Rapid Determination of *Corynebacterium diphtheriae* Toxigenicity by Counterimmunoelectrophoresis

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A method for detecting toxin production in *Corynebacterium diphtheriae* cultures by counterimmunoelectrophoresis is described. Precipitin lines were observed in toxigenic strains in 30 min.

The determination of the toxigenicity of isolates of *Corynebacterium diphtheriae* is a time-consuming procedure. In vitro methods include passive agar gel diffusion (3, 5, 10, 13, 14) and tissue culture (11, 15). Since most clinical microbiology laboratories have limited access to animals or cell cultures, the passive agar gel method has been the most widely used. This procedure usually takes at least 48 h after the organism has been isolated, and interpretation is often difficult (1, 16).

Counterimmunoelectrophoresis (CIE) not only increases the sensitivity 10- to 100-fold over passive agar gel diffusion, but decreases the reaction time as well by actively producing antigen-antibody contact in an electric field. CIE has been used to detect microbial antigens of *N. meningitidis* (2), *S. pneumoniae* (4), *H. influenzae* (9), *S. aureus* (12), and hepatitis virus (6) in body fluids and to identify cultures of group B streptococci (8). It occurred to us that CIE might appropriately be applied to the detection of toxin in cultures of *C. diphtheriae*.

Cultures of Park-Williams (ATCC 13812), Park 8 (ATCC 296), Gravis (ATCC 9059), Intermedius (ATCC 8032), Mitis (ATCC 8024), and an avirulent (ATCC 11913) strain of *C. diphtheriae* were used. In addition, *C. hemolyticum* and three unspcciated diphtheroid isolates were also tested. Modified Columbia broth, pH 7.3, and Frobisher Proteose peptone broth, pH 7.8 (5), were evaluated as basal media. Horse serum, rabbit serum, and the combination of Casamino Acids and Tween 80 (7) (K-L enrichment, Difco) were each evaluated as supplements at a concentration of 20%. Complete medium was dispensed in 10-ml amounts in 125-ml Erlenmeyer flasks. Cultures were incubated at 35°C under 7% CO₂ for varying periods of time. The effect of shaking was compared with stationary incubation.

CIE was performed on whole broth cultures with a kit obtained from Hyland Laboratories which consisted of disposable electrophoresis base units with foil electrodes and sponge wicks, prepunched agar gel plates, barbital buffer solution (pH 8.6), and a power supply. Samples were run at room temperature for 30 min at 40 mA. Diphtheria antitoxin was obtained from Merrill National Laboratories. (This antitoxin is now produced by Connaught Laboratories and may be obtained from Elkins-Sinn, Richmond, Va.; the undiluted antitoxin has a 5-year shelf life at 2 to 8°C.) Varying dilutions of the antitoxin ranging from 1:30 to 1:2,000 were prepared with barbital buffer and contained from 1 to 64 U of antitoxin per ml. Plates were examined for precipitin lines immediately after the current was turned off.

The effect of the basal medium, supplement, and length of incubations is shown in Table 1. Lines first appeared in proteose peptone broth supplemented with horse or rabbit serum at 16 h and became distinct by 24 h. No advantage was gained by shaking the cultures or by extending the incubation beyond 48 h. Better lines were obtained with proteose peptone broth than with Columbia broth, but this may have been due to differences in the pH of these media.

Proteose peptone broth, pH 7.8, supplemented with 20% horse serum was adopted as the standard medium. Cultures were incubated under stationary conditions for 24 h prior to testing by CIE. The optimal dilution of antitoxin was found to be 1:125 and contained approximately 16 U of antitoxin per ml.

The results of testing the various strains are shown in Fig. 1. Distinct lines were obtained with the Park-Williams, Park 8, Gravis, and Intermedius strains in 24-h cultures; lines were not detected with the Mitis strain until 48 h of incubation. We were subsequently advised by the American Type Culture Collection that this Mitis strain was "barely toxigenic." No lines occurred with the avirulent strain, *C. hemolyti-
studies on toxigenic and nontoxigenic clinical isolates are certainly indicated.

LITERATURE CITED