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Accuracy of *Burkholderia pseudomallei* Identification Using the API 20NE System and a Latex Agglutination Test[▽]

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In an evaluation of the API 20NE for the identification of *Burkholderia* spp., 792/800 (99%) *Burkholderia pseudomallei* and 17/19 (89%) *B. cepacia* isolates were correctly identified but 10 *B. mallei* and 98 *B. thailandensis* isolates were not correctly identified. A latex agglutination test was positive for 796/800 (99.5%) *B. pseudomallei* isolates and negative for 120 other oxidase-positive gram-negative bacilli.

The gram-negative bacillus *Burkholderia pseudomallei* is a category B biothreat agent and the cause of melioidosis. Endemic infection is most common in northeast Thailand and northern Australia, with endemic and imported melioidosis being increasingly recognized around the world (2). When diagnostic laboratory staff are familiar with *B. pseudomallei*, basic screening tests (Gram stain, oxidase test, gentamicin and polymyxin susceptibility, and colonial characteristics on a differential agar medium) are inexpensive and accurate for the identification of *B. pseudomallei* (3). Elsewhere, diagnostic laboratories rely on kits or automated systems already in place for the identification of other gram-negative bacilli. One kit used worldwide for the identification of oxidase-positive gram-negative bacilli is the API 20NE (bioMérieux sa, Marcy l'Etoile, France).

An evaluation of API 20NE for the identification of *B. pseudomallei* was first reported in 1989; 390 of 400 (97.5%) predominantly clinical *B. pseudomallei* isolates from patients in Ubon Ratchathani, northeast Thailand, were correctly identified on first testing (3). A subsequent evaluation of API 20NE using 103 *B. pseudomallei* isolates, the majority of which were from cases of human disease in northern Australia, demonstrated correct identification of 98% (10). Other studies, however, have reported poorer results. In an evaluation of 50 clinical *B. pseudomallei* isolates from Singapore, API 20NE correctly identified 40 isolates, gave unacceptable or uninterpretable results for 4 isolates, and misidentified 6 isolates as other species, the commonest being *Chromobacterium violaceum* (7). Two further studies in 2005 reported correct identification of only 37% for 71 *B. pseudomallei* isolates from Western Australia (8) and 60% of 58 *B. pseudomallei* isolates tested at CDC, Atlanta, that were predominantly isolated from cases of imported melioidosis (5).

One possible reason for the interstudy variability is that *B. pseudomallei* isolates from different geographical areas are phenotypically distinct and clinical isolates are distinct from those from the environment. The first aim of this study was to reexamine the accuracy of API 20NE using a large collection (n = 800) of *B. pseudomallei* isolates obtained from clinical cases, the environment, and animals from seven Asian countries and northern Australia, together with 127 isolates representing other *Burkholderia* species.

Many investigators have reported the development of latex agglutination tests for the identification of *B. pseudomallei*; these have been evaluated using bacterial colonies growing on solid agar or in liquid such as blood culture or urine (1, 4, 11–15, 17). The antibodies used to coat the latex beads have varied and included polyclonal antibodies raised in rabbits (13, 14) and monoclonal antibody to exopolysaccharide (1, 12, 15, 17), lipopolysaccharide (4), or a 30-kDa antigen (11). These have a high sensitivity and specificity overall, although not all assays can distinguish between different species of *Burkholderia* (including *B. mallei* and *B. thailandensis*). A commercial agglutinating-antibody test has been evaluated; this correctly identified 82% of 71 isolates from Western Australia on first testing (8). Our laboratory has previously described a latex agglutination test based on a monoclonal antibody to exopolysaccharide (17). The second aim of this study was to determine its sensitivity and specificity when applied to this large strain collection.

The study was undertaken at a single laboratory at the Wellcome Unit, Faculty of Tropical Medicine, Mahidol University, in Bangkok, Thailand. The 927 *Burkholderia* sp. isolates tested were identified by, and obtained from, multiple laboratories as described in Table 1. An additional collection of 120 isolates representing a range of other oxidase-positive gram-negative species were obtained from diagnostic laboratories in Thailand (Sappasithiprasong Hospital in Ubon Ratchathani, Udon Thani General Hospital, and The Neurology Institute, Bangkok) (Table 1). Bacterial identification was performed by the originating laboratory.

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TABLE 1. Bacterial organisms used in this evaluation

Bacterial species (*n*)	No. of isolates	Source	Country(ies)
Burkholderia spp. (927)			
B. pseudomallei (800)	450	Human	Thailand[a]
	86	Environment	Thailand[b]
	5	Human	Cambodia
	14	Environment	Cambodia
	58	Human	Lao PDR
	48	Environment	Lao PDR
	15	Environment	Vietnam
	11	Animal	Malaysia
	20	Human	Singapore
	23	Animal	Hong Kong
	3	Environment	Hong Kong
	45	Human	Australia
	17	Environment	Australia
	5	Animal	Australia
B. mallei	10	Laboratory strains	Various
B. thailandensis	98	Environment	Thailand, Cambodia, and Vietnam[c]
B. cepacia	19	Laboratory strains	Various
Oxidase-positive spp. (120)			
Pseudomonas aeruginosa	86	Human	Thailand
Pseudomonas spp.	9	Human	Thailand
Stenotrophomonas maltophilia	3	Human	Thailand
Achromobacter xylosoxidans	2	Human	Thailand
Aeromonas spp.	20	Human	Thailand

[a] Isolates were from Ubon Ratchathani (n = 291), Udon Thani (100), Khon Kaen (49), and Phangnga (10).
[b] Isolated in Ubon Ratchathani province.
[c] Eighty-six isolates were from Thailand, 3 were from Cambodia, and 9 were from Vietnam.

These 120 isolates were used to assess the specificity of the latex agglutination test. All isolates were stored at −80°C in Trypticase soy broth with 15% glycerol. Prior to testing, isolates were subcultured twice on Columbia agar and incubated at 37°C in air for 18 to 24 h. Isolates were allocated a unique number in order to blind the operator to the bacterial species and randomized to ensure that all isolates were tested in random order.

API 20NE was performed as recommended by the manufacturer by technical staff with previous experience in its use. The result for each test cupule was recorded as positive or negative. API 20NE results were recorded by two independent operators. In the event of a disagreement, a third person checked for transcription errors; the API 20NE was repeated if the result remained discrepant between the two operators. Numerical profiles were interpreted using the APILAB Plus software, version 1.21.

Latex beads coated with a monoclonal antibody specific to a 200-kDa exopolysaccharide of *B. pseudomallei* were prepared as described previously (1, 17). The test was performed by mixing bacterial cells picked from a single colony on Columbia agar with 10 μl of latex suspension on a glass slide. Agglutination was detected visually after rotation for 2 min. A positive control (*B. pseudomallei* K96243) and negative control (*Escherichia coli* ATCC 25922) were performed on each day the test was used.

API 20NE correctly identified 792 of 800 (99.0%) isolates of *B. pseudomallei* (sensitivity, 99.0%; 95% confidence interval [CI], 98.0 to 99.6%) (Table 2). The remaining eight isolates were misidentified as *Chromobacterium violaceum* (four isolates), *Burkholderia cepacia* (two isolates), *Pseudomonas aeruginosa* (one isolate), and *Pseudomonas fluorescens* (one isolate). All misidentifications were reproducible on repeat testing. Twenty-two profiles were obtained (Table 2). The two most common profiles (1156576 for 453 isolates and 1156577 for 264 isolates) were different only for citrate, while 1556576 (n = 39) and 1556577 (n = 10) were different only for esculin hydrolysis. The two most common profiles predominated in collections from each country. None of the 10 *B. mallei* isolates were correctly identified by API 20NE: 6 gave an unacceptable profile, 2 were misidentified as *Chromobacterium violaceum*, and 1 each was misidentified as *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes.* All 98 *B. thailandensis* isolates were misidentified by API 20NE as follows: *B. cepacia*, 63 (64%); *B. pseudomallei*, 31 (32%); *Pseudomonas fluorescens*, 4 (4%). Of the *B. cepacia* isolates, 17 of 19 (89%) were correctly identified (2 with a low % identification of 66.7%) and 2 isolates were misidentified as *Pseudomonas fluorescens*. A discrepant result occurred between the two readers on a single occasion. This occurred due to a recording error rather than a difference in opinion over interpretation of the API 20NE. This suggests that a single experienced operator is sufficient to interpret this test.

The latex agglutination test was positive for 796 of 800 *B. pseudomallei* isolates (sensitivity, 99.5%; 95% CI, 98.7 to 99.9%). The four false negatives were reproducible on repeat testing. All four isolates were from Hong Kong (animal strains) and were identified as a single clone (ST 70) on multilocus sequence typing (16). There were no false positives among the 120 non-*Burkholderia* oxidase-positive con-

TABLE 2. API 20NE profiles for 800 isolates of *B. pseudomallei*

Result (no. of isolates)	Identity (%)	Profile no. (no. of isolates identified)
B. pseudomallei (792)	>80	1156576 (453), 1156577 (264), 1556576 (39), 1556577 (10), 1156477 (1), 1356577 (1), 1156574 (11), 1156575 (1), 0156576 (1), 1156556 (1), 1154576 (3), 1056576 (3), 1056577 (1)
	<80	1056574 (1), 1157576 (1), 1157577 (1)
Other species (8)		
Chromobacterium violaceum	>80	1150554 (1), 1150514 (2)
Chromobacterium violaceum	<80	1156554 (1)
Burkholderia cepacia	<80	1576576 (2)
Pseudomonas aeruginosa	<80	1154554 (1)
Pseudomonas fluorescens	<80	1156757 (1)

trol organisms (specificity, 100%; 95% CI, 97.0 to 100%). All 98 *B. thailandensis* isolates and all 19 *B. cepacia* isolates were negative. However, 9 of 10 *B. mallei* isolates were positive by latex agglutination, indicating that the test cannot reliably differentiate between *B. pseudomallei* and *B. mallei*.

Testing with the largest *B. pseudomallei* isolate collection ever assessed by identification kits proved that API 20NE is highly suitable for this purpose. It is unclear why the accuracy of API 20NE for the identification of *B. pseudomallei* reported by previous studies varied from 37% to 99% (3, 5, 7, 8, 10). We did not find any evidence for segregation of biochemical profiles between different geographic areas. One possible reason for this interstudy variability may lie with the interpretation of assimilation tests, which can be difficult to read.

API 20NE is not suitable for the identification of *B. thailandensis*. This organism is usually nonpathogenic, reports of disease caused by this organism being limited to two cases (6, 9). Incorrect identification of *B. thailandensis* as *B. pseudomallei* could lead to treatment with a prolonged course of unnecessary antimicrobials, a possibility that should be born in mind in geographic areas where both exist in the environment. In practice, *B. thailandensis* is rarely present in clinical specimens, but alternative methods of identification are required if this species is suspected.

All studies that utilize existing strain collections to assess the accuracy of commonly used bacterial identification tools are potentially biased since isolates are not a naive, untested bacterial population but have already been successfully identified. The vast majority of the strains tested here have not been identified previously using API 20NE, since identification of *B. pseudomallei* in endemic areas commonly relies on a panel of biochemical tests, including arabinose assimilation, together with the susceptibility pattern.

The latex agglutination test was highly sensitive and specific for the identification of *B. pseudomallei*, although it failed to differentiate between this and *B. mallei*. The latex test is not directly comparable to API 20NE since it does not provide an alternative bacterial identification for negative isolates but rather represents a cheap, easy-to-perform test in endemic settings that regularly culture *B. pseudomallei*. Low-cost commercial production could facilitate widespread access to this useful reagent.

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