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Inflight Microbial Monitoring- an alternative method to culture based detection currently used on the International Space Station

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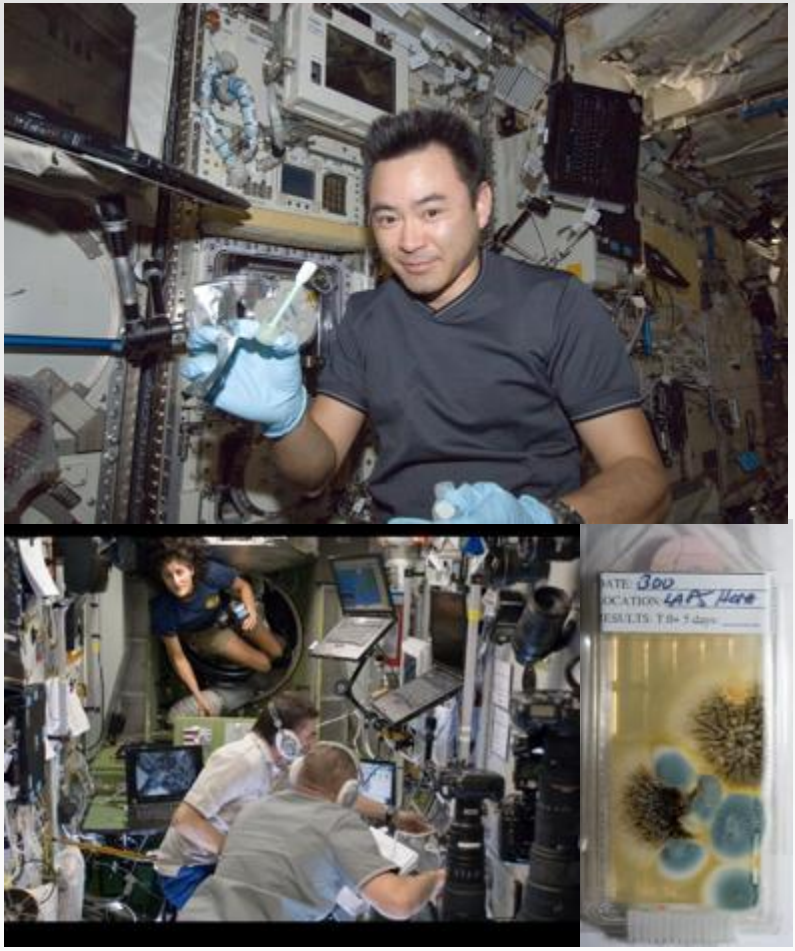
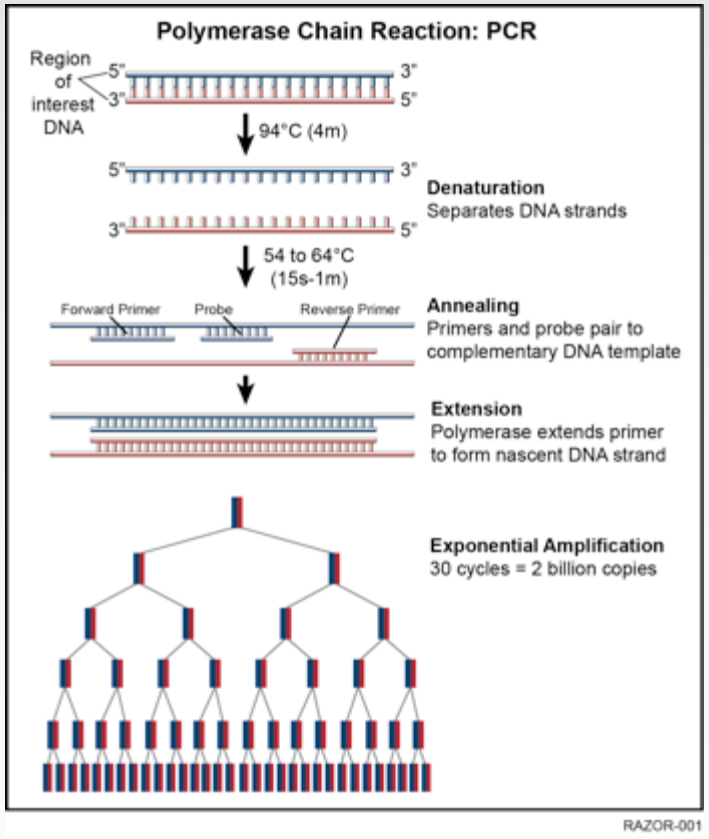
Introduction

- Microorganisms including potential human pathogens have been detected on the International Space Station (ISS).
- The potential to introduce new microorganisms occurs with every exchange of crew or addition of equipment or supplies.
- Current microbial monitoring methods require enrichment of microorganisms and a 48-hour incubation time resulting in an increase in microbial load, detecting a limited number of unidentified microorganisms.
- An expedient, low-cost, in-flight method of microbial detection, identification, and enumeration is warranted.



Pictures courtesy of NASA

- The current approach has sufficiently protected ISS crew members from infection, many subsystems on the ISS have been negatively impacted by microbial biofouling.
- In 2011, microbiologists and other subject matter specialists recommended implementing molecular-based technologies, such as real-time polymerase chain reaction (PCR), to evaluate if qPCR could replace current culture-based technologies.



- The RAZOR EX, a ruggedized, commercial off the shelf, real-time PCR field instrument was tested for its ability to detect microorganisms at low concentrations within one hour.

Goals

- To test the capabilities of a COTS PCR instrument for use as a potential microbial monitoring system.
  - The RAZOR EX® (Biofire Defense, Inc, Salt Lake City, UT) is a compact, lightweight, ruggedized, automated, PCR instrument.
  - Provides rapid microbial identification and relative enumeration without the need to grow or culture microorganisms from samples.

- To customize and test assays for detection of specific microorganisms and determine:
  - Lowest level of detection for targeted organisms.
  - Effects of media, solutions and complex matrices on detection of microorganisms.
  - Whether communities of microorganisms interfere with the detection of each individual type.

Experiments

- **Bacterial detection**
  - *Escherichia coli, Salmonella enterica* Typhimurium*, Pseudomonas aeruginosa, and Enterobacter aerogenes* were cultured and tested independently to determine detection limit for each.
  - A combination of the four bacteria was used to determine competition or interference of multiple organisms co-located in the same media.
  - PBS and culturing media were tested as diluent to determine any effects in downstream reactions.

➢ PCR chemistries

- HybProbe.

  HybProbe and pre-formulated water and food pouches were used to determine the low level of detection (LLOD) for *Salmonella enterica* Typhimurium in both water and food samples.

- TaqMan.

  Allowed for the development of customized species specific assays. Isolated RNA also tested

Table 1. Target microorganisms and genes selected for detection on RAZOR EX TaqMan System

Target Microorganism	Gene	Amplicon (bp)	Reference
Salmonella enterica Typhimurium	*invA*	119	Hoofar, et al., 2000[3]
Escherichia coli K-12	*uid*A	84	Frahm, E. and Obst, U., 2003[4]
Pseudomonas aeruginosa	*gyr*B	67	Lee, et al., 2011[5]
Enterobacter aerogenes	*kpc*	184	Swayne, R. L. et al., 2010[6]
Universal bacterial primers	16S	123	Suzuki, et al. 2000[7]

HybProbe

S. enterica typhimurium. (10^8-10^2)



Food and water specific pouches

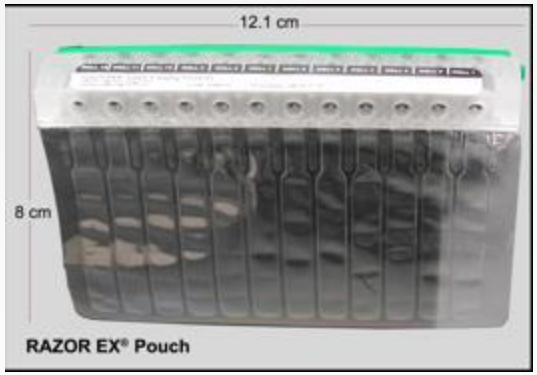
TaqMan

E. coli, S. enterica,

P. aeruginosa, E. aerogenes,

4 combined

(10^8-10^2)



Custom pouch

Results

- HybProbe:
  - Salmonella was detected in 100% of samples when cell number was ≥ 500 cells mL^{-1} in food and water samples.
  - Detection level in mixed culture was significantly higher; 10,000 cells mL^{-1}

Table 2. Lower limit of detection (LLOD) for *Salmonella* on the RAZOR EX HyBProbe Microbial Monitoring System

Salmonella Cells/reaction	N	% Detected in Replicate Assays
0		ND*
1,000	4	100
100	18	100
50	2	100
25	2	50
10	18	56
1	12	8

➢ TaqMan:

- All bacteria with the exception of *E. aerogenes* were detected when cell number was $\geq$ 1000 mL^{-1}
- All genes tested were specific to the microorganism and no cross amplification with other organisms was evident.
- PBS and broth media interfered with the PCR reaction with both HybProbe and TaqMan chemistries.

Table 3. PCR results of serial diluted cultures for the 4 targeted microorganisms diluted in sterile water. Template was 100 µl of each concentration run in duplicate in each pouch. Each culture was prepared in triplicate. N=no amplification detected, Y=amplification did occur prior to the last 5 PCR amplification cycles, NT = not tested.

Microorganism	**Concentrations – Serial dilutions in sterile water (100 µl*0.667)**						
100 µl in 150 µl volume	1.0E+01	1.0E+2	1.0E+03	1.0E+04	1.0E+05	1.0E+06	1.0E+07
Salmonella enterica Typhimurium	N	Y	Y	Y	Y	NT	NT
Pseudomonas aeruginosa	N	Y	Y	Y	Y	NT	NT
E. coli K12	N	Y	Y	Y	Y	Y	Y
Enterobacter aerogenes	N	N	N	N	N	N	N

Trade study

- Voice of the customer trade study indicated favorability of the RAZOR EX system when compared to other COTS systems based on safety, engineering and capabilities.

Instrument	VOC Total Score
RAZOR EX	37.24
iCubate	21.04
Cepheid Smartcycler	20.53
LOCAD	17.42

Table 4. Total VOC Critical Customer Requirements (CCR) scores for the top 4 COTS instruments considered for microbial monitoring qPCR instruments.

RNA detection

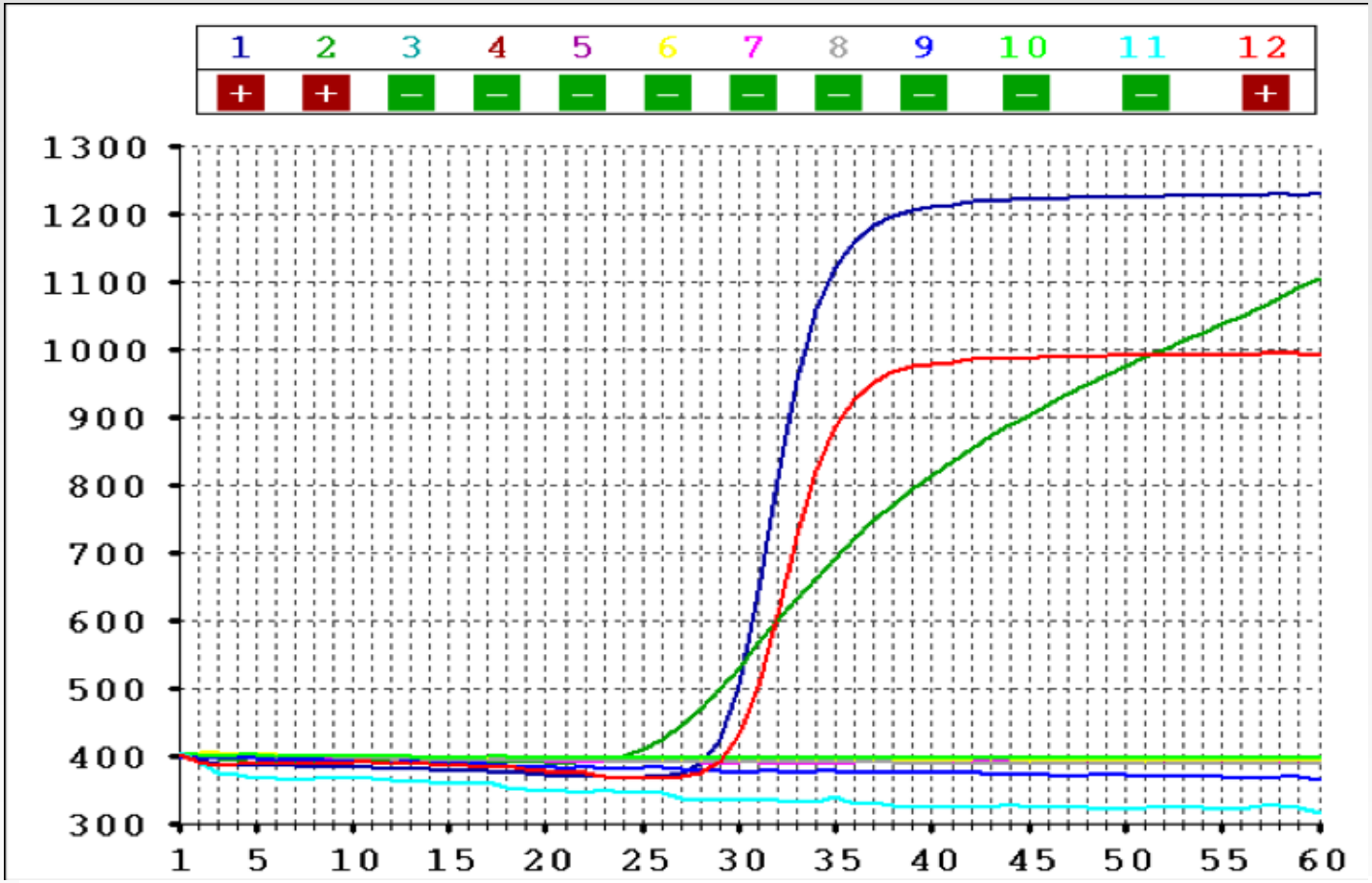


Figure 1. RAZOR EX reverse transcription reaction for *Salmonella*. Positive control (10 ng RNA) is red, L12; negative control, light blue in L11; Lanes 1 & 2, dark blue and green are different concentrations of the RNA template.

Summary

- This work demonstrates the ability of the RAZOR EX PCR platform to detect target microorganisms in a variety of samples.
- An in-flight microbial monitoring system such as RAZOR EX would enable rapid assessment of the microbial environment on ISS with minimal sample prep and enrichment.

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Questions?