510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I. Background Information:

A. 510(k) Number:

K211181

B. Applicant

Biocartis NV

C. Proprietary and Established Names

Idylla™ MSI Test

D. Regulatory Information

Product Code	Classification	Regulation Section	Panel
PZJ	Class II	21 CFR 864.1866 - Lynch Syndrome Test Systems	88 Pathology

II. Submission/Device Overview:

A. Purpose for Submission:

New Device

B. Measurand:

Seven microsatellite alleles (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A and SULF2) in DNA in formalin fixed, paraffin-embedded (FFPE) colorectal tissue sections.

C. Type of Test:

Polymerase chain reaction (PCR)-based nucleic acid amplification and subsequent melt-curve analysis

III. Intended Use/Indications for Use:

A. Intended Use

The Idylla MSI Test, for use on the Idylla System, uses formalin-fixed, paraffin-embedded (FFPE) tissue sections of human colorectal cancer (CRC) tumor, from which nucleic acids are liberated, then analyzed using PCR amplification of seven monomorphic biomarkers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A and SULF2) and subsequent melt-curve analysis. The Idylla MSI Test reports results as either microsatellite stable (MSS), or microsatellite instability high (MSI-H) or invalid.

Idylla MSI Test is indicated for use by healthcare professionals for the qualitative identification of microsatellite instability (MSI) in CRC tumors, indicative of mismatch repair deficiency, as an aid in the identification of potential Lynch syndrome to help identify patients that would benefit from additional genetic testing to diagnose Lynch syndrome.

The Results from the Idylla MSI Test should be interpreted by healthcare professionals in conjunction with other clinical findings, family history, and other laboratory data. The Idylla MSI Test should not be used for diagnosis of CRC.

The clinical performance of this device to guide treatment decision for MSI high patients has not been established.

B. Special conditions for use statement(s)

Rx – For prescription use

For *in vitro* diagnostic use

C. Special instrument requirements

Biocartis Idylla System with Idylla MSI Test TTP.

IV. Device Description

1. Principle of Operation

The Idylla MSI Test is an automated test on the Idylla System. The Biocartis Idylla System covers the entire process from sample to result with fully integrated sample preparation followed by real time polymerase chain reaction (PCR) amplification and fluorescence detection of the targeted sequences. The Idylla System consists of the Idylla Console connected to up to eight Idylla Instruments. Idylla Cartridges, designed for specific applications, are processed by the Idylla System using test specific software. The test specific software, referred to as Test Type Package (TTP), is available for download to the Idylla Console. The Test procedure and data analysis are validated for FFPE tissue sections.

The Idylla MSI Test detects a novel panel of seven monomorphic biomarkers which are listed in the Table 1 below.

Table 1. Seven biomarkers in the Idylla MSI Test

Biomarkers
ACVR2A
BTBD7
DIDO1
MRE11
RYR3
SEC31A
SULF2

The process steps in the Idylla MSI Test are:

- **Sample Processing** (FFPE liquefaction and cell lysis): After insertion of the FFPE tissue section into the cartridge, a combination of chemical reagents, enzymes, heat, and high intensity focused ultrasound (HIFU) induces deparaffinization, disruption of the tissue and lysis of the cells. The nucleic acids are liberated for subsequent PCR amplification. There is no DNA extraction step in the procedure.

- **Amplification, Detection and Analysis**: All necessary PCR reagents are present in a stable formulation and are used to amplify seven biomarkers. After amplification of the specific regions of the seven (7) biomarkers, the molecular beacons bind to the target regions. Contact quenching no longer takes place and the beacons will produce a fluorescence signal. These mutant-specific beacons differentially melt from the wild type or mutated amplicons with increasing temperature. The fluorescence differences at melting temperatures are further analyzed by the MSI TTP and translated into genetic calls on biomarker level and MSI status on sample level.

- **Reporting**: At the end of the run, a result report outlining the MSI status along with the number of positive MSI biomarkers detected in the analyzed sample is displayed on the console screen. The MSI status of the sample can be determined if at least five valid marker-specific fluorescence profiles could be fully analyzed (otherwise the MSI status is called 'Invalid'). The presence of at least two mutant markers results in an 'MSI-H' call (Microsatellite Instability-High), otherwise the status is reported as 'MSS' (Microsatellite Stable).

2. Specimen Handling/Requirement

Standard formalin-fixation and paraffin-embedding procedures should be followed. Tissue samples should be fixed as quickly as possible and fixation times limited to preferably less than 72 hours to reduce the risk of severe DNA fragmentation. 4 µm to 10 µm FFPE tissue

sections can be used as specimen in the Idylla MSI Test. Specimens should be macrodissected if less than 33% of the section is tumor.

3. Interpretation of Results

The Idylla System automatically interprets the test results and makes them available for viewing on the Console. The Idylla MSI Test reports a result for the MSI status of the sample, the MSI Score Range of the sample, the number of positive biomarkers and an overall quality status. A sample is called Microsatellite Instability-High or MSI-H if the sample has ≥2 mutant biomarkers and an MSI Score Range ≥ 0.15. A sample is called Microsatellite Stable or MSS if the sample has <2 mutant biomarkers and an MSI Score Range <0.15 (Table 2).

Table 2. Result Interpretation

Number of Positive Biomarkers	MSI Score Range	MSI Status
<2	**<0.15**	**MSS**
≥2	**≥0.15**	**MSI-H**

An 'Invalid' call for a biomarker is observed when no PCR product was formed in a particular sample and therefore no characteristic fluorescent profile could be analyzed. This may occur when using poor quality samples. A Cartridge is considered valid if ≥ 5 out of 7 MSI biomarkers show a valid marker result for the sample. An invalid Cartridge will report the MSI status being 'Invalid'. An 'Invalid' result is reported if >2 out of 7 MSI biomarkers show an invalid biomarker result for a particular sample (Table 3).

Table 3. Invalid Reporting

Number of Invalid Biomarkers	THE QUALITY STATUS SHOWN ON THE RESULT REPORT IS...
0 Invalid biomarkers	**7 MSI biomarkers have been properly amplified and therefore the Test result is VALID**
1 Invalid biomarkers	**6 of the 7 MSI biomarkers have been properly amplified and therefore the Test result is VALID**
2 Invalid biomarkers	**5 of the 7 MSI biomarkers have been properly amplified and therefore the Test result is VALID**
3 or more Invalid biomarkers	**Less than 5 MSI biomarkers have been properly amplified and therefore the Test result is INVALID**

4. Materials Provided

The following materials are provided when purchasing the Idylla MSI Test:

- Idylla MSI Test Cartridges

Each Cartridge contains the following:

- Liquefaction buffer: 1.7 mL
- Dilution buffer: 2.2 mL
- Enzymes, Primers and Probes (dried)

Electronic Components required to perform an Idylla MSI Test

- MSI Test Type Package (TTP): MSI_IVD/2.0
- Idylla MSI Test Instructions
- Idylla Operator Manual

5. Materials Required But Not Provided

The following materials are not provided, but are required to perform an Idylla MSI Test:

Table 4. Materials required but not provided .

ITEM	SPECIFICATION
Idylla Instrument	Cat. No. P0010
Idylla Console	Cat. No. P1010
Idylla MSI FFPE Reference Set	SENSID-GMBH
Nuclease-free water	

V. Quality Control

Blank or No Template Controls are not included in the design of the Idylla MSI Test.

Idylla MSI FFPE Reference Set, manufactured by SensID GmbH, is intended as qualitative control material for MSI markers in FFPE format based on human cell lines. It consists of MSI FFPE MSI-H control with all MSI markers at a frequency of 1x LOD and MSI FFPE MSS control with 0% frequency for all MSI markers. This Reference set should be used as the external controls to demonstrate that the reagents and the assay procedure perform properly. The quality control results should pass before testing patient samples. If the controls do not perform as expected, the test must be repeated and/or contact Biocartis technical support before testing patient samples. Controls should be tested:

- At the beginning of the study before the first specimen test.
- Before each new lot of Biocartis ldylla MSI Test (IUO) cartridges were placed into use.
- After every 24 tests per ldylla™ console.
- After each major service of the instrument and/or critical instrument part replacement.
- At the end of testing.
- As required by laboratory's standard quality control procedures.
- As required by federal, state and local guidelines.

VI. Substantial Equivalence Information

A. Predicate Device Name:

OncoMate MSI Dx Analysis System

B. Predicate 510(k) Number:

K200129

C. Comparison with Predicate:

Item	Subject Device: Idylla MSI Test K211181	Predicate Device: OncoMate MSI Dx Analysis System K200129
	Similarities	
Intended Use	The Idylla MSI Test, for use on the Idylla System, uses formalin-fixed, paraffin-embedded (FFPE) tissue sections of human CRC tumor, from which nucleic acids are liberated, then analyzed using PCR amplification of seven monomorphic biomarkers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A and SULF2) and subsequent melt-curve analysis. The Idylla MSI Test reports results as either microsatellite stable (MSS), or microsatellite instability high (MSI-H) or invalid. Idylla MSI Test is indicated for use by healthcare professionals for the qualitative identification of microsatellite instability (MSI) in colorectal cancer (CRC) tumors, indicative of mismatch repair deficiency, as an aid in the identification of potential Lynch syndrome to help identify patients that would benefit from additional genetic testing to diagnose Lynch syndrome. The results from the Idylla MSI Test should be interpreted by	The OncoMate MSI Dx Analysis System is a qualitative multiplex polymerase chain reaction (PCR) test intended to detect the deletion of mononucleotides in 5 microsatellite loci (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) using matched tumor and normal DNA obtained from formalin fixed, paraffin-embedded (FFPE) colorectal tissue sections. The OncoMate MSI Dx Analysis System is for use with the Applied Biosystems® 3500Dx Genetic Analyzer and OncoMate MSI Dx Interpretive Software. The OncoMate MSI Dx Analysis System is indicated in patients diagnosed with colorectal cancer (CRC) to detect microsatellite instability (MSI) as an aid in the identification of probable Lynch syndrome to help identify patients that would benefit from additional genetic testing to diagnose Lynch syndrome. Results from the OncoMate MSI Dx Analysis System should be interpreted by healthcare

Item	Subject Device: Idylla MSI Test K211181	Predicate Device: OncoMate MSI Dx Analysis System K200129
	healthcare professionals in conjunction with other clinical findings, family history, and other laboratory data. The Idylla MSI Test should not be used for diagnosis of CRC. The clinical performance of this device to guide treatment decision for MSI high patients has not been established.	professionals in conjunction with other clinical findings, family history, and other laboratory data. The clinical performance of this device to guide treatment decision for MSI high patients has not been established.
Special Conditions for Use Statements	Rx- For prescription use. For *In Vitro* Diagnostic use.	Same
Specimen	FFPE Tumor Tissue	Same
Results	MSS or MSI-H	Same
Target Population	Patients diagnosed with CRC	Same
Differences		
Technology	PCR followed by melting point analysis	PCR followed by capillary electrophoresis
Software	MSI TTP	OncoMate MSI Dx Interpretive Software
Targets	ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A, and SULF2	5 Mononucleotide tracts BAT25, BAT26, MONO27, NR21 and NR24
External Controls	2 cell line-based FFPE sections designed to represent MSS and MSI-H	DNA extracted from MSS human cell line
Internal Control	The melt analysis result of the sample processing control in each of the PCR reactions is used to check for adequate execution of the complete process from sample to result.	None
Instrument	Idylla System	Applied BioSystems 3500 Dx Genetic Analyzer

VII. Standards/Guidance Documents Referenced:

Table 5. Consulted Standards

STANDARD ORG.	STANDARD DESIGNATION NUMBER	TITLE OF STANDARD
CLSI	EP05-A3	Evaluation of Precision of Quantitative Measurement Procedures, 3rd Edition

STANDARD ORG.	STANDARD DESIGNATION NUMBER	TITLE OF STANDARD
CLSI	EP07-A2	Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
CLSI	EP17-A2	Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline -- Second Edition
CLSI	EP25-A	Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline
CLSI	EP14-A3	Evaluation of Commutability of Processed Samples; Approved Guideline --Third Edition
CLSI	EP12-A2	User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition.
CLSI	MM13-A	Collection Transport Preparation and Storage of Specimens for Molecular Methods; Approved Guideline
AAMI ANSI IEC	62304:2006/Amd 1:2015	Medical device software - Software life cycle processes
IEC	TR 80002-1 Edition 1.0 2009-09	Medical Device Software – Part 1: Guidance on the Application of ISO 14971 to Medical Device Software
AAMI	TIR-45:2012	Guidance on the Use of AGILE Practices in the Development of Medical Device Software
ISO	15223-1 Fourth Edition 2021-07	Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements
IEC	61326-2-6 Edition 3.0 2020-10	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment
ANSI AAMI IEC	62366-1:2015	Medical devices - Part 1: Application of usability engineering to medical devices
ANSI UL	61010-1 3rd Ed, dated May 12, 2012 with revision through July 19, 2019	Standard for Safety for Electrical Equipment For Measurement, Control and Laboratory Use; Part 1: General Requirements
ISO	14971 Third Edition 2019-12	Medical devices - Application of risk management to medical devices

Applicable FDA Guidance Documents:

- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests
- Guidance for the Content of Premarket Submission for Software Contained in Medical Devices - Guidance for Industry and FDA Staff, May 2005
- General Principles of Software Validation; Final Guidance for Industry and FDA Staff, January 2002

- Content of Premarket Submissions for Management of Cybersecurity in Medical Devices- Guidance for Industry and Food and Drug Administration Staff (2014)
- Postmarket Management of Cybersecurity in Medical Devices- Guidance for Industry and Food and Drug Administration Staff (2016)

VIII. Performance Characteristics

A. Analytical Performance:

1. Precision/Reproducibility

The inter-lab reproducibility study was conducted at three laboratory sites. Seven individual clinical specimens [MSI-H and MSS FFPE colorectal (CRC) tissue sections] were tested at all three sites with three lots of Idylla MSI Test Cartridges, on the Idylla Platform. Samples were selected to cover a range of repeats, size, and biomarker status as shown in Table 6.

Table 6. Details of FFPE clinical samples used in the inter-lab reproducibility study

SAMPLE ID (IN SLIMS)	SECTION ON SIZE (µm)	PER PATHOLOGY REPORT		Idylla MSI 2.0 FINAL CALL AND INDIVIDUAL MARKER STATUS (MUT or WT)								PROMEGA RESULTS
		TISSUE SIZE (mm^2)	%NEOPLASTIC CELLS IN TISSUE	MSI Status	BTBD7	RYR3	SEC31A	ACVR2A	DIO1	MRE11	SULF2	
Sample 1	10 µm	75	30 - 40	MSI-H	MUT	WT	WT	MUT	MUT	MUT	MUT	MSI
Sample 2	10 µm	30	50 - 60	MSI-H	WT	WT	WT	MUT	MUT	MUT	WT	MSI
Sample 3	4 µm	160	30 - 40	MSI-H	MUT	MUT	MUT	MUT	MUT	MUT	MUT	MSI
Sample 4	10 µm	125	40	MSI-H	MUT	MUT	MUT	MUT	MUT	MUT	WT	MSI
Sample 5	10 µm	37	35	MSS	WT	WT	WT	WT	WT	WT	WT	MSS
Sample 6	10 µm	35	50	MSS	WT	WT	WT	WT	WT	WT	WT	MSS
Sample 7	4 µm	110	40	MSS	WT	WT	WT	MUT	WT	WT	WT	MSS

Note: MUT = Positive for MSI marker; WT (wild type) = Negative for MSI marker

This study was designed to evaluate lot-to-lot, instrument/operator, inter-day and inter-lab variability on the Idylla MSI Test. At each site, each sample was tested by one operator on four instruments with one replicate per instrument per day, for three non-consecutive days using three lots of Idylla MSI Test Cartridges, resulting in a total of twelve replicates per sample per site. Overall, this study produced 36 results (tests) in total per sample from all three sites. All 252 valid runs from the three sites were used for data analysis.

Table 7. Reproducibility study results

CLINICAL SAMPLE	NUMBER OF REPLICATES TESTED	REPRODUCIBILITY RESULTS	
		NUMBER OF CORRECT MSI STATUS CALLS	CONCORDANCE RATE (%)
Sample 1 (MSI-H)	36	36/36	100%
Sample 2 (MSI-H)	36	36/36	100%
Sample 3 (MSI-H)	36	36/36	100%
Sample 4 (MSI-H)	36	36/36	100%
Sample 5 (MSS)	36	36/36	100%
Sample 6 (MSS)	36	36/36	100%
Sample 7 (MSS)	36	36/36	100%
WITHIN SITE			
Site 1	84	84/84	100%
Site 2	84	84/84	100%
Site 3	84	84/84	100%
All Sites	252	252 / 252	100%

Table 8. Summary of PPA (MUT) and NPA (WT) and 95% CI for MSI status and individual biomarkers in FFPE clinical samples

SAMPLE	REFERENCE MSI STATUS	AGREEMENT TO REFERENCE STATUS % PPA (MUT) AND NPA (WT) (HIT RATE), 95% CI	INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE (HIT RATE*), 95% CI)						
			BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 1	MSI-H	100% (36/36)	100% (36/36 MUT)	100% ** (36/36 MUT)	100% (36/36 WT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)
		90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%
Sample 2	MSI-H	100% (36/36)	77.78% (28/36 WT)	97.22% ** (35/36 WT)	100% (36/36 WT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	86.11% (31/36 WT)
		90.36-100%	61.92-88.28%	85.83-99.51%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	71.34-93.92%

SAMPLE	REFERENCE MSI STATUS	AGREEMENT TO REFERENCE STATUS % PPA (MUT) AND NPA (WT) (HIT RATE), 95% CI	INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE (HIT RATE*), 95% CI)						
			BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 3	MSI-H	100% (36/36)	80.56% (29/36 MUT)	97.22% (35/36 MUT)	97.22% (35/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	94.44% (34/36 MUT)	97.22% (35/36 MUT)
		90.36-100%	64.97-90.25%	85.83-99.51%	85.83-99.51%	90.36-100%	90.36-100%	81.86-98.46%	85.83-99.51%
Sample 4	MSI-H	100% (36/36)	100% (36/36 MUT)	66.67% (24/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	86.11% (31/36 MUT)	97.22% (35/36 WT)
		90.36-100%	90.36-100%	50.33-79.79%	90.36-100%	90.36-100%	90.36-100%	71.34-93.92%	85.83-99.51%
Sample 5	MSS	100% (36/36)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)
		90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%
Sample 6	MSS	100% (36/36)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)
		90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%
Sample 7*	MSS	100% (36/36)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	88.89% ** (32/36 MUT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)
		90.36-100%	90.36-100%	90.36-100%	90.36-100%	74.69 – 95.59	90.36-100%	90.36-100%	90.36-100%

* Borderline samples per Idylla Test v2.0 initial screening results

** While reproducibility for this marker across 36 replicates is > 85%, the biomarker call observed is discordant to the biomarker status of the clinical sample that was obtained during sample characterization.

All three sites generated correct MSI status calls across the sites for all seven clinical samples tested (Table 7). The reproducibility across 36 replicates for each biomarker per sample is between 77.8 and 100%, for some occasions a biomarker call is observed that is discordant to the initial biomarker status of the clinical sample (** indicated in Table 8).

2. MSS and MSI-H FFPE Control Reproducibility

The reproducibility of the external controls was evaluated at three laboratory sites. The test panel consisted of 2 types of contrived reference sample types (MSI-H and MSS),

manufactured in 3 different sample lots by supplier SenslD. At the individual sites, each sample was tested on a set of 4 instruments, by 2 operators and with samples originating from 3 lots during 6 non-consecutive days with 2 replicates. It was observed that the call rate per sample lot was at least 35/36 (97%) and overall call rate on for all replicates was 216/216 (100%) in this study. A call rate of at least 105/108 was observed with a point estimate of 97% for individual biomarkers (Tables 9 and 10).

Table 9. Overview of primary analysis

DESCRIPTION CRITERION	HIT RATE	POINT ESTIMATE	CI
Within sample lot	36/36	100%	[90.36% -100.00%]
Within day	36/36	100%	[90.36% - 100.00%]
Within site	72/72	100%	[94.93% -100.00%]
Within operator	108/108	100%	[96.57% -100.00%]
Within study	216/216	100%	[98.25% - 100.00%]

Table 10. The correct call rates at marker level per sample type and corresponding 95% confidence interval

SAMPLE TYPE	TARGET	CORRECT CALL	TOTAL	CALL RATE	POINT ESTIMATE	LOWER LIMIT	UPPER LIMIT
MSI-H	ACVR2A	108	108	108/108	1	0.9657	1
	BTBD7	108	108	108/108	1	0.9657	1
	DIDOl	108	108	108/108	1	0.9657	1
	MREll	105	108	105/108	0.97	0.9215	0.9905
	RYR3	108	108	108/108	1	0.9657	1
	SEC31A	108	108	108/108	1	0.9657	1
	SULF2	108	108	108/108	1	0.9657	1
MSS	ACVR2A	108	108	108/108	1	0.9657	1
	BTBD7	108	108	108/108	1	0.9657	1
	DIDOl	108	108	108/108	1	0.9657	1
	MREll	108	108	108/108	1	0.9657	1
	RYR3	108	108	108/108	1	0.9657	1
	SEC31A	108	108	108/108	1	0.9657	1
	SULF2	108	108	108/108	1	0.9657	1

3. Linearity

Not Applicable

4. Analytical Sensitivity

4.1 Limit of Blank

The limit of blank (LoB) study was conducted to evaluate the non-specific amplification and cross-reactivity between the mutant and wild type targets and consisted of two parts.

Part 1: The no template control (NTC) study wherein the level of contamination with human DNA was determined in MSI cartridges produced in the manufacturing facilities.

Part 2: Wild Type (WT) study wherein the background signals caused by potential cross-reactivity of the MSI mutation amplicons with MSI WT DNA were evaluated.

For the NTC study, 60 cartridges from three lots were tested to examine the contamination in the investigational use only (IUO) MSI Test cartridges. The results obtained from NTC study were calculated for the proportion of invalid calls (# of invalid/60). For the WT study, 20 FFPE MSS clinical samples were tested in duplicate using three different lots of IUO MSI Test cartridges to evaluate the analytical specificity of the test.

The LoB study results demonstrate that the Idylla MSI Test can correctly generate an 'Invalid' MSI status call when there is no sample in the Cartridge and the MSI status 'MSS' when a wild type sample is tested with the Idylla MSI Test.

4.2 Limit of Detection

The Limit of Detection (LoD) is defined as the lowest average mutant/total allele ratio (of weighted combined MSI biomarkers based on prevalence) generating a positive MSI status in 95% of cases (at a 95% confidence level).

Estimation of LoD with reference samples: The LoD estimation study was performed using reference cell lines (0% and 100% allelic frequency (AF) samples), by a titration experiment using varying levels of MSI-H allelic frequency (5% - 50%) at two different sample input levels (high and low input) representative for clinical samples. This study result showed the estimated LoD was 30% allelic frequency, at which the Idylla MSI Test generated correct MSI status calls for all the biomarkers (Table 11 and 12).

Table 11. Estimation of LoD results using reference (contrived) samples.

ALLELIC FREQUENCY (% MSI-H)	NUMBER OF REPLICATES TESTED	LOD ESTIMATION RESULTS	
		LOW SAMPLE INPUT	HIGH SAMPLE INPUT
		NUMBER OF CORRECT CALLS (%)	NUMBER OF CORRECT CALLS (%)
50%	24	24/24 (100%)	24/24 (100%)
30%	24	24/24 (100%)	24/24 (100%)

ALLELIC FREQUENCY (% MSI-H)	NUMBER OF REPLICATES TESTED	LOD ESTIMATION RESULTS	
		LOW SAMPLE INPUT	HIGH SAMPLE INPUT
		NUMBER OF CORRECT CALLS (%)	NUMBER OF CORRECT CALLS (%)
20%	24	24/24 (100%)	24/24 (100%)
15%	24	24/24 (100%)	24/24 (100%)
10%	24	22/24 (91.7%)	24/24 (100%)
5%	24	4/24 (16.7%)	0/24 (0%)

Table 12: LoD estimation hit rates* and 95% confidence interval per biomarker and per sample in high and low input (contrived samples)

SAMPLE DILUTION SERIES (AF%)		INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE OR HIT RATE), 95% CI)						
		BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
high input background	5% MSI-H	4% (1/24)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)
		0.74-20.24%	0.00-13.80%	0.00-13.80%	0.00-13.80%	0.00-13.80%	0.00-13.80%	0.00-13.80%
	10% MSI-H	100% (24/24)	88% (21/24)	100% (24/24)	100% (24/24)	75% (18/24)	12% (3/24)	83% (20/24)
		86.20-100%	69.00-95.66%	86.20-100%	86.20-100%	55.10-88.00%	4.34-31.00%	64.15-93.32%
	15% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	92% (22/24)	42% (10/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	74.15-97.68%	24.47-61.17%	86.20-100%
	20% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	92% (22/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	74.15-97.68%	86.20-100%
	30% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%
	50% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%
low input background	5% MSI-H	17% (4/24)	8% (2/24)	4% (1/24)	17% (4/24)	4% (1/24)	0% (0/24)	8% (2/24)
		6.68-35.85%	2.32-25.85%	0.74-20.24%	6.68-35.85%	0.74-20.24%	0.00-13.80%	2.32-25.85%
	10% MSI-H	83% (20/24)	50% (12/24)	88% (21/24)	75% (18/24)	88% (21/24)	17% (4/24)	75% (18/24)
		64.15-93.32%	31.43-68.57%	69.00-95.66%	55.10-88.00%	69.00-95.66%	6.68-35.85%	55.10-88.00%
		88% (21/24)	88% (21/24)	100% (24/24)	96% (23/24)	92% (22/24)	33% (8/24)	83% (20/24)

SAMPLE DILUTION SERIES (AF%)		INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE OR HIT RATE), 95% CI)						
		BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
	15% MSI-H	69.00-95.66%	69.00-95.66%	86.20-100%	79.76-99.26%	74.15-97.68%	17.97-53.29%	64.15-93.32%
	20% MSI-H	100% (24/24)	96% (23/24)	100% (24/24)	100% (24/24)	96% (23/24)	58% (14/24)	92% (22/24)
		86.20-100%	79.76-99.26%	86.20-100%	86.20-100%	79.76-99.26%	38.83-75.53%	74.15-97.68%
	30% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	96% (23/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	79.76-99.26%	86.20-100%
	50% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%

Clinical LoD estimation: The LoD using clinical samples was estimated using four FFPE samples by a titration experiment using varying levels of MSI-H neoplastic cell content (5-50%). The call rates for sample 4 were variable: this sample had a droplet digital PCR (ddPCR) measurement which was well below the validated linear range of the ddPCR method before dilutions were made. This sample was also originally characterized as a challenging sample with only 2 positive markers. Therefore, the call rate for this sample could also be indicative for the performance of challenging samples (Table 13).

Table 13. Estimation of LoD results using clinical samples

%NEOPLATIC CELL CONTENT	CORRECT MSI-H CALL (HIT RATE)				RESULTS (CORRECT CALLS)
	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	
50%	6/6	6/6	6/6	6/6	24/24
40%	6/6	6/6	6/6	6/6	24/24
30%	6/6	6/6	6/6	4/6	22/24
20%	6/6	6/6	6/6	5/6	23/24
10%	6/6	6/6	6/6	5/6	23/24
5%	0/6	3/6	6/6	1/6	10/24

Clinical LOD confirmation: The LOD was confirmed using seven FFPE clinical samples (MSI-H and MSS including challenging samples) at approximately 33% neoplastic cell content, and the minimum required tissue level (25 mm² per 10 µm section). The samples were tested across two Cartridge lots in ten replicates per lot (n=20) over five days on multiple Instruments (total n=140 from all seven samples). As

presented in Table 14 below, the results revealed that all seven (7) samples tested (three (3) MSS and four (4) MSI-H) generated 100 % correct MSI status calls. This study confirms the clinical analytical sensitivity (LoD) of the Idylla MSI Test at ≤33% neoplastic cell content in clinical samples at the lowest sample input size (25 mm^2 per 10 µm section).

Table 14. Clinical LoD confirmation study results (clinical samples tested at approximately 33 % neoplastic cell content and the lowest sample input)

SAMPLE ID	CARTRIDGE LOT	TOTAL RUNS	MSI STATUS (IDYLLA RESULTS)	CONCORDANT (CORRECT) CALL %
Sample 1 (MSI-H)	Lot 1	40	MSI-H	100% (80/80)
Sample 2 (MSI-H)				
Sample 3 (MSI-H)				
Sample 4 (MSI-H)	Lot 2	40		
Sample 5 (MSS)	Lot 1	30	MSS	100% (60/60)
Sample 6 (MSS)				
Sample 7 (MSS)	Lot 2	30		

Table 15. LOD confirmation study: Point estimate and 95% CI presented per biomarker and per sample for MSI-H samples (clinical specimens)

SAMPLE	INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE OR HIT RATE*) 95% CI AT 33% NEOPLASTIC CELL CONTENT						
	BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 1 (MSI-H)	100% (20/20 mut)	95.00% (19/20 wt)	100% (20/20 mut)	100% (20/20 mut)	70% (14/20 mut)	95.00% (19/20 wt)	100% (20/20 mut)
	83.89-100%	76.39-99.11%	83.89-100%	83.89-100%	48.1-85.45%	76.39-99.11%	83.89-100%
Sample 2 (MSI-H)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	95.00% (19/20 mut)	65% (13/20 mut)
	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	76.39-99.11%	43.29-81.88%
Sample 3 (MSI-H)	100% (20/20 mut)	100% (20/20 mut)	85% (17/20 wt)	100% (20/20 wt)	100% (20/20 mut)	100% (20/20 wt)	100% (20/20 wt)
	83.89-100%	83.89-100%	63.96-94.76%	83.89-100%	83.89-100%	83.89-100%	83.89-100%

SAMPLE	INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE OR HIT RATE*) 95% CI AT 33% NEOPLASTIC CELL CONTENT						
	BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 4 (MSI-H)	100% (20/20 mut)	100% (20/20 wt)	35% (7/20 wt)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)
	83.89-100%	83.89-100%	18.12-57.00%	83.89-100%	83.89-100%	83.89-100%	83.89-100%

Table 16. LOD confirmation study: mutant hit rates and 95% CI presented per biomarker and per sample for MSS samples

SAMPLE	INDIVIDUAL BIOMARKER RESULTS (POINT ESTIMATE OR HIT RATE*) 95% CI AT 33% NEOPLASTIC CELL CONTENT						
	BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 5 (MSS)	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%
Sample 6 (MSS)	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%
Sample 7 (MSS)	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%	100% (20/20 wt) 83.89-100%

*Hit rates are provided as fraction of calls observed (n/N: ratio of calls) with as reference the call observed in the initial screening
**MSI-H borderline sample per Idylla MSI Test v2.0 initial screening results
Note: 'mut' refers to a mutant biomarker and 'wt' refers to a wild type biomarker

For MSS samples, individual biomarkers score consistently wild type (Table 16). For clinical MSI-H samples, a concordance of ≥ 95%-point estimate can be observed for 4 biomarkers (BTBD7, RYR3, ACVR2A and MRE11) across all 4 MSI-H samples. The remaining biomarkers showed concordance below 95% for some samples. For SEC31A, two samples had hit rates below 95%, Sample 3 at 85% and Sample 4 at 35%. DIDO1 demonstrated a 70% hit rate in sample 1, and SULF2 demonstrated a hit rate of 65% with sample 2 (Table 15).

5. Analytical Specificity/Interference

Interference testing was performed using seven FFPE colorectal cancer clinical samples (MSI-H and MSS), including commonly encountered interfering substances such as

hemoglobin, triglycerides, and paraffin. For hemoglobin and triglycerides, interference was tested at the highest possible concentration levels (2 mg/mL and 37 mmol/L, respectively). To assess the paraffin interference, one additional 10 µm paraffin blank section was added to the cartridge along with the sample. Each sample was tested with an interfering substance in five replicates using a single lot of Idylla MSI Cartridges. Additionally, the samples were tested without an interfering substance as a control. The results obtained for each interfering substance were checked for a correct MSI status call and compared with the control results (runs without interfering substance) for the proportion (%) of concordance with control results.

Table 17. Interference results for hemoglobin, triglycerides and paraffin

INTERFERENCE SUBSTANCE	TOTAL RUN* WITH EACH INTERFERENCE SUBSTANCE	INTERFERENCE TEST RESULTS	
		CORRECT MSI STATUS CALLS (%)	CONCORDANCE WITH CONTROL RESULTS (%)
Control (No Interferent)	35	35 / 35 (100%)	-
Hemoglobin (2 mg / mL)	35	35 / 35 (100%)	100%
Triglycerides (37 mM)	35	35 / 35 (100%)	100%
Paraffin (1 x 10 µm section)	35	35 / 35 (100%)	100%

* Total runs from all seven clinical samples tested (at five runs per sample)

All seven samples tested with interference substances i.e., hemoglobin, triglycerides and excess paraffin generated correct MSI status calls, showed 100% concordance with control results (Table 17).

Mucin (i.e., % mucinous cells in FFPE sample) impact on the Idylla MSI Test was evaluated using nineteen individual FFPE clinical samples with various levels of mucinous cell content (0% - 60%). These samples included both MSI-H and MSS samples that met sample requirement criteria for the test. Each mucinous sample was tested in three replicates using one lot of Idylla MSI Test Cartridges. All nineteen mucinous samples results showed correct MSI calls for approximately 96.5% runs (55 of 57 runs) performed, except one sample which generated 'Invalid' results for 2 out of 3 runs performed. This study indicated that samples with mucinous cell content up to 60% have no impact on the performance of the Idylla MSI Test, and can still generate correct MSI status calls.

The impact of necrosis content >50% on the Idylla MSI Test was evaluated using four FFPE clinical samples with various levels of necrosis content (66-73%). The samples included both MSI-H and MSS samples. Each sample was tested in triplicate with and without necrosis content. All four samples generated a correct MSI status call for testing with and without necrosis. Therefore, necrosis content up to 73% has no impact on the Idylla MSI Test.

6. Assay Reportable Range

Seven samples consisting of four MSI-H and three MSS clinical FFPE samples (including one each of MSI-H and MSS borderline samples) were used for this study. In this study, borderline samples refer to MSI mutant samples with one positive marker (MSS borderline, negative) or 2/3 positive markers (MSI-H borderline, positive). The minimum and maximum tissue requirement was determined by a titration experiment using six different levels of input material (12.5 mm^2 – 300 mm^2) per sample using 10 µm FFPE clinical specimens containing the minimum required tumor content (i.e., ≥ 33%) per the product requirements. Sufficient FFPE MSI-H/MSS sections from each sample were liquefied using MSI Test split protocol Part 1, and these liquefactions were diluted with buffer to prepare the desired six level tissue input samples (300, 200, 100, 50, 25 and 12.5 mm^2). Each dilution sample was tested in six replicates using three different MSI Test cartridge lots. The results obtained were analyzed for proportion of correct calls and checked against the acceptance criteria specified in the protocol.

Data analysis showed that overall, the results met the acceptance criteria of ≥95% concordance (correct MSI status calls) for tissue input levels (mm^2) 300, 200, 100, 50, and 25. However, input level 12.5 mm2 did not meet the acceptance criteria with an 85.7 % (36/42) concordance (correct MSI status calls).

The results demonstrated that the Idylla MSI Test can achieve valid and correct MSI status calls with tissue input levels from minimum of 25 mm^2 to maximum of 300 mm^2 for 10 µm sections, equivalent for 4 µm sections from minimum of 62.5 mm^2 to maximum 750 mm^2 tissue input.

7. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods)

7.1 Shelf Life

Four verification lots and one validation lot were tested in support of the Idylla MSI Test shelf life. The product lots were stored at temperatures of 5±3°C and 30±2°C representing the worst-case scenarios. A limited number of cartridges were stored at temperature of 25±2°C to be tested if the instability trend is observed at 30±2°C (fallback scenario). One product lot (#3505) was subjected to the mixed thermal program prior to the real time stability testing as described in the Shipping Stability section.

The real time stability testing results support the current claimed shelf life and the unopened Idylla MSI Test is stable up to 18 months (Table 18).

Table 18. Stability testing plan

LOT #	STUDY	CONDITIONS	TIME POINTS (MONTHS)							
			T_0	T_0'	3	7	10	12	13	16	19
3505	Thermocycling followed by long term	5±3°C	x	x	-	x	-	x	x	-	x
		25±2°C			fallback scenario for 30 °C storage						
		30±2°C			x	x	x	x	x	x	x
3507	long term	5±3°C	x	-	-	x	-	x	x	-	x
		25±2°C			fallback scenario for 30 °C storage						
		30±2°C			x	x	x	x	x	x	x
3509	long term	5±3°C	x	-	-	x	-	x	x	-	x
		25±2°C			fallback scenario for 30 °C storage						
		30±2°C			x	x	x	x	x	x	x
3504	long term	5±3°C	x	-	-	x	-	x	x	-	x
		25±2°C			fallback scenario for 30 °C storage						
		30±2°C			x	x	x	x	x	x	x
3794	long term	5±3°C	x	-	-	x	-	x	x	-	x
		25±2°C			fallback scenario for 30 °C storage						
		30±2°C			x	x	x	x	x	x	x

7.2 Shipping Stability

Cartridges underwent temperature cycling between -20°C for 72 hours and +25°C for 24 hours across a total of two cycles. The third winter cycle of 48 hours at -20°C and 24 hours at +25°C hours followed immediately. After completion of winter cycling, the cartridges were exposed to the temperature of 40°C for 72 hours.

Immediately after temperature cycles, 9 MSI cartridges were tested with 6 FFPE MSI slices and 3 FFPE MSS slices to generate the time point 0' data. The reference baseline for data analysis was time point 0 data (QC release). The cartridges exposed to the thermal cycling have been stored at 30 ± 2 °C and further tested according to the real time stability study protocol.

The results show that exposing the Idylla MSI Test to high (up to 40°C) and low temperatures (to -20°C) for a limited time up to 72 hours does not impact the product performance.

7.3 In Use Stability

For this study, 84 MSI cartridges were removed from the pouch and kept in the incubator at 30 ± 2 °C and 75 % RH for 6 days. First 12 cartridges were removed from the incubator

after two hours (= day 0) and tested with 6 MSI-H samples and 6 MSS samples. The remaining cartridges were run on day 1 to 6 (six cartridges per day). In-use stability study (without pouch, without specimen) is verified at the beginning (within 3 months after T0) and at the end of shelf life.

For in-use study 1, MSI cartridges were removed from their pouches and placed in an incubator (at 30 ± 2 °C and 75 ± 5 %RH) for up to 6 days. The first cartridges were tested with MSS and MSI FFPE slices after 2 hours (= day 0) in the incubator, and the remaining cartridges were tested at the 5 subsequent time points, i.e., day 2 to day 6. Six cartridges were tested per time point: 3 cartridges with FFPE MSS slices and 3 cartridges with FFPE MSI slices.

For In-use study 2, MSI cartridges were removed from their pouches, loaded with a MSS FFPE slice or MSI slice. Six cartridges loaded with the two input sample types were run on Idylla immediately after the sample was loaded (hour 0). The other loaded cartridges were placed in an incubator (at 30 ± 2 °C and 75 ± 5 %RH) for 3 and 9 hours before running them on Idylla. Six cartridges were tested per time point: 3 cartridges with FFPE MSS slices and 3 cartridges with FFPE MSI slices.

The in-use stability data obtained at beginning and end of shelf life support that MSI cartridges can be used up to:

- 5 days after removed from the pouch and stored on the bench (at laboratory ambient conditions) without sample
- 8 hours after removed from pouch, loaded with a sample and stored at laboratory ambient conditions.

8. Assay Cut-Off

Seven novel individual MSI markers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A and SULF2) will be tested.

- A sample is called Microsatellite Instability-High or MSI-H if the sample has ≥2 mutant biomarkers and an MSI Score Range ≥ 0.15.
- A sample is called Microsatellite Stable or MSS if the sample has <2 mutant biomarkers and an MSI Score Range <0.15.

9. Cartridge Accuracy for Minimal Number of Expansions

The test panel to assess cartridge accuracy consisted of synthetic targets (ST), representing all seven MSI biomarkers, containing the repeat sequence with one additional nucleotide (+1) or one deleted nucleotide (-1). Both reference sample and spiked MSS samples were tested in triplicate in cartridges resulting from one cartridge lot (Table 19).

Table 19. Overview analytical accuracy testing results

PANEL	MSI STATUS	CALL RATE
MSS	MSS	3/3

PANEL	MSI STATUS	CALL RATE
MSS + ST cocktail 1 (-1)	MSI-H	3/3
MSS + ST cocktail 2 (+1)	MSS	3/3

Table 20. Overview individual calls on marker level

	MSI BIOMARKERS – CURVE CALL						
PANEL	ACVR2A	BTBD7	DIDO1	MRE11	RYR3	SEC31A	SULF2
MSS	0/3	0/3	0/3	0/3	0/3	0/3	0/3
MSS + ST cocktail 1 (-1)	3/3	3/3	3/3	3/3	3/3	3/3	3/3
MSS + ST cocktail 2 (+1)	0/3	0/3	0/3	0/3	0/3	0/3	0/3

All nine Idylla runs resulted in valid test results. Three cartridges tested with the control panel, MSS contrived sample, resulted in an MSS call. Three cartridges tested with MSS sample spiked with ST cocktail 1 (-1 nucleotide) resulted in an MSI-H call. Cartridges tested with MSS sample spiked with ST cocktail 2 (+1 nucleotide) resulted in three MSS calls (Table 20).

10. Carry-Over

The Idylla MSI Test is a unitized cartridge test system. Therefore, this study is not applicable.

11. **Curls versus Slides of FFPE Specimens**

Ten paired (curls and slides) FFPE MSS and ten paired (curls and slides) FFPE MSI-H samples from CRC patients that fulfilled the sample requirements per Idylla MSI Test Instructions For Use were tested. A total of 40 samples (20 curls and 20 slides) were tested. The agreement of the final MSI status (genetic) call results between curls and slides for each sample tested (10 MSS and 10 MSI-H) were compared.

The data analysis revealed that of the 20 samples tested (10 MSS and 10 MSI-H), one MSI-H sample had discordant results between curl and slide, and the remaining 19 samples showed agreement for results between curls and slides.

B. Comparison Studies:

A method comparison study was conducted to clinically validate the accuracy of the Idylla MSI Test against a predicate device, the OncoMate MSI Dx Analysis System for the detection of MSI status. A comparison of the Idylla MSI Test result to results of a germline Next Generation Sequencing (NGS) for DNA mismatch repair genes (MMR) was performed for identification of Lynch syndrome (LS) cases.

The study was conducted by testing 123 sequentially enrolled colorectal samples representative of the intended use population. The sequential sample set was supplemented with 20 confirmed Lynch cases (enrichment cohort) obtained from the Colorectal Cancer Family Registry (CCFR). The study used retrospective FFPE tissue samples collected from CRC patients in the US representative of the intended use population. All stages of CRC (stages I – IV), and tumors from different sites of the colorectal area were represented.

Of the total 143 samples included in the study (123 sequential and 20 confirmed Lynch), valid test results were obtained for all 143 with Idylla MSI Test, for 140 with the predicate and 133 with germline NGS of MMR genes.

The agreement parameters PPA, NPA and OPA were calculated between Idylla MSI Test and the predicate for MSI status calls and between Idylla MSI Test and germline NGS of MMR genes for Lynch identification.

1. Idylla MSI Test vs Predicate

The concordance and agreement analysis between Idylla MSI Test and the predicate for the 143 samples is presented in Table 21 and Table 22, respectively. A total of 32 samples were detected as MSI-H by the predicate out of which 31 were also detected as MSI-H by the Idylla MSI Test. Of the total 108 samples detected as MSS by the predicate, 107 were also MSS by Idylla MSI Test. The PPA was 96.88% and the NPA was 99.07% with OPA of 98.57% between the two methods.

Table 21. Concordance for MSI status between Idylla MSI Test and the predicate for all samples.

IDYLLA MSI TEST	ONCOMATE MSI				
	MSI-H	MSS	INVALID	NO CALL	TOTAL
MSI-H	31	1**	0	3	35
MSS	1*	107	0	0	108
Invalid	0	0	0	0	0
Total	32	108	0	3	143

*: One (1) sample that tested MSS by Idylla MSI Test and MSI-H by the OncoMate MSI Dx Analysis System is a confirmed Lynch case by NGS.
**: One (1) sample that tested MSI-H by Idylla MSI Test and MSS by the OncoMate MSI Dx Analysis System is a confirmed Lynch case by NGS.

Table 22. Percent agreement for Idylla MSI test and the predicate for all samples.

MEASURE	RATE	POINT ESTIMATE (%)	95% CI
PPA	31/32	96.88	83.78 – 99.92
NPA	107/108	99.07	94.95 – 99.98
OPA	138/140	98.57	94.93 – 99.83

Concordance and agreement analysis categorized by sequential and enrichment cohorts are provided in Table 23 - Table 26.

Table 23. Concordance for MSI status between Idylla MSI Test and the predicate for sequential cohort.

IDYLLA MSI TEST	ONCOMATE MSI				
	MSI-H	MSS	INVALID	NO CALL	TOTAL
MSI-H	15	1	0	0	16
MSS	0	107	0	0	107
Invalid	0	0	0	0	0
Total	15	108	0	0	123

Table 24. Concordance for MSI status between Idylla MSI Test and the predicate for sequential cohort.

MEASURE	RATE	POINT ESTIMATE (%)	95% CI
PPA	15/15	100	78.2 – 100.00
NPA	107/108	99.07	94.95 – 99.98
OPA	122/123	99.19	95.55 – 99.98

Table 25. Concordance for MSI status between Idylla MSI Test and the predicate for enrichment cohort.

IDYLLA MSI TEST	ONCOMATE MSI				
	MSI-H	MSS	INVALID	NO CALL	TOTAL
MSI-H	16	0	0	3	19
MSS	1	0	0	0	1
Invalid	0	0	0	0	0
Total	17	0	0	3	20

Table 26. Concordance for MSI status between Idylla MSI Test and the predicate for enrichment cohort.

MEASURE	RATE	POINT ESTIMATE (%)	95% CI
PPA	16/17	94.12	71.31 – 99.85
NPA*	0/0	N/A	N/A
OPA	16/17	94.12	71.31 – 99.85

*: All samples in the enrichment cohort are confirmed Lynch cases expected to be MSI-H.

The analysis demonstrated the agreement of the Idylla MSI Test with the predicate device, the predicate to detect MSI status from samples collected from CRC patients representative of the intended use population.

2. Idylla MSI Test Vs Germline NGS for MMR Genes

Based on the detection of germline pathogenic mutations by NGS in the MMR genes (MLH1, MSH2, MSH6 or PMS2), a total of 25 samples were Lynch syndrome positive. Table 27 and Table 28 present concordance and agreement results, respectively, between the Idylla MSI Test and germline NGS of MMR genes for all samples. Of the total 25 confirmed Lynch cases by germline NGS, the Idylla MSI Test correctly identified 23 samples as MSI-H. Two (2) samples typed as MSS were confirmed by NGS to have pathogenic mutations in the MSH2 gene.

The PPA between Idylla MSI Test and NGS for identification of Lynch syndrome was 92%. The NPA is less informative since Lynch syndrome negative samples by germline NGS can still exhibit MSI-H due to sporadic somatic mutations in one or more of the MMR genes (sporadic dMMR).

Table 27. Concordance for MSI status between Idylla MSI Test and germline NGS for MMR genes for all samples.

IDYLLA MSI TEST	GERMLINE NGS RESULTS			
	LYNCH POSITIVE	LYNCH NEGATIVE	INVALID	TOTAL
MSI-H	23	11	1	35
MSS	2	97	9	108
Invalid	0	0	0	0
Total	25	108	10	143

Table 28. Agreement for MSI status between Idylla MSI Test and germline NGS for MMR genes for all samples.

MEASURE	RATE	POINT ESTIMATE (%)	95% CI
PPA	23/25	92%	73.97 – 99.02
NPA	97/108	89.81%	82.50 – 94.80
OPA	120/133	90.22%	83.99 – 94.20

The concordance and agreement result between the Idylla™ MSI Test and germline NGS of MMR genes categorized by sequential cohort and enrichment cohort is presented in Table 29 - Table 32 below. The PPA for identification of Lynch syndrome cases was 80% and 95% with sequential and enrichment cohorts, respectively.

Table 29. Concordance for MSI status between Idylla MSI Test and germline NGS for MMR genes for sequential cohort.

IDYLLA MSI TEST	GERMLINE NGS RESULTS			
	LYNCH POSITIVE	LYNCH NEGATIVE	INVALID	TOTAL
MSI-H	4	11	1	16
MSS	1	97	9	107
Invalid	0	0	0	0
Total	5	108	10	123

Table 30. Agreement for MSI status between Idylla MSI Test and germline NGS for MMR genes for sequential cohort.

MEASURE	RATE	POINT ESTIMATE (%)	95% CI
PPA	4/5	80%	28.36 – 99.49
NPA	97/108	89.81%	82.51 – 94.80
OPA	101/113	89.38%	82.18 – 94.39

Table 31. Concordance for MSI status between Idylla MSI Test and germline NGS for MMR genes for enrichment cohort.

IDYLLA MSI TEST	GERMLINE NGS RESULTS			
	LYNCH NEGATIVE	LYNCH NEGATIVE*	INVALID	TOTAL
MSI-H	19	N/A	0	19
MSS	1	N/A	0	1
Invalid	0	N/A	0	0
Total	20	N/A	0	20

Table 32. Agreement for MSI status between Idylla MSI Test and germline NGS for MMR genes for enrichment cohort.

MEASURE	RATE	POINT ESTIMATE (%)	95% CI
PPA	19/20	95%	75.13 – 99.87
NPA	N/A	N/A	N/A
OPA	N/A	N/A	N/A

IX. Instrument Name

Biocartis Idylla System

X. System Description:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?
   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
   Yes _____ or No ____X____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No_____

3. Level of Concern:

   Moderate

4. Specimen Handling:

   Please refer to Device Description section above.

5. Calibration and Quality controls:

   In contrast to standard PCR equipment, the ldylla System is calibrated at the time of manufacturing. No user performed system calibration is needed. The instrument automatically initiates a self-test before a cartridge is processed. This test checks individual instrument modules. If the self-test fails, a fatal error is reported and the instrument is disabled. On a monthly basis, the system requires a self-diagnostic test to check for potential hardware issues. The system notifies the user of the test need by displaying a warning icon on the console. Self-diagnostic test failure leads to a fatal error and requires the intervention of authorized service personnel.

XI. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

XII. Proposed labeling:

The labeling is sufficient, and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

XIII. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

XIV. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.