Is the Future of Phenotypic-based Identification of Biothreat Agents in Jeopardy of the Rule Out or Refer Practice?

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OBJECTIVES

- Identify three reasons that contributed to decline in utilization of the Sentinel Level Protocols in support of the Rule Out/Refer Concept
- What is the cost comparison between conventional rule out tests versus newer technologies?
- Identify the primary training resource that nonpublic health clinical laboratories rely on for training related to biothreat and emerging infectious disease agents

Contributing Factors to the Decline in the use of LRN Sentinel Level Protocols

- **New Technology and Personnel Shortages**

  - Mass Spectrometry (MALDI-TOF)

  - Some media not being retained in laboratory inventory (e.g. Urea, Motility)

  - Lack of well trained, skilled laboratory scientists

    “the dumbing down process”

Shortage of Trained Personnel

Administrators Belief that Automation is the Final Solution

- Aging (Avg. age: 56 years)
- Clinical Laboratory Science Programs:
- **not teaching basic concepts/fundamentals**
  - clinical laboratory rotation relied upon to prepare students for microbiology
  - students not exposed to organism culture characteristics and Gram stain morphology
  - "you will have an automated or conventional system making the ID for you"
  - accepting the ID as 100% correct without question
  - little or no training of Biothreat Agents
  - 18 months or more of training to work independently

Media and Reagents Required in Conventional Rule Out/Refer Protocols

Media

- Blood, MacConkey, Chocolate, Urea, Motility

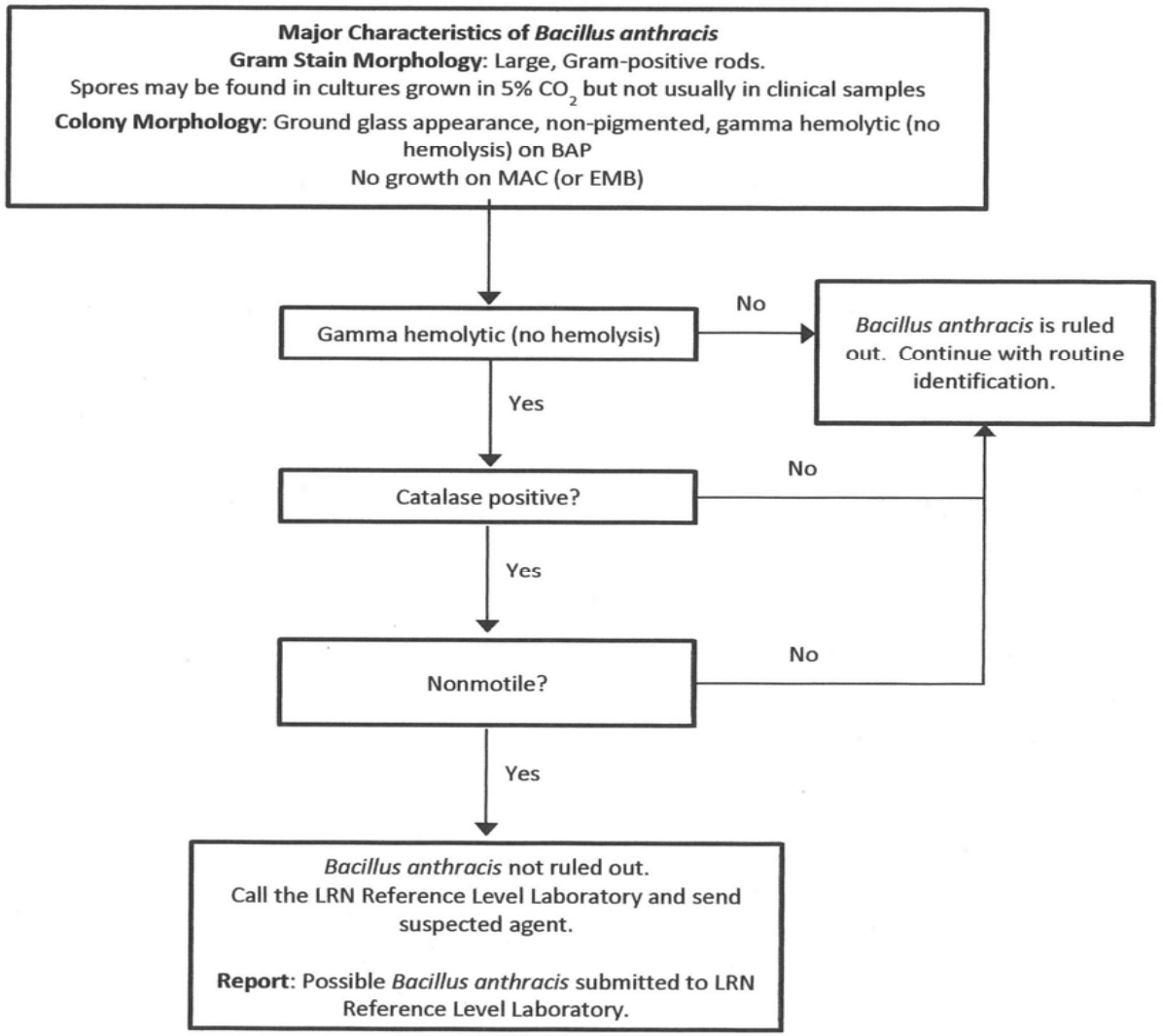
Reagents

- Gram stain, Catalase, Oxidase, Indole, Cefinase, *Stapylococcus aureus* ATCC 25923

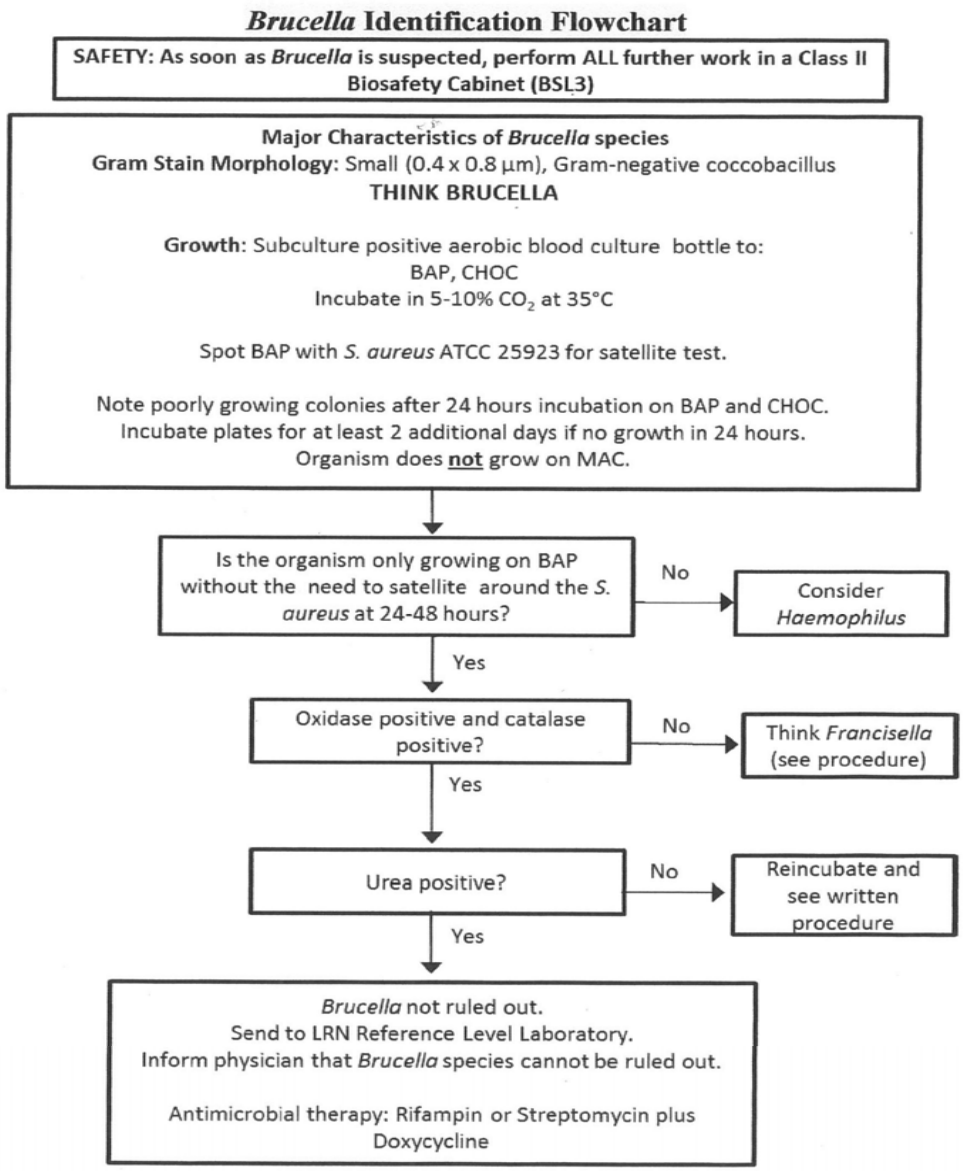
Antibiotic Disks

- Colistin, Amoxicillin/Clavulanate, Penicillin

Bacillus anthracis Identification Flowchart



Note: Biochemical test procedures and quality control instructions can be found at the end of the General Recommendation and Biochemical Testing Procedures document.



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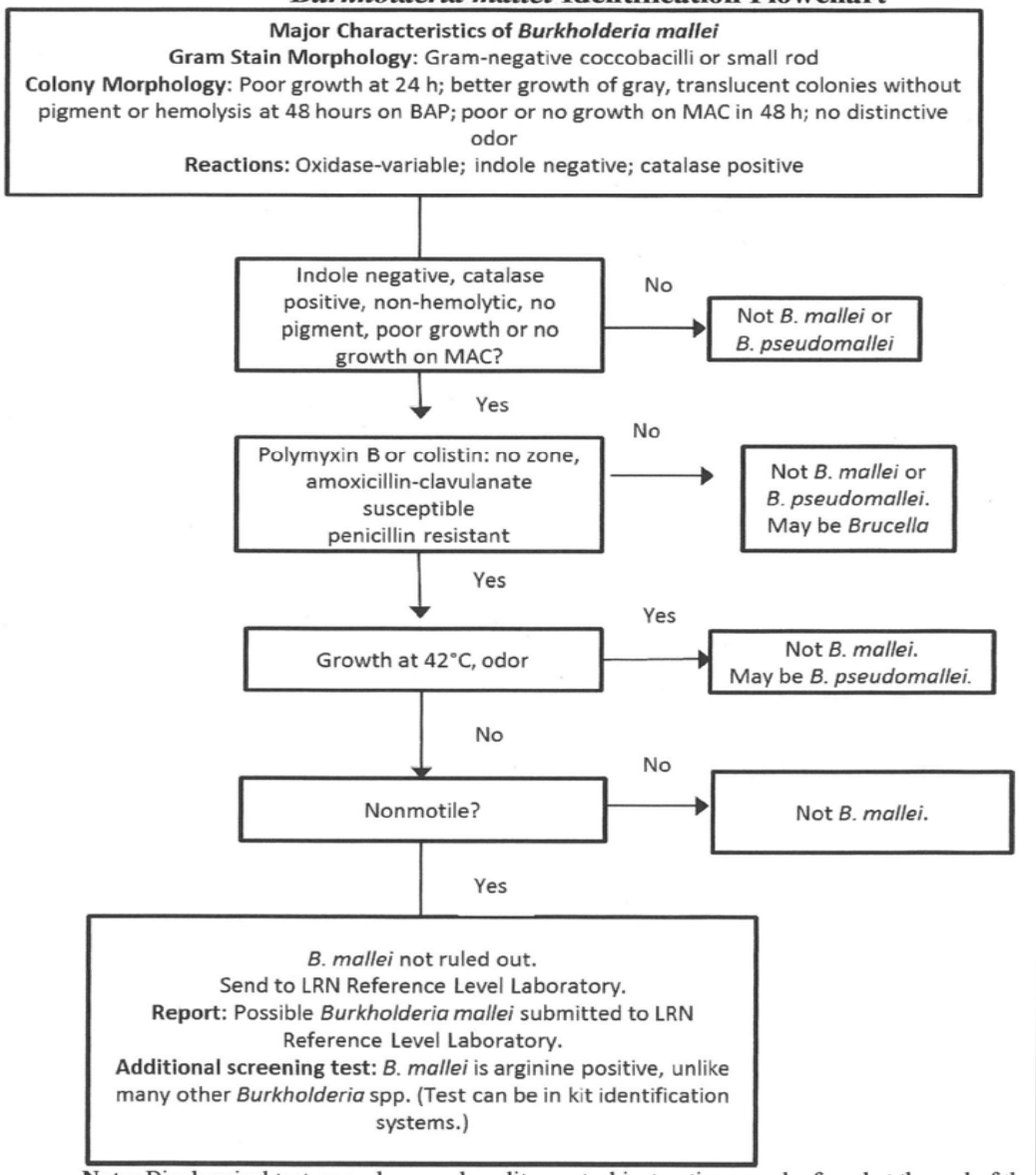
Burkholderia mallei Identification Flowchart



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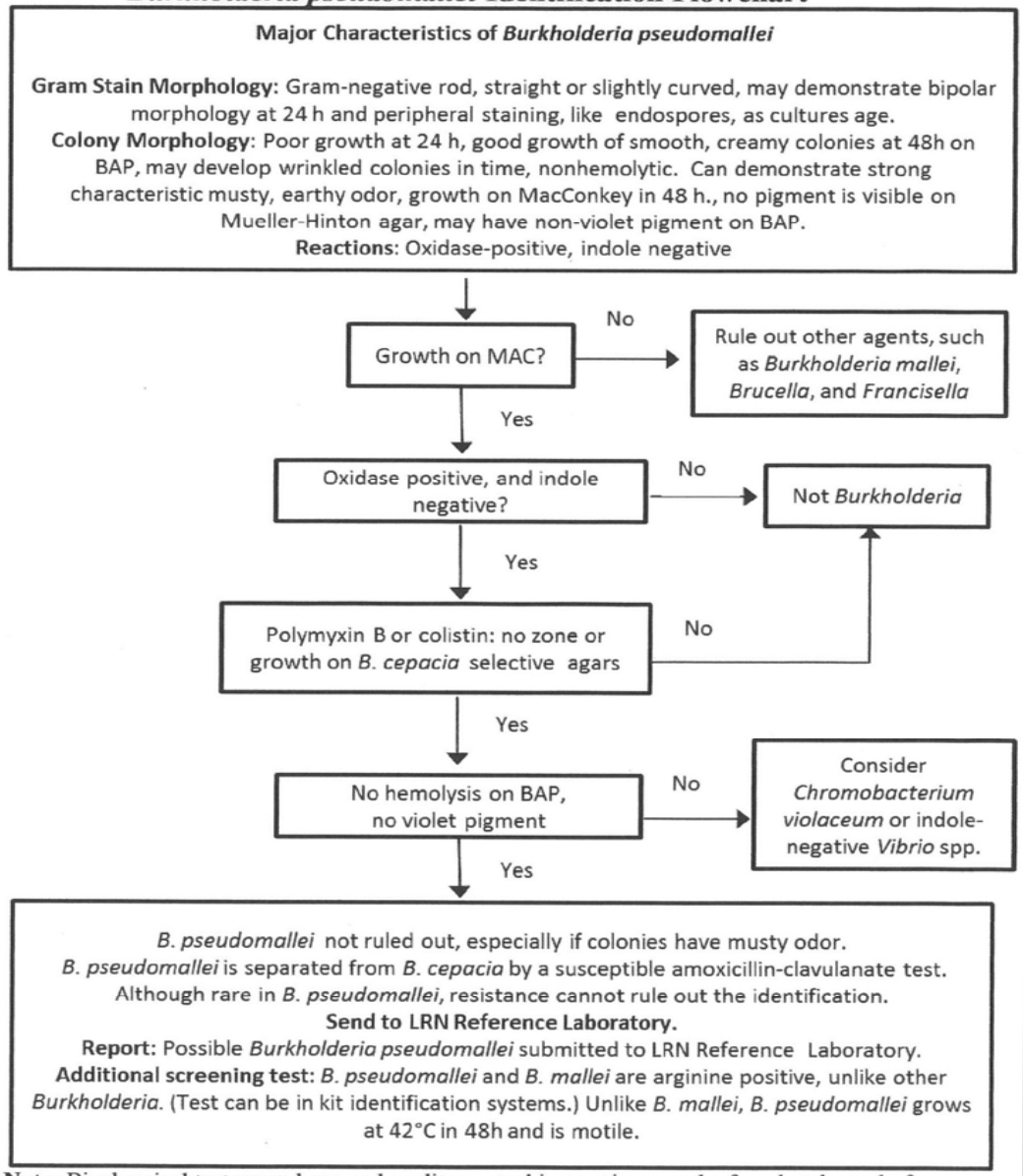
Burkholderia pseudomallei Identification Flowchart



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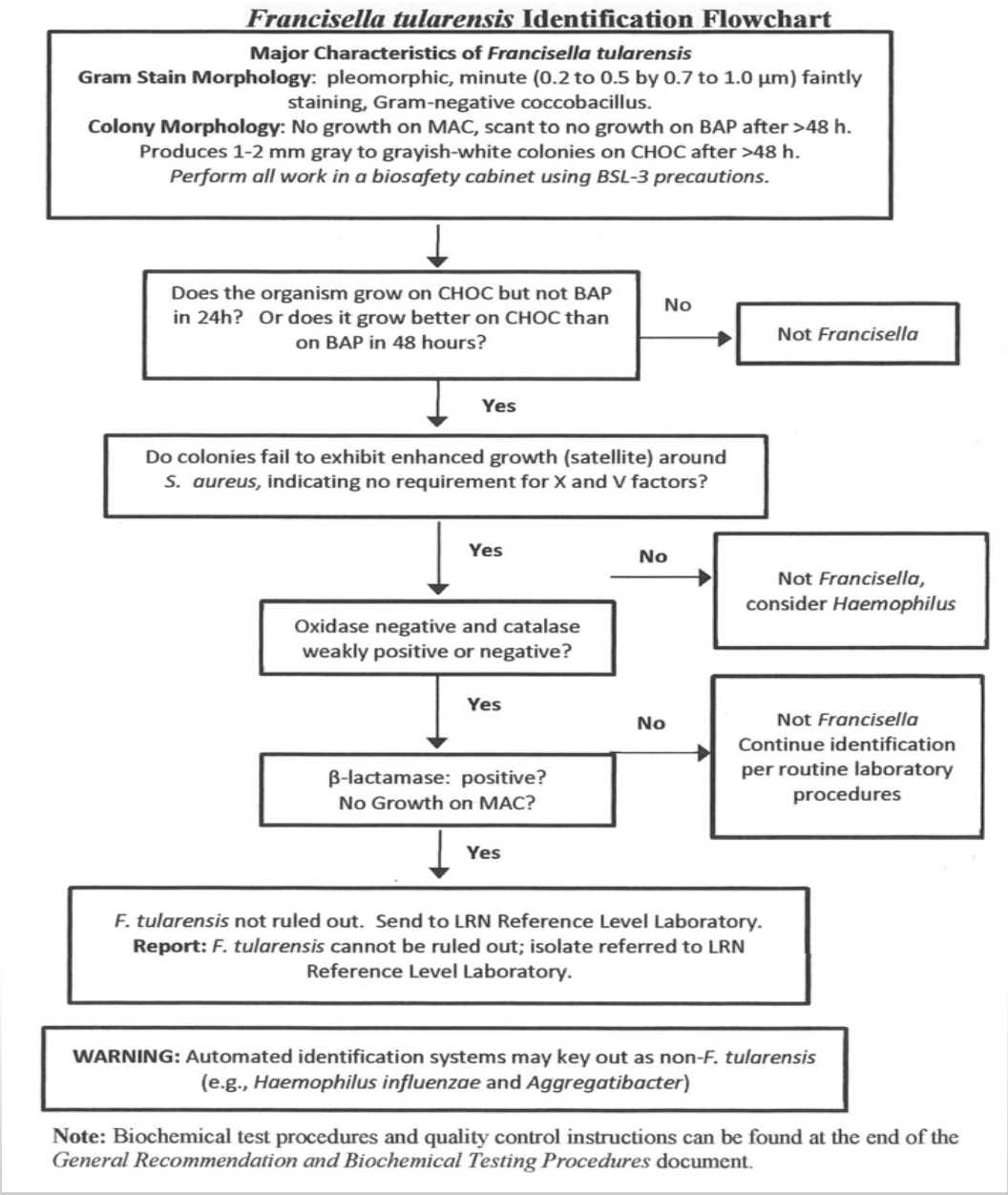

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Yersinia pestis Identification Flowchart

Major Characteristics of *Yersinia pestis*

Gram Stain Morphology: Gram-negative, plump rods, 0.5 x 1-2 µm.

Colony Morphology: Slow growing at 35°C with either pinpoint colonies or no growth on SBA after 24 h; colonies after 48 h are 1-2 mm, gray-white to slight yellow and opaque; non-lactose fermenter on MAC/EMB.

Specimen is blood, sputum, or lymph node aspirate

↓

Oxidase: Negative
Catalase: Positive
Indole: Negative
Urease: Negative

→ No → Not *Yersinia pestis*. Continue identification per routine laboratory procedures. May be other *Yersinia spp.*

↓ Yes

Y. pestis not ruled out. Send to LRN Reference Level Laboratory.
Report: *Y. pestis* cannot be ruled out; isolate referred to LRN Reference Level Laboratory.

WARNING: Some of the automated identification systems do not identify *Y. pestis* adequately. *Y. pestis* has been falsely identified as *Y. pseudotuberculosis*, *Shigella*, H_2S negative *Salmonella*, *Acinetobacter*, and *Pseudomonas* species.

Note: Biochemical test procedures and quality control instructions can be found in the *General Introduction, Recommendations and Biochemical Procedures* document.

Cost Comparison between Standard Rule Out and MALDI-TOF

Agent	*Rule Out Protocol*		*MALDI Protocol*	
	Reagent Cost	Labor Cost	Reagent Cost	Labor Cost
Bacillus anthracis	$2.58	$5.13	$2.18/test*	
Brucella	$1.73	$5.64		
Burkholderia mallei	$3.86	$6.14		
Burkholderia pseudomallei	$1.47	$5.60		
Francisella tularensis	$1.37	$4.54		
Yersinia pestis	$1.65	$5.64		

*Cost includes labor, reagents, template. Does NOT include cost of instrumentation.

Issues Regarding MALDI-TOF

- **Data Bases**
  - Clinical Application (CA)
  - Research Use Only (RUO)
  - Security Software*
- Incorrect Identifications of Biothreat Agents
  - *Yersinia pestis* ID as *Y. pseudotuberculosis*
  - *Bacillus anthracis* ID as *B. cereus*
  - *Burkholderia pseudomallei* ID as *B. Thailandensis*

* Most clinical laboratories do not have Security Software

MALDI-TOF Issues

- **Lack of focus on safety in processing isolates for ID**
  - risk assessment/mitigation absent
  - spotting of isolate
  - solutions potentially a biohazard
  - effective disinfection of reusable templates
  - *Bacillus cereus* biovar *anthracis*??
  - ionization chamber/flight tube under vacuum with air expelled from the instrument; incomplete inactivation of organism is a risk for dispersal of viable bacteria via exhaust
  - most laboratories routinely perform direct colony testing, not tube-based extraction
  - presence of a hazardous bacterium is often not recognized until it has been identified

Cunningham, S. A. and R. Patel. 2015. J. Clin Microbiol. 53: 2788

The Critical Need for Training of Laboratory Personnel for Responding to BT agents

- Public Health has a major role
- Clinical Laboratory Science Programs
- Medical School
- Support for Continuing Education Programs

Summary

- Essential Media in support of rule out/refer not being maintained by the laboratory
- Reduction in skilled and qualified personnel
- New technology: faster, simple, requires less labor, and generally accurate
- MALDI: Cheaper and faster vs conventional rule out/refer methods
- Noncultural methods slowly replacing culture-based methods
- The days of phenotypic-based Rule Out/Refer are nearing an end
- Safety a major concern

NEW TECHNOLOGY HAS COME AND IS REPLACING WHAT WE HAVE BEEN DOING FOR A CENTURY OR MORE