

Myriad EndoPredict® Technical Specifications

Myriad Genetic Laboratories, Inc. Effective Date: December 10, 2019

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

Indications and Use

Intended Use

This assay is intended for *in vitro* analysis of FFPE resection and biopsy specimens of primary female invasive breast cancer (estrogen receptor positive, HER2 negative), for the determination of the 10-year risk of distant recurrence (metastatic disease), the likelihood of distant recurrence 5-15 years after diagnosis, and the estimated absolute benefit of chemotherapy at 10 years.

For resected tissue, a 12-Gene Molecular Score is combined with tumor size and lymph node status to generate an EPclin Risk Score, associated recurrence risks and estimated absolute benefit of chemotherapy.¹⁻⁶ For biopsy specimens, the molecular score and its associated 10-year risk of distant recurrence, which is reported as a risk category, are provided. A table for calculating the EPclin score and associated 10-year risk of distant recurrence, as both a percentage risk and as a risk category are also provided for reference, once nodal status and tumor stage are available. The EPclin Risk Score is a more significant predictor of the 10-year risk of distant recurrence than the molecular score alone.

Summary and Explanation

Breast cancer is the most frequently diagnosed cancer in U.S. women (246,000 new cases annually), and is the second leading cause of cancer-related death.⁷ Approximately 1 in 8 U.S. women, or 12%, will develop invasive breast cancer over the course of their lifetime.⁷ Breast cancer is a heterogeneous disease and is often classified based on the status of the human epidermal growth factor receptor 2 (HER2) protein, as well as two hormone receptors (HR), which are the estrogen receptor (ER) and progesterone receptor. Based on the expression of these proteins, which can inform medical management, newly diagnosed patients with breast cancer are stratified into three groups: HR-positive/HER2-negative, HER2-positive, or triple negative (HR-negative and HER2-negative). Patients with HR-positive/HER2-negative invasive breast cancer have the most favorable 5-year outcomes, followed by patients with HER2-positive, and then triple negative disease.⁸

Patients specifically with ER-positive and HER2-negative (ER+/HER2-) invasive breast cancer respond well to endocrine therapy. Additional use of adjuvant chemotherapy can be considered for patients with high risk features; however, not all patients have the same potential for chemotherapy benefit.⁹ EndoPredict® is a molecular test that identifies a large population of patients (55%-65% of patients tested in clinical trials) with excellent 10-year outcomes that can safely forgo adjuvant chemotherapy.¹⁻⁴ The EndoPredict Clinical (EPclin) Risk Score is generated from the combination of molecular and clinical data (described in more detail below) and has been shown to be a significant and independent predictor of breast cancer distant recurrence and adds significant prognostic and chemotherapy benefit information to clinical features alone.¹⁻⁶

Description of Method

Acceptable sample types for testing are formalin-fixed paraffin-embedded (FFPE) tissue from blocks and/or slides of primary female invasive breast tumor resection or biopsy specimens (ER+/HER2-). Blocks must have a portion of the lesion with ≥30% invasive tumor on the diagnostic H&E slide (>50% invasive tumor is recommended for biopsy specimens), with at least 20 μm of remaining unstained tissue for testing. In cases where blocks are not available, one 3-5 μm H&E slide followed by 2 consecutive 10 μm unstained slides may be acceptable. Samples frozen prior to fixation are not

appropriate for analysis. Samples 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 sample. Unused tissue is returned to the provider upon the completion of testing.

For testing, the H&E slide from each case is evaluated by an anatomic pathologist, who determines the location and amount of tumor per slide. Using the H&E stained slides as a guide, tumor tissue is macrodissected from the unstained sections and total RNA is extracted from the excised tissue. Quantitative RT-PCR is then used to measure, in triplicate, the expression of 8 signature genes, and 3 normalization genes, with 1 control gene to assess for DNA contamination. These molecular data are used to generate a 12-Gene Molecular Score. If tumor size and lymph node status are unavailable at the time of testing (e.g. biopsy specimens obtained prior to resection), the 10-year risk of distant recurrence is reported as a risk category (i.e. High Risk or Low Risk). If these clinical features are available, the 12-Gene Molecular Score is combined with tumor size and nodal status to generate the EPclin Risk Score, the 10-year risk of distance recurrence, which is reported as both a percentage risk and a risk category, the likelihood of distant recurrence 5-15 years after diagnosis, and the estimated absolute benefit of chemotherapy at 10 years.^{1,5,6}

Performance Characteristics

Both the 12-Gene Molecular Score and EPclin Risk Scores were trained in a large, multi-site cohort of 964 treatment-naïve FFPE primary breast resection samples.¹ A Cox proportional hazards model was used to fit a linear combination of the 12-Gene Molecular Score, tumor stage and lymph node status, generating an EPclin Risk Score. The EPclin Risk Score is calculated, according to the model, as: EPclin Risk Score = (0.35 * tumor size) + (0.64 * lymph node status) + (0.28 * 12-Gene Molecular Score) [tumor size: 1 (T1a, ≤1 cm), 2 (T1c, >1 but <2 cm), 3 (T2, >2 but <5 cm), 4 (T3, >5 cm); lymph node status: 1 (no positive nodes), 2 (1-3 positive nodes, or micrometastases)]. Both the 12-Gene Molecular Score and EPclin Risk Score have been subsequently validated in multiple independent cohorts.¹⁻⁶

Clinically Reportable Range

Based on the analysis of 1,702 treatment-naïve FFPE primary breast resection tumor samples, we determined a 12-Gene Molecular Score range from 0.0-15.0 and an EPclin Risk Score range from 1.0-8.2.

Analytical Precision, Linearity and Detection Limit

A set of 18 tumor samples, consisting of 14 resections and 4 biopsies, was tested 3 times, and the standard deviation of the 12-Gene Molecular Score was determined to be 0.21 score units.¹² The standard deviation of the EPclin Risk Score was determined to be 0.06 score units.^{10,12} The signature was originally validated to require a minimal input of RNA sufficient to generate a crossing threshold (C_t) value less than 40 for normalization and target genes.¹ In regards to RNA input linearity, the test reproduces valid results from an average C_t of 19-27 for the housekeeper genes.¹⁰ Samples with an average housekeeper value <19 or >27 are invalid and will lead to test cancellation.

Quality Control Measures

A minimum of one no-RNA control and one normal human RNA control (with a previously determined score) are analyzed concurrently with each sample. Additionally, one control that is positive for DNA contamination is measured

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with each sample. Controls are analyzed to verify technical consistency and samples are only reported when all controls tested with the sample perform within specifications.

Test Interference

Systemic treatment prior to biopsy or resection of the primary breast tumor (*e.g.*, neoadjuvant chemotherapy, radiation treatment) may affect test performance, potentially resulting in inaccurate test results. Therefore, patient samples exposed to systemic treatments prior to surgery, are not suitable for testing.

Limitations and Sample Rejection Criteria

The reported 10-year distant recurrence risk, the likelihood of distant recurrence 5-15 years after diagnosis, and the estimated absolute benefit of chemotherapy at 10 years are based on analysis of multiple cohorts of pre/post-menopausal women with resected ER+/HER2- invasive female breast cancer who have not been treated prior to resection with systemic neo-adjuvant therapy (*e.g.*, chemotherapy, radiation therapy, endocrine therapy) and who do not have a current or prior diagnosis of an additional cancer.^{1,5,6} Risks may differ for individuals who do not meet the aforementioned clinical characteristics. This test result is invalid if the patient has already experienced a distant recurrence.

Performance characteristics for the test have not been established in non-invasive samples, tumors that are not ER+/HER2-, patients currently undergoing hormone replacement therapy, patients that received systemic treatment or localized radiation prior to resection, nor in male breast cancer tumors.¹ However, local radiation therapy after resection does not interfere with the test result. Patients that received hormone replacement therapy after menopause are suitable for testing, provided that the therapy was halted after diagnosis of breast cancer. Samples in non-validated tissues (*i.e.*, non-breast tissue) will not be accepted for testing. This test was validated in multiple cohorts of post-menopausal women,^{1,2} and a mixed cohort of pre/post-menopausal women.⁴

Retrospective analysis of resection samples, up to 10 years after surgery, may be suitable, provided that the patient received uninterrupted endocrine therapy for 5 years (or until time of sample testing if it has been less than 5 years since the time of surgery). The test result may not accurately reflect the patient's risk of recurrence if the patient received endocrine treatment for longer than 5 years, or had interruption in their 5 year endocrine treatment regimen.

Two clinical parameters are required for calculation of the EPclin Risk Score, which are tumor size/stage and the number of positive lymph nodes. Only primary tumors that are T stage ≤ 3 are acceptable for testing. Tumors with grade G1-G3 or Gx are suitable for testing. Samples with 0-3 positive lymph nodes are currently accepted for testing. Samples with clinical variables that are unsuitable for testing may lead to test cancellation and/or follow-up with the referring healthcare provider. Samples with less than the 30% minimum invasive tumor may also lead to test cancellation.

Primary breast tumors with invasive ductal, invasive lobular, mixed histology (with both invasive ductal and invasive lobular characteristics), and primary invasive breast tumors with no specified histology are all acceptable for testing. Samples with micrometastatic disease in lymph nodes are also acceptable for testing. Samples that are recurrences of previous primary breast cancers or patients with synchronous cancer are not suitable for testing.

The FFPE tissue preservation process may cause RNA degradation resulting in insufficient quantity or quality of

RNA for analysis. Samples with insufficient RNA quantity and/or quality may lead to test cancellation and/or follow-up with the referring healthcare provider.

Results of this analysis should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Analytically equivalent results between breast tumor biopsy and paired surgical resection samples were demonstrated by a published study¹¹ and an independent validation.¹² However, resections are the recommended sample type for this assay due to sampling variation observed in biopsy samples (Pearson correlation 0.95).

Interpretive Criteria

The following criteria should be used to interpret the reported score.

EPclin Risk Scores from 1.0 through 3.3

Scores within this range are clinically validated and will be reported. Patients with scores in this range have a low 10-year risk for distant recurrence. The estimated 10-year risk of recurrence, the likelihood of distant recurrence 5-15 years after diagnosis, and the estimated absolute benefit of chemotherapy at 10 years will be provided, based upon the EPclin Risk Score.

EPclin Risk Scores from 3.4 through 6.0

Scores within this range are clinically validated and will be reported. Patients with scores in this range have a high 10-year risk for distant recurrence. The estimated 10-year risk of recurrence, the likelihood of distant recurrence 5-15 years after diagnosis, and the estimated absolute benefit of chemotherapy at 10 years will be provided, based upon the EPclin Risk Score.

EPclin Risk Scores less than 1.0 or greater than 6.0

Based upon 1,702 samples, a reportable range for EPclin Risk Scores and their associated 10-year likelihood of distant recurrence was established for scores from 1.0 through 6.0.¹ EPclin Risk Scores from >6.0 to 8.2 will be reported with their corresponding EPclin Risk Class (high); however, an associated 10 year likelihood of distant recurrence, the likelihood of distant recurrence 5-15 years after diagnosis, and the estimated absolute benefit of chemotherapy at 10 years will not be provided.

12-Gene Molecular Scores from 0.0 through 4.9

Scores within this range are clinically validated and will be reported. Patients with scores in this range have a low 10-year risk for distant recurrence.

12-Gene Molecular Scores from 5.0 through 15.0

Scores within this range are clinically validated and will be reported. Patients with scores in this range have a high 10-year risk for distant recurrence.

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