Investigation into an Alternative Method for Microbial Monitoring on ISS

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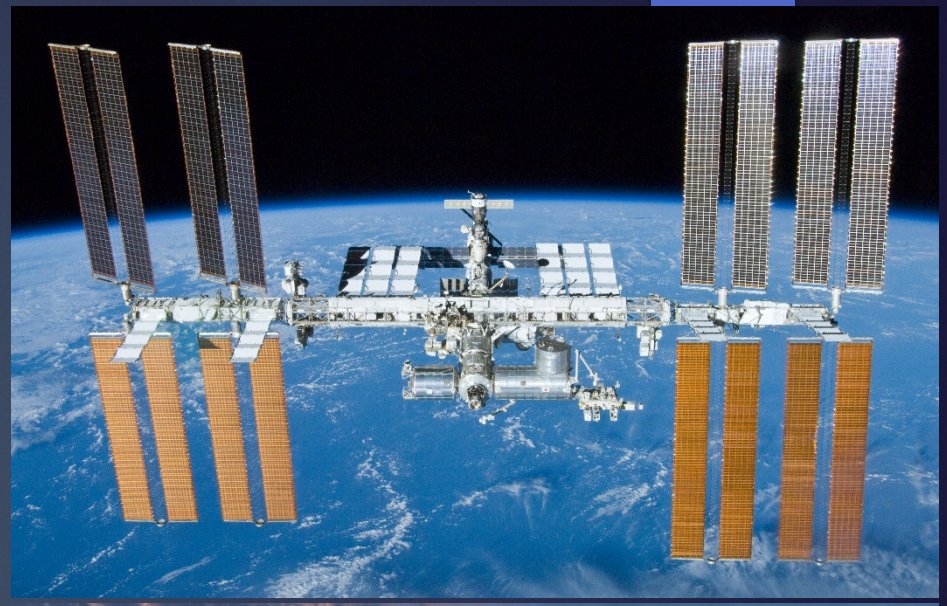
History

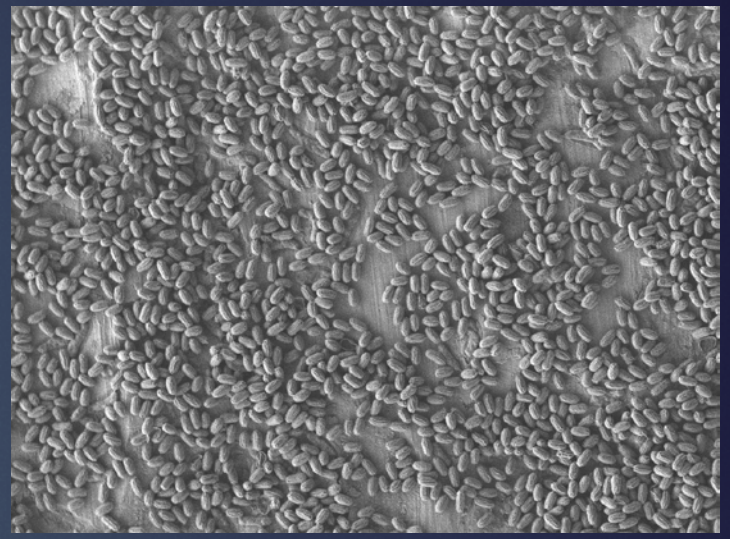
- Microorganisms have been detected on the International Space Station (ISS)
  - Potential human pathogens
- Introduce new microorganisms with every exchange of crew or addition of equipment or supplies



Background

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

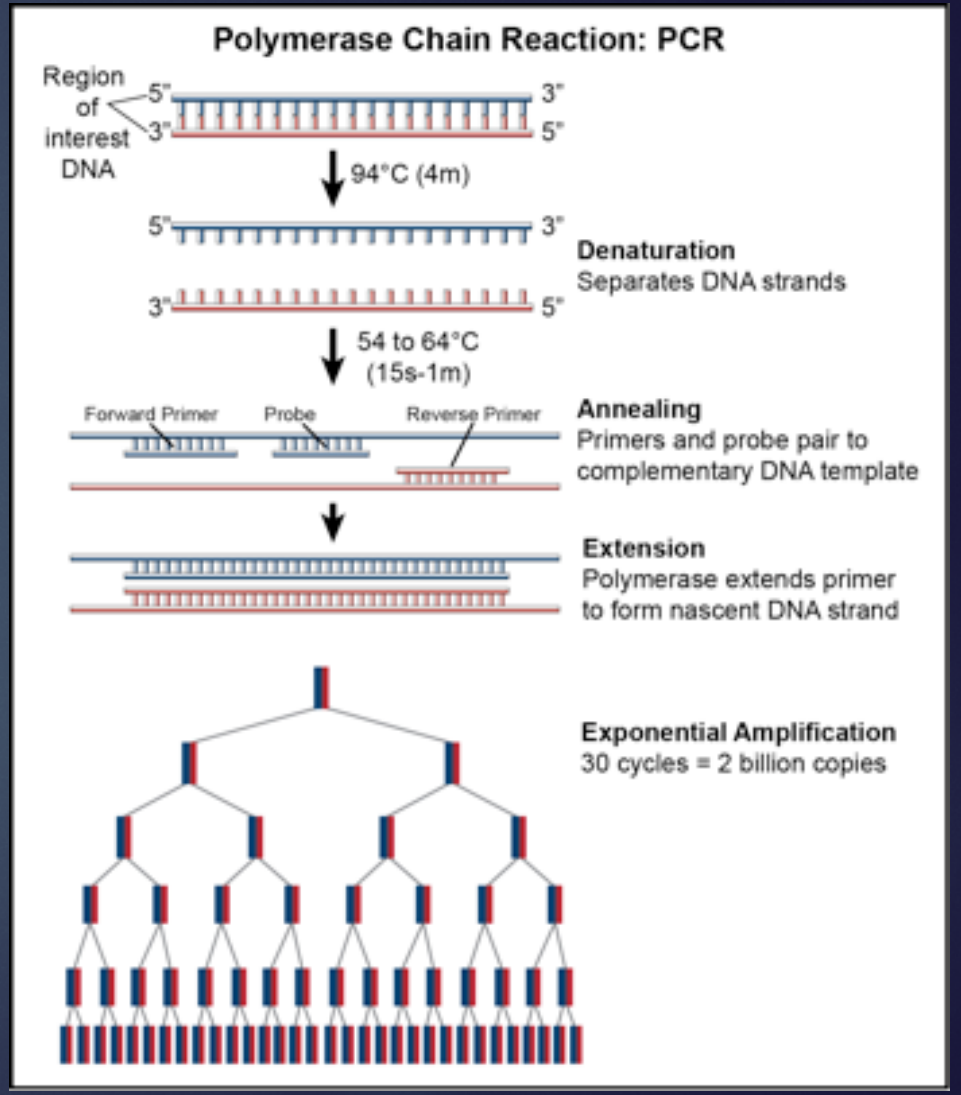
- Workshop determination
  - Replace or supplement current culture based practices on ISS
  - Selected molecular based quantitative Polymerase Chain reaction (qPCR)



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



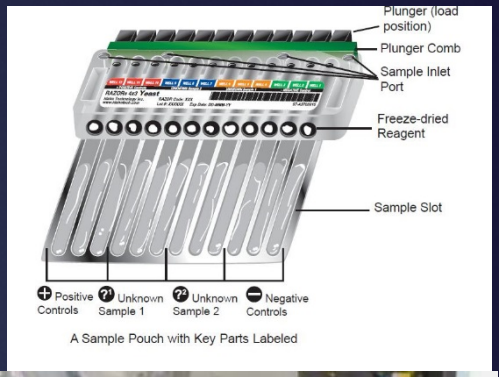
Benefits of Molecular-based Methods

- 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

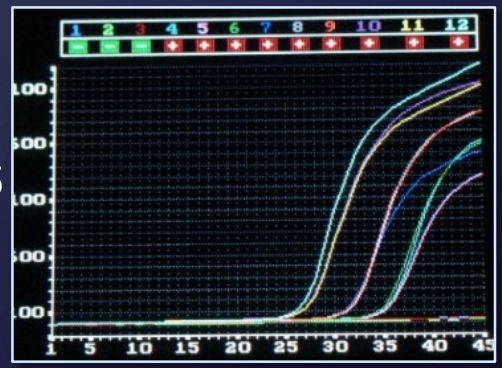
Purpose and Benefits

- 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



A Sample Pouch with Key Parts Labeled



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

NASA 2x 2015
DEVELOP | FLY | EXPLORE

- 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

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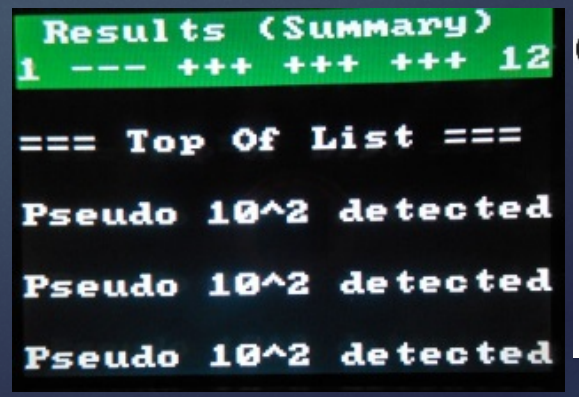
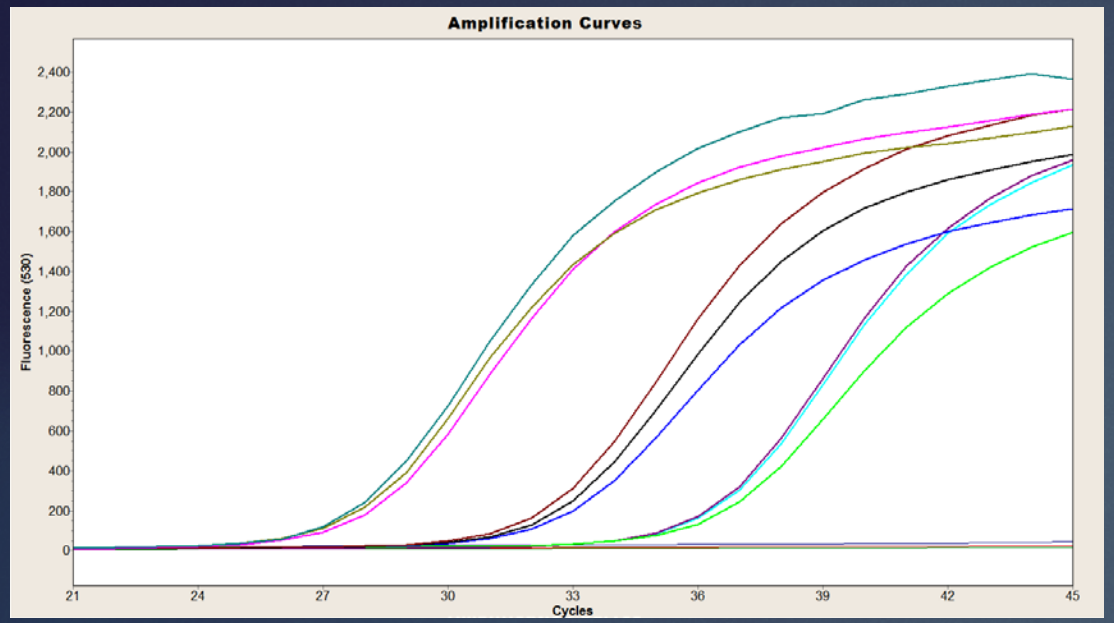
Methodology

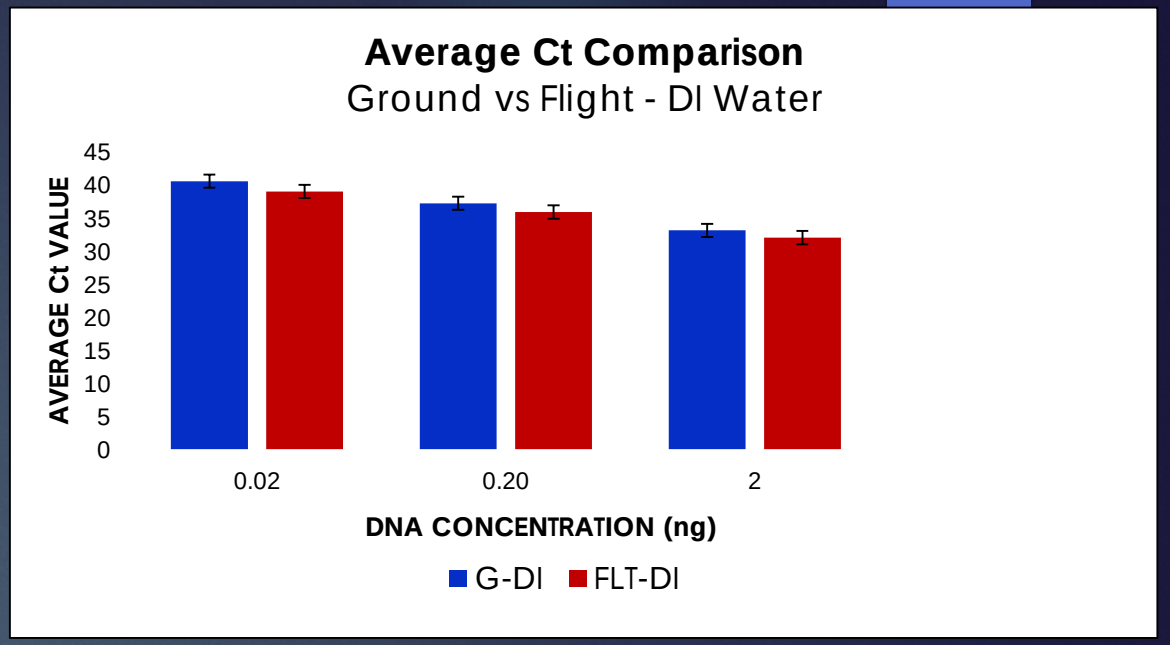
- BioFire Defense, RAZOR EX
  - Uses raw or prepared samples
  - Pouch system contains customizable, optimized freeze-dried reagents
    - *Pseudomonas aeruginosa*
    - 5 varying concentrations
  - Sample to answer in approximately 1 hour

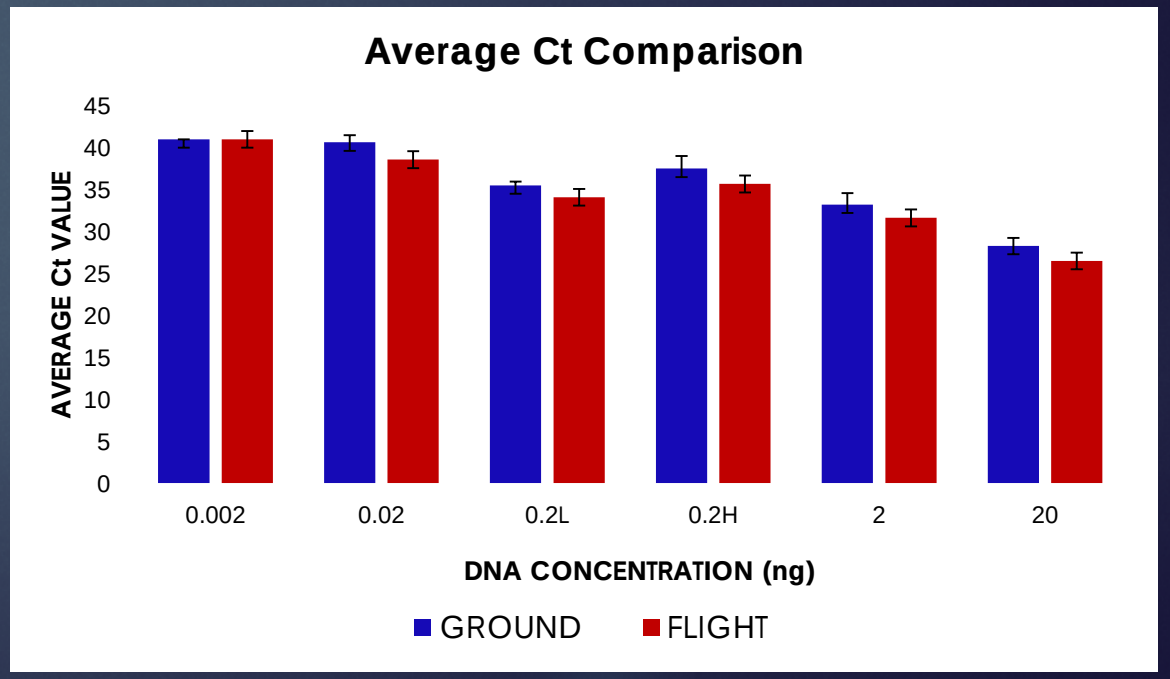
On-Orbit Operations	Molecular Grade Water	Unfiltered PWD Water	Filtered PWD Water
September 20, 2016	X		
September 22, 2016	X	X	
October 3, 2016		X	
October 18, 2016	X		
November 22, 2016		X	
December 14, 2016			X
January 17, 2017			X
March 14, 2017			X



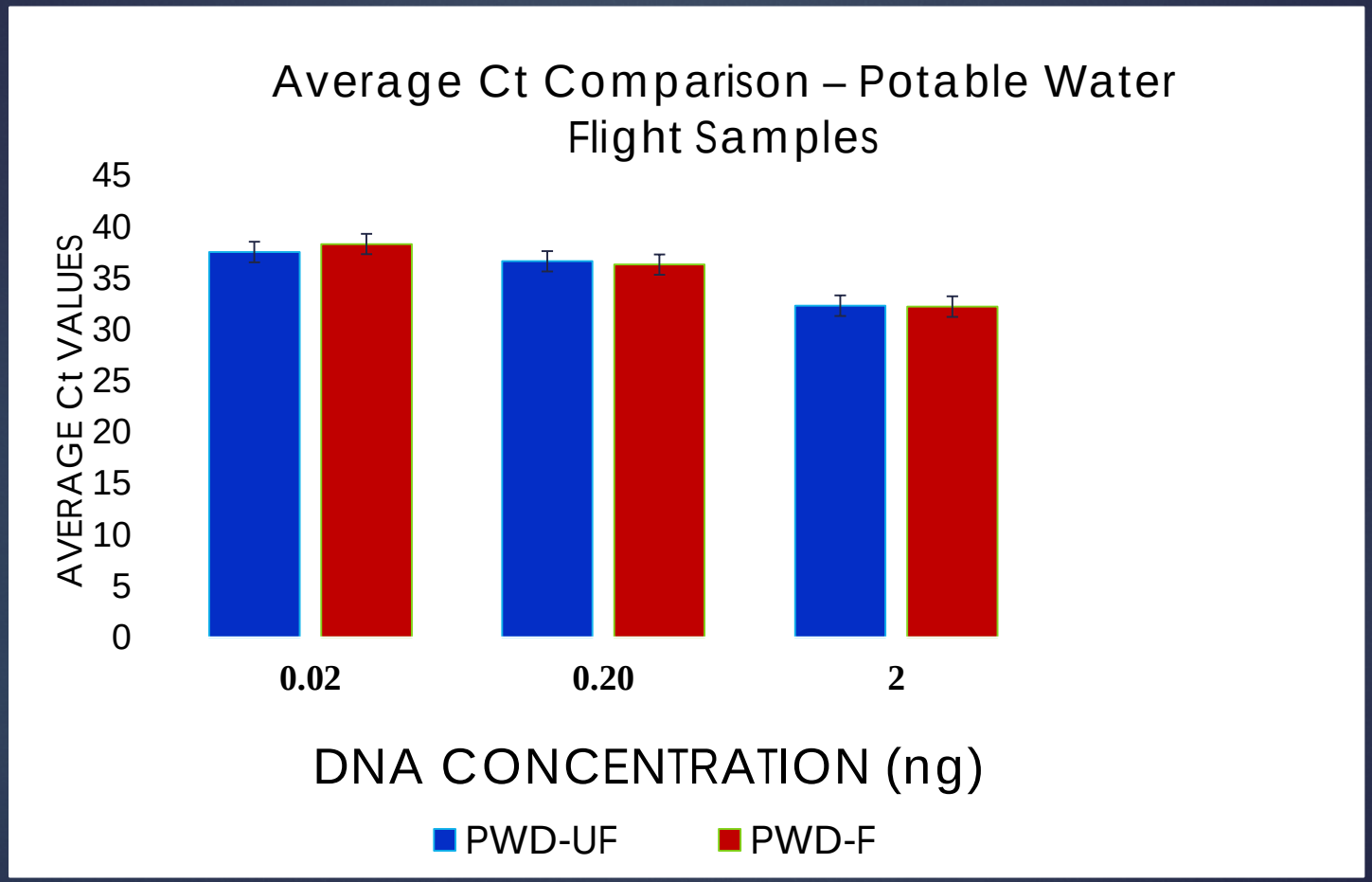
Results


Amplification Curves
Fluorescence (530)
Cycles
2,400
2,200
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
21
24
27
30
33
36
39
42
45

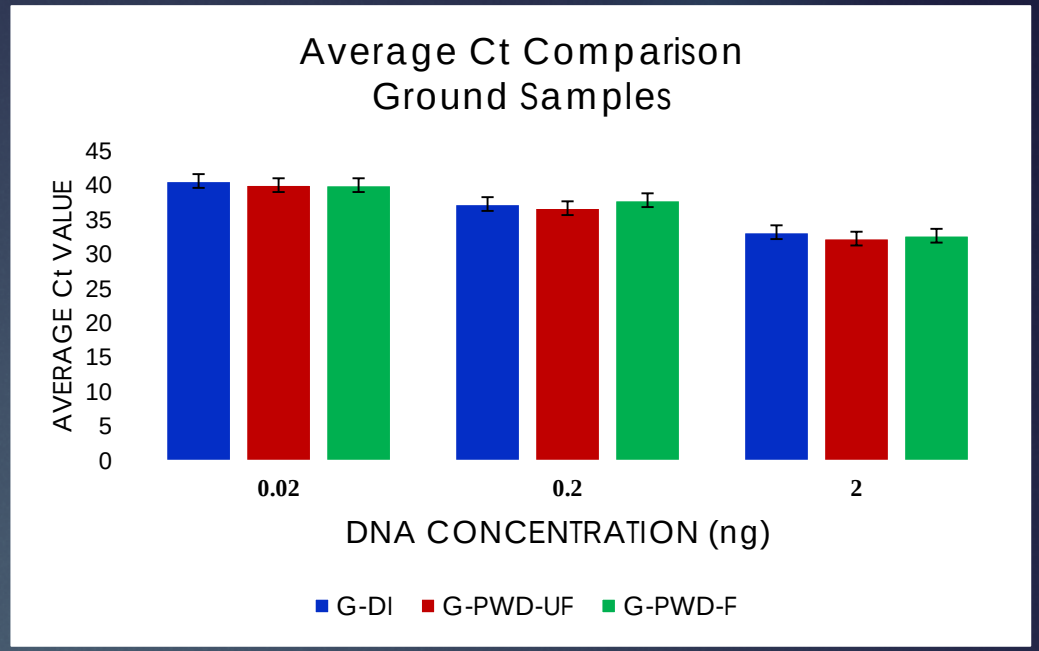

Average Ct Comparison
Ground vs Flight - DI Water
AVERAGE Ct VALUE
45
40
35
30
25
20
15
10
5
0
0.02
0.20
2
DNA CONCENTRATION (ng)
G-DI
FLT-DI

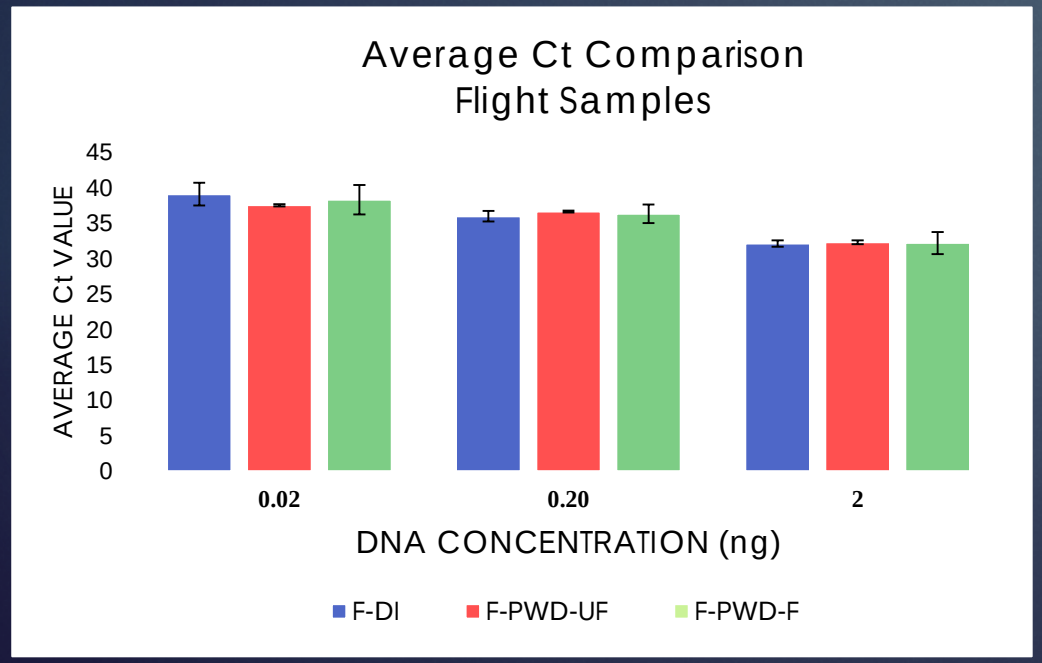

Average Ct Comparison
AVERAGE Ct VALUE
45
40
35
30
25
20
15
10
5
0
0.002
0.02
0.2L
0.2H
2
20
DNA CONCENTRATION (ng)
GROUND
FLIGHT

Results–ISS Potable Water Samples



Results


Average Ct Comparison
Ground Samples
AVERAGE Ct VALUE
45
40
35
30
25
20
15
10
5
0
0.02
0.2
2
DNA CONCENTRATION (ng)
G-DI
G-PWD-UF
G-PWD-F


Average Ct Comparison
Flight Samples
AVERAGE Ct VALUE
45
40
35
30
25
20
15
10
5
0
0.02
0.20
2
DNA CONCENTRATION (ng)
F-DI
F-PWD-UF
F-PWD-F

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

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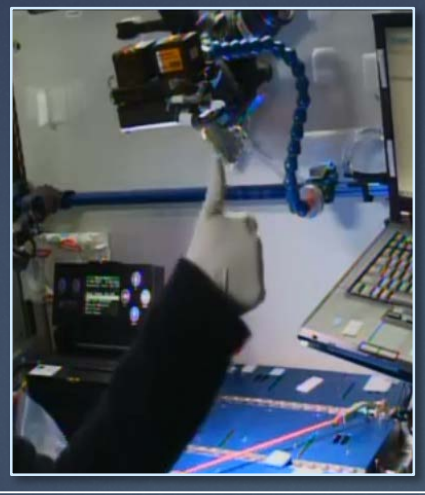

I want to say how fantastic the procedures are and how easy this is to use. It is phenomenal that you have taken a piece of commercial hardware and … made it work in space the first time and is very user friendly. I think this is going to be a fantastic capability for the space station.

-Kate Rubins, Ph.D.


Microbial Monitoring System
Birmele Flint Khodadad Ott SpaceX9 Castro Oubre Jones Roman

Questions