Large Area Surface Sampling for Contamination Control of Critical Environments using the Concentrating Pipette

Application Note

Revision A*

Introduction:

Traditional surface sampling methods provide notoriously poor results. Common surface sampling swabs limit users to very small surface areas and collection efficiency is often poor. Most swab kits are made for culture-based analysis requiring incubation of several hours or days and many pathogens of interest can be viable, though not culturable. Recovery of a surface sample from a swab for rapid molecular methods requires additional fluid for rinsing that often dilutes the target beyond the limit of detection of the assay.

NASA's Jet Propulsion Laboratory, California Institute of Technology, Biotechnology and Planetary Protection Group, Pasadena, CA, USA developed the following large area surface sampling method, for sampling bacteria and fungi on the International Space Station's surfaces. This new method uses the InnovaPrep Concentrating Pipette to concentrate the final sample into sub-milliliter volumes (see referenced publications below).

> "Culture-based analysis limits understanding of the diversity of microbes that grow and thrive on surfaces because only a small fraction of organisms in a given environment can be cultured under standard laboratory conditions. Molecular methods, such as quantitative polymerase chain reaction (qPCR) and targeted amplicon sequencing, which can identify and quantify both culturable and unculturable organisms provide a more thorough assessment of what is actually present and in what amounts." - NASA

The InnovaPrep Concentrating Pipette System quickly concentrates microorganisms, achieving up to 10,000 fold concentration from liquid samples. The system filters the sample and then employs a Wet Foam Elution method to quickly recover the microorganisms into a much smaller volume. The system's ease of use and ability to deliver exceptionally high concentration factors make it an ideal approach for rapid analysis of large area surface samples.

The Concentrating Pipette System is appropriate for bacteria, molds, spores, and viruses. The method is suitable for a variety of applications including food safety, outbreak investigations, disease monitoring including hospital-acquired infections, animal health, and cleanroom monitoring.

The method uses standard sterile wetted polyester wipes to sample large area surfaces or objects up to one square meter. The wipes are placed in a bottle with 200 ml of buffer, shaken or vortexed, and then the fluid is concentrated (in minutes) from 200 mL to a final volume of approximately 250 µL using the Concentrating Pipette. The concentration factor achieved using this procedure is approximately 800X, multiplied by the efficiency (usually 50-90%).

Materials Required:

- **Instrument: CP Select (InnovaPrep)**
- **Consumables:**

CP Select Consumables (choose from the following):

- **Elution Buffer:**
  - **Tris** Item # HC08001 (for molecular methods)
  - **PBS** Item # HC08000 (for culture)
- **Concentrating Pipette Tips (CPTs):** (see Consumable Selection Guide for more information)
  - **Ultrafilter CPTs** – Item # CC08003-10
  - **0.05 µm CPTs** – Item # CC08020-10
  - **0.2 µm CPTs** – Item # CC08022-10
- **CP Storage fluid** – item # HC08558 for system decontamination

- **Tween 20 -** for sample addition
- **Sterile polyester wipes**
  - Available in both dry and sterile formats - See examples below from Texwipe
  - STX1709 9 x 9" sterile dry Revolve series
  - STX1712 12 x 12" sterile dry Revolve series
  - STX1709P 9 x 9" sterile, pre-wetted, 70% IPA Revolve series
  - STX1712P 12 x 12" sterile, pre-wetted, 70% IPA Revolve series
  - TX3225 12 x 12" sterile dry Textra 10
- Sterilized forceps (optional)
- **500 mL Sample Bottle** – for sample recovery
- **Sterile phosphate-buffered saline** - (PBS; pH 7.4) – 200 mL per wipe for elution

If using dry wipes, additional materials are required:

- **Sterile molecular-grade water**
- **Sealable zip lock style plastic bags**
  - Recommend quart size to accommodate wipes and buffer.

Analysis Methods Compatibility for Viruses using Wet Foam Elution™

The Wet Foam Elution™ technology was developed as a front-end to virtually any analysis method, whether modern molecular methods or classical culture. There is a caveat, however, regarding viruses.

The foaming agent, Tween 20, a component in the FluidPrep elution buffer, has a different effect depending on whether the virus is enveloped or non-enveloped. Non-enveloped viruses are surrounded by a protein coating, commonly referred to as a capsid. The capsid is quite robust and not easily damaged by the Tween, therefore both molecular and plaque assays are compatible for non-enveloped viruses. However, the Tween 20 may affect host mammalian cells used for plaque assays or TCID50 assays, so a dilution of the concentrate might be necessary.

Studies have indicated that the addition of 0.5% Tween 20 can improve recovery of viruses using Wet Foam Elution and that up to 0.05% is not likely to disrupt the virus. Enveloped viruses also have a protein coat, but this capsid is encased within an outer lipid membrane. Although effects can vary widely depending on virus, it is possible that Tween 20 can disrupt the viral envelope, or prevent the virus from attaching to, and entering host cells. As a result, in some cases only molecular assays are compatible for enveloped viruses.

SAFETY:

Due to the potential presence of infectious pathogens in samples, users should work with their organization's occupational safety team to ensure that methods and safety measures are appropriate and approved. Unless working with samples known to be non-infectious, InnovaPrep recommends that CP Select operations be performed in a biosafety cabinet.

STEP 1 - Prepare Sample Wipes (Skip this step if using prewetted wipes and proceed to Step #2)

STEP 1 - Prepare the Sample Wipes (if using pre-wetted, skip to Step 2)

- Select preferred dry wipe and fold in half twice.
- Soak each wipe in 15 mL of sterile molecular grade water for 30 min.

  Note: The addition of 0.05% Tween 20 to the "soak water" and/or the "sample collection buffer" may provide improved recovery of SARS-CoV-2 and other target organisms.
- Transfer each wipe to a separate sterile, sealable plastic bag.

STEP 2 - Sampling Method

- Using gloved hands, or sterilized forceps, remove one of the pre-soaked wipes from the packaging or plastic bag.
- Use the wipe to sample surfaces up to one square meter, including knobs, handles, crevices, or items (plastics, glass, metals, smooth wood, etc.).
- After sample collection, transfer the wipe to a 500 mL bottle containing 200 mL of sterile phosphate-buffered saline (PBS; pH 7.4).

STEP 3 - Sample Processing

- Shake or vortex the bottle with the wipe for 2 minutes.
- Concentrate the liquid sample using the InnovaPrep CP Select™ following the menu recommendations in the user guide.

Step 4 – Sample Analysis

- Samples are suitable for a variety of culture and rapid methods, including digital PCR/qPCR, digital RT-PCR/RT-qPCR, NGS, etc. The samples concentrated by the CPT are now ready for analysis and any further downstream processing steps.
- Store pre-concentrated or post-concentrated liquid samples at 4 °C.

Notes:

Concentrating Pipette Tips come in a variety of pore sizes. Users can access the Consumable Selection Guide on the InnovaPrep website to determine which tip will be optimal for their target of interest and application.

The final eluate can be analyzed using classical culture or rapid molecular methods of your choice.

Pooling and concentrating multiple samples into a single concentrated sample can be useful for certain applications, such as cleanroom monitoring, as it can lower analysis costs and improve method sensitivity. When using this approach, it is important to note that identification of the exact location of a positive result may require additional sampling or analysis of reserved aliquots.

*Please check our website for the most current methodology as updates are published periodically.

References:

2022 - Genomic Characterization of Parengyodontium torokii sp. nov., a Biofilm-Forming Fungus Isolated from Mars 2020 Assembly Facility

2022 - Microbial Burden Estimation of Food Items, Built Environments, and the International Space Station Using FilmMedia

2022- Draft Genome Sequences of Fungi Isolated from Mars 2020 Spacecraft Assembly Facilities

2022 - The Isolation and Characterization of Rare Mycobiome Associated With Spacecraft Assembly Cleanrooms

2021 - Clean room microbiome complexity impacts planetary protection bioburden

2020 - Description of Chloramphenicol Resistant Kineococcus Rubinsiae sp. nov. Isolated from a Spacecraft Assembly Facility

2020 - Crewmember microbiome may influence microbial composition of ISS habitable surfaces

2020 - Characterization of Spacesuit Associated Microbial Communities and their Implications for NASA Missions

2019 - Characterization of the Total and Viable Bacterial and Fungal Communities Associated with the International Space Station Surfaces

2017 - Human presence impacts fungal diversity of inflated lunar/Mars analog habitat

2016 - Characterization of Aspergillus Fumigatus Isolates from Air and Surfaces of the International Space Station

2016 - Microbial succession in an inflated lunar/Mars analog habitat during a 30-day human occupation