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, HER2Type, 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.

BluePrint predicts the magnitude of systemic therapy benefit to guide treatment decisions in patients with breast cancer.

The BluePrint genes measure the characteristics of the multiple step development of breast cancer cells to survive, proliferate, disseminate and metastasize as covered within the hallmarks of cancer[5].

The test is performed for female breast cancer patients, with Stage I, Stage II or Stage III disease. The BluePrint® FFPE result is indicated for use by physicians and should be interpreted along with other clinico-pathological factors.

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.

Procedure

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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: MKT-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, HER2Type, 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 and reproducibility, reportable range, analytical specificity, and limit of detection

Analytical performance

The concordance of the results from the Fresh and FFPE tissues was assess in two studies where it was found to be 97% (n=413) and 90% (n=55), with no bias in BluePrint results between tissue types.

BluePrint is also stable over time with a reproducibility between 97.6% and 98.9% and reproducible between different isolations of the same tissue where no significant difference was observed in subtype (Cochran's Q-test, p=1.0) and indices (ANOVA Luminal p=0.850, HER2 p=0.725, and Basal p=0.400). Reproducibility of BluePrint between different locations was 100% and between different scanners was also 100%. Furthermore, the high precision (98.6%) and repeatability (99%) were observed (6).

Additionally for the FG-array, no difference was found between the results of repeated RNA isolations, the precision (98.98%, 98.87%, and 98.84% for Luminal, HER2, and Basal, respectively), reproducibility (98.71%, 98.43%, and 98.24%, for Luminal, HER2, and Basal, respectively), and repeatability (99.08%, 99.03%, and 98.96% for Luminal, HER2, and Basal, respectively), and the agreement between 8pack and FG-array results of two separate datasets was found to be 98.91% (n = 92) and 99.86% (n = 698).

The reportable range of the BluePrint FFPE index is the correlation of the expression profile to the templates (the average mRNA expression levels of Luminal-, Basal- and HER2-type tumors). The molecular subtype of the sample is then determined by the one with the highest BluePrint index.

An interference study was conducted on the full genome array (FG-array) to assess whether four relevant substances interfere with the BluePrint FFPE results. The results showed DNA contamination up to 2.5x the background levels and protein spikes during RNA isolation as well as, ethanol and AMPure XP Beads carry-over during cDNA purification did not impact BluePrint results. Overall, interfering tissue components do not influence the BluePrint result, since original tumor series has been representative for breast cancer specimens and here also, the other tissue components were randomly distributed and were not related to disease outcome[(1)].

To assess the limit of detection, BluePrint FFPE results were compared over the different dilutions and showed very stable results even at very low inputs of cDNA on the array. Based on the results the limit of detection for input of cDNA is 900ng. Additionally, the LoD for RNA input derived from a separate study was found to be 19.98 ng.

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. Haan et. Al. Genes Chromosomes Cancer 2021; (61):148-160
6. 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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Basic UDI-DI: 0850024841BlueprintBB

MKT-518-2
Date of issue: February 2025

Modifications to previous version

Version 2 - update of intended use, the reference section, performance characteristics and warnings and precautions

Initial release – November 2022