

Division of Laboratory Systems



Biosafety Practices and Reporting Occupational Exposures for Select Agents and Toxins

Tarsha L. Harris, PhD

Michael J. Perry MS, MS Ed

May 31, 2023



Agenda

- Introduction
 - *Today's Presenters*
- *Biosafety Practices and Reporting Occupational Exposures for Select Agents and Toxins*
- Q&A
- Upcoming Events

Division of Laboratory Systems

- Slide decks may contain presentation material from panelists who are not affiliated with CDC. Presentation content from external panelists may not necessarily reflect CDC's official position on the topic(s) covered.



Division of Laboratory Systems

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Presenter



Tarsha L. Harris, PhD

Microbiologist/Form 3 Coordinator,
Division of Select Agents and Toxins (DSAT),
Office of Readiness and Response (ORR),
CDC

Presenter



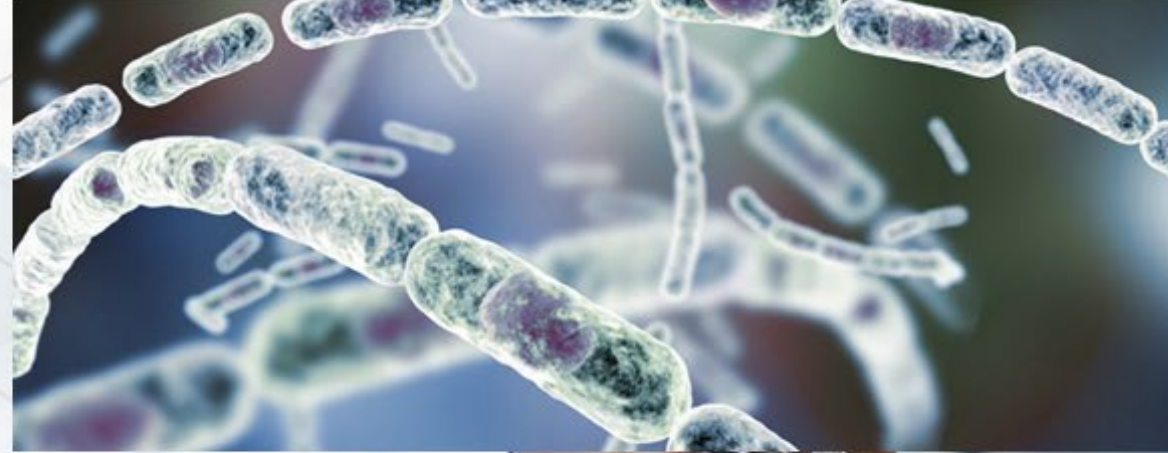
Michael J. Perry, MS, MS Ed

Associate Director,
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New York State Department of Health (NYS DOH) -
Wadsworth Center

Report of a Select Agent or Toxin Release/Loss/Theft (APHIS/CDC Form 3)

Tarsha Harris, PhD
Division of Select Agents and Toxins
Office of Readiness and Response, CDC

OneLab Network Presentation
May 2023



Federal Select Agent Program (FSAP)

- **Regulates the possession, use, and transfer of biological select agents and toxins (BSAT) with the potential to pose a severe threat to public, animal or plant health, or to animal or plant products**
- **Managed jointly by:**



- The Division of Select Agents and Toxins (DSAT), Centers for Disease Control and Prevention (CDC), U.S. Department of Health and Human Services (HHS)



- The Division of Agricultural Select Agents and Toxins (DASAT), Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA)



APHIS/CDC Form 3 Purpose, Regulations, Statistics, and Reporting



APHIS/CDC Form 3 Purpose

- The APHIS/CDC Form 3, Report of a Select Agent or Toxin Release/Loss/Theft, is used by entities to report a theft, loss, or release of a select agent or toxin
- Reports to FSAP any theft, loss, or release involving a select agent and toxin which have the potential to pose a severe threat to public health and safety, animal health or animal products, or plant health or plant products

The image shows the top portion of the APHIS/CDC Form 3, 'Report of a Release/Loss/Theft of a Select Agent or Toxin'. It includes the USDA and CDC logos, the title, and contact information for both agencies. The form is divided into Section A (Entity Information) and Section B (Incident Information). Section A includes fields for Name of Entity, Physical Address, City, State, Zip Code, Name of Responsible Official, and Name of Principal Investigator. Section B includes fields for Date and Time of Incident, Date of Immediate Notification, Type of notification, Strain designation, Severity of the incident, and Biosafety Level. It also includes checkboxes for Release/Potential Exposure, Loss, and Theft, and a note to complete Appendix 1.

USDA
REPORT OF A RELEASE/LOSS/THEFT OF A SELECT AGENT OR TOXIN APHIS/CDC FORM 3

FORM APPROVED
 OMB NO. 0520-0576
 EXP DATE: 01/31/2024

Detailed instructions are available at <http://www.selectagents.gov/form3.html>.
 This report must be signed and submitted to either DASAT or DSAT:

Animal and Plant Health Inspection Service
 Division of Agricultural Select Agents and Toxins
 4700 River Road Unit 2, Mailstop 22, Cubicle 1A07
 Riverdale, MD 20737
 FAX: (301) 734-3652
 Email: DASAT@usda.gov

Centers for Disease Control and Prevention
 Division of Select Agents and Toxins
 1600 Clifton Road NE, Mailstop H21-4
 Atlanta, GA 30329
 FAX: (404) 471-8375
 Email: form3@cdc.gov

Submit completed form only once by either eFSAP, fax, or email

SECTION A – ENTITY INFORMATION

1. Name of Entity: _____

2. Physical Address (NOT a post office box): _____ 3. City: _____ 4. State: _____ 5. Zip Code: _____
 (Select) (Select)

6. Name of Responsible Official or Laboratory Supervisor: _____ 7. Name of Principal Investigator: _____

8. Telephone Number of Responsible Official: _____ 9. Email address of Responsible Official: _____

SECTION B – INCIDENT INFORMATION

1. Date and Time of Incident: _____ 2. Date of Immediate Notification to CDC or APHIS: _____ 3. Type of notification to CDC or APHIS:
 E-mail Fax Telephone eFSAP 4. Location of Incident (bldg., room, equipment, etc.): _____

5. Name of Select Agent or Toxin: _____ (Select) 6. Strain designation of Select Agent or Toxin:
 Recombinant Agent 7. Quantity (Unit (vial, plates, etc.): _____
 (Select) Recombinant Agent
 (Select) Recombinant Agent

8. Type of Incident:
 Release/ Potential Exposure (After completing Section B. Go to Section C)
 Loss (After completing Section B. Go to Section D)
 Theft (After completing Section B. Go to Section E)
 Note: Please complete Appendix 1, event timeline, to provide details on the theft/loss/release incident.

9. Severity of the incident:
 Negligible
 Low
 Moderate
 High

10. What Biosafety Level did the incident occur?
 BSL2 ABLS2
 BSL3 ABLS3
 BSL4 ABLS4
 ACL 2 ABLS3Ag
 ACL 3 Storage area
 ACL 4 Other _____

11. Is this incident associated with an APHIS/CDC Form 2 (Transfer):
 Yes, APHIS/CDC Form 2 transfer #: _____
 No

12. Is this incident associated with an APHIS/CDC Form 4 (Identification):
 Yes, APHIS/CDC Form 4 clinical ID#: _____
 No

Image of APHIS/CDC Form 3 (page 1/4)



APHIS/CDC Form 3 Purpose (continued)

Additional Benefits of Reporting:

- | | | |
|---|---|--|
| <ul style="list-style-type: none">• Compliance with regulatory requirements | <ul style="list-style-type: none">• Connection to Subject Matter Experts (SMEs) for guidance on occupational exposure treatment and/or surveillance | <ul style="list-style-type: none">• Communication with FSAP to assess practices to mitigate recurrence |
|---|---|--|



Select Agent and Toxin Regulations

- [7 C.F.R. Part 331: Agriculture](#)
 - [9 C.F.R. Part 121: Animals and Animal Products](#)
 - [42 C.F.R. Part 73: Public Health](#)
-
- All entities
 - 7 CFR 331.19
 - 9 CFR 121.19
 - 42 CFR 73.19

[Federal Select Agent Program \(selectagents.gov\)](https://selectagents.gov)



Image of microorganism from Federal Select Agent Program website



APHIS/CDC Form 3 Requirements

- 19 (a): Upon **discovery of the theft or loss** of a select agent or toxin, an individual or entity must **immediately notify** CDC or APHIS and appropriate Federal, State, or local law enforcement agencies. Thefts and losses must be reported even if the select agent or toxin is subsequently recovered or the responsible parties are identified.
- 19 (a)(2): A completed APHIS/CDC Form 3 must be submitted within **seven calendar days**.



Image of thumbtack on calendar day 7



APHIS/CDC Form 3 Requirements (continued)

- 19 (b): Upon **discovery of the release** of an agent or toxin causing occupational exposure, or release of the select agent or toxin outside of the primary barriers of the biocontainment area, an individual or entity must **immediately notify** CDC or APHIS.
- 19 (b)(2): A completed APHIS/CDC Form 3 must be submitted within **seven calendar days**.



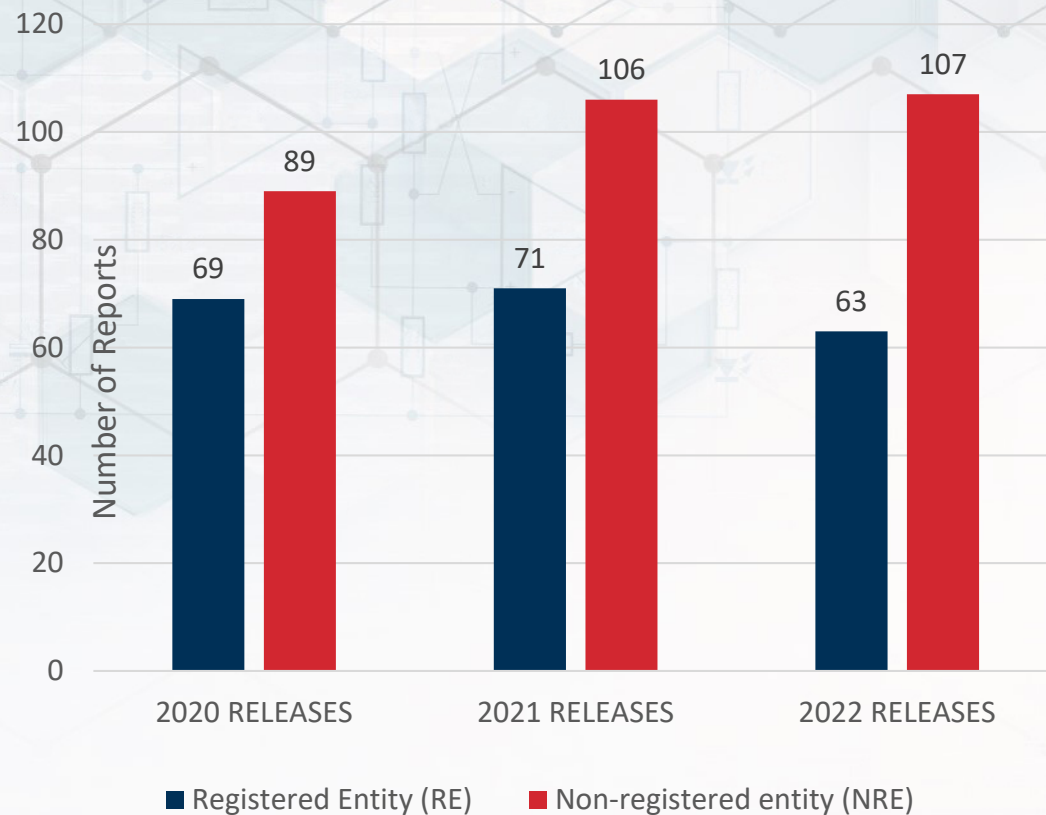
Image of thumbtack on calendar day 7

42 CFR §73.19, 7 CFR §331.19, 9 CFR §121.19

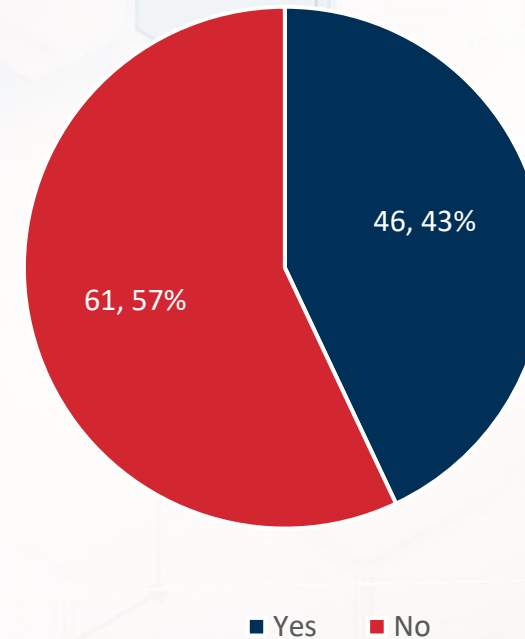


APHIS/CDC Form 3 Reported Release Statistics

FSAP Releases, RE vs NRE, 2020-2022



First Time Submissions, NREs, 2022

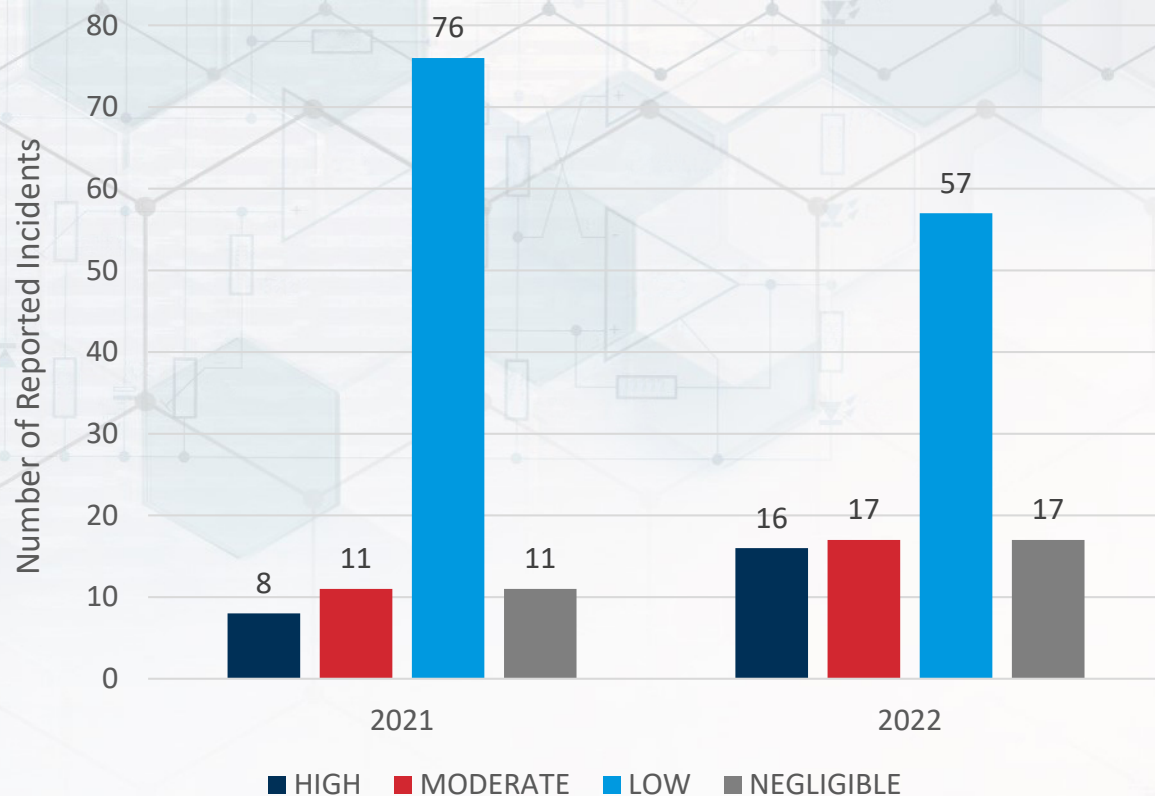


The non-registered entity includes those that are not registered for possession of BSAT, but have identified BSAT in specimens for diagnosis, verification, or proficiency testing.

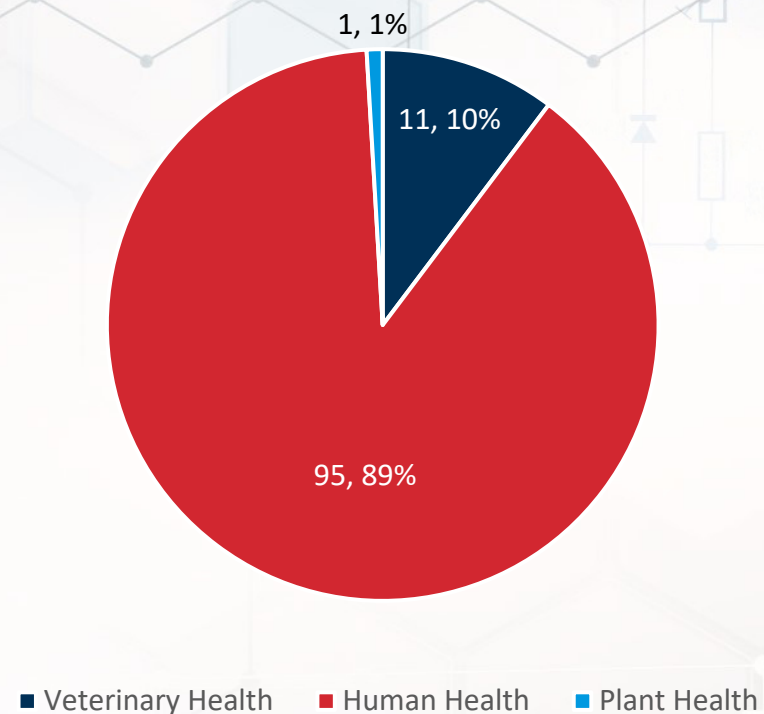


APHIS/CDC Form 3 Reported Release Statistics (continued)

Reported Incident Severity by Year (NRE)



Type of NRE Submitters in 2022



APHIS/CDC Form 3 Reported Release Statistics (continued 2)



APHIS/CDC Form 3 Helpful Information – Date of Immediate Notification

- Immediate Notification – Question B2
 - Immediate Notification (IN) is required for all APHIS/CDC Form 3 reports
 - Date of IN should be within 24 hours of the incident or date the laboratory was notified of a select agent or toxin identified in the specimen manipulated outside of primary containment
 - This is the date that FSAP was notified of the incident
- Strain designation of Select Agent or Toxin – Question B6
 - Provide the strain designation that was identified, if known. Alternatively, enter "Unknown"

| SECTION B – INCIDENT INFORMATION | | | |
|---|---|--|---|
| 1. Date and Time of Incident: _____ | 2. Date of Immediate Notification to CDC or APHIS: _____ | 3. Type of notification to CDC or APHIS: <input type="checkbox"/> E-mail <input type="checkbox"/> Fax <input type="checkbox"/> Telephone <input type="checkbox"/> eFSAP | 4. Location of Incident (bldg., room, equipment, etc.): _____ |
| 5. Name of Select Agent or Toxin: {Select} | 6. Strain designation of Select Agent or Toxin: <input type="checkbox"/> Recombinant Agent | | 7. Quantity (Unit (vial, plates, etc.)): |
| {Select} | <input type="checkbox"/> Recombinant Agent | | |
| {Select} | <input type="checkbox"/> Recombinant Agent | | |



APHIS/CDC Form 3 Helpful Information – Location of Incident and Biosafety Level

- Location of incident– Question B4
 - This should be the building and room name or number where the incident occurred
- What biosafety level did the incident occur – Question B10
 - Select the biosafety level for the space where the incident occurred
 - If a non-laboratory space, select "other"

| SECTION B – INCIDENT INFORMATION | | | |
|---|---|--|--|
| 1. Date and Time of Incident: _____ | 2. Date of Immediate Notification to CDC or APHIS: _____ | 3. Type of notification to CDC or APHIS: <input type="checkbox"/> E-mail <input type="checkbox"/> Fax <input type="checkbox"/> Telephone <input type="checkbox"/> eFSAP | 4. Location of Incident (bldg., room, equipment, etc.): _____ |
| 5. Name of Select Agent or Toxin: {Select} _____ | 6. Strain designation of Select Agent or Toxin: <input type="checkbox"/> Recombinant Agent | | 7. Quantity (Unit (vial, plates, etc.)): |
| {Select} _____ | <input type="checkbox"/> Recombinant Agent | | |
| {Select} _____ | <input type="checkbox"/> Recombinant Agent | | |
| 8. Type of Incident: <input type="checkbox"/> Release/ Potential Exposure (After completing Section B. Go to Section C) <input type="checkbox"/> Loss (After completing Section B. Go to Section D) <input type="checkbox"/> Theft (After completing Section B. Go to Section E) Note: Please complete Appendix 1, event timeline, to provide details on the theft/loss/release incident. | | 9. Severity of the incident: <input type="checkbox"/> Negligible <input type="checkbox"/> Low <input type="checkbox"/> Moderate <input type="checkbox"/> High | 10. What Biosafety Level did the incident occur? <input type="checkbox"/> BSL2 <input type="checkbox"/> ABSL2 <input type="checkbox"/> BSL3 <input type="checkbox"/> ABSL3 <input type="checkbox"/> BSL4 <input type="checkbox"/> ABSL4 <input type="checkbox"/> ACL 2 <input type="checkbox"/> ABSL3Ag <input type="checkbox"/> ACL 3 <input type="checkbox"/> Storage area <input type="checkbox"/> ACL 4 <input type="checkbox"/> Other _____ |



APHIS/CDC Form 3 Helpful Information – Association with an APHIS/CDC Form 4

- Associated with an APHIS/CDC Form 4 (Reporting the Identification of a Select Agent or Toxin) – Question B12
 - Provide the APHIS/CDC Form 4 number (CID-F4-#####)
 - If your laboratory did not identify the select agent or toxin, the laboratory that identified the select agent or toxin can provide the number

| | | | | | | | | | | | | | | |
|--|--|---|-------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------|----------------------------------|--------------------------------|---------------------------------------|--------------------------------|--------------------------------------|
| <p>8. Type of Incident:</p> <p><input type="checkbox"/> Release/ Potential Exposure (After completing Section B. Go to Section C)</p> <p><input type="checkbox"/> Loss (After completing Section B. Go to Section D)</p> <p><input type="checkbox"/> Theft (After completing Section B. Go to Section E)</p> <p>Note: Please complete Appendix 1, event timeline, to provide details on the theft/loss/release incident.</p> | <p>9. Severity of the incident:</p> <p><input type="checkbox"/> Negligible</p> <p><input type="checkbox"/> Low</p> <p><input type="checkbox"/> Moderate</p> <p><input type="checkbox"/> High</p> | <p>10. What Biosafety Level did the incident occur?</p> <table><tr><td><input type="checkbox"/> BSL2</td><td><input type="checkbox"/> ABSL2</td></tr><tr><td><input type="checkbox"/> BSL3</td><td><input type="checkbox"/> ABSL3</td></tr><tr><td><input type="checkbox"/> BSL4</td><td><input type="checkbox"/> ABSL4</td></tr><tr><td><input type="checkbox"/> ACL 2</td><td><input type="checkbox"/> ABSL3Ag</td></tr><tr><td><input type="checkbox"/> ACL 3</td><td><input type="checkbox"/> Storage area</td></tr><tr><td><input type="checkbox"/> ACL 4</td><td><input type="checkbox"/> Other _____</td></tr></table> | <input type="checkbox"/> BSL2 | <input type="checkbox"/> ABSL2 | <input type="checkbox"/> BSL3 | <input type="checkbox"/> ABSL3 | <input type="checkbox"/> BSL4 | <input type="checkbox"/> ABSL4 | <input type="checkbox"/> ACL 2 | <input type="checkbox"/> ABSL3Ag | <input type="checkbox"/> ACL 3 | <input type="checkbox"/> Storage area | <input type="checkbox"/> ACL 4 | <input type="checkbox"/> Other _____ |
| <input type="checkbox"/> BSL2 | <input type="checkbox"/> ABSL2 | | | | | | | | | | | | | |
| <input type="checkbox"/> BSL3 | <input type="checkbox"/> ABSL3 | | | | | | | | | | | | | |
| <input type="checkbox"/> BSL4 | <input type="checkbox"/> ABSL4 | | | | | | | | | | | | | |
| <input type="checkbox"/> ACL 2 | <input type="checkbox"/> ABSL3Ag | | | | | | | | | | | | | |
| <input type="checkbox"/> ACL 3 | <input type="checkbox"/> Storage area | | | | | | | | | | | | | |
| <input type="checkbox"/> ACL 4 | <input type="checkbox"/> Other _____ | | | | | | | | | | | | | |
| <p>11. Is this incident associated with an APHIS/CDC Form 2 (Transfer):</p> <p><input type="checkbox"/> Yes, APHIS/CDC Form 2 transfer #: _____</p> <p><input type="checkbox"/> No</p> | <p>12. Is this incident associated with an APHIS/CDC Form 4 (Identification):</p> <p><input type="checkbox"/> Yes, APHIS/CDC Form 4 clinical ID#: _____</p> <p><input type="checkbox"/> No</p> | | | | | | | | | | | | | |



APHIS/CDC Form 3 Helpful Information – Personal Protective Equipment (PPE) Worn

- What PPE was worn at the time of the incident – Question C3
 - Select the PPE worn by all individuals involved with the incident
 - Respiratory protection designed to protect the wearer from airborne hazards (e.g., N95, N100, PAPR)
 - Surgical masks and non fit-tested or ill-fitting respirators may not provide protection against airborne hazards

| SECTION C- REPORT OF RELEASE | |
|---|--|
| <p>1. Type of Potential Exposure/Release (choose all that apply):</p> <p><input type="checkbox"/> Animal bite/scratch</p> <p><input type="checkbox"/> PPE failure</p> <p><input type="checkbox"/> Spill</p> <p><input type="checkbox"/> Needle stick/Sharps</p> <p><input type="checkbox"/> Inactivation failure</p> <p><input type="checkbox"/> Equipment/mechanical failure</p> <p><input type="checkbox"/> Package damaged in transit (complete B-11)</p> <p><input type="checkbox"/> Decontamination failure</p> <p><input type="checkbox"/> Unintended exposure of animal or plants</p> <p><input type="checkbox"/> Work performed on an open bench</p> <p><input type="checkbox"/> Other: _____</p> | <p>2. Was there a release outside containment barriers?</p> <p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p> <p>If yes, (choose all that apply)</p> <p><input type="checkbox"/> Release outside primary containment (e.g., biosafety cabinet)</p> <p><input type="checkbox"/> Release beyond secondary containment (e.g., laboratory)</p> <p><input type="checkbox"/> Release outside all containment barriers of the facility (e.g., resulting in possible agricultural/environmental/public health threat)</p> |
| <p>3. What PPE was worn at the time of the incident (choose all that apply)?</p> <p><input type="checkbox"/> Hand Protection (e.g., gloves)</p> <p><input type="checkbox"/> Head Protectors/Covers</p> <p><input type="checkbox"/> Body Protection(e.g.,lab coat, BSL4 suit)</p> <p><input type="checkbox"/> Eye/Face Protection (e.g., goggles, face shield)</p> <p><input type="checkbox"/> Foot Protection (e.g., booties, shoe covers)</p> <p><input type="checkbox"/> Respiratory Protection (e.g.,PAPR, N95): Type _____</p> <p><input type="checkbox"/> Other: _____</p> | <p>4. Did the release result in potential exposure(s)?</p> <p><input type="checkbox"/> No</p> <p><input type="checkbox"/> Yes</p> <p>4a. If yes, how many individuals/animals/plants were exposed? _____</p> <p>4b. Of the number in 4a, how many individuals were laboratory staff: _____</p> |



APHIS/CDC Form 3 Helpful Information – Potential Exposure

- Did the release result in potential exposure(s) – Question C4
 - Indicate how many individuals/animals/plants were exposed
 - Indicate how many individuals were laboratory staff

| SECTION C- REPORT OF RELEASE | | | | | | | | | | | | | |
|---|---|---|---|---|--|--|---|--|--|--|--|---------------------------------------|--|
| <p>1. Type of Potential Exposure/Release (choose all that apply):</p> <table><tr><td><input type="checkbox"/> Animal bite/scratch</td><td><input type="checkbox"/> Equipment/mechanical failure</td></tr><tr><td><input type="checkbox"/> PPE failure</td><td><input type="checkbox"/> Package damaged in transit (complete B-11)</td></tr><tr><td><input type="checkbox"/> Spill</td><td><input type="checkbox"/> Decontamination failure</td></tr><tr><td><input type="checkbox"/> Needle stick/Sharps</td><td><input type="checkbox"/> Unintended exposure of animal or plants</td></tr><tr><td><input type="checkbox"/> Inactivation failure</td><td><input type="checkbox"/> Work performed on an open bench</td></tr><tr><td></td><td><input type="checkbox"/> Other: _____</td></tr></table> | <input type="checkbox"/> Animal bite/scratch | <input type="checkbox"/> Equipment/mechanical failure | <input type="checkbox"/> PPE failure | <input type="checkbox"/> Package damaged in transit (complete B-11) | <input type="checkbox"/> Spill | <input type="checkbox"/> Decontamination failure | <input type="checkbox"/> Needle stick/Sharps | <input type="checkbox"/> Unintended exposure of animal or plants | <input type="checkbox"/> Inactivation failure | <input type="checkbox"/> Work performed on an open bench | | <input type="checkbox"/> Other: _____ | <p>2. Was there a release outside containment barriers?</p> <p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p> <p>If yes, (choose all that apply)</p> <p><input type="checkbox"/> Release outside primary containment (e.g., biosafety cabinet)</p> <p><input type="checkbox"/> Release beyond secondary containment (e.g., laboratory)</p> <p><input type="checkbox"/> Release outside all containment barriers of the facility (e.g., resulting in possible agricultural/environmental/public health threat)</p> |
| <input type="checkbox"/> Animal bite/scratch | <input type="checkbox"/> Equipment/mechanical failure | | | | | | | | | | | | |
| <input type="checkbox"/> PPE failure | <input type="checkbox"/> Package damaged in transit (complete B-11) | | | | | | | | | | | | |
| <input type="checkbox"/> Spill | <input type="checkbox"/> Decontamination failure | | | | | | | | | | | | |
| <input type="checkbox"/> Needle stick/Sharps | <input type="checkbox"/> Unintended exposure of animal or plants | | | | | | | | | | | | |
| <input type="checkbox"/> Inactivation failure | <input type="checkbox"/> Work performed on an open bench | | | | | | | | | | | | |
| | <input type="checkbox"/> Other: _____ | | | | | | | | | | | | |
| <p>3. What PPE was worn at the time of the incident (choose all that apply)?</p> <table><tr><td><input type="checkbox"/> Hand Protection (e.g., gloves)</td><td><input type="checkbox"/> Foot Protection (e.g., booties, shoe covers)</td></tr><tr><td><input type="checkbox"/> Head Protectors/Covers</td><td><input type="checkbox"/> Respiratory Protection (e.g., PAPR, N95):</td></tr><tr><td><input type="checkbox"/> Body Protection (e.g., lab coat, BSL4 suit)</td><td>Type _____</td></tr><tr><td><input type="checkbox"/> Eye/Face Protection (e.g., goggles, face shield)</td><td><input type="checkbox"/> Other: _____</td></tr></table> | <input type="checkbox"/> Hand Protection (e.g., gloves) | <input type="checkbox"/> Foot Protection (e.g., booties, shoe covers) | <input type="checkbox"/> Head Protectors/Covers | <input type="checkbox"/> Respiratory Protection (e.g., PAPR, N95): | <input type="checkbox"/> Body Protection (e.g., lab coat, BSL4 suit) | Type _____ | <input type="checkbox"/> Eye/Face Protection (e.g., goggles, face shield) | <input type="checkbox"/> Other: _____ | <p>4. Did the release result in potential exposure(s)?</p> <p><input type="checkbox"/> No</p> <p><input type="checkbox"/> Yes</p> <p>4a. If yes, how many individuals/animals/plants were exposed? _____</p> <p>4b. Of the number in 4a, how many individuals were laboratory staff: _____</p> | | | | |
| <input type="checkbox"/> Hand Protection (e.g., gloves) | <input type="checkbox"/> Foot Protection (e.g., booties, shoe covers) | | | | | | | | | | | | |
| <input type="checkbox"/> Head Protectors/Covers | <input type="checkbox"/> Respiratory Protection (e.g., PAPR, N95): | | | | | | | | | | | | |
| <input type="checkbox"/> Body Protection (e.g., lab coat, BSL4 suit) | Type _____ | | | | | | | | | | | | |
| <input type="checkbox"/> Eye/Face Protection (e.g., goggles, face shield) | <input type="checkbox"/> Other: _____ | | | | | | | | | | | | |



APHIS/CDC Form 3 Helpful Information – Medical Surveillance and/or Treatment

- What medical surveillance and/or treatment was provided to individuals – Questions C6 and C6a
 - Select the type of medical surveillance and treatment provided and include the number of individuals receiving surveillance and/or treatment

| | | | | | | | | | | | | | |
|--|---|--|--|---|--|---|---|---|--|--|--|-------------------------------|---------------------------------------|
| <p>5. Did the release result in a laboratory acquired infection or an infection/outbreak in agriculture or in the environment?</p> <p><input type="checkbox"/> Yes</p> <p><input type="checkbox"/> No</p> <p><input type="checkbox"/> Not currently known</p> | <p>6. What medical surveillance and/or treatment was provided to individuals, if any? (choose all that apply)</p> <p><input type="checkbox"/> None</p> <p><input type="checkbox"/> Physical evaluation</p> <p><input type="checkbox"/> Fever/symptom watch</p> <p><input type="checkbox"/> Serology screening</p> <p><input type="checkbox"/> Antibiotics or other prophylaxis</p> <p><input type="checkbox"/> Other: _____</p> <p>6a. Total number of individuals medical surveillance and/or treatment provided to: _____</p> | | | | | | | | | | | | |
| <p>7a. Has an internal investigation been initiated to lessen the likelihood of recurrences of incident involving the select agents and toxins at this entity?</p> <p><input type="checkbox"/> No</p> <p><input type="checkbox"/> Yes (If yes, please provide additional details below)</p> <p>Describe the internal investigation initiated following the incident (if any), and any root cause(s) identified.</p> <p>7b. What corrective actions have been initiated to lessen the likelihood of recurrence of incident involving the select agents and toxins at this entity? (choose all that apply)</p> <table border="0"><tr><td><input type="checkbox"/> Retraining on existing policy</td><td><input type="checkbox"/> New/modified policy</td><td><input type="checkbox"/> New training developed</td><td><input type="checkbox"/> New/updated SOP</td></tr><tr><td><input type="checkbox"/> New PPE provided</td><td><input type="checkbox"/> New equipment provided</td><td><input type="checkbox"/> Equipment repair</td><td><input type="checkbox"/> Review/revise risk assessment</td></tr><tr><td><input type="checkbox"/> Audit/remove faulty PPE</td><td><input type="checkbox"/> Audit/remove faulty equipment</td><td><input type="checkbox"/> None</td><td><input type="checkbox"/> Other: _____</td></tr></table> | | <input type="checkbox"/> Retraining on existing policy | <input type="checkbox"/> New/modified policy | <input type="checkbox"/> New training developed | <input type="checkbox"/> New/updated SOP | <input type="checkbox"/> New PPE provided | <input type="checkbox"/> New equipment provided | <input type="checkbox"/> Equipment repair | <input type="checkbox"/> Review/revise risk assessment | <input type="checkbox"/> Audit/remove faulty PPE | <input type="checkbox"/> Audit/remove faulty equipment | <input type="checkbox"/> None | <input type="checkbox"/> Other: _____ |
| <input type="checkbox"/> Retraining on existing policy | <input type="checkbox"/> New/modified policy | <input type="checkbox"/> New training developed | <input type="checkbox"/> New/updated SOP | | | | | | | | | | |
| <input type="checkbox"/> New PPE provided | <input type="checkbox"/> New equipment provided | <input type="checkbox"/> Equipment repair | <input type="checkbox"/> Review/revise risk assessment | | | | | | | | | | |
| <input type="checkbox"/> Audit/remove faulty PPE | <input type="checkbox"/> Audit/remove faulty equipment | <input type="checkbox"/> None | <input type="checkbox"/> Other: _____ | | | | | | | | | | |



APHIS/CDC Form 3 Helpful Information - Corrective Actions

- What corrective actions have been initiated to lessen the likelihood of recurrence of an incident involving a select agent or toxin at this entity – Question C7b
 - Indicate any changes in practices, policies, or procedures

| | | | | | | | | | | | | | |
|--|--|--|--|---|--|---|---|---|--|--|--|-------------------------------|---------------------------------------|
| <p>5. Did the release result in a laboratory acquired infection or an infection/outbreak in agriculture or in the environment?</p> <p><input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> Not currently known</p> | <p>6. What medical surveillance and/or treatment was provided to individuals, if any? (choose all that apply)</p> <p><input type="checkbox"/> None <input type="checkbox"/> Physical evaluation <input type="checkbox"/> Fever/symptom watch <input type="checkbox"/> Serology screening <input type="checkbox"/> Antibiotics or other prophylaxis <input type="checkbox"/> Other: _____</p> <p>6a. Total number of individuals medical surveillance and/or treatment provided to: _____</p> | | | | | | | | | | | | |
| <p>7a. Has an internal investigation been initiated to lessen the likelihood of recurrences of incident involving the select agents and toxins at this entity?</p> <p><input type="checkbox"/> No <input type="checkbox"/> Yes (If yes, please provide additional details below)</p> <p>Describe the internal investigation initiated following the incident (if any), and any root cause(s) identified.</p> | | | | | | | | | | | | | |
| <p>7b. What corrective actions have been initiated to lessen the likelihood of recurrence of incident involving the select agents and toxins at this entity? (choose all that apply)</p> <table border="0"><tr><td><input type="checkbox"/> Retraining on existing policy</td><td><input type="checkbox"/> New/modified policy</td><td><input type="checkbox"/> New training developed</td><td><input type="checkbox"/> New/updated SOP</td></tr><tr><td><input type="checkbox"/> New PPE provided</td><td><input type="checkbox"/> New equipment provided</td><td><input type="checkbox"/> Equipment repair</td><td><input type="checkbox"/> Review/revise risk assessment</td></tr><tr><td><input type="checkbox"/> Audit/remove faulty PPE</td><td><input type="checkbox"/> Audit/remove faulty equipment</td><td><input type="checkbox"/> None</td><td><input type="checkbox"/> Other: _____</td></tr></table> | | <input type="checkbox"/> Retraining on existing policy | <input type="checkbox"/> New/modified policy | <input type="checkbox"/> New training developed | <input type="checkbox"/> New/updated SOP | <input type="checkbox"/> New PPE provided | <input type="checkbox"/> New equipment provided | <input type="checkbox"/> Equipment repair | <input type="checkbox"/> Review/revise risk assessment | <input type="checkbox"/> Audit/remove faulty PPE | <input type="checkbox"/> Audit/remove faulty equipment | <input type="checkbox"/> None | <input type="checkbox"/> Other: _____ |
| <input type="checkbox"/> Retraining on existing policy | <input type="checkbox"/> New/modified policy | <input type="checkbox"/> New training developed | <input type="checkbox"/> New/updated SOP | | | | | | | | | | |
| <input type="checkbox"/> New PPE provided | <input type="checkbox"/> New equipment provided | <input type="checkbox"/> Equipment repair | <input type="checkbox"/> Review/revise risk assessment | | | | | | | | | | |
| <input type="checkbox"/> Audit/remove faulty PPE | <input type="checkbox"/> Audit/remove faulty equipment | <input type="checkbox"/> None | <input type="checkbox"/> Other: _____ | | | | | | | | | | |



APHIS/CDC Form 3 Helpful Information - Events Timeline

- Events Timeline – Appendix 1
 - Fully describe the events leading up to and immediately following the incident
 - Include dates, times, equipment used, type of testing, others in the area, etc.

APPENDIX 1 EVENTS TIMELINE

Provide a detailed summary of events, including a timeline of what occurred.

- Who was involved?
- What happened?
- When did it happen?
- Where did it happen?
- Why and how (root cause) did it happen?



APHIS/CDC Form 3 Scenarios



APHIS/CDC Form 3 Scenario A

On Friday afternoon, your laboratory received a reference laboratory notification of a *Burkholderia pseudomallei* identification from the culture isolate your laboratory tried to identify a few days before. Two technicians performed subculturing and additional testing on the open bench at your laboratory. What should your laboratory do?

- A. Nothing, because it's Friday afternoon and it can wait until Monday
- B. Call the laboratory to verify the results, because *Burkholderia pseudomallei* is not known in your area
- C. Immediately notify FSAP, because the two technicians worked on the open bench with a select agent
- D. I do not know



APHIS/CDC Form 3 Scenario A Response

On Friday afternoon, your laboratory received a reference laboratory notification of a *Burkholderia pseudomallei* identification from the culture isolate your laboratory tried to identify a few days before. Two technicians performed subculturing and additional testing on the open bench at your laboratory. What should your laboratory do?

- A. Nothing, because it's Friday afternoon and it can wait until Monday
- B. Call the laboratory to verify the results, because *Burkholderia pseudomallei* is not known in your area
- C. Immediately notify FSAP, because the two technicians worked on the open bench with a select agent**
- D. I do not know



APHIS/CDC Form 3 Scenario B

While performing diagnostic testing, **Laboratorian A** opened a blood agar plate on the open bench and observed tiny colonies. The next day, **Laboratorian B** took the plate to the biosafety cabinet to perform a Gram stain and other assays. **Laboratorian C** later opened the plate on the bench to collect a colony to spot a MALDI-TOF slide. The isolate was identified as select agent. All laboratorians wore a lab coat and gloves and no others were present in the laboratory.

Which laboratorians should be counted on the Form 3 as an occupational exposure to a select agent?

- A. Laboratorian A only
- B. Laboratorians B and C
- C. Laboratorians A and C
- D. All Laboratorians



APHIS/CDC Form 3 Scenario B Response

While performing diagnostic testing, **Laboratorian A** opened a blood agar plate on the open bench and observed tiny colonies. The next day, **Laboratorian B** took the plate to the biosafety cabinet to perform a Gram stain and other assays. **Laboratorian C** later opened the plate on the bench to collect a colony to spot a MALDI-TOF slide. The isolate was sent to and identified as a select agent by the state reference laboratory. All laboratorians wore a lab coat and gloves and no others were present in the laboratory.

Which laboratorians should be counted on the Form 3 as an occupational exposure to a select agent?

- A. Laboratorian A only
- B. Laboratorians B and C
- C. Laboratorians A and C opened the plate or worked with the select agent outside of primary containment**
- D. All Laboratorians



APHIS/CDC Form 3 Scenario C

Which do you think is the most frequently reported select agent by non-registered entities (NREs) for incidents involving a release and/or occupational exposure?

- A. *Brucella melitensis*
- B. Avian influenza virus
- C. *Francisella tularensis*
- D. Botulinum neurotoxin producing species of *Clostridium*
- E. SARS-associated coronavirus (SARS-CoV)



APHIS/CDC Form 3 Scenario C Response

Which do you think is the most frequently reported select agent by non-registered entities (NREs) for incidents involving a release and/or occupational exposure?

- A. *Brucella melitensis*
- B. Avian influenza virus
- C. *Francisella tularensis*
- D. Botulinum neurotoxin producing species of *Clostridium*
- E. SARS-associated coronavirus (SARS-CoV)



www.selectagents.gov

CDC Contact Information
Division of Select Agents and Toxins
Irsat@cdc.gov
404-718-2000

APHIS Contact Information
Division of Agricultural
Select Agents and Toxins
DASAT@usda.gov
301-851-2070



The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Animal and Plant Health Inspection Service.





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Safely Implementing MALDI-TOF MS Commonly Used In Clinical Laboratories

**Michael Perry, MS, MSED
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Overview

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

Objectives

- Identify biosafety practices to minimize the release or exposure to select agents or toxins while using the MALDI-TOF instrument.
- Recognize a release or exposure incident while handling a select agent or toxin.



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)



Ion Detector

Separation in Flight Tube

Electrostatic Field

Matrix/Analyte

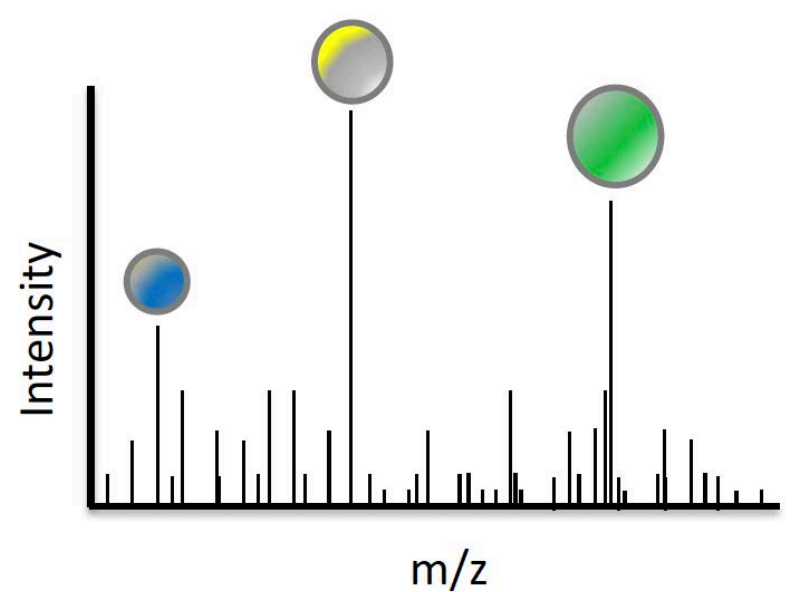
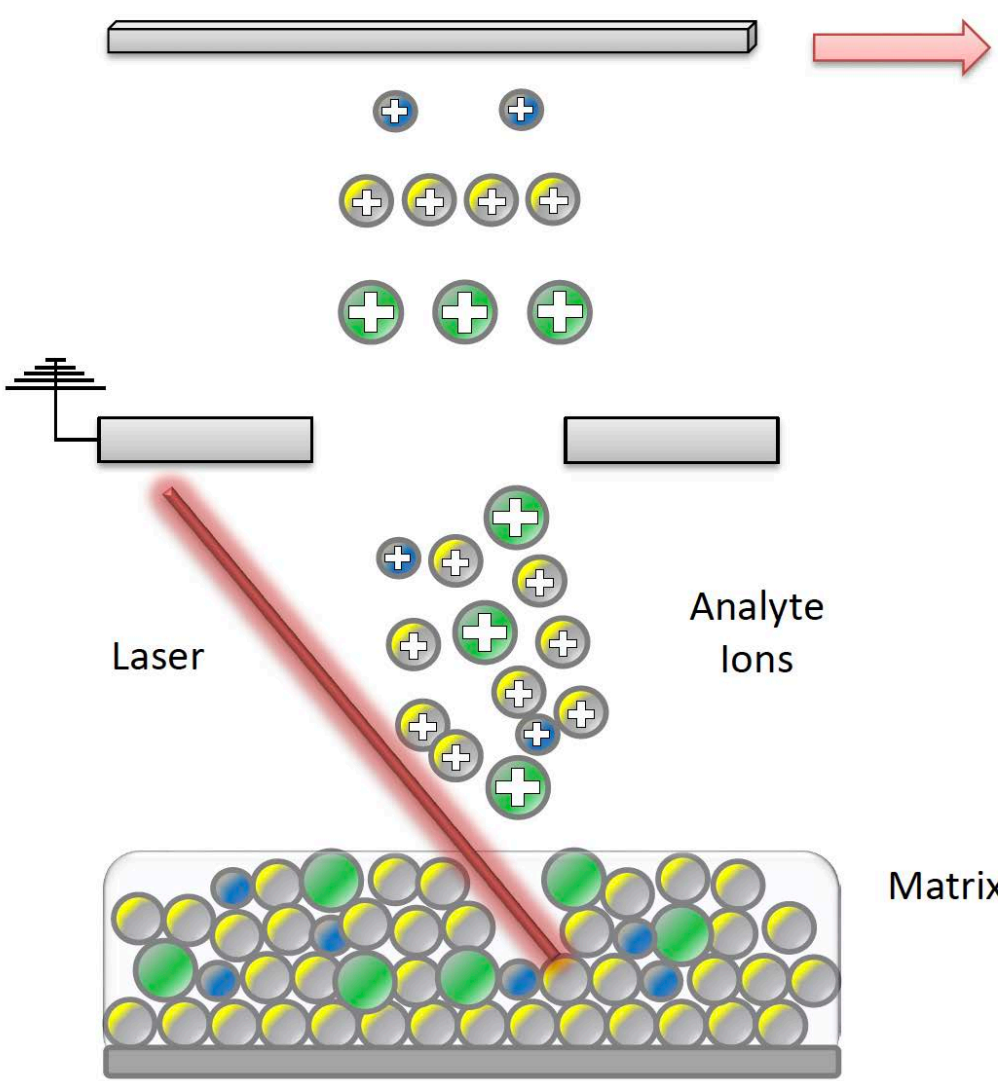


Figure created by Wadsworth Center



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Facility and Safety – General Safety Considerations

- 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)



<https://www.pngegg.com/en/png-bbeki/download>
<https://www.vecteezy.com/free-vector/biohazard>



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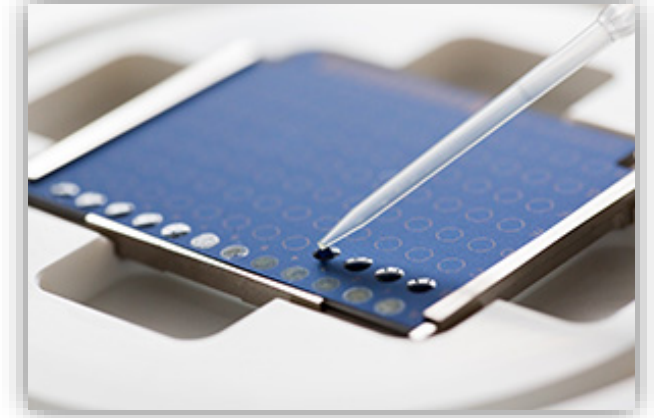
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Chemical Hazard Considerations

- 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



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

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



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MALDI MS TOF Method – Inactivation Efficiency



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. Soehrlen,^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)



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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

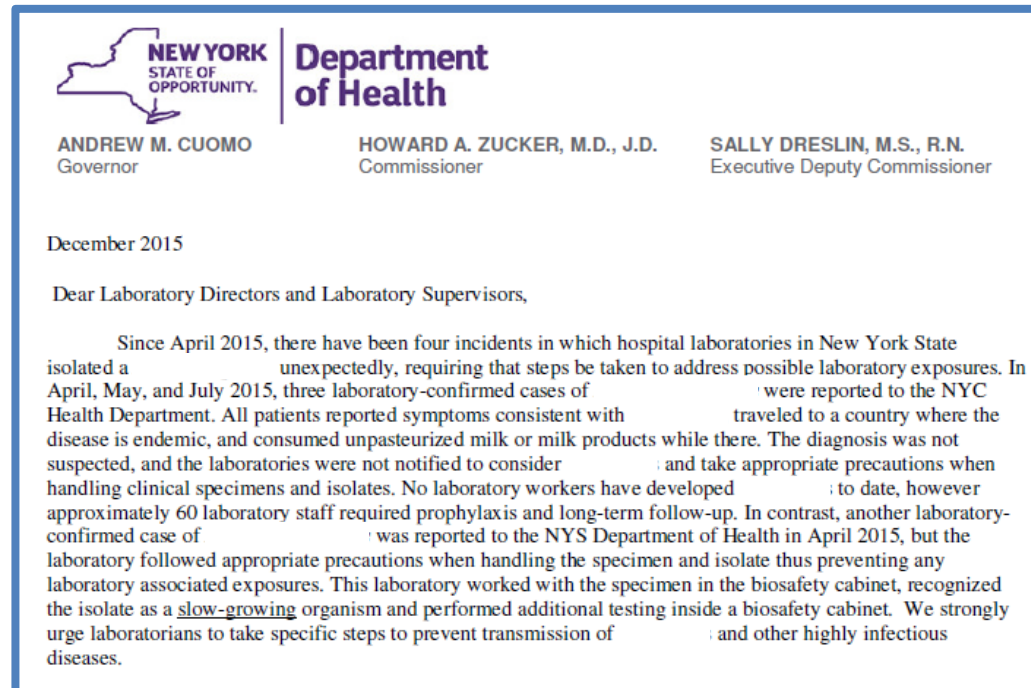


Clinical Cases



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



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**

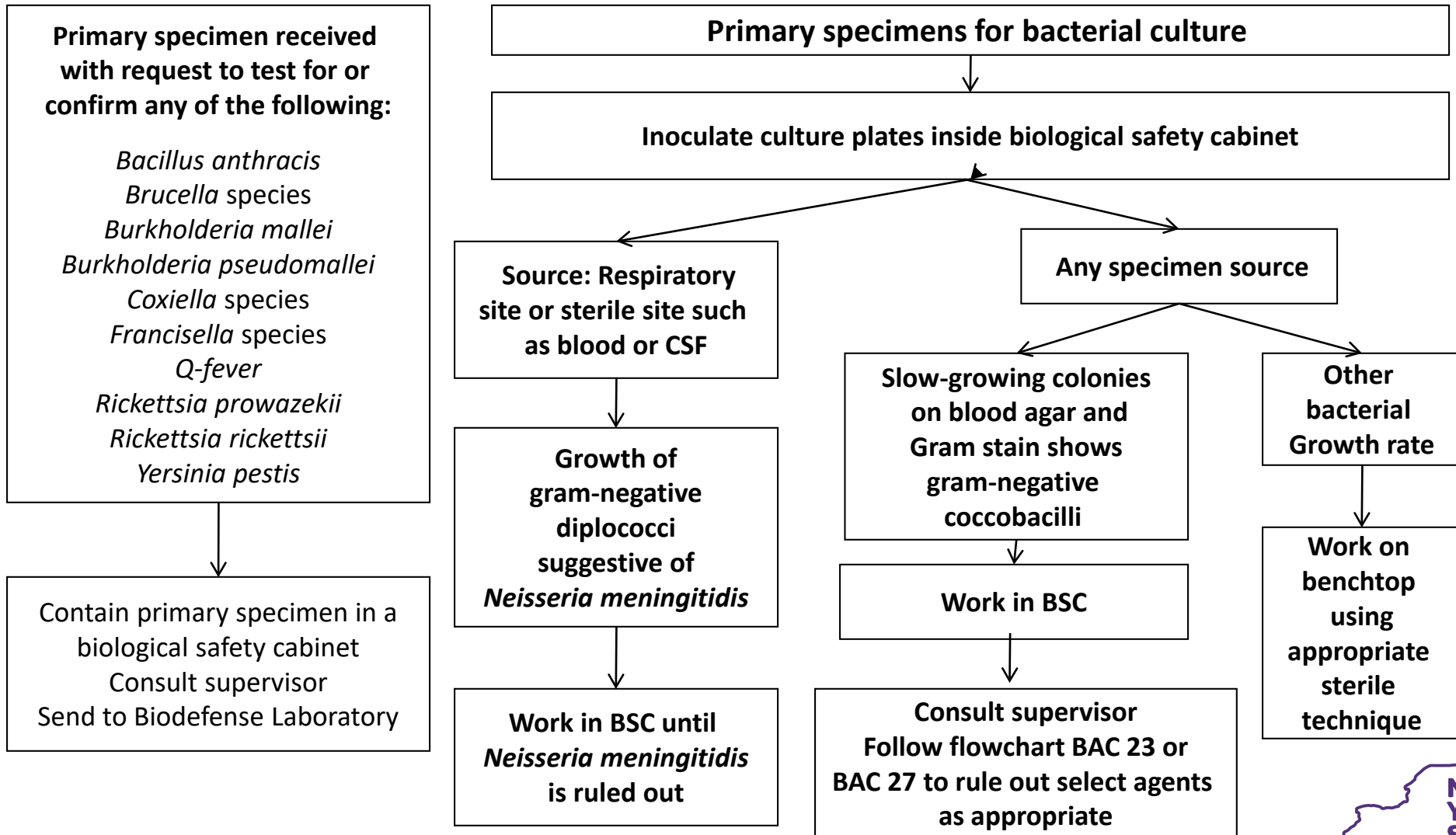
- Isolate from same patient from hospital 2
- ID'ed at hospital 2 as unknown Gram-negative coccobacillus
- Worked up in BSC but initial biochemical did not rule-in *Brucella*
- Re-plated and prepared for MALDI-TOF MS on the benchtop.
- MALDI-TOF ID'ed high match to *Brucella*

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



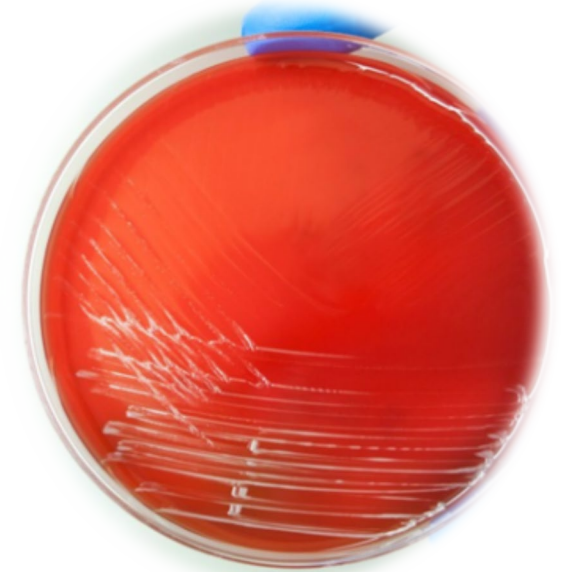
Wadsworth Center Workflow Pre-Exposure

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



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>



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Start all work in the biological safety cabinet

Flowchart for processing unknown Gram-negative or Gram-variable isolates

Steps added Post Exposure in red

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

Gram stain, colony morphology and growth rate not suspicious

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

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



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 stain 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





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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?





Questions?

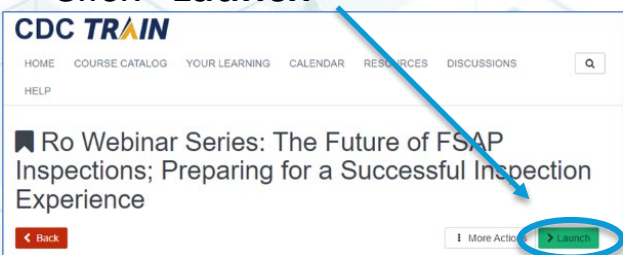
Continuing Education

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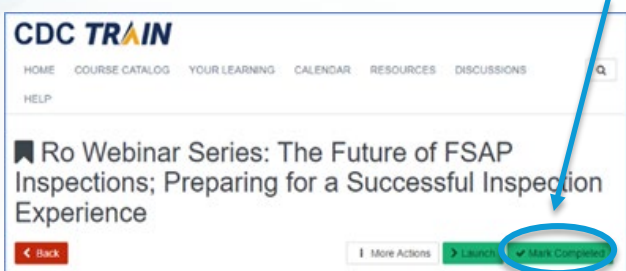
1. Attend entire webinar/**register** for course on TRAIN

- Register for the course in TRAIN
- Registration passcode: **S827**
- Select **"PACE"** credit type

- Click **"Launch"**

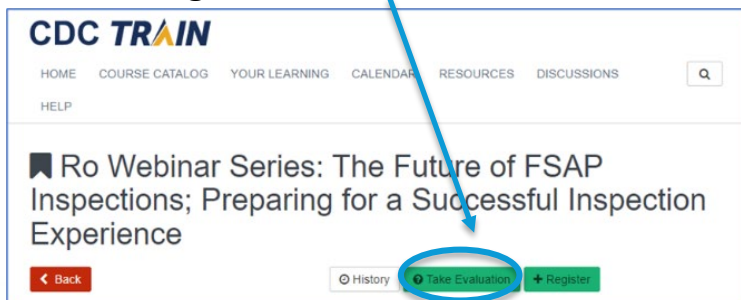


- Click on green **"Mark Complete"**



2. Complete webinar evaluation

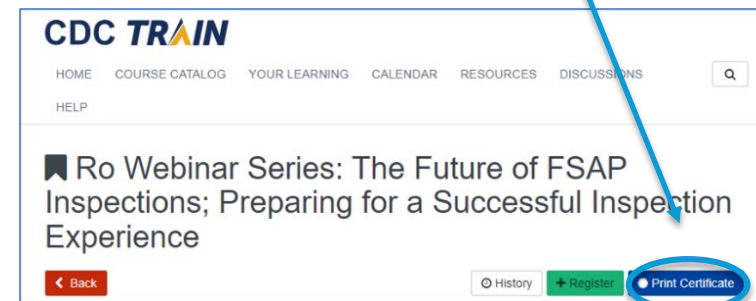
- Click green **"Take Evaluation"** button



- **Complete the evaluation**

3. Obtain P.A.C.E Certificate

- Click on the blue **"Print Certificate"** button to download





Thank You!

