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

James T. Rudrik, Ph.D.
Michigan Department of Health and Human Services

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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 –20ºC
  - *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 (%)**
S. aureus	2.357	2.368	2.209	3.9
P. aeruginosa	2.049	2.056	1.956	2.8
C. perfringens	2.451	2.444	2.393	1.3

		Range	**Mean score**	**CV (%)**
S. pneumoniae	Replicates (9)	2.082-2.248	2.180	2.9
	Pool	2.131-2.267	2.210	3.2
B. cepacia	Replicates (9)	2.215-2.359	2.317	1.8
	Pool	2.296-2.392	2.338	2.1
M. catarrhalis	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. insidosa*, 1 *A. baumannii*, 1 *P. aeruginosa*, 1 *A. xylosoxidans*, 1 *V. cholarae*, 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

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

Bruker: Near Neighbors

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

Bruker: Near Neighbors

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