

Melioidosis Reference Manual



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Foreword: This manual was written to support clinical and laboratory colleagues.

“Could my patient have melioidosis?” “What investigations can help with diagnosis?”

“What clinical samples should I collect and how?” “How can the microbiology

laboratory isolate, identify and confirm *Burkholderia pseudomallei*?” “How can we

undertake environmental sampling?”. Rapid advances are occurring in these areas

and some content will become out of date and need future revisions. Readers are

recommended to look at the most recent literature for new diagnostics and reviews.

Bart Currie, Menzies School of Health Research and Royal Darwin Hospital, Darwin

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Chapter 1: Overview of Melioidosis

Chapter Overview and Introduction to Melioidosis

This chapter provides a brief overview of melioidosis, a neglected tropical disease (NTD) endemic throughout many tropical and subtropical regions of the world. It was first described in a series of 38 patients in Myanmar in 1912. Preliminary autopsies of the patients showed a “peculiar cheesy consolidation” of the lungs, gross subcutaneous or splenic abscesses with “non-Gram-staining- [*Burkholderia mallei*]-like bacilli” (Whitmore & Krishnaswami 1912). The infection was termed melioidosis in 1921 (Stanton & Fletcher 1921). Recent modeling has since estimated that melioidosis kills more people every year globally than diseases that are considerably better recognized, including dengue and leptospirosis. The disease still frequently goes undiagnosed in resource-poor settings, meaning that the true burden of infection is likely greater than what is currently acknowledged (Limmathurotsakul et al. 2016).

Etiology

Melioidosis is caused by the environmental bacterium, *Burkholderia pseudomallei* (Cheng & Currie 2005). *B. pseudomallei* is a gram-negative, oxidase-positive, motile bacillus with a characteristic “safety pin” (bi-polar) appearance upon staining. The bacterium grows well under aerobic conditions in an optimal temperature of 37-42°C on routine culture media, though selective Ashdown or TBSS-C50 media is typically required to isolate the bacterium from sites with normal microbiota or from the environment (Wuthiekanun et al. 1990). Colonies have a characteristically wrinkled morphology and metallic appearance with a characteristic earthy odor (Foong et al. 2014; Wiersinga et al. 2018). *B. pseudomallei* is innately resistant to many antibiotics, including macrolides, narrow-spectrum cephalosporins, the majority of penicillins, polymyxins, and aminoglycosides (Currie 2015).

For environmental specimens, differentiation from the avirulent but closely related species, *B. thailandensis*, is done based on the ability to assimilate L-arabinose (Chaiyaroj et al. 1999).

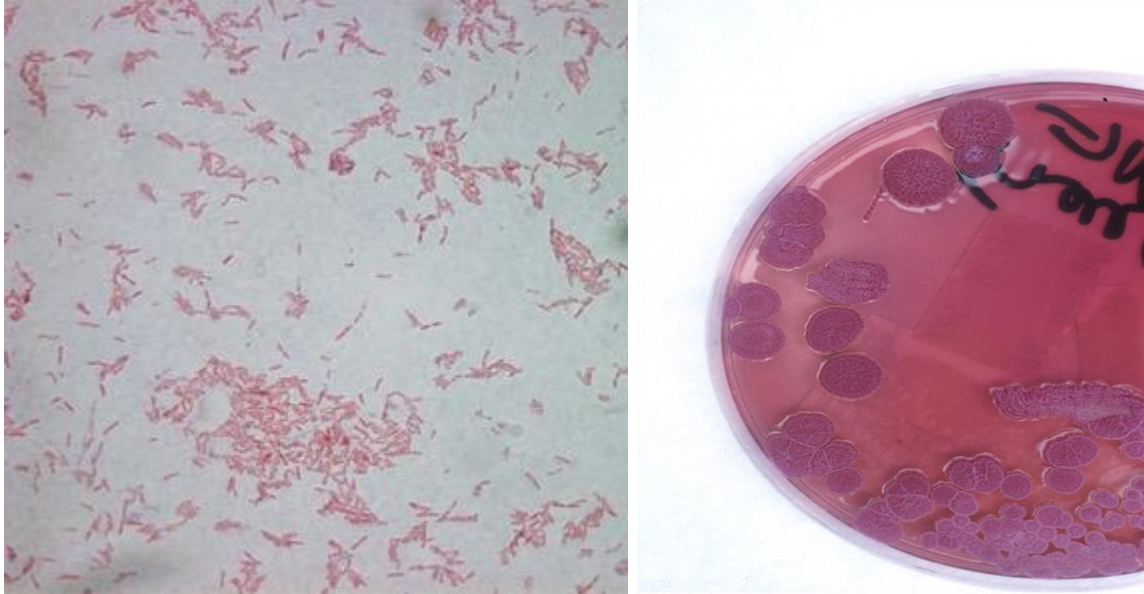


Figure 1- Typical Gram stain with Gram-negative bipolar appearance and characteristic dry, wrinkled morphology on Ashdown agar (Images courtesy of Menzies School of Health Research).

Epidemiology

Global Known and Predicted Occurrence

Melioidosis has recently been recognized as a significant source of global morbidity and mortality, with one recent model estimating 165,000 human cases and 89,000 deaths to occur annually worldwide (Limmathurotsakul et al. 2016). The vast majority of cases are diagnosed in Southeast Asia and Northern Australia, with these two regions representing hyper-endemic areas of the world (Currie et al. 2008). Thailand has by far the largest number of culture-confirmed cases of melioidosis, with 2,000 to 3,000 individuals diagnosed with the infection each year, most of which are reported in the northeastern provinces (Chaowagul et al. 1989; Limmathurotsakul et al. 2010). With recent advancements in diagnostic facilities

and better awareness of the disease, the *B. pseudomallei* endemic region has now been expanded to include parts of southern China, Taiwan, Malaysia, Indonesia, as well as a significant portion of the Indian subcontinent (Cheng & Currie 2005; Dance 1991). Here, recent modelling by Limmathurotsakul et al. (2016) projected more than 50,000 cases to occur per annum. Escalating numbers of endemic infections also continue to be reported from the Philippines, Papua New Guinea, and the Pacific Ocean islands of New Caledonia. Cases of melioidosis are increasingly being documented from elsewhere outside the classic endemic region as a result of improved clinical surveillance and laboratory diagnosis (Dance & Limmathurotsakul 2018), with occasional clinical cases and environmental isolates of *B. pseudomallei* reported in the Middle East, Africa, several Indian Ocean and Caribbean islands, as well as Central and South America (Benoit et al. 2015; Currie 2015; Hall et al. 2019; Sarovich et al. 2016). Sporadic cases from Europe and the USA are mostly considered to be travel-related, though recent human cases reported in Texas, USA suggest possible endemicity in the region (Birnie et al. 2019; Cossaboom et al. 2020). Tourists from nonendemic regions of the globe with diabetes and cystic fibrosis are at an increased at risk, particularly if exposed to rainy season soil and severe storms (Currie 2003; Geake et al. 2015; O'Sullivan et al. 2011).

Despite recent advancements, melioidosis still frequently goes undiagnosed in resource-poor settings, meaning that the true burden of disease is likely greater than what is currently acknowledged (Limmathurotsakul et al. 2016). Global climate change and extreme weather, combined with urbanization and alteration to soil composition, may cause an increase in the incidence and the geographical spread of melioidosis.

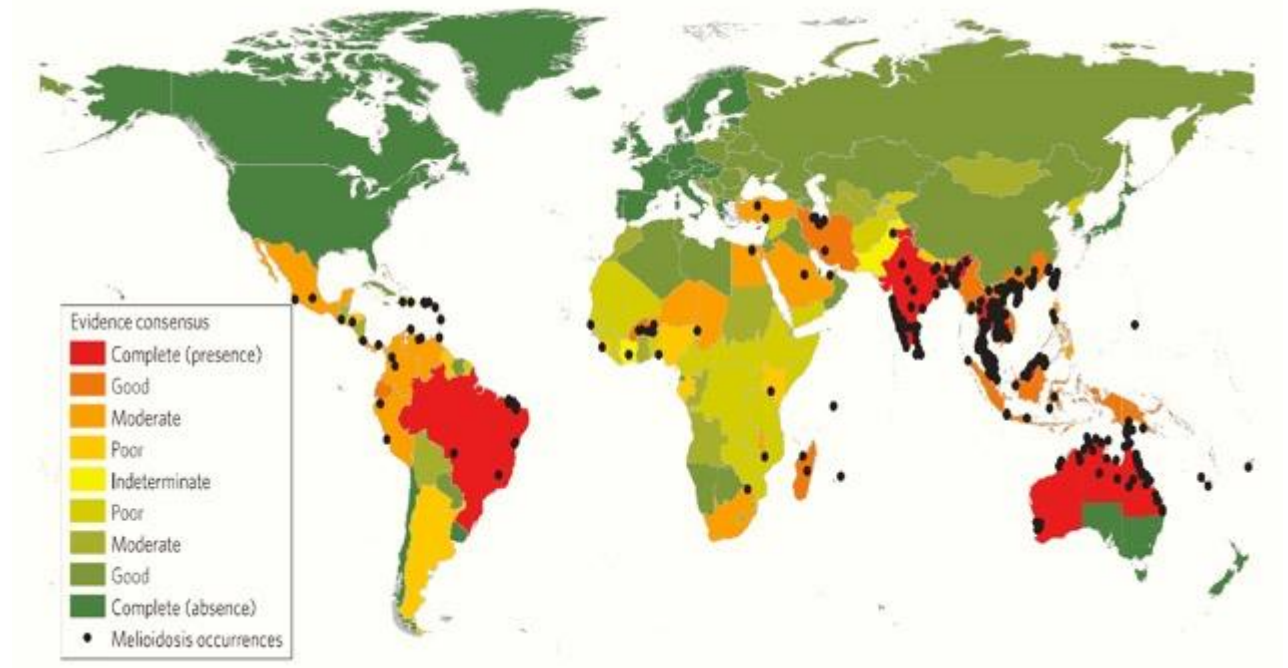


Figure 2- Evidence-based consensus for presence of *B. pseudomallei* and recorded geographic locations of melioidosis infection (Limmathurotsakul et al. 2016).

Routes of Exposure

Infection with *B. pseudomallei* typically occurs via percutaneous inoculation, inhalation, or ingestion of contaminated soil or water (Cheng & Currie 2005). In most regions, the disease is highly seasonal, with rainy season peaks corresponding with higher infection rates.

Vertical and sexual transmission (Thatrimontrichai & Maneenil 2012), zoonotic transmission from animals with melioidosis (Low Choy et al. 2000) and transmission to laboratory staff are very uncommon but have been documented (Benoit et al. 2015; Green & Tuffnell 1968; Schlech et al. 1981).

Inoculation with *B. pseudomallei*-contaminated soil is considered to be the primary route of melioidosis infection in humans, though further habitats for the bacterium and other potential sources of infection have been revealed in recent years (Currie et al. 2010).

Ingestion is now recognized as being a common route of infection in animals and studies from Southeast Asia have suggested that ingestion of *B. pseudomallei* from unchlorinated drinking water supplies and other freshwater sources may be more common in humans than previously acknowledged (Currie et al 2015; Limmathurotsakul et al. 2013; Zimmermann et al. 2018). Outbreaks of melioidosis in Australia have been associated with *B. pseudomallei* contaminated drinking-water supplies, with the implicated water sources shown to be unchlorinated or containing inadequate levels of chlorine (Currie et al. 2001; Inglis et al. 2000; McRobb et al. 2015). Though human-to-human transmission is rare, a reported case of transmission to an infant from a mother with melioidosis mastitis via ingestion of breast milk has been described (Ralph et al. 2004), as has an instance of possible sexual transmission (McCormick et. al 1975).

Inhalation via contaminated aerosols has also been recognized as a significant source of infection (Currie et al. 2015). These cases typically follow severe weather events, such as cyclones and typhoons, and may be associated with increased disease severity (Currie & Jacups 2003; Parameswaran et al. 2012). During severe weather events such as tropical monsoonal storms or cyclones, inhalation may become a more prominent route of *B. pseudomallei* transmission and infection (Cheng et al. 2006; Mu et al. 2014). Studies from northern Australia and Taiwan have demonstrated an association between the occurrence of cases and the timing and severity of rainfall-related events (Kaestli et al. 2016; Lam et al. 2012). Melioidosis pneumonia was also documented in patients after the December 2004 Indian Ocean tsunami. While these cases were thought to be primarily associated with aspiration events, inoculation via lacerations in the skin may have also occurred (Allworth 2005; Chierakul et al. 2005). Collectively, this suggests that cases of inhalational melioidosis could increase if current predictions about the growing number and severity of weather-related events are realized (Chen et al. 2015; Liu et al. 2015).

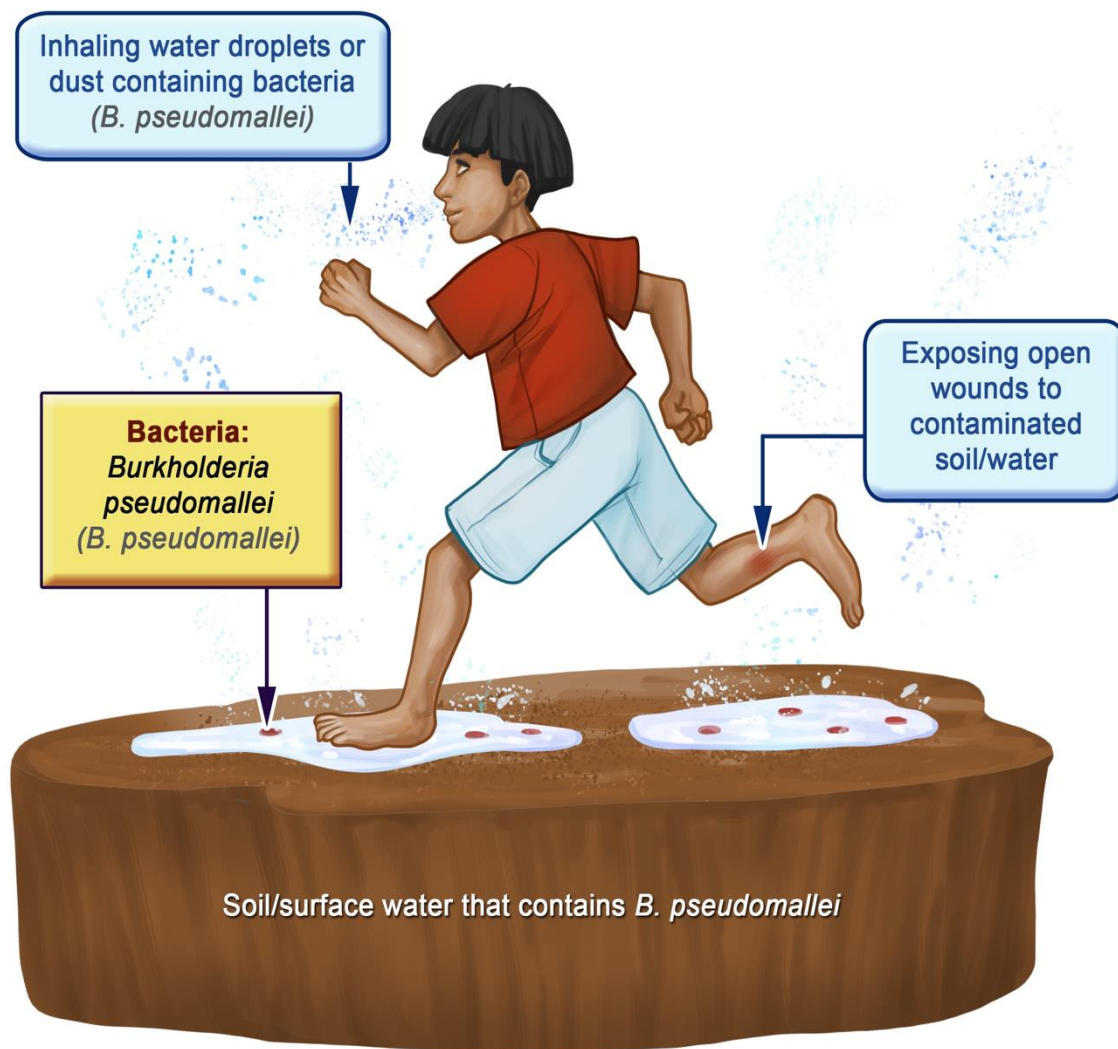


Figure 3- Routes of exposure to *B. pseudomallei* in the environment.

Risk Factors

Approximately 80% of patients diagnosed with melioidosis have one or more risk factor for the disease, however in children, who make up 5-15% of cases, identified risk factors are much less frequent (McLeod et al. 2014). Diabetes mellitus is the most common risk factor predisposing individuals to melioidosis and is present in >50% of all patients. Those with

diabetes mellitus are thought to have a 12-fold higher risk of melioidosis (after adjusting for age, sex and other disease-specific risk factors) (Currie et al. 2004; Limmathurotsakul et al. 2010; Wiersinga et al. 2018). Individuals with regular exposure to mud and surface water, such as rice farmers, are particularly susceptible, especially during the rainy season (Limmathurotsakul et al. 2013).

People with the following conditions are at increased risk for developing melioidosis (Currie 2008):

1. Diabetes (the strongest risk factor)
2. Excessive alcohol use
3. Chronic lung diseases, especially cystic fibrosis
4. Chronic liver disease
5. Chronic kidney disease
6. Malignancy, especially if on chemotherapy
7. Other forms of immunosuppression, especially long-term corticosteroid use
8. Thalassemia

Disease severity and clinical manifestations are influenced by the route of infection, virulence of the infecting *B. pseudomallei* strain, bacterial load, and most significantly, host risk factors. Infection with HIV (human immunodeficiency virus) does not appear to predispose an individual to development of the disease (Wiersinga et al. 2018). Death from melioidosis, particularly in those without known risk factors, is extremely uncommon with prompt diagnosis, appropriate antimicrobial therapy and effective sepsis management.

Chapter Summary

This preceding chapter gives a brief overview of melioidosis and its causative agent, *Burkholderia pseudomallei*. Melioidosis is still frequently underdiagnosed in many settings,

meaning that the true burden of disease is likely greater than what is currently acknowledged. The reasons for this are numerous, including the fact that it mainly affects the disadvantaged rural poor in areas without the necessary diagnostic facilities. The disease is likely to become more frequent in the years to come as risk factors, such as the prevalence of diabetes, as well the number and severity of weather-related events begin to increase.

Chapter 2: Clinical Presentations and Disease Progression

Chapter Overview

The following chapter discusses common clinical manifestations of melioidosis in humans and animals and the current recommended treatment guidelines. Control measures for the disease are also described. Since public awareness of melioidosis is limited, particularly in poorly-resourced countries, primary prevention should involve education about minimizing exposure to wet season soils, surface water, and aerosols generated during severe weather events. This is especially important for high-risk groups, such as diabetics, patients on immunosuppressive therapy and those on long-term steroid treatment.

Disease Progression in Humans

Infection occurs in all age groups, although only a small percentage of cases are diagnosed in children, many of whom have relatively mild, localized infections (McLeod et al. 2014). The majority of cases are diagnosed in adults aged 40 to 70 years, who tend to present with more severe infections (Currie et al. 2010; Suputtamongkol et al. 1994). The incubation period of acute infections is usually between 1 and 21 days, with a median of nine days (Currie et al. 2000). More severe disease often has shorter incubation times and is thought to occur following inhalation or aspiration of contaminated water (Chierakul et al. 2005). Melioidosis mortality rates have been demonstrated to be 10-50% with proper treatment (Wuthiekanun & Peacock 2006) and death can occur within the first 48 hours of hospital presentation as a result of septic shock (Limmathurotsakul et al. 2010).

While rare, it is possible for the disease to remain latent in some individuals, with clinical symptoms delayed by several months to years before melioidosis develops. Activation from latency has been estimated to account for <5% of all melioidosis cases (Currie et al. 2010).

Reported latency periods have ranged from 19 years to 29 years (Gee et al. 2017), suggesting *B. pseudomallei* may enter a dormant state to evade immune surveillance (Adler et al. 2009). The site of latency and mechanisms by which *B. pseudomallei* survives undetected remain uncertain, although there is evidence to suggest that some immunocompromising conditions may lead to activation of a latent infection (Gan 2005; Jabbar & Currie 2012).

Clinical Presentations in Humans

Disease manifestations vary from localized abscess or pneumonia to acute septicaemia, or may present as a chronic infection, though specific clinical presentations and severity may differ depending on the route of exposure, host immune system, as well as bacterial strain-specific virulence factors and inoculating dose (Wiersinga et al. 2018). While those infected frequently present with a septic illness, including pneumonia and disseminated hepatic, splenic and other organ abscesses, the disease can exhibit a diverse range of symptoms, giving rise to its nickname as the “great mimicker” (Yee et al. 1988). Initial bacterial multiplication at the site of entry may lead to a local lesion such as skin sore, abscess formation, or to pneumonia following inhalation. The progression of the disease is very variable, with some patients developing acute, fulminating infection and others developing chronic, indolent, granulomatous lesions, which may occasionally be asymptomatic (Kingsley et al. 2016).

Cutaneous melioidosis often presents with a solitary lesion at the site of inoculation and failure to respond to standard anti-staphylococcal/anti-streptococcal antibiotics (Currie et al. 2010; Suputtamongkol et al. 1999). Visceral abscesses are commonly documented in the spleen, liver, adrenals, parotids, kidneys, and the prostate. Skin involvement is seen more frequently in children (60%) than in adults (13%) (Currie et al. 2010; McLeod et al. 2014).

Cutaneous melioidosis may resolve spontaneously in some patients, but in others the organism may spread via the bloodstream or lymphatic system, leading to pneumonia and secondary abscesses (McLeod et al. 2014).



Figure 4- An ulcerated cutaneous lesion on the right ankle of a patient with melioidosis (Image courtesy of Menzies School of Health Research, Australia).

Most cases (85%) of melioidosis result in an acute infection. Apart from those with cutaneous-only melioidosis, the majority of these patients (regardless of the route of infection) present with bacteremia with or without pneumonia, and/or localized abscesses. Of these, approximately 20% develop septic shock (mortality up to 90%). In endemic regions acute pneumonia, particularly with upper lobe consolidation, may justify further investigation of melioidosis (Meumann et al. 2012; Wiersinga et al. 2018). While

rare, osteomyelitis and septic arthritis have been described resulting from penetrating injury or haematogenous dissemination. Additionally, mycotic vascular aneurysms, pericarditis, mediastinal masses, orchitis, and scrotal abscesses have been reported as clinical manifestations of the disease (Cheng & Currie 2005; Currie et al. 2010).

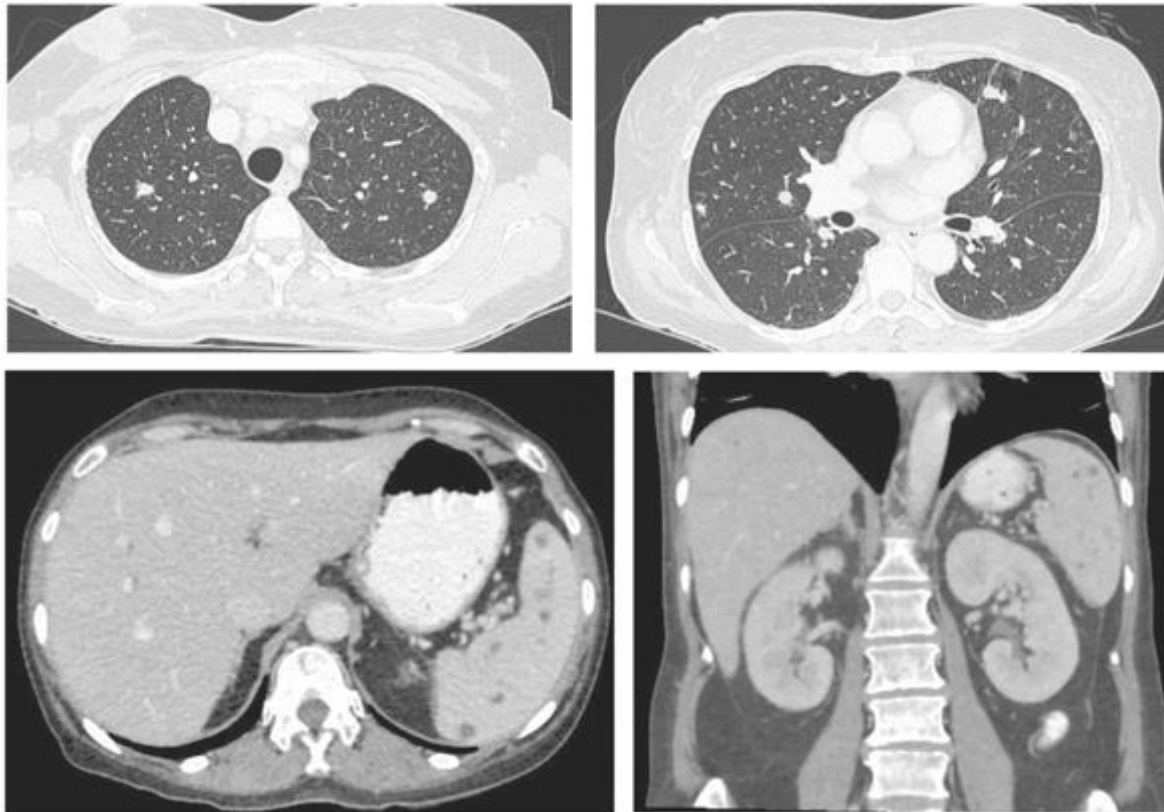


Figure 5- CT imaging showing multiple nodules in both lungs (top) and hypoechoic nodules in the liver and spleen (bottom) (Sukauichai & Pattarowas 2020).

There are several notable differences in presentation between patients in northern Australia and Southeast Asia. Suppurative parotitis, accounting for up to 40% of cases of melioidosis in children in Thailand and Cambodia, is very rare in Australia (Cheng & Currie 2005). In Australia, prostatic melioidosis is a more common clinical manifestation and is present in up to 20% of male patients. Neurologic melioidosis also occurs more frequently in Australian

patients and typically manifests as brainstem encephalitis, often with cranial-nerve palsies (especially cranial nerve VII), or as myelitis with peripheral motor weakness (Currie et al. 2010; McLeod et al. 2014). Current mortality rates for melioidosis are approximately 40% in northeast Thailand (35% in children) (Limmathurotsakul et al. 2010) and less than 14% in Australia (Currie 2015). This difference is likely due to a combination of access to diagnostic facilities and melioidosis-specific antibiotics, as well as state-of-the-art intensive care and severe sepsis management of patients.

While most patients develop acute melioidosis following recent infection, some individuals can develop chronic melioidosis (symptoms >2 months). Chronic melioidosis can mimic pulmonary tuberculosis in that the infection may or may not be disseminated and cavitary lesions in the upper lobes of the lungs can be seen in disseminated infections. In a 20-year prospective Australian study, chronic infection occurred in 11% of cases (Currie 2016).

Recurrent melioidosis can also occur, often in the first year after initial presentation (Currie 2015). Recurrent melioidosis following therapy can be defined either as relapse (with molecular typing showing the same strain) or re-infection with a new strain. Relapse correlates with duration of and compliance with the eradication therapy as well as severity of initial infection. In the past, recurrence was found to occur in as many as 13–23% of melioidosis patients. Improved antibiotic therapy and longer courses of treatment have significantly reduced the overall recurrence rate to as low as 1.2–7% (Chetchotisakd et al. 2014; Rachlin et al. 2016; Sarovich et al. 2014). Approximately a quarter of recurrences are due to reinfection while the remainder are due to relapse from a persistent focus of infection (Limmathurotsakul 2008).

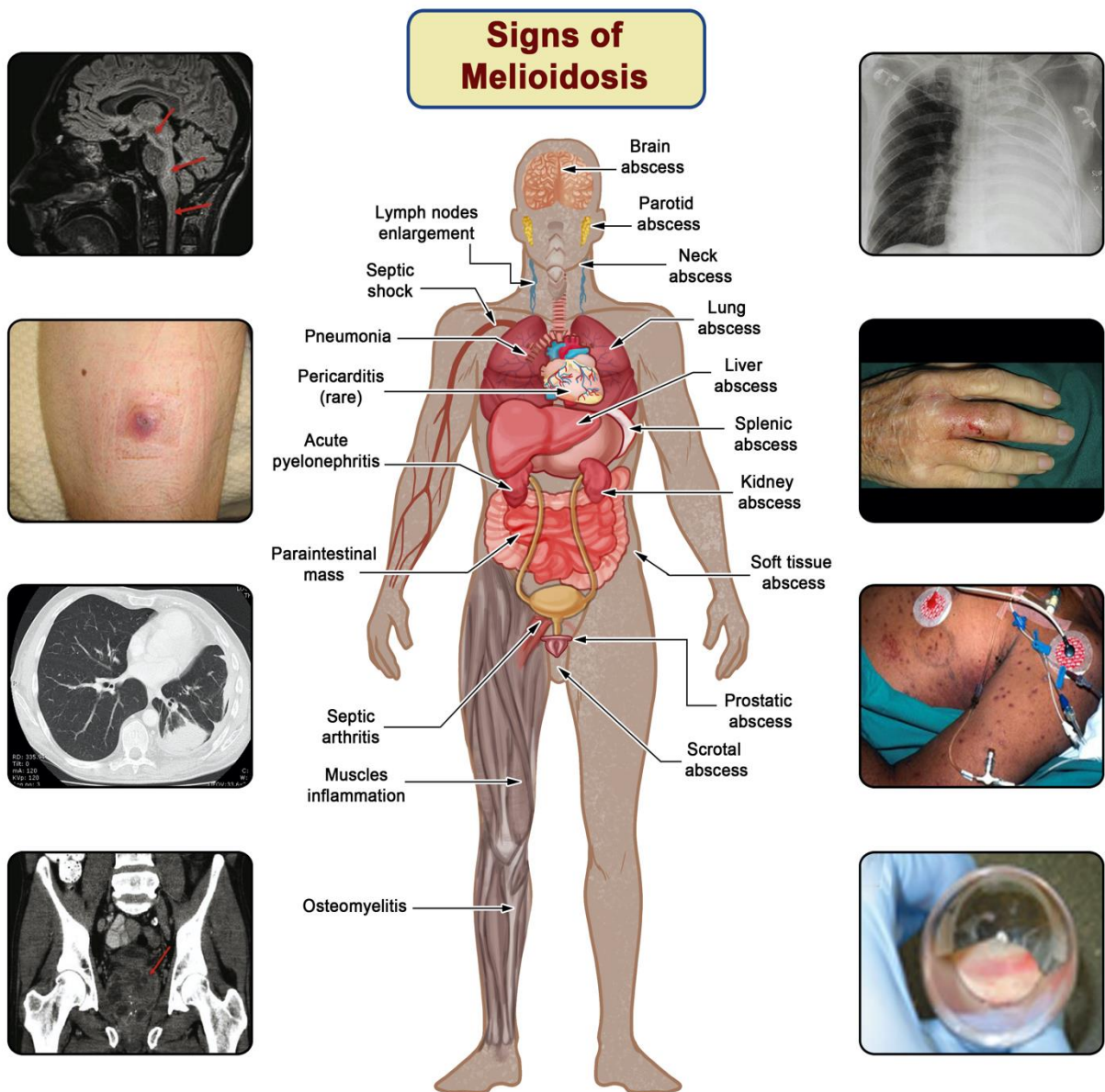


Figure 6- Possible clinical manifestations of melioidosis in humans (images courtesy of Bart Currie, Menzies School of Health Research, Darwin, Australia).

Disease Progression and Clinical Presentations in Animals

In melioidosis-endemic areas, the disease has been identified in a wide array of species including livestock, domestic pets, and exotic imported animals (Limmathurotsakul et al. 2012; Low Choy et al. 2000; Sprague & Neubauer 2004). While infection can occur in native wildlife, these animals are typically resistant to disease, with an underlying immunosuppressive condition usually accountable for infection (Asche 1991; Egerton 1963; Low Choy et al. 2000).

Certain species are acknowledged to be particularly susceptible to infection and disease, including goats (Tonpitak et al. 2014), sheep (Cottew 1950), camels (Forbes-Faulkner et al. 1992), alpacas (Low Choy et al. 2000), and marine mammals (Hicks et al. 2000). Exotic animals imported to endemic regions appear especially at risk, most notably non-human primates, including iconic species such as gorillas and meerkats (Rachlin et al. 2019; Sim et al. 2018). Several cases have also been documented in green iguanas imported to the USA and Europe from disease-endemic regions, highlighting the potential role the species may play as a reservoir for *B. pseudomallei* (Elschner et al. 2014; Hellebuyck et al. 2018; Zehnder et al. 2014). Although rare, outbreaks have also been described in endemic and non-endemic regions, including piggeries in Queensland, Australia (Ketterer et al. 1986), in imported sheep, goats and pigs from Aruba (Netherlands Antilles) and in a Paris zoo (Dance et al. 1992). Regular outbreaks described in intensive livestock farms and in zoos indicate that stressful conditions may lead to the initiation of disease onset, especially in non-native species. While cases of animal melioidosis are frequently described throughout Northern Australia, cases from Southeast Asia and other endemic countries lacking contemporary diagnostic facilities are very rarely reported (Limmathurotsakul et al. 2012).

Similar to humans, melioidosis is most likely to occur after heavy rainfall or soil disturbance, resulting in exposure to the bacterium. Animals can also become infected by ingesting contaminated water or soil and inhaling contaminated aerosols during heavy rainfall or strong winds. Additionally, infected animals may further spread the bacterium through contact with wounds, nasal secretions, milk, faeces and urine (Sprague & Neubauer 2004).

Clinical presentations of melioidosis in animals varies based on species. Symptoms can include depression, fever, weight loss, respiratory symptoms such as heavy breathing and sneezing, lameness and swelling of the joints, and death. Symptoms of melioidosis in particular species include (Merck Vet Manual 2016):

Animal	Susceptibility	Common Symptoms
Sheep	Very susceptible	Respiratory disease is common. Signs include cough, respiratory distress and discharge from nose and eye. Some sheep become lame and have swollen joints.
Goats	Very susceptible	Pneumonia is common with symptoms including severe cough, respiratory distress and discharge from nose and eye. Lameness, anorexia, neurological symptoms and mastitis are also frequently reported.
Camelids (camels, llamas, alpacas):	Very susceptible	Respiratory disease with coughing, nasal discharge and difficulty breathing is common. Hindleg weakness, lack of coordination, wasting and acute disease with death have all been reported.
Pigs	Moderately susceptible	Adults typically develop few clinical symptoms. Wasting, nervous signs such as lack of coordination, skin ulcers and diarrhoea have been all been reported. Juvenile pigs may develop acute infection with fever, anorexia, coughing and discharge from the nose and eye.
Horses	Moderately susceptible	Symptoms may include weakness, wasting, swelling of the limbs, colic, diarrhoea, coughing and nasal discharge. Skin infections resembling fungal eczema can later become

		<p>papular (blister-like lumps). Acute disease with high fever, limb swelling, diarrhoea and death have also been reported.</p>
Cat	Less susceptible	<p>Abscesses have been reported in various organs in cats. Jaundice, anemia, neurological disease and osteomyelitis have also been described.</p>
Dog	Less susceptible	<p>Curd-like nodules or abscesses in any organ. Blood infection, fever, diarrhea, and pneumonia have been described. Skin lesions and inflammation of the lymphatic system can occur, as well as loss of appetite, muscle pain, and swelling of the limbs.</p>
Cattle	Less susceptible	<p>Melioidosis is rarely reported in cattle. Signs in those reported include fever, difficulty breathing and nervous symptoms.</p>
Birds	Less susceptible	<p>Birds appear to be relatively resistant to infection. Cases with lethargy, anorexia and diarrhoea leading to death have been described.</p>

Table 1- Clinical symptoms of melioidosis in common domesticated and farmed animals (Low Choy et al. 2000; Merck Vet Manual 2016; Sprague & Neubauer 2004).



Figure 7- Gross pathology of melioidosis in meerkats. (a.) Typical ulcerative skin lesions (arrows). (b.) Stomach (asterisk) showing severe haemorrhage and purulent exudate in adjacent mesentery. (c.) Multifocal suppurative lesions throughout the liver. (d.) Lung with abundant coalescing red foci, representing severe, diffuse embolic showering of the lung (Rachlin et al 2019).

Treatment in Humans

Treatment for melioidosis consists of an initial intravenous intensive phase (Table 2) that lasts for approximately 14 days (though this can be longer when clinically indicated) and an oral eradication phase (Table 3). Appropriate oral eradication can dramatically decrease the risk of relapse, which can occur when intravenous antimicrobial drugs only are used.

Without eradication therapy, patients have a high risk of relapse and may develop serious disease with high mortality rates similar to those for primary infection. The relapse rate after completing the full eradication treatment is approximately 10%, but this can increase to 30% if oral treatment regimen is taken for less than eight weeks (Chaowagul et al. 1999; Lipsitz et al. 2012). Other studies have found that failure to complete at least 12 weeks of treatment is a primary determinant in disease relapse (Chetchotisakd et al. 2014). Recent studies from Australia support longer initial intravenous therapy (4 to 8 weeks or longer) for complicated pneumonia, deep-seated infection including prostatic abscesses, neurological melioidosis, osteomyelitis and septic arthritis (Pitman et al. 2015).

Intensive-Phase Antibiotic Treatment

Intravenous ceftazidime or meropenem are the preferred agents used during the intensive phase of treatment. Duration of therapy should be determined by the nature and severity of clinical presentations but should be given for a minimum of 10–14 days (Currie 2015). This should be increased for severely ill patients, including those with extensive pulmonary disease, deep-seated collections or organ abscesses, osteomyelitis, septic arthritis, or neurological melioidosis (Table 4) (Smith et al. 2018). In resource-poor settings it may not be affordable to extend intensive-phase treatment duration, but a minimum of 10 to 14 days is recommended (Dance 2014; Lipsitz et al. 2012). In this situation, completing a full course of oral eradication therapy is essential.

In patients with a collection (including skin ulcers/abscesses and abscesses in internal organs), and in bone/joint, genitourinary, or in CNS infections (but not for pneumonia), intravenous or oral trimethoprim/sulfamethoxazole may be added (Chierakul et al. 2005; Currie 2015). If trimethoprim/sulfamethoxazole is included, it should be continued for the entire duration of the intensive-phase treatment.

Repeat blood cultures in any bacteremic patient on day 7 of treatment and then weekly until negative. Patients with persistent bacteremia should have repeat blood cultures every 2 days after the start of antimicrobial therapy until negative. A repeat positive blood culture after >1 week of antimicrobial therapy typically indicates extensive bacterial burden or possible treatment failure, as does deterioration of clinical condition (worsening sepsis with organ dysfunction after 48 hours of therapy) (Lipsitz et al. 2012). Patients with persisting bacteremia or failure to respond to therapy should be examined for any undrained abscesses, and a switch from ceftazidime to meropenem should be considered for those on ceftazidime. Treatment failure is not normally related to the emergence of antimicrobial drug resistance (Lipsitz et al 2012). MICs should be performed where there is persistent or recurrent culture positivity (such as by E-test), in order to assess whether acquired resistance has developed.

Table 2- Recommended antimicrobials and dosages for initial intravenous intensive phase treatment of melioidosis. *Asterisk denotes agent of first choice. **NOTE-** Meropenem is often restricted to those requiring intensive care therapy, with ceftazidime used for all others. ¹In neurological melioidosis meropenem dose should be doubled to 40mg/kg/dose up to 2g 8-hourly.

Drug	Patient	Dosage/Route	Frequency
*Ceftazidime	Adult	Up to 2 g/kg intravenous	Every 8 h, or 6 g/d by continuous infusion after a 2-g bolus for at least 14 days
	Child	50 mg/kg intravenous	
OR ¹ Meropenem	Adult	Up to 1 g/kg intravenous	Every 8 h for at least 14 days
	Child	25 mg/kg intravenous	

For neurological melioidosis, osteomyelitis and septic arthritis, genitourinary infection including prostatic abscesses, and skin and soft tissue infections: ADD Trimethoprim/Sulfamethoxazole AND Folate (0.1mg/kg up to 5mg) PER DAY	Adult, >60 kg	320/1600 mg orally/intravenously	Twice daily
	Adult, 40–60 kg	240/1200 mg orally/intravenously	Three times daily
	Adult, <40 kg	160 /800 mg orally/intravenously	Twice daily
	Child	6 mg/30 mg/kg orally/intravenously	Twice daily

Eradication-Phase Antibiotic Treatment

Oral trimethoprim/sulfamethoxazole is the treatment of choice during the eradication phase of treatment (Chetchotisakd et al. 2014; Dance 2014; Lipsitz et al. 2012). Eradication therapy should be continued for a minimum of 3 months post cessation of IV therapy but can be extended to 6 months if neurological melioidosis, osteomyelitis or vascular mycotic aneurysms are present. Some patients with localized disease (should be confirmed by imaging to exclude other foci) who are systemically well may be safely treated with oral eradication therapy alone (Dance 2014; McLeod et al. 2014). Simultaneous administration of folic acid should be considered when giving trimethoprim/sulfamethoxazole in high doses for a long duration to reduce potential adverse effects associated with the drug’s anti-folate effect (e.g., bone marrow toxicity). Alternative but inferior eradication therapy for these patients is doxycycline or amoxicillin/clavulanic acid (Cheng et al. 2008).

Table 3- Recommended antimicrobials and dosages for oral eradication phase treatment of melioidosis. *Asterisk denotes agent of first choice.

Drug	Patient	Recommended Dosage/Frequency
Trimethoprim/sulfamethoxazole* AND Folate (0.1mg/kg up to 5mg) PER DAY	Adult, >60 kg	160 mg/800 mg tablets: 2 tablets every 12 h
	Adult, 40–60 kg	80 mg/400 mg tablets: 3 tablets every 12 h
	Adult, <40 kg	160 mg/800 mg tablets: 1 tablet every 12 h OR 80 mg/400 mg tablets: 2 tablets every 12 h
	Child	6 mg/30 mg/kg; maximum dose 320 mg/1,600 mg every 12 h
OR Doxycycline adults & children 12 years or older 100mg every 12 hours		
OR Amoxicillin/clavulanic acid (co-amoxiclav)	Adult, ≥60 kg	500 mg/125 mg tablets: 3 tablets every 8 h
	Adult, <60 kg	500 mg/125 mg tablets: 2 tablets every 8 h
	Child	20 mg/5 mg/kg every 8 h; maximum dose 1,000 mg/250 mg every 8 h

Table 4- Recommended duration of antibiotic treatment of melioidosis. ^a Use clinical judgement to guide duration if improvement is slow or blood cultures are still positive after 7 days. ^b Enlargement of any hilar or mediastinal lymph node >10mm diameter. ^c Abscess anywhere other than skin, lungs, bone, CNS or vasculature. ^d Time is the date of most recent drainage of collection where culture of the drainage grew *B. pseudomallei* or where no specimen was sent for culture; time is not reset if drainage is culture-negative. ^e Where simultaneous oral therapy is not indicated during the intensive phase, oral eradication therapy should begin during the start of the last week of intensive intravenous therapy. ^f May require life-long suppressive antibiotic treatment if vascular prosthetic surgery is performed (Sullivan et al. 2020).

Site of Infection	Minimum Intensive Phase Duration (Weeks) ^a	Eradication Phase Duration (Months) ^e
Skin abscess	2	3
Bacteraemia without focus	2	3
Pneumonia ICU admit OR mediastinal lymphadenopathy ^b OR bacteremia and bilateral or unilateral multi-lobar pneumonia	4	3
Pneumonia with bacteremia and pneumonia but only a single lobe involved OR bilateral or unilateral multi-lobar pneumonia but not bacteremia	4	3
All other pneumonias	2	3
Deep seated collection ^c	4 ^d	3
Septic arthritis	4 ^d	3
Osteomyelitis	6	6
Central nervous system infection	8	6
Mycotic aneurysm or other arterial infection ^f	8	6 ^f

Post-Exposure Prophylaxis

If high-risk laboratory exposure to *B. pseudomallei* occurs, post-exposure prophylaxis (PEP) is recommended. The first-line recommended PEP is oral trimethoprim–sulfamethoxazole (Table 5) (Peacock et al. 2008). Prophylaxis should last for 21 days. It is important that the potential benefits of PEP are considered before commencing, since trimethoprim–sulfamethoxazole can have severe adverse side effects. For low-risk exposure incidents, a decision to begin prophylaxis should be based on the presence of known risk factors for melioidosis in the exposed staff. Individuals with disease risk factors should be advised to start PEP. If staff have no known risk factors, monitoring for the development of symptoms or seroconversion is normally sufficient (Lipsitz et al. 2012; Peacock et al. 2008). See Chapter 4 for more detail about *B. pseudomallei* biosafety and high and low risk exposure incidents in the laboratory.

Table 5- Recommended *B. pseudomallei* post-exposure antibiotic prophylaxis. *Asterisk

Drug	Dosage	Frequency
Trimethoprim sulfamethoxazole*	TMP-SMX orally 8-40 mg/kg 2 x 160-800mg (960mg) tablets if more than 60kg, 3 x 80-400 (480mg) tablets if 40kg-60kg, and 1 x 160-800mg (960mg) OR 2 x 80-400 (480mg) tablets if adult less than 40kg PLUS Folate 0.1mg/kg up to 5mg PER DAY	Every 12 hours
OR Doxycycline	Adults & children 12 years and older 100mg	Every 12 hours
OR Amoxicillin clavulanic acid	20/5 mg/kg/dose (or 3 x 500/125 tabs) if >60 kg, and 2 x 500/125 tabs if ≤ 60kg	Every 8 hours

denotes first choice treatment agent (Peacock et al. 2008).

Antimicrobial Drug Resistance

B. pseudomallei is naturally resistant to many antimicrobial agents and should be considered when selecting an appropriate therapy. Antimicrobial agents that are considered unsuitable for treatment include penicillin, ampicillin, first and second generation cephalosporins, gentamicin, tobramycin, streptomycin, macrolides and polymyxins. Of note, clonal groups of isolates susceptible to gentamicin are common in Sarawak, Malaysia (Podin et al. 2014; Wiersinga et al. 2018). Primary ceftazidime resistance is thought to be rare but can occur naturally (<1%) (Lipsitz et al. 2012).

Prevention and Control in Humans

Public awareness of melioidosis, particularly in poorly-resourced countries with limited diagnostic capabilities, may be limited and preventive approaches are not always adopted. Primary prevention in endemic regions should involve education about minimizing exposure to wet season soils, surface water, and aerosols generated during severe weather events.

This is especially important for high-risk groups, such as diabetics, patients on immunosuppressive therapy and those on long-term steroid treatment. Patients with cystic fibrosis should also consider avoiding travel to areas of high endemicity during the rainy season (Boyd et al. 2016; Currie 2016). Closed footwear and gloves should be recommended to gardeners in highly endemic areas, and guidelines for the prevention of melioidosis in Thailand suggest that certain occupations such as rice farmers as well as travellers to the region should wear protective equipment if direct contact with soil or water is unavoidable (e.g., boots and gloves) (Suntornsut et al. 2020). Wounds or sores that have come into direct contact with soil or water should be thoroughly rinsed and cleaned to prevent infection. In higher risk individuals with underlying medical conditions, the cessation of smoking is advised, and the application of herbal remedies or organic substances to wounds is discouraged (Limmathurotsakul et al. 2013).



Figure 8- Various types of boots recommended for the prevention of melioidosis in rice farmers: Wellington boots (a), over-the-knee boots (b), hip boots (c) and half-body waders (d) (Suntornsut et al. 2016).

While appropriate chlorination of drinking water has been shown to be effective at preventing melioidosis outbreaks in Northern Australia, in resource-poor countries where water is untreated, drinking supplies should be boiled before consumption (Boyd et al. 2016; Howard & Inglis 2003). In high-income countries, ultraviolet light treatment can be implemented for the remediation of *B. pseudomallei*-contaminated private domestic water supplies, especially if household individuals are at an increased risk of infection (McRobb et al. 2013).

Prevention and Control in Animals

In animals, strategies to prevent potential infection with melioidosis are crucial for highly susceptible non-native species imported into endemic regions. This is particularly relevant for exotic species on display in zoos and wildlife parks. Adequate water hygiene is the predominant method of melioidosis prevention in endemic areas and animals should have their water supplies chlorinated (Low Choy et al. 2000) or sterilized using ultraviolet light filtration (McRobb et al. 2013). Additional strategies to prevent infection include limiting access to high-risk areas and providing adequate drainage of pens or enclosures to prevent surface water accumulation. Pens should preferably be concreted or made on dry solid ground while unpenned animals should be removed from the area of contamination (Fitzpatrick & Kearney 2008). Animals, such as non-human primates imported from melioidosis-endemic areas for laboratory purposes also present a potential occupational health and safety risk. Laboratory facilities importing animals from melioidosis-endemic regions should use precautionary measures to avoid potential exposures, including staff training in appropriate procedures for handling potentially infected animals, proper use of PPE and storage of baseline serum samples (Johnson et al. 2013).

Where animals are bred for human consumption, stringent control and disposal of sewage is necessary to prevent the spread of *B. pseudomallei* and the possibility of animal-to-human transmission (Inglis & Sousa 2009). Wearing gloves and disinfecting contaminated knives are recommended to prevent transmission during the processing of meat products (Ketterer et al. 1986). Since milk from goats and dairy cows may contain *B. pseudomallei*, pasteurization is recommended (Low Choy et al. 2000).

Case Definition Recommended by the U.S. Centers for Disease Control and Prevention

Clinical Description- Clinical presentation of melioidosis varies on a case-by-case basis. The following characteristics are typical of melioidosis.

- a) An acute or chronic localized infection which may or may not include symptoms of fever and muscle aches. Such infection often results in ulcer, nodule, or skin abscess; rarely as parotid abscess.
- b) An acute pulmonary infection with symptoms of high fever, headache, breathlessness, chest pain, anorexia, and general muscle soreness.
- c) A bloodstream infection with symptoms of fever, headache, respiratory distress, abdominal discomfort, joint pain, muscle tenderness, and/or disorientation.
- d) A disseminated infection with symptoms of fever, weight loss, stomach or chest pain, muscle or joint pain, and/or headache or seizure. Abscesses in the liver, lung, spleen, and prostate are often observed in patients diagnosed with disseminated infections; less frequently, brain abscesses may be seen.
- e) A neurological infection with symptoms of severe and persistent headache and fever, with or without neck stiffness, confusion and altered sensorium.

Laboratory Criteria for Diagnosis

Confirmed cases:

- a) Isolation of *B. pseudomallei* from a clinical specimen of a case of severe febrile illness: Culture of the bacterium may be done from various clinical samples such as blood, sputum or other respiratory secretions (like endotracheal aspirate), urine, pus, body fluids (like pericardial, pleural or peritoneal fluid), throat swab, or swabs/aspirates from organ infection (bone and joint) or abscesses or wounds.

Probable:

- a) Evidence of a fourfold or greater rise in *B. pseudomallei* antibody titer between acute- and convalescent-phase serum specimens obtained greater than or equal to 2 weeks apart.
- b) Evidence of *B. pseudomallei* DNA (for example, by validated polymerase chain reaction) in a clinical specimen collected from a normally sterile site (blood) or lesion of other affected tissue (abscesses, wound).

Case Classifications

Probable:

A case that meets the clinical case definition, one or more of the probable lab criteria, and one of the following epidemiologic findings:

- a) History of travel to a melioidosis-endemic region, OR
- b) Known exposure to *B. pseudomallei* as a result of intentional release or occupational risk (lab exposure).

Confirmed:

A case that is laboratory confirmed with clinical evidence.

Chapter Summary

The following chapter discusses common clinical presentations observed in human and animal cases of melioidosis. Current recommended treatment guidelines and recommendations for PEP are also described. Since public awareness of melioidosis is

limited, particularly in under-resourced countries with limited diagnostic capacity, primary prevention and control of the disease should aim to increase education about the infection.

This includes information about minimizing exposure to wet season soil, surface water and aerosols generated during severe weather events.

Chapter 3: Handling in the Field and Personal Protective Equipment (PPE)

Chapter Overview

B. pseudomallei is considered a biothreat agent by the U.S. Centers for Disease Control and Prevention (CDC) due its high case fatality rate, difficulty in diagnosis and treatment in regions where it is not endemic, intrinsic antibiotic resistance, and potential for aerosol spread. Use of appropriate personal protective equipment (PPE) is essential to prevent accidental exposure, whether in the field, or handling culture specimens in the laboratory. The following chapter describes the minimum PPE recommended for handling potentially contaminated material or live cultures containing *B. pseudomallei*.

Minimum PPE for Specimen Collection in the Field

Personal protective equipment (PPE) should be used to protect yourself from accidental exposure to *B. pseudomallei* and is an essential part of safely working with the bacterium. Individuals should wear PPE when working with wet soil and muddy water. This includes gloves, protective clothing and enclosed footwear. A well-fitted mask should be worn if exposed to aerosols generated from muddy water and wet soil. Proper respiratory protection, such as an N95 respirator, should be considered for areas like water storage tanks that may contain biofilms or sludge. A surgical-type mask does not protect against infection. Prior to sample collection, any skin abrasions (e.g., cuts, scratches, lesions, etc.) present on skin should be covered using water resistant bandaging. Always avoid any skin contact with contaminated materials, soil or water. If available, wear long sleeves and long pants to cover exposed skin. Wash hands as soon as possible after handling or collecting specimens or samples and after removing gloves (Peacock et al. 2008).

PPE for melioidosis clinical and/or environmental specimen collection includes but is not limited to: long sleeved gowns or aprons, eye goggles and gloves, shoe covers/boots, and a fitted respirator. The minimal PPE necessary depends on the activity being performed (Table 6).

Table 6- Recommended minimum PPE for specimen collection.

Activity	Minimum PPE
Specimen collection from humans and animals	Use standard precautions as per local routine practice (e.g., disposable gloves).
Environmental sample collection (soil, water, air, plants, biofilms, sludge)	Disposable gloves, boots or closed shoes that can be disinfected.

PPE for Laboratory Diagnostics

Laboratory personnel, particularly those who may handle live cultures of *B.*

pseudomallei, are at risk of laboratory-acquired melioidosis, since the procedures required to prepare specimens for culture may cause particles to aerosolize and release *B.*

pseudomallei into the air.

Adequate PPE in the laboratory is important to avoid accidental exposure to *B. pseudomallei* and prevent contamination of the laboratory space. Lab coats and gloves should be worn at all times when handling live cultures to prevent any skin from becoming exposed. Lab coats with a cuff are preferred and should extend the full length of the arm to the wrist. This ensures the glove can be pulled past the wrist and over the cuff, covering all skin. If a cuffed lab coat is not available there are several other ways to ensure the glove extends over the wrist. Using longer wristed gloves or fastening the end of the lab coat to the wrist with a tie or tape are both options. Eye and respiratory protection should also be used if there is any possibility of aerosolization or splashing. Procedures that may generate aerosols include

centrifugation, shaking/vortexing, pouring liquid, opening tubes that contain liquid and streaking plates. Sealed cups should be used in all centrifuges, and these should be opened only in a biological safety cabinet (BSC). When working with larger quantities of sample or with liquid specimens, eye protection and respiratory protection should be considered. Respiratory protection is essential when centrifuging or performing work on *B. pseudomallei*-suspected or confirmed cultures outside of a BSC (WHO 2020; BMBL 6th ed. 2020). Good laboratory practices and use of appropriate PPE will prevent most laboratory accidents involving exposure to *B. pseudomallei*. See Chapter 4 for further detail on *B. pseudomallei* biosafety in the laboratory.

Putting on and Taking off PPE

It is important to use safe lab practices while wearing PPE to protect yourself and prevent contamination. These include keeping hands away from the face, restricting the number of surfaces touched and changing gloves regularly. Hand hygiene should be performed between steps if hands become contaminated and immediately after removing all PPE, including gloves. Using the correct techniques for putting on and taking off PPE is extremely important to ensure optimal protection. PPE should be put on and taken off in a certain sequence to limit the spread of contamination.

Sequence for putting on PPE (see diagram below):

1. Gown (or long sleeves and pants)
2. Boots or boot covers (if required-see guidelines above)
3. Mask or respirator (if required-see guidelines above)
4. Goggles or face shield (if required-see guidelines above)
5. Gloves

Sequence for taking off PPE (see diagram below):

1. Boots or boot covers (if wearing)
2. Gloves
3. Goggles or face shield (if wearing)
4. Gown
5. Mask or respirator (if wearing)
6. Wash hands or use an alcohol-based hand sanitizer immediately after removing all

PPE

Additional Tips for Glove Use:

- Change gloves if torn or heavily soiled
- Discard gloves after use, never wash or re-use disposable gloves

Disposable items should be placed in a biohazard bag and sealed for disposal, while reusable items should be decontaminated in a 10% bleach or 70% ethanol solution. Reusable items typically include rubber boots, face shields and eye protection. These items should be soaked for at least 15 minutes before being rinsed off with water and allowed to dry.

SEQUENCE FOR PUTTING ON PERSONAL PROTECTIVE EQUIPMENT (PPE)

The type of PPE used will vary based on the level of precautions required, such as standard and contact, droplet or airborne infection isolation precautions. The procedure for putting on and removing PPE should be tailored to the specific type of PPE.

1. GOWN

- Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back
- Fasten in back of neck and waist



2. MASK OR RESPIRATOR

- Secure ties or elastic bands at middle of head and neck
- Fit flexible band to nose bridge
- Fit snug to face and below chin
- Fit-check respirator



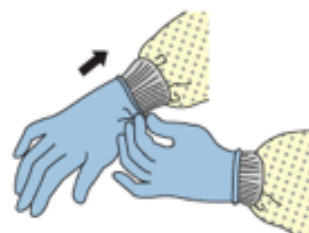
3. GOGGLES OR FACE SHIELD

- Place over face and eyes and adjust to fit



4. GLOVES

- Extend to cover wrist of isolation gown



USE SAFE WORK PRACTICES TO PROTECT YOURSELF AND LIMIT THE SPREAD OF CONTAMINATION

- Keep hands away from face
- Limit surfaces touched
- Change gloves when torn or heavily contaminated
- Perform hand hygiene

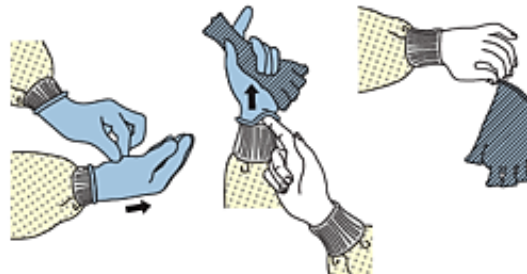


HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE) EXAMPLE 1

There are a variety of ways to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. Here is one example. **Remove all PPE before exiting the patient room** except a respirator, if worn. Remove the respirator **after** leaving the patient room and closing the door. Remove PPE in the following sequence:

1. GLOVES

- Outside of gloves are contaminated!
- If your hands get contaminated during glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Using a gloved hand, grasp the palm area of the other gloved hand and peel off first glove
- Hold removed glove in gloved hand
- Slide fingers of ungloved hand under remaining glove at wrist and peel off second glove over first glove
- Discard gloves in a waste container



2. GOGGLES OR FACE SHIELD

- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band or ear pieces
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container



3. GOWN

- Gown front and sleeves are contaminated!
- If your hands get contaminated during gown removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Unfasten gown ties, taking care that sleeves don't contact your body when reaching for ties
- Pull gown away from neck and shoulders, touching inside of gown only
- Turn gown inside out
- Fold or roll into a bundle and discard in a waste container

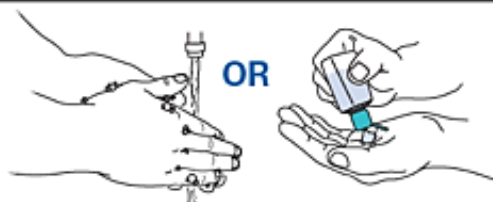


4. MASK OR RESPIRATOR

- Front of mask/respirator is contaminated — **DO NOT TOUCH!**
- If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front
- Discard in a waste container



5. WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE



PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS BECOME CONTAMINATED AND IMMEDIATELY AFTER REMOVING ALL PPE



Figure 9- Instructional diagrams/sequences for putting on and taking off PPE (CDC 2014).

Chapter Summary

The preceding chapter describes the minimum recommended PPE when handling potentially infectious material or specimens contaminated with *B. pseudomallei*. Use of PPE is essential to prevent accidental exposure to *B. pseudomallei*, whether in the field or in the laboratory.

Chapter 4: *B. pseudomallei* Laboratory Biosafety

Chapter Overview and Introduction to Biosafety

Biosafety refers to the safe handling and containment of infectious microorganisms and hazardous biological materials (BMBL 6th ed. 2020). It involves the principles, technologies and practices that are necessary to prevent unintentional exposure to pathogens and toxins, or their accidental release. In the context of *B. pseudomallei*, biosafety typically relates to the safe handling of the organism and samples in order to minimize the risk of exposure and what to do should exposure occur.

Biosecurity is complementary to biosafety and refers to security measures that are designed to prevent the loss, theft, misuse, or release of pathogens and toxins. This includes measures that address access to facilities, storage and accountability of materials and data as well as publication policies (BMBL 6th ed. 2020). *B. pseudomallei* is currently listed as a Tier 1 select agent by the U.S. Centers for Disease Control and Prevention (CDC) due to the current lack of a vaccine, the high mortality rate, potential for aerosolization, and significant antibiotic resistance profile. Appropriate biosafety is thus a primary concern when handling *B. pseudomallei* in the laboratory.

Risk Categories and Biosafety Levels

There are four risk groups for microorganisms. Risk Group 1 includes microorganisms that do not or are unlikely to cause disease in humans or animals, while Risk Group 4 includes pathogens that usually cause serious disease in humans or animals and can be transmitted from person to person. These organisms pose a high risk for both the individual and community and usually do not have effective treatment or preventative measures. *B.*

pseudomallei is listed as a Risk Group 3 pathogen by the WHO, since it does not normally spread by casual contact from one individual to another but can still result in serious disease with an increased risk of aerosol spread.

The design of the laboratory relates to the classification of microorganisms being brought into the space. There are four levels of laboratory design: Biosafety Levels (BSL) 1, 2, 3 and 4. The assignment of a microorganism to a biosafety level takes both the risk level and several other factors into consideration including:

1. Pathogenicity of the organism and infectious dose.
2. Mode of transmission and host range of the organism.
3. Immunity, density and standards of environmental hygiene of the local population.
4. Presence of appropriate vectors.
5. Reports of laboratory acquired infections.
6. Local availability of prophylaxis by immunization or effective treatment.

For example, a pathogen assigned to risk group 2 will usually require a BSL-2 facility.

However, if the experiments being conducted generate a high-concentration of aerosols, then a BSL-3 facility would be more appropriate (WHO 2020; BMBL 6th ed. 2020). Where biosafety facilities are available, BSL-2 practices and containment facilities are recommended when handling potentially infectious clinical materials and primary cultures of *B. pseudomallei* (BMBL 6th ed. 2020). It is recommended that if handling large volumes of *B. pseudomallei* culture or there is the potential for aerosolization to occur (such as during centrifugation or vortexing) that the specimen or culture be transferred to a BSL-3 facility as soon as possible.

In the United States, researchers and facilities handling select agents (including *B. pseudomallei*) must be registered with the Federal Select Agent Program (FSAP), inspected and approved by the relevant federal agencies before they can obtain the microorganism. BSL-3 containment equipment and facilities are recommended for all manipulations of suspect cultures, animal necropsies, and for experimental animal studies. BSL-3 practices are also recommended when preparing cultures and contaminated materials for automated identification systems, or when performing any procedure that can generate an aerosol (where possible). Elsewhere, practices should follow appropriate national biosafety guidelines (BMBL 6th ed. 2020). All laboratory workers, regardless of location, should undergo organism- and site-specific training. This should aim to include training for new workers and refresher training for all current workers done annually (Peacock et al. 2008).

***B. pseudomallei* Risk Assessment**

All handling of *B. pseudomallei* or specimens that may potentially contain it should be the subject of a risk assessment prior to starting any work with the organism. This should take into account the type of work, local circumstances, the nature of routine specimen handling, local melioidosis endemicity, and any predisposing risk factors for melioidosis in staff who may handle the organism. Laboratories should always take any local or national guidelines into account (Gassiep et al. 2021).

Staff with risk factors for the disease should be educated about their increased risk and alternative work arrangements should be discussed and provided if available. All laboratory staff working with *B. pseudomallei* with an identifiable risk factor should be examined for *B. pseudomallei* infection if they have any febrile illness symptoms, regardless of whether there is any history of laboratory exposure. Examples of high and lower risk work with *B. pseudomallei* are listed below and are shown in Table 7 (from Peacock et al. 2008).

High Risk:

- Predisposing condition without use of appropriate PPE, including diabetes mellitus, chronic liver or kidney disease, heavy alcohol use, chronic lung disease (including cystic fibrosis), thalassemia, immunosuppression or long-term steroid use.
- Needle stick or any other injury where skin is penetrated by an instrument contaminated with *B. pseudomallei*.
- Splash event resulting in contamination of mouth or eyes.
- Production of aerosols outside of a BSC through sonication, centrifugation, etc.

Low Risk:

- Unintentional opening of the lid of an agar plate growing *B. pseudomallei* outside a BSC.
- Unintentional sniffing of agar plate growing *B. pseudomallei*.
- Splash event amounting to contact of gloved hand/protected body part with *B. pseudomallei*.
- Spillage of small volume of liquid culture (<1mL) within a BSC.
- Contact of intact skin with bacterial culture.

LOW RISK	HIGH RISK
Accidental opening of agar plate lid growing <i>B. pseudomallei</i> outside a biological safety cabinet	The presence of any predisposing melioidosis risk factor without appropriate personal protective equipment (PPE)
Unintentional sniffing of agar plate growing <i>B. pseudomallei</i> in the absence of contact with bacterium	Needle prick or penetrating injury with any instrument contaminated with <i>B. pseudomallei</i>
Contact of <i>B. pseudomallei</i> with gloved hand or protected body following splash event, in the absence of any visible evidence of aerosol production	Bite or scratch by experimental animal infected with <i>B. pseudomallei</i>
Spillage of small volume of liquid culture (<1ml) in a biological safety cabinet	Splash event leading to contact with the mouth or eyes

Contamination of intact skin with culture	Production of aerosols outside biological safety cabinet (e.g. centrifuge incident)
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Table 7- Risk assessment of laboratory incidents involving *B. pseudomallei* (Peacock et al. 2008).

Laboratory-Acquired Infection with *B. pseudomallei*

Laboratory-acquired infections (LAIs) occur when a laboratory worker becomes infected.

This can happen when there are lapses in good laboratory practice (GLP), such as inappropriate specimen handling or waste decontamination. Poor laboratory staff training can also increase the risk of LAIs or other biological accidents in the laboratory, and may also contribute to improper pathogen storage, labelling and transportation (WHO 2020).

GLP will prevent most laboratory accidents involving exposure to *B. pseudomallei*. The organism has been routinely handled with minimal precautions in many laboratories in endemic areas without staff becoming infected, and while LAI with *B. pseudomallei* has been reported previously, these cases have typically followed lapses in GLP. This suggests that the risk of LAI with *B. pseudomallei* is lower than several other hazard group 3 pathogens, including *Brucella species* or *Francisella tularensis* (Dance et al. 2017; Gassiep et al. 2021; Green & Tufnell 1968; Schlech et al. 1981). This should be taken into account when conducting a risk assessment and when deciding on the need for post-exposure prophylaxis (PEP).

Laboratories should prepare a written protocol that describes how staff will be managed if an exposure incident occurs, including the arrangements available for immediate medical assessment. Where possible, a physician with experience in the treatment of melioidosis should be specified in advance. Where possible, baseline serum samples should also be

obtained from workers before starting work with *B. pseudomallei* and stored at -80°C .

Testing of serum is only required if a suspected exposure incident occurs (Peacock et al. 2008). A well-documented log of these samples should be kept.

If accidental exposure to *B. pseudomallei* is believed to have occurred, the site of contamination should be washed with clean water immediately, followed by appropriate disinfection of the area. The specified laboratory safety officer should be informed as soon as possible. The specific bacterial strain, its antimicrobial drug susceptibility (if known) and other pertinent information should be documented. While high-risk exposure events warrant increased attention and monitoring, all exposure events should be taken seriously. A risk assessment should be carried out that examines whether the exposure incident poses a low or high risk and if PEP is required (see Chapter 2 for further guidance on PEP dosage). Due to the significant health risks associated with PEP, it is advised that only those who have underlying risk factors that predispose them to melioidosis and are involved in high-risk exposure events are given treatment if exposure to *B. pseudomallei* has occurred (Peacock et al. 2008).

***B. pseudomallei* Laboratory Biosafety**

GLP will prevent most exposure to *B. pseudomallei* and suspected specimens should always be handled with appropriate Personal Protective Equipment (PPE), including gloves, gowns, closed shoes and goggles (see Chapter 3 for further detail regarding appropriate PPE).

Depending on the facilities available, handling of *B. pseudomallei* or suspected specimens should be done in a Biosafety Cabinet (BSC). BSCs are essential for safe laboratory work. BSCs are different from polymerase chain reaction (PCR) clean cabinets in that they provide protection both for the person performing the work and for the work being performed. The

risk for laboratory acquired infection is significantly decreased when a BSC used. However, work performed within a BSC should still be done carefully and safely. Initial primary culture inoculations do not require a BSC, however, where available, work should be transferred to one once *B. pseudomallei* is identified or is suspected or where there is the potential for aerosolization. If the bacterial identity is confirmed the risk of potential exposure to laboratory staff should be assessed.

All testing in the BSC should be done while wearing gloves to protect from infections through the skin (Peacock et al. 2008). Centrifugation and vortexing should be avoided outside of the BSC and plates and tubes should be taped shut. All suspected plates and tubes should be put inside of a well-marked sealable container and transferred to an incubator after appropriate disinfection once manipulations have been undertaken in a BSC. The

container should be transferred back to the BSC before it is opened to examine plates and bacterial cultures.



Checklist for Safe Use of Biological Safety Cabinets

This checklist is a template you can edit and modify as necessary to incorporate your laboratory-specific standard operating procedures (SOPs). Use this checklist as a daily reminder of the activities/tasks needed for safely working in a Biological Safety Cabinet (BSC), as a training tool, or for annual audit of operational protocols.

Biological Safety Cabinet (BSC) Checklist

Preparing for Work in a BSC:

- Put on PPE according to your laboratory SOP.
- Turn UV light OFF (if used).
- Turn fluorescent light ON.
- Turn the cabinet ON, allow it to run for 4 minutes (or Manufacturer's recommended time) to purge the BSC of particulates. Some cabinets may alarm until purge is complete.
- Verify proper sash height and that sash alarm is ON.
- Verify drain valve underneath the cabinet is closed (valve handle is perpendicular to valve body).
- Check cabinet's certification sticker expiration date is within 1 year.
- Record the pressure differential gauge reading (if present), compare it against the calibration set point.
- Verify inward airflow according to your laboratory SOP (e.g., tissue test, smoke test).
- Schedule uninterrupted work time, if possible.
- Decontaminate all surfaces of the cabinet, according to your laboratory SOP.
- Collect all materials needed for the work.

Safe Use of a BSC:

- Place absorbent plastic-backed material to protect the work surface, if required by your lab SOP.
- Wipe the external surfaces of any equipment or supplies that you will need to place in the BSC.
- Place materials as far back in the cabinet as practical without blocking front and rear grilles.
- Separate clean/sterile items from dirty/potential contaminated items inside the BSC.
- Work from clean to dirty to minimize cross-contamination.
 - One person at a time should work in a cabinet. If two people need to work in a BSC, use a 6' cabinet and document the protocol-driven need in a risk assessment completed by the Lab Supervisor prior to starting work.
- Move your arms slowly in and out of the BSC. Do not move arms in sweeping, sideways movements.
- If using the vacuum system, protect the vacuum line with a filter.
- Do not use open flame without approval from the Safety Office.
- Adjust the stool/bench height so your face is above the bottom of the sash and your arms enter the cabinet with elbows at 90° angles, armpits level with the bottom of the sash. Use a footrest if your feet do not touch the floor.
- Discard all waste in a biohazard bag **inside** of the BSC.

After Completing Work in a BSC:

- Do NOT turn cabinet OFF while removing items and decontaminating the cabinet.
- Close and surface decontaminate ALL containers and lab materials before removal.
- Surface decontaminate the exterior of the biohazard waste bag/container before removal and disposal.
- Decontaminate cabinet interior, including sidewalls, back wall, inside of sash and work surface.
- Remove the gloves you were using in the BSC and dispose of them according to your laboratory SOP.
- Turn fluorescent light and blower motor switches OFF.
- If required by your lab SOP, turn UV light ON. Keep the sash closed while the UV light is on.
- Wash your hands.

Working with *B. pseudomallei* Outside of a Biosafety Cabinet

In laboratories without access to a BSC and biosafety facilities, it is essential that appropriate GLPs are used, including training, competency assessments, and use of PPE (wearing lab gowns, gloves, goggles and closed footwear). Sniffing of open plates should be avoided. If *B. pseudomallei* is identified or is highly suspected in any specimen, staff should put on a well-fitted respirator (e.g. N95 mask) before continuing work. Respiratory protection is especially important when centrifuging or performing other procedures that may generate aerosols outside of a biological safety cabinet (BSC). Exposure to aerosols presents the greatest *B. pseudomallei* biohazard, potentially resulting in inhalation, ingestion, and mucous membrane contact. Staff should be trained in the appropriate use of equipment and equipment should be available at all times. PPE should be checked, maintained and restocked regularly. If working with confirmed or suspected *B. pseudomallei* outside of a BSC, laboratory staff in higher risk categories (e.g. members with diabetes, lung or kidney disease) should be identified and assigned to alternative tasks.

Laboratory and Biosafety Cabinet Decontamination

Steam autoclaving is the preferred method of sterilization. Materials for decontamination and disposal should be placed in containers such as autoclavable plastic bags that are color-coded according to whether the contents are to be autoclaved and/or incinerated. Work surfaces of the BSC and bench should be decontaminated after each diagnostic test and immediately following any spills of infectious material. All contaminated materials, such as gloves, paper products, etc., should be sterilized before disposal, for example by autoclaving (WHO 2020). Small spills within BSCs can be handled using a towel dampened with decontaminating solution. Large spills that result in liquids flowing through BSC grills require more extensive decontamination. All items inside the cabinet should be decontaminated and

then removed from the BSC. Once removed, decontaminating solution can be poured into the workspace, ensuring that the drain valve is closed. Thirty minutes is typically adequate for decontamination, but this may vary depending on the disinfectant used. Any spillage and disinfectant solution should be wiped up with paper towels and discarded into a biohazard bag (BMBL 6th ed. 2020; Wuthiekanun et al. 2011).

B. pseudomallei is susceptible to numerous disinfectants, including benzalkonium chloride, iodine, 10% bleach solution, 70% ethanol and 2% glutaraldehyde and is effectively killed by the commercial disinfectants, Perasafe and Virkon using a contact time of approximately 30 minutes (Wuthiekanun et al. 2011). The pathogen can also be destroyed by heating to above 74 °C for 10 min or by ultraviolet irradiation (Rose & O'Connell 2009).

Additional Technical Resources

General international biosafety guidelines are provided by the biosafety manual of the World Health Organization and can be found here:

<https://www.who.int/publications/i/item/9789240011311>.

For additional information regarding standard practices, safety equipment and facility specifications recommended for biosafety laboratories in the USA, see the Biosafety in Microbiological and Biomedical Laboratories- 6th Edition (BMBL 6th ed. 2020) available from:

https://www.cdc.gov/labs/pdf/SF_19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf.

Chapter Summary

B. pseudomallei is currently listed as a Tier 1 select agent by the U.S. Centers for Disease Control and Prevention (CDC) due to the current lack of a vaccine, the high mortality rate,

potential for aerosolization, and significant antibiotic resistance profile. Appropriate biosafety is thus a primary concern when handling *B. pseudomallei* in the laboratory. Researchers and facilities handling *B. pseudomallei* should always follow appropriate national biosafety guidelines when handling the bacterium in the laboratory.