

Supplemental Information for:

A Portable, Pressure Driven, Room Temperature Nucleic Acid Extraction and Storage System for Point of Care Molecular Diagnostics

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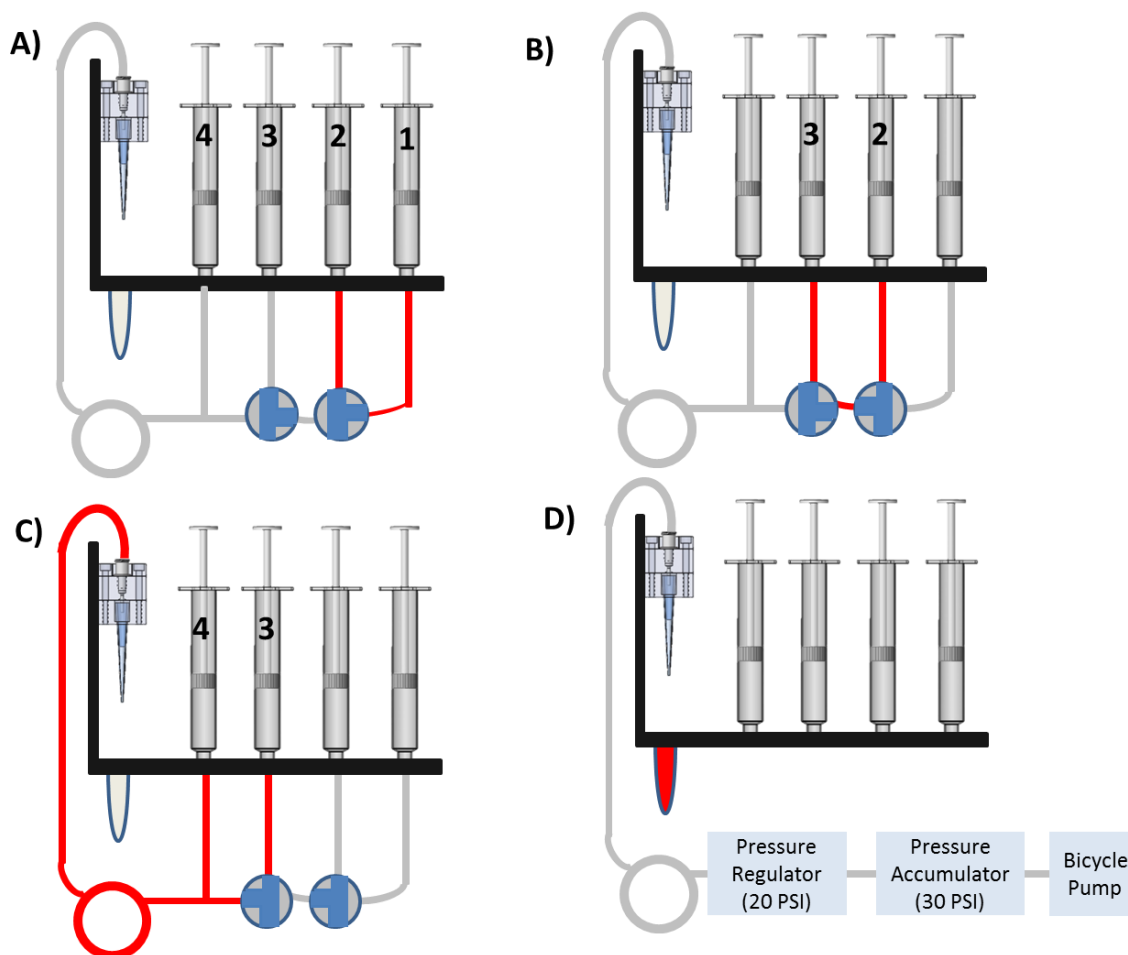


Figure S1. SNAP System Operation Schematic.

A) Sample and Reagents are loaded into 3 mL plastic syringes to allow extra space for in-syringe mixing, and connected to the SNAP system as follows.

Syringe 1) 70 μ L Blood Sample

Syringe 2) 500 μ L Boom D Lysis Buffer

Syringe 3) 550 μ L Precipitation Buffer

Syringe 4) 1 mL Wash Buffer

Valves are initially configured to connect syringes 1 and 2. Blood and lysis buffer are thoroughly mixed by iteratively dispensing each syringe into the other 10 times. Sample lysis occurs over a 15 minute incubation period.

B) With the resulting lysed sample held in syringe 2, valves are reconfigured to connect syringes two and three. Lysed sample is mixed with precipitation buffer by iteratively dispensing syringes into each other 10 times.

C) With the lysed and precipitated sample in syringe 3, valves are reconfigured to connect syringe 3 to the buffer coil. Sample solution from syringe 3 is dispensed into the coil, followed by the wash solution in syringe 4.

D) The onboard air accumulator is pressurized to 30 PSI via a standard bicycle pump connection. An in-line pressure regulator steps the effective pressure down to 20 PSI, ensuring a consistent operating pressure for the duration of the process. Air pressure is applied to the back end of the buffer coil, sequentially forcing the sample solution, the wash buffer, and pressurized air through the SPE Cartridge over the course of approximately 20 minutes.

Elution

Once detached from the SNAP system, purified RNA is eluted for the SPE cartridge by connecting a plastic syringe containing a suitable elution buffer directly to the luer connection on the top of the cartridge.

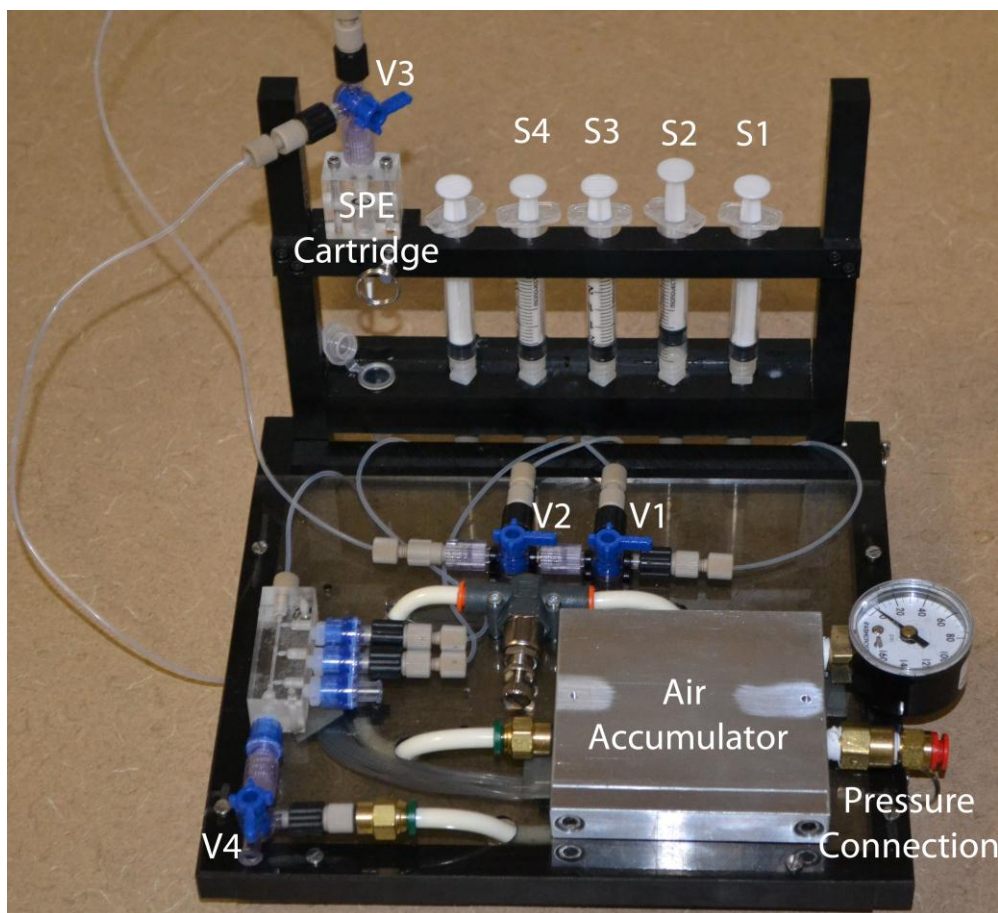


Figure S2. Close up of SNAP device with important operational components labeled. Syringes S1 through S4 are loaded with sample and reagents as previously described. Valves V1 and V2 are used to connect the sample, lysis buffer, and precipitation buffer for mixing and dispensation to the buffer coil (located on the underside of the device). Valve V3 serves as an alternate input port for the buffer coil system (for cleaning and pressure release), and valve V4 acts as a release port for excess air pressure before detachment of the SPE Cartridge. Structural components were milled from acetal homopolymer (Delrin), while the air accumulator was milled from Aluminum alloy 6061. Pressure input is achieved through an interchangeable fitting allowing for either a standard schrader valve bicycle pump connection, or a 1/4" OD push to connect fitting.

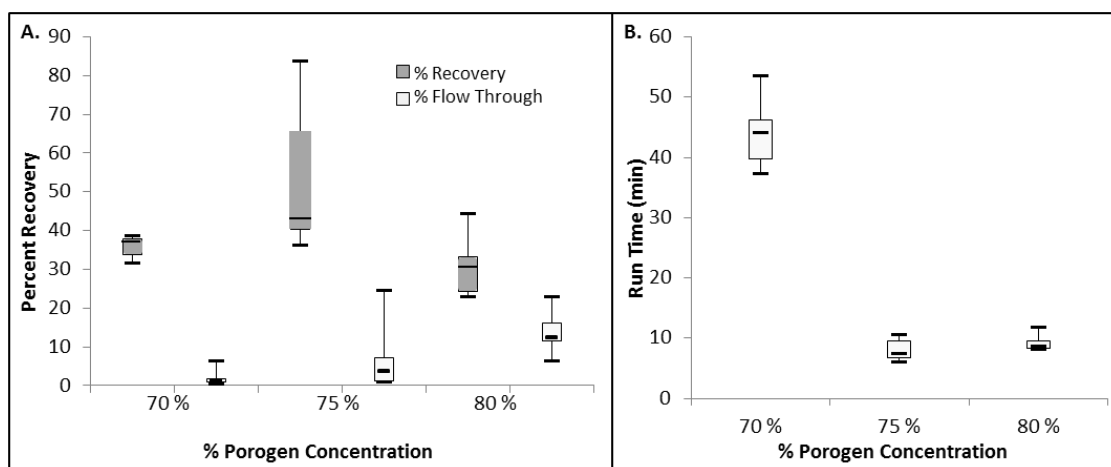


Figure S3. SPE formula optimization. There may be evidence for RNA degradation because combined recovery of the column and flow-through does not equal 100%. **A)** Extraction and flow-through yields for three monomer/porogen ratios at 50 psi (n=6). A significant increase in uncaptured RNA was observed with SPE columns containing porogen concentrations greater than 75%. **B)** Average extraction times for the three porogen percentages in **A** (n=6). A significant decrease in overall time was observed for porogen concentrations greater than 75%. Results indicate that SPE columns containing a 75% porogen concentration represent the optimum balance between extraction efficiency and assay runtime.

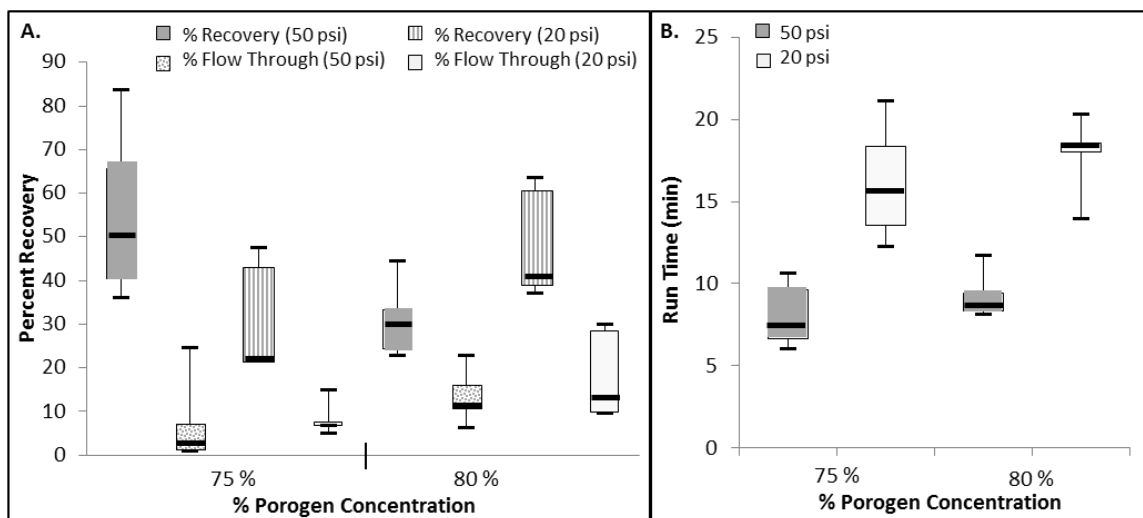


Figure S4. Operating Pressure Optimization. **A)** Extraction and flow through yields for SPEs made with 75% and 80% porogen concentrations at 20 and 50 psi (n=5). For the 75% porogen concentration, there is no difference in RNA loss in the flow through at either pressure. Recovery for the 80% porogen SPE increases significantly when the pressure is decreased from 50 to 20 psi. **B)** Average extraction run-times for the 75% and 80% porogen SPEs at 20 and 50 psi (n=5).

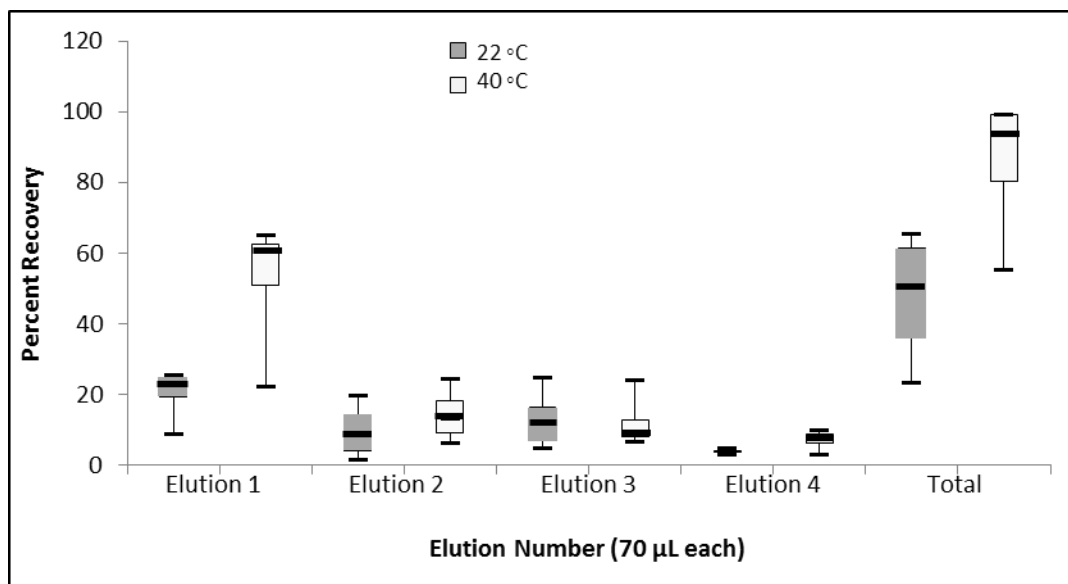


Figure S5. The elution profile for SPE Cartridges made with 75% porogen concentrations at elution temperatures of 22°C and 40°C (n=4). Four elution volume fractions were collected from each SPE. Both the first fraction recovery and the total recovery of RNA over all fractions were higher for elevated elution temperature.