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Evaluation of the RapID CB Plus System for Identification of *Corynebacterium* Species and Other Gram-Positive Rods

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Due to the difficulty of identifying *Corynebacterium* spp. with standard methods, we compared them with the RapID CB Plus system (Remel, Lenexa, Kans. [formerly Innovative Diagnostic Systems, Norcross, Ga.]), which consists of 4 carbohydrate and 14 preformed enzyme tests, for the identification of 98 clinical isolates of *Corynebacterium* sp., other coryneforms, *Listeria monocytogenes*, and 17 ATCC strains. Forty (95%) of 42 strains of *Corynebacterium* spp. were accurately identified to the species level by the RapID CB Plus system, and two additional strains of *C. striatum* were identified with one additional conventional test for lipid requirement. Twenty-seven (75%) of the 36 coryneform strains tested were identified correctly to the species level. However, three of four strains of *Brevibacterium* sp. and all seven of the *L. monocytogenes* strains were identified to the genus level only. *Actinomyces* strains had variable results, and the one strain of *Arcanobacterium haemolyticum* tested was not identified. Overall, the RapID CB Plus system compared favorably with the conventional methods, was easy to inoculate and interpret, and is promising as a new method for identification of gram-positive bacilli.

In recent years, the incidence and recognition of *Corynebacterium* species and coryneform bacteria as etiologic agents of serious infections, such as bacterial sepsis, endocarditis, and indwelling catheter infections, has increased, especially in immunocompromised patients (1, 4, 11, 17). Treatment of these infections has been problematic because of the increase in resistance to antibiotics (10, 11, 18). Therefore, it has been suggested that susceptibility testing be performed on isolates obtained from serious infections. Serious and sometimes fatal clinical outcomes due to inappropriate therapy, possibly due to the difficulty in identifying these organisms and failure to recognize their significance, have been reported (1, 3, 4, 7, 9, 11). Because conventional methods are expensive and time-consuming to set up, require prolonged incubation (7 to 10 days), and have problems with overlapping biochemical reactions for different species, we evaluated a 4-h rapid identification panel, the RapID CB Plus system (Remel, Lenexa, Kans. [formerly Innovative Diagnostic Systems, Norcross, Ga.]). We used the RapID CB Plus system, which contains substrates for 14 enzyme and 4 carbohydrate tests, to identify *Corynebacterium* spp. and other gram-positive bacilli and compared it to conventional biochemical methods.

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MATERIALS AND METHODS

The following reference strains from the American Type Culture Collection (ATCC) were studied: *Actinomyces pyogenes* ATCC 19411, *A. bernardiae* ATCC 51727, *Arcanobacterium haemolyticum* ATCC 9345, *Erysipelothrix rhusiopathiae* ATCC 19414, *Corynebacterium pseudodiphtheriticum* ATCC 10700 and 10701, *Bacillus polymyxa* ATCC 842, *Oerskovia xanthineolytica* ATCC 27402, *Brevibacterium epidermidis* ATCC 35514, *C. diphtheriae* ATCC 13812, *C. pseudotuberculosis* ATCC 19410, *C. jeikeium* ATCC 43734 and 43216, *C. minutissimum* ATCC 23348, *C. striatum* ATCC 6940, *C. urealyticum* ATCC 43042, and *C. xerosis* ATCC 373. The 98 clinical isolates were as follows: *C. accolans* (1 strain), *C. amycolatum* (4 strains), "*C. aquaticum*" (10 strains), *C. jeikeium* (11 strains), *C. kutscheri* (1 strain), *C. minutissimum* (12 strains), *C. striatum* (2 strains), *C. propinquum* (1 strain), *C. urealyticum* (1 strain), *Rothia* sp. (2 strains), *Dermabacter hominis* (2 strains), *E. rhusiopathiae* (2 strains), *Listeria monocytogenes* (7 strains), *Actinomyces odontolyticus* (8 strains), *A. viscosus* (5 strains), *A. israelii* (4 strains), *A. naeslundii* (6 strains), *Arcanobacterium haemolyticum* (1 strain), *Brevibacterium* sp. (5 strains), Centers for Disease Control and Prevention (CDC; Atlanta, Ga.) group F (2 strains), CDC group G (1 strain), and *Bacillus* sp. (10 strains). The strains were previously isolated from clinical specimens and identified by conventional methods (6, 11). Isolates were obtained from various sources, as follows: blood cultures, 11; bone, 4; cat bites, 18; dog bites, 26; human bites, 1; other wounds, 12; abscesses, 4; urine, 2; tissue, 2; pelvic sample, 1; respiratory samples, 2; ear, 1; appendix, 3; unknown sources, 11. Isolates were stored in 20% skim milk at −70°C until use. Cultures were initiated from frozen stocks. Strains of *Actinomyces* were incubated in an anaerobic chamber (Anaerobe Systems, San Jose, Calif.), subcultured twice on tryptic soy blood agar (Hardy Diagnostics, Santa Maria, Calif.), incubated aerobically at 37°C in 5 to 7% CO_2 for 24 to 48 h, and retested. All other strains were transferred twice on tryptic soy agar with 5% sheep blood and incubated at 37°C in 5 to 7% CO_2 for 24 to 48 h.

The test inoculum for the RapID CB Plus panel (Remel) was prepared by using a cotton-tipped swab and suspending sufficient growth in 2 ml of inoculation fluid (provided with the kit) to produce a turbidity equal to a no. 4 McFarland standard or 1.2×10^9 CFU/ml. The panel was inoculated by first peeling back the upper right-hand corner of the panel and pouring the inoculation fluid into the panel. The panel was then tilted backward and from side to side to evenly fill the rear baffles in the top of the panel. The panel was then tilted forward to simultaneously inoculate all of the biochemical wells in the front of the panel. The inoculated panel was incubated at 37°C for 4 h in ambient air.

After incubation, the first nine wells of the panel were interpreted according to the color reactions as described by the manufacturer. Two drops of the RapID CB Plus reagent were added to each of the five wells that contain substrates for the aminopeptidase tests, and two drops each of nitrate reagents A and B were added to the nitrate well. After 30 s, the reactions were interpreted (Table 1). All ATCC strains were tested at least twice with the RapID CB Plus kit for reproducibility. Based on the results of the RapID CB Plus kit, a profile number was generated for each strain, which was then compared to a code compendium provided by the manufacturer. The organisms included in the RapID CB Plus database are listed in Table 2.

Conventional tests included both enzymatic and carbohydrate tests. Enzymatic tests were performed by using WEE-TABS (Key Scientific Products, Co., Round Rock, Tex.). These are plastic tubes (13 by 50 mm) that contain a tablet composed of one or more substrates. They are inoculated with 0.5 ml of a heavy (greater than or equal to a no. 3 McFarland standard) suspension of the test organism in water and incubated aerobically at 37°C for 2 to 4 h. The enzymes tested with the WEE-TABS were urease, pyrazinamidase, alkaline phosphatase, and *o*-nitrophenyl-β-D-galactopyranosidase. Carbohydrate tests were chosen as

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TABLE 1. Components and interpretation of the RapID CB Plus panel tests

Test or substrate	Positive reaction	Negative reaction
Carbohydrate utilization tests		
Glu (glucose)	Yellow, gold, or light yellow-orange	Red, dark orange, or orange
Suc (sucrose)		
Rib (ribose)		
Mal (maltose)		
Substrates for glycosidase tests		
α-GLU (*p*-nitrophenyl-α-D-glucoside)	Yellow	Clear, tan, or very pale yellow
β-GLU (*p*-nitrophenyl-β-D-glucoside)		
NAG (*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide)		
GLY1 (*p*-nitrophenylglycoside)		
ONPG (*o*-nitrophenyl-β-D-galactopyranoside)		
Substrates for aminopeptidases		
PRO (proline-β-naphthylamide)		
TRY (tryptophan-β-naphthylamide)		
PYR (pyrrolidine-β-naphthylamine)	Purple, red, or very dark purple	Yellow, light orange, or very light pink
LGLY (leucyl-glycine-β-naphthylamide)		
LEU (leucine-β-naphthylamide)		
Single-enzyme tests		
URE (urease)	Red or dark red-orange	Yellow or orange
NIT (nitrate reductase)	Red or pink	Clear
PHS[a] (phosphatase)	Yellow	Clear, tan, or very pale yellow
LIP (lipase)	Yellow or yellow-orange	Red or orange

[a] PHS is also a *p*-nitrophenyl test.

specified by the CDC, except that Andrades carbohydrates for glucose, sucrose, mannitol, and xylose (Remel [formerly Carr Scarborough, Decatur, Ga.]) were used instead of CDC carbohydrates, as these are commercially unavailable (6, 11, 15). The carbohydrate tests were inoculated by using a sterile stick and incubated aerobically at 37°C for 7 to 10 days. Strains that did not appear to utilize any of the carbohydrates were supplemented with 0.1% Tween 80, as some strains are known to require lipid supplementation for growth. Tests for hydrolysis (esculin and gelatin), nitrate reduction, and carbohydrate fermentation of mannose, lactose, and maltose were performed with aerated, prereduced, anaerobically sterilized PRAS medium (Remel). In addition to tests for carbohydrates, motility was determined by wet mount and motility media (Hardy Diagnostics). All isolates were tested for catalase production (by using 10% H_2O_2) and examined for yellow pigment production.

RESULTS

Forty (95%) of 42 strains of *Corynebacterium* spp. were correctly identified by the RapID CB Plus system without supplemental tests. However, both of the *C. striatum* strains required an additional test for lipid requirement (0.1% Tween) to generate a profile number that matched the code compendium identification (Table 3). Unlike the RapID CB Plus system, the conventional method required addition of 0.1% Tween 80 to the carbohydrate tests for lipophilic strains. Specifically, Tween 80 was required in the conventional carbohydrate tests of two strains of *C. jeikeium*, one strain that resembled *C. minutissimum*, and all four strains of *Brevibacterium* sp. Strains of *C. amycolatum* and *C. striatum* were difficult to identify to the species level by using the conventional tests due to similarity of the biochemical reactions. The only characteristics differentiating the strains of *C. striatum* were that the colonies were white and moist while those of *C. amycolatum* were white and dry. Also, *C. striatum* strains were maltose negative and CAMP test positive while *C. amycolatum* strains were maltose positive and CAMP test negative.

All seven strains of *L. monocytogenes* were identified only to the genus level by the RapID CB Plus system (Table 4). These seven strains were beta-hemolytic, positive by the CAMP test with *Staphylococcus aureus*, and alanyl peptide hydrolysis negative and matched the biochemical profile of *L. monocytogenes* obtained by using the conventional method.

Twenty-seven (75%) of the 36 coryneforms were identified by the RapID CB Plus system without supplemental tests (Table 4), except for one strain of *Arcanobacterium haemolyticum*, which was identified by the conventional method but not by the RapID CB Plus system. Also, the strains of *Actinomyces* sp. had variable results. All eight *Actinomyces odontolyticus* strains were identified correctly by the RapID CB Plus system, whereas all five *A. viscosus* strains were identified but required supplemental tests for motility and pigment production because of profile numbers that overlapped those of *Oerskovia* sp. Three of four *A. israelii* strains were identified to the species level, but one strain produced a profile number that had no match with the code compendium. Two of six *A. naeslundii* strains were identified to the species level, while the other four strains were identified to the genus level only. The *Actinomyces* sp. strains were tested after anaerobic and aerobic incubation and showed different results. Specifically, all strains of *A. odontolyticus* were esterase and ribose positive when incubated in an anaerobic environment and negative when incubated in CO_2; also, some strains of *A. naeslundii* were ribose positive, tryptophan positive, and L-glycine positive when incubated anaerobically but were negative when incubated in a CO_2 environment. The *Brevibacterium* strains were identified only to the genus level by both methods, except for one strain, which the RapID CB Plus system was able to identify as *B. casei* (Table 4).

Ten strains of *Bacillus* sp. were included in the study to confirm that the kit will not identify strains of bacteria not included in the code compendium. The profile numbers obtained for these showed no match. ATCC strains were all tested at least twice by using the RapID CB Plus system, and all of the strains produced essentially the same reactions each time, with the exception of the substrate for phosphatase, which was variable with *Bacillus polymyxa* ATCC 842 and *Actinomyces pyogenes* ATCC 19411.

TABLE 2. Organisms included in the database of the RapID CB Plus system

Bacterium
Corynebacteria
C. accolans
C. afermentans subsp. *afermentans*
C. afermentans subsp. *lipophilum*
C. amycolatum
"*C. aquaticum*"
C. argentoratense
C. auris
C. bovis
C. cystitidis
C. diphtheriae
C. glucuronolyticum
C. jeikeium (JK)
C. kutscheri
C. matruchotii
C. minitissimum
C. propinquum (ANF3)
C. pseudodiphtheriticum
C. pseudotuberculosis
C. renale
C. striatum
C. ulcerans
C. urealyticum (D-2)
C. xerosis
Taxa
CDC group F-1
CDC group G (G/LD)
CDC group I1
Listeriae
L. grayi/*murrayi*
L. innocua
L. ivanovii
L. monocytogenes
L. seeligeri
L. welshimeri
Irregular gram-positive bacilli
Actinomyces bernardiae (group 2)
Oerskovia sp.
Rhodococcus equi
Rothia sp.
Turicella otitidis
A. israelii
A. naeslundii
A. neuii (group 1)
A. odontolyticus
A. pyogenes
A. viscosus
Arcanobacterium haemolyticum
Bifidobacterium sp. (group E)
Brevibacterium casei
Brevibacterium sp. (group B)
Cellulomonas (group A3/A4)
Dermabacter hominis
Erysipelothrix rhusiopathiae
Exiguobacterium acetylicum

DISCUSSION

Corynebacterium spp. and other gram-positive bacilli are difficult to identify with the existing systems. In addition, methods such as the API Coryne system (API-BioMerieux, Inc., St. Louis, Mo.) and the conventional method have incubation periods of at least 24 h and 7 days, respectively. The long incubation period required for biochemical tests and fastidious growth requirements for some strains of these organisms can be a problem as well. Moreover, the previous methods are problematic for identification of some strains of *Corynebacterium* due to overlapping biochemicals or requirement of additional tests for identification of most strains. Therefore, the RapID CB Plus system is helpful in the identification of these organisms because it is a 4-h test for preformed enzymes and does not depend on growth of the organism for identification.

The RapID CB Plus system compared favorably with conventional methods for identification of *Corynebacterium* sp. and most of the other gram-positive bacilli tested. The RapID CB Plus system did not require additional tests for the identification of most strains of *Corynebacterium* spp. and other gram-positive rods, unlike the API Coryne kit, which requires additional tests for most of these strains (8, 13). The only strains requiring additional tests for identification to the species level with the RapID CB Plus system were *C. striatum* and *A. viscosus*. In addition, the RapID CB Plus system identified several species that are difficult to distinguish by other methods. Previous reports indicate confusion regarding the descriptions of *C. xerosis*, *C. striatum*, and *C. amycolatum* (7, 11, 16), which were based on carbohydrate fermentation. There were several enzymatic tests that helped to identify these strains in the RapID CB Plus system. The RapID CB Plus system percentage charts included in the kit indicated that tests for *C. amycolatum* were 80% maltose positive, 88% esterase positive, 9% L-glycine positive, and 11% leucine positive, while those for *C. striatum* were 0% maltose positive, 37% esterase variable, 70% L-glycine positive, and 82% leucine positive. The results of these enzyme tests help to differentiate *C. amycolatum* from *C. striatum* (8, 11, 13, 14). Other tests previously reported to be helpful in the identification of *C. amycolatum*, *C. striatum*, and *C. xerosis* include the requirement for lipids, the presence of mycolic acids, propionic acid as a major end product of glucose metabolism, and rRNA gene sequencing (2, 11). The latter four tests are not suitable for clinical laboratories.

Because there is confusion regarding the taxonomy of several species of *Corynebacterium* and coryneforms, the identification of some of these strains may change in the future (7, 9, 11, 12). For example, proper classification of "*C. aquaticum*" and *Aureobacterium* sp. is still being considered. Therefore, we called all 10 of our strains "*C. aquaticum*" as their biochemical profiles, obtained by using conventional methods, fit this identification. Although the RapID CB Plus system also identified these 10 strains as "*C. aquaticum*" (Table 4), *Aureobacterium* sp. is not included in the database of the kit. A similar problem with taxonomic differentiation exists between *Brevibacterium*

TABLE 3. Comparison of conventional method with RapID CB Plus system for identification of *Corynebacterium* spp.

Corynebacterium sp.	No. of isolates tested	No. identified to species level
C. accolans	1	1
C. amycolatum	4	4
"*C. aquaticum*"	10	10
C. jeikeium	11	11
C. kutscheri	1	1[a]
C. minutissimum	11	11
C. striatum	2	2[b]
C. propinquum	1	1
C. urealyticum	1	1

[a] Isolated from a cat bite wound.
[b] Identified by using extra tests.

TABLE 4. Comparison of conventional method with RapID CB Plus system for identification of gram-positive bacilli other than *Corynebacterium* spp.

Organism	No. of strains				
	Tested	Genus level identification	Species level identification	Identified with extra tests	Not identified
Listeria monocytogenes	7	7			
Irregular gram-positive bacilli					
CDC group F-1	2		2		
CDC group G	1		1		
Actinomyces odontolyticus	8		8		
A. viscosus	5			5	
A. israelii	4		3		1
A. naeslundii	6	4	1		1
Arcanobacterium haemolyticum	1				1
Brevibacterium sp. (group B)	3	3			
Brevibacterium casei	1		1		
Dermabacter hominis	2		2		
Erysipelothrix rhusiopathiae	2		2		
Rothia sp.	2		2		
Bacillus sp.[a]	10				10

[a] Not in RapID CB Plus database.

sp. and *Arthrobacterium* sp. Many species of *Arthrobacterium* were defined based on the biochemical reactions of single ATCC strains. We called our four strains *Brevibacterium* based on the lack of clear data regarding *Arthrobacterium* sp. The RapID CB Plus system identified these strains as *Brevibacterium* sp., as *Arthobacterium* sp. strains are also not included in the database of the kit.

The RapID CB Plus kit was designed for all strains (including strains such as *Actinomyces* sp., which are often grouped with anaerobes) to be grown aerobically. We found that when strains were incubated anaerobically, some results did not match the species profile numbers in the code compendium. Although most of the coryneforms listed on Table 2 were identified to the species level, some coryneform species had erroneous results. For instance, the seven *L. monocytogenes* isolates were misidentified by the RapID CB Plus system as *L. welshimeri* because the test results were similar for both species. Therefore, *Listeria* spp. can be identified only to the genus level with this system. An improvement to the kit to identify *Listeria* spp. may be to add alanyl peptide hydrolysis as a supplemental test (5). In general, the *Actinomyces* sp. strains were identified, except for some strains that required additional tests for motility and pigmentation (Table 4). Strains of *A. naeslundii* were the most difficult to identify, as four of six could only be identified to the genus level, one was not identified, and only one was identified to the species level.

The conventional method using carbohydrates and some enzymatic tests is often cumbersome, since all tests are individual tests and have to be inoculated separately. In addition, they may require supplementation and a long incubation period (7 to 10 days) for growth. Furthermore, these results are sometimes difficult to achieve. A comparison of the results obtained with the conventional method and the RapID CB Plus system determined that these two methods had comparable results for corynebacteria and most strains of coryneforms (Tables 3 and 4). Because the RapID CB Plus system contains mostly substrates for enzymatic tests, it may be more accurate than carbohydrate utilization tests for identification of these types of organisms.

Overall, the RapID CB Plus panels compared favorably with the conventional method, are easy to inoculate and interpret, and are promising as a new method for identification of *Corynebacterium* sp. and other gram-positive bacilli.

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