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Cross-Reactions of Reagents from Streptococcal Grouping Kits with *Streptococcus porcinus*

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Streptococcus porcinus **is usually associated with swine. Because we have received several isolates from human sources that had cross-reacted with commercial group B streptococcal reagents, we examined several commercial kits to determine the extent of this cross-reaction. Fifteen reference and 15 clinical strains of *S. porcinus* were tested for cross-reactions with group B streptococcal reagents from 12 different commercial kits. Cross-reactions were detected with all group B reagents, but the number of cross-reactions varied with each kit. We recommend that manufacturers of reagents designed to identify group B streptococci by serologic methods test their reagents for cross-reactions with selected *S. porcinus* cultures or antigens.**

Current recommendations for establishing the specificity of streptococcal grouping reagents, including the rapid antigen tests for detecting group A and B streptococci, include strains representing group A, B, C, D, F, and G streptococci (2, 3, 5). The beta-hemolytic strains of *Streptococcus porcinus* (groups E, P, U, and V) were not included because these strains are seldom isolated from human sources (4). *S. porcinus* is usually associated with cervical lymph node infections in swine and is rarely reported from human infections. In clinical settings, the beta-hemolytic streptococci are most often identified with commercial grouping kits. Identification is by either coagglutination or latex agglutination with extraction of the antigen from the organism with either an enzyme or nitrous acid.

We previously reported an increased incidence of isolates of *S. porcinus* from the genitourinary tracts of reproductive-age women (4). Recently, several *S. porcinus* cultures have been submitted to the Centers for Disease Control and Prevention (CDC) Streptococcus Reference Laboratory because they had reacted with group B slide agglutination reagents of commercial streptococcal grouping kits. Since antimicrobial therapy generally is provided to pregnant women with group B streptococci identified from the genitourinary tract, incorrectly identifying *S. porcinus* as a group B streptococcus may have some clinical significance. We examined several commercially available streptococcal grouping kits for agglutination reactions with *S. porcinus*. All strains of *S. porcinus* used in this study were obtained from the Streptococcus Reference Laboratory, CDC. Fifteen reference strains, which included representatives of groups E, P, U, and V and new groups 1, 2, and 3 (NG1, NG2, and NG3), were used (1). Also included were 15 clinical strains of *S. porcinus* (2 group P, 12 NG1, and 1 with no group antigen). All clinical strains were extracted by the Lancefield extraction procedure and tested with CDC-prepared group antisera in a capillary precipitin test (6). Eight commercial streptococcal grouping kits and four kits designed to identify only group B streptococci were tested for cross-reactions with *S. porcinus*. Meritec-Strep (Meridian Diagnostics, Cincinnati, Ohio) is a coagglutination test. Bacto Strep (Difco Laboratories, Detroit, Mich.) is a latex agglutination kit with and without acid extraction. Prolex (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada), PathoDx (Diagnostic Products Corp., Los Angeles, Calif.), and RIM (Remel, Lenexa, Kans.) are acid extraction-latex agglutination kits. Streptex (Murex Diagnostics Ltd., Dartford, United Kingdom), BBL Strep (Becton Dickinson, Cockeysville, Md.), and Streptolex-OD (Orion Diagnostica Inc., Somerset, N.J.) are enzyme extraction-latex agglutination kits. Strep B OIA (Biostar, Boulder, Colo.) is an acid extraction optical immunoassay for group B streptococci. ICON Strep B (Hybritech Inc., San Diego, Calif.) and Equate Strep B (Binax, Inc., Portland, Maine) are acid extraction enzyme-linked immunosorbent assays (ELISA) for group B streptococci. The Directigen Group B Test (Becton Dickinson) is a heat extraction-latex agglutination test for group B streptococci. Strains were grown overnight in a CO_2 incubator on Trypticase soy–5% sheep blood agar plates (BBL). Each culture was grouped with each of the kits according to the manufacturers' instructions.

Results are shown in Table 1. No reactions were observed with any group A, C, D, F, or G reagent in the kits tested. One clinical strain (1256-95 [NG1]) reacted with all group B reagents, including CDC's group B capillary precipitin antiserum. This was also the only clinical strain to react with the group B reagents of the BBL Strep, Prolex, and Streptex kits. The PathoDx group B reagent and Strep B OIA tests reacted with the most clinical strains, while the group B reagents in the Meritec-Strep, Bacto Strep, and Streptolex-OD kits reacted with about half of these strains. The first lot of the RIM group B reagent reacted with several reference strains and all 12 of the NG1 clinical strains. The manufacturer provided us with a second lot of the reagents containing a group B reagent absorbed by *S. porcinus*. The absorbed lot of group B reagent did not react with any of the reference strains and reacted with only two of the clinical strains. The only other strains to react with CDC's group B antiserum were the reference strains SS-995 (NG1) and SS-996 (NG1). They reacted very weakly with the CDC group B antiserum.

Two unabsorbed lots of CDC streptococcal group B grouping antisera were available for testing. Lancefield extracts of *S. porcinus* SS-995, SS-996, and 1256-95 showed cross-reactions with one lot. Adsorption experiments failed to remove the *S. porcinus* cross-reaction in CDC unabsorbed group B antiserum. Aliquots of unabsorbed group B antiserum were absorbed with *S. porcinus* cells of strains SS-995, SS-996, and

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TABLE 1. Reactions of 30 strains of *S. porcinus* with the group B reagents of 12 commercial streptococcus test kits and one CDC capillary precipitin antiserum

Group (no. of strains)	No. of strains reacting with kit[a]:													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Reference strains														
E (3)	0	0	0	0	0	0 (0)[b]	0	0	0	0	0	0	0	0
P (2)	0	2	0	2	1	2 (0)	0	0	1	0	2	2	0	0
U (2)	2	0	0	1	2	2 (0)	0	0	1	0	2	2	0	0
V (2)	0	1	2	2	2	2 (0)	0	0	2	0	2	2	0	0
NG1 (2)	1	1	2	2	2	2 (0)	1	0	2	2	2	2	2	0
NG2 (2)	1	2	1	1	1	1 (0)	1	0	1	0	1	1	1	0
NG3 (2)	0	1	2	2	2	2 (0)	0	0	1	0	2	2	0	0
Clinical strains														
P (2)	1	1	1	0	1	1 (1)	0	0	1	0	1	1	1	1
NG1 (12)	6	5	5	1	10	12 (2)	1	1	8	1	12	11	2	2
No group antigen (1)	0	0	0	0	0	0 (0)	0	0	0	0	0	0	0	0

[a] 1, Meritec-Strep, no extraction, coagglutination; 2, Bacto Strep, no extraction, latex agglutination; 3, Bacto Strep, acid extraction, latex agglutination; 4, Prolex, acid extraction, latex agglutination; 5, PathoDx, acid extraction, latex agglutination; 6, RIM, acid extraction, latex agglutination; 7, Streptex, enzyme extraction, latex agglutination; 8, BBL Strep, enzyme extraction, latex agglutination; 9, Streptolex-OD, enzyme extraction, latex agglutination; 10, CDC capillary precipitin antiserum; 11, Strep B OIA, acid extraction, optical immunoassay for group B streptococci; 12, Directigen, heat extraction, latex agglutination for group B streptococci; 13, ICON Strep B, acid extraction, ELISA for group B streptococci; 14, Equate Strep B, acid extraction, ELISA for group B streptococci.

[b] Numbers in parentheses are numbers of strains that reacted with the RIM experimental lot with additional absorption.

1256-95 in ratios of 1 part cells to 5, 10, and 20 parts antiserum. Cells and antisera were mixed by vortexing and held at room temperature for 30 min before being separated by centrifugation. Only absorption at the 1:5 ratio of cells to antiserum completely removed all the cross-reactions, but absorptions with each strain at all three ratios of cells to antiserum removed or greatly reduced the sensitivity of the reaction of the group B antiserum. This indicates some sharing of antigenic entities between *S. porcinus* and group B streptococci. This was seen in agar gel diffusion studies in which Lancefield extracts of *S. porcinus* (strain 1256-95) and group B streptococci (strain SS-1073) showed lines of partial identity when tested with unabsorbed group B antisera.

Some confusion can occur in distinguishing between *S. porcinus* and group B streptococci. Both are bacitracin negative, CAMP positive (7), and beta-hemolytic. Zones of hemolysis are much more pronounced with *S. porcinus* than with group B streptococci (Fig. 1). The two types of streptococci can be differentiated phenotypically with the pyrrolydonlarylamidase (PYRase) and Voges-Proskauer tests; *S. porcinus* is positive in both tests, while group B streptococci are negative in both.

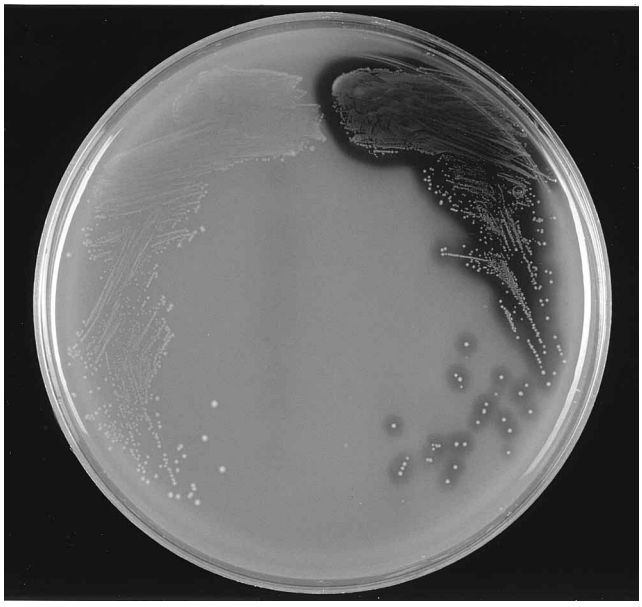

FIG. 1. Comparison of zones of hemolysis produced by group B streptococci (left) and *S. porcinus* (right).

Our results indicate that strains that react with the group B reagents of commercial kits, are CAMP positive and bacitracin negative, and produce a wide zone of hemolysis on 5% sheep blood agar may be *S. porcinus*. The identification of these strains may be confirmed by the PYRase and Voges-Proskauer tests.

Not all lots of CDC-prepared or commercially prepared group B streptococcal grouping antisera cross-react with *S. porcinus* strains. We do not know the incidence of *S. porcinus* strains in humans. We expect that it is currently quite low, but no studies seeking to identify these streptococci have been done. We recommend that all future lots of group B reagents be tested for potential cross-reactions with *S. porcinus* group E, P, U, V, and NG1 strains. Strains with representative *S. porcinus* group antigens are available from us upon request.

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