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Research investigates the best temperatures to store sea turtle blood samples

Storing samples in ideal temperatures can lead to higher quality data for RNA and plasma protein analysis for sea turtle health assessments

Using samples such as blood and tissue to collect data is a common practice among researchers. But in order to have repeatable and accurate results, the most reliable samples must be used. Delicate molecules like RNA and plasma proteins are both great indicators for health in loggerhead sea turtles. The RNA/DNA ratio indicates growth potential while the concentration of plasma proteins indicates the health status of loggerheads. These blood components are used often to assess the health of the endangered species, but it is critical to determine what the ideal conditions are such as the temperature or vial used to preserve the molecules.

Kelly Townsend is an intern through the College of Charleston's summer NSF REU program. Townsend's research with the National Institute of Standards and Technology under Dr. Jennifer Lynch is investigating RNA and plasma protein degradation in different storage conditions over time. By knowing what happens on a molecular level to blood when storage conditions go wrong, we hope to eliminate the use of low quality samples or promote the use of adequate samples.

Whole blood was obtained from seven loggerhead sea turtles off the coast of South Carolina. The blood was collected in either Vacutainer blood collection tubes containing sodium heparin or PAXgene tubes containing an RNA preservative. The sodium heparin tubes used for plasma were spun down on the boat to separate the blood components (i.e, plasma, white blood, and remaining cells) while the PAXgene tubes for RNA were left unspun. Once in the lab, the tubes were divided out in order to subject them to different treatments. Plasma was used for the plasma protein treatments while whole blood was used for the RNA treatments (Figure 1 and 2). There were also three samples from 2001 and 2008 that were selected from an archive for plasma analysis. At the end of the treatments, plasma was analyzed for protein concentrations and the whole blood was analyzed for RNA quality.

The results of this study will determine the storage conditions at which plasma proteins and RNA are most stable and begin to lose stability. The preserved samples can then be used to trace back either the origin of a disease or environmental contaminants that have entered the ocean. Due to having the ability to store samples for longer periods of time, it allows for new opportunities to ask unique questions by either eliminating variables or discovering new trends. Knowing the correct conditions will hopefully guide others in deciding how to store samples along with which ones are best suited for a variety of analyses.

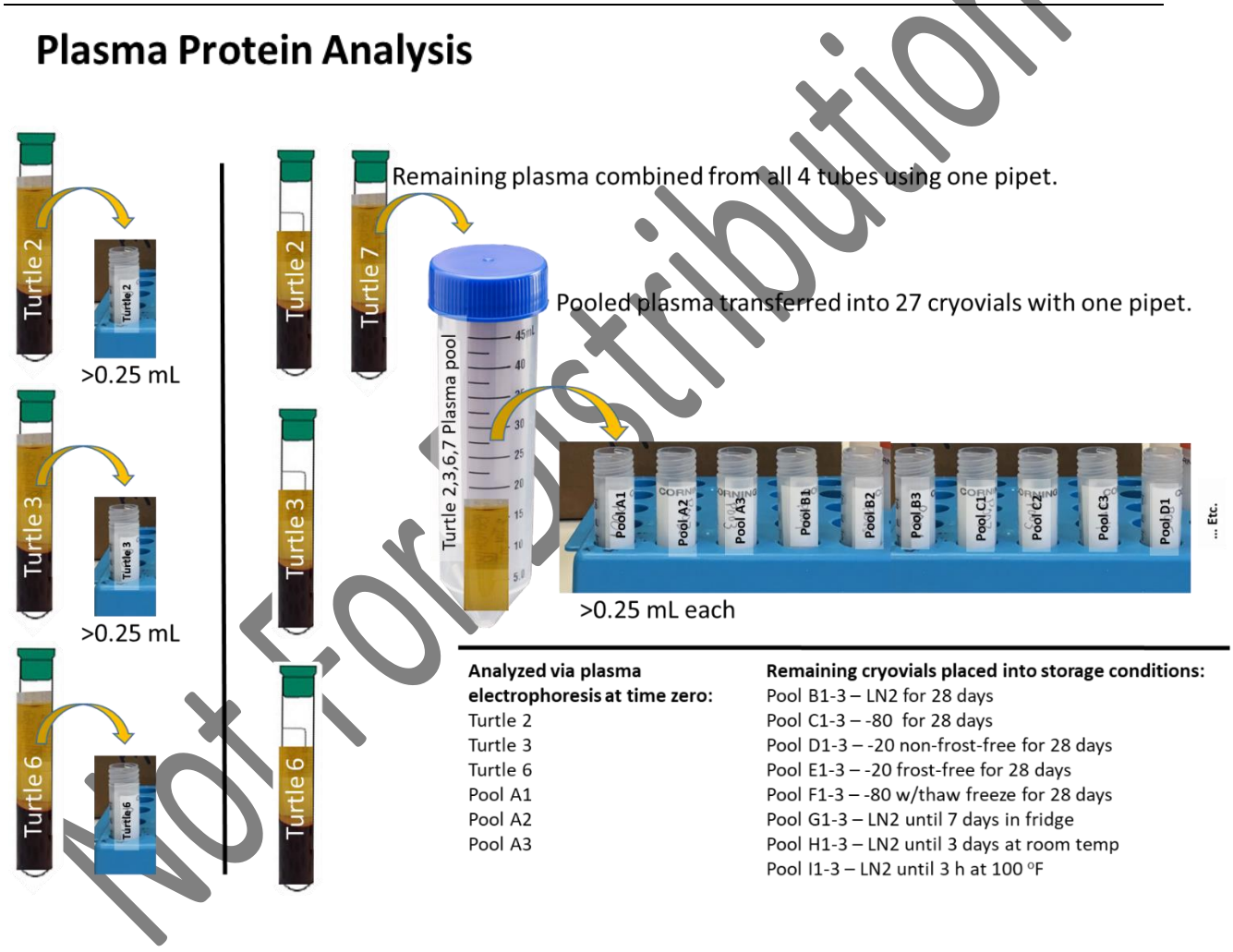

Figure 1: 0.25 mL of plasma from turtles 2, 3, and 6 was pipetted into individual vials before combining the remaining plasma from the vials and plasma from turtle 7 into one tube. Then the pooled plasma was separated between 27 cryovials and sent to their proper storage condition while the individual plasma and A1-A3 of the pooled plasma was analyzed via plasma electrophoresis as a time zero measurement.

RNA Analysis

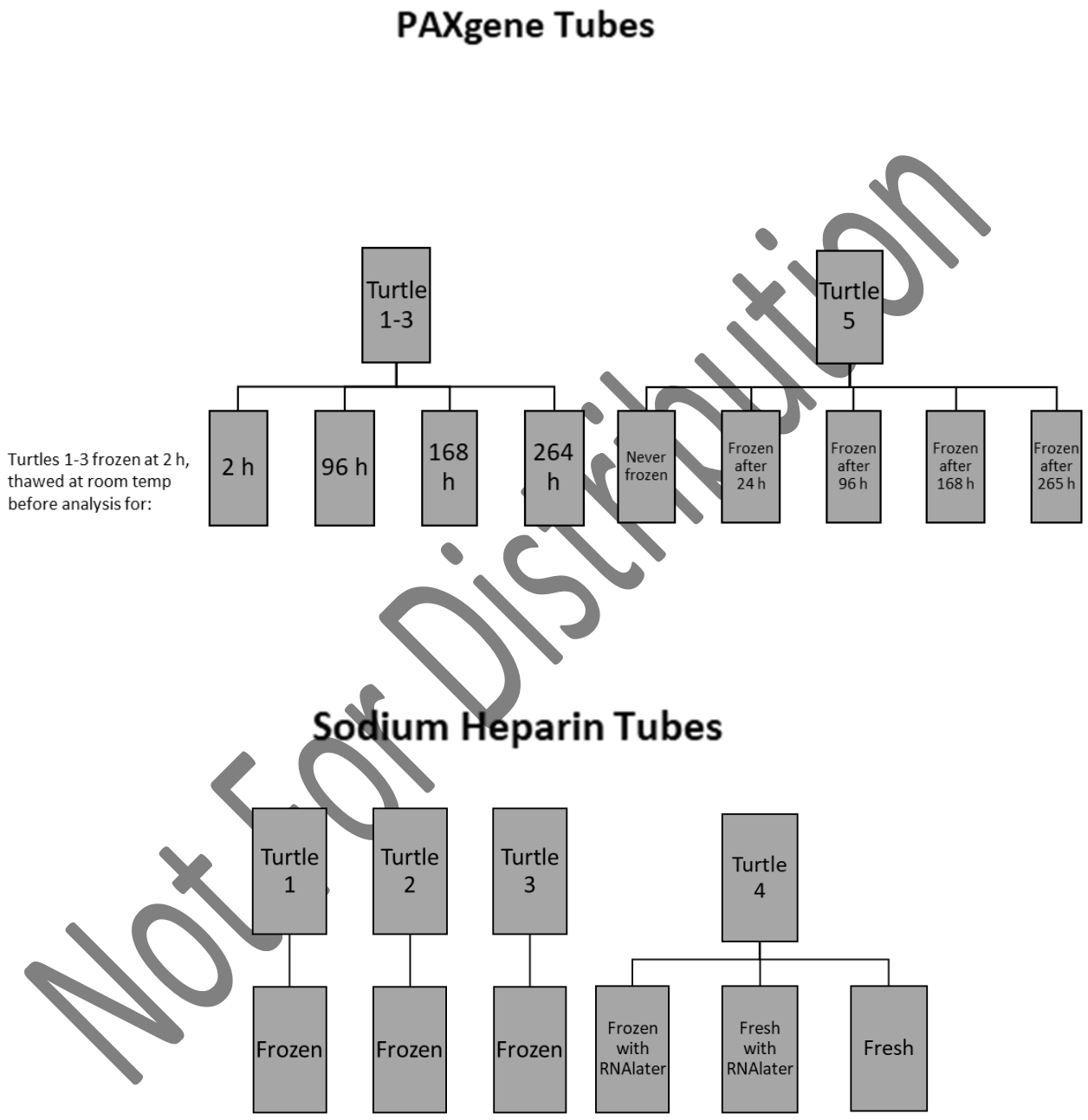

Figure 2: List of storage conditions whole blood underwent from each turtle. PAXgene tubes were treated to different freeze/thaw times while sodium heparin tubes were either frozen once, frozen with an RNA preservative (RNAlater), fresh with an RNA preservative, or fresh.

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