Comparison of Continuous and Discontinuous Counterimmunoelectrophoresis with Immunodiffusion in Identification of *Candida* Antibody Using HS Antigen

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We have compared continuous counterimmunoelectrophoresis (CCIE) and discontinuous counterimmunoelectrophoresis (DCIE) with immunodiffusion (ID) in the identification of *Candida albicans* antibody using HS antigen. Eighteen sera with a titer of 1/1 to 1/32 by DCIE were titrated by CCIE and ID. Fewer were positive by CCIE and ID (13 of 18 and 9 of 18, respectively). Furthermore, the titers were lower when measured by these two methods in comparison to DCIE (4 of 18 and 3 of 18 sera had titers $\geq 1/8$ in comparison with 7 of 18 $\geq 1/8$ by DCIE). Finally, DCIE was found to be much faster than CCIE and ID. After 20 min of electrophoresis, 17 of 21 sera were positive by DCIE, whereas none were positive by CCIE. Even after 90 min, only 10 of 21 were positive by CCIE, whereas 4 of 21 were positive by ID. The increased sensitivity and speed of DCIE over CCIE and ID suggest that DCIE is preferable to these other methods in the measurement of candida antibody using HS antigen.

Counterimmunoelectrophoresis (CIE) has been widely used to identify viral (1, 2, 6, 7, 12, 14), bacterial (3, 5, 8, 9, 16), and fungal (4, 10, 13) antigens and antibody. Wallis and Melnick (17) have reported that the sensitivity of CIE is affected by the relative ionic strengths of the gel and tank buffers. These investigators found that sensitivity of CIE for hepatitis antigen and antibody was improved if the ionic strength of the gel buffer was lower than the ionic strength of the tank buffer, that is, if a discontinuous buffer system was used.

We have compared the usefulness of this type of CIE, termed discontinuous CIE (DCIE), with conventional or continuous CIE (CCIE) and immunodiffusion (ID) in the measurement of candida antibody. We have determined that the sensitivity and speed of CIE for candida antibody may be greatly improved by means of a discontinuous buffer system and that both types of CIE are more sensitive than ID in the measurement of this type of antibody.

MATERIALS AND METHODS

CIE plates were prepared by precoating 7.8-cm glass plates with 4 ml of 1% ion agar in distilled water. After drying, the plates were coated with 14 ml of 1% agarose in barbital buffer. The ionic strength (I) of the gel buffer was varied from 0.015 to 0.075 I in different experiments, whereas the tank buffer was held constant at 0.075 I. Pairs of wells, 2 mm in diameter and separated by 4 mm horizontally and 2 mm vertically, were cut in the gel through a plastic template. Antigen

purchased from the Hollister Stier Laboratories and designated HS antigen was placed in the cathodal (right-hand) row, and dilutions of serum (expressed as $-\log_2$ in Fig. 1) were placed in the anodal (left-hand) row (Fig. 1). In preliminary studies it was determined that a range of antigen dilutions from 1/4 to 1/64 ($-\log_2$ 2 to 6) was required to identify antibody in low and high dilutions of positive sera. Two lots of "HS" antigen were used in this study. Comparisons of CCIE with DCIE were made only with the same lot of antigen. A positive control (human reference serum) was included on each plate to ensure correct orientation of the plate in the electric field and reactivity of the antigen. Current was set at 30 mA. Voltage varied according to the ionic strength of the buffer in the gel. Plates were subjected to electrophoresis in different experiments for periods ranging from 10 to 180 min. After electrophoresis, the plates were examined under indirect light, held in a saline bath overnight to wash away nonspecific precipitate, dried, stained with amido Schwartz dye, and sprayed with acrylic. The appearance of a precipitin band at any dilution of antigen was defined as a positive result at that dilution of serum.

ID plates were prepared by precoating glass plates (7.8 by 10.2 cm) with 1% ion agar in distilled water. After drying, the plates were coated with 14 ml of 1% agarose in phosphate-buffered saline (pH 7.4) containing 0.02% sodium azide. Wells were cut in the gel through a plastic template. In different experiments, different-size wells (2-, 3-, and 4-mm diameters) and different well separations (1, 2, and 4 mm) in all combinations were compared. Dilutions of sera were placed around the center well containing dilutions of antigen from 1/4 to 1/16. ID plates were incubated at 37°C for 90 min, examined under indirect light, and

FIG. 1. Format of DCIE plate. Dilutions of serum ($-\log_2$) were placed in the left-hand (anodal) well, and dilutions of antigen ($-\log_2$) were placed in the right-hand (cathodal) well.

held at 4°C overnight. After re-examination, the ID plates were washed and stained as above.

In preliminary studies it was determined that maximum sensitivity of ID was achieved under the following conditions: serum well size, 4 mm in diameter; antigen well size, 2 mm in diameter; well separation, 2 mm; and antigen dilution, 1/16.

Sera from patients with different types of candidiasis were selected for comparison, including patients with systemic (10), gastrointestinal (4), and superficial (4) infections as defined elsewhere (11).

RESULTS

In the first experiment the effect of the gel and tank buffer ionic strength on the sensitivity of CIE was determined and compared with ID (Table 1). Eighteen sera with a CIE titer of undilute to 1/32 in the 0.015 I gel were titrated in the 0.075 I gel and by ID. The cumulative number positive (single bands only) at each titer

TABLE 1. Relative sensitivities of DCIE, CCIE, and ID for candida antibody (18 patients)

Reciprocal antibody titer	Cumulative no. of patients positive by:		
	DCIE (0.015 I) ^a	CCIE (0.075 I) ^b	ID ^c
32	3	0	0
16	4	2	0
8	7	2	3
4	9	8	6
2	14	11	7
1	18	13	9

^a Gel/tank ionic strength, 0.015/0.075 I; current, 30 mA; voltage, 18 V/cm.

^b Gel/tank ionic strength, 0.075/0.075 I; current, 30 mA; voltage, 6 V/cm.

^c Antiserum well size, 4 mm in diameter; antigen well size, 2 mm in diameter; antigen dilution, 1/16; and well separation, 2 mm.

beginning with 1/32 was compared. Titers were lower, and fewer specimens were positive, in the 0.075 I gel and lower yet with fewer specimens positive by ID.

In a second experiment the effect of the gel and tank buffer ionic strength on the speed of CIE was determined and compared with ID. Precipitin bands (single bands only) appeared more rapidly in the lower-ionic-strength gel. After 10 min of electrophoresis, 11 of 21 sera were positive (titer $\geq 1/1$) in the lower-ionic-strength-gel, and after 20 min 17 of 21 sera were positive, whereas none was positive in the higher-ionic-strength gel. Electrophoresis was continued in the higher-ionic-strength gel, and by 90 min 10 of 21 were positive. In contrast, only 4 of 21 sera were positive by ID after 90 min of incubation. It was observed that weakly reactive sera may not be positive immediately after electrophoresis but may become so over a period of 1 to 18 h. This phenomenon was seen in both types of gels. At 18 h after electrophoresis, all 21 sera were positive in the lower-ionic-strength gel, whereas 16 were positive in the higher-ionic-strength gel compared with 9 positive by ID. It was observed that voltage, endosmosis, and heat build-up were greater in the lower-ionic-strength gel as might be expected because there are fewer ions to carry current. This limited electrophoresis time in the lower ionic strength gel to 20 min.

DISCUSSION

Identification of candida antibody by CCIE and ID utilizing HS antigen has been advocated as one way of diagnosing systemic candidiasis (4, 13, 15). Some patients with systemic candidiasis, however, may be negative by these methods because of either immunosuppression or the

timing of the antibody determinations in question (4, 13).

In other studies we have reported that identification of candida antibody by DCIE is useful in the diagnosis of candidiasis (11). In this study we have demonstrated that DCIE is faster and more sensitive than CCIE and ID in the identification of candida antibody. The increased sensitivity of DCIE for candida antibody might permit a diagnosis of candidiasis in some patients negative by CCIE or ID.

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