The Idylla™ MSI Test (IVD): An Automated PCR-Based System for the Determination of MSI Status as an Aid for Identification of Probable Lynch Syndrome in Colorectal Cancer Patients

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Introduction

Over the past several decades, screening for DNA mismatch repair deficiency (dMMR) in colorectal cancer (CRC) has evolved to the point where universal testing is now recommended for all patients by the National Comprehensive Cancer Network (NCCN). The move from widely implemented clinical algorithms such as the revised Bethesda guidelines to universal screening for hereditary nonpolyposis colorectal cancer (a.k.a. Lynch syndrome) is largely driven by increased access and reduced cost of testing. Testing for dMMR typically relies on either immunohistochemical (IHC) demonstration of deficient protein expression or molecular evidence of microsatellite instability (MSI). Commercially available FDA-cleared IHC panels include BOND MMR (Leica Biosystems, UK) and VENTANA MMR IHC (Roche, Switzerland) while OncoMate™ MSI Dx (Promega Inc., USA) was the only PCR-based screening test cleared by the FDA prior to the work represented herein. The Idylla™ MSI Test[#] (Biocartis NV, Belgium) is a PCR-based fully automated system for the detection of MSI from FFPE CRC tissue, with no matching normal tissue required. This study was performed to determine the diagnostic accuracy of the Idylla™ MSI Test as part of a 510(k) premarket submission to the FDA, to demonstrate non-inferiority to OncoMate™ MSI Dx in screening for MSI in CRC tumor. Idylla™ MSI Test performance was also determined through comparison with TumorNext-*Lynch*™ next generation sequencing (NGS) results (REALM IDx, Inc., USA).

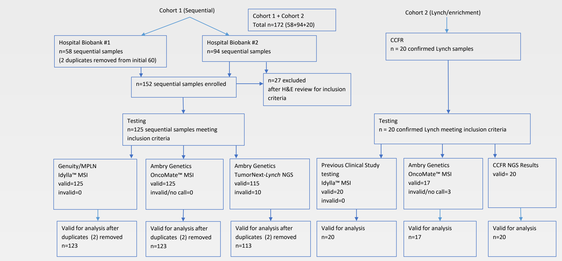
*510k cleared in the US

Materials and Methods

Specimens:

- 172 samples were enrolled under two study cohorts: sequential (Cohort 1) and enrichment (Cohort 2).
- Of 152 samples from Cohort 1, 125 met all criteria for inclusion into the study.
- Cohort 2 was comprised of 20 samples with corresponding NGS data obtained from the Colon Cancer Family Registry (CCFR).

Figure 1: Study Design



Materials and Methods (cont.)

Molecular Assays:

- The Idylla™ MSI Test, for use on the Idylla™ System, uses 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.
- Two comparator methods were utilized to evaluate the diagnostic accuracy of the Idylla™ MSI Test.
  1) OncoMate™ MSI Dx is the only previously FDA-approved method for the investigation of microsatellite status based on multiplex PCR of five microsatellite loci using paired tumor and normal FFPE samples.
  2) TumorNext-*Lynch*™ is a validated probe-based NGS panel targeting all exons and select intronic regions of the MMR genes from paired FFPE tumor and peripheral blood samples.

Statistical Analysis:

- Positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were calculated for the Idylla™ MSI Test against each comparator method for both cohorts combined.
- Acceptable diagnostic accuracy was defined as point estimates ≥90% for PPA utilizing both comparator methods and a point estimate ≥90% for NPA using OncoMate™ MSI Dx data.

Figure 2: Currently available FDA-approved testing workflows for the identification of dMMR/MSI in screening for Lynch Syndrome: VENTANA or BOND MMR panels (top), OncoMate™ MSI Dx (center), and Idylla™ MSI Test (bottom)

Table 1: Tumor site data for study samples

Tumor site	Count (Cohort 1)	Percentage (Cohort 1)	Count (Cohort2)	Percentage (Cohort 2)
Duodenum	1	0.81	0	0
Ileum	1	0.81	0	0
Appendix	1	0.81	0	0
Cecum	10	8.13	3	15
Ascending/ Right Colon	29	23.6	7	35
Transverse Colon	6	4.89	1	5
Descending/ Left Colon	8	6.50	1	5
Rectosigmoid	25	20.3	5	25
Unknown/NOS	42	34.1	3	15
Total	123	100	20	100

Results

- Invalid/No Call rates for each method utilized in this study were as follows:
  - 0/145 or 0% for Idylla™ MSI
  - 3/145 or 2.07% for OncoMate™ MSI
  - 10/145 or 6.89% for NGS.
- When compared to OncoMate™ MSI Dx, the PPA and NPA for the Idylla™ MSI Test were 96.88% (31/32) and 99.07% (107/108), respectively (Table 3).
  - Both discordant samples were confirmed Lynch cases by NGS with each of the above two methods indicating MSI in one of the two cases.
- When compared to germline NGS, the PPA and NPA for the Idylla™ MSI Test were 92.00% (23/25) and 89.81% (97/108), respectively (Table 3).
  - Two cases of germline NGS confirmed Lynch Syndrome were typed as MSS by the Idylla™ MSI Test with both samples having mutations in the *MSH2* gene (Table 2).
  - NPA is less informative when using NGS as a comparator method because Lynch syndrome negative samples by germline NGS can still be MSI-H due to sporadic somatic mutations in MMR genes.

Conclusions

- All point estimates for percentage agreement exceeded the 90% benchmark established a priori.
- The Idylla™ MSI Test is non-inferior to the OncoMate™ MSI Dx, the predicate device and only other FDA-approved PCR method for the determination of MSI status in the CRC population.
- The Idylla™ MSI Test is a clinically valid methodology for dMMR screening in CRC patients, and results of this testing can be used to guide further evaluation of individuals for Lynch Syndrome.

Table 2: MSI status and germline mutations in Lynch Syndrome cases

Cohort	Idylla™ MSI	OncoMate™ MSI	Lynch Mutations
Sequential	MSS	MSS	MSH2, c.2253_2283del31(p.R752*)
Sequential	MSI-H	MSI-H	MLH1, c.350C>T (p.T117M)
Sequential	MSI-H	MSS	MLH1, c.1614G>A (p.W538*)
Sequential	MSI-H	MSI-H	MSH6, c.2999delA (p.K1000Rfs*20)
Sequential	MSI-H	MSI-H	MSH6, c.1190_1191delAT (p.Y397Cfs*3)
Enrichment	MSI-H	MSI-H	MLH1, c.793C>A (p.R265S)
Enrichment	MSI-H	No Call	MLH1, c.793C>T (p.R265C)
Enrichment	MSS	MSI-H	MSH2, 1705_1706delGA f/shift
Enrichment	MSI-H	MSI-H	MSH2, c.942+3A>T (p.V265_Q314del)
Enrichment	MSI-H	MSI-H	MLH1, c.199G>A (p.G67R)
Enrichment	MSI-H	MSI-H	PMS2, c.1939A>T (p.K647X)
Enrichment	MSI-H	MSI-H	MSH2, ex1_8del
Enrichment	MSI-H	MSI-H	MSH2, c.1661G>A (p.S554N)
Enrichment	MSI-H	MSI-H	MSH2, M2x1-x2 deletion
Enrichment	MSI-H	MSI-H	MSH2, c.679ins37 fshift
Enrichment	MSI-H	MSI-H	MSH2, c.136_164del29 fshift
Enrichment	MSI-H	MSI-H	MLH1, c.793C>T (p.R265C)
Enrichment	MSI-H	MSI-H	MSH2, c.942+3A>T
Enrichment	MSI-H	MSI-H	MLH1, c.350C>T (p.T117M)
Enrichment	MSI-H	MSI-H	MSH2, c.1216C>T (p.R406X)
Enrichment	MSI-H	No Call	MSH2, c.1165C>T (p.R389X)
Enrichment	MSI-H	MSI-H	MLH1, c.1667_1668insA
Enrichment	MSI-H	MSI-H	MSH2, c.942+3A>T (p.V265_Q314del)
Enrichment	MSI-H	No Call	MSH6, 2731C>T (p.R911X)
Enrichment	MSI-H	MSI-H	PMS2, c.1492del11

Table 3: Idylla™ vs. OncoMate™/NGS

Measure	Rate	Point Estimate (%)	95% CI
PPA	31/32	96.88	83.78-99.92
	23/25	92.00	73.97-99.02
NPA	107/108	99.07	94.95-99.98
	97/108	89.81	82.50-94.80
OPA	138/140	98.57	94.93-99.83
	120/133	90.22	83.99-94.20

References

1) Referenced (permission waived) from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Colon Cancer V.3.2023. © National Comprehensive Cancer Network, Inc 2023. All rights reserved. Accessed [Oct 1, 2023]. To view the most recent and complete version of the guideline, go online to NCCN.org.
2) Revised Bethesda Guidelines for Hereditary Nonpolyposis Colorectal Cancer (Lynch Syndrome) and Microsatellite Instability. *JNCI: Journal of the National Cancer Institute*, Volume 96, Issue 4, 18 February 2004, Pages 261-268

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