Letters to the Editor

Proline-Aminopeptidase Test for Rapid Screening of Clostridium difficile

In a recent article (1), Fedorko and Williams reported the usefulness of the PRO Disc test as a screening method for the identification of Clostridium difficile isolates growing on cycloserine-cefoxitin-fructose agar (CCFA). We feel their results could improve the investigation of this pathogen. Our experience agrees with that of the above-mentioned authors.

In our laboratory, we routinely perform CCFA cultures of those specimens for which investigation of C. difficile is requested. Over a 2-year period a total of 50 isolates (30 toxigenic and 20 nontoxigenic) of C. difficile showing both typical morphologies on CCFA (yellowish, flat colonies with a ground-glass appearance) and compatible Gram stain results have been isolated. Final identification of C. difficile was achieved with the API RapidID 32A kit (BioMérieux, Balmes-les-Grottes, France). In addition, an L-proline–aminopeptidase (PRO Disc) test was done with diagnostic tablets (Rosco A/S, Taastrup, Denmark). All 50 isolates were PRO Disc positive.

Moreover, 10 Clostridium innocuum isolates growing on CCFA in the beginning of the study and showing colony morphology similar (but not equal, in our experience) to that of the C. difficile strains were identified by the API method and tested by the PRO Disc test: all gave PRO Disc-negative results. In addition, we initially tested 16 stored clinical isolates of different clostridial species (1 Clostridium bifermantans isolate, 3 Clostridium perfringens isolates, 4 C. innocuum isolates, 4 Clostridium ramosum isolates, 3 Clostridium sordellii isolates, and 1 Clostridium septicum isolate). Only C. sordelli and C. bifermantans strains were found to be PRO Disc positive, but none of these strains grew in CCFA. Consequently, we systematically use the PRO Disc test for screening and identification of suspicious colonies growing on this medium.

Besides its interest for epidemiological purposes, selective culture for C. difficile improves the performance of toxin detection on stool samples, and both tests together are now considered the “gold standard” for the investigation of this pathogen in the clinical laboratory (2). However, suspicious colonies on CCFA require further identification and confirmation of their toxigenicity. Our experience agrees with the screening protocol proposed by Fedorko and Williams, which could be remarkably advantageous due to its ability to simplify the identification method. By using this protocol together with rapid detection of toxin A or B genes by PCR-based assays, it is possible to identify toxigenic C. difficile strains growing on CCFA during a normal working day. We are now applying a simple PCR method, based on DNA heating extraction followed by amplification of the toxin B gene and electrophoretic detection, that can be done in 7 to 8 h. By this screening method for the identification of cultures and PCR, we are able to establish the final laboratory diagnosis in about the same turnaround time as that for the classic cell culture toxin detection.

REFERENCES


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Ed. Note: The authors of the published article declined to respond.