

Non-Invasive Gene Expression Testing to Rule Out Melanoma

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ABSTRACT

The Pigmented Lesion Assay (PLA) is a gene expression test that helps rule out melanoma and has the potential to reduce the need for surgical biopsies of atypical pigmented skin lesions. Utilizing a new technological platform for the non-invasive profiling of skin, the assay analyzes samples collected from adhesive patches for expression of two key genes (PRAME and LINC00518) known to be overexpressed in melanoma. The test result is binary (positive/negative) based on the detection of one or both genes. PLA positive cases are generally biopsied to establish the histopathologic diagnosis, while PLA negative cases are considered for ongoing monitoring. The combination of visual inspection with histopathology, the current gold standard for melanoma diagnosis, has a relatively low negative predictive value (NPV) of approximately 83%, meaning that 17% of melanomas will be interpreted as benign lesions. In contrast, the PLA has a very high NPV (>99%). Further, with its high specificity (69-91%), use of the PLA can reduce the number of false positive samples subjected to histopathology review. By adding the PLA to the current care pathway, the number of surgical biopsies needed to find a melanoma (number needed to biopsy) is markedly reduced from 20-25 biopsies for dermatologists and 39 biopsies for physician assistants, to an average of 2.7. To date, unnecessary surgical procedures of benign lesions have been reduced by 88% based on a sample of more than 20,000 analyzed cases. This has resulted in fewer missed melanomas and significant cost savings to health care systems.

Key words: Pigmented Lesion Assay, non-invasive, melanoma, gene expression, test

Introduction and Current Care Pathway

Management of atypical pigmented lesions involves ruling out melanoma via visual and/or dermoscopic assessment followed by surgical biopsy and histopathologic examination (Figure 1A).^{1,2} Ideally, when melanomas are identified, they are found at the earliest stages (melanoma *in situ* [MIS]/Stage 1a) when a high cure rate is possible by wide excision.^{3,4} While the purpose of the visual assessment/surgical biopsy paradigm is to rule out melanoma, this approach has relatively poor performance metrics with an estimated 3-10% specificity for visual examination alone.⁵ This, coupled with the low sensitivity of 64-84% for histologic assessment^{6,7} and the estimated in-office prevalence of around 5-10%, leads to a low NPV for early stage disease (83%, Figure 1). Thus, during histopathologic assessment, a small number of melanomas are identified from a large pool of biopsied pigmented lesions. Perhaps even more concerning is the risk of false negative histologic diagnoses resulting from a significant overlap in the histopathologic criteria between atypical nevi and

early stage melanoma.⁶⁻⁸ Elmore et al. concluded the diagnosis of early stage melanoma was not accurate after finding 187 pathologists misinterpreted 35% of slide interpretations for MIS/Stage 1a melanomas.⁶ Given the prevalence of early stage melanoma in biopsied lesions is approximately 5-10%, the NPV can be approximated as between 75-89%.^{5-7,9,10}

The number of surgical biopsies needed to identify one melanoma (NNB, number needed to biopsy) averages around 20-25 and ranges from 8 to >30 depending on the clinical setting.^{5,9,11-13} Further complicating the issue is that the histopathologic assessment of routinely biopsied lesions, without serial sectioning, is limited. With routine step sectioning of the tissue block providing less than 2% of the material for evaluation, there remains uncertainty as to what is present in the rest of the specimen. With the current diagnostic approach, it is estimated that, in the United States, 3 million pigmented lesion surgical biopsies were performed in the year 2017 alone, yielding <200,000 melanoma diagnoses.^{14,15}

The purpose of this review is to briefly summarize and compare the current pathway for pigmented lesion management to a novel diagnostic pathway that includes the incorporation of a non-invasive Pigmented Lesion Assay (PLA) to help guide biopsy decisions.

Pigmented Lesion Assay Overview

The PLA is a gene expression test that helps clinicians rule out melanoma and avoid the need for a surgical biopsy of concerning pigmented lesions (Figure 1B).¹⁶⁻²² The PLA is based on a new technology for non-invasive skin testing that permits gene expression analysis of skin samples collected with adhesive patches.¹⁹ In order to retrieve enough genetic material, the lesion is sampled consecutively four times, each time with a different patch. For each patch, the clinical margin of the lesion is delineated in pen and then the outlined tissue is dissected from the surrounding sample at the processing lab. Finally, the recovered RNA is extracted and analyzed for two indicator genes. The indicator genes used are PRAME (Preferentially Expressed Antigen in Melanoma) and LINC00518 (Long Intergenic Non-Coding RNA 518), both of which are overexpressed in melanoma. These genes were categorized as the key factors driving test performance in a microarray-based gene expression screen that identified a group of 312 genes differentially expressed in melanoma versus non-melanoma pigmented lesion samples.^{16,21}

Sampling of the most superficial skin layers contains information from deeper epidermal cells as a result of normal skin physiology in which basal cells migrate to the surface of the skin as they differentiate into squamous cells. During this process, keratinocytes acquire melanosomes from melanocytes through a phagocytic process of the melanocyte dendrite. In addition, some melanocytes migrate to the skin surface by a process known as pagetoid spread. Consequently, epidermal sampling with an adhesive patch yields molecular material from a variety of cells, including melanocytes, keratinocytes, and immune cells.¹⁹ In contrast to histopathologic sectioning, this method of genetic tissue sampling allows for the collection of material from the entire lesion. The PLA is intended for use in patients 18 years of age or older with pigmented lesions measuring 5 mm or larger and suspicious for, but without obvious clinical features of melanoma. It is not intended for use on non-melanocytic lesions (e.g., seborrheic keratoses), non-melanoma skin cancers (e.g., basal cell carcinomas) and bleeding or ulcerated lesions. Further, at present, the PLA cannot be used on palms, soles, nails, or mucous membranes.

Importantly, the PLA is intended to aid clinicians in surgical biopsy decisions but not to be used as a diagnostic test for melanoma. Positive PLA tests should be followed with a surgical biopsy, while patients with a negative test can have the lesion monitored per standard of care.

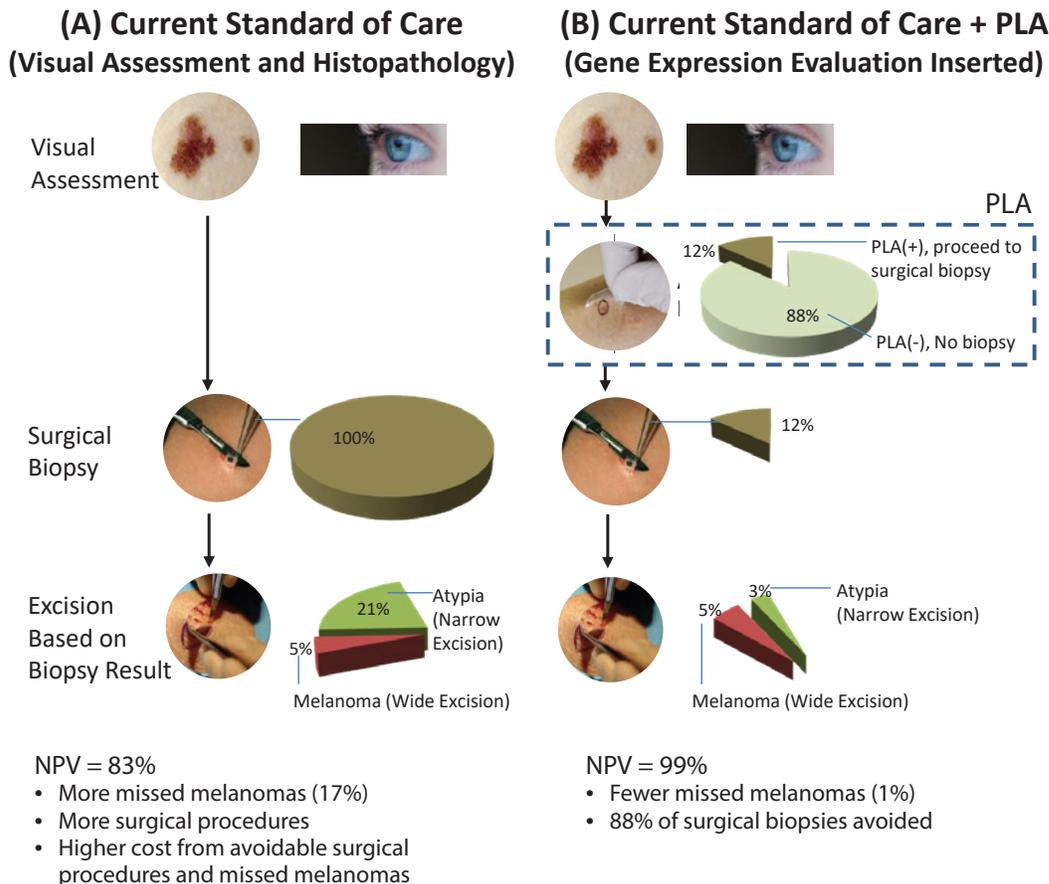


Figure 1: Comparison of the current standard of care for pigmented lesion management using visual assessment followed by surgical biopsy and histopathology (A) to a pathway that includes non-invasive gene expression testing by PLA (B).

	Current Standard of Care	PLA
Test purpose	Rule out melanoma	Rule out melanoma
Type	Visual assessment with surgical biopsy/histopathology	Noninvasive gene expression
Negative predictive value (NPV)	83%	99%
Probability of missed melanoma	17%	1%
Number needed to biopsy	20	2.7

Table 1: Comparison of the key performance metrics of the PLA versus the current standard of care (visual assessment and surgical biopsy/histopathology) for ruling out melanoma.

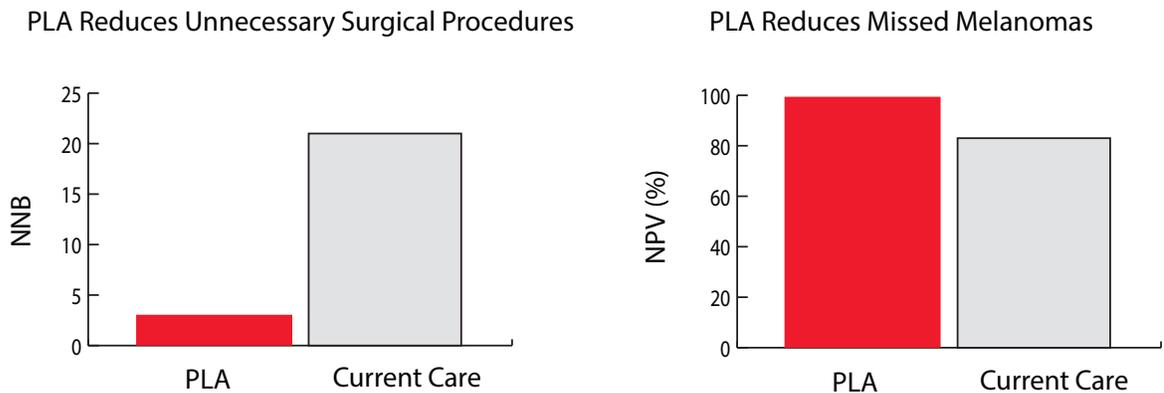


Figure 2: Summary of the key improvements that the PLA demonstrates over the current standard of surgical biopsy and histopathology. The PLA reduces the number of biopsies needed to identify a melanoma (NNB, number needed to biopsy). With a negative predictive value (NPV) >99%, the PLA ensures few melanomas will be missed.

PLA Versus Current Standard of Care

Table 1 compares the key performance metrics of the PLA against the current standard of care (visual assessment and surgical biopsy/histopathology) for pigmented lesion management. In contrast to the current standard of care, the PLA has a very high NPV (>99%) coupled with a high sensitivity (91-95%), ensuring a very low probability of missing a melanoma.^{16,17} The relatively high specificity of the PLA (69-91%) also helps to effectively reduce the number of lesions that would require subsequent histopathology review.¹⁶ Consequently, using the PLA, the number of lesions needed to be biopsied to find one melanoma is reduced from 20-25 to 2.7 (Table 1, Figure 2).^{5,9,11-13,17}

By utilizing the PLA, unnecessary surgical procedures may be reduced by as much as 88%.¹⁸ The findings of this internal data set is consistent with a recently published review of 18,715 biopsied pigmented lesions where 83% of those lesions were either benign or mildly atypical nevi and did not require additional treatment.¹⁰ Thus, about 90% of surgical biopsies performed on pigmented lesions in the general community may be avoidable.

Conclusion

In the current diagnostic pathway for pigmented lesions, the relatively low specificity of the clinical examination has resulted in a large number of biopsies to ensure the detection of melanoma. The addition of PLA to this diagnostic pathway, can lead to fewer surgical procedures and would provide significant benefits to patients such as reduced pain, infections and

scarring. In addition, significant benefits accrue to the healthcare system because the PLA can reduce the costs associated with unnecessary surgical procedures.²² Most important, however, is the lower probability of missing a melanoma compared with the current standard of care. The PLA provides a unique and disruptive technology for the assessment of pigmented lesions that may soon transform the current diagnostic pathway to one that is less often invasive, highly reproducible, and a cost savings to the health care system.¹⁶⁻²⁰

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