

# Prolaris<sup>®</sup> Biopsy Technical Specifications

## Myriad Genetic Laboratories, Inc. Effective Date: Sept 21, 2016

### 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 prostate tumor biopsies for determination of prostate cancer-specific mortality risk within 10 years of diagnosis.

##### Summary and Explanation

Approximately 220,800 men in the U.S. will be diagnosed with prostate cancer in 2015.<sup>1</sup> Prostate screening allows for the early detection of cancer. However, many tumors detected through screening will be indolent, while other seemingly low-risk cancers can progress rapidly and become fatal. Most men diagnosed with prostate cancer will die of other causes. Current diagnostic tools are imperfect at distinguishing between indolent and aggressive tumors.

The majority of diagnosed individuals are older than 65 and disease often progresses slowly. While these individuals have the option to pursue active surveillance, many individuals with localized disease choose more aggressive clinical treatments, including radical prostatectomy and radiation treatment, which are associated with morbidity. Prognostic markers that can further stratify indolent versus aggressive prostate cancer can aid in the treatment decision-making process. The Prolaris<sup>®</sup> assay has been shown to be an independent predictor of prostate cancer-specific mortality risk for patients.

#### Description of Method

Acceptable sample types are limited to formalin-fixed paraffin-embedded (FFPE) tissue from blocks or slides of prostatic adenocarcinoma biopsies. Ideally, blocks should include at least 0.5 mm of linear tumor (with >75% tumor) on diagnostic H&E slides for sample processing and RNA extraction. In cases where blocks are not available, one 3-5  $\mu$ m H&E slide followed by five consecutive 4-5  $\mu$ m unstained slides 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 the 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 RT-PCR to generate a Prolaris Score<sup>TM</sup>, which is used to estimate the 10-year risk of prostate cancer-specific mortality.

#### Performance Characteristics/Limitations

The Prolaris Score was trained on a pooled sample of 1,059 prostate cancer patients with complete clinical data.<sup>2</sup> The Prolaris Score was then clinically validated on 1,106 FFPE prostate tumor biopsy samples from two British cohorts of conservatively managed (watchful waiting) patients.<sup>3,4</sup> The distribution of Prolaris Scores in the U.S. population was estimated using 1,174 patients tested at Myriad and may be adjusted in the future to reflect additional observations (data on file).

##### Clinically Reportable Range

Prolaris Scores are calculated as previously described.<sup>3</sup> 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 analysis of 1,106 FFPE prostate tumor biopsy samples from two cohorts of conservatively managed patients,<sup>3,4</sup> a clinically reportable adjusted Prolaris Score range of 1.9 to 8.7 was established. Scores outside of this range may be reported, but risk estimates of prostate cancer-specific mortality will not be provided.

A scale of Prolaris Scores will be reported for the American Urological Association (AUA) 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 AUA risk category in the U.S. population. There is approximately a 1.9-fold change in risk of prostate cancer mortality between intervals, which is the hazard ratio corresponding to a 1-unit change in the Prolaris Score, when adjusted for the 3 AUA risk categories.

##### Analytical Precision and Linearity of the Prolaris Score

A set of 12 biopsy samples was tested, with 3 biological replicates for each sample, and the standard deviation of the Prolaris Score was determined to be 0.20 score units (95% CI: 0.14, 0.25).<sup>5</sup> The linearity of the RNA input, with respect to C<sub>T</sub> values, was determined to be 3-780 ng of input RNA (RNA concentration: 0.24-62.5 ng/ $\mu$ L),<sup>6</sup> and clinical samples are tested within the clinically validated range of 25-500 ng of input RNA (RNA concentration: 2-40 ng/ $\mu$ L).<sup>3,4</sup>

##### Dynamic Range of the Prolaris Score

The dynamic range of the Prolaris Score component was determined to be from -13 to 14.<sup>6</sup> In clinical validations, adjusted Prolaris Scores were observed from 1.9 to 8.7.<sup>3,4</sup> Only Prolaris Scores within the clinically validated range will be reported with prostate cancer-specific mortality risk estimates.

##### Quality Control Measures

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

##### Interference

Neoadjuvant hormonal therapy and radiation treatment can affect Prolaris Scores, potentially resulting in incorrect test interpretation. Patients receiving these treatments prior to biopsy 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 frozen, samples not fixed in neutral buffered formalin, samples from patients that received chemotherapeutics or radiation treatments prior to biopsy, or transurethral resection of the

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prostate (TURP) samples. Samples with insufficient clinical information provided may be canceled. Samples of insufficient tumor quantity (<0.5 mm linear tumor and/or <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 1.9 and 8.7

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

Prolaris Scores less than 1.9 but greater than 1.0

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 1.9 to 8.7. An estimated prostate cancer-specific mortality risk will not be provided, but the U.S Distribution Percentile for patients in the same AUA risk category (low, intermediate, or high) will be reported.

Prolaris Scores greater than 8.7 but less than 11.0

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 1.9 to 8.7. An estimated prostate cancer-specific mortality risk will not be provided, but the U.S Distribution Percentile for patients in the same AUA risk category (low, intermediate, or high) will be reported.

Prolaris Scores less than 1.0 or greater than 11.0

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 March 12, 2015.
2. Brawer MK, Cooperberg MR, Freedland SJ, et al. Development and validation of a multivariate model combining cell cycle progression score with CAPRA to predict prostate cancer mortality in a conservatively managed cohort. *ASCO Meeting Abstracts*. 2013; 31(6\_suppl):67.
3. Cuzick J, Berney DM, Fisher G, et al. Prognostic value of a cell cycle progression signature for prostate cancer death in a conservatively managed needle biopsy cohort. *Br J Cancer*. 2012; 106(6):1095-9.
4. Cuzick J, Stone S, Fisher G, et al. Validation of an RNA cell cycle progression score for predicting death from prostate cancer in a conservatively managed needle biopsy cohort. *Br J Cancer*. 2015; 113(3):382-9.
5. Data on file. April 2016.
6. 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.