Assessment of Availability and Efficacy of Commercial *Salmonella* Grouping Antisera

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Salmonella somatic antisera for groups A to E were purchased from four commercial producers directly by the Center for Disease Control (CDC) and indirectly through two hospitals. CDC specifications and methods were used to evaluate antisera shipped directly to CDC. To assess the performance of the products under simulated user conditions, we used the commercial antisera purchased indirectly through the hospitals to group coded cultures. Of the 23 antisera ordered by CDC and the hospitals, the CDC received all 23, a large medical complex received 20, and a private hospital received 9. Similar results were obtained with producer and CDC test methods. Forty-five different lots of antisera were evaluated, of which 20% did not meet CDC specifications. The CDC specifications and latest revisions are discussed.

The Diagnostic Products Evaluation Branch, Center for Disease Control (CDC), evaluates commercial *Salmonella* reagents submitted voluntarily by producers before they are marketed and also evaluates marketed reagents. Most of the consumer problems conveyed to the Diagnostic Products Evaluation Branch have concerned the reactivity of antisera, but difficulties in obtaining reagents have also been reported. To assess their quality, as judged by CDC specifications, and availability, CDC purchased *Salmonella* somatic (O) antisera (i) directly from commercial producers, (ii) through a large university teaching medical center, and (iii) through a large private hospital.

Commercial reagents are also tested by the Diagnostic Products Evaluation Branch at the request of the Food and Drug Administration. These evaluations are done according to the producers' instructions, which are packaged with the reagents. The differences between these methods and those in the CDC specifications vary depending upon the particular reagent and the producer. The results obtained by using CDC and commercial testing procedures were compared by using the antisera purchased directly from the producers.

In addition to evaluating with CDC methods the antisera purchased through the hospitals, we used these products to group coded *salmonella* cultures. The latter procedure provided results which (i) could be used to assess the types of identification that would be obtained with these products in clinical laboratories and

(ii) could confirm the regular evaluation procedure results.

MATERIALS AND METHODS

Antisera. Purchase orders for *Salmonella* somatic antisera groups A, B, C₁, C₂, D, and E were submitted by CDC and the two laboratories directly to BBL, Lederle Diagnostics, Lee Laboratories, Inc., and Difco Laboratories. Group D antiserum was not ordered from Difco because it was not listed in the Difco catalog. Orders not filled within 2 months were cancelled. The products received were stored at 4°C at the hospitals for this 2-month period and then were shipped to CDC under the same conditions as they would have been by the producer. Forty-three of the 52 reagents thus obtained had 1978 through 1980 expiration dates, with the other 9 expiring in 1977. All were evaluated before their expiration dates. Reagents are identified by group, company, and lot number in Table 1.

Grouping and subgrouping antisera produced by the Biological Products Division, CDC, were used to verify the reactivity and smoothness of test antigens.

Somatic antigens. Antigens used to evaluate the antisera by CDC test methods were produced from freeze-dried cultures of strains that had been originally serotyped in the Enteric Unit, National *Salmonella* Reference Center, CDC. The coded cultures were recent isolates from groups A to H, K, N, O, R, T, 51, and 54, serotyped in the Enteric Unit, CDC. All antigens were dense suspensions of living organisms in 0.85% NaCl as described previously (2). These antigens were used for both CDC and producer test methods because they were specified in the instructions accompanying the commercial antisera purchased directly by CDC in 1975. In addition, Lederle package inserts suggested using boiled organisms suspended in saline,

and Lee literature gave instructions for emulsifying a colony in saline directly on the slide.

Four different inserts accompanied the Difco reagents. The three dated May 1970, October 1974, and June 1975 accompanied reagents purchased directly by CDC and recommended using a dense suspension of the organisms in saline. The April 1976 package insert, which accompanied the reagents purchased through the hospitals, recommended that a loopful of growth be emulsified directly in the antiserum on a slide, and that antigen suspensions be reserved for testing that required boiled organisms. Furthermore, the 1976 Difco brochure stated "They [Difco antisera] have not been tested employing antigen suspension in saline or alcohol-treated cultures."

Each antigen listed in Tables 1 and 2 was made from a different culture and used in serological assays performed the day it was prepared. Since the serogroups of the cultures shown in Table 2 were unknown, criteria for selecting antigen suspensions differed from those for antigens shown in Table 1. Antigens in Table 1 were first tested with various dilutions of CDC homologous grouping or subgrouping antiserum. Those that agglutinated at 3+ or stronger with the routine test dilution of homologous antiserum were further tested for smoothness with saline and groups A, B, C₁, C₂, D, E₁, E₂, E₃, and E₄ heterologous antisera. Those antigens that reacted normally were used. The antigen suspensions of the coded cultures were pre-tested only with saline to simulate the process of serogrouping salmonella isolates in the clinical laboratory.

Flagellar (H) antigens. These antigens were stock Formalinized infusion broths prepared as described in the CDC specifications (2). Somatic antisera purchased directly by CDC were tested for the presence of agglutinins to the H antigens of the immunizing strains used to produce the O antiserum. The immunizing strains were identified for us by each company except BBL, whose antisera were tested with H antigens of serotypes most likely to have been used as immunizing strains. The commercial antisera purchased indirectly through the hospitals were not assayed for H agglutinins because inadequate quantities were available.

Antiserum evaluation procedure. Two persons experienced in evaluating commercially produced *Salmonella* antisera did all the testing. Antisera were tested with all homologous antigens listed in Table 1, and limited heterologous antigens of groups A to 67 were selected on the basis of known antigenic relationships. The coded cultures (Table 2) were used only with those antisera purchased through the hospitals and with CDC antiserum.

The commercial antisera were diluted according to manufacturers' instructions and tested, at this dilution only, for the presence of O agglutinins. The major difference between the CDC testing procedure and those of the producers was the amount of reagents used. Difco and Lederle literature specified using 1 drop (0.05 ml) each of antigen and antiserum. The Lee Laboratories brochure specified that the dropper supplied with the vial be used to dispense 1 drop of antiserum and stressed that the drop of antigen must be of comparable volume. BBL instructions specified

using a small drop of each reagent. Since the 0.05-ml amount of each reagent satisfied the requirements of each company except Lee Laboratories, it was used for all antisera except theirs. A Clay Adams Micro-Selectapette (set at 50 μ l) was used to dispense the 0.05-ml amounts, and the Lee Laboratories droppers, which dispensed about 0.05 ml, were used with their antisera.

Droplets of 0.01 ml of each reagent were used in the CDC method for evaluating all antisera by CDC specifications and in grouping coded cultures.

All reactions were read at 1 min, and Difco antisera reactions were also read at 2 min as specified in their instructions. The remainder of the slide test procedure, reading reactions, and testing for H agglutinins was done as described previously (2), except that larger areas on the slides had to be marked off because of the larger volumes of reagents specified in the commercial procedures.

Performance criteria. CDC performance requirements (see below) were used to judge the acceptability of each reagent evaluated (2).

An acceptable group-specific antiserum must agglutinate at 3+ or greater with homologous antigens within 1 min. It must not react within 1 min with heterologous *Salmonella* O groups. Cross-reactions with antigenically related salmonellae may occur, but must not exceed 1+ agglutination within 1 min. However, group H antisera may agglutinate at higher than 1+ with strains from other groups which contain factor 14, and group E antisera may agglutinate at higher than 1+ with group 54 strains which contain factor 3. Many antigenic relationships are not expressed in the schema. An antiserum for a subgroup of group C must not react with heterologous members of group C. A somatic antiserum must not contain flagellar agglutinins.

RESULTS

Of the 23 reagents ordered by each institution, CDC received 23, the medical complex 20, and the private hospital 9. Two lots of antisera purchased directly by CDC were also obtained through the medical complex. Of the nine lots bought through the private hospital, four from Difco and one from Lederle were from the same lots obtained by the medical complex. The private hospital received no reagents from BBL and Lee Laboratories. Since sets of duplicates reacted comparably, only the results on the duplicate products sold directly to CDC and to the medical complex are shown in Table 1. Three reagents of the 23 (13%) purchased directly from the producer by CDC, 5 of the 20 (25%) purchased through the medical complex, and 4 of the 9 (44%) purchased through the private hospital did not meet CDC specifications. Of 45 different lots tested, 9 (20%) were unacceptable. Fifteen of the 45 (33%) had been submitted to CDC for premarket evaluation. No H agglutinins were detected in the antisera.

Only results obtained with the CDC method are presented in Table 1, because the reactions

[illegible]

^aThe reaction of the antigen with the routine test dilution of CDC homologous grouping or subgrouping antiserum is given.

Antigen controls for the testing of antisera purchased directly by CDC from the producer are given in the first column.

Those for the testing of antisera purchased through hospitals are given in the second column.

^bAntiserum purchased directly from the producer by CDC.

^cAntiserum purchased directly from the producer by CDC.

^dAntiserum purchased through a large private hospital by CDC.

^aDesignated according to the antigenic schema presented by Edwards and Ewing (4).

with the CDC and the producer's method differed only slightly. These minor variations affected the final assessment only of Lederle group C₁ as described below. Reactions with Difco antisera read at 1 min usually remained the same or intensified after 2 min (e.g., a 3+ became a stronger 3+ or a weak 2+ became a routine 2+), an insufficient change to have any effect on the acceptability of the Difco reagents.

Group A. Among the group A antisera (see Table 1), the Lederle product lot 395-267 was of better quality than the others, since it reacted strongly with all homologous strains and had only one weak, but acceptable, heterologous reaction. BBL group A antiserum lot 17GAED cross-reacted weakly with many group B strains, a fact which is acceptable because factors 1 and 12 are common to both groups.

Group B. The 2+ cross-reaction of Difco group B lot 615879 with *S. typhi* did not render the product unacceptable, because it did not cross-react with seven other group D strains. The bulk product from which Lederle group B lot 433-749 was packaged had been previously submitted to CDC for premarket evaluation and did not meet specifications because of heterologous reactions with three of five group C₂ strains. The bulk product had cross-reacted at stronger than the 1+, whereas the purchased packaged product reacted only faintly. Although these two weak heterologous reactions do not render the product unacceptable, stronger cross-reactions might occur with some group C₂ isolates.

Group C. When tested with the CDC method, Lederle antiserum group C₁ lot 418-794 reacted strongly with three of the six homologous strains. In addition, one 3+ reaction with the CDC method was only 2+ with the Lederle method, making the product unacceptable with four of the six homologous strains.

Group D. Lederle group D lot 418-795 antiserum was inadequate because it did not identify the (9),46 strain, a problem which was corrected in subsequent lots submitted voluntarily for evaluation.

Group E. Difco antiserum group E lot 624848 reacted at barely acceptable levels with 3,10 and 3,15 strains and did not identify (3),(15),34 strains. Lederle antiserum group E lot 475-717 reactions with E₃ strains were weaker than desirable, but the product was classified as acceptable because the 3 and 15 factors in these strains are partial antigens. Two lots of group E antisera reacted strongly with a group 54 strain because some of the O54 strains possess factor 3 (8). Because group 54 strains are isolated infrequently relative to group E isolates, it is impractical to require the absorption of factor 3 agglutinins from group E antisera.

Table 2 shows the results obtained when sets of group A to E antisera from each producer were used to identify coded cultures. Two antigens reacted with saline and could not be grouped. The CDC antisera readily identified groups A, B, C₁, D, and E strains but not the C₂ strains. BBL antisera detected groups A, B, C₁, C₂, D, and E strains, but group D antiserum did not distinguish between B and D strains.

No group C₁ and D Difco antisera were available for testing. The Difco set identified A, B, and C₂ cultures, but group A antiserum misidentified six strains of groups B, D, E, G, and R. The Difco C₂ antiserum reacted in a misleading manner with a C₁ strain, and group E antiserum did not detect an E₃ strain.

The variety of lots of antisera received from Lederle allowed two sets of tests to be run with coded samples. The sets identified group B, C₁, D, and E strains, but reacted inconsistently with groups A and C₂.

The Lee Laboratories set of antisera correctly identified all strains.

DISCUSSION

Practical considerations for production and use were the basis for the definitions and the potency and specificity requirements for *Salmonella* reagents in the CDC specifications (2). Antisera for some O antigens may be specific without absorption, whereas antisera for others such as 2 or 19 require extensive absorption, which generally lowers the homologous titer. By separating these, we can require strong homologous reactions for antisera that are usable with limited absorption and permit weaker homologous reactions for the others. This approach provides reliable reagents for the clinical laboratory without imposing unnecessary production requirements.

In grouping cultures, it is unnecessary to use antisera that have been absorbed so that they react with only one antigenic factor in the slide agglutination test. Single-factor antisera are needed in determining serotype rather than serogroup. The immunizing strain for a grouping antiserum should be carefully selected to contain as little as possible of antigens that are common to other groups, to render the resulting antiserum more specific (3). Although cross-reactions may occur, they should not be as strong as the homologous reaction (e.g., an unabsorbed group A antiserum at the routine test dilution reacted 4+ with a 1,2,12 strain, but reacted 1+ with strains of groups D, G, and H and 2+ with an E₄ strain, as shown by Ewing [6]).

The Difco package insert accompanying group A lot 622771 dated April 1977 explained, "A

serum prepared from a 1,2,12 culture, if not absorbed, will react with cultures of serogroups B and D in varying degrees, depending upon the concentration of the commonly shared 1 and 12 factors." This same insert recommended using a single-factor antiserum in serogrouping. The package insert received with Difco group A lot 615979 antiserum, which expired in February 1978, is dated October 1974 and does not have this explanation. It is interesting that the two lots of Difco group B antisera evaluated do not cross-react with strains of groups A and D.

In 1975 the CDC specifications (1) for *Salmonella* antisera were revised as follows: *Salmonella* O subgroups except C₁ and C₂ were deleted from the reagents covered. The proper evaluation of subgrouping antisera depends upon the interaction of the antisera within a set. For example, antiserum for subgroup E₁ (3,10) may have enough factor 3 agglutinins present to react with E₂ (3,15), E₃ [(3),(15),34], and E₄ (1,3,19) strains. This crossing is acceptable if the E₁ product reacts more rapidly and strongly with E₁ strains. If the antiserum has been absorbed so that it reacts only with 3,10 strains, it should be called a single-factor 10 antiserum. This mutual dependency within the subgroup makes it impractical to test subgrouping antisera in the CDC premarket evaluation program.

Groups C₁, C₂, C₃, and C₄ can be treated differently. Edwards and Ewing (4) separated these strains but did not label 8,20 and 6,7,14 strains as C₃ and C₄. Kauffmann (7) denoted 8 and 8,20 strains as group C₃ and 6,7,14 strains as C₄, with the notation that the latter are C₁ lysogenized by phage 14. In the context of producing and using grouping antiserum, it is practical to retain 6,7,14 agglutinins in group C₁ and 8 and 8,20 in group C₂ antisera, thus allowing cultures without factor 6 to be used as immunizing strains for group C₂ antiserum. For groups C₁ and C₂ (if a 6,8 strain is used), immunizing strains that have minimal amounts of the 6₁ factor should be used so that cross-reactions are not apparent in the slide agglutination test (3).

In the 1975 CDC specifications, antisera were no longer required to meet potency specifications with antigens made by heating the organisms in alcohol, centrifuging, and resuspending in saline. If the manufacturer's instructions specify using living organisms only, the product is evaluated only with living antigens. In general, more dilute antisera can be used with living antigens than with alcohol-treated antigens (4). All U.S. commercial producers recommend using living antigens.

Realizing that some antisera are more difficult to produce than others, in the 1972 specifications (1) we had eliminated requirements for mini-

mum homologous O titers so that each product could be diluted appropriately. Many commercial *Salmonella* antisera give neither very strong nor very weak reactions at the routine test dilution. A less diluted product might provide an antiserum of good rather than mediocre quality. However, commercial producers have chosen to restrict themselves, since all recommend a 1:3 dilution for every somatic antiserum, irrespective of the difficulties peculiar to the production of individual reagents.

Three antisera that met CDC specifications failed to identify the coded cultures adequately. They were Lederle group A lot 451-704, CDC group C₂, and Lederle group C₂ lot 431-739. Lederle group C₁ lot 457-758 met specifications in premarket testing but was reported to have "borderline homologous reactions." These discrepant results probably result from form variation of the cultures and mediocre antisera.

Culture variations make evaluation results and clinical laboratory results difficult to interpret. Consider, for example, the homologous results for group A in Tables 1 and 2. Lederle group A lot 466-730 did not meet CDC potency specifications when evaluated, but lot 451-704 did. Yet, when used with antigens selected as they would be in a clinical setting, the lots reacted comparably (Table 2). Although lot 451-704 did meet CDC specifications, Lederle lot 395-267 would be used with more confidence because it allows margin for form variation. The other two lots reacted at levels ranging from barely meeting specifications with one set of antigens to not quite meeting with another.

Failure to identify the Lederle group C₂ antiserum as unacceptable probably stems from failure to recognize CDC group C₂ as unacceptable. The C₂ antigens used in evaluating the antisera were selected by testing them with various dilutions of CDC group C₂ antiserum. Only those that reacted at 3+ with the routine test dilution of the antiserum were used. The coded cultures were not screened in this manner, and only one reacted at an adequate level. To prevent these problems recurring, we made three changes: CDC group C₂ antiserum is no longer being distributed; to meet potency specifications, homologous reactions must be definite rather than marginal 3+ reactions; and routine test dilutions for several reference antisera have been lowered so that evaluation antigens will be more like clinical isolates and not a select population. Even with these changes, a product that causes problems in the clinical laboratory may meet CDC specifications because testing cannot be all-encompassing, and the antigenic composition of isolates cannot be foreseen.

When *Salmonella* O antisera are evaluated,

in addition to form variation, roughness of cultures is an inherent problem that is difficult to handle in a manner equitable to both producer and consumer. Kauffmann (7) noted that rough *Salmonella* cultures do not always react with saline and do not contain the complete O antigen. Obviously, rough cultures should not be used to evaluate antisera, since they may have strong homologous reactions with poor-quality antisera or, conversely, weak reactions with good-quality antisera. Therefore, despite the fact that clinical laboratories use only saline controls in detecting rough cultures, CDC methods include testing the evaluation antigen for roughness with nine antisera (groups A to E₄) before using it. This careful screening of evaluation antigens may prevent detecting rough agglutinins in the antiserum being tested.

In the clinical laboratory the unknown culture is tested with group A to E antisera as in the CDC screening method. Therefore, when the antigen-saline control is negative, the clinical laboratorian must distinguish between reactions caused by expected antigenic relationships and those caused by roughness of the culture, a process which is, at best, educated guessing. According to Kauffmann (7), the only way to determine whether the strain is rough is to immunize a rabbit and test the resulting antiserum for the presence of rough agglutinins, a procedure which would not be performed routinely. Even the CDC method of testing is quite time-consuming and limits the number of cultures processed because each antigen is made, evaluated, and used in 1 day.

It would be helpful for the laboratorian to establish the adequacy of a set of antisera and then use it over a long period so that the idiosyncrasies of the individual antisera are familiar. Unfortunately, expiration dates interfere with this approach. Many *Salmonella* antisera distributed by CDC were made over 15 years ago and still retain their potency. The extension of expiration dates on *Salmonella* antisera should be considered to avoid wasting antisera and the time spent evaluating them.

The part of the study comparing CDC and producer methods was completed in 1976 before the use of antigen suspensions was excluded from Difco recommended methods. In addition to saline suspensions of living organisms, other antigens such as saline suspensions of boiled organisms were recommended in the commercial company brochures, but it was not feasible to use each procedure. Emulsifying a colony in antiserum on a slide may be practical for preliminary work with a polyvalent antiserum, but when testing with six or more grouping antisera, making an antigen suspension is logical. How-

ever, using a drop of antigen suspension instead of only bacterial growth might dilute the antiserum to the extent that homologous reactions are diminished. Not only is the method of emulsifying bacterial growth on a slide tedious, but using different colonies as antigens would have introduced bias into comparing reagents.

Using 0.05 ml of a reagent when less will suffice not only increases the cost of the test but is more hazardous, because of the increased risk of spills when the slide is rocked and the reactions read.

In some laboratories, it would be difficult to do basic *Salmonella* serogrouping because of the difficulty of obtaining satisfactory reagents. The question of the extent of serotyping of salmonellae that should be done in a particular laboratory has been debated for years. According to Edwards and Ewing (4), the personnel of large or small laboratories should be able to identify completely *S. typhi*, *S. cholerae-suis*, and *S. enteritidis* bioaser. Paratyphi A, ser. Paratyphi B, and ser. Typhimurium. For this purpose, they recommended using a basic set of antisera consisting of a polyvalent antiserum, O antisera for groups A to E, H antisera for antigens a, b, c, d, i, and 1 (one) complex, single-factor H antisera for factors 2 and 5, and a Vi antiserum.

As stated previously by Evins et al. (5), it is ludicrous to expect the clinical laboratory to do the type of reagent evaluation presented here, yet the careful checking of test antigens makes the evaluation meaningful. Use of a preevaluated reagent should allay concern over its initial sensitivity and specificity, but does not annul the obligation of the user to control quality.

The most elemental quality control for *Salmonella* antisera for groups A to E requires the maintenance of 14 cultures in the smooth form. The proper use of even 14 strains is an unrealistic requirement in most clinical laboratories since serogrouping of salmonellae is only one task among many.

When purchasing reagents, it is advisable for consumers to request reagents that have been evaluated by CDC and found to meet CDC specifications. Even though the scarcity of the antisera impedes the effectiveness of this policy, the requirement should eventually improve the quality of the reagents that are available. Laboratory Licensure and Food and Drug Administration regulations that recognize realistic extension of expiration dates would help to alleviate the availability problem.

Because of the current emphasis on preventive medicine, and on the epidemiological significance of salmonellosis and the fact that *Salmonella* serotyping has been a practical labora-

tory procedure for over 30 years, it seems reasonable to expect that a basic set of *Salmonella* antisera be easily obtained and consistently reliable.

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