New River Labs MelaPro Dx™ Technical Specifications

TEST RESULT SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS

Effective Date: February 14, 2019

Intended Use

MelaPro Dx is a clinically validated test for patients whose cutaneous melanocytic lesion is not definitively malignant or benign based solely on clinical and histopathological evaluations. The test utilizes histology guided mass spectrometry (HGMS) profiling for molecular differentiation of malignant melanomas from benign melanocytic nevi in formalin fixed paraffin embedded (FFPE) tissues. MelaPro Dx is the first ancillary diagnostic test to evaluate the proteomic contents of melanocytic cells through molecular profiling. MelaPro Dx provides additional scientific data to help the ordering physician more accurately diagnose ambiguous cutaneous melanocytic lesions. The ordering physician should consider the test result, along with all other histopathological data, clinical examinations, and other relevant findings, to make an overall diagnosis.

Summary and Explanation

Melanoma incidence in the US has risen considerably since the early 2000s with an estimated 1% increase in cases each year.[1] Accordingly, the number of skin biopsies in the US has more than doubled within that same time period with a current annual estimate of over four million biopsies per year.[1,2] Up to 14% of biopsies are classified as ambiguous based on histopathological evaluations, and a high degree of diagnostic uncertainty and discordance can be observed even among experienced dermatopathologists.[3,4,5] In response to these challenges, MelaPro Dx was developed as an ancillary test to augment histopathological evaluations, aiding in the accurate diagnosis of melanocytic lesions when histopathology alone is insufficient.

Description of Methods

Physicians may request a MelaPro Dx test kit at their convenience. Either FFPE tissue sections or FFPE tissue blocks of melanocytic lesions are required for testing. Specimens must contain sufficient melanocytic component for a minimum of five (5), 100-micron regions to be targeted for analysis. If FFPE tissue sections are provided, three (3), 5-micron thick serial sections of tissue must be mounted onto individual test kit slides according to the Test Kit Instructions. Completed test kits, along with the completed Test Requisition Form, are then shipped to New River Labs, LLC (New River Labs).

Upon test kit receipt, the specimen will be inspected to ensure the specimen requirements are met. The conventional microscope slide will be stained with hematoxylin and eosin (H&E), and a high resolution digital image of the tissue section will be produced. An experienced, board certified dermatopathologist will review the pathology report, any molecular test results, and the digital image of the H&E stained tissue section to confirm the specimen is suitable for testing. If the specimen is accepted for testing, the dermatopathologist will identify and annotate the areas of melanocytic component to be targeted for proteomic analysis. Digital images of the annotated H&E stained section and the unstained section on one of the indium-tin oxide (ITO) slides are then merged, allowing for relevant tissue regions to be targeted for HGMS

profiling. ITO slides are deparaffinized and antigen retrieved, followed by on-tissue tryptic digestion and CHCA matrix application. Proteomic fingerprints of targeted regions are acquired via a Bruker Matrix Assisted Laser Desorption/Ionization (MALDI) Time of Flight (TOF) mass spectrometer. Molecular data are then analyzed via the MelaPro Dx algorithm, and results are populated into the ordering physician's online portal account.

Interpreting Results

The MelaPro Dx algorithm assigns a score based on statistical analysis of the proteomic fingerprints obtained from the mass spectrometer. Scores are determined based on the relative abundance and overall distribution of 1,075 mass spectral peaks generated from the targeted melanocytic cells. Test scores range from -10 to +10 and are classified into one of the following groups:

Benign: -10.000 to -0.371

Indeterminant: -0.370 to +0.370

Malignant: +0.371 to +10.000

Clinical Validation

Algorithm training was performed with 211 melanocytic lesions composed of 100 malignant melanomas and 111 benign nevi.[7] All benign specimens received a definitive clinical diagnosis with a minimum of five (5) years of patient clinical follow up information. Malignant samples required at least two (2) dermatopathologists to concur on a histopathological diagnosis of malignant melanoma. Seven (7) malignant melanoma subtypes (acral lentiginous, desmoplastic, lentigo maligna, nevoid, nodular, Spitzoid, and superficial spreading), three (3) benign nevus subtypes (acral, conventional, and Spitz), and metastatic melanoma lesions were collected and analyzed.

Algorithm validation was performed using 545 melanocytic lesions, including 257 malignant melanomas and 288 benign nevi, and resulted in a sensitivity of 98% and a specificity of 99%. An indeterminant rate of less than 5% was observed among specimens analyzed. Indeterminant specimens were excluded from the sensitivity and specificity calculations. Validation specimens consisted of 165 biopsies and 380 tissue cores that were part of tissue microarrays (TMAs). If multiple tissue sections or cores pertaining to a single case were present in either the biopsy or TMA specimens, the associated tissue sections or cores were aggregated and analyzed as a single specimen. Specimens were received from multiple clinical laboratories across the US as well as from other countries and, in some cases, were more than 20 years old. Validation studies show that specimen collection variability among different laboratories does not adversely affect the ability of HGMS to correctly classify melanocytic lesions.[6,7]

Interferences

Formalin fixation causes methylene crosslinks between reactive groups of amino acid residues. Fixatives other than neutral buffered formalin will likely result in different protein crosslink patterns, which may yield inaccurate results.

Limitations

MelaPro Dx is validated for seven (7) melanoma subtypes (acral lentiginous, desmoplastic, lentigo maligna, nevoid, nodular, Spitzoid, and superficial

spreading), three (3) benign nevus subtypes (acral, conventional, and Spitz), and metastatic melanoma. MelaPro Dx has not been validated for non-cutaneous neoplasms, non-melanocytic neoplasms, re-excision specimens, specimens directly exposed to radiation therapy, or specimens from patients currently, or recently, receiving chemotherapy. Therefore, these specimens are not suitable for testing and will be rejected.

MelaPro Dx is an ancillary test intended to provide additional scientific data to aid the ordering physician in the diagnostic process. This data is not intended to be used as diagnostic information without the review of the ordering physician. The ordering physician should consider the test result, along with all other histopathological data, clinical examinations, and other relevant findings, to make an overall diagnosis.

Specimen Rejection Criteria

New River Labs, in its sole discretion, may reject a specimen if, for any reason, it believes the specimen does not meet the test requirements. Specimens may be rejected for the following reasons:

- Specimen is not a cutaneous melanocytic lesion, based solely on a review of the documentation submitted by the ordering physician
- Specimen is a cutaneous melanocytic lesion for which MelaPro Dx has not been validated, based solely on a review of the documentation submitted by the ordering physician
- Specimen contains insufficient melanocytic cells for testing or is of inadequate quality to allow for required annotation of melanocytic cells, based solely on a review of the digital image of the H&E stained tissue section
- Specimen is preserved in a fixative other than neutral buffered formalin
- If FFPE tissue sections, extra paraffin was added to the slides
- Specimen was overheated, resulting in paraffin that is visibly melted
- Specimen sections are improperly adhered to the slides or adhered to the wrong side of the slides
- Specimen sections are not serial
- Specimen or slides are visibly damaged
- Test Requisition Form was not submitted through the New River Labs online physician portal, Test Requisition Form is incomplete, and/or a paper copy of the Test Requisition Form was not also placed in the test kit when submitted for testing
- Copies of the pathology report and/or any molecular test results were not submitted through the New River Labs online physician portal or placed in the test kit when submitted for testing
- Copy of the patient insurance card, including secondary insurance, if applicable, was not submitted through the New River Labs online physician portal or placed in the test kit when submitted for testing

Certification

MelaPro Dx was developed, and its performance characteristics determined, by New River Labs. The test has not been cleared or approved by the FDA. New River Labs meets the requirements under CLIA to perform high complexity clinical laboratory testing. Patent pending.

References

1. Weinstock MA, et al. 2017. Skin biopsy utilization and melanoma incidence among Medicare beneficiaries. Br J Dermatol 176(4): 949-954.

2. Wang DM, et al. 2018. An ecological study of skin biopsies and skin cancer treatment procedures in the United States Medicare population, 2000 to 2015. J Am Acad Dermatol 78(1):47-53.

3. Shoo BA, et al. 2010. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. J Am Acad Dermatol 62(5):751-756.

4. Farmer ER, et al. 1996. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Pathol 27(6):528- 531.

5. Elmore JG, et al. 2017. Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. BMJ 357: j2813.

6. Lazova R, et al. 2016. Imaging mass spectrometry assists in the classification of diagnostically challenging atypical Spitzoid neoplasms. J Am Acad Dermatol 75(6): 1176- 1186.

7. Data on file.