

Emerging Technologies in Preparedness: View from the Sentinel Level Laboratory

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DISCLOSURES

BioFire Defense, LLC Proxy Board

OpGen, Inc; Advisory Board

OBJECTIVES

- Review role of Sentinel Laboratories in the Laboratory Response Network
- Current technology
- Future BT Diagnostic technology designed for DoD using a common instrument found in Sentinel Laboratories
- SAFETY

***RedLine Alert*[™] Test**

Principles of Test

- **Antigen Capture**
- **Antibody Sandwich Format**
 - **Monoclonal Ab as Detector (colloidal gold labeled)**
 - **Polyclonal Ab as Capture**
- **Lateral Flow Device**
- **Visual Detection of Colored Line for Positive**



Intended Use

The Tetracore *RedLine Alert*TM Test is an immunochromatographic test intended for the rapid, *in vitro* qualitative presumptive identification of *Bacillus anthracis* from non-hemolytic *Bacillus* colonies cultured on sheep blood agar plates. The test is intended for use in clinical, public health, and hospital laboratories in conjunction with other markers and testing for the presumptive identification of *Bacillus anthracis*.



Interpretation of Results

If the test is positive and the bacterial colony is non-hemolytic, *B. anthracis* cannot be ruled out. Submit the sample to the State Public Health Laboratory or State Public Health Department for confirmation.



Summary of Additional Biothreat Alert Test Strips

- Plague
 - Tularemia
 - Ricin
 - Botulinum Toxin
 - Staph Enterotoxin
 - Orthopox
- Brucella
 - Abrin
 - Proficiency Test
 - Sample Collection Kit
 - BW Simulants

RedLine Alert

- Inoculum dependent
- Expensive (\$22 – 25)
- Presumptive
- Cost-effective?
- Low utilization in Sentinel Laboratories
- Not a substitute for LRN Sentinel Protocols
- Results **MUST** be confirmed by a certified laboratory (LRN Reference Laboratory)

Current Technologies

- BT Agents

1. Reverse Transcription-PCR-Electrospray Ionization Mass Spectrometry (ESI-MS)
 - Amplicons generated via (RT)-PCR

2. MALDI-TOF MS

- used in 10-15% (estimated) Sentinel Laboratories

RT-PCR ESI-MS

- *Bacillus anthracis*
- *Yersinia pestis*
- *Francisella tularensis*
- *Brucella* spp.
- *Burkholderia* spp.
- *Rickettsia prowazekii*

Mass Spectrometry MALDI-TOF

Bruker

Kiester, BD

bioMurieux

Microscan



MALDI-TOF MS

- Revolutionizing Clinical Microbiology
- Recently FDA approved
- Rapid, Inexpensive (initial capital expense)
- Bacteria, Fungi, AFB, Antimicrobial Resistance
- Separate Biothreat Package
 - *B. anthracis*
 - *Brucella*
 - *Burkholderia mallei*
 - *Francisella*
 - *Yersinia pestis*
 - *C. botulinum*
- *Shigella, Salmonella, Vibrio, Xanthomonas albilineans*

MALDI-TOF MS

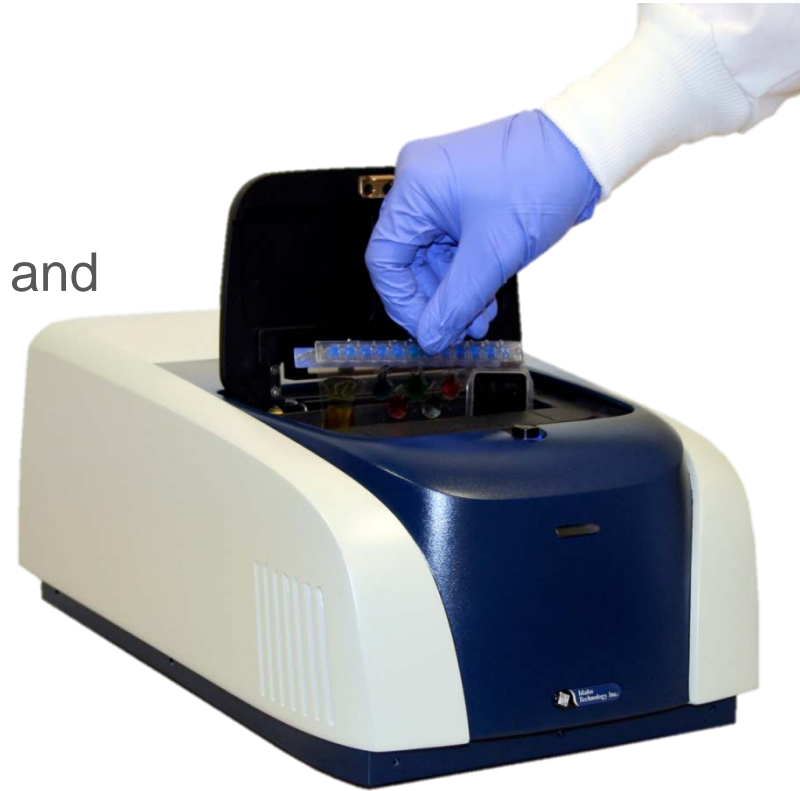
- Protein Extraction
 - trifluoroacetic
 - chloroform
 - acetonitrile
 - ethanol-formic acid
 - **ethanol, formic acid, acetonitrile**
- Standardization? (Growth Conditions?, Density of test Inoculum)
- BSL-3 or BSL-2/BSL-3?

Rule-Out Testing and MALDI-TOF

- Recommended Extraction Method to ensure safety of testing personnel and instrument contamination in a BSL-2 environment?
- Laboratory acquired infections via aerosols or incomplete decontamination of instrument
 - current safety study by APHL/ASM
 - organism viability (matrix treatment, extraction)?
 - sporocidal?
- Extent of, and reliability of data bases for BT agents?
- LRN Reference Labs responsible for confirmatory testing
- Sentinel Protocols for Rule-Out
- Reference Labs proactive in training/educating Sentinel Labs

FilmArray - Simple, Easy, Fast

- Laboratory Platform
- Commercial BioThreat Panel Available
- DTRA Funded Development of Dengue and Malaria Assays (modified BWA panel)
- Down select US DoD Next Generation Diagnostic System (NGDS)
 - (JBAIDS Replacement)
- 125 Systems in US DoD
- UK MOD >20 Systems
 - 2014-2015 Programs around FilmArray



FilmArray Commercial BioThreat Panel*

Viral Pathogens	Bacterial Pathogens	Toxin Gene Targets
Ebola virus	<i>Bacillus anthracis</i> (pXO1, pXO2, chromosomal)	Ricin toxin gene from <i>Ricinus communis</i>
Marburg virus	<i>Yersinia pestis</i>	SEB gene from <i>Staphylococcus aureus</i>
Eastern Equine Encephalitis virus	<i>Francisella tularensis</i>	Botulinum toxin from <i>Clostridium botulinum</i>
Western Equine Encephalitis virus	<i>Brucella</i> (<i>melitensis</i> species)	
Venezuelan Equine Encephalitis virus	<i>Burkholderia</i> species (<i>mallei</i> , <i>pseudomallei</i>)	
Variola major virus	<i>Coxiella burnetii</i>	
Orthopox virus	<i>Rickettsia</i> species (spotted fever group, typhus group)	

*Restricted to Department of Defense

SUMMARY

- Sentinel Labs desire to remain on “cutting edge”
- MALDI-TOF biggest challenge
- Filmarray BT technology and Sentinel Labs?
- Strengthen Public Health and Private Sector partnership
- SAFETY, SAFETY, SAFETY

THANK YOU!

QUESTIONS?