Serogroup Identification of Meningococci by a Modified Antiserum Agar Method

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Modifications in the antiserum agar method for serogroup identification of meningococci, which reduce the amount of group-specific antisera required and increase long-term storage of prepoured antiserum agar plates, are described.

Meningococci are serogrouped on the basis of immunologically and chemically distinct capsular polysaccharides (1). Eight serogroups, designated A, B, C, D X, Y, Z, W135, and 29E, are recognized, of which serogroups A, B, C, Y, W135, and 29E account for most disease. Several laboratories interested in serogrouping meningococci have found that the antiserum agar (ASA) method is rapid and easy to perform, and that the results compare favorably with those of bacterial slide agglutination (1, 3). ASA has also been useful for direct serogroup identification of meningococcal carriers (D. E. Craven, C. E. Frasch, L. F. Mocca, F. B. Rose, and R. Gonzalez, submitted for publication), and for identifying bacteria containing antigens cross-reactive with the meningococcal capsular polysaccharides (2).

The major limitations of the ASA technique are the large amounts of antisera required and difficulties related to long-term storage of prepoured ASA plates. Described herein are modifications of the ASA technique which make this method more suitable for laboratories serogrouping small numbers of meningococcal isolates.

ASA was prepared as previously described (1), using antisera prepared in horses and burros immunized with Formalin-inactivated meningococci of serogroups A, B, C, Y, W135, and 29E. Antisera were added at 50°C to autoclaved flasks of tryptic soy broth (Difco, Detroit, Mich., 30 g/liter) and Noble agar (Difco; 14 g/liter). Agarose (Sigma Chemical Co., St. Louis, Mo.), used in place of Noble agar, yielded easier halo visualization but did not alter final serogroup results. Dilutions of each antiserum added to the tryptic soy-Noble agar medium ranged from 1:10 to 1:40, depending on the strength of the antiserum used. Using a 24-well Costar tissue culture dish (Bellco Glass, Inc., Vineland, N.J.), 0.5 ml of ASA for each serogroup was pipetted into one of six vertical rows. Unused ASA plates were

stored at 4°C in "zipper-seal" plastic bags (Fisher Scientific, Silver Spring, Md.).

Meningococci to be serogrouped, taken either directly from frozen vials or from growth on brain heart infusion agar (Difco) containing 1% normal horse serum, were inoculated with a sterile wooden applicator stick into wells containing the different ASA. The plates were incubated at 37°C in the presence of 5% CO₂ and examined at 24 h and 48 h for group-specific halos (Fig. 1). Halos were usually visible at 24 h, but in the presence of good bacterial growth halos were sometimes visible 8 h after inoculation.

Two cross-reactions characteristic of the antiserum agar method occurred (1). These result from the use of horse serum and from the similar chemical compositions of the meningococcal capsular polysaccharides, but neither impaired correct serogrouping. First, group Y strains inoculated onto ASA produced strong halos with group W135 and group Y ASA, whereas group W135 strains produced halos only on the group W135 ASA. A similar reaction was present among group 29E and group Z strains. The group Z horse antiserum for ASA was kindly provided by B. B. Diena, Bureau of Bacteriology, Laboratory Center for Disease Control, Ottawa, Canada. Group Z strains produced halos on both the group Z and group 29E ASA, whereas serogroup 29E strains produced halos only on the group 29E ASA. Thus, with the absence of group Z ASA in the tissue culture ASA plates, group Z strains were identified as group 29E. This is of minor consequence, since group Z strains are uncommon and rarely produce disease.

The use of tissue culture plates greatly reduces the amount of antiserum necessary for ASA serogrouping. The average dilution of antiserum to agar is 1:20, and therefore 1 ml of antiserum will serogroup approximately 40 meningococcal strains. Although our antisera are prepared in horses and burros, the use of tissue culture plates

FIG. 1. A 24-well tissue culture dish, containing antiserum agar to meningococcal serogroups A, B, C, Y, W135, and 29E in wells 1 to 6, respectively, is shown after the inoculation of three different meningococcal strains in rows A, B, and C. Halos at 48 h seen in wells A-1, B-3, and C-3 identify the three strains as serogroups A, C, and C, respectively.

permits the use of antisera prepared in rabbits. The use of rabbit antisera for ASA may eliminate the cross-reactivity problem observed with horse and burro antisera. Although we have not used the ASA method with commercial antisera, such sera may be employed in the ASA plates, provided they have sufficiently high antibody concentrations. Four different meningococcal strains can be serogrouped on each plate, but if only one or two strains are serogrouped, the plate can be replaced in its plastic bag, sealed, and stored at 4°C for future use. We have stored ASA in tissue culture plates more than 9 months and have had no problems with the ASA drying and no apparent decrease in antibody activity.

The ASA method described herein is now routinely used in our laboratory, and over 100 fresh isolates have been serogrouped without difficulty. Although the ASA method was origi-

nally recommended for use in laboratories serogrouping numerous strains of meningococci (1), we feel that the modifications reported here make this method useful for hospitals and state reference laboratories wishing to serogroup small numbers of meningococci.

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