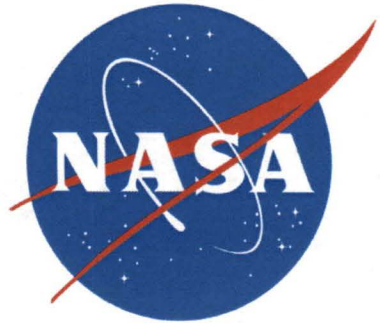
International Conference on Environmental Systems

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

Monserrate C. Roman and Kathy U. Jones
Marshall Space Flight Center
Cherie M. Oubre, Victoria Castro, and C. Mark Ott
Johnson Space Center
Michele N. Birmele
Kennedy Space Center
Kasthuri J. Venkateswaran and Parag A. Vaishampayan
Jet Propulsion Laboratory



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

DEDICATION

This presentation is dedicated

In honor of

Angela Johnston

Marshall Space Flight Center

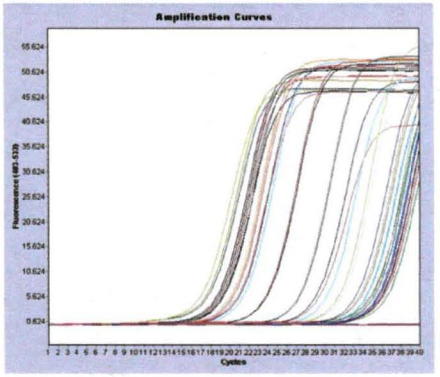
AIAA

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

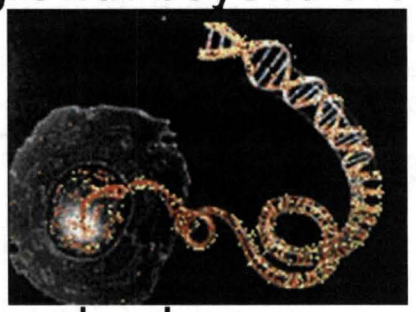
Introduction:

- 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

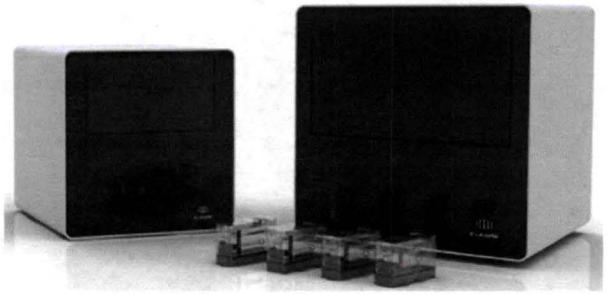
Materials and Methods : Market Survey

- 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

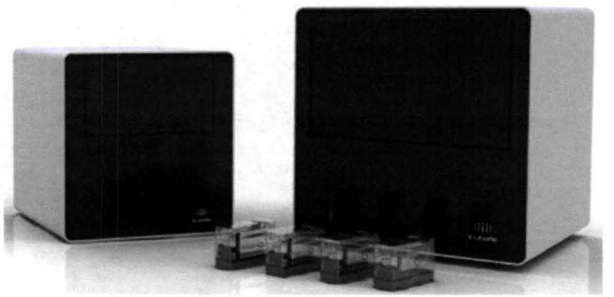


- BioFire, RAZOR EX and Film Array, Salt Lake City, UT - KSC

- Cepheid Smartcycler, Sunnyvale, CA - JPL

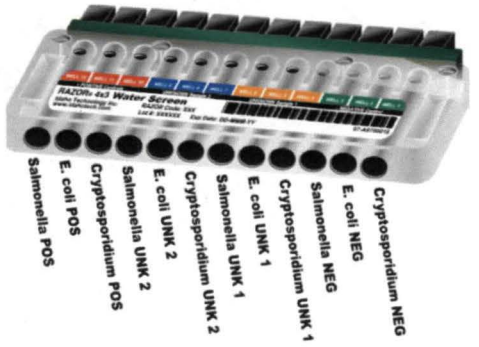
Materials and Methods: Attributes of PCR-based platforms

- 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



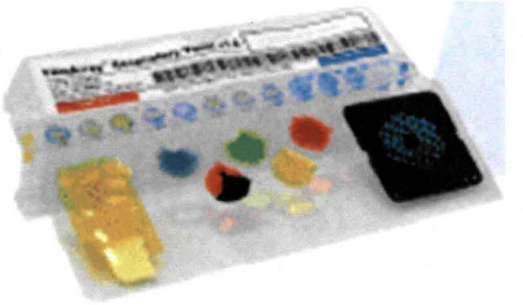
Materials and Methods: RAZOR EX

- 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?

AIAA
The World's Forum for Aerospace Leadership