Polyvalent Antiserum Agar System for the Detection of Staphylococcal Enterotoxins A, B, C, and E

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A polyvalent antiserum agar system in capillary tubes was developed and evaluated for the detection of enterotoxins A, B, C, and/or E present in culture supernatant fluids.

A capillary tube technique for detection of enterotoxins A, B, and C in culture supernatant fluids was described by Fung and Wagner (2). The technique was successfully used in studying various aspects of staphylococcal enterotoxigenesis in liquid media and liquid foods (1, 3, 4). Since, to date, six types of toxins were identified, it will be useful to develop systems that can detect the presence of any one type of toxin in cultures and in foods. This report describes a polyvalent antiserum system for the detection of enterotoxins A, B, C, and E in culture supernatant fluids and was presented in part at the 73rd Annual Meeting of the American Society for Microbiology in Miami Beach, Florida, 6–11 May 1973.

The enterotoxigenic strains of *Staphylococcus aureus* tested in this study are listed in Table 1. With the exception of strain 494, which was obtained from R. W. Bennett (Food and Drug Administration, Washington, D.C.), all *S. aureus* cultures, enterotoxins A, B, C, and E, of known concentrations, and specific antiserum A, B, C, and E were obtained through the courtesy of M. S. Bergdoll (Food Research Institute, University of Wisconsin). Enterotoxin B and antiserum B were also obtained from Makor Co., Jerusalem, Israel. Eight coagulase-positive staphylococcal isolates obtained from cheese thought to be the vehicle of a food-borne intoxication outbreak and 39 confirmed clinical isolates of *S. aureus* were also tested for their toxin production abilities.

Growth conditions of cultures, quantitative and qualitative detection of crude toxin samples from culture supernatant fluids, and procedures for preparation of monovalent capillary tubes were previously described (1–4). In preparing polyvalent antiserum agar, the only modification was the incorporation of appropriate volumes of all four antisera (A, B, C, and E) into the agar. The dilutions for antisera A, B, C, and E used were 1:18, 1:20, 1:40, and 1:40, respectively. Standard curves (toxin concentrations versus resultant precipitin band lengths) of each toxin type were established for quantitative estimation of individual toxins in the culture supernatant fluids (2). Tests on all toxins showed that precipitin bands in the polyvalent system migrated more slowly than in the monovalent system after the same incubation time. However, the bands obtained in the polyvalent system were more distinct.

Toxin detection of culture supernatant fluids of seven enterotoxigenic strains of *S. aureus* was made by the polyvalent as well as the monovalent antiserum tubes (Table 1). All culture supernatant fluids containing A, B, C, and/or E showed precipitin bands in the polyvalent antisera A, B, C, and E tubes, except the supernatant fluid of *S. aureus* 494, which produces enterotoxin D. Upon testing in monovalent tubes, all corresponding monovalent antiserum tubes showed precipitin bands.

Table 1 also shows that two of the eight coagulase-positive staphylococcal isolates showed positive reactions in the polyvalent system. Further testing in monovalent tubes revealed that isolate number 3 produced enterotoxin B, whereas number 25 produced enterotoxin C.

A survey of clinical staphylococcal isolates from local hospitals made by the polyvalent system showed that out of 39 isolates tested, 25 were positive (Table 2). Further identification using monovalent tubes showed that the number of isolates producing enterotoxin A, B, and C were 7, 14, and 4, respectively. None pro-
Table 1. Detection of enterotoxins from isolates and strains of Staphylococcus aureus

| S. aureus strains | Polyvalent antiserum | Monovalent antiserum |
|------------------|----------------------|---------------------|
|                  | A, B, C,             | A | B | C | E |
|                  | and/or E             |   |   |   |   |
| Strain 100 (A)   | 0.4                  | 1.6 (12)*    | - | - | - |
| Strain 196E (A)  | 0.1                  | 0.2 (3)      | - | - | - |
| Strain S-6 (A, B)| 7.0                  | 0.1 (2)      | 9.0 (200) | - | - |
| Strain 137 (C)   | 4.0                  | -d           | - | 6.0 (200) | - | - |
| Strain 217 (C)   | 0.1                  | -            | - | 0.1 (2.5) | - | - |
| Strain 494 (D)   | 0                    | -            | - | -  | - | - |
| Strain 326 (E)   | 0.2                  | -            | - | -  | - | 0.3 (2) |
| Isolate no. 3*   | 2.0                  | -            | 4.0 (30) | - | - |
| Isolate no. 25*  | 0.5                  | -            | 1.5 (12) | - | - |

* Precipitin bands measured in millimeters.
* Numbers in parentheses indicate expected toxin.
* Numbers in parentheses indicate values in micrograms per milliliter.
* - toxins not detectable.
* A total of eight isolates were tested.

Table 2. Detection of enterotoxins from clinical staphylococcal isolates

| No. of isolates | Enterotoxin(s) in culture supernatant fluid* | Polyvalent antiserum | Monovalent antiserum |
|-----------------|-----------------------------------------------|----------------------|---------------------|
|                  |                                               | A, B, C,             | A | B | C | E |
|                  |                                               | and/or E             |   |   |   |   |
| 7               | +                                              | -                    | - | - | - |
| 14              | +                                              | -                    | + | - | - |
| 4               | +                                              | -                    | - | + | - |
| 25              | -                                              | -                    | - | - | - |

* Isolates were obtained from local hospitals and confirmed in the laboratory as Staphylococcus aureus.
* Supernatant fluids were obtained from 24-h culture of isolates grown in 3% PHP-NZ-Amine NAK medium at 40 C.

duced enterotoxin E and none produced more than one toxin.

The polyvalent system has the potential for use in large-scale screening of toxigenic S. aureus isolates from various health-related environments and suspected foods. Although preliminary work (data not shown) indicated that this polyvalent system was able to detect about 0.1 µg of toxin per ml of liquid food (after appropriate extraction and concentration), further investigation is needed before this system could be utilized for detection of minute quantities of toxin in foods (<1 µg/100 ml).

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