

Yeast Cells as a Candidate Reference Material to Support Training in On-Site Biological Agent Sampling and Detection

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NIST
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MATERIAL MEASUREMENT LABORATORY

Acknowledgement and Disclaimers

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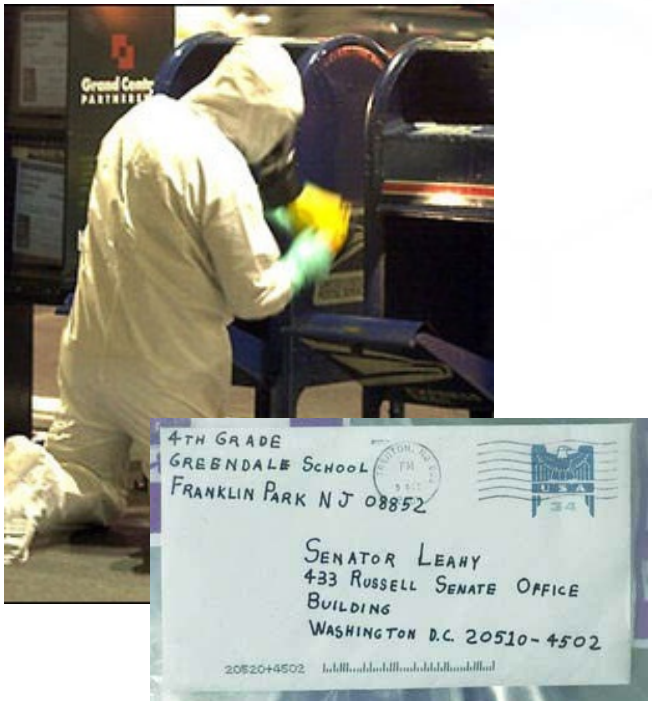
- *Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendations or endorsement by NIST, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.*

“The biological threat is real and growing…”

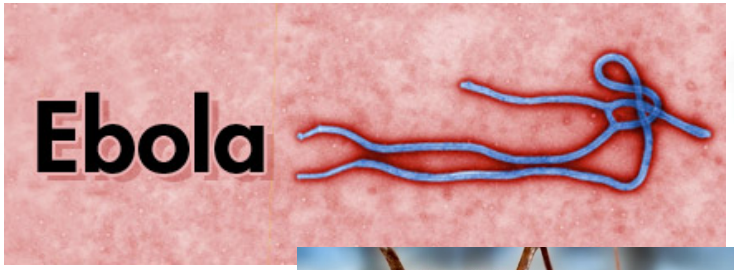
A National Blueprint for Biodefense, Oct. 2015

Bipartisan Report of the Blue Ribbon Study Panel on Biodefense

Malicious intent



Benign human activity or simple change of nature



Standards to Support Field Biological Agent Detection

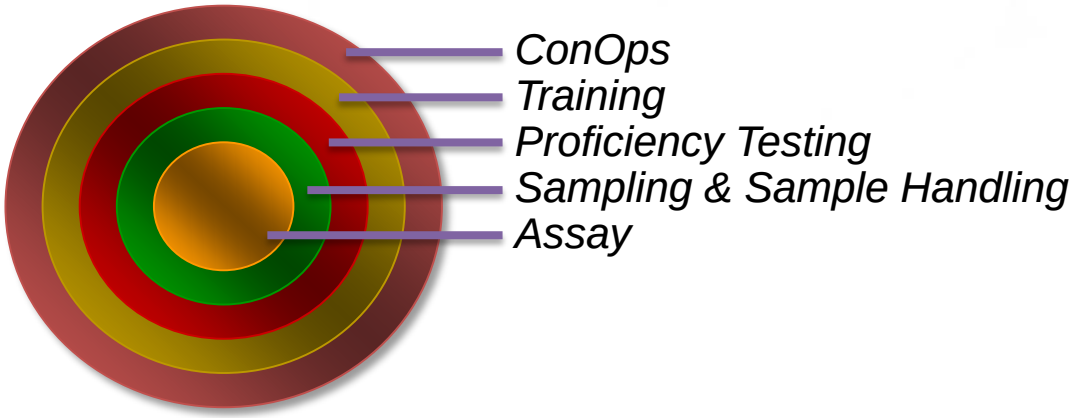
DHS S&T – NIST Interagency Agreement

Goal: Develop standards and methods to support field biothreat detection and biosurveillance

- Microbial reference materials for training
- Methods, metrics and standards to characterize biological test materials
- Documentary standards to support field response mission capability

Impact: Increasing confidence in field results and improving National ability to detect and respond to suspected biological incidents

Components of a Biothreat Field Response Capability



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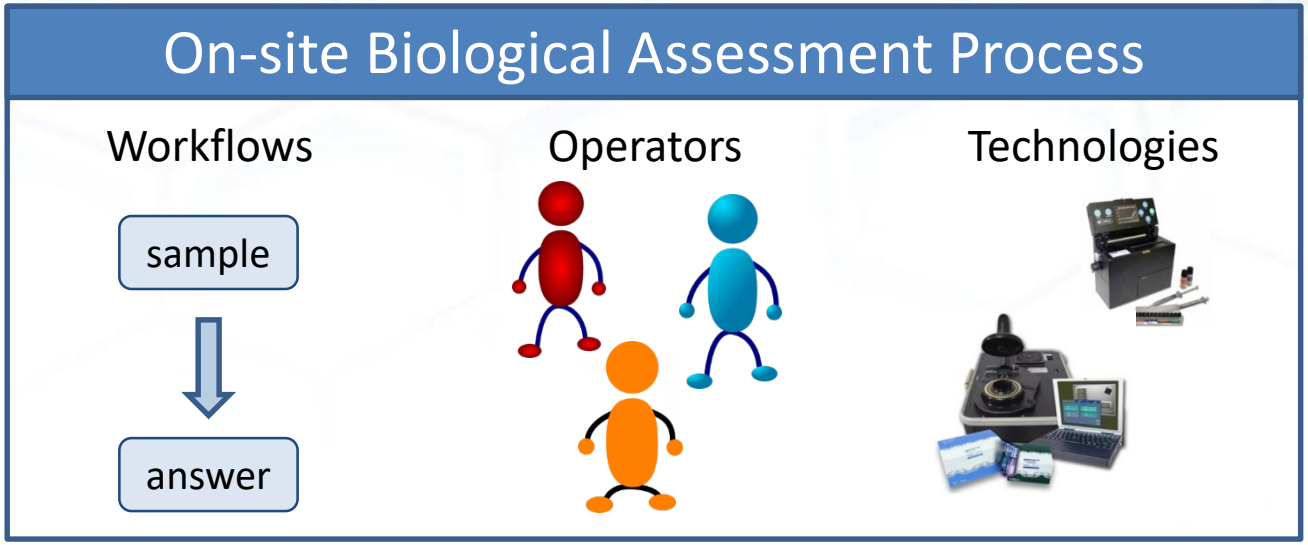
Poster by Nate Olson

"Challenging a bioinformatic tool's ability to detect microbial contaminants using *in silico* whole genome sequencing data."

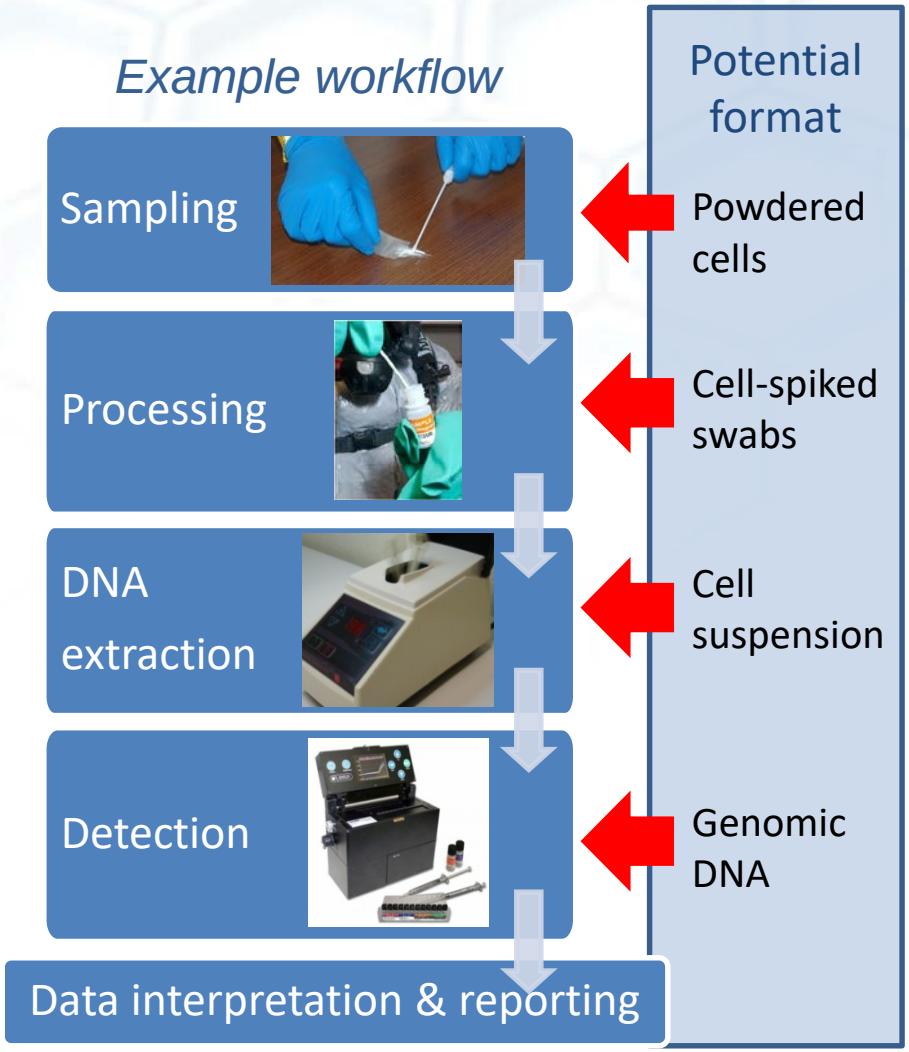
Most Training Uses Biothreat Agents or Near Neighbor Organisms

Surrogates Needed: Non-threat, biological materials

Evaluate, challenge, and establish confidence in biological assessment in the field



Format of a Surrogate Material



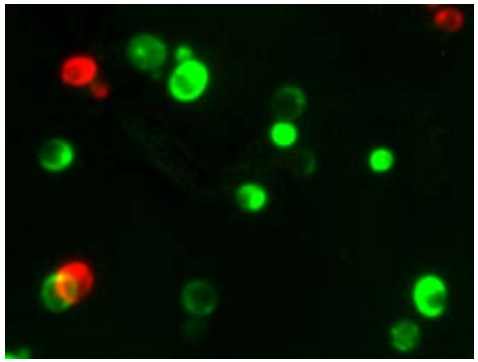
Desirable Properties

- Well-characterized, quantified
- High concentration (>10^7 cells)
- Long (multi-year) shelf-life
- Stable at 25 °C or 4 °C
- Amenable to powder formation, aerosolization, etc.
- Low cost to end users
- Inactivated?

Objective

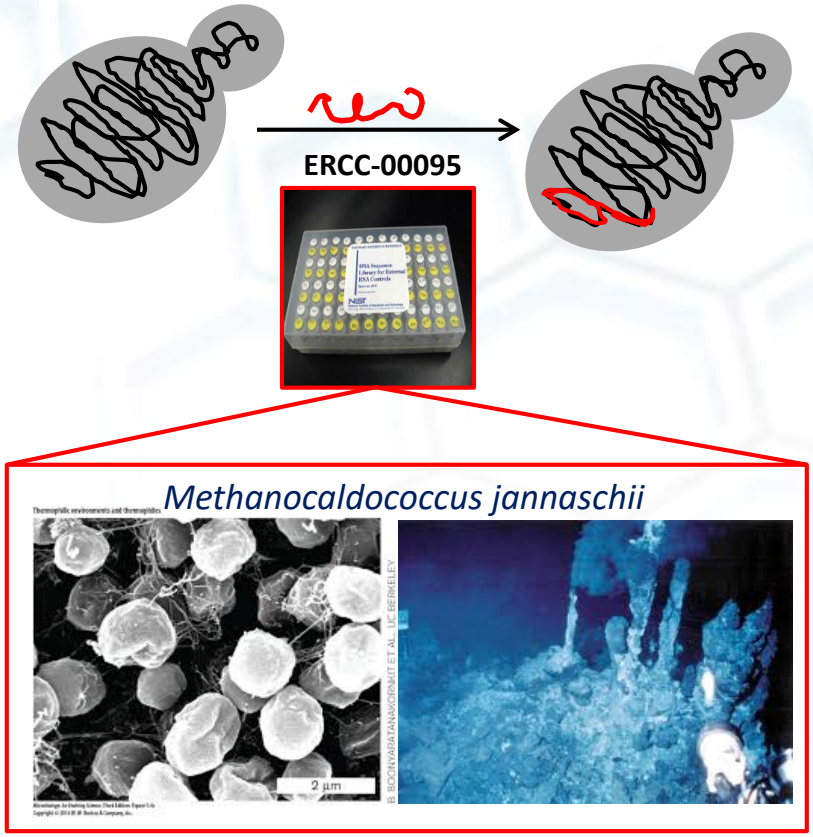
Develop, characterize, and demonstrate modified yeast cells as a surrogate for biothreat agents

- Stable, versatile whole cell material
- Quantification of total cells for DNA-based detection
- Relevant protocols that enable users to expect positive detection



Develop: Modified *Saccharomyces cerevisiae* NE095

- Designed to challenge nucleic-acid based detection technologies
- Procured lyophilized yeast pellets from Microbiologics, Inc.
  - Verified no PCR inhibition due to lyophilization matrix



*External RNA Controls Consortium (ERCC)

- *External RNA Controls Consortium (ERCC) DNA sequences are part of NIST Standard Reference Material (SRM) 2374: DNA Sequence Library for External RNA Controls.*
- *ERCC-00095 corresponds to the latter three (of eight total) open reading frames in the phosphate specific transport complex component of M. jannaschii.*

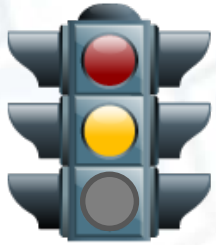
Lyophilized *S. cerevisiae* NE095

Minimized real and perceived risk

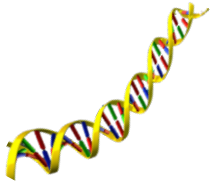
Readily available, can use almost anywhere

No false positives in real events from equipment contamination

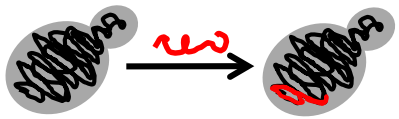
Indicator of a broken process



Low DNA extraction efficiency to challenge the process

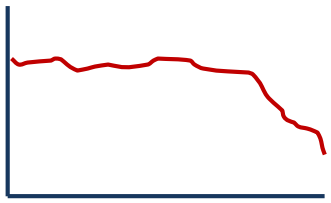


DNA target eliminates environmental false positives



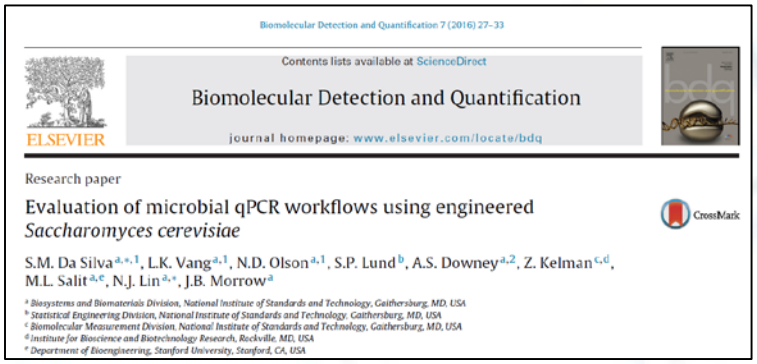
Lyophilized yeast can be crushed into a powder

Quantitative material can track performance



Initial Studies Demonstrated Successful Detection by Potential End Users

- Interlaboratory study with potential end users detected yeast cells in suspension*
- 4th CST confirmed DNA extraction from the yeast via multiple methods


Biomolecular Detection and Quantification 7 (2016) 27–33

Contents lists available at ScienceDirect

Biomolecular Detection and Quantification

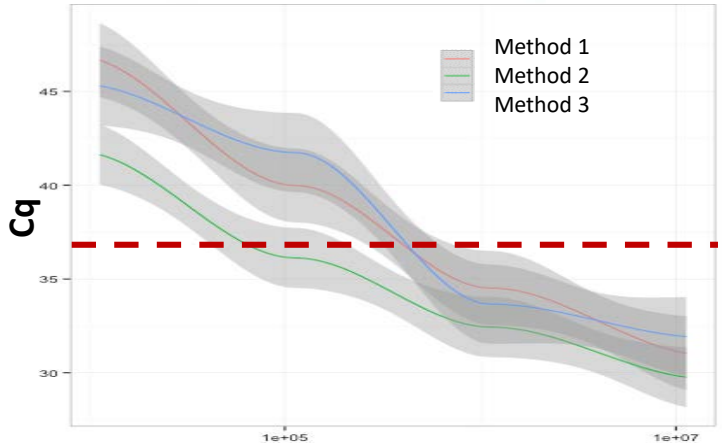
journal homepage: www.elsevier.com/locate/bdq

Research paper

Evaluation of microbial qPCR workflows using engineered *Saccharomyces cerevisiae*

S.M. Da Silva, L.K. Vang, N.D. Olson, S.P. Lund, A.S. Downey, Z. Kelman, M.L. Salit, N.J. Lin, J.B. Morrow

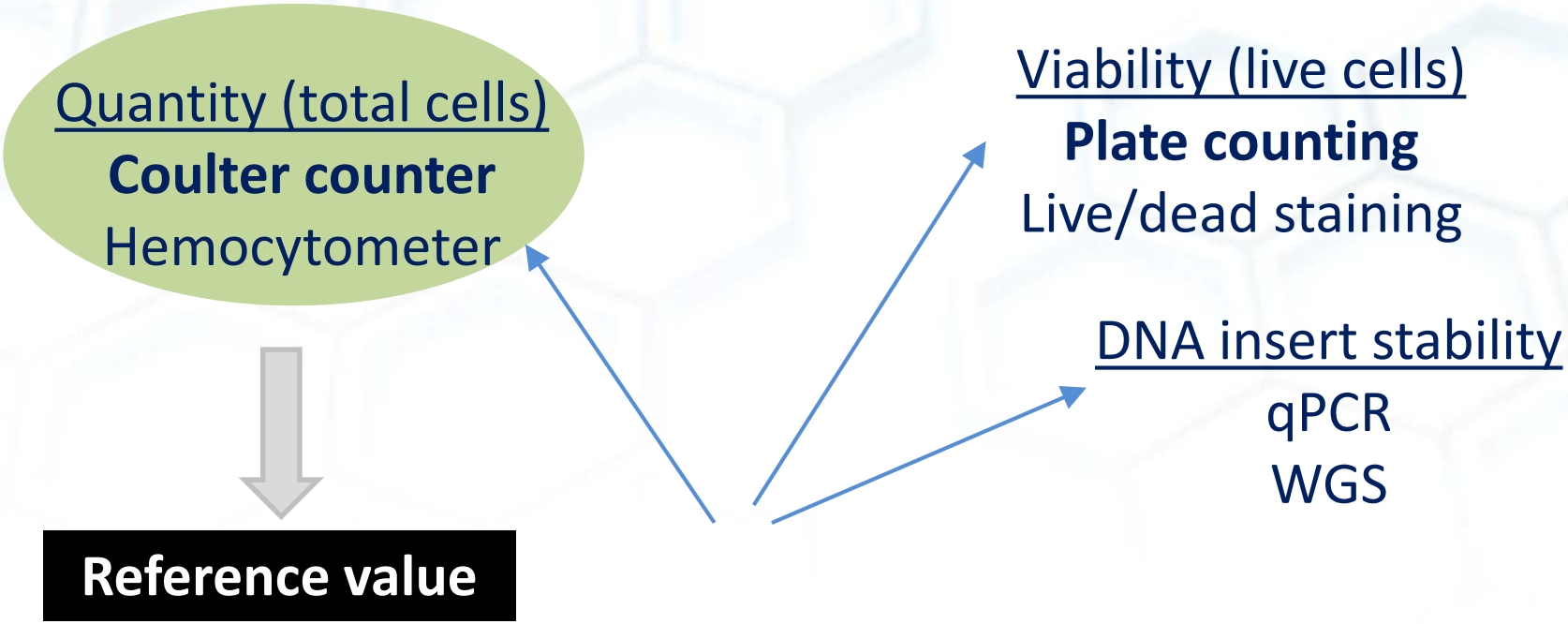
Da Silva et al, Biomol Detect Quant, 2016



Interlaboratory study participants

- *Florida Dept. of Health*
- *Michigan Dept. of Community Health*
- *Minnesota Dept. of Health*
- *New York Department of Health*
- *Washington Dept. of Health*
- *4th Civil Support Team (CST), Georgia Army National Guard*

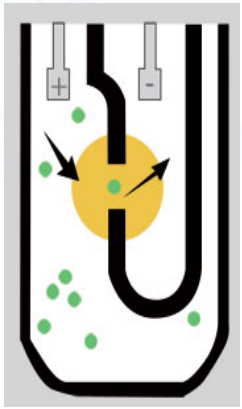
Characterize: Quantify Yeast Cells



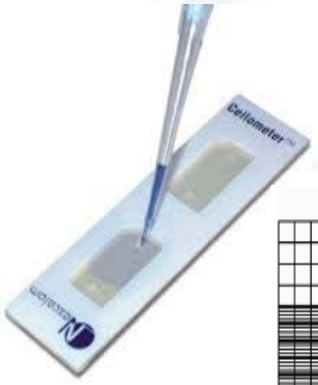
- Homogeneity
- Stability
- Fitness for Purpose

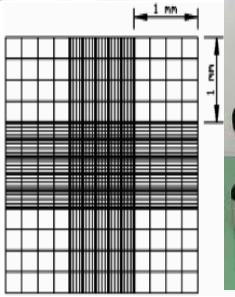
Total Cells per Vial

Coulter Technology



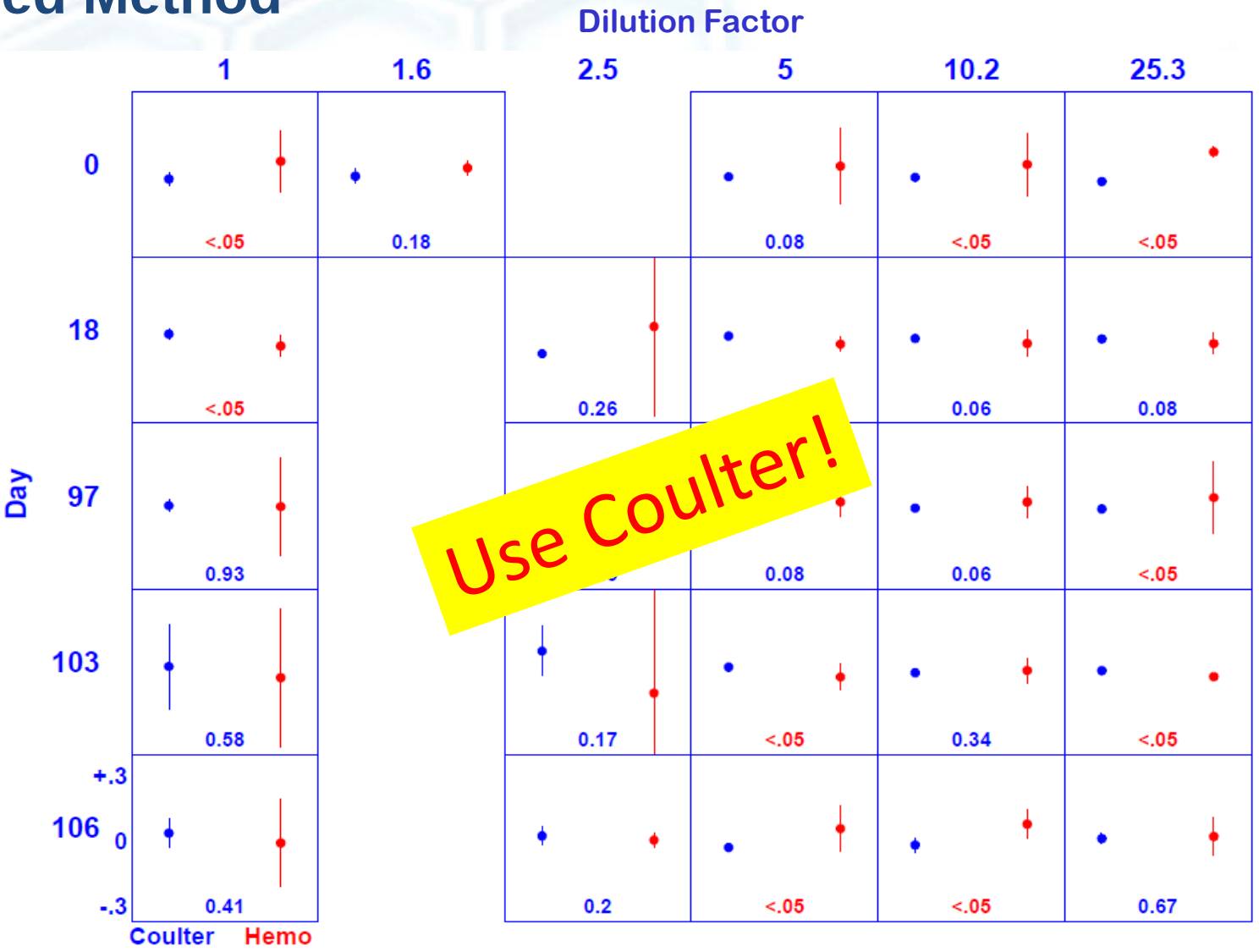
Imaging-Based





No significant difference in the mean values from the two methods.

Significantly Higher Standard Deviation for Imaging-Based Method



Characterization of Prototype Batch

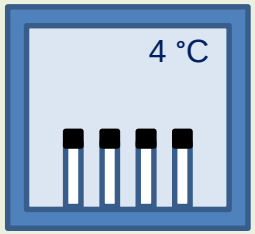
Homogeneity

Measurement	Cells per vial x 10^7	Vials	Reps
Total cells (Coulter)	3.81 ± 0.51 (13.3 %)	28	2
Viable cells (Plating)	0.095 ± 0.018 (18.9 %)	14	1

Viability = 2.50 ± 0.58 %

Real-time stability (>2 years)

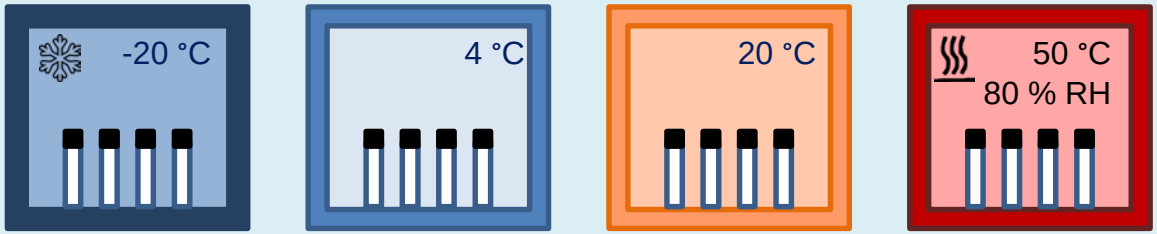
Time (d) 0 178 365 472 758



Total and viable cell number unchanged

Accelerated stability (~4 months)

Time (d) 0 26 61 117



Total cell number unchanged

At 50 °C, viability drops to ≈ 0

Demonstrate: Operation Vigilant Sample IV – July 2015

- Sample collection and biological detection exercise conducted in real-time
- Designed to help define a national exercise template for NGB CST Commanders
- Obtained EPA approval to use the yeast
- Led by CPT Bryon Marsh, 4th CST
  - 4th and 48th NGB CSTs
  - FL, GA Dept. of Health (CDC LRN)
  - BioWatch (DHS OHA)
  - EPA
  - Local responders

Yeast Incorporation into the Exercise

Day 1

On-site yeast prep by CSTs

Day 2

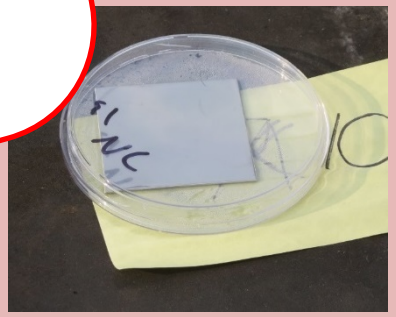
Team assembly and JIT training

Plume model

Yeast successfully detected.

Day 3

Sample Placement



Sample collection

Decon and chain of custody

On-site DNA extraction and qPCR

On-site results

Ongoing Activities

Yeast as a surrogate powder

- LOD study with PHLs - data being analyzed
- Interlab study on sampling and detection of yeast powder with first responders and LRN (upcoming)

Yeast as a NIST RM

- Reference value based on total (not viable) cells
- EPA Microbial Commercial Activity Notice required

Toxic Substances Control Act

ASTM standards

- WK42642 guidance on surrogate materials
- Draft standard for specific applications

ASTM INTERNATIONAL
Standards Worldwide

Summary

- Demonstrated that yeast material can be used in place of biothreat agents for training and workflow assessments in the field
- Validated a method to quantify total yeast cells (Coulter)
- Identified a yeast format that is stable and versatile (lyophilized)
- Developed a robust protocol for field training using yeast dried onto a surface
- Paving the way for a first-of-its-kind NIST RM
- The yeast material is a critical part of the developing Quality Assurance infrastructure to support reliable, consistent results from the First Responder Community

Broader Applicability

- Need for whole-cell based reference materials
- Lessons learned from the yeast apply to other microorganisms, with some caveats
- Next steps:
  - Validate methods to quantify total bacterial cells
  - Develop reference materials based on whole bacterial cells?
  - Extend the mixed microbial gDNA candidate RM for biosurveillance applications

Sandra Da Silva

Jim Filliben

Jayne Morrow

Nate Olson

Lindsay Harris

Supported by
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Steve Lund (NIST)
Zvi Kelman (NIST)
Capt. Bryon Marsh (4th CST, ret.)
SFC David Rygmyr (4th CST)
Jeremy Clancy (Howard Co. Fire Dept.)

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CDC Laboratory Response Network (LRN)
Rich Ozanich (PNNL)
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