Detection of bacterial antigens by counterimmunoelectrophoresis (CIE)

Both electrophoresis and immunodiffusion are utilized, hastening and focusing the diffusion of antigens and antibodies in agarose gel. On the electric field, at a pH between 8.2 and 8.6, negatively-charged antigen which may be present in the patient's specimen migrates toward the anode. Commercially prepared antisera drifts toward the cathode via electroendosmosis. Where antigen and antibody meet, a precipitin line is formed.

CIE is a method used to detect bacterial antigens within various body fluids. It is used most often as an adjunct in the diagnosis of bacterial meningitis. If antigen is present in large quantity, a positive result may be detected within one to two hours; when small amounts of antigen are present, specimen concentration or more prolonged incubation may be necessary to detect a precipitin line. This test may be particularly useful when a Gram-stained smear and culture have failed to reveal the organism, possibly because the patient has been started on therapy prior to specimen collection.

Generally, any body fluid may be tested for antigen. A body fluid that might clot should be allowed to do so before testing. Certain bacterial antigens (e.g., those of group B streptococci) are apparently detected more frequently in a concentrated urine than in serum or cerebrospinal fluid from patients with systemic sepsis or meningitis. Antigenuria, however, does not localize an infection and is not specific for meningitis. Antisera are available to detect pneumococcal antigen ("Omniserum"), meningococci (groups A, B, C, X, Y, Z, and W135), group B streptococci, Haemophilus influenzae type b, and E. coli K1.

Intraspecies and intergeneric cross-reactions have given erroneous results in clinical use. For example, E. coli antigen may cross-react with N. meningitidis A antiserum. These cross-reactions may be due to sharing of immunochemically similar capsular or non-capsular antigens, or contamination of the antigen preparations employed for
antiserum production with non-specific media constituents. Positive and negative antigen controls must be used with each CIE run. Latex agglutination tests have also been developed for detection of most of these same bacterial antigens and appear to be more sensitive than CIE with equal specificity.

REFERENCES


TEST EVALUATION
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TEST

Detection of *Clostridium difficile* cytotoxin (stool)

METHOD

Tissue culture assay on stool extract. Stool extract is added to tissue culture which is examined for cytopathic effect. A positive finding is confirmed by neutralization with clostridia antitoxin, usually *Clostridium sordellii* antitoxin.

CLINICAL APPLICATIONS

*Clostridium difficile* has been implicated in the etiology of most cases of antimicrobial-associated pseudomembranous colitis and approximately 25% of cases of antimicrobial-associated diarrhea. All of the major classes of antimicrobial agents with activity against normal intestinal flora, except vancomycin and probably parenteral aminoglycosides, are capable of inducing *C. difficile*-associated intestinal disease. Exposure to these antimicrobial agents alters the flora of the gastrointestinal tract so as to permit *C. difficile* to proliferate, elaborate toxin, and cause colitis.

Bolton et al. have reported that *C. difficile* toxin may be associated with exacerbations of inflammatory bowel disease or with nonspecific diarrhea in patients with no history of recent antimicrobial therapy. *Clostridium* species, including *C. difficile*, have also been implicated in the pathogenesis of necrotizing enterocolitis. However, results of stool cultures for *C. difficile* and assays for the organism's toxin in cases of necrotizing enterocolitis have varied among authors.

CLINICAL AND TECHNICAL LIMITATIONS

*Clostridium difficile* has been recovered from the stools of 80–95% of patients with antimicrobial-associated pseudomembranous colitis. The organism is not usually present in the colonic flora of adults although it has been detected in the stools of up to 3% of healthy adults. Higher carriage rates for the organism have been reported in some hospitals in antibiotic-treated adults. In contrast, the organism is a frequent component of the fecal flora of healthy newborn infants. Thus, recovery of *C. difficile* from stool cultures is suggestive but not diagnostic of *C. difficile*-induced intestinal disease.

The tissue culture assay is based on the observation that stools from patients with antimicrobial-associated pseudomembranous colitis contain a cytopathic toxin whose characteristics are identical to those of toxin produced by *C. difficile*. The amount of toxin in the stool does not necessarily correlate with the severity of symptoms or pathological changes. The toxin produces cytopathic changes on a number of cell types, the particular susceptibility and cytopathic effect varying with the cell line. The time of appearance of the changes varies inversely with the amount of toxin.
present in the cell culture. Cytotoxicity is usually apparent within 4 hours with specimens of high titer; the majority of specimens show typical changes at 24 hours, although occasional specimens do not become positive until 48 hours. Stools from most patients with pseudomembranous colitis produce cytopathic changes at dilutions of $10^{-2}$ to $10^{-5}$. Thus, false negative results could be obtained if the tissue culture is not observed for a long enough time or if toxin is not present in high enough concentration to cause cytopathic changes. Several authors have noted that in preparation of the stool specimen for the tissue culture assay, specimens containing small concentrations of toxin may be diluted beyond the detection limit of the assay. The criterion for a positive assay is the demonstration that the cytopathic substance in the stool can be neutralized by clostridial antitoxin, either partially purified \textit{C. difficile} antitoxin or more frequently, \textit{C. sordellii} antitoxin, since it is more readily available and appears to crossreact antigenically.

Bartlett et al. have reported that the cytotoxicity assay is 98\% sensitive for antimicrobial-associated pseudomembranous colitis. This group found the test was positive in 96\% of 114 patients with antimicrobial-associated pseudomembranous colitis and in 2\% of 248 individuals without gastrointestinal complications related to antimicrobial usage. In contrast, a Swedish study reported that the toxin test was positive in 27\% of patients with antimicrobial-associated enterocolitis and that a positive toxin titer had an apparent predictive value of 69\% for pseudomembranous enterocolitis or other serious colitis while a negative titer had a 74\% predictivity for non-serious enteric disease. A British study by Burdon found the cytotoxin test failed to detect as many as 20\% of patients with pseudomembranous colitis. George notes limited instances of toxin detection in the feces of asymptomatic adults receiving antimicrobial therapy and of infants who do not have diarrhea. Donta and Myers detected \textit{C. difficile} toxin in the feces of 10.5\% of normal newborn infants and 55\% of neonates in the intensive care unit, less than one-third of whom had signs of enteric illness. Therefore, results of the tissue culture assay for \textit{C. difficile} cytotoxin must be interpreted in conjunction with clinical information, especially when dealing with infants. Reports have appeared that \textit{C. difficile} produces a second toxin in addition to the cytotoxin. The second toxin has somewhat different characteristics and may be important in the clinical expression of intestinal disease.

Alternatives to the tissue culture assay for detection of the \textit{C. difficile} cytotoxin have been introduced, including counterimmunoelectrophoresis (CIE) and enzyme linked immunosorbent assay (ELISA). The advocates of these newer methods find them more rapid, more convenient, and more sensitive than the tissue culture method of toxin detection. However, several reports have appeared regarding cross-antigenicity between antitoxins used in these newer methods and other \textit{C. difficile} antigens, in addition to the toxin produced by the organism. Thus, further experience with these newer alternative methods is needed to evaluate them properly.
REFERENCES


