had been tested for MSI status using either PCR-based methods or IHC. Based on their MSI status, we divided the samples into 2 groups: a positive group with 20 MSI-H samples and a negative group including 20 MSS samples and 2 MSI-L samples. We analyzed all samples through the Idylla MSI test system. Nineteen of 20 MSI-H tumors were detected as MSI-H by the Idylla MSI test system, with a concordance rate of 95%. All 22 samples from the negative group were identified as MSS by the Idylla MSI test system, with a 100% concordance rate (Table 1). The overall concordance rate was 97.62%. To check the reproducibility of the Idylla MSI test system, a few samples were run in duplicate, and the same results were observed. We also ran a small-scale blind test and observed a 100% concordance rate between the Idylla MSI test and the original test (see Supplemental Table 1 in the online version).

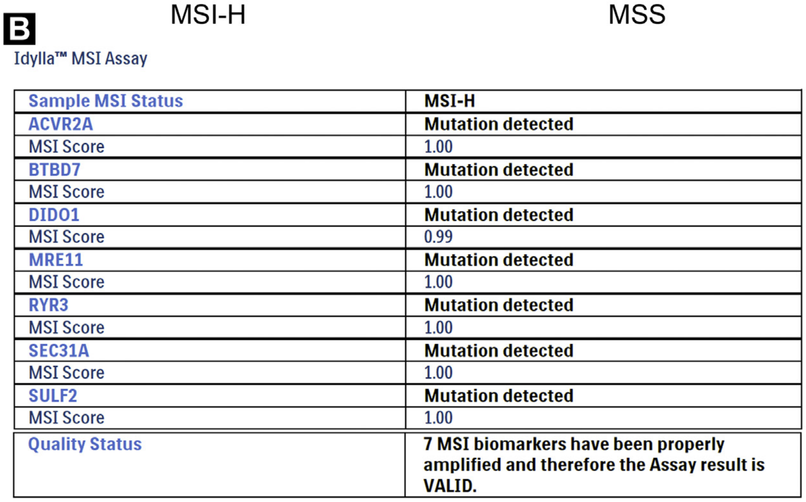
The MSI test results produced by the Idylla MSI test system were highly concordant with results derived from conventional methods, yet the total test procedure was largely simplified. This fully automated platform enables rapid and accurate MSI status tests with no other instruments or molecular biology preparation needed.

Discussion

Tumor MSI status has emerged as an important molecular marker for tumor prognosis and treatment. The accuracy and turnaround time of an MSI status assay is critically important when choosing and implementing treatment plans for patients with cancer. In this study, we used 42 FFPE tumor tissues to test the accuracy of a fast, fully automated MSI test system. We found a high concordance rate (97.62%) when comparing the samples' MSI status as determined using the Idylla MSI system and conventional methods. Conventional PCR-based MSI status workflows consist of deparaffinization, tissue lysis, DNA extraction, and PCR. The whole process takes ∼9 hours and requires extensive molecular biology operation (Figure 1B). The IHC method that is widely used by clinical pathology laboratories requires 2 hours to overnight incubation prior to deparaffinization and at least 5 hours hands-on time for deparaffinization, rehydration, antigen retrieval, and staining steps. In contrast, the automated Idylla MSI system only takes 2.5 hours to produce accurate results without any molecular biology or immunohistology preparation. The total hands-on time is less than 5 minutes. In addition to a largely simplified workflow, the per-sample cost of the Idylla MSI system is close to 50% of the IHC method (equipment and maintenance costs not included). The Idylla MSI system provides a great platform for pathology labs to quickly detect tumor MSI status without DNA extraction. Even though this system is currently for research use only, it shows great potential for future clinical testing purposes.

Figure 2 Idylla MSI Assay Data Presentation. A, MSI Status Is Decided by the Number of Mutated biomarkers. A Qualitative Score is Generated for Each biomarker Based on the Melting Curve Analysis. The Score Expresses the Probability of Mutations in Each Valid Marker. Zero Means No Mutation and 1.00 Means a 100% Chance of Mutation. Left Panel, MSI-H; Right Panel, MSS. B, An Example MSI Assay Report From the Idylla^{TM} Console




B

Idylla™ MSI Assay

Sample MSI Status	MSI-H
ACVR2A	Mutation detected
MSI Score	1.00
BTBD7	Mutation detected
MSI Score	1.00
DIDO1	Mutation detected
MSI Score	0.99
MRE11	Mutation detected
MSI Score	1.00
RYR3	Mutation detected
MSI Score	1.00
SEC31A	Mutation detected
MSI Score	1.00
SULF2	Mutation detected
MSI Score	1.00
Quality Status	7 MSI biomarkers have been properly amplified and therefore the Assay result is VALID.

Abbreviations: MSI = microsatellite instability; MSI-H = microsatellite instability-high; MSS = microsatellite stable.

The Idylla MSI assay is a closed system, so no alterations to the assay or reporting algorithm can be made. To make an MSI determination, the assay uses a dedicated post-processing algorithm. First, a mathematical transformation algorithm will transform fluorescence measurements generated input data by the Idylla platform into a characteristic pattern of 16 output parameters per curve. This mathematical transformation algorithm takes into account variability that might exist between instruments. Then, a dedicated classification algorithm is used to classify the characteristic pattern produced for each biomarker by the mathematical transformation algorithm. A neural network optimized on several thousand wild-type and mutant fluorescence profiles will then calculate a probability/similarity score (MSI Score) for any given valid biomarker-specific profile, reflecting the status of each biomarker.

The 7 novel Idylla MSI biomarkers are a robust selection from an exclusively licensed broader set of 59 MSI biomarkers previously described by Zhao and colleagues.[38] The 7 biomarkers detect somatic single nucleotide mutation differences in short homopolymers, consistently observed in several ethnic groups, including Caucasian, African American, and Asian. The final selection of 7 biomarkers results from several selection criteria: (1) high frequency markers in MSI-H CRC and endometrial cancers; (2) optimization of the number of biomarkers that can be combined in 1 cartridge; (3) stable and robust assay that is compatible with beacon technology; (4) stable markers throughout different ethnicities; (5) multiplex capabilities with maximization of final PCR end product; (6) compatibility of assay components with buffers and stability requirements; and (7) compatibility of read-out with mathematical transformation algorithm to maximize classification of MSS versus MSI-H profiles.

Detection of these specific targets is performed using fluorescently labeled molecular beacons after PCR amplification. These beacons differentially melt from the wild-type or mutated amplicons with increasing temperature. The output is detected by fluorescence and analyzed by a dedicated and automated Idylla MSI Detection workflow via a mathematical transformation algorithm and a

Table 1 MSI Test Results From Idylla MSI Test System

Sample	MSI Status	Idylla MSI Assay Results	Tumor Cell Content, %	Idylla MSI Biomarkers With Mutation Detected
CRC-1	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, RYR3, SEC31A, SULF2,
CRC-2	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,
CRC-3	MSI-H[a]	MSI-H	>50	ACVR2A, DIDO1, MRE11, SULF2,
CRC-4	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, RYR3,
CRC-5	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,
CRC-6	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,
CRC-7	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, SULF2,
CRC-8	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,
CRC-9	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, SEC31A, SULF2,
CRC-10	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SULF2,
CRC-11	MSI-H[a]	MSI-H	>50	ACVR2A, DIDO1, MRE11, SEC31A, SULF2,
CRC-12	MSI-H[a]	MSI-H	>50	BTBD7, DIDO1, MRE11, RYR3,
CRC-13	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, SEC31A, SULF2,
CRC-14	MSI-H[a]	MSI-H	>50	ACVR2A, DIDO1,RYR3,
CRC-15	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,
CRC-16	MSI-H[b]	MSI-H	>20	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,
CRC-17[c]	MSI-H[b]	MSI-H	>20	BTBD7, DIDO1, MRE11, SULF2,
CRC-18	MSI-H[b]	MSI-H	>20	DIDO1, MRE11,
CRC-19	MSI-H[a]	MSI-H	>50	ACVR2A, BTBD7, DIDO1, MRE11, SEC31A, SULF2,
CRC-20	MSI-H[a]	MSS	>50	No mutation detected
	Concordance	95%		
CRC-21	MSS[b]	MSS	>20	No mutation detected
CRC-22	MSS[b]	MSS	>20	No mutation detected
CRC-23	MSS[b]	MSS	>20	No mutation detected
CRC-24	MSS[b]	MSS	>20	No mutation detected
CRC-25	MSS[b]	MSS	>20	No mutation detected
CRC-26	MSS[b]	MSS	>20	No mutation detected
CRC-27[c]	MSS[b]	MSS	>20	No mutation detected
CRC-28	MSS[b]	MSS	>20	No mutation detected
CRC-29	MSS[b]	MSS	>20	No mutation detected
CRC-30[c]	MSS[b]	MSS	>20	No mutation detected
CRC-31	MSI-L[b]	MSS	>20	No mutation detected
CRC-32	MSS[b]	MSS	>20	No mutation detected
CRC-33	MSS[b]	MSS	>20	No mutation detected
CRC-34	MSS[b]	MSS	>20	No mutation detected
CRC-35	MSI-L[b]	MSS	>20	No mutation detected
CRC-36	MSS[b]	MSS	>20	No mutation detected
CRC-37	MSS[b]	MSS	>20	No mutation detected
CRC-38	MSS[b]	MSS	>20	No mutation detected
CRC-39	MSS[b]	MSS	>20	No mutation detected
CRC-40	MSS[a]	MSS	>50	No mutation detected
CRC-41	MSS[a]	MSS	>50	No mutation detected
CRC-42	MSS[a]	MSS	>50	No mutation detected
	Concordance	100%		
	Overall concordance	97.62%		

Abbreviations: CRC = colorectal cancer; MSI = microsatellite instability; MSI-H = microsatellite instability-high; MSI-L = microsatellite instability-low; MSS = microsatellite stable.

[a] The original MSI test results were determined by the immunohistochemistry method.

[b] The original MSI test results were determined by the polymerase chain reaction-based method.

[c] These samples were tested in duplicate. The same results were observed.

classification algorithm. The report will show an overall result that classifies the sample as MSI-H, MSS, or invalid. The report will also show an individual mutant or non-mutant result for each separate marker with an MSI score per marker.

In our study, one sample from the positive MSI-H group returned a discordant result from the Idylla MSI system. We closely looked at the 7 biomarkers tested by the Idylla system in this sample. None of these markers showed a mutation score. We speculate this might be because of tumor heterogeneity.[39] The FFPE tissue may have had a relatively low amount of neoplastic cell content that was below the Idylla MSI kit's detection threshold (> 20%). Owing to the limited amount of this sample, we did not have enough tissue to repeat the test or confirm the result with a conventional PCR method. Nonetheless, the results still reached a 97.62% concordance rate, which was very close to the manufacturer's published number (98.7% with Promega, 97.6% with IHC, European Society for Medical Oncology 2018 Congress, October 19-23, 2018, Munich, Germany).

The Idylla MSI test result does not include a MSI-L classification. This may limit its clinical utility for patients with MSI-L tumors. However, the biological significance of MSI-L status is still controversial. Several studies indicated MSI-L status may be associated with a higher frequency of KRAS mutations and more common in serrated neoplasms.[40,41] Other studies demonstrated that MSI-L and MSS tumors shared many genetic and pathologic properties and were not distinguishable from each other.[42-44] Further, there is a lack of evidence linking the MSI-L phenotype to any known diseases caused by a dMMR system. Regardless, the MSI-H phenotype is the most clinically relevant phenotype in the majority of cancer types.

Conclusion

The Idylla MSI test system generates reliable results with minimal hands-on preparation and short turnaround time. It provides a great platform for the increasing need for tumor MSI testing.

Clinical Practice Points

- Conventional MSI detection methods require multistep molecular biology preparation. The process is long and not easily accessible for many clinical pathology laboratories.
- The new Idylla MSI detection platform that we introduced in this manuscript is fast and fully automated. The accuracy of this new platform is comparable to the traditional methods. The turnaround time is much shorter, plus no molecular biology preparation is needed.
- The Idylla MSI assay platform provides a reliable and convenient tool for MSI testing under clinical settings.

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Disclosure

The authors have stated that they have no conflicts of interest.

Supplemental Data

Supplemental table accompanying this article can be found in the online version at https://doi.org/10.1016/j.clcc.2019.05.006.

References

1. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; 138:2073-87.
2. Fabio G, Monica B, Andrea S, Giuseppe P, Stefano C. The evolving role of microsatellite instability in colorectal cancer: a review. *Cancer Treat Rev* 2016; 51:19-26.
3. Kawakami H, Zaanan A, Sinicrope FA. Microsatellite instability tesing and its role in the management of colorectal cancer. *Curr Treat Options Oncol* 2015; 16:30.
4. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology* 2008; 135:1079-99.
5. Ladabaum U, Wang G, Terdiman J, et al. Strategies to identify the Lynch syndrome among patients with colorectal cancer: a cost-effectiveness analysis. *Ann Intern Med* 2011; 155:69-79.
6. Toyota M, Issa JP. CpG island methylator phenotypes in aging and cancer. *Semin Cancer Biol* 1999; 9:349-57.
7. Hughes LA, Khalid-de Bakker CA, Smits KM, et al. The CpG island methylator phenotype in colorectal cancer: progress and problems. *Biochim Biophys Acta* 2012; 1825:77-85.
8. Sinicrope FA, Rego RL, Halling KC, et al. Prognostic impact of microsatellite instability and DNA ploidy in human colon carcinoma patients. *Gastroenterology* 2006; 131:729-37.
9. Gafa R, Maestri I, Matteuzzi M, et al. Sporadic colorectal adenocarcinomas with high-frequency microsatellite instability. *Cancer* 2000; 89:2025-37.
10. Lanza G, Gafa R, Santini A, Maestri I, Guerzoni L, Cavazzini L. Immunohistochemical test for MLH1 and MSH2 expression predicts clinical outcome in stage II and III colorectal cancer patients. *J Clin Oncol* 2006; 24:2359-67.
11. Samowitz WS, Curtin K, Ma KN, et al. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. *Cancer Epidemiol Biomarkers Prev* 2001; 10:917-23.
12. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; 23:609-18.
13. Jover R, Zapater P, Castells A, et al. Gastrointestinal Oncology Group of the Spanish Gastroenterological Association. The efficacy of adjuvant chemotherapy with 5-fluorouracil in colorectal cancer depends on the mismatch repair status. *Eur J Cancer* 2009; 45:365-73.
14. Kim GP, Colangelo LH, Wieand HS, et al, National Cancer Institute. Prognostic and predictive roles of high-degree microsatellite instability in colon cancer: a National Cancer Institute-National Surgical Adjuvant Breast and Bowel Project Collaborative Study. *J Clin Oncol* 2007; 25:767-72.
15. Hemminki A, Mecklin JP, Jarvinen H, Aaltonen LA, Joensuu H. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. *Gastroenterology* 2000; 119:921-8.
16. Elsaleh H, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumor site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000; 355:1745-50.
17. Tejpar S, Saridaki Z, Delorenzi M, Bosman F, Roth AD. Microsatellite instability, prognosis and drug sensitivity of stage II and III colorectal cancer: more complexity to the puzzle. *J Natl Cancer Inst* 2011; 103:841-4.
18. Arnold CN, Goel A, Boland CR. Role of hMLH1 promoter hypermethylation in drug resistance to 5-fluorouracil in colorectal cancer cell lines. *Int J Cancer* 2003; 106:66-73.
19. Fischer F, Baerenfaller K, Jiricny J. 5-Fluorouracil is efficiently removed from DNA by the base excision and mismatch repair systems. *Gastroenterology* 2007; 133:1858-68.
20. Quasar Collaborative Group, Gray R, Barnwell J, McConkey C, et al. Adjuvant chemotherapy versus observation in patients with colorectal cancer: a randomised study. *Lancet* 2007; 370:2020-9.
21. Sargent DJ, Shi Q, Yothers G, et al. Prognostic impact of deficient mismatch repair (dMMR) in 7,803 stage II/III colon cancer (CC) patients (pts): A pooled individual pt data analysis of 17 adjuvant trials in the ACCENT database. *J Clin Oncol* 2014; 32(15 Supp):3507.
22. Fink D, Nebel S, Aebi S, et al. The role of DNA mismatch repair in platinum drug resistance. *Cancer Res* 1996; 56:4881-6.
23. Gavin PG, Paik S, Yothers G, Pogue-Geile KL. Colon cancer mutation: prognosis/prediction—response. *Clin Cancer Res* 2013; 19:1301.
24. Gavin PG, Colangelo LH, Fumagalli D, et al. Mutation profiling and microsatellite instability in stage II and III colon cancer: an assessment of their prognostic and oxaliplatin predictive value. *Clin Cancer Res* 2012; 18:6531-41.
25. Flejou JF, Andre T, Chibaudel B, et al. Effect of adding oxaliplatin to adjuvant 5-fluorouracil/leucovorin (5FU/LV) in patients with defective mismatch repair (dMMR) colon cancer stage II and III included in the MOSIAC study. *J Clin Oncol* 2013; 31(15 Supp):3524.
26. Sinicrope FA, Mahoney MR, Smyrk TC, et al. Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. *J Clin Oncol* 2013; 31:3664-72.
27. Westdorp H, Fennemann FL, Weren RD, et al. Opportunities for immunotherapy in microsatellite instable colorectal cancer. *Cancer Immunol Immunother* 2016; 65:1249-59.

28. Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov* 2015; 5:43-51.
29. Kroemer G, Galluzzi L, Zitvogel L, Fridman WH. Colorectal cancer: the first neoplasia found to be under immunosurveillance and the last one to respond to immunotherapy? *Oncoimmunology* 2015; 4:e1058597.
30. Reck M, Rodríguez-Abreu D, Robinson AG, et al, KEYNOTE-024 Investigators. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016; 375:1823-33.
31. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. *N Engl J Med* 2017; 377:1345-56.
32. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; 260:816-9.
33. Boland CR, Thibodeau SN, Hamilton SR, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; 58:5248-57.
34. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004; 96:261-8.
35. Buhard O, Cattaneo F, Wong YF, et al. Multipopulation analysis of polymorphisms in five mononucleotide repeats used to determine the microsatellite instability status of human tumors. *J Clin Oncol* 2006; 24:241-51.
36. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. *J Clin Oncol* 2002; 20:1043-8.
37. Vanderwalde A, Spetzler D, Xiao N, Gatalica Z, Marshall J. Microsatellite instability status determinded by next-generation sequencing and compared with PD-L1 and tumor mutational burden in 11,348 patients. *Cancer Med* 2018; 7: 746-56.
38. Zhao H, Thienpont B, Yesilyurt BT, et al. Mismatch repair deficiency endows tumors with a unique mutation signature and sensitivity to DNA double-strand breaks. *Elife* 2014; 3:e02725.
39. Prieto-Potin I, Montagut C, Bellosillo B, et al. Multicenter Evaluation of the Idylla NRAS-BRAF mutation test in metastatic colorectal cancer. *J Mol Diagn* 2018; 20: 664-76.
40. Jass JR, Biden KG, Cummings M, et al. Characterisation of a subtype of colorectal cacner combining features of the suppressor and mild mutator pathways. *J Clin Pathol* 1999; 52:455-60.
41. Konishi M, Kikuchi-Yanoshita R, Tanaka K, et al. Molecular nature of colon tumors in hereditary nonpolyposis colon cancer, familial polyposis and sporadic colon cancer. *Gastroenterology* 1996; 111:307-17.
42. Thibodeau SN, French AJ, Cunningham JM, et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principle involvement of hMLH1. *Cancer Res* 1998; 48:1713-8.
43. Gonzalez-Garcia I, Moreno V, Navarro M, et al. Standardized approach for microsatellite instability detection in colorectal carcinomas. *J Natl Cancer Inst* 2000; 92:544-9.
44. Wright CM, Dent OF, Barker M, et al. Prognostic significance of extensive microsatellite instability in sporadic clinicopathological stage C colorectal cancer. *Br J Surg* 2000; 87:1197-202.

Supplemental Table 1 Blind Test Results From Idylla MSI Test System

Sample	Idylla MSI Assay Results	Idylla MSI Biomarkers With Mutation Detected	Tumor Cell Content, %	Original MSI Status
Blind test S1	MSI-H	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, SULF2,	>20	MSI-H[a]
Blind test S2	MSS	No mutation detected	>20	MSS[a]
Blind test S3	MSS	No mutation detected	>20	MSS[a]
	Concordance	100%		

Abbreviations: MSI = microsatellite instability; MSI-H = microsatellite instability-high; MSS = microsatellite stable.

[a] The original MSI test results were determined by the polymerase chain reaction-based method.