JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1988, p. 144–146
0095-1137/88/010144-03$02.00/0

Vol. 26, No. 1

Comparison of API ZYM System with API AN-Ident, API 20A, Minitek Anaerobe II, and RapID-ANA Systems for Identification of *Clostridium difficile*

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Received 9 June 1987/Accepted 30 September 1987

The API ZYM system was compared with four anaerobe identification systems for the definitive identification of *Clostridium difficile* by using 88 cultures of *C. difficile* grown on Mueller-Hinton blood agar medium. The API ZYM system yielded a distinct and consistent enzyme profile for all test strains, whereas the sensitivities of the other systems in identifying *C. difficile* ranged from 78 to 96% (AN-Ident, 77.9%; RapID-ANA, 88.6%; Minitek Anaerobe II, 90.9%; and API 20A, 95.5%). The API ZYM system is highly reliable in identifying *C. difficile* accurately, is rapid, and is relatively simple to use.

Clostridium difficile is a well-recognized human pathogen, and most clinical laboratories now routinely process specimens for this agent. Isolation by culturing is a common approach, and although *C. difficile* can be presumptively identified in many instances by its culture characteristics and cellular morphology, definitive identification requires additional tests. Several anaerobe identification systems are now commercially available in convenient kit forms. The ability of these kits to identify anaerobes in general has been evaluated by several workers, but their usefulness specifically for the identification of *C. difficile* has not been fully assessed (3, 5, 8, 12). The API ZYM system (Analytab Products, Plainview, N.Y.) has recently been reported to be a rapid and reliable method for the identification of *C. difficile* (10, 11). This system, however, has not been evaluated in comparison with other systems available for identifying anaerobes. Also, at present, there is no database for the ready identification of anaerobes by this system. We carried out a study to further define the usefulness of the API ZYM system in identifying *C. difficile* specifically in comparison with four other commercial anaerobe identification systems.

The API ZYM system is a semiquantitative micromethod consisting of 20 microcupules, 19 of which contain dehydrated chromogenic substrates for detecting 19 preformed-enzyme activities. The test strips are inoculated and incubated aerobically at 37°C for 4 h, and then two reagents are added to develop the chromogenic substrates. The resultant colorimetric reactions are indicative of the degree of enzyme activity and are graded on a scale of 0 to 5 in comparison with the control well and a color chart. The other kits evaluated in the study included RapID-ANA (Innovative Diagnostic Systems, Decatur, Ga.), AN-Ident and API 20A (Analytab Products), and Minitek Anaerobe II (BBL Microbiology Systems, Cockeysville, Md.). Both RapID-ANA and AN-Ident are similar to API ZYM in design and function and detect a series of preformed enzymes. The substrates for carbohydrate fermentation and enzyme detection used in API 20A and Minitek are similar to those used in conventional tests and require 24 to 48 h of incubation before results are read. With the exception of the API ZYM system, all of the systems generate numerical profiles which are based on test reactions and which are then compared with a database.

A total of 88 clinical isolates of *C. difficile* obtained in our laboratory and elsewhere in Canada were used to evaluate the kits. In addition, 19 strains of *C. perfringens* also were tested with the API ZYM system. The identity of all test strains was established by conventional culture and biochemical parameters and through the analyses of volatile fatty acid profiles by gas-liquid chromatography in accordance with standard procedures (2). All cultures were maintained on Mueller-Hinton II agar base (BBL 11438; BBL Microbiology Systems) containing 5% sheep blood.

Test strains were grown on Mueller-Hinton blood agar medium anaerobically at 37°C for 48 h. The growth was emulsified in an appropriate inoculum fluid to yield a suspension of uniform turbidity, as recommended by each manufacturer. The panels were inoculated and incubated, and the results were read and interpreted in accordance with the instructions of the manufacturers; however, when supplementary tests were indicated, these were not carried out. As indicated in a recent report (6), the recommended 24-h incubation of API 20A was extended to 48 h. To determine the reproducibility of the test reactions in individual panels, we picked seven isolates of *C. difficile* at random and tested them three times with each of the five systems in accordance with the above-described protocol. To determine the effect of culture media on the outcome of the API ZYM reactions, we compared the growth of 18 isolates of *C. difficile* from Mueller-Hinton blood agar with the growth of isolates from Columbia agar (code CM331; Oxoid Canada Ltd., Nepean, Ontario, Canada), brucella agar (catalog no. M08400; GIBCO Canada, Burlington, Ontario, Canada), and brain heart infusion agar (Difco Laboratories, Detroit, Mich.), all of which contained 5% sheep blood.

In the comparative evaluations of the identification systems, the AN-Ident system correctly identified 60 (77.9%) of the 77 *C. difficile* strains tested to the species level and 15 to the genus level, with two incorrect identifications; the RapID-ANA system correctly identified 78 (88.6%) of the 88

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TABLE 1. Comparison of commercial anaerobe identification systems with the API ZYM system for identification of *C. difficile*

Identification system	Incubation time (h)	Interpretation of test results[a]	Simplicity of overall procedure[a]	Sensitivity (%)	Reproducibility (%)	Cost/test ($)[b]
AN-Ident	4	+	++	77.9	98.1	5.20
RapID-ANA	4	++	++++	88.6	100	7.00
Minitek	48	++	+	90.9	95.2	5.68
API 20A	48	+	++	95.5	98.8	4.38
API ZYM	4	++++	+++	100	100	5.60

[a] Degree of satisfaction: +, poor; ++, fair; +++, good; and ++++, best.
[b] List price in Canadian dollars as of May 1987.

strains tested to the species level and 1 to the genus level, with nine incorrect identifications. The Minitek system yielded correct identifications for 80 (90.9%) of the 88 strains tested, with 2 being identified to the genus level; the remaining 6 were misidentified. The API 20A system correctly identified 84 (95.5%) of the 88 strains tested, with three incorrect identifications; 1 was identified to the genus level. The API ZYM system consistently yielded a uniform enzyme profile for all 88 *C. difficile* strains tested: a range of low to high positive reactions (grades 1 to 5; 5 to ≥40 nmol) for esterase (C-4) and leucine aminopeptidase activities, a weak to a low positive (<5 to ~20 nmol) variable reaction for esterase lipase (C-8) activity, a weak reaction (<5 nmol) for phosphohydrolase activity, and a uniform negative reaction for the remaining 15 enzyme activities. There is no database to interpret the API ZYM results, but the consistent API ZYM profile obtained for all 88 isolates of *C. difficile* tested can probably be considered as 100% correct identification of the organism by this system (Table 1). The reproducibility of the test reactions varied only minimally and was generally high with the RapID-ANA and API ZYM systems (100%) (Table 1). The growth of *C. difficile* was slightly more luxuriant on brain heart infusion medium; however, there were no significant differences between the API ZYM activities for cultures on different media, including brain heart infusion medium. The API ZYM system yielded a distinctly different enzyme profile for the 19 strains of *C. perfringens* tested. The API ZYM profiles of *C. difficile* and *C. perfringens* obtained in the present study are summarized in Table 2, along with the results obtained for *C. difficile* and other

TABLE 2. API ZYM profile of *C. difficile* in comparison with those of other *Clostridium* species reported in the literature[a]

API ZYM test	Result[b] for:												
	C. difficile (88) [PR]	*C. difficile* (95) [10]	*C. difficile* (6) [11]	*C. bifermentans* (7) [10, 11]	*C. butyricum* (7) [10]	*C. cadaveris* (6) [11]	*C. glycolicum* (13) [10]	*C. innocuum* (6) [10]	*C. paraputrificum* (8) [10, 11]	*C. perfringens* (31) [10, 11; PR]	*C. septicum* (6) [11]	*C. sordellii* (5) [10]	*C. sporogenes* (9) [10, 11]
Aklaline phosphatase	−	V	−	+	−	V	+	−	−	+	−	+	−
Esterase (C-4)	+	+	+	+	+	V	+	+	+	+	V	+	+
Esterase lipase (C-8)	V	+	+	+	+	V	+	+	+	+	V	+	+
Lipase (C-14)	−	−	−	−	−	−	−	−	−	−	−	−	−
Leucine aminopeptidase	+	+	V	V	−	−	+	−	−	V	−	V	−
Valine aminopeptidase	−	−	−	−	−	−	−	−	−	−	−	−	−
Cystine aminopeptidase	−	−	−	−	−	−	−	−	−	−	−	−	−
Trypsin	−	−	−	−	−	−	−	−	−	−	−	−	−
Chymotrypsin	−	−	−	−	−	−	−	−	−	−	−	−	−
Acid phosphatase	−	−	−	+	−	+	+	+	W	+	+	+	+
Phosphohydrolase	W	W	V	+	−	V	V	+	+	+	+	V	+
α-Galactosidase	−	−	−	−	+	−	−	−	−	+	−	−	−
β-Galactosidase	−	−	−	−	+	−	−	−	+	+	+	−	−
β-Glucuronidase	−	−	−	−	−	−	−	−	−	V	−	−	−
α-Glucosidase	−	−	−	−	+	−	−	−	V	+	V	−	−
β-Glucosidase	−	−	−	−	−	−	−	−	−	−	−	−	−
N-Acetyl-β-glucosaminidase	−	−	−	−	−	+	−	−	+	+	+	−	−
α-Mannosidase	−	−	−	−	−	−	−	−	−	−	−	−	−
α-Fucosidase	−	−	−	−	−	+	−	−	−	−	−	−	−

[a] Discrepant enzyme profiles of some species were not included or were modified when combined with data obtained for a larger number of strains in separate studies.
[b] +, Positive; −, negative; W, weak; V, variable. Numbers in parentheses indicate the numbers of strains tested; numbers in brackets represent references; PR, present report.

Clostridium spp. in previous evaluations of the API ZYM system (10, 11).

A recent study compared the API AN-Ident, Minitek, and RapID-ANA systems for the identification of *C. difficile* and found a sensitivity of 9% for the API AN-Ident system (4). Among the five systems we evaluated, the API AN-Ident system was found to have the lowest sensitivity (78%), but this was far higher than the sensitivity of 9% previously reported. Similarly, we found a higher sensitivity for Minitek (91%) than the 66% previously reported (4). However, in contrast to the 100% sensitivity previously reported for the RapID-ANA system (4), we found the sensitivity to be only 89%. These discrepancies are difficult to explain. Whether the differences reflect improvements or changes in the identification systems concerned is not known. Nevertheless, our observation of a 95.5% sensitivity for the API 20A system in identifying *C. difficile* was almost identical to that in a recent report (6).

The API ZYM system previously has been reported to be a rapid and reliable method for the identification of a wide variety of microorganisms (7, 9, 13–16). Our results indicate that the API ZYM system is an excellent method for the identification of *C. difficile* as well and confirm and further extend the earlier observations on the application of this system in specifically identifying *C. difficile* (1, 10, 11). We did not test the specificity of this system in identifying *C. difficile*; nevertheless, it is evident from previous studies that the API ZYM profile of *C. difficile* is sufficiently characteristic to allow for a definitive differentiation of this organism from other members of the genus *Clostridium* (Table 2). The compositions of culture media influence API ZYM enzyme activities, and Columbia and brain heart infusion media have been found previously to yield uniform results (10). We compared Columbia, brain heart infusion, and brucella media with Mueller-Hinton medium and found no significant differences; it should be pointed out that we tested only 48-h cultures. In previous evaluations of the API ZYM system for identifying *C. difficile*, Columbia blood agar and Centers for Disease Control anaerobe blood agar cultures were used (10, 11). Whether differences in the culture medium used could be reasons for the discrepancies observed between individual evaluations of the API ZYM system, including our own, is not known (1, 10, 11) (Table 2). Variations between batches of API ZYM strips, however, have been observed (11), and these could account for the discrepancies.

In conclusion, we consider the API ZYM system to be highly reliable in identifying *C. difficile*. It is also rapid and relatively simple to use, and the test reactions are highly reproducible and easy to interpret.

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