

Modified Method for Serological Identification of Group B Streptococci

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Extracts from 10- and 40-ml cultures compared favorably for serological identification of group B streptococci. Typing by gel diffusion proved reliable and efficient. These modifications save time, media, and antisera.

The preferred methods for serological classification of beta-hemolytic streptococci remain those described by Lancefield and co-workers (4, 5, 11). Epidemiological studies of group B streptococcal infections, requiring the serotyping of many isolates, prompted us to compare acid extracts prepared by the conventional (11) and a modified method for typing in capillary precipitin and agar gel diffusion tests.

Beta-hemolytic streptococci from infectious material (27 strains), newborns (45 strains), and pregnant women (65 strains) were presumptively identified as group B streptococci by cultural characteristics and CAMP reactions (1). Two acid extracts of each strain were prepared, one as recommended from a 40-ml Todd-Hewitt broth (THB) culture and one from a 10-ml THB culture. The hot-acid extraction procedure was identical except for a reduction of N/5 HCl from 0.4 to 0.25 ml for the 10-ml culture. Paired extracts were coded to permit examination in double-blind fashion.

Reference antisera were obtained from the Center for Disease Control, Atlanta, Ga., courtesy of Hazel Wilkinson. Serological grouping was by capillary precipitation (3). Typing was done in capillary pipettes (12) and by our modification (2) of the double-diffusion technique in Noble agar gel used by Rotta et al. (10). Immuno-plate diffusion plates (Hyland) were prepared with four sets of circular wells to accommodate the four type-specific group B streptococcal antisera on a single slide. Two sets of paired extracts were examined per slide.

All 137 presumptive group B streptococci were identified serologically as members of group B by capillary precipitation; there was 100% agreement between the grouping of extracts prepared from 10- and 40-ml THB cultures. Precipitin reactions were quantitatively similar in most pairs (73%) but stronger with the modified (10 ml) extracts of 18 (13%) strains.

Serotyping results are presented in Table 1. Complete agreement between the two extracts being compared was observed in capillary precipitin tests. Quantitative reactions were uniformly strong with either extract for types Ia, II, and III, being somewhat weaker with the Ib antiserum. However, all reactions were 2+ (moderate) or greater.

In agar gel typing, precipitin bands were sharp and distinct with each of the extracts of strains identified as type Ia, II, or III; Ib bands were somewhat diffuse and hazy, especially those obtained with extracts from 10-ml THB cultures. Agreement between paired extracts was quite good, however, with discrepancies limited to three type Ib strains. Two extracts from 10-ml cultures and one from a 40-ml THB culture failed to react. Each of the paired extracts of these three strains gave equally strong capillary precipitin reactions with the Ib antiserum. Serotyping by agar gel diffusion alone, with extracts prepared from the 10-ml THB cultures, thus resulted in proper identification of 134 of 136 group B streptococcal strains.

Methods for the serological classification of beta-hemolytic streptococci into groups and specific serotypes evolved from a common line of investigation by Rebecca Lancefield (4-6, 11). The acid extraction method elutes both polysaccharide and protein cellular antigens in soluble form. The results now reported demonstrate that growth from 10-ml THB cultures compares favorably with that from 40-ml THB cultures for preparing acid extracts suitable for both grouping and typing of group B streptococci.

Others have shown that the extraction of streptococcal-group carbohydrate antigens can be accomplished by various means using bacterial sediment from as little as 5 to 10 ml of culture volume or from blood agar plates (3). The methods we are now reporting provide additional practical and economical advantages for

TABLE 1. *Typing of group B streptococci from 10- or 40-ml broth cultures by capillary precipitin or by gel diffusion tests*

Serotype	No. positive by precipitin test			
	Capillary		Gel diffusion	
	40 ml	10 ml	40 ml	10 ml
Ia	17	17	17	17
Ib	28	28	27	26
Ic	15	15	15	15
II	40	40	40	40
III	36	36	36	36
NT ^a	1	1	2	3

^a Nontypable.

the laboratory concerned with both group and type identification of group B streptococci. Culture media, antisera, and technical time are all conserved.

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