Instructions for use – MammaPrint FFPE Microarray

Introduction

The MammaPrint test is a gene expression profile to predict clinical outcome of breast cancer patients. It was developed with the rationale that the natural history of a tumor is determined by its underlying regulatory gene pathways; therefore, gene expression profiles, in addition to standard clinical pathology, can more accurately predict the prognosis and response to treatment.

Using an unbiased approach, the MammaPrint gene signature was determined by running a genome-wide analysis of 25,000 genes in untreated primary breast tumor samples and comparing the gene activity profiles of patients who did not have distant metastasis within 5 years with those who did. This resulted in a 70-gene profile that could distinguish patients at high risk of developing metastases from those whose long term distant metastasis risk was sufficiently low that systemic therapy would provide little to no absolute clinical benefit. [1,2]

Risk of recurrence - The MammaPrint test analyzes the expression of 70 specific genes in a tissue sample by running RNA isolated from breast tumor samples on custom glass microarray slides. The expression profile is then used in a proprietary algorithm to categorically classify the patient as being at either High or Low Risk of breast cancer recurrence.

Intended Use

MammaPrint® FFPE is a qualitative, non-automated in vitro diagnostic test, performed in Agendia's Diagnostic Service Laboratory, using the gene expression profile obtained from formalin-fixed paraffin embedded (FFPE) breast cancer tissue samples to assess a patient's risk for distant metastasis within 5 years.

The test is performed for female breast cancer patients with Stage I or Stage II disease who are lymph node negative or lymph node positive with up to 3 positive nodes, with a tumor size less than or equal to 5.0 cm, and for patients with Stage III disease. The MammaPrint® FFPE result is indicated for use by physicians as a prognostic marker only, along with other clinico-pathological factors.

Intended User

MammaPrint FFPE microarray is intended to be requested by a health care provider of the breast cancer patient. By ordering the MammaPrint FFPE microarray test, the health care provider requests Agendia to execute the test on their sample in Agendia's Diagnostic Service Laboratory.

Test Principle

The analysis is based on several non-automated processes: isolation of RNA from FFPE breast cancer tissue sections; reverse transcription of RNA resulting in cDNA; amplification and labeling of the cDNA; hybridization of the amplified and labeled cDNA to the diagnostic microarray; washing and scanning the diagnostic microarray and data acquisition (feature extraction); calculation and determination of the risk of recurrence (MammaPrint).

The MammaPrint analysis is designed to determine the gene activity of specific genes in a tissue sample. The result is an expression profile, or "fingerprint", of the sample. Using this expression profile, the MammaPrint Index is calculated and the molecular prognosis profile of the sample is determined (Low Risk, High Risk).

Warnings and Precautions

The patient identification on the request form must correctly match the identification of the specimen within the LIMS system which creates an internal identifier that should be correctly matched to the specimen in the lab process.

Fill out the test request form with the appropriate information.

The specimen selected for MammaPrint testing should match the intended use population characteristics such as, but not limited to, female breast cancer, early stage and tumor cell of at least 30%.

MammaPrint results are indicated for use by physicians as a prognostic marker in addition to standard clinico-pathological factors. The test is not intended to determine the outcome of disease, nor to suggest or infer an individual patient's response to therapy.

A MammaPrint Low Risk result does not guarantee that distant recurrence will not occur within five years. Similarly, a High Risk result does not guarantee that distant recurrence will occur. Test results should be used in conjunction with clinico-pathological factors.

Procedure

a) Patient selection

Patients are eligible if they are female and diagnosed with Stage I, Stage II or Stage III breast cancer lymph node negative and lymph node positive (up to 3 positive nodes), with a tumor size of ≤ 5.0 cm, independent of estrogen receptor status (ER+/-).

b) Sample Collection, Registration and Shipment.

Conditions for collection, handling and preparation of the sample are provided to the customer through IFU: M-ROW-067. This kit includes the following:

- 10 Microscopic slides
- 2 Five-slide carriers
- Small and large zip-style plastic bag
- Specimen sampling Instructions
- Test Request Form
- Label sheet with barcode labels
- Shipping materials

Sample registration is initiated by notification from the ordering health care provider. This notification (Test Request Form- TRF) can take place by online customer portal or other communication channel where Agendia registers all related sample and patient information. The TRF is for single patient use only. The sample is shipped directly to Agendia's Diagnostic Service Laboratory by the ordering health care provider, at ambient temperature, using the courier transportation materials provided.

c) Sample analysis at Agendia

FFPE tissue sections, either provided by the customers on glass slides or prepared from FFPE tumor blocks using a standard microtome, are used. Total RNA is extracted from the tissue sections using a standard commercially available isolation kit. The RNA sample is purified, amplified and labeled with a cyanine-CTP/ dUTP fluorescent dye.

The RNA/cDNA sample is hybridized on a specifically designed diagnostic microarray (8-pack, Agilent Technologies). An Agilent microarray scanner is used for scanning the diagnostic microarray and the result is a scan file (TIFF). This file is used by the Agilent Feature Extraction Software. The Feature Extraction Software analyzes the scan file (TIFF) by determining the relative fluorescent intensities of the individual features against the diagnostic microarray chip design file as a template in order to identify control features, normalization features and reporter gene features. The

fluorescent intensities of the features are a measure for the expression of particular genes.

d) Data Analysis

Data analysis is performed according to the MammaPrint algorithm, which calculates the MammaPrint Index, and determines the molecular prognosis profile of the sample (Low Risk, High Risk). Samples with a MammaPrint index value greater than 0 are classified as Low Risk, and samples with a value less than or equal to 0 are classified as High Risk.

The MammaPrint Index of a sample can fall within a pre-defined area around the classification threshold between a MPI of > -0.05 and <0.05, in which the MammaPrint result has <90% classification accuracy but not lower than 50% (i.e., borderline sample).

Extensive Quality Controls (>25) are implemented in order to ensure the correct analytical result. QCs, together with the result, are reviewed and approved internally by the Laboratory Director.

e) Reporting

The ordering health care provider receives a Patient Report as well as a Summary of Results for each ordered MammaPrint FFPE microarray test. With respect to personal data of patients, please refer to our data processing terms in Agendia's Privacy Policy.

Limitations of the Procedure

MammaPrint has been validated for use only with female breast cancer tumor tissue. Testing of other specimen types may result in incorrect results or no results. Reliable results are dependent on adequate specimen collection and transport procedures.

MammaPrint has been specifically validated for tumors that are invasive ductal carcinoma or lobular carcinoma. Testing of other specimen types (e.g. lymph nodes) has not been evaluated.

Expected Values

The MammaPrint result is provided as a binary result and is categorized as either "Low Risk" or "High Risk" of recurrence. The prognostic profile of a sample is determined by calculating the MammaPrint Index (MPI) on a scale of -1.000 to +1.000.

As with any other test, our test also has an established classification threshold. For MammaPrint, the threshold for Low and High Risk has been set at zero in order to reach the highest possible sensitivity, and values >0.000 are classified as Low Risk, and values ≤ 0.000 are classified High Risk. Overall average MPI accuracy is 98.5%, with scores < 0.1685 or >0.168 having an accuracy of >99%.

"Borderline" MPI values

While the technical accuracy of MammaPrint is extremely high, samples with an MPI value very close to the zero threshold have a slightly lower accuracy percentage than samples further away from the threshold. Specifically, around the zero threshold, between a MPI of > -0.05 and <0.05, also referred to as borderline region, the test accuracy drops below 90%. For example, at an MPI of 0.04, there is 86% certainty that the MP result is in the positive range (Low Risk), and 14% possibility it is in the negative range (High Risk). It is important to note that samples with MPI values outside of the "borderline" region have an average >99% accuracy.

Performance Characteristics

The performance characteristics investigated for MammaPrint comprise: precision, reproducibility, threshold, sensitivity, specificity, Limit of Detection and accuracy.

MammaPrint

Analytical performance

MammaPrint precision and repeatability was assessed by an independent inter-laboratory study in Agendia's two Clinical Laboratory Improvement Amendments (CLIA) certified laboratories in The Netherlands and the USA. [3]
Based on the analytical performance of MammaPrint, the precision of classifying a sample as High Risk or Low Risk is 97.3% with a repeatability of the measurement being 97.8%. [3] Reproducibility was measured in over 300 control samples and shown to be 99%. [4] The performance characteristics are based on the studies and papers listed below.

Clinical Validation

Results are based on previously published data for the 70-gene molecular prognosis profile[1] in breast tumors. Good outcome patients are classified as Low Risk (i.e. no distant metastasis within at least 5 years). Poorer outcome patients are classified as High Risk (i.e. higher likelihood of distant metastasis within 5 years). The numerical MammaPrint Index value is provided.

MammaPrint was developed using adjuvant-untreated, lymph node negative, mainly European patients to capture the biology of the primary tumor in a gene expression profile. [1,2] In January 2013, the 5 year outcome results of the prospective, observational RASTER study were published[4]. This impact study was a 'first of its kind' biomarker-centric trial in which the MammaPrint Fresh assay was performed and reported on 427 early stage breast cancer patients aged 18-61 years old, pT1 and pT2, lymph node negative, ER+/-, HER 2 +/- prior to the physician-patient decision for adjuvant therapy. Patients were treated according to standard of practice guidelines taking into account all relevant clinico-pathological factors and the MammaPrint signature results.

Subsequently, MammaPrint was also performed on FFPE tissue from the RASTER patients. MammaPrint results were compared using 345 paired fresh and FFPE RASTER samples with 5 year outcome data. Not accounting for any covariates other than the patient's MammaPrint test result, patients classified as 'Low Risk' by MammaPrint FFPE (71 adjuvantly treated and 108 not adjuvantly treated), demonstrated a 1.3% (95% CI 0 – 3.1) chance of cancer recurrence within 5 years[5].

Patients classified as 'High Risk' by MammaPrint FFPE (145 adjuvantly treated and 21 not adjuvantly treated), demonstrated an 11.7% (95% CI 6.6 – 16.8) chance of cancer recurrence within 5 years[5].

MammaPrint has been independently validated in studies in over 75,000 breast cancer patients with results published in leading peer-reviewed medical and scientific journals internationally and shown to provide information independent of clinico-pathological risk assessment [56].

Ordering of the test

A sample collection kit will be provided by your Agendia contact person. Using the kit add the tumor sample to the slides according to the instructions, or prepare an FFPE block. Place your order via our online portal or by completing the test request form that you find in the Specimen Collection kit. Details can be found in the IFU of this kit.

If you require any additional support or information please contact us at Customerservice@agendia.com or +31 (0)20 462 1510.

References

1. van 't Veer et al. Nature 2002; 415(31): 530-536
2. van de Vijver et al. New Engl J Med 2002; 347(25): 1999-2009
3. Sapino et al. J Mol Diagnostics 2014; 16: 190-197
4. Drukker et al. Int J Cancer 2013; 133(4): 929-936
5. Beumer et al. Breast Cancer Res Treat 2016; DOI 10.1007/s10549-016-3764-5
6. Piccart et al. Lancet Oncol. 2021;22(4):476-488

Advisory Notice:
Report any serious incident related to MammaPrint FFPE to the manufacturer and to the competent authority of the Member State. The manufacturer will report the serious incident to the competent authority of the Member State in which the user/patient is established.

Clinical Laboratory Improvement Amendments (CLIA)
Certificates of Accreditation:
Agendia, Inc.: 05D1089250

Manufacturing Details

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