

Prolaris[®] Post Prostatectomy Technical Specifications

Myriad Genetic Laboratories, Inc. Effective Date: October 15, 2018

TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

Indications and Use

Intended Use

This assay is intended for *in vitro* diagnostic analysis of FFPE resected prostate tumors for determination of biochemical recurrence risk within 10 years of post-prostatectomy.

Summary and Explanation

Approximately 164,690 men in the U.S. will be diagnosed with prostate cancer in 2018.¹ Approximately 30% of men undergoing prostatectomy experience biochemical recurrence. Adjuvant therapies such as radiation treatment, hormonal ablation, medical castration with an LHRH agonist, and bilateral orchiectomy significantly reduce recurrence risk but are associated with significant morbidity and adverse side effects. Prognostic markers identifying individuals at high risk for recurrence can be used to provide more individualized treatment post-prostatectomy.

The Prolaris[®] assay provides a rapid and reliable method of determining the risk of post-prostatectomy biochemical recurrence.

Description of Method

Acceptable sample types are limited to formalin-fixed paraffin-embedded (FFPE) tissue from prostatectomy blocks of prostatic adenocarcinoma. Ideally, blocks should include at least 5 mm of tumor (with >75% tumor) on diagnostic H&E slides for sample processing and RNA extraction. At the discretion of the Myriad pathologist, samples containing less tumor content may also be accepted. In cases where blocks are not available, one 3-5 μ m H&E slide followed by five consecutive 4-5 μ m unstained slides and a final H&E slide may be acceptable. Blocks (or slides) are shipped overnight with an ice pack to Myriad Genetic Laboratories, Inc. for analysis. Upon receipt, sample barcodes, which are scanned and tracked, are applied to each block (or slide). The H&E slides from each case are evaluated by a pathologist who determines the location and amount of tumor per slide. Using the H&E stained slides as a guide, tumor tissue is removed from five unstained slides and total RNA is extracted from the tissue.

The expression of 31 cell cycle genes, normalized by 15 housekeeper genes, is then measured in triplicate by quantitative PCR to generate a Prolaris Score[™], which is used to estimate the 10-year risk of biochemical recurrence.

Performance Characteristics/Limitations

The Prolaris Score was validated in two independent cohorts of post-prostatectomy patients.^{2,3} The first cohort consisted of FFPE tumor samples from 366 men, 138 of whom demonstrated biochemical recurrence.² The second patient cohort consisted of FFPE tumor samples from 413 men, 82 of whom demonstrated biochemical recurrence.³ The predicted risk of biochemical recurrence was estimated from the more contemporary cohort.³

The distribution of Prolaris Scores in the U.S. population was estimated using 998 patients tested at Myriad and may be adjusted in the future to reflect additional observations (data on file).

Clinically Reportable Ranges

Prolaris Scores are calculated as previously described,² and the score is then adjusted by +4 units in order to convert it to a more understandable range (approximately 0 to 10). The adjusted Prolaris Score is reported to the patient. The

Interpretive Criteria in this document reference the adjusted Prolaris Score.

Based on observed scores in the 779 FFPE post-prostatectomy tumor samples from the two patient cohorts,^{2,3} a clinically reportable Prolaris Score range of 2.4 to 7.7 was established. Prolaris Scores outside this range may be reported, but risk estimates for biochemical recurrence will not be provided.

A scale of Prolaris Scores will be reported for the CAPRA-S risk category of the individual patient. The scale will consist of five 1-unit intervals, with the middle interval being centered at the median Prolaris Score for that specific CAPRA-S risk category in the U.S. population. There is approximately a 1.8-fold change in risk of biochemical recurrence between intervals, which is the hazard ratio corresponding to a 1-unit change in the Prolaris Score, adjusted for the 3 CAPRA-S risk categories.

Analytical Precision and Linearity the Prolaris Score

A set of 13 biopsy samples and 2 radical prostatectomy samples was tested, with 3 biological replicates for each sample. The mixed sample set was representative of the distribution of sample types tested by MGL, and the standard deviation of the Prolaris Score was determined to be 0.22 score units (95% CI: 0.16, 0.35).⁴ In regards to RNA input linearity, the maximum RNA input concentration is 40 ng/ μ l (500 ng) and consistent results are obtained when samples are diluted until the average housekeeper gene Ct value exceeds 24.⁴ Samples with an average housekeeper value >24 are invalid and will lead to test cancellation.

Dynamic Range of the Prolaris Score

The dynamic range of the Prolaris Score was determined to be -13 to 14.⁵ In clinical validations, adjusted Prolaris Scores were observed from 2.4 to 7.7.^{2,3} Only Prolaris Scores within the clinically validated range will be reported with biochemical recurrence risk estimates.

Quality Control Measures

A minimum of one no-RNA control and one normal human RNA control with a previously determined Prolaris Score is analyzed within each sample run. Controls are analyzed to verify expected results.

Interference

Neoadjuvant hormonal therapy and radiation treatment may affect Prolaris Scores, potentially resulting in incorrect test interpretation. Patients receiving these treatments prior to surgery are not suitable candidates for testing.

Limitations

Performance characteristics for the Prolaris assay have not been established for tissues other than human FFPE prostate tumor specimens. Thus, other tissue types will not be accepted for analysis. This test is not validated for the analysis of tumor samples from individuals with PSA levels >100 ng/ml. The FFPE tissue preservation process may cause RNA degradation resulting in insufficient RNA quality or quantity for analysis. Results of this analysis should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Sample Rejection Criteria

Inappropriate sample types can cause cancellation of the test. Inappropriate sample types include: tumors other than prostatic acinar adenocarcinoma, samples that were previously

Prolaris[®] Post Prostatectomy Technical Specifications

Myriad Genetic Laboratories, Inc. Effective Date: October 15, 2018

TEST RESULTS SHOULD BE USED ONLY AFTER REVIEW OF THE FOLLOWING SPECIFICATIONS:

frozen, samples not fixed in neutral buffered formalin, or samples from patients that received chemotherapeutics or radiation treatments prior to biopsy. Samples with insufficient clinical information provided may be canceled. Samples of insufficient tumor quantity (<5 mm linear tumor and/or less than 75% tumor), or insufficient quality may also be canceled. Insufficient quality may be due to damage during shipping or insufficient RNA yields. A test may also be canceled if the Prolaris Score is outside of the validated range of scores.

Interpretive Criteria

Prolaris Scores between 2.4 and 7.7

Prolaris Scores within this range are clinically validated and will be reported as the calculated Prolaris Score. The estimated biochemical recurrence risk will be provided for patients with the same CAPRA-S score. In addition, the U.S. Distribution Percentile will be provided for patients in the same CAPRA-S risk category (low, intermediate, or high).

Prolaris Scores less than 2.4 but greater than -4

Linearity of Prolaris Scores within this range has been established. Thus, the calculated Prolaris Score will be reported. However, these scores lie outside of the clinically validated Prolaris Score range of 2.4 to 7.7. Estimated biochemical recurrence risk will not be provided, but the U.S. Distribution Percentile for patients in the same CAPRA-S risk category (low, intermediate, or high) will be reported.

Prolaris Scores greater than 7.7 but less than 12.0

Linearity of Prolaris Scores within this range has been established. Thus, the calculated Prolaris Score will be reported. However, these scores lie outside of the clinically validated Prolaris Score range of 2.4 to 7.7. Estimated biochemical recurrence risk will not be provided, but the U.S. Distribution Percentile for patients in the same CAPRA-S risk category (low, intermediate, or high) will be reported.

Prolaris Scores less than -4.0 or greater than 12.0

These scores may represent an artifact or technical error. Thus, these scores will not be reported and the test will be canceled.

References

1. American Cancer Society; www.cancer.org. Revised January 4, 2018.
2. Cuzick J, Swanson GP, Fisher G, et al. Prognostic value of an RNA expression signature derived from cell cycle proliferation genes in patients with prostate cancer: a retrospective study. *Lancet Oncol.* 2011; 12:245-55.
3. Cooperberg MR, Simko JP, Cowan JE, et al. Validation of a cell-cycle progression gene panel to improve risk stratification in contemporary prostatectomy cohort. *J Clin Oncol.* 2013; 10(11):1428-34.
4. Data on File. September 2018.
5. Warf MD, Reid JE, Brown KL, et al. Analytical validation of a cell cycle progression signature used as a prognostic marker in prostate cancer. *J Mol Biomark Diagn.* 2015; 6:239.