DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

January 23, 2015

Agendia NV
Mr. Guido Brink
Vice President Regulatory Affairs & Compliance
Science Park 406, 1098XH
Amsterdam, The Netherlands

Re: K141142
Trade/Device Name: MammaPrint® FFPE
Regulation Number: 21 CFR §866.6040
Regulation Name: Gene Expression Profiling Test System for Breast Cancer Prognosis
Regulatory Class: Class II
Product Code: NYI
Dated: December 8, 2014
Received: December 10, 2014

Dear Mr. Guido Brink:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set

forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Reena Philip -S

Reena Philp, Ph.D.
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Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K141142

Device Name
MammaPrint® FFPE

Indications for Use *(Describe)*
MammaPrint® FFPE is a qualitative in vitro diagnostic test, performed in a central 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 breast cancer patients, with Stage I or Stage II disease, with tumor size ≤ 5.0 cm and lymph node negative. The MammaPrint® FFPE result is indicated for use by physicians as a prognostic marker only, along with other clinico-pathological factors.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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510(k) Summary

I. Submitter

Agendia NV
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The Netherlands
Telephone: 31 20 462 1500

Contact person: Guido Brink, VP Regulatory Affairs and EU Market Access

Date Prepared: January 22nd, 2015

II. Device

Name of Device: MammaPrint® FFPE

Common or Usual Name: Multivariate device for cancer prognosis

Classification Name: Gene expression profiling test system, for breast cancer prognosis (21 CFR 866.6040)

Regulatory Class: Class II

Product Code: NYI

III. Predicate Device

Agendia NV's MammaPrint (k101454)

IV. Device Description

The MammaPrint® FFPE test is a microarray based gene expression analysis of a tumor. The analysis is based on several processes: isolation of RNA from FFPE breast cancer tissue sections; elimination of gDNA, reverse transcription of RNA resulting in cDNA; amplification of the cDNA, purification and labeling of 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.

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

V. Intended Use

MammaPrint® FFPE is a qualitative in vitro diagnostic test, performed in a central 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 breast cancer patients, with Stage I or Stage II disease, with tumor size ≤ 5.0 cm and lymph node negative. The MammaPrint® FFPE result is indicated for use by physicians as a prognostic marker only, along with other clinico-pathological factors.

VI Comparison of technological characteristics with the predicate device

MammaPrint FFPE shares the same principles of operation, overall technical and functional capabilities as the predicate device covered under k101454. The only difference concerns the pre-analytical sample preparation; the use of formalin-fixed paraffin embedded (FFPE) breast tumor tissue in addition to fresh breast tumor tissue as is used in the predicate device.

VII. Performance Testing (Bench)

Analytical performance

MammaPrint analytical (i.e., non-clinical) performance characteristics investigated comprise concordance between FFPE tissue samples and Fresh as well as Precision and Reproducibility performance assessment. Also inter-laboratory and microarray scanner comparisons are included.

1 - Concordance FFPE tissue samples and Fresh

First independent validation: n=122

In order to extend the intended use of MammaPrint to FFPE samples as well as Fresh a concordance comparison was performed. A selection of 122 tumor samples was used from which one section of the tumor was preserved as Fresh and a second part as FFPE. The Fresh section of the tumor was subjected to latest design controlled version (internal version US09.1 / EU09.1) of the latest FDA cleared MammaPrint assay (k101454) and the FFPE sections were subjected to MammaPrint FFPE (internal version US01.1 / EU01.1). The MammaPrint results from Fresh and FFPE sections for each sample were compared.

The overall concordance in outcome between MammaPrint Fresh and FFPE is 89.34 %. If only the true positives and negatives are considered (non-borderline samples), there is a concordance of 92.7%. For this comparison the NPA is equal to 88.4% (95%CI: 80.5 to 95) and the PPA was 90.6% (95%CI: 79.8 to 95.9).

Second independent validation: n=345 (Raster study)

A second concordance comparison between MammaPrint Fresh and FFPE was performed using the Raster study. A selection of 345 samples was used from which Fresh RNA as well as FFPE tissue was available. The Fresh RNA was subjected to MammaPrint Fresh version US09.1/EU09.1 and the FFPE tissues were subjected to MammaPrint FFPE version US01.1/EU01.1.

The concordance in this Raster sample set was 89.28%. If only the true positives and negatives (non-borderlines) are considered the concordance is equal to 93.5%. For this comparison the NPA is equal to 91.5% (95%CI: 86.7 to 94.7) and the PPA was 86.6% (95%CI: 80.4 to 91.1).

2 - Test Performance

Test performance of MammaPrint FFPE was assessed on different levels.

I. Reproducibility was assessed on multiple isolations from the same sample (report VR-TR-206a). For 30 FFPE samples four isolations were performed: I1, I2, I3 and I4. All 4 isolations were further processed to generate MammaPrint FFPE results.

A repeated Measurements ANOVA was used to determine if there was a difference in MammaPrint Indices over all four isolations. The results show that there is no significant difference (*p=0.994*).
A Cochran's Q test was used to assess the difference in MammaPrint Outcome over the four isolations. The results of this test show that there is no significant difference in MammaPrint Outcome over the different isolations (*p=0.290*).

II. Reproducibility was assessed on multiple labeling/hybridizations by measuring FFPE control samples overtime (report VR-TR-233a):

- PHTR: **P**araffin, **H**igh risk
- PLEP: **P**araffin, **L**ow risk
- PHHE: **P**araffin, **H**igh risk

This was performed on a daily basis in order to obtain overtime MammaPrint FFPE results for all three controls. The standard deviations passed the predefined acceptance criteria (stdevs: PHTR=0.045 (n=54), PLEP=0.056 (n=52), PHHE=0.072 (n=52)).

III A Precision and Evaluation (P&E) experiment (*NCCLS, Evaluation of Precision Performance of Quantitative Measurement methods, EP5-A2*) was performed to determine the Repeatability and Method Precision of MammaPrint FFPE test (report VR-TR-223a).
This experiment set up consists of repeated runs over 20 days in which all test outcome levels are represented by one test sample. After collecting the data from 20 successive days, statistical analysis was performed to determine the repeatability and within-lab precision per sample level:

		Repeatability (Within-Run)		Method Precision (within-Laboratory)	
Sample ID	Sample level: outcome	Standard deviation	Variance	Standard deviation	Variance
11003290	High Risk	0.036	0.0013	0.044	0.0019
11003347	High Risk	0.046	0.0021	0.057	0.0033
11003810	Low Risk	0.042	0.0018	0.050	0.0025
11003860	Low Risk - Borderline	0.049	0.0024	0.066	0.0044

The P&E results for Repeatability and Method Precision meet the predefined acceptance criteria.

3 - Inter-laboratory comparison of MammaPrint FFPE between Irvine and Amsterdam laboratories

For this validation 25 FFPE samples were selected from which MammaPrint FFPE results were previously generated using standard FFPE protocols in Amsterdam. From these samples sections were taken and processed from isolation onwards by the diagnostic departments in both Agendia Irvine and Amsterdam according to standard protocols.

The MammaPrint FFPE results generated for these 25 samples at Irvine and Amsterdam locations were compared to the previously generated results. These results meet the predefined acceptance criteria (Irvine: Kappa score = 0.90, Amsterdam: Kappa Score = 0.9).

Furthermore, the Passing and Bablok regression analysis also showed that there is high agreement in MammaPrint Index within this inter-laboratory comparison of Amsterdam and Irvine Operations. For both locations the intercept is close to zero and the slope is close to 1.

4 - Validation of use of multiple microarray scanners for MammaPrint FFPE Irvine and Amsterdam

For the validation of both micro-array scanners in central lab in Amsterdam, 25 samples were hybridized two times; first on the originally validated scanner, serial number US810R3210 and in addition using the scanner with serial number US22502555. MammaPrint Indices were compared between both scanners. The comparison of MammaPrint indices between both scanners meet the predefined acceptance criteria *(Pearson correlation = 1.0)*. Also the comparison of MammaPrint Outcome meet the predefined acceptance criteria *(Kappa score =1.0, NPA= 100% (95%CI: 67.6 to 100), PPA= 100% (95%CI:81.6 to 99.0))*.

Furthermore, the Passing and Bablok regression analysis also showed that there is high agreement in MammaPrint Index between both scanners located in Amsterdam. For this comparison the intercept is close to zero and the slope is close to 1.

For the validation of both micro-array scanners in central lab in Irvine, 27 samples were hybridized two times; first on the originally validated scanner, serial number US811R3213 and in addition using the scanner with serial number US45103019. MammaPrint Indices were compared between both scanners.

The comparison of MammaPrint indices between both scanners meet the predefined acceptance criteria *(Pearson correlation = 1.0)*. Also the comparison of MammaPrint Outcome meet the predefined acceptance criteria *(Kappa score =1.0 NPA= 100% (95%CI: 77.2 to 100), PPA= 100% (95%CI:78.5 to 100))*.

Furthermore, the Passing and Bablok regression analysis also showed that there is high agreement in MammaPrint Index between both scanners located in Irvine. For this comparison the intercept is close to zero and the slope is close to 1.

5 – Determine minimum input in hybridization of MammaPrint FFPE

A dilution study was performed to determine the minimum input of labeled cDNA in a hybridization of MammaPrint FFPE. In this dilution study three samples as well as three MammaPrint FFPE control samples were each labeled five times and per sample to generate sufficient amount of labeled material. Samples were then diluted in 8 steps with water to reach the specific yield (ng),in which step 8 reflects a hybridization with no labeled material.

MammaPrint FFPE results were compared over the different dilution and showed very stable results even at low input of cDNA.

VIII. Performance testing-Clinical

As part of this submission of MammaPrint FFPE the clinical performance of MammaPrint FFPE was compared to MammaPrint Fresh. For this comparison samples were used that were part of the Raster study that were processed on MammaPrint Fresh between 2004 and 2007 and currently have a clinical follow-up of 5 years. For the clinical performance evaluation of MammaPrint FFPE on RASTER FFPE samples, endpoints were defined as Distant Recurrence Free Interval (DRFI) as presented for the Raster study[1] as well as the 5 year Distant Metastasis as first event (DM1st).

Comparison based on DRFI

For both the Fresh version of MammaPrint as well as the FFPE version, Kaplan-Meier curves showed a significant difference in DRFI the Low and High Risk groups LogRank p=0.001)
(Figure 5.1 A and B respectively).

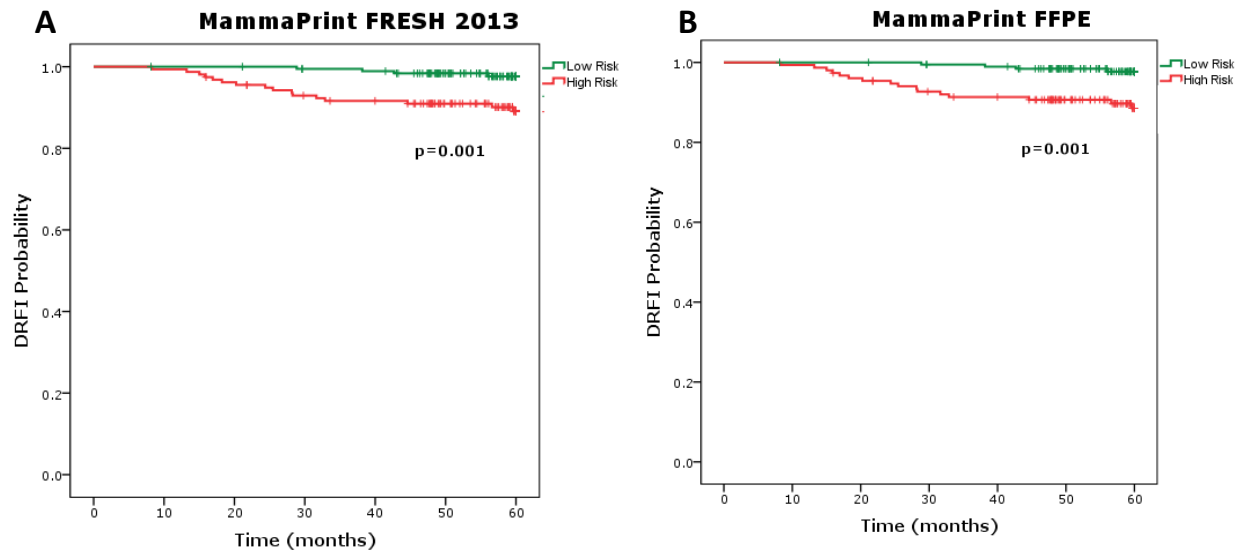

Figure 5.1: *Kaplan-Meier analysis of the 5 year DRFI probability among 345 patients with LN0 breast cancer that were part of the Raster study for* ***A)*** *MammaPrint Fresh and* ***B)*** *MammaPrint FFPE*

The 5-year DFRI of both MammaPrint Fresh and FFPE is shown in below. The performance of MammaPrint FFPE falls within the 95% CI of MammaPrint Fresh and therefore complies with the predefined acceptance criteria.

	MammaPrint Fresh 2013			***MammaPrint FFPE***		
		95% CI			*95% CI*	
Low Risk Signature	*0.976*	*0.952*	*1.000*	*0.977*	*0.955*	*0.999*
High Risk Signature	*0.891*	*0.840*	*0.942*	*0.885*	*0.830*	*0.940*

Based on the results shown MammaPrint FFPE is clinically equivalent to the predicate device MammaPrint Fresh (k101454).

Comparison based on DM 1st

The 5-year DM1st survival of both MammaPrint Fresh and FFPE is shown below. The performance of MammaPrint FFPE falls within the 95% CI of MammaPrint Fresh and therefore complies with the predefined acceptance criteria.

MammaPrint Fresh 2013				***MammaPrint FFPE***			
		95% CI				*95% CI*	
Low Risk Signature	*0.976*	*0.952*	*1.000*	*Low Risk Signature*	*0.977*	*0.955*	*0.999*
High Risk Signature	*0.907*	*0.860*	*0.954*	*High Risk Signature*	*0.903*	*0.854*	*0.952*

Prognostic assessment of MammaPrint® FFPE was further investigated using a univariate and multivariate analysis. In the univariate analysis MammaPrint FFPE is significantly associated with risk recurrence. Multivariate analysis did not conclusively demonstrate prognostic significance for MammaPrint FFPE beyond that of other clinicopathological factors. This is attributable to the RASTER study design, in which MammaPrint result was included along with all relevant clinic-pathological factors, and treatment decisions were guided by assessed prognostic risk and the standard of practice. In this real–world context, the overall cohort experienced a low event rate which, despite the favorable trend, diminishes independent contribution of MammaPrint.

IX. Conclusion

MammaPrint FFPE and its predicate device MammaPrint Fresh (K101454) are a clinically and analytically accurate prognostic marker for providing a risk assessment of distant metastasis of breast cancer when performed in either Agendia's European or US central laboratory.

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