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SENTINEL LABORATORY GUIDELINES FOR SUSPECTED AGENTS OF BIOTERRORISM

Burkholderia mallei and *B. pseudomallei*

American Society for Microbiology

Credits: *Burkholderia mallei* and *B. pseudomallei*

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Table of Contents: *Burkholderia mallei* and *B. pseudomallei*

I. GENERAL INFORMATION

A. Description of organisms

Burkholderia mallei is a nonmotile, aerobic gram-negative coccobacillus, which may or may not be oxidase positive or grow on MacConkey agar. *Burkholderia pseudomallei* is an oxidase-positive, aerobic gram-negative bacillus that is straight or slightly curved. The organism will grow on most standard laboratory media, such as sheep blood and chocolate and MacConkey agars, and it produces a characteristic musty odor (8).

B. History

Burkholderia mallei is the etiologic agent of glanders, a febrile illness typically seen in equids: i.e., horses, mules, and donkeys (1). During World War I, the German military used this agent as a biological weapon against horses and mules, the primary form of transportation during that conflict (2). Naturally occurring human infection is likely due to exposure to an infected animal. The last naturally acquired human case of glanders in the United States was seen in 1945 (1). A case of laboratory-acquired glanders occurred in 2000 (1).

Burkholderia pseudomallei is an environmental organism found in soil and water and is most likely obtained naturally by direct contact with, or aerosols from, environmental sources. For many years, this bacterial species was classified as a member of the genus *Pseudomonas*, but in 1992, it was reclassified into the genus *Burkholderia* (15). This organism was first reported as causing human infections in 1911 by Whitmore from individuals living in Rangoon, Burma (now Yangon, Myanmar) (14) and in earlier medical literature was called "Whitmore's disease." These patients were described as septicemic with widespread abscesses in the lungs, liver, spleen, and kidneys. *Burkholderia* was one of the first organisms reported as a cause of infection in intravenous drug users (14). Because the organism was thought to cause a glanders-like illness in humans, it was called "*pseudomallei*" by Stanton and Fletcher (5). The glanders-like disease in humans due to *B. pseudomallei* is now referred to in the medical literature as "melioidosis," from the Greek word "melis," which was the term for distemper in donkeys (11). It was found to cause disease in soldiers from both Australia and the United States during the Vietnam War and has been referred to as the "Vietnam time bomb" because the disease, much like tuberculosis, can reactivate after remaining latent for decades (5).

C. Geographic distribution

B. mallei was eradicated from the United States and Western Europe due to a program of compulsory slaughter of infected or seropositive horses or other animals. Equines are the primary reservoir of the rare cases of glanders still seen in Eastern Europe, the Middle East, Asia, and Africa (1).

Melioidosis is a disease endemic in the tropical regions of the world, with the majority of cases in the medical literature being reported from rice-growing regions of Southeast Asia and the tropical, northern regions of Australia. The organism has

been detected in very high concentrations in water found in rice paddies in both Vietnam and Thailand (8). There are data to suggest that this organism is also endemic in both the Philippines and the Indian subcontinent (5). There is little known about the prevalence of this organism in tropical regions of Africa and the Americas. Infections with this organism in the United States and Western Europe are almost certainly imported from regions of endemicity, and imported cases are well documented in the medical literature (8). Laboratory-acquired infections have also been reported (5). The recognition of multiple cases of melioidosis in North America or Western Europe in patients without an appropriate travel history requires a thorough investigation of the possibility of a bioterrorism attack.

D. Clinical presentation

1. Glanders. Glanders can present as either a cutaneous or systemic disease (1). The incubation period is typically 1 to 14 days. Patients with the cutaneous infection will have nodules with accompanying localized lymphadenitis. The systemic illness usually manifests itself either as broncho- or lobar pneumonia. Bacteremia may also occur, resulting in lesions being seen in the liver and spleen. Infection in humans with *B. mallei* is often fatal without antimicrobial treatment.

2. Melioidosis. The illness can manifest as an asymptomatic, acute, subacute, or chronic process.

Asymptomatic infection. Serosurveillance indicates that the majority of those infected remain asymptomatic.

Acute infection. The typical presentation of acute infection is pneumonia. The incubation period of this infection is 2 to 5 days. The disease presents with high fever, dyspnea, and pleuritic chest pain. Sputum is usually purulent, and hemoptysis may be observed. The most severe manifestation of acute melioidosis is septicemic pneumonia. Mortality in those patients is approximately 40%; patients with fulminant septicemia, as evidenced by >100 CFU of *B. pseudomallei* per ml or blood culture showing growth in the first 24 h of incubation, have a mortality approaching 90% (5). Genitourinary infections are well described, and given the number of prostatic infections detected, all male patients with melioidosis should have their abdomens imaged, for example, by computerized tomography (CT) (4). Neurological melioidosis exists, but rather than presenting as a meningitis, the disease is more consistent with a brainstem encephalitis displaying peripheral weakness or flaccid paralysis (4).

Subacute infection. Subacute infections can mimic those of *Mycobacterium tuberculosis*. Patients can have low-grade fevers, malaise, anorexia, and weight loss, which occur over a period of months (11). Like *M. tuberculosis*, *B. pseudomallei* can survive within phagocytes (12) and produce nodular or cavitary lesions visible on a chest radiograph. The disease may lay quiescent for many years only to later reactivate, a more common finding than reinfection (3). Reactivation is most likely to occur in immunosuppressed individuals.

Chronic infection. Chronic infection is similar to miliary tuberculosis in that the infection is disseminated, and granulomatous lesions can be seen in a variety of tissues. Patients may have minimal symptoms, but most have symptoms similar to miliary tuberculosis, including fever, cough, and weight loss (11).

Risk factors for developing melioidosis include alcoholism, diabetes, renal failure, or penetrating wounds (8). The role of inoculum size in impacting whether infections are subacute, acute, fulminant, or chronic is unknown. Person-to-person spread has been documented via direct contact, but there are no data to suggest that person-to-person spread occurs via the respiratory route.

B. pseudomallei may also cause a disease similar to glanders in animals, with animal-to-animal spread being documented (11).

E. Treatment

1. ***Burkholderia mallei.*** There is essentially no clinical experience with treating *B. mallei* with the modern armamentarium of antimicrobial agents. It is likely that treatment strategies used for *B. pseudomallei* will be effective against *B. mallei*. For prophylaxis, limited animal studies suggest that both ciprofloxacin and doxycycline could be used (7).

2. ***Burkholderia pseudomallei.*** There is fairly extensive experience treating *B. pseudomallei* infections. For acute or chronic infections, parenteral administration of imipenem or ceftazidime for 2 to 4 weeks followed by oral therapy with amoxicillin-clavulanate or a combination of doxycycline and trimethoprim-sulfamethoxazole for 3 to 6 months is recommended (9). Because resistance has developed with ceftazidime therapy, combination therapy is usually recommended for initial treatment. There are no data published on prophylactic agents for *B. pseudomallei*, although it is likely that the agents used for oral eradication therapy would be useful prophylactically. Of note, *B. pseudomallei* organisms are frequently resistant to aminoglycosides, first and second generation cephalosporins, and fluoroquinolone antimicrobials in vitro, and so drugs in these classes, would likely not be good prophylactic agents.

3. There is no vaccine currently available for either *B. mallei* or *B. pseudomallei*.

II. PROCEDURES

A. General

The procedures described below are to be used to rule out the presence of *B. mallei* and *B. pseudomallei* in clinical specimens or as isolates.

B. Precautions

1. Level A (Sentinel) laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the State Health Laboratory.

2. **Laboratory-acquired infections have been documented.** All patient specimens and culture isolates should be handled while wearing gloves and gowns in a biosafety cabinet. Plates should be taped shut when incubating.

C. Specimens

1. Blood or bone marrow

2. Sputum or bronchoscopically obtained specimens

3. Abscess material and wound swabs

4. Urine

5. Serum (1 ml). Both acute- and convalescent-phase (obtained 14 days after the acute-phase specimen) specimens should be collected if serologic diagnosis of *B. pseudomallei* infection is being considered. Currently, no serology for *B. mallei* is available in the United States.

D. Materials

1. Blood and bone marrow cultures can be done using:

a. Standard automated blood culture system

b. Lysis centrifugation system

2. Media for isolation from other clinical specimens:

a. Chocolate agar (CHOC)

b. Sheep blood agar (SBA)

c. MacConkey agar (MAC)

d. Selective agars for *Burkholderia pseudomallei* (8)

3. Reagents

a. Gram stain reagents

b. Oxidase reagent

c. Hydrogen peroxide (3%) for catalase test

d. Spot indole reagent (5% *p*-dimethylaminobenzaldehyde or 1% paradimethylaminocinnamaldehyde in 10% [vol/vol] concentrated HCl.)

e. Colistin (10 μg) or polymyxin B (300 U) disk

f. Motility semisolid medium with 2,3,5-triphenyltetrazolium chloride (TTC) indicator (Remel, Inc.; catalog no. 061414) or broth for wet mount motility.

g. Optional reagents

i. Arginine dihydrolase and Moeller base control

ii. Triple sugar iron agar (TSI) or Kligler's iron agar (KIA)

iii. Nitrate test

a. Nitrate broth with gas indicator tube

b. Nitrite reduction reagent 1 (sulfanilic acid)

c. Nitrite reduction reagent 2 (dimethyl-α-naphthylamine)

d. Zinc dust

h. Alternately, API 20NFT, also called 20NE (BioMerieux, Durham, N.C.), or Vitek 1 gram-negative identification panels (BioMerieux) can be used for preliminary identification. These organisms are not in the databases of all commercial identification systems; consult the manufacturer.

NOTE: While only the API 20NFT and the Vitek system have been studied extensively (10, 13), other systems that have arginine as one of the biochemical tests may work well. *B. pseudomallei* is in the database of the Microscan overnight system, but not the rapid, gram-negative rod panel, although the sensitivity and specificity of the overnight product have not been studied extensively.

4. Equipment and supplies

1. Blood culture instrument (optional)

2. 35°C (and 42°C [optional]) incubators

3. Light microscope with ×100 objective and ×10 eyepiece

4. Microscope slides, disposable bacteriologic inoculating loops

5. Glass tubes, sterile pipettes

6. Biological safety cabinet (BSC)

E. Quality control

Document all quality control (QC) for the following tests per standard laboratory procedure/protocol.

F. Stains and smears. Gram stain

1. Procedure. Perform Gram stain procedure/QC per standard laboratory protocol.

2. Interpretation

a. *B. mallei* is a small gram-negative coccobacillus.

b. *B. pseudomallei* is a small gram-negative rod (Fig. 1).

c. The organisms may be observed in direct gram stain from respiratory specimens or abscess material/wounds. They can also be seen in smears of positive blood culture bottles.

G. Cultures

1. Inoculation and plating procedures. Inoculate and streak the following media for isolation of the respective specimen types. NOTE: Standard media should be used according to normal laboratory procedures.

a. Blood cultures. Process according to standard laboratory procedure.

b. Respiratory specimens, abscess material/wounds. Plate directly onto SBA and MacConkey agar; enrichment broth can be used for wound/abscess material. Ashdown medium (8) is a selective medium specifically designed

for recovery of *B. pseudomallei*. This medium is not likely to be available in most Sentinel laboratories.

For improved isolation, a colistin disk or polymyxin B disk may be placed in the initial inoculation area of the SBA if isolation of *Burkholderia* spp. is specifically requested.

2. Incubation

a. Temperature. 35 to 37°C

b. Atmosphere. Ambient; CO_2 acceptable

c. Length of incubation. Hold primary plates for a minimum of 5 days; read daily. *B. pseudomallei* will reliably grow with 5 days of incubation from blood cultures, so extended incubation of either broth or plated blood cultures (lysis-centrifugation) is not necessary. *B. mallei* will not grow as rapidly as *B. pseudomallei* and may require extended incubation.

3. Growth and colony characteristics

All cultures suspected of containing *B. mallei* or *B. pseudomallei* should be handled in a biological safety cabinet.

a. Fulminant sepsis. The recovery of *B. pseudomallei* from blood culture within the first 24 h of incubation indicates fulminant sepsis, which has a very high (90%) mortality rate.

b. Growth characteristics

1. *B. mallei*

i. On SBA, the organism shows smooth, gray, translucent colonies in 2 days, without pigment or distinctive odor. *B. mallei* will grow without any inhibition around the colistin or polymyxin B disk.

ii. Colonies may or may not be present on MacConkey agar.

2. *B. pseudomallei*

i. On SBA, the organism often reveals small, smooth creamy colonies in the first 1 to 2 days, which gradually change after a few days to dry, wrinkled colonies similar to *Pseudomonas stutzeri*. Colonies are neither yellow nor violet pigmented. *B. pseudomallei* will grow without any inhibition around the colistin or polymyxin B disk.

ii. Colonies are present on both SBA and MacConkey agars.

iii. *B. pseudomallei* often produces a distinctive musty or earthy odor that is very pronounced on opening a petri dish growing the microorganism or even opening an incubator door when a positive plate is present**. "Sniffing" of plates containing *B. pseudomallei* is dangerous and should not be done.** However, the odor will be apparent without sniffing.

4. Screening tests

a. The following biochemical tests can be used to rule out an isolate as *B. mallei*: oxidase, indole, catalase (not needed if isolate is growing on MacConkey agar), resistance to colistin or polymyxin B, and motility. Isolates suspected to be *B. mallei* based on colony and Gram stain morphology should have these tests performed.

b. The following biochemical tests can be used to rule out an isolate as *B. pseudomallei*: oxidase, indole, and resistance to colistin or polymyxin B. Isolates suspected to be *B. pseudomallei* based on colony, odor, and Gram stain morphology should have these tests performed.

1. Catalase test. Perform catalase test/QC following standard laboratory procedure, **if the isolate is *not* growing well on MacConkey agar in 48 h:** *B. mallei* and *B. pseudomallei* are catalase positive.

2. Oxidase test. Perform oxidase test/QC following standard laboratory procedure: *B. mallei* may be **oxidase positive or negative.** *B. pseudomallei* is **oxidase positive.**

3. Indole test. Perform indole test/QC following standard laboratory procedure: *B. mallei* and *B. pseudomallei* are **indole negative.**

4. Colistin or polymyxin B resistance

i. Procedure

a. Streak either an SBA or Mueller-Hinton agar plate with growth, using a swab dipped into a broth culture corresponding to a no. 0.5 McFarland turbidity standard.

b. Place disk in the inoculated area of the plate.

c. Incubate for 24 to 48 h.

d. Examine for zone of inhibition around the disk.

ii. Interpretation

1. No zone around the disk indicates resistance to polymyxin B or colistin. *Burkholderia*, *Chromobacterium violaceum*, and some *Vibrio*, and *Ralstonia* are resistant; *Pseudomonas* species are susceptible.

2. *B. mallei* and *B. pseudomallei* are **resistant to polymyxin B and colistin.**

3. As an alternative, growth on *B. cepacia* selective agars or modified Thayer Martin may substitute for the disk test, because these media contain polymyxin B or colistin. However, the lack of growth on these media should be confirmed by the disk test.

iii. Quality control

Polymyxin B: 300 U on MH	*Pseudomonas aeruginosa* ATCC 27853	**17-21 mm (6)**
	Escherichia coli ATCC 25922	**17-20mm (6)** **12-16 mm (BD package insert)**
Colistin: 10 µg on MH	*Pseudomonas aeruginosa* ATCC 27853	**15-19 mm (6)**
	Escherichia coli ATCC 25922	**16-20 mm (6)** **11-15 mm (BD package insert)**

5. Motility test

a. Procedure

1. The motility test should be performed if the isolate has the colony morphology and Gram stain reaction of *B. mallei* and is resistant to colistin or polymyxin B. Because of the danger of laboratory-acquired infection, the wet mount motility should be performed with caution in a BSC wearing protective equipment; the tube test is recommended.
2. Inoculate medium with a stab down the center of the tube, to within 0.5 in. from the bottom of the tube.
3. Incubate tubes at 35°C (30°C is preferred).

b. Interpretation

1. A diffusible red-colored growth spreading away from the stab line indicates motility.
2. *B. mallei* is nonmotile, and *B. pseudomallei* is motile.

c. Quality control

1. Since motility can be difficult to demonstrate among glucose-nonfermenting rods, use *Pseudomonas aeruginosa* ATCC 27853 as the positive control for the test.
2. *Klebsiella pneumoniae* ATCC 10031 is nonmotile.

6. Additional screening tests

If available, TSI or KIA, arginine dihydrolase, and nitrate may be performed to further exclude similar organisms.

a. TSI or KIA slant

1. Procedure

Perform TSI or KIA QC following standard laboratory procedure.

2. Interpretation

B. mallei and *B. pseudomallei* are **glucose-nonfermenting rods or coccobacilli,** and the results that will be observed will be an alkaline (red or no change) in tube butt/no H_2S (no black color). *B. pseudomallei* may or may not produce an acid (yellow) slant, due to oxidation of lactose.

b. Arginine dihydrolase with Moeller's base control

1. Procedure

i. Inoculate a tube of both arginine dihydrolase and Moeller's base with an isolated colony of the suspected isolate.

ii. Overlay the contents of both tubes with sterile mineral oil or Vaspar, liquid paraffin, or petroleum jelly, maintained at 56°C in liquid form.

iii. Tighten caps on tubes.

iv. Incubate for 1 to 2 days at 35°C.

2. Interpretation

i. Compare colors in the Moeller's base control to that in the tube containing arginine.

a. Positive result. Arginine tube is clearly purple, while Moeller base control has not changed color or has become yellow.

b. Negative result. No difference between the tube containing arginine and the tube containing base.

ii. *B. mallei* and *B. pseudomallei* are **positive** for arginine dihydrolase.

3. Quality control using glucose-nonfermenting strains:

i. Positive control strain. *Pseudomonas aeruginosa* ATCC 27853

ii. Negative control strain. *Acinetobacter lwoffi* ATCC 15309

4. This test is available in many identification systems used routinely in laboratories.

c. Nitrate reduction

1. Procedure

i. Inoculate the nitrate broth with 1 to 2 isolated colonies.

ii. Incubate aerobically at 35 to 37°C for up to 48 h.

iii. After 24 h of incubation, observe for turbidity and gas in the Durham tube.

a. *Do not add reagents if there is no visible growth in the tube.*

b. If gas is present and the isolate is glucose nonfermenting (alkaline butt in TSI or KIA), do not add reagents, as the test is positive.

c. If there is growth and no gas production, remove 1 ml of broth from tube with a sterile pipette. Place broth in a small glass tube.

i. Add 1 or 2 drops of nitrite-reduction reagents 1 and 2 to broth. If a red color forms after the addition of nitrite-reduction reagents 1 and 2, the organism is positive for nitrate reductase activity.

ii. If no red color forms, add a pinch of zinc dust. If red color forms after addition of zinc dust, nitrate has not been reduced. If no color change is observed after addition of zinc dust, nitrate has been reduced to nitrogen gas.

d. If the test is negative after 24 h (i.e., a red color is observed after addition of zinc dust), the remaining broth should be reincubated and retested at 48 h.

2. Interpretation

i. *B. mallei* typically reduces nitrate without gas and will give a red color with the addition of reagents 1 and 2 and is therefore **nitrate reductase positive without gas**.

ii. *B. pseudomallei* typically reduces nitrate to nitrogen gas and is therefore **nitrate reductase positive with gas.** *B. pseudomallei* often produces only one small bubble of gas.

3. Quality control

i. Positive control strains. *Pseudomonas aeruginosa* ATCC 27853 reduces nitrate to nitrogen gas. *Escherichia coli* 25922 reduces nitrate to nitrite with no gas.

ii. Negative control strain. *Acinetobacter lwoffi* ATCC 15309. No red color develops until the zinc dust is added.

iii. Use of commercial systems for the identification of *B. pseudomallei.* The API 20E and 20NE systems and Vitek 1 (but NOT Vitek 2) (BioMerieux, Durham, N.C.) have been found to be reasonably reliable systems for the identification of *B.*

pseudomallei (10, 13). Some strains of *B. pseudomallei* have been misidentified by the API 20E and 20NE systems as *C. violaceum* (10, 13). Therefore, strains that are not violet colored but are identified as *C. violaceum* by the API system should also be referred to a Level B (LRN Reference) laboratory for confirmation. All isolates identified as *B. pseudomallei* should be referred to a LRN Reference laboratory for confirmation.

H. Susceptibility testing

Due to the danger in working with *B. mallei* and *B. pseudomallei,* susceptibility testing should be performed only in laboratories with biosafety level 3 containment and personnel precautions.

I. Interpretation and reporting

1. Presumptive identification criteria

a. *Burkholderia mallei*

i. Gram stain reaction. Small, straight or slightly curved gram-negative coccobacilli. Cells are arranged in pairs, parallel bundles, or the Chinese-letter form.

ii. Colony characteristics. Colonies are gray, translucent, and have no pigment or distinctive odor. They may or may not grow on MacConkey agar.

iii. Oxidase variable, catalase positive, indole negative, nonmotile, colistin resistant.

iv. Optional: TSI-alkaline/alkaline, KIA-alkaline/alkaline, arginine dihydrolase positive, and nitrate reductase positive without gas.

v. Kit identification. Check with the manufacturers. The databases may not include *B. mallei*, but arginine and colistin tests may be present in some kits.

b. *Burkholderia pseudomallei*

i. Gram stain reaction. Small, straight, or slightly curved gram-negative rod; may demonstrate bipolar morphology in direct specimens (Fig. 1).

ii. Colony characteristics. Colonies are initially cream colored. After 3 or 4 days of incubation, *B. pseudomallei* colonies will have a dry, wrinkled appearance on MacConkey agar (Fig. 2). The colonies will also emit a strong, musty or dirt-like odor.

iii. Oxidase positive, indole negative, motile, colistin resistant

iv. Optional: TSI-acid or alkaline/alkaline, KIA-acid or alkaline/alkaline, arginine dihydrolase positive, and nitrate reductase positive with gas.

v. Kit identification. Isolates with appropriate gram stain reaction and colony morphology that give a “very good” to “excellent” probability score for *B. pseudomallei* should be referred to a LRN Reference

laboratory for confirmation. Additionally nonpigmented isolates identified as *Chromobacterium violaceum* should be checked for glucose fermentation in the butt of TSI. If acid production in the butt is lacking, these strains should also be referred to a LRN Reference laboratory for confirmation of identification.

2. Referral of presumptive *B. mallei* or *B. pseudomallei* isolates to LRN Reference laboratory

a. When to refer:

i. Naturally occurring cases of *B. mallei* are extremely rare in humans and should be referred to LRN Reference laboratories in all cases.

ii. Naturally occurring cases of *B. pseudomallei* may be observed in any of the following situations. Confirmation of the identification of these isolates may be requested from a LRN Reference laboratory, but they are unlikely to represent a bioterrorism event. Patients who cannot be classified into any of the following patient populations may represent a bioterrorism event.

1. Patients with acute infection who have a recent history of travel to the region of endemicity. This includes Southeast Asia (in particular Thailand, Vietnam, Mynamar, or Taiwan), the Philippines, the Indian subcontinent, or the northern coast of Australia.

2. Recent immigrants or visitors from the region of endemicity.

3. Patients with recent onset of diabetes, renal failure, or immunosuppressed states who have travelled in the region of endemicity mentioned above even if that travel occurred decades before.

4. Individuals who work with animals (such as zoo employees) that have recently been imported from regions of endemicity.

5. Individuals who work in laboratories where they may be exposed to this organism.

b. Whom to notify and when to notify them:

i. Further epidemiologic investigation is needed whenever a presumptive identification of *B. mallei* or *B. pseudomallei* is made.

ii. Within the hospital setting, the infectious disease service and/or infection control department should be notified so further investigation of the patient's history can be made so that naturally occurring infections can be ruled out.

iii. The State Laboratory Director (or designate) should be notified of the presumptive identification of *B. mallei* or *B. pseudomallei*.

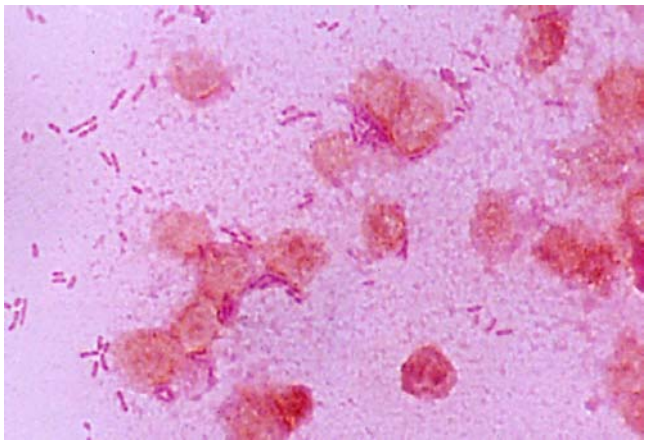
iv. If a criminal investigation is to be considered, a chain of custody should be followed when referring the isolate to the LRN Reference laboratory. In

particular the appropriate material, including blood culture bottles, tubes and plates, and actual clinical specimens (aspirates, biopsies, sputum specimens) should be saved as evidence.

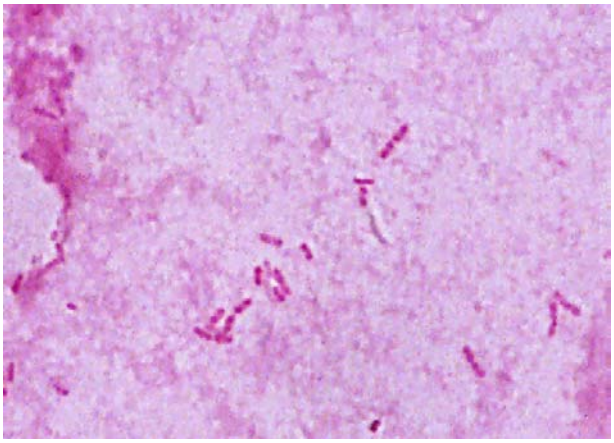
v. Any environmental specimens collected for detection of *B. mallei* or *B. pseudomallei* as part of an epidemiologic/criminal investigation should be referred directly to the State Public Health Laboratory.

vi. The State Public Health Laboratory Director will coordinate notification of the Centers for Disease Control and Prevention and State and Federal law enforcement agencies.

Figure 1

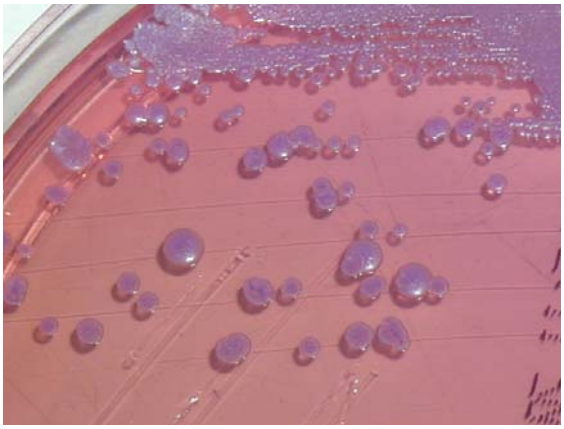


Gram stain of *B. pseudomallei* in a blood culture (8)



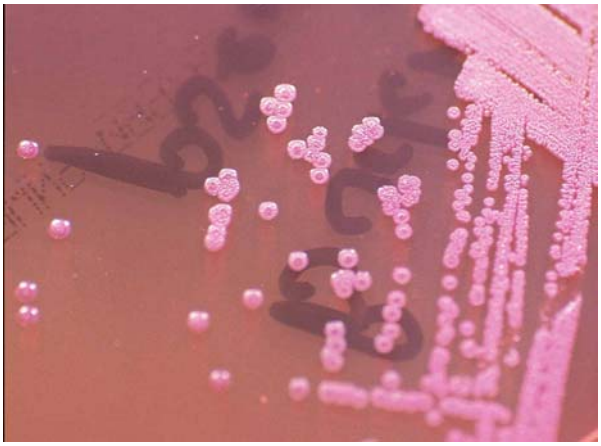
Gram stain of *B. pseudomallei* from a colony on blood agar (8)

Figure 2



B. pseudomallei **colonies on MacConkey agar (8)**

B. pseudomallei **colonies on blood agar (8)**



B. pseudomallei **colonies on Ashdown medium agar (8)**

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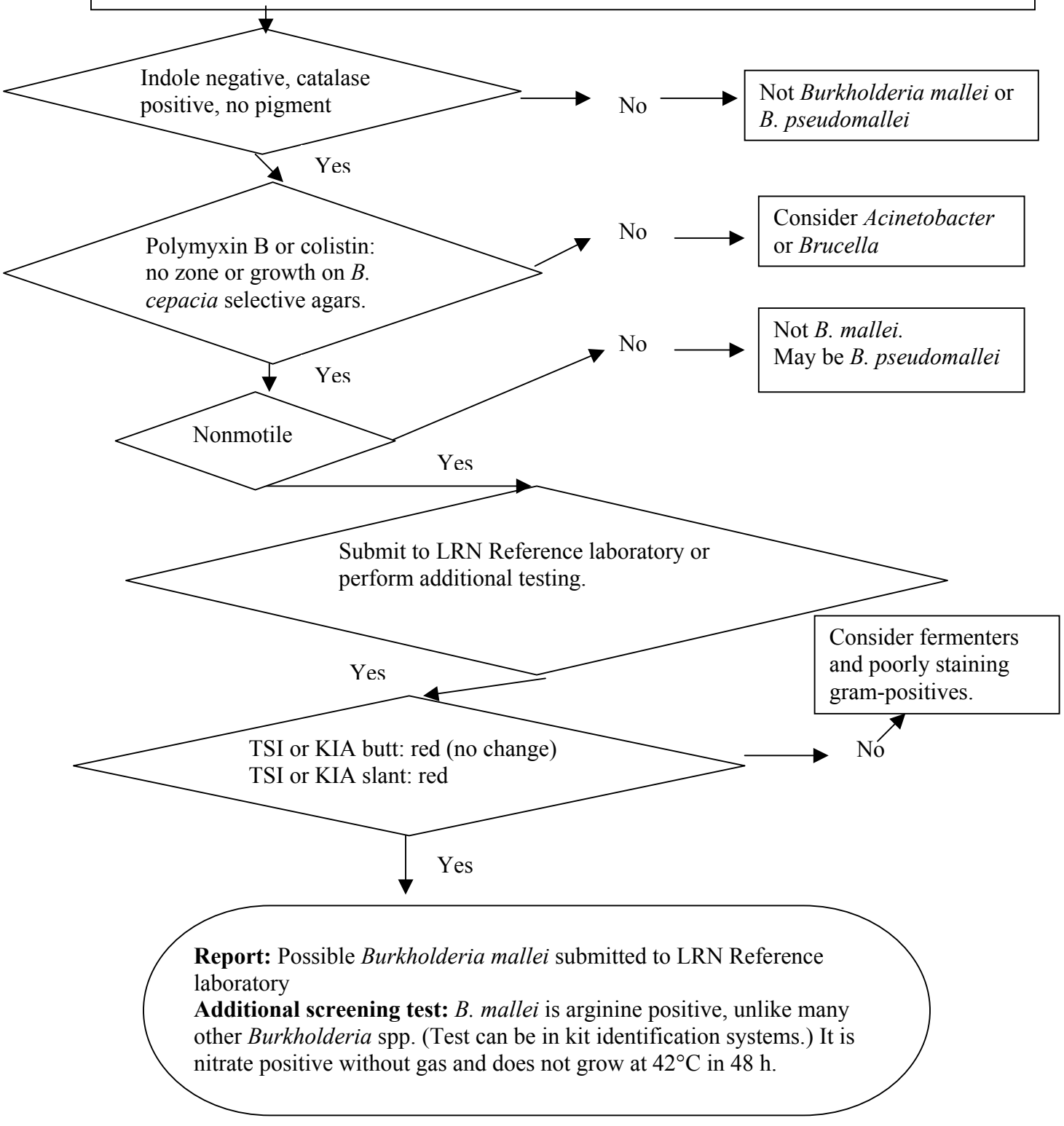
Burkholderia mallei Flowchart
Sentinel Laboratory

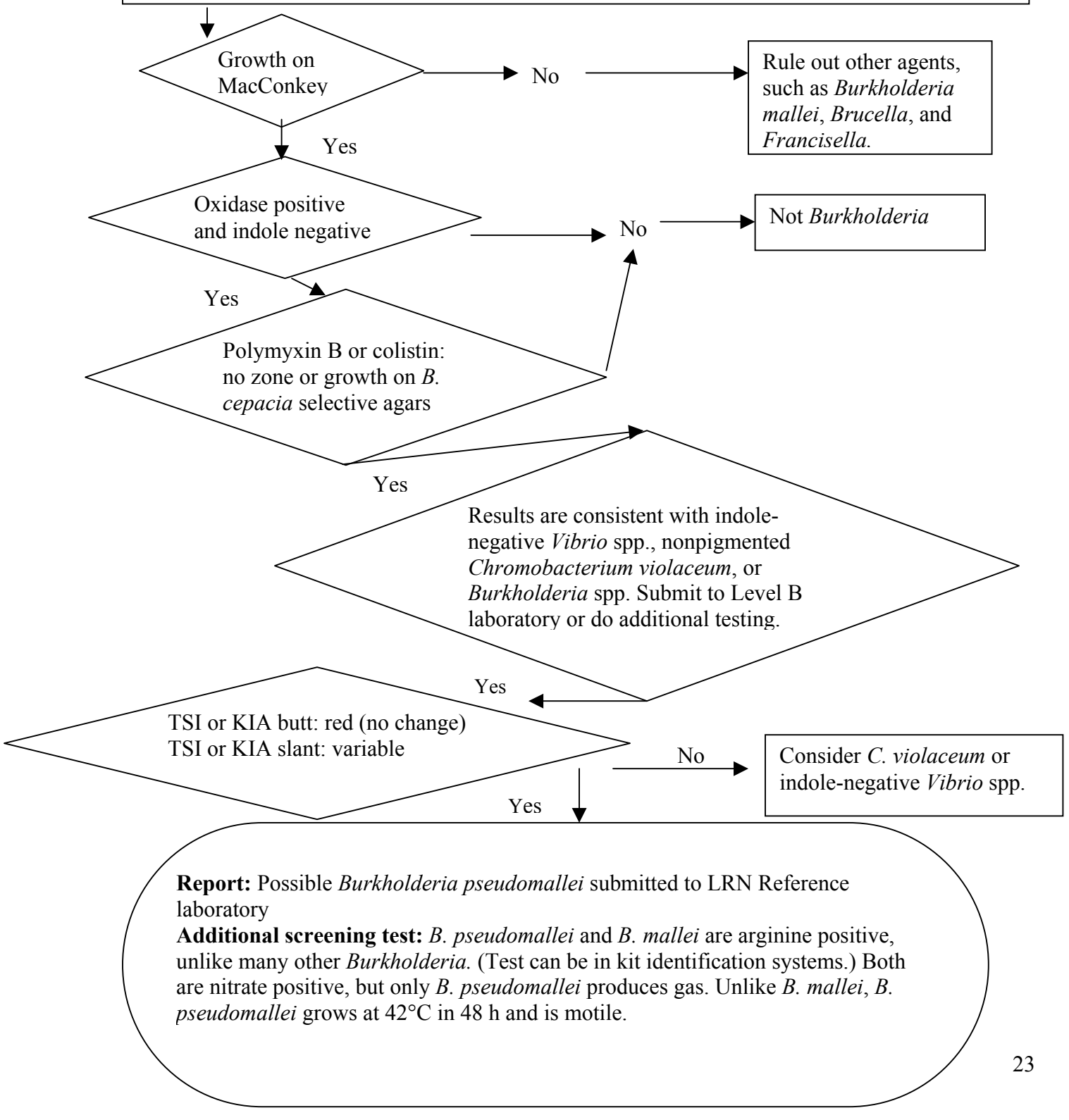
Morphology: Gram-negative coccobacilli or small rod
Growth: Poor growth at 24 h; better growth of gray, translucent colonies at 48 h on SBA; may or may not grow on MAC; no distinctive odor
Reactions: Oxidase-variable, variable growth on MAC, indole negative, catalase positive



Report: Possible *Burkholderia mallei* submitted to LRN Reference laboratory
Additional screening test: *B. mallei* is arginine positive, unlike many other *Burkholderia* spp. (Test can be in kit identification systems.) It is nitrate positive without gas and does not grow at 42°C in 48 h.

Burkholderia pseudomallei Flowchart
Sentinel Laboratory

Morphology: Gram-negative rod, small, straight or slightly curved, may demonstrate bipolar morphology at 24 h and peripheral staining, like endospores, when cultures are older.
Growth: Poor growth at 24 h, good growth of white colonies at 48 h on SBA, may develop wrinkled colonies in time, nonpigmented. Often demonstrates strong characteristic musty, earthy odor.
Reactions: Oxidase-positive, growth on MacConkey in 48 h, indole negative



Report: Possible *Burkholderia pseudomallei* submitted to LRN Reference laboratory
Additional screening test: *B. pseudomallei* and *B. mallei* are arginine positive, unlike many other *Burkholderia.* (Test can be in kit identification systems.) Both are nitrate positive, but only *B. pseudomallei* produces gas. Unlike *B. mallei*, *B. pseudomallei* grows at 42°C in 48 h and is motile.