DESAL: REDUCING SALT CONTENT FOR IN-SITU AUTOMATED DNA EXTRACTIONS K. L. Craft[1], M. Hagedon, J. Tiffany, and C. Bradburne, Johns Hopkins University Applied Physics Laboratory (11100 Johns Hopkins Rd., Laurel, MD 20723) [1]Kate.Craft@jhuapl.edu

Introduction: The DESALination project tackles a challenge in medical and astrobiological life detection sample preparation: reducing salinity of salty samples to enable detection and characterization of the viruses or organisms within. High salt content can confound techniques for DNA extraction and sequencing. Clinical samples such as blood have high salinity (up to 6,000 uS/cm), and planetary samples are also expected to have high salt content (examples include soils at the recently observed near-surface briny slope linea on Mars [*1*], a salt-water plume, or the crust of an icy moon). Therefore, automated sample preparation process for detection and characterization of organisms within medical and planetary samples will require some form of desalination.

The desalination process can be completed with special care in a laboratory, but requires human handling at the macro scale. Performing the task within an automated, small chip-sized (~ 5 x 4 cm^2) device enables a great leap forward in the capabilities of doctors to diagnose viruses and sickness in patients on-site as well as in-situ testing (such as by a rover or probe) for life outside of Earth. As an example, a DNA concentration and extraction process called SCODA (Synchronous Coefficient of Drag Analysis), which applies electric fields to isolate the DNA from other inhibitors and complete the extraction, requires a salinity level of < 300uS/cm in 5mL [2]. Our microfluidics chip desalination process has the potential to achieve this level of salinity autonomously, thus solving the problem of high-salt content sample processing in extraterrestrial and biomedical samples.

Additionally, the DESAL chip will prevent environmental and human contamination, a major problem for the validity of extraterrestrial life detection and accurate biomedical diagnoses. Similarly, discovery of extraterrestrial life requires assurance that contamination is not the "new life" being detected. The DESAL chip will be self contained and automated, preventing external contamination and human contact that could compromise results.

Our group has previously performed successful DNA characterizations from salty soils and ice containing low biomass, proving the capability of this technique to desalinate samples, yet a smaller, automated system is still required for application in the field. Miniaturizing and automating the desalination system onto a microfluidics chip will allow movement of the sample through several chambers to cleanse and remove salts and inhibitors. Here we present results of the chip function through validation tests for salinity reduction and transport of DNA/cellular material.

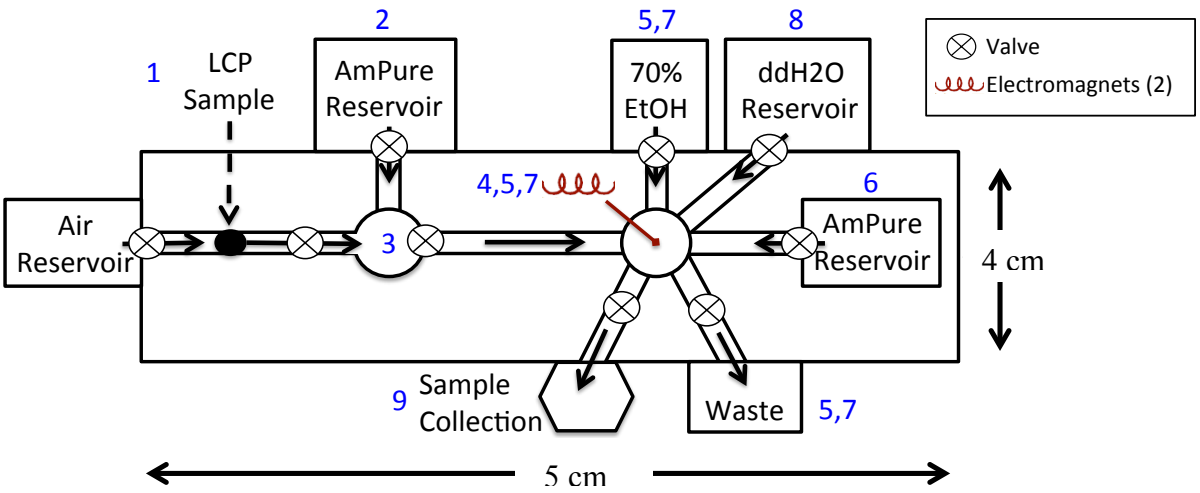
Methods:

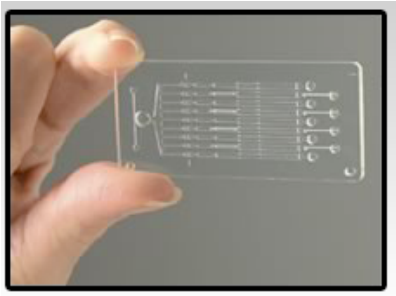
A microfluidics chip with channels that can pass the sample fluid through and remove the salts was designed and built. **Figure 1** below shows an example chip and the flow process applied. Process steps in blue: To remove salts with minimal polymer loss, the microfluidics system passes the sample (1) in solution to a chamber containing silica coated carboxyl beads (under magnets). A strongly charged solution is then added (2) which repels any similarly charged polymers (e.g. DNA), causing it to cling to the beads. Next, a magnetic field is applied that captures and immobilizes the bead/polymer complexes (3). The solution is then flushed several times (4-7), reducing salinity and inhibitors. Removal of the magnetic field then allows resuspension of the ploymers and transfer into the SCODA system for further contaminant removal and polymer concentration (8-9).

Validation tests were then performed on controls with known biomass and spiked material up to the level expected in blood for clinical samples, which is one of the worst case clinical samples for salinity. Resulting salinity levels of the samples that exited were tested and compared to the 300uS/cm in 5mL qualifying level. Biomass levels were also quantified at exit to measure the amount lost in processing.

Discussion: The miniaturization and automation of a process for removing salts from blood and environmental samples is a game-changer for performing analyses "in the field" whether standing next to a medical patient, a sick soldier on the front or attached to a planetary probe on Mars. The high salt content of samples of interest nominally prevents further amplification of the nucleic acids for determination of what organisms are present. The DESAL salt removal chip will enable, "in the room" or "in the field" clinical diagnostics and in-situ DNA extraction from planetary samples. The self-contained system will also prevent outside environmental or human contamination.

References: [1]Ojha et al., 2015, *Nature Geoscience.* [2]Boreal Genomics Aurora User Manual (BG-2002-07-004 v2.09).





http://www.pulsemdm.com/prototyping/

Figure 1. Left: Example of a microfluidics chip **Above**: DESAL microfluidics schematic showing process steps and inputs. Sodium Hypochlorite = NaClO, 70% Ethanol = EtOH, and ddH_2O is double distilled, purified water