**Materials and Methods**

- **Context:** Laser capture microdissection is a well-established technique for procuring pure populations of cells from clinical tissue samples for gene expression analysis. Since RNA is very labile, a frequent question by investigators is the maximum amount of time they have to complete microdissection after tissue preparation. Since RNA degradation is primarily due to endogenous RNases that are activated in aqueous environments, the staining process potentially leads to the greatest loss of RNA. This study will compare the efficacy of three commercially available RNase inhibitors added to hematoxylin and eosin staining reagents to preserve RNA in tissue for laser capture microdissection.

**Design:** Anonymized snap-frozen colon cancer tissue was used for this study. All tissue sections for microdissection were cut on glass slides that were autoclaved and treated with RNase inhibitor. Tissue sections were hematoxylin and eosin stained without RNase inhibitor and with inhibitor using one of three different inhibitors including ProtectRNA (Sigma Aldrich, St. Louis, Missouri, USA); ProtectRNase (Rock, Indianapolis, Indiana, USA), or RNase Inhibitor (Qiagen, Valencia, California, USA). Fifteen thousand cells were microdissected after being set at room temperature for 0, 2, 24, and 48 hours. RNA was extracted, quantitated, and quality determined by 28S:18S ratio, RNA integrity number, and quantitative reverse transcription polymerase chain reaction for the ratio of Ct’s for β-actin and 3’ end.

**Results:** There was essentially no difference in the quantity and quality of RNA recovered from samples with and without RNase inhibitor treatment for all time points. RNA quality was excellent for 0 and 2 hours with and without RNase inhibitor treatment. After 24 hours, there was no statistical difference in RNA quality for RNase inhibitor-treated and untreated samples. After 48 hours, all samples showed poor quality RNA. 

**Background:** This study suggests that one has up to 15 hours to microdissect stained colon cancer tissue derived from frozen tissue with or without RNase inhibitor treatment to obtain RNA of suitable quality for gene expression analysis.

**Conclusions:**

- There is no relationship between quantity of recovered RNA and presence of RNase inhibitors or time. 
- RNA was well preserved in H&E stained frozen colon cancer tissue sections for up to approximately 15 hours.
- Treatment of tissue sections with RNase inhibitors did not significantly improve RNA preservation in tissue.

**Future Directions:**

Will examine other tissue types including tissues that contain high levels of RNases (e.g. pancreas).

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**Reference**