Microbial Monitoring of Common Opportunistic Pathogens by Comparing Multiple Real-time PCR Platforms for Potential Space Applications

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DEDICATION

This presentation is dedicated
In honor of
Angela Johnston
Marshall Space Flight Center
History

- Current methods adequate for monitoring & safeguarding short-term spaceflight missions and ISS
- Will not be sufficient for long term spaceflight missions
  - Keep air & water free of microbes
  - Keep crew healthy
  - Be autonomous & robust for long spacecraft missions
- 2011 Workshop at JSC reviewed cutting edge technology
  - Environmental microbiology
  - Infectious diseases/Pathogens
  - Food Safety
History

• JSC Conference determination
  - Should replace or supplement the current practices

• Reviewed current methods
  - Real-time qPCR
  - ATP bioluminescence
  - Flow cytometry
  - Matrix assisted laser desorption/ionization (TOF)
  - Microscopy
Challenges

- Challenges ahead for long-term spaceflight
  - No COTS units to fulfill the needs

Recommendations for Instrument or Method

- Easy to use
- High throughput
- Effects of microgravity
- Cost
- Phylogenetic resolution
- Live vs Dead
- Quantitative
- Easy to interpret data
- Multipurpose
- Real time information
- Compact
- Short time from sample to answer
- Work with multiple samples
Introduction:

- **Current methods for microbial detection**
  - Labor & time intensive cultivation-based approaches that can fail to detect or characterize all cells present
  - Requires collection of samples on orbit and transportation back to ground for analysis

- **Disadvantages to current detection methods**
  - Unable to perform quick and reliable detection on orbit
  - Lengthy sampling intervals
  - No microbe identification
Background:

- Molecular-based technology
  - Polymerase Chain Reaction (PCR) for real-time quantification and characterization
  - Identifies specific targets or total heterotrophic growth beyond the current capabilities aboard ISS
  - Provide rapid assessments of environment
  - High reproducibility and accuracy
  - Low detection limits on culturable & unculturable microbes

- Utilize commercial off the shelf (COTS) PCR units
  - Operational under microgravity conditions
  - Meet ISS interface and safety conditions
Goals:

- Develop a rapid microbial identification system
  - Reduce crew time & expedite operational decisions
  - Provide an in-flight identification system
  - Increase monitoring of crew health
  - Monitor air, water and surfaces for potential pathogens
  - Reduce or eliminate reliance on ground support
  - Provide independent system for long-term space flight
Materials and Methods: Evaluate Commercial Off the Shelf Units (COTS)

- Market survey of available platforms

- Evaluate technologies & initial proof of concept
  - Flight feasibility

- Determine LLOD for each platform
  - Using identical cultures prepared at KSC

- Capability to monitor ISS potable water system
Materials and Methods: Market Survey

- Platform overview including size, weight, ease of operation
- Number of reactions/samples that can be processed simultaneously
- Reagents required for sample to answer
- Platform and hardware components
- Power, data, refrigeration requirements

See ppr appendix B
Materials and Methods: Proof of Concept on 3 PCR-based instruments

- iCubate, iCubate 2.0 system, Huntsville, AL – JSC
- BioFire, RAZOR EX and Film Array, Salt Lake City, UT - KSC
- Cepheid Smartcycler, Sunnyvale, CA - JPL
iCubate, 2.0 System
- Multiplex, semi-quantitative system
- Sample to answer
- Self-contained cassette pre-loaded with all PCR reagents
- Evaluate up to 30 microorganisms simultaneously
- Ability to customize reactions for additional organisms
Materials and Methods: RAZOR EX

- **BioFire RAZOR EX**
  - Field-portable, real-time PCR unit
  - Semi-quantitative
  - Uses raw or prepared samples
  - Pouch system contains optimized freeze dried reagents
  - Customizable designs for additional microbes
  - Sample to answer in less than 1 hour
Materials and Methods: Film Array

- **BioFire Film Array**
  - Multi-plex PCR all-in-one integrated system
  - Windows-based instrument
  - Automated analyses
  - Freeze-dried reagent format
  - Sample to answer in less than 1-hour
Materials and Methods: Cepheid Smartcycler

- Cepheid Smartcycler
  - Modular real-time PCR instrument
  - Barcode scanners
  - Solid-state optical system
  - Smart-tube sample processing
  - Software capable of real-time analysis
Materials & Methods: Other platforms reviewed

- **LOCAD**
  - Lab-on-a-chip Application Development
  - Biomarkers for bacteria or fungi

- **WETLAB 2 – NASA Ames Research Center**
  - Considered 9 platforms for in-flight
  - Smartcycler selected for deployment

- **MIDASS – European Commission & ESA**
  - Microbial detection in air system for space
  - PCR based detection system for air & surfaces
Proof of Concept: LLOD Determination

- Tested three of the PCR-based platforms
- Single target in vendor's reagent assay kit
  - Challenge organism - *Salmonella enterica* (ATCC 14028)
  - Functional negative control - *Pseudomonas aeruginosa* (ATCC 700888)
    - 1 x 10^5 to 1 x 10^2 CFU/mL serial dilutions
    - LLOD determined for each platform
- Mixed culture of both organisms
  - Varied based on LLOD
Materials and Methods: Proof of Concept Testing

- All testing completed under identical environmental conditions
  - Ambient room temperature
  - Test organisms cultured at one location and shipped to each test site
  - DNA extracted from Salmonella at JPL, evaluated on Nanodrop 1000 and tested on each platform
## Results: Market Survey

<table>
<thead>
<tr>
<th>Instrument Attribute</th>
<th>iCubate 2.0</th>
<th>RAZOR EX</th>
<th>Film Array</th>
<th>Smartcycler</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of samples</td>
<td>4 x 12</td>
<td>12</td>
<td>102</td>
<td>16</td>
</tr>
<tr>
<td>Volume</td>
<td>40 µl</td>
<td>100 µl</td>
<td>100 µl</td>
<td>1 µl</td>
</tr>
<tr>
<td>Size (in)</td>
<td>14 x 15 x 14 &amp; 17 in³</td>
<td>25.4 x 11.4 x 19</td>
<td>10 x 15.5 x 6.5</td>
<td>12 x 12 x 10</td>
</tr>
<tr>
<td>Weight (lb)</td>
<td>177</td>
<td>11</td>
<td>20</td>
<td>22</td>
</tr>
<tr>
<td>Power</td>
<td>Standard</td>
<td>24V 4A power supply &amp; battery</td>
<td>Standard</td>
<td>Standard</td>
</tr>
<tr>
<td>Reagents</td>
<td>Pre-loaded cassettes</td>
<td>Pre-loaded pouches</td>
<td>Pre-loaded pouches</td>
<td>Sealed, preloaded SmartTube</td>
</tr>
<tr>
<td>Time to answer</td>
<td>6 - 8 h</td>
<td>30 m</td>
<td>30 m</td>
<td>Labor intensive</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Raw or DNA</td>
<td>Raw or DNA</td>
<td>Raw or DNA</td>
<td>DNA only</td>
</tr>
</tbody>
</table>
## Results: Proof of Concept

<table>
<thead>
<tr>
<th>Instrument</th>
<th>iCubate 2.0</th>
<th>RAZOR EX</th>
<th>Smartcycler</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella</strong></td>
<td>1 x 10⁴</td>
<td>1 x 10⁴</td>
<td>1 x 10³</td>
</tr>
<tr>
<td><strong>LLOD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Combined culture</strong></td>
<td>1 x 10⁵</td>
<td>1 x 10⁵</td>
<td>1 x 10⁴</td>
</tr>
<tr>
<td><strong>LLOD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Minimum cells needed</strong></td>
<td>400</td>
<td>50</td>
<td>94</td>
</tr>
<tr>
<td><strong>per reaction</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion & Conclusions:

- Three platforms had capability to detect $\leq 400$ cells *Salmonella enterica*
- Two platforms considered for further testing
  - iCubate 2.0 system & RAZOR EX
  - SmartCycler removed from future testing
    - Wetlab2 Project
- Further requirements developed for technologies to be used in competitive proposal process
Further Research: Microbial Monitoring System

• Platforms will be simultaneously analyzed
  - Quantification AND Identification abilities
  - 20 targeted microbe populations in water samples
  - Culture independent technology

• Quantitative & qualitative matrix developed
  - Science
  - Engineering
  - Functionality
## Further Studies: Quantitative & Qualitative Matrix

<table>
<thead>
<tr>
<th>VOC</th>
<th>CCR</th>
<th>Description</th>
<th>Criteria (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Safety: ensure safety of flight crew, ground personnel, public, flight vehicles, and environment</td>
<td>S: amount of potential hazards produced by the system</td>
<td>Number of hazards</td>
<td>11</td>
</tr>
<tr>
<td>Performance: system can identify target microbes within a sample</td>
<td>P1: ability of system to accurately identify problematic microbes in a sample when present above detection limit</td>
<td>Number of microbes identified; Time to results</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>P2: system uses molecular methods independent of culturing</td>
<td>Number of microbes identified; Time to results</td>
<td>16</td>
</tr>
<tr>
<td>Operability: crew is able to operate system in ambient conditions both on the ground and in the spacecraft</td>
<td>O1: ability of system to operate in ambient conditions both on the ground and in the spacecraft</td>
<td>Number of environmental conditions met</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>O2: ease of use for operator</td>
<td>Number of steps operator performs</td>
<td>19</td>
</tr>
<tr>
<td>Functionality: system is physically capable of performing required functions</td>
<td>F1: ability of system to function with minimal resources</td>
<td>Number of functional requirements met</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>F2: ability of system to store and transmit data to crew and ground personnel</td>
<td>Number of software requirements met</td>
<td>13</td>
</tr>
<tr>
<td>Manufacturability: system can be modified for space flight</td>
<td>M: ability of manufacturer to meet requirements</td>
<td>Number of requirements met</td>
<td>9</td>
</tr>
</tbody>
</table>
### Further studies: Quantitative & Qualitative Matrix

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Task</th>
<th>Status</th>
<th>Outcome</th>
</tr>
</thead>
</table>
| **Phase 1** | Define top-level VOCs                          | Complete | 1. Safety  
|           |                                                |         | 2. Performance  
|           |                                                |         | 3. Operability  
|           |                                                |         | 4. Functionality  
|           |                                                |         | 5. Manufacturability |
| **Phase 2** | Prioritize VOCs based on customer input (ISS Office) | Complete | VOCs weighted |
| **Phase 3** | Define Critical Customer Requirements (CCRs)     | Complete | 8 CCRs defined and weighted |
| **Phase 4** | Data collection                                 | In-work | Pending (collecting data for 133 total criteria) |
| **Phase 5** | Analysis using VOC software                     | Awaiting data | Data will be transformed into bins based on weights from MMS team; scores generated by Pugh Matrix method |
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Questions?