Evaluation of the Rapid ID 32A system for identification of anaerobic Gram-negative bacilli, excluding the *Bacteroides fragilis* group

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Objective: To evaluate the Rapid ID 32A system (bioMérieux, Marcy-l'Etoile, France) for the identification of anaerobic Gram-negative bacilli, excluding the *Bacteroides fragilis* group.

Methods: Five hundred and twenty-eight identified clinical isolates of non-*B. fragilis* group anaerobic Gram-negative bacilli were tested in the Rapid ID 32A system, and identifications were compared with those obtained with conventional biochemical tests and gas–liquid chromatography.

Results: The Rapid ID 32A system correctly identified 280 (60.9%) of the 460 isolates tested for which taxa were included in the database, without the need for additional testing. A further 97 (21.1%) isolates were correctly identified to species level following the performance of complementary tests recommended by the manufacturer. Fifty-nine (12.8%) isolates were identified at the genus level only, and 21 (4.6%) were misidentified at the species level. Three isolates of *Prevotella* were not identified by the system. Of the 68 isolates belonging to taxa not included in the database, no identification was obtained for 33 (48.5%), while 35 (51.5%) were misidentified.

Conclusions: The Rapid ID 32A system provided a rapid and reliable method for the identification of non-*B. fragilis* group, anaerobic Gram-negative bacilli to the genus level, while the success of species-level identification varied with different taxa. There was poor discrimination between *Fusobacterium nucleatum* and *F. necrophorum,* between *Porphyromonas asaccharolytica* and *Porphyromonas endodontalis,* and between *Prevotella buccalis, Prevotella denticola, Prevotella loescheii, Prevotella melaninogenica* and *Prevotella oralis.* The need to perform conventional complementary tests on 149 (32.4%) of the 460 isolates compromised the usefulness of the system for rapid species identification.

Key words: Rapid ID 32A, identification, anaerobic Gram-negative bacilli

INTRODUCTION

The anaerobic Gram-negative bacilli are well recognized as significant clinical pathogens, and it is therefore important that they can be isolated from clinical specimens and identified as rapidly and accurately as possible [1]. The identification of anaerobic bacteria by conventional methods involving biochemical tests and gas–liquid chromatography is, however, a slow, time-consuming and labor-intensive process requiring considerable technical expertise. Although used in reference laboratories for anaerobe identification, these techniques are not practical in a diagnostic clinical laboratory trying to minimize workload and costs and wanting rapid results for effective patient management.

Commercial identification systems are now available for the identification of anaerobic bacteria isolated from clinical specimens. The original commercial systems were based on conventional biochemical tests and therefore relied on organism growth within the system after 24–48 h of anaerobic incubation. More recently, several identification systems have become available that utilize preformed bacterial enzymes for the hydrolysis of chromogenic substrates. Such systems include the RapID ANA system (Innovative Diagnostic Systems Inc., Atlanta, Ga, USA), the AN-Ident system (Analytab Products, Plainview, NY, USA) and the Rapid ID 32A system (bioMérieux, Marcy-l'Etoile, France). These systems are growth independent, allowing an identification to be achieved after only 4 h of

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incubation under aerobic conditions. They also allow for the identification of the asaccharolytic, traditionally non-reactive species, which can demonstrate high levels of reactivity in these systems.

Previous studies have evaluated the Rapid ID 32A system for the identification of anaerobic cocci and the *Bacteroides fragilis* group [2–5]. Other studies have also evaluated the API system for the identification of anaerobic Gram-negative bacilli and some other anaerobic bacteria recovered from clinical specimens [6–8]. However, in these studies only 22–25 strains of *Prevotella* spp., zero to eight strains of *Porphyromonas* spp. and 10–22 strains of *Fusobacterium* spp. were tested in the system [6–8]. While it is important to evaluate an identification system using taxa in the proportions in which they are recovered from clinical specimens, it is also important to challenge the system with larger numbers of less frequently isolated species to assess its true performance. There are 80 anaerobic taxa represented in the Rapid ID 32A database, of which 21 are Gram-negative bacilli, not including the *Bacteroides fragilis* group. We applied the rapid method to clinical isolates of this group of organisms to evaluate the ability of the Rapid ID 32A system to identify these Gram-negative bacilli accurately, compared with conventional methods. We also tested taxa not included in the database to determine whether the system would accurately classify them as unidentifiable strains or incorrectly assign them to other taxa in the database with similar enzyme profiles.

MATERIALS AND METHODS

Bacterial strains

Five hundred and twenty-eight identified clinical isolates of anaerobic Gram-negative bacilli, excluding the *Bacteroides fragilis* group, were tested in the present study. Of these 528 isolates, 460 represented taxa that were included in the Rapid ID 32A database (Table 1) and 68 were from taxa not included in the database (Table 3). A further 15 isolates that we were unable to identify by conventional methods were also tested in the system, although these results do not figure in the final analysis. All were clinical isolates associated with infection and were selected from a total of 813 collected in 15 European medical and dental laboratories over an 18-month period (January 1995 to June 1996) for a study of antibiotic susceptibility [9]. The isolates were selected to give representative numbers of the different taxa included in this study. The following seven ATCC reference strains were also tested in the Rapid ID 32A system: *Bacteroides fragilis* 25285, *Bacteroides thetaiotaomicron* 29741, *Bacteroides thetaiotaomicron* 29742 and *Bacteroides ureolyticus* 33387, which represent taxa included in the database, and *Prevotella corporis* 33547, *Porphyromonas levii* 29147 and *Campylobacter gracilis* 33236, which represent taxa not included in the database. All strains were stored in 7% glycerol broth at −70°C and subsequently passaged twice before testing. Incubation was in an anaerobic cabinet with an atmosphere of 80% N_2, 10% H_2 and 10% CO_2.

Conventional identification

Identification was performed according to standard criteria [10,11]. Conventional tests, with Fastidious Anaerobe Agar (FAA) (Lab 90, Lab M, Bury, UK) as the culture medium, included Gram stain and colony morphology, pigmentation, disk susceptibility to antimicrobials (kanamycin, 1000 μg; vancomycin, 5 μg; colistin, 10 μg), spot indole test with paradimethylaminocinnamaldehyde, catalase reaction with 15% (vol/vol) hydrogen peroxide and sensitivity to 20% bile on bile-esculin agar. Where appropriate, further tests included the lipase reaction on egg yolk agar, indole production and nitrate reduction in indole-nitrate broth (Becton Dickinson Microbiology, Cockeysville, MD, USA), pigmentation on laked rabbit blood agar, stimulation of growth by formate and fumarate or pyruvate, and motility from broth culture. Enzyme reactions for α-fucosidase, β-*N*-acetylglucosaminidase, β-xylosidase, α-glucosidase, trypsin, esculin hydrolysis, β-galactosidase (ONPG) and urease were determined with Rosco tablets (Rosco Diagnostica, Taastrup, Denmark). Rosco tablets were added to a heavy bacterial suspension in 0.25 mL of saline and reactions read after 4-h and overnight incubation at 37°C, according to the manufacturer's instructions.

Carbohydrate-fermentation tests were performed in prereduced anaerobically sterilized (PRAS) media for glucose, arabinose, cellobiose, lactose, salicin, sucrose and xylose [10]. A peptone–yeast broth without carbohydrate was included with each set as a fermentation blank. Control plates for each PRAS set were inoculated and incubated anaerobically and aerobically to check for purity and aerotolerance. Short-chain fatty acid analysis by gas–liquid chromatography (GLC) was performed on the PRAS peptone–yeast–glucose broth culture.

In this study, *Prevotella intermedia* represents both *Prevotella intermedia* and *Prevotella nigrescens*, as there are no reliable phenotypic tests to differentiate these two species, and the molecular techniques required to do so were not available for this study. Likewise, the type strain of *Porphyromonas levii* is of animal origin, but catalase-negative strains with phenotypic characteristics similar to those of *Porphyromonas levii* have been recovered from humans [11]. Ten such isolates are included in this study as *Porphyromonas levii*-like strains.

Rapid ID 32A identification

The Rapid ID 32A system is a 4-h identification system based on enzymatic degradation of chromogenic substrates by preformed bacterial enzymes. The system consists of disposable plastic strips containing 29 dehydrated substrates for the following reactions: urease (URE), arginine dihydrolase (ADH), α-galactosidase (αGAL), β-galactosidase (βGAL), β-galactosidase 6-phosphate (βGP), α-glucosidase (αGLU), β-glucosidase (βGLU), α-arabinosidase (αARA), β-glucuronidase (βGUR), β-*N*-acetylglucosaminidase (βNAG), mannose (MNE) and raffinose (RAF) fermentation, glutamic acid decarboxylase (GDC), α-fucosidase (αFUC), nitrate reduction (NIT), indole production (IND), alkaline phosphatase (PAL), arginine arylamidase (ArgA), proline arylamidase (ProA), leucyl glycine arylamidase (LGA), phenylalanine arylamidase (PheA), leucine arylamidase (LeuA), pyroglutamic acid arylamidase (PyrA), tyrosine arylamidase (TyrA), alanine arylamidase (AlaA), glycine arylamidase (GlyA), histidine arylamidase (HisA), glutamyl glutamic acid arylamidase (GGA) and serine arylamidase (SerA).

The Rapid ID 32A strips were inoculated according to the manufacturer's instructions. A 48-h anaerobic culture on Columbia agar (Oxoid, Basingstoke, UK) with 7% (vol/vol) horse blood was suspended in 3 mL of suspension medium (bioMérieux) and the turbidity adjusted to diode number 30 on the densitometer supplied with the system (equivalent to a 4 McFarland turbidity standard). The panels were inoculated with 55 μL of suspension per cupule, and the urea cupule was overlayed with mineral oil. Panels were incubated aerobically for 4 h at 37°C. Reagents supplied by the manufacturer were then added to the following cupules: Nit 1 and Nit 2 reagents to nitrate, James reagent to indole and FB (fast blue) reagent to PAL to SerA inclusive. The test reactions were read visually between 5 and 10 min after the addition of reagents, according to the manufacturer's instructions. For the carbohydrate-degradation enzymes, any development of yellow color, except for a faint tinge, was recorded as a positive test, and color reactions for the aminopeptidase tests were graded from 0 to 5, with grades 0–2 representing negative test reactions and grades 3–5 positive reactions (as instructed by bioMérieux). Equivocal color reactions could be coded as a question mark, and the computer program would disregard these tests when determining the identification of the isolate. A 10-digit profile number was generated and an identification obtained with the computerized database (software version 3.0). Each isolate was listed with a percentage of identification (% ID), which is an estimate of how closely its enzyme profile corresponded to the given taxon relative to all the other taxa in the database. When there was low discrimination between taxa with similar enzyme profiles, complementary tests, as suggested by the manufacturer, were used to differentiate the taxa listed. In this study, these consisted of Gram stain morphology, spore production, hemolysis, motility, lecithinase production, hydrolysis of esculin, starch and gelatin, and fermentation of arabinose, cellobiose, fructose, glucose, maltose and salicin.

Interpretation of results

The Rapid ID 32A results were compared to conventional identifications. When the species identification was the same by both methods, the strain was classified as correct to species level, either with or without complementary tests. When the genus identification was the same by both methods, but the Rapid ID 32A system was unable to assign a species identification, the strain was classified as correct to genus level, either with or without complementary tests. Incorrect identification meant that the species identification obtained by the Rapid ID 32A system differed from the conventional identification. Strains with enzyme profiles that gave unacceptable or non-valid identifications in the Rapid ID 32A system were classified as not identified.

RESULTS

The four ATCC reference strains tested that were included in the database were all correctly identified in the Rapid ID 32A system, while *Prevotella corporis* 33547 was identified as *Prevotella* sp., and *Porphyromonas levii* 29147 and *Campylobacter gracilis* 33236 were, correctly, not assigned an identification by the system. These ATCC strains were not included in the analysis of the clinical isolates.

The results for the 460 isolates whose taxa are included in the Rapid ID 32A database are shown in Table 1. Of these 460, the Rapid ID 32A system correctly identified 280 (60.9%) to the species level without complementary tests, and a further 97 (21.1%) to species level with complementary tests recommended by the manufacturer. Identification to correct genus level only occurred for 59 (12.8%) isolates, of which 39 required complementary tests. Twenty-one (4.6%) isolates were misidentified by the Rapid ID 32A system (Table 2), while the system was unable to identify three *Prevotella* isolates.

Eighty-nine *Fusobacterium* isolates were tested, of which only 27 (30.3%) were identified to species level without complementary tests. Twenty *F. necrophorum* isolates (all IND and PAL positive and either positive or negative for GDC) required Gram stain and spore test to differentiate them from *Clostridium tetani*. Four

Table 1 Identification of anaerobic, Gram-negative bacilli included in the Rapid ID 32A database

Isolate	No. tested	No. correct to species level: Without complementary tests	No. correct to species level: With complementary tests	No. correct to genus level only: Without complementary tests	No. correct to genus level only: With complementary tests	No. incorrect at species level: Without complementary tests	No. incorrect at species level: With complementary tests	No. not identified
Bacteroides capillosus	1	1						
Bacteroides ureolyticus	24	24						
Fusobacterium necrophorum	27		20		3	4		
F. nucleatum	48	21			27			
F. mortiferum	7	4		3				
F. varium	7	2	5					
Leptotrichia buccalis	1	1						
Porphyromonas asaccharolytica	24	8		16				
Porphyromonas endodontalis	2	1		1				
Porphyromonas gingivalis	26	21				1	4	
Prevotella bivia	85	66	17			1	1	
Prevotella buccae	33	32						1
Prevotella buccalis	6		1		4		1	
Prevotella denticola	33		31			2		
Prevotella disiens	31	30			1			
Prevotella intermedia[a]	67	66						1
Prevotella loescheii	10	1	5		4			
Prevotella melaninogenica	10		3				7	
Prevotella oralis	18	2	15					1
Total	460	280	97	20	39	8	13	3

[a]*Prevotella intermedia* includes both *Prevotella intermedia* and *Prevotella nigrescens*.

Table 2 Misidentification of isolates included in the Rapid ID 32A database

Conventional identification	No. of isolates	Rapid ID 32A identification
Fusobacterium necrophorum	4	*F. nucleatum*
Porphyromonas gingivalis	5	*Porphyromonas endodontalis*
Prevotella bivia	2	*Prevotella oralis* (1), *Prevotella loescheii* (1)
Prevotella buccalis	1	*Prevotella loescheii*
Prevotella denticola	2	*Prevotella melaninogenica*
Prevotella melaninogenica	7	*Prevotella denticola* (6), *Prevotella bivia* (1)

F. necrophorum isolates (all IND and GDC positive but PAL negative) were misidentified as *F. nucleatum* by the system (Table 2). Three isolates of *F. necrophorum* and 27 of *F. nucleatum* were positive only for IND in the Rapid ID 32A system, which resulted in low discrimination between *F. nucleatum*, *Clostridium tetani*, *Clostridium bifermentans* and *F. necrophorum*. The complementary tests of Gram stain, spore test, lecithinase and motility were able to discriminate them from the *Clostridium* spp. but unable to differentiate *F. nucleatum* and *F. necrophorum*, resulting in a final identification of *Fusobacterium* sp. Three *F. mortiferum* isolates could be identified only as *Fusobacterium* sp., as no complementary tests were suggested by the system to differentiate the two choices of *F. mortiferum* and *F. necrogenes*. Five *F. varium* isolates required Gram stain and spore test to differentiate them from *Clostridium histolyticum*, *Clostridium tyrobutyricum* and *Clostridium sporogenes*.

Of the 52 *Porphyromonas* isolates tested, 30 (57.7%) were identified to species level without complementary tests, while 17 (32.7%) could be identified to the genus level only as *Porphyromonas* sp. All of the 24 *Porphyromonas asaccharolytica* isolates tested were positive for IND, PAL, LGA, AlaA and αFUC in the Rapid ID 32A panel. Eight of the 24 were also positive for GGA and identified as *Porphyromonas asaccharolytica*, but the remaining 16 were GGA negative and could be identified to the genus level only as *Porphyromonas* sp.,

because the system could not differentiate them from *Porphyromonas endodontalis*. The two isolates of *Porphyromonas endodontalis* tested were also positive for IND, PAL, LGA and AlaA, but negative for αFUC. One isolate was GGA negative and identified as *Porphyromonas endodontalis*, while the other was GGA positive and could be identified only as *Porphyromonas* sp. Five isolates of *Porphyromonas gingivalis* were incorrectly identified as *Porphyromonas endodontalis* (Table 2).

Two hundred and ninety-three *Prevotella* isolates were tested, of which 197 (67.2%) were identified to species level without complementary tests. A further 72 (24.6%), all requiring fermentation of cellobiose and salicin, with some strains also requiring fermentation of maltose, and detection of hemolysis or hydrolysis of esculin, starch or gelatin, were identified to species level following the performance of these complementary tests. Seventeen isolates of *Prevotella bivia* required complementary tests to differentiate them from *Prevotella oralis*. All 31 *Prevotella denticola* isolates requiring additional testing for correct species identification had *Prevotella oralis* listed as an alternative identification option, while 14 also had *Prevotella melaninogenica*, 10 *Prevotella loescheii*, four *Prevotella bivia*, two *Prevotella buccalis* and one *Bacteroides capillosus* as possible identifications. Five *Prevotella loescheii* isolates required complementary tests to differentiate them from either *Prevotella oralis*, *Prevotella denticola*, *Prevotella melaninogenica* or *Bacteroides capillosus*, while three isolates of *Prevotella melaninogenica* required differentiation from *Prevotella oralis* and *Prevotella denticola*. Of the 18 *Prevotella oralis* isolates tested, 15 required complementary tests to discriminate between one or more of *Prevotella denticola* (10), *Prevotella loescheii* (nine), *Prevotella melaninogenica* (three), *Prevotella bivia* (two) and *Bacteroides capillosus* (three), before species-level identification could be achieved. Nine of the 293 *Prevotella* isolates tested were identified to genus level only as *Prevotella* sp., because the complementary tests suggested could not differentiate between the given options. Twelve isolates were misidentified at the species level (Table 2), while, for three isolates of *Prevotella*, no identification was obtained with the Rapid ID 32A system.

The results for the 68 isolates whose taxa are not included in the Rapid ID 32A database, are shown in Table 3. The system, correctly, did not assign any identification to 33 (48.5%) isolates including 11 of the 12 *Bilophila wadsworthia* isolates, four *Campylobacter gracilis* isolates and one *Campylobacter rectus* isolate that were all non-reactive in the test strips. The remaining 35 (51.5%) isolates were assigned an identification, with or without complementary tests; of these, 34 were given a species identification, while one *Prevotella corporis* isolate was identified as *Prevotella* sp. The 10 isolates identified as *Bacteroides ureolyticus* consisted of six *Campylobacter rectus* isolates and two *Campylobacter gracilis* isolates that were LeuA positive and identified as *Bacteroides ureolyticus* following complementary tests (spore test, lecithinase, fructose and Gram stain) to differentiate them from *Clostridium difficile* and *Clostridium bifermentans*. Also, one isolate of *Bilophila wadsworthia* that was URE positive in the Rapid ID 32A system and one of *F. russii* that was PAL and GDC positive were both identified as *Bacteroides ureolyticus*.

The 15 isolates tested for which a conventional identification could not be obtained consisted of two distinct phenotypic groups of eight and seven isolates each. The first eight isolates were all positive for βGAL, αGLU, αARA, PAL, ArgA, LGA, AlaA and GGA, with variable reactions to βGLU, βGUR and βNAG. All eight strains had unacceptable profiles in the Rapid ID 32A system, so no identification was obtained. They

Table 3 Identification of anaerobic, Gram-negative bacilli not included in the Rapid ID 32A database

Isolate	No. tested	No. not identified	No. misidentified	Rapid ID 32A identifications for 35 misidentified isolates
Bacteroides putredinis	1	1		
Bacteroides splanchnicus	5	4	1	*Prevotella buccae*
Bilophila wadsworthia	12	11	1	*Bacteroides ureolyticus*
Campylobacter gracilis	8	5	3	*Bacteroides ureolyticus* (2), *Peptostreptococcus prevotii* (1)
Campylobacter rectus	7	1	6	*Bacteroides ureolyticus*
Fusobacterium naviforme	1		1	*Fusobacterium nucleatum*
Fusobacterium russii	1		1	*Bacteroides ureolyticus*
Porphyromonas levii-like[a]	10	9	1	*Prevotella oralis*
Prevotella corporis	4		4	*Prevotella disiens* (3), *Prevotella* sp. (1)
Prevotella dentalis	1		1	*Prevotella buccae*
Prevotella heparinolytica	1		1	*Prevotella buccae*
Prevotella oris	17	2	15	*Prevotella buccae* (9), *Prevotella oralis* (4), *Prevotella loescheii* (2)
Total	68	33	35	

[a]Human isolates with phenotypic characteristics similar to those of *Porphyromonas levii*.

were most similar to *Prevotella buccae* and *Prevotella oralis*, although, for all isolates, between one and three tests were in total disagreement with these options. For the second group, six of the seven isolates had identical profiles, being positive for βGAL, βGP, αGLU, βNAG, PAL, LGA and AlaA. This profile gave a very good identification to genus level as *Prevotella* sp., with species options *Prevotella oralis* (90.0% ID) and *Prevotella denticola* (9.6% ID). A final identification of *Prevotella denticola* was obtained with the complementary tests of cellobiose and salicin fermentation. The seventh isolate was positive for RAF and GGA in addition to the seven tests already mentioned. This resulted in a good identification to the genus level as *Prevotella* sp., with species options *Prevotella oralis* (54.6% ID) and *Prevotella denticola* (41.9% ID). Once again, complementary tests for cellobiose and salicin fermentation resulted in a final identification of *Prevotella denticola*. However, conventional tests do not confirm this identification as these isolates are non-pigmented and α-fucosidase and sucrose negative, whereas *Prevotella denticola* should be positive for these tests [11]. Molecular studies need to be carried out on these isolates to determine their true identification.

DISCUSSION

This study evaluated the ability of the Rapid ID 32A system to identify the anaerobic Gram-negative bacilli, excluding the *Bacteroides fragilis* group. Of the 460 isolates belonging to taxa included in the database, 377 (82%) were correctly identified to the species level, 280 (60.9%) without complementary tests and 97 (21.1%) with complementary tests. Fifty-nine (12.8%) isolates with low discrimination were identified to the correct genus level only without species identification, as the complementary tests suggested by the manufacturer were unable to differentiate between the options listed. Twenty-one (4.6%) isolates were misidentified at the species level, although the genus identification was correct in all cases. The Rapid ID 32A system was unable to identify three *Prevotella* isolates.

The Rapid ID 32A system was simple to use, and the interpretation of most test reactions was not difficult. In the present study, the βGP test and, to a lesser extent, the αGAL test could be difficult to interpret when they gave a pale yellow color reaction. The aminopeptidase tests ArgA and GGA could also be difficult to interpret when they gave borderline color reactions. According to the manufacturer's instructions, a negative GDC reaction is green and a positive GDC reaction is blue. In the present study, however, we found that 40% of our *F. nucleatum* isolates gave a blue–green color reaction which we interpreted as a weak positive, resulting in a correct identification. If these weak GDC reactions had been interpreted as negative, then these *F. nucleatum* isolates would have been identified to the genus level only as *Fusobacterium* sp. All 27 isolates of *Bilophila wadsworthia*, *Campylobacter gracilis* and *Campylobacter rectus* tested were positive for nitrate reduction by conventional methods, but with the exception of one *Campylobacter gracilis* isolate, they were all nitrate negative in the Rapid ID 32A system. There were also discrepancies with the urea test for 11 isolates of *Bilophila wadsworthia* that were urea positive by conventional methods, while only one was urea positive in the Rapid ID 32A system.

The preformed enzyme systems rely on different taxa having species-specific enzyme profiles to provide a species-level identification. However, when several taxa have similar enzyme profiles, resulting in low discrimination between strains from these taxa, the ability of the system to provide a species identification may be compromised. Low discrimination between *Porphyromonas asaccharolytica* and *Porphyromonas endodontalis* meant that 16 of the 24 isolates of *Porphyromonas asaccharolytica* tested could be identified only as *Porphyromonas* sp. Also, the failure of the Rapid ID 32A system to provide complementary tests to differentiate between *F. nucleatum* and *F. necrophorum* meant that 27 of the 48 isolates of *F. nucleatum* tested could be identified only to the genus level as *Fusobacterium* sp. These 16 isolates of *Porphyromonas asaccharolytica* and 27 isolates of *F. nucleatum* accounted for 72.9% of the 59 isolates identified to genus level only with the Rapid ID 32A systems. We also found low discrimination in the system between some of the *Prevotella* spp., namely *Prevotella buccalis, Prevotella denticola, Prevotella loescheii, Prevotella melaninogenica* and *Prevotella oralis*, with only three of the 77 isolates tested being correctly identified to the species level without complementary tests, and a further 55 being identified to species level with complementary tests. Eight isolates from these taxa could be identified only to the genus level as *Prevotella* sp., while 10 were misidentified at the species level. It should be noted that, even by conventional tests, these closely related *Prevotella* spp. have few tests to differentiate them.

In the present study, 149 (32.4%) of the 460 isolates included in the database required complementary tests. Some tests, such as the Gram stain, which was required to differentiate *Fusobacterium* spp. from *Clostridium* spp., are simple and easy to perform. However, all of the 90 *Prevotella* spp. requiring complementary tests needed fermentation of cellobiose and salicin, with further additional tests being required for some strains (maltose fermentation, detection of hemolysis, and hydrolysis of esculin, starch or gelatin). The use of such conventional

biochemical tests as complementary tests diminishes the usefulness of the system by increasing the workload and delaying identification by days. However, 71 of these 90 *Prevotella* spp. were from an oral source and would therefore be less frequently isolated in a routine diagnostic laboratory. In addition, they could probably be identified to the genus level only as *Prevotella* sp. with minimal clinical impact, thereby making the Rapid ID 32A system more practical in many diagnostic laboratories.

In previous studies evaluating the Gram-negative bacilli included in the database, excluding the *Bacteroides fragilis* group, Kitch and Appelbaum [7] identified 19 (55.9%) of their 34 isolates, Looney et al. [8] identified 27 (55.1%) of their 49 isolates, and Arzese et al. [6] correctly identified 29 (58%) of their 50 isolates tested to the species level without complementary tests, which is comparable to the 60.9% achieved in the present study. However, for the identification to species level including complementary tests, Kitch and Appelbaum [7] achieved correct species identification for 30 (88.2%) isolates, Arzese et al [6] for 45 (90%) isolates and Looney et al [8] for 48 (98%) isolates, versus 82% species identification in the present study. The higher percentage of species-level identification in these studies could in part be due to the selection of taxa tested. In the study of Looney et al [8] for example, 16 of the 21 *Prevotella* spp. tested were either *Prevotella bivia*, *Prevotella buccae* or *Prevotella intermedia*, all taxa which we found that the Rapid ID 32A system could successfully identify. The smaller number of isolates tested from each taxon in these previous studies may also affect the outcome of their evaluations. We tested 24 isolates of *Porphyromonas asaccharolytica*, of which 16 could be identified only as *Porphyromonas* sp., while Looney et al [8] tested only two and Arzese et al [6] four isolates of *Porphyromonas asaccharolytica*, and Kitch and Appelbaum [7] did not test any *Porphyromonas* spp. Another contributing factor to the lower rate of species-level identification in the present study was the fact that 27 isolates of *F. nucleatum* could be identified only to the genus level. However, in these previous studies, 30 isolates of *F. nucleatum* were tested, and even though they all required complementary tests, 29 were identified as *F. nucleatum* and one as *F. varium* [6–8].

Of the isolates tested whose taxa are not included in the Rapid ID 32A database, the non-reactivity of *Bilophila wadsworthia* in the test strips means that it would not be possible to incorporate this taxon into the database. Six of the seven *Campylobacter rectus* isolates tested were positive for LeuA and misidentified as *Bacteroides ureolyticus*. *Campylobacter rectus* could be included in the database if complementary tests were added to differentiate these two taxa. All nine unidentified *Porphyromonas levii*-like isolates, as well as the ATCC *Porphyromonas levii* strain, were positive for βGAL, βNAG, PAL, LGA, AlaA and GGA, while one was also positive in the Rapid ID 32A system for αFUC, although it was negative for this test with the Rosco tablets. This unique and distinctive enzyme profile should make it easy to incorporate *Porphyromonas levii* into the Rapid ID 32A database. However, the one *Porphyromonas levii*-like isolate that was misidentified as *Prevotella oralis* was positive for αGLU in addition to the other six tests previously mentioned. The manufacturers would therefore need to include complementary tests to differentiate αGLU-positive *Porphyromonas levii*-like strains from *Prevotella oralis*. For *Prevotella corporis* and *Prevotella oris*, however, inclusion in the database may not be as successful, because of the low discrimination from other *Prevotella* spp. already in the database, namely *Prevotella disiens* and *Prevotella buccae*.

In conclusion, we found the Rapid ID 32A system to be a simple, rapid and reliable method for the identification of the non-*Bacteroides fragilis* group, anaerobic Gram-negative bacilli to the genus level, while the success of species-level identification varied with different taxa. The low discrimination between *F. nucleatum* and *F. necrophorum*, as well as the lack of adequate complementary tests to distinguish them, meant that many isolates of *F. nucleatum* could be identified to the genus level only as *Fusobacterium* sp. Similarly, because the system could not readily discriminate between *Porphyromonas asaccharolytica* and *Porphyromonas endodontalis*, the majority of *Porphyromonas asaccharolytica* isolates were identified only as *Porphyromonas* sp. The system performed very well with *Prevotella buccae*, *Prevotella disiens*, *Prevotella intermedia* and, to a lesser extent *Prevotella bivia*, without the need for complementary tests. However, the low discrimination between *Prevotella buccalis*, *Prevotella denticola*, *Prevotella loescheii*, *Prevotella melaninogenica* and *Prevotella oralis* meant that 71 of the 77 isolates tested from these taxa required complementary tests. To make the system more practical, these taxa could be identified to genus level only as *Prevotella* sp. The system would be suitable for routine use in clinical laboratories when identification is considered necessary. Its usefulness in normal flora studies, particularly in oral microbiology, is debatable, as the database is limited and may not include all the species encountered.

Overall, some of the problems encountered could be alleviated by changes in the database and the addition of appropriate complementary tests, but the need to perform conventional biochemical tests to complement the system for some species compromises its usefulness as a rapid system of identification for these taxa.

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