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Letters to the Editor

False-Negative Results in Typing of Group B Streptococci by the Standard Lancefield Antigen Extraction Method

Group B streptococci (GBS) are generally known to be pathogenic for humans, particularly for newborn infants, but they are also recognized as a cause of bacteremia in parturient women, elderly people, and immunocompromised patients (2). At present, GBS strains are typed in reference laboratories around the world by use of typing schemes based on serotyping, followed, if necessary, by phage typing (3). The primary serologic test used for classification in the laboratories is the capillary precipitation test introduced by Lancefield in 1934 (5). This test is based on the presence of capsular antigens extracted with hot hydrochloric acid (HCl) and allows classification of GBS strains into nine serotypes (Ia, Ib, II, III, IV, V, VI, VII, and VIII) (2, 3, 7). Most GBS strains can be classified as belonging to one of these serotypes, but always a certain percentage of strains remains nontypeable (NT) (2, 4). We have observed that the normality of the HCl used in the extract antigen can affect the number of strains that are determined to be NT in surveillance studies.

Routinely, the microbiology departments of various institutions in Denmark submit GBS strains (confirmed to be such by pure culture) for typing in our facility (the Streptococcus Unit of the Statens Serum Institut). However, before we carry out the serotyping for a given strain, we always verify that it is a GBS strain by evaluating the strain visually on a blood-agar plate and by testing it with GBS antiserum by the Lancefield method.

Of the 383 human isolates of GBS received at the Streptococcus Unit during the years 1997 to 2000, 154 were classified as serotype III. Of these, 103 isolates reacted with the specific antiserum when extracted with both 0.1 and 0.2 N HCl, and 51 isolates reacted only when extracted with 0.2 N HCl. A total of 26 of the isolates did not react when extracted with either HCl concentration. Based on this observation, we decided that, beginning in the year 2001, we would test all GBS isolates with both 0.1 and 0.2 N HCl to determine if we could reduce the number of NT GBS isolates. We had previously used only one extract concentration (0.2 N HCl) for typing GBS isolates.

The antisera used for typing the GBS isolates as belonging generally to group B and more specifically to one of the serotypes (Ia to VIII) were produced at the Streptococcus Unit. The Lancefield extracts used as controls were based on reference strains of GBS serotypes Ia to VIII, as well as of group B, that were obtained within the Streptococcus Unit, which serves as a national reference center for streptococci. A modified version of the Lancefield method (which modification is used as the standard serotyping method in the Streptococcus Unit) was performed as follows. A freeze-dried culture of GBS was suspended into serum broth. From this suspension, a control for purity was made by plating a droplet onto a 5% horse blood-agar plate. The agar plate was incubated at 36°C overnight. The next day, a few colonies from the agar plate were added to 5 ml of glucose broth, and the bacterial suspension was incubated at 36°C overnight. After incubation, the suspension was centrifuged and the supernatant was removed. A 0.1-ml volume of either 0.1 or 0.2 N hydrochloric acid was added to the bacteria pellet. The suspension with the acid added was held at 100°C (i.e., placed in boiling water) for 15 min and thereafter cooled under tap water. After cooling, 0.2 N NaOH was added to adjust the pH to 7.0 (it was also recommended that a droplet of phenol red be added to the acid suspension as an indicator). The suspension was then centrifuged for 10 min to obtain a crystal-clear supernatant, and serotyping was performed as described by Lancefield (3, 5).

We tested all the GBS isolates that we received from January to mid-November 2001. During this period, a total of 94 isolates were identified as GBS and typed. Of these, 82 (87.2%) could be identified with both normalities. Of the remaining isolates, 10 (10.6%) could be identified only with the 0.2 N extract and 2 (2.1%) could be identified only with the 0.1 N extract.

Serotyping of GBS is essential for surveillance studies as part of the development and formulation of protective human GBS vaccines. There may be many reasons why some isolates are NT, and one of the explanations could be that some of the NT isolates represent new serotypes that still remain to be described. However, many studies (1, 2, 3, 6) of the distribution of GBS serotypes among human isolates are mainly based on serotyping by use of the capillary precipitation method with only one standard extract antigen normality (5). Given the results described above, if different extract normalities had been used, it is possible that fewer NT isolates would have been found.

A suggestion for better use of the precipitation test in the future is to retest all the strains defined as NT, using the standard 0.2 N HCl extract and other, different extract normalities (such as 0.1 N HCl) in order to reduce the number of false-negative responses.

REFERENCES

1. **Blumberg, H. M., D. S. Stephens, M. Modansky, M. Erwin, J. Elliot, R. R. Facklam, A. Schuchat, W. Baughman, and M. M. Farley.** 1996. Invasive group B streptococcal disease: the emergence of serotype V. J. Infect. Dis. **173:**365–373.
2. **Ferrieri, P., and A. E. Flores.** 1997. Surface protein expression in group B streptococcal invasive isolates, p. 635–637. *In* T. Horaud, A. Bouvet, R. Leclercq, H. de Montclos, and M. Sicard (ed.), Streptococci and the host. Proceedings of the 13th Lancefield International Symposium on Streptococci and Streptococcal Diseases. Plenum Press, New York, N.Y.
3. **Heard, S. R., and J. A. Mawn.** 1993. New phenotypic scheme for group B streptococci. J. Clin. Pathol **46:**145–148.
4. **Lachenauer, C. S., D. L. Kasper, J. Shimada, Y. Ichiman, H. Ohtsuka, M. Kaku, L. C. Paoletti, P. Ferrieri, and L. C. Madoff.** 1999. Serotypes VI and VIII predominate among group B streptococci isolated from pregnant Japanese women. J. Infect. Dis. **179:**1030–1033.
5. **Lancefield, R. C.** 1934. Serological differentiation of specific types of bovine haemolytic streptococci (group B). J. Exp Med. **59:**441–458.
6. **Perch, B., E. Kjems, and J. Henrichsen.** 1979. New serotypes of group B streptococci isolated from human sources. J. Clin. Microbiol. **10:**109–110.

7. **Rýc, M., J. Jelínková, J. Motlová, and M. Wagner.** 1988. Immuno-electron-microscopic demonstration of capsules on group-B streptococci of new serotypes and type candidate. J. Med Microbiol **25:**147–149.

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