**SUMMARY**

- RNARetain® is a pre-analytical solution* manufactured under cGMP that protects nucleic acids in freshly collected tissues or cells**
- RNARetain® is compatible with the collection, storage and shipping of preoperative pancreatic or thyroid FNAs for direct extraction of total RNA or total nuclear DNA
- Analysis of specific DNA, mRNA or miRNA biomarkers in RNARetain®-preserved FNAs might be useful in the future to facilitate the molecular characterization and preoperative differential diagnosis of suspicious cancer FNAs

**INTRODUCTION**

Cytopathological diagnosis of fine needle aspirates (FNA) is challenging and can be inconclusive. Molecular testing for relevant markers associated with specific cancers can improve the preoperative diagnosis of FNAs. For example, we previously showed that a 2-miRNA classifier, initially developed and validated on FFPE (formalin-fixed, paraffin-embedded) tissue, can help identify benign versus malignant pancreatic FNAs (Saitabaka et al. Oncogene 2007 and J Clin. Chem, 2008). Testing for the presence of specific DNA, mRNA or miRNA (miRNA) biomarkers has also been shown to improve the diagnostic value of thyroid nodules or FNAs.

RNARetain® is a solution* that preserves nucleic acids in tissue (see Figure 1) allowing nucleic acid extraction and molecular analysis at a later time and different location**. RNARetain® is manufactured under cGMP and is cleared in the US as an accessory to the Agendia's MammaPrint® (A260/A280) technology (performed on an ABI 7500 Fast or 7900 instrument) or multiplex qualitative endpoint PCR assays based on Signature® technology platforms. These procedures were developed so that the cell-free nucleic acids released during the FNA collection process could be recovered together with the cellular nucleic acids. Results for control extractions in the absence of nucleic acid were not observed for the samples with the lowest total RNA concentration.

**MATERIALS AND METHODS**

RNARetain® (RUS) was used as 1 mL aliquots in 2 mL single-use vials allowing collection, storage, shipment, and recovery of 1 to 3 FNA per vial. Ultrastratage® (US) guided FNAs were collected at various sites, immersed in RNARetain® and processed as described in Figure 2. All human specimens in this study were de-identified and evaluated by a local IRB (Institutional Review Board) and in accordance with institutional guidelines.

Purified nucleic acids were subjected to several quality controls including concentration/purity (optical spectrophotometry) and integrity (highest Bioanalyzer) expression of specific biomarkers was determined using simple quantitative real-time PCR assays based on TaqMan® technology (performed on an ABI 7500 or 7900 instrument) or multiplex qualitative endpoint PCR assays based on Signature® technology (performed on a Lumex 200 instrument).

**RESULTS**

**Nucleic Acid Extraction from RNARetain®**

(B) Representative example of quantitative real-time PCR analysis for 3 types of total nucleic acid samples tested at 20 or 40 ng input per DNA- or RNA-based assay, with and without reverse transcription step. This approach is currently used at Asuragen to identify novel sets of miRNAs that may be useful in the future to facilitate the molecular characterization and preoperative differential diagnosis of suspicious cancer FNAs.

**CONCLUSIONS**

These results demonstrate that a 1 mL research use formulation of RNARetain®* is compatible with the collection, storage, and transport of preoperative FNAs from different tissue types. Total nucleic acid was efficiently recovered from the pre-analytical solution and successfully tested for known DNA and RNA biomarkers as well as novel miRNA candidates**. This process could facilitate the future complement to the cytological diagnosis of changing neoplastic diseases such as pancreatic and thyroid cancers. Ultimately, validation of standardized collection and testing protocols might improve the differential diagnostic of suspicious cancer FNAs and facilitate preoperative decision making.

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*For research use only. Not for use in diagnostic procedures.

**Preliminary research data: the performance characteristics of this reagent have not yet been established.**