

# Capillary Tube Immunological Assay for Staphylococcal Enterotoxins

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A simple assay is reported in which 1  $\mu\text{g}$  of staphylococcal enterotoxins A, B, and D per ml was detected in less than 1 hr. Interfacial reaction of antisera and enterotoxin solutions in a 1-ml internal diameter capillary tube allowed rapid detection of sera type.

Casman et al. (2) reviewed the advantages of the microslide gel double-diffusion test for assaying staphylococcal enterotoxins. Bennett et al. (*Bacteriol. Proc.*, p. 6, 1970) reported assay results in 24 hr using thinner gels. Additional advantages over other diffusion assays included greater economy of reagents, greater specificity, simplicity, and applicability to detection of enterotoxins in foods. More sensitive assays (4, 6) were ruled out because of the unavailability of sufficient antisera free of nonspecific antibodies.

This paper summarizes successful application of an interfacial test in capillary tubes which produced results in 5 to 40 min depending upon antigen concentration.

## MATERIALS AND METHODS

One-millimeter internal diameter capillary tubes were flame sealed at one end. Five microliters of antisera was introduced into the tubes using Hamilton 10- $\mu\text{liter}$ , fixed needle syringes. Teflon- or silicone-treated needles prevented wetting wall of tube above antiserum. Identical syringes were used to transfer 5  $\mu\text{liters}$  of purified and concentrated extracts from cheese (7) or staphylococcal cultures onto the surface of the antisera (3). Bubble formation and interface disruption were avoided. Tubes were incubated vertically at 25 C and evaluated visually with black background and white sidelight.

## RESULTS

Faint precipitate bands formed at the antibody-antigen interface. Using antisera of 1:80 titer, 1  $\mu\text{g}$  of enterotoxin B/ml produced visible precipitate in 40 min. The figure shows the precipitate formation that occurred 15 min after preparation of solutions containing 30, 3, and 0  $\mu\text{g}$  of enterotoxin B/ml. Enterotoxin levels of

0.5  $\mu\text{g}/\text{ml}$  were detected after 12 hr at 5 C. Precipitate settled and concentrated in the V-shaped bottom of the tubes during this time. No visual changes were evident in either antisera or enterotoxin control tubes.

During enterotoxin B purification, we successfully followed toxin elution from columns with the capillary test. The test was also used to follow antibody titer changes during rabbit immunization (5).

## DISCUSSION

Rapid enterotoxin immunological assays (4, 6) require relatively large volumes of reagents and approximately 3 hr to reach a detectable end point. Microgel diffusion tests (R. W. Bennett et al., *Bacteriol. Proc.*, p. 6, 1970; 2, 7) require longer incubation times for diffusion and special techniques in preparing the apparatus. Careful interface formation in small capillary tubes eliminates some of these disadvantages. Capillary tube tests have been routinely used in Lancefield grouping of the streptococci (1). However, we experienced interface disruption using capillarity fill of an open tube.

Five to six capillary tube tests can be completed using the antisera volume required in one well of the microslide gel double-diffusion test (2). If 0.5-mm internal diameter capillary tubes are used, even smaller volumes may be possible; however, bubble entrapment and droplets above antisera prevented our adapting the narrow tube.

The capillary test is less subject to the suppression effects resulting in false negatives that plague gel diffusion methods. Preparation of special equipment, glassware, or agar media is not required. Only the syringes were reused

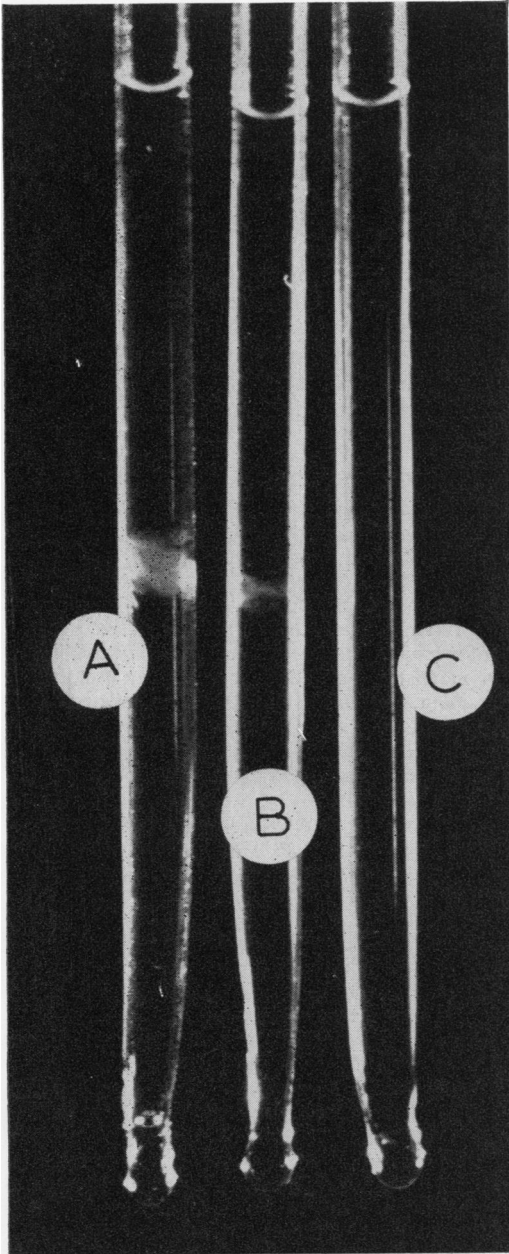


FIG. 1. Photograph of precipitate 15 min after preparation of enterotoxin B solution, 1:80 titer anti-

The capillary test should be applicable whenever enhancement procedures are not possible (2).

A 250- $\mu$ liter repeating dispenser Hamilton syringe would allow rapid inoculation of numerous tubes with one antiserum. Tubes could be sealed, frozen, and stored. Purified food extract could be rapidly evaluated by thawing tubes containing A, B, C, and D antisera, opening, and layering with the purified food extract. After toxin identification, several more frozen antisera tubes could be used for rapid estimation of enterotoxin concentration. The micro-slide diffusion assay (2), however, is required for evaluating intersection reactions.

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serum interface in 1-mm internal diameter capillary tubes. Five microliters of each solution used. A = 30  $\mu$ g of enterotoxin B/ml, B = 3  $\mu$ g of enterotoxin B/ml, C = 0  $\mu$ g of enterotoxin B/ml.