February 27, 2023

Biocartis NV
Elnaz Jokar
Regulatory Affairs Manager
Generaal De Wittelaan 11 B3
Mechelen, Antwerpen 2800
Belgium

Re: K211181
Trade/Device Name: Idylla MSI Test
Regulation Number: 21 CFR 864.1866
Regulation Name: Lynch syndrome test systems
Regulatory Class: Class II
Product Code: PZJ
Dated: October 21, 2022
Received: October 21, 2022

Dear Elnaz Jokar:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR

803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Zivana Tezak-fragale -S

Zivana Tezak, PhD
Branch Chief
Division of Molecular Genetics
and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

# Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
*See PRA Statement below.*

510(k) Number *(if known)*
K211181

Device Name
Idylla™ MSI Test

Indications for Use *(Describe)*
For in vitro diagnostic use.
For use on the Biocartis Idylla™ System only.

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 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

**CONTINUE ON A SEPARATE PAGE IF NEEDED.**

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# 510(k) Summary

| | |
|---|---|
| Applicant | Biocartis NV<br>Generaal De Wittelaan 11 B<br>2800 Mechelen, Belgium |
| Contact Person | Elnaz Jokar<br>Manager, Regulatory Affairs |
| Email: | ejokar@biocartis.com |
| Preparation Date: | February 27, 2023 |

## 1. Device

| | |
|---|---|
| Device Trade Name: | Idylla™ MSI Test |
| Common Name: | Idylla™ MSI Test |
| Regulatory Section: | 21 CFR §864.1866 – Lynch Syndrome test systems |
| Classification: | II |
| Product Code: | PZJ – Lynch Syndrome test system |
| Review Division: | 88 – Pathology |
| Proposed Intended Use: | For *in vitro* diagnostic use.<br>For use on the Biocartis Idylla™ System only.<br><br>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.<br>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.<br>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.<br>The clinical performance of this device to guide treatment decision for MSI high patients has not been established. |
| Special Instrument Requirements | Idylla™ System manufactured by Biocartis, NV |
| Proposed Predicate Device | OncoMate™ MSI Dx Analysis System K200129 |

## 2. Device Description

The Biocartis Idylla™ System covers the entire process from sample to result with fully integrated sample preparation followed by PCR amplification and high-resolution melting detection of the targeted sequences. The Idylla™ System consists of the Idylla™ Console connected to one or more Idylla™ Instruments (up to eight instruments). Idylla™ Cartridges, designed for specific applications, can be processed by the Idylla System using test specific software (Test Type Package, MSI TTP). The Idylla™ MSI Test procedure and data analysis are validated for FFPE tissue sections.

The Idylla™ MSI Test detects a novel panel of seven monomorphic biomarkers.
The Idylla™ MSI Test Cartridges are ready-for-use and contain the necessary reagents to perform sample preparation, PCR amplification and high-resolution detection, starting from insertion of FFPE tissue sections. The MSI TTP directs the processing of the sample within the cartridge.

The process steps in the Idylla™ MSI Test are:

- **FFPE liquefaction and cell lysis:** After insertion of the FFPE tissue section into the cartridge, a combination of chemical reagents, enzymes, heat, and High Frequency Ultrasound (HIFU) induces deparaffinization, disruption of the tissue and lysis of the cells. The nucleic acids are liberated for subsequent PCR amplification.
- **PCR using biomarker-specific primers:** All necessary PCR reagents are present in a stable formulation and are used to amplify seven biomarkers indicative for MSI status.
- **Detection and analysis:** 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 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, the result, reporting the MSI status, the number of mutated biomarkers, and an MSI score range in the analyzed sample is displayed on the console screen.

The biomarkers detected by the Idylla™ MSI Test are listed in the Table 1 below.

**Table 1: Biomarkers**

| Biomarkers |
|---|
| ACVR2A |
| BTBD7 |
| DIDO1 |
| MRE11 |
| RYR3 |
| SEC31A |
| SULF2 |

## 3. Comparison to Predicate Device

**Table 2: Comparison to Predicate Device**

| Item | Subject Device:<br>Idylla™ MSI Test<br>K211181 | Predicate Device:<br>OncoMate™ MSI Dx Analysis System<br>K200129 |
|---|---|---|
| **Similarities** | | |
| **Regulation** | 21 CFR §864.1866 | 21 CFR §864.1866 |
| **Product Code** | PZJ | PZJ |
| **Device Class** | Class II | Class II |
| **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. | 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.<br>The OncoMateTM MSI Dx Analysis System is for use with the Applied Biosystems® |

| Item | Subject Device:<br>Idylla™ MSI Test<br>K211181 | Predicate Device:<br>OncoMate™ MSI Dx Analysis System<br>K200129 |
|---|---|---|
| | 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.<br>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.<br>The clinical performance of this device to guide treatment decision for MSI high patients has not been established. | 3500Dx Genetic Analyzer and OncoMate™ MSI Dx Interpretive Software.<br>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.<br>Results from the OncoMate™ MSI Dx Analysis System should be interpreted by healthcare professionals in conjunction with other clinical findings, family history, and other laboratory data.<br>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.<br>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 based microsatellite measurement in tumor DNA | PCR based microsatellite measurement in normal and tumor DNA |
| **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 |

| Item | Subject Device:<br>Idylla™ MSI Test<br>K211181 | Predicate Device:<br>OncoMate™ MSI Dx Analysis System<br>K200129 |
|---|---|---|
| **Instrument** | Idylla™ System | Applied BioSystems 3500 Dx Genetic Analyzer |

# 4. Analytical Performance Data

## 4.1 Analytical Specificity

The Idylla™ MSI Test qualitatively detects a novel panel of seven monomorphic biomarkers. Specifically, the 1-bp deletion of a certain biomarker is considered the mutant allele, while the normal length (no deletion) is considered the wild type (WT) allele. These mutants are frequently present in samples with MSI-H status.

In silico analysis of the human genome sequence did not identify reactivity for any of the oligonucleotide primers outside the MSI marker genes that could possibly result in non-specific detection. Therefore, any cross- reactivity of the MSI primers can be excluded.

Moreover, in silico analysis of the human genome sequence did not identify significant interference of known mutations with any of the oligonucleotide primers or beacons of the Idylla™ MSI Test that could possibly result in non-specific detection. Therefore, any cross-reactivity due to mutations can be excluded.

Limit of Blank (LoB): The LoB study was conducted to evaluate the non-specific amplification and cross-reactivity between the mutant and wild type targets. 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. This study also demonstrates that there is no cross-reactivity between the MSI mutant primers and beacons with MSI wild type DNA. The Test can correctly generate the MSI status 'MSS' when a wild type sample is tested with the Idylla™ MSI Test.

## 4.2 Analytical Sensitivity

Analytically 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). The initial LoD estimation was determined using contrived samples from cell lines designed to be representative of an average heterozygous clinical sample.

For clinical samples, LoD has been defined as the proportion of cells minimally required to obtain a correct MSI call. Estimation was done using four clinical MSI-H samples, diluted to various neoplastic cell contents with MSS samples. Final LoD confirmation studies were performed using seven clinical samples (MSI-H and MSS) at approximately 33% neoplastic cell content 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 biomarker allelic frequency (5% - 50% mimicking heterozygous clinical samples with 10- 100% neoplastic

cell content) 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. Additionally, the study results showed that the Idylla™ MSI Test generated correct MSI status calls with estimated allelic frequency LoD using contrived specimens, ranging from 15 to 30% for low input background in the individual biomarkers as demonstrated in Table 4 below.

**Table 3: Estimation of LoD Study Results**

| 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%) |
| **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 4: LoD Estimation Hit Rates and 95% CI per Biomarker and per Sample in High and Low Input**

| 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% |
| | 15% MSI-H | 88% (21/24) | 88% (21/24) | 100% (24/24) | 96% (23/24) | 92% (22/24) | 33% (8/24) | 83% (20/24) |
| | | 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 clinical LoD 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 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 5: Estimation of Clinical LoD Results**

| % Neoplastic 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 clinical LoD was confirmed using seven FFPE clinical samples (MSI-H, MSS and borderline samples) at approximately 33% neoplastic cell content, and the minimum required tissue level (25 $mm^2$ 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 6 below, all seven clinical samples (MSI-H and MSS) including those with neoplastic cell content of approximately 33%, and the lowest sample input size of 25 $mm^2$ per 10 µm section, generated reports with 100% correct calls. This study confirms the clinical analytical sensitivity (LoD) of the Idylla™ MSI Test at around 33% neoplastic cell content in clinical samples at the lowest sample input size (25 $mm^2$ per 10 µm section). A secondary analysis of the data was performed on biomarker level as shown in Table 7 for the MSI-H samples and in Table 8 for the MSS samples.

**Table 6: 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 7: 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 | 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 | 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 | 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 4 | 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% |

*Hit rates of biomarkers n/N: mutant detected/total observations

**Table 8: 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 | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) |
| | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% |
| Sample 6 | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) |
| | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% |
| Sample 7 | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) | 100% (20/20 wt) |
| | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% | 83.89-100% |

*Hit rates of biomarkers n/N: mutant detected/total observations

### 4.3 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) that satisfied the sample requirement conditions were tested at all three sites with three lots of Idylla™ MSI Test Cartridges, on the Idylla™ Platform. This study was designed to evaluate the impact of 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. As presented in Table 9 below, all three sites generated identical and correct MSI status calls across the sites for all seven clinical samples tested. Reproducibility data showed no relevant effects of the different variation sources analyzed (inter-lab, inter-Instrument, inter-lot Cartridge and inter-day) in this study. A secondary analysis of the data from the multi-site reproducibility study using FFPE clinical samples was performed for every biomarker and for each of the specimens. Table 10 provides a global overview of the analysis for every sample, including the agreement for both MSI status and individual biomarkers with 95% CI.

**Table 9: 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 10: Summary of PPA (MUT) And NPA (WT) and 95% CI for MSI Status and Individual Biomarkers in FFPE Clinical Samples**

| Sample | Reference MSI status | Tissue Size ($mm^2$) | % Neoplastic Cells in tissue | Agreement to reference status | BTBD7 | RYR3 | SEC31A | ACVR2A | DIDO1 | MRE11 | SULF2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | % PPA (MUT) and NPA (WT) (Hit rate), 95% CI | Individual biomarker results (Point estimate (Hit rate), 95% CI) | | | | | | |
| **Sample 1** | MSI-H | 75 | 30 - 40 | 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 | 30 | 50 - 60 | 100% (36/36) | 77.78% (28/36 WT) | 97.22% ** (35/36 MUT) | 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 3** | MSI-H | 160 | 30-40 | 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 | 125 | 40 | 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 | 37 | 35 | 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 | 35 | 50 | 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 | 110 | 40 | 100% (36/36) | 100% (36/36 WT) | 100% (36/36 WT) | 100% (36/36 WT) | 88.89% ** (32/36 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% | 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.

### 4.4 Interfering Substances

Interference testing was performed using seven FFPE colorectal cancer clinical samples (MSI-H and MSS) that met the sample requirement criteria, 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) per CLSI guidelines Interference Testing in Clinical Chemistry, EP07-A2. 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. As presented in Table 11 below, 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 (with no interference substance). Therefore, hemoglobin, triglycerides, and paraffin have no impact on the Idylla™ MSI Test.

**Table 11: Interference Results for Hemoglobin, Triglycerides and Paraffin**

| Interference Substance | Total Runs* 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% |

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. The results obtained were summarized for the mucinous cell content for each sample tested and their valid MSI status call. The highest mucinous cell content (%) tested, at which level the sample still generated a valid and correct MSI status call is considered as the tested interference limit for mucinous content impact on the Idylla™ MSI Test on Idylla™ Systems. 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 also 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.

## 5. Clinical Performance Data

A method comparison study design was utilized to demonstrate the diagnostic accuracy of the Idylla™ MSI Test against the OncoMate™ MSI Dx Analysis System for the detection of MSI status. A comparison of the Idylla™ MSI Test results with results from a germline Next Generation Sequencing (NGS) for DNA mismatch repair (MMR) genes was performed to confirm identification of Lynch cases. The study analysis included a total of 143 samples; of which 123 were sequentially selected (sequential cohort) from two hospital biobanks and 20 (enrichment cohort) were confirmed Lynch cases obtained from the Colorectal Cancer Family Registry. Statistical analysis was performed to calculate PPA, NPA and associated Clopper-Pearson 95% confidence intervals (CI) with valid results from testing 143 samples on the Idylla™ MSI Test, OncoMate™ MSI and germline NGS.

### Idylla™ MSI Test Vs OncoMate™ MSI Dx Analysis System

For MSI status, MSS and MSI-H, point estimates of agreements calculated were PPA of 96.88% (95% CI, 83.78 – 99.92), and NPA of 99.07% (95% CI, 94.95 – 99.98), between the Idylla™ MSI Test and the OncoMate™ MSI for all samples, i.e., sequential and enrichment cohorts combined. The concordance and percent agreement results are presented in Tables 12 and 13 below.

**Table 12: Concordance for MSI status between Idylla™ MSI Test and the OncoMate™ MSI Dx Analysis System for all samples.**

| Idylla™ MSI Test | ONCOMATE™ MSI DX | | | | |
|---|---|---|---|---|---|
| | 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 13: Percent Agreements between Idylla™ MSI Test and the OncoMate™ MSI Dx System for all samples tested.**

| 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 Tables 14 to 17 below.

**Table 14: Concordance for MSI status between Idylla™ MSI Test and the OncoMate™ MSI Dx Analysis System 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 15: Percent agreement for MSI status between Idylla™ MSI Test and the OncoMate™ MSI Dx Analysis System 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 16: Concordance for MSI status between Idylla™ MSI Test and the OncoMate™ MSI Dx Analysis System 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 17: Percent agreement for MSI status between Idylla™ MSI Test and the OncoMate™ MSI Dx Analysis System 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.

## Idylla™ MSI Test Vs Germline NGS for MMR Genes

The PPA between the Idylla™ MSI Test against germline NGS of MMR genes for all samples, i.e., sequential and enrichment cohorts combined, was 92%, and NPA was 89.81%. The NPA is less informative since Lynch syndrome negative samples by germline NGS can still exhibit microsatellite instability (MSI-H) due to sporadic somatic mutations in one or more of the MMR genes (sporadic dMMR) (Chen, W. et al. 2017 Diagn. Pathol. 12, 24). Concordance and agreement result between the Idylla™ MSI Test and germline NGS for MMR genes is presented in Tables 18 and 19 below.

**Table 18: 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 19: Percent Agreements Between Idylla™ MSI Test and Germline NGS For All Samples**

| Measure | Rate | Point Estimate | 95% CI |
|---|---|---|---|
| **PPA** | 23/25 | 92.00% | 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 Tables 20 - Table 23 below. The PPA for identification of Lynch syndrome cases was 80% and 95% with sequential and enrichment cohorts, respectively.

**Table 20: 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 |

**Table 21: Percent 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 22: 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 |

*: All samples from enrichment cohort are confirmed Lynch cases.

**Table 23: Percent 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 |

## 6. Conclusion

The Idylla™ MSI Test demonstrates substantially equivalent performance to the predicate OncoMate™ MSI Dx Analysis System.