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Identification of Group C Streptococcal Antigen Extracts with Lectin-Bound Polystyrene Particles

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Crude extracts of *Dolichos biflorus* can be coupled to polystyrene particles to yield an agglutination reagent for the detection of group C streptococcal antigen extracts. The reagent is relatively inexpensive and simple to prepare and can be employed for the definitive identification of this beta-hemolytic streptococcus.

The lectins *Dolichos biflorus* (1–3, 5), *Wistaria floribunda* (4), and *Helix pomatia* (5) were previously shown to agglutinate group C beta-hemolytic streptococci. Confirmation as to the specificity of the *Dolichos* lectin in agglutinating the cells of group C streptococci and not cells of group A, B, F, or G streptococci from primary colonies dervied from throat cultures was recently demonstrated (7). In this paper we describe a unique and highly specific agglutination test which enables the serogrouping of nitrous acid extracts of group C streptococci with *D. biflorus*-sensitized polystyrene spheres.

Group C streptococci strains used in this investigation were obtained from our own stock reference cultures and from clinical specimens. Clinical specimens received on transport swabs (Culturette II; Marion Scientific Corp., Rockford, Ill.) and stock cultures were streaked on Columbia sheep blood agar plates (Scott Laboratories, Fiskville, R.I.) to obtain isolated colonies. The plates were incubated at 35°C under anaerobic conditions in type A Bio-Bags with catalyst and anaerobic generator (Marion Scientific Corp., Kansas City, Mo.) for 12 to 18 h. Gram staining and the catalase test were performed on all beta-hemolytic colonies to presumptively identify them as beta-hemolytic streptococci. Five or more isolated colonies of beta-hemolytic streptococci from each of the primary isolation plates and stock cultures were selected and serogrouped by a micronitrous acid extraction method (8) employing the Meritec streptococcal coagglutination test reagents (Meridian Diagnostics, Inc., Cincinnati, Ohio) as the reference method.

Polystyrene spheres (CX-Covaspheres; Covalent Technology Corp., Ann Arbor, Mich.) were coupled with a preparation of *D. biflorus* lectin (E-Y Laboratories, Inc., San Mateo, Calif.). For this procedure, 100 μl of Covasphere CX particles, 0.7 μm diameter, were added to 1 ml of distilled water containing 0.1 mg of crude *D. biflorus* lectin. Coupling was effected with the addition of 0.1 mg of 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide hydrochloride (Sigma Chemical Co., St. Louis, Mo.) as a spacer arm (6). The mixture was then incubated at ambient room temperature overnight. The preparation was then washed three times with FA buffer (Difco Laboratories, Detroit, Mich.) containing 1% bovine serum albumin for 10 minutes at 10,000 × *g* and 4°C. The pellet was suspended in 1 ml of this buffer and stored at 4°C.

A drop of a nitrous acid extract of bacteria was placed on a glass microscope slide. The drop was mixed with 1 drop of the polystyrene-*Dolichos* reagent. The slide was rocked for 1 min and examined for agglutination of the polystyrene particles with transillumination against a dark background. Unlabeled polystyrene spheres were used as a control.

A trial was conducted to examine the *Dolichos*-polystyrene reagent for its ability to identify group C streptococci prepared as nitrous acid, autoclave, and enzyme extracts (8). The extracts of all 40 strains of group C streptococci prepared by each of the three methods were agglutinated with the *Dolichos*-polystyrene preparation. The agglutination was observed within 20 to 30 s of examination. No agglutination was observed when extracts prepared by all three methods of 15 strains each of groups A, B, F, and G beta-hemolytic streptococci, *Streptococcus pneumoniae*, and *Klebsiella pneumoniae* were tested with the lectin-polystyrene reagent.

This is the first report demonstrating that group C streptococcal antigen extracts can be specifically agglutinated by a lectin bound to a carrier particle. Accordingly, extracts as well as the cells of these bacteria can be tested with *Dolichos* lectin as a definitive serogrouping method for the identification of group C streptococci isolated on blood agar plates. Cross-reactive coagglutination response was previously demonstrated when autoclave and enzyme extracts derived from *S. pneumoniae* were mixed with a coagglutination reagent for group C streptococcus. However, no serological cross-reactions occurred with single colonies of this organism when the group C coagglutination reagents and nitrous acid extracts were used (8). In contrast, the present investigation demonstrated that there was no reactivity with autoclave, enzyme or nitrous acid extracts of *S. pneumoniae* or *K. pneumoniae* with *Dolichos*-polystyrene.

It appears that the *Dolichos* lectin can be successfully used in place of antisera to group C streptococci.

The cost for the crude preparation of lectin and reagents required to prepare 1 ml of the final *Dolichos*-polystyrene reagent is ca. $8.00. Certainly, the preparation of the reagent does not involve the relative complexity associated with the production of an antibody preparation derived from an animal source. Accordingly, due to the ease of production and the relatively low cost of reagents required in its preparation, the *Dolichos*-polystyrene reagent could be incorporated into diagnostic kits for the serogrouping of beta-hemolytic streptococci. The advantage of this reagent is not only in its cost and ease of production but also in its high specificity compared with antisera to group C streptococci.

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