Rapid, Sensitive Assay for Staphylococcal Enterotoxin A by Reversed Immuno-osmophoresis

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Reversed immuno-osmophoresis using phenylsulfonated immune gamma2-globulin is a rapid sensitive method of assaying for staphylococcal enterotoxin A. The technique is compared with other serological methods.

Significant progress has been made in the methodology of detection procedures for staphylococcal enterotoxin. Angelotti (1) reviewed the methods of toxin detection in detail. This paper describes a modification of the immuno-osmophoretic technique suitable for detection of staphylococcal enterotoxin A.

Immuno-osmophoresis is the simultaneous electrophoresis of proteins and their homologous antibodies in agar, resulting in the formation of precipitin lines. The method is dependent on the endosmotic flow to the cathode and the net negative charge on the antigens. Endosmosis depends to some extent on the concentration and composition of the buffer solution, but the purity of the agar employed is the prime contributory factor. Antigens with an absolute electrophoretic mobility below \(-1.38 \times 10^{-8}\) cm² per V per sec are carried toward the cathode by the strong liquid flow in Noble agar. This technique was shown to be a rapid and sensitive method for evaluating certain viral antigens (6). It has also been used to assay tuberculo-proteins in agar columns (2). We report here successful attempts to make this technique applicable to the detection of enterotoxin A antigen. The adaptation of this technique to enterotoxin A detection was accomplished by introducing negative phenylsulfonate groups into the immune gamma2-globulins to reverse their electrophoretic mobility in agar without the loss of serological activity. In addition to saving a great deal of time, this method required very small amounts of antiserum and antigen sample.

Coupled with entrapment procedures (8), it is a convenient means for conducting multiple sample analyses. The homologous anti-enterotoxin A serum was prepared by using purified enterotoxin A in goats (C. E. Kimble, Ph.D. thesis, Oregon State Univ., Corvallis, 1970) and the gamma2-globulin fraction separated by alcohol precipitation (5). The negative net charge of the immune goat gamma2-globulin was increased by reacting the free amino groups on the protein with p-isothiocyanobenzenesulfonic acid. Purified gamma2-globulin (1 mg) was mixed with 8 ml of 0.2 M carbonate-bicarbonate buffer, pH 8.7, and 0.5 ml of 0.21 M sodium p-isothiocyanobenzenesulfonate in water. The solution was then incubated for 35 min at 37 C. The solution was then dialyzed for 2 h against 0.015 M tris(hydroxymethyl) aminomethane (Tris)-maleate buffer, pH 7.7, at 4 C. The solution was finally dialyzed for 2 h against 0.015 M Tris-maleate buffer, pH 6.5, containing 2.5 g of NaCl per liter.

A 1.5% agar (Noble; Difco) was prepared in Tris-maleate buffer, pH 6.0, and poured to a depth of approximately 3 mm on glass slides (2.5 by 7.5 cm). Sample wells (6 mm in diameter) were cut in the agar and filled with enterotoxin A antigen. The antibody slots were 1 by 10 mm. Contrary to the usual procedure, the modified antibody solution was added to the well closest to the cathode instead of the anode, hence the term "reversed immuno-osmophoresis" (RIO). A potential of 5 V/cm of agar path length at a current of 12 mA per slide was applied for 30 to 90 min at 4 C.

The slides were examined under a binocular stereomicroscope against a black background under low magnification and oblique lighting. Since only the active gamma2-globulin fraction
of the antiserum was used, rinsing with physiological saline solution was unnecessary before staining.

RIO was compared with the Oakley double-diffusion method (3) for efficiency (Table 1). RIO demonstrated comparable sensitivity to the Oakley method but suffers the disadvantage of being essentially a qualitative means of analysis. However, it possessed a distinct advantage in the time necessary to complete analysis. It lacks the sensitivity of “reversed passive hemagglutination” (7); but, there are substances in food which affect the specificity of the latter method. Several samples were assayed by gel diffusion, hemagglutination inhibition, and RIO. The results are shown in Table 2. The RIO procedure proved to be more sensitive than the Oudin procedure and less sensitive than hemagglutination inhibition techniques.

RIO is potentially a good method for screening food samples for staphylococcal enterotoxin. It offers the advantages of speed and requires minimal quantities of highly specific antisera. However, it is not a quantitative method, and direct analysis of food samples, particularly meats and cheese, make precipitin lines difficult to visualize.

### Table 1. Sensitivity of the double-diffusion and reversed immuno-osmophoresis assay systems for enterotoxin A

| Assay system            | Assay period (days) | Enterotoxin concn (µg/ml) | Results  |
|-------------------------|---------------------|---------------------------|----------|
| Double diffusion        | 4                   | 1.0                       | 3+*      |
|                         | 3                   | 0.75                      | 4+       |
|                         | 4                   | 0.50                      | 3+       |
|                         | 10                  | 0.25                      | 2+       |
| Reversed immuno-        | 65                  | 1.0                       | 3+       |
| osmophoresis            | 65                  | 0.75                      | 3+       |
|                         | 90                  | 0.50                      | 2+       |
|                         | 120                 | 0.25                      | 1+       |

*Relative clarity of precipitin lines.

### Table 2. Comparison of methods for detection of enterotoxin A in Tris diluent*

| Sample | Enterotoxin added (µg/ml) | Hemagglutination inhibition | Immuno-diffusion (Oudin) | RIO* |
|--------|---------------------------|-----------------------------|--------------------------|------|
| 1      | 50                        | 48.0                        | 45.0                     | +    |
| 2      | 10                        | 7.0                         | 6.3                      | +    |
| 3      | 5                         | 2.0                         | 3.8                      |      |
| 4      | 1                         | 0.8                         |                          |      |

* Results are the average of three determinations.
* Hemagglutination experiments performed with sheep red blood cells preserved with formaldehyde.
* Positive precipitin test indicated by +.

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