OPTIMIZING PROTOCOLS FOR RNA ISOLATION FROM FIXED TISSUE
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Detection of pathogens in environmental specimens is challenging due to the rapid degradation of nucleic acids based on preservation techniques used. A study was designed to evaluate two protocols for specimen preparation for optimized preservation and integrity of cellular and viral RNA: 1) formalin-fixed paraffin embedded (FFPE) sample processing and 2) optimal cutting temperature (OCT) embedding compound for fixed and frozen tissue specimens. Soybeans in stage 1 of germination were fixed with 10% formalin for 24 to 48 hours prior to embedding in either FFPE or OCT. RNA was then isolated from 5μm thick slices using a Qiagen RNeasy FFPE Kit. RNA quantity was measured with Qubit™ RNA High Sensitivity (HS) Assay Kit and RNA integrity was assessed using Qubit™ RNA IQ Assay Kit.

The objective of this study was to determine the optimal protocol for isolation of RNA from environmental samples. This study focused on the FFPE and OCT protocols. Each protocol had its own timeframe, challenges, and implications. FFPE required longer sample processing time, more reagents for step-wise tissue dehydration, and instrumentation to facilitate embedding specimens into hot paraffin wax. The OCT protocol required less time and reagents to process samples. FFPE and OCT protocols for RNA isolation were used to determine the optimal RNA concentrations and integrity of 24hr and 48hr fixed tissues.

In conclusion, the RNA concentrations for both FFPE and OCT were not significantly different (ns, p-value >0.05). Therefore, there was not a statistically significant difference between the embedding processes evaluated or for formalin fixation of 24 or 48 hours.
Furthermore, no significant difference was observed between RIQ score between FFPE and OCT methods or for fixation of 24 or 48 hours. Both FFPE and OCT samples rendered a similar RNA concentration and RIQ score. The hours invested in each tissue embedding protocol were significantly different. FFPE requires a total of 96 hours to process the tissues, while the OCT protocol only requires 2 hours to process the tissues. For the future, the aim is to compare the FFPE and OCT protocols and observe if RNA is retained \textit{in situ}. 