Detection of microsatellite instability in a panel of solid tumours with the Idylla MSI Test using extracted DNA

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ABSTRACT

Aim During the last few years, determination of microstatellite instability (MSI) status has become a routine part of clinical practice, essentially to detect Lynch syndrome. Recently, MSI testing has increased with the development of immunotherapy and has expanded to a large panel of solid tumours. The aim of our work was to evaluate a fully automated system developed by Biocartis, the Idylla MSI Test, which performs an MSI analysis within 150 min.

Methods A comparison between pentaplex PCR, immunohistochemistry and Idylla MSI Test was performed in 53 colorectal carcinoma samples, 7 small intestine adenocarcinomas, 15 duodenal and pancreatic adenocarcinomas, 16 gastric tumours, 15 endometrial adenocarcinomas, 5 ovarian carcinomas and 4 cases of urinary tract tumours using extracted DNA. Limit-of-detection (LOD) experiment was also done using a commercial DNA known to harbour MSI phenotype.

Results The overall sensitivity was 94% and the overall specificity was 100%. Two invalid and three false-negative results were observed. Our experiments showed that the amount of DNA loaded into the cartridge was decisive and should be superior to 25 ng. LOD comprised between 4% and 8%.

Conclusion Overall, we have demonstrated that the Idylla MSI Test is a rapid and valid option to detect MSI phenotype which can be used in a large panel of solid tumours.

INTRODUCTION

Microsatellites are short repeated sequences in the genome that often cause errors during replication. DNA sequence mismatches due to the repeated structure of microsatellites are recognised and corrected by the mismatch repair system (MMR) consisting of four proteins encoded by four genes: MLH1, MSH2, MSH6 and PMS2. Microstatellite instability (MSI), which is characterised by a variation in the length of the repeat region, is a marker of MMR system failure. It occurs in 15% of colorectal carcinomas (CRC) and in a large panel of cancers such as ovarian, endometrial, gastric, urothelial or pancreatic cancers.[1] MSI is most commonly sporadic (70%–90% of MSI CRC), caused by MLH1 promoter hypermethylation.[2] It can also be inherited and caused by Lynch syndrome, an autosomal dominant tumour predisposition syndrome, caused by a germline mutation of one or more genes encoding the proteins involved in the MMR system.[3] In CRC, some clinical and pathological features, such as right colic localisation, colloid mucosal type or intratumorous lymphocyte richness, may be suggestive of MSI.

In the last few years, the analysis of microsatellite status was essentially performed to detect Lynch syndrome. Thereby, MSI DNA testing was mainly done for young patients and for patients with a suspecting Lynch syndrome considering their personal or family history. Furthermore, MSI was also investigated in stage II CRC because some studies, which remain controversial, had shown that these MSI tumours were found to be less responsive to 5-fluorouracil (5-FU)-based adjuvant chemotherapy.[4] Recently, MSI testing has increased with the development of immunotherapy. Indeed, MSI phenotype is a marker of excellent response to antiprogrammed cell death protein 1 (anti-PD1) in metastatic CRC, independently of programmed death-ligand 1 (PD-L1) expression.[5] This response can be explained by the fact that the MSI phenotype leads to a high mutational and neoantigenic load, thus inducing a strong immune response. Since 2017, the US Food and Drug Administration has approved anti-PD1 immunotherapy for all MSI cancers regardless of PD-L1 expression.[6 7] MSI can be diagnosed by two main methods according to the European Society for Medical Oncology recommendations published in 2019.[8]

The first one is molecular and requires tumour DNA: it consists in the amplification of microsatellite sequences (at least five markers including Bat25 and Bat26) by PCR, followed by a fragment size analysis. The other method is based on immunohistochemistry (IHC) and it consists in highlighting a loss of expression of MLH1, MSH2, MSH6 or PMS2, which also helps distinguish between the sporadic and hereditary character of the MSI phenotype. Some recent studies have shown that the analysis of MSI status can also be performed by next-generation sequencing (NGS).[9 10] However, this method is time consuming and requires trained laboratory technicians and bioinformatics.

The aim of our work was to evaluate a fully automated system developed by Biocartis, the Idylla MSI Test, which performs a microsatellite instability analysis within 150 min. This method is based on PCR amplification followed by analysis of the fusion profiles of seven biomarkers. These markers are short homopolymers located on the frequently mutated *ACVR2A, BTBD7, DIDO1, MRE11, RYR3,*

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SEC31A and *SULF2* genes in MSI cancers. Idylla is CE-IVD validated by the manufacturer for formalin-fixed paraffin-embedded (FFPE) tissue sections, but the results of several studies showed that it can be used with extracted DNA directly deposited into the cartridge.[11–13] Pertinently, biopsies are rapidly exhausted and it is important to store the material to search for all biomarkers assuring better therapeutic management of the patient. This study was performed on a series of 53 CRCs DNA whose MSI status had previously been defined by multiplex PCR and/or IHC. A panel of other tumours likely to have a MSI phenotype such as endometrial, stomach, oesophageal and pancreatic tumours was also analysed.

MATERIALS AND METHODS

Sample selection

This study was designed to assess the relative performances of the fully automated Idylla MSI Test to detect microsatellite instability using extracted DNA. To do so, 53 CRC samples, 7 small intestine adenocarcinomas, 15 duodenal and pancreatic adenocarcinomas, 16 gastric tumours, 15 endometrial adenocarcinomas, 5 ovarian carcinomas and 4 cases of urinary tract tumours were selected. They had previously undergone pentaplex PCR and IHC (for non-CRC tumours) for the detection of MSI status in our Department of Pathology between 2012 and 2019 and in compliance with French regulations. The prerequisite for sample selection was the availability of at least 10 µL of residual archival extracted DNA. Each sample had previously undergone routine PCR high-resolution melting (HRM) analysis, targeting exon 2 of Kirsten rat-sarcoma viral oncogene to determine DNA quality, and was accorded a grade of A–D, with A being the best, based on the crossing threshold (Ct) observed during that PCR. An enrichment of MSI samples was made during sample selection.

DNA extraction and quantification

DNA extractions were performed, after macrodissection when necessary, from FFPE tissue sections (usually seven sections, 5 µm thick). From 2012 to 2014, DNA was extracted using the EZ1DNATissue Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions, and quantified by spectrophotometry with Nanodrop ND-1000 (ThermoFisher Scientific, Waltham, Massachusetts, USA). From 2014 to 2019, DNA was extracted using the Maxwell 16 FFPE Plus LEV DNA Purification Kit IVD (Promega, Charbonnières-les-Bains, France), according to the manufacturer's instructions, and quantified using a Qubit fluorimeter in combination with the Qubit dsDNA HS Array Kit (ThermoFisher Scientific).

Fluorescent pentaplex PCR

Pentaplex PCR was performed using a single fluorescent multiplex system comprising five quasimonomorphic mononucleotide repeats (BAT25, BAT26, NR21, NR22 and NR24) as previously described.[14]

Briefly, PCR reactions were carried out in 18 µL reaction volume containing 2 µL of 10X PCR Gold reaction buffer (Applied Biosystems, ThermoFisher Scientific), 1.2 µL of $MgCl_2$ (25 mM), 0.3 µL of deoxynucleoside triphosphate (10 mM), 1.1 µL of both labelled and unlabelled primer (20 µm) (Eurofins genomic, Ebersberg, Germany) and 0.15 µL of TaqPolymerase (5 UI/µL) (ampliTaq, Applied Biosystems, ThermoFisher Scientific) with 2 µL of DNA at 20 ng/µL. The following thermocycling conditions were performed on 2720 thermal cycler (Applied Biosystems, ThermoFisher Scientific): 10 min at 95°C; 35 cycles of 95°C for 30 s, 55°C for 45 s and 72°C for 30 s; and a final extension at 72°C for 7 min. PCR products were then submitted to capillary electrophoresis on a 3130XL Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific). The size of PCR products were analysed using Genescan Analysis software (ThermoFisher Scientific) to measure the length of the fragments (in base pairs). Tumours presenting two or more unstable markers are considered unstable (MSI) and those presenting none or one marker unstable are considered stable (microsatellite stable (MSS)). For CRC tumours, the analysis was only performed on tumours, as recommended by the National institute of Cancer (Institut National du Cancer, INCa, 2016). For non-CRC tumours, the analysis was also done on non-tumorous tissue and the migration profile obtained was compared with that of the tumour.

Immunohistochemistry

IHC analysis of MMR proteins was performed in all non-CRC tumours. For CRC tumours, IHC was carried out for young patient (<40 years), for patient with doubtful result in pentaplex PCR and for patient harbouring an MSI phenotype. IHC was done on FFPE tissue sections using antibodies against MLH1 (clone G168.728, Microm Micotech, Brignais, France), anti-PMS2 (clone A16-4 1:100, BD Pharmingen, Le Pont de Claix, France), anti-MSH2 (clone FE11, 1:100, Biocare Medical, Pacheco, California, USA) and anti-MSH6 (clone EP49 1:100, CliniSciences, Nanterre, France). FFPE tissue sections were deparaffinised with BOND Dewax Solution (Leica Biosystems, Nanterre, France) for 30 min at 72°C then dehydrated through alcohol and subjected to antigen retrieval using BOND Epitope Retrieval Solution 2 (Leica Biosystems) for 20 min at 100°C. Blockage of endogenous peroxide activity was carried out with the Peroxide Block solution. After tissue section incubation with post primary rabbit antimouse IgG for 10 min, then with polymer antirabbit Poly-HRP-IgG for 10 min, bound primary antibodies were detected using DAB (Mix DAB Refine) for 8 min. Amplification signal was realised with BOND DAB Enhancer for 5 min (Leica Biosystems) only for MSH2 and MSH6 detection. Nuclei were counterstained with haematoxylin. IHC was performed on a BOND III or a BOND-MAX (Leica Biosystems) automated stainer platform. Reagents used came from BOND Polymer Refine Detection (Leica Biosystems).

The expression of the four MMR proteins defined a stable phenotype (MSS). Staining pattern consisted in nuclear staining within tumour cells with infiltrating lymphocytes, as positive internal controls. The loss of one or more proteins characterised by a total absence of nuclear staining within tumour cells with a positive labelling of non-tumorous cells, defined microsatellite unstable phenotype (MSI).

Idylla MSI Test

Each sample was retested using Idylla: 10 µL of the original sample-DNA preparation was directly deposited into the Idylla cartridge regardless of the concentration or quality of DNA. The Idylla System (Biocartis, Malines, Belgium) covers the entire process from sample to result with a run of approximately 150 min.[15] The Idylla MSI Test detects a novel panel of seven monomorphic biomarkers (*ACVR2A*, *BTBD7*, *DIDO1*, *MRE11*, *RYR3*, *SEC31A* and *SULF2*). These biomarkers are amplified and their detection is performed using fluorescently labelled molecular beacons after PCR amplification. These beacons differentially melt from the wild type or mutated amplicons with increasing temperature. The fluorescence differences at melting temperatures are further analysed by a specific software (MSI

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Table 1 Characteristics of the 53 samples of CRC assessed in the Idylla performance study to detect MSI phenotype

Cases	Tumour cells (%)	DNA input (ng)	DNA quality score*	Pentaplex PCR testing	IHC testing	Conclusion of Idylla MSI Test	Mutated/Valid microsatellites	*ACVR2A*	*BTBD7*	*DIDO1*	*MRE11*	*RYR3*	*SEC31A*	*SULF2*
1	20	180	A	MSI	MLH1 and PMS2 lost	MSI	2/7	+	–	+	–	–	–	–
2	30	130	B	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	–	+
3	30	680	B	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	+	–
4	40	990	A	MSI	MLH1 and PMS2 lost	MSI	5/7	+	+	+	+	–	+	–
5	40	360	B	MSI	PMS2 lost	MSI	4/7	+	+	+	+	–	–	–
6	40	70	C	MSI	MLH1 and PMS2 lost	MSI	5/7	+	+	+	–	+	+	–
7	40	500	A	MSI	MLH1 and PMS2 lost	MSI	5/7	+	+	–	+	+	–	+
8	50	470	A	MSI	MSH6 lost	MSI	5/7	+	+	–	+	+	–	+
9	50	20	NE	MSI	MSH6 lost	MSS	1/7	–	–	+	–	–	–	–
10	50	310	C	MSI	MLH1 and PMS2 lost	MSI	7/7	+	+	+	+	+	+	+
11	60	170	B	MSI	MSH2 and MSH6 lost	MSI	6/7	+	+	+	+	–	+	+
12	60	130	A	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	–	+	+
13	60	660	A	MSI	MLH1 and PMS2 lost	MSI	5/7	+	–	–	+	+	+	+
14	70	260	C	MSI	MSH2 and MSH6 lost	MSI	5/7	+	+	+	+	+	–	–
15	80	800	B	MSI	MSH2 and MSH6 lost	MSI	6/7	+	+	–	+	+	+	+
16	80	640	A	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	–	+
17	50	420	A	MSI	MSH2 and MSH6 lost	MSI	7/7	+	+	+	+	+	+	+
18	<10	70	A	MSS	NA	MSS	0/6	–	–	–	NC	–	–	–
19	15	260	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
20	20	500	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
21	20	140	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
22	30	290	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
23	30	410	A	MSS	NA	MSS	1/7	–	–	+	–	–	–	–
24	40	520	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
25	40	370	C	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
26	50	840	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
27	50	40	NE	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
28	60	260	B	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
29	60	840	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
30	70	970	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
31	80	420	B	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
32	90	570	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
33	50	70	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
34	50	260	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
35	40	710	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
36	40	390	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
37	30	180	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
38	70	NE	NE	MSS	NA	Invalid test	0/2	–	NC	NC	NC	NC	–	NC
39	30	850	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
40	60	1410	B	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
41	25	700	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
42	80	70	B	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
43	40	1160	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
44	40	940	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
45	20	200	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
46	50	1080	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
47	20	1040	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
48	20	580	B	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
49	30	1100	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
50	30	240	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
51	40	60	A	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
52	30	310	C	MSS	NA	MSS	0/7	–	–	–	–	–	–	–
53	30	620	B	MSS	NA	MSS	0/7	–	–	–	–	–	–	–

*Quality graded from A to D, with A being the best.
CRC, colorectal carcinoma; IHC, immunohistochemistry; MSI, microstatellite instability; MSS, microsatellite stable; NA, not applicable; NC, not contributory; NE, not evaluated.

Test Type Package, MSI TTP). At the end of the run, the result, reporting the presence, absence of mutation or invalidity for each biomarker and the MSI status, in the analysed sample is displayed on the Console screen. The MSI status of the sample can be determined with high confidence if at least five valid marker-specific fluorescence profiles could be fully analysed (otherwise the MSI status will be called 'Invalid'). At least two mutant markers will result in a status being 'MSI', otherwise the status will be scored as 'MSS'.[16]

Determination of LOD

For LOD assessment, 56 ng of a commercial MSI DNA (16%) was used (MSI FFPE DNA HD830, Horizon, Horizon Discovery,

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Table 2 Characteristics of the 38 samples of digestive system tumours assessed in the Idylla performance study to detect MSI phenotype

Cases	Tumour cells (%)	DNA input (ng)	DNA quality score*	Pentaplex PCR testing	IHC testing	Conclusion of Idylla MSI Test	Mutated/Valid microsatellites	*ACVR2A*	*BTBD7*	*DIDO1*	*MRE11*	*RYR3*	*SEC31A*	*SULF2*
Adenocarcinoma of the small intestine														
54	70	1200	A	MSI	MSH2 and MSH6 lost	MSI	7/7	+	+	+	+	+	+	+
55	30	50	B	MSI	MSH2 and MSH6 lost	MSS	0/5	–	NC	–	–	–	NC	–
56	99	640	NA	MSI	MLH1 and PMS2 lost	MSI	2/7	+	–	+	–	–	–	–
57	80	280	B	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	+	–
58	30	560	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
59	60	300	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
60	40	1060	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
Duodenal and pancreatic adenocarcinoma														
61	65	910	A	MSI	MSH2 and MSH6 lost	MSI	7/7	+	+	+	+	+	+	+
62	20	110	A	MSI	MSH2 lost and doubtful MSH6	MSI	7/7	+	+	+	+	+	+	+
63	60	50	C	MSI	MLH1 and PMS2 lost	MSI	4/6	NC	+	+	–	+	–	+
64	80	470	B	MSI	MSH2 and MSH6 lost	MSI	5/7	–	+	+	+	–	+	+
65	60	510	A	MSI	MLH1 and PMS2 lost	MSI	6/7	+	–	+	+	+	+	+
66	30	890	A	MSI	PMS2 lost	MSI	5/7	+	+	+	+	–	–	+
67	30	140	A	MSI	MSH6 lost and doubtful PMS2	MSI	3/7	+	+	+	–	–	–	–
68	60	900	B	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	–	+
69	90	690	A	MSI	MSH2 and MSH6 lost	MSI	7/7	+	+	+	+	+	+	+
70	30	200	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
71	30	130	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
72	30	70	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
73	50	160	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
74	40	20	NE	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
75	60	4	NE	MSS	PMS2 doubtful	Invalid test	0/3	–	NC	NC	–	NC	NC	–
Gastric tumour														
76	80	350	A	MSI	MLH1 and PMS2 lost	MSI	5/7	+	+	+	+	–	–	+
77	40	120	A	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	+	–
78	30	230	A	MSI	MLH1 and PMS2 lost	MSI	5/7	+	+	+	+	–	–	+
79	60	120	B	MSI	MLH1 and PMS2 lost	MSI	5/7	+	–	+	–	+	+	+
80	60	30	NE	MSI	MLH1 and PMS2 lost	MSI	4/5	NC	–	+	+	+	NC	+
81	70	1160	B	MSI	MLH1 and MSH2 lost	MSI	7/7	+	+	+	+	+	+	+
82	50	450	A	MSI	MLH1 and PMS2 lost	MSI	6/7	+	+	+	+	+	–	+
83	80	530	B	MSI	PMS2 lost and doubtful MLH1	MSI	5/7	+	+	+	+	–	–	+
84	40	200	A	MSI	Doubtful MLH1 and PMS2	MSI	4/7	+	–	+	+	–	–	+
85	50	200	B	MSI	MLH1 and PMS2 lost	MSI	5/7	–	+	+	+	+	–	+
86	50	720	B	MSI	MLH1 and PMS2 lost	MSI	4/7	+	–	+	+	–	–	+
87	50	390	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
88	15	80	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
89	70	350	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
90	20	490	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
91	30	40	NE	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–

*Quality graded from A to D, with A being the best.
IHC, immunohistochemistry; MSI, microstatellite instability; MSS, microsatellite stable; NA, not applicable; NC, not contributory; NE, not evaluated.

Cambridge, UK). That DNA was serially diluted in MSS DNA (MSS FFPE DNA HD831, Horizon) to obtain a 3-point scale: 16%, 8%, 4% of MSI DNA.

RESULTS

Evaluation of Idylla's ability to detect MSI phenotype in CRC

The aim of the first part of our study was to evaluate Idylla's ability to detect MSI phenotype in CRC using DNA. In our series, 17 cases were MSI, according to pentaplex PCR and IHC, and 36 were MSS according to pentaplex PCR (table 1). An average of 480 ng of DNA was deposited in the cartridge and those samples contained an average of 46% tumour cells. Among the 53 cases, one (number 38) was rendered invalid due to the low concentration (<2 ng/µL) of DNA used for the test. In this series, IHC was performed for only 22 patients; sensitivity was thus calculated using pentaplex PCR as reference. Idylla sensitivity to detect MSI phenotype was 94% (16/17). Its specificity was 100%. One false-negative result was obtained for case number 9 (quantity of DNA used for the test: 20 ng/µL).

Evaluation of Idylla's ability to detect MSI phenotype in digestive system tumours

In the second part of our work, we evaluated the performance of Idylla system in detecting MSI phenotype in digestive system tumours (except CRC) using DNA. MSI status has been previously analysed in those tumours using pentaplex PCR and IHC. The analysis was performed on 7 small intestine adenocarcinomas (4 MSI and 3 MSS), 15 duodenal and pancreatic adenocarcinomas (9 MSI and 6 MSS) and 16 gastric tumours (11 MSI and 5 MSS) (table 2). The average of DNA deposited in the

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Table 3 Characteristics of the 20 samples of gynaecological tumours assessed in the Idylla performance study to detect MSI phenotype

Cases	Tumour cells (%)	DNA input (ng)	DNA quality score*	Pentaplex PCR testing	IHC testing	Conclusion of Idylla MSI Test	Mutated/Valid microsatellites	*ACVR2A*	*BTBD7*	*DIDO1*	*MRE11*	*RYR3*	*SEC31A*	*SULF2*
Endometrial carcinoma														
92	40	100	B	MSI	MLH1 and PMS2 lost	MSI	4/7	–	–	+	–	+	+	+
93	60	400	A	MSI	MLH1 and PMS2 lost	MSI	7/7	+	+	+	+	+	+	+
94	60	480	B	MSI	MLH1 and PMS2 lost	MSI	5/7	–	+	+	+	–	+	+
95	70	1140	A	MSI	MLH1 and PMS2 lost	MSI	5/7	–	+	+	+	–	+	+
96	70	360	B	MSI	MLH1 lost	MSI	5/7	+	+	+	+	+	–	–
97	60	120	A	MSI	MLH1 and PMS2 lost	MSI	5/7	+	+	+	+	–	–	+
98	70	2080	C	MSI	MLH1 and PMS2 lost	MSI	5/7	–	+	+	+	+	–	+
99	90	1020	C	MSI	MLH1 and PMS2 lost	MSS	1/7	–	–	–	+	–	–	–
100	70	580	A	MSI	MLH1 and PMS2 lost	MSI	4/7	–	–	+	+	–	+	+
101	80	640	B	Doubtful	No loss	MSS	0/7	–	–	–	–	–	–	–
102	60	320	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
103	40	240	A	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
104	30	20	NE	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
105	60	120	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
106	30	10	NE	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
Ovarian cancer														
107	60	60	B	MSI	MSH6 lost	MSI	3/7	+	–	+	–	–	+	–
108	50	460	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
109	60	190	C	Doubtful	No loss	MSS	0/7	–	–	–	–	–	–	–
110	70	350	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–
111	30	520	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–

*Quality graded from A to D, with A being the best.
IHC, immunohistochemistry; MSI, microstatellite instability; MSS, microsatellite stable; NA, not applicable; NC, not contributory; NE, not evaluated.

cartridge was 389 ng. Those tumours contained an average of 50% tumour cells. Again, one case (number 75) was rendered invalid due to the very low amount of DNA (4 ng) used for the test. In those digestive system tumours, Idylla sensitivity to detect MSI phenotype was 96% (23/24) and its specificity was 100%. One false-negative result was obtained for case number 55. This false negative was observed on a small intestine adenocarcinoma with 30% of tumour cells. In that case, DNA quality was pretty good (quality B) and the quantity of the DNA used for the test was 50 ng.

Evaluation of Idylla's ability to detect MSI phenotype in endometrial and ovarian carcinomas

We next assessed the Idylla MSI Test performance on a series of gynaecological tumours including 15 endometrial adenocarcinomas and 5 ovarian carcinomas from extracted DNA. Among these cases, 10 were MSI (9 endometrial tumours and 1 ovarian tumour) and 10 were MSS (6 endometrial tumours and 4 ovarian tumours) according to pentaplex PCR and IHC results (table 3). An average of 580 ng of DNA was deposited in the cartridge and those samples contained an average of 46% tumour cells. The sensitivity was 90% (9/10) and the specificity was 100%. Among the 10 MSI cases, one (number 99) was rendered MSS with the Idylla system. The quality of this DNA was poor, as evaluated by HRM PCR (quality C), which could account for this failure. To note, one of the markers, *MRE11*, was mutated for this case whereas other MSS samples did not reveal any mutation in the seven markers. Interestingly, two samples (number 101 and 109) that were doubtful in pentaplex PCR with no loss of MMR proteins observed by IHC, were confirmed MSS using Idylla system.

Evaluation of Idylla's ability to detect MSI phenotype in urinary tract tumours

Finally, we tested four cases of urinary tract tumours with the Idylla MSI cartridge using extracted DNA. In this little series,

Table 4 Characteristics of the four samples of urinary tract tumours assessed in the Idylla performance study to detect MSI phenotype

Cases	Tumour cells (%)	DNA input (ng)	DNA quality score*	Pentaplex PCR testing	IHC testing	Conclusion of Idylla MSI Test	Mutated/Valid microsatellites	*ACVR2A*	*BTBD7*	*DIDO1*	*MRE11*	*RYR3*	*SEC31A*	*SULF2*
112	60	510	C	MSI	MSH6 lost	MSI	5/7	–	+	+	+	–	+	+
113	80	110	A	MSI	MSH2 and MSH6 lost	MSI	5/7	–	+	+	+	+	+	–
114	80	170	B	Doubtful	No loss	MSS	0/7	–	–	–	–	–	–	–
115	80	540	B	MSS	No loss	MSS	0/7	–	–	–	–	–	–	–

*Quality graded from A to D, with A being the best.
IHC, immunohistochemistry; MSI, microstatellite instability; MSS, microsatellite stable; NA, not applicable; NC, not contributory; NE, not evaluated.

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Table 5 Determination of the LOD of the Idylla MSI Test

% of MSI DNA	Conclusion of Idylla MSI Test	Mutated/Valid microsatellites	*ACVR2A*	*BTBD7*	*DIDO1*	*MRE11*	*RYR3*	*SEC31A*	*SULF2*
16	MSI	7/7	+	+	+	+	+	+	+
8	MSI	5/7	+	+	+	−	−	+	+
4	MSS	1/7	−	+	−	−	−	−	−

LOD, limit of detection; MSI, microstatellite instability; MSS, microsatellite stable.

two cases were MSI and two were MSS (table 4). An average of 332.5 ng of DNA was deposited in the cartridge and those samples contained an average of 65% tumour cells. Results obtained with Idylla system were concordant with those obtained by pentaplex PCR and IHC. For case number 115, pentaplex PCR results were difficult to interpret but Idylla and IHC both found an MSS phenotype. Again, one sample (number 114) that was doubtful in pentaplex PCR with no loss of MMR proteins observed by IHC, was confirmed MSS using Idylla system.

Determination of the Idylla LOD for MSI Test using extracted DNA

Our last objective was to evaluate the Idylla LOD for the MSI Test using a commercial MSI DNA known to harbour 16% of MSI DNA. LOD was measured using 56 ng of this commercial DNA, serially diluted in control MSS DNA to obtain a 3-point Variant Allele Frequency scale: 16%, 8% and 4% (table 5). The MSI phenotype was detected in samples with 16% and 8% of MSI DNA. Interestingly, in sample with 16% of MSI DNA, 7/7 markers were found mutated whereas in sample with 8% of MSI DNA only 5 markers were mutated. For sample with 4% of MSI DNA, Idylla did not detect the MSI phenotype and it was reported to be MSS. In this sample, only one of the seven markers was rendered mutated. Thus, the Idylla MSI Test LOD was estimated to be between 4% and 8% using 56 ng of this commercial DNA lot.

DISCUSSION

This study was undertaken to evaluate Idylla's ability to detect MSI using extracted DNA. The study included a total of 53 CRC, 7 small intestine adenocarcinomas, 15 duodenal and pancreatic adenocarcinomas, 16 gastric tumours, 15 endometrial adenocarcinomas, 5 ovarian carcinomas and 4 cases of urinary tract tumours previously subjected to pentaplex PCR and IHC. In addition, LOD experiment aimed to determine this method's sensitivity for extracted DNA. Idylla results were concordant with those previously obtained by pentaplex PCR for 107/110 (97%) samples analysed (case numbers 101, 109 and 114 with doubtful result by pentaplex PCR are excluded) and for 81/84 cases analysed by IHC. The overall sensitivity was 94% and the overall specificity was 100% (table 6). In total, with the Idylla MSI Test two invalids and three false-negative results were observed. Our experiments showed that the amount of DNA loaded into the cartridge was decisive. In fact, the two invalid samples (case number 38 (<2 ng/µL) and 75 (4 ng/µL)) as well as two false-negative results obtained (case numbers 9 (20 ng/µL) and 55 (50 ng/µL)) could be explained by a lower amount of DNA used for the test. These results are in agreement with those previously published by our team concerning the Idylla *EGFR*-Mutation Assay in which we have shown that Idylla system failed to detect EGFR T790M mutation for samples with an amount of DNA used for the test <2.5 ng/µL.[17] For sample number 99, neither the amount of DNA used for the test nor the percentage of tumorous cells can explain the failure to detect the MSI phenotype. Nevertheless, the poorer quality of this DNA sample could account for Idylla's failure, highlighting the fact that DNA quality can affect its performance. Our LOD results are in concordance with the recommendation of the manufacturer as we observed that LOD comprised between 4% and 8% using a commercial DNA of very good quality.

Idylla's ability to detect MSI phenotype has been evaluated in other studies. Lee *et al* tested Idylla's performance on 133 CRC samples whose MSI status had been validated by standard PCR and/or NGS panel using FFPE slide. Consistently with our results, they found good diagnostic performance of the Idylla system with 100% sensitivity and 98.94% specificity.[18] In another study, Li *et al* evaluated the system on 42 CRCs which were clinically tested for MSI status using PCR or IHC from FFPE slides.[19] They founded an overall 97.62% concordance with previously used methods with one false-negative result. They speculated that the failure might be because of tumour heterogeneity or because of the limited amount of material available for the test. This observation is in agreement with our results, suggesting that a low quantity of DNA available for the test can affect assay performance. Recently, Zwaenepoel *et al* compared the clinical performance of the Idylla MSI Test and the Promega MSI Analysis system in a large panel of 330 CRC.[16] In this study, an overall agreement, sensitivity and specificity of 99.7%, 98.7% and 100% was reached, respectively. A low number of invalid results were observed. Again, this work demonstrates that Idylla MSI Test is an accurate assay for the detection of MSS. Samaison *et al* selected 12 tumour samples: 10 CRC, 1 endometrial adenocarcinoma and 1 ovarian carcinoma, to study the performance of the Idylla MSI Test in the diagnosis of MSI or MSS phenotype. In this study, concordance with the initial genetic testing by PCR and IHC and Idylla MSI Test was perfect.[20] This was the only study published with data obtained in other cancer than CRC. Our work is the first to confirm these results in a large series of non-CRC (62 cases) and indicates that Idylla MSI Test is able to detect MSI or MSS phenotype in these tumours.

To conclude, our results showed that the Idylla MSI Test is a rapid and valid option to detect MSI phenotype. They, like those reported by Evrard *et al*[21] and Gilson *et al*,[12] demonstrated that Idylla provides adequate findings when run on extracted

Table 6 Summary of the sensitivity and specificity of the Idylla MSI Test in several types of cancers

Histological type	Number of cases in the series	Sensitivity (%)	Specificity (%)
Colorectal carcinoma	53	94	100
Carcinoma of the small intestine	7	75	100
Duodenal and pancreatic carcinoma	15	100	100
Gastric tumour	16	100	100
Endometrial carcinoma	15	89	100
Ovarian cancer	5	100	100
Urinary tract carcinoma	4	100	100
Overall	115	94	100

MSI, microstatellite instability.

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DNA, which allows specimen storage. Our work also demonstrated that this test is not restricted to CRC and can be used in other solid tumours like endometrial, gastric and pancreatic cancers. Furthermore, the Idylla fully automated procedure has the advantage of eliminating the analysis of healthy tissue which save time and labour for laboratory technician. Although it is more expensive than pentaplex PCR, this test is therefore a simple, fast and reliable technique for assessing MSI status in CRC and non-CRC.

Take home messages

- Idylla MSI Test is a rapid and valid option to detect microstatellite instability (MSI) phenotype using DNA.
- Idylla MSI Test can be used for colorectal cancer and for other solid tumours.
- The amount of DNA loaded into the cartridge and the percentage of tumorous cells are decisive to avoid false-negative results.

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