

MALDI-TOF: It's Effect on Rule Out and Refer, Workflow, Safety, and Accuracy

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Objectives

- Discuss the implementation of new technology in the clinical lab using MALDI-TOF as an example
- Discuss the limitations of MALDI-TOF for the identification of BT agents
- Describe the critical workflow steps needed for the identification of BT agents using new technologies

New Test Check List

- Specimen collection directions and test interpretation posted to lab website
- LIS configured to report results
- Proficiency testing plan
- Establish calibration and control procedures
- SOP written and approved
- Employee training and competency assessment
- Test validation completed. Reviewed and approved
- Pre-validation risk assessment

Risk Assessment

- Handling chemicals
 - Biosafety cabinet or fume hood
 - Disposal of chemicals contaminated with infectious agents
- Equipment issues
 - Heat
 - Laser
 - Location in BSL-2 or BSL-3
 - Sample preparation of Risk Group 3 organisms for testing in a BSL-2 environment

Sample Preparation 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
- *B. anthracis*, *B. thuringiensis*, *B. cereus*, *B. subtilis* survived tube extraction in other studies
- Use tube extraction AND 0.1 μm filter for “hazardous organisms”

Validation/Verification Study

- Accuracy of identification
 - Sample preparation method(s)
 - Number of isolates tested
 - Age of isolate
 - Effect of media
 - Software libraries to validate
 - Bruker: IVD (210), RUO (2,500), Security (12)
 - Vitek: IVD (193), RUO (1,388)
 - Unclaimed
 - Resolution of discrepancies
- What is an acceptable identification?
 - Bruker: Sample score ≥ 2.0
 - Vitek: Identification score $\geq 60\%$

Study Design

- Determine ability of Bruker and Vitek software libraries to accurately identify BT agents
 - Test IVD, ROU, and Security Libraries independently
- Panel of 6 strains and 6 near neighbors for each agent
 - *B. anthracis*, *Y. pestis*, *F. tularensis* (5), *C. botulinum* (4), *B. mallei*, *B. pseudomallei*, *Brucella* spp.
 - Select isolates from AOAC Stakeholder Panel on Agent Detection Assays (SPADA) – inclusivity and exclusivity panels
 - Each isolate tested in triplicate
- Challenges
 - No SPADA panels for *C. botulinum* or *Brucella* spp.
 - Isolate availability and shipping

Organism (# of strains tested)	Reported Identification	IVD Library		RUO Library		Security Library	
		Mean Score (# replicates)	Species level ID % (>2.0)	Mean Score (# replicates)	Species level ID % (>2.0)	Mean Score (# replicates)	Species level ID % (>2.0)
<i>B. anthracis</i> (6)	No reliable ID	1.13 (90)		1.38 (45)		1.1 (40)	
	<i>B. cereus</i>			1.88 (62)	8.3		
	<i>B. anthracis</i>					2.08 (68)	50
	<i>B. pseudomycooides</i>			1.56 (1)			
<i>B. thuringiensis</i> (1)	No reliable ID	1.1 (12)		1.33 (9)		1.12 (9)	
	<i>B. cereus</i>			2.01 (9)	22.2		
	<i>B. anthracis</i>					2.06 (9)	38.9
<i>B. circulans</i> (1)	No reliable ID	1.06 (12)		1.33 (5)		1.01 (18)	
	<i>B. circulans</i>			1.87 (13)	16.7		
<i>B. cereus</i> (1)	No reliable ID	1.15 (12)		1.35 (9)		1.23 (9)	
	<i>B. cereus</i>			2.08 (9)	44.4		
	<i>B. anthracis</i>					2.14 (9)	50
<i>B. mycooides</i> (1)	No reliable ID	1.15 (15)		1.61 (7)			
	<i>B. mycooides</i>			1.77 (7)			
	<i>B. anthracis</i>					1.77 (18)	
	<i>B. weihenstephanensis</i>			1.19 (4)			
<i>B. megaterium</i> (1)	No reliable ID	1.12 (12)		1.35 (3)		1.06 (18)	
	<i>B. megaterium</i>			1.98 (15)	55.6		
<i>B. subtilis</i> (1)	No reliable ID	1.04 (15)		1.47 (13)		0.98 (18)	
	<i>B. subtilis</i>			1.68 (5)			

Organism (# of isolates tested)	Reported Identification	IVD Library		RUO Library	
		Mean Score (# replicates)	Species level ID % ($\geq 60\%$)	Mean Score (# replicates)	Species level ID % ($\geq 60\%$)
<i>Y. pestis</i> (6)	No Identification	0 (2)		0 (23)	
	<i>Y. pseudotuberculosis/Y. frederiksenii</i>	51.7/48.2 (7)			
	<i>Y. ruckerii/Y. pseudotuberculosis/Y. frederiksenii</i>	33.3/33.4/33.2 (1)			
	<i>Y. pseudotuberculosis</i>	99.9 (17)	58.6	87.8 (18)	33.3
	<i>Y. pestis</i>	99.9 (1)	3.4		
	<i>Yersinia</i> spp.			88.8 (13)	
<i>Y. ruckeri</i> (1)	No Identification	0 (5)			
	<i>Y. ruckerii</i>	99.7 (4)	44.4		
	<i>Yersinia</i> spp.			85.7 (9)	
<i>Y. pseudotuberculosis</i> (3)	No ID	0 (1)			
	<i>Y. pseudotuberculosis</i>	99.6 (20)	95.2	91.9 (20)	95.2
	<i>Y. enterocolitica</i>			94.1 (1)	4.8
<i>Y. enterocolitica</i> (2)	No Identification	0 (1)			
	<i>Y. enterocolitica</i>	99.9 (8)	66.7	97.5 (11)	91.7
	<i>Y. pseudotuberculosis</i>			86 (1)	
	<i>Y. pseudotuberculosis/Y. enterocolitica</i>	49.1/50.9 (3)			

Identification “Errors”

Organism	Concerns
<i>B. anthracis</i>	<i>B. cereus</i> <i>B. cereus</i> group Split identifications
<i>B. thuringiensis</i>	<i>B. anthracis</i>
<i>B. cereus</i>	<i>B. anthracis</i>
<i>Y. pestis</i>	<i>Y. pseudotuberculosis</i> <i>Yersinia</i> spp. Split identifications
<i>Y. pseudotuberculosis</i>	<i>Y. pestis</i>
<i>Y. enterocolitica</i>	<i>Y. pestis</i>
<i>F. philomiragia</i>	<i>F. tularensis</i>

Organism	Concerns
<i>B. mallei</i>	<i>B. pseudomallei</i>
<i>B. pseudomallei</i>	<i>B. mallei</i>
<i>B. thailandensis</i>	<i>B. pseudomallei</i> <i>B. mallei</i>
<i>C. botulinum</i>	<i>C. sporogenes</i>
<i>B. melitensis</i>	<i>Brucella</i> spp.
<i>B. abortus, suis, canis, ovis, ceti pinnipedialis</i>	<i>B. melitensis</i> <i>Brucella</i> spp.

Study Summary

- Software libraries for BT agents need improvement
 - Currently not reliable for BT agents
 - Addition of BT agents to IVD software
 - Availability of alternative libraries i.e. MicrobeNet
- Laboratories must be aware of software limitations
 - Partial or incorrect identifications
 - Additional training by manufacturer or BT coordinator
- Continue use of the rule out and refer protocols

Laboratory Workflow

- Use the Gram Stain
- Apply simple tests
 - Growth rate
 - Colony morphology
 - Catalase
 - Oxidase
- DOES THE RESULT MAKE SENSE!!!!!!