**Breast Cancer Test Suite Physician’s Brochure**

*For In Vitro Diagnostic Use*

### Caution: US Federal law restricts this device to sale by or on the order of a physician

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**Introduction**

The Agendia Breast Cancer Test Suite consists of three molecular diagnostic tests: MammaPrint®, BluePrint® and TargetPrint®, each providing unique information about breast cancer to help make more informed treatment decisions.

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**Intended Use**

All tests are qualitative, in vitro diagnostic test services and performed in a central laboratory, using the gene expression profile of fresh or formalin-fixed paraffin embedded (FFPE) breast cancer tissue samples.

MammaPrint assesses a patient’s risk for distant metastasis (up to 10 years for patients less than 61 years old, up to 5 years for formalin-fixed samples) and informs if a patient is at high risk for distant metastasis to up to 5 years.

BluePrint assesses the molecular subtype of breast cancer and informs if tumors are Basal-type, Luminal-type or HER2-type.

TargetPrint is a single gene read-out expression analysis for ER, PR and HER2.

The Agendia Breast Cancer Test Suite is performed in the US for breast cancer patients, with Stage I or Stage II disease, with a tumor size of ≤ 5.0 cm, independent of estrogen receptor status (ER+/−) and lymph node negative. Outside the US, also to be used for breast cancer patients with up to 3 positive lymph-nodes.

The result is indicated for use by physicians as a prognostic marker only, in conjunction with other clinic-pathological factors.

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**Summary**

The analysis is based on several processes: isolation of RNA from fresh frozen or FFPE breast cancer tissue sections; elimination of gDNA; linear amplification and labeling of cDNA (FFPE only); 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) or determination of the molecular subtype (BluePrint) or quantification of gene expression levels (TargetPrint).

The MammaPrint analysis (for both fresh and FFPE tissue) 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 with fresh tissue. MammaPrint FFPE assesses a patient’s risk for distant metastasis up to 5 years.

BluePrint assesses the molecular subtype of breast cancer and informs if tumors are Basal-type, Luminal-type or HER2-type.

TargetPrint is a single gene read-out expression analysis for ER, PR and HER2.

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**Procedure**

- **a) Patient selection**
  - In the US, patients are eligible if they are diagnosed with breast cancer, Stage I or Stage II, lymph node negative, with a tumor size of ≤ 5.0 cm, independent of estrogen receptor status (ER+/−). Outside the US, patients are eligible also with up to 3 positive lymph-nodes.

- **b) Sample collection, registration and shipment.**
  - Samples will be collected by providing the customers with sample collection kits. These kits consist of:  
    - Sturdy outer box or container
    - Sample receptacles and transportation tube (Fresh)
    - BioPly Punch for sub-sampling on surgical specimens (Fresh)
    - Tissue RNA preservative solution (Fresh)
    - Slides for hybridization (including 10x glass slides (FFPE))
    - 2 zip-lock bags (FFPE)
    - Courier transportation materials
    - Test Request Form (optional)
    - Identification stickers
    - Sampling Instruction Folder
    - Physician’s Brochure

Registration is initiated by notification from the ordering health care provider. This notification (Test Request Form) can take place by fax, online customer portal or other communication channel. Agendia registers all related sample and patient information. The sample is shipped directly to Agendia’s central laboratory by the ordering health care provider, at ambient temperature, using the courier transportation materials provided. For non-US / non-Latin America requests, samples should be shipped to Amsterdam, The Netherlands. For all US, Puerto Rico and Latin America requests, samples should be shipped to Irvine, California, USA.

- **c) Sample analysis at Agendia**
  - To assess the gene activity in a fresh sample, frozen tissue sections are made using a freeze microtome, and are collected in a receptacle. For FFPE tissue samples, the provided glass slides with FFPE tissue sections are used or slides are made from the FFPE tumor block using a standard microtome. Total RNA is extracted from the tissue sections using a standard commercial ready available isolation kit. The RNA sample is purified, amplified, and labeled with a cyanine-CTP/3′UTP fluorescent dye.

  The mRNA/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.

  The results for ER, PR, and HER2 expression are quantitative and will also be presented in a binary mode (positive /negative).

  A positive result by TargetPrint for ER and PR is equivalent to a 1% or higher IHC positively stained tumor.

  For HER2 TargetPrint, a HER2 positive result is equivalent to an IHC score of 3+ or to an IHC score of 2+ that has been confirmed to be HER2 positive by in-situ hybridization (FISH, CISH).

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**Warnings and Precautions**

MammaPrint, BluePrint and TargetPrint are not indicated as stand-alone tests to determine the outcome of disease, nor to suggest or infer an individual patient’s likely response to therapy. Results should be taken in the context of other relevant clinicopathological factors and standard practice of medicine.

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**Expected Values**

**MammaPrint and MammaPrint FFPE**

The MammaPrint result is given as “Low Risk” or “High Risk” for risk of recurrence.

The MammaPrint Index of a sample can fall within a predefined area around the classification threshold in which the MammaPrint result has <90% classification accuracy (i.e., borderline sample). When a sample is considered to be “borderline”, it is clearly indicated on the MammaPrint analysis report.

**BluePrint**

Basal-type basal-type breast cancers are characterized by gene expression of the basal/myoepithelial cells of origin. The Basal-type cancers are typically triple-negative for ER, PR and HER2 (basal-like) with a specific gene expression profile. Hormone therapy and anti-HER2 therapies, such as trastuzumab and lapatinib, are not believed to be effective against these cancers, although chemotherapy is thought to be helpful. A Basal-type BluePrint result means that the tumor phenotype most closely resembles the Basal-type intrinsic subtype.

**Luminal-type**

Luminal-type breast cancers are characterized by gene expression of the luminal epithelial cells that line the breast ducts and glands. The Luminal-type cancers are typically hormone receptor positive tumors and are likely responsive to hormonal therapy.
A Luminal-type blue Print result means that the tumor phenotype most closely resembles the Luminal-type intrinsic subtype.

TargetPrint

TargetPrint provides qualitative results supported by quantitative measurements that give health care providers additional insight into the biology of each individual tumor and assists in treatment decisions. A positive ER prediction measures a tamoxifen benefit (16). HER2

Quantitative data of the HER2 expression level in an individual tumor can affect the choice of treatment. HER2 is an important target of the monoclonal antibody, trastuzumab (Herceptin®). Trastuzumab is only effective in breast cancer patients where breast cancer screening programs are implemented.

MammaPrint was developed using adjuvant treatment. The HER2 amplified or overexpressed tumors have a higher risk of developing distant metastases up to 5 years: “Low Risk” had a 7% chance to develop distant metastases at 5 years without any adjuvant treatment. Patients classified as MammaPrint Low Risk, defined as “Low Risk” by MammaPrint FFPE (71% adjuditantly treated and 108 adjuditantly not treated), demonstrated a 1.3% (95% CI 0 – 3.1) chance of cancer recurrence within 5 years.

In January 2013, the 5 year outcome results of the prospective observational RASTER study were published (17). 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 clinicopathological factors and the MammaPrint Fresh signature results.

Subsequently MammaPrint FFPE was also performed on FFPE tissue of the RASTER patients. Results from MammaPrint-Fresh and MammaPrint FFPE were compared for the 345 paired fresh and FFPE samples with 5 year outcome data from the 427 RASTER patients (18). Not accounting for any covariates other than the patient’s MammaPrint FFPE status, patients classified as ‘Low Risk’ by MammaPrint FFPE (71% adjuditantly treated and 108 adjuditantly not treated), demonstrated a 1.3% (95% CI 0 – 3.1) chance of cancer recurrence within 5 years.

Patients classified as ‘High Risk’ by MammaPrint FFPE (145 adjuditantly treated and 21 adjuditantly not treated), demonstrated an 11.7% (95% CI 6.6 – 16.8) chance of cancer recurrence within 5 years (11).

Prognostic assessment of MammaPrint® FFPE was further investigated using univariate and multivariate analyses (19). In the univariate and multivariate analyses, a MammaPrint FFPE High/Low Risk result is significantly associated with high/low risk for recurrence.

Univariate analysis: DRFI (Distance Recurrence Free Interval) at 5 years n=345

Table 1: DRFI at 5 years n=345

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Low Risk</th>
<th>High Risk</th>
<th>P-Value</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>342</td>
<td>2.430</td>
<td>1.879</td>
<td>0.173</td>
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<td>Tumor size (cm)</td>
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Multivariate analysis: DRFI at 5 years n=345

Table 2: DRFI at 5 years n=345

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MammaPrint has been independently validated in studies on over 12,000 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.

MammaPrint precision and repeatability was assessed by independent laboratory study in Agendia’s two manufacturing laboratories in the Netherlands and the USA (20). MammaPrint FFPE status, MammaPrint FFPE status, and MammaPrint Fresh status were compared to IHC and FISH equivalence.

The performance characteristics are based on the studies and papers listed below.

References

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Agenda NV: 991D030869

Manufacturing address Agenda NV:
Science Park 406
1098 XH Amsterdam, The Netherlands
Phone: +31 (0)20 462 1210
Fax: +31 (0)20 462 1205

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