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Comparison of Three Commercial Rapid Identification Systems for the Unusual Gram-Positive Cocci *Dolosigranulum pigrum*, *Ignavigranum ruoffiae*, and *Facklamia* Species

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We evaluated three rapid identification systems—The Biomerieux rapid ID 32 STREP (ID32), the BBL Crystal rapid gram-positive identification (Crystal), and the Remel IDS RapID STR (IDS) systems—for their ability to identify 7 strains of *Alloiococcus otitidis*, 27 strains of *Dolosigranulum pigrum*, 3 strains of *Ignavigranum ruoffiae*, and 18 strains of 4 different *Facklamia* species. Since none of these six species of gram-positive cocci are included in the identification databases for these systems, the correct identification for the strains tested should be "unacceptable ID" for the ID32 and Crystal systems or "no choice" for the IDS system. The ID32 system identified all 27 strains of *D. pigrum*, 6 of 18 *Facklamia* species, and 2 of 3 cultures of *I. ruoffiae* as "unacceptable ID." The Crystal system identified 10 of 27 *D. pigrum*, 2 of 18 *Facklamia* species, and 2 of 3 *I. ruoffiae* strains as "unacceptable ID." The IDS system identified only 1 culture of *D. pigrum* as "no choice," but it also identified 2 cultures of *D. pigrum* as a "questionable microcode" and 19 cultures of *D. pigrum* as an "inadequate ID, *E. faecalis* 90%, *S. intermedius* 9%." A total of 2 of the 18 cultures of *Facklamia* and all 3 of the *I. ruoffiae* cultures were correctly identified as "no choice." The most common misidentifications of *Facklamia* species by the ID32 and IDS systems were as various *Streptococcus* species and as *Gemella* species. In the Crystal system, the most common erroneous identification was *Micrococcus luteus*. These data indicate the need for the commercial manufacturers of these products to update their databases to include newly described species of gram-positive cocci.

Since the description of the first *Facklamia* species in 1997, four additional species of this genus have been described (5–8, 17). The species *Facklamia hominis*, *F. ignava*, *F. sourekii*, and *F. tabacinasalis* are most often arranged in chains, whereas *F. languida* is most often arranged in clusters with very little chaining. *Dolosigranulum pigrum* was described in 1993 (2). This bacterium is arranged in pairs, tetrads, and clusters. *Ignavigranum ruoffiae*, recently described (9), is arranged primarily in chains. Information on the identification of these newly described bacteria is minimal, and in most cases the authors initially describing these species have used a combination of molecular characterization, conventional tests, and miniaturized rapid tests such as the API 50CH (Biomerieux, Inc., Hazelwood, Mo.). The identification of these bacteria is problematic since none of the rapid testing systems have them in their databases. We also included seven strains of *Alloiococcus otitidis* for comparison purposes since the phenotypic characteristics of this organism resemble those of *Dolosigranulum*, *Facklamia*, and *Ignavigranum* strains at the genus level.

In a previous study examining 120 strains of unidentified gram-positive cocci with phenotypic characteristics that eliminated them from the known genera of gram-positive bacterial genera, e.g., *Aerococcus*, *Enterococcus*, *Gemella*, *Lactococcus*, and *Streptococcus*, we identified 18 strains as *Facklamia* species, 27 strains of *D. pigrum*, and 3 strains of *I. ruoffiae* by using conventional test methods (11). Rapid identification test systems for gram-positive cocci have been used for nearly 20 years. We evaluated three systems for their capability to correctly identify the *A. otitidis*, *D. pigrum*, *I. ruoffiae*, and *Facklamia* species: the rapid ID 32 STREP (ID32; Biomerieux, Inc., Hazelwood, Mo.) (15), the BBL Crystal Rapid Gram-Positive ID Kit (Crystal; BD Bioscience, Cockeysville, Md.) (20), and the RapID STR (Remel, Inc., Lenexa, Kans.) (3, 16, 19). The ID32 system is a modification of the API 20S system (16, 19). The Remel RapID STR system is the same test kit previously distributed by Innovative Diagnostics, Inc., as the IDS RapID STR system and is referred to here simply as IDS.

MATERIALS AND METHODS

The 55 strains tested were taken from the culture collection of the *Streptococcus* Laboratory at the Centers for Disease Control and Prevention. All strains were identified to the genus level by previously described conventional tests (11) (see Tables 1 and 2). Seventeen of the strains were additionally identified by 16S rRNA sequencing. The sources of these gram-positive cocci were similar to those of the viridans streptococci; 24 cultures were isolated from blood cultures, 6 were isolated from cultures of the eye, 4 were isolated from nasopharyngeal swabs, 2 cultures each were from cerebrospinal fluid and abscesses, and one each was from cultures of bone, ear, gall bladder, gastric contents, sinus, sputum, urine, vagina, wound, and unknown. All seven cultures of *A. otitidis* were isolated from inner ear cultures. Antimicrobial susceptibilities, sources, clinical diagnosis, and other demographic information on these organisms will be reported elsewhere (L. L. LaClaire and R. R. Facklam, submitted for publication).

The ID32 kit and reagents, the Crystal kit, and the IDS system were all used according to the manufacturer's instructions provided. Most strains were tested at least two different times. Some strains were tested four times.

RESULTS AND DISCUSSION

Conventional tests. Strains were first identified as potential *Dolosigranulum*, *Facklamia*, and *Ignavigranum* spp. by determining unique phenotypic characteristics in conventional tests used to identify the different genera of catalase-negative, gram-positive cocci. These three genera are characterized as suscep-

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TABLE 1. Phenotypic characteristics of catalase-negative, gram-positive cocci

Genus table	Phenotypic characteristics[a]									
	Gram stain[b]	Van	GAS	PYR	LAP	NaCl	10°C	45°C	Mot	Hem
Leuconostoc[c]	ch	R	+	−	−	v	+	+	−	α/γ
Weisella[c]	ch	R	+	−	−	+	v	v	−	α/γ
Enterococcus	ch	S/R	−	+	+	+	+	+	v	α/γ
Lactococcus	ch	S	−	+	+	v	+	v	−	α/γ
Vagococcus	ch	S	−	+	+	+	+	−	+	α/γ
Streptococcus	ch	S	−	−	+	v	−	v	−	α/β/γ
Abiotrophia	ch	S	−	+	+	−	−	v	−	α/γ
Globicatella	ch	S	−	+	−	+	−	−	−	α
Dolosicoccus	ch	S	−	+	−	−	−	−	−	α
Pediococcus	cl/t	R	−	−	+	v	−	+	−	α
Tetragenococcus	cl/t	S	−	−	+	+	−	+	−	α
Aerococcus urinae[d]	cl/t	S	−	−	+	+	−	−	−	α
Aerococcus viridans	cl/t	S	−	+	−	+	−	−	−	α
Helcococcus	cl/t	S	−	+	−	+	−	−	−	γ
Gemella	cl/t/ch	S	−	+	v	−	−	−	−	α/γ
Facklamia	cl/ch	S	−	+	+	+	−	−	−	γ
Alloiococcus	cl/t	S	−	+	+	+	−	−	−	γ
Ignavigranum	cl/ch	S	−	+	+	+	−	−	−	γ
Dolosigranulum	cl/t	S	−	+	+	+	−	−	−	γ

[a] Van, vancomycin sensitivity screening test; GAS, gas production in MRS broth; PYR, production of PYRase; LAP, production of LAPase; NaCl, growth in broth containing 6.5% NaCl; 10°C and 45°C, growth at 10 and 45°C; Mot, motile; Hem, hemolytic activity on Trypticase soy 5% sheep blood agar. v, variable; S, susceptible; R, resistant.

[b] Cell arrangement in Gram strain: cl, clusters; t, tetrads; ch, chains.

[c] *Leuconostoc* and *Weisella* are often coccobacillary, sometimes appearing rod-like in chains.

[d] *Aerococcus christensenii* has the same phenotypic characteristics as *A. urinae*.

tible to vancomycin, with negative gas production, positive L-pyrrolidonyl-β-naphthylamide (PYR) reactions, positive leucine aminopeptidase (LAP) reaction, growth in 6.5% NaCl broth, negative bile esculin reaction, and negative growth at 10°C and 45°C, are nonmotile, and are either alpha-hemolytic or gamma-hemolytic on 5% sheep blood agar (Table 1). Since the arrangement of cells into chains or clusters is not reliable and depends on the medium from which the cells were taken for the Gram strain, identification to the genus level must rely on the combination of tests listed in Table 1. The combination of reactions listed above is not unique to the genus level; *Alloiococcus otitidis*, *D. pigrum*, *Facklamia* spp., and *I. ruoffiae* all have the same phenotypic characteristics. *A. otitidis* does not grow anaerobically, which differentiates it from the other three genera (1, 13, 18). The cultures we described as "*Alloiococcus*-like" in a previous study (18) have since been identified as *D. pigrum*.

Facklamia species are differentiated from each other and from *D. pigrum* and *I. ruoffiae* by the deamination of arginine (Arg), by hydrolysis of hippurate (Hip) and esculin (Esc), and by acid formation in sucrose (Suc) and sorbitol broth (Sbl) (Table 2). *D. pigrum* is positive only for Esc; all other species, including *I. ruoffiae*, are negative for Esc. *I. ruoffiae* and *F. languida* are both negative in all five tests; however, *I. ruoffiae* has a distinctive sauerkraut odor on blood agar, which helps to identify the species. *F. hominis* is positive for Arg and Hip and variable in Suc. *F. ignava* is positive only for Hip. *F. sourekii* is positive for Hip, Suc, and Sbl. The newest species, *F. tabacinasalis*, not yet documented from humans, is positive for Suc and Sbl only. This combination of tests identifies all of these strains to the species level.

ID32. Since *A. otitidis* gave the same genus identification as the *Dolosigranulum*, *Facklamia*, and *Ignavigranum* cultures, we included seven strains in this study for comparison purposes (Table 1). None of the *A. otitidis* strains were correctly identified by the ID32, Crystal, or IDS systems. *A. otitidis* strains presented difficulty in preparing satisfactory inocula for the test systems because of poor growth and adherence properties. These strains must be grown on heart infusion rabbit blood agar for at least 48 to 72 h.

The ID32 system correctly identified 7 (39%) of the 18 *Facklamia* species as "unacceptable ID." Those that were correctly identified were 3 of 4 strains of *F. hominis*, 3 of 5 strains of *F. ignava*, none of 6 strains of *F. languida*, and 1 of 3 strains of *F. sourekii* (Table 3). The *F. languida* strains were most often misidentified as *Gemella morbillorum*, as was one strain of *F. ignava*. This misidentification is somewhat understandable since there is little difference between the *Gemella* spp. and *Facklamia* spp.; however, the correct division of the two genera can be made with growth in 6.5% sodium chloride. Only the *Facklamia* species grow in broth containing 6.5% sodium chloride. Another common misidentification was *Facklamia* species being identified as *S. acidiminimus*; this occurred with three different strains of *Facklamia*. This, too, may be understandable since the Centers for Disease Control and Prevention (CDC) *Streptococcus* Laboratory has distributed several strains previously identified as *S. acidiminimus*, which were reidentified as *Facklamia* species. In fact, we included these strains in previous studies of rapid identification kits (10, 12, 22). This points out a need to update the identification of strains obtained from the CDC *Streptococcus* Laboratory and to update the commercial databases as well.

Although 46 profiles were generated from testing 27 strains of *D. pigrum* in the ID32 test system, all 46 profiles resulted in an "unacceptable ID" (Table 3). These results were very encouraging since the product's manufacturer would only have to include profiles we have generated for *D. pigrum* in its database to accurately identify this species.

Two of the three strains of *I. ruoffiae* were correctly identified by the ID32 system. The third strain tested had a doubtful profile. Not enough numbers of this species have been tested to conclude that the system can be adjusted to identify the species accurately; however, these results are encouraging.

Crystal. As stated above, *A. otitidis* was included in this study because of the similar genus identification. Six of the seven strains tested in the Crystal system were identified as *Micrococcus* species. The seventh was identified as *Streptococcus pneumoniae* (Table 3). The identification of *A. otitidis* may have been compromised because of the difficulties in preparing the inoculum as previously stated; however, its identification may point out a more serious problem for this system. *Micrococcus* species are catalase positive, whereas most of the cultures we included in this study were catalase negative. Some of

TABLE 2. Phenotypic characteristics of *D. pigrum*, *I. ruoffiae*, and *Facklamia* species as shown by conventional biochemical tests

Species	Phenotypic characteristics				
	Arginine	Hippurate	Esculin	Sucrose	Sorbitol
D. pigrum	−	−	+	−	−
I. ruoffiae	−	−	−	−	−
F. hominis	+	+	−	v[a]	−
F. ignava	−	+	−	−	−
F. sourekii	−	+	−	+	+
F. languida	−	−	−	−	−
F. tabacinasalis	−	−	−	+	+

[a] v, variable.

TABLE 3. Identification of *A. otitidis*, *D. pigrum*, *Facklamia* species, and *I. ruoffiae* by three commercial rapid identification systems

Species	No. of strains	Identification results (no. of profiles: identification data)[a] with:		
		ID32	Crystal	IDS
A. otitidis	7	7 profiles: 6 strains, 3 different *S. acidiminimus* profiles (98.7, 99.8, 99.9); 1 strain, 4 different *S. oralis* profiles (99.5, 98.2, 94.1, 75.3)	4 profiles: 3 strains, 1 profile *M. luteus* (99.5); 2 strains, 1 profile *M. luteus* (99.9); 1 strain, 1 profile *S. pneumoniae* (99.9); 1 strain, 1 profile *M. roseus* (58.6)	4 profiles: 4 strains, probable overlap between *G. morbillorum* and *A. viridans*; 2 strains implicit *G. morbillorum*; 1 strain implicit *S. mitis*; 1 strain, probable *S. mitis*
F. hominis	4	8 profiles: 3 strains, 6 different "unacceptable ID"; 1 strain, 1 profile *S. oralis* (99.7)[b]; 1 strain, 1 profile *S. acidiminimus* (97)	5 profiles: 3 strains, 1 profile *M. luteus* (75.7); 2 strains, 1 profile *M. luteus* (89.7); 1 strain, 1 profile *M. luteus* (99.99); 1 strain, 2 "unacceptable ID" profiles	2 profiles: 3 strains, 1 profile *S. acidiminimus* (99); 3 strains, 1 profile *S. acidiminimus* (76)
F. ignava	5	8 profiles: 3 strains, 4 different "unacceptable ID" profiles; 1 strain, 2 different *S. acidiminimus* profiles (99.9, 96.3); 1 strain, 1 profile *S. oralis* (86.2); 1 strain, 1 "doubtful ID" profile *G. morbillorum* (71)	7 profiles: 3 strains, 5 different "unacceptable ID" profiles; 3 strains, 1 profile *M. luteus* (75.7); 1 strain, 1 profile *M. luteus* (99.7)	6 profiles: 2 strains, 3 different "no choice" profiles; 2 strains, 2 different *S. acidiminimus* profiles (99 and 76); 1 strain, 1 profile *S. constellatus* (99)
F. languida	6	3 profiles: 4 strains, one "doubtful ID" profile *G. morbillorum* (86.8); 1 strain, 1 profile *G. morbillorum* (85.5); 1 strain, 1 "doubtful ID" profile *G. morbillorum* (55)	7 profiles: 7 strains, 5 different *M. luteus* profiles (99.9); 1 strain, 1 profile *M. luteus* (75.7); 1 strain, 1 profile *M. roseus* (59)	3 profiles: 4 strains, 1 profile *G. morbillorum* (99); 1 strain, 1 profile *G. morbillorum* (98.8); 1 strain, 1 profile *G. morbillorum* (99)
F. sourekii	3	3 profiles: 1 strain, 1 profile *S. acidiminimus* (99.9); 1 strain, 1 "doubtful ID" profile *A. viridans* (94.5); 1 strain, 1 "unacceptable ID" profile	6 profiles: 3 strains, 5 different "unacceptable ID" profiles; 1 strain, 1 profile *M. luteus* (99.5)	5 profiles: 2 strains, 3 *E. avium* profiles (99); 1 strain, 1 profile *G. morbillorum* (99); 1 strain, 1 "no choice" profile
D. pigrum	27	46 profiles: 2 strains, 4 different "unacceptable ID" profiles; 2 strains, 3 different "unacceptable ID" profiles; 9 strains, 2 different "unacceptable ID" profiles; 14 strains, 1 "unacceptable ID" profile each	36 profiles: 2 strains, 6 different profiles, *M. cristinae* (66.1), *M. roseus* (99.6, 67.2), *S. sanguis* (89.7), and two "unacceptable ID" profiles; 3 strains, 6 different profiles, *S. agalactiae* (58.2), *M. roseus* (99.4, 93.4), *L. lactis* (72), and two different "unacceptable ID" profiles; 1 strain, 2 different profiles, *M. roseus* (93.4) and 1 "unacceptable ID" profile; 10 strains, 12 different "unacceptable ID" profiles	8 profiles: 19 strains, same profile, "inadequate ID" *E. faecalis* (90); 2 strains, same profile, presumptive *S. mitis* (99.8); 2 strains, same "questionable code" profile, *S. intermedius*; 1 strain, 2 different "no acceptable choice" profiles; 1 strain, group A streptococcus (99); 1 strain, *Enterococcus* sp.
I. ruoffiae	3	2 profiles: 2 strains, 1 "unacceptable ID"; 1 strain, 1 "doubtful profile" *G. vaginalis* (99.8)	3 profiles: 2 strains, two different profiles, *M. luteus* (60.5, 79.6); 1 strain, "unacceptable ID" profile	3 profiles: 1 strain, "no choice profile"; 1 strain, unreliable profile; 1 strain, "inadequate identification" profile

[a] Most strains were tested two times; consequently, there are more profiles than the total number of strains. The percentage values, given in parentheses, were provided by the manufacturer of each product and refer to the confidence levels of identification.

[b] The percentage value refers to the confidence level of identification provided by the manufacturer of each product.

TABLE 4. Potentially useful tests included in the ID32 system for differentiating unusual gram-positive cocci

Species	% Strains positive[a] with:													
	Adh	β-Gar	α-Gal	Man	Sbl	Tre	Sac	Darl	APPA	β-Gal	GTA	Hip	Mal	Ure
A. otitidis	— (−)	30 (v)	— (−)	— (−)	— (−)	40 (v)	— (−)	— (−)	10 (v)	100 (+)	— (−)	80 (+)	10 (v)	— (−)
D. pigrum	88 (+)	94 (+)	13 (v)	59 (v)	38 (v)	88 (+)	97 (+)	6 (−)	13 (v)	97 (+)	88 (+)	50 (v)	100 (+)	— (−)
F. hominis	75 (v)	100 (+)	75 (v)	— (−)	— (−)	— (−)	25 (v)	— (−)	100 (+)	100 (+)	100 (+)	75 (v)	25 (v)	75 (v)
F. ignava	60 (v)	20 (v)	40 (v)	20 (v)	20 (v)	20 (v)	40 (v)	— (−)	80 (+)	60 (v)	80 (+)	80 (+)	40 (v)	40 (v)
F. languida	— (−)	— (−)	— (−)	— (−)	— (−)	100 (+)	— (−)	— (−)	— (−)	— (−)	100 (+)	— (−)	— (−)	— (−)
F. sourekii	— (−)	— (−)	— (−)	67 (v)	67 (v)	100 (+)	67 (v)	67 (v)	33 (v)	— (−)	33 (v)	100 (+)	100 (+)	— (−)
F. tabacinasalis[b]	— (−)	— (−)	100 (+)	100 (+)	— (−)	100 (+)	100 (+)	— (−)	100 (+)	— (−)	— (−)	— (−)	— (−)	— (−)
I. ruoffiae	67 (v)	— (−)	— (−)	67 (v)	— (−)	67 (v)	67 (v)	33 (v)	— (−)	— (−)	— (−)	100 (+)	33 (v)	67 (v)

[a] The number given is the percentage of strains found to be positive; the interpretation of the test result as positive (+), negative, or variable (v) is indicated in parentheses. Abbreviations: Adh, arginine dihydrolysis; Man, mannitol, Sbl, sorbitol; Tre, trehalose; Sac, saccharose; Darl, D-arabitol; Hip, hydrolysis of hippurate; Mal, maltose; Ure, urease. —, 0%.

[b] Type strain only, no human isolates tested.

the *A. otitidis* cultures are catalase positive, but we have found that this is not true in every case. Most of the *A. otitidis* cultures we have examined have been very weak-to-negative catalase producers.

Like the ID32 system, the Crystal system identified seven (39%) strains of *Facklamia* species as "unacceptable ID" (Table 3). One of four strains of *F. hominis*, three of five strains of *F. ignava*, none of six strains of *F. languida*, and three of three strains of *F. sourekii* were correctly identified. Of the 18 strains of *Facklamia* tested, 13 were identified as various *Micrococcus* species. These misidentifications indicate the need to include catalase reactions in the identification schemes for gram-positive cocci.

Among the 27 strains of *D. pigrum*, 17 "unacceptable ID" profiles were observed with 14 different strains (Table 3). This indicates that half of the cultures could be accurately identified by incorporating these newly generated profile numbers into the Crystal database. As with the *Facklamia* species (indicating a problem that arises by not including the catalase test into the identification process), eight strains of *D. pigrum* generated eight profile numbers that were identified as various *Micrococcus* species. Eleven different profile numbers identifying strains as various streptococcal species were observed with 11 different strains of *D. pigrum*. The majority of these profiles had low percentages of confidence for identification (Table 3).

We observed three profiles with the three strains of *I. ruoffiae*: one was an "unacceptable ID" and two were low-level confidence *M. luteus*. These misidentifications again indicate the need to include catalase in the identification scheme.

IDS. None of the *A. otitidis* strains were correctly identified in the IDS system. Four strains were identified as a probable overlap between *G. morbillorum* and *Aerococcus viridans*, two strains were identified as implicit identification for *G. morbillorum*, one was identified as an implicit identification of *S. mitis*, and one was identified as a probable *S. mitis*. Only 3 of 18 (17%) *Facklamia* strains were identified as "no choice" (Table 3). The identification of *F. hominis*, *F. ignava*, and *F. sourekii* as *S. acidiminimus* with high levels of confidence with the IDS kit was not surprising. As explained above for the ID32 kit, strains originally identified as *S. acidiminimus* have been reidentified as *F. hominis*. It is probable that some of the strains that the CDC *Streptococcus* Laboratory had identified and distributed as *S. acidiminimus* were used to generate databases for the IDS system (22). Also, the identifications of the various *Facklamia* species as *G. morbillorum* and as various *Streptococcus* species may be interpreted as poorly growing *Streptococcus* species or misidentified strains as *Gemella* that have been used to generate databases. *Gemella* species do not grow in 6.5% NaCl broth, so strains with profiles identical to the ones described here should be retested for growth in 6.5% sodium chloride. We observed only one "no choice" profile in 27 strains of *D. pigrum* tested. The majority of *D. pigrum* strains tested (19 of 27 [70%]), generated one profile number: "inadequate ID, *Enterococcus faecalis*, 90%." This is an erroneous identification, even though the confidence level is quite low (Table 3). Other misidentifications of *Streptococcus*, including group A streptococci and *Enterococcus* species, are inexplicable.

A discouraging point for all three test kits is the incidence of generated profile numbers shared among different species and genera. The ID32 had one instance in which a single strain of *F. hominis* and *F. ignava* had the same profile. This phenomenon occurred twice with the Crystal system among two different strains of *F. ignava* and among three different strains of *F. hominis*. The Crystal system also generated shared profiles between different genera: *F. ignava* and *F. sourekii* with *Alloiococcus* sp. The IDS system had three instances in which *F. sourekii* and *F. ignava* shared profiles, as well as two instances in which three different strains of *F. hominis* and *F. ignava* had the same profiles.

Previous investigators have used a combination of conventional tests and reactions from one or more of the rapid identification systems to describe and differentiate the newly identified genera and species (2, 4–9, 17). We explored the possibility that each one of the three systems we evaluated could be used to identify the species of *Alloiococcus*, *Dolosigranulum*, *Facklamia*, and *Ignavagranulum*. When we used conventional tests first to identify these four genera with common phenotypic traits, we then tested these rapid kits to determine whether they could identify all the species of these genera. Although we have included *Alloiococcus* strains in these investigations, it is unlikely that microbiologists would confuse this bacterium with the other genera in Tables 4, 5, and 6. *A. otitidis* is a strict aerobe and, to our knowledge, has been isolated only from inner ear cultures from patients with otitis. The results of using the ID32 for differentiation of *D. pigrum* and *I. ruoffiae* from the *Facklamia* species are presented in Table 4. Although not perfect, combinations of reactions can identify most of the species. *D. pigrum* can be identified by positive reactions in arginine and β-galactosidase (β-Gar), by acidification of trehalose and maltose, and by negative reaction in alanine-phenylalanine-proline aryalamidase (APPA). *F. hominis* can be identified by positive reactions in β-Gar, APPA, β-galactosidase (β-Gal), and glycyl-tryptophane arylamidase (GTA) and by negative reactions in trehalose. *F. ignava* is problematic because there are too many variable reactions to the majority

TABLE 5. Potentially useful tests included in the Crystal system for identifying unusual gram-positive cocci

Species	% Strains positive[a] with:							
	FPR	FPY	FTR	MAL	AGN	MTT	BGL	PPG
A. otitidis	12 (v)	88 (+)	100 (+)	— (−)	— (−)	— (−)	— (−)	25 (v)
D. pigrum	12 (v)	88 (+)	100 (+)	64 (v)	64 (v)	52 (v)	97 (+)	55 (v)
F. hominis	100 (+)	— (−)	100 (+)	25 (v)	25 (v)	25 (v)	— (−)	100 (+)
F. ignava	60 (v)	20 (v)	100 (+)	40 (v)	40 (v)	40 (v)	25 (v)	40 (v)
F. languida	— (−)	67 (v)	100 (+)	— (−)	— (−)	— (−)	— (−)	— (−)
F. sourekii	— (−)	100 (+)	33 (v)	33 (v)	— (−)	— (−)	100 (+)	— (−)
F. tabacinasalis	100 (+)	— (−)	— (−)	— (−)	— (−)	— (−)	100 (+)	100 (+)
I. ruoffiae	33 (v)	100 (+)	67 (v)	33 (v)	— (−)	33 (v)	— (−)	— (−)

[a] The number given is the percentage of positive results for each strain; a positive (+), negative (−), or variable (v) reaction for each test interpretation is indicated in parentheses. Abbreviations: FPR, L-proline–AMC; FPY, L-pyroglutamic acid–AMC; FTR, L-trytophan–AMC, MAL, maltose; AGN, *N*-acetyl-D-glucosamine; MTT, maltotriose; BGL, *p-n-p*-β-D-glucoside; PPG, *p-n-p*-β-D-galactoside and *p-n*-α-D-galactoside. —, 0%.

of tests in the ID32 system. *F. languida* is easily identified because it is positive in only 2 of the 14 tests: acid formation in trehalose and GTA. *F. sourekii* is identified by acidification of mannitol, sorbitol, trehalose, maltose, D-arabitol, and saccharose and by hydrolysis of Hip. Only a single nonhuman isolate of *F. tabacinasalis* is represented in Table 4, so these results should be cautiously interpreted. *I. ruoffiae* is identified by positive reactions in Arg, acidification of mannitol, trehalose, and saccharose, and hydrolysis of hippurate and by negative reactions in β-Gar, acidification of sorbitol, APPA, β-Gal, and GTA.

The same process can be used for the Crystal system (Table 5). The reactions of *A. otitidis* overlap somewhat with the reactions of some of the *D. pigrum* and *Facklamia* species. *D. pigrum* is FPY (L-pyroglutamic acid-AMC), FTR (L-trytophan–7-amino-4-methylcoumarin [AMC]) and BGL (*p-n-p*-β-D-glucoside) positive. *F. hominis* is FPR (L-proline-AMC), FTR, and PPG (*p-n-p*-β-galactoside and *p-n*-α-D-galactoside) positive. *F. ignava* is problematic because of the number of variable reactions. *F. languida* is identified by a positive reaction in FPY and negative reactions in FPR, MTT (acid formation in maltotriose), BGL, and PPG. *F. sourekii* is identified by positive reactions in FPY and BGL and negative reactions in MTT and PPG. Again, only a single strain of *F. tabacinasalis* (nonhuman) was tested, so these reaction percentages should be interpreted cautiously. *I. ruoffiae* is identified by positive reactions in FPY and FTR and by negative reactions in BGL and PPG.

Attempting to apply the same interpretation to the IDS system is more difficult because of the limited number of tests with positive reactions (Table 6). Cultures of *F. hominis* and *I. ruoffiae* appear to have unique profiles, but the cultures of *D. pigrum* and the remainder of the *Facklamia* species have a common profile.

We have previously compared various reactions included in rapid identification tests with conventional tests and have indeed found differences (14). Comparing the individual tests in these systems to the conventional tests resulted in several interesting observations. In the conventional disk test we use for the PYR test (L-pyrrolidonyl-β-naphthylamide) all of the *D. pigrum*, *Facklamia* species, and *I. ruoffiae* cultures were positive. However, in the ID32 test, two strains of *D. pigrum*, one strain of *F. hominis*, four strains of *F. ignava*, two strains of *F. languida*, and one strain *I. ruoffiae* were negative. These numbers were similar to those for the Crystal and IDS PYR tests. In the conventional test for deamination of Arg, only the *F. hominis* and *I. ruoffiae* strains were positive. But in the ID32, Crystal, and IDS systems, 88, 79, and 79% of the *D. pigrum* cultures, respectively, were positive. Of the *F. ignava* cultures, three of five, four of five, and three of five strains were positive for Arg in the ID32, Crystal, and IDS systems, respectively; none of these cultures were positive in the conventional Moeller's decarboxylase tests. These results were similar to those of West et al. (21), who also reported major differences in Arg hydrolysis by viridans streptococci, depending on the rapid test used.

It is apparent that the manufacturers of these rapid identification kits need to update their databases by including the identification of newly described genera and species of gram-positive cocci, including the *Dolosigranulum*, *Facklamia* and *Ignavigranum* species.

TABLE 6. Potentially useful tests included in the IDS system for identification of unusual gram-positive cocci

Species	% Strains positive[a] with:					
	Arg	GAL	PO_4	TYR	LYS	PYR
D. pigrum	79 (v)	9 (–)	6 (–)	93 (+)	97 (+)	91 (+)
F. hominis	100 (+)	100 (+)	100 (+)	100 (+)	100 (+)	— (−)
F. ignava	60 (v)	40 (v)	40 (v)	100 (+)	100 (+)	20 (v)
F. languida	— (−)	— (−)	17 (v)	100 (+)	100 (+)	67 (v)
F. sourekii	— (−)	— (−)	— (−)	67 (v)	67 (v)	100 (+)
F. tabacinasalis[b]	— (−)	100 (+)	— (−)	— (−)	— (−)	— (−)
I. ruoffiae	67 (v)	— (−)	— (−)	— (−)	100 (+)	67 (v)

[a] The number is the percentage of strains found to be positive; the interpretation of the test result is indicated in parentheses as positive (+), negative (−), or variable (v). Abbreviations: Arg, arginine; GAL, *p*-nitrophenyl-α-galactoside; PO_4, *p*-nitrophenyl phosphate; TYR, tyrosine β-naphthylamide; LYS, lysine β-naphthylamide. —, 0% positive.

[b] Type strain only, no human isolates tested.

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