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Safety and Accuracy Assessment of MALDI-TOF Mass Spectrometry Platforms for the Detection of Biological Threats

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Sample Preparation Safety Study

- Determined viability of BT agents using direct, extended direct, and tube extraction
 - *Bacillus anthracis* Sterne, *Brucella abortus* Strain 19, *Francisella tularensis* LVS, *Yersinia pestis* A1122, *Burkholderia thailandensis* ATCC 70038, *Clostridium* spp (*botulinum* types A, B, E, and *perfringens*)
- Some isolates of *B. anthracis*, *C. botulinum*, *F. tularensis*, *Y. pestis*, and *B. abortus* survived the direct and extended direct prep methods
- None survived the tube extraction

Safety Study

- CDC – *B. anthracis*
- Lash *et al.* – *B. cereus*, *B. subtilis*
 - Anal Chem 80:2026-2034
- Drevinek *et al.* – *B. anthracis*
 - Lett Appl Microbiol 55:40-46
- Tracz *et al.* – *B. anthracis*, *B. thuringiensis*
 - J Clin Microbiol 54:764-767

Recommendations

- **Use tube extraction AND 0.1 μm filter for “hazardous organisms”**
- **Conduct risk assessment as part of MALDI-TOF validation**
- **ASM Sentinel Lab Procedures - use tube extraction and filtration if MALDI-TOF is used for suspect BT agents**

Laboratory Validation of MALDI-TOF

- Challenges

Software Library	Bruker Micro Flex	Vitek MS
CA	210 (955)	193 (562)
ROU	2,371	1,388
Security	18	NA

Study Design

- Determine ability of Bruker and Vitek software libraries to accurately identify BT agents
 - Test CA, ROU, and Security Libraries independently
- Panel of 6 strains and 6 near neighbors for each agent
 - *B. anthracis*, *Y. pestis*, *F. tularensis*, *C. botulinum*, *B. mallei*, *B. pseudomallei*, *Brucella* spp.
 - Select isolates from AOAC Stakeholder Panel on Agent Detection Assays (SPADA) – inclusivity and exclusivity panels
- Challenges
 - No SPADA panels for *C. botulinum* or *Brucella* spp.
 - Isolate availability and shipping
 - No tube extraction procedure for Vitek MS

Organism selection

Inclusivity

- *Y. pestis* CO92
- *Y. pestis* Nepal 516
- *Y. pestis* UG05-0454
- *Y. pestis* Pestoides F
- *Y. pestis* Angola
- *Y. pestis* Harbin35

Exclusivity

- *Y. ruckeri* YERS063
- *Y. pseudotuberculosis* YPIII
- *Y. pseudotuberculosis* Pa3606
- *Y. pseudotuberculosis* IB
- *Y. enterocolitica* WA
- *Y. enterocolitica* 2516-87

Pre-study Considerations

- Stability of extracts stored at -20°C
 - *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *C. perfringens* ATCC 13124
 - Test extracts in triplicate at day 0, 30 , and 45
- Affect of pooling on identification
 - *S. pneumoniae* ATCC 49619, *B. cepacia* ATCC 17765, *M. catarrhalis* C11-11811
 - Extract in triplicate, test in triplicate, pool extracts and test in triplicate

Results for extract storage and pooling

	Mean score day 0	Mean score day 30	Mean Score day 45	CV across time (%)
<i>S. aureus</i>	2.357	2.368	2.209	3.9
<i>P. aeruginosa</i>	2.049	2.056	1.956	2.8
<i>C. perfringens</i>	2.451	2.444	2.393	1.3

		Range	Mean score	CV (%)
<i>S. pneumoniae</i>	Replicates (9)	2.082-2.248	2.180	2.9
	Pool	2.131-2.267	2.210	3.2
<i>B. cepacia</i>	Replicates (9)	2.215-2.359	2.317	1.8
	Pool	2.296-2.392	2.338	2.1
<i>M. catarrhalis</i>	Replicates (9)	2.436-2.485	2.465	1.0
	Pool	2.43-2.458	2.447	0.6

Comparison of Direct vs. Tube Extraction for Vitek MS

- 50 random isolates submitted for ID
 - 5 *Shigella* spp., 4 *E. coli*, 6 *K. pneumoniae*, 2 *E. cloacae*, 9 *Salmonella* spp., 3 *R. insidiosa*, 1 *A. baumannii*, 1 *P. aeruginosa*, 1 *A. xylosoxidans*, 1 *V. cholerae*, 1 *C. fetus*, 3 *N. gonorrhoeae*, 3 *S. mitis*, 7 *S. aureus*, 1 *S. pneumoniae*, 1 *E. faecalis*, 1 *S. pyogenes*
- 48/50 (96%) gave same ID for extract and direct colony method (1 wrong ID each)
- Tube extraction appears to be a satisfactory sample prep method for Vitek

Organism	n	# replicates	Identification	CA Library		ROU Library		Security Library	
				Mean Score	Species level ID % (>2.0)	Mean Score	Species level ID % (>2.0)	Mean Score	Species level ID % (>2.0)
<i>Bacillus anthracis</i>	2	6	No reliable ID	1.19	0	-	-	-	-
			B. cereus	-	-	2.11	100	-	-
			B. anthracis	-	-	-	-	2.47	100
<i>Brucella abortus</i>	2	6	No reliable ID	1.00	0	1.27	0	-	-
			<i>B. melitensis</i>	-	-	-	-	2.16	100
<i>Brucella melitensis</i>	2	6	No reliable ID	1.09	0	1.28	0	-	-
			<i>B. melitensis</i>	-	-	-	-	2.22	100
<i>Brucella suis</i>	1	3	No reliable ID	1.04	0	1.24	0	-	-
			<i>B. melitensis</i>	-	-	-	-	2.11	100
<i>Brucella canis</i>	1	3	No reliable ID	1.00	0	1.26	0	-	-
			<i>B. melitensis</i>	-	-	-	-	2.20	100
<i>Yersinia pestis</i>	2	6	<i>Y. pseudotuberculosis</i>	2.30	100	2.32	100	-	-
			<i>Y. pestis</i>	-	-	-	-	2.40	100
<i>Francisella tularensis</i>	5	15	No reliable ID	1.01	0	1.32	0	1.38 (12)	0
			<i>F. tularensis</i>	-	-	-	-	1.77 (3)	0
<i>Burkholderia pseudomallei</i>	6	18	No reliable ID	1.31	0	1.48 (3)	0	-	-
			<i>B. thailandensis</i>	-	-	1.89 (15)	13	-	-
			<i>B. pseudomallei</i>	-	-	-	-	2.22	89
<i>Burkholderia mallei</i>	6	18	No reliable ID	1.28	0	1.58 (9)	0	-	-
			<i>B. thailandensis</i>	-	-	1.92 (9)	11	-	-
			<i>B. pseudomallei</i>	-	-	-	-	1.98 (1)	6
			<i>B. mallei</i>	-	-	-	-	2.24 (17)	94
<i>Clostridium botulinum</i>	5	15	No reliable ID	1.11	0	1.46 (3)	0	1.39 (3)	0
			<i>C. sporogenes</i>	-	-	1.85 (9)	-	-	-
			<i>L. rhamnosis</i>	-	-	2.20 (3)	20	-	-
			<i>C. botulinum</i>	-	-	-	-	1.95 (12)	53

Bruker: Near Neighbors

Organism	n	# replicates	Identification	CA Library		ROU Library		Security Library	
				Mean Score	Species level ID % (>2.0)	Mean Score	Species level ID % (>2.0)	Mean Score	Species level ID % (>2.0)
<i>Brucella neotomae</i>	1	3	No reliable ID	1.15	0	1.23	0	-	-
			<i>B. melitensis</i>	-	-	-	-	1.91	0
<i>Brucella ovis</i>	1	3	No reliable ID	1.06	0	1.22	0	1.36 (1)	0
			<i>B. melitensis</i>	-	-	-	-	1.87 (2)	0
<i>Ochrobactrum anthropi</i>	1	3	No reliable ID	0.99	0	-	-	1.06	0
			<i>O. anthropi</i>	-	-	1.92	0	-	-
<i>Brucella pinnipedialis</i>	1	3	No reliable ID	1.15	0	1.61	0	1.64 (2)	0
			<i>B. melitensis</i>	-	-	-	-	1.79 (1)	0
<i>Brucella ceti</i>	1	3	No reliable ID	1.15	0	1.45	0	-	-
			<i>B. melitensis</i>	-	-	-	-	1.93	0
<i>Oligella ureolytica</i>	1	3	No reliable ID	-	-	-	-	1.08	0
			<i>O. ureolytica</i>	2.00	33	1.81	0	-	-
<i>Burkholderia thailandensis</i>	1	3	No reliable ID	1.34	0	-	-	-	-
			<i>B. thailandensis</i>	-	-	2.15	100	-	-
			<i>B. pseudomallei</i>	-	-	-	-	2.03	66
<i>Burkholderia cepacia</i>	1	3	No reliable ID	-	-	-	-	1.12	0
			<i>B. cepacia</i> complex	2.20	100	-	-	-	-
			<i>B. cenocepacia</i>	-	-	2.17	100	-	-
<i>Burkholderia multivorans</i>	1	3	No reliable ID	-	-	-	-	1.47	0
			<i>B. multivorans</i>	2.44	100	2.31	100	-	-

Bruker: Near Neighbors

Organism	n	# replicates	Identification	CA Library		ROU Library		Security Library	
				Mean Score	Species level ID % (>2.0)	Mean Score	Species level ID % (>2.0)	Mean Score	Species level ID % (>2.0)
<i>Stenotrophomonas maltophilia</i>	1	3	No reliable ID	-	-	-	-	0.94	0
			<i>S. maltophilia</i>	2.16	100	2.29	100	-	-
<i>Clostridium perfringens</i>	1	3	No reliable ID	-	-	-	-	1.14	0
			<i>C. perfringens</i>	2.39	100	2.40	100	-	-
<i>Clostridium difficile</i>	1	3	No Reliable ID	-	-	-	-	1.15	0
			<i>C. difficile</i>	2.41	100	2.21	100	-	-
<i>Clostridium septicum</i>	1	3	No reliable ID	1.05	0	-	-	1.13	0
			<i>C. septicum</i>	-	-	2.41	100	-	-
<i>Clostridium sordellii</i>	1	3	No reliable ID	1.11	0	-	-	1.19	0
			<i>C. sordellii</i>	-	-	2.21	100	-	-
<i>Clostridium innocuum</i>	1	3	No reliable ID	1.29	0	-	-	1.08	0
			<i>C. innocuum</i>	-	-	2.24	100	-	-
<i>Francisella philomiragia</i>	1	3	No reliable ID	1.11	0	-	-	1.29	0
			<i>F. philomiragia</i>	-	-	1.74	0	-	-
<i>Francisella novocida</i>	2	6	No reliable ID	1.04	0	1.37	0	1.36 (4)	0
			<i>F. tularensis</i>	-	-	-	-	1.74 (2)	0
<i>Haemophilus influenzae</i>	1	3	No reliable ID	1.51	0	-	-	0.59	0
			<i>H. influenzae</i>	-	-	1.88	0	-	-

Conclusions - Preliminary

- CA and ROU libraries may misidentify organisms
 - *B. anthracis* as *B. cereus*
 - *Y. pestis* as *Y. pseudotuberculosis*
 - *B. pseudomallei* as *B. thailandensis*
 - *C. botulinum* as *L. rhamnosis*
- Security library may misidentify organisms
 - *Brucella* spp. as *B. melitensis*
 - *B. thailandensis* vs *B. pseudomallei*
- Unclaimed ID from CA may be misleading
- Results for BT agents and some near neighbors require additional testing
- Software libraries need improvement

Study Participants

- Michigan Department of Health and Human Services
- New York Department of Health, Wadsworth Center, Biodefense Laboratory
- North Carolina State Laboratory of Public Health
- New York City Department of Health and Mental Hygiene
- Florida Department of Health, Bureau of Public Health Laboratories – Jacksonville Branch
- Minnesota Department of Health
- State Hygienic Laboratory at the University of Iowa
- Texas Department of State Health Services, Laboratory Services Section, Austin
- Center for Microbial Genetics and Genomics, Northern Arizona University

Questions

