

Myriad myPath[®] Melanoma Technical Specifications

Myriad Genetic Laboratories, Inc. Effective Date: Oct, 2015

TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

Indications and Use

Intended Use

This gene expression signature is intended for the *in vitro* analysis of melanocytic neoplasms to aid in the diagnosis of the lesion as benign or malignant. This is an adjunctive assay and should be used in conjunction with clinical data and histopathological features.

Summary and Explanation

Melanoma is the 5th most common cancer in men and the 6th most common cancer in women. There is a 1 in 50 chance for a person living in the United States to develop melanoma in their lifetime. It is estimated that in 2015 approximately 73,800 cases of melanoma will be diagnosed and 9,940 people will die from melanoma.¹

Melanoma is highly curable if diagnosed and treated in early stages. There is a marked difference in survival between localized and metastatic disease, with 10 year survival rates of 86-95% for patients with the localized disease, versus 10-68% for patients with advanced metastatic melanoma.¹ Therefore, diagnostic markers that facilitate accurate diagnosis of melanoma at earlier stages could help prevent progression of the disease and reduce patient mortality. The Myriad myPath[®] Melanoma assay features 23 unique molecular biomarkers whose gene expression profile has been shown to differentiate benign lesions from malignant melanoma with high sensitivity and specificity in multiple independent cohorts.²

Description of Methods

Acceptable samples are formalin-fixed paraffin-embedded (FFPE) tissue from blocks or slides of melanocytic lesions. Myriad requires one 3-5 μ m H&E slide representative of the lesion, followed by 5-8 consecutive 4-5 μ m unstained slides. Melanocytic lesions that are at least 0.5 mm x 0.1 mm with a minimum of 10% cells of interest should be present on the H&E slide. Samples containing less material may be accepted at the discretion of the Myriad pathologist. If submitting FFPE block(s) for processing, the block(s) should contain the tissue most representative of the lesion. Multiple blocks may be submitted if more than one block is necessary for adequate representation of the lesion. Slides or block(s) should be shipped overnight to Myriad Genetic Laboratories, in the Myriad myPath Melanoma test kit according to the included Specimen Instructions.

Upon receipt of a sample, sample barcodes are applied to each slide or block and are scanned and tracked. The H&E slides from each case are evaluated by a board certified pathologist who determines the location and amount of the lesion to be tested. Using the H&E slide as a guide, the corresponding tissue is macrodissected from the unstained slides and total RNA is extracted from the tissue. The expression of 14 genes within the diagnostic signature are measured and normalized by 9 housekeeping genes. The gene signature includes 13 genes with known immune

functions and one gene that regulates cellular differentiation. Gene expression is measured in triplicate, using quantitative reverse transcription polymerase chain reaction (qRT-PCR), and is analyzed to generate a Myriad myPath Melanoma Score.

Development and Validation

The training of the signature was conducted on 464 melanocytic lesions consisting of all major histological subtypes and derived from two independent sources.² The signature was validated in a second cohort of 437 melanocytic lesions, consisting of all major histological subtypes and derived from four different independent sources.² The signature performance was determined by comparing the Score (as determined by the expression of the gene signature) to consensus histopathologic diagnosis (as determined by comparing the pathology report and one independent, blinded review by a board-certified dermatopathologist with discordant diagnoses adjudicated by a third independent board certified dermatopathologist).

Score thresholds were established that would classify a sample as malignant, benign or indeterminate.² For Scores outside the indeterminate zone in the validation cohort, the gene expression signature had a sensitivity of 94% (95% CI of 90%-97%) and a specificity of 90% (95% CI of 85%-94%). Furthermore, 9% of total lesions were classified as indeterminate.

Interpretive Criteria

Scores between -16.7 and -2.1

Scores from -16.7 to -2.1 will be reported as consistent with a benign nevus.

Scores between -2.0 and -0.1

Scores from -2.0 to -0.1 will be reported as indeterminate.

Scores between 0 and 11.1

Scores from 0 to 11.1 will be reported as consistent with a malignant melanoma.

Scores less than -16.7 or greater than 11.1

Based on an analysis of 437 melanocytic lesions in the validation study,² a reportable Score range of -16.7 and 11.1 was established and Scores within this range will be reported. Scores outside of the validated range may lead to test cancellation or follow-up with the referring healthcare provider.

Performance Characteristics and Limitations

Analytical Precision, Detection Limit and Linearity

A set of 30 samples was each tested in triplicate and the standard deviation of the Score in this dataset was 0.69 (95% CI: 0, 0.82)³, which represents 2.5% of the range of scores observed in the clinical validation.² The signature is validated for samples with RNA concentrations ranging

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from 2-40 ng/ μ L (RNA input of 25-500 ng).² The linearity of the signature has been verified by testing samples with RNA yields of 50 ng/ μ L (RNA input of 625 ng), and performing serial dilutions of the RNA to yields as low as 0.5 ng/ μ L (RNA input of 6.25 ng).³ Scores were observed to remain constant throughout this concentration range, due to normalization by housekeeper genes. Samples with RNA concentrations >40 ng/ μ L (RNA input >500 ng) are diluted to 40 ng/ μ L; samples with concentrations <2 ng/ μ L (RNA input of 25 ng) are not tested because 2 ng/ μ L is the limit of detection for RNA concentration.

Interference

Immunosuppressant therapy, radiation treatment or re-excised specimens may lead to inaccurate results for this signature. Thus, patients receiving immunosuppressant therapy or radiation treatment prior to biopsy and re-excised lesions are not suitable for testing.

Melanin can be a potent inhibitor of PCR, but melanin sufficient to inhibit the assay has not been observed to accrue during RNA extraction.³ Thus, even highly pigmented melanocytic lesions are acceptable for testing.

Quality Control Measures

A minimum of one no-RNA control and one human RNA control (with a previously determined Score) are run with each sample and analyzed to verify expected results. Housekeeper genes are also used as internal controls to measure the quality of a sample.

Limitations of the Assay

Performance characteristics of the Myriad myPath[®] Melanoma gene expression signature have not been established for tissue other than FFPE melanocytic lesions. The signature has not been validated on re-excision specimens, melanomas known to be metastatic, non-melanocytic neoplasms, or lesions subjected to prior treatment. Analysis of these samples may result in incorrect test interpretation. Therefore, these specimens are not suitable for testing and will be canceled.

The FFPE preservation process or improper sample storage may cause RNA degradation within a lesion, resulting in insufficient quality or quantity of RNA for analysis.

Results from this test should be used in conjunction with other information from clinical evaluation, histopathological features and other diagnostic procedures.

Sample Rejection Criteria

Inappropriate sample types can cause cancellation of the test. Inappropriate sample types include re-excision specimens, melanomas known to be metastatic, non-melanocytic lesions, samples not fixed in neutral buffered formalin or samples from patients that received immunosuppressant or radiation treatments prior to biopsy. Samples of insufficient quantity of neoplastic cells, or

insufficient quality may also be canceled (which may be due to insufficient neoplastic cells within the sample, damage during shipping, or insufficient RNA yields). A test may also be canceled if the Score is outside of the validated range of Scores (see Interpretive Criteria).

References

1. American Cancer Society; www.cancer.org. Revised March 20, 2015.
2. Clarke LE, Warf MB, Flake DD, et al. Clinical validation of a gene expression signature that differentiates benign nevi from malignant melanoma. *J Cutan Pathol.* 2015; 42:244-252.
3. Warf MB, Flake DD, Adams D, et al. Analytical validation of a melanoma diagnostic signature using formalin-fixed paraffin-embedded melanocytic lesions. *Biomark Med.* 2015; 9(5):407-416.