Division of Laboratory Systems

OneLab™

A Unified Response to Training Needs

Recognizing, Identifying, and Reporting the Identification of Select Agents and Toxins

Susanna Schmink, MPH CPH

John R. McQuiston, PhD

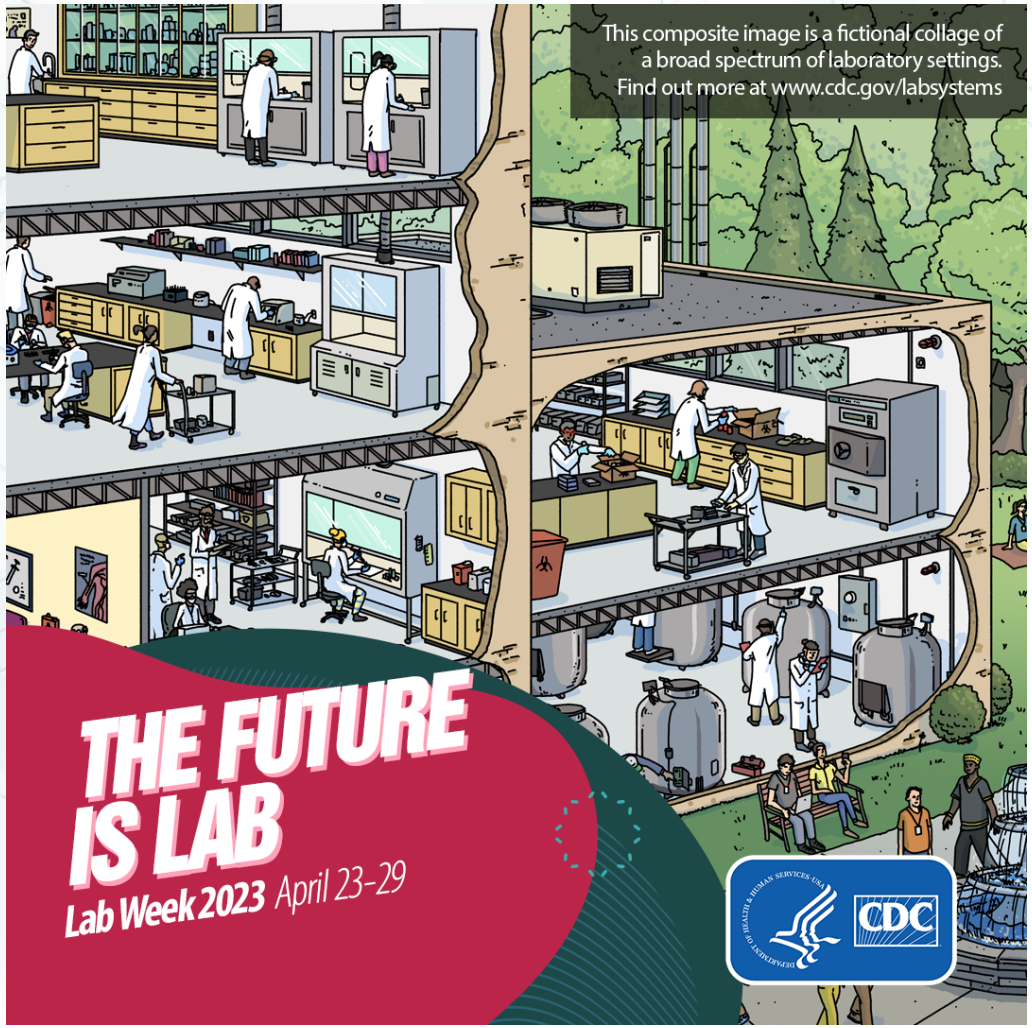
With Opening Remarks from Victoria Olson, PhD

April 26, 2023

Agenda

- Happy Lab Week!
- Opening Remarks
  - Dr. Victoria Olson, PhD
- Introductions
  - *Today's Presenters*
- ***Recognizing, Identifying, and Reporting the Identification of Select Agents and Toxins***
- Q&A
- P.A.C.E Credit Instructions
- Upcoming Events

Medical Laboratory Professionals Week: April 23-29



- We celebrate laboratory professionals who protect our future by skillfully adapting to meet today's evolving patient care and public health challenges with **resilience**, **innovation**, and **expertise**.

- Join DLS in celebrating Lab Week 2023 by
  - Showing thanks to a laboratory professional
  - Participating in DLS's Lab Week activities
  - Accessing our digital toolkit and content

www.cdc.gov/csels/dls/lab-week/

- CDC, our planners, and our presenters wish to disclose they have no financial interests or other relationships with the manufacturers of commercial products, suppliers of commercial services, or commercial supporters.

Opening Remarks

Dr. Victoria Olson, PhD

Deputy Director

Office of Laboratory Science and Safety (OLSS)

Centers for Disease Control and Prevention (CDC)

Presenter

Susanna Schmink, MPH CPH

Microbiologist, Form 4 Technical Advisor
Division of Select Agents and Toxins (DSAT)
Office for Readiness and Response(ORR)
Centers for Disease Control and Prevention (CDC)

Presenter

Dr. John R. McQuiston, PhD

Team Lead, Special Bacteriology Reference Laboratory (SBRL)
Bacterial Special Pathogens Branch
Division of High Consequence Pathogens and Pathology
Centers for Disease Control and Prevention (CDC)

Reporting the Identification of a Select Agent or Toxin (APHIS/CDC Form 4)

Susanna Schmink, MPH CPH
Division of Select Agents and Toxins,
Center for Preparedness and Response, CDC

OneLab Network Presentation
April 2023

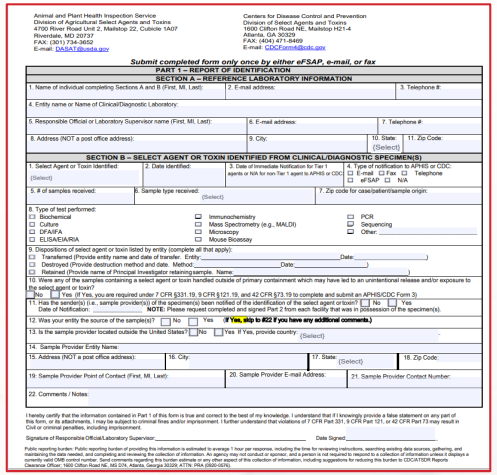
Federal Select Agent Program (FSAP)

- **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 4 Purpose, Regulations, and Reporting

APHIS/CDC Form 4 Purpose

- The APHIS/CDC Form 4, Reporting the Identification of a Select Agent or Toxin, is used by clinical or diagnostic laboratories and other entities to notify the Federal Select Agent Program of the identification of a select agent or toxin as the result of diagnosis, verification, or proficiency testing and of the final disposition of that identified agent or toxin.



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
E-mail: 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) 471-8489
E-mail: CDCForm4@cdc.gov

Submit completed form only once by either eFSAP, e-mail, or fax

PART 1 – REPORT OF IDENTIFICATION

SECTION A – REFERENCE LABORATORY INFORMATION

1. Name of individual completing Sections A and B (First, MI, Last): | 2. E-mail address: | 3. Telephone #:

4. Entity name or Name of Clinical/Diagnostic Laboratory:

5. Responsible Official or Laboratory Supervisor name (First, MI, Last): | 6. E-mail address: | 7. Telephone #:

8. Address (NOT a post office address): | 9. City: | 10. State: {Select} | 11. Zip Code:

SECTION B – SELECT AGENT OR TOXIN IDENTIFIED FROM CLINICAL/DIAGNOSTIC SPECIMEN(S)

1. Select Agent or Toxin Identified {Select} | 2. Date Identified: | 3. Date of Immediate Notification for Tier 1 agents or N/A for non-Tier 1 agent to APHIS or CDC: | 4. Type of notification to APHIS or CDC: ☐ E-mail ☐ Fax ☐ Telephone ☐ eFSAP ☐ N/A

5. # of samples received: | 6. Sample type received: {Select} | 7. Zip code for case/patient/sample origin:

8. Type of test performed:
☐ Biochemical ☐ Immunochemistry ☐ PCR
☐ Culture ☐ Mass Spectrometry (e.g., MALDI) ☐ Sequencing
☐ DFA/IFA ☐ Microscopy ☐ Other:
☐ ELISA/EIA/RIA ☐ Mouse Bioassay

9. Dispositions of select agent or toxin listed by entity (complete all that apply):
☐ Transferred (Provide entity name and date of transfer. Entity: Date:)
☐ Destroyed (Provide destruction method and date. Method: Date:)
☐ Retained (Provide name of Principal Investigator retaining sample. Name:)

10. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?
☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)

11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
Date of Notification: NOTE: Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

12. Was your entity the source of the sample(s)? ☐ No ☐ Yes **(If Yes, skip to #22 if you have any additional comments.)**

13. Is the sample provider located outside the United States? ☐ No ☐ Yes If Yes, provide country: {Select}

14. Sample Provider Entity Name:

15. Address (NOT a post office address): | 16. City: | 17. State: {Select} | 18. Zip Code:

19. Sample Provider Point of Contact (First, MI, Last): | 20. Sample Provider E-mail Address: | 21. Sample Provider Contact Number:

22. Comments / Notes:

I hereby certify that the information contained in Part 1 of this form is true and correct to the best of my knowledge. I understand that if I knowingly provide a false statement on any part of this form, or its attachments, I may be subject to criminal fines and/or imprisonment. I further understand that violations of 7 CFR Part 331, 9 CFR Part 121, or 42 CFR Part 73 may result in Civil or criminal penalties, including imprisonment.

Signature of Responsible Official/Laboratory Supervisor: Date Signed:

Public reporting burden: Public reporting burden of providing this information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering, and maintaining the data needed, and completing and reviewing the collection of information. An agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to CDC/ATSDR Reports Clearance Officer, 1600 Clifton Road NE, MS D74, Atlanta, Georgia 30329; ATTN: PRA (0920-0576)

Image of APHIS/CDC Form 4

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

- Nonregistered entities
  - 7 C.F.R. 331.5
  - 9 C.F.R. 121.5-6
  - 42 C.F.R. 73.5-6

Federal Select Agent Program (selectagents.gov)

Image of microorganism from Federal Select Agent Program website

APHIS/CDC Form 4 Requirements

- Clinical or diagnostic laboratories and other entities that possess, use, or transfer a select agent or toxin that is contained in a specimen presented for diagnosis or verification will be exempt from the requirements of the select agent regulations, provided that:
  - A completed and signed APHIS/CDC Form 4A is submitted within **seven calendar** days after identification
  - The select agent or toxin is secured against theft, loss, or release during the period between identification of the select agent or toxin and transfer or destruction

APHIS/CDC Form 4 Requirements (continued)

- The clinical or diagnostic specimens collected from a patient infected with a select agent are transferred in accordance with § 73.16* or destroyed on-site by a recognized sterilization or inactivation process within **seven calendar** days
- The identification of the agent or toxin is reported to CDC or APHIS, the specimen provider, and to other appropriate authorities when required by Federal, State, or local law

* Per the HHS regulations

See 7 CFR §331.5(a), 9 CFR §121.5(a) and 121.6(a), and 42 CFR §73.5(a) and 73.6(a)

Image of thumbtack on calendar day 7

Select Agents and Toxins Requiring Immediate Reporting

- *Bacillus anthracis*
- *Bacillus cereus* Biovar *anthracis*
- Botulinum neurotoxins
- Botulinum neurotoxin producing species of *Clostridium*
- *Burkholderia mallei*
- *Burkholderia pseudomallei*
- Ebola viruses
- Foot-and-mouth disease virus
- *Francisella tularensis*
- Marburg virus
- Rinderpest virus
- Variola major virus (Smallpox virus)
- Variola minor (Alastrim)
- *Yersinia pestis*

APHIS/CDC Form 4A Helpful Information – Date of Immediate Notification

- Immediate Notification – Question B3
  - Immediate Notification (IN) is required for Tier 1 select agents and toxins
  - Date of IN is the date the laboratory identifying the select agent or toxin notified CDC or APHIS

SECTION B – SELECT AGENT OR TOXIN IDENTIFIED FROM CLINICAL/DIAGNOSTIC SPECIMEN(S)

1. Select Agent or Toxin Identified:	2. Date identified:	3. Date of Immediate Notification for Tier 1 agents or N/A for non-Tier 1 agent to APHIS or CDC:	4. Type of notification to APHIS or CDC: ☐ E-mail ☐ Fax ☐ Telephone ☐ eFSAP ☐ N/A
{Select}			

5. # of samples received:	6. Sample type received: {Select}	7. Zip code for case/patient/sample origin:

8. Type of test performed:

☐ Biochemical	☐ Immunochemistry	☐ PCR
☐ Culture	☐ Mass Spectrometry (e.g., MALDI)	☐ Sequencing
☐ DFA/IFA	☐ Microscopy	☐ Other: _____
☐ ELISA/EIA/RIA	☐ Mouse Bioassay	

APHIS/CDC Form 4A Helpful Information – Date Sample Provider Notified

- Sample Provider Notification – Question B11
  - Sample Provider is the doctor, veterinarian, treatment facility, laboratory, etc. where the sample came from
  - Date the Sample Provider was notified will be a date after the identification date

11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
Date of Notification: _____ **NOTE:** Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

12. Was your entity the source of the sample(s)? ☐ No ☐ Yes **(If Yes, skip to #22 if you have any additional comments.)**

13. Is the sample provider located outside the United States? ☐ No ☐ Yes If Yes, provide country: {Select}

14. Sample Provider Entity Name:

15. Address (NOT a post office address):	16. City:	17. State: {Select}	18. Zip Code:

19: Sample Provider Point of Contact (First, MI, Last):	20. Sample Provider E-mail Address:	21. Sample Provider Contact Number:

22. Comments / Notes:

APHIS/CDC Form 4A Helpful Information – Date Notified of the Select Agent or Toxin Identification

- Date notified by reference laboratory of select agent or toxin identification that was reported to APHIS or CDC – Question D2
  - This date should match the date provided by the reference laboratory

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY

1. Select Agent or Toxin Identified: {Select}

2. Date notified by reference laboratory of select agent or toxin identification reported to APHIS or CDC:

3. # of samples shipped:

4. Sample type provided: {Select}

5. Zip code for case/patient/sample origin:

6. Date sample(s) shipped to Reference Laboratory:

7. Name of Reference Laboratory:

8. Disposition of any remaining select agent or toxin listed by entity:
- ☐ Destroyed (Provide destruction method and date. Method:_____ Date:_____)
- ☐ Retained (Provide name of Principal Investigator retaining sample. Name:_____)
- ☐ Not applicable, the entire specimen was transferred to the Reference Laboratory.

9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?
☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)

10. Was your entity the source of the sample(s)? ☐ No ☐ Yes (**If Yes, skip to #21 if you have any additional comments.**)

11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
NOTE: Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

APHIS/CDC Form 4A Helpful Information – Number of Samples Shipped

- Number of samples shipped – Question D3
  - This number may not match the number of samples received provided by the reference laboratory on the APHIS/CDC Form 4A Sections A&B, Question B5

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY

1. Select Agent or Toxin Identified: {Select}

2. Date notified by reference laboratory of select agent or toxin identification reported to APHIS or CDC:

3. # of samples shipped:

4. Sample type provided: {Select}

5. Zip code for case/patient/sample origin:

6. Date sample(s) shipped to Reference Laboratory:

7. Name of Reference Laboratory:

8. Disposition of any remaining select agent or toxin listed by entity:
- ☐ Destroyed (Provide destruction method and date. Method:_____ Date:_____)
- ☐ Retained (Provide name of Principal Investigator retaining sample. Name:_____)
- ☐ Not applicable, the entire specimen was transferred to the Reference Laboratory.

9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?
☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)

10. Was your entity the source of the sample(s)? ☐ No ☐ Yes (**If Yes, skip to #21 if you have any additional comments.**)

11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
NOTE: Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

APHIS/CDC Form 4A Helpful Information – Zip Code for Sample Origination

- Zip code for case/patient/sample origin – Question D5
  - The zip code is the only patient-related information required on the form
  - The zip code is important

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY

1. Select Agent or Toxin Identified: {Select}
2. Date notified by reference laboratory of select agent or toxin identification reported to APHIS or CDC:
3. # of samples shipped:
4. Sample type provided: {Select}
5. Zip code for case/patient/sample origin:
6. Date sample(s) shipped to Reference Laboratory:
7. Name of Reference Laboratory:
8. Disposition of any remaining select agent or toxin listed by entity:
   - ☐ Destroyed (Provide destruction method and date. Method:_____ Date:_____)
   - ☐ Retained (Provide name of Principal Investigator retaining sample. Name:_____)
   - ☐ Not applicable, the entire specimen was transferred to the Reference Laboratory.
9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?
   ☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)
10. Was your entity the source of the sample(s)? ☐ No ☐ Yes (**If Yes, skip to #21 if you have any additional comments.**)
11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
    NOTE: Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

APHIS/CDC Form 4A Helpful Information- Sample Disposition – Sections A&B

- Disposition of select agent or toxin – Question B9
  - An entity not registered with the Federal Select Agent Program cannot retain a select agent or toxin
  - Transfer or Destroy **onsite**

8. Type of test performed:

☐ Biochemical	☐ Immunochemistry	☐ PCR
☐ Culture	☐ Mass Spectrometry (e.g., MALDI)	☐ Sequencing
☐ DFA/IFA	☐ Microscopy	☐ Other: _____
☐ ELISA/EIA/RIA	☐ Mouse Bioassay	

9. Dispositions of select agent or toxin listed by entity (complete all that apply):

☐ Transferred (Provide entity name and date of transfer. Entity:_____ Date:_____)

☐ Destroyed (Provide destruction method and date. Method:_____ Date:_____)

☐ Retained (Provide name of Principal Investigator retaining sample. Name:_____)

10. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?

☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)

APHIS/CDC Form 4A Helpful Information – Sample Disposition – Sections C&D

- Disposition of any remaining select agent or toxin – Question D8
  - Destroy **onsite** or entire sample sent to reference laboratory
  - An entity not registered with the Federal Select Agent Program cannot retain a select agent or toxin

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY

1. Select Agent or Toxin Identified: {Select}		2. Date notified by reference laboratory of select agent or toxin identification reported to APHIS or CDC:
3. # of samples shipped:	4. Sample type provided: {Select}	5. Zip code for case/patient/sample origin:
6. Date sample(s) shipped to Reference Laboratory:		7. Name of Reference Laboratory:

8. Disposition of any remaining select agent or toxin listed by entity:
- ☐ Destroyed (Provide destruction method and date. Method:_____ Date:_____)
- ☐ Retained (Provide name of Principal Investigator retaining sample. Name:_____)
- ☐ Not applicable, the entire specimen was transferred to the Reference Laboratory.

9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?
☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)

10. Was your entity the source of the sample(s)? ☐ No ☐ Yes (**If Yes, skip to #21 if you have any additional comments.**)

11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
NOTE: Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

APHIS/CDC Form 4A Helpful Information – Release and/or Exposure

- Unintentional release and/or exposure – Questions B10 and D9
  - A 'Yes' response requires submission of an APHIS/CDC Form 3

SECTION D – SPECIMEN(S) CONTAINING SELECT AGENT OR TOXIN PROVIDED TO REFERENCE LABORATORY

1. Select Agent or Toxin Identified: {Select}
2. Date notified by reference laboratory of select agent or toxin identification reported to APHIS or CDC:
3. # of samples shipped:
4. Sample type provided: {Select}
5. Zip code for case/patient/sample origin:
6. Date sample(s) shipped to Reference Laboratory:
7. Name of Reference Laboratory:
8. Disposition of any remaining select agent or toxin listed by entity:
   - ☐ Destroyed (Provide destruction method and date. Method:_____ Date:_____)
   - ☐ Retained (Provide name of Principal Investigator retaining sample. Name:_____)
   - ☐ Not applicable, the entire specimen was transferred to the Reference Laboratory.
9. Were any of the samples containing a select agent or toxin handled outside of primary containment which may have led to an unintentional release and/or exposure to the select agent or toxin?
   ☐ No ☐ Yes (If Yes, you are required under 7 CFR §331.19, 9 CFR §121.19, and 42 CFR §73.19 to complete and submit an APHIS/CDC Form 3)
10. Was your entity the source of the sample(s)? ☐ No ☐ Yes (**If Yes, skip to #21 if you have any additional comments.**)
11. Has the sender(s) (i.e., sample provider(s)) of the specimen(s) been notified of the identification of the select agent or toxin? ☐ No ☐ Yes
    NOTE: Please request completed and signed Part 2 from each facility that was in possession of the specimen(s).

APHIS/CDC Form 4 Scenarios

APHIS/CDC Form 4 Scenario A

Your hospital laboratory received two serum tubes, and a wound swab from a patient that the doctor suspects has botulism. Your laboratory appropriately packages and ships the samples to the State Health Department Laboratory (SHDL). Three days later the SHDL contacts you with a positive identification of Botulinum neurotoxins from the wound swab. Is your hospital laboratory required to submit an APHIS/CDC Form 4 Sections C/D?

A. No, because the State Health Department Laboratory identified the Botulinum neurotoxins
B. Yes, because a select toxin was identified
C. No, because Botulinum neurotoxins is not a select agent if it is from a wound swab
D. Not sure

APHIS/CDC Form 4 Scenario A Response

Your hospital laboratory received two serum tubes, and a wound swab from a patient that the doctor suspects has botulism. Your laboratory appropriately packages and ships the samples to the State Health Department Laboratory (SHDL). Three days later the SHDL contacts you with a positive identification of Botulinum neurotoxins from the wound swab. Is your hospital laboratory required to submit an APHIS/CDC Form 4 Sections C/D?

A. No, because the State Health Department Laboratory identified the Botulinum neurotoxins
B. Yes, because a select toxin was identified
C. No, because Botulinum neurotoxins is not a select agent if it is from a wound swab
D. Not sure

APHIS/CDC Form 4 Scenario B

From Scenario A: Your hospital laboratory received two serum tubes, and a wound swab from a patient that the doctor suspects has botulism. Your laboratory appropriately packages and ships the samples to the State Health Department Laboratory (SHDL). Three days later the SHDL contacts you with a positive identification of Botulinum neurotoxins from the wound swab. What number of samples would you indicate for question D3 on the APHIS/CDC Form 4 Sections C/D?

A. 3, because that is the total number of samples shipped
B. 3, because all the samples came from the same patient
C. 1, because only the wound swab was tested and positive for the select toxin
D. Not sure

APHIS/CDC Form 4 Scenario B Response

From Scenario A: Your hospital laboratory received two serum tubes, and a wound swab from a patient that the doctor suspects has botulism. Your laboratory appropriately packages and ships the samples to the State Health Department Laboratory (SHDL). Three days later the SHDL contacts you with a positive identification of Botulinum neurotoxins from the wound swab. What number of samples would you indicate for question D3 on the APHIS/CDC Form 4 Sections C/D?

A. 3, because that is the total number of samples shipped
B. 3, because all the samples came from the same patient
C. 1, because only the wound swab was tested and positive for the select toxin
D. Not sure

APHIS/CDC Form 4 Scenario C

Your hospital laboratory identifies *Brucella abortus* RB51 in a patient's blood sample. Should your laboratory submit a completed APHIS/CDC Form 4A Sections A/B?

A. Yes, because the identification indicated a 99.9 % accuracy for *Brucella abortus* RB51
B. No, because the remainder of the blood sample was sent to the State Health Department Laboratory for identification confirmation
C. No, because *Brucella abortus* RB51 is an attenuated vaccine strain excluded from the Federal Select Agent Program regulations
D. Not sure

APHIS/CDC Form 4 Scenario C Response

Your hospital laboratory identifies *Brucella abortus* RB51 in a patient's blood sample. Should your laboratory submit a completed APHIS/CDC Form 4A Sections A/B?

A. Yes, because the instrument identification indicated *Brucella abortus* RB51

B. No, because the remainder of the blood sample was sent to the State Health Department Laboratory for identification confirmation

C. No, because *Brucella abortus* RB51 is an attenuated vaccine strain excluded from the Federal Select Agent Program regulations

D. Not sure

www.selectagents.gov

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

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

Recognizing, Identifying, and Reporting the Identification of Select Agents and Toxins

MALDI-TOF

Limitations, Misidentifications and Safety

John R. McQuiston Ph.D.

Special Bacteriology Reference Laboratory

Bacterial Special Pathogens Branch

Division of High Consequence Pathogens and Pathology

Centers for Disease Control and Prevention

Atlanta, GA USA

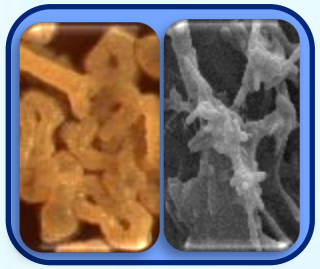
MALDI-TOF

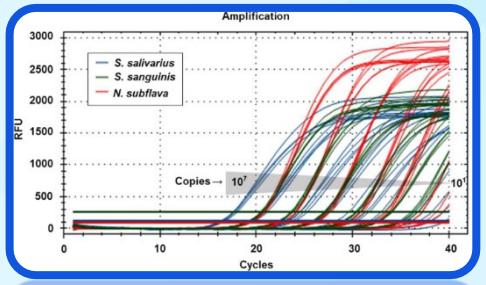
Limitations, Misidentifications and Safety

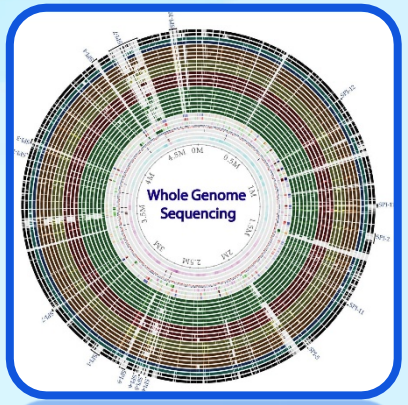
- MALDI-TOF Overview
- Taxonomy- Why does it have to be so confusing?
- Safe handling.
- Database importance
- MicrobeNet overview
- Conclusions

Bacterial Clinical Identification Testing

- Biochemical / Phenotypic Tests – Too slow, too expensive.
- PCR – Too specific
- Genomics – Too slow, too expensive, too much expertise needed (for now)
- MALDI – TOF ….just right

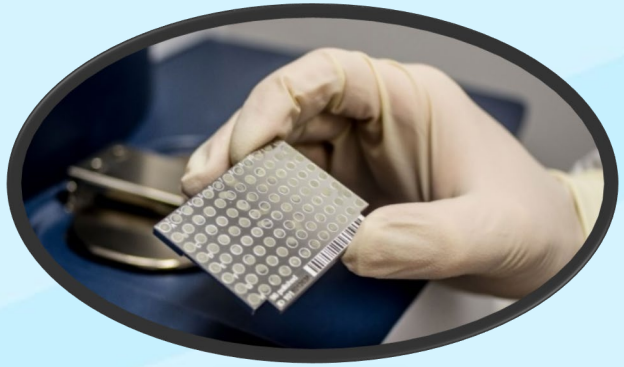






MALDI-TOF

- **MALDI-TOF is one of the fastest, easiest and cheapest 'specimen to result' laboratory tests ever developed for thousands of bacterial and fungal species.**
  - ≤ 10 min per sample prep time (full extraction).
  - Hundreds of specimens per day.
  - Accurate results within minutes.
  - ~$0.50 per isolate (excluding equipment and staff)
  - Accurate at the genus and species level.
    - A few publications on strains of *E.coli*.
    - New IR technology will go to the strain level.
  - Can ID some Antimicrobial Resistance based on markers



MALDI-TOF sample plate

Vitek MS

… it's not perfect.

Bruker MS

MALDI-TOF: “Why can’t I differentiate …?”

Limitations

- Really only accurate to the species level (with a few recent exceptions).
- Results are only as good as your database representation and quality.
- Taxonomists get in the way.

Misidentifications

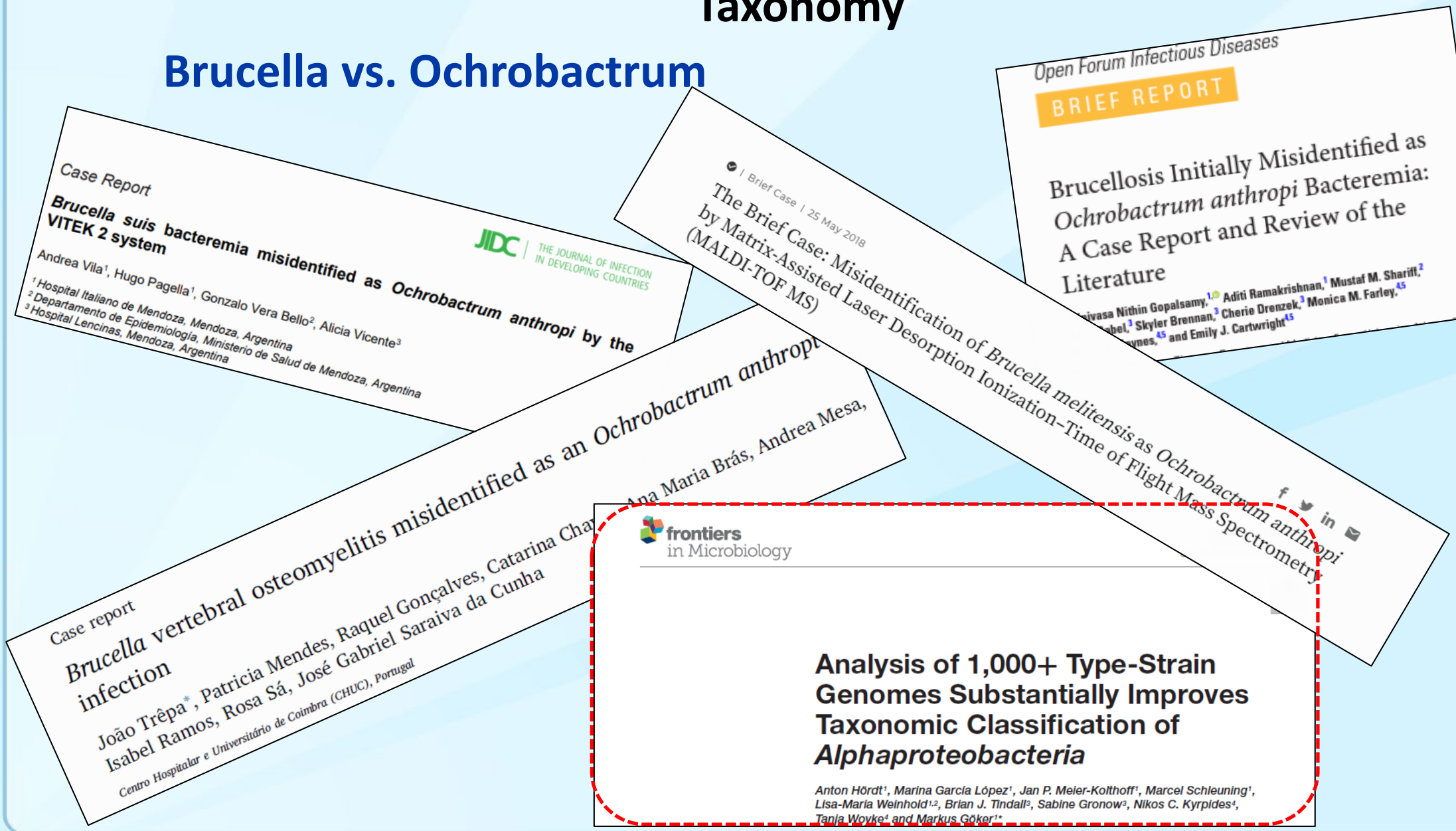
Misidentifications of species on biological tests occur for different reasons:

- Cross reactivity (PCR)
- Contamination (Genomics)
- Lateral gene transfer (Molecular tests)
- Taxonomic nomenclature issues (MALDI)
- Species relatedness (MALDI)

Taxonomy : Species Relatedness

Taxonomy

Brucella vs. Ochrobactrum



Case Report

Brucella suis bacteremia misidentified as Ochrobactrum anthropi by the VITEK 2 system

JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Andrea Vila[1], Hugo Pagella[1], Gonzalo Vera Bello[2], Alicia Vicente[3]

[1] Hospital Italiano de Mendoza, Mendoza, Argentina
[2] Departamento de Epidemiología, Ministerio de Salud de Mendoza, Argentina
[3] Hospital Lencinas, Mendoza, Argentina

Brief Case | 25 May 2018

The Brief Case: Misidentification of *Brucella melitensis* as *Ochrobactrum anthropi* by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS)

Open Forum Infectious Diseases

BRIEF REPORT

Brucellosis Initially Misidentified as *Ochrobactrum anthropi* Bacteremia: A Case Report and Review of the Literature

Srivasa Nithin Gopalsamy,[1] Aditi Ramakrishnan,[1] Mustaf M. Shariff,[2] Isabel,[3] Skyler Brennan,[3] Cherie Drenzek,[3] Monica M. Farley,[4,5] ynes,[4,5] and Emily J. Cartwright[4,5]

Case report

Brucella vertebral osteomyelitis misidentified as an *Ochrobactrum anthropi* infection

João Trêpa*, Patricia Mendes, Raquel Gonçalves, Catarina Cha... Ana Maria Brás, Andrea Mesa, Isabel Ramos, Rosa Sá, José Gabriel Saraiva da Cunha

Centro Hospitalar e Universitário de Coimbra (CHUC), Portugal

frontiers in Microbiology

Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of *Alphaproteobacteria*

Anton Hördt[1], Marina García López[1], Jan P. Meier-Kolthoff[1], Marcel Schleuning[1], Lisa-Maria Weinhold[1,2], Brian J. Tindall[3], Sabine Gronow[3], Nikos C. Kyrpides[4], Tanja Woyke[4] and Markus Göker[1]

Taxonomy

Brucella vs. *Ochrobactrum*

[Brucella and Ochrobactrum Taxonomic Updates for Laboratories | ASM.org]

AMERICAN SOCIETY FOR MICROBIOLOGY

Brucella and *Ochrobactrum* Taxonomic Updates for Laboratories

Frequently Asked Questions (FAQ) for Clinical Laboratories

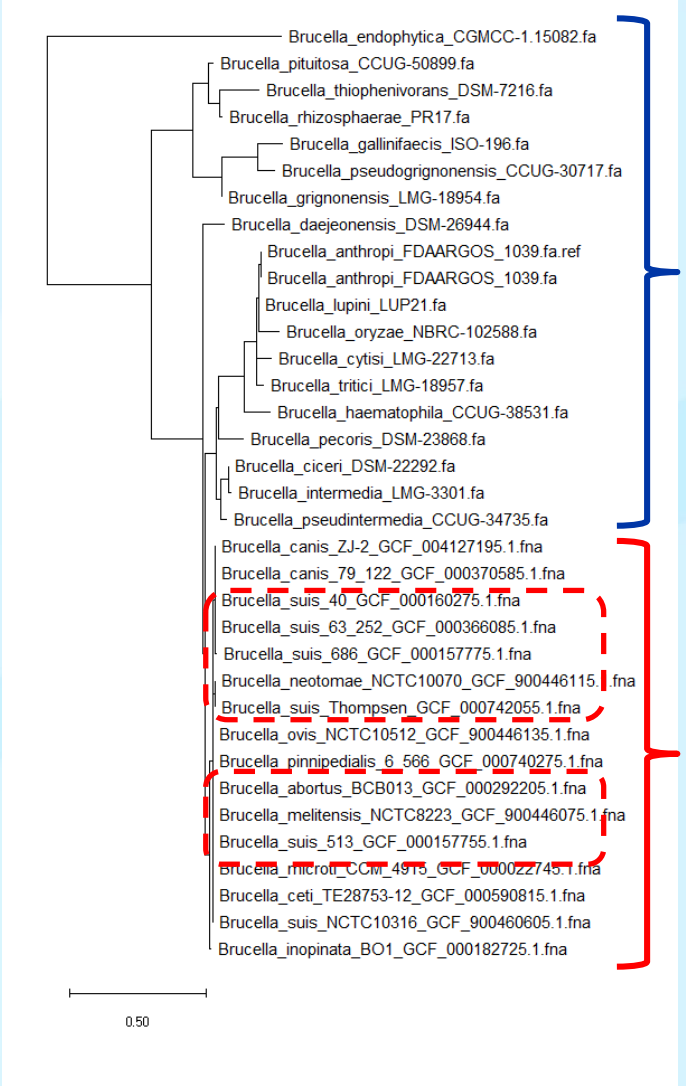
Authors: Rosemary She, Carrie Anglewicz, Kurt Jerke, Ryan Relich, Mark Glazier, Laura Filkins*, Audrey Schuetz*

**Co-corresponding authors*

On behalf of the American Society for Microbiology Clinical and Public Health Microbiology Committee, Laboratory Practices Subcommittee

Background

In 2020, Hördt et al. proposed the reclassification of *Ochrobactrum* species to the genus *Brucella* based on recent gene-content analysis studies (1). The past taxonomic distinction between *Ochrobactrum* and *Bru-*



Taxonomy

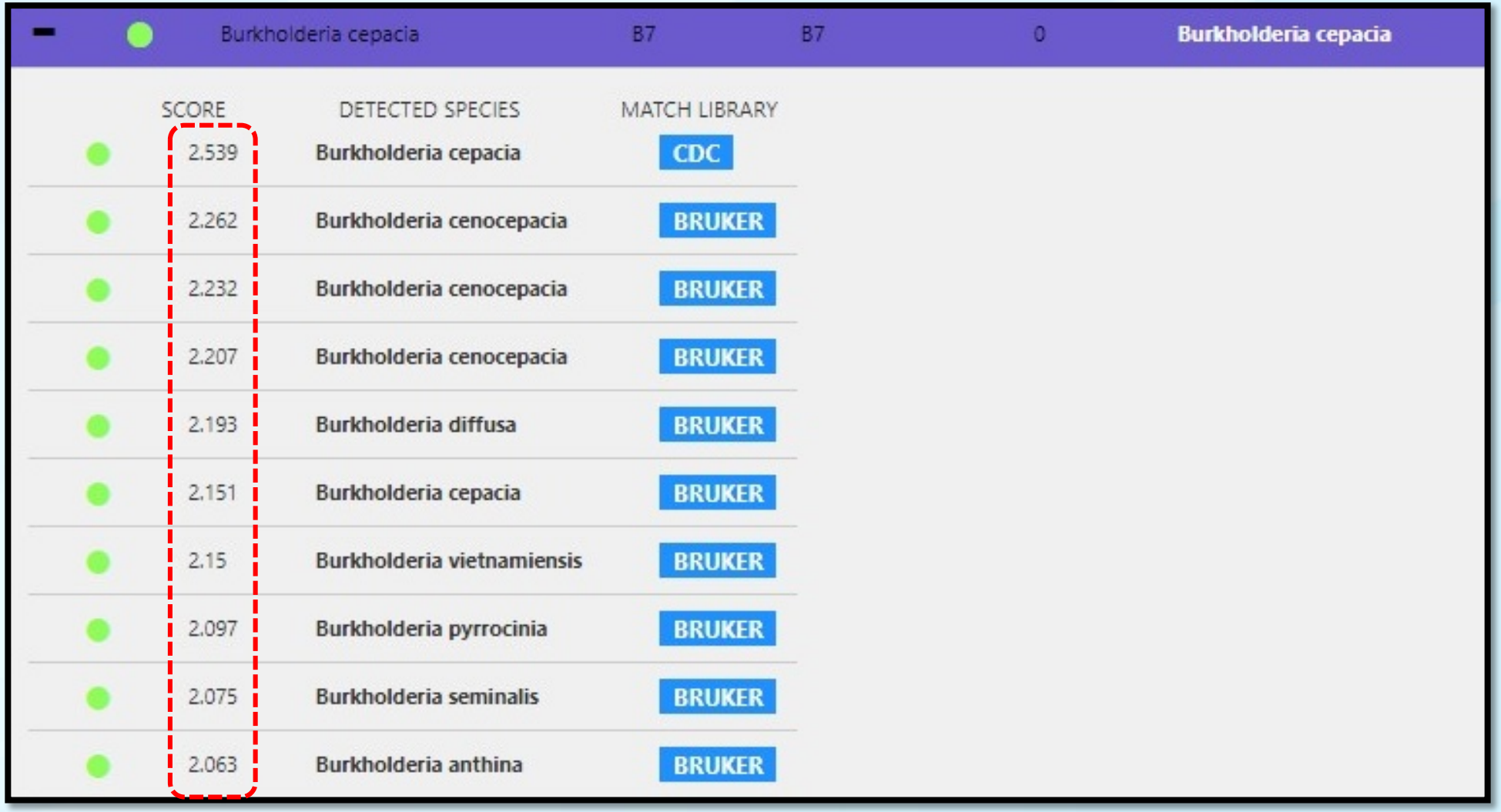
…even within *Ochrobactrum/Brucella*

Brucella lupini

According to Gazolla-Volpiano et al. (2019), this species is a later heterotypic synonym of *Ochrobactrum anthropi* Holmes *et al.* 1988. **Publication:** Gazolla Volpiano C, Hayashi Sant'Anna F, Ambrosini A, Brito Lisboa B, Kayser Vargas L, Passaglia LMP. Reclassification of *Ochrobactrum lupini* as a later heterotypic synonym of *Ochrobactrum anthropi* based on whole-genome sequence analysis. *Int J Syst Evol Microbiol* 2019; **69**:2312-2314.

According to Hoerdt et al. (2020), this species is not a later heterotypic synonym of *Brucella anthropi* (Holmes *et al.* 1988) Hördt *et al.* 2020. **Publication:** Hordt A, Lopez MG, Meier-Kolthoff JP, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, Goker M. Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of *Alphaproteobacteria*. *Front Microbiol* 2020; **11**:468.

Species Relatedness: the Burks



Solutions

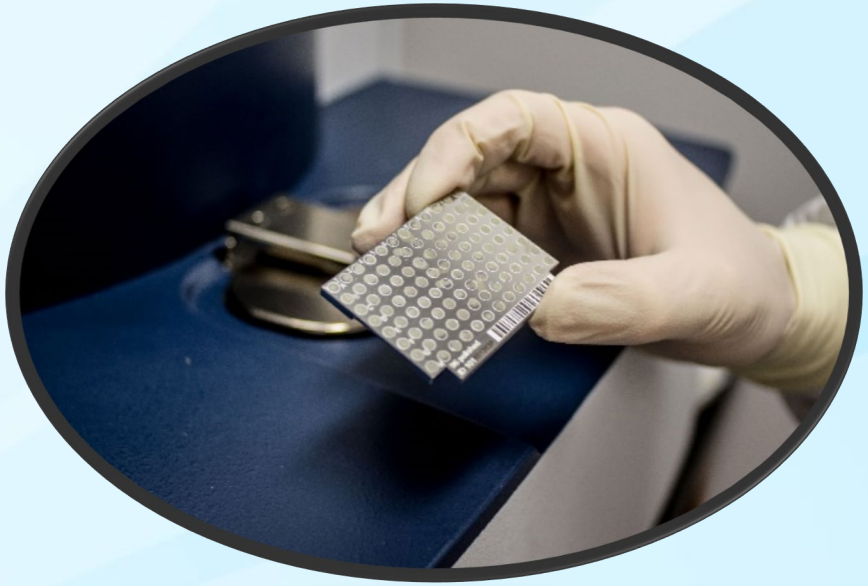
What is the MALDI misidentification solution?

1. Check that your database is up to date and includes representatives of Select Agents.
2. *Be wary* when two or more species are above a 2.0 score in MALDI-TOF results and within 10%.
3. Be aware of the taxonomic synonyms of the bacterial species.
   - Check with LRN, LPSN, SPHL, CDC or MicrobeNet (MicrobeNet@cdc.gov)
4. Use MicrobeNet for Bruker MALDI as it is kept up to date, combined with Bruker releases and contains SA's.
5. ***Look out for your own safety!***

***LPSN - List of Prokaryotic names with Standing in Nomenclature**
https://www.bacterio.net/

MALDI-TOF and Safety

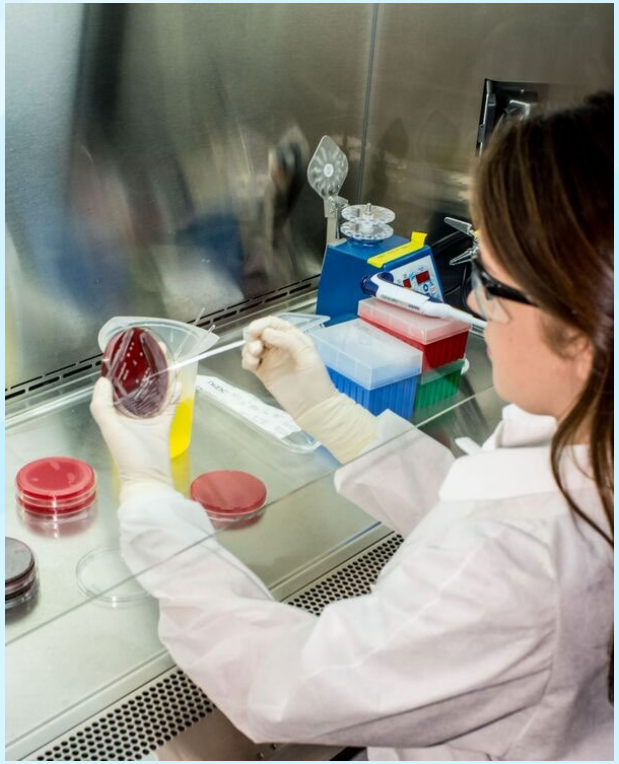
The biggest risk of MALDI-TOF is open culture exposure of an unknown.



MALDI-TOF and Safety

- Treat all isolates as unknowns until identified.
- In BSPB bacterial culture work is performed in a ***BSC with PPE***.
- Think of BSC space as bench space.

"Full tube extraction (10 min) is the best proven method for safety" (Rudrick et.al 2017)



MALDI-TOF and Safety

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

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

James T. Rudrik,[a] Marty K. Soehnlen,[a] Michael J. Perry,[b] Maureen M. Sullivan,[c] Wanda Reiter-Kintz,[d] Philip A. Lee,[e] Denise Pettit,[f] Anthony Tran,[g] Erin Swaney[h]
Bureau of Laboratories, Michigan Department of Health and Human Services, Lansing, Michigan, USA[a]; Biodefense Laboratory, Wadsworth Center, New York State Department of Health, Albany, New York, USA[b]

[Safety and Accuracy of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry for Identification of Highly Pathogenic Organisms - PubMed (nih.gov)]

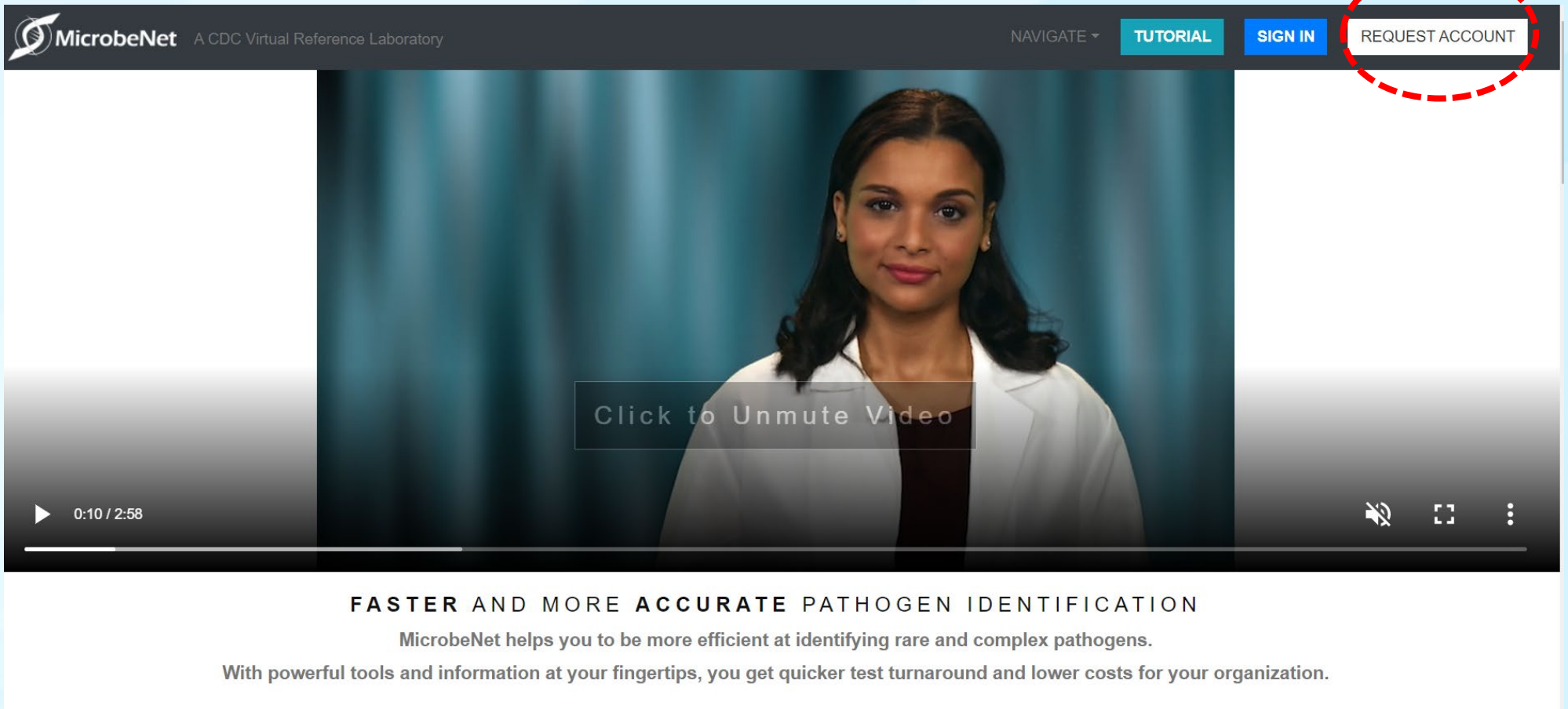
TABLE 1 Viability of BT agents following MALDI-TOF sample preparation

Organism(s)	No. of tubes with growth using indicated sample preparation method/no. tested								
	Direct colony			On-plate formic acid			Tube extraction		
	Target	Spot + Matrix	Spot	Target	Spot + Matrix	Spot	Target	Spot + Matrix	Spot
Bacillus anthracis	3/5	5/5	5/5	1/5	5/5	5/5	0/5	1/5	5/5
Burkholderia thailandensis	0/5	5/5	5/5	0/5	5/5	5/5	0/5	0/5	5/5
Clostridium botulinum/Clostridium perfringens	1/5	1/5	3/5	1/5	0/5	2/5	0/5	1/5	4/5
Francisella tularensis	1/5	2/5	4/5	1/5	2/5	5/5	0/5	1/5	5/5
Yersinia pestis	0/4	3/4	4/4	1/4	4/4	4/4	0/4	0/4	3/4
Brucella abortus	0/4	3/4	4/4	1/4	4/4	4/4	0/4	0/4	3/4
Total	5/28	19/28	25/28	5/28	20/28	25/28	0/28	3/28	25/28

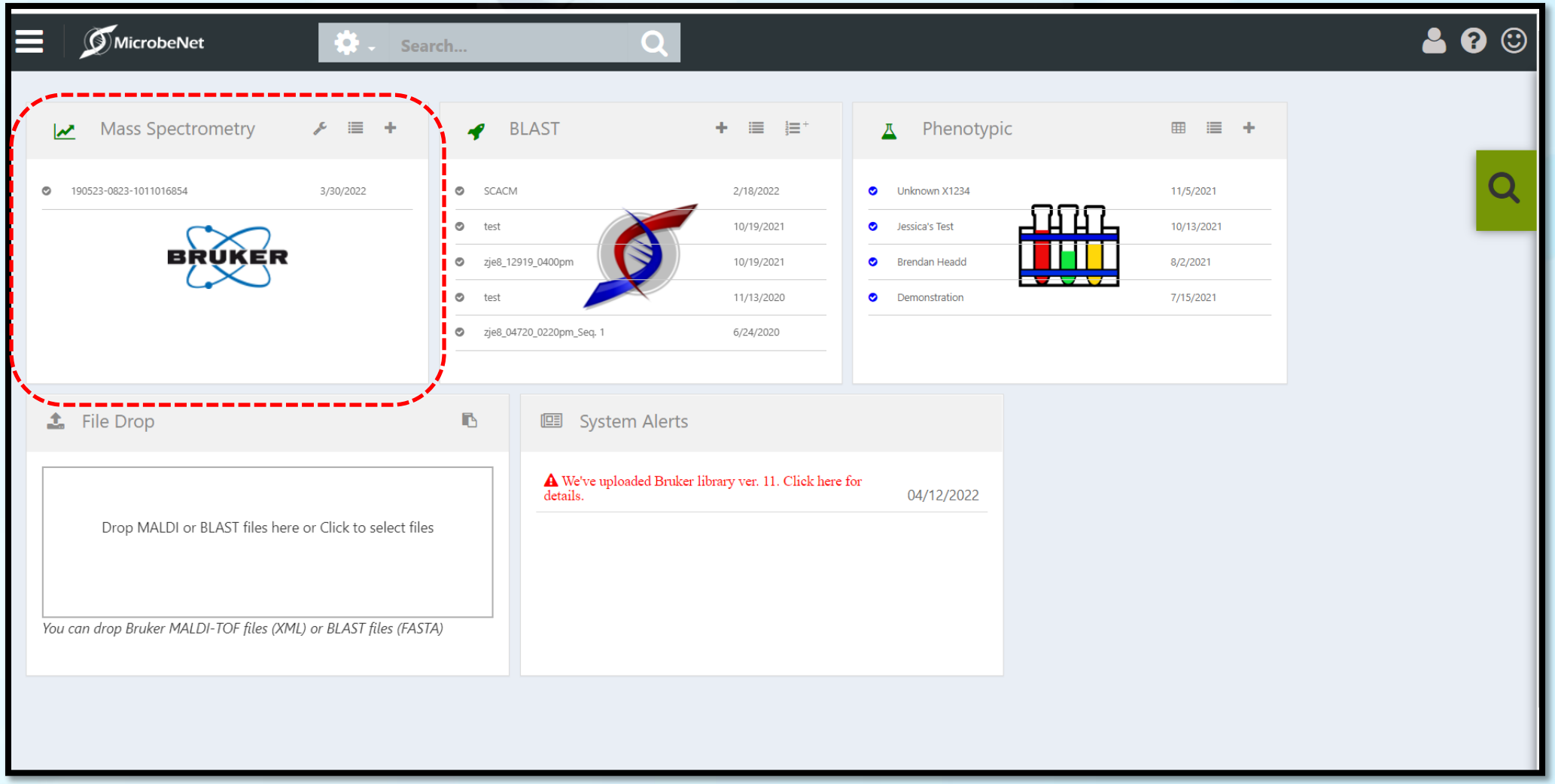
Databases

- **Quality and curation of databases**
  - Keep databases up to date.
  - Check for accurate taxonomic curation of strains added to the database
- **Taxonomic confusion misleading the user.**
  - Former *Ochrobactrums* are now identified as: *i.e.* "*Brucella anthropi* (form. *Ochrobactrum*)" in many commercial databases.
- **Representation**
  - Misidentifications if the correct species data is NOT in the database
    - Low scores, make sure scores are acceptable to the species level.
    - Don't just pick the top result.

https://microbenet.cdc.gov/



MicrobeNet™


MicrobeNet
Search...
Mass Spectrometry
190523-0823-1011016854 3/30/2022
BRUKER
BLAST
SCACM 2/18/2022
test 10/19/2021
zje8_12919_0400pm 10/19/2021
test 11/13/2020
zje8_04720_0220pm_Seq. 1 6/24/2020
Phenotypic
Unknown X1234 11/5/2021
Jessica's Test 10/13/2021
Brendan Headd 8/2/2021
Demonstration 7/15/2021
File Drop
Drop MALDI or BLAST files here or Click to select files
You can drop Bruker MALDI-TOF files (XML) or BLAST files (FASTA)
System Alerts
We've uploaded Bruker library ver. 11. Click here for details. 04/12/2022

Bacterial Identification by MALDI-TOF

Classification Result

cbdd0f1b-631c-4384-a7c8-33e3135a11be

Created: 3/30/2022 11:53:56 AM
By: John McQuiston
Libraries Selected:

- MICROBENET - 2021.1.0 (v 2021.1.0 published 2/11/2021, MSP Count: 11366)
- ARGENTINA (v 1 published 6/23/2020, MSP Count: 36)

Meaning of Sco

Range
2.00 - 3.00
1.70 - 1.99
0.00 - 1.69

Showing 1 to 4 of 4 entries CSV

		ID	NAME	POSITION	CHIP	DETECTED SPE
−	●	19-112A	A5	A5	0	Elizabethkingia miricola

SCORE	DETECTED SPECIES	MATCH LIBRARY
2.267	Elizabethkingia miricola	CDC
2.23	Elizabethkingia miricola	CDC
2.21	Elizabethkingia miricola	CDC
2.162	Elizabethkingia miricola	BRUKER
2.153	Elizabethkingia miricola	BRUKER
2.133	Elizabethkingia miricola	CDC
2.085	Elizabethkingia miricola	BRUKER
2.057	Elizabethkingia miricola	CDC
2.053	Elizabethkingia miricola	BRUKER
2.051	Elizabethkingia miricola	BRUKER

+	●	19-112A	A6	A6	0	Elizabethkingia miricola
+	●	19-112B	A7	A7	0	Staphylococcus hominis

MicrobeNet Search...

Elizabethkingia

Type Species: ***Elizabethkingia meningoseptica***

GenusOverview

On most commercially-available media, colonies are white or very pale yellow. Cells are rod shaped and Gram-staining-negative. Strains grow optimally at 25-37°C, pH 7.0, and aerobically. Most of the species are halotolerant. Menaquinone-6 is the only or major respiratory quinone. Species from this genus are inherently resistant to many antibiotics, and the type strains from most species were isolated from human clinical specimens, and are often found in hospital environments, particularly sinks. Certain strains are the causative agent of a contagious disease among farmed frogs. *Elizabethkingia* isolates have been derived from a variety of animals, including insects, horses, and fish. DNA G+C content (mol%): 35.0-38.2 (HPLC). The range calculated from whole genome sequence (WGS) data suggests a narrower range, from 35.5 to 36.5%. The type species is *Elizabethkingia meningoseptica*.

Contact Information:

Species Contact Info:
The Special Bacteriology Reference Lab (SBRL/BSPB)
SBRL@cdc.gov
(404) 639-0270

a
m
miricola

Elizabethkingia miricola

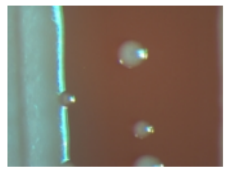
Taxonomy /Strain

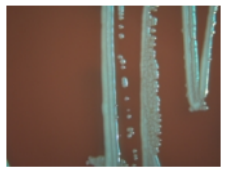
Strains in MicrobeNet:
DSM14571

Overview

Rods, approximately 0.5 µm in diameter and 1.0–2.5 µm in length. Good growth occurs on tryptic soy, blood, BHI, and nutrient agars. Very slow growth occurs on cetrimide agar. Growth occurs at 22–37°C but not at 5 or 42°C. Colonies are white–yellow, circular, entire, smooth, and very sticky on solid media. Oxidase- and catalase-positive. Indole and β-galactosidase are produced. Urea is hydrolyzed, and gelatinase activity is strain dependent. 2-Naphthyl butyrate is hydrolyzed in the API ZYM gallery. The type strain forms a precipitate when grown on egg yolk agar. D-Mannitol, sodium citrate, and N-acetyl-D-glucosamine are assimilated, but tyrosine is not degraded. Source: The type strain was isolated from condensation water in the space station Mir; a second strain was recently identified from the contaminated commercial preparation of an enzyme. DNA G +C content (mol%): 35.3±0.3 (type strain: 35.0) (HPLC). 35.9 (WGS). Type strain: : W3-B1, DSM 14571, JCM 11413, GTC 862.

Images


SBRL


SBRL


SBRL

Conclusions

1. *Your safety must come first!*
2. Work in a BSC – think of it as more bench space and they are 1/25th the cost of the MALDI-TOF.
    - And much less than a lab shutdown or staff comp time.
3. Do full extractions! (c'mon it's only 10 min)
4. Taxonomy will always be a challenge and you need to be aware that something could be a select agent.
5. When two species results are ≥ 2.0, this should be a alert of a possible taxonomy issue.
6. When in doubt, use MicrobeNet!!
7. If you have questions ask! MicrobeNet@cdc.gov. (or microbenetlatam@anlis.gob.ar for Latin America)

Acknowledgements

CDC Special Bacteriology Reference Laboratory

Melissa Bell
Ainsley Nicholson
Ben Humrighouse
Adam Szewc
Chris Gulvik
Alli Gombolay
Joe Rehfus

MicrobeNet

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Reliant Global Technologies

ANLIS Argentina (Coordinating Center for MicrobeNet Latin America)

Dr. Monica Prieto
Florencia Rocca
Dra. Claudia Perandones
Dr. Carlos Vay

Bruker Daltonics

Markus Kostrzewa
Karl Otto Kraeuter
Justin Clark
Gongyi Shi

BRUKER

The.Yeasts

ThermoFisher SCIENTIFIC

Thank you!

Please contact us at:

MicrobeNet@cdc.gov

or

https://microbenet.cdc.gov/

Questions?

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May 31, 2023

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   - Register for the course in TRAIN
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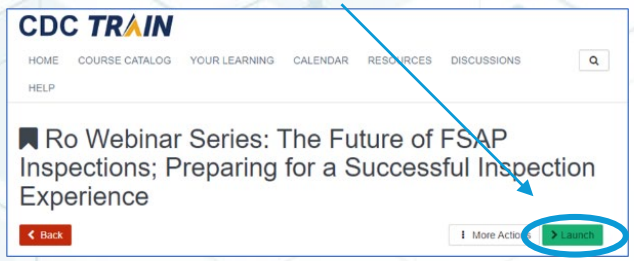
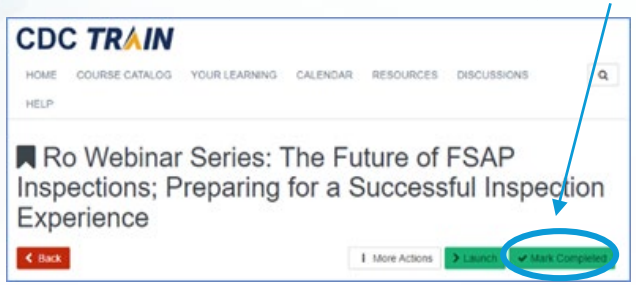
   - Select **"PACE"** credit type

   - Click "**Launch**"



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



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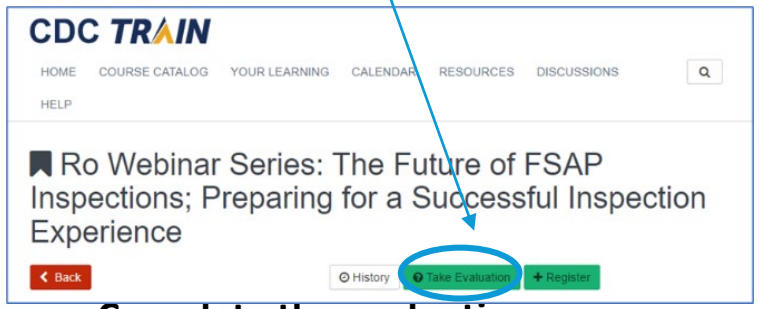
   - Click green **"Take Evaluation"** button



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