International Conference on Environmental Systems

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

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43nd ICES, 14–18 July 2013, Vail, Colorado

DEDICATION

This presentation is dedicated

In honor of

Angela Johnston

Marshall Space Flight Center

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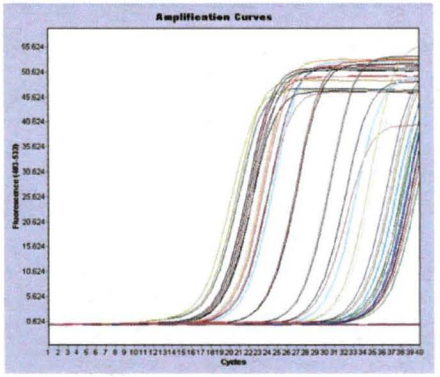
History

- 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

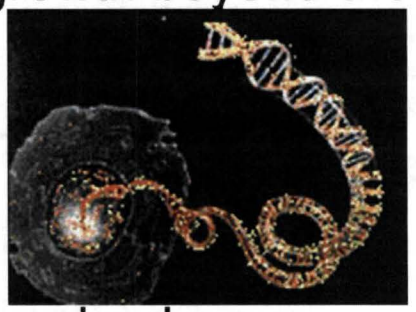
Introduction:

- 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

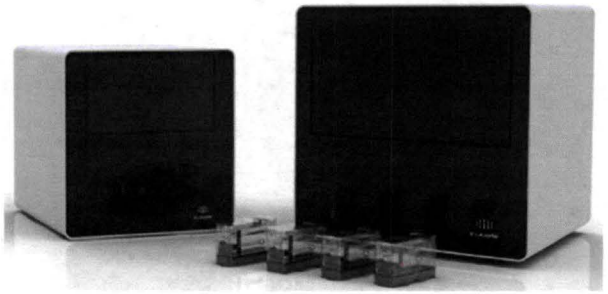
Materials and Methods : Market Survey

- 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

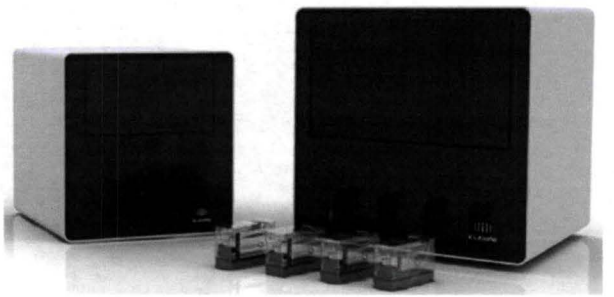


- BioFire, RAZOR EX and Film Array, Salt Lake City, UT - KSC

- Cepheid Smartcycler, Sunnyvale, CA - JPL

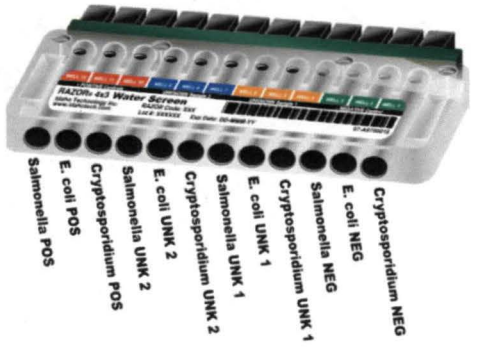
Materials and Methods: Attributes of PCR-based platforms

- 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



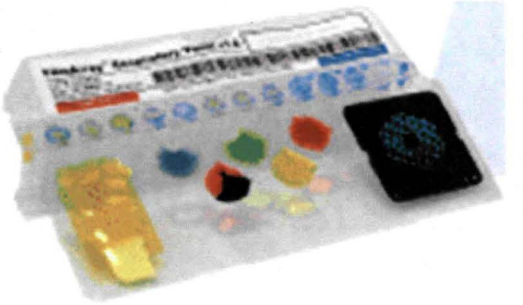
Materials and Methods: RAZOR EX

- 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

Instrument Attribute	iCubate 2.0	RAZOR EX	Film Array	Smartcycler
No. of samples	4 x 12	12	102	16
Volume	40 µl	100 µl	100 µl	1 µl
Size (in)	14 x 15 x 14 & 17 in^3	25.4 x 11.4x19	10 x 15.5 x 6.5	12 x 12 x 10
Weight (lb)	177	11	20	22
Power	Standard	24V 4A power supply & battery	Standard	Standard
Reagents	Pre-loaded cassettes	Pre-loaded pouches	Pre-loaded pouches	Sealed, preloaded SmartTube
Time to answer	6 - 8 h	30 m	30 m	Labor intensive
Sample Type	Raw or DNA	Raw or DNA	Raw or DNA	DNA only

Results: Proof of Concept

Instrument	iCubate 2.0	RAZOR EX	Smartcycler
Salmonella LLOD	1×10^4	1×10^4	1×10^3
Combined culture LLOD	1×10^5	1×10^5	1×10^4
Minimum cells needed per reaction	400	50	94

Discussion & Conclusions:

- Three platforms had capability to detect ≤ 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

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

Further studies: Quantitative & Qualitative Matrix

Milestone	Task	Status	Outcome
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

Acknowledgements

The authors would like to thank
Adesh Singhal – Marshall Space Flight Center, Huntsville, AL
Angela Johnston – Marshall Space Flight Center, Huntsville, AL
Tamra Ozbolt – Emerald City Initiatives, Inc., Grant, AL
Jerry Owens - Marshall Space Flight Center, Huntsville, AL
Daniel Jett – Teledyne Brown Engineering, Huntsville, AL
Darrell Jan – Jet Propulsion Lab & Ames Research Center, CA
Michael Roberts – Consolidated Safety Services, Inc, Dynamac, KSC, FL
Gerard Newsham – ESC-Team QNA, KSC, FL
Megan Morford – NASA, NE-Surface Systems, KSC, FL
Janicce Caro – ESC-Team QNA, KSC, FL
Mary Hummerick – ESC-Team QNA, KSC, FL
Airan Yoets - Enterprise Advisory Services, Inc. (Easi), JSC, Houston, Tx

Research Support Acknowledgements

Research support was provided by

- KSC Research & Technology Review Board CTC Grant for In-flight Monitor.
- Bioastronautics Contract Number NAS9-02078 (JSC).
- Part of the research described in this study was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under contract with the National Aeronautics and Space Administration.

Questions?

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