JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2003, p. 4445–4447 Vol. 41, No. 9
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.9.4445–4447.2003
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Latex Assay for Serotyping of Group B *Streptococcus* Isolates

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Received 10 April 2003/Returned for modification 17 May 2003/Accepted 16 June 2003

We developed a group B streptococcus (GBS) latex serotyping kit that reduces the numbers of GBS nontypeable isolates by nearly 50%. A total of 232 isolates were tested, and 203 isolates were serotyped by the GBS latex test, while the capillary precipitation test serotyped 184 isolates.

At present, group B streptococcus (GBS; *Streptococcus agalactiae*) strains are serotyped in reference laboratories primarily by use of the capillary precipitation test (the Lancefield method) (6, 11), which is a time-consuming method that requires experienced staff. Other techniques have been proposed, but they either are labor intensive or have inadequate sensitivity or specificity (8, 15).

Latex agglutination tests (hereinafter called latex tests) have been used for identification and grouping of many clinically important microorganisms, including different streptococcal groups (1, 7, 14, 16), and a GBS urine latex test has been described previously (18).

The purpose of this study was to investigate the possibility of creating a latex test that can be used to serotype the nine GBS serotypes (Ia to VIII).

Reference strains of GBS serotypes Ia to VIII were obtained from the Streptococcus Unit, Statens Serum Institut (SSI) (Table 1).

A total of 132 invasive GBS isolates from all age groups and 100 collection strains were included in the study (Table 2). Latex solutions of type VI and VII were also tested on reference strains.

Strains were cultured on 5% blood agar plates and incubated overnight at 33 to 37°C, and when used for latex typing, a culture of Todd-Hewitt (TH) broth (SSI) was made from the plate and incubated overnight at 33 to 37°C. TH broth was used because it generates a high concentration of free antigen (9).

GBS type-specific antiserum (Ia to VIII) (SSI) was absorbed by standard procedures (10) before being used to test the 232 isolates.

The latex reagent was based on a description by Severin (13). The latex suspension was made by mixing 1 to 50 μl of absorbed antiserum plus 500 μl of 1% latex suspension (6 ml of 10% latex solution plus 54 ml of glycine-buffered saline [pH 8.2]) plus 450 to 499 μl of glycine-buffered saline (pH 8.2). The solution was incubated in a 55°C water bath for 20 min and resuspended every 5 min. After incubation, the suspension was cooled to room temperature, and 20 μl of bovine serum albumin was added per ml. The suspension was stored at 5°C.

The latex test was performed by mixing 10 μl of latex suspension and 10 μl of TH broth culture with GBS on a glass plate. The plate was rocked, and the agglutination reaction could be seen within 10 to 15 s, as weaker false-positive reactions could have appeared if the test was read after more than 30 s.

Different concentrations of type-specific antiserum were tested, and for all latex solutions (serotypes), 10 μl/ml was chosen, except for serotype V, for which 15 μl/ml was used. All isolates were tested with both 0.1 and 0.2 N HCl extracts (Lancefield method) (15). Serum broth (SSI) and Mueller-Hinton (MH) broth (SSI) were tested with nine reference strains to determine whether they could be used to grow the bacteria for the latex test.

Of the nine different GBS latex solutions, seven initially showed cross-reactions when tested with all nine reference strains (data not shown). The cross-reactions were removed by standard absorption (10), except for one type: the reaction between the latex solution for serotype III and the type VI strain could not be removed. This reaction was also observed by another type VI reference strain and one of the two invasive isolates (Table 3 and 4).

Of the 232 isolates, 203 (87.5%) could be serotyped by the GBS latex method. Only 184 (79.3%) of the isolates could be serotyped by the Lancefield method (Table 2). Eight of the isolates that could be serotyped with the latex reagents had

TABLE 1. Reference GBS strains used to test and adjust the GBS latex

Serotype	Strain designation
Ia	O90 (ATCC 12400)
Ib	H36 (NCTC 8187)
II	18 RS 21 (NTCT 11079)
III	M 781 USA 27/10-93
IV	12351
V	SS 1169
VI	NT6
VII	7271 SSI
VIII	130013 Colindale

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TABLE 2. Total number of GBS strains tested with both Lancefield method and latex agglutination

GBS serotype	No. serotyped by:	
	0.1 and 0.2 N Lancefield method[a]	Latex agglutination method
Ia	42	42
Ib	12	12
II	11 (69)	16
III	82 (99)	83
IV	10 (50)	20
V	17 (94)	18
VI	2	3
VII	1	2
VIII	7	7
NT	48 (166)	29
Total[b]	232 (20.7%)	232 (12.5%)

[a] Percentage of GBS strains that were serotyped with the Lancefield method (compared to the number serotyped by the latex agglutination method) is shown in parentheses.
[b] For each typing method, the percentage of the total that was NT is shown in parentheses.

cross-reactions; however, these eight isolates only reacted with one serotype using the Lancefield method. Of the 29 isolates that could not be typed using the latex method, six could not be typed because of cross-reactions. Cross-reactions were not seen with use of the Lancefield method. The greatest differences between the Lancefield and latex agglutination methods occurred when serotyping serotypes II and IV. The Lancefield method only serotyped 50% of serotype IV strains and 69% of serotype II strains.

We also tested the effect of different media used to grow the bacteria on the latex method. We found that different media do have an effect on the latex typing results (Table 4). MH broth culture showed a weaker agglutination reaction than did TH broth. Cross-reactions between the latex solution for serotype III and strain VI were observed when using MH and TH broth, although this reaction was not observed with serum broth (Table 4).

Latex agglutination is used to serotype pneumococci (1), but ours is the first report to describe the use of this procedure to serotype GBS. The test is simple to perform and is less time-consuming than previously described procedures for typing bacteria (1, 4, 17), and the latex test does not require specific laboratory conditions (1, 14). Compared to PCR typing of GBS (8), the presently described latex method is very simple to carry out.

Of the 232 GBS isolates tested, 14 isolates showed cross-reaction by use of the GBS latex test, but 8 of these isolates showed single serotypes when tested by the Lancefield method. Because of this finding, we routinely retest strains that have cross-reactions by latex agglutination by using the capillary precipitation method. Nineteen nontypeable (NT) isolates could be serotyped using latex agglutination but would not serotype with the Lancefield method (Table 2). We believe the latex procedure accurately serotyped these GBS strains that are NT by the Lancefield method because the antibodies used to produce the latex reagents have been made specific for each serotype of GBS with few cross-reactions; however, we plan to test these isolates by the PCR procedure described by Kong et al. (8).

The latex reagents have not been tested for cross-reactions toward other species. Rare cross-reactions between GBS types and other species, such as certain serotypes of *S. pneumoniae*, have previously been described (5); therefore, this method should be used only on isolates that are confirmed GBS.

The selection of TH broth was based on the work of Lafong and Crothers (9), who observed that TH broth gave a high concentration of free antigen. TH broth is also frequently used for GBS in other tests (2, 3, 12). Small differences in the formulation of the TH broth (data not shown), or the use of other media, may have an effect on the agglutination reactions (Table 4). An example is the cross-reaction between latex type III and serotype IV strain that appeared when TH broth or MH broth was used but was not observed with serum broth. The modification of media to alter GBS culture and thereby improve typing results has been described previously (3). It is therefore necessary to use a standard broth that has been tested for cross-reactions and strong positive reactions.

In summary, the Lancefield method is the generally accepted typing method for GBS isolates (6); however, the growing numbers of NT strains make this method less suitable for large-scale serotyping. The present study shows that the GBS latex method can reduce the number of NT strains by up to 50%. It is simple to carry out and is very suitable for large-scale serotyping. Due to these results, we will implement the latex assay in our laboratory and use the Lancefield method only

TABLE 3. Test for cross-reactions by use of GBS reference strains

Latex suspension type	Cross-reaction with reference strain[a]:								
	Ia (O90)	Ib (H36)	II (18RS21)	III (M781)	IV (12351)	V (SS1169)	VI (NT6)	VII (7271SSI)	VIII (130013 Colindale)
Ia	+	−	−	−	−	−	−	−	−
Ib	−	+	−	−	−	−	−	−	−
II	−	−	+	−	−	−	−	−	−
III	−	−	−	+	−	−	+[b]	−	−
IV	−	−	−	−	+	−	−	−	−
V	−	−	−	−	−	+	−	−	−
VI	−	−	−	−	−	−	+	−	−
VII	−	−	−	−	−	−	−	+	−
VIII	−	−	−	−	−	−	−	−	+

[a] +, positive reaction; −, negative reaction.
[b] Cross-reaction between reference strain VI and latex suspension III.

TABLE 4. Effect of different broths on latex antiserum reaction against the GBS reference strains

Latex type	Response of reference strain to latex broth[a]								
	Ia (O90)	Ib (H36)	II (18RS21)	III (M781)	IV (12351)	V (SS1169)	VI (NT6)	VII (7271SSI)	VIII (130013 Colindale)
Ia	+#¤	—	—	—	—	—	—	—	—
Ib	—	+#¤	—	—	—	—	—	—	—
II	—	—	+#¤	—	—	—	—	—	—
III	—	—	—	+#¤	—	—	+-¤	—	—
IV	—	—	—	—	+#¤	—	—	--¤	--¤
V	—	—	—	—	—	+#¤	—	—	—
VI	—	—	—	—	—	—	+#¤	—	—
VII	—	—	—	—	—	—	—	+#¤	—
VIII	—	—	—	—	—	—	—	—	+#¤

[a] Symbols: +#¤, positive responses with TH, serum, and MH broths; —, negative response with all three broths; +-¤, positive responses with TH and MH broths but negative response with serum broth; --¤, negative responses with TH and serum broths but positive response with MH broth.

when we obtain cross-reactions or are not able to serotype the GBS isolates.

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