Microbial Contaminant Detection Across Rapid Sterility Testing Methods - Preliminary Interlaboratory Study Findings

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National Institute of Standards and Technology
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Outline

- WG03 Background
- ILS#1 examining off-the-shelf material suitability w/ qPCR
- ILS#2 surveying sterility testing methods using a common sample set

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Background on WG03

Why is WG03 needed?

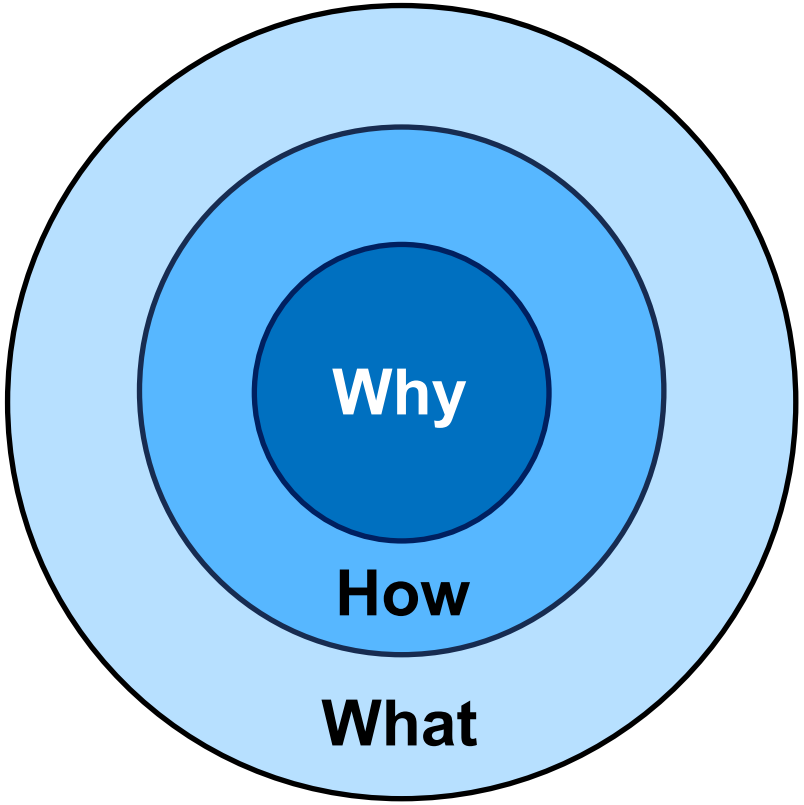
- Investigate questions on RMTM suitability for sterility testing

How do we do it?

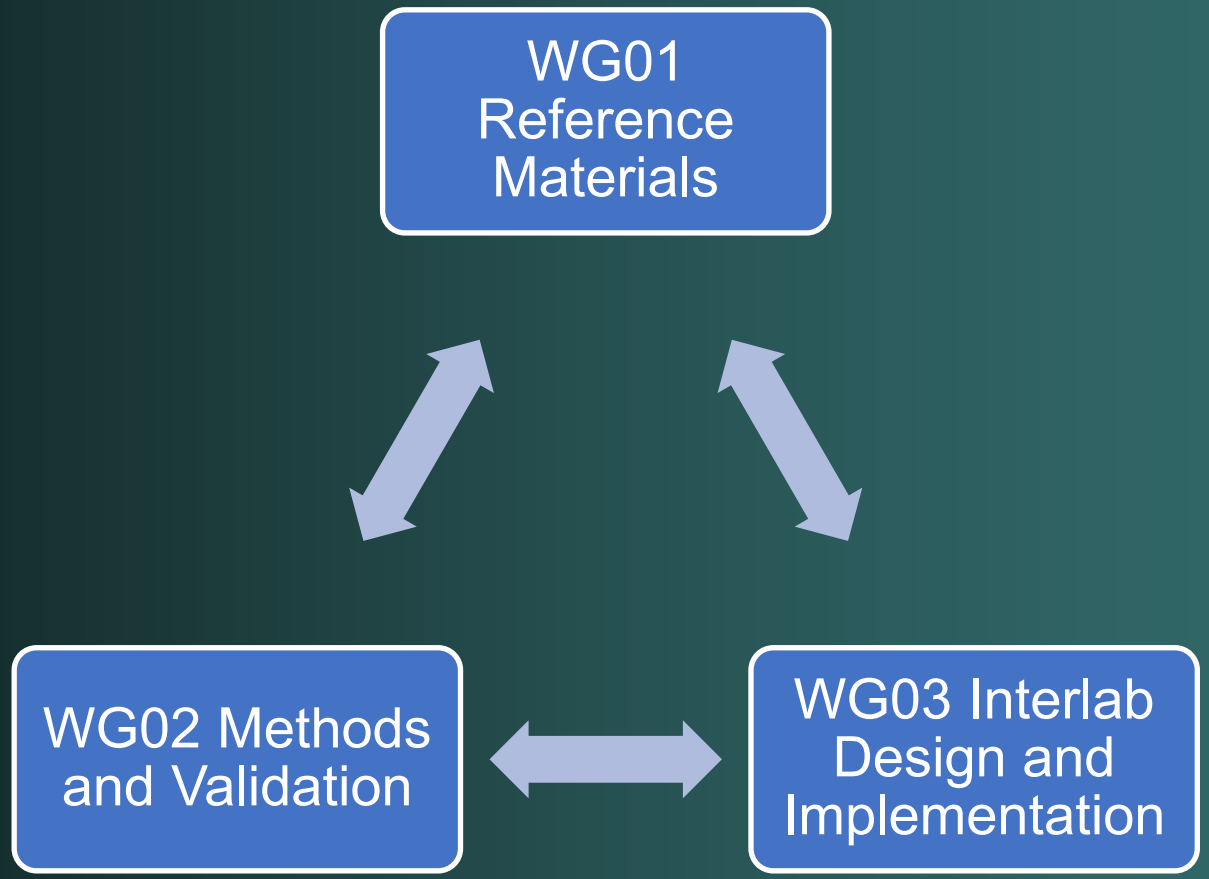
- Use member strengths & expertise to define the critical needs in RMTMs

What do we do?

- **Interlaboratory studies** on materials and methods to assess current and prospects in RMTMs



Interactions of the WGs



Question 12: What rapid microbial measurement technologies are you most hopeful to be adopted in your industry? (multiple answers permitted, 98 responses)

First Questions from WG01

- Are off-the-shelf materials (OTS) fit for purpose wrt qualifying/validating rapid measurements?
- ILS #1: Proof-of-concept test of OTS *E. coli* materials using a qPCR sterility-testing method

Study Overview – ILS #1 qPCR of *E. coli*

Purpose: Evaluate qPCR method performance and determine Genome Copy numbers of selected Reference Material as a prelude to a broader comparison of RMTMs

Do these laboratories, using the same materials and method, generate the same results?

Defining the first Interlab Study:

- **Materials:** M-S *E. coli* Vitroid & Biomerieux Bioball (at 10^5 -10^7 cell/mL); *E. coli* DNA from NIST
- **Common Method:** Sartorius Microsart ATMP Extraction & RESEARCH Bacteria Kit
- **Read-outs** from Interlab Study #1:
  - Compare Method performance
  - Compare Copy Number

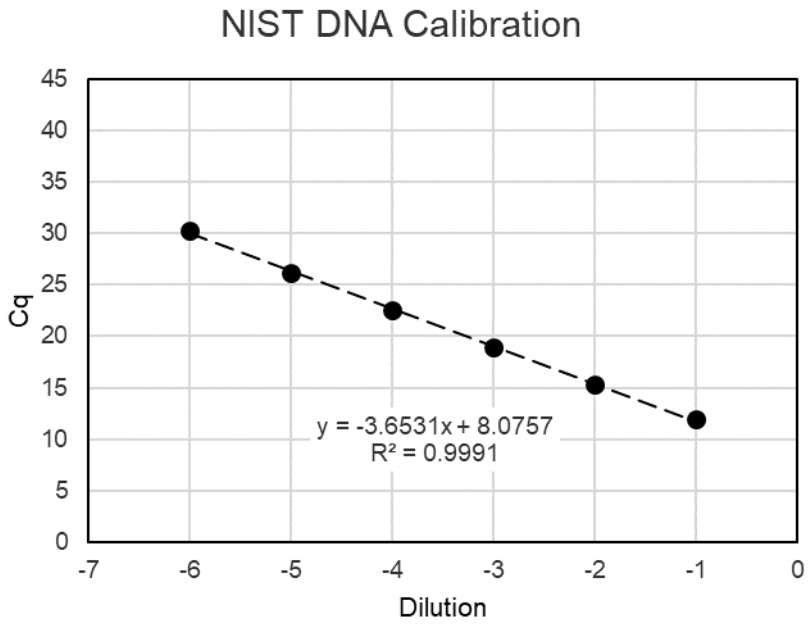
Assess reproducibility across test sites

not test all variables, pick a method, nor assign value

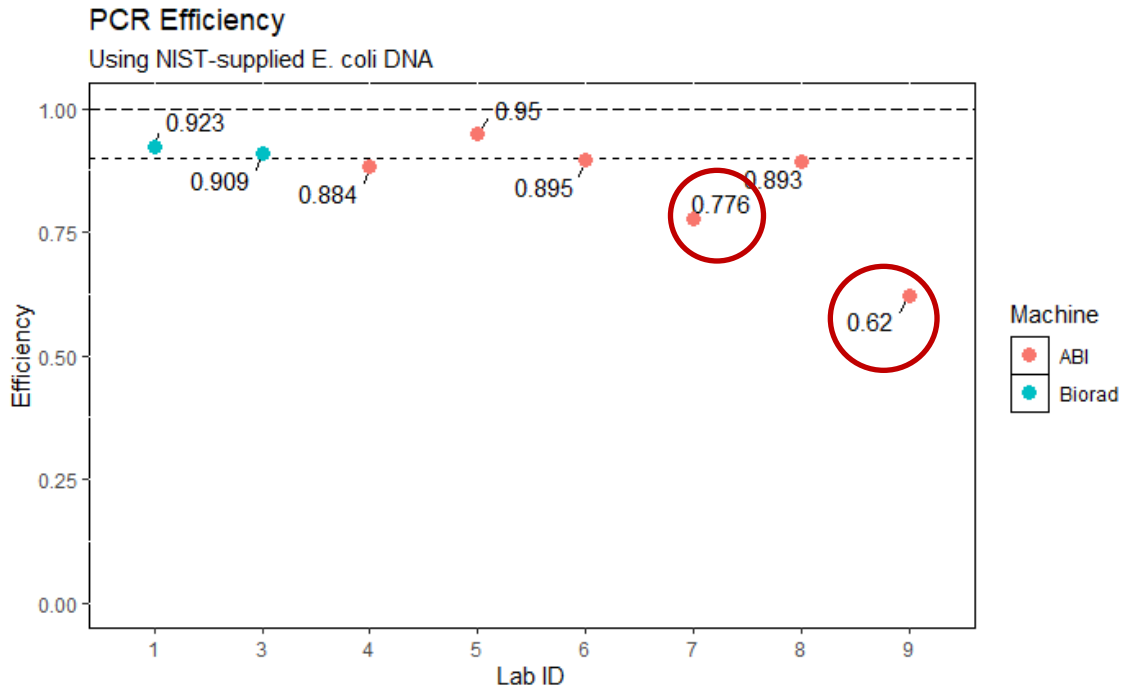
The DNA Standard Curve – Checkpoint

Assaying dilutions of the *E. coli* DNA enables

1. Intercomparability of system response
2. QA/QC of sample & assay preparation

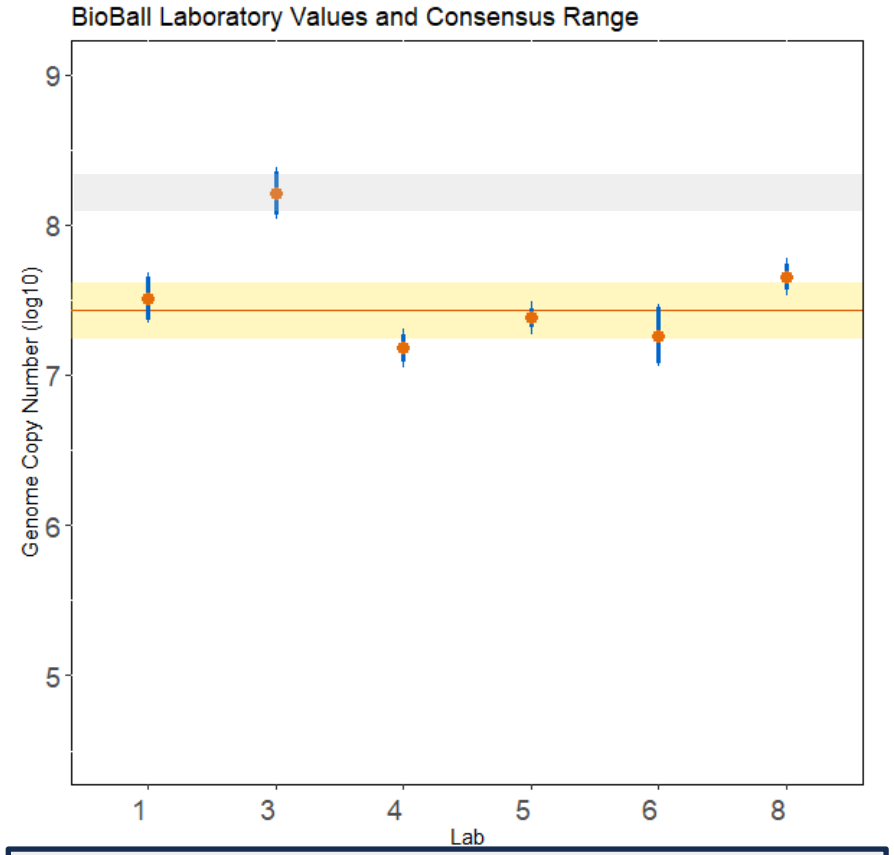


Efficiency 94%



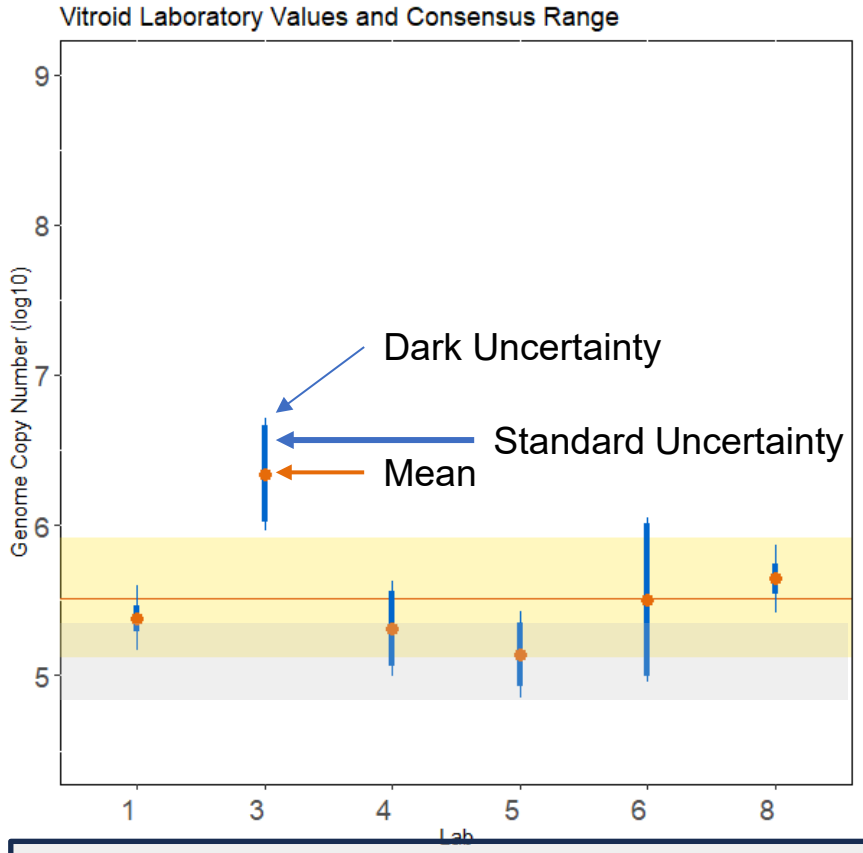
Consensus Ranges

Range: $(1.8 \cdot 10^7$ to $4.1 \cdot 10^7)$ GC/unit
Mean = $2.7 \cdot 10^7$ GC/unit



Label value: $1.4 \cdot 10^8 \pm 2.8 \cdot 10^7$ CFU/unit

Range: $(1.3 \cdot 10^5$ to $8.2 \cdot 10^5)$ GC/unit
Mean = $3.3 \cdot 10^5$ GC/unit



Label value: $5.1 \cdot 10^4$ to $2.1 \cdot 10^5$ CFU/unit

Conclusions ILS #1

- BB & Vitroid *E. coli* materials (CFU-certified) appear fit-for-purpose as qPCR controls
  - The relative range of consensus values for each material were consistent with those for CFU (vitroid matched, BB was lower)
  - Intra-laboratory values were consistent
  - Data rejection due to calibration curves, NOT material failures or assay

Lessons Learned

- Familiarity with method was important
  - Unfamiliarity leads to challenging results
  - Exploring new methods may require longer studies

- Centralizing materials and data
  - Complex studies could be delayed by single material/reagent
  - 1 Point of Contact = easier on participants

Effort 2: Interlab Study #2

NIST Lead: Jason Kralj (jason.kralj@nist.gov)

Goal: To generate data that will support adoption of RMTMs, specifically a reference that can be used USP in developing compendial methods or as support when going to the FDA

Option 1: Demonstration of equivalency testing between established method (CFU) with PMA-qPCR using cell lines and/or existing reference materials

Option 2: Develop a dataset using **common reference samples** provided by NIST to support comparability assessment of different platforms (RMTM or traditional)

Effort 2: Interlab Study #2 (continued)

NIST Lead: Jason Kralj (jason.kralj@nist.gov)

Goal: To generate data that will support adoption of RMTMs, specifically a reference that can be used by USP in developing compendial methods or as support when going to the FDA

Option 2: Develop a dataset using common reference samples provided by NIST to support comparability assessment of different platforms (RMTM or traditional)

Potential Impact: Shared dataset to compare the performance of multiple sterility testing modalities. This can be a direct comparison because common materials were used and could help end users select methods based on needs. (Study could be designed to address specific questions like comparing LOD, compare different matrices)

Needs/Timeline: 10-15 laboratories, ~6-18 mo. (**Representative samples**, experimental design, shipping, lab work)

Goals of ILS #2

- Survey of sterility testing methods using common sample set
  - NOT proficiency testing!

Questions

1. Assess comparability between compendial, common, and new methods for microbial testing
2. Demonstrate fitness-for-purpose of test materials

Benefit: manufacturers & regulators to highlight testing capabilities in pre-competitive space

Methods included

Growth/metabolic

- Colony forming units (CFU)
- BacT/ALERT (pH/CO_2)
- Bactec (turbidity + CO_2)
- Celsis (ATP)
- Microcalorimetry (heat)
- USP <71> Compendial (turbidity)

Molecular/chemical

- RNAseq
- qPCR
- Monocyte activity (LPS)

12 Labs
9 Methods
18 Data sets

Thank you BioFabUSA (Kurtis & Chelas)!

Study Plan– Consensus Choice

Background: CD3+ T-cells @ 1M cells/mL

Organisms: *S. aureus, P. aeruginosa, C. albicans, A. brasiliensis*

Levels: 0, 10 CFU/mL, 100 CFU/mL

Replicates: 3

TOTAL SAMPLES: 27

	Live						
Organism	CFU/mL						Controls
A. brasiliensis	100	100	100	10	10	10	
P. aeruginosa	100	100	100	10	10	10	0
S. aureus	100	100	100	10	10	10	0
C. albicans	100	100	100	10	10	10	0

Proposed Sample Sets

Human Cells

- Lifeline Cell Technology https://www.lifelinecelltech.com/
- CD3+ T-cells, expanded for ~2 weeks to 600M+

Microbes

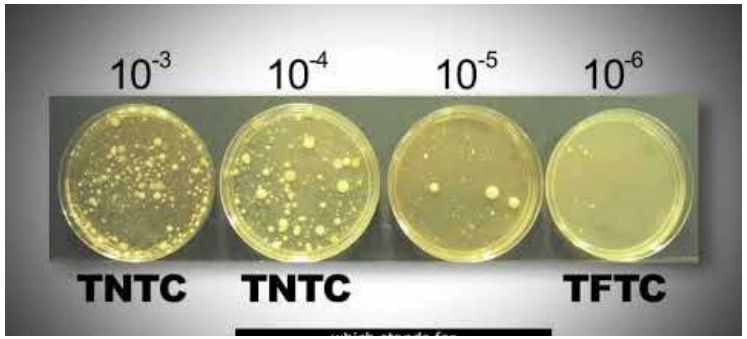
- *S. aureus, C. albicans, P. aeruginosa, A. brasiliensis*
- Culture, NIST counting (CFU, flow cyto., BactoBox, Coulter, Haemocyto.)

Samples

- 1 mL of human cells @ 1 M/mL
- 0, 10, 100 microbes per sample
- Coded for testing

ILS Sample Preparation

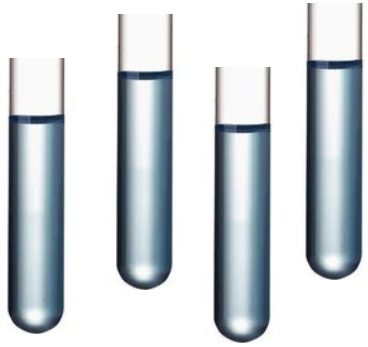
Dilutions for CFU



Quantifying Stocks

SampleID	Date	Group	10^-5	10^-6	10^-7
Sa37-1	1/24/2025	S	tntc	21	3
Sa37-2	1/24/2025	S	tntc	27	4
Sa37-3	1/24/2025	S	tntc	52	6
Pa25-1	1/24/2025	P	tntc	tntc	28
Pa25-2	1/24/2025	P	tntc	tntc	28
Pa25-3	1/24/2025	P	tntc	tntc	19
Ca37-1	1/24/2025	C	49	11	0
Ca37-2	1/24/2025	C	54	5	0
Ca37-3	1/24/2025	C	70	9	2

Dilutions For Samples



~10,000 CFU/mL

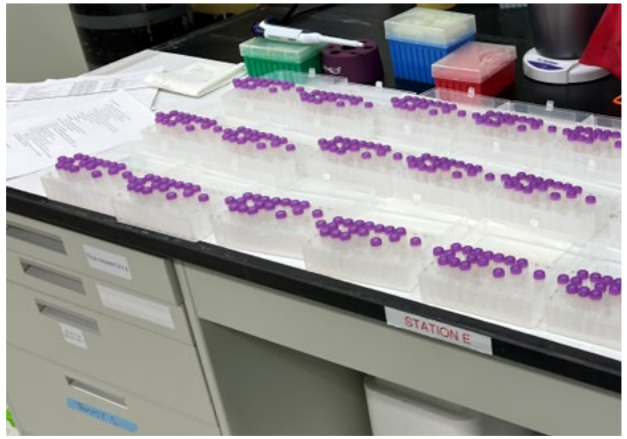
70 uL

700 uL

0 CFU

0 CFU

Aliquot



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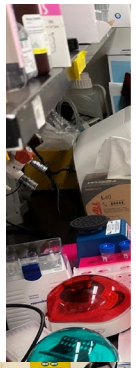
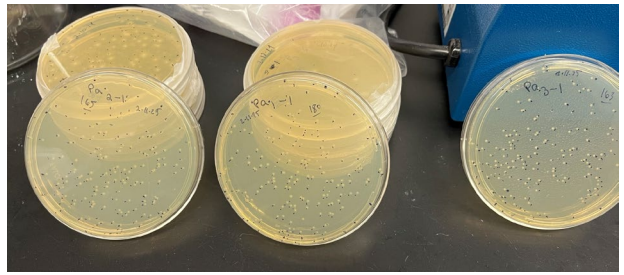
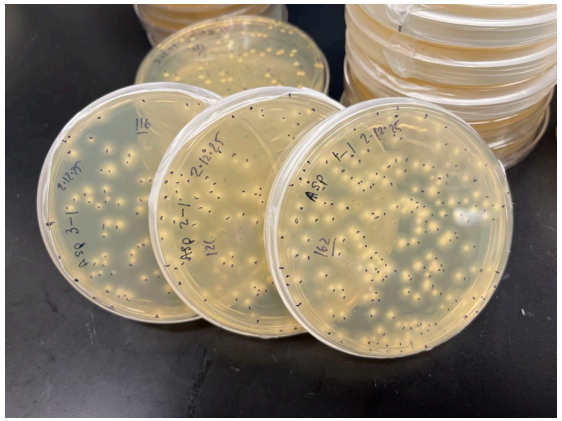






Details

- All samples shipped on 11.Feb.2025
  - T-cells harvested, microbes diluted, samples aliquoted, packed, and shipped @ 10am
- All samples received 12.Feb (US) or by 14.Feb (Intl)
  - All received in good condition w/o significant delays
- 12/12 labs have returned results
  - Meeting one-on-one to review as needed
  - Sub-group mtgs with replicate methods

Pre-ILS Sample Analysis

Microbial Stocks

Cultured Microbes
- Overnight broth
- Refrigerated ~72 h
- Counts / mL

Organism	CFU	Bactobox	Coulter	Flow	Hemocyt.
C. albicans	2.9e7	5.6e7	3.4e7	3.4e7	--
S. aureus	1.3e8	8.0e8	NR	8.5e8	--
P. aeruginosa	1.6e9	6.7e8	NR	3.4e9	--
A. brasiliensis	1.5e6	--	--	7.9e6	1.2e6

Accurate counts enable accurate spike-ins

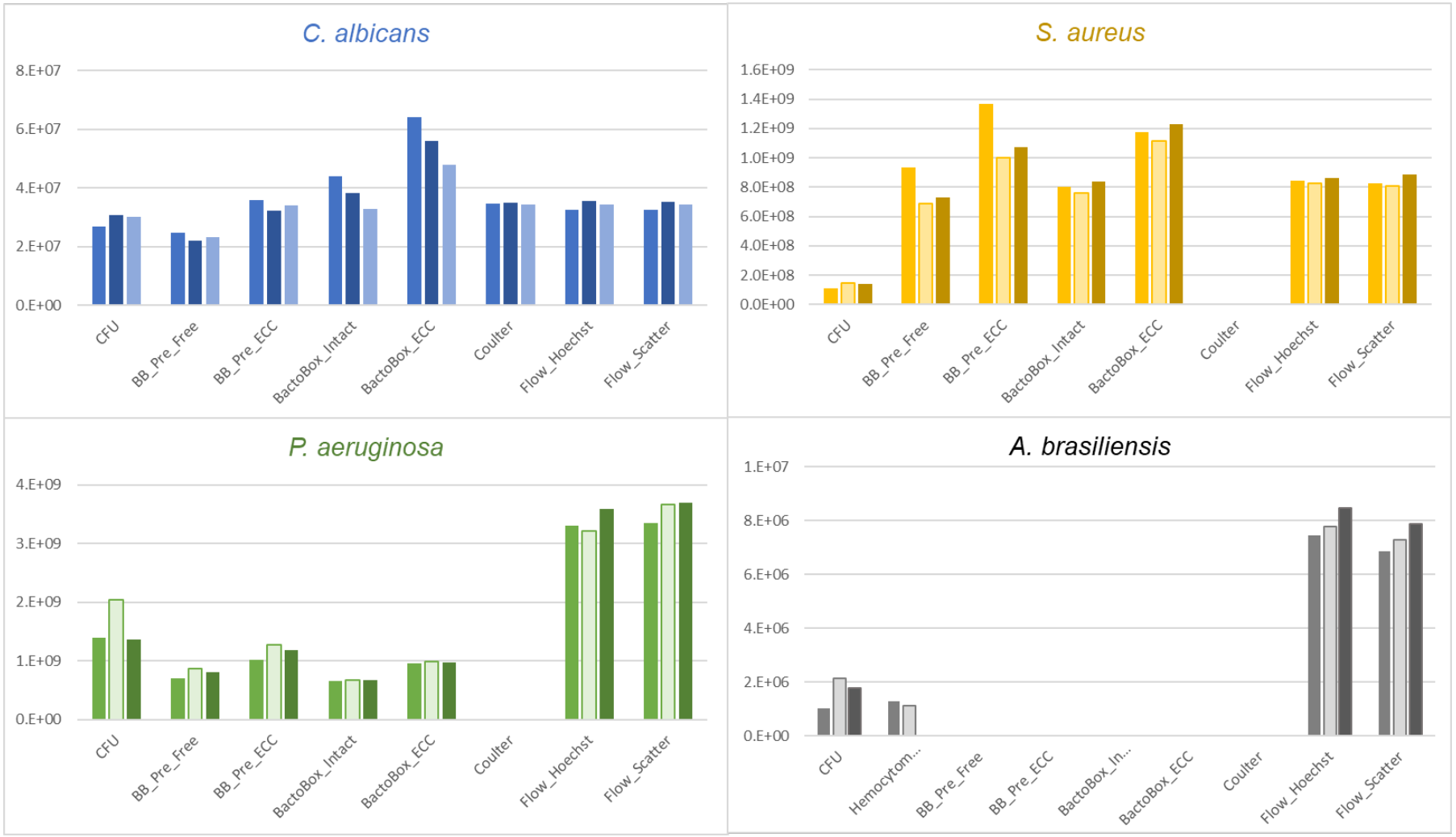
For spiking into T-cells

Master Sample Spike-Ins
- CFU triplicate
- Target 10k/mL

Organism	Mean ± (sd) CFU/mL
C. albicans	9000 (2248)
S. aureus	7230 (1194)
P. aeruginosa	16300 (2135)
A. brasiliensis	12300 (3262)

Special Thanks:
K. Parratt
A. Logan
S. da Silva
I. Hines
M. Hunter

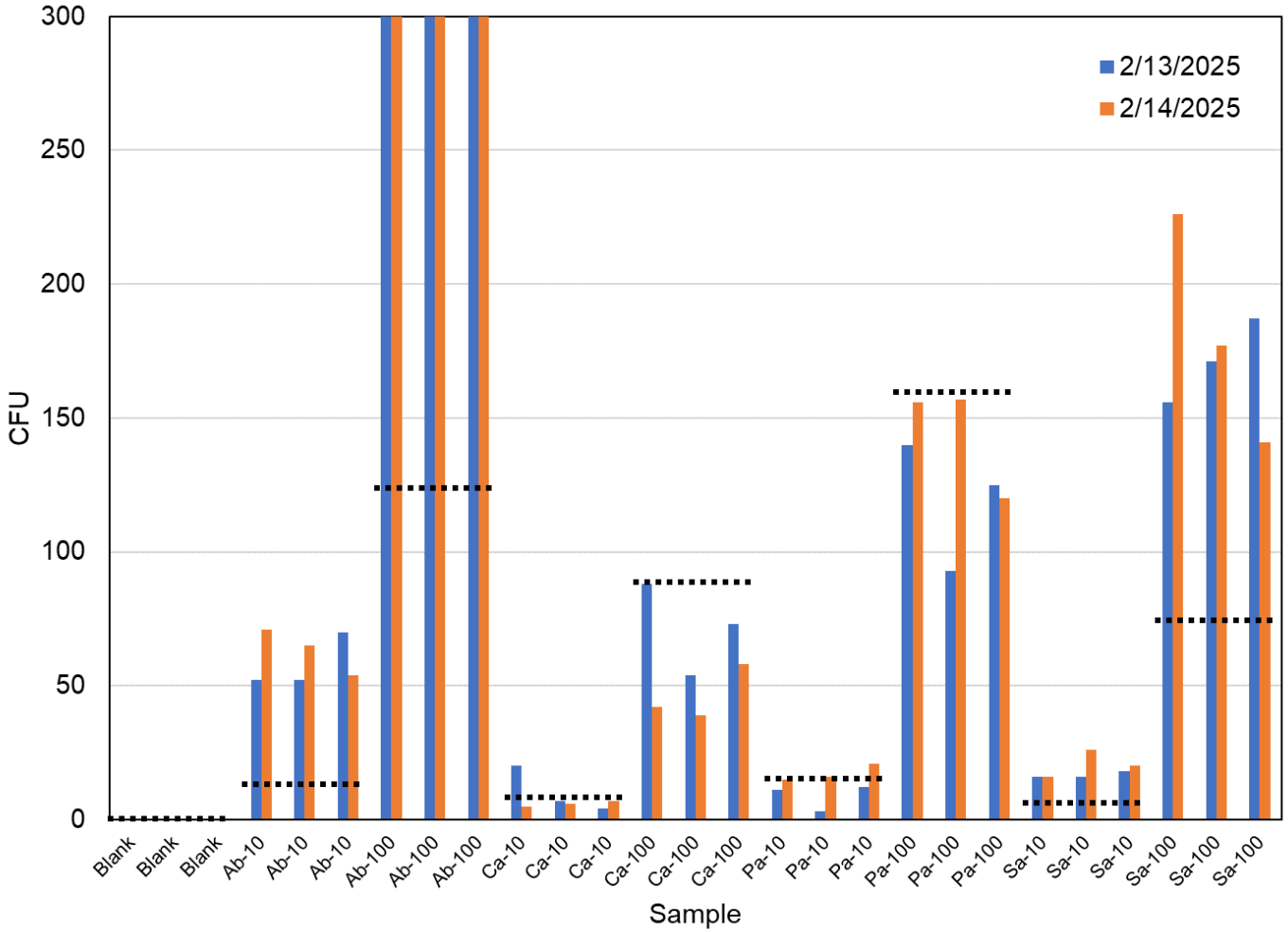
Sample Analysis



Special Thanks:
K. Parratt
A. Logan
S. da Silva
I. Hines
M. Hunter

NIST CFUs to Track Sample Stability

- CFU measures on repeat days
  - Tracking **microbial** stability
  - No evidence of degradation
  - Good repeatability
- Incubation 5 or 4 days @RT
  - Good agreement w/ predicted
  - *Aspergillus* spores re-seeding?



Results Sheets

Sample IDs still blinded

Sample Results Sheet

Rapid Microbial Testing Methods Interlaboratory Study #2

POCs: Jason Kralj jason.kralj@nist.gov
Stephanie Servetas stephanie.servetas@nist.gov

Sample	Date Rec'd	Date Result	Batch	Operator(s)	Method	Result	Contaminant ID	Signal	Signal Units	Comments
1	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		34.87	cycles	
2	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		31.35	cycles	
3	2/12/2025	2/16/2025	#1	MH	qPCR	Negative			cycles	
4	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		36.2	cycles	
5	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		34.25	cycles	
6	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		30.32	cycles	
7	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		34.79	cycles	
8	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		29.76	cycles	
9	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		31.17	cycles	
10	2/12/2025	2/16/2025	#1	MH	qPCR	Negative			cycles	
11	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		33.17	cycles	
12	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		35.45	cycles	
13	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		36.24	cycles	
14	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		29.98	cycles	
15	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		29.32	cycles	
16	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		34.77	cycles	
17	2/12/2025	2/16/2025	#1	MH	qPCR	Negative			cycles	
18	2/12/2025	2/16/2025	#1	MH	qPCR	Negative			cycles	
19	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		31.61	cycles	
20	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		29.65	cycles	
21	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		34.94	cycles	
22	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		34.46	cycles	
23	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		33.18	cycles	
24	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		29.72	cycles	
25	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		35.56	cycles	
26	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		33.15	cycles	
27	2/12/2025	2/16/2025	#1	MH	qPCR	Positive		36.72	cycles	

Required

Optional (rec'd)

Preliminary Results

Excellent performance from most methods

- Replication of multiple methods
- NIST samples were good surrogate

Sample	Organism	Level	CFU	CFU	USP <71>	USP <71>	BacT/ALERT	BacT/ALERT	BacT/ALERT	BacT/ALERT	BD Bactec	BD Bactec	CalScreener	Celsis	RNAseq
Blank			Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Blank	Blank	0	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Blank			Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Positive	Negative
Ab-10			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive
Ab-10		10	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ab-10	A. brasiliensis		Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ab-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive
Ab-100		100	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ab-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ca-10			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ca-10		10	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Ca-10	C. albicans		Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Ca-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ca-100		100	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Ca-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Pa-10			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Pa-10		10	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Pa-10	P. aeruginosa		Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Pa-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Pa-100		100	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Pa-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Sa-10			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Sa-10		10	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Sa-10	S. aureus		Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Sa-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Sa-100		100	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Sa-100			Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive

All methods

Sens	99%
Spec	95%
Prec	99%
Acc	98%

Time to positive result (days):		1	1	14	2	1	2	2	1	1	0.6	1	4	4
Time to negative result (days):		5	4	14	14	15	8	7	14	14	5	4	4	4
Sens		100%	100%	100%	100%	100%	100%	100%	100%	92%	100%	100%	92%	100%
Spec		100%	100%	100%	100%	100%	67%	100%	100%	100%	100%	100%	67%	100%
Prec		100%	100%	100%	100%	100%	96%	100%	100%	100%	100%	100%	96%	100%
Acc		100%	100%	100%	100%	100%	96%	100%	100%	93%	100%	100%	89%	100%

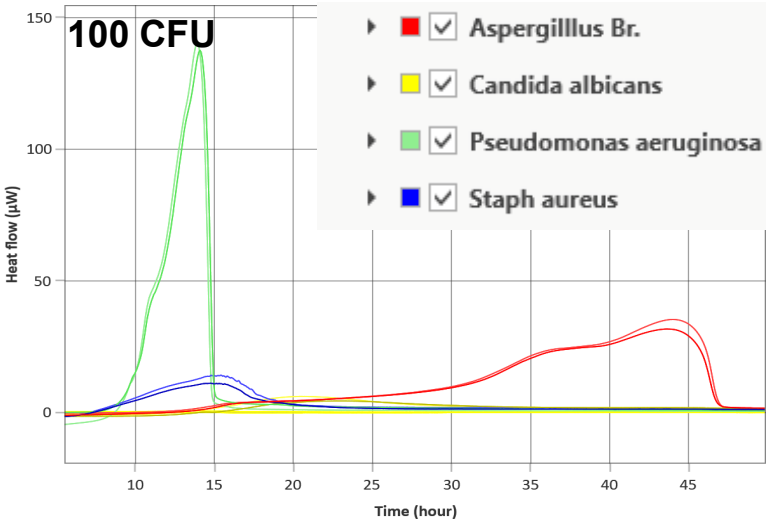
Microcalorimetry

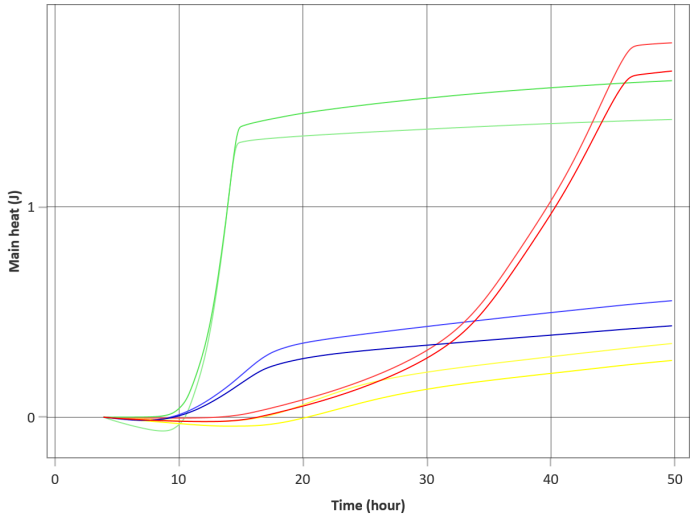
Experiment

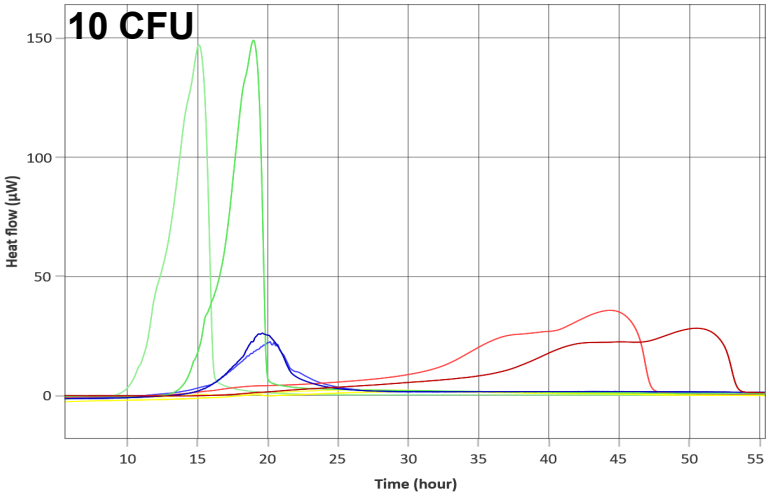
- 9 unblinded sample stocks from ILS2
- 100 µL/sample in TSB
- 2 replicates ea.

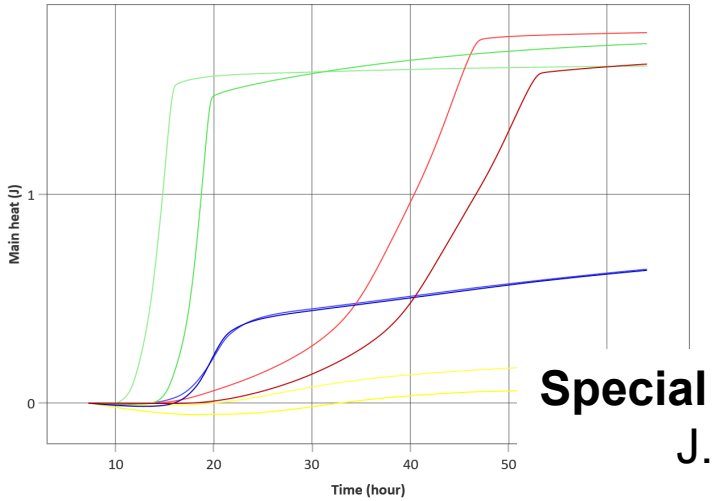
Results

- 9/9 correct
- Unique heat profiles by organism
- Good reproducibility

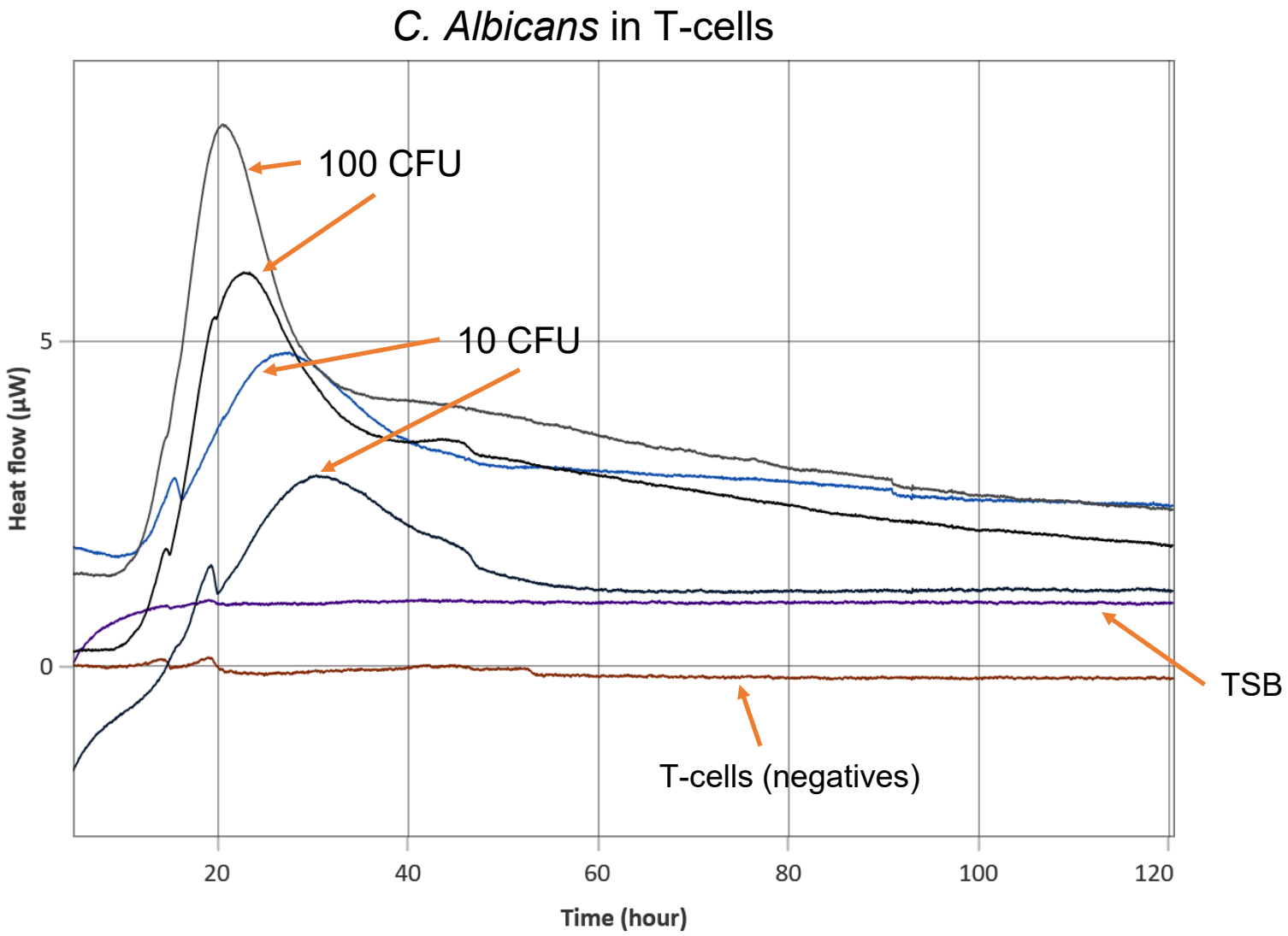








Special Thanks:
J. Dunkers


C. Albicans in T-cells
100 CFU
10 CFU
TSB
T-cells (negatives)
Heat flow (µW)
5
0
20
40
60
80
100
120
Time (hour)

Molecular Only

- RNAseq – excellent results
- MAT (endotoxin)
  - Significant LPS in the albumin

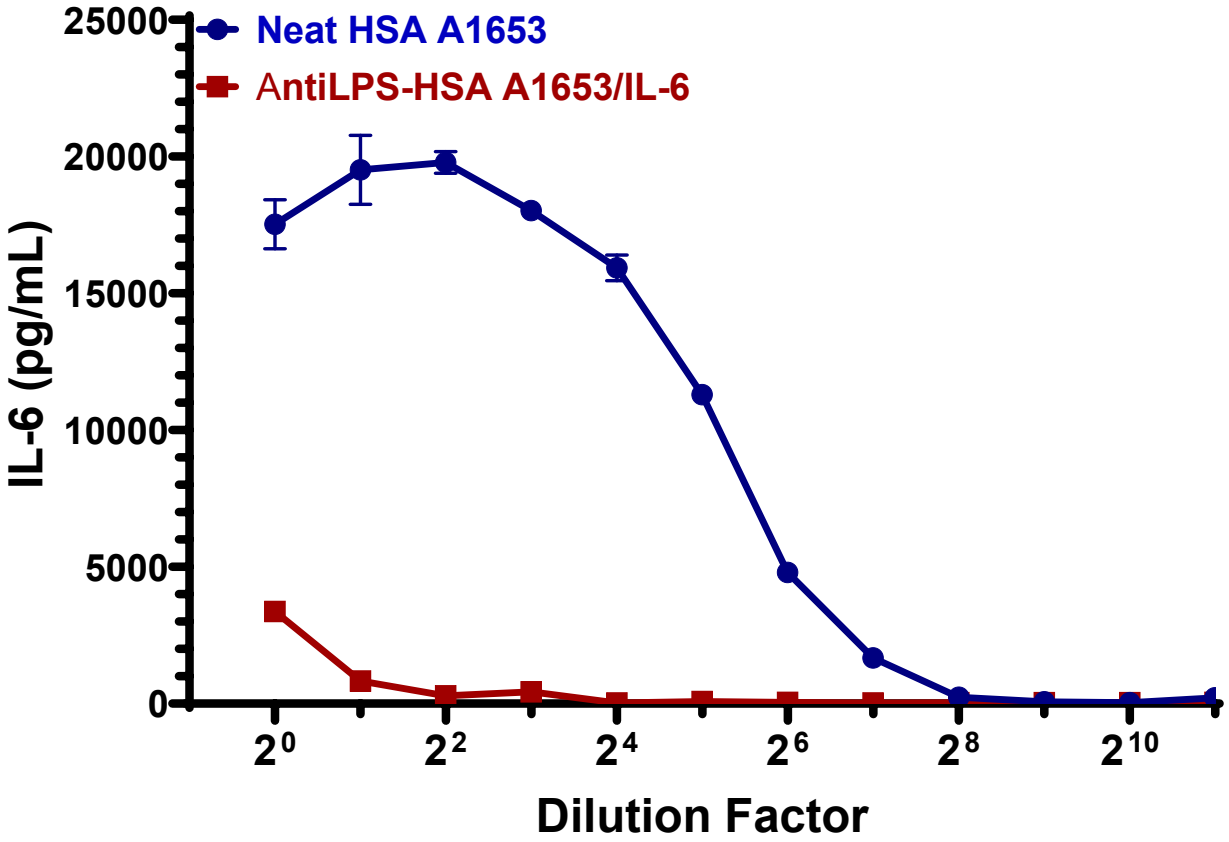
Sample	Organism	Level	RNAseq
Blank			Negative
Blank	Blank	0	Negative
Blank			Negative
Ab-10			Positive
Ab-10		10	Positive
Ab-10			Positive
Ab-100	A. brasiliensis		Positive
Ab-100		100	Positive
Ab-100			Positive
Ca-10			Positive
Ca-10		10	Positive
Ca-10			Positive
Ca-100	C. albicans		Positive
Ca-100		100	Positive
Ca-100			Positive
Pa-10			Positive
Pa-10		10	Positive
Pa-10			Positive
Pa-100	P. aeruginosa		Positive
Pa-100		100	Positive
Pa-100			Positive
Sa-10			Positive
Sa-10		10	Positive
Sa-10			Positive
Sa-100	S. aureus		Positive
Sa-100		100	Positive
Sa-100			Positive

Time to positive result (days):	4
Time to negative result (days):	4

PyroDex

Courtesy of Djik Maouyo

Human Serum Albumin Pyrogenicity Profile



Human serum albumin

- Vendor: Sigma-Aldrich
- Product#: A1653
- Batch: 0000415240
- Reconstituted in WFI
  - 50mg/mL
  - Adjusted tonicity to 45 mg/mL for MAT
- **HSA final test results:**
  - Total pyrogen: 3.328 EEU/ml
  - **LPS-specific: 3.251 EU/mL**
  - Non-LPS Pyrogen: 0.077EEU/mL

Molecular Only

- RNAseq – excellent results
- MAT (endotoxin)
  - Significant LPS in the albumin
- qPCR (DNA)
  - Extremely sensitive and fast
  - Detected background DNA contamination
  - Performance testing not possible
- Examination and validation on individual product basis
  - Collect additional data on components not observed with culturing
  - ID potential steps w/ contamination

Sample	Organism	Level	RNAseq	MAT	qPCR	qPCR	qPCR	qPCR
Blank			Negative	Positive	Positive	Positive	Positive	Negative
Blank	Blank	0	Negative	Positive	Positive	Positive	Negative	Negative
Blank			Negative	Positive	Positive	Positive	Negative	Negative
Ab-10			Positive	Positive	Positive	Positive	Positive	Positive
Ab-10		10	Positive	Positive	Positive	Positive	Negative	Negative
Ab-10	A. brasiliensis		Positive	Positive	Positive	Positive	Negative	Negative
Ab-100			Positive	Positive	Positive	Positive	Positive	Positive
Ab-100		100	Positive	Positive	Positive	Positive	Positive	Positive
Ab-100			Positive	Positive	Positive	Positive	Negative	Negative
Ca-10			Positive	Positive	Positive	Positive	Positive	Negative
Ca-10		10	Positive	Positive	Positive	Positive	Negative	Negative
Ca-10	C. albicans		Positive	Positive	Positive	Positive	Negative	Negative
Ca-100			Positive	Positive	Positive	Positive	Positive	Positive
Ca-100		100	Positive	Positive	Positive	Positive	Negative	Negative
Ca-100			Positive	Positive	Positive	Positive	Positive	Negative
Pa-10			Positive	Positive	Positive	Positive	Positive	Positive
Pa-10		10	Positive	Positive	Positive	Positive	Positive	Positive
Pa-10	P. aeruginosa		Positive	Positive	Positive	Positive	Positive	Negative
Pa-100			Positive	Positive	Positive	Positive	Positive	Positive
Pa-100		100	Positive	Positive	Positive	Positive	Positive	Positive
Pa-100			Positive	Positive	Positive	Positive	Positive	Positive
Sa-10			Positive	Positive	Positive	Positive	Positive	Negative
Sa-10		10	Positive	Positive	Positive	Positive	Positive	Negative
Sa-10	S. aureus		Positive	Positive	Positive	Positive	Positive	Negative
Sa-100			Positive	Positive	Positive	Positive	Positive	Positive
Sa-100		100	Positive	Positive	Positive	Positive	Positive	Positive
Sa-100			Positive	Positive	Positive	Positive	Positive	Negative
Time to positive result (days):			4	2	0.5	0.5	0.5	0.5
Time to negative result (days):			4	2	0.5	0.5	0.5	0.5

Current Status

- Meet 1-on-1 with labs to review results
- Examining reagent contamination
- Meet as working group to discuss data
  - Highlight what the sample set could (& couldn't) show us
  - Make recommendations on sterility testing mock-samples
    - What is good enough?
  - Provide big-picture message to the community on RMTMs
- Manuscript drafting

Conclusions

ILS#2 is a resounding success!

Question #1: Survey method comparability

- Many new methods' performance comparable to USP <71>, even at 10 CFU level
- Several faster, with identification

Question #2: NIST sample set suitability (as-is) for testing

- Growth/Metabolic activity : yes
- RNA-seq : yes
- MAT and qPCR : reagent contamination, unable to eval.

OLD/NEW BUSINESS

ANNOUNCEMENTS

MEETING WRAP-UP

Pre-ILS2 Samples

w/ A. Lau (NIH)

Microbial Cells

- *C. albicans* (fungi)*, S. aureus* (Gram +), *P. aeruginosa* (Gram -)
- Sample prep
  - Characterized bulk materials w/ Bactobox and Coulter counter
  - Add volumes for 10 CFU and 100 CFU into samples

Plating of the 10 and 100 CFU Volumes

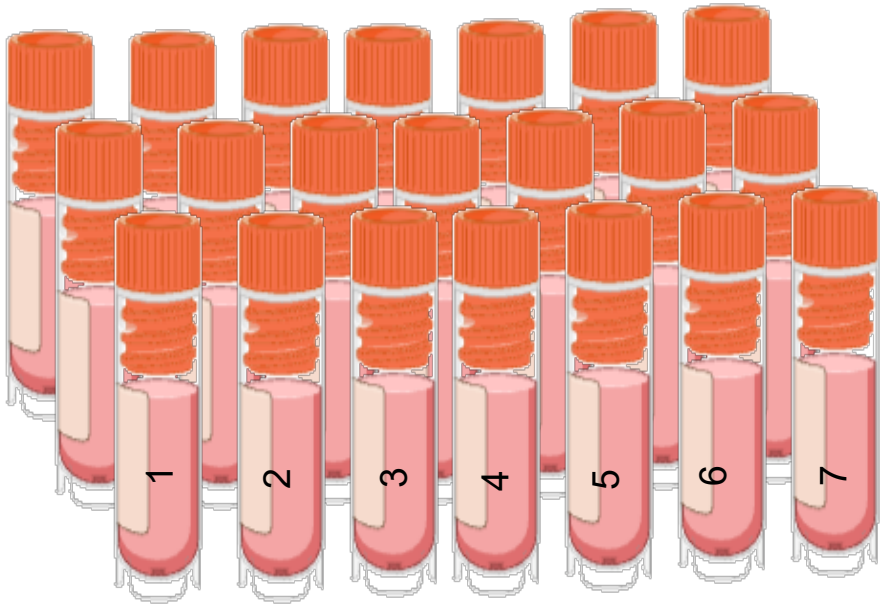
ID	100 CFU	10 CFU
S. aureus	136	18
P. aeruginosa	126	9
C. albican	224	18

Sample Characterization @ 24h (Plate)

- Centrifuge 10k for 2’ collect
- Plate ~100 uL

Name	Spike-in (CFU)	Measured (CFU)
Blank	0	0
Blank	0	0
S. aureus	10	1
S. aureus	10	5
S. aureus	100	33
S. aureus	100	38
P. aeruginosa	10	1
P. aeruginosa	10	1
P. aeruginosa	100	45
P. aeruginosa	100	43
C. albicans	10	4
C. albicans	10	5
C. albicans	100	40
C. albicans	100	53

Proposed Test Set v1.0



Set 24 Vials with **T-cells + viable microbes or control** (buffer alone) - blinded

Receive vials (on ice, overnight) > sample with your in-house method

Sample Characterization w/ Lau Lab

24 Samples (blinded)

- 3 bacterial strains, neg. controls
- Delivered overnight, cold packs

BacT/ALERT

- Incubate 2 weeks (start 8/28/24)
- Aerobic & anaerobic cartridges

What it tests

- Sample fitness for purpose
- Potential adjustments

BacT/ALERT

BacT/ALERT (Lau Lab)

- Results
  - 100% (24/24) Accuracy
  - “I suspect you may want to go even lower in organism concentration.” –AL

Aerobic

iFA+	Time to detection (day)	
	Spike-in	
Organism	**10**	**100**
Pseudomonas aeruginosa	0.80	0.79
Candida albicans	1.24	1.18
Staphylococcus aureus	0.83	0.77

Anaerobic

iFN+	Time to detection (day)	
	Spike-in	
Organism	**10**	**100**
Pseudomonas aeruginosa	2.81	2.82
Candida albicans	ND	ND
Staphylococcus aureus	1.08	1.02

Decoded		Sample	Result		Contaminant ID	
Organism	Spike-in (CFU)		iFA+	iFN+	iFA+	iFN+
P. aeruginosa	126	1	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
P. aeruginosa	126	2	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
C. albicans	18	3	Positive	Negative	*Candida albicans*	N/A
C. albicans	18	4	Positive	Negative	*Candida albicans*	N/A
P. aeruginosa	9	5	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
P. aeruginosa	9	6	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
S. aureus	136	7	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*
S. aureus	136	8	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*
C. albicans	224	9	Positive	Negative	*Candida albicans*	N/A
C. albicans	224	10	Positive	Negative	*Candida albicans*	N/A
S. aureus	18	11	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*
S. aureus	18	12	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*
Blank	0	13	Negative	Negative	N/A	N/A
Blank	0	14	Negative	Negative	N/A	N/A
C. albicans	100	15	Positive	Negative	*Candida albicans*	N/A
C. albicans	10	16	Positive	Negative	*Candida albicans*	N/A
P. aeruginosa	126	17	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
P. aeruginosa	18	18	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
S. aureus	136	19	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*
S. aureus	18	20	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*
Blank	0	21	Negative	Negative	N/A	N/A
P. aeruginosa	100	22	Positive	Positive	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
C. albicans	224	23	Positive	Negative	*Candida albicans*	N/A
S. aureus	100	24	Positive	Positive	*Staphylococcus aureus*	*Staphylococcus aureus*

Discussion

- ✓ Fitness for purpose
- Adjustment Options
  - ✓ Increase T-cell density — 500k/mL → 1M+/mL
  - ✓ Addition of HSA — 2.5-5%
  - Volume change — 3 mL → ?
  - Microbes
    - Concentrations — add a ~1 CFU/sample (very tricky)
    - Add'l compendial strains — *B. subtilis, C. sporogenes, A. brasiliensis, B. vulgatus*