FINAL REPORT

Patient: John Doe
Sex: Male
DOB: 00/00/0000
Client: Institution
Clinician: Provider Name

Tumor Site: Left ankle
Specimen ID: S00-00-00000
Collected: 00/00/0000
Received: 00/00/0000
Reported: 00/00/0000

DecisionDx® DiffDx™-Melanoma Result

Benign

Gene expression profile suggestive of benign neoplasm

TEST DESCRIPTION

The proprietary **DecisionDx-DiffDx-Melanoma (DiffDx-Melanoma)** test is an empirically derived multi-analyte algorithmic assay (e.g. MAAA). The **DiffDx-Melanoma** test is a 35-gene qRT-PCR assay that employs a neural network algorithm comprised of 2 gene expression signatures inclusive of 32 discriminant and 3 control genes[1]. The algorithm was trained on a set of patients with definitive diagnosis of either benign nevi or malignant melanoma. The test yields one of 3 results: benign, intermediate-risk of malignancy or malignant.

TEST VALIDATION AND PERFORMANCE METRICS IN ADULTS

Test Validation: The **DiffDx-Melanoma** test was validated in an independent cohort totaling 503 pigmented lesions. Within this clinical validation study, adult patients represented 478 (230 melanomas and 248 nevi) cases[1]. Test performance was determined in this patient subset through comparison of probability scores to consensus diagnosis via histopathologic review by board-certified dermatopathologists. The table below shows accuracy metrics for lesions in patients ≥18 years of age.

Accuracy metrics*	Sensitivity	Specificity	PPV	NPV
	99.1%	96.2%	96.1%	99.1%

*Accuracy metrics were calculated without the inclusion of lesions identified as intermediate risk (3.8% of the total samples).

BACKGROUND AND INTENDED USE

Background: Current methods used for definitive diagnosis of melanoma are sufficient for the majority of lesions. However, histopathologic assessment can be challenging, even for experienced dermatopathologists. High rates of diagnostic discordance have been reported.[2–6] The **DiffDx-Melanoma** test refines the diagnosis of nevi and melanoma by providing an objective tool to aid in classification of pigmented lesions.

Intended use: The **DiffDx-Melanoma** gene expression test is intended for the in vitro analysis of primary cutaneous melanocytic lesions for which malignant potential is uncertain. This ancillary test aids in characterizing these lesions as benign or malignant and should be interpreted in the context of other clinical, laboratory and histopathologic information.

Castle Biosciences, Inc. Sherri Borman, PhD, HCLD, Laboratory Director

PERFORMANCE IN ALL AGES

Test Validation: The **DiffDx-Melanoma** test was validated in an independent cohort of pigmented lesions totaling 503 (230 melanomas and 273 nevi)[1]. The algorithmic score provided three classifications: benign, intermediate-risk of malignancy and malignant. Test performance was determined through comparison of probability scores to consensus diagnosis via histopathologic review by board-certified dermatopathologists. The table below shows accuracy metrics for lesions in patients ≥2 years of age.

Accuracy metrics*	Sensitivity	Specificity	PPV	NPV
	99.1%	**94.3%**	**93.6%**	**99.2%**

*Accuracy metrics were calculated without the inclusion of lesions identified as intermediate risk (3.6% of the total samples).

ADDITIONAL INFORMATION ABOUT THE TEST

The 32 discriminating genes are: ABLIM1, ANXA8L1, ATP6V0E2, BAP1, BCL2A1, BTG1, CLCA2, CST6, CSTA, CXCL14, DCT, DSP, DUSP4, GATA3, GJA1, GPR143, HAL, KLF5, KRT17, KRT2, MGP, NES, PPL, PTN, RPL37A, RPS16, S100A8, S100A9, SAP130, SFN, TP63, WIPI1. Three control genes consist of YKT6, HNRNPL, FXR1.

All data shown in this report were collected and verified under an IRB approved multi-center study to establish and validate the test's diagnostic accuracy in benign nevi versus malignant melanoma.

REFERENCES

1. Estrada, S. I. *et al.* Development and validation of a diagnostic 35-gene expression profile test for ambiguous or difficult-to-diagnose suspicious pigmented skin lesions. *SKIN J. Cutan. Med. In press*, (2020).
2. Elmore, J. G. *et al.* Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. *BMJ* **357**, j2813 (2017).
3. Shoo, B. A., Sagebiel, R. W. & Kashani-Sabet, M. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. *J. Am. Acad. Dermatol.* **62**, 751–756 (2010).
4. Farmer, E. R., Gonin, R. & Hanna, M. P. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. *Hum. Pathol.* **27**, 528–531 (1996).
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6. Warycha, M. A. *et al.* Changes in the Presentation of Nodular and Superficial Spreading Melanomas Over 35 Years. *Cancer* **113**, 3341–3348 (2008).