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Identification of *Salmonella* O Antigens by Coagglutination

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This study concerns the preparation of reagents for identifying the somatic O antigens of *Salmonella enteritidis*. Coagglutination reagents (COAGs) with antibody fixed to killed and stabilized protein A-bearing staphylococci were prepared with antisera which were used for identifying the somatic O antigens of *S. enteritidis* by the slide agglutination test. The reactions of the COAGs were compared with those obtained with the grouping antisera in routine slide agglutination tests in which 41 or more serologically different *Salmonella* strains, representing most of the known groups, were used. One-third of the COAGs gave identical reactions to those of the slide agglutination antisera. The reactions of the other COAGs varied from the slide agglutination antisera results, some by many reactions and others by only a few. The coagglutination procedure was more reactive than the routine slide agglutination test and resulted in cross-reactions which were not observed in the original grouping antisera. More COAGs were specific when they were tested with alcohol-treated cultures than with live cultures. Coagglutination conserves antiserum, allowing about 12 times as many tests for a given volume of group-specific glycerolized antiserum as does the slide agglutination method.

The slide agglutination method for identification of the somatic O antigens of *Salmonella enteriditis* requires the use of about 40 absorbed antisera (3, 4). The preparation of these antisera is difficult and cumbersome. In the interest of improving methods for the identification of salmonellae and of conserving antiserum, the coagglutination procedure, in which antibody attached to protein A-bearing staphylococci is used (1, 5), was investigated. Some preliminary experimentation with identification of *Salmonella* O antigens by coagglutination has been reported by Edwards and Hilderbrand (2) and Sanborn et al. (6). These papers reported rapid identification of some *Salmonella* groups. However, only a few heterologous strains were tested with coagglutination reagents (COAGs), so the specificity of these reagents was not clearly demonstrated. The application of coagglutination to *Salmonella* grouping will depend on demonstrating that the COAGs have the same specificity and the same or greater potency as the slide agglutination grouping antisera.

MATERIALS AND METHODS

Antisera. The *Salmonella* antisera used for attachment to protein A-bearing staphylococci were absorbed and were group specific by standard slide agglutination tests. All were obtained from the Biological Products Program, Centers for Disease Control, Atlanta, Ga., except for four antisera from the Enteric Bacteriology Laboratory, Centers for Disease Control. These four antisera were highly absorbed. All of the antisera used were prepared as previously described (4).

Preparation of staphylococci. *Staphylococcus aureus* Cowan 1 was grown on tryptic soy agar at 37°C for about 18 h. The cells were harvested, washed in phosphate buffer, exposed to 0.5% formaldehyde, and heated to 80°C and held for 5 min by the procedure of Edwards ad Hilderbrand (2).

Attaching O antisera to staphylococci. One milliliter of the 10% suspension of stabilized, killed staphylococci was added to 0.2 ml of non-glycerolated, specific antiserum or to 0.4 ml of glycerolated antiserum, mixed thoroughly, and allowed to react at room temperature for 3 h, with occasional gentle shaking (2). The suspension was centrifuged at 800 × *g* for 30 min and washed twice with 0.02 M phosphate (pH 7.3)-buffered 0.85% saline (PBS). The cells were then suspended in PBS to a final volume of 5 ml, and 1 drop of 5% sodium azide was added (2). The antibody-conjugated staphylococci made up a COAG. The COAGs were stored at 4°C until they were used to test *Salmonella* cultures by coagglutination on slides (or on a glass pane). The COAGs remained active after storage at 4 to 8°C for at least 1 year.

Testing *Salmonella* strains by coagglutination. *Salmonella* strains were grown overnight on blood agar base (BBL Microbiology Systems). To test for agglutination, we placed 1 drop of COAG on a slide or a glass pane. Growth was taken from a slant with a bacteriological loop and was mixed into the COAG drop. Strong homologous reactions usually occurred immediately. If there was no reaction, the slide was rocked gently for 1 min and then read under fluorescent light against a black background. The amount of agglutination was read as 4+, 3+, 2+, or 1+. A trace was recorded as +/−. Agglutination by the slide agglutination antisera was read the same way.

Suspensions of killed, alcohol-treated salmonellae (3) were tested by placing 1 drop of antigen near 1 drop of COAG and then mixing the two together with a bacteriological needle, rocking the glass panel, and reading the degree of agglutination as described above.

RESULTS AND DISCUSSION

Cultures (antigens) were tested in the slide coagglutination test as both alcohol-treated and live antigens. Fewer cross-reactions were seen with the alcohol-treated antigens than with live cultures. The treatment of *Salmonella* cultures with heat and alcohol destroys the antigenicity of some surface proteins. In coagglutination, antigens that are not agglutinins may react. Such antigens might be denatured in alcohol-treated preparations (3; Fig. 1 and 2). The cross-testing with heterologous strains was essential for the evaluation of the COAGs because tests with COAGs are more sensitive than routine tests with fluid antisera. Only about one-third of the COAGs both gave strong specific homologous reactions and showed complete agreement with the reaction pattern ob-

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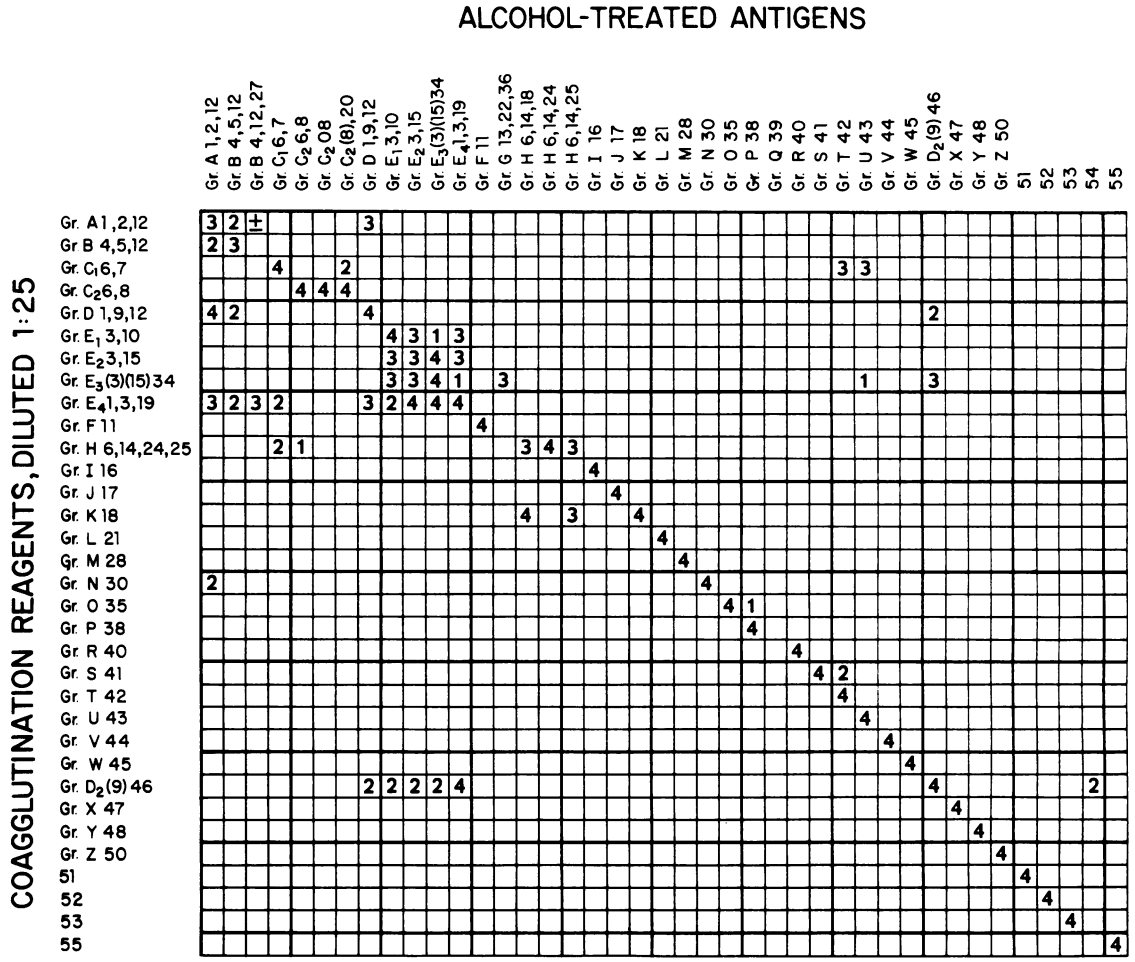

FIG. 1. Reactions of *Salmonella* COAGs with alcohol-treated antigens. A blank space indicates that a test was done but that no agglutination was observed.

tained by routine slide agglutination tests with live antigens.

In some instances when an original COAG behaved unsatisfactorily by exhibiting cross-reactions (where the original antiserum did not react), a new COAG was prepared from a different, more highly absorbed lot of grouping antisera (Table 1). Four of five of these new COAG lots were specific and exhibited 4+ homologous reactions. These results indicate that it is possible to achieve the correct reaction spectrum by absorption or selection of antiserum or by both. Whether it is practical to replace the entire system with COAGs depends on further experimentation to show both the possibility and practicality of producing suitable COAGs routinely. The main advantage of using coagglutination for grouping salmonellae is the conservation of serum; 12 times as many tests are achieved with a given volume of specific antiserum as are possible with the usual slide agglutination procedure. This investigation points out the importance of extensive testing of an enteric antiserum when it is to be used in a test such as coagglutination, which is more reactive than the routine slides agglutination test.

Because of the similarity of the testing procedures for routine slide agglutination and for coagglutination, one could devise a grouping system for *Salmonella* strains in which available specific COAGs are used, but in which the routine slide agglutination antisera are used for factors for which a specific COAG is not available. This would still make possible the conservation of antiserum in situations in which the availability of antiserum is the limiting element.

TABLE 1. Cross-reactions of *Salmonella* reagents with live cells by using COAGs prepared from more than one lot of antiserum[a]

Group	Antiserum	Homologous reaction	No. of cross-reactions
c_1	Lot 1	4+	6
c_1	Lot 6	4+	0
H	Lot 1	4+	15
H	2938-34[b]	4+	6
J	Lot 1	4+	10
J	1-6-49[b]	4+	0
L	Lot 1	4+	25
L	c2816-R13[b]	4+	0
O43	Lot 1	4+	8
O43	3-14-69[b]	4+	0

[a] Each lot was tested with 48 serologically different *Salmonella* strains.

[b] A highly absorbed lot of antiserum obtained from the Enteric Bacteriology Laboratory of the Centers for Disease Control.

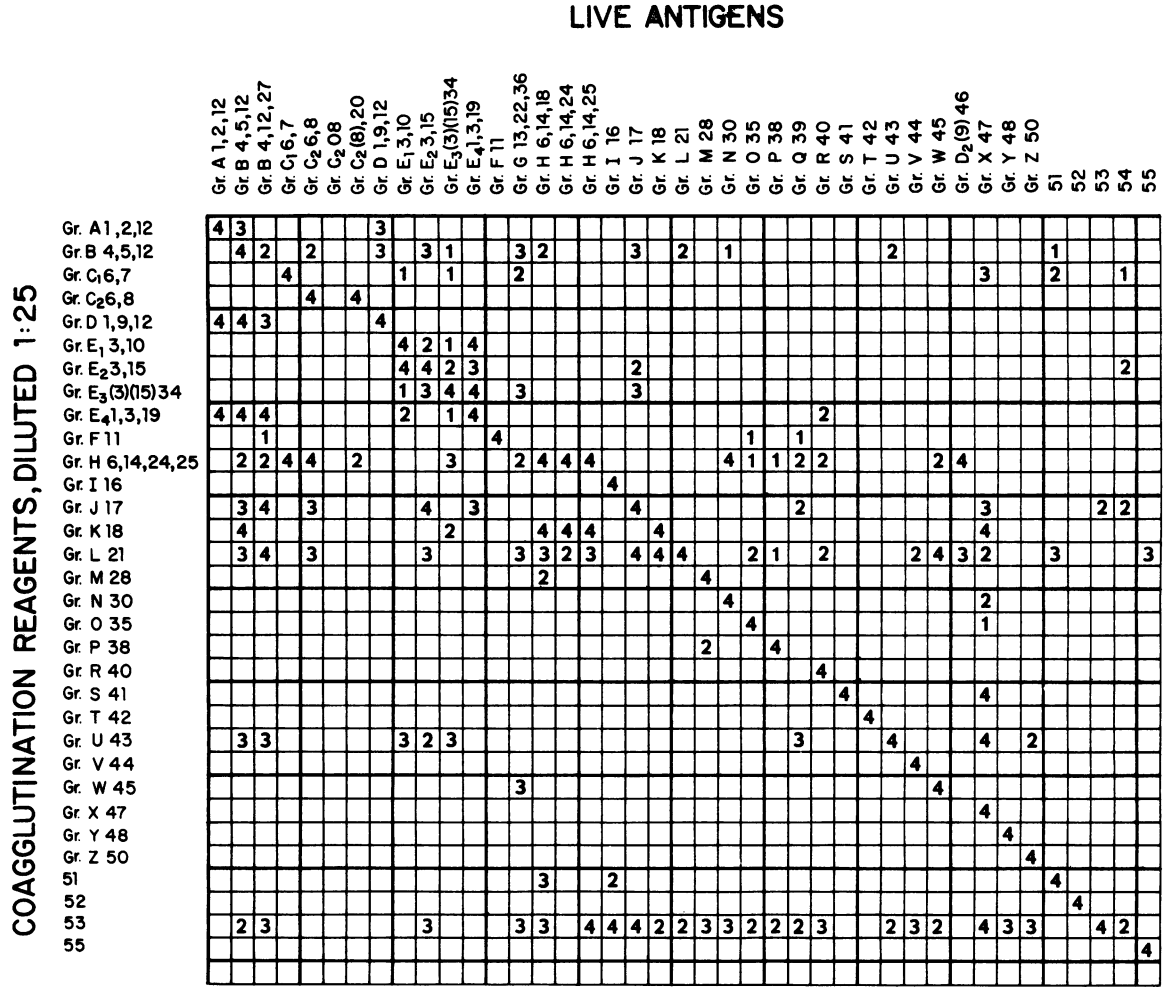

FIG. 2. Reactions of *Salmonella* COAGs with live antigens. A blank space indicates that a test was done but that no agglutination was observed.

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