Meeting #1 with Function Engineering
Time: September 4, 2019 – 10:30am
Place: MBARI--Harbor Conference Room

Overall goals—Phase 1--Preliminary Brainstorming and Conceptual Development

MBARI's immediate needs are three-fold: (a) develop a reliable Lyse and Go (LG) cartridge, (b) conceptualize methods for reducing the cost-of-manufacture for both Archival (AR) and LG cartridge types, and (c) simply the user experience, from cartridge assembly to usage to cleaning.

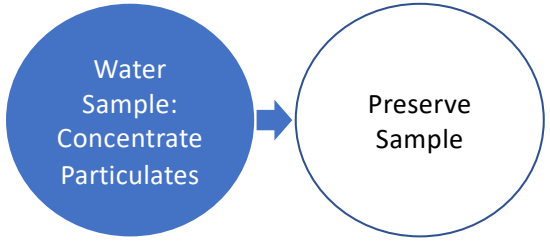
Goals of First Meeting

1. Review what we hope to accomplish through the MBARI/Function relationship
2. Transfer MBARI's AR and LG cartridge working-knowledge (e.g., parts, function, processing) to Function staff. Protocol details are included at end of this document.
3. Describe common failure points and issues with LG cartridges.
4. Provide all parts and BOM to build a LG and AR that Function staff can take away for study.
5. Provide a design envelope – defined by functional requirements, electromechanical and fluid interfaces, and metrics (see agenda)

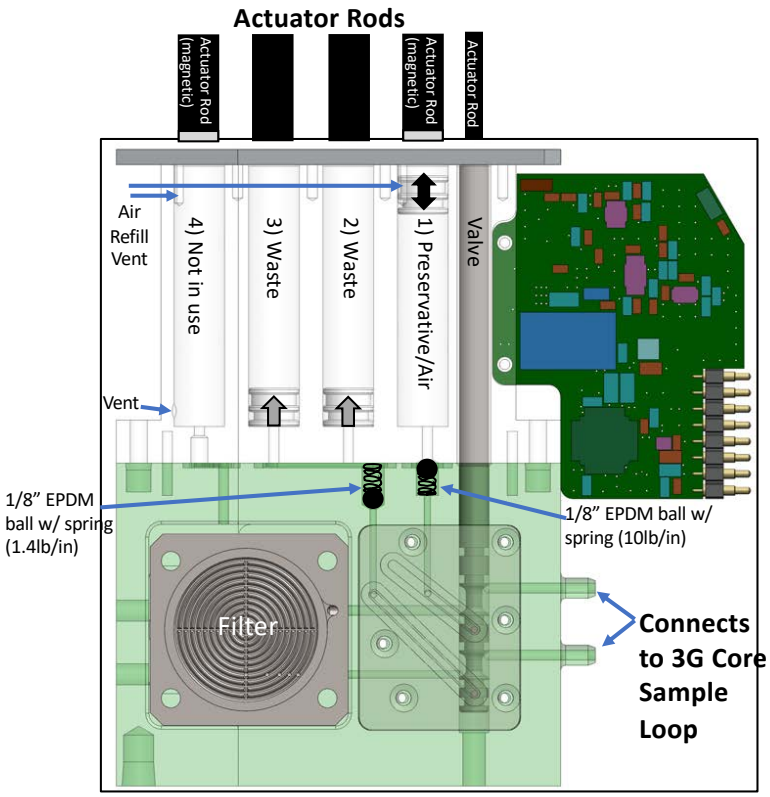
Agenda for Sep 4

1. Discuss overall goals of the project
2. Methods
    a. Review the attached protocols for both AR and LG cartridge processing.
    b. Disassemble/reassemble a cartridge and discuss cleaning requirements.
    c. Observe complete protocols for AR and LG cartridges (on benchtop machine).
    d. Identify common failure points during LG protocol.
    e. Identify common failure points during Archive protocol.
    f. Discuss material compatibility and why we are using what we are currently using
3. Next steps

Archive Process

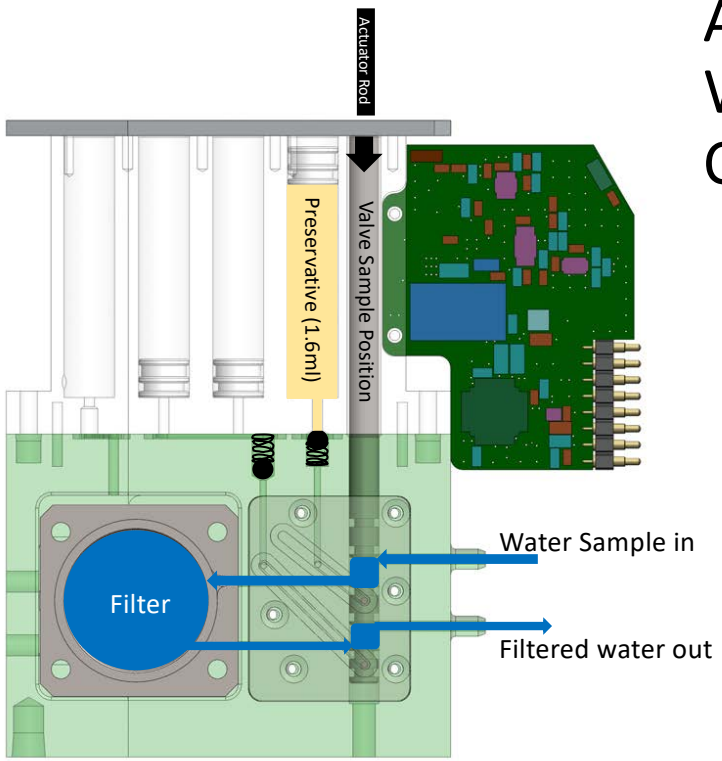


- Requirements
  - Pressure range 0-500 psi
  - Temperature 3C – 30C
  - Multiple 25 mm filter porosities/types
    - 30 µm – 0.1 µm
  - Chemicals: seawater, freshwater, 10% bleach, 10% HCl, silicone oil
  - Deliver fixative across/through whole filter (No bubbles)



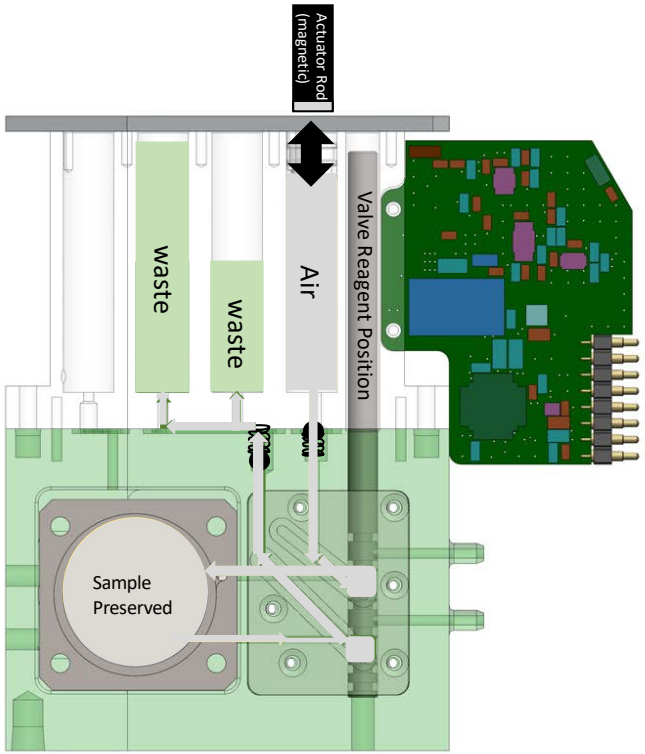
Archive Cartridge -- Features

- Connection to 3G core Sample Loop
- 5 Actuator Rods Actuate
  - 4 Syringes
  - 1 Valve (sample or reagent)
- 4 Syringes
  - Reagent/Air or waste



Archive – Water Filtration Concentrate Particulates

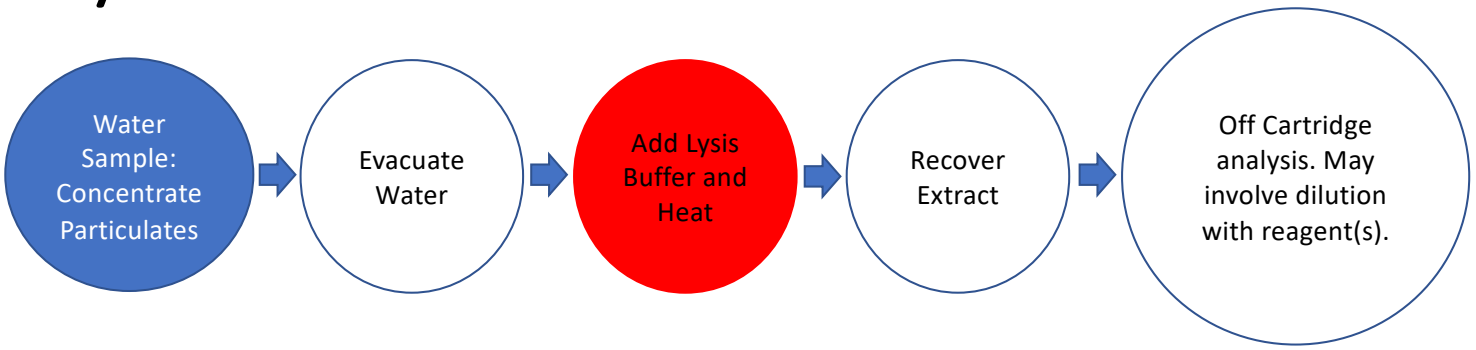
- Sampling (surface to 300m)
  - Test psi range 0-500 psi
  - Multiple 25 mm filter porosities/types
  - Sample path under vacuum in 3G/LRAUV.
  - Sample enters top of filter first before full fluid path through cartridge is opened.
- When sampling is complete move valve to reagent position.



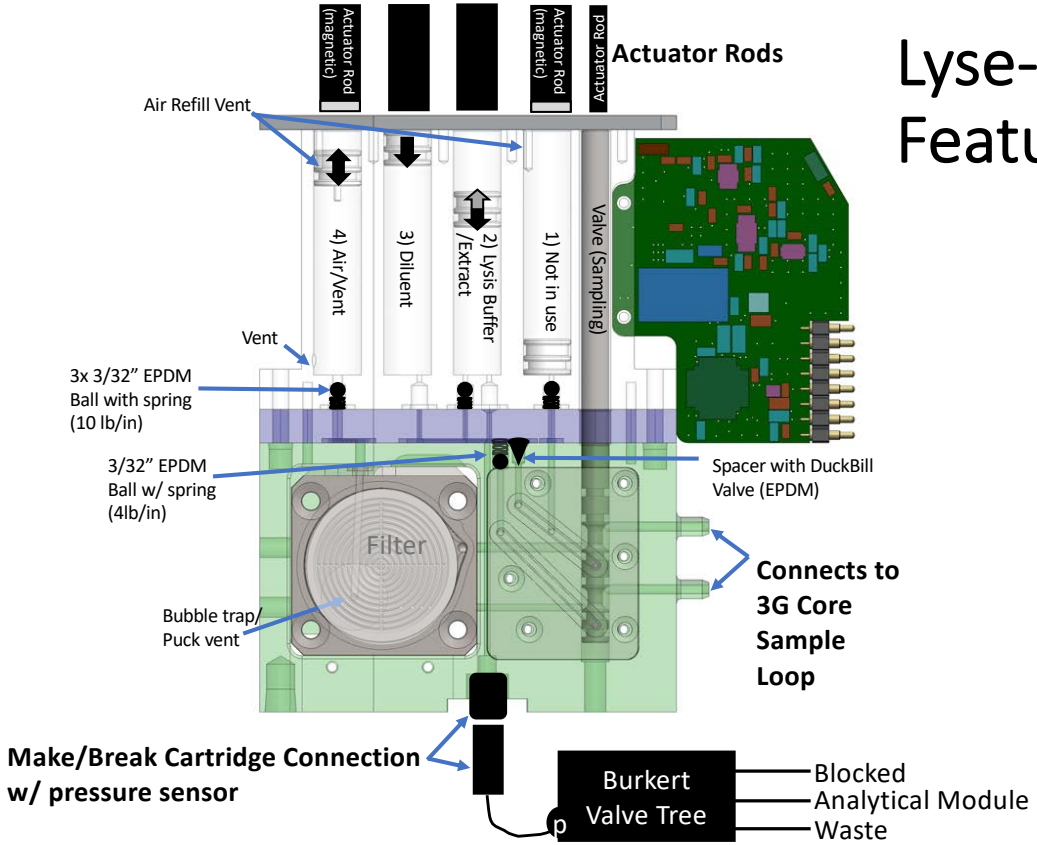
Archive – Preserve Sample

- Preservative pushed in and displaces the water to waste. Preservative reaches >95% concentration.
- Incubate
- Refill Syringe 1 with air and displace preservative

Lyse-n-Go Process



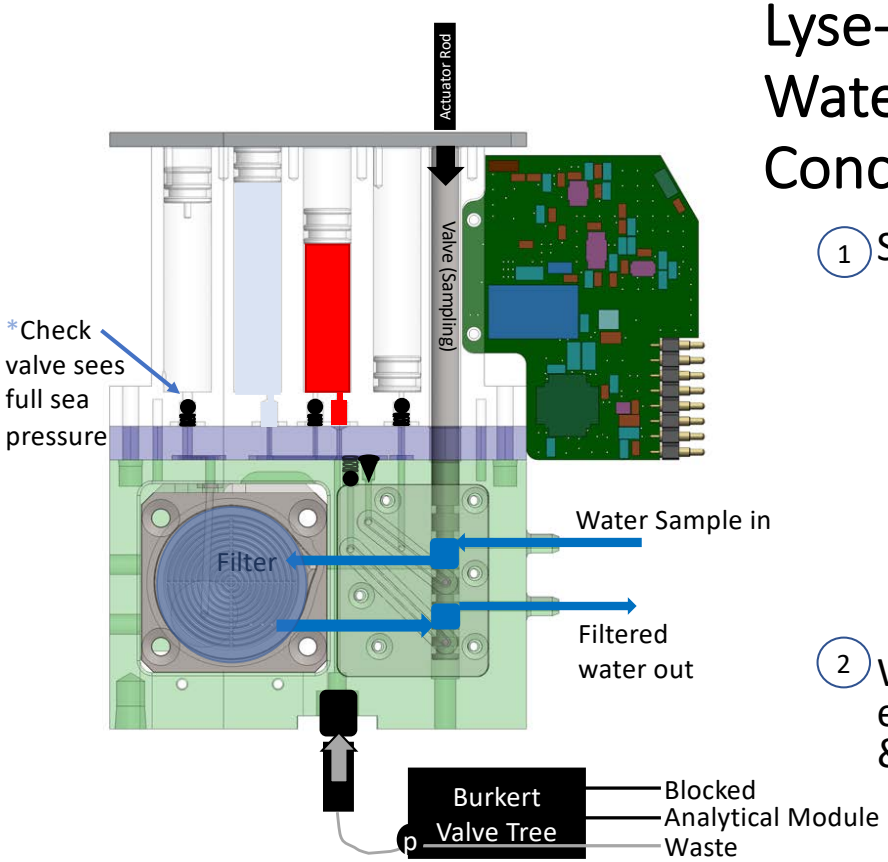
- Requirements
  - Pressure range 0-500 psi
  - Temperature 3C – 30C
  - Multiple 25 mm filter porosities/types
    - 30 µm – 0.1 µm
  - Chemicals: seawater, freshwater, 10% bleach, 10% HCl, silicone oil
  - Deliver lysis buffer and recover cell lysate.
  - Perform dilution
  - Deliver a bubble free extract off cartridge for analysis.



Lyse-n-Go Cartridge -- Features

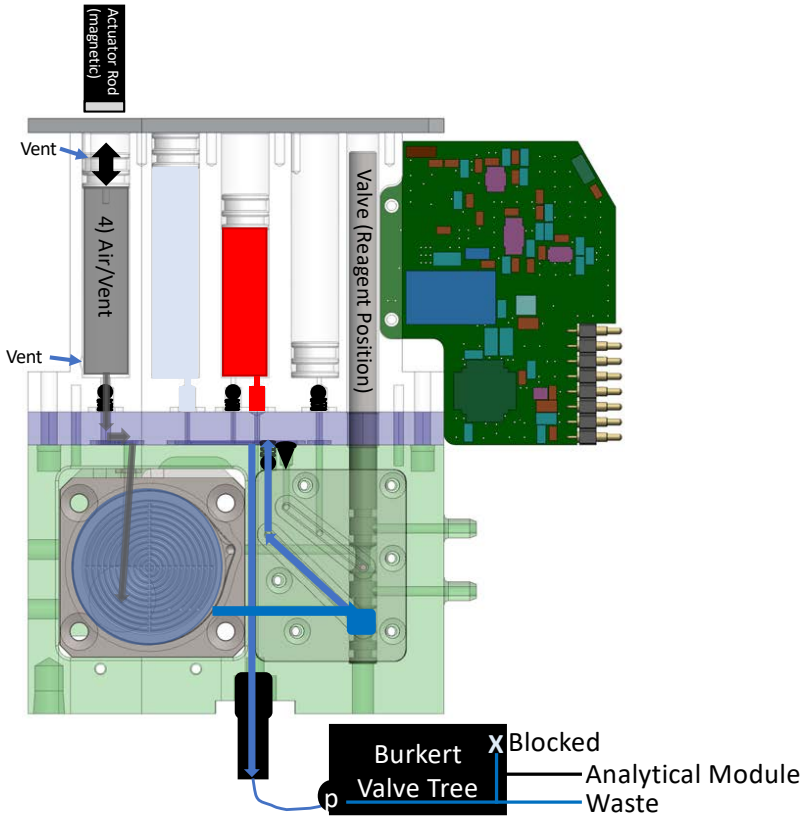
- Connection to Sample Loop
- 5 Actuator Rods
- 4 Syringes
- 1 Valve (sample or reagent)
- Make/Break cartridge connection to downstream components (waste, analytical module)

Lyse-n-Go Cartridge – Water Filtration Concentrate Particulates



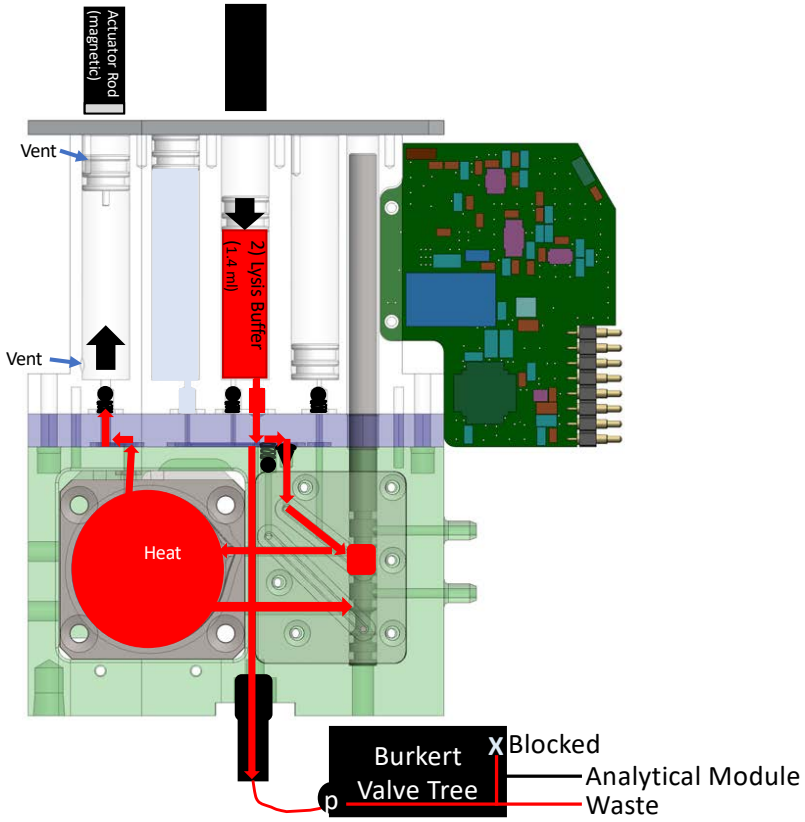
1. Sampling (surface to 300m)
   - Test psi range 0-500 psi
   - Multiple 25 mm filter porosities/types
   - Sample path under vacuum in 3G/LRAUV.
   - Sample enters top of filter first before full fluid path through cartridge is opened.

2. When sampling is complete engage handoff (open to waste) & move valve to reagent position.



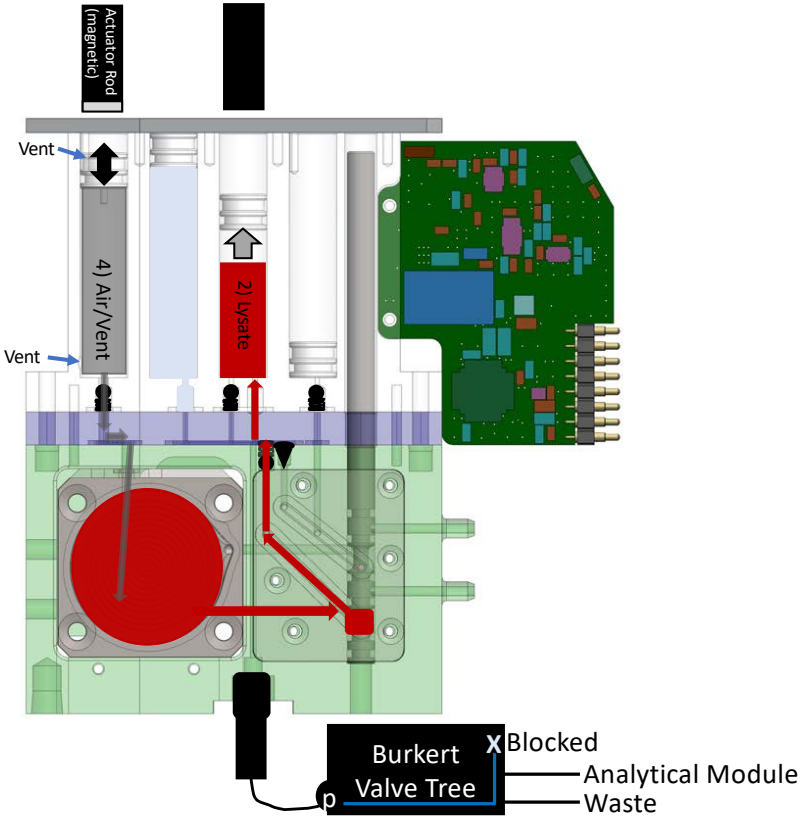
Lyse-n-Go Cartridge – Evacuate Water

3. Refillable air/vent syringe (4) used to push water to waste
   - All other plungers in syringes backed up with actuator rods
4. To remove remainder of water, must break bubble point of the filter.
   - Burkert tree at blocked
   - Repeated cycles with air/vent syringe (4) until reach specified psi
5. Drain Water to waste
6. Vent top of puck
   - Plunger below bottom vent & boss of plunger displaces check valve



Lyse-n-Go Cartridge – Add Lysis Buffer & Heat

7. Fill off cartridge line with lysis buffer (300 µl)
   - Puck top vented, open to waste
8. Fill majority of filter chamber with lysis buffer (syringe 2)
   - Puck top Vented (syringe 4)
   - Burkert tree at blocked
9. Pressurize filter chamber & Heat
   - Burkert tree at blocked
   - Puck top sealed. Plunger moved above vent with pressure balanced move with Syringe 4 & Syringe 2.
   - Drive syringe 2 to empty.
   - No bubble over filter
   - Heat to 85°C, 14 minutes



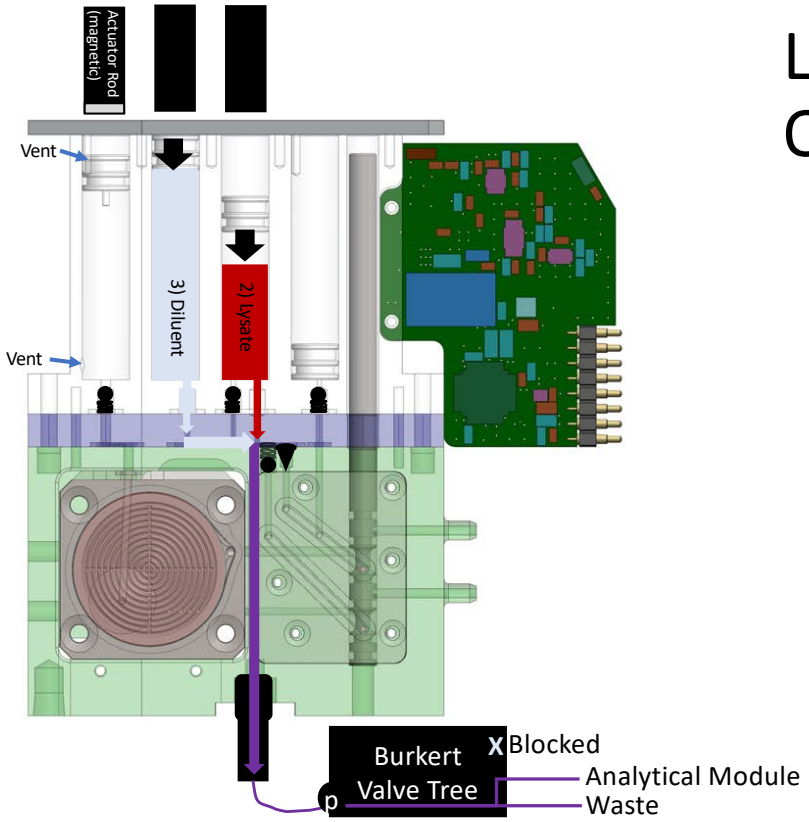
Lyse-n-Go Cartridge – Recover Extract

10 Recover 900 µl of Extract

- Pressure filter chamber with Air/Vent Syringe
- Draw back on actuator rod (2) when pressure exceeds threshold. Plunger moves due to pressure.

11 Relieve pressure in system

- Open to Waste
- Vent Puck Top



Lyse-n-Go Cartridge – Off Cartridge Analysis

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- Syringes 2 & 3 push at different rates to “mix” reagents at appropriate dilution
  - Initial mixture pushed to waste before pushing to analytical module