International Space Station Smart Sample Concentrator for Microbial Monitoring of Potable Water

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Potable water produced by the International Space Station (ISS) Water Processor Assembly requires routine microbial monitoring to ensure crew safety. Existing methods are culture-based and present significant operational challenges in micro-gravity, as well as a potential health risk in the enclosed ISS environment. Fortuitously, molecular detection technologies have come of age in the last two decades and now provide the potential for overcoming these challenges. However, despite the theoretical ability to detect single bacteria, small assay volumes preclude the use of molecular detection technologies for monitoring drinking water without a concentration step. Molecular detection technologies routinely use only a few microliters of sample per reaction, therefor water samples must be concentrated by three orders of magnitude to approach the necessary detection limits. In response to this need, InnovaPrep LLC, of Drexel, MO has worked under Phase I and II NASA SBIR projects to develop the ISS Smart Sample Concentrator (iSSC). Development now continues under the Phase II-X Program with funding from the Advanced Exploration Systems' Life Support Systems Project and the NASA SBIR program. The iSSC is capable of processing one-liter water samples through a hollow fiber membrane filter concentration cell in less than ten minutes, capturing microorganisms in the fiber lumen. Following capture, the microorganisms are eluted using a novel Wet Foam Elution process in which a viscous, wet, carbonated foam is used to sweep the captured particles from the membrane surface and dispense them in a concentrated sample volume of less than 500 µL. A novel Capillary Flow Assisted Container then allows the concentrated liquid sample to be removed, while in the microgravity environment, and transferred to an assay tube for subsequent molecular detection. Physical and microbiological test results and the latest progress towards an ISS deployable instrument will be presented.

Nomenclature

BB	=	Bead Beat lysis
BL	=	Boiled Lysis (95° C extended hold)
BOV	=	Bag-on-Valve
CFAC	=	Capillary Flow Assisted Container
CFU	=	Colony Forming Unit
CP	=	Concentrating Pipette
CPT	=	Concentrating Pipette Tip
HHE	=	Hand Held Elution Canister
ISS	=	International Space Station
iSSC	=	International Space Station Smart Sample Concentrator
JPL	=	Jet Propulsion Laboratory
LLOQ	=	Lower Limit of Quantification

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NASA = National Aeronautics and Space Administration
ND = Non-Detect
NTC = No Template Control
qPCR = quantitative Polymerase Chain Reaction
SBIR = Small Business Innovation Research

I. Introduction

Providing a safe and reliable supply of drinking water to the International Space Station crew, and other present and future spacecraft crew, is of the highest importance. Systems are now available for efficient recovery and purification of water – including the current ISS Water Recovery System – and improved systems are under development. Regardless of the design or principal of operation, all water treatment systems are susceptible to microbial contamination due to release of particles from biofilms or component failures.[1,2] Therefore, microbial testing is required to ensure the safety of water produced with these systems. Because the ISS Water Recovery System is used for treatment of wastewater and condensate it is a highly complex system with an increased potential for failure or growth of biofilms. As reported by Shkedi et al., there have been multiple instances of microbial contamination or significant biofilm growth that have occurred in the ISS water system.[3] Further, Yamaguchi et al. noted that crewed habitats in space can result in altered human-microbe relationships and lead to increased pathogenicity and virulence of microbes.[4] The potential for water treatment system failures, coupled with the potentially high penalty of sickness to deployed space crew, creates a significant need for a rapid way to determine when these failures occur.

Significant time to detection, labor requirements, and biosafety concerns, related to NASA's current microbial monitoring methods, led to a recommendation to implement rapid molecular-based technologies such as real-time polymerase chain reaction (PCR).[5] Lower limit of detection of 50 to 400 cells per reaction were demonstrated for commercially available real-time PCR systems evaluated for potential use aboard ISS by Oubre et al. However, even for the best performing of these systems, small reaction volumes result in a lower limit of detection of 500 CFU/mL, at best, or an order of magnitude greater than the 50 CFU/mL maximum total bacteria concentration specified in the International Space Station Medical Operations Requirements Document (ISS MORD).[6] Further, the ISS MORD specifies that coliform bacteria must be non-detectable in 100 mL of drinking water. Thus, a lower limit of detection of 1 CFU/100 mL, or 50,000 times lower than currently achievable by qPCR, must be attained in order to test to this requirement.

Several factors have led to current rapid microbiological detection methods using small – generally less than 100 µL – reaction volumes, including the high cost of reagents and the inherit difficulty with quickly and efficiently thermo-cycling larger volumes. Due to these small qPCR analysis volumes, a method of mechanically concentrating pathogens from large water samples into volumes closely matching the reaction volume is necessary to achieve the required lower limit of detection.

InnovaPrep LLC (Drexel, MO) has been awarded Phase I and Phase II NASA Small Business Inovation Research (SBIR) grant awards, and more recently Phase II Expanded and Phase III SBIR awards, in an effort to address this need through development of a novel system for rapid, mechanical concentration of microorganisms. The International Space Station Smart Sample Concentrator (iSSC) (Figure 1) is based on underlying technologies of the commercially available InnovaPrep Concentrating Pipette Select instrument, with improvements to enable the concentration process to be performed in microgravity environments. The iSSC is a prototype system that will be used to verify the efficacy of the concentration approach in the ISS environment. Following successful ISS flight demonstration, a fully automated system will be developed for integration into water recovery systems, including the current ISS Water Processor Assembly. In this way, the iSSC can be used along with qPCR, or other rapid microbiological methods, to enable simple and rapid microbial monitoring of drinking water during human space missions.

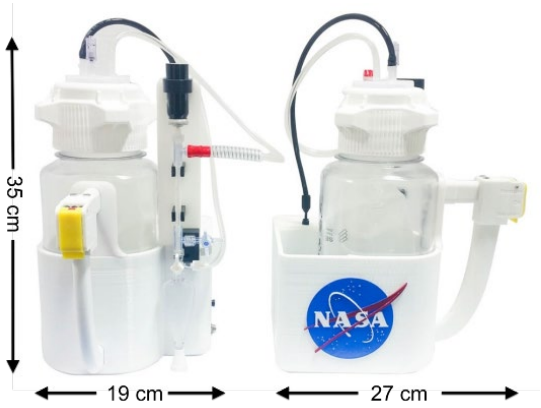

Figure 1. International Space Station Smart Sample Concentrator (iSSC)

II. System Description

The iSSC relies on technologies developed by InnovaPrep for earth-based microbial concentration from large volumes of aqueous samples. The technologies were commercialized as the Concentrating Pipette instrument (CP-150 and CP Select, Figure 2) and Concentrating Pipette Tips (CPTs), and to date over 150 instruments have been sold. While the early success of the CP instrument has demonstrated its usefulness on earth, significant innovation is required to enable its use in microgravity environments.

Figure 2. Concentrating Pipette Select

The CP uses flat membrane filter or hollow fiber membrane filter CPTs to capture microbes from up to 5 liters of sample. Following capture, the microbes are eluted from the CPT using InnovaPrep's Wet Foam Elution process. A buffered elution fluid, containing Tween 20 as a foaming agent, is stored under a nominal carbon dioxide head pressure of 120 psi in small aerosol canisters. During the elution process, the elution fluid is released through a timed valve to atmospheric pressure, causing carbon dioxide to come out of solution and form microbubbles in the solution. Because nearly 6 volumes of carbon dioxide are contained in each volume of the elution fluid an expanded wet foam is produced. The viscous wet foam is approximately 6-times the volume of the starting fluid. As the foam is released gas pressure pushed the foam tangentially down the retentate side of the membrane – recovering the captured microbes into a small volume of foam. The foam then catastrophically fails and breaks down into a small, concentrated liquid sample of 0.2 to 0.5 mL in volume.

The CP concentration process, and especially the wet foam elution process, create unique challenges that must be overcome to enable use in the ISS microgravity environment. In contrast to the CP operational environment, the microgravity environment requires that all fluids be contained and under full control at all times. This includes the starting water sample, the elution fluid and wet foam, as well as the final concentrated sample. Additionally, in microgravity, gas and gas bubbles are difficult to separate from fluids and can create issues during transfer and other processes. To enable operation of the iSSC in a microgravity environment, InnovaPrep has developed four key modifications to maintain fluid control and to separate out gas and gas bubbles, these are: (1) use of a bag for delivery of the sample to the concentrator, (2) a bladder bag contained within a vacuum reservoir for capture of the processed sample, (3) a bag-on-valve (BOV) aerosol canister for control of the elution fluid prior to release, and (4) a Capillary Flow Assisted Container (CFAC) for control of the concentrated sample. These components as well as the hollow fiber membrane filter concentration cell are identified in Figure 3 and Figure 4.

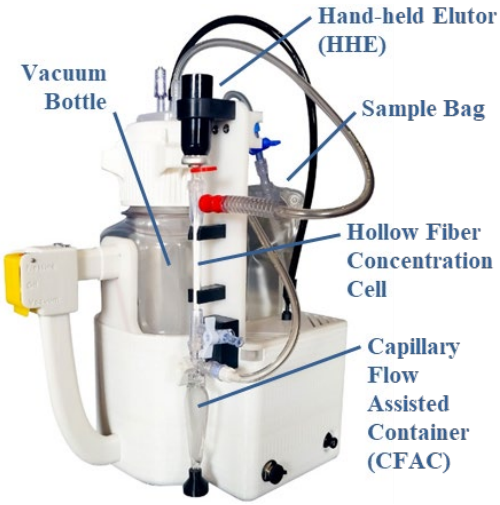

Figure 3. iSSC Components

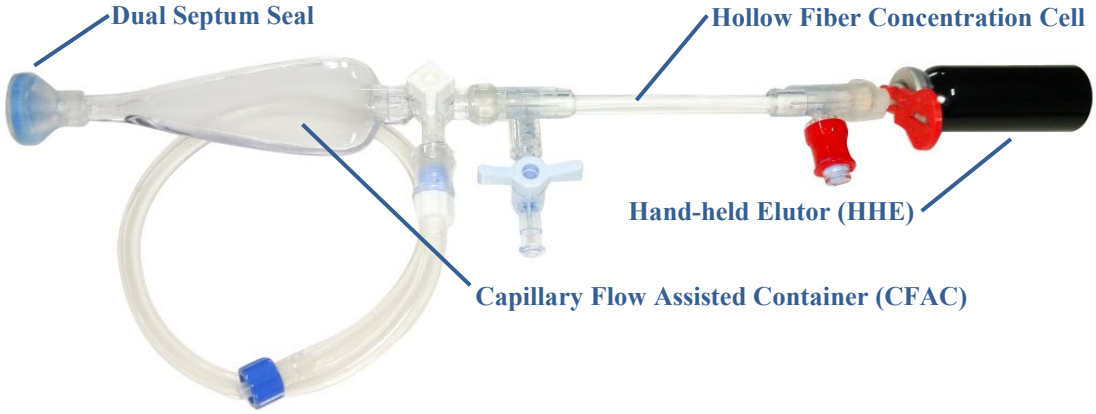

Figure 4. iSSC Consumable Assembly

The small pore size and hydrophilic nature of the hollow fiber membrane filters used in Hollow Fiber Concentration Cell causes the consumable assembly to be impermeable to gasses after it has been wetted. Therefore, to enable to iSSC system to process large water samples in a microgravity environment it is important that gasses and gas bubbles be excluded from the sample container. The sample bag allows a 1 L water sample to be loaded with a minimum of gas and gas bubbles – allowing the entire sample to be processed.

A diaphragm pump is used to draw the water sample through the Hollow Fiber Concentration Cell. In a microgravity environment it is important to maintain control of the sample during and after processing. To accomplish this the water sample is pumped indirectly by using the pump to draw negative pressure on a vacuum bottle that contains a bladder bag that the sample is drawn into. In addition to keeping the processed sample from being drawn into the pump in a microgravity environment, this approach also maintains control over the sample and after processing is complete it allows the pump to be reversed to dispense the processed sample from the container into the ISS urinal funnel for waste disposal.

During sample processing the entire 1 L water sample is drawn through the hollow fiber membrane filter Concentration Cell. More specifically, the sample fluid enters the lumens of seventy-two 0.2 μm polysulfone hollow fiber membrane filters and exits through the wall of filters and into the bladder bag. Particles larger than the filter pore size are retained in the fiber lumen. After the entire sample has been passed the particles are eluted into a small concentrated volume using the Wet Foam Elution process.

In standard earth-based use the elution fluid contained in a standard aerosol canister and fluid (containing dissolved carbon dioxide) is dispensed by inverting the canister, so that none of the carbon dioxide head space gas is released. For use in microgravity a method of controlling the fluid had to be developed. In this case, a small volume of carbonated elution fluid is stored in a set-volume silicone "bag" which is bonded to the aerosol valve and contained within the Hand-Held Elutor (HHE) aerosol canister. Larger commercially sold versions of this design are sold at Bag-on-Valve (BOV) aerosol devices. A cut-away render of the iSSC BOV is provided in Figure 5. During manufacturing gas is injected between the BOV and the inside-wall of the aerosol canister to support the pressurized bag. This method allows a small set volume of carbonated elution fluid to be stored and later released in a single burst. The elution fluid then quickly expands into a wet foam and is swept through the hollow fiber lumen to recover the captured particles.

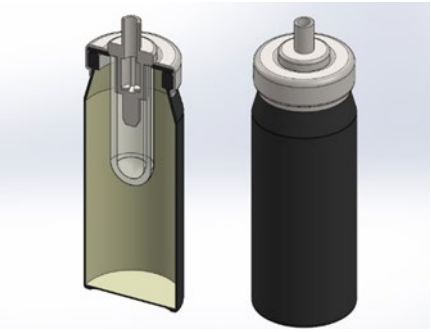

Figure 5. Hand-Held Elutor (HHE) and Bag-on-Valve (BOV) Assemblies

Figure 6. Orthographic View of the CFAC Design

Figure 7. CFAC Sample Extraction using Repeater Pipette

Prior to elution, the diaphragm pump is used to create negative pressure in the CFAC. During elution, the wet foam passes through the hollow fiber membrane filter lumens and is dispensed into the CFAC. The negative pressure acts to draw the eluate into the CFAC and also initiates rapid and complete breakdown of the foam. Specifically, the action of taking the elution fluid from a pressurized carbon dioxide environment to a low pressure first triggers release of carbon dioxide in the form of microbubbles – creating the wet foam – then film thinning, bubble coalescence, and bubble bursting create instability and finally complete failure and breakdown of the foam. In a carbonated wet foam, these mechanisms are largely not driven by gravity, and are therefore anticipated to take place as readily in microgravity as they do at 1-g.

After the foam has broken down, the CFAC design takes advantage of capillary forces to passively separate the fluid phases. The surface tension, wetting conditions, and container geometry are used to deliver the liquid to a port to be withdrawn with a Repeater pipette or other transfer device. The final concentrated sample is then removed from the CFAC using a Repeater pipette and transferred to a quantitative Polymerase Chain Reaction (qPCR) instrument or other rapid detection system for detection of the organisms of interest.

III. Results and Discussion

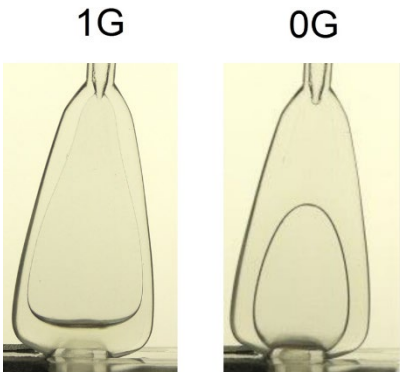

Figure 6. CFAC at 1G and 0G

To date, the iSSC has been tested to insure proper and safe operation in microgravity environments and to verify efficient and reproducible concentration of bacteria for subsequent detection by qPCR. Operational testing to verify microgravity performance has included drop tower testing of the CFAC, parabolic flight testing of the full iSSC, and, more recently, ISS flight test of the CFAC aboard overseen by the WetLab-2 team. Each test was specifically related to operation of the iSSC with the exception of the Wet Lab 2 team test, which was related to potential replacement of the current WetLab-2 debubbler with the CFAC.

CFAC drop tower testing was performed by IRPI LLC at the Dryden Drop Tower at Portland State University. Images are provided below in Figure 8 showing sample wicking to the CFAC outlet port (at top) at 0G (as well as a control at 1G). This testing demonstrated excellent wicking performance that is anticipated to provide bubble free samples at the CFAC outlet in microgravity environments.

Full operation of the iSSC was tested in micro gravity during a 30-parabola flight test performed by Zero Gravity Corporation on March 21, 2017. Specifically, this included the following iSSC operations: start-up, concentration, sample depletion, sample-bag operation, elution, and withdrawal of the sample in microgravity. Start-up and concentration were performed during the first parabola. Sample depletion and sample-bag operation were performed during the second parabola. Sample elution, including breakdown of the wet foam, was performed during the third parabola. Withdrawal of the eluted sample was performed during the fourth parabola. A photograph of iSSC operation during the parabolic test flight is provided in Figure 9.

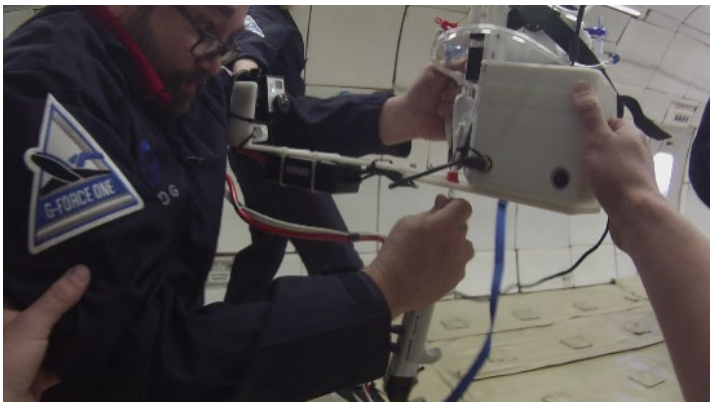

Figure 7. iSSC Operation During Parabolic Test Flight

All iSSC operations were successfully verified in a microgravity environment. Successful foam breakdown and movement of the concentrated sample to the sample-out port on the CFAC, were both observed, but were slightly slower than anticipated. This was believed to be due to poor crimps on some HHE causing some leakage of gas pressure – an issue which is currently being addressed through improvements to the crimper setup. The slower than anticipated foam breakdown and movement to the CFAC port created some difficulty during the parabolic test flight, due to the short time period of each parabola, but are not anticipated to be of concern during ISS use.

The NASA WetLab-2 test released a report on October 19, 2018 detailing testing of the InnovaPrep CFAC performed aboard the ISS.[7] A series of test runs were performed initially by drawing dye-colored buffer solutions from an air/dye-colored buffer filled CFACs and then dispensing to SmartCycler tubes. A second series of test runs was then performed in which samples of DNA solutions were drawn from air/solution filled CFACs, then dispensed to Smart Cycler tubes containing lyophilized reagents and then analyzed in the SmartCycler. The quality of the data was then judged by the smoothness of the qPCR curves and the uniformity of qPCR results from replicate tubes. A photograph is provided in below Figure 10 of the CFAC during testing aboard ISS. The WetLab-2 report notes that results of the flight testing showed that the CFAC can be used instead of the current WetLab-2 Pipette Loader and will result in lower crew time needs, lower up-mass and lower costs. Because this work was performed with buffers that do not contain a surfactant – while the iSSC elution fluid contains 0.075% Tween 20 – the data does not provide direct evidence that the CFAC will perform as required. However, these data do provide additional indirect evidence that the CFAC will perform as currently anticipated when used as part of the iSSC during the future ISS test flight.

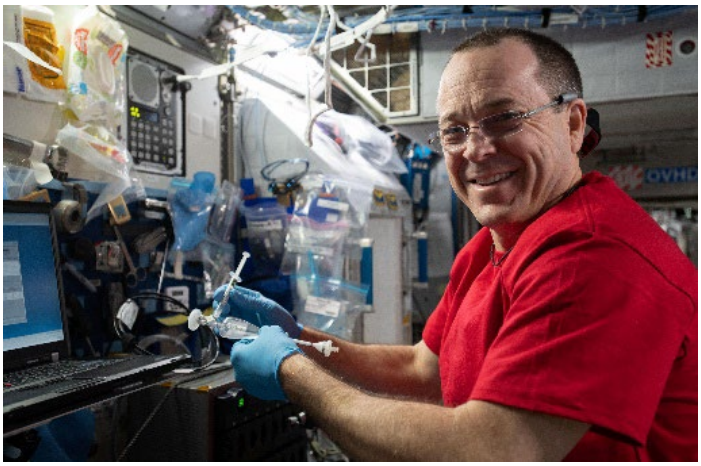

Figure 8. ISS Flight Test of the CFAC

The HHE canisters and Consumable Assemblies are manufactured in a cleanroom. Following liquid filling, the HHEs are gassed with carbon dioxide and are then e-beam irradiated. After receipt from irradiation, the HHEs are attached to the iSSC Consumable Assemblies and packaged. The packaged iSSC Consumable Assemblies are then Revox treated – a room-temperature vaporized peracetic acid sterilization process - as the final step in the manufacturing process.

Microbiological challenge testing of the iSSC was performed independently at the Southern California Coastal Water Research Project (SCCWRP) using 1 L samples of sterile, deionized water spiked with *Escherichia coli* (*E. coli*). One-liter sterile water samples were spiked with *E. coli* cells at concentrations of 10^4, 10^3, 10^2, 10^1, and 10^0 cells/100 mL. Spiked samples were then concentrated using the iSSC and elution fluid was transferred to the appropriate assay tube using a repeater pipette. concentration was run five times for a total of 25 runs. In addition, 9 blanks were processed alongside the spiked samples on the iSSC. Each blank test run consisted of 1 L non-spiked sterile water.

From each concentrated sample (including the 9 blanks), 5 qPCR reactions were performed using a 95° C extended hold for 5 minutes to lyse the concentrated cells (referred to as "boiled") and 5 qPCR reactions were performed using bead beating to lyse the cells (n=356 reactions total; 250 test concentration; 88 blanks; 18 NTCs). A standard curve was also run the morning of the testing. *E. coli* was quantified in each sample using BioGX *E. coli* (uidA) lyophilized reagents and a Cepheid Smart Cycler qPCR system. Preliminary work performed prior to initiating this study, revealed inhibition of the PCR reaction when using 95° C lysis and the BioGX *E. coli* (uidA) lyophilized reagents and a Cepheid Smart Cycler qPCR system. Inhibition was overcome using a 2X dilution of the concentrated sample in each reaction.

Frequency of detection

E. coli detection ranged from 100% at the higher concentrations tested (10^4 and 10^3 cells/100 mL) to 0%-12% detection at the lowest concentration tested (10^0 cells/100 mL). At each concentration tested, frequency of detection was higher when samples underwent bead beating. *Two* out of 88 blanks were detected, but at levels below the lower limit of quantification (BLLOQ) and 0 of the 18 NTCs were detected. The frequency of detection for bead beat (BB) and boiled (BL) (95° C extended hold) lysis are presented below in Table 1.

Table 1. Frequency of detection of *E. coli* by test concentration level and cell lysis method

Target	Conc	Method	% Detection
EC	1.E+00	BB	12%
EC	1.E+00	BL	8%
EC	1.E+01	BB	72%
EC	1.E+01	BL	24%
EC	1.E+02	BB	100%
EC	1.E+02	BL	84%
EC	1.E+03	BB	100%
EC	1.E+03	BL	100%
EC	1.E+04	BB	100%
EC	1.E+04	BL	100%
Blank	0.E+00	BB	0%
Blank	0.E+00	BL	4%

***E. coli* quantification**

E. coli concentrations measured were consistent between replicate runs for each concentration (A-E), with higher concentrations measured (~35% higher) in samples that underwent a bead beating cell lysis step. *E. coli* levels were quantifiable at the higher concentrations tested (10^4, 10^3, 10^2 cells/100 mL). At the lower concentrations (10^1 and 10^0 cells/100 mL), 100% of the samples were either below the lower limit of quantification (BLLOQ) or not detected. Measured *E. coli* concentrations versus the cell lysis method are presented below in Figure 11. Replicate runs are illustrated with different colors (Rep A-E). The same data presented above in Figure 11 is presented below in Figure 12 to provide a more detailed look at variability between sample replicates.

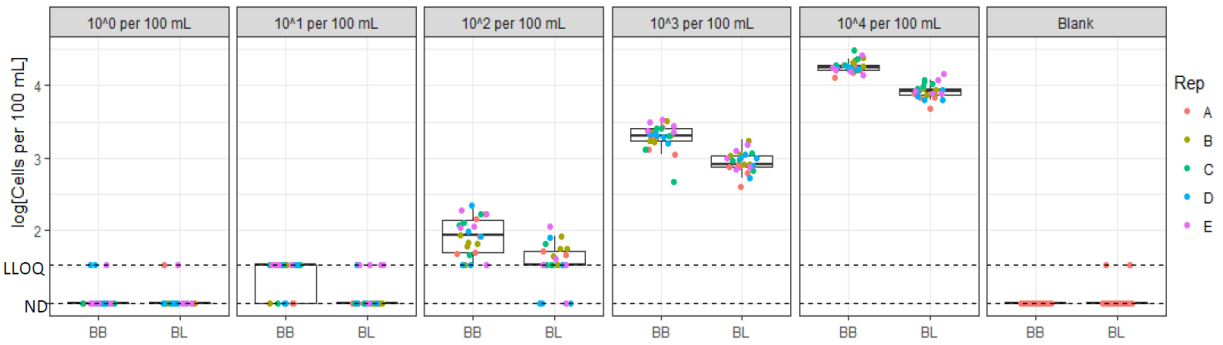

Figure 11. *E. coli* Concentration Versus Cell Lysis Method

Threshold at 5

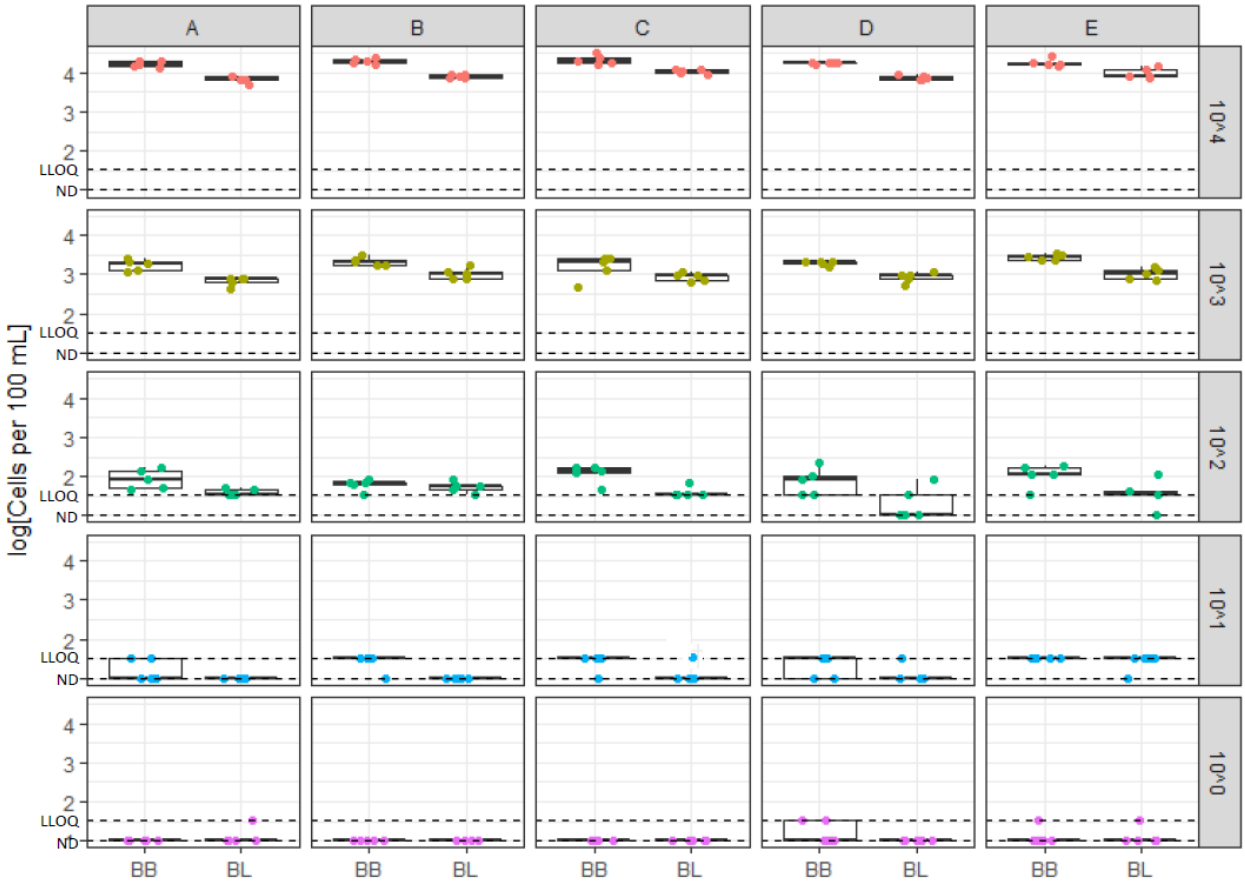

Figure 12. *E. coli* Concentration Versus Cell Lysis Method– Detailed Look at Replicate Test Runs (A-E)

IV. Conclusion

With NASA SBIR program support, InnovaPrep is developing the iSSC, a prototype microbial concentration system, for use in microgravity environments. The iSSC will be used to verify the efficacy of mechanical concentration, coupled with qPCR, to enable rapid and reliable detection of microbial contamination in ISS potable water. Following successful ISS flight demonstration, the system may be implemented in its current state, and a fully automated system may also be developed for integration into the current ISS Water Processor Assembly and future water recovery systems allowing crew to easily and rapidly monitor drinking water for microbiological contaminates.

Components of the iSSC system, as well as the full system, have been demonstrated by InnovaPrep and partners through drop tower tests, 1G laboratory tests, a parabolic test flight, and more recently an ISS test flight demonstration of the CFAC. We have successfully demonstrated that the iSSC is capable of processing one-liter water samples through a hollow fiber membrane filter concentration cell in less than ten minutes, capturing microorganisms in the fiber lumen. Following capture, the microorganisms are eluted using a novel Wet Foam Elution process in which viscous, wet foam is used to sweep the captured particles from the membrane surface and dispense them in a concentrated sample volume of less than 500 µL.

Drop tower testing performed by IIRP at the Dryden Drop Tower first demonstrated successful gas/liquid separation in a microgravity environment using the CFAC. This capability, along with microgravity operational capability of the entire iSSC system, was then demonstrated during a 30-parabola flight test performed by Zero Gravity Corporation. In this test flight, each iSSC operation, including: start-up, concentration, sample-depletion, sample-bag operation, elution, and withdrawal of the sample were demonstrated successfully in a microgravity environment. The WetLab-2 program later demonstrated successful debubbling of buffer samples, prior to qPCR analysis, during an ISS test flight.

Independent 1G testing was performed by SCCWRP using *E. coli* spiked 1 L water samples. The samples were concentrated using the iSSC and then analyzed using BioGX *E. coli* (uidA) lyophilized reagents and a Cepheid Smart Cycler real-time PCR instrument. A total of 356 qPCR reactions were performed, including 250 test concentration samples; 88 blank samples, and 18 NTCs. The test results demonstrated 100% detection at 10^4 and 10^3 cells per 100 mL (i.e. 100 cells/mL and 10 cells/mL) for both bead beat and 95° C lysis methods; exceeding the LOD that is required to meet the ISS MORD requirement of 50 CFU/mL maximum total bacteria concentration.

Using 95° C lysis, the iSSC testing demonstrated 84% detection frequency at 100 cells per 100 mL and 24% at 10 cells per 100 mL. Several means are anticipated to be available for lowering the LOD. While this testing was performed using the SmartCycler, the currently understood use case calls for 95° C lysis and qPCR analysis performed using the Biofire Razor real-time PCR instrument. Per Oubre et. al., the Razor detection limit is approximately 50% lower than the SmartCyler. Ongoing work, under the Phase II Expanded and Phase III efforts, to improve the ease of operation and performance of the iSSC are anticipated to further improve upon the system concentration efficiency and concentration factor, and thus also leading to an improved LOD.

It is anticipated that in order to meet the ISS MORD requirement for coliforms, improvements to the iSSC and the available real-time qPCR instruments will be required. Further, integration of the iSSC process into the ISS Water Processor Assembly will enable larger sample volumes to be processed – further increasing iSSC concentration factors. The overriding objective is for the concentrator to be coupled with a detection platform that together allow for simple, rapid detection at 1 CFU/100 mL.

Through the recently initiated Phase II Expanded and Phase III efforts, InnovaPrep is working to improve the reliability and usability of the iSSC, meet the system performance requirements and schedule the future ISS Test Flight an perform necessary Safety Review. This work is scheduled for completion by March 8th, 2020 where upon InnovaPrep anticipates nearing an ISS Test Flight for the iSSC system.

Acknowledgments

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