DLS ECHO Biosafety Session: March 22, 2023

Safely Implementing New Diagnostics Platforms Commonly Used in Clinical Laboratories

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CDC Centers for Disease Control and Prevention

UNM Health Sciences

Project ECHO

Agenda

- Didactic and Case Presentation
- Discussion
- Summary of Discussion
- Closing Comments and Reminders

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Safely Implementing New Diagnostic Platforms Commonly Used In Clinical Laboratories

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Overview

- Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)
- Automated Microbial Identification Systems
- Facility/Safety Considerations
- Examples of High-Risk Pathogens in Clinical Labs
- Clinical Cases
- Best Practices

Objectives

- Identify effective biosafety practices that strengthen laboratory systems and advance laboratory safety
- Examine biosafety concepts that apply to conducting risk management when performing laboratory activities.

MALDI-TOF MS Technology

- **Ionization Source**
  - Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI MS)
  - Ions are created in the sample as a result of pulsed laser irradiation

- **Mass Analyzer**
  - **Time of flight (TOF)**
    - Uniform electromagnetic force is applied to all ions at the same time, causing them to accelerate down a flight tube
    - Lighter ions travel faster, arrive at the detector first (m/z)

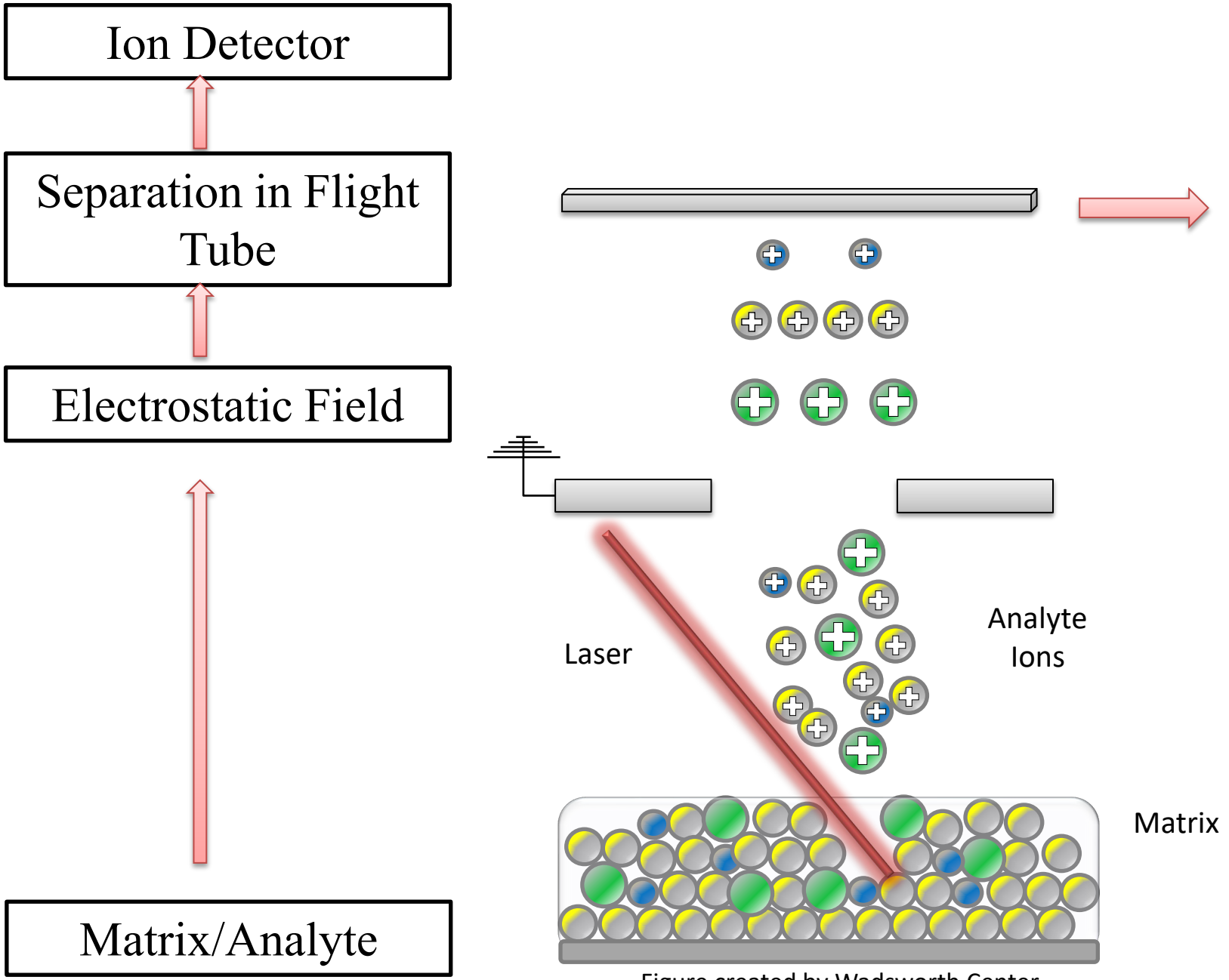

Figure created by Wadsworth Center

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Facility and Safety – General Safety Considerations

https://www.pngegg.com/en/png-bbekj/download;
https://www.vecteezy.com/free-vector/biohazard

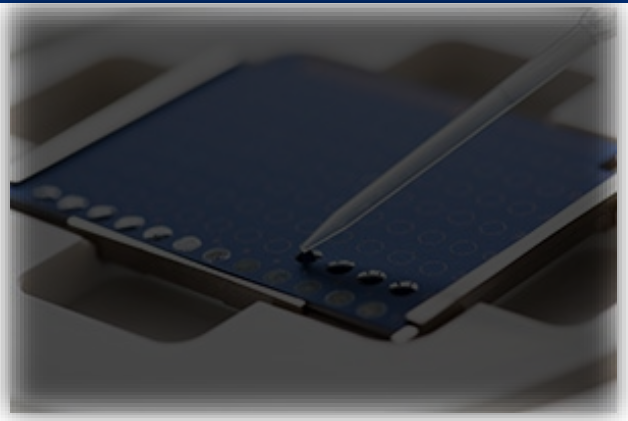
- Direct contact with reagents
- Exposure to chemical fumes
- Examining or manipulating cultured microorganisms
- Handling prepared target slides or plates
- Safe handling of primary patient specimen cultured microorganism to prevent Laboratory Acquired Infections (LAIs)

Chemical Hazard Considerations

- **Phenolic acid matrix (typically α-Cyano-4-hydroxycinnamic acid) that is solubilized with organic solvents**
- **Additional chemicals**
  - Acetonitrile
  - Ethanol
  - Formic Acid
  - Trifluoroacetic acid
- **Small aliquots of matrix and FA solutions can be handled safely on the benchtop**
  - Gloves, protective clothing, well-ventilated room
- **Other processes may involve larger volumes and more hazardous chemicals**
  - Should be performed in a chemical fume hood

Safety Considerations: Biohazards

- **Highest risk:**
  - Handling/manipulation of specimens
  - Disposal of primary specimens
  - Cultured microorganisms before analysis



https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/microbial-identification/consumables-accessories-for-gp-and-ruo-systems.html

- **Direct transfer onto a MALDI-TOF target should be performed with caution using BSL-2 practices and facilities**
  - However, manipulation should use more stringent biosafety practices
  - At minimum, spotting should be performed within a laminar flow BSC.
    - If not, a face shield should be used

- **Inactivation with Matrix**
  - Biomass thickness
  - Encompassing the spot

- **Every lab should adopt and verify recommended inactivation protocols**

MALDI MS TOF Method – Inactivation Efficiency

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

Safety and Accuracy of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry for Identification of Highly Pathogenic Organisms

James T. Rudrik,[a] Marty K. Soehnlen,[a] Michael J. Perry,[b] Maureen M. Sullivan,[c] Wanda Reiter-Kintz,[d] Philip A. Lee,[e] Denise Pettit,[f] Anthony Tran,[g] Erin Swaney[h]

https://pubmed.ncbi.nlm.nih.gov/29021156/

Article Conclusion:

- Direct and extended direct methods – may contain viable organisms
- Tube extraction method – no viable organisms
- Exposure to air decreased the viability of *C. botulinum / C. perfringens*
- Used surrogates or attenuated strains, results for wild type strains might vary

Recommendations:

- Suspected highly pathogenic organisms – use tube extraction method
- Ideally, sample preparation in a BSL-3 or minimally BSC
- Filter tube extraction (0.1 µM filter)

Inadvertent Analysis of High-Risk Pathogens

- Despite enhanced biosafety education and improved lab practices, LAI continue to pose a risk to personnel
- If a high-risk infectious agent is suspected, labs should consult with appropriate reference lab before beginning any testing
- Analysis of known high-risk pathogens should be avoided

Picture courtesy of Mike Wren, NYSDOH

Inadvertent Analysis of High-Risk Pathogens

High-risk or select agent on a MALDI-TOF MS

- Immediately report incident
- Follow institutions biosafety and infection control procedures

Risk management steps should include:

- Determining who was potentially exposed
- What safety measures were taken
- Post exposure prophylaxis and/or health monitoring
- Sequester materials
- Autoclave contaminated disposables
- Thoroughly clean affected bench areas

Picture courtesy of Mike Perry, NYSDOH

Inadvertent Analysis of High-Risk Pathogens

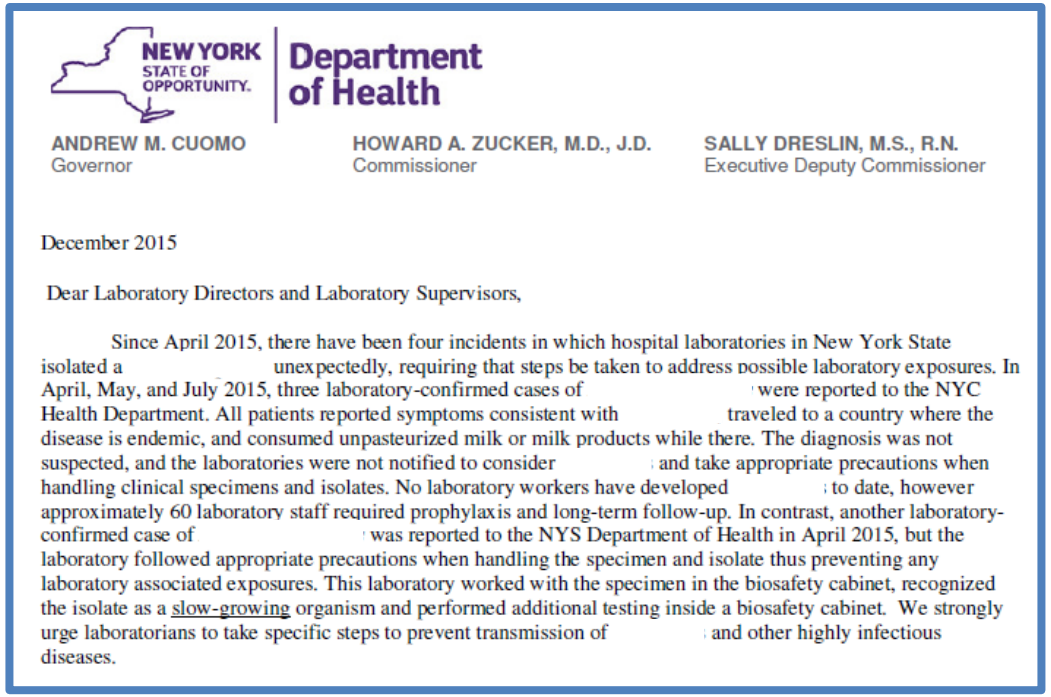
- **Additional safety measures are needed because database limitations exist**
  - Closely related ID or “no identification” may indicate the presence of a high-risk pathogen

- **Early indicators should be used to prevent inadvertent analysis of high-risk pathogens**
  - Gram Stain
  - Biochemical Results
  - Travel History

Clinical Cases

High Risk Pathogen Exposure

- In 2015, NYS had 4 **High Risk Pathogen** cases in a 3-month period
- 3 cases resulted in laboratory acquired exposures
- NYC and NYS sent out alerts to clinical labs and physicians to remind them about proper lab protocols involving isolation of High Risk Pathogens and alerting the lab if physicians are suspicious



NEW YORK STATE OF OPPORTUNITY. Department of Health

ANDREW M. CUOMO
Governor

HOWARD A. ZUCKER, M.D., J.D.
Commissioner

SALLY DRESLIN, M.S., R.N.
Executive Deputy Commissioner

December 2015

Dear Laboratory Directors and Laboratory Supervisors,

Since April 2015, there have been four incidents in which hospital laboratories in New York State isolated a unexpectedly, requiring that steps be taken to address possible laboratory exposures. In April, May, and July 2015, three laboratory-confirmed cases of were reported to the NYC Health Department. All patients reported symptoms consistent with traveled to a country where the disease is endemic, and consumed unpasteurized milk or milk products while there. The diagnosis was not suspected, and the laboratories were not notified to consider and take appropriate precautions when handling clinical specimens and isolates. No laboratory workers have developed to date, however approximately 60 laboratory staff required prophylaxis and long-term follow-up. In contrast, another laboratory-confirmed case of was reported to the NYS Department of Health in April 2015, but the laboratory followed appropriate precautions when handling the specimen and isolate thus preventing any laboratory associated exposures. This laboratory worked with the specimen in the biosafety cabinet, recognized the isolate as a slow-growing organism and performed additional testing inside a biosafety cabinet. We strongly urge laboratorians to take specific steps to prevent transmission of and other highly infectious diseases.

Health Commerce Communication to NYS Permitted Labs, Dec 2015

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Brucella Exposure

- Several exposures related to new instrumentation in the lab (MALDI-TOF)
- Public Health Laboratory Response Network (LRN) works with labs to provide information on prevention of lab acquired exposures and infections
- Evaluate MALDI-TOF for biosafety concerns

Picture courtesy of Mike Perry, NYSDOH

Brucella Exposure at 3 Labs from 1 Patient

Isolates Received at WC (05/2016) – Patient traveled from Mexico to US in 12/2015

- **Isolate 1**
  - Isolate received for confirmation from hospital 1
  - Hospital 1 ID'ed isolate as *Haemophilus influenzae*, gram negative coccobacillus
  - Sub-cultured on bench, PCR ruled out *Haemophilus influenzae*
  - Re-plated and prepared for MALDI-TOF MS on the benchtop
  - No indication this isolate was *Brucella* until 2nd isolate (below) was identified at Wadsworth Center

- **Isolate 2**
  - Isolate from same patient from hospital 2
  - ID'ed at hospital 2 as unknown Gram-negative coccobacillus
  - Worked up in BSC but initial biochemical did not rule-in *Brucella*
  - Re-plated and prepared for MALDI-TOF MS on the benchtop.
  - MALDI-TOF ID'ed high match to *Brucella*

- **Isolate was moved to the BSL-3 where confirmatory methods ID'ed *Brucella melitensis***

Wadsworth Center Workflow Pre-Exposure

Wadsworth Center Flowchart for processing primary specimens (Pre-Exposure)

Primary specimen received with request to test for or confirm any of the following:

Bacillus anthracis
Brucella species
Burkholderia mallei
Burkholderia pseudomallei
Coxiella species
Francisella species
Q-fever
Rickettsia prowazekii
Rickettsia rickettsii
Yersinia pestis

↓

Contain primary specimen in a biological safety cabinet
Consult supervisor
Send to Biodefense Laboratory

Primary specimens for bacterial culture

↓

Inoculate culture plates inside biological safety cabinet

↓

Source: Respiratory site or sterile site such as blood or CSF

↓

Growth of gram-negative diplococci suggestive of *Neisseria meningitidis*

↓

Work in BSC until *Neisseria meningitidis* is ruled out

Any specimen source

↓

Slow-growing colonies on blood agar and Gram stain shows gram-negative coccobacilli

↓

Work in BSC

↓

Consult supervisor
Follow flowchart BAC 23 or BAC 27 to rule out select agents as appropriate

Other bacterial Growth rate

↓

Work on benchtop using appropriate sterile technique

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Why did exposures occur?

- **Rule-out algorithm was too complex**
  - Needs to be simplified
- **Use of Bunsen burner to sterilize metal inoculating loop – generates aerosols**
  - Use microincinerators
- **Spotting MALDI plates on open bench without facial barrier**
  - Use face shield, benchtop shield, or BSC

https://www.aphl.org/aboutAPHL/publications/Documents/PHPR-2020-Biothreat-Rule-Out.pdf

Start all work in the biological safety cabinet

Isolate sent by submitter as an unknown Gram negative rod (GNR), Gram variable rod (GVR), Gram negative coccobacilli (GNCB) or Gram variable coccobacilli (GVCB)

Work inside BSC to plate original to BAP.
Read growth on BAP at 24 hours and 48 hours.
Prepare Gram stain slide

Growth: Slow growing or suspicious for slow growth rate
Gram Stain: GNR or GVR or GNCB or GVCB

Gram stain, colony morphology and growth rate not suspicious

Send to Biodefense Laboratory to rule out *Brucella* species and *Francisella tularensis*

Continue work inside BSC. Setup oxidase, urea, TSI, and motility to rule out select agents

If suspicious for select agent:
Review Gram stain, colony morphology, and growth rate
Consult supervisor and send to Biodefense Laboratory if needed

If organism is *Brucella* species or *F. tularensis,* contain all plates and slants with growth of this organism in safety carrier and send to the Biodefense Laboratory

If isolate is not *Brucella* species or *Francisella* species Biodefense laboratory sends isolate to Bacteriology Lab

Suspicious for: *B. mallei*
•Oxidase +/-
•TSI: No change
•Motility -

Suspicious for: *Y. pestis*
•Oxidase -
•Urea -
•Motility -

Suspicious for: *B. pseudomallei*
•Oxidase +
•TSI: NC or slight oxid.
•Motility +

After select agents are ruled out can work with isolate outside the BSC. Proceed with MALDI-TOF algorithm for processing isolates submitted to the Bacteriology Laboratory

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Recent *Brucella* Exposure case in NYS

04/2017

- **Large network micro lab**
  - Patient traveled to Mexico
  - Blood culture bottle indicated positive after 3 days
  - Oxidase, urease, catalase positive – Open Bench
  - Small faint colonies on blood plate after 24 hrs, lab decided to run it on MALDI
    - *Prepared slide on open bench*
    - *MALDI – No Identification*
  - Gram strain from blood plate indicated gram negative coccobacilli
  - Lab consists of a large open room with many technologists resulting in numerous laboratory exposures

Success!...Someone is listening

- Sentinel lab received positive *Brucella* specimen in 2016
- Multiple laboratory exposures in 2016
- Lab received a specimen for identification in 2017
- All work was in a BSC
  - Positive blood culture bottle
  - Gram stain – gram negative coccobacilli
  - Culturing and subbing
- No additional work was conducted
- Secured subbed plates and blood culture bottle
- Two hospital labs, both received samples from the same patient

Success!...Someone is listening

***Number exposures = 0**

Risk Assessment: Things to Consider

Primary Concerns for MALDI-TOF

- Extraction Method
- Initial spotting
- Matrix application
- MALDI target transfer/removal to instrument
- Target cleaning

Risk Assessment: Things to Consider

- Sample Preparation Considerations
- Decontamination Considerations

Risk Assessment: Things to Consider

Sample Preparation Considerations

- Review the culture handling steps when picking colony
- Which extraction/processing sample preparation method was used?
  - Direct transfer
    - Recommended by manufacturers, but can result in viable organism handling
  - On-plate formic acid
  - Ethanol and Formic Acid tube extraction
- Filtration step used?
- How was the application/smear step performed?
- How was the matrix added?
- Was the sample(s) loaded into the MALDI properly
- Was a standard inoculum used
  - Inoculum biomass may play a role in inactivation
- Correctly following procedural steps

Risk Assessment: Things to Consider

Decontamination Considerations

- Don appropriate PPE
- Use appropriate disinfectant
- Decontaminate outside of instrument and any space around it
- Decontaminate the inside of the tray and sample door
- Decontaminate any other involved areas
  - BSC
  - Secondary containment
  - Transfer equipment
- Change filters
- **Do not attempt to disinfect or decontaminate the inside of the instrument without consulting with the manufacturer**
  - Call manufacturer to explain the incident and request their input for decontamination response

AUTOMATED MICROBIAL IDENTIFICATION SYSTEMS

Automated Microbial Identification Systems

Examples of Microbial Identification Systems

www.api.org

https://www.biomerieux-usa.com/vitek-2

Based on use of dehydrated substances to detect enzymatic activity and fermentation of sugars which produce a pH change resulting in a color change

Based on the use of miniaturized fluorescent biochemical tests that can produce results in hours

Safety Considerations: Biohazards

- **Highest risk during:**
  - Handling/manipulation
  - Cultured microorganisms before analysis

- **Preparation of bacterial suspensions should be performed using BSL-2 practices and facilities**

- **There should be no open tube vortexing of bacterial suspensions**

- **Tubes should be carefully opened as to avoid any aerosol generation**

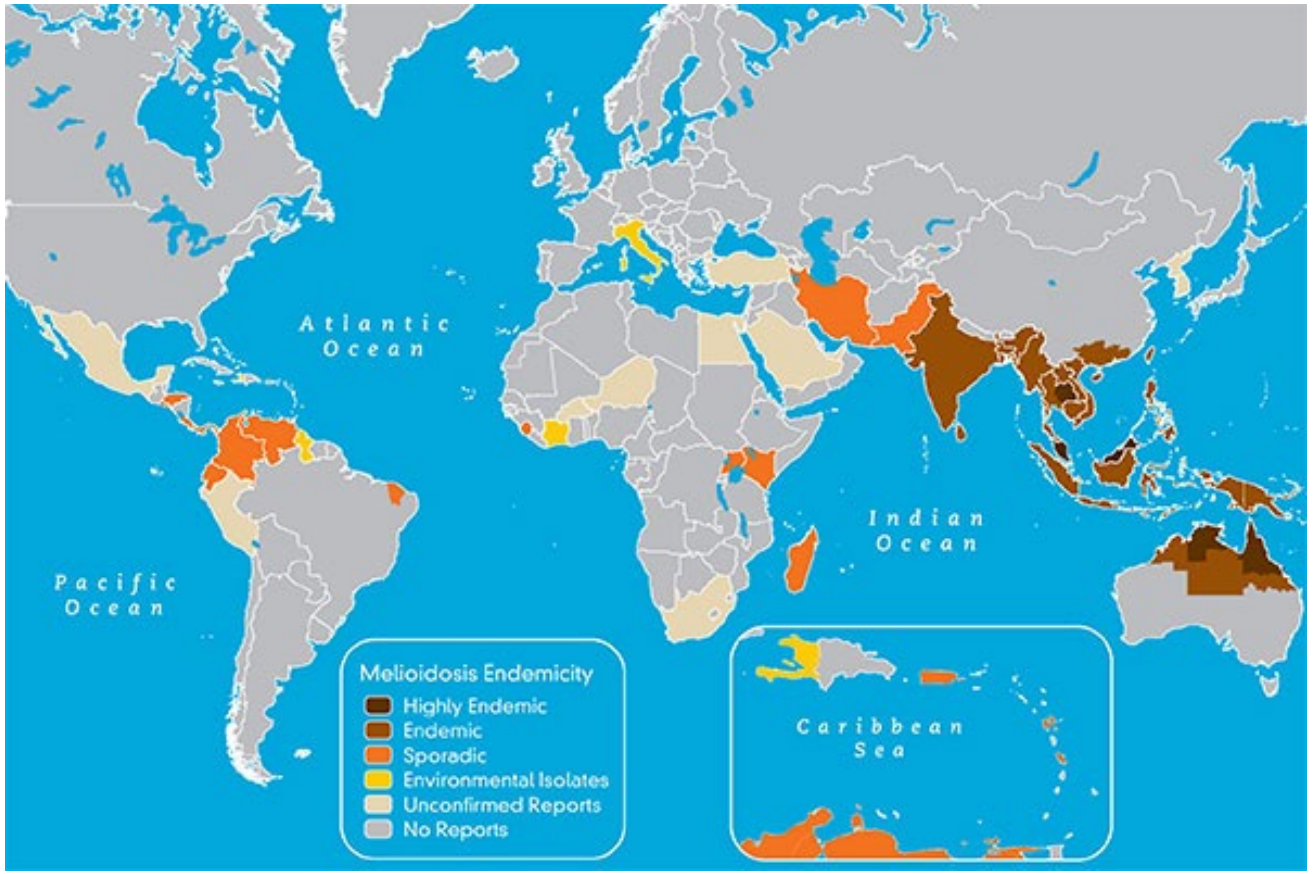
High Risk Pathogens in Clinical Microbiology Laboratory

High Risk Pathogens in Clinical Microbiology Laboratory

- *Brucella spp.*
- *Francisella tularensis*
- *Neisseria meningitidis*
- *Mycobacteria tuberculosis*
- *Burkholderia pseudomallei-* *newer pathogen of concern in USA

Burkholderia pseudomallei

- **Causes melioidosis**
- **Saprophytic, aerobic, gram-negative bacillus**
- **Found in soil and surface water**
  - Tropics, especially Southeast Asia and northern Australia



https://www.cdc.gov/melioidosis/prevention.html

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Human Melioidosis

- 0-5 US cases annually, acquired overseas
- Diabetes, chronic kidney/liver disease, and immunosuppression are risk factors
- Manifestations
  - Bacteremia and fulminant sepsis
  - Pneumonia, often with abscesses
  - Cutaneous infection
  - Solid organ abscesses and granulomas
- Can be chronic, smoldering, or recurrent
- Incubation period can be long
- Treatment – IV followed by oral antibiotics, 3-6 months total
- Case fatality rate 15-40%
  - Depending on promptness of recognition and treatment initiation

Melioidosis Locally Endemic in Areas of the Mississippi Gulf Coast after *Burkholderia pseudomallei* Isolated in Soil and Water and Linked to Two Cases – Mississippi, 2020 and 2022

Print

HAN HEALTH ALERT NETWORK

This is an official CDC HEALTH ADVISORY

Distributed via the CDC Health Alert Network
July 27, 2022, 3:30 PM ET
CDCHAN-00470

Summary

The Centers for Disease Control and Prevention (CDC) identified the bacterium *Burkholderia pseudomallei (B. pseudomallei)* for the first time in the environment in the continental United States. This bacterium causes a rare and serious disease called melioidosis. *B. pseudomallei* was identified through environmental sampling of soil and water in the Gulf Coast region of southern Mississippi during an investigation of two human melioidosis cases.

It is unclear how long the bacterium has been in the environment prior to 2020 or how widespread the bacterium is in the continental United States; modeling suggests that the environmental conditions found in the Gulf Coast states are conducive to the growth of *B. pseudomallei* [1]. Extensive environmental sampling is needed to answer these questions.

This Health Alert Network (HAN) Health Advisory serves to alert clinicians and public health officials throughout the country to consider melioidosis in patients whose clinical presentation is compatible with signs and symptoms of the disease, regardless of travel history to international disease-endemic regions, as melioidosis is now considered to be locally endemic in areas of the Gulf Coast region of Mississippi.

https://emergency.cdc.gov/han/2022/han00470.asp?ACSTrackingID=USCDC_511-DM86587&ACSTrackingLabel=HAN%20470%20-%20General%20Public&deliveryName=USCDC_511-DM86587

Case Of Deadly Tropical Disease In MN Linked To Aromatherapy

Walmart recalled Better Homes and Gardens aromatherapy room sprays after bacteria sickened four people, with two of them later dying.

William Bornhoft, Patch Staff

Posted Thu, Mar 3, 2022 at 2:33 pm CT | Updated Thu, Mar 3, 2022 at 2:36 pm CT

Replies (2)

A Minnesotan is among the four cases of a rare tropical disease — typically found in South Asia — that was linked to an imported aromatherapy spray product sold at Walmart stores. (Image via U.S. Consumer Product Safety Commission)

MINNEAPOLIS — A Minnesotan is among the four cases of a rare tropical disease — typically found in South Asia — that was linked to an imported aromatherapy spray product sold at Walmart stores.

The Minnesota patient survived, but the same disease recently killed a 5-year-old boy in Georgia and a 53-year-old woman in Kansas.

https://patch.com/minnesota/southwestminneapolis/case-rare-tropical-disease-mn-linked-aromatherapy

Clinical Cases

2 *Burkholderia pseudomallei* Lab Exposures (November 2022 & January 2023)

- 67 yr old male with travel to Honduras presented at hospital 24 hrs after return to US
- Treated for meningitis and given antibiotics
- Blood cultures obtained and sent out to reference laboratory
- Blood cultures were positive the next day
- Work was performed outside the BSC including automated ID system and MALDI-TOF
- Preliminary ID was *B. pseudomallei*
- Exposures in reference lab: **13**
  Exposures in original lab: **0**

- 63 yr old female with recent travel to Africa
- 3 weeks of cough, dyspnea, anorexia, nausea, fever while in Africa
- Lab processed specimen/isolate for automated identification and manipulated the isolate on the bench
- Preliminary ID was *B. pseudomallei*
- *Exposures in the lab:* ***10***

Automated Systems and Misidentification of *B. pseudomallei* and *B. mallei*

- Automated systems may misidentify *B. pseudomallei* as another bacterium
- Misidentifications may include *Burkholderia spp.* (specifically *B. cepacia* and *B. thailandensis*), *Chromobacterium violaceum, Ochrobactrum anthropi*, and often *Pseudomonas* spp., *Acinetobacter* spp., and *Aeromonas* spp.

https://www.clinmicronow.org/doi/10.1128/9781683670438.CMPH.ch16.9-2

- In a study that describes the reliability of commercial systems for the identification *B. mallei,* 3 systems either incorrectly identified or failed to identify 23 *B. mallei* isolates
- In general, commercial systems may misclassify *B. pseudomallei* isolates as *B. cepacia* complex and *C. violaceum*

https://pubmed.ncbi.nlm.nih.gov/15635021/

Inadvertent Analysis of High-Risk Pathogens

- **Analysis should be performed with caution**
  *There are database limitations for some automated instruments including only containing a small number of certain high-risk pathogens

- **Samples with a closely related ID or "no identification" should not be worked on the open bench**

- **Samples that are misidentified can lead to additional laboratory exposures or illness**

- **Early indicators should be utilized within laboratory**
  - Gram stain
  - Biochemical Results
  - Travel History

Risk Assessment: Things to Consider

- Use of systems that are totally automated or allow for front-end processing in a BSC
- Use of totally automated systems that allow for tele-microbiology through viewing images of culture plates eliminating risk when opening and examining plates
- Organism growth rate - slow growing?
- Perform work in a BSC until deemed safe for benchtop

Risk Assessment: Things to Consider

Organism Considerations

- **Gram positive, negative, spore former, etc**
- **Organism growth rate**
- **Were other lab tests performed besides the MALDI?**
- **Were the organism characteristics taken into consideration?**
  - Growth characteristics on different media
  - Colony morphology
  - Gram stain characteristics
  - Motility
  - Urease
  - Oxidase
  - Common rapid tests
- **Were the ASM Sentinel lab protocols followed for the safe and presumptive recognition of suspected select agents?**

https://asm.org/Articles/CPHMC/Laboratory-Response-Network-LRN-Sentinel-Level-C

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Conclusion

What biosafety steps can help to prevent exposures?

- **Blood culture bottles vented in BSC**
- **BSC used when working with unknowns**
  - Slow growing
  - Gram negative/variable organisms
- **Reviewing ASM protocols for ruling-out and referring potential BT agents**
- **Contacting LRN lab before starting work with potential high-risk pathogens**
- **Limiting the use of automated ID systems**
- **Implementing use of benchtop shields and/or face protection**

What questions can labs ask when implementing new platforms?

- **Any potential aerosol generating steps?**
- **Any potential spills or splashes, or other areas of contamination concern?**
- **Any facility specific concerns?**
  - Staff performing the procedures
  - Area where work was conducted
- **Appropriate PPE used?**
- **Appropriate/current training provided?**
- **Inactivation method previously verified?**

DLS ECHO Biosafety Session: April 25, 2023

Decontamination of Laboratory Equipment

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