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NOTES

Evaluation of the 24-h API 20A Anaerobe System for Identification of *Clostridium difficile*

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Accurate identification of *Clostridium difficile* is important when antibiotic-associated diarrhea or pseudomembranous colitis is suspected. Presumptive identification of *C. difficile* was made on the basis of microscopic features and colony characteristics on cycloserine, cefoxitin, fructose, and egg yolk agar medium. We studied the reliability of the 24-h API 20A anaerobe system for definitive identification of *C. difficile*. This system showed low dependability after the recommended 24 h of incubation by confirming the identity of only 54% of the isolates presumptively identified as *C. difficile*. There was a marked improvement in the system's capability after 48 h of incubation, when the identity of 95% of the isolates was confirmed.

Clostridium difficile has been implicated in antibiotic-associated diarrhea and pseudomembranous colitis. Presumptive identification of *C. difficile* by growth characteristics on a selective and differential medium is rapid and useful in many cases. When definitive identification is desired, conventional biochemical tests and gas-liquid chromatography are usually performed. The purpose of this report is to evaluate the performance of the 24-h API 20A anaerobe system (Analytab Products, Plainview, N.Y.) in definitively identifying strains of *C. difficile*.

Thirty-seven separate isolates from fecal specimens obtained from patients at the Veterans Administration Medical Center, Minneapolis, Minn., and the University of Minnesota, Minneapolis, were presumptively identified as *C. difficile*. The isolates were collected over a 9-month period and stored in prereduced chopped meat-glucose broth (Scott Laboratories, Fiskeville, R.I.) The presumptive tests consisted of a Gram stain of this chopped meat-glucose broth and subculture to a selective and differential medium containing cycloserine, cefoxitin, and fructose in egg yolk base (Diagnostics, Inc., St. Paul, Minn.), prepared by the method of George et al. (1). The selective medium plates were incubated at 35°C for 48 h in an anaerobic glove box (model 3650; National Appliance Co., Portland, Oreg.) or GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) and were then observed for flat to low umbonate yellow colonies with ground-glass appearance and slightly filamentous edges, color change of the medium around the colonies to yellow, and yellow fluorescence under long-wavelength UV light. A Gram stain was performed on an isolated colony, which was then observed for large, gram-positive bacilli.

After presumptive identification characteristics were confirmed, an isolated colony was subcultured from the selective medium to a brain heart infusion base sheep blood plate supplemented with menadione, hemin, and yeast extract for the growth of anaerobes (medium I; Diagnostics, Inc.). After 24 to 48 h of incubation in an anaerobic glove box at 35°C, an isolated colony was subcultured to a second medium I plate and incubated for 48 h. Growth on this medium I plate was examined for purity and for the colonial morphology of ground-glass appearance, slightly filamentous edges, and flat to low umbonate colonies that is typical of *C. difficile*.

The manufacturer's instructions were followed for the use of the API 20A system. Since the importance of a heavy inoculum for accurate results in this system was emphasized, half of the 7-ml portion of Lombard-Dowell medium provided (supplied under N_2) was removed before the medium was inoculated. A sterile dry swab was used to harvest all the growth from a 48-h medium I culture and to suspend the growth in the basal medium. Before the API 20A strip was inoculated, the same swab was used to inoculate a plate of medium I which served as a purity check. The strips were inoculated according to the manufacturer's instructions and incubated anaerobically in a glove box or GasPak jar for 24 h at 35°C. When the positive reactions which occurred after 24-h incubation were insufficient for species identification, the strips were allowed to incubate for an additional 20 to 24 h, with no reagents added until the end of this time. As catalase and indole tests are uniformly negative for *C. difficile*, they were only performed after 48 h. This evaluation therefore consisted of a determination of the time required to obtain the positive results that are necessary to identify *C. difficile*. The strips were read as specified by the manufacturer. Profile numbers were interpreted by using the 1981 analytical Profile Index provided by API or by using the API computer service when profile numbers were not listed.

The 37 isolates were also identified by standard methods (3), including analysis of volatile fatty acids by gas-liquid chromatography and tests for lecithinase and lipase production, gelation liquefaction, the presence of indole, esculin and starch hydrolysis, and fermentation of glucose, fructose, maltose, mannitol, mannose, sucrose, and xylose in prereduced anaerobically sterilized media.

The API 20A identification is based on the results of 21

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biochemical tests (16 carbohydrates, indole, urease, gelatin liquefaction, esculin hydrolysis, and catalase). In this study, it was assumed that the indole and catalase tests would be negative at both 24 and 48 h. According to the table given in the package insert, the percentages of positive tests expected after 24 h for *C. difficile* are: glucose, 99%; mannitol, 83%; salicin, 22%; xylose, 5%; cellobiose, 5%; mannose, 66%; melezitose, 83%; gelatin liquefaction, 44%; and esculin hydrolysis, 11%. The percentages of positive reactions we obtained after 24 to 48 h of incubation were: glucose, 100%; mannitol, 90%; salicin, 10%; xylose, 0%; cellobiose, 0%; mannose, 85%; melezitose, 95%; gelatin liquefaction, 70%; and esculin hydrolysis, 10%. These reactions were adequate to identify only 20 of 37 (54%) isolates after 24 h of incubation. Of the 37, 35 (95%) were definitively identified as *C. difficile* when incubation was extended to 48 h. Strains not identified as *C. difficile* by the API 20A were given as either "no ID" or "*Clostridium* species" by the analytical profile index. All 37 strains were positively identified as *C. difficile* when tested by gas-liquid chromatography and conventional biochemical methods.

Although evaluations of the API 20A system for identification of *Clostridium* species have been made (2, 4), we specifically studied its performance in identifying isolates of *C. difficile*. The manufacturer currently recommends a 24-h incubation for the API 20A strips. We found that 24 h was not adequate for identifying *C. difficile*. Only 54% of the strains were definitively identified as *C. difficile* after the recommended 24-h incubation, because an insufficient number of tests (other than indole and catalase) gave positive results within that period. When the strips were incubated for 48 h, however, there was a dramatic increase in definitive identifications from 54 to 95%. The major problem in using the system to identify isolates of *C. difficile* was the insufficient number of positive reactions (not enough for species identification) after 24 h. The API 20A system was not reliable in identifying *C. difficile* after the currently recommended 24 h of incubation, but gave results that agreed well with those of presumptive and conventional tests when the incubation time was extended to 48 h.

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