A performance characterization of microsatellite instability and mismatch repair testing methods in endometrial samples

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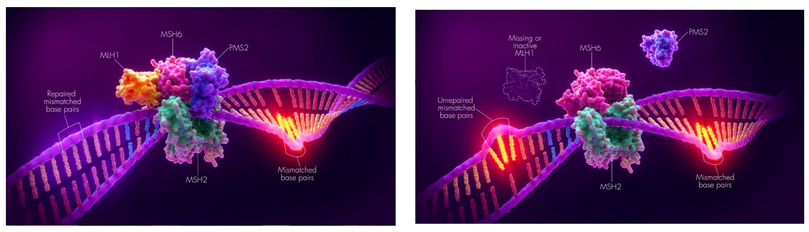
Abstract #4251

1. Introduction

Microsatellite instability (MSI), a functional measure of mismatch repair (MMR) deficiency, is a well-established technology traditionally used for Lynch Syndrome screening in colorectal cancer. Recently testing for MSI has expanded from hereditary cancer risk testing to a new role predicting immunotherapeutic response in all solid tumors. With this increase in utility and awareness, mismatch repair deficiency is now relevant in a variety of other cancer tissue types. However, little data is available for the performance of these testing techniques in non-colorectal cancer samples. In this study, we examine performance of methods for detecting MSI in endometrial cancer samples.

MSS (pMMR) **MSI-H (dMMR)**



2. Background: MSI Analysis by PCR

To detect MSI, specific sites in the genome are amplified and separated by size. Changes in size between the tumor and normal sample indicate that there is microsatellite instability present.

A panel of five mononucleotide repeats for MSI analysis, has been established as the most sensitive and specific way to detect mismatch repair deficiency in colorectal cancers. However, with the expansion of this testing into other tissue types, there is discussion as to whether a pentaplex is sufficient. In this study, we investigate the value of additional loci by comparing a pentaplex versus a 13-plex assay for detection of MSI in solid tumor samples.

5 quasi-monomorphic mononucleotide panel

Mononucleotides are more sensitive and specific than dinucleotides for detecting dMMR

Locus	Repeat	Sensitivity (%)	Specificity (%)	
D2S123	Di	91	86	
D17S250	Di	88	93	
D5S346	Di	81	98	
BAT-25	Mono	96	99.5	NCI Panel Aka "Bethesda Panel"
BAT-26	Mono	93	100	
MONO-27	Mono	98	100	Promega Panel [2,3]
NR-21	Mono	99	100	
NR-24	Mono	92	100	

[2]Bacher et al. 2004
[3]Murphy et al. 2006

- Measured using the Promega MSI Analysis System, v1.2
- Loci chosen for ability to identify dMMR
- 5 highly specific and sensitive mononucleotide markers measured[2,3]
- 16 years of data to support use of these loci

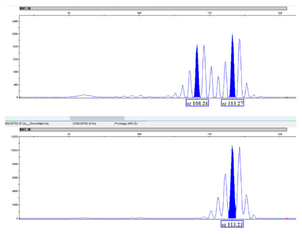
13–plex panel

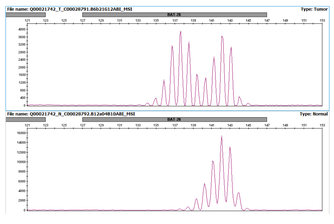
- BAT-25
- BAT-26
- BAT-40
- CAT-25
- NR-21
- NR-22
- NR-24
- NR-27
- ABI-16
- ABI-17
- ABI-19
- ABI-20A
- ABI-20B

- Measured by the TrueMark™ MSI Assay
- 4 established loci
- 5 unknown proprietary loci

3. Experimental Methods

51 matched tumor and normal endometrial cancer samples were obtained from a biorepository for testing with MSI by PCR followed by capillary electrophoresis and immunohistochemistry for MMR protein expression. Loss of immunostaining for one or more MMR protein was considered dMMR. MSI panels were run according to the manufacturer's recommended protocol. MSI was defined for each panel as outlined below.





Pentaplex Calling:
Sample analysis was performed for the Promega MSI Analysis System using automated peak calling by the GeneMapper™ Software (example shown above).

- 2+/5 markers unstable = MSI-H
- 1/5 markers unstable =MSI-L
- 0/5 markers unstable = MSS

Instability at each marker locus was defined as a 2.75bp or greater difference between the smallest peak called with GeneMapper between the Normal and Tumor sample.

13-plex Calling:
Sample analysis was performed for TrueMark MSI Assay using the TrueMark MSI Analysis Software with default conditions (example shown above).

- 4+/13 markers unstable= MSI-H
- 1-3/13 markers unstable=MSI-L
- 0/13 markers unstable =MSS

Mechanism of calling instability at each marker has not been disclosed.

No normal is required for this method but was included for this study.

All Reagents Mentioned are for Research Use Only.

4. MSI by PCR methods compared to dMMR IHC

Testing for MSI was performed with the standard mononucleotide pentaplex panel and a 13-plex microsatellite panel. A 3bp cutoff for marker instability was used for the Promega assay based on the optimal calling method previously established for colorectal cancer samples. In addition to MSI analysis, each sample was tested for MMR protein expression with immunohistochemistry. Agreement to IHC was assessed by calculating positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA).

Agreement with DNA Mismatch Repair Protein Immunohistochemistry

Promega MSI Analysis System, 1.2

	dMMR	pMMR
MSI-H	6	0
MSI-L	4	0
MSS	7	34

PPA:	59%
NPA:	100%
OPA:	86 %

TrueMark MSI Assay

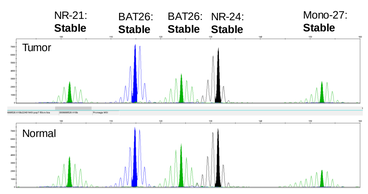
	dMMR	pMMR
MSI-H	10	5
MSI-L	1	6
MSS	2	16
No Call	7	4

PPA:	55%
NPA:	58%
OPA:	53%

Example Sample 900017163

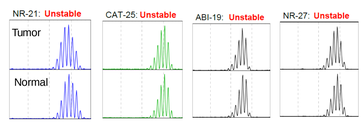
Promega MSI Analysis System, 1.2

Overall Call: **MSS** Unstable **0 of 5**



TrueMark MSI Assay

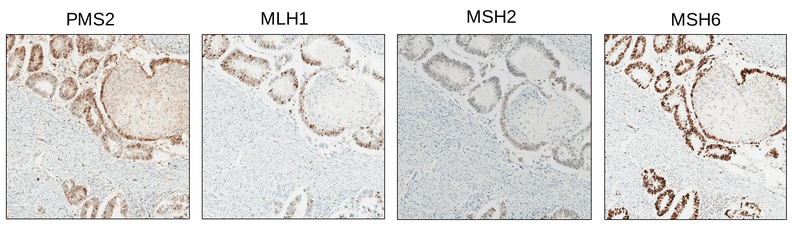
Overall Call: **MSI-High** | Unstable: **4 of 13**



*Shown are only the loci called as unstable by the TrueMark MSI Analysis Software.

Immunohistochemistry for Sample 900017163

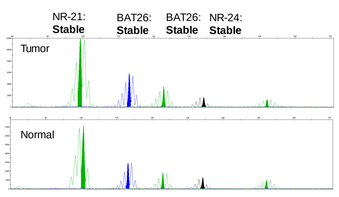
Overall Call: **pMMR, No Loss of Expression**



Example Sample Q00026959

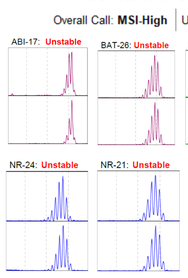
Promega MSI Analysis System, 1.2

Overall Call: **MSS** Unstable **0 of 5**



TrueMark MSI Assay

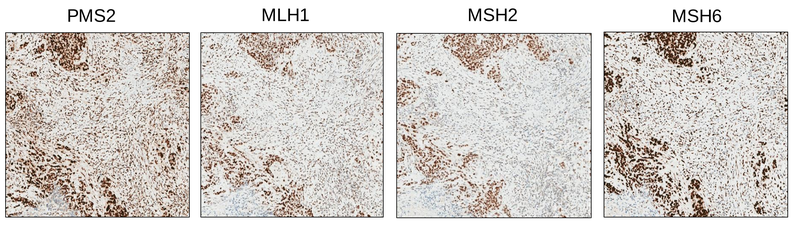
Overall Call: **MSI-High** | Unstable: **5 of 13**



*Shown are only the loci called unstable by the TrueMark MSI Analysis Software.

Immunohistochemistry for Sample Q00026959

Overall Call: **pMMR, No Loss of Expression**



- The MSI Analysis System, V1.2 exhibited high sensitivity (59%) and specificity(100%) in endometrial samples, with overall percent agreement at 86%.
  - No false positive samples were called with this method.
  - No assay failures were observed with this method
- The TrueMark MSI Assay exhibited similar sensitivity (55%) but decreased specificity (58%) with an overall percent agreement of 53%.
  - 11 false positives were called with this method using the automated software.
  - 11/51 (20%) samples were not called due to assay/analysis failure.

Conclusion:

These results show high concordance between IHC and a pentaplex panel of mononucleotides. A 13-plex with automated calling showed lower concordance to IHC and a high rate of false positives.

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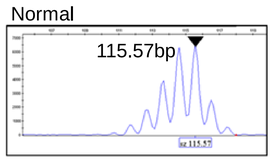
5. 2 Base Pair vs. 3 Base Pair Shift Definition

Determining stability at each marker is a crucial step in MSI analysis. The definition of a shift between the tumor and normal allele determines how small a change can be detected. Detecting shifts that are too small can confuse analytical variability for real instability, resulting in false positives. A very conservative definition can cause a decrease in sensitivity. These two parameters must be balanced for an assay to perform optimally. Endometrial samples are known to exhibit smaller shifts compared to colorectal samples; therefore, we tested the use of a 3bp vs. a 2bp cutoff for calling marker instability with the Promega MSI System, v1.2 and examined performance. A similar comparison could not be done with the TrueMark™ MSI Assay, since instability calling uses an undisclosed algorithm.

Marker Instability Calling Definitions Used

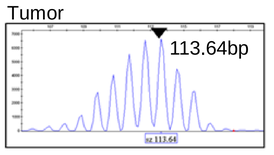
Shift = Normal peak size (bp) – Tumor peak size (bp)
- If Shift ≥ Cutoff, then marker is unstable.
- If Shift < Cutoff, then marker is stable.

Normal



3bp Shift Definition
Cutoff defined as a 2.75bp (3 bp + 0.25bp allowance based on the variability of the capillary electrophoresis instrument used)

Tumor



2bp Shift Definition
Instability at each locus was defined as a 1.75bp (2 bp + 0.25bp allowance based on the variability of the capillary electrophoresis instrument used)

BAT26 example above:
115.57 - 113.64 = 1.93bp Shift
3bp cutoff – **stable**
2bp cutoff - **unstable**

Performance of 2bp vs. 3bp Cutoff for Endometrial Samples

The 51 sample endometrial cohort was reanalyzed using a 2bp calling cutoff and 3bp cutoff for marker instability.

Promega MSI Analysis System, 1.2

2bp Calling Performance

	dMMR	pMMR
MSI-H	9	0
MSI-L	5	0
MSS	5	32

PPA:	74%
NPA:	100%
OPA:	90 %

3bp Calling Performance

	dMMR	pMMR
MSI-H	6	0
MSI-L	4	0
MSS	7	34

PPA:	59%
NPA:	100%
OPA:	86 %

- Lowering the threshold for instability at each marker from 3bp to 2bps slightly increases sensitivity from 59% to 74% and overall percent agreement from 86% to 90%.
  - No false positive samples were called with either call threshold.

Conclusion:

The Promega MSI Analysis System, v1.2 exhibits slightly higher concordance with IHC when a 2bp cutoff is used vs. a 3bp. The method of analysis may be important for optimal performance in endometrial samples.

6. Conclusions

MSI by pentaplex panel and immunostaining for MMR proteins showed high concordance. Decreased specificity and increased assay failure was observed with 13-plex panel utilizing non-standard loci and a proprietary undisclosed analysis software algorithm.

Using an MSI assay with an increased rate of false positives could lead to costly unnecessary testing and incorrect study results. This study supports earlier work establishing the importance of what loci are included in a panel when performing MSI analysis, as they can greatly affect the performance of the assay.

Further experiments were performed to characterize how the definition of instability at the marker level can effect performance in endometrial samples. More studies are needed but this evidence suggests that a lower (2bp vs 3bp) definition may increase sensitivity of a pentaplex panel in extra-colonic cancers.

The markers (loci) and analysis method used for MSI testing should be carefully considered for maximum sensitivity and specificity in endometrial cancer samples.