



GLANDERS AND MELIOIDOSIS

GUIDELINES FOR ACTION IN THE EVENT OF A DELIBERATE RELEASE

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Note: Comments are welcome from healthcare, laboratory and public health professionals, and should be sent to DRcomments@hpa.org.uk. These guidelines may be subject to change as comments are received, so please ensure that you have the latest version available through the HPA website at: [http://www.hpa.org.uk/deliberate accidental releases/biological](http://www.hpa.org.uk/deliberate_accidental_releases/biological)

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1 BACKGROUND

These guidelines are intended for healthcare, laboratory and public health professionals to guide clinical and public health action in the event of a deliberate release of glanders or melioidosis.

1.1 Introduction

Burkholderia mallei and *Burkholderia pseudomallei* are the causative agents of glanders and melioidosis respectively.

Glanders is primarily a disease of equine species that may occasionally be transmitted to humans. Glanders is rare in most of the world, but is believed still to be endemic in parts of Africa, the Middle East, South and South East Asia, and Turkey.

Melioidosis is caused by an environmental saprophyte found in mud and water in tropical and sub-tropical regions, particularly South and South East Asia and northern Australia.

1.1.1 Deliberate release of glanders/melioidosis

Both *B. mallei* and *B. pseudomallei* are included in Category B of the CDC list of 'critical biological agents'. They have a number of features that make them candidates for deliberate release including:

- Ability to cause severe, rapidly fatal invasive infections
- Ability to initiate infections via aerosols, inoculation and, possibly, ingestion
- Intrinsic resistance to many antibiotics
- Ability to infect a wide range of animals as well as humans
- Long term persistence in the environment under suitable conditions (*B. pseudomallei*)

As a result, a number of countries, including the Soviet Union, Japan, and the USA, have conducted research into the possible weaponisation of these organisms, and *B. mallei* was actually used deliberately to infect animals by German agents during the First World War. Attempts to genetically modify *B. mallei* to render it more resistant to antimicrobials were also carried out in the Soviet Union.

1.2 Epidemiology

1.2.1 Transmission

For both organisms, it is likely that the natural mode of transmission is mainly by inoculation into small abrasions or by inhalation e.g. during heavy rains. Infection by inhalation is also the most likely route by which infection would occur following deliberate release. There is circumstantial evidence that glanders may be acquired by ingestion. This is less convincing for melioidosis, although two outbreaks in Australia were linked to contaminated potable water supplies. Person-to-person spread is extremely rare.

1.2.2 Infectious dose

The infectious dose of *B. pseudomallei* for humans is not known, but probably varies considerably according to host resistance. Most infections with *B. pseudomallei* in previously healthy individuals have followed gross contamination (e.g. near-drowning episodes, contaminated war wounds, inoculation with contaminated medications, laboratory accidents). However, other cases are thought to have occurred following relatively minor exposure (e.g. inhalation of aerosols by helicopter crew). In some susceptible animals, the lethal dose (LD₅₀) by inoculation is less than 10 organisms.

Even less is known about the infectious dose of *B. mallei* for humans, although the high incidence of infection amongst laboratory workers in the past implies that it may be even more infectious than *B. pseudomallei*. Work by Japan's 'Unit 731' estimated the infectious

dose (ID₅₀) of *B. mallei* as 0.2 mg by subcutaneous inoculation, although the equivalent inoculum in colony forming units is unclear.

1.2.3 Incubation period

Incubation period is extremely variable and probably depends on the size and route of the inoculum, as well as the intrinsic virulence of the strain concerned and the immune status of the host. The mean incubation period for melioidosis following defined inoculation injuries in Australia was 9 days with a range of 1-21 days (Currie *et al.*, 2000). Following near-drowning episodes, septicaemia usually occurs within 4 days. However, latent intervals as long as 62 years have been reported in natural melioidosis (Nguay *et al.* 2005).

1.2.4 Period of communicability

The infection is not highly communicable and there are only two well documented instances of person-to-person spread of melioidosis. However, excretion of the organism (e.g. in sputum) may continue for many weeks despite appropriate treatment, and both clinical and microbiological relapses are common.

Spread of glanders from person-to-person has been reported rarely, but since most naturally occurring human cases result from close contact with infected animals, it may be more communicable than melioidosis.

1.3 Clinical features

These are also very variable, but the spectrum is similar for both organisms. There is far more recent clinical experience of human melioidosis than glanders. The extent to which manifestations might vary according to the route of infection is unclear. Pneumonia may follow inoculation, but appears particularly common following heavy rains, presumably as a result of inhalation (Currie & Jacups, 2003). It is almost impossible to diagnose either infection on clinical grounds alone.

One form of disease may progress to another, and infections may present acutely with rapid progression and death, or run a chronic and relapsing course. It should also be remembered that strains used in deliberate attacks might have been modified in order to enhance or alter virulence.

1.3.1 Overwhelming sepsis

The clinical picture is typical of sepsis syndrome resulting from other infections. There will probably be evidence of primary local inflammation according to the route of infection (e.g. most likely pneumonia, but pharyngitis, skin or soft tissue infection are also possible). Metastatic foci of infection are established rapidly during bacteraemia, particularly in the lungs (multifocal pneumonia, which may cavitate), liver, spleen and kidneys (multiple abscesses), skin and soft tissues (cellulitis, pustules), bones and joints, lymph nodes and prostate, although any site may be affected.

1.3.2 Pyrexia of unknown origin

The fever is usually high and swinging. Bacteraemia may be present intermittently, and deep-seated visceral abscesses (especially in the liver, spleen, kidney and prostate) should be sought.

1.3.3 Localised infection

The commonest site for localised melioidosis is the lung. Areas of consolidation, particularly in the upper lobes but with relative apical sparing, may be single or multiple,

and often progress to cavitation. This is often confused with pulmonary tuberculosis. Other well-described forms include skin or soft tissue infection, visceral abscesses (as above), lymphadenitis, osteomyelitis and septic arthritis, and brain abscesses. Parotid abscesses are common manifestations of melioidosis in children.

1.4 Mortality

The overall mortality of severe/ bacteraemic melioidosis approaches 100% if untreated, but can be reduced to around 20-40% with optimal management and aggressive intensive care. Localised melioidosis has a much lower mortality rate (4-5%). There have been too few human cases of glanders in the post-antibiotic era to make an accurate estimate of mortality rate, but it is likely to be similar to that of melioidosis.

1.5 Organism survival

Although *B. pseudomallei* does not form spores, it is able to survive for several years in moist soil and water at tropical ambient temperatures. The organism was also reported to have survived for several years in soil in France during an outbreak in the 1970's.

B. mallei is generally less robust than *B. pseudomallei*, but it has been reported to survive for up to 4 weeks in water.

Both organisms are susceptible to killing by a variety of disinfectants (e.g. sodium hypochlorite at 500 ppm chlorine for 5 minutes or 5% phenol for 10-15 minutes), although little recent work has been done on this subject. There is no reason to believe that they would be more resistant than other Gram-negative bacteria (e.g. *Pseudomonas aeruginosa*) to orthodox disinfection regimens. Soil decontamination using hypochlorite was attempted during the French outbreak, but it is unclear whether this contributed at all to the ultimate disappearance of the organism from contaminated areas.

1.6 Antimicrobial susceptibilities

B. pseudomallei is intrinsically resistant to the earlier beta-lactams, all aminoglycosides, colistin and polymyxin, and relatively resistant to fluoroquinolones. It is susceptible *in vitro* to chloramphenicol, tetracyclines, cephalosporins such as ceftazidime, carbapenems, ureidopenicillins, and co-amoxiclav. *B. mallei* has a similar antibiogram, but is susceptible to aminoglycosides.

Fifty isolates of *B. pseudomallei* and 15 of *B. mallei* were tested for their susceptibilities to 35 antimicrobial agents (Thibault *et al.*, 2005) - lower MICs were obtained with imipenem, ceftazidime, piperacillin, doxycycline and minocycline. Generally fluoroquinolones and aminoglycosides had poor activities. One isolate of *B. pseudomallei* was found to be resistant to ceftazidime, co-amoxiclav and doxycycline but sensitive to imipenem. The results underline the importance of resistance in both species.

Clinical trials have shown that beta-lactams such as ceftazidime and imipenem or meropenem are the treatments of choice during the acute phase of melioidosis. The addition of co-trimoxazole to ceftazidime does not reduce the acute mortality rate (Chierakul *et al.*, 2006). A prolonged course of oral antibiotics ('eradication phase') is also required to reduce the risk of relapse, either as a combination regimen comprising doxycycline and co-trimoxazole (Chetchotsakd *et al.* 2001; Chaowagul *et al.* 2005), or co-amoxiclav (amoxicillin-clavulanate) alone. Chloramphenicol, was originally included in the combination regimen, but appears to offer no therapeutic advantage and increases the risks of toxicity. On the basis of *in vitro* susceptibility and limited clinical experience, *B. mallei* is assumed to respond to similar regimens.

Recent consensus guidelines based on available pharmacokinetic evidence, clinical trials and clinical experience recommend ceftazidime or carbapenem as first line treatment followed by a prolonged oral eradication course of cotrimoxazole (with or without doxycycline). The guidelines also recommend co-amoxiclav at 20/5mg/kg orally three times daily, as a second line agent in eradication treatment (Cheng *et al*, 2008).

Fluoroquinolones do not provide good post-exposure prophylaxis (PEP) of experimental *B. pseudomallei* in mice (Steward *et al*. 2005). Doxycycline alone (Chaowagul *et al.*, 1999) and ciprofloxacin in combination (Chetchotisakd *et al.*, 2001) have proved disappointing during the eradication phase of treatment, whereas co-trimoxazole has been successfully used alone for eradication in Australia (Cheng & Currie, 2005). A recent study of pre- and post-exposure prophylaxis with doxycycline, co-amoxiclav and co-trimoxazole in mice exposed to aerosolised *B. pseudomallei* through the inhalational route found that oral co-trimoxazole is an effective prophylactic particularly if it is given within 48 hours of exposure (Sivalingam *et al*, 2008). Peacock *et al* (2008) suggest co-trimoxazole as the agent of first choice for PEP, with doxycycline and co-amoxiclav as alternatives, for a period of three weeks.

2 CLINICAL PROCEDURES

2.1 Diagnosis and collection of samples

It is very difficult to make a specific diagnosis of melioidosis or glanders on the basis of clinical features alone. Rapid diagnostic tests, most notably immunofluorescent microscopy on pus or secretions (Wuthiekanun *et al.*, 2005), are of value in endemic areas but are not currently available within the UK. A specific diagnosis thus usually depends on culture and identification of *Burkholderia* spp. from clinical material. The organisms may be isolated from virtually any sample, but particularly blood cultures, sputum, pus or swabs and urine. Serological testing may be useful later in the course of disease, and is available from the Reference Laboratory (see section 3.4).

2.1.1 Precautions for sampling

The samples outlined below should be taken to confirm the diagnosis. These must be taken using Universal Precautions and with the utmost care to avoid inoculation injuries. The procedures for transporting samples to the laboratory are outlined in section 3.6. The receiving laboratory should be telephoned to expect arrival. Chain of evidence documentation should also accompany all specimens; however in larger incidents this would only be required for several of the initial cases.

2.1.2 Samples to be taken from acutely ill humans

Specimens to be obtained should include:

- Blood cultures
- Sputum (if evidence of pneumonia)
- Pus (if suppurative lesions present)
- Urine
- Serum - acute and convalescent
- Other samples as indicated by clinical features

2.1.3 Post-mortem specimens

Samples may also be taken post-mortem to assist diagnosis. However, full autopsy examinations are strongly discouraged if either glanders or melioidosis are suspected (see 2.3.5).

2.1.4 Transport of samples

Strict procedures should be followed for the transport of samples of suspected melioidosis or glanders both from the clinical environment to the laboratory, and from local laboratories onto the reference laboratory. These are outlined in section 3.5.

B. mallei and *B. pseudomallei* samples fall into Category A for the purposes of transport. Samples and request forms should be identified as 'High risk', specifically mentioning the suspicion of melioidosis or glanders, and transported according to procedures for dealing with samples suspected of containing Category A pathogens. All samples should be transported as per UN 602 as described in "Appendix 1.2 Transport of infectious substances" in "Biological agents: Managing the risks in laboratories and healthcare premises." Advisory Committee on Dangerous Pathogens (ACDP), Health and Safety Executive (HSE) May 2005 accessed at <http://www.advisorybodies.doh.gov.uk/acdp/managingtherisks.pdf>.

Chain of evidence documentation should also accompany all specimens; however in larger incidents this would only be required for several of the initial cases.

2.2 Treatment

Resuscitation and supportive treatment according to normal practice are essential. This will include restoration of blood volume and correction of metabolic abnormalities. Septic patients may need to be managed in an Intensive Care Unit. Abscesses should be drained where possible.

The following treatment recommendations should be adjusted in the light of *in vitro* susceptibility profiles of any isolates obtained.

In the event of a deliberate release of these bacteria, the possibility of genetic manipulation to increase antibiotic resistance should be borne in mind.

2.2.1 Melioidosis

Initial treatment for patients with severe melioidosis should be with one of the following:

- **Ceftazidime** 120mg/kg/day as 3 IV doses (adults usually 2g tds)
- **Meropenem** 50mg/kg/day as 3 IV doses (adults usually 1g tds)
- **Imipenem with cilastatin** 50mg/kg/day as 3 IV doses (adults usually 1g tds)

All doses should be adjusted appropriately in patients with renal impairment. If these agents are unavailable due to shortages, treatment with co-amoxiclav and cefoperazone-sulbactam have also been shown to be effective, but expert advice (see section 5.0) should be sought. The duration of intravenous treatment will depend on the site of infection and clinical response; **the minimum duration for all patients is 14 days.**

The recommended **oral 'eradication'** regimens, to complete a total of **20 weeks** treatment, are:

- **Doxycycline** 4mg/kg/day plus **co-trimoxazole** (sulfamethoxazole 40mg/kg and trimethoprim 8mg/kg) oral daily
- or if doxycycline and/or co-trimoxazole are contra-indicated (especially for children and pregnant women) **co-amoxiclav** 20/5mg/kg orally three times daily (usual adult dose two co-amoxiclav 625 mg tablets every 6 hr)

Prolonged intensive phase parenteral therapy is generally used for deep-seated infections such as osteomyelitis, multiple undrained abscesses, or CNS infection. Patients with mild infections may be treated with the oral regimens alone for 12-20 weeks. The combination regimen results in a lower relapse rate but is associated with more side effects.

2.2.2 Glanders

There is little evidence available on antibiotic treatment of glanders, and only anecdotal reports have led to a recommendation to use sulphonamides in the past. Cases of glanders should be treated with the same regimens used for melioidosis. Alternatively, for *B. mallei* only **Gentamicin** 5mg/kg once daily for **2 weeks** plus oral **Co-trimoxazole** 40/8 mg/kg/day continued for 2 weeks (or longer depending on response) is likely to be effective. Antibiotic doses should be adjusted according to renal function, and Gentamicin serum levels should be monitored.

2.2.3 Follow-up

In view of the recalcitrant nature of these infections and their tendency to relapse, long term follow-up is required. In addition to clinical and radiological monitoring of resolution of focal infection, inflammatory markers such as C-reactive protein (CRP) may give an

early indication of relapse. Positive cultures should be repeated regularly (weekly during parenteral treatment and monthly thereafter) during convalescence in order to detect the emergence of resistance to the antibiotics used.

Patients should be followed up regularly for at least 5 years following recovery, including regular measurement of inflammatory markers such as CRP and convalescent serology. Patients should be warned that there may be a lifelong risk of relapse, and should be told to alert health care staff to their previous history if they subsequently develop a severe febrile illness.

2.3 Infection Control Practice

2.3.1 Decontamination of exposed persons

The risk of acquiring infection from contaminated clothing of exposed persons is thought to be low. Heavily contaminated individuals should be instructed to remove outer clothing, which should then be treated as High Risk (Infected) linen according to local policies. Such individuals should also be instructed to shower with soap and water, ideally in a special decontamination facility if available. An incident specific risk assessment will be required.

2.3.2 Isolation of patients

Although the risk of person-to-person spread is extremely low, it has been reported, and contamination of the environment from a case is also possible. **Cases should be nursed in Standard Isolation, wherever possible, until no longer culture-positive.**

2.3.3 Cleaning, disinfection and waste disposal

Normal procedures for Standard Isolation are appropriate. Contaminated environmental surfaces should be cleaned with hypochlorite solution.

2.3.4 Special precautions

Melioidosis is an opportunistic infection, to which immunocompromised patients, (particularly diabetics, patients with chronic renal disease, and those on steroid treatment) are especially susceptible. Patients with cystic fibrosis are also vulnerable to pulmonary melioidosis. **Health care staff known to be immunocompromised, including diabetics, should not have direct contact with cases of melioidosis and glanders.**

2.3.5 Post-mortem Procedures

Autopsy

The risk of acquiring melioidosis following contact with the body of a person who has died from the disease is negligible, because person-to-person transmission is very rare and there is no evidence of autopsy transmission. However, the risk for glanders is low because both person-to-person and autopsy transmission can occur.

Autopsy examinations are strongly discouraged if either melioidosis or glanders is suspected, as the body fluids and tissues in a patient who has died of either disease are likely to have large numbers of the causative bacteria present. If an autopsy is necessary expert advice must be sought from the HPA. The Pathologist must be informed of the known or suspected diagnosis. Standard precautions for post-mortem examinations on patients infected with Containment Level 3 organisms are appropriate. Instruments should be autoclaved.

Similarly, body preparation should be carried out with normal control of infection procedures. Standard precautions for the disposal of bodies infected with Containment Level 3 pathogens should be observed, and the undertaker should be informed. **Cremation** is the preferred method for disposal of the deceased. **Embalming** of bodies should not be undertaken because the body fluids are likely to contain large numbers of the causative bacteria and therefore the process of embalming exposes the embalmer to an unacceptable risk.

Pacemaker removal

Pacemaker removal is permitted. Pacemaker should be treated with hypochlorite solution (10,000 ppm available chlorine), bagged and disposed of appropriately (not by incineration).

2.4 Prophylaxis of persons exposed to glanders/ melioidosis

Although there is no evidence of the protective efficacy of post-exposure antibiotic prophylaxis in preventing human melioidosis or glanders, on the basis of animal experiments the following 7 day regimen is currently recommended for those known to have been exposed to heavy contamination:

Doxycycline 100mg twice daily

or

Co-trimoxazole 960mg oral twice daily (for children under 12 years of age sulfamethoxazole 40mg/kg and trimethoprim 8mg/kg oral daily)

Other exposed persons should be monitored and asked to self-record temperature twice daily for 21 days and to report immediately for medical attention in the event of febrile illness (temperature >38°C) or development of cough.

2.4.1 Immunisation

There is no human vaccine available for either agent at present.

2.4.2 Monitoring of exposed persons

Anyone who is known to have been exposed to a deliberate release of either organism may be at lifelong risk and should be advised always to bring this to the attention of any doctor they consult subsequently for a febrile illness.

2.4.3 Contacts of cases

There is no need to provide antibiotic prophylaxis to contacts of patients unless there is concern they were also exposed to the initial release.

2.5 Environmental Decontamination

Although prolonged survival of both organisms in the environment has been reported the risks of secondary aerosolisation or infection from a contaminated surface are probably low. In temperate climates, the level of contamination is likely to decline over time as a result of drying and exposure to sunlight.

Environmental decontamination is not recommended, except for highly localised contamination (e.g. in the laboratory) in which case standard antibacterial disinfection according to local policies should be used.

It would be prudent, however, to monitor the extent and level of contamination sequentially, and exclude non-essential personnel from the contaminated environment as far as possible. Expert advice should be sought.

2.6 Protection of frontline workers

This includes all emergency staff involved in management at the scene of a release, and staff involved in the care of patients.

2.6.1 Protective clothing

In the event of a known deliberate release of *Burkholderia* spp., emergency service staff entering a heavily contaminated area or **exposed zone** should wear that presents a high risk. Any personnel entering this zone should wear a biologically-resistant suit with outer gloves and boots (for example a CR1, PRPS or gas-tight suit), and a correctly fitting high-efficacy particulate respirator of FFP3 standard **AT ALL TIMES**.

Healthcare workers will not normally be asked to enter this zone, but may be called into it to treat casualties, for example if an explosive device has accompanied the release of biological agent. In this case the appropriate protective clothing should be worn.

Exposed persons will normally be moved from the exposed zone, through decontamination if necessary, and into a place of safety for medical assessment. Those involved in decontamination, and others who have any contact with contaminated clothing and fomites should wear the appropriate protective clothing and equipment. Emergency staff who attend exposed persons after decontamination has been completed do not need to take special precautions other than observing standard Universal Precautions.

For healthcare workers involved in the management of hospitalised patients, standard Universal Precautions (gloves, gowns, masks and hand washing) provide sufficient protection, and mortuary staff should use similar barrier protection.

2.6.2 Antibiotic prophylaxis

Frontline workers entering the **exposed zone** and others in the exposed area should be offered antibiotic prophylaxis (see 2.4).

Although person-to-person transmission is rare and there is little evidence of autopsy transmission (glanders only), antibiotic prophylaxis may be considered for workers involved in decontamination of exposed persons or management of patients, or mortuary staff who handle the deceased. Decisions about who should receive prophylaxis should be taken on an individual basis according to duration and degree of potential exposure, and taking into account the availability and side effects of prophylactic treatments.

In addition frontline workers involved at the scene of release and healthcare workers and mortuary staff involved in the management of cases should be advised that if they develop febrile disease they should always mention their exposure on seeking medical attention (see 2.4.2).

2.7 Other Considerations

Since glanders is primarily a disease of horses, and melioidosis can infect many animal species, there could be veterinary consequences of any deliberate release of these organisms. Infected animals could also act as an ongoing source of potential human infection. Close co-ordination with veterinary colleagues is thus essential.

3 LABORATORY PROCEDURES

3.1 Risk Assessment

Both organisms are Containment Level 3 pathogens, and should thus be covered by existing risk assessments for handling such organisms in diagnostic laboratories. In fact, very few laboratory-acquired infections with either organism have ever been reported, and these have usually followed gross lapses in technique.

3.1.1 Receipt and handling of specimens and suspected isolates

Samples should have been labelled as 'High risk' by submitting staff and should be handled according to local protocols for or 'Danger of Infection' samples. All procedures should be performed in a Containment Level 3 laboratory.

In some circumstances, the emergency services may submit samples with chain-of-evidence documentation, in which case the instructions should be followed carefully.

3.2 Isolation and Identification

B. pseudomallei is not difficult to isolate, but *B. mallei* is rather less robust. For isolation, standard procedures should detect both organisms in samples from normally sterile sites. Microscopy may show bipolar or unevenly staining Gram-negative bacilli, but this is neither specific nor sensitive. Isolation rates for *B. pseudomallei* from sites with a normal flora (superficial wound swabs, throat swabs, stool or rectal swabs) may be increased by the use of selective media (e.g. Ashdown's medium, which may be prepared locally according to the recipe in the Appendix). Commercial selective medium for the isolation of *B. cepacia* has been found to perform as well as Ashdown's medium, and is more likely to be available in most diagnostic laboratories (Peacock *et al.*, 2005).

3.2.1 Culture

Samples should be processed according to usual isolation procedures. If melioidosis is suspected, all samples from sites with a normal flora (see above) should be cultured on *B. cepacia* or Ashdown's medium in addition to routine culture media. All media should be incubated for a minimum of 48 hours before being discarded.

In difficult cases, the yield of cultures from sites with a normal flora (e.g. throat swabs, sputum) may be further increased by a pre-enrichment in a specific selective broth incubated at 42°C (contact the Reference Laboratory for details – see 3.4). Unfortunately, no selective medium for *B. mallei* has been developed.

The appearance of *B. pseudomallei* on non-selective media is quite variable. Often there is a metallic surface sheen and a sweet, earthy smell. After 48 hours incubation, some strains form the characteristic rugose colonies described in text books, but other strains are smooth and shiny. Colonies on Ashdown's agar are usually rough and wrinkled and violet to purple after 48 hours, irrespective of the strain. Colonies on *B. cepacia* medium resemble *B. cepacia*. *B. mallei* grows more slowly in culture, and colonies are nondescript.

Laboratory pictures of *B. mallei* and *B. pseudomallei* are available on the HPA website at: <http://www.hpa.org.uk/webw/HPAweb&Page&HPAwebAutoListName/Page/1204031511601?p=1204031511601>

3.2.2 Confirmation and Additional tests

Most isolates will be identified by routine laboratory methods, including commercial kits, of which the API20NE is best validated. The commonest profiles for *B. pseudomallei* using this kit are 1156577 and 1556577. The API20E and Vitek 1 automated systems will also

correctly identify 96-99% of isolates, but the Vitek 2 automated system currently misidentifies a high proportion of *B. pseudomallei* isolates (Lowe *et al.*, 2002). Other useful clues to the identity of *B. pseudomallei* include the characteristic antibiogram (resistance to gentamicin, ampicillin and colistin, susceptibility to chloramphenicol, tetracyclines and co-amoxiclav), although isolates used in a deliberate release might have been rendered more resistant.

In the event of a deliberate release, screen suspect colonies of *B. pseudomallei* by simple tests:

- **Oxidase (positive)**
- **Growth right up to 10 µg gentamicin and colistin discs on any susceptibility testing agar**
- **Susceptibility to co-amoxiclav**
- **Characteristic morphology on Ashdown's medium**

Confirm suspect isolates by biochemical test kits (e.g. API 20NE)

Send initial isolates from any incident to the Reference Laboratory (see below 3.4 and 3.5), having warned them in advance that isolates are being sent.

3.2.3 Serology

Acute and convalescent sera should be sent to the Reference Laboratory for testing. The laboratory should be warned in advance that sera are being submitted.

3.3 Waste Disposal

Waste should be disposed of according to local procedures for Laboratory Containment Level 3.

3.4 Reference Laboratory

Isolates for confirmation of identity and serum samples should be sent to:

Dr Tyrone Pitt
Laboratory of Healthcare Associated Infection
Epidemiology Typing Unit
HPA Centre for Infections
61 Colindale Avenue
London NW9 5HT
020 8327 7224
tyrone.pitt@hpa.org.uk

3.5 Transportation of samples

Strict procedures apply for transport of samples to the laboratory. Biological agents, or materials that contain or may contain them, are allocated to UN Division 6.2 – infectious substances. Infectious substances are divided into Category A or Category B. Full details are given in Appendix 1.2 Transport of infectious substances in *Biological agents: Managing the risks in laboratories and healthcare premises*. ACDP HSE May 2005, available at <http://www.advisorybodies.doh.gov.uk/acdp/managingtherisks.pdf> and in the Department of Health's guidance, available at <http://www.dh.gov.uk/assetRoot/04/11/48/13/04114813.pdf>

Cultures of *B. mallei* and *B. pseudomallei* are Category A infectious substances capable of causing disease in humans or animals and are therefore assigned to UN2814 and must be packaged in accordance with UN Packaging Instructions PI620 (road/rail) /PI602 (air).

P620 and P602 are identical specifications but given different codes in ADR and ICAO regulations respectively (for a full description of PI see <http://www.unece.org/>). Category A transfers should be individually requested through an approved courier. **The service will be a next day, tracked door-to-door delivery, which must be signed for at collection and receipt.**

The current HPA courier arrangements for transportation of samples can be found at http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1202487028723

Clinical samples are generally classified as Category B and are assigned to UN3373 (Diagnostic and Clinical specimens) and should be packaged in accordance with UN PI650. Clinical samples may be posted.

Packaging must meet with UN performance requirements i.e. UN-type approved packaging for Division 6.2 substances. The packaging should consist of an inner package (watertight receptacle, watertight secondary packaging, an absorbent material in sufficient quantity to absorb the entire contents placed between the receptacle and the secondary packaging) and a rigid outer package of adequate strength for capacity, mass and intended use. Packages should be marked with the proper shipping name (e.g. UN 2814), and the appropriate warning label (i.e. the danger sign for infectious substances).

The following procedures should be adopted for the transport of all specimens, and also all cultures for confirmation. These apply within hospitals and laboratories as well as for specimens sent to the reference laboratory:

- The primary container (bijoux or similar) should be screwed tight, labelled and placed in an intact plastic bag.
- A 'High Risk' label should be affixed to both specimen and request form. The latter should include any other relevant information and include adequate clinical details to indicate level of suspicion.
- Under no circumstances should the request form be placed in the same bag as the specimen.
- The bag should be sealed, using tape or heat sealer. Pins, staples and metal clips should not be used. A separate bag should be used for each specimen.
- Each specimen must then be placed in a leak-proof secondary container with sufficient absorbent material to absorb all the contents should leakage occur.
- Each specimen must be packaged individually - i.e. three specimens, three separate packages.
- The secondary container should be externally disinfected – e.g. by wiping with hypochlorite (1,000ppm).

3.5.1 Samples sent to the reference laboratory

Samples should be transported according to local arrangements for High Risk specimens. Precautions should include:

- Secondary containers should be placed within a final outer tertiary packaging.
- This packaging **must** comply with UN-type approved packaging for the transport of infectious substances.
- The package should be certified to this standard and carry the appropriate UN certification numbers on the tertiary packaging along with the following information:
 - 1 BIOHAZARD – danger of infection symbol Class UN 6.2.
 - 2 Instructions not to open if found.
 - 3 Telephone number of a responsible person - e.g. Consultant Microbiologist, Laboratory Manager.

- The container should be transported either by an approved courier for cultures (UN 2814) or by post for clinical samples (UN 3373), without delay, directly to the reference laboratory.

3.5.2 Samples sent within hospitals and laboratories

- Secondary containers should be placed in a good quality box, which is well taped up and clearly labelled "Pathological Specimen – Open only in Laboratory".
- Specimens should be transported by hand by a responsible person using the above packaging.
- Vacuum-tube systems should **not** be used for transportation of specimens within hospitals or laboratories.
- Extra care should be taken to ensure that laboratory records are kept to a high standard.

3.6 Protection of laboratory staff

The risk of laboratory-acquired infections with these agents is low. Staff who have handled the organisms on the open bench prior to the recognition of their identity would not normally require antimicrobial prophylaxis. However, individual cases should be referred for an occupational health risk assessment, which should particularly consider host immune status and likely exposure to aerosols or inoculation incidents. If exposure is considered sufficiently significant, prophylaxis (described in section 2.4) may be offered.

In view of the opportunistic nature of melioidosis, it is recommended that laboratory staff known to be immunocompromised, including diabetics, should not work with the organism.

4 PUBLIC HEALTH PROCEDURES

4.1 Surveillance and detection

A deliberate release may be overt with an announcement and/or confirmation by environmental sampling. However, it is also possible that a deliberate release may be covert and will not be identified until the first cases of disease arise.

Glanders and melioidosis are rare. Since they are not found in the UK, deliberate release should be considered in the event of a single confirmed case of glanders or melioidosis in a person who has not travelled to an endemic area, or clusters of clinically suspicious cases that are linked in time and place.

Close co-ordination with veterinary colleagues is essential: horses are the natural hosts of *B. mallei*, and *B. pseudomallei* can infect many species of mammals and birds. Cases arising in animals may therefore provide an early warning system or additional epidemiological information during an outbreak due to a deliberate release.

4.2 Case Definition

Since the symptoms of glanders and melioidosis are rather non-specific, it is likely to be difficult to identify single cases on the basis of clinical suspicion. However, clusters of cases of severe febrile illness, with or without obvious foci of infection, that present together should be considered suspicious and immediately reported to the local Consultant in Communicable Disease Control (CCDC).

Clinical microbiology laboratories should also be alert to the possibility of *Burkholderia* spp. and should immediately report suspicious isolates to the local CCDC and send specimens to the Reference laboratory for confirmation.

4.2.1 Suspected cases

A suspected case is either:

1. A patient with severe febrile illness in whom an organism suspected of being *B. pseudomallei* or *B. mallei* has been isolated before the identity has been confirmed by the Reference Laboratory or
2. A patient with severe febrile illness in whom antibodies to *B. pseudomallei* or *B. mallei* have been detected but from whom no isolate has been obtained or
3. A patient with severe febrile illness who is known to have been exposed to *B. pseudomallei* or *B. mallei*

4.2.2 Confirmed cases

A case or cases of severe febrile illness, with or without obvious foci of infection from whom reference laboratory confirmed *B. mallei* or *B. pseudomallei* has been isolated.

4.3 Public Health Action

4.3.1 Procedure for handling exposed persons

Depending on the site and method of release, organisms may be dispersed over a wide area. Expert advice will be provided to define an **exposed zone** in time and space. All individuals who have been present in the exposed zone need to be identified.

In the event of an overt release, some of them will still be at the scene when emergency services respond to the incident. This group will be decontaminated and then referred to health workers at a nearby place of safety (this will be a clinical area just outside the exposed zone and within the cordon that will be established at the scene of the incident) for assessment and administration of prophylactic antibiotics. Others will have left the

scene before emergency services arrive and will be identified later when they approach GPs and A&E departments after details of the incident have been made public. Procedures need to ensure that these individuals are appropriately decontaminated, receive prophylaxis, and have their details collected for follow-up.

4.3.2 Extent of post-exposure prophylaxis

Prophylactic antibiotics (see 2.4) are indicated for all individuals exposed to a release, including health and emergency workers called to the scene (see 2.6.2). The parameters of the exposed zone should be reviewed in the light of emerging epidemiological information from new cases as they arise.

It is not advisable to give antibiotics to people who do not have a clear history of having been present at the time and site of release. It is inappropriate to provide antibiotics to large numbers of people who have not been exposed, but who are generally concerned or have non-specific mild illnesses.

4.3.3 Follow-up of exposed persons

After an overt release, a basic set of personal details needs to be collected from all persons present in the exposed zone. Instructions will be given to seek immediate medical attention should symptoms develop (see also 2.4).

4.3.3 Case finding

If cases of glanders or melioidosis arise and a covert release is suspected, health services should be contacted to raise awareness of the possibility of further cases and to determine whether other suspicious cases have presented.

4.3.5 Preventing secondary spread

Person-to-person spread of these infections is negligible, and therefore there is no specific treatment or advice required for secondary contacts. There is no requirement for quarantine of infected patients.

4.4 Epidemiological investigation

If a case is strongly suspected or confirmed, HPA Centre for Infections should be notified immediately (020 8200 4400 or 6868, 24 hours). If cases arise due to a covert release, or following an overt release in people who have not been present in the exposed zone, it is important to collect some epidemiological details in addition to a basic set of personal details. This is in order to define or redefine the exposed zone and aid identification of others at risk of infection. Details should be as thorough as possible, whilst recognising that in the event of a large release with multiple exposed persons or cases, it may not be possible to collect comprehensive information from everyone.

The aim of epidemiological investigations may be:

- Following a covert release to assist definition and ongoing review of the temporal and spatial parameters of the exposed zone so that post exposure prophylaxis can be distributed appropriately.
- Following an overt release to guide review of the exposed zone if cases arise in persons who were not present within it.

4.4.1 Environmental sampling

Microbiological samples may be taken from the environment around the release by the police and tested in designated laboratories. Information from environmental microbiology may be used to complement epidemiological information to determine who is at risk of infection.

5 LIST OF NATIONAL SPECIALISTS

Expert advice on any aspect of human melioidosis and glanders, including diagnosis, management, and public health aspects can be obtained from:

Dr David AB Dance
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Laboratory Expert

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Out of hours contact details are held at the HPA Centre for Infections by the on call duty doctor; Tel: (+44) 020 8200 6868 or 020 8200 4400

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7 APPENDIX: PREPARATION OF ASHDOWN'S AGAR

Materials

- i. 475ml distilled water
- ii. 7.5g agar (bacteriological or technical grade, for example, Oxoid Agar No. 1 #LPO011)
- iii. 5g Tryptone Soy Broth (Oxoid CMO129)

- iv. 20ml warmed glycerol
- v. 2.5ml 0.1% crystal violet
- vi. 2.5ml 1.0% neutral red

Freshly prepared 100ug/ml gentamicin solution

Glass universal containers

1 litre glass flask

Plastic Petri dishes

Method

1. Mix ingredients (i) to (vi) in a 1 litre glass flask.
2. Steam to dissolve, and dispense in 19ml amounts in glass universal containers, leaving the caps loose.
3. Autoclave at 15psi for 15 minutes. When cool, tighten caps hand tight and store at +4°C.

4. When required for use, loosen cap and place in a boiling water bath to melt.
5. After melting, cool to 56°C and add 1ml 100ug/ml gentamicin. Mix carefully and pour into a plastic Petri dish. Flame any bubbles and allow to set.
6. Label plates "ASH" with date of preparation, and store at +4°C for no more than one week.