# MICROSATELLITE ANALYSIS: COMPARISON BETWEEN BETHESDA AND HAMELIN PANEL

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## INTRODUCTION

Microsatellites are non-coding DNA sequences and their instability, which indicates a damaged mismatch repair (MMR) system, is used as marker for assessing prognosis and treatment decisions in many cancer types, expecially in colorectal cancer (CRC). Immune checkpoint inhibitors have demonstrated great efficacy in cancer patients with microsatellite instability-high (MSI-H). There are many panels available on the market used to establish the microsatellite status, but the only one approved by international authorities and the most diffused one remains the Bethesda assay, which is based on the analysis of 2 mononucleotide (BAT25 and BAT26) and 3 dinucleotide (D2S123, D5S346 and D17S250) repeats and requires the analysis of paired tumor and healthy tissues. However, there are emerging data showing that other assays can better describe the microsatellite status compared to the Bethesda panel. A novel, one-instrument, fast and objective method for detection of MSI (MicroSight® MSI) was developed by the Danish company PentaBase A/S. The assay does not depend on the use of a paired healthy reference and analyzes five well-described mononucleotide microsatellite sequences (BAT25, BAT26, NR22, NR24 and MONO27) by real-time PCR followed by high-resolution melt (HRM). The microsatellite length is evaluated via PCR product melting profiles.

## AIM

The aim of this study was to evaluate the efficacy of the new MicroSight® MSI panel by comparing the results obtained with the fragment analysis PlentiPlex™ MSI PentaBase Panel.

## PATIENTS AND METHODS

A PCR fragment analyisis using the ready-to-use PlentiPlex™ MSI PentaBase Panel, which includes the mononucleotide loci BAT25, BAT26, NR22, NR24 and MONO27, was conducted in order to characterize the MSI status of 185 patients affected by CRC. The results were considered as reference and were used to calculate sensitivity and specificity for the MicroSight® MSI assay. Subsequently, all 185 patients were tested with MicroSight® MSI panel. From these samples, 30 cases were selected and analyzed in four other laboratories located in Italy (Legnano, Novara, Turin and Terni). Furthermore, the assay was validated by analyzing a validation cohort comprising at least 30 in-house CRC samples. In addition, a universal reference was set up to be used in the absence of normal tissue.

## RESULTS

The presence of normal and tumor cells affect the microsatellite length leading to a change in the shape of the melting curve. This fact make it possible to differentiate between microsatellite stability (MSS) and MSI. Using a universal reference, a temperature shift was applied in order to differentiate unstable from stable samples. (Figure 1).

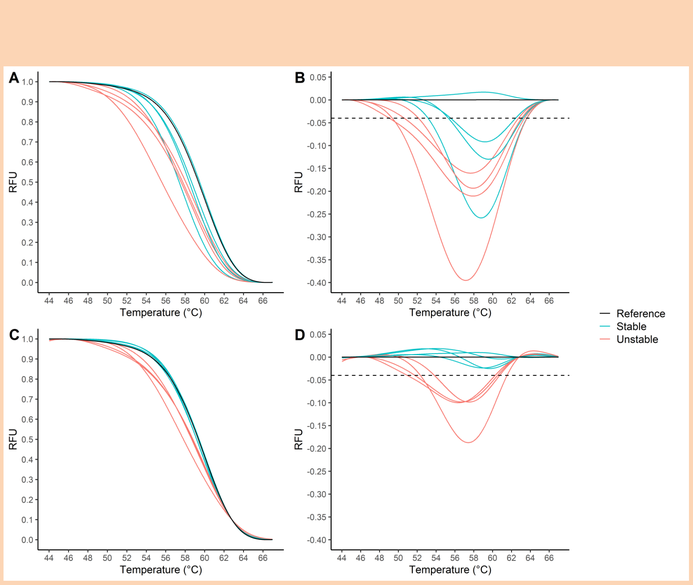


Figure 1:MicroSight® MSI analysis using universal reference. Temperature shift makes it possible to differentiate unstable from stable samples. A) Normalized HRM curves without temperature shift. B) Difference plot without temperature shift. Threshold was set at -0.04 RFU. C) Normalized HRM curves with temperature shift at 0.1 RFU. D) Difference plot with temperature shift at 0.1 RFU. Threshold was set at -0.04 RFU. MSI: microsatellite instability; HRM: high resolution melt; RFU: relative fluorescence unit.

In laboratory 1, all samples gave the same MSI-classification as the PCR-fragment analysis using both paired samples and universal reference. Stability was observed in 151 cases and MSI-H in 34. Concerning the remaining four laboratories, all MSS and MSI-H patients selected from the initial 185 were also correctly classified using both paired samples and universal reference. Stability was observed in 25 cases and MSI-H in 5 (Table 1).

| Type | Laboratory | MSS | MSI-L | MSI-H | Cohen's Kappa [95% CI] |
|---|---|---|---|---|---|
| Paired | 1 | 151/151 | 0/0 | 34/34 | 1.000 |
| Paired | 2 | 25/25 | 0/0 | 5/5 | 1.000 |
| Paired | 3 | 25/25 | 0/0 | 5/5 | 1.000 |
| Paired | 4 | 25/25 | 0/0 | 5/5 | 1.000 |
| Paired | 5 | 25/25 | 0/0 | 5/5 | 1.000 |
| Universal | 1 | 151/151 | 0/0 | 34/34 | 1.000 |
| Universal | 2 | 25/25 | 0/0 | 5/5 | 1.000 |
| Universal | 3 | 25/25 | 0/0 | 5/5 | 1.000 |
| Universal | 4 | 25/25 | 0/0 | 5/5 | 1.000 |
| Universal | 5 | 25/25 | 0/0 | 5/5 | 1.000 |

Table 1. MicroSight® MSI agreement to the PCR fragment analysis.

## CONCLUSIONS

The development and validation of the novel MicroSight® MSI assay for the detection of MSI in cancer patients showed high agreement with PCR fragment analyisis and demonstrated high sensitivity and specificity using both paired samples and universal reference. MicroSight® MSI demonstrated very similar performance to the gold standard, but is superior in ease-of-use, instrument cost and turn-around-time. MicroSight® MSI obtains results in less than 90 minutes, there is no need for the evaluation of normal mucosa biopsy for paired samples which leads to a decrease of time and cost for DNA preparation, and the analysis is objective and does not require highly trained personnel. Therefore, MicroSight® MSI can be easily implemented in every laboratory, even in an institute without a background of MSI evaluation.