Myriad myPath® Melanoma Technical Specifications
Myriad Genetic Laboratories, Inc. Effective Date: Dec 3, 2018

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

Indications and Use

Intended Use

The Myriad myPath® Melanoma gene expression signature is intended for the *in vitro* analysis of primary cutaneous melanocytic neoplasms to aid in the diagnosis of those neoplasms as benign or malignant. This is an adjunctive test and should be used in conjunction with clinical examination, histopathological findings, and any other relevant information.

Summary and Explanation

Melanoma is the 5th most common cancer in men and the 6th most common cancer in women. It is estimated that in 2018 approximately 91,270 cases of melanoma will be diagnosed and 9,320 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 signature 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.[2-5]

Description of Methods

Acceptable samples are formalin-fixed paraffin-embedded (FFPE) tissue from blocks or slides of melanocytic lesions. Blocks should contain melanocytic lesions that are at least 0.5 mm x 0.1 mm, with a minimum of 10% cells of interest. In cases where blocks are unavailable, one 3-5 µm H&E slide representative of the lesion, followed by at least 20 µm of unstained tissue on slides may be acceptable. 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 the expression of 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 gene expression signature was conducted on 464 melanocytic lesions, from two independent sources, representing a wide variety of clinical-histological melanocytic neoplasm subtypes, using concordance with histopathologic diagnosis by expert pathologists.[2] Score thresholds were established that would classify a sample as malignant, benign or indeterminate. Performance of the signature was assessed in three independent clinical validation studies, two using concordance with histopathologic diagnosis,[2,3] and in one clinical validation study using clinical outcomes.[4] In these validation studies, for Scores outside the indeterminate zone, the signature had a sensitivity of 90-94% and a specificity 91-96%. The signature has a lower sensitivity of approximately 80% when assessing desmoplastic melanomas.[5]

Interpretive Criteria

Scores from -16.7 through -2.1

These Scores will be reported as consistent with a benign nevus.

Scores from -2.0 through -0.1

These Scores will be reported as indeterminate.

Scores from 0 through 11.1

These Scores 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 first validation study, a reportable Score range of -16.7 and 11.1 was established.[2] Samples with Scores outside of the validated range are canceled.

Performance Characteristics and Limitations

Analytical Precision, Detection Limit and Linearity

A set of 14 samples was each tested in triplicate and the standard deviation of the Score in this dataset was 0.7 (upper 95% CI: 1.0),[6] which represents 2.5% of the reportable range of scores from -16.7 to 11.1.[2] In regards to RNA input linearity, the maximum RNA input concentration is 40 ng/µl (500 ng) and consistent results are obtained when samples are diluted until the average housekeeper gene Ct value exceeds 24.[6-7] Samples with an average housekeeper value >24 are invalid and will lead to test cancelation.

Interference

Immuno-suppressant therapy, radiation treatment or re-excision of a lesion may lead to inaccurate results for this signature. Thus, patients receiving immune-suppressant 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 testing has not been observed to accrue during RNA extraction.[6,7] 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 each sample.

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Limitations

Performance characteristics of the signature have not been established for tissue other than formalin-fixed paraffin-embedded (FFPE) primary cutaneous melanocytic lesions. The signature has not been validated on re-excised lesions, metastatic lesions (*i.e.* non-primary melanomas), non-melanocytic neoplasms, or lesions subjected to prior treatment (*e.g*. radiation therapy). Therefore, these samples are not suitable for testing.

RNA degradation may occur as a result of the FFPE preservation process, or improper sample storage/transport, 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 test cancelation. Inappropriate sample types include re-excised lesions, metastatic lesions (*i.e.* non-primary melanomas), non-melanocytic lesions, samples not fixed in neutral buffered formalin, or samples from patients that received treatment prior to biopsy. Samples of insufficient quantity of neoplastic cells, or insufficient quality may also be canceled. 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 Oct, 2018.
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. Clarke LE, Flake DD, Busam K, *et al*. An independent validation of a gene expression signature to differentiate malignant melanoma from benign melanocytic nevi. *Cancer*. 2017; 123(4):617-628.
4. Ko J, Matharoo-Ball B, Billings SD, *et al*. Diagnostic distinction of malignant melanoma and benign nevi by a gene expression signature and correlation to clinical outcomes. *Cancer Epidemiol Biomarkers Prev*. 2017; 26(7):1107-1113.
5. Clarke LE, Pimental K, Kimbrell H, Busam K. Gene expression signature as an ancillary method in the diagnosis of desmoplastic melanoma. 2017. *American Soc Dermatopathology Annual Meeting*.
6. Data on File. Oct 2018
7. 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.