

Latex agglutination test for identification of *Pseudomonas pseudomallei*

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Abstract

A latex agglutination test was developed and evaluated for the rapid presumptive identification of *Pseudomonas pseudomallei*, the causative organism of melioidosis. The test was 100% sensitive for 52 isolates of *Ps pseudomallei* and 100% specific when tested with other medically important *Pseudomonas* species and Enterobacteriaceae. A subsequent field trial, with clinical specimens from patients with suspected melioidosis, confirmed the sensitivity and specificity of the test.

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Melioidosis occurs primarily in South East Asia and northern Australia^{1,2} and is a major cause of morbidity and mortality in north eastern Thailand.³ The disease has a wide range of clinical manifestations and definitive diagnosis is made by isolation of the causative organism, *Pseudomonas pseudomallei*. Primary culture of this organism is not difficult, although it may be overlooked or discarded as a contaminant. Conventionally, definitive identification of *Ps pseudomallei* has required an extensive range of biochemical tests.⁴ However, accurate identification of the species can be achieved with the use of simple screening tests and the commercial API 20NE biochemical kit.⁵ Latex agglutination tests are popular, rapid, and sensitive methods for the routine identification of common organisms.⁶ We have developed a latex agglutination test for the rapid identification of *Ps pseudomallei* and evaluated its usefulness in clinical practice.

Methods

PREPARATION OF THE LATEX TEST

A rabbit serum prepared against whole boiled cells of a reference strain of *Ps pseudomallei* (NCTC 1688) was used.⁷ To one volume of serum (5 ml), two volumes of acetate buffer (pH 4.0) were added at room temperature and mixed. Caprylic acid (N-octanoic acid; BDH, Poole) was added (375 µl) dropwise with continuous stirring.

After 30 minutes of stirring, the solution was centrifuged at 8000 × g for 20 minutes and the supernatant fluid was retained and filtered through Whatman No 54 paper. The filtrate was dialysed against six changes of saline over three days and the volume was

reduced to 3 ml by pressure dialysis. After dialysis against glycine saline buffer the solution was filtered through non-sterile membranes (Millipore) of 8.0, 3.0, 1.2, 0.8, 0.45 and 0.22 µm pore size, respectively. The globulin concentration was measured at 280 nm and adjusted by dilution to give 15-25 mg/ml. Latex particles (0.2 U; Rhone-Poulenc, Manchester) were coated with a range of dilutions of globulin, at 56°C for one hour, before bovine serum albumin (Sigma) was added to 1% (w/v). The optimal coating concentration was determined by mixing a drop of the latex suspension with a boiled overnight broth culture of the immunising strain on a white card and recording the presence or absence of agglutination. The control latex was coated with normal rabbit serum and tested in parallel with the test latex. Suspensions were stored at 4°C for up to one year and brought to room temperature before use.

The test was performed by placing 20 µl of test and control latex suspensions on an agglutination card or a clean glass slide. A small portion of a colony was emulsified directly into the suspension, using a wooden applicator stick, and rocked gently for 1 minute. Rapid agglutination (less than 5 seconds) of the test latex with no agglutination in the control indicated a positive result; no agglutination of either latex indicated a negative result and agglutination in both test and control latex was interpreted as a non-specific (negative) reaction.

PRELIMINARY EVALUATION

Initial evaluation was performed using 52 strains of *Ps pseudomallei* previously isolated from patients in north eastern Thailand and identified definitively using API 20NE (API, bioMerieux) and supplementary tests.⁵ The following other Gram negative bacteria were also tested (table): *Pseudomonas* species (n = 35) including *Ps cepacia* and *Ps aeruginosa*, and Enterobacteriaceae (n = 43) including *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* species and *Enterobacter* species. All organisms were grown on Columbia agar and incubated aerobically for 18 hours.

CLINICAL EVALUATION

Subsequently the latex agglutination test was used on suspect colonies isolated from clinical specimens, as described previously.⁸ Isolates obtained from normally sterile sites, such as blood, were grown on horse blood agar incubated overnight. Isolates from non-

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Strains (No tested)	Latex test		
	Positive	Non-specific	Negative
<i>Pseudomonas pseudomallei</i> (52)	52	0	0
<i>Pseudomonas</i> species			
<i>Ps cepacia</i> (6)	0	2	4
<i>Ps aeruginosa</i> (4)	0	0	4
<i>Ps acidovorans</i> (4)	0	1	3
<i>Ps mesophilica</i> (3)	0	1	2
<i>Ps pickettii</i> (3)	0	0	3
<i>Ps stutzeri</i> (3)	0	1	2
<i>Ps testosteronei</i> (3)	0	2	1
<i>Ps alcaligenes</i> (2)	0	1	1
<i>Ps fragi</i> (2)	0	1	1
<i>Ps fluorescens</i> (1)	0	0	1
<i>Ps pseudoalcaligenes</i> (1)	0	1	0
<i>Ps putida</i> (1)	0	1	0
<i>Ps vesicularis</i> (1)	0	0	1
<i>Shewanella putrefaciens</i> (1)	0	0	1
Enterobacteriaceae			
<i>Escherichia coli</i> (12)	0	3	9
<i>E fergusonii</i> (1)	0	1	0
<i>Klebsiella</i> sp (9)	0	4	5
<i>Proteus mirabilis</i> (8)	0	0	8
<i>Enterobacter</i> sp (4)	0	2	2
<i>Citrobacter</i> sp (3)	0	2	1
<i>Serratia</i> sp (3)	0	1	2
<i>Morganella morganii</i> (2)	0	0	2
<i>Hafnia alvei</i> (1)	0	1	0

Results of latex agglutination test with various bacterial species

sterile sites, such as sputum, were taken from Ashdown's agar⁹ as soon as there was visible growth (18–48 hours' incubation). The clinical specimens were collected as part of our ongoing clinical trials situated in Ubon Ratchatani, north eastern Thailand.¹⁰

Results

The table shows that all 52 laboratory isolates of *Ps pseudomallei* gave a positive result with the test latex suspension. Eleven of 35 isolates of various other *Pseudomonas* species gave non-specific reactions compared with 14 of 43 enterobacterial isolates. The species which most frequently gave these reactions were invariably difficult to emulsify and produced stringy non-uniform suspensions. As the same reaction was evident in the control latex these results did not represent false positives.

A total of 188 suspect colonies from clinical specimens were tested by latex agglutination and 126 (from 72 patients) gave a positive reaction. All were subsequently verified as *Ps pseudomallei*. Forty nine colonies gave negative reactions. These were a variety of Gram negative bacilli, including *Ps aeruginosa* and *Ps cepacia*; none was identified as *Ps pseudomallei*. Thirteen organisms, mucoid forms of *Klebsiella* sp (n = 8), *Acinetobacter* sp (p < 2), *Enterobacter* sp (n = 3) and one strain of *Ps cepacia* gave non-specific reactions. Any isolate picked from selective media which gave a non-specific reaction was retested after subculture to Columbia agar, but the results remained unchanged.

Discussion

Melioidosis is a potentially lethal infection. Severe disease carries a high mortality¹ and does not respond to conventional treatment for severe sepsis in these endemic areas (gentamicin combined with benzyl penicillin or ampicillin). It is critical, therefore, that *Ps pseudomallei* be identified as early as possible so that patients with melioidosis receive appropriate antibiotics.

Current methods for the identification of *Ps pseudomallei* are relatively time consuming. Using selective media, such as Ashdown's agar, small colonies appear after 24 hours but the typical colonial morphology appears only after another 24–48 hours' incubation. The use of API 20NE kits also requires a further 48 hours and would be too expensive for routine use in laboratories situated in endemic areas. Simple tests such as Gram stain appearance, colonial characteristics on differential agar, and resistance to gentamicin and colistin, do permit reliable identification,⁵ but also require more time.

Slide agglutination tests have been used before,¹¹ but commercial antiserum is no longer available. The latex agglutination test, as described, is 100% sensitive and specific for the identification of *Ps pseudomallei*, provided the control latex is always used in parallel. This test therefore offers a very rapid and effective method for the early screening of colonies suspected to be *Ps pseudomallei*, obtained from patients in an endemic area.

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