WetLab-2: Providing Quantitative PCR Capabilities on ISS

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WetLab-2 Objectives

• To place on the ISS a research platform to facilitate space biology gene expression research.
  
  – Capability to process samples and perform qRT-PCR

• Facility will support multiple sample types (bacteria, cells, tissue)

• The analyzer will remain on ISS, while the experiment-specific disposable hardware will launch with the experiments.

• Also capable of supporting analysis of air, surface, water, and crew health.

• Validation Flight: SpaceX-7
WetLab-2 Operations Overview
Sample Transfer Tool

• WL2 uses a Luer-lok connection to accept samples
• Techshot Analytical Containment Transfer Tool (ACT2)
  – Uses luer-lok
  – Provides two levels of containment
  – Maintained throughout sample transfer process
• Standard syringe can be used if two containment levels are not needed

For Validation Flight:
• Will use the 5ml configuration of the ACT2
• Sample (E. coli) will be frozen at -80C after loading in unit
• Crew will thaw the sample then use it as input to the SPM
• Will use a standard luer-lok syringe to introduce the tissue
Sample Preparation Module (SPM)

- Function of SPM
  - Breaks open cells and binds RNA to column
  - Washes RNA
  - Elutes RNA into removable RNA syringe

- Two versions: mammalian, bacterial
- Closed system
- Fluids are pre-loaded in syringes
- No alcohols or organic solvents
- Disposable one time use
- Designed to be run in Disposable Glove Bag (DGB)
- Crew manipulations consist of:
  - Attaching and removing syringes
  - Turning OmniLyser on and off
  - Pushing syringe plungers
  - Switching valves

System has been successfully tested on the ground with bacterial and mammalian cells
SPM runs give comparable results to those from the already proven ClaremontBio bench procedure.

**E. coli**

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**Mouse liver**

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Pipette Loader

• Function of RAM (Pipette Loader)
  – Removes air bubbles from the RNA sample
  – Loads pipette tip

• Closed system
• Designed to be run in the Disposable Glove Bag (DGB)
• No fluids pre-loaded (long shelf life)
• Sample must be driven slowly through the bubble trap to be effective
• Loads the sample into the Repeater Pipette Tip for downstream tube loading
Reaction Tube Loading and Rotor

• Reaction Tube Loading
  – Pipette is used to load 25ul into each Reaction Tube
  – Loading occurs through septum of modified cap
  – Prototypes tested on Parabolic Flight

• Reaction Tube Rotor
  – Holds 8 Reaction Tubes
  – Rotor is attached to drill on ISS
  – Spin to get RNA into the sample window
  – Prototypes used on Parabolic Flight – design changes made based on experience
Pre-filled Reaction Tubes

- These are COTS SmartTube with a custom septa cap for loading in microgravity.
- They contain lyophilized primers, probes, enzymes and Master Mix.
- They will be foil packed to protect from moisture and light.
- Lyophilized Reagents give comparable data to commercial wet chemistry reagents.
• SmartCycler
  – qRT-PCR system
  – Instrument is designed for field work
  – Will fly as a COTS item
  – Will be used in the aisle
  – Mounted on a Bogun Arm on the rack
  – Provides 16 wells and multiplexing capability
  – Thermal programs can be uploaded from the ground
  – Data can be downlinked to ground after run
On-Orbit Configuration

OCAMS Used for Uplink/Downlink Data

Pantry Laptop

Disposable Glove Bag

SmartCycler (15A Fuse)

ISS Inverter (12A Fuse)

To Rack 120 VDC
Goal of Validation Flight: On-orbit test and check-out of the WetLab-2 system in a systematic way to ensure it will return valid data to future researchers

Objectives of Validation Flight:

• Install software and set-up hardware (Session 1)
• Does real-time PCR data generated on-orbit match data on earth? (Session 2)
  – No convection or other microgravity related issues
  – Validate SmartCycler, RAM, tube loading and rotor functions
• Does the Sample Processing Module function correctly on-orbit? (Session 3)
  – All fluidic manipulations function properly
  – Prove out system with first sample type (*E. coli*)
  – Test system using on-orbit isolated RNA as input to SmartCycler
• Does system function correctly on-orbit with tissues? (Session 4)
  – All fluidic manipulations function properly
  – Prove out system with second sample type: mouse tissue

Flight results from each session will be compared to results from ground controls

Ground controls will be run with a 2-24 hour delay from the flight samples
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**Successful singleplex, duplex and triplex results**

**This data is typical for post-optimization runs**
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Successful singleplex, duplex and triplex results

This data is typical for post-optimization runs
Conclusion

- WetLab-2 will facilitate space biology gene expression research
  - Establishes a qRT-PCR analytical instrument on the ISS.
  - Provides a Standard Transfer System for sampling among Wetlab-2 and other bioprocessing & analytical systems (Techshot ACT2)
  - Sample preparation of minimal complexity, can be completed by crew in <2 hours
- Reduce need for downmass of samples due to on-orbit analysis
- Allow researchers to begin to utilize the ISS as a fully working laboratory
  - Results will be available to researchers within hours of run completion allowing for the potential for interactivity with experiments driven by the analysis of results
- Provide on-orbit analysis of air, surface, water, and clinical samples to monitor environmental contaminants and crew health.
  - Results would be available in as little as 90 min compared to current testing that takes 3-6 months due to the need for sample return
- Looking for users of the system after completion of validation flight
WetLab-2 Team

**Management and Systems Engineering**
- Julie Schonfeld
- Mark Mallinson
- Eddie Uribe
- Gary Hiatt

**Science Team**
- Eduardo Almeida
- Macarena Parra
- Jimmy Jung
- Luan Tran

**S&MA**
- Leonard Hee
- Dean Chacon

**Configuration Management**
- Mike Henschke

**Engineering**
- Peter Tong
- Youssef Mohamedaly
- Tori Chinn
- Liz Hyde
- Tony Chen
- Sean Sharif

**Fluidics**
- Travis Boone

**Software**
- Matt Chin
- Matt Everingham

**Test Leads**
- Dzung Hoang
- Jennifer Murphy

**Operations**
- Jessica Hauss
- Cindy Harris

**Finance**
- Veny Jubilo

**Manufacturing**
- Emmett Quigley
- Ron Strong

**PIM:** Laura Holcomb
**Ops POC:** Lisa Prendergast
**RIM:** Melissa Wallace (Brienne Shkeddi)
**RPM:** Jessica Curry

Note: Wetlab-2 = Wet Lab RNA SmartCycler
Backup Slides
## Session 2: q-PCR using QC DNA

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No carryover from tube to tube

This data is typical
Post-Validation/Post-Flight

- Fluidics components are disposable
- SmartCycler to remain on board ISS
- SLPS Principle Investigators who propose to use the SmartCycler with launch fluidics components with science hardware
  - Current estimates indicate SmartCycler use 2-4 investigations per year
  - Reagents loaded in SPM and SmartTubes to be experiment-specific