Instructions for use – BluePrint FFPE Microarray

Introduction

The BluePrint test is a molecular subtyping assay that classifies breast cancer into three distinct subtypes: Luminal-Type, HER2-Type, and Basal-Type based on the true biological profile of the tumor using gene expression. Molecular subtyping improves treatment allocation, as tumors that may appear identical based on histopathological features can in fact have very different clinical outcomes. [1,2,3]

Using 200 initial breast cancer patient samples and four independent validation cohorts (n=748), the 80-gene BluePrint profile was established. [1] BluePrint discriminates between the three intrinsic breast cancer subtypes; Luminal-Type, HER2-Type, and Basal-Type. These intrinsic subgroups are more accurately identified using gene expression panels compared to standard IHC/FISH assessment. [2,4]

The BluePrint test analyzes the expression profile of 80 specific genes in a tissue sample by running isolated RNA from tumor tissue samples on custom glass microarray slides. Using a proprietary algorithm, BluePrint determines the correlation index of the 80-gene profile with each of three distinct molecular subtyping centroids: Luminal-Type, HER2-Type, and Basal-Type.

Intended Use

BluePrint 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 determine the molecular subtype.

The test is performed for female breast cancer patients, with Stage I, Stage II or Stage III disease.

Intended User

BluePrint FFPE microarray is intended to be requested by the treating health care provider of the female breast cancer patient. By ordering the BluePrint FFPE microarray test the health care provider requests Agendia to execute the test on the 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 molecular subtype (BluePrint).

The BluePrint analysis is designed to determine the activity of specific genes in a tissue sample. The result is an expression profile, or "fingerprint", of the sample. The correlation of the expression profile to the templates (the average mRNA expression levels of Luminal-, Basal- and HER2-Type tumors) is calculated (BluePrint Index) and the molecular subtype of the sample is determined (i.e. Basal-Type, Luminal-Type, HER2-Type).

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 BluePrint testing should match the intended use population criteria such as, but not limited to, female breast cancer, early stage, tumor cell content of at least 30%.

BluePrint results are indicated for use by physicians 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.

Procedure

a) Patient selection
Patients are eligible if they are diagnosed with breast cancer, Stage I, Stage II or Stage III disease.

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 carrier
- 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) can take place by online customer portal or other communication channel. Agendia registers all related sample and patient information. 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
For FFPE tissue samples, the customer provided glass slides with FFPE tissue sections are used or slides are made from the customer provided FFPE tumor block using a standard microtome. 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 and Reporting

Data analysis is performed according to a specific BluePrint algorithm which calculates the BluePrint Indices and determines the molecular subtype of the sample (Basal-Type, Luminal-Type or HER2-Type).

Extensive Quality Controls (>25) are implemented in order to ensure the correct analytical result. QC's together with the result are reported and approved internally by the Laboratory Director.

e) Reporting

The ordering healthcare provider receives a Patient Report as well as a Summary of Results for each ordered BluePrint 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

BluePrint 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.

BluePrint 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 BluePrint FFPE test classifies breast cancer tissue samples into three distinct subtypes: Luminal-Type, HER2-Type, and Basal-Type. The subtype with the highest index score determines the molecular subtype of the tumor.

Luminal-Type

Luminal-Type breast cancers are characterized by gene expression of the luminal epithelial cells that line the breast ducts and glands.

HER2-Type

The HER2-Type breast cancers are characterized by amplification or over-expression of the HER2 locus.
The HER2-Type cancers are typically HER2-positive tumors by IHC or FISH (HER2/neu positive). A HER2-Type BluePrint result means that the tumor phenotype most closely resembles the HER2-Type intrinsic subtype.

Basal-Type

Basal-Type breast cancers are characterized by gene expression of the basal/myoepithelial cells of origin. Basal-Type cancers are typically triple-negative for ER, PR and HER2 with a specific gene expression profile.
A Basal-Type BluePrint result means that the tumor phenotype most closely resembles the Basal-Type intrinsic subtype.

Performance Characteristics

The performance characteristics investigated for BluePrint comprise: precision, reproducibility, Limit of Detection and accuracy.

The technical validity of BluePrint is determined on multiple individual validation experiments and a comprehensive inter-laboratory comparison study. [5] Based on the analytical performance of BluePrint, the precision of classifying as Luminal-Type, Basal–Type or Her2-Type is 98.6%, with the repeatability of the measurement being 99.0%. The reproducibility was determined to be 98.3%.

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

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. Krijgsman et al. Breast Cancer Res Treat 2011; 133(1): 37-47
2. Glück et al. Breast Cancer Res Treat 2013; 139(3): 759-767
3. Whitworth et al. Ann Surg Oncol 2017; 24(3): 669-675
4. Beitsch et al. Ann Surg Oncol 2017; 24(9): 2539-2546
5. Mittempergher et al. Transl Oncol 2020; 13(4): 100756

Advisory Notice:

Report any serious incident related to BluePrint FFPE to the manufacturer and 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

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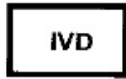
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