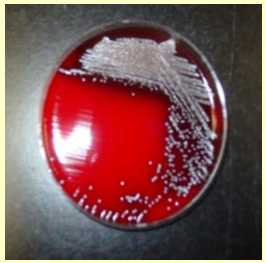
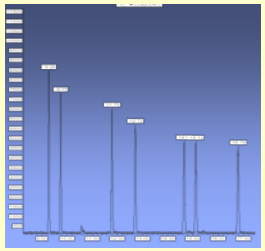




Biosafety Implications of New Technologies and Emerging Pathogens

James T. Rudrik, Ph.D.

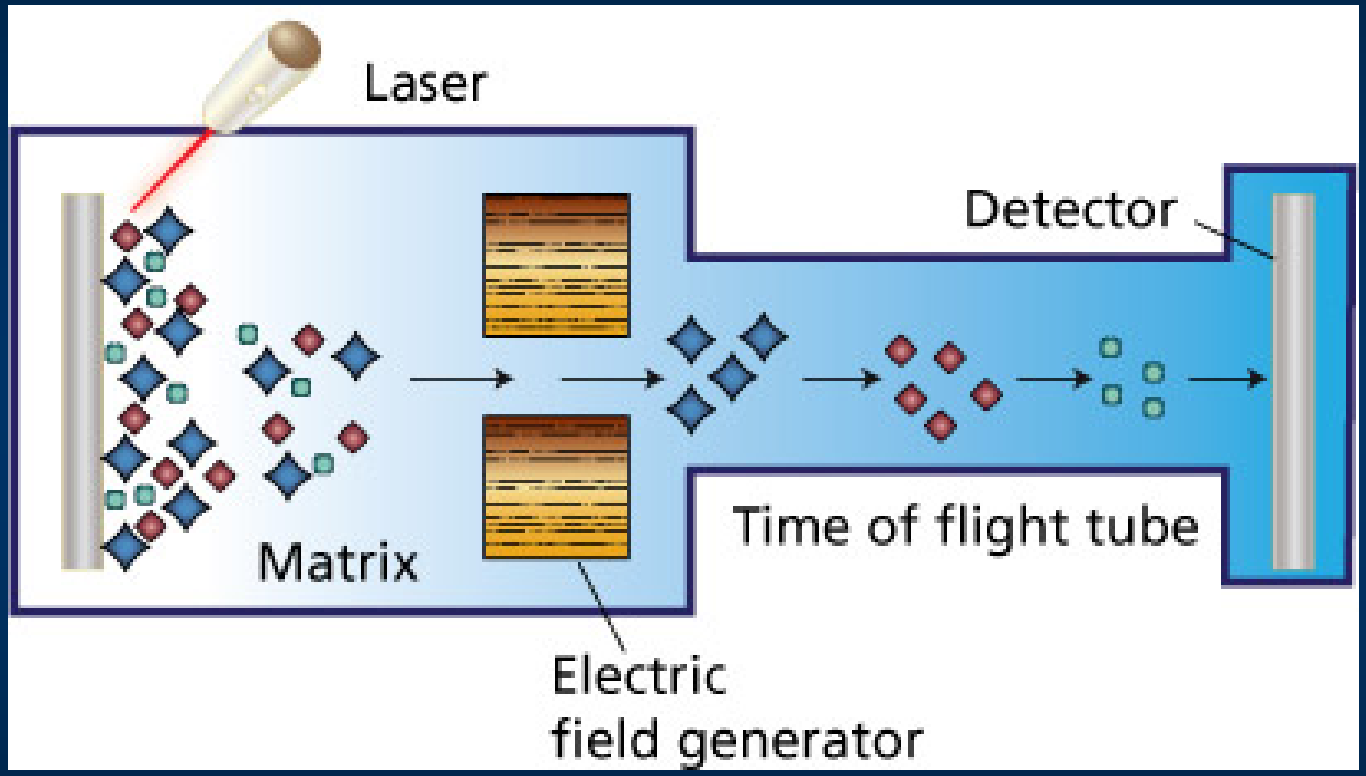
Michigan Department of Health and Human Services

Report on the Potential Exposure to Anthrax

Centers for Disease Control and Prevention

7/11/2014

MDHHS



sigmaaldrich.com

Concerns with MALDI-TOF

- Safety
  - Viability of organisms before and after testing
    - BLS-3 considerations
  - Creation of aerosols by instrument
- Accuracy
  - Ability of the instrument to accurately identify hazardous organisms
    - ROU vs. FDA approved libraries
    - Vitek vs. Bruker

MDHHS

Agents Tested

- *Brucella abortus* Strain 19
- *Bacillus anthracis* Sterne
- *Burkholderia thialandensis* ATCC 70038
- *Francisella tularensis* LVS
- *Yersinia pestis* A1122
- *Clostridium* spp.
  - *botulinum* types A, B, and E; *perfringens*

Sample Preparation - Direct

1. Smear biological material (single colony) as a thin film directly onto a cleaned MALDI target. **IMPORTANT** After the sample has dried, matrix must be added within 10 minutes.
2. Carefully overlay each sample position (= spot) with 1 µL HCCA matrix solution.
3. Allow the sample positions to dry at room temperature.

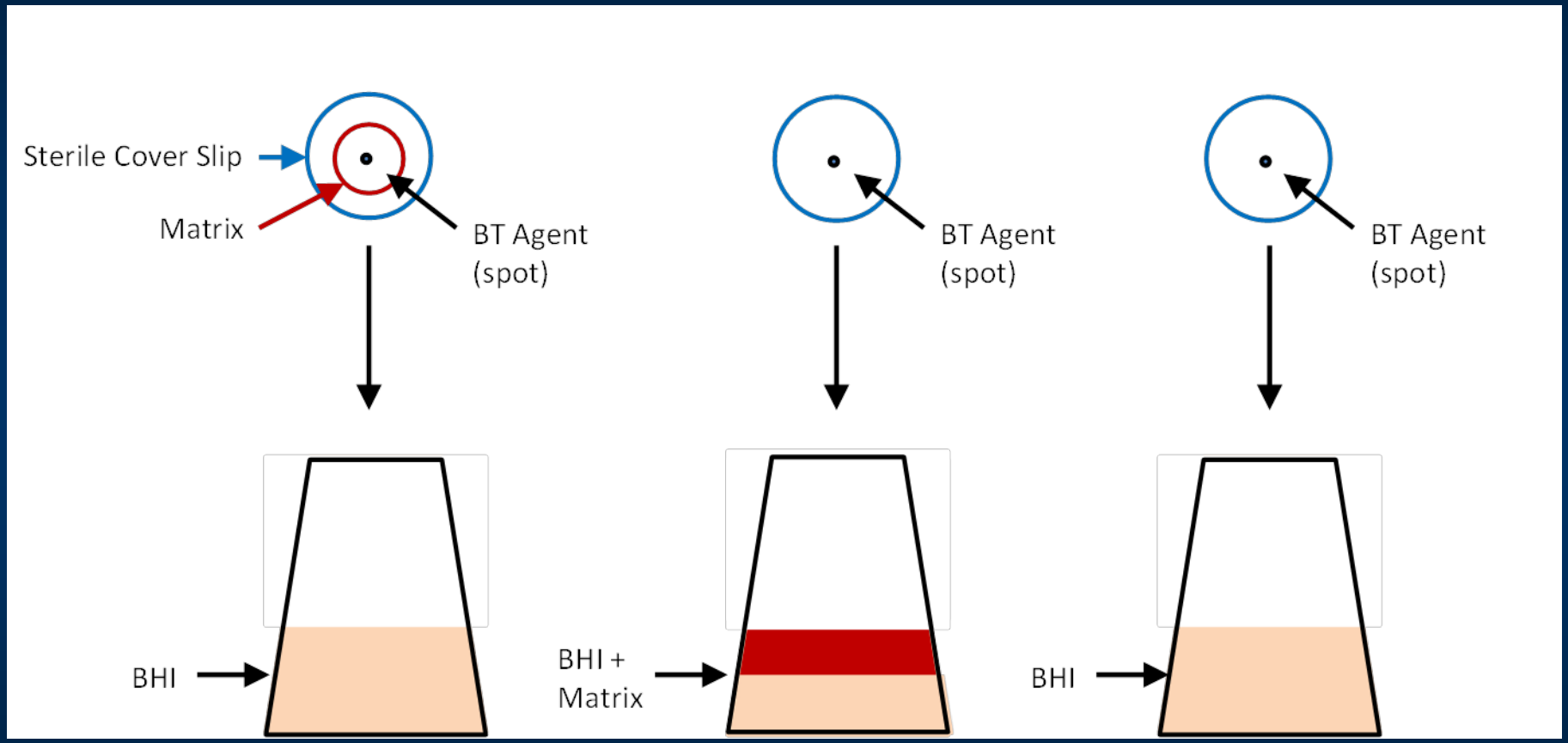
Sample Preparation – Extended Direct

1. Smear biological material (single colony) as a thin film directly onto a cleaned MALDI target. **IMPORTANT** After the sample has dried, matrix must be added within 10 minutes.
2. Cover smear with 1 µL of 70% formic acid and allow to dry completely.
3. Carefully overlay each sample position (= spot) with 1 µL HCCA matrix solution.
4. Allow the sample positions to dry at room temperature.

Sample Preparation –Tube Extraction

1. Pipet 300 µL ultra pure water into a clean Eppendorf tube. Transfer a single colony of biological material into the tube. Vortex for at least one minute.
2. Add 900 µL of pure ethanol into the tube and vortex the suspension for at least one minute.
3. Centrifuge the tube for 2 minutes at 13,000 rpm and remove the supernatant.
4. Repeat step 3. All residual ethanol should be removed.
5. Add 50 µL of 70% aqueous formic acid, mix thoroughly and let stand at least 5 minutes.
6. Add 50 µL acetonitrile to the tube and mix carefully.
7. Centrifuge the tube for 2 minutes at 13,000 rpm.
8. Pipette 1 µL of microorganism extract supernatant onto a cleaned MALDI target.
9. Allow the sample positions to dry at room temperature. **IMPORTANT** After the sample has dried, matrix must be added within 10 minutes.
10. Carefully overlay each sample position (= spot) with 1 µL HCCA matrix solution.
11. Allow the sample positions to dry at room temperature.

MDHHS


Sterile Cover Slip
Matrix
BT Agent
(spot)
BT Agent
(spot)
BT Agent
(spot)
BHI
BHI +
Matrix
BHI

Results

Organism	Direct Method			Extended Direct			Tube Extraction		
	Target	Spot + Matrix	Spot	Target	Spot + Matrix	Spot	Target	Spot + Matrix	Spot
B. anthracis	3/5	5/5	5/5	1/5	5/5	5/5	0/5	1/5	5/5
B. thailandensis	0/5	5/5	5/5	0/5	5/5	5/5	0/5	0/5	5/5
Clostridium spp.	1/5	1/5	3/5	1/5	0/5	2/5	0/5	1/5	4/5
F. tularensis	1/5	2/5	4/5	1/5	2/5	5/5	0/5	1/5	5/5
Y. pestis	2/5	5/5	5/5	0/5	4/5	5/5	0/5	1/5	4/5
B. abortus	0/4	3/4	4/4	1/4	4/4	4/4	0/4	0/4	3/4

MDHHS

Study Conclusions

- Drying may result in loss of viability
- Some viable organisms are present on the target using the direct and extended direct sample prep methods
- No viable organisms were found following the tube extraction
- Study is limited by the number and types of strains tested

Safety Considerations

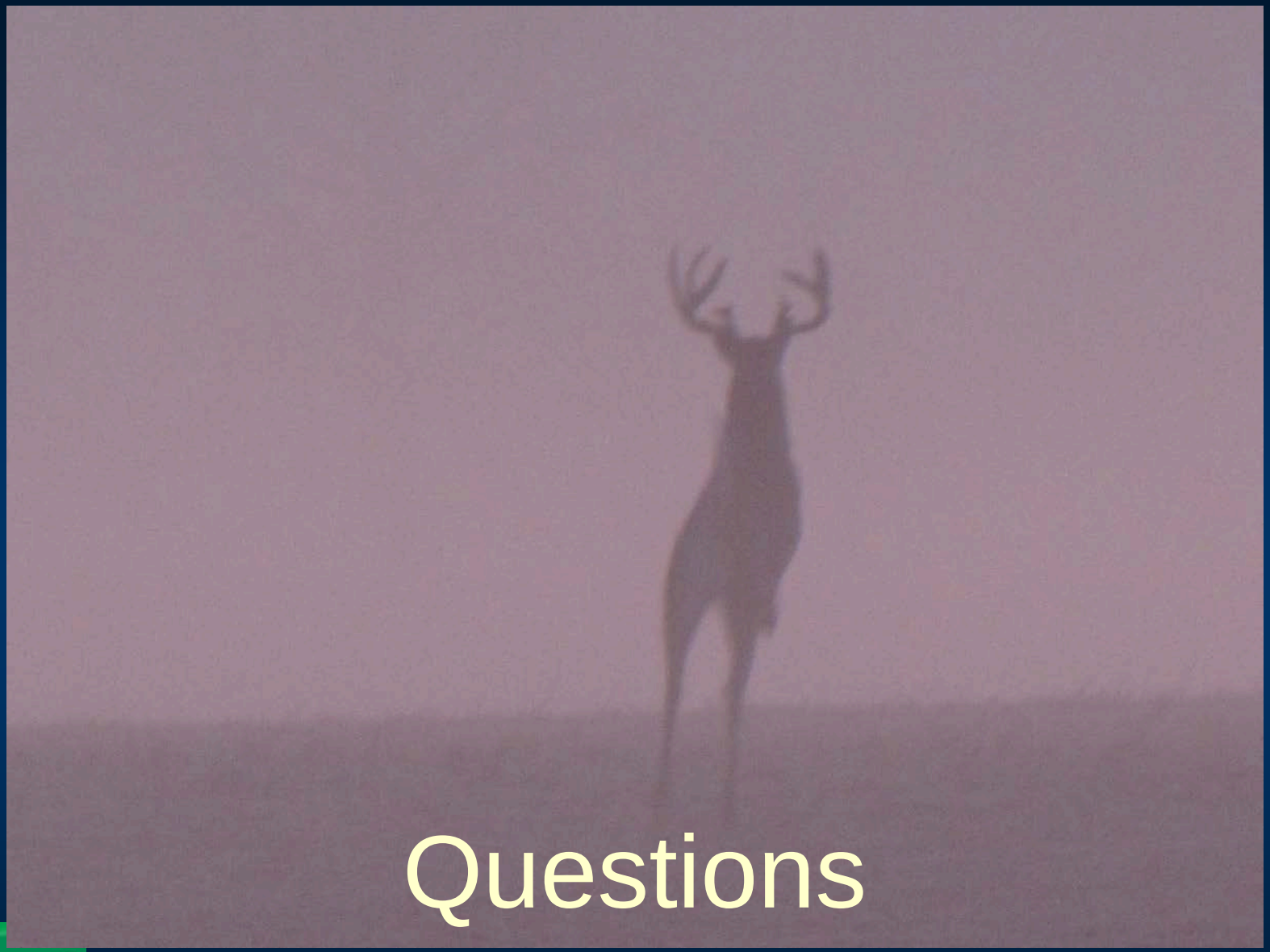
- Clean target often
- Make sure matrix completely covers “spot”
- Use tube extraction AND 0.1 µm filter for “hazardous organisms”

Addressing safety

- New Equipment Purchase Checklist
  - Biosafety level
- New Test Implementation Checklist
  - Samples
  - Sample prep
  - Sample transport
  - Waste disposal

Participating Laboratories

- Michigan Department of Community Health
- Wadsworth Center, New York Department of Health
- Texas Department of State Health Services
- Wisconsin State Laboratory of Hygiene
- North Carolina State Laboratory of Public Health
- State Hygienic Laboratory at the University of Iowa



Questions