The current 23- and 35-gene expression profile (GEP) ancillary diagnostic testing workflow for difficult-to-diagnose melanocytic lesions increases the rate of actionable results to 99%

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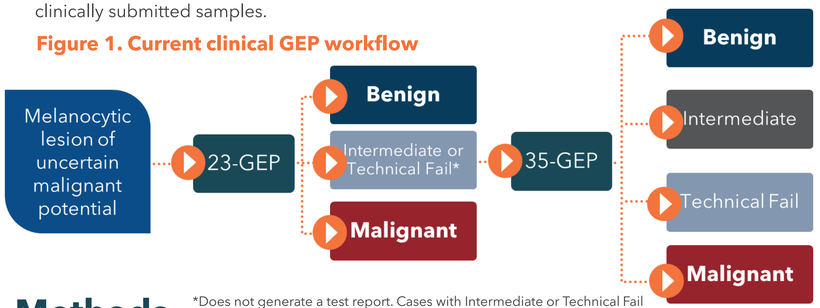
Background

- Diagnostic discordance in suspicious cutaneous melanocytic lesions is well documented and particularly prevalent among difficult-to-diagnose cases, for which histopathology may be insufficient for a definitive diagnosis.[1-4]
- The **23-gene expression profile** (**GEP**) and **35-GEP** tests are clinically available objective ancillary tools that facilitate diagnosis of melanocytic lesions with ambiguous histopathology. The tests use proprietary algorithms to produce results of: **suggestive of benign neoplasm**; **intermediate** (**cannot rule out malignancy**); or **suggestive of malignant neoplasm**.[5-7]
- The 23-GEP has shown accuracy metrics of over 90% sensitivity in multiple clinical studies that included patient outcomes.[8-10] The 23-GEP historically has resulted in ~23% of cases receiving a technical failure or an intermediate result, which can be perceived as **nonactionable**.[6,11-13]
- The 35-GEP test addresses this shortcoming, showing both an increased sensitivity[7] and a decreased nonactionable rate of 8.5% in clinical orders.
- Clinical utility has been demonstrated with benign and malignant GEP test results;[11,14] therefore, those test results are defined as **actionable**.

Objective

- Today, both the 23- and 35-GEP are offered from a single laboratory. Under the current laboratory workflow, unless preferred otherwise by the ordering clinician, clinical samples are processed first through the 23-GEP test, and if a technical failure or intermediate result is received, processed through the 35-GEP (**Figure 1**). However, both are run independently of one another and can be ordered as stand-alone tests.
- Here, we report accuracy metrics from a Performance Cohort and actionable results from clinically submitted samples.

Figure 1. Current clinical GEP workflow



*Does not generate a test report. Cases with Intermediate or Technical Fail results from the 23-GEP undergo testing with the 35-GEP. **GEP**, gene expression profile.

Methods

- Melanocytic lesions and associated de-identified clinical data from patients ≥18 years of age were included in this study. Samples were acquired under an IRB-approved protocol, including those previously submitted for clinical testing for the 31-GEP. Performance Cohort samples were independently reviewed (blinded to the original diagnosis) by at least 3 total dermatopathologists for adjudication and included if they received at least 2 out of 3 diagnostic concordance (**Table 1**). The study also included clinical cases submitted for GEP testing with results reported since implementation of the described workflow from 3 June – 3 December 2021 (**Table 2**).
- All cases not receiving a benign or malignant result from the 23-GEP were run on the 35-GEP, except for pediatric cases (<18 years), which were only run on the 23-GEP and excluded from analysis. Technical failure included samples with insufficient quantity of RNA and/or control or discriminant gene amplification failure based on the requirements for each test.

Results

- The Performance Cohort was comprised of 350 FFPE archival biopsy samples from adults ≥18 years of age with a cutaneous melanocytic lesion with a consensus diagnosis. All samples were run on the 23-GEP, and any intermediate or technical fail samples were subsequently run on the 35-GEP per the current clinical protocol (**Figure 1**). Accuracy metrics demonstrated high performance of the GEP workflow (**Table 1**).

Table 1. Performance Cohort accuracy metrics from the current GEP workflow

Performance Cohort, n=350		
	GEP	**95% CI**
Sensitivity	96.0%	92.0% – 99.0%
Specificity	87.8%	80.8% – 93.8%
PPV	89.0%	83.8% – 94.1%
NPV	95.6%	91.1% – 98.9%
Intermediate	*1.5%*	

CI, confidence interval; **GEP**, gene expression profile; **NPV**, negative predictive value; **PPV**, positive predictive value.

- Clinical test results were analyzed over a 6-month period. The 23-GEP test gave an actionable result of benign or malignant in 77.1% of cases (**Table 2**), which is comparable to past reporting in ambiguous cases for this test.[6,11]
- Nonactionable classifications of the 23-GEP test were 22.9% (13.3% intermediate and 9.6% technical failure). These cases then underwent testing with the 35-GEP, and an additional 22.2% of originally submitted cases received an actionable result. Only 0.6% of cases received a final intermediate result (i.e., from both tests); the technical failure rate was 0.1% (**Table 2**).
- This GEP workflow increased the rate of an actionable report from 77.1% to 99.3% when compared with 23-GEP testing alone (**Table 2**). The GEP test results overall were 60.2% benign, 39.1% malignant, 0.6% intermediate, and 0.1% technical failure.
- The median turnaround time for sample processing was 4 business days, (**Table 2**) and was only increased by 1 day when both GEP tests were run.

Table 2. Clinically actionable GEP test results

	Actionable*	**Nonactionable‡**
23-GEP only	77.1%	22.9%
Subsequent 35-GEP	22.2%	0.7%
Overall Results	**99.3%**	**0.7%**
	Turnaround Time#	
Median	4 days	
≤ 3 days	27.6%	
≤ 5 days	90.9%	

*Actionable: sum of benign and malignant test results; ‡Nonactionable: sum of intermediate and technical failure test results
#From the date of receipt of tissue by the lab. **GEP**, gene expression profile

Conclusions

- Though either GEP test can be run individually, the current GEP workflow collectively leverages the strengths of both independent GEP assays
- The GEP workflow demonstrated a high rate of accuracy in the Performance Cohort cases, with 96.0% sensitivity and 87.8% specificity
- The current GEP workflow for ambiguous melanocytic lesions has substantially improved reporting of **clinically actionable results** from a historic rate of ~77% for the 23-GEP alone to over 99%

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