Biosafety and Biosecurity in the Clinical Bacteriology Laboratory

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Learning Objectives

Understand how clinical labs are connected to Public Health for Microbiology

Identify which bacterial pathogens represent a biosecurity or biosafety risk

Detail how a clinical bacteriology lab can identify select agents

Outline the principles of Biosafety

Describe pre-exposure activities, exposure mitigation, and procedure controls

Lecture Outline

Laboratory Response Network

Select Agents: A Clinical Description

Lab Identification of Select Agents

Principles of Biosafety

Preexposure Activities and Exposure Mitigation

Procedure Controls

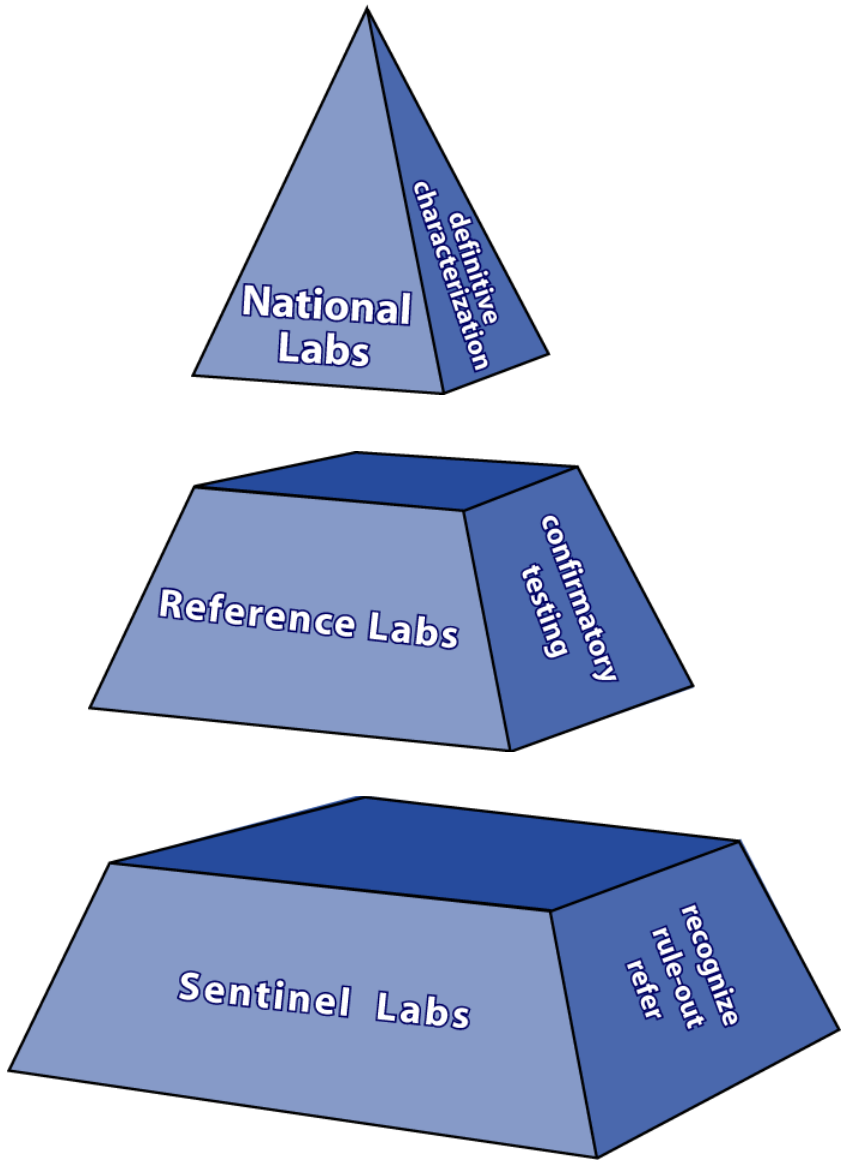
Laboratory Response Network (LRN)

Network of local, state and federal laboratories

Public health, hospital-based, food testing, veterinary, and environmental testing laboratories

Provide laboratory diagnostics and the capacity to respond to public health emergencies.

Structure of the LRN



CDC, USAMRIID
Strain characterization
Bioforensics
Handling highly infectious agents / toxins

Public Health Laboratories
Responsible for investigations
Referred specimens for confirmatory testing

Sentinel Laboratories
Routine diagnostic services
Rule-out and referral steps
UCLA is considered an advanced sentinel laboratory capable of analyzing specimens or samples that may contain microbiology select agents or biological toxins

The Sentinel Laboratory

Notify LA County Public Health if:

1. We presumptively isolate one of CDC's high priority pathogens (select agents):

   Bacillus anthracis
   Francisella tularensis
   Yersinia pestis
   Brucella
   Clostridium botulinum
   Coxiella burnetii
   Burkholderia mallei
   Burkholderia pseudomallei

2. A physician requests a culture for one of these agents based on a high index of suspicion (not just ruling out)
3. Large numbers of identical isolates are isolated from persons with similar syndromes (eg, *Salmonella*)
4. The lab isolates an uncommon organism that causes a disease not normally present in Los Angeles
5. An organism exhibits and unusual biochemical reaction or antibiogram

Select Agents by Category

Category A: Top priority.

Easily disseminated or transmitted and have potential for major public health impact and high mortality.

Category B: 2nd highest priority agents.

Moderately easy to disseminate with high morbidity and low mortality

Category C: emerging pathogens that can be possibly engineered for mass dissemination due to availability, ease of production, and potential for high morbidity and mortality

Examples of Category A Agents

Bacteria

- Anthrax (*Bacillus anthracis*)
- Plague (*Yersinia pestis*)
- Tularemia (*Francisella tularensis*)

Viruses

- Smallpox (*Variola major*)
- Viral Hemorrhagic Fevers (filoviruses)

Biotoxins

- Botulism (*Clostridium botulinum* toxin)

Clinical Case (October 2001)

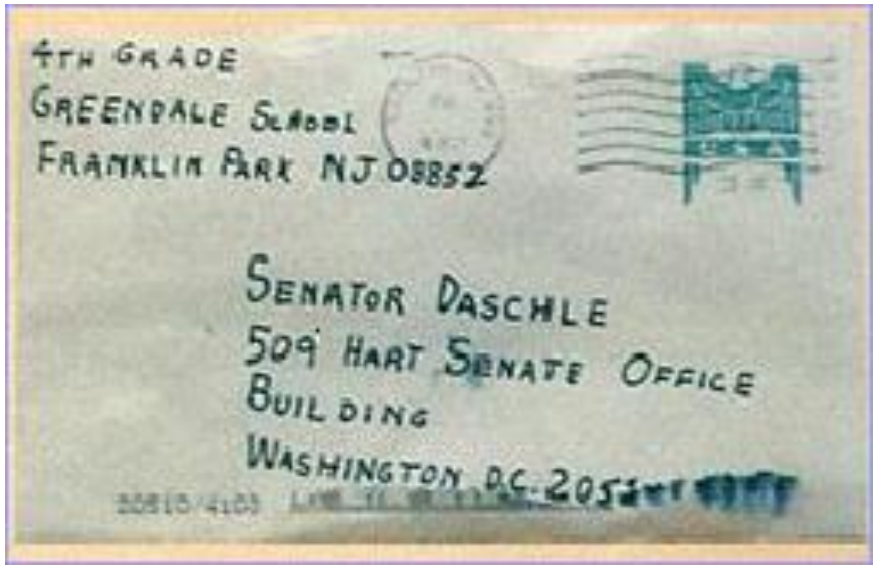
63 year-old man presented to clinic with fever, confusion, and respiratory symptoms

Blood & CSF gram stain = gram-positive bacilli

Died despite antibiotic therapy

Last U.S. case of inhalational anthrax: 1976

Exposure:
Anthrax spores sent in mail
18 cases – 11 inhalational, 7 cutaneous



7 cases seen by physician and unrecognized

Mortality
- 65% in patients presenting critically ill
- No deaths in patients treated early

More than 30,000 placed on prophylaxis (none became ill)

Anthrax Clinical Presentations

Primary clinical forms:

Inhalational

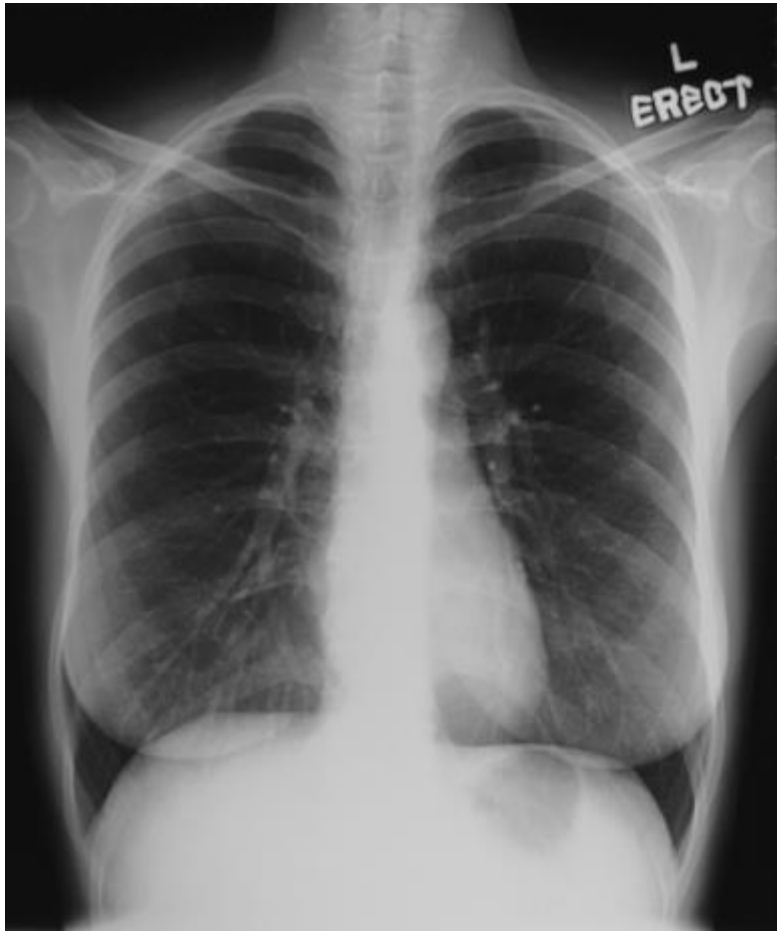
Has a rare natural occurrence
Present within 1-2 weeks of exposure
Nonspecific febrile prodrome (hours to days) then severe pulmonary disease
CXR shows mediastinal widening (may be subtle)

Anthrax Meningitis manifests in 50% of Inhalational Anthrax patients

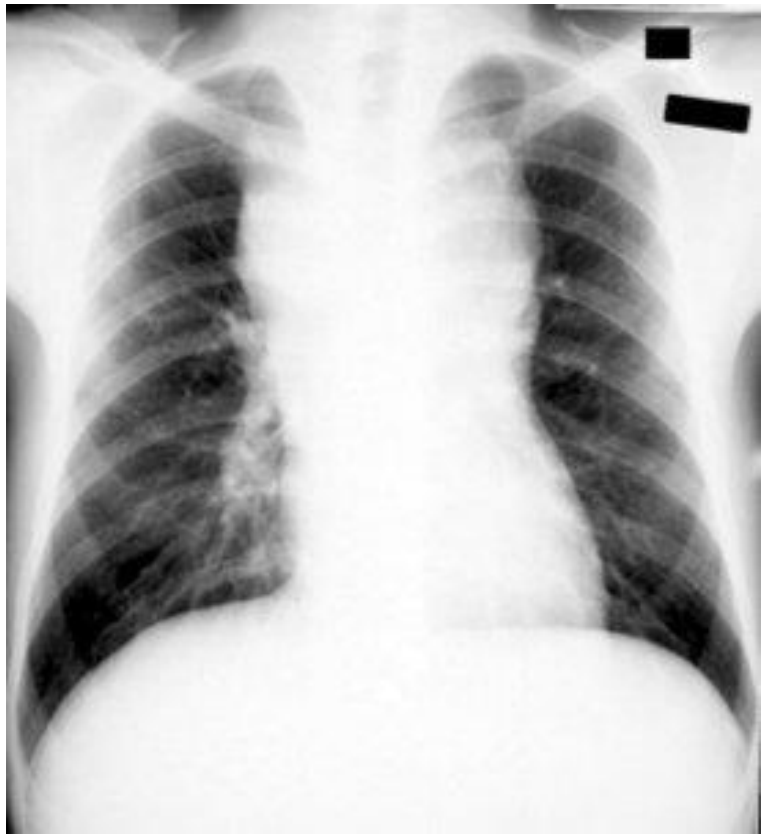
Cutaneous

Most common form in naturally occurring cases (animal exposure)
Characteristic lesion, low mortality if treated

Normal vs. Anthrax Chest X-RAY



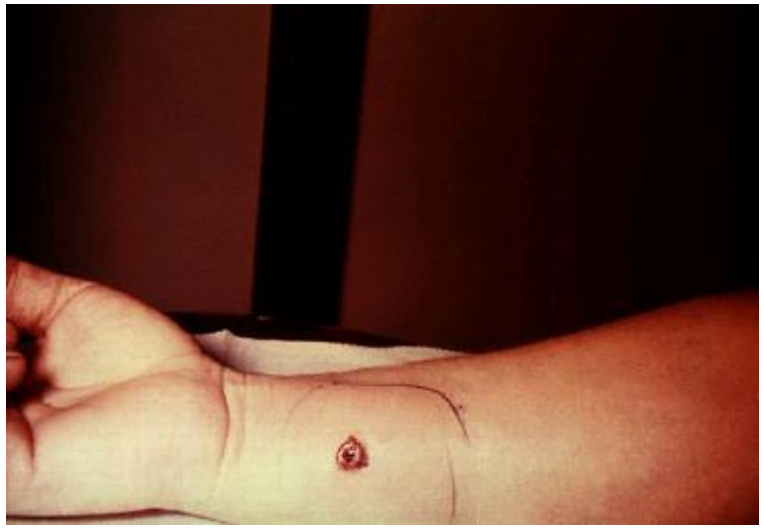
Normal CXR

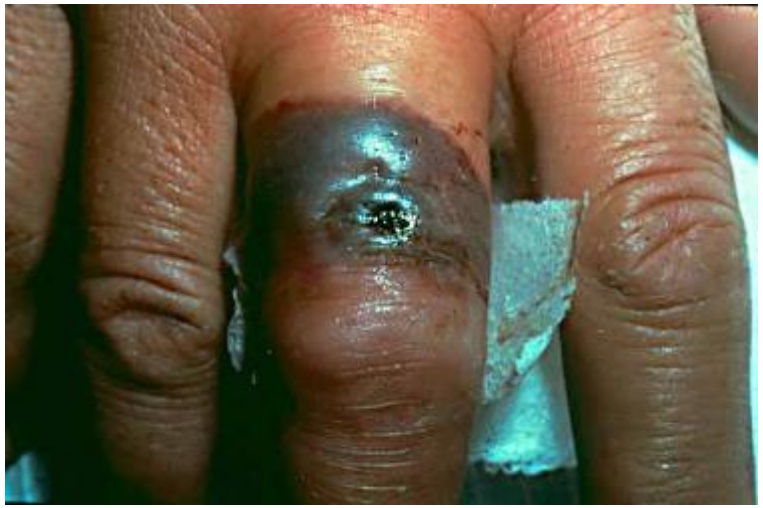


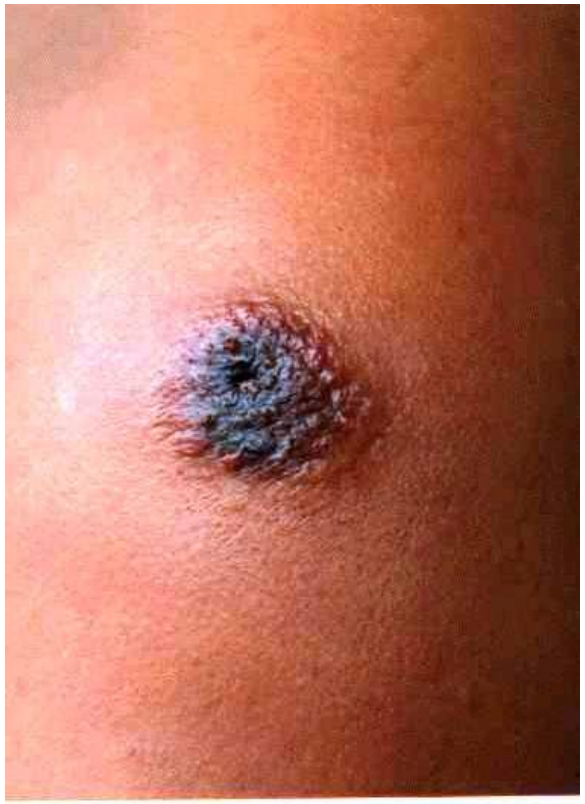
The classic appearance of inhalation anthrax on a chest x-ray is mediastinal widening with clear lungs.

Source: American College of Physicians

Cutaneous Anthrax







The case fatality rate of cutaneous anthrax is 20% without antibiotic treatment, and <1% with antibiotics.

Examples of Category B Agents

Brucellosis

Epsilon toxin of Clostridium perfringens

Food safety threats

Salmonella species, Escherichia coli O157:H7, Shigella

Glanders

Melioidosis (Burkholderia pseudomallei)

Psittacosis (Chlamydia psittaci)

Q Fever

Ricin toxin

Staphylococcal enterotoxin B

Typhus fever,

Viral encephalitis (alphaviruses)

Water safety threats

Vibrio cholerae

Cryptosporidium parvum

Select Agent Reporting

(Revised 10/10/16)

Please Post

County of Los Angeles • Department of Public Health

County of Los Angeles Public Health

REPORTABLE DISEASES AND CONDITIONS

Title 17, California Code of Regulations (CCR), § 2500

It is the duty of every health care provider, knowing of or in attendance on a case or suspected case of any diseases or conditions listed below, to report to the local health officer for the jurisdiction where the patient resides. "Health care provider" encompasses physicians (surgeons, osteopaths, oriental medicine practitioners), veterinarians, podiatrists, physician assistants, registered nurses (nurse practitioners, nurse midwives, school nurses), infection control professionals, medical examiners/coroners, dentists, and chiropractors, as well as any other person with knowledge of a case or suspected case.

Note: This list is specific to Los Angeles County and differs from state and federal reporting requirements ★

For laboratory reporting: www.publichealth.lacounty.gov/lab/index.htm For veterinary reporting: www.publichealth.lacounty.gov/vet/index.htm

Urgency Reporting Requirements

☎ = Report **immediately** by telephone ❶ = Report by telephone **within 1** working day

✉ = Report by electronic transmission (including FAX), telephone or mail within **1 working day** from identification

⑦ = Report by electronic transmission (including FAX), telephone or mail within **7 calendar days** from identification

REPORTABLE DISEASES

- ✉ Amebiasis
- ⑦ Anaplasmosis
- ☎ Anthrax, human or animal +
- ✉ Babesiosis
- ☎ Botulism: infant, foodborne, or wound
- ⑦ Brucellosis, animal; except infections due to *Brucella canis* +
- ☎ Brucellosis, human +
- ✉ Campylobacteriosis
- ⑦ Chancroid ■
- ☎ Chickenpox (Varicella), only hospitalizations, deaths, and outbreaks (≥3
- ✉ Hantavirus Infection
- ☎ Hemolytic Uremic Syndrome
- ✉ Hepatitis A, acute infection
- ⑦ Hepatitis B, specify acute or chronic
- ⑦ Hepatitis C, specify acute or chronic
- ⑦ Hepatitis D (Delta), specify acute or chronic
- ⑦ Hepatitis E, acute infection
- ⑦ Human Immunodeficiency Virus (HIV) infection, stage 3 (AIDS) ■ (§2641.30-2643.20)
- ❶ Human Immunodeficiency Virus (HIV), acute infection ■ (§2641.30-2643.20)
- ⑦ Rocky Mountain Spotted Fever
- ⑦ Rubella (German Measles)
- ⑦ Rubella Syndrome, Congenital
- ✉ Salmonellosis, other than Typhoid Fever +
- ✉ Scabies, atypical or crusted ★
- ☎ Scombroid Fish Poisoning
- ☎ Shiga Toxin, detected in feces
- ✉ Shigellosis
- ☎ Smallpox (Variola)
- ☎ Streptococcal Infection, outbreaks any type
- ✉ Streptococcal Infection, individual case in a food handler or dairy worker
- ✉ Streptococcal Infection, Invasive Group A

How do we identify select agents?

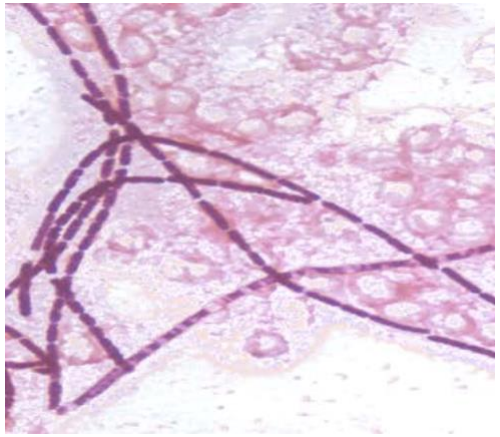
Do NOT fully identify (protects staff, avoids errors)

All LRN protocols rely recognition of:

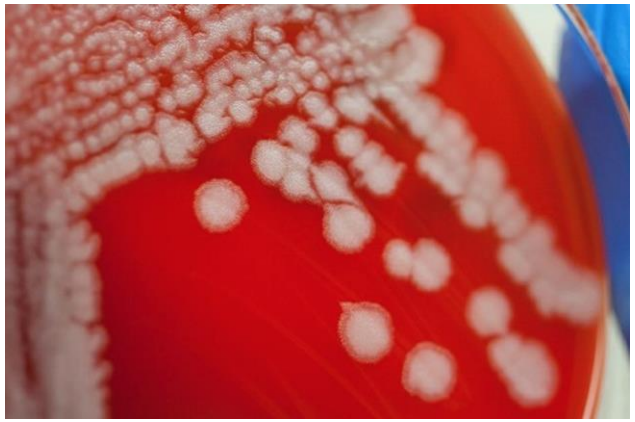
- Growth morphology
- Gram stain
- Spot tests
  - Oxidase
  - Catalase
  - Urea
  - Arginine
  - β-lactamase
  - Polymixin B resistance

Examples

Large gram-positive rods that look like this:



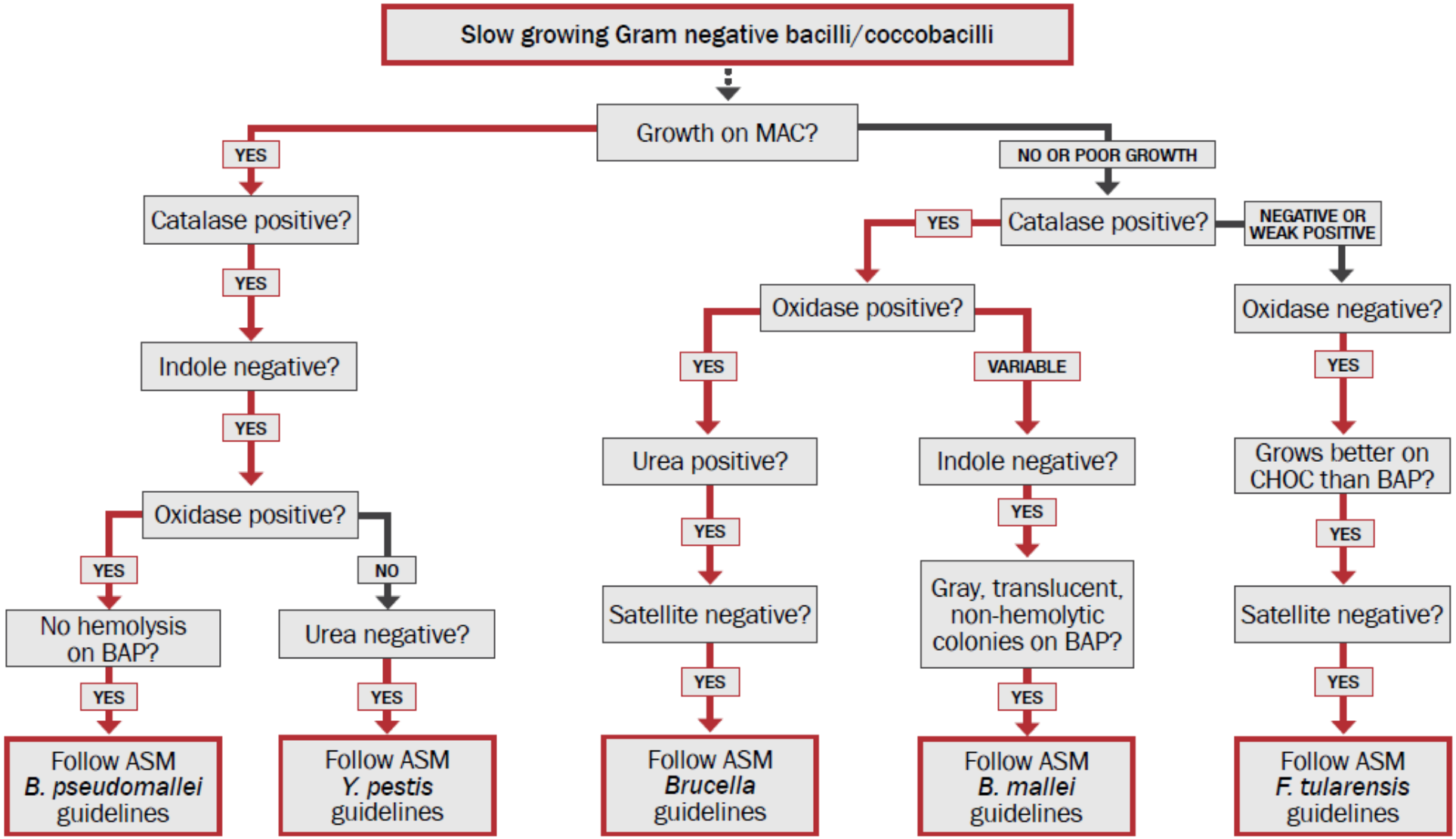
Tenacious, non-hemolytic colonies that look like this:



Rule out *Bacillus anthracis*

BIOTHREAT AGENT IDENTIFICATION

Gram Negative Bacilli/Coccobacilli Rule-Out Algorithm



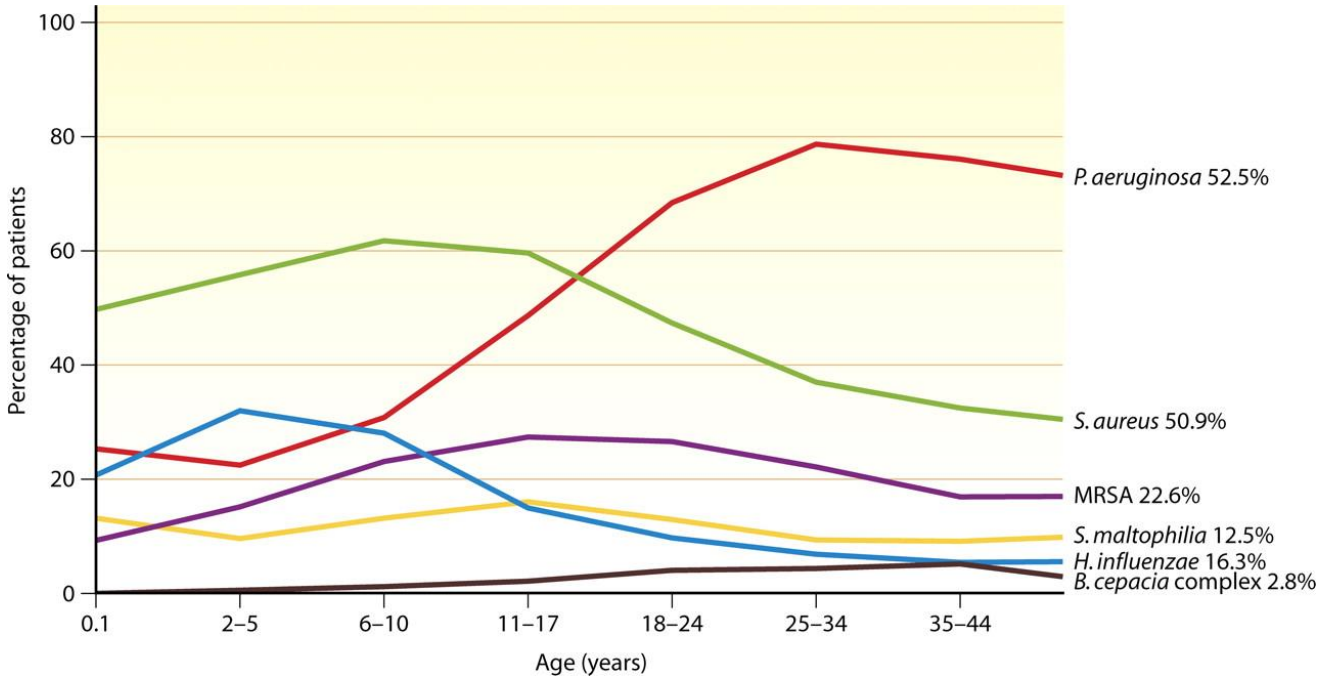
Select Agent Exposure Case Study

23 yr old female, history of Cystic fibrosis and pancreatic insufficiency

Presented with exacerbation of CF symptoms including shortness of breath and worsening cough, chest pain and fever
CT imaging showed signs of pneumonia

Bacterial Respiratory Culture:
Staphylococcus aureus, Stenotrophomonas maltophilia, Mucoid *Pseudomonas aeruginosa*

1 colony of an isolate identified as *Burkholderia cepacia* complex on the Vitek MS



Select Agent Exposure Case Study

To expedite treatment decisions, the lab began set up of broth micro dilution for the presumed *Burkholderia cepacia*.

At the same time, per protocol, the isolate was tested on the Vitek 2 and identified as *Burkholderia pseudomallei* (a select agent).

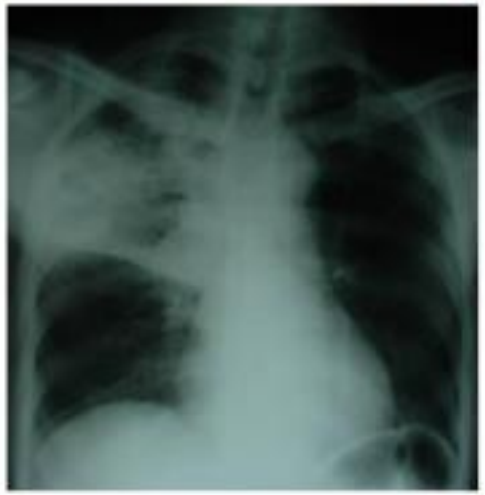
At this point all work was stopped and we began rule out testing for *B. pseudomallei*. Previous to this, all *B. pseudomallei* isolated at UCLA came from sterile sources (blood).

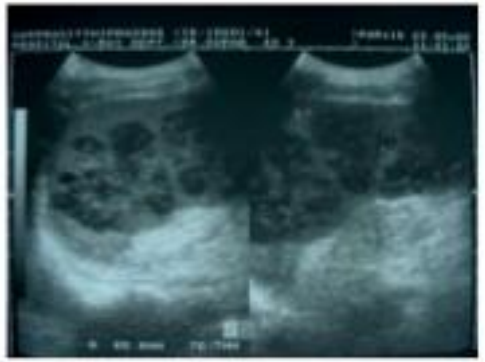
Couldn’t rule out. Sent to LA County. Confirmed as *Burkholderia pseudomallei* by PCR.

Select Agent Exposure Case Study

Melioidosis

- An infectious disease caused by Gram-negative bacilli, *Burkholderia pseudomallei*
- Organism is present in soil and water in the endemic areas
- Difficult to diagnose
- No specific clinical presentation.
- Common presentations include sepsis, severe sepsis, septic shock, pneumonia, and abscesses in any organs
- May present acute, sub-acute and chronic
- May mimic other diseases, such as TB





Select Agent Exposure Case Study

2 weeks before presentation to UCLA, patient had traveled to Thailand with friends for vacation. Felt well during the trip.

This information was very difficult to find in the case notes. A nutritionist was the only health care worker who mentioned this. (Thanks to the micro staff for hunting this down).

Because of the immediate antimicrobial susceptibility testing set up (for the optimal patient care turn around time), 19 staff were found to be exposed to the isolate.

6 personnel were high risk: directly manipulated cultures performing either MALDI-TOF or susceptibility testing
13 personnel were low risk: Working within close proximity (5-8 feet) of open cultures

All 19 staff received prophylaxis of either Doxycycline or Bactrim for a 3 week course
1 staff member had an allergic reaction (rash) to Bactrim and was switched to Doxy

All staff had baseline serologies drawn with follow up controlled by OHF and infectious disease

Why didn’t the MALDI work?

JCM
Journals.ASM.org

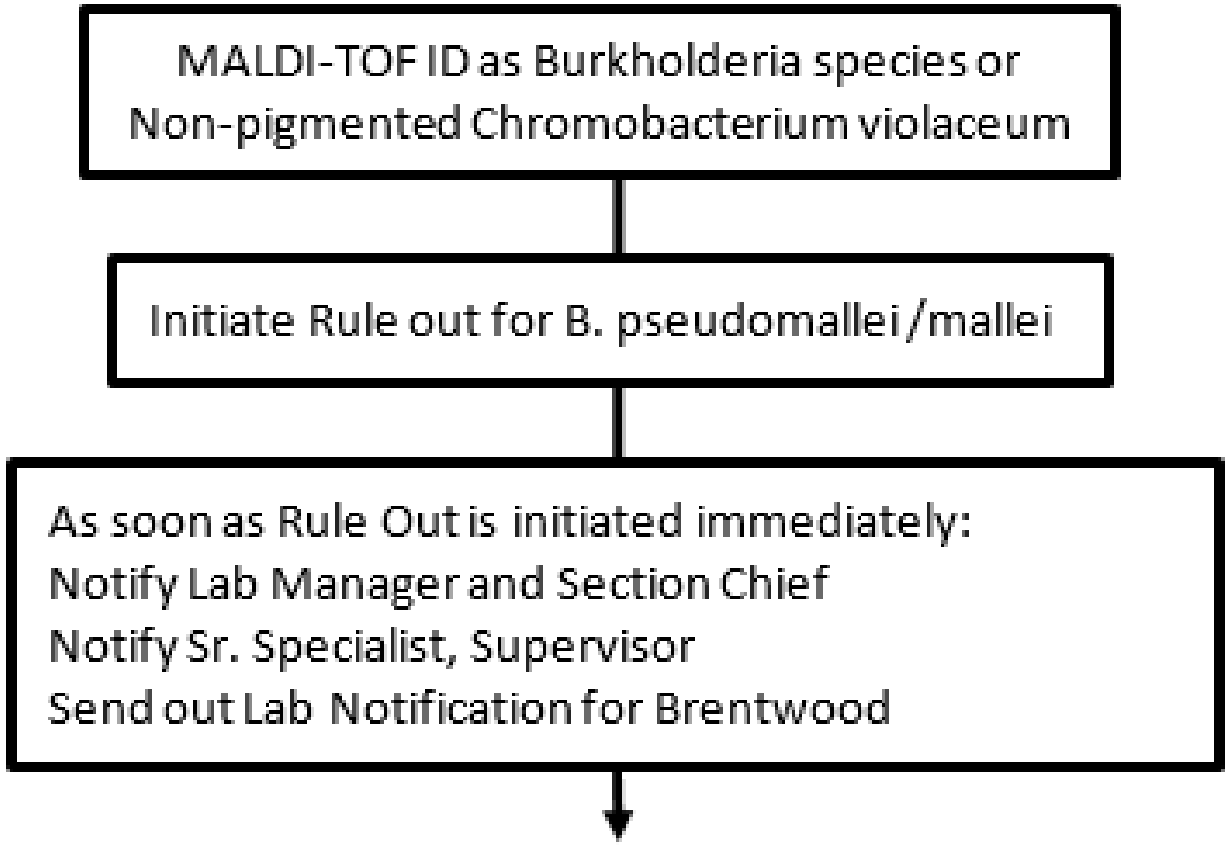
LETTER TO THE EDITOR

Accidental Exposure to *Burkholderia pseudomallei* in the Laboratory in the Era of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry

Tanis C. Dingle, Susan M. Butler-Wu, April N. Abbott
Department of Laboratory Medicine, University of Washington, Seattle, Washington, USA

Select agents are not in the FDA-cleared databases
Regulatory requirement due to Select Agent rules
Many other examples of this being a problem.

Work Flow for Burkholderia Select Agent Rule Out



Rule Out Test	B. cepacia	B. mallei	B. pseudomallei	Test Isolate Results
Growth on MAC 24 hours	+	–*	+	
Catalase	+	+	+	
Indole	-	-	-	
Oxidase	+ V	V	+	
Hemolysis on BAP	Gamma	Gamma	Gamma	
Growth at 42°C	V	No Growth	Growth	
Colistin or Polymyxin B **	Resistant	Resistant	Resistant	
Susceptible to Amox/Clav KB (≥18mm)	<18mm (R)	≥18mm(S)	≥18mm(S)	
Penicillin	Resistant	Resistant	Resistant	
Pigmented on Muller Hinton Agar	- V	-	-	
Arginine ***	-	+	+	
Wrinkled Colony	-	-	+	
Motility	+	-	+	
If unable to Rule Out for B. pseudomallei/mallei, then send out to LA County Public Health. Notify Supervisor, Sr. Specialist, Lab Manager and Section Chief immediately.				

* B. mallei may grow poorly on MacConkey agar at 48 hours incubation

** Growth on selective agar (i.e. B. cepacia selective agar, or Modified Thayer Martin by substitute for colistin or Polymyxin B susceptibility test.

*** Arginine may be performed by tube or commercial kit system (i.e. API 20NE). Biochemical test by API 20NE must be heavily inoculated and incubated a full 48 hours. Tube methods must be held for up to 4 days for negative result.

Table 1. Common laboratory routes of exposure to infectious agents

Route	Microbiological practices/accident
Inhalation	Procedures that produce aerosols:
	Centrifugation
	Mixing, sonication, vortexing, blending
	Spills and splashes
	Pouring/decanting culture fluids
	Manipulation of inoculating loop
Inoculation	Needlestick
	Lacerations from sharp objects (e.g., blades, broken glass)
Ingestion	Splashes to the mouth
	Placing contaminated articles/fingers in mouth
	Consumption of food in the laboratory
	Mouth pipetting
Contamination of skin and mucous membranes	Splashes Contact with contaminated fomites

FROM: SEWELL DL. (2006) LABORATORY-ACQUIRED INFECTIONS: ARE MICROBIOLOGISTS AT RISK? CLINICAL MICROBIOLOGY NEWSLETTER 28:1

Principles of Biosafety

- Biosafety Level 1-4
  - Lab practices and techniques
    - Standard practices
    - Special practices
  - Safety equipment (primary barriers)
  - Lab facilities (secondary barriers)
- Knowledgeable supervisor
- Personnel
  - Aware of potential hazards
  - Proficient in practices and techniques
- Biosafety procedures manual specific to the lab

Provide increasing levels of personnel and environmental protection

Guidelines for working safely in microbiological and biomedical laboratories

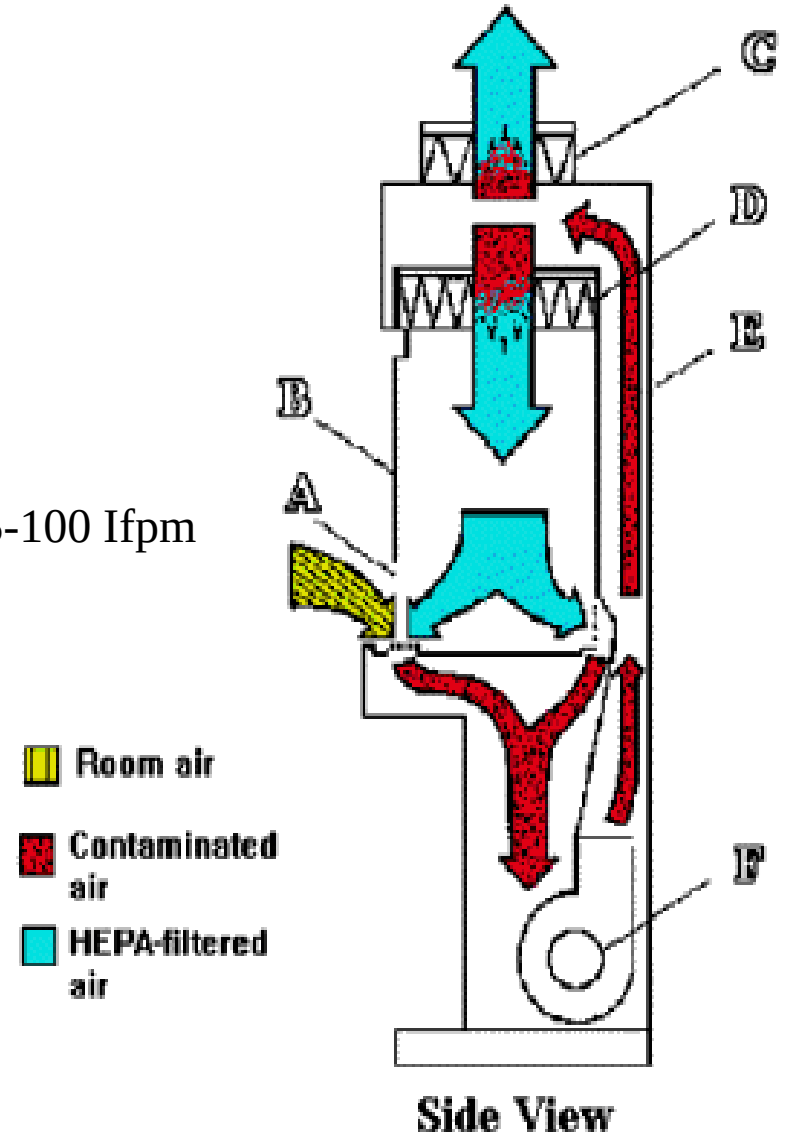
Safety Equipment (Primary Barriers)

- Biosafety cabinets (BSCs)
- Personal protective equipment
  - Gloves and gowns
  - Eye and face protection
  - Respiratory protection (BSL-3)
- Pipetting devices
- Safety centrifuge cups and rotors

BSC Class II, Type A*

A. Front opening
B. Sash
C. Exhaust HEPA filter
D. Rear plenum
E. Supply HEPA filter
F. Blower

75-100 lfpm

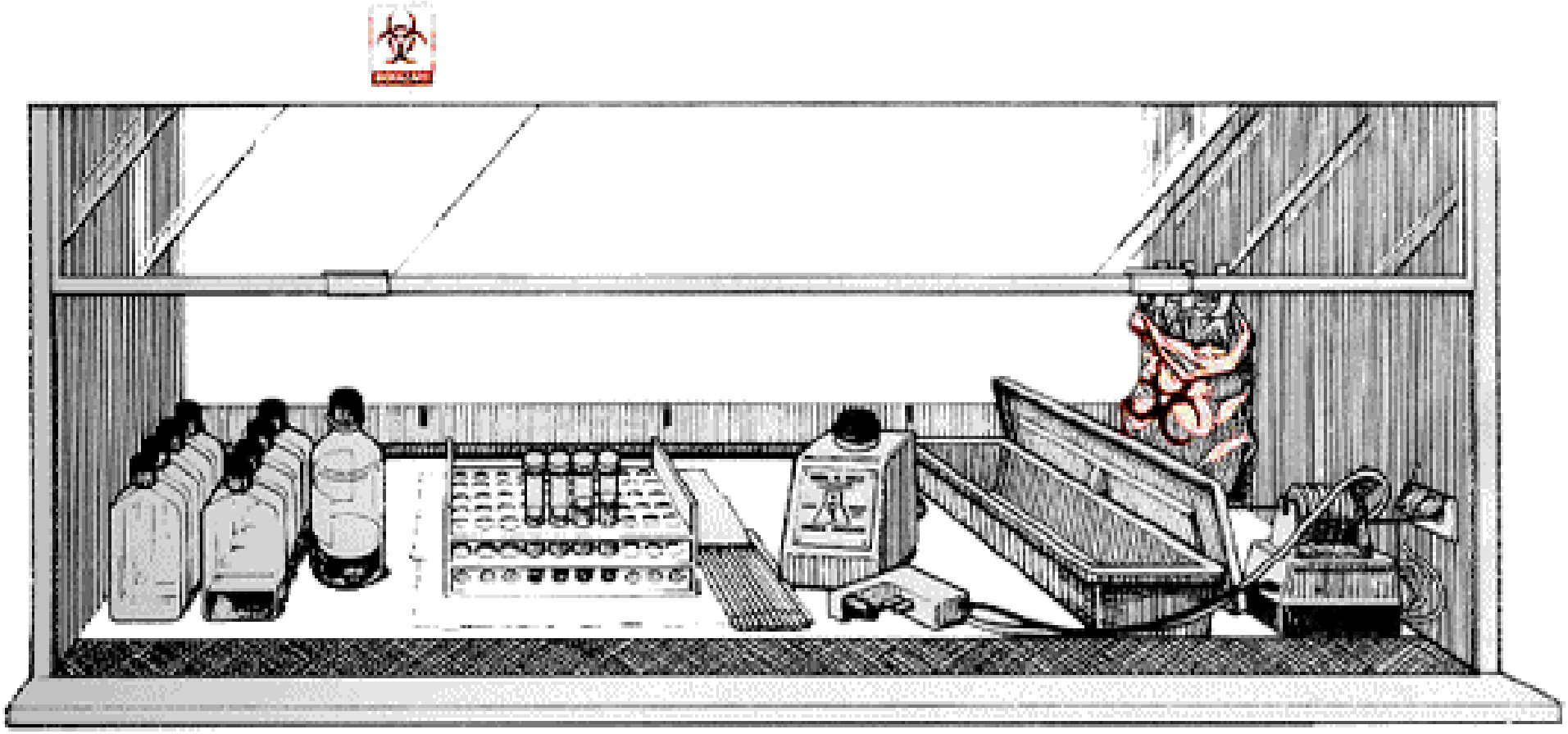


*Class II BSC's are classified into two types (A&B) based on construction.

The air is recirculated within the cabinet in Type A cabinets (not suitable for volatile or radionucleotides).

Type A BSC's can be exhausted into the lab or to outdoors via a "thimble" connection to building exhaust system.

Unidirectional Workflow Under A BSC: A typical layout for working “clean to dirty” within a Class II BSC. Clean cultures (left) can be inoculated (center); contaminated pipettes can be discarded in the shallow pan and other contaminated materials can be placed in the biohazard bag (right).



Pre-exposure activities

Risk assessment

Education

Communication

Vaccination

Periodic assessments

<u>Vaccines all microbiologists should seriously consider</u>

HBV

HAV

MMR

Tdap

Pneumococcus

Meningococcus

Influenza

MMWR Quick Guide to Adults, 2007 56(41)

Exposure mitigation

- Hand hygiene
- Universal precautions
- PPE for BSL-2
- Aerosol protection for BSL 2.5
- Surface hygiene
- Waste management
- Procedural controls

Can we do better at hand hygiene? [1]

- 100% of microbiologists wash hands at end of duty
- BUT:
  - 37% wear rings
  - 47% wear a watch
  - 6% wear a bracelet

Pathogens recovered exclusively from this group!

Hand hygiene among laboratory workers. ICHE 2006 27(9): 978

Universal → Standard Precautions

Universal Precautions- blood and certain body fluids of all patients are considered potentially infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other bloodborne pathogens.

Standard Precautions, replaced Universal Precautions and assumes blood and body fluids of any patient could be infectious, recommends PPE as well as other infection control practices to prevent transmission in any healthcare setting, Decisions about PPE use determined by the type of interactions with the patient.

Procedure controls

- High Risk Activities:
  - Manipulating cultures outside of BSC
  - Spot tests
  - Streaking
  - Sniffing plates
  - Aerosol generating activities
- Low Risk Activities:
  - In lab but not in high risk category
- No Risk Activities:
  - All procedures within BSC but ONLY if used correctly

Aerosol Generating Activities

- Splashing
- Pipeting
- Centrifuging
- Grinding
- Blending
- Shaking
- Mixing
- Sonicating
- Opening container of liquid
- Flaming loop

High risk aerosol protocol

Follow select agent protocols for how to rapidly identify these agents

Suspect case → all work performed in BSC II

Emails / huddles with staff on new patient suspect/confirmed high risk agent

LIS marker put on patients with these infections

Plates taped / labeled with high risk aerosol risk

Participate in LPX

Annual Talk for all staff to review biosafety

What happens when we ID a select agent

Must report to CDC

- Routed through LA County, as they perform the final ID
- Cultures MUST be destroyed within 7 days
- Suspicious cases reported to I.P., Lab Medical Director, Staff, LA County, UCLA Emergency Preparedness

If staff exposed, this is considered a “release” of a select agent

- More paperwork and follow-up with CDC

Biohazardous waste

- Biohazardous waste:
  - Anything with a biohazard symbol must be disposed of in biohazardous waste
  - Containers must be closed when not in active use
  - Waste is double bagged, and tied with a gooseneck knot.
- Sharps containers:
  - Only place sharps in sharps containers (no labels, kimwipes, etc.)
  - If the item has the potential to puncture a bag – it has to go into sharps waste
  - Do not fill the container more than 2/3 to 3/4 full.
  - Dispose of sharps containers in biohazard barrels – ensure they are placed right side up – the containers are not leak proof.

PPE reminders

- Lab coats must be worn when working in the lab, and buttoned to protect your clothing
- Only 1 glove may be worn in hallways. Do not open/close doors, push elevator buttons, or make copies using the gloved hand.
- Face shields must be worn anytime there is a potential for splashes.
- PPE must not be worn in clean areas – this includes restrooms, the admin office, conference room, or break room.

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REVIEW

Clinical Laboratory Biosafety Gaps: Lessons Learned from Past Outbreaks Reveal a Path to a Safer Future

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Biosafety and Biosecurity Gaps in the Clinical Lab

Laboratories lack direct control over how specimens are collected and transported

Our knowledge of instrument contamination during routine use, or during use with highly pathogenic microbes, is limited, and these risks may be under appreciated.

There are discrepancies between the current designation of Category A infectious substances and the actual wide range of waste materials generated during clinical laboratory testing. These impact waste management before and after waste leaves the laboratory.

There is inadequate guidance or training for clinical laboratory professionals in use of PPE. The availability of PPE in clinical laboratories is often insufficient. There remains confusion between the differences in using PPE for directpatient care and the processes for PPE use in a clinical laboratory and testing environment.

It is often challenging for providers of laboratory safety training to collect evaluation data beyond learner satisfaction.

Data are lacking on to what extent laboratories conduct monitoring and evaluation of biosafety practice.