

1. Please clarify the overall throughput requirements for ASAP:

- Should the system be designed to meet throughput (phase 1 = 32 samples in 8hrs, phase II = 80 samples in 8hrs) at steady state?
  - PNNL Response: Not sure what is meant by this question.
- Or should it be designed to complete the entire workflow on all samples (from beginning of sieving on first sample to completion of solid phase extraction on the last sample) in 8 hours?
  - PNNL Response: Not sure what is meant by this question, but the 8 hours does not include sieving. Sieving is done by hand prior to being loaded on the system. Samples can be interleaved to maximize efficiency in the smallest footprint. The idea is that samples begin being loaded at the start of the 8 hours and they can be loaded over time either after prior samples have completed or they can load after prior samples go on to subsequent stages. Multiple samples could be loaded at the same time and processed in parallel, etc. All samples should be completed by the end of the day so they can be loaded on the Mass Spec.
  - If so, this will increase the amount of equipment required, resulting in increased cost and floor space needed.
  - If so, please provide the timing assumptions that we should use to calculate throughput and equipment selection/quantities.
    - Sample preparation time ahead of aliquot and weigh step per soil sample
      - PNNL Response: Sample Prep Time ahead of aliquoting: Core cutting, core sectioning, sieving, tube labelling, etc. should take about an hour. Note that these steps are manual and can be accomplished before the “8 hour workday” with regards to the throughput.
    - Solid phase extraction time per filtered extractant sample using current manual process
      - PNNL Response: SPE time per filtered sample using current manual process: done at scale ~20 samples/ 1.5 hours (samples are run in parallel)
  - Please confirm that the time associated with mass spectrometry read and data analysis are not to be considered when calculating throughput.
    - PNNL Response: Throughput is only to produce the soil aliquots for other testing and the final extracted samples in MicroSolv tubes ready to be loaded onto mass spec.

2. Please clarify soil sample introduction requirements:

- Are all soil samples (phase 1 = 32 samples, phase II = 80 samples) to be introduced to the process (i.e begin sieving) at the beginning of the day?
  - PNNL Response: No (see below).
  - If so, this will increase the amount of equipment required, resulting in increased costs and floor space needed.
- Or will soil samples be introduced gradually throughout the day?
  - If so, please provide the soil sample introduction schedule/rate that we should use to calculate throughput and equipment selection/quantities

- PNNL Response: Sample cores will be received into the lab all at once and to be processed same day for biotic measurements. Cores are generally processed one at a time (approximately 1 hour processing time) with all cores being stored at 4C until their turn to be processed.
3. Please clarify the sample agitation requirements for solvent extraction:
- From SOW: *Gently rock or rotate the tubes to mix the soil and solvent for 1 hour.*
  - Can you describe the ideal motion profiles required for this step? For example:
    - At what rate does it need to be rocked? e.g 180 degrees of motion in 1 second.
    - When rotating, does the sample need to be completely inverted?
    - PNNL Response: Currently: sample lie on their sides on an orbital shaker. Speed is 150 rpm, throw (orbital radius) is typically 5 cm, but it varies by shaker and isn't critical.
    - PNNL Response: The idea is that PNNL doesn't want the sample to stay in a static, upright position and have soil pellet down to the bottom. We want soil to fully contact with solvent.
    - PNNL Response: The model of the typical shaker is: New Brunswick Innova 2180 Orbital Shaker
    - PNNL Response: Tilting is an acceptable option instead of orbital shaking, and they do not need to be fully inverted. Use the guidance above to determine angle and speed. Not vigorous, but enough to ensure good soil-solvent contact.
4. Is it acceptable or desirable to provide software toolsets that empower PNNL to develop their own solutions, such as LIMS integrations, samples level tracking, and custom user interfaces, rather than deployment of a turn-key solution by the vendor? Some clients find benefits in being able to easily re-configure and maintain solutions themselves, rather than constantly requiring the involvement of a third-party vendor.
- a. PNNL Response: We would like the solution to be ready to run, but with the access and training so that we can handle the process validation changes and future improvements on our own as much as possible. In other words, we don't want a blank slate that we have to program before we can use, but we also don't want a black box that prevents us from making changes.
5. Please confirm which data connections must be encrypted? Does that apply to connections within the private network?
- a. PNNL Response: Our assumption is that the system and all software live in our private network, so nothing needs to be encrypted. If software needs to be hosted outside of PNNL, we would need to look into what security steps are required.
6. The endpoint for this automated assay appears to be FTICR mass spectrometry. Should the mass spectrometry device and associated results be managed by the ASAP system? Or is that considered out of scope for this project? Although physical integration of mass spectrometry devices is typically difficult, we have software solutions that might enable to software integration.

- a. PNNL Response: Scope is limited to producing microsolv vials ready to be loaded by hand onto the mass spec. No need to integrate hardware-wise or software-wise at this time.
7. When performing solid-phase extraction, there are a few different ways to move the mobile phase through the solid phase. Some examples are negative pressure (vacuum), positive pressure, and centrifugation. Do you have a preferred technique? Or are you open to recommendations on techniques that facilitate automation? Please note that these would be recommendations, not guarantees.
- a. PNNL Response: PNNL doesn't have a preference and are open recommendations as to whichever is most automation friendly.
8. Can you please confirm that there is no requirement for automated storage below +4degC?
- a. PNNL Response: Confirmed.
9. Does an unguarded, collaborative robot meet ASAP safety requirements? Collaborative robots meet industry standards for safety without guarding and largely improve user experience when interacting with work cells. Or do all work cells need to be fully enclosed to prevent operators from hazards regardless of this?
- a. PNNL Response: "Collaborative robotics are acceptable for use provided they have warning stickers to identify pinch points and are listed by an OSHA approved NRTL. Electrical equipment that is not listed by an OSHA approved NRTL (Nationally Recognized Testing Laboratory - e.g. UL, CSA, TUV) must be inspected by an approved third party evaluator either prior to arrival or upon receipt, prior to use.  
<https://lni.wa.gov/licensing-permits/electrical/electrical-installation-information/electrical-product-testing-and-engineering-evaluation#accredited-product-testing-laboratories>"