Standard Operating Procedure (SOP)

Latex Agglutination for the detection of

Burkholderia pseudomallei

Compiled by:

Ms Vanaporn Wuthiekanun (VW), Dr Narisara Chantratita (NC),

Dr David Dance (DD), Dr Direk Limmathurotsakul (DL),

and Prof Sharon Peacock (SP)

on behalf of

www.melioidosis.info Steering Committee

1. Background

Melioidosis is increasingly reported from many countries across south and east Asia as well as parts of South America, Papua New Guinea and the Caribbean. It is apparently rare in Africa [1]. However, melioidosis may pass unrecognized because diagnostic confirmation relies on microbiological culture, which is often unavailable in resource-restricted regions of the world. Even with such facilities, *B. pseudomallei* may be dismissed as a culture contaminant [2], or be misidentified by standard identification methods including API 20NE and automated bacterial identification systems [3,4].

2. Objective

To identify *B. pseudomallei* colonies isolated from clinical samples

3. Application

Any Gram-negative bacillus that is oxidase positive and not *Pseudomonas aeruginosa* isolated from any clinical specimens (including blood, sputum, urine, pus and fluid collected from normally sterile sites) should ideally be screened by latex agglutination, particularly if it is found to be resistant to aminoglycosides and colistin or polymyxin but susceptible to co-amoxiclav. This is because isolation of even a single colony of *B. pseudomallei* from any clinical specimen (including urine) provides a definite diagnosis of melioidosis.

B. pseudomallei colonies are usually cream coloured with a metallic sheen and may become dry and wrinkled after >24 hours' incubation on blood agar, although considerable variation is seen, and may be discarded as a contaminant. On MacConkey agar, *B. pseudomallei* colonies will be white and opaque with a metallic sheen (non-lactose fermenter) and become pink and rugose after 48 hours.

After exclusion of *Pseudomonas aeruginosa*, many microbiology laboratories may identify other oxidase-positive Gram-negative bacteria as '*Pseudomonas* spp.', and consider them as contaminants. In all tropical regions with a high rainfall, and especially countries where melioidosis is known or suspected to occur (Table 1), we recommended that at least all '*Pseudomonas* spp.' isolated from blood cultures should be tested with latex agglutination to rule out the possibility of them being *B. pseudomallei.*

Latex agglutination may also be used to screen suspect colonies isolated from patients travelling from those areas and from patients in other regions in whom melioidosis is suspected.

Table 1. List of countries where melioidosis is known or suspected to occur [5]

Continents	Countries
Africa	Burkina Faso, Chad, Gambia, Kenya, Madagascar, Niger, Nigeria, Sierra Leone, South Africa and Uganda
Asia	Bangladesh, Brunei, Burma (Myanmar), Cambodia, South of China, Egypt, Hong Kong, India, Indonesia, Iran, Japan, Lao PDR, Malaysia, Pakistan, Philippines, Saudi Arabia, Singapore, Sri Lanka, Taiwan, Thailand and Vietnam
Europe	Turkey
North America	Costa Rica, El Salvador and Panama
Oceania	Australia, Fiji and Papua New Guinea
South America	Brazil, Columbia, Costa Rica, Ecuador, El Salvador, Honduras, Mexico, Panama, Peru, Puerto Rico and Venezuela
Others	Aruba, Cote d'Ivoire, Fiji, Guadeloupe, Guam, Haiti, Mauritus, Martinique, New Caledonia and Puerto Rico

4. Latex agglutination test

Latex agglutination test is a screening test to identify *B. pseudomallei*, using latex particles coated with monoclonal antibodies specific for the 200-kDa exopolysaccharide of *B. pseudomallei* [6]. Anyone interested in obtaining this reagent should contact VW at the Mahidol-Oxford Tropical Medicine Research Unit (lek@tropmedres.ac) or Dr Surasak at Melioidosis Research Center (sura_wng@kku.ac.th).

5. Procedures

5.1 Use a toothpick to pick a suspected colony, and mix the bacterium with 5-10 µl latex reagent on a glass slide.

5.2 Gently rock the slide, to keep the fluid suspension in constant movement for 2 minutes.

5.3 Observe for positive agglutination: fine, but readily discernible granularity against clear background.

5.4 Confirm each batch of tests with positive and negative control reagents provided (Figure 1), or with a positive (e.g. *B. pseudomallei* NR 8071 - see http://www.beiresources.org) and negative (e.g. *B. thailandensis* ATCC 700388) control strain according to local availability.

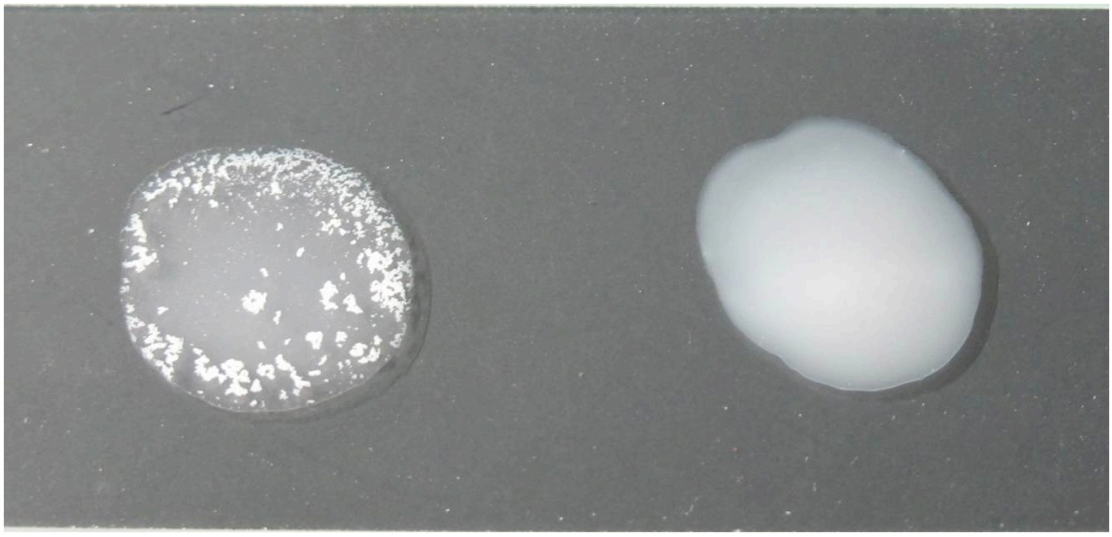

Figure 1: Positive latex agglutination test (left) after mixing 10 µl latex reagent with 10 µl positive control reagent provided and negative latex agglutination test (right) after mixing 10 µl latex reagent with 10 µl negative control reagent provided.

6 Definitive identification of *B. pseudomallei*

Any oxidase-positive Gram-negative bacillus that has a colonial appearance typical of *B. pseudomallei* can be presumptively identified as *B. pseudomallei* if it is positive for latex agglutination. However, occasional false positives have been seen (e.g. with *B. mallei* [7] and some strains of *B. cepacia* complex (Bcc) bacteria). The antibiogram may be helpful in distinguishing *B. pseudomallei*, which is usually resistant to aminoglycosides and colistin or polymyxin but susceptible to co-

amoxiclav, a pattern which is very unusual in other organisms. On Triple Sugar Iron agar, *B. pseudomallei* may give either 'no change' or 'slight oxidation'. As the chance of false positivity by latex agglutination is very low, we recommend that isolates presumptively identified as *B. pseudomallei* for the first time in a given country or region should be referred to a national or international reference laboratory for definitive identification.

Several methods are applicable for definitive identification of isolates presumptively identified as *B. pseudomallei*, including biochemical test kits such as the API 20NE or molecular identification tests depending on what is available locally. These are described in more detail in the review and consensus guidelines.

Please note that typical API 20 NE (also called 20 NFT) (BioMerieux, Durham, N.C.), profiles for *B. pseudomallei* are 1156577, 1556577 or 1156576. With commercial systems, *Burkholderia pseudomallei* may sometimes be misidentified as *Burkholderia cepacia*, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, or *Chromobacterium violaceum.*

7. Safety considerations

All procedures should follow local safety rules and national regulations and should be subject to local risk assessment. The following guidance would normally be applicable.

7.1 All inspection of culture plates and manipulations of microbiological organisms should be carried out in a biological safety cabinet (Class I or II).

7.2 Once *B. pseudomallei* is suspected, all subsequent laboratory procedures should be carried out at biosafety level 3 (BSL3), if possible.

7.3 Isolates presumptively identified as *B. pseudomallei* for the first time in a given country or region should be referred to a national or international reference laboratory for definitive identification. In addition, please contact Dr Direk Limmathurotsakul (direk@tropmedres.ac) on behalf of the www.melioidosis.info steering committee, who can report these findings on the website when the local investigator approves this or after publication of any resulting article.

8. References:

1. Currie BJ, Dance DA, Cheng AC (2008) The global distribution of *Burkholderia pseudomallei* and melioidosis: an update. Trans R Soc Trop Med Hyg 102 Suppl 1: S1-4.
2. John TJ, Jesudason MV, Lalitha MK, Ganesh A, Mohandas V, et al. (1996) Melioidosis In India: the tip of the iceberg? Indian J Med Res 103: 62-65.
3. Weissert C, Dollenmaier G, Rafeiner P, Riehm J, Schultze D (2009) *Burkholderia pseudomallei* misidentified by automated system. Emerg Infect Dis 15: 1799-1801.
4. Deepak RN, Crawley B, Phang E (2008) *Burkholderia pseudomallei* identification: a comparison between the API 20NE and VITEK2GN systems. Trans R Soc Trop Med Hyg 102 Suppl 1: S42-44.
5. Limmathurotsakul D, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, et al. (2012) Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics. A re-evaluation of five diagnostic tests for leptospirosis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.
6. Anuntagool N, Naigowit P, Petkanchanapong V, Aramsri P, Panichakul T, et al. (2000) Monoclonal antibody-based rapid identification of *Burkholderia pseudomallei* in blood culture fluid from patients with community-acquired septicaemia. J Med Microbiol 49: 1075-1078.
7. Anuntagool N, Sirisinha S (2002) Antigenic relatedness between *Burkholderia pseudomallei* and *Burkholderia mallei*. Microbiology and immunology 46: 143-150.