

Challenges Integrating Bioforensics and Biosafety

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**Location: 54th Annual
Biological Safety
Conference**

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**Homeland
Security**

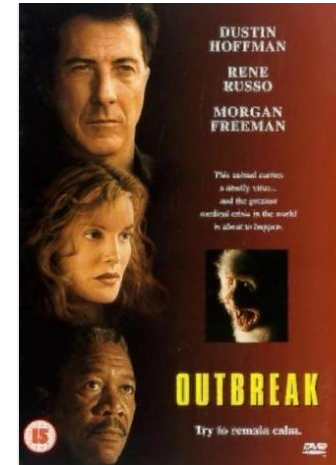
Science and Technology

Myths



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- Forensic work is glamorous
- Forensic work is performed in the dark
- Anyone can enter the laboratory
- Cases solved by single technician
- Personal Protective Equipment is optional
- Results are available immediately
- Results easily displayed and interpreted



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waterborne
the next attack



www.thenextattack.com

©Drops Entertainment



©National Broadcasting Company



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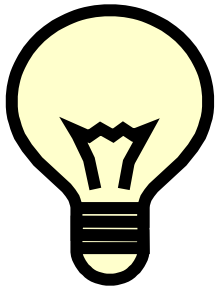


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Reality



- Forensic work is performed when necessary
- Lights are always on
- The laboratory is accessible by badge only
- Coordination of multiple technicians and departments
- Personal Protective Equipment is always used
- Results rely on the time required for procedure
 - Documentation is tedious
- Results are presented by scientist, not automatically



Anthrax Attacks - 2001

Florida, New York and Washington, DC

- The anthrax attacks in 2001 demonstrated the need for a dedicated national bioforensic capability

Confirmed Anthrax Cases

Following are cases of anthrax confirmed by the Centers for Disease Control and Prevention. Other suspected cases remain unconfirmed by the CDC. They include two workers at the New York Post and a second NBC worker.

| VICTIM | KIND | LOCATION | CONFIRMED | STATUS |
|--------------------------|---------|--------------------------------------|-----------|---------------|
| Bob Stevens, 63 | Inhaled | American Media, Boca Raton | Oct. 4 | Died Oct. 5 |
| Erin O'Connor, 38 | Skin | NBC in Manhattan | Oct. 12 | Recovering |
| Boy, 7 months | Skin | ABC in Manhattan | Oct. 15 | Recovering |
| Ernesto Blanco, 73 | Inhaled | American Media, Boca Raton | Oct. 15 | Left hospital |
| Claire Fletcher, 27 | Skin | CBS in Manhattan | Oct. 18 | Recovering |
| Teresa Heller, 32 | Skin | West Trenton post office | Oct. 18 | Recovering |
| Patrick O'Donnell, 35 | Skin | Hamilton Township mail center, N.J. | Oct. 19 | Recovering |
| Leroy Richmond, 57 | Inhaled | Brentwood mail center, D.C. | Oct. 21 | Hospitalized |
| Unnamed man | Inhaled | Brentwood mail center, D.C. | Oct. 22 | Hospitalized |
| Thomas L. Morris Jr., 55 | Inhaled | Brentwood mail center, D.C. | Oct. 23 | Died Oct. 21 |
| Joseph Curseen Jr., 47 | Inhaled | Brentwood mail center, D.C. | Oct. 23 | Died Oct. 22 |
| Unnamed worker, 59 | Inhaled | State Department mail center, D.C. | Oct. 25 | Hospitalized |
| Unnamed woman, 56 | Inhaled | Hamilton Township mail center, N.J. | Oct. 28 | Hospitalized |
| Non-postal worker | Skin | Works near Trenton, N.J. | Oct. 29 | Left hospital |
| Unnamed worker | Inhaled | Hamilton Township mail center, N.J. | Oct. 30 | Left hospital |
| Kathy Nguyen, 61 | Inhaled | Manhattan Eye, Ear & Throat Hospital | Oct. 30 | Died Oct. 31 |

SOURCES: Centers for Disease Control and Prevention, staff and wire reports

THE WASHINGTON POST

Ricin as a Biocrime Agent

An “Inappropriate Valentine’s Day Present”



NBFAC's Operational Mission Is HSPD-10 Directed



NBFAC is... “the lead Federal facility to conduct and facilitate the technical forensic analysis and interpretation of materials from biocrime and bioterror investigations or that recovered following a biological attack in support of the appropriate lead Federal agency.”

Presidential Directive, Biodefense for the 21st Century, April 2004

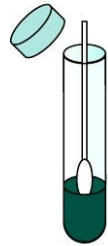
NBFAC provides a national capability to conduct/coordinate analyses of evidence from biocrime and bioterror investigations

New NBACC Building



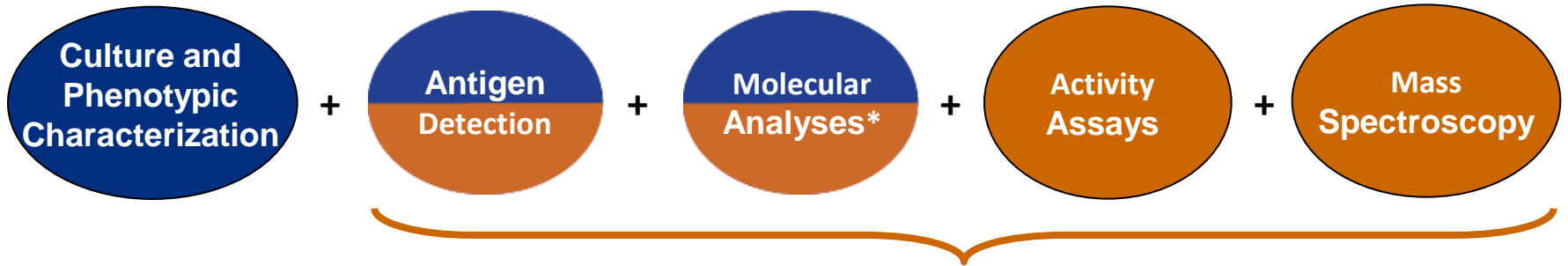
- **Allows for biocontainment and signature containment—“Bioforensic BSL -3”— design based upon “work flow” and “types of analyses” to avoid live agent or signature cross contamination**
- **Dedicated casework analysis laboratories and separate casework support laboratories**
- **Large “flexible bays” for large evidence receipt and use with law enforcement analytical equipment**
- **Individual casework analysis and support laboratories can be decontaminated with Vaporized Hydrogen Peroxide (VHP)**

Overlapping Agent Based Techniques for Complementary Identification – “High Confidence Results”



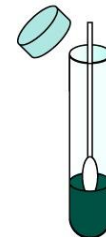
Live Agent
(Bacteria/Virus)

Identification/Characterization

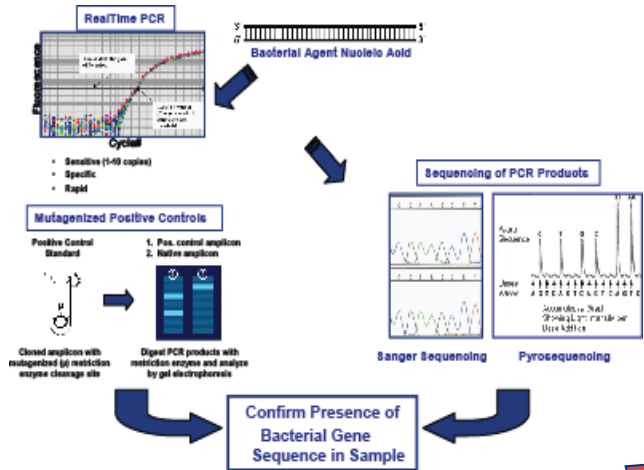


Identification/Characterization

Non-Replicating
Agent (Toxin)

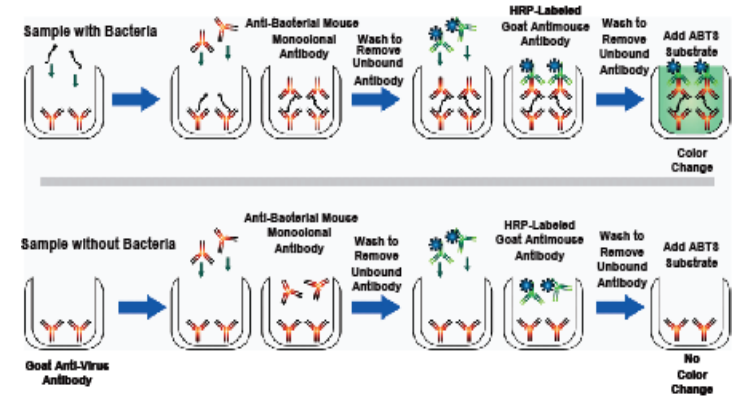
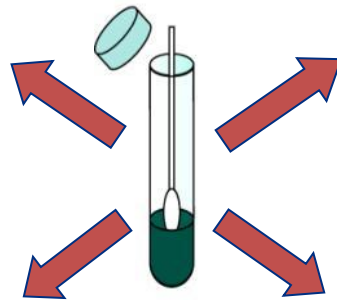


Identification and Characterization of Bacteria Utilizing Complementary and Overlapping Techniques

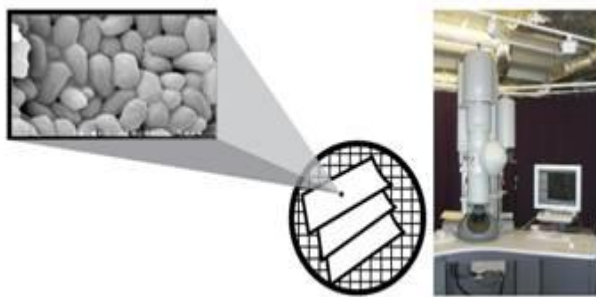


Molecular Identification
(RT-PCR, Sequencing, Genotyping)

Unknown Sample



Antigen Detection
(Antigen Capture ELISA, IFA, Slide Agglutination)

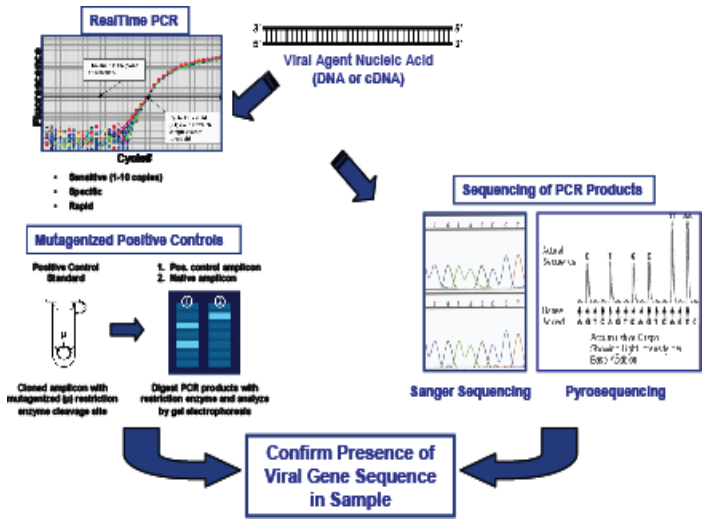


Electron Microscopy

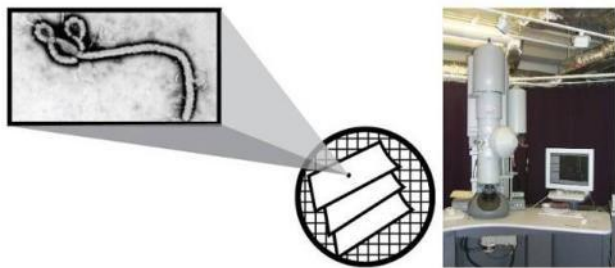


Culture/ID/Phenotypic Characterization
(culture, Gram stain, Biochemical identification, Motility, Phage susceptibility, Antibiotic tests)

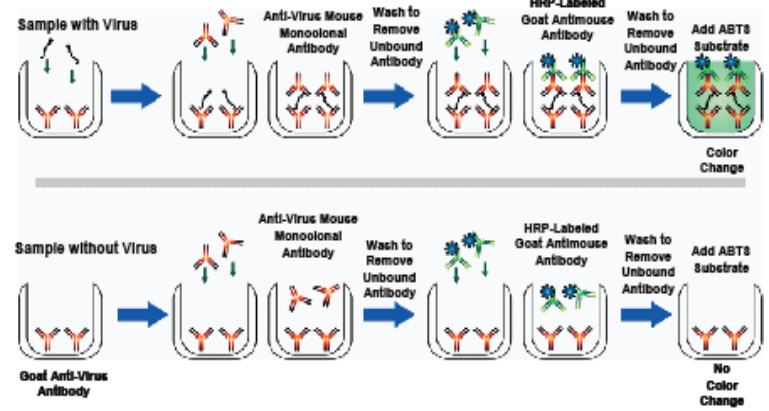
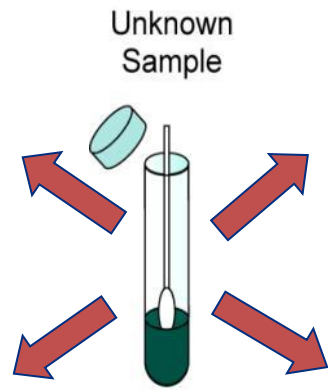
Identification and Characterization of Viruses Utilizing Complementary and Overlapping Techniques



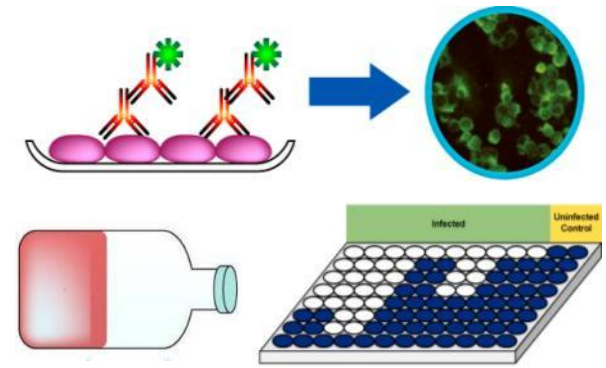
Molecular Identification
(RT-PCR, Sequencing, Genotyping)



Electron Microscopy

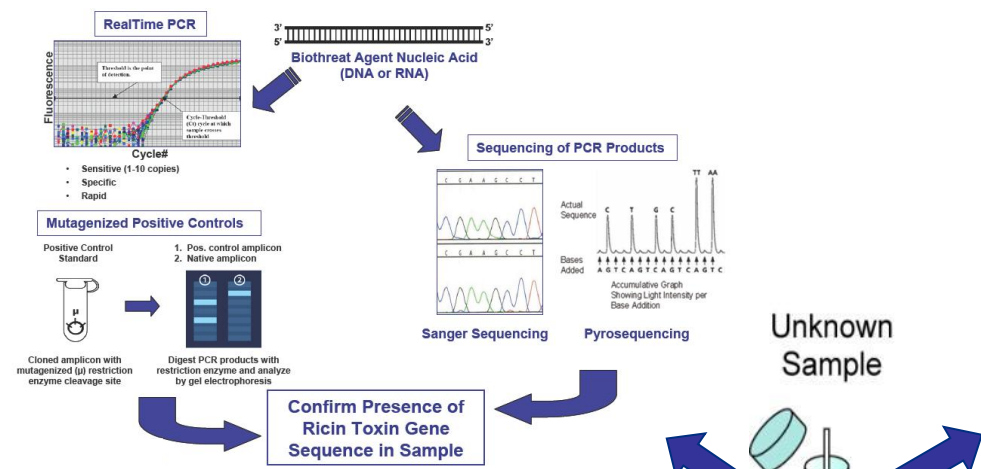


Antigen Detection (Antigen Capture ELISA, IFA)

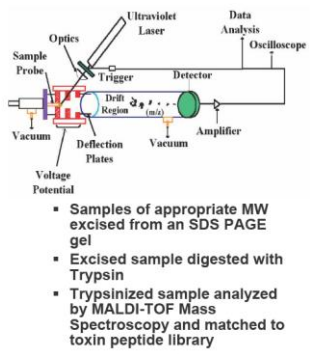
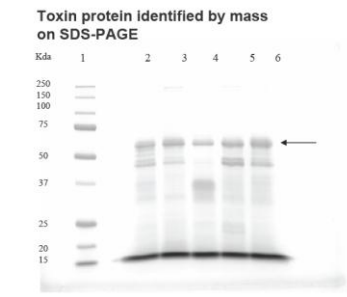


Culture/ID and Enumeration
(culture, plaque assay, TCID₅₀)

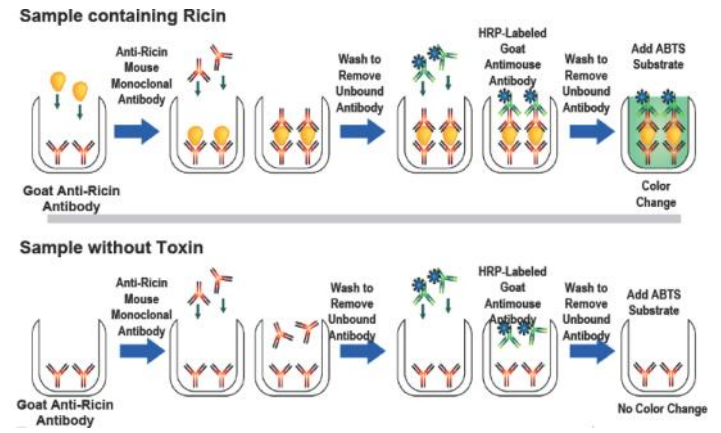
Identification and Characterization of Toxins Utilizing Complementary and Overlapping Techniques



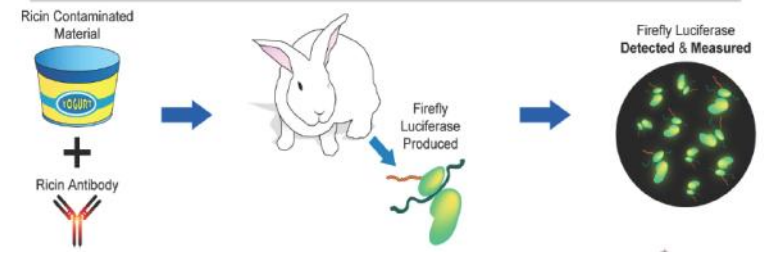
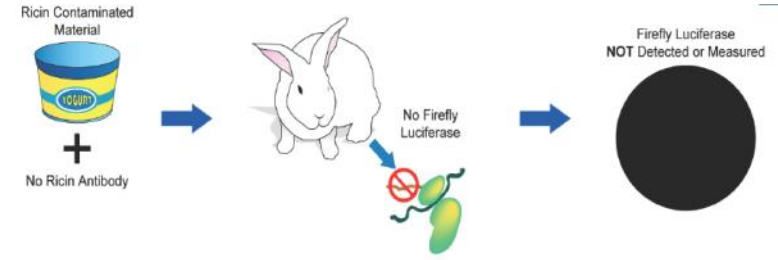
Molecular Identification



Mass Spectroscopic ID



Antigen Capture ELISA



Cell Free Translation Assay

ISO/IEC 17025:2005

- **An international quality standard for testing and calibration laboratories that specifies technical competence to perform tests, in addition to maintenance of a management system.**
- **Critical to our program success, by providing an objective and internationally recognized measure of our technical competence and commitment to doing good science.**
- **A program that supports a total approach - not just assays, sampling, and testing methods - defines an entire management system that includes:**
 - **Maintaining the physical plant** (lab, environment, equipment)
 - **Supply chain** (vendors, material qualifications, storage)
 - **Personnel** (training, competency, proficiency)
 - **Management** (conflict of interest, authority, service to the customer)



The interrelationship of all of these factors has a direct impact on the quality of the science program.

Comparison of Quality Standards

| Attributes of Laboratory | Good Lab Practices | ISO 9001 | ISO 17025 |
|--|---------------------------|-----------------|------------------|
| Personnel Education and Experience | • | • | • |
| Standard Operating Procedures | • | • | • |
| Equipment Maintenance, Calibration and Records | • | • | • |
| Reagent Labeling, Acceptance, Storage and Expiration | • | • | • |
| Specimen Tracking | • | • | • |
| Record Retention | • | • | • |
| Reporting of Results | • | • | • |
| Quality Management System | | • | • |
| Document Control | | • | • |
| Record Control | | • | • |
| Corrective Action | | • | • |
| Preventative Action | | • | • |
| Internal Audit | | • | • |
| Management Requirements | | | • |
| Technical Requirements | | | • |
| Method Validation | | | • |
| Competency Testing of Laboratory | | | • |
| Proficiency Testing of Staff | | | • |
| Quality Controls | | | • |
| International Recognition | | | • |
| Customer Confidence in Results | | | • |

Why ISO 17025?

- **It is the international quality standard for testing and evaluation validations**
- **A well implemented 17025 Quality Management System:**
 - **Supports maintenance of a state of readiness for the laboratory**
 - **Provides a mechanism to grow capabilities and manage change—continuous improvement**
 - **Meets FBI standards for forensic laboratories, and assures production of reliable and defensible scientific evidence**

ISO 17025

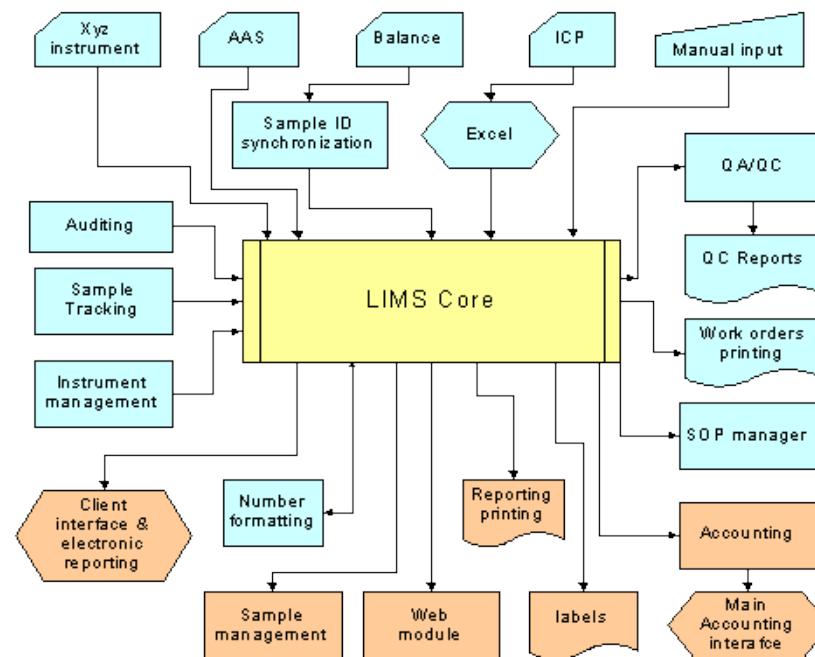
ACCREDITED LABORATORY

Quality at NBACC

- Quality Assurance programs in multiple disciplines with many different data types require rigorous data and document control



- Control programs add to overall cost and training requirements



Challenges in Bioforensics

I. Unknown Sample

- Samples can be a wide range of sizes and materials

II. Personnel

- Trained, reliable personnel available to support 24/7 operations

III. Environment

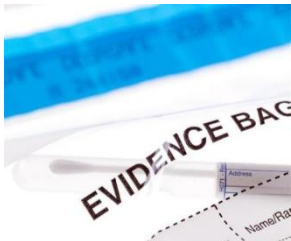
- BSL-2/3/4 laboratory and equipment maintained for bioforensic analysis

IV. Time Sensitivity and Pressures

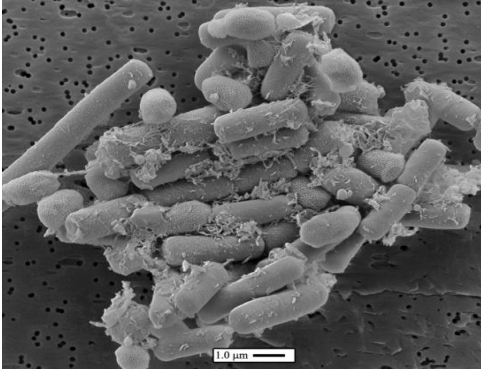
- National security and/or law enforcement are dependent on bioforensic analyses

Unknown Sample

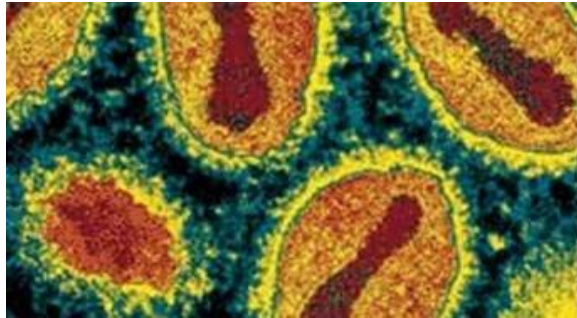
- Must be ready for anything
- Information regarding sample can be difficult to obtain in advance



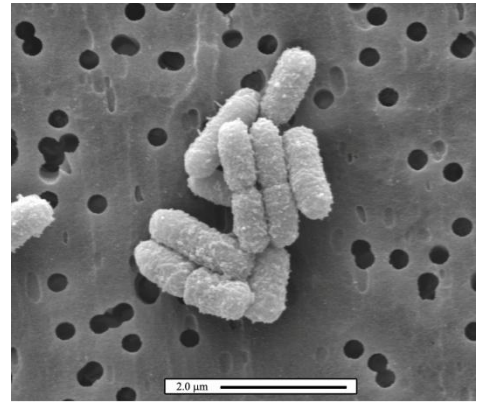
Agents



Bacillus anthracis



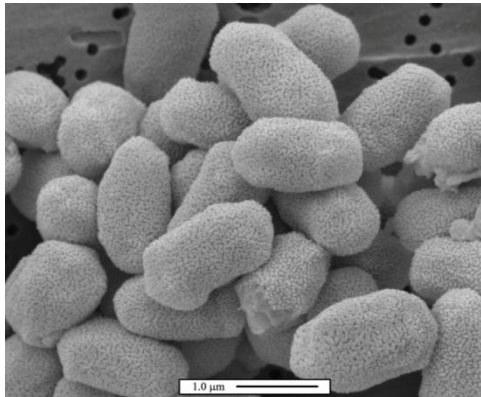
Orthopox virus



Yersinia pestis



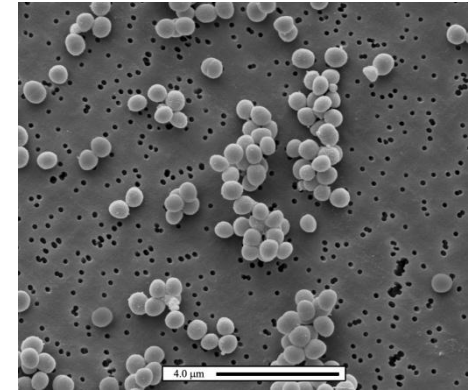
Rosary Peas



Bacillus anthracis
spores



Castor Beans



Staphylococcus aureus

Personnel

- **Sample identification is not an automated process**
 - **Requires highly trained, experienced, and reliable personnel to implement the analysis plans**
 - Personnel must understand the importance of the mission, security and safety
 - **Occupational health requirements need to be considered and constantly monitored:**
 - General health status
 - Immunization status
 - Ability to wear PPE
- **Cross-training is essential to support the mission**
- **Obtain and maintain a security clearance, PRP certification, ability to work with BSAT**



"They hate it when you carry the testtubes that way."

All of this has a cost associated with it

Personnel Reliability Program (PRP)

- **Purpose:**

- To create an operational environment where work with BSAT and radioactive materials is conducted in a safe, secure, and reliable manner



- **Objective:**

- Serves as a tool for management to make risk-based assessment decisions to ensure that persons with access to BSAT and radioactive materials meet high standards of reliability

PRP

- **Must look at all stressors**
- **To provide a safe reporting mechanism for staff to self-report and relay concerns about other staff members**
 - *Mechanism in place to opt out of lab work if not physically or emotionally able to work safely (ex. family emergency, mild illness).*
- **To foster a culture where staff members watch out for each other and take responsibility for both their own performance and that of others**
- ***NBACC PRP Certification: provides assurance that high expectations have been met and are being maintained***



Life Safety Considerations

- **Must look at all stressors**
 - **Life threatening agents, containment work**
 - Always aware of agents manipulated
 - Monitor and report current health status
 - **Long hours during casework**
 - On-call rosters, shifted schedules
 - **High-profile work due to impact of results**
 - **Provide the results as soon as possible**
 - High quality demands
 - Accuracy is critical
 - Efficiency is key
 - **Personal life issues may impact job performance**

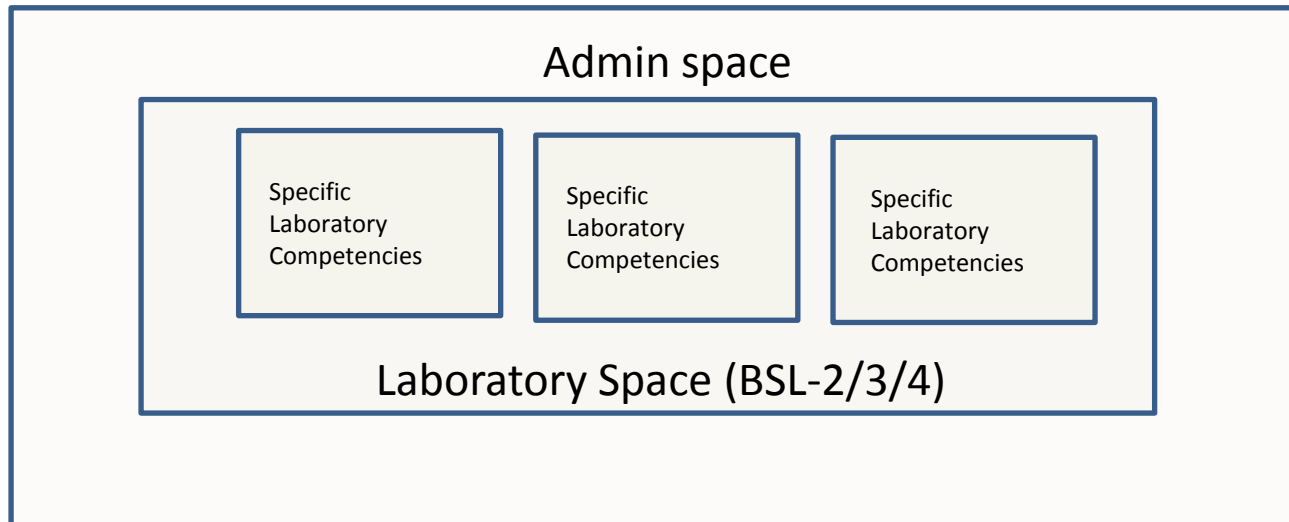
Immunizations

- **Personnel must be considered for all immunizations for agents which they may encounter**
 - Are staff medically able to receive vaccination?
 - Are staff willing to receive vaccination?
 - Must thoroughly understand risk/benefit
 - Employer cannot require IND vaccines
 - What if personnel are unable or unwilling to receive vaccination?
 - Additional PPE? Waiver process?
 - Reassign or limit duties (no direct work with agent)?
 - If immunized:
 - What is time to immunity?
 - What are known and expected side effects?
 - Is access to laboratory impacted by vaccination?

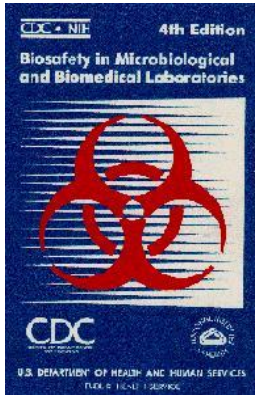


Training

- **General Safety and Biosafety**
- **Security and Biosurety**
- **Quality**
- **Method/Procedure**
 - **Competency Testing → Authorization**



Environment: Containment

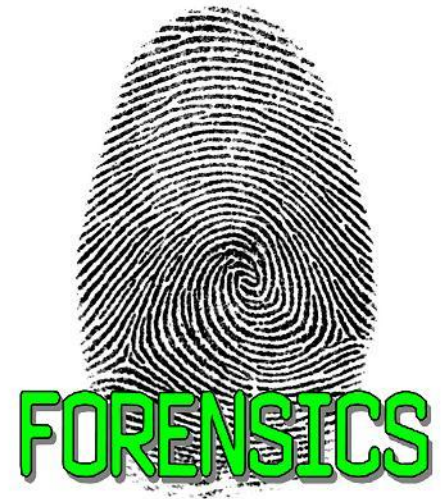


- Nearly all analysis at NBACC is done in biocontainment (BSL-2/3/4) according to CDC BMBL guidelines
- Everything, even simple tasks, becomes more complicated



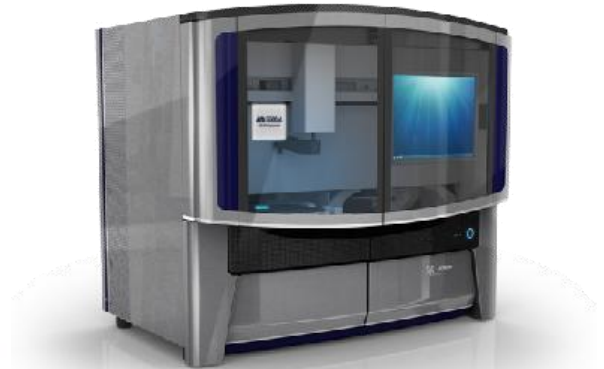
Environment: Containment

- **The mindset for working in containment must be overlaid on the requirements of forensic casework:**
 - **Slower, deliberate physical movement**
 - **Additional PPE requirements (PAPRs, positive pressure suits in BSL-4)**
 - **Increased safety requirements (change of clothes and shower out, use of primary containment devices, decontamination of samples prior moving them)**
 - **Increased reliance on pre-planning**
 - **Supplies**
 - **Staff available**
 - **Maintenance of equipment**
 - **Documentation**
 - **Movement of materials takes longer**
 - **Sterility testing of evidence and samples**
 - **Shipping requirements increased**
 - **BSAT regulations and reporting requirements**



Unique Instrumentation In Containment

- Mission relies on cutting edge technology not necessarily designed with containment work in mind
 - Issues with maintenance, calibration, decontamination
- Examples—RealTime molecular analysis, electron microscopy, and high-throughput sequencing



Instruments--Requirements

- **Example—Pacific Biosciences RS sequencer**
 - Capable of generating 10^5 sequence reads averaging 2,000 nt or longer in ~30 min, but....
 - Requires
 - N_2 generator (uses 2-3 standard cylinders per week)
 - ~60 sq ft footprint
 - Multiple dedicated circuits for power
 - Dedicated cooling (RS generated ~50,000 BTU at peak use)
 - Stringent ambient temperature control (no deviations, 68-76 F)
 - Ultra high speed data lines and high capacity storage system for transferring data (system has enough memory for a single run)
 - Very little vibration or deviation from flatness in floor
 - Positive pressure environment



How do you do this at BSL-3 AND protect the sample?

Protecting The Sample

- **Must protect the sample to ensure reliable analysis**
 - Some processes are sensitive to any and all external contaminants (e.g. Whole Genome Amplification)
- **Combination of controlled workflow, dedicated space, and single-use consumables allows the sample to be processed with no contact from other samples or environmental contaminants**

Warning—this is an
ultraclean room!

Do not enter if you
have been in any other
laboratory space today



NBFAC Contamination Control— Setting a New National Standard



- Total access control
- “Signature control”
- Continuous environmental surveillance

Disposable
Sleeve

Disposable
Glove

Disposable
Lab Coat

Disposable
Shoe Cover



Functional Dedicated Facility



Time Sensitivity and Pressure

■ Unknown Sample

- Unknown timing of event
- Must assess upon arrival for final details
- Sample analysis plan must be drafted, reviewed, approved

■ Personnel

- Support 24/7 operations with limited staff that do not work 24/7
- Laboratory access
- Training

■ Environment

- Containment
- Quality rigor
- Forensic evidence requirements
- BSAT documentation (2 person)



Results are expected in a timely manner, despite the challenges

Challenges in Bioforensics



I. Unknown Sample

II. Personnel

III. Environment

IV. Time Sensitivity and Pressures

Execution of Sample Analysis



- **Notification of services needed**
- **Coordinate with law enforcement and schedule receipt**
- **Alert staff – assess time of day, hours worked already, expected workload, outside factors**
- **Develop Sample Analysis Plan**
- **Execute sample analysis under the established ISO 17025 framework**
 - **Analysis plan and SOPs are strictly followed**
 - **All sample manipulations are documented**
 - **Non-conforming work or deviations are documented and investigated**
- **Compile, Review, and Report Results**

Despite the Challenges, At NBACC We Are Making It Happen!



- **How?**
 - **Dedicated facility controls for environment and staff**
 - **Staff from multiple specialties supporting the mission**
 - **All critical functions have someone on call**
 - **Quality Management System**
 - **Standard Operating Procedures (SOPs)**
 - **Risk Assessments**
 - **Robust Occupational Health Program**
 - **Planning and Close Coordination Across Multiple Divisions**
 - **Learn and Improve Based on Each Case**





This Document was prepared for the Department of Homeland Security (DHS) by the Battelle National Biodefense Institute, LLC (BNBI) as part of contract HSHQDC-07-C-00020 to manage and operate the National Biodefense Analysis and Countermeasures Center (NBACC), a Federally Funded Research and Development Center. In no event shall the DHS, BNBI or NBACC have any responsibility or liability for any use, misuse, inability to use, or reliance upon the information contained herein. **In addition, no warranty of fitness for a particular purpose, merchantability, accuracy or adequacy is provided regarding the contents of this document.**



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