

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k080252

B. Purpose for Submission:

The addition of a second scanner and also replacing low density microarray with high density microarray.

C. Measurand:

70 gene expression profile

D. Type of Test:

Expression microarray

Test service performed in a single laboratory in Agendia's Amsterdam facility.

E. Applicant:

Agendia BV

F. Proprietary and Established Names:

MammaPrint®

G. Regulatory Information:

1. Regulation section:

21 CFR 866.6040 Gene expression profiling test system for breast cancer prognosis

2. Classification:

Class II

3. Product code:

NYI, Classifier, prognostic, recurrence risk assessment, RNA gene expression, breast cancer

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

MammaPrint® is a qualitative in vitro diagnostic test service, performed in a single laboratory, using the gene expression profile of fresh breast cancer tissue samples to assess a patients' risk for distant metastasis.

The test is performed for breast cancer patients who are less than 61 years old, Stage I or Stage II disease, with a tumor size ≤ 5.0 cm and lymph node negative. The MammaPrint® result is indicated for use by physicians as a prognostic marker only, along with other clinicopathological factors.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

MammaPrint® is not intended for diagnosis, or to predict or detect response to therapy, or to help select the optimal therapy for patients.

4. Special instrument requirements:

Agilent 2100 Bioanalyzer: Serial number DE54700497 en DE24802382

Agilent DNA microarray scanner: Serial numbers US22502555 and US45103019

Note: The scanner and bioanalyzer are components of this assay and are cleared only for this assay and not for any other application. In addition, clearance is only limited to the bioanalyzer and scanners with the serial numbers as specified above.

I. Device Description:

The MammaPrint® test is performed and provided as a service by Agendia Laboratory. The test is a microarray based gene expression analysis of RNA extracted from breast tumor tissue. The test is a custom-designed array chip manufactured by Agilent Technologies using the Agilent oligonucleotide microarray platform which assesses the mRNA expression of the 70 genes in triplicate. The MammaPrint® microarray features eight 1900-feature subarrays per glass slide which can each be individually hybridized. Per subarray 232 reporter genes are printed in triplicate, including the 70 genes which make up the MammaPrint® prognostic profile. Each subarray additionally includes 465 normalization genes and 289 spots for hybridization and printing quality control.

The analysis is based on several processes: isolation of RNA from fresh tumor tissue sections, DNase treatment of isolated RNA, linear amplification and labeling of DNase treated RNA, cRNA purification, hybridization of the cRNA to the MammaPrint® microarray, scanning the MammaPrint® microarray and data acquisition (feature extraction), calculation and determination of the risk of recurrence in breast cancer patients.

The MammaPrint® analysis is designed to determine the gene activity of specific genes in a tissue sample compared to a reference standard. The result is an expression profile, or fingerprint, of the sample. The correlation of the sample expression profile to a template (the mean expression profile of 44 tumors with a known good clinical outcome) is calculated and the molecular profile of the sample is determined (Low Risk, High Risk, Low Risk Borderline, High Risk Borderline).

J. Substantial Equivalence Information:

1. Predicate device name(s):

Agendia BV's MammaPrint®

2. Predicate 510(k) number(s):

k070675

3. Comparison with predicate:

The device is the same as the predicate, except for the addition of a microarray scanner and replacement of the low density (LD) microarray with high density (HD) microarray. Similar to the LD microarray, the HD microarray is manufactured under QSR design control. The differences between the HD and the LD microarrays are:

- HD array has more concentrated spots on the array and the 70 signature genes are printed in nine-fold instead of three-fold
- Feature extraction software uses Version 9.5, which uses the triplicate printings of the 465 normalization genes, rather than single print of 465 normalization genes in Version 8.5
- X-Print analysis software calculates average of LogRatios of probes printed in nine-fold instead of three-fold for the LD array.

K. Standard/Guidance Document Referenced (if applicable):

None

L. Test Principle:

The MammaPrint® service is a microarray based gene expression analysis of a tumor. Refer to k062694 for detailed description.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

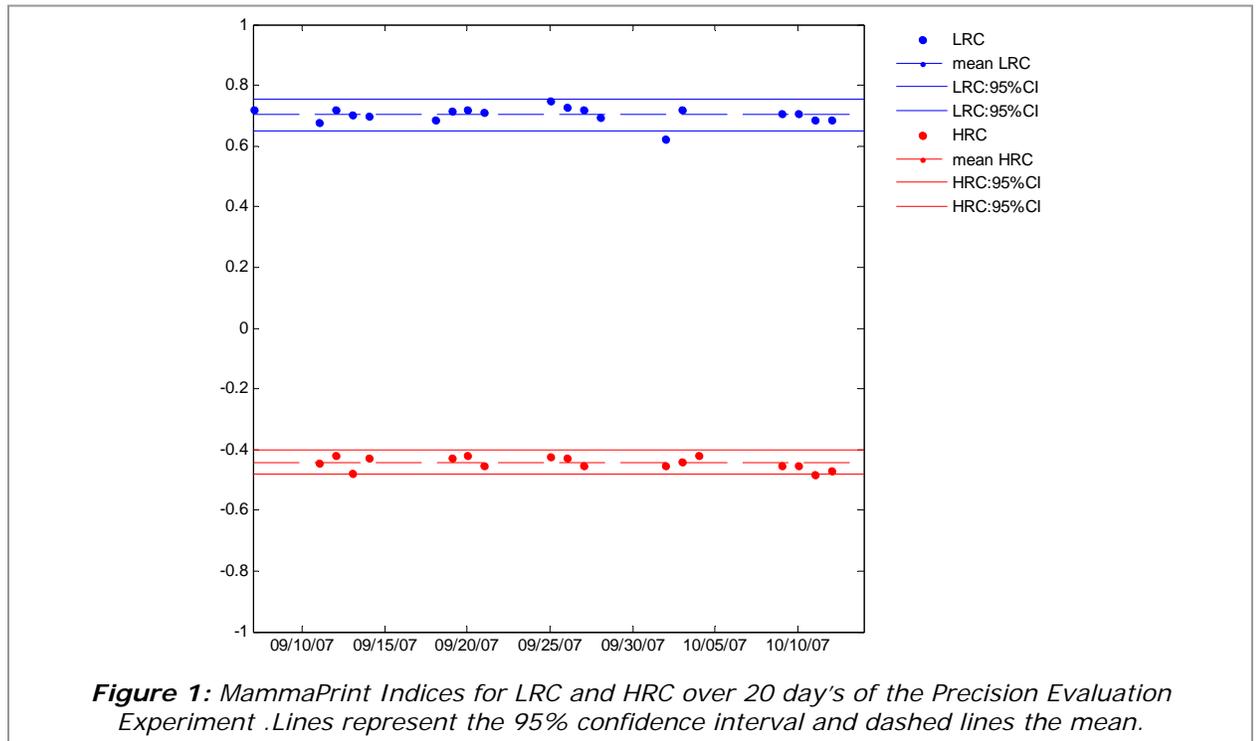
a. *Precision/Reproducibility:*

i. Reproducibility of high density microarray:

The experimental design consisted of repeated runs over 20 days in which 3 samples with different outcome levels, high, low and borderline, were performed on MammaPrint index High Density 8-pack arrays. For each day, one run is performed. For each run, each sample level was assayed in duplicate.

Additionally both control samples high and low risk control (HRC, LRC) as used for MammaPrint were also run along with one of the replicate Runs. The

repeatability and precision of the MammaPrint index of all outcome levels were at least as good as the performance of the predicate device (Standard Deviation and Variance of 0.030 and 0.001 respectively).



b. *Linearity/assay reportable range:*

Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Same as previous submission.

d. *Detection limit:*

Same as previous submission.

e. *Analytical specificity:*

Same as previous submission.

f. *Assay cut-off:*

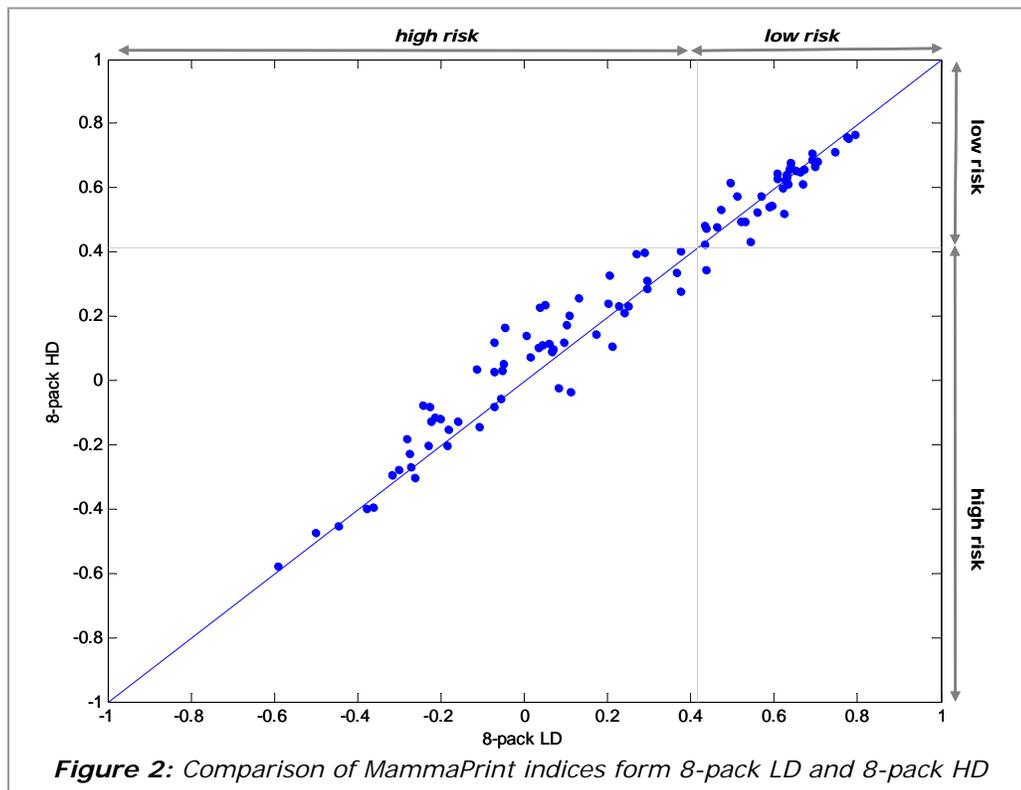
Same as previous submission.

2. Comparison studies:

a. *Method comparison with predicate device:*

i. Comparison between the HD and LD microarrays

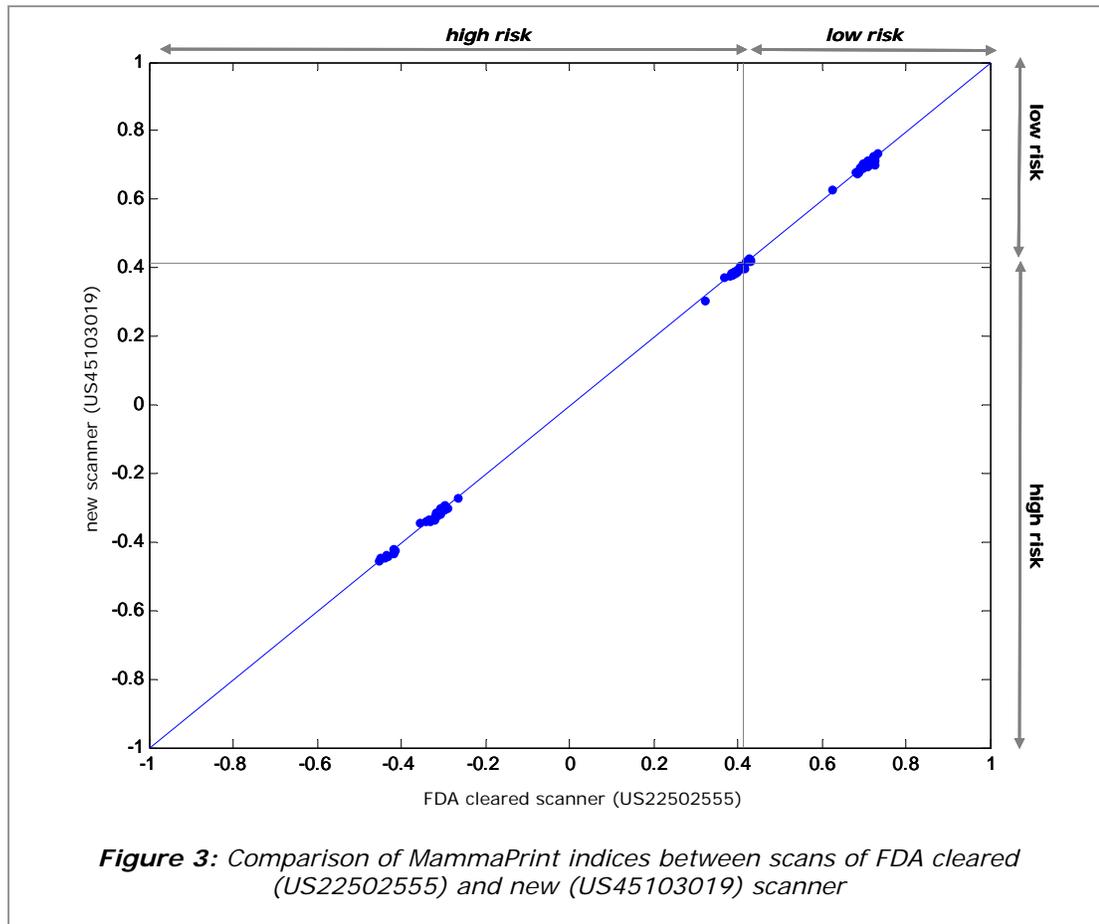
Ninety-eight (98) samples were selected for MammaPrint service from the period 2004 through 2007. Samples originating from 2004 through 2005 were relabeled and hybridized to both LD and HD 8-pack arrays. However, for samples from 2006 to 2007, the labeled cRNAs were only re-hybridized on HD 8-pack MammaPrint arrays and the original MammaPrint Index from previous LD hybridizations were used. Comparison results showed a 98.9% concordance in MammaPrint outcome between HD and LD microarray which falls within the 97.7% technical precision of the predicate device.



ii. Comparison between the previously cleared scanner and the additional scanner

The same samples from the precision study were used to evaluate the performance between the scanners. A set of 26 newly hybridized slides (104 samples) were scanned first on the FDA cleared scanner (serial nr: US22502555, Agendia DPd Id: 002) and subsequently on the new scanner (serial nr: US45103019 Agendia DPd Id: 112). MammaPrint indices were compared between both scans using Passing and Bablok regression analysis and a comparison of the variance per scanner. The difference between the mean, median and standard deviation for all

samples levels between both scanners fell within the accepted variance of the predicate device of 0.059 (1.96*0.030).



b. *Matrix comparison:*

Not applicable

3. Clinical studies:

Same as previous submission.

a. *Clinical Sensitivity:*

Same as previous submission.

b. *Clinical specificity:*

Same as previous submission.

c. *Other clinical supportive data (when a. and b. are not applicable):*

Same as previous submission.

4. Clinical cut-off:

Same as Assay cut-off

5. Expected values/Reference range:

Same as previous submission.

N. Instrument Name:

Agilent DNA microarray scanner (This scanner is not cleared for any other application.)

O. System Descriptions:

1. Modes of Operation:

Automated

2. Software:

MammaPrint analysis involves the use of scanner software, feature extraction software, and data analysis software. The scanner- and feature extraction software are off the shelf software (OTS) developed by Agilent Technologies. The data analysis software (X-Print analysis software) is custom software developed by Agendia.

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes x or No _____

3. Specimen Identification:

Barcode

4. Specimen Sampling and Handling:

Batch

5. Calibration:

Installation and calibration are performed by the instrument manufacturer. No user calibration required.

6. Quality Control:

QC protocol uses a fluorescently labeled reference sample complimentary to every oligo on the QC microarray.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

None

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.