

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION

DECISION SUMMARY

A. 510(k) Number:

k113420

B. Purpose for Submission:

New device

C. Measurand:

Ribonucleic acid (RNA)

D. Type of Test:

Collection, storage and transportation of fresh breast tissue specimens for subsequent RNA isolation and further molecular diagnostic testing

E. Applicant:

Asuragen, Inc.

F. Proprietary and Established Names:

RNA*Retain*®

G. Regulatory Information:

1. Regulation section:

21 CFR §866.4070 RNA Preanalytical Systems

2. Classification:

Class II

3. Product code:

OZF, Tissue RNA preservative for collection, storage, and transportation

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

The RNA*Retain*® device is a single-use, prefilled container intended for the collection, storage, and transportation of fresh breast tissue specimens for subsequent RNA isolation and further molecular diagnostic testing.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For professional use only

4. Special instrument requirements:

None

I. Device Description:

The RNA*Retain*® device consists of an aqueous, hypertonic tissue preservation solution that is provided in a single-use, non-sterile vial intended to serve as the container for the collection, storage and transport of breast tissue specimens.

RNA*Retain*® comes in three configurations: (1) 6mL RNA*Retain*® solution in 8 mL tube that has a maximum tissue capacity of 0.6 mL or 0.6 g; 18 vials/box; (2) 5 mL RNA*Retain*® solution in 6 mL tube that has a maximum tissue capacity of 0.5 mL or 0.5 g; 18 vials/box, and (3) 1mL RNA*Retain*® solution in 2mL tube that has a maximum tissue capacity of 0.1 mL or 0.1 g; 24 vials/box. (1) and (2) use leak-proof polyethylene caps and (3) uses leak-proof polyethylene cap with O-ring.

J. Substantial Equivalence Information:

1. Predicate device name(s):

PAXgene® Blood RNA System

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

k042613

3. Comparison with predicate:

| Similarities | | |
|--------------|---|-----------|
| Item | Device | Predicate |
| Technology | Deactivation of ribonucleases and preservation of RNA molecules | Same |
| Format | Single-use, specimen container prefilled with preservation solution | Same |

| Differences | | |
|--------------|--|--|
| Item | Device | Predicate |
| Intended Use | The RNA <i>Retain</i> ® device is a single-use, prefilled container intended for the collection, storage, and transportation of fresh breast tissue specimens for subsequent RNA isolation and further molecular diagnostic testing. | The PAXgene Blood RNA System is intended for the collection, storage, and transport of blood and stabilization of intracellular RNA in a closed tube and subsequent isolation and purification of host RNA from whole blood for RT-PCR used in molecular diagnostic testing. |
| Sample type | Breast tissue | Whole blood |
| Sterility | Non-sterile | Sterile |

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

Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: RNA Preanalytical Systems (RNA Collection, Stabilization and Purification Systems for RT-PCR used in Molecular Diagnostic Testing) August 25, 2005.

L. Test Principle:

A breast tissue sample is collected into RNA*Retain*® solution in a single-use, prefilled container and can be stored in reagent for up to 3 days at 35 to 39°C, 7 days at 18 to 25°C, 30days at 2 to 8°C, or 3 years at -15 to -30°C before RNA extraction. Tissue should be submerged in RNA*Retain*® for at least 12 hours prior to freezing. The RNA*Retain*® solution can rapidly permeate tissue to protect cellular nucleic acids from nucleases that would otherwise rapidly degrade the nucleic acids within the specimen. RNA can be extracted from preserved breast tissue and utilized in downstream molecular testing.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision and reproducibility of breast tissue specimens preserved using the RNA*Retain*® device was demonstrated using the MammaPrint® device in submission k070675. Five previously analyzed tumor samples (one borderline, two high-risk samples, and two low-risk samples) were processed in duplicate for RNA isolation over multiple days according to standard MammaPrint® protocols. No statistically significant difference in MammaPrint® risk group assignment or MammaPrint® index between the two separate RNA isolations was observed.

A subsequent repeatability study compared RNA integrity, purity and yield using breast tissues from each of the 3 subjects that were sectioned into 20 sections with adjacent sections split between storage in RNA*Retain*® and fresh frozen. Small variations were observed among replicates in RNA purity as determined by A_{260}/A_{280} (1.51 to 6.96% CV), quantitative RT-PCR (qRT-PCR) of a housekeeping gene (1.33 to 3.97% CV), and RNA integrity as measured by either the ratio of 28S:18S RNA (10.3 to 18.8% CV) or RNA Integrity Number (RIN) values. A variation is noted in RNA yield and is likely due to the tissue composition (*e.g.*, the amount of fat and stroma present vs. epithelial tissue).

b. *Linearity/assay reportable range:*

Not applicable.

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

Stability of tissues stored in RNA*Retain*®

The effect of shipment of a tumor in RNA*Retain*® on stability of MammaPrint® results was demonstrated in k070675. The tumor was selected from which both immediately snap-frozen and RNA*Retain*® preserved sections were available. Both sections of the tumor had a similar tumor cell percentage and similar RNA quality. Both samples were labeled 5 times and hybridized on MammaPrint® microarrays according to standard protocols. MammaPrint® Indices were compared to determine if samples shipped in RNA*Retain*® have a greater stability than tumor sections which are immediately stored at -70°C after excision. Results showed that the incorporation of both Cy5 and Cy3 were significantly higher for samples shipped in RNA*Retain*® (Unpaired T-tests, $p=0.018$ and $p=0.001$ respectively). The variance in MammaPrint® indices was smaller for samples that were stored in RNA*Retain*® compared to the samples that were frozen immediately (SDev 0.022 vs. 0.042). An unpaired T-test of the MammaPrint®

index revealed no significant difference in the actual MammaPrint® indices for the RNARetain® and frozen samples (p=0.24). Based on these experiments, the stability in MammaPrint® index is greater in samples stored in RNARetain® than in samples that were immediately frozen. The difference in MammaPrint® index between RNARetain® and frozen tissue ($\Delta 0.027$) was within the previously determined acceptable limit of index variation.

In another subsequent study, human breast cancer cell line MCF-7 were grown and resuspended in RNARetain®, incubated overnight at 2 to 8°C, then subjected to up to 7 days at 35 to 39°C, up to 15 days at 18 to 25°C, up to 60 days at 2 to 8°C, and up to 3 years at -15 to -30°C. RNA Yield (A260), Purity (A260/A280), and Integrity (28S:18S ratios) were compared to Fresh Frozen cells. Samples are stable up to 3 days at 35 to 39°C, 15 days at 18 to 25°C, 60 days at 2 to 8°C, and up to 3 years at -15 to -30°C.

Table 1. Quality of total RNA recovered from MCF-7 cells stored in RNARetain® over multiple temperatures and time-points

| Temp(°C) | Time | Integrity (28S:18S) | Purity (A260/A280) |
|------------|---------|---------------------|--------------------|
| 35 to 39 | 3 days | 1.3 | 1.94 |
| 18 to 25 | 7 days | 1.6 | 2.02 |
| 2 to 8 | 30 days | 1.2 | 1.98 |
| -15 to -30 | 3 years | 1.7 | 1.99 |

RNARetain® reagent stability

Stability studies were performed with 3 lots of the 8 mL vial (6 mL volume) up to 20 months with storage at room temperature (18 to 25°C) and showed acceptable performance. Additional stability studies with the 6 mL vial (5 mL volume) and 2 mL vial (1 mL volume) configurations were also performed and demonstrated acceptable stability of up to 36 months at room temperature.

d. *Detection limit:*

Not applicable.

e. *Analytical specificity:*

Not applicable.

f. *Assay cut-off:*

Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

The performance of RNA*Retain*® was demonstrated in k070675 based on a comparison to results obtained from fresh frozen tissue using the MammaPrint® device. The samples for this study were collected in 2003 as a pilot study for the Dutch Raster clinical trial sponsored by the Dutch Health Insurance Council where tumor tissue samples were shipped in RNA*Retain*® from 20 hospitals. One set consisted of 33 breast tumor samples of which one part of the sample was immediately snap-frozen in liquid nitrogen and stored at -70°C, another part was stored in RNA*Retain*® for 3 to 5 days at room temperature and subsequently removed from the preservation solution, snap frozen and stored at -70°C. Another set comprised of 18 tumors of which two parts were available for research that were immediately snap frozen and stored at -70°C. RNA isolation and DNase treatment were performed in this same period. H&E stained sections were re-examined by a pathologist to confirm invasive ductal carcinoma and sufficient tumor cell content. All samples were hybridized on MammaPrint® microarrays, and passed all sample, labeling and hybridization QCs. Analysis was performed using Feature Extraction version 8.5 and XPrint version 1.40. MammaPrint® indices of paired RNA*Retain*® and frozen samples have a median difference of 0.070. The Pearson correlation (0.94) and regression analysis indicate a high similarity ($R^2 = 0.90$). This finding is similar to the results of a series of tumors of which two frozen samples were available and were collected in the same time period. The median difference in MammaPrint® Index was 0.105. A comparison of the differences in both series (RNA*Retain*®-frozen vs. frozen-frozen) showed no significant difference (*t-test*, $p=0.57$) indicating no variation is introduced by RNA*Retain*®.

b. *Matrix comparison:*

As described above.

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Not applicable

N. Proposed Labeling:

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

O. Conclusion:

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