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Evaluation of Four Commercial Test Systems for Identification of *Actinomyces* and Some Closely Related Species

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We evaluated four commercially available kits for rapid identification of *Actinomyces* and related species. The kits identified correctly 26 to 65% of "classical" *Actinomyces* strains to the species level and 13 to 49% of newly described *Actinomyces* strains to the genus level, thus indicating relatively poor applicability and a need to update these kits.

The genus *Actinomyces* embraces a heterogeneous group of facultatively anaerobic, gram-positive, mainly branching rods, which occur as frequent inhabitants on mucosal surfaces but also as common opportunistic pathogens in various infectious processes (3, 5, 7, 10, 15, 17). Improvements in identification methods have resulted in expanded recognition of new *Actinomyces* species from clinical specimens (3, 4, 6, 8, 13, 18). On the other hand, some former *Actinomyces* species have been moved to other genera, such as *Arcanobacterium* and *Actinobaculum* (11, 14). Over 80% of the problematic isolates sent to our reference laboratory turn out to belong to the genus *Actinomyces*, indicating that clinical microbiology laboratories need updated information on these slowly growing, asporogenous rods. Recently, Sarkonen et al. published an extensive identification scheme for *Actinomyces* species for the purpose of clarifying their accurate identification on the basis of phenotypic testing alone (16); however, many laboratories rely solely on commercial kits because of lack of manpower and facilities. In the present study, we demonstrated the performance of four commercial test kits in the identification of "classical" (i.e., described before 1994) *Actinomyces* species and tested the reactivity of some newly described species not yet included in the databases.

The RapID ANA II system (Remel, Lenexa, Kans.) consists of 10 wells (including dehydrated reactants for 18 biochemical reactions) and the Rapid ID 32 A system (bioMérieux, Marcy-l'Etoile, France) consists of 32 cupules (including 29 containing dehydrated test substrates) for the testing of anaerobic bacteria. The RapID CB Plus system (Remel) consists of 18 wells (including 4 for carbohydrate utilization and 14 for single-substrate enzyme tests) for the testing of coryneform and related bacteria. The BBL Crystal ANR ID system (Becton Dickinson Microbiology System, Cockeysville, Md.) consists of 30 wells (including 17 wells with fluorescent substrates, 12 wells with chromogenic substrates, and a fluorescence control well); the assay includes tests for fermentation, oxidation, degradation, and hydrolysis of various substrates.

A total of 21 reference strains from international culture collections and 86 clinical *Actinomyces* strains (65 of infectious origin and 21 from human resident floras) were included in this study. All strains were previously identified by conventional methods (16) or by the ARDRA (PCR-restriction fragment length polymorphism) method (8) or by both. The organisms were revived from frozen (−70°C) stocks, grown twice in subcultures on brucella blood agar, and incubated anaerobically at 35°C for 3 to 4 days before testing was conducted according to the instructions of the manufacturers. In the RapID ANA II assay, a 6-digit microcode was generated; the identification was performed using the RapID ANA II microcode compendium. In the Rapid ID 32 A assay, the 10-digit code was checked using APILAB software. In the RapID CB Plus assay, the results were derived in the form of a 7-digit microcode, which was entered into the identification differential chart electronic code compendium containing the RapID CB Plus database. In the BBL Crystal assay, the reading was done visually with the BBL Crystal Panel Viewer, and the generated 10-digit profile number was interpreted using the BBL Crystal ID System electronic codebook.

Approximately half of the classical *Actinomyces* strains were correctly identified to the species level (Table 1): 40% by RapID ANA II, 58% by Rapid ID 32 A, 26% by RapID CB Plus, and 65% by BBL Crystal. Previously, various levels of species identification have been reported for RapID ANA II (24% [12] and 85% [1]), RapID CB Plus (52% [74% with extra tests]) (9), and BBL Crystal (97%) (2). When the present results obtained by different kits were combined, *A. israelii* proved to be the species most successfully identified, the identification being correct for 72% of the strains examined. Except for the BBL Crystal kit, the applicability of the kits for *A. naeslundii* and *A. viscosus* identification was poor. The main problem with these two species was the negative urease reaction. According to the differential charts of the RapID ANA II and RapID CB Plus systems, 90 to 94% of *A. naeslundii* and >80% of *A. viscosus* strains should be urease positive; how-

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TABLE 1. Performance of four commercial test kits in correctly identifying (i) classical *Actinomyces* to the species level and (ii) newly described *Actinomyces* and related species to the genus level

Organism	No. of strains tested	Result for kit							
		RapID ANA II		RapID 32 A		RapID CB Plus		BBL Crystal ANR ID	
		Proportion (%) of samples correctly identified	Strain included in kit database	Proportion (%) of samples correctly identified	Strain included in kit database	Proportion (%) of samples correctly identified	Strain included in kit database	Proportion (%) of samples correctly identified	Strain included in kit database
Classical *Actinomyces* strains									
A. israelii	8	25	Yes	100	Yes	75	Yes	88	Yes
A. meyeri	5	100	Yes	100	Yes		No	0	Yes
A. naeslundii	12	0	Yes	17	Yes	8	Yes	67	Yes
A. odontolyticus	15	73	Yes	60	Yes	20	Yes	80	Yes
A. viscosus	13	0	Yes	15	Yes	0	Yes	92	Yes
Newly described *Actinomyces* and related species strains									
A. europaeus	5	0	No	80	No	20	No	60	No
A. georgiae	4	0	No	0	No	0	No	25	No
A. gerencseriae	4	0	No	100	No	25	No	50	No
A. graevenitzii	10	0	No	0	No	0	No	60	No
A. neuii subsp. *anitratus*	6	0	No	0	No	33	No	83	No
A. neuii subsp. *neuii*	6	0	No	17	No	100	Yes	0	No
A. radicidentis	1	0	No	100	No	0	No	100	No
A. radingae	6	0	No	17	No	0	No	0	No
A. turicensis[a]	5	100	No	40	No	0	No	80	No
A. urogenitalis	1	0	No	100	No	0	No	0	No
Arcanobacterium pyogenes	1	0	No	0	No	0	No	100	No
Arcanobacterium bernardiae	1	0	No	0	No	100	Yes	100	No
Actinobaculum schaalii	4	50	No	50	No	0	No	25	No

[a] *A. turicensis* is included in the latest RapID ANA II database.

ever, none of the strains examined in the present study produced urease in either system. Most *A. viscosus* strains did not produce urease, whereas *A. naeslundii* strains were urease positive when tested using individual Rosco diagnostic tablets (16). In addition, negative or weak enzymatic reactions in tests using RapID ANA II and RapID CB Plus gave false identifications with *A. naeslundii*. Hudspeth et al. (9) also reported difficulties in the identification of *A. naeslundii* by RapID CB Plus. Rapid ID 32 A was hardly more successful with the two species: together with a negative urease reaction, a negative glycine reaction was the main reason for misidentification.

In general, BBL Crystal revealed to be most successful system for the rapid identification of classical *Actinomyces* species. This system contains 29 reactants (in similarity to the Rapid ID 32 A system), whereas the RapID ANA II and RapID CB Plus systems contain only 18 reactants. Consequently, the BBL Crystal system is more discriminatory with respect to identifying different species. *A. meyeri* was the only classical *Actinomyces* species misidentified, but 75% of the clinical strains were correctly identified to the genus level. Cavallaro et al. (2) found difficulties only with *A. naeslundii*, reporting one strain as *Bifidobacterium dentium-A. israelii*. In our study, one *A. naeslundii* strain was identified as *A. israelii* (with 92.5% agreement), the second choice being *B. dentium* (7.0% agreement). This strain gave a positive β-D-xylosidase reaction, whereas *A. naeslundii* should give a negative reaction according to the BBL Crystal differential chart. Two *A. naeslundii* strains misidentified at 4 h were correctly identified after 24 h due to a positive furanose reaction in one strain and positive furanose, pyranose, α-D-galactosidase, and α-D-glucosidase reactions in another strain. BBL Crystal identified the *A. pyogenes* type strain as *A. israelii*. According to the code compendium, *A. pyogenes* should give negative results for isoleucine, β-D-xyloside, disaccharide, furanose, pyranose, β-D-galactosidase, α-D-glucosidase, and β-D-glucosidase; however, for the tested strains those reactions were positive, as reported previously (16). Poor identifications in the BBL Crystal system were due to weak fluorescence after 4 h of incubation. Notably, the reactions were more intense after 24-h incubation and thus easier to interpret as positive. It can be recommended that the incubation of BBL Crystal panels be continued overnight after the 4-h reading time point.

New *Actinomyces* strains (except for *A. neuii* and *A. bernardiae* in the RapID CB Plus database) are not included in the databases of the examined kits; however, the highest percentage of correct identification to the genus level was obtained with the BBL Crystal system (Table 1). By phenotypic testing, a nitrate reduction differentiated *A. neuii* subsp. *neuii* from *A. neuii* subsp. *anitratus*, a positive arabinose reaction (and colony morphology) differentiated *A. gerencseriae* from *A. israelii*, and a positive esculin reaction differentiated *A. europaeus* from *A. pyogenes* (negative reaction) (16). Recently, Clarridge and Zhang (3) correctly identified some newly described *Actinomyces* species using the RapID ANA II system; one clinical *A. turicensis* strain in our study had an identical code number (020671).

In conclusion, none of the examined commercial test systems identifies *Actinomyces* strains correctly; thus, additional tests or corrections to their databases are needed. BBL Crystal proved to be the most reliable kit for the rapid identification of classical *Actinomyces*. Commercial kits should be developed to identify the newly described *Actinomyces* species as well.

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