



Viability Retention of *Yersinia pestis* Cells in Environmental Samples



TECHNOLOGY DRIVEN. WARFIGHTER FOCUSED.

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Overview



- Motivation
- Background
- Experimental Design
- COTS Screening
- Results
- Sampling from Concrete Blocks
- Genomic DNA Integrity
- Conclusions and Future Direction



Motivation



- Following a BW release, biological sampling from the environment is critical to map contaminated zones and to assess the effectiveness of decontamination process
- In the field, the samples often are stored under non-ideal conditions, resulting in a rapid loss of viability, especially Gram-negative vegetative cells, rendering lab analyses inconclusive
- Two key objectives of this study were as follows:
 - **Can viability of *Yersinia pestis* cells be prolonged under storage at non-permissive temperatures, i.e. (40 or 37 °C)?**
 - **Do non-viable cells of *Y. pestis* retain genomic integrity stored under non-permissive temperatures?**



Background



- *Yersinia pestis* is the etiological agent of plague, which can manifest as bubonic, septicemia, or pneumonic forms
- Three historical pandemics have been caused by this agent, and even in current times, the pathogen causes several thousands of human cases each year world-wide
- Cells are non-motile, Gram-negative, are coccobacillus
- Cells contain three plasmids – pPCP1, pCaD, pMT1, in addition to a 4.38-mbp size chromosome
- Under lab conditions, at low temperature, cells are known to enter in 'viable but non-culturable' state in water



Experimental Design



1. Viability Assessment

- Grow *Y. pestis* (A1122) in BHI broth, and wash cells thoroughly (3x) washes in Butterfield buffer
- Suspend washed cells in one of the 15 test solutions (commercial-of-the-shelf) marketed for clinical samples
- Store each cell suspension at 4, 22, and 40 (or 37) °C
- Assess viability of aliquots at 0, 1, 3, 7, and 14 days after storage
- Count colony-forming units (CFUs)

2. Sampling from Concrete and Steel Surface

- 1-ml of *Y. pestis* broth (grown for 48 hours) spiked on 2x2-inch painted concrete blocks
- Cells sampled from surface (CDC method) using Puritan foam-tipped applicators
- Cells stored at non-permissive temperatures and viability assessed



Experimental Design



3. Genomic DNA Integrity

- Non-viable samples (at 14-day after storage) extracted for genomic DNA
- Real-time PCR performed using 96-well FastBlock format on the Applied Biosystems 7900HT
- Three genomic targets, YPT-1FB-K, YPT-2FB-K, and YPT-4FB-K, were amplified using DBPAO reagents
- Total run 45 cycles
- Samples deemed positive if Ct value - 15-35



COTS & Experimental



	Preservation System	Manufacturer	Cat #
1	Butterfield's Phosphate Buffer	U.S. FDA Formulation	-
2	All-In-One Sample Collection Swab	QuickSilver Analytics, Inc.	10193
3	Biomatrix [®] Custom Form. #1	Biomatrix [®] , Inc.	-
4	Biomatrix [®] Custom Form. #2	Biomatrix [®] , Inc.	-
5	BBL [™] CultureSwab [™]	Becton Dickinson & Co.	220099
6	BD ESwab	Becton Dickinson & Co.	220245
7	Buffered Peptone Water	Sigma-Aldrich Co., LLC	77187
8	Buffered Peptone Water	Sigma-Aldrich Co, LLC	77187
9	Copan ESwab [™]	Copan Diagnostics, Inc.	480C
10	Copan Swab-Rinse-Kit (SRK)	Copan Diagnostics, Inc.	R4160
11	Puritan [™] Liquid Amies kit	Puritan Medical	LA-116
12	Remel Sanicult [™] Transport	ThermoFisher Scientific, Inc.	R723140
13	Skim Milk (Filtered)	Cloverland Farms Dairy	-
14	Spent Tryptic Soy Broth	ThermoFisher Scientific, Inc.	R112731
15	Tryptic Soy Broth (diluted 1/50)	ThermoFisher Scientific, Inc.	R112731
16	Tryptic Soy Broth (dil 1/100)	ThermoFisher Scientific, Inc.	R112731
17	Tryptic Soy Broth (dil 1/1000)	ThermoFisher Scientific, Inc.	R112731

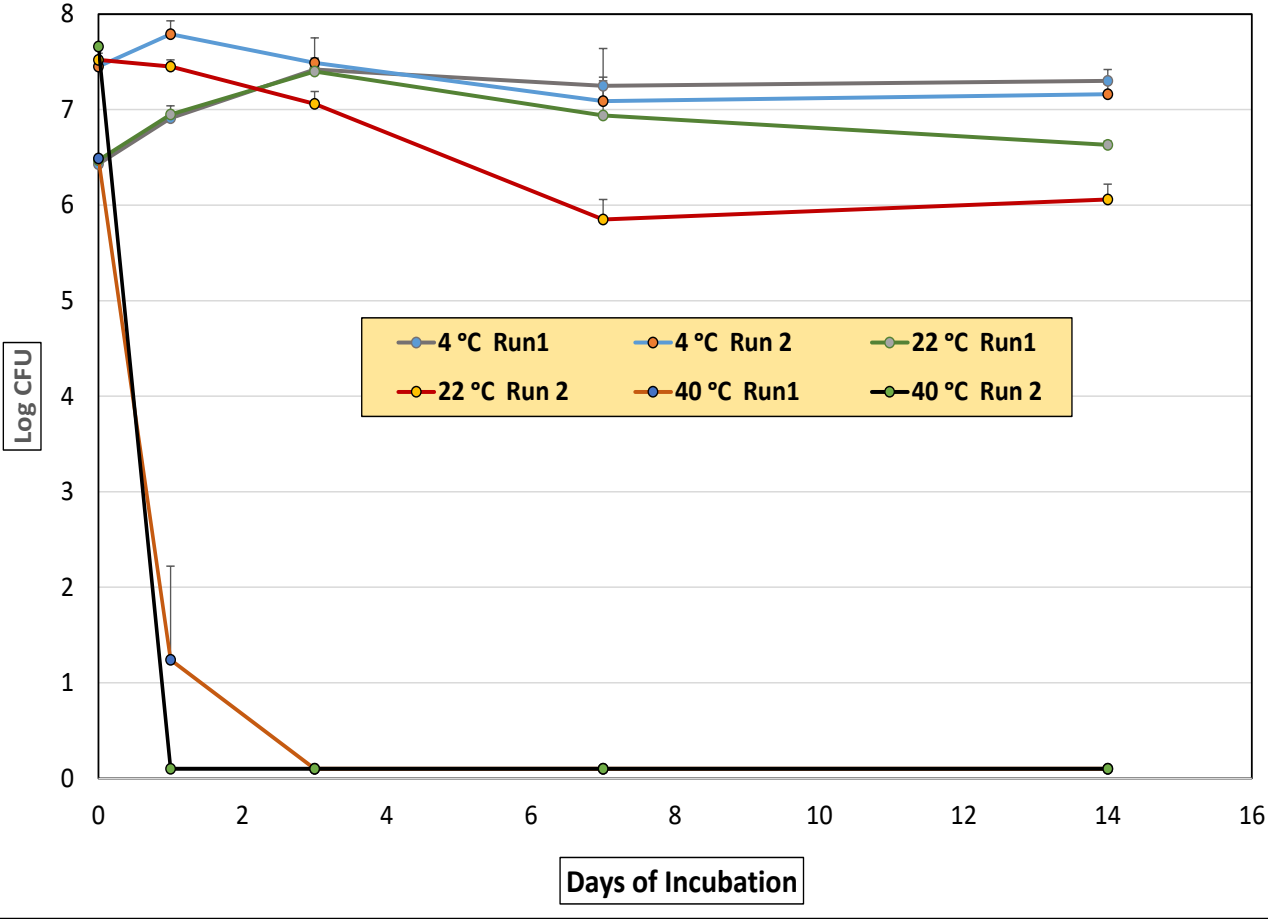
- None of the tested material sustained viability at 40 °C
- Four of tested material sustained viability at 37 °C



Results - Butterfield



Yersinia pestis A1122 Cell Numbers in Butterfield Solution over 14 days at Three Temperatures



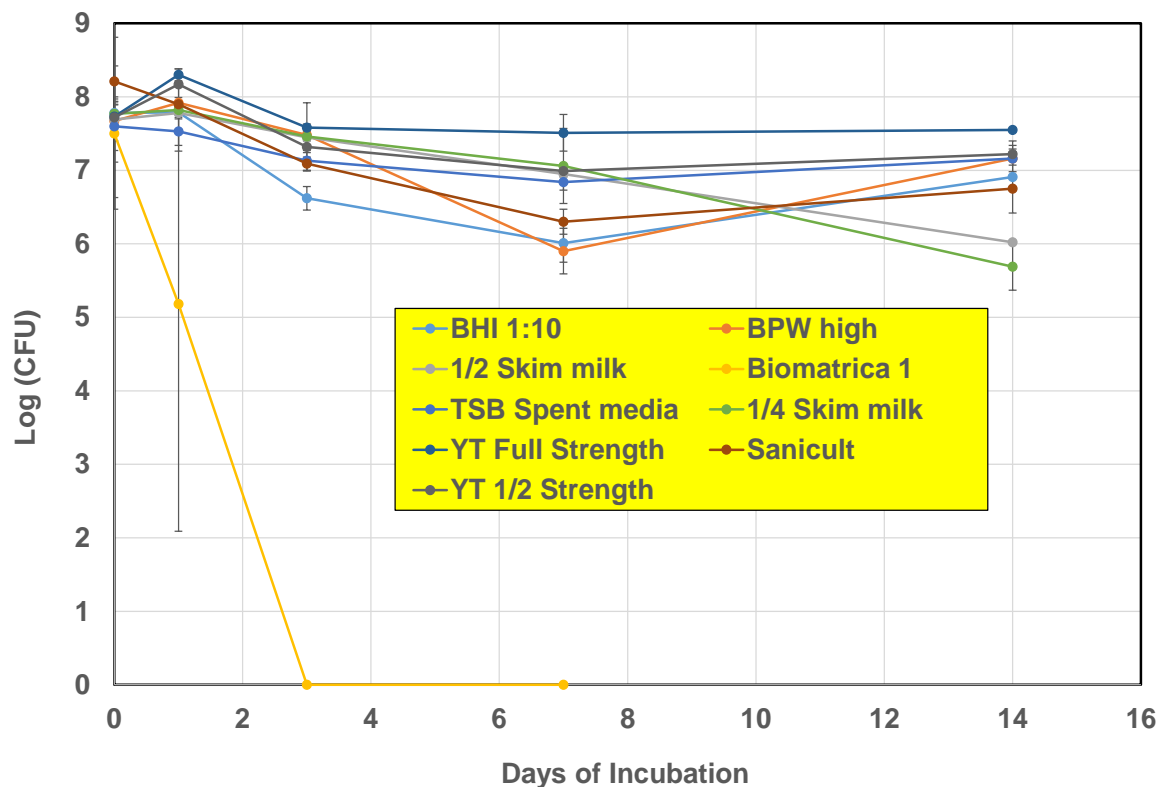
- Viability was retained for over two week period at low and room temperatures
- Viability was lost within 3-4 days at 40 °C



Results – 37 °C



Yersinia pestis A1122 Cell Numbers over a Two week Period at 37 °C



- Viability was retained for over two week period at 37 °C in YT, skim milk, spent TSB media, and diluted BHI
- Viability was lost at 37 °C within 2-3 days in commercial Biomatrica 1



Results – Summary

Preservative	# Runs	4°C - 7d	22°C - 7d	40°C - 7d	4°C - 14d	22°C - 14d	40°C - 14d	35°C - 7d	35°C - 14d	37°C - 7d	37°C - 14d
All-in-One Swab Kit	2	Green	Green	Red	Green	Green	Red				
BBL Culture Swab	2	Green	Green	Red	Green	Green	Red				
BD Eswab	2	Green	Green	Red	Green	Green	Red				
Biomatrica #1	1									Red	Red
Biomatrica #2											
BPW (5g/L) - low	2	Green	Green	Red	Green	Green	Red				
BPW (20g/L) - high	2	Green	Green	Red	Green	Green	Red	Green	Green	Green	Green
Butterfield's Buffer	2	Green	Green	Red	Green	Green	Red				
Copan Eswab	2	Green	Green	Red	Green	Green	Red				
Copan SRK	1	Don't have 7 day data		dead at 3 day		Yellow	Red				
Puritan Liquid Amies	2	Green	Green	Red	Green	Green	Red				
Sanicult Transport Swab	2	Green	Green	Red	Green	Green	Red	Green	Green	Green	Green
TSB (1/50)	1	Green	Green	Red	Green	Green	Red				
TSB (1/100)	1	Green	Green	Red	Green	Green	Red				
TSB (1/1000)	1	Green	Green	Red	Green	Green	Red				
BHI 1:50	1	Green	Green	Red	Green	Green	Red				
4% BPW	1	Green	Green	Red	Green	Green	Red				
BHI spent media	1			Red			Red				
1/2 Skim milk	1			Yellow			Red	Green	Green	Green	Green
TSB Spent media	1			Red			Red	Green	Green	Green	Green
1/4 skim milk	1			Red			Red	Green	Green	Green	Green
YT Full Strength	1			Red			Red	Green	Green	Green	Green
YT 1/2 strength	1			Red			Red	Green	Green	Green	Green
BHI 1:10	1							Green	Green	Green	Green
TSB 1:10	1							Yellow	Red		

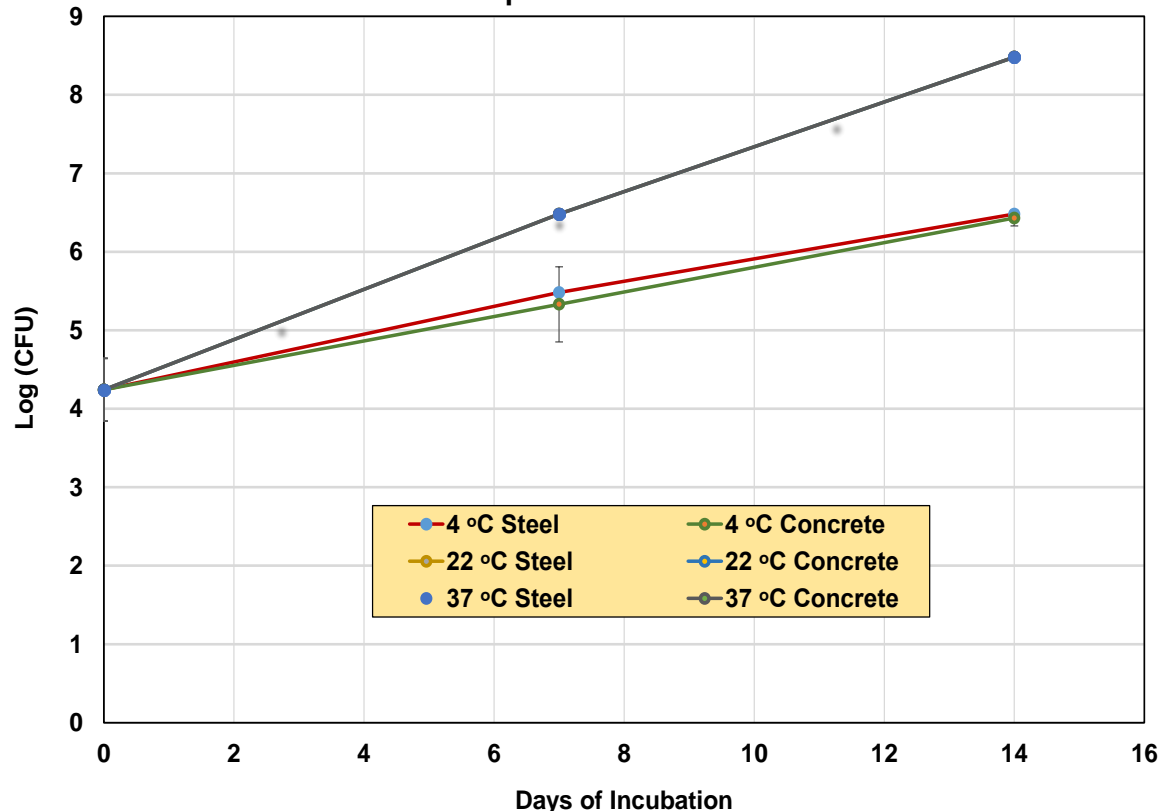
Key
>3-Logs
<3-Logs
No Viability



Results – Sampling



Yersinia pestis A1122 Cell Numbers over Two Week Period at three Temperatures in 1/2 Skim Milk



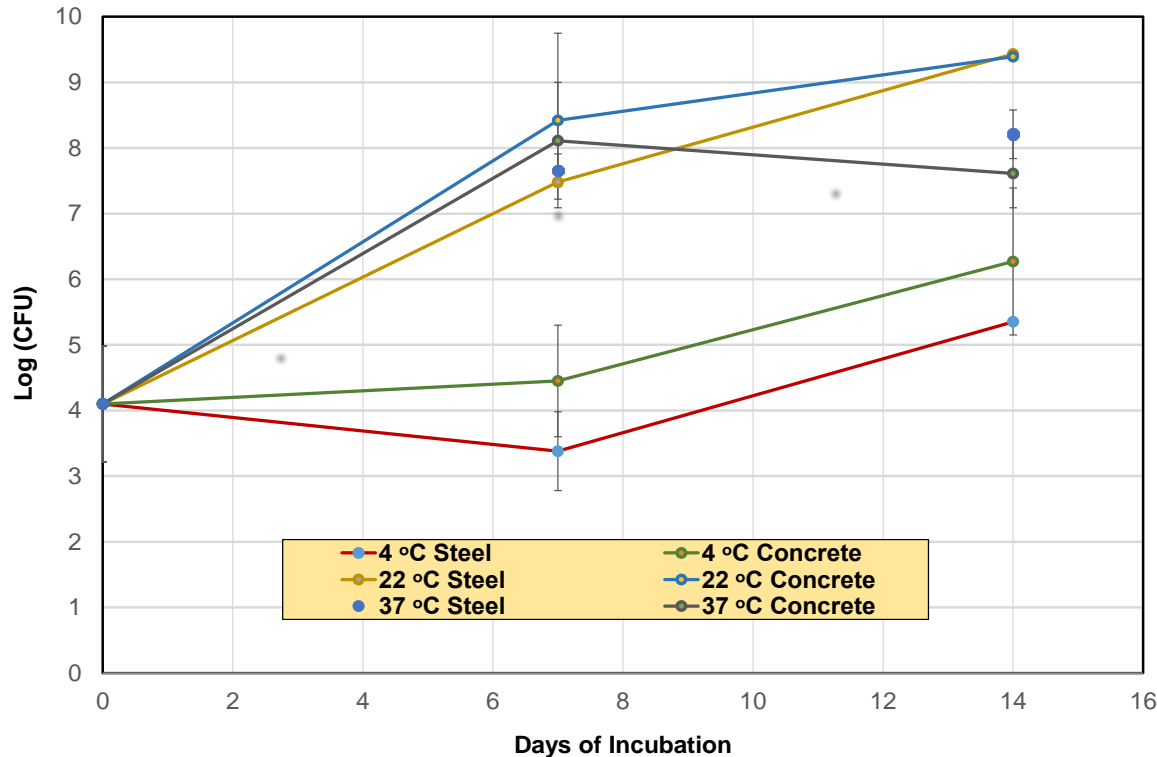
- *Y. pestis* cells sampled from concrete and steel surfaces retained viability after drying and storage in 1/2 strength skim milk
- Cells appear to grow most rapidly at 37 °C over a two-week period

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Sampling – Arizona Dust



Yersinia pestis A1122 Cell Numbers over Two Week Period at Three Temperatures in 1/2 Skim Milk (Cells mixed with Arizona Dust)



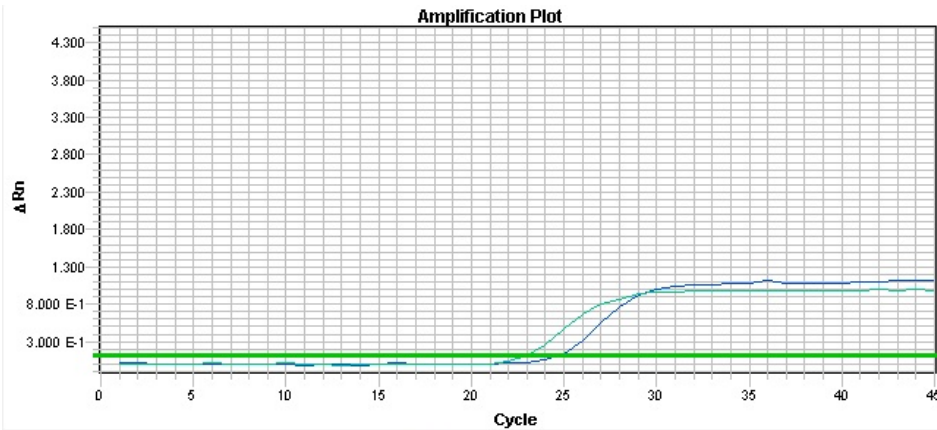
- Arizona dust appear to not affect the viability retention after sampling off steel and concrete surfaces
- Cells appear to grow at most rapidly at 37 °C over a two-week period



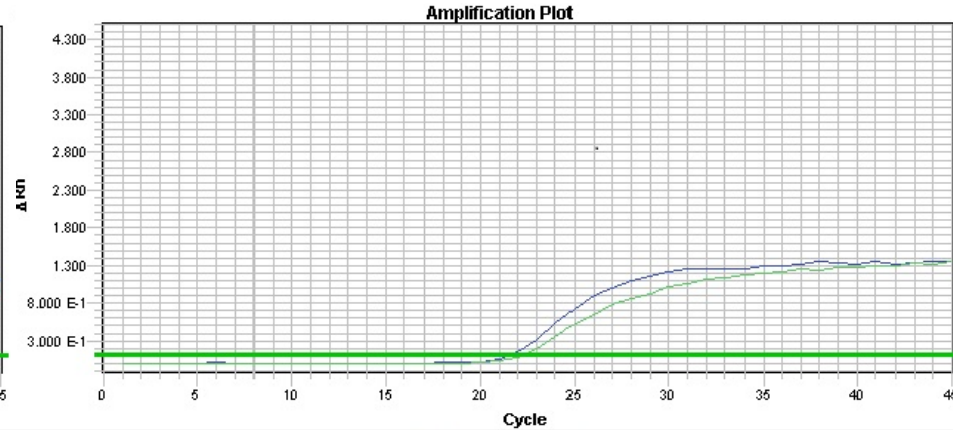
Genomic DNA Integrity



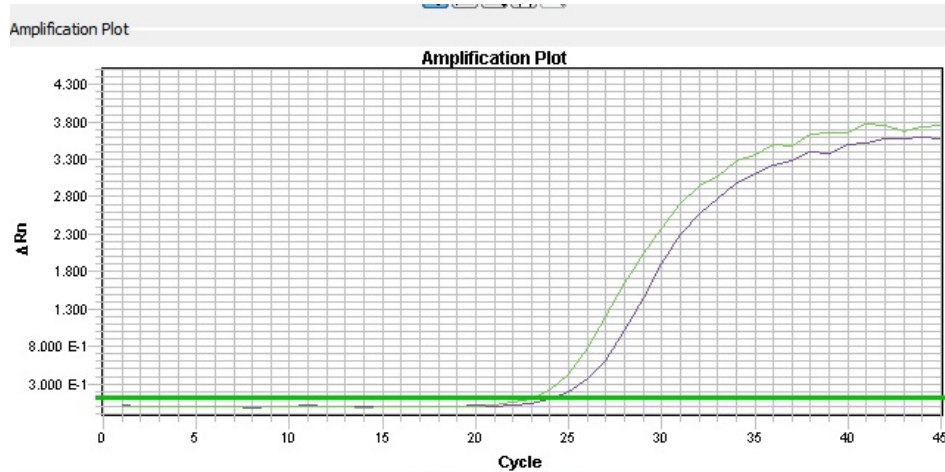
• Target 1



• Target 2



• Target 4



- ALL three targets amplified from genomic DNA from ALL non-viable samples
- Ct value was between 25-30, indicating recovery of high genomic DNA from non-viable cells



Conclusions



- *Y. pestis* cells lose viability when stored at 40 °C in all commercial and experimental solutions tested
- The cells retained their viability at 37 °C in spent TSB, skim milk, and YT media
- All non-viable samples retained integrity of genomic DNA, as evidenced by PCR amplifications of three targets
- Cells sampled from concrete and steel surface retained their viability, irrespective of presence or absence of Arizona dust
- Cells appear to grow at 22 and 37 °C in skim milk and other tested media



Future Work



- Screen viability retention of other vegetative BW agent cells in spent media and other solutions at 37 and 40 °C
- Screen other environmental surfaces to assess sampling and storage of sampled cells at non-permissive temperatures
- Screen viability retention of pathogenic counterparts of *Yersinia pestis*, *Burkholderia mallei*, *B. pseudomallei*, and *Fransicella tularensis*, from surfaces for storage under non-permissive temperatures



Credits



BioDefense Branch

Daniel Angelini, Ph.D. -	Co-PI
Lisa S. Smith, M.S. -	Analyst/Performer (now with U.S. EPA)
Jackie Harris, B.S. -	Performer

Excet, Inc.

Savannah Hurst, M.S. -	Performer
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ORISE

Laura Burton, B.S. -	Performer
Pooja Rastogi, B.S. -	Performer

DTRA (Funding)

Kristen O'Connor, Ph.D. -	PM
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