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**SENTINEL LEVEL CLINICAL MICROBIOLOGY  
LABORATORY GUIDELINES  
FOR  
SUSPECTED AGENTS OF BIOTERRORISM  
AND  
EMERGING INFECTIOUS DISEASES**

***Burkholderia mallei* and *B. pseudomallei***

American Society for Microbiology

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

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## 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 (13). The recent sequencing of the genomes of these two organisms suggest that *B. mallei* is a recently evolved clone of *B. pseudomallei*. *B. mallei* has a smaller genome which makes it much less environmentally adaptable (14).

### B. History

*Burkholderia mallei* is the etiologic agent of glanders, a febrile illness typically seen in equines: i.e., horses, mules, and donkeys (2). 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 (4). 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 (2). A case of laboratory-acquired glanders occurred in 2000 (2).

*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 as well as *B. mallei* were classified as a member of the genus *Pseudomonas*, but in 1992, they were reclassified into the genus *Burkholderia* (28). *B. pseudomallei* was first reported as causing human infections in 1911 by Whitmore from individuals living in Rangoon, Burma (now Yangon, Myanmar) (27) 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 (27). Because the organism was thought to cause a glanders-like illness in humans, it was called “*pseudomallei*” by Stanton and Fletcher (8). 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 (19). 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 (8).

Two organisms which are very similar to *B. pseudomallei* phenotypically have recently been described in the literature (9,10). *Burkholderia thailandensis* is an environmental organism found in rice paddy water and soil in Thailand. It has been shown to be of comparatively low virulence in animal models and has been infrequently reported to be a cause of human disease (9,10). In the clinical laboratory, it is most easily differentiated from *B. pseudomallei* on the basis of its

ability to assimilate arabinose and test for which *B. pseudomallei* is negative. The second organism is *Burkholderia oklahomensis*. It has been reported from two cases in the US and it has been found to be essentially avirulent in animal models (9). These two organisms will not be differentiated from *B. pseudomallei* by the algorithm supplied in this protocol. However given the rarity of isolation of these two organisms in clinic settings, it is unlikely that they will be encountered in critically ill individuals.

### 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 (2).

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 (13). There are data to suggest that this organism is also endemic in both the Philippines and the Indian subcontinent (8). There is little known about the prevalence of this organism in tropical regions of Africa and the Americas although cases have been recently documented in both Brazil and Honduras (3,18). This paucity of knowledge may be due to the inability to recognize this organism in part due to poor performance of commercial identification systems (11,17). 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 (13). Laboratory-acquired infections have also been reported (8). 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 (2).

The incubation period is typically 1 to 14 days.

**Cutaneous.** Patients with the cutaneous infection will have nodules with accompanying localized lymphadenitis.

**Systemic.** 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% (8). 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) (7). 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 (7).

**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 (19). Like *M. tuberculosis*, *B. pseudomallei* can survive within phagocytes (20) and produce nodular or cavitory lesions visible on a chest radiograph. The disease may lay quiescent for many years only to later reactivate, a more common finding than reinfection (6). 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 (19).

Risk factors for developing melioidosis include alcoholism, diabetes, renal failure, or penetrating wounds (13). 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 (19).

## E. Treatment

- 1. *Burkholderia mallei*.** There is essentially no clinical experience with treating *B. mallei* with modern 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 (12).
- 2. *Burkholderia pseudomallei*.** There is fairly extensive experience treating *B. pseudomallei* infections. For acute or chronic infections, parenteral administration of imipenem, meropenem 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 (15). 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. These procedures will not differentiate *B. pseudomallei* from *B. thailandensis* or *B. oklahomensis*. Because the 2 species share >99% homology at the nucleotide level, differentiation between them at the molecular level is challenging (1). Biochemical differentiation can be more productive since they have unique characteristics.

### B. Precautions

1. Sentinel laboratories should not accept environmental or animal specimens; such specimens should be forwarded directly to the State Public Health Laboratory. (A list of State Public Health Laboratory contacts can be downloaded at [http://www.aphl.org/programs/emergency\\_preparedness/Documents/EPrep110707\\_SP\\_HL\\_contact\\_list.pdf](http://www.aphl.org/programs/emergency_preparedness/Documents/EPrep110707_SP_HL_contact_list.pdf))
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* including *Burkholderia cepacia* selective agars (13, 25)

**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.:Lenexa Ks) 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- $\alpha$ -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 of *Burkholderia pseudomallei* but not *B. mallei* with reasonable accuracy. For a review of the performance of commercial systems, see reference 24.

NOTE: While only the API 20NFT and the Vitek system have been studied extensively (16, 22), 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. *Burkholderia pseudomallei* is not in the Phoenix (BD) database and has been reported to call the isolates *B. cepacia* (21).

#### 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; a standard enrichment broth can be used for wound/abscess material. Ashdown medium (13) is a selective medium specifically designed for recovery of *B. pseudomallei*. This medium is not likely to be available in most Sentinel laboratories. If available, *Burkholderia cepacia* selective agars can also be used to for the isolation of *B. pseudomallei* (25). It is unlikely that BCSA selective medium could be used for isolation of *B. mallei* since isolates are typically not resistant to aminoglycosides, a selective agent in this medium (26).

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<sub>2</sub> 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; most *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 (5)

<b>Polymyxin B: 300 U on Mueller Hinton agar</b>	<i>Pseudomonas aeruginosa</i> ATCC 27853	<b>14-18 mm</b>
	<i>Escherichia coli</i> ATCC 25922	<b>13-19 mm</b>
<b>Colistin: 10 µg on Mueller Hinton agar</b>	<i>Pseudomonas aeruginosa</i> ATCC 27853	<b>11-17 mm</b>
	<i>Escherichia coli</i> ATCC 25922	<b>11-17 mm</b>

## 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 not be performed; 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 30°C.

### 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<sub>2</sub>S (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 lwoffii* 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 after 10 minutes, 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 lwoffii* ATCC 15309. No red color develops until the zinc dust is added.

## 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. Routine antimicrobial susceptibility testing is not recommended.

## I. Interpretation and reporting

### 1. Presumptive identification criteria

#### a. *Burkholderia mallei*- see figure 3

- 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.** There is only one study that describes the reliability of commercial systems for the identification *B. mallei*. Both API 20 NE and RapID NF Plus (Remel, Lenexa, KS) either incorrectly or failed to identify 23 *B. mallei* isolates. (11) Therefore isolates that screen as potentially *B. mallei* and are not identified by commercial systems should be referred to the LRN Reference Laboratory for identification.

#### b. *Burkholderia pseudomallei* – see Figure 4

- 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. **In many cases a Kit identification is performed before B. pseudomallei is suspected (see Figure 5 for guidelines for supplemental tests when kits are used).** Initial studies suggested that the API 20E and 20NE systems and VITEK 1 (but NOT VITEK 2) (BioMerieux, Durham, N.C.) were reasonably reliable systems for the identification of *B. pseudomallei* (16, 22). More recent studies suggest that the API 20NE is not as reliable for identification of *B. pseudomallei* when a greater variety of isolates were tested (11,17). In a recent study, when colorimetric rather than fluorometric based identification system was used in the VITEK 2, the identification of *B. pseudomallei* was still not

particularly accurate (23). These data suggest that organisms identified as *B. pseudomallei* by commercial systems should be referred to an LRN Reference Laboratory for confirmation. In addition glucose non-fermenters which screen as potential *B. pseudomallei* using the above listed screening tests and give “no identification” by commercial systems should also be referred. Finally, some strains of *B. pseudomallei* have been misidentified by the API 20E and 20NE systems as *C. violaceum* (16, 22). Therefore, strains that are not violet colored but are identified as *C. violaceum* by the API system should also be referred to a LRN Reference laboratory for confirmation.

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

### **a. When to refer:**

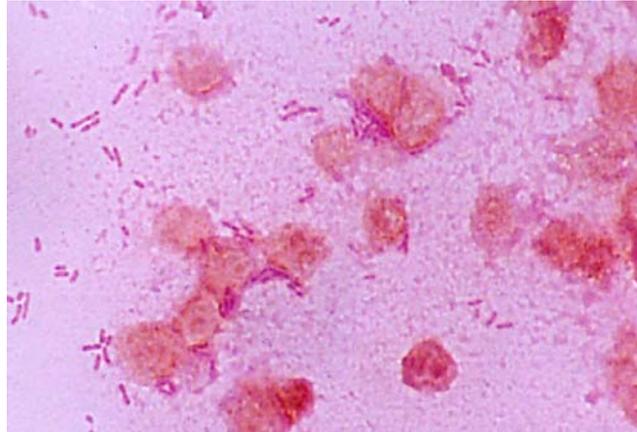
- i.** Naturally occurring cases of *B. mallei* are extremely rare in humans and should be referred to LRN Reference laboratories in all cases.
- ii.** Naturally occurring cases of *B. pseudomallei* may be observed in any of the following situations. Confirmation of the identification of these isolates 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, Myanmar, or Taiwan), the Philippines, the Indian subcontinent, or the northern coast of Australia. Recent US cases have also been imported from Honduras and cases have been reported in Brazil so *B. pseudomallei* may also be considered, for biothreat reasons, as endemic in tropical regions of Central and South America.
  - 2.** Recent immigrants or visitors from the region of endemicity.
  - 3.** Patients with recent onset of diabetes, renal failure, or immunosuppressed states who have traveled 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:**

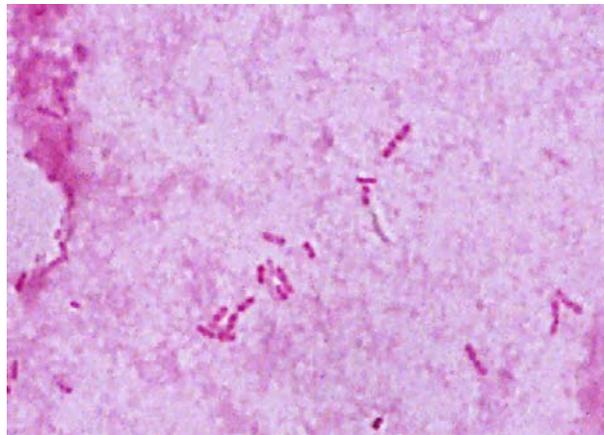
- i.** Further epidemiologic investigation is needed whenever a presumptive identification of *B. mallei* or *B. pseudomallei* is made.

- 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. When referring the isolate to the LRN Reference laboratory, the Reference Laboratory should provide the referring laboratory with guidance and recommendations for retaining the specimen or isolate. Once the identification is confirmed, and the isolate is confirmed as either *B. mallei* or *B. pseudomallei*, the Sentinel Lab is required to destroy it (e.g. autoclaving). In particular the appropriate material, including blood culture bottles, tubes and plates, and actual clinical specimens (aspirates, biopsies, sputum specimens) should be saved until the Reference Laboratory confirms the identification.
- 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**

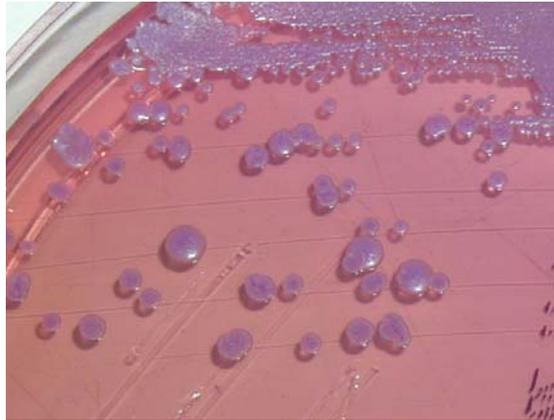


**Gram stain of *B. pseudomallei* in a blood culture (8)**

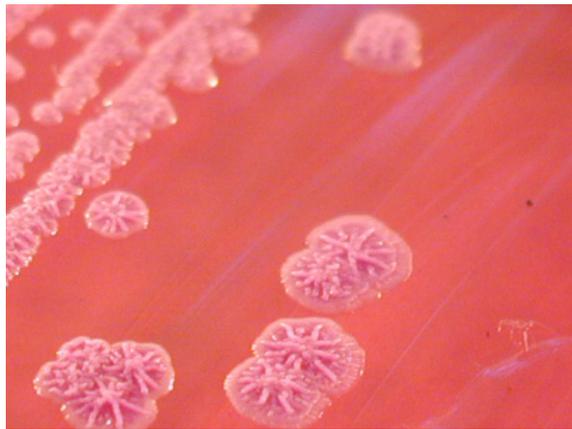


**Gram stain of *B. pseudomallei* from a colony on blood agar (13)**

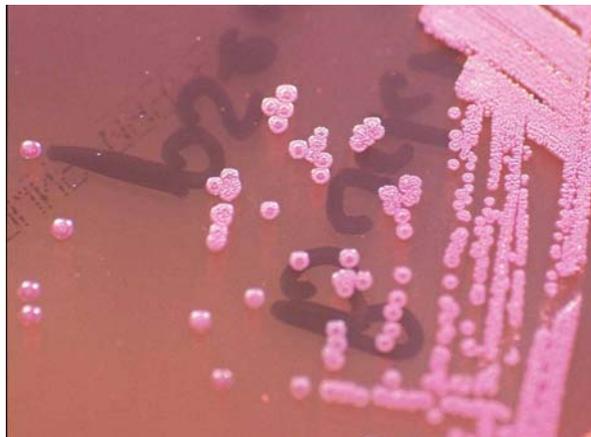
**Figure 2**



***B. pseudomallei* colonies on MacConkey agar (13)**



***B. pseudomallei* colonies on blood agar (13)**



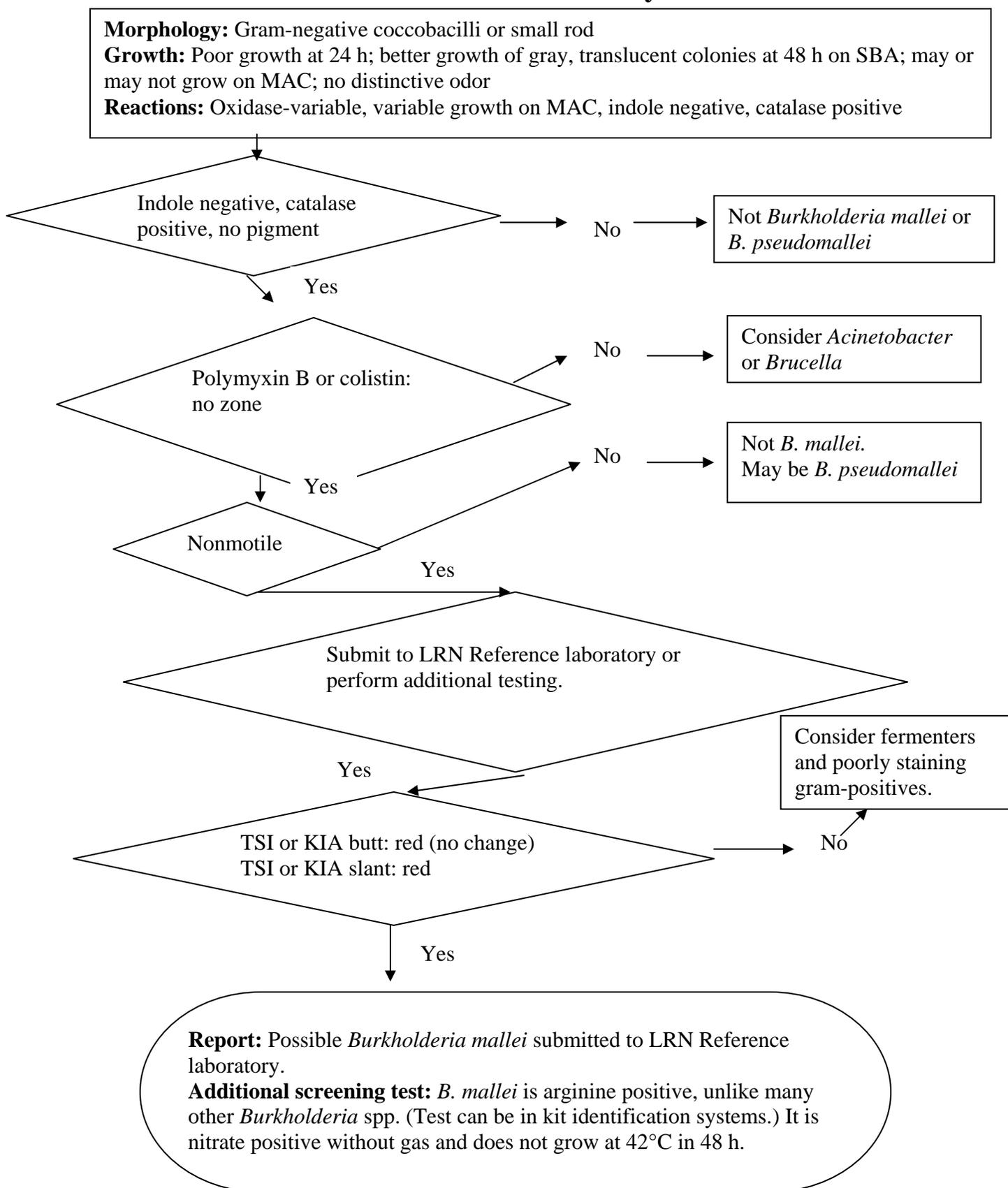
***B. pseudomallei* colonies on Ashdown medium agar (13)**

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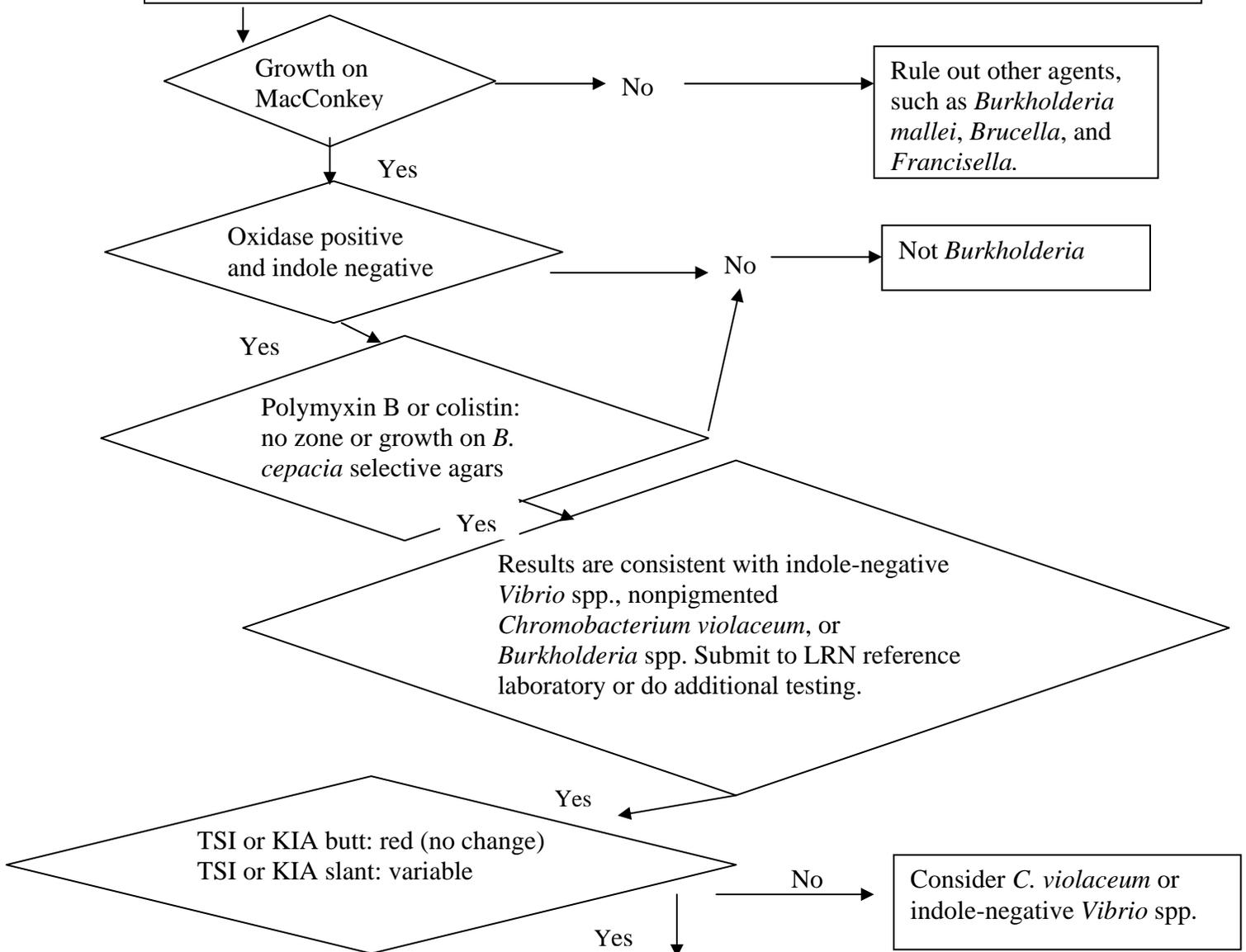
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**Figure 3. *Burkholderia mallei* Flowchart  
Sentinel Laboratory**



## Figure 4. *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



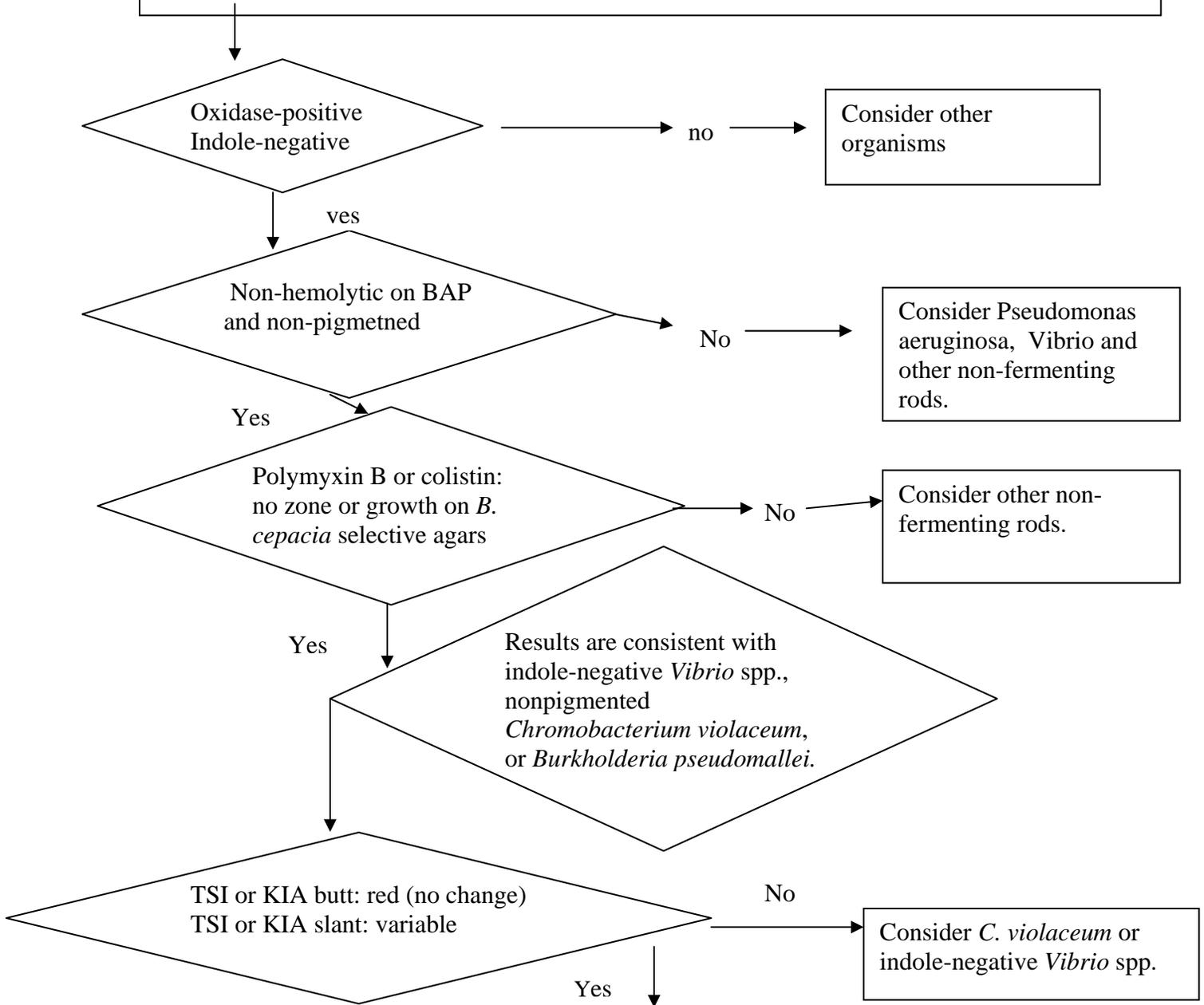
**Report:** Possible *Burkholderia pseudomallei* submitted to LRN Reference laboratory

**Additional screening test:** *B. pseudomallei* and *B. mallei* are arginine positive, unlike 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. Neither species is hemolytic on blood agar, which easily differentiates them from hemolytic *Pseudomonas aeruginosa*.

## Figure 5. *Burkholderia pseudomallei* Flowchart Sentinel Laboratory doing automated or kit methods

**Growth:** Poor growth at 24 h, good growth of white colonies at 48 h on SBA, growth on MacConkey in 48 hr; may develop wrinkled colonies in time, nonpigmented. Often demonstrates strong characteristic musty, earthy odor.

**Reaction:** arginine positive on kit



**Report:** Possible *Burkholderia pseudomallei* submitted to LRN Reference laboratory.  
**Additional screening test:** *B. pseudomallei* is nitrate positive, and produces gas. *B. pseudomallei* grows at 42°C in 48 h and is motile. Note: rare *Achromobacter* may be resistant to polymyxin B or colistin and share these positive reactions.