

Investigation into an Alternative Method for Microbial Monitoring on ISS

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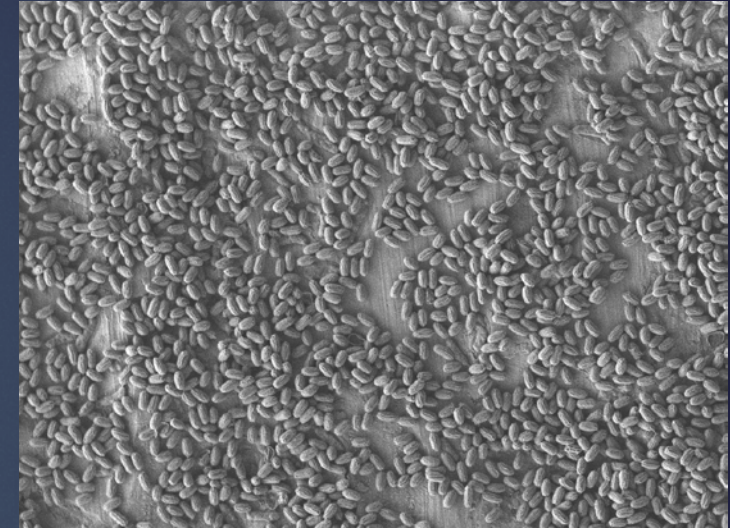




Background

- 2011 Workshop at JSC reviewed cutting edge technology (NASA/CP-2012-217460, 2011)
 - Environmental Microbiology
 - Infectious Diseases/Pathogens
 - Food and Water Safety

- Workshop determination
 - Replace or supplement current culture based practices on ISS
 - Selected molecular based quantitative Polymerase Chain reaction (qPCR)

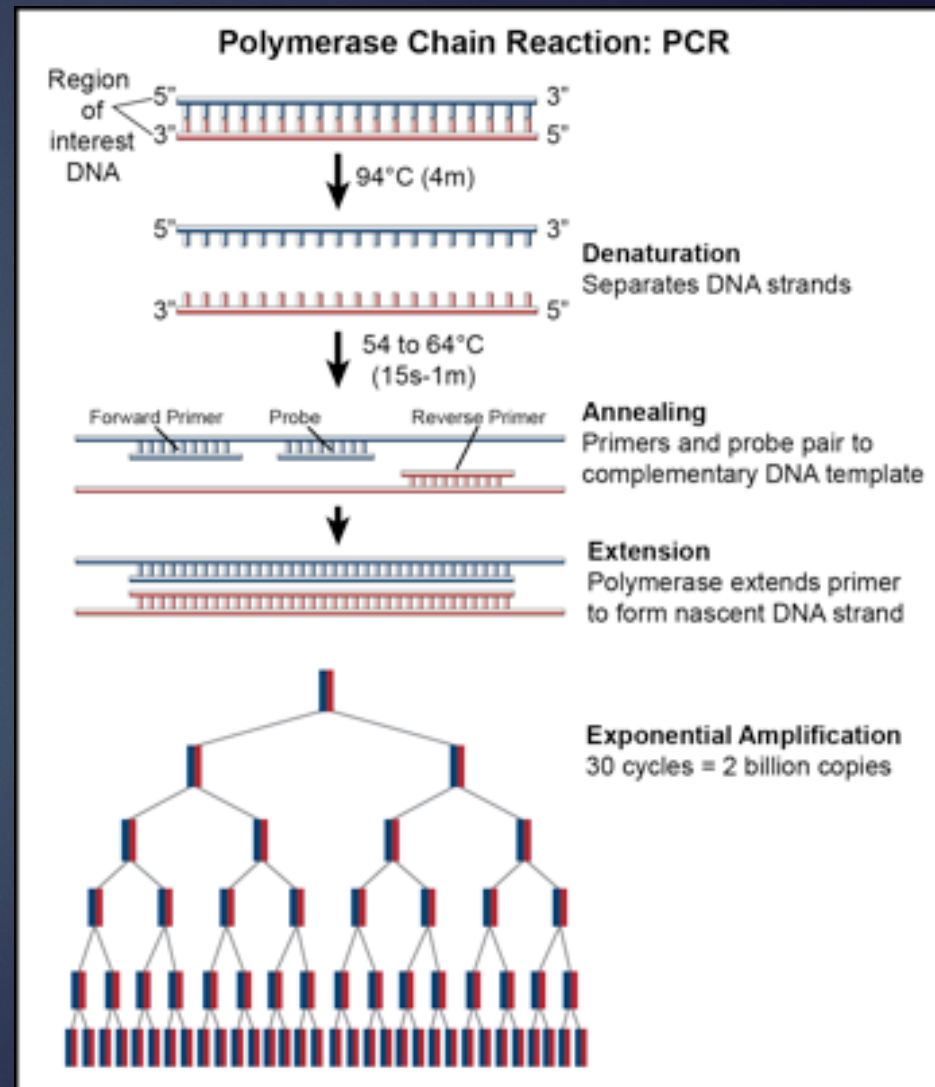


Will add a picture of Aspergillus too



Background

- Molecular-based technology
 - Polymerase Chain Reaction (PCR)
 - Real-time quantification and characterization
 - Identifies specific targets
 - Total heterotrophic growth
 - Rapid assessment of the environment
 - High reproducibility and accuracy
 - Low detection limits on culturable and unculturable microbes
- Utilize COTS PCR units
 - Operational in microgravity
 - Meet ISS interface and safety conditions





Benefits of Molecular-based Methods

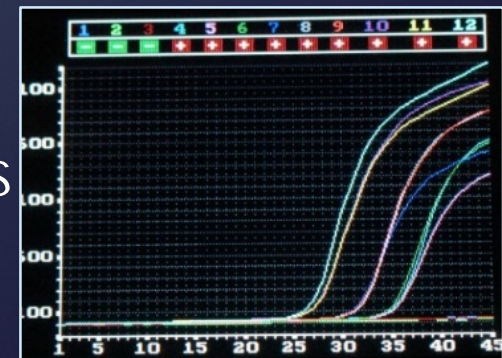
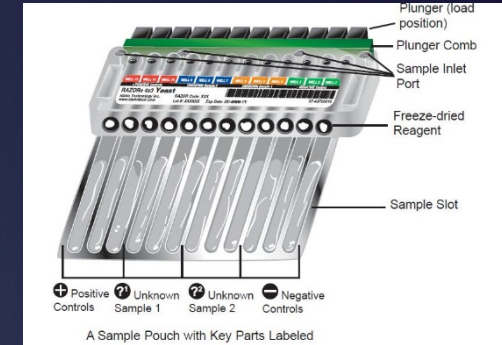
- Current methods for microbial detection
 - Labor and time intensive cultivation-based approach
- Requires collection of samples on orbit and transportation back to ground
- Disadvantages to current detection methods
 - Unable to perform quick, reliable detection on orbit
 - Lengthy sampling intervals
 - Minimal identification capabilities
 - Can fail to detect or characterize all cells present





Purpose and Benefits

- Develop a rapid microbial identification system
 - Reduce crew time & expedite operational decisions
 - Provide an in-flight microbial identification system
 - Increase monitoring of crew health
 - Monitor air, water, food, & surfaces for potential pathogens
 - Reduce or eliminate reliance on ground support
 - Provide an independent system for long-term space flight
 - Provide flexibility
 - development of customized species specific assays
 - Determine viability with RNA possible





Challenges

- Find a COTS, PCR instrument
 - Effective in microgravity
 - Compact
 - Low cost
 - Identify to species level
 - Live vs Dead cell via RNA
 - Quantitative
 - Easy to use and interpret data
 - Real-time information
 - Short time from sample to answer
 - Work with multiple sample types



- RAZOR EX, BioFire Defense, Inc, was tested & integrated as one of three instruments into the 2 x 2015 Water Monitoring Suite
 - developed as an SBIR with DoD

- Completed engineering/safety evaluations and launched to ISS, SpaceX-9, July 2016





Goals of Tech Demonstration

- To perform in-flight and ground-based real-time quantitative Polymerase Chain Reaction (qPCR) validation using a commercial off-the-shelf system
- The proposed technology demonstration on ISS was aimed at determining:
 1. If qPCR testing will work in the spaceflight environment
 2. If qPCR testing during a flight operation yields results similar to those from a controlled experiment on Earth, thereby validating the instrument for potential use as a monitoring tool aboard the ISS.
 3. Develop a CONOPS for water testing
 4. Verify that qPCR is successful using ISS water samples





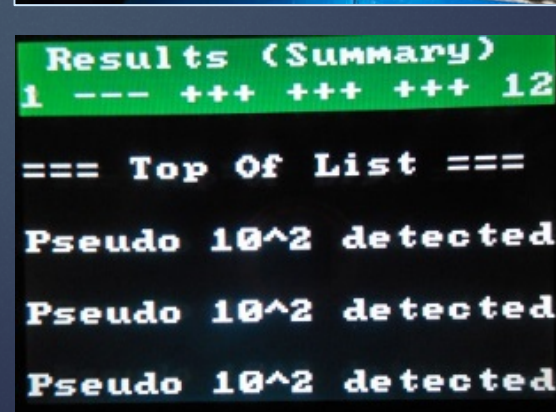
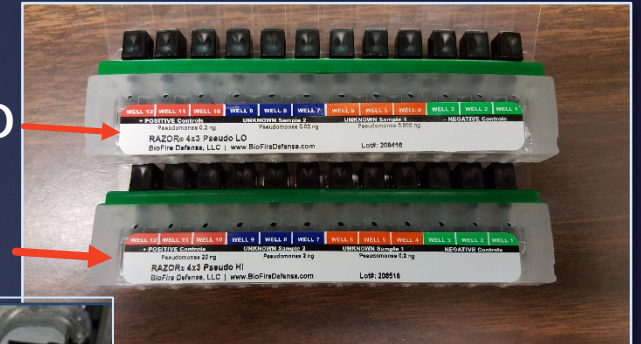
Methodology

- BioFire Defense, RAZOR EX
 - Uses raw or prepared samples
 - Pouch system contains customizable, optimized freeze-dried reagents
 - *Pseudomonas aeruginosa*
 - 5 varying concentrations
 - Sample to answer in approximately 1 hour

On-Orbit Operations	Molecular Grade Water	Unfiltered PWD Water	Filtered PWD Water
September 20, 2016	X		
September 22, 2016	X	X	
October 3, 2016		X	
October 18, 2016	X		
November 22, 2016		X	
December 14, 2016			X
January 17, 2017			X
March 14, 2017			X

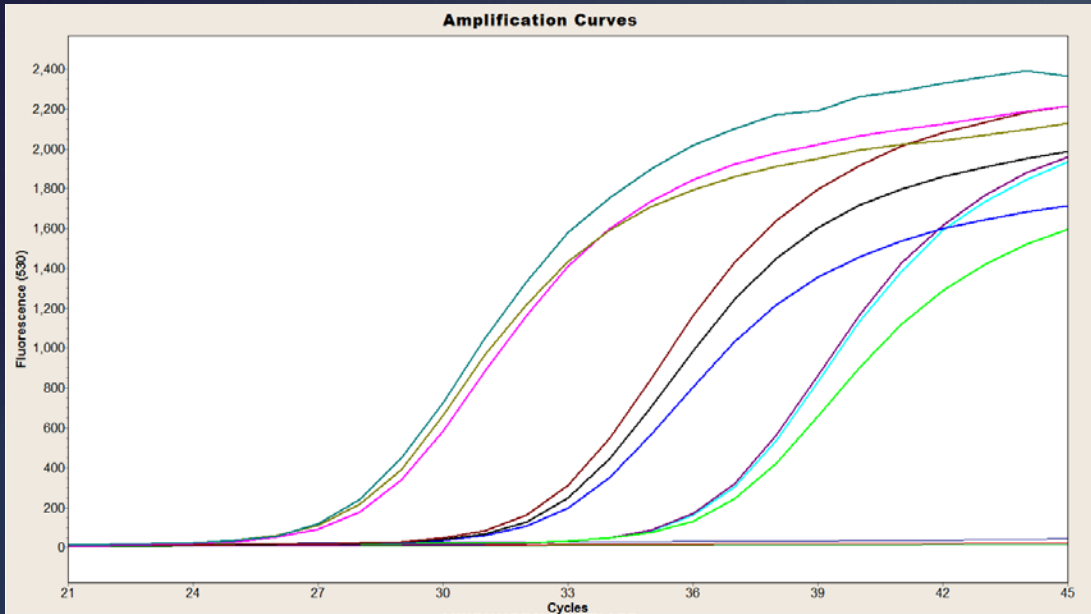
Pseudo LO

Pseudo HI

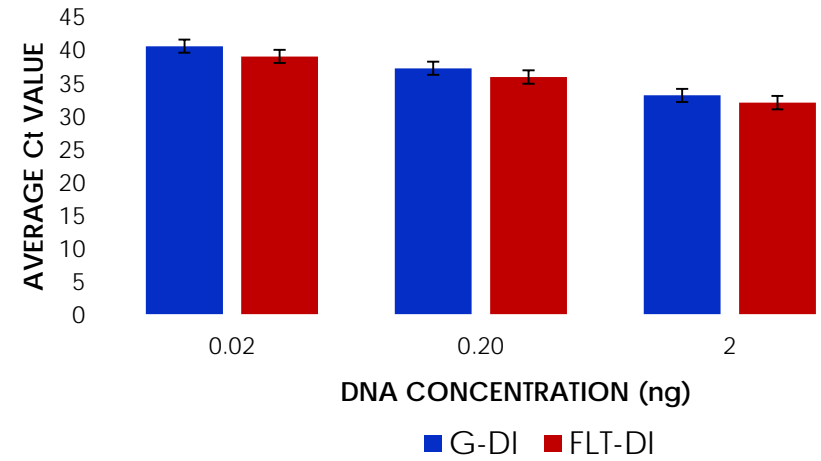




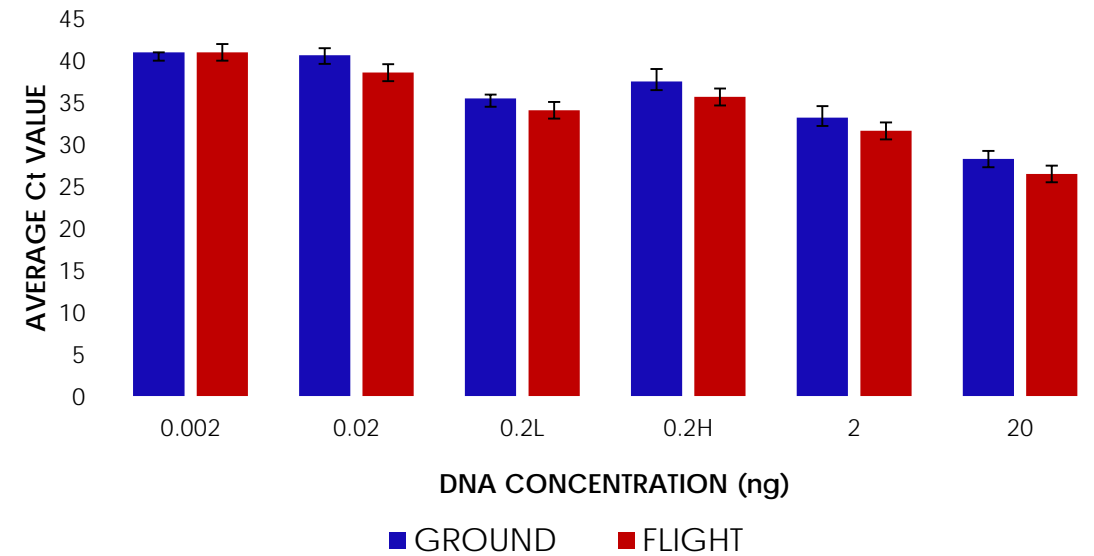
Results



Average Ct Comparison
Ground vs Flight - DI Water

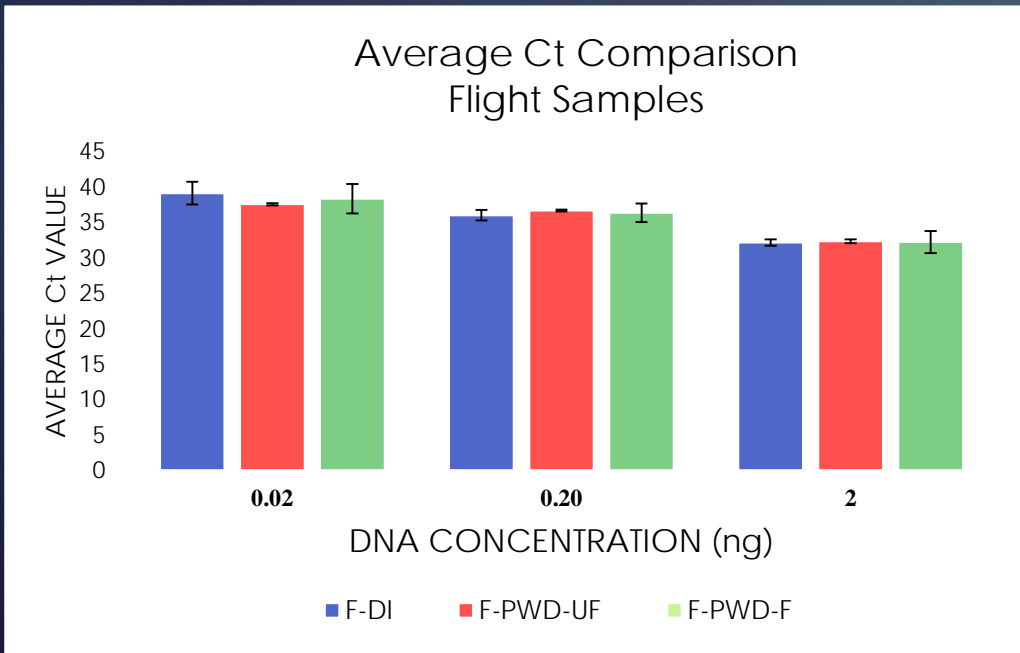
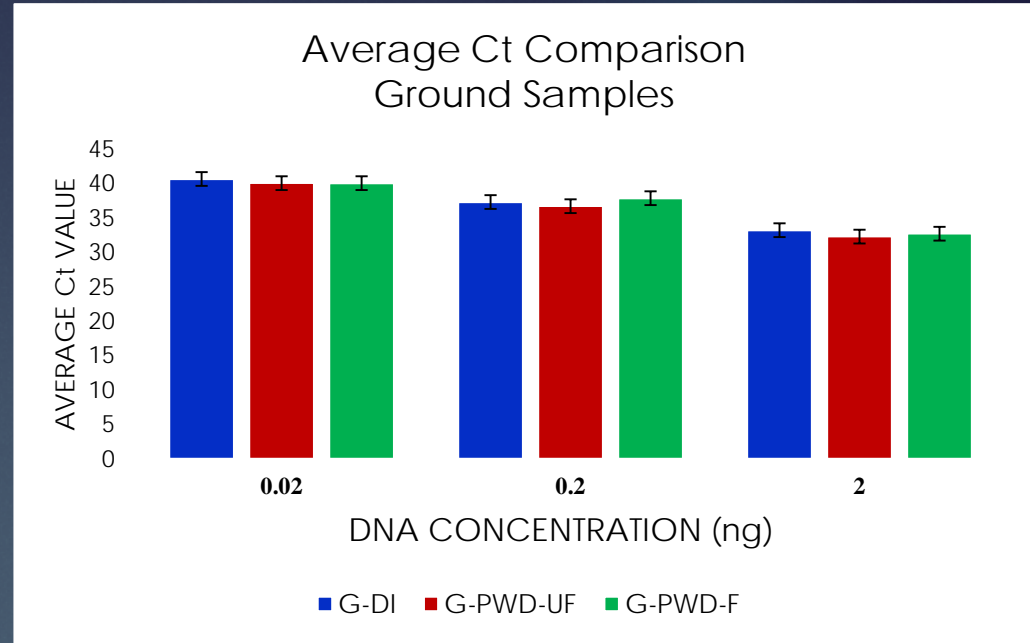


Average Ct Comparison





Results





Conclusions

- Microbial Monitor is the first device to successfully and accurately perform quantitative PCR using ISS water samples in the microgravity environment of space providing a fast sample to answer.
- The residual chemicals in the potable water had no effect on the PCR chemistry
 - There was no difference between the molecular grade water and the ISS potable water
 - There was no difference between the filtered and unfiltered ISS potable water samples
- The flight samples were not significantly different from the ground controls



What is proposed for future work with the Microbial Monitoring System

- Optimize additional assays for real-time detection
- Develop methods of sampling additional environments on ISS, i.e. Air, surfaces, food
- Equate genetic equivalents to colony forming units (CFU)
- Develop methodology to recover amplicons for further investigations
- Optimize Microbial Monitoring for future exploration



The team would like to thank:

Crissy Canerday
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Katy Larner
Logan Godfrey
William Therrien
Susan Hanley
Joseph Benjamin
Ray Wheeler



I want to say how fantastic the procedures are and how easy this is to use. It is phenomenal that you have taken a piece of commercial hardware and ... made it work in space the first time and is very user friendly. I think this is going to be a fantastic capability for the space station.

-Kate Rubins, Ph.D.



Thomas Pesquet [@Thom_astro](#) · 5h

We don't want microbes to proliferate on ISS! We clean it carefully but we also monitor microbial growth in water and on all surfaces...



7

84

384



Questions

