

Pigmented Lesion Assay

Non-invasive gene expression analysis of pigmented skin lesions



Performance and Development Notes

Overview

The DermTech Pigmented Lesion Assay (PLA) provides physicians with readily interpretable, non-invasively obtained gene expression information for clinically atypical pigmented skin lesions. The PLA provides two results: the PLA MAGE (Melanoma Associated Gene Expression) and the PLA Score. Both are used to provide additional clinical information about pigmented lesions to aid the decision for surgical biopsy. The PLA captures the current state of the specimen's dynamic melanoma-associated gene expression profile, and gives an objective evaluation of a pigmented lesion's behavior at the time of the patient visit

The PLA test begins with the non-invasive collection of a stratum corneum specimen using a proprietary adhesive-patch collection and shipping kit provided by DermTech. Detailed Instructions for Use and all materials necessary are contained in the kit. Once a specimen is received in DermTech's laboratory, RNA is isolated from the epidermal material on the patches and the expression levels of 6 genes are measured.

The PLA MAGE (Melanoma Associated Gene Expression), which detects the presence or absence of expression for two specific genes (*PRAME* and *LINCO0518*), is used as the primary profile assessment. An additional algorithmic combination of the gene expression levels, when either of the primary genes are expressed, is used to calculate the specimen's PLA Score, a single number ranging from 0 to 100 that supplements the PLA MAGE. The PLA Report displays both results and facilitates the physician's rapid assessment of the specimen's similarity to melanomas or non-melanomas.

Performance

Clinical performance measures for the PLA were determined from multiple rigorously controlled studies by comparing the PLA MAGE and the PLA Score to specimens' histopathologic diagnoses established by a panel of expert dermatopathologists^{1,2}. The clinical validation study on 319 pigmented lesions (83 melanomas and 236 non-melanomas) established the ability of the PLA to discriminate melanomas from non-melanomas in specimens collected with adhesive patches. Thirty seven percent (37%) of the specimens had a melanoma in situ or lentigo maligna diagnosis and the average thickness of invasive melanomas was 0.64mm at biopsy.

Concordance DX	PLA MAGE	
	positive	negative
All Melanoma N=83	92%	8%
All Non-Melanoma N=236	36%	64%

The table above shows the performance of the PLA MAGE for discrimination between melanomas and non-melanomas. Sensitivity is 92%, specificity is 64%, and the prevalence-adjusted Negative Predictive Value (NPV) is > 99%.

The PLA MAGE performance is enhanced by incorporating the PLA Score to further discriminate among melanomas and non-melanomas that have a positive PLA MAGE. Higher PLA scores are seen in melanomas (both in situ and invasive), while lower PLA scores are seen in atypical nevi, conventional nevi and other non-melanoma lesions. Atypical nevi with *LINCO0518* and/or *PRAME* expression typically score lower than melanomas, with conventional nevi and other lesions typically scoring lower still.

The PLA Score when applied post-hoc to PLA MAGE-positive specimens can increase the specificity as shown in the table below.

	Sensitivity	Specificity
PLA MAGE only	92%	64%
PLA MAGE with PLA Score \geq 8	92%	77%

The combined PLA MAGE and PLA Score interpretation performs well across lesion subtype categories, and may allow characterization of the lesion subtype as shown below.

Diagnosis Subtype	Median PLA Score
Melanoma	78
Atypical Nevi	41
Conventional Nevi	5
Other Non-Melanoma	16

The Negative Predictive Value of a test is an indicator of how often a negative result is correct in a real-life situation in clinical practice (a patient with an unclear pigmented lesion walking through the door). The PLA has an NPV of greater than or equal to 99% when adjusted to account for a prevalence of 7% melanomas in a typical pigmented lesion dermatology practice setting.

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DermTech developed the PLA by first recognizing dermatologists' unmet need for an objective and cost-effective method of examining pigmented lesions that are clinically suspicious. DermTech's scientists screened the entire human genome for differential gene expression between melanoma and non-melanoma pigmented skin lesions³. The PLA development program has studied over 650 pigmented lesions including over 250 melanomas.

The expression levels of all 30,000 human genes were measured in adhesive-patch sampled melanomas and non-melanomas, and by using advanced machine-learning statistical analyses, a refined set of 6 genes was ultimately found whose RNA levels provide the power needed to reliably differentiate between melanomas and non-melanomas. RNA expression for the PLA Detection and Score is measured for the genes *PRAME*, *LINC00518*, *CMIP*, *B2M*, *ACTB*, and *PPIA*. *PRAME* (PReferentially expressed Antigen in MElanoma) is known to have a role in oncogenesis, and *LINC00518* (Long Intergenic Non-protein Coding RNA518) is a member of a newly described class of regulatory RNA molecules; both are elevated in melanomas compared to the other genes. The other genes act to provide necessary normalization values for laboratory processes.

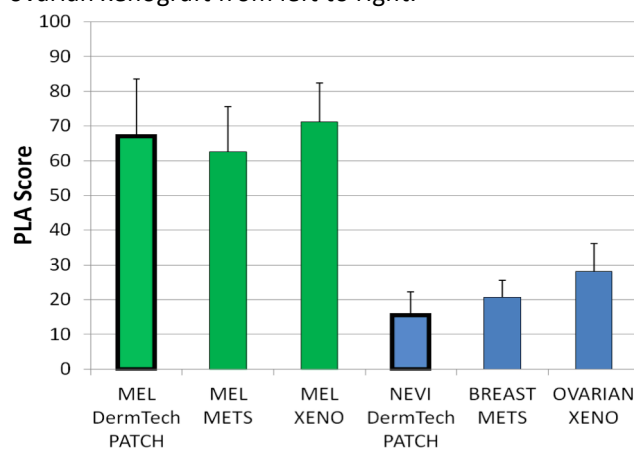
The PLA MAGE and PLA Score were trained on specimens ascertained under the same protocols and inclusion criteria as the validation specimens. The clinical performance characteristics shown were evaluated using the locked algorithm on validation sets that were separate and distinct from the training sets. All of DermTech's studies were performed under rigorous conditions, including full Institutional Review Board approvals for research involving human subjects. The dermatopathologists on the panel were blinded to each other's diagnoses, and the laboratory staff was blinded to all clinical details and diagnoses.

PLA in Tumor Surgical Biopsies

The PLA reflects the underlying tumor biology. In a study that compared PLA Scores from adhesive-patch sampled melanomas and biopsied metastatic tumor tissues⁴, our data showed no statistical differences between adhesive-patch sampled melanomas and biopsied melanoma metastases. In addition, the study demonstrated that the PLA scores distinguished all melanoma specimens from adhesive patch sample non-melanoma skin lesions and biopsied metastases (breast and ovarian cancers).



PLA scores were measured for metastatic melanomas and breast cancers by isolating RNA directly from biopsy tissue. Additionally, PLA scores were measured in metastatic melanomas and ovarian cancers from patient derived xenograft tissues that were propagated in nude mice. The tissue and xenograft PLA scores were compared with PLA scores from adhesive patch sampled melanoma and non-melanoma lesions. The graph below shows the mean PLA score for specimens in each tumor category, with the standard deviation indicated by the error bars. Melanoma specimen types are shown in green, with adhesive patch, metastatic tumor biopsy, and xenograft tumor biopsy from left to right, and non-melanoma specimen types are shown in blue, with adhesive patch, metastatic breast biopsy, and metastatic ovarian xenograft from left to right.



These results indicate that the PLA Score can effectively differentiate melanomas from non-melanomas in the underlying tumor tissue, including metastatic sites, and not just in the adhesive patch sampled stratum corneum.

References

1. Development of a novel noninvasive adhesive patch test for the evaluation of pigmented lesions of the skin. (2014) *J. Amer. Acad. Dermatol.* 71(2):237-44.
2. Data on file at DermTech, pending publication.
3. Noninvasive Genomic Detection of Melanoma (2011) *Br. J. Dermatol.* 164(4):797-806. Stratum Corneum RNA Levels Are Diagnostic for Melanoma, commentary. (2011) *Br. J. Dermatol.* 164(4):693-4.
4. American Academy of Dermatology, 2015, 73rd annual meeting. Poster available at www.dermtech.com.

About DermTech

DermTech is a commercial stage dermatology company developing non-invasive gene expression tests to aid the clinical diagnosis of skin cancer and other skin conditions. DermTech operates a CLIA licensed laboratory (CLIA ID #05D2073519) located at the company's La Jolla, CA headquarters. DermTech's patented technology allows the genomic analysis of skin samples collected non-invasively using an adhesive patch rather than a scalpel. DermTech provides highly accurate, objective information to physicians that improves patient care and reduces healthcare costs. For additional information visit:

www.dermtech.com or call toll-free 1-866-450-4223