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Preliminary Evaluation of the API 20NE and RapID NF Plus Systems for Rapid Identification of *Burkholderia pseudomallei* and *B. mallei*

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We evaluated the API 20NE and the RapID NF Plus systems with 58 *Burkholderia pseudomallei* and 23 *B. mallei* strains for identification of these agents, but neither was reliable for confirmatory identification, with only 0 to 60% strains identified accurately. A greater diversity of strains in the system databases would be beneficial.

Burkholderia pseudomallei and *B. mallei* are classified as category B biological threat agents due to their potential for aerosol dissemination and severe impact on human health (10). *B. pseudomallei*, an environmental pathogen causing melioidosis, is endemic in areas of Southeast Asia and Australia. Humans typically become infected through contact with contaminated soil and water. Infection with *B. mallei* causes glanders, primarily a disease of horses. Eradicated from North America 50 years ago by effective testing, restrictions, and animal slaughter, *B. mallei* historically infected humans who worked alongside afflicted animals. In recent years, *B. mallei* laboratory exposure and infection have been reported (2, 11).

Rapid and reliable confirmatory identification of *B. pseudomallei* and *B. mallei* is crucial because of their potential public health impact if used as biothreat agents. Since no human vaccine is available, the sole intervention available is the timely administration of appropriate antimicrobial therapy (6). Conventional confirmatory identification of *B. pseudomallei* and *B. mallei* presently relies on an extensive set of biochemical tests that may require up to 7 days before results are obtained. Consequently, manual and automated identification systems may offer a rapid alternative, especially in first-line laboratories unequipped to perform molecular approaches such as diagnostic PCR or 16S rRNA gene sequencing. We selected the API (bioMérieux, Hazelwood, Mo.) and RapID (Remel, Lenexa, Kans.) systems because of their common use in first-line diagnostic laboratories. Both systems contain profile codes and are approved for use with *B. pseudomallei*, but neither contains profile codes or is approved to identify *B. mallei*. We used a geographically and temporally diverse collection of *B. pseudomallei* and *B. mallei* strains to preliminarily assess the potential of the API and RapID systems as standalone tools for identification of these species.

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Bacterial strains. Fifty-eight *B. pseudomallei* and 23 *B. mallei* strains were selected for their geographical origin and temporal diversity (Table 1). Confirmatory identification for all strains was carried out by standard biochemical testing (13) and 16S rRNA gene sequencing (4). Isolates were stored at −70°C in defibrinated rabbit blood until tested. All work was performed according to the manufacturer's instructions and took place in a biological safety cabinet in a biosafety level 3 environment. Oxidase testing was carried out with Bactidrop oxidase (Remel, Lenexa, Kans.). Prior to testing, all strains were subcultured twice on Trypticase soy agar with 5% defibrinated sheep blood (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C for 18 to 24 h. All tests were performed once, and no retesting or additional testing was performed. Control strains were used as recommended by the manufacturer of each rapid system.

API 20NE. Each strain was inoculated into 0.85% NaCl, and turbidity was adjusted to 0.5 MacFarland standard (bioMerieux, Hazelwood, Mo.). The inoculum was distributed into test strips which were incubated at 30°C and read at 24 and 48 h. Quality control testing was performed with every test. Biochemical reactions were read as positive or negative, translated into numerical profiles, and interpreted with the manufacturer's software (APILAB Plus update 3.3.3).

RapID NF Plus. Each strain was inoculated into the RapID inoculation fluid, and turbidity was adjusted to between 1.0 and 3.0 MacFarland standard (Remel, Lenexa, Kans.). Strips were inoculated and read after a 4-h incubation at 37°C. Quality control tests were performed with each test. Reactions were read as positive or negative, translated into a biocode, and interpreted with the IDS Electronic Code Compendium V1.3.97.

***B. pseudomallei* results with API 20NE.** Thirty-one different profiles were obtained with the API 20NE; 35 (60%) of the 58 *B. pseudomallei* strains were identified correctly, 18 (31%) were misidentified, and 5 (9%) were classified as not identifiable (Table 2). Adipate, mannose, and mannitol assimilation and gelatin hydrolysis were most frequently associated with incorrect or unidentifiable strains resulting in a number of

TABLE 1. Designations of 58 *B. pseudomallei* and 23 *B. mallei* isolates used in this study

Species (no. of strains)	CDC identifier	Other identifier	Origin[a]
B. pseudomallei (58)	2000032024		Human, US, 2000
	2000032025		Human, US, 2000
	2000032026		India, 1995
	2000032027		US, 1968
	2000032028		Human, US, 2000
	2000032029		Human, US, 1994
	2001029240		Human, US, 2001
	2002721090		Human, US, 1980
	2002721096		Human, US, 1981
	2002721102		Human, US, 1983
	2002721103		Human, Netherlands, 1985
	2002721108		Human, US, 1988
	2002721114		Human, US, 1991
	2002721115		Human, US, 1992
	2002721116		Human, US, 1992
	2002721123		Human, Puerto Rico, 1998
	2002721124		Human, US, 1999
	2002721145		Human, Philippines, 1969
	2002721146		Human, US, 1969
	2002721161		Human, US, 1970
	2002721162		Human, Australia, 1970
	2002721166		Human, US, 1973
	2002721171		Human, Venezuela, 1976
	2002721177		Human, US, 1977
	2002721181		Human, US, 1979
	2002721184		Human, Ecuador, 1962
	2002721186		Human, US 1966
	2002721209		Monkey, US, 1069
	2002721617	NCTC 8016	Sheep, Australia, 1949
	2002721618		Monkey, Philippines, 1990
	2002721619		Monkey, Indonesia, 1990
	2002721620		Horse, France, 1976
	2002721622		Sheep, Australia, 1984
	2002721623		Cow, Australia, 1985
	2002721624		Goat, Australia
	2002721625		Environment, Singapore, 1991
	2002721626		Environment, Thailand, 1990
	2002721628		Environment, Madagascar, 1977
	2002721629		Environment, Kenya, 1992
	2002721630		Environment, France, 1976
	2002721631		Environment, Australia
	2002721632		Environment, Australia
	2002721633		Human, Thailand, 1987
	2002721634		Human, Thailand, 1992
	2002721635		Human, Singapore, 1988
	2002721636	NCTC 10276	Human, Bangladesh, 1960
	2002721637		Human, Pakistan, 1988
	2002721638		Human, Vietnam 1963
	2002721639		Human, Kenya, 1980
	2002721640		Human, Papua New Guinea, 1989
	2002721641		Human, Fiji, 1992
	2002721642		Human, Malaysia
	2002721646		Human, Holland, 1999
	2002721647		Human, UK, 1999
	2002734325		Monkey, US, 2003
	2003000540		Human, US, 2002
	2003021442		Human, US, 2003
	2003021443		Human, US, 2003
B. mallei (23)	2000031063	ATCC15310	Horse, Hungary, 1961
	2000031064		India
	2000031065		Turkey
	2000031066		India
	2000031304		Human, US, 2000
	2002721273		US, 1956
	2002721274		US, 1956
	2002721275	ATCC 10399	Horse, China, 1956
	2002721276		US, 1956
	2002721277		US, 1956
	2002721278		Human, US, 1964
	2002721279		Human, US, 1964

Continued on facing page

TABLE 1—*Continued*

Species (no. of strains)	CDC identifier	Other identifier	Origin[a]
	2002721280		France, 1972
	2002721648	ATCC 23344	Human, China
	2002734299	NCTC 10229	Hungary, 1961
	2002734300	NCTC 10247	Turkey, 1960
	2002734301	NCTC 10260	Human, Turkey, 1949
	2002734302		Turkey
	2002734303	NCTC 3709	Horse, India, 1932
	2002734304	NCTC 10248	Human, Turkey, 1950
	2002734305	NCTC 3708	Mule, India, 1932
	2002734306	NCTC 120	UK, 1920
	2002734307		

[a] The source of the isolate is given when available. US, United States; UK, United Kingdom.

different numerical profiles. In previous studies, this system was reported to identify 80 to 98% of strains correctly (3, 5, 9), but the *B. pseudomallei* strains used were primarily from clinical specimens in areas where *B. pseudomallei* is endemic and so lacked geographical, temporal, and source diversity. In another study testing 114 geographically diverse clinical, environmental, and reference *Burkholderia* spp. and closely related strains (but no *B. pseudomallei*), API 20NE correctly identified 77% of strains (12). Our study emphasizes the importance of including a greater diversity of strains in the API 20NE database.

TABLE 2. Results of testing 58 *B. pseudomallei* strains by the API 20NE system

Result (*n*)	Identity (%)	Profile no. (no. of isolates identified)
Correct identification[a] (35)	>80	1156574 (2)
		1156575 (3)
		1156577 (2)
		1554577 (1)
		1556535 (1)
		1556557 (2)
		1556574 (10)
		1556575 (5)
		1556576 (1)
		1556577 (4)
	<80	1056574 (1)
		1056575 (1)
		1456575 (1)
		5156575 (1)
Incorrect identification[a] (18)	>80	0156574 (1)[c]
		1112444 (1)[d]
		1150054 (1)[d]
		1150475 (1)[e]
		1154574 (2)[e]
		1540554 (1)[f]
		1554574 (2)[e]
	<80	1146575 (2)[c]
		1154554 (1)[e]
		1454554 (1)[f]
		1550554 (2)[g]
		1556554 (3)[c]
Not identifiable[b] (5)	NA[h]	0554554
		1044576
		1446574
		1556154
		5744554

[a] Profile identification response was excellent, very good, good, or acceptable.
[b] Profile indentification response was unacceptable, indeterminate, or invalid or no species indentification could be determined.
[c] Identified as *Pseudomonas fluorescens*.
[d] Identified as *Comamonas testosterone*/*Pseudomonas alcaligenes*.
[e] Identified as *Pseudomonas aeruginosa*.
[f] Identified as *Aeromonas salmonicida*.
[g] Identified as *Chromobacterium violaceum*.
[h] NA, not applicable.

***B. pseudomallei* results with RapID NF Plus.** None of the 58 *B. pseudomallei* strains was identified correctly with the RapID NF Plus, 30 (52%) were misidentified, and 28 (48%) were classified as not identifiable by the 13 microcodes obtained (Table 3); tests for arginine hydrolysis, *p*-Nitrophenyl-*N*-acetyl-β-D-glucosaminide, and *N*-nezyl-arginine-β-napthylamide were most frequently associated with incorrect or nonidentifiable strains. In a previous study, Rapid NF Plus correctly identified 80 to 90% of nonfermenting gram-negative bacilli (7, 8); however, no reports are available to date on use of this system for identification of *B. pseudomallei*. Kiska et al. (7) tested 150 nonfermenting strains and reported difficulties in using this system to identify members of the genus *Burkholderia*, con-

TABLE 3. Results of testing 58 *B. pseudomallei* strains by the RapID NF Plus system

Result (*n*)	Profile no. (no. of isolates identified)
Incorrect identification[a] (30)	430014 (4)[c]
	430016 (9)[c]
	430204 (1)[c]
	430216 (5)[c]
	530016 (2)[d]
	610016 (2)[e]
	630014 (3)[e]
	630236 (4)[f]
Not identifiable[b] (28)	510016 (1)
	630016 (7)
	630017 (2)
	630216 (14)
	730016 (4)

[a] Correct identifications registered an excellent, very good, good, implicit, satisfactory, or adequate biocode. Probability, >95%.
[b] Not identifiable biocodes were the result of a probability overlap between two or more possibilities; additional tests were required or gave an incorrect or unidentified response.
[c] Identified as *Burkholderia cepacia*.
[d] Identified as *Chromobacterium violaceum*.
[e] Identified as *Comamonas testosteroni*.
[f] Identified as *Shewanella putrefaciens*.

TABLE 4. Results of testing 23 *Burkholderia mallei* strains by API 20NE

Result (*n*)	Identity (%)	Profile no. (no. of isolates identified)
Incorrect identification[a] (6)	>80	1040400 (2)[c]
	<80	1000000 (1)[c]
		1040404 (1)[d]
		1040500 (2)[c]
Not identifiable[b] (17)	NA[e]	0040500 (1)
		1041500 (1)
		1042500 (3)
		1042520 (1)
		1042521 (1)
		1044420 (1)
		1140500 (2)
		1140504 (1)
		1140520 (1)
		1144501 (1)
		1146520 (4)

[a] Profile identification response was excellent, very good, good, or acceptable.
[b] Profile indentification response was unacceptable, indeterminate, or invalid or no species indentification could be determined.
[c] Identified as *Pasteurella* sp.
[d] Identified as *Aeromonas salmonicida masoucida/achromogenes*.
[e] NA, not applicable.

cluding that the conventional biochemical identification is still preferable.

Neither system incorporates *B. mallei* in its diagnostic algorithm, but both use the same biochemical tests commonly used to identify this agent by conventional methods. Consequently, we also evaluated both systems for the ability to confirm *B. mallei*.

***B. mallei* results with API 20NE.** Six (26%) of the *B. mallei* strains were identified as other organisms, and 17 (74%) were not identifiable (Table 4). With 15 profiles generated from 23 strains, this system was unable to present a cohesive identification for *B. mallei*. However, it shows potential in that the majority of those profiles were not identifiable and would not cause a misidentification if encountered.

TABLE 5. Results of testing 23 *Burkholderia mallei* strains by RapID NF Plus

Result[a] (*n*)	Probability (%)	Profile no. (no. of isolates identified)
Incorrect identification (11)	>95	030010 (1)[c]
		400012 (1)[c]
		410002 (1)[d]
		420012 (1)[c]
		430012 (5)[c]
		430212 (1)[c]
		630212 (1)[e]
Not identifiable	NA[f]	030012 (1)
		430002 (1)
		630006 (1)
		630002 (1)
		630012 (8)

[a] Correct identifications registered an excellent, very good, good, implicit, satisfactory, or adequate biocode.
[b] Not-identifiable biocodes were the result of a probability overlap between two or more possibilities; additional tests were required or gave an incorrect or unidentified response.
[c] *Burkholderia cepacia*.
[d] CDC NO-1.
[e] *Stenotrophomonas maltophilia*.
[f] NA, not applicable.

***B. mallei* results with rapID NF Plus.** Eleven (48%) of the *B. mallei* strains were identified as other organisms, and 12 (52%) were not identifiable (Table 5); these 23 strains produced 12 profiles. Microcodes 430012 and 630012 were the most commonly identified.

The strains of *B. pseudomallei* and *B. mallei* that were correctly identified, misidentified, or not identified by either system were not associated by common geography, source, or time period.

Conclusion. In this study, all test results were intentionally based upon a single test, and no additional testing was performed. Other studies reported retesting and/or supplementing these rapid tests with additional traditional biochemical tests (1, 3, 5). This preliminary evaluation did not find either of these systems in the current format to be promising for confirmatory identification of potential *B. pseudomallei* or *B. mallei*, and therefore, we did not pursue a further major validation study. In addition to the poor performance of both the API 20NE and RapID NF Plus systems, we encountered other problems while working with them. While the RapID NF Plus requires only a 4-h incubation, an extensive (48-h) incubation of the API test strips was required, which is a disadvantage in terms of rapid response (1, 3). However, as it did not correctly identify any *B. pseudomallei* isolate, the speed of the RapID NF Plus systems confers no real advantage over the API 20NE. Safety was also a concern. The potential aerosolization from the manipulation of suspensions, the open-reaction cupules on the test strips, and the sharp edges generated from snapping open glass tube API reagents present opportunities for laboratory-acquired infection or injury (5).

To be beneficial in the detection of *B. pseudomallei* and *B. mallei*, these systems need to expand their databases to include a wider diversity of strains and/or adjust problematic biochemical tests within the test panels. Consequently, we continue to recommend the use of traditional biochemical methods for preliminary identification of these agents, followed by submission of suspicious isolates to a laboratory capable of confirmatory identification (1).

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