Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

The Importance of Critical Thinking in Organism Identification Using Current Technology

Alana Sterkel, PhD, D(ABMM), SM(ASCP)^{CM}
Associate Director
Communicable Disease Division, WSLH
Assistant Professor
Path. And Lab Medicine, UW-Madison

Clicker Warm-Up

- Turn the power on
- Wait for the timer to start
- Select your answers promptly
- All answers are anonymous

Test Question

Which of these best describes you

A) Laboratorian

B) Administrator

C) Infection Preventionist

D) Public Health Department Member

E) Other

Case 1

- Linda the laboratorian is working on Blood cultures Tuesday afternoon. She notices tiny pinpoint colonies growing on the blood agar plate.

A) Send it to MALDI

B) Gather more information

C) Put it back in the incubator to wait for more growth

Case 1

- Linda does not detect any hemolysis.
- There are similar tiny colonies on the chocolate agar and no growth on MacConkey agar.

A) Send it to MALDI

B) Gather more information

C) Put it back in the incubator to wait for more growth

Case 1

- Linda reviews the gram stain and sees that it was reported as small, faintly staining gram positive rods.
- The culture showed no growth yesterday and was put back into the incubator. It's now 48 hours out of the bottle.

A) Send it to MALDI

B) Gather more information

C) Put it back in the incubator to wait for more growth

Case 1

- Since the gram stain was positive she decides to run the MALDI.
- The VITEK MALDI returns the result of *Ochrobactrum anthropi*

A) Report as *O. anthropi*

B) Gather more information

C) Ask for help

Stop and ask “Does this make sense?”

What fits?

- There are case reports of blood stream infections with *O. anthropi*

What doesn't fit?

- *O. anthropi* is a gram negative coccobacillus
- *O. anthropi* grows on MacConkey

What else could it be?

What additional information could you seek?

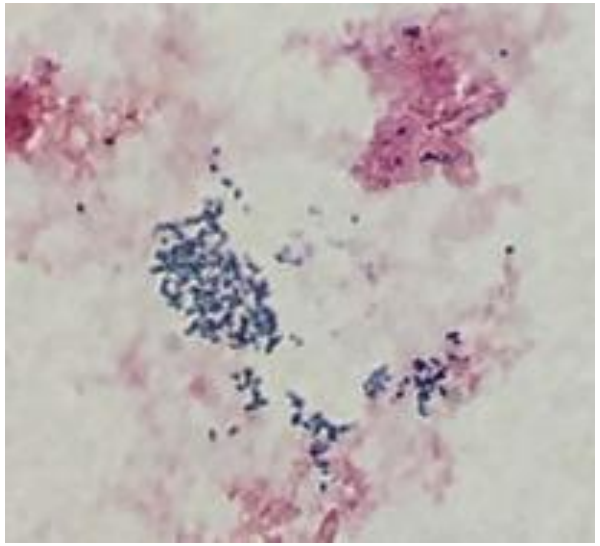
- Biochemical testing doesn't distinguish the two

	Catalase	Oxidase	Urease	EMB/MaC
Brucella	+	+	+	-
O. anthropi	+	+	+	+

- Cases of *O. anthropi* blood stream infections are very rare and only in immune compromised people. Check the patient's chart.
  - Immune competent, History of feral hog hunting
- Brucella antibody testing works
- 16S sequencing can distinguish these, but this could be dangerous if it is *Brucella*. (https://pubmed.ncbi.nlm.nih.gov/18222618/)
- An LRN lab can tell them apart! We use a *Brucella* specific PCR in a BSL3 lab.

What should have triggered different action?

- *Brucella* can sometimes appear gram positive.



- Slow growing coccobacillus, No growth on MacConkey, biochemicals (Cat +, Ox +, Urea +)
  - If you can't rule out a Select Agent, send it to an LRN lab!

This has happened before

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

Srinivasa Nithin Gopalsamy ✉, Aditi Ramakrishnan, Mustaf M Shariff, Julie Gabel, Skyler Brennan, Cherie Drenzek, Monica M Farley, Robert P Gaynes, Emily J Cartwright

Open Forum Infectious Diseases, Volume 8, Issue 10, October 2021, ofab473,
https://doi.org/10.1093/ofid/ofab473
Published: 01 October 2021 **Article history** ▾

Table 1. Cases of brucellosis initially misdiagnosed as *Ochrobactrum anthropi* infection

Case	*O. anthropi* Identification Method	*Brucella* Antibody	AgglutinationTest	PCR
Elsaghir et al., 2003 [28]	API 20NE	Positive	Positive	*B. melitensis*
Horvat et al., 2011 [7]	RapID NF Plus			*B. suis*
Carrington et al., 2012 [8]	RapID NF Plus VITEK 2	Positive		*B. suis*
Vila et al., 2016 [29]	VITEK 2	Positive	Positive	*B. suis*
Trêpa et al, 2018 [30]	VITEK MS		Positive	*B. melitensis*
Poonawala et al., 2018 [31]	VITEK MS	Positive		*B. melitensis*
Khaliulina Ushakova et al., 2020 [32]	MALDI-TOF MS Bruker[a]		Positive	
Current case	VITEK 2	Positive	Positive	*B. suis*

Identification method refers to the modality used that initially identified the isolate as *O. anthropi*. Agglutination test includes serum agglutination test (including tube, plate, Rose-Bengal and Wright tests) and *Brucella* microagglutination test. PCR testing includes 16S rRNA sequencing.

Abbreviation: PCR, polymerase chain reaction.

[a]Later changed to *B. melitensis*

https://academic.oup.com/ofid/article/8/10/ofab473/6378660

Consequences

***A delayed or missed diagnosis can lead to increased morbidity or mortality for our patients and increased risks to laboratorians**

Emerg Infect Dis. 2004 Oct; 10(10): 1848–1850.
doi: 10.3201/eid1010.040076

PMCID: PMC3323255
PMID: 15504276

Laboratory-acquired Brucellosis

Stephanie Noviello,[✉*†] Richard Gallo,[*] Molly Kelly,[*] Ronald J. Limberger,[*] Karen DeAngelis,[‡] Louise Cain,[‡] Barbara Wallace,[*] and Nellie Dumas[*]

Keep Learning, Stay Informed

- All *Ochrobactrum* species were recently reclassified into the *Brucella* genus to align taxonomical nomenclature with phylogenetic analyses.
  - CDC LOCS message 12/19/22**:** https://www.cdc.gov/locs/2022/12-19-2022-Lab-Update-Reclassification_Ochrobactrum_species_Brucella_genus.html
  - Review of journals:
    - July 2020- List of new names and new combinations previously effectively, but not validly, published | Microbiology Society (microbiologyresearch.org)
    - April 2020- Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of *Alphaproteobacteria* – PubMed (nih.gov)
  - WCLN Lab messages: Jan. 10, 2023
  - WCLN Conferences and Webinars

What this means

- **The big change- *Brucella* is no longer a pure select agent genus**.

- Once software is updated with this name change automated instrument results of *Brucella* should still trigger select agent rule out procedures.

Case 1 - Summary

- The evolving nature of science will result in gaps between agreed upon naming conventions and electronic databases.
- It is important to be aware of instrument limitations and to question results that don’t make sense.
- A strong educational base, career experience, and lifelong learning provide the tools needed for effective critical thinking.

Critical Thinking

- *Def*: The objective analysis and evaluation of an issue in order to form a judgment.

- We use it all the time
  - Crossing the street
  - Cooking a new recipe
- If you ever think “This doesn’t seem right” investigate further!
- Ultimate goal- accurate testing!

Case 2

- Linda the laboratorian is back from a spring break vacation and is getting ready to report out gonorrhea results from an automated instrument.
- She sees that 10 of the 50 (20%) results from today's run are positive.

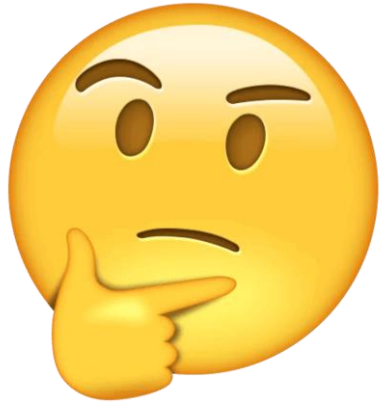
A) Report results

B) Gather more information

C) Ask for help

Case 2

- The number of positives seems abnormally high to Linda. She normally only sees 1-3 positives a day
- She knows that most of their testing comes from low risk asymptomatic screening in a clinic setting.



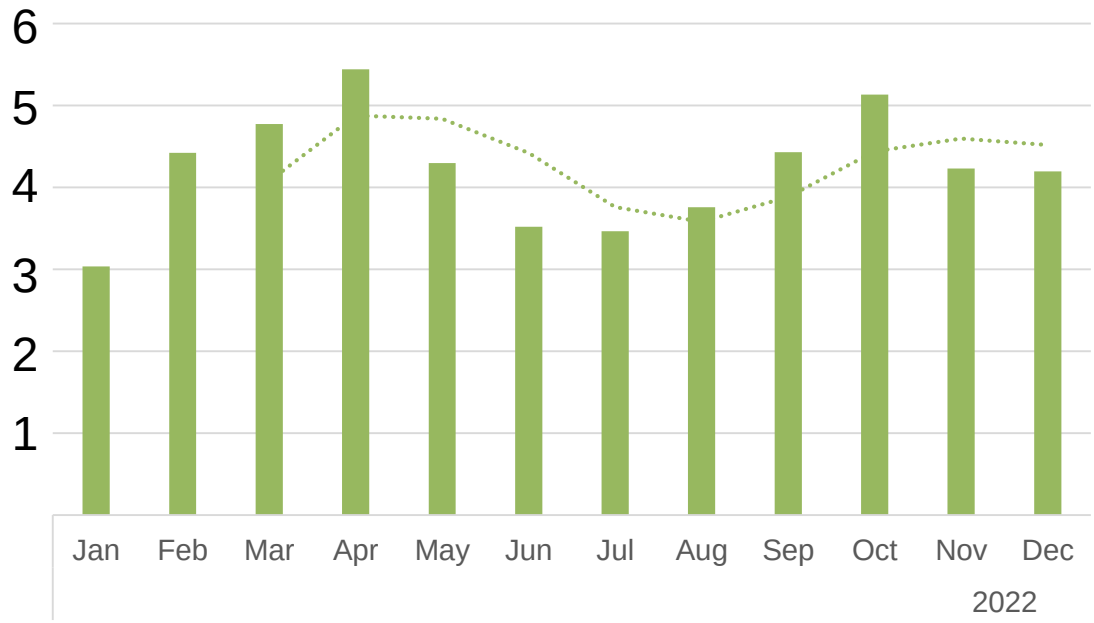
A) Report results

B) Gather more information

C) Ask for help

Case 2

- Linda decides to review the average percent positivity of this test and sees the rate was 3.8% last year.
- However, the lab routinely sees higher rates after spring break.



A) Report results

B) Gather more information

C) Ask for help

Case 2

- Linda tells her colleague Larry that she has a really high positivity in this run. Larry agrees that it is very high and mentions that they had a large influx of specimens from a post spring break university testing event.

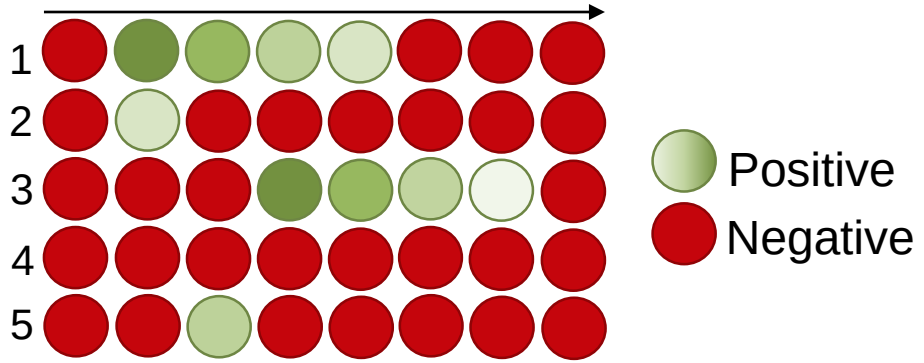
A) Report results

B) Gather more information

C) Ask for help

Case 2

- The rate still seems unusually high even for a higher risk patient population. Linda decides to review the pattern of positive samples in the run.



A) Report results

B) Gather more information

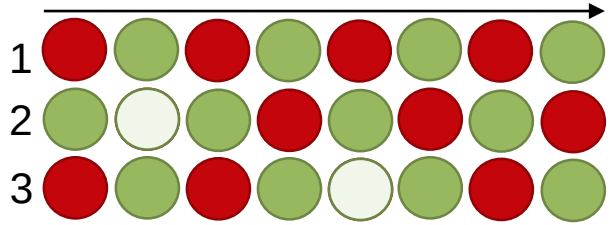
C) Ask for help

Case 2

- Linda decides the high positivity rate and pattern is suspicious for contamination and does not report the results.
- She shares her suspicions with the Lab Manager and Director.

Case 2- Follow-up

- Testing is halted during the investigation
- Wipe testing is performed and identifies contamination in the instrument.
- The Director asks for a checkerboard test to be performed and contamination is observed.



- Maintenance is requested and identifies an alignment issue that resulted in carry over and splashing within the instrument.

Case 2 - Conclusion

- After repairs are completed and the instrument in cleaned the wipe test is repeated and tests negative.
- The checkerboard test is repeated and passes.
- Past results are reviewed to see if the error impacted any other patients and suspicious patterns are seen in 3 other runs.
- All positive specimens from the impacted runs are repeated and corrected reports issued if needed.
- If specimens are no longer available for re-testing clinicians are contacted to explain the issue and recommend re-testing if appropriate.
- Thanks to Linda's critical thinking several patients did not receive false positive gonorrhea results!

Case 2 - Summary

- Instruments can fail
- Know your patient population
- Be aware of seasonal trends and positivity
- Investigate when results are outside of normal ranges
- Know how to recognize patterns of contamination
- The experience and expertise of your colleagues can help you troubleshoot during an investigation.

Summary

- Critical thinking is necessary for accurate testing
- Be informed- conferences, webinars, patient populations, test limitations
- Don’t go on autopilot thinking instrumentation is fool proof
- Stop and ask “Does this make sense?”
- Trust your instincts