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Method for Identifying *Salmonella* and *Shigella* Directly from the Primary Isolation Plate by Coagglutination of Protein A-Containing Staphylococci Sensitized with Specific Antibody[1]

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A technique is described that allows presumptive identification of either *Salmonella* or *Shigella* organisms directly upon the original isolation plate, in this case, MacConkey agar. This was accomplished by applying a drop of specifically sensitized protein A-containing *Staphylococcus aureus* over a "suspected" colony or several colonies of organisms grown on MacConkey agar. The plate is tilted to and fro to allow mixing of the particles with specific antigen that is readily solubilized from the colony and observing for agglutination of the sensitized particles by use of a dissecting microscope. The agglutination can frequently be seen within 15 s, increasing in intensity over a 2-min period. The polyvalent *Salmonella* antiserum was slower in developing strong agglutination (1.5 to 2 min) compared to particles sensitized with group-specific antisera (15 to 45 s). A high-titer antiserum was important for a test reagent to have the required sensitivity.

The agglutination of specifically sensitized protein A-containing staphylococci by antigens of pneumococci (5), streptococci (1, 4), gonococci (3), meningococci, and *Haemophilus influenzae*, type b (4, 5), has provided new dimensions to diagnostic bacteriology. The reagent particles, in this case, *Staphylococcus aureus* Cowan I strain, were sensitized by the Fc portion of the immunoglobulin G antibody molecule, thereby making the Fab portion free to combine with antigen. *S. aureus* sensitized with specific antibodies were agglutinated by the corresponding antigen only, thus allowing specific identification of bacterial species and types.

The classical method of identifying *Salmonella* and *Shigella* organisms has been by a slide agglutination test and/or biochemical studies when a suspicious colony of bacteria was observed on differential or selective media.

This is to report the direct identification of *Salmonella* and *Shigella* on a primary isolation plate. The method is simple and the reactions occur within 1.5 to 2 min.

MATERIALS AND METHODS

Preparation of protein A-containing staphylococci. The preparation of protein A-containing staphylococci was that described by Kronvall (5) as modified by Edwards and Larson (4). Briefly, the method was as follows: *S. aureus* Cowan I strain (ATC 12598) was grown overnight in Trypticase soy broth (BBL). The bacteria were washed five times with phosphate-buffered saline (PBS; 0.03 M phosphate, 0.12 M NaCl, pH 7.3, with a final concentration of 0.1% sodium azide added as a preservative). The bacteria were then suspended in 0.5% formaldehyde (0.5 ml of 37% formaldehyde added to 36.5 ml of PBS) and allowed to stand at room temperature for 3 h. The treated bacteria were washed three times with PBS and reconstituted to a final concentration of 10% in PBS. This suspension was heated at 80 C over a hot plate with continual stirring with a magnetic stirrer for 1 h. The heat-treated bacteria were then washed three times with PBS, reconstituted to 10% in PBS, and stored at 6 C until used.

Preparation of sensitized staphylococci. One milliliter of the 10% formaldehyde and heat-treated staphylococci was transferred to a test tube (15 by 75 mm). A 0.1-ml amount of specific antiserum was added and thoroughly mixed, and the mixture was allowed to set at room temperature for 3 h, with gentle hand shaking at approximately 0.5-h intervals. The suspension was centrifuged at 5,000 × *g* in a refrigerated centrifuge for 30 min, the supernatant was discarded, and the pellet was reconstituted to a 0.5% suspension with PBS for use in this study. However, either a 1 or 0.5% suspension appeared to give equal sensitivity.

Antisera. The following antisera were used: *Salmonella* polyvalent antiserum, lot no. 439-750 (Lederle), containing antitypes 1-11, 13-16, 19, 22, 23, and Vi; *Salmonella* group C_1, lot no. 392-341 (Lederle); *Salmonella* C_2, lot no. 392-342 (Lederle); *Salmonella* group B, lot no. 392-340 (Lederle), and *Salmonella* group A, lot no. 392-339 (Lederle); *Shigella* group B, lot no. 439-754 (Lederle), and

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Shigella group D, lot no. 439-764 (Lederle); *Shigella* group B and D pool, same lot number as above.

Bacterial strain and clinical samples. To test for specificity of the various sensitized staph reagent used in this study, the following strains of the family *Enterobacteriaceae* were obtained from C. R. Lissner, MSC, USN, Naval Regional Medical Center, San Diego, Calif.: *Salmonella* group A *(S. enteritidis)*, group B *(S. typhimurium)*, group C_1 *(S. cholerae-suis)*, group C_2 *(S. newport)*, and group D *(S. typhi)*, and *Salmonella munich*. *Shigella* serogroups were group B *(S. flexneri)* and D *(S. sonnei)*. *Enterobacteriaceae* serotypes for which antiserum are not generally available were *Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Enterobacter hafniae, Proteus mirabilis, Serratia liquefaciens, Citrobacter freundii, Arizona hinshawii, Klebsiella pneumoniae,* and *Citrobacter diversus*. Clinical samples were kindly made available to us by Charles Davis and Michael Hinesman, University Hospital, University of California Medical School, San Diego. They consisted of an original MacConkey streaked plate and a Selenite F broth transfer onto a MacConkey plate. Both had been incubated for 15 to 24 h at 37 C.

Plating of the stock *Enterobacteriaceae* for identification and specificity of reactions. MacConkey agar plates (BBL) were used throughout this pilot study. Plates were streaked for colony isolation by the conventional streak method. All plates were incubated at 37 C for 18 h before testing.

Identification of the organisms. A paraffin ring as previously described (4) was made around isolated colonies or bacterial growth that developed after stab inoculum. A drop of staph reagent was added within the paraffin ring. The plate was rocked to and fro from 1 to 3 min. Periodic observation was made of the reaction using a dissecting microscope, as agglutination could frequently be detected within 15 s. The aggregates usually became larger as mixing continued up to about 3 min. It must be emphasized that continuous tilting of the plate was important to improve the sensitivity of the test. A control of nonsensitized particles of the same lot of staphylococci dropped onto a suspected colony was used to rule out nonspecific agglutination. In addition, a paraffin ring was made on an area of the plate that was bacteria free, and a drop of sensitized staph reagent was added to insure that the staph reagent did not show spontaneous agglutination.

RESULTS

Typical examples of the coagglutination of sensitized staph reagent when applied to colonies or a stab transfer for either *Salmonella* or *Shigella* organisms are shown in Fig. 1 and 2. Continual tilting of the plate to and fro will result in gross coagglutination that may be detected visually by holding the plate against a dark background with proper lighting. However, the use of a dissecting microscope is recommended because weak reactions may not be detected by direct visualization.

The reactions between sensitized staph reagent and a battery of *Enterobacteriaceae* species and/or serotypes are shown in Table 1. Except for a very weak reaction between the polyvalent *Salmonella* antiserum and *Salmonella* group B antiserum with *A. hinshawii*, there was excellent specificity demonstrated with the commercially prepared antisera without absorption to increase specificity. The cross-reactions between *Salmonella* groups A and B were not unexpected because of the relative broad sharing of antigens between these two serogroups.

Of 16 samples from patients who were cultured for enteric pathogens, 10 were positive on both the original 15- to 24-h MacConkey plate and the Selenite broth transfer plate. Six failed to show any reaction with any of the staph reagent used. These results were confirmed by the University Hospital laboratory findings. Positive reactions were observed in as short a time as 15 s. Some reactions, especially those using the *Salmonella* polyvalent antisera, took from 1.5 to 2 min before definite reactions could be observed. At no time did it require more than 2 min for reactions to occur.

DISCUSSION

Recent advances in immunochemistry have provided new approaches to rapid identification of microbial agents. By using specifically sensitized protein A-containing *S. aureus*, methods and interpretation of reactions for the identification of several microorganisms have been simplified (4, 5). The data in this report extend this technique to the *Salmonella* and *Shigella* genera of *Enterobacteriaceae*. Preliminary results from a limited clinical trial indicate that identification can be made directly on an original (MacConkey) streaked plate or the transfer from an enrichment media, in this case, Selenite F broth. If larger field trials confirm these data, this method would eliminate the need for the technician to transfer the suspected colony to other differential/selective media or carry out other biochemical studies if only *Salmonella* or *Shigella* are of primary interest. Its use in identifying the other *Enterobacteriaceae* will depend upon the availability of potent and specific antiserum. However, for the purpose of rapidly identifying *Salmonella*, the polyvalent antiserum containing agglutinins for the O antigens of serological groups A through E and for Vi antigen should detect approximately 95% of the serotypes of *Salmonella* isolated from man (2). The use made here of the *Salmonella* polyvalent antiserum-sensitized staphylococci appears to have the sensitivity and specificity that allows an early presumptive report to be made to the physician, so appropriate treat-

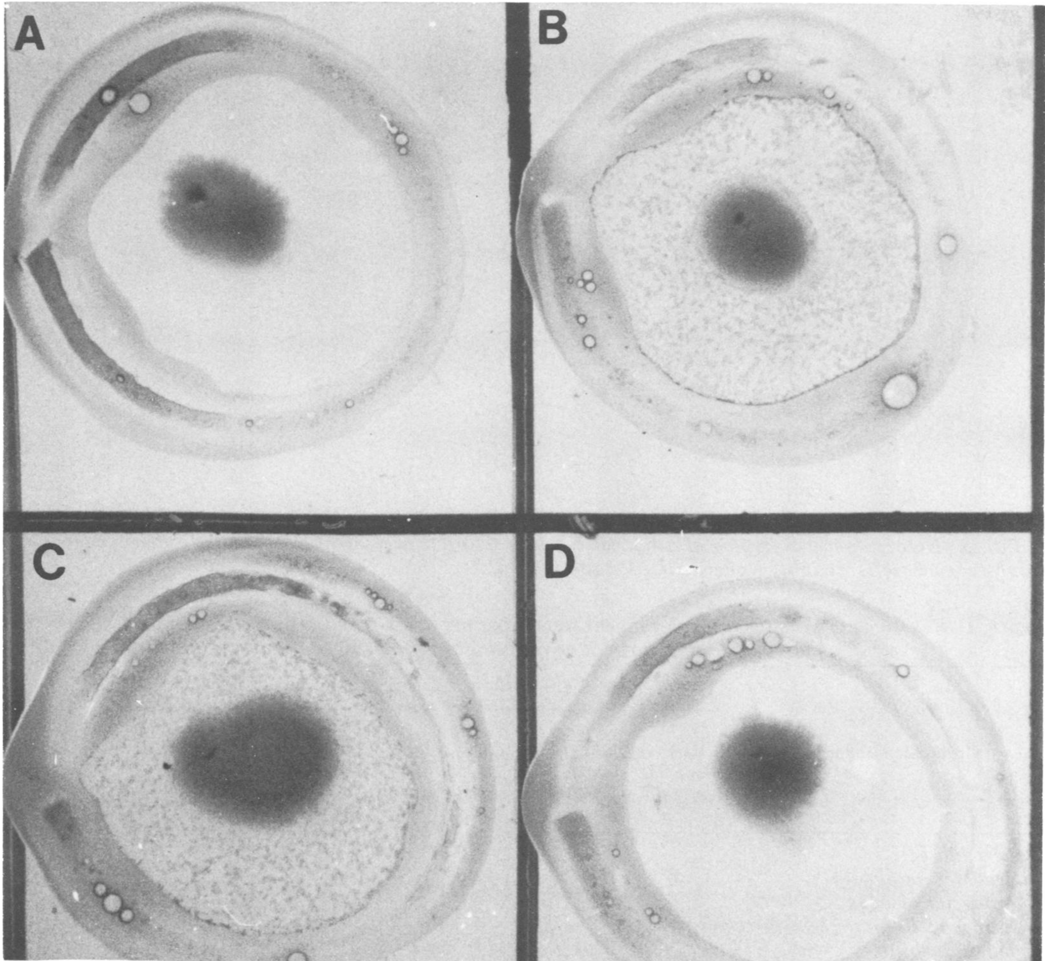

FIG. 1. *Reaction seen when a suspected enteric pathogen is "stab" transferred to a MacConkey plate. Large aggregates start forming from 15 to 30 s after applying the sensitized staph reagent. (A) Salmonella serogroup A, Shigella polygroup D-sensitized staph reagent; (B) Shigella sonnei, Shigella polygroup D-sensitized staph reagent; (C) Salmonella serogroup A, Salmonella polyvalent antisera-sensitized staph reagent; (D) Shigella sonnei, Salmonella polyvalent antisera-sensitized staph reagent.*

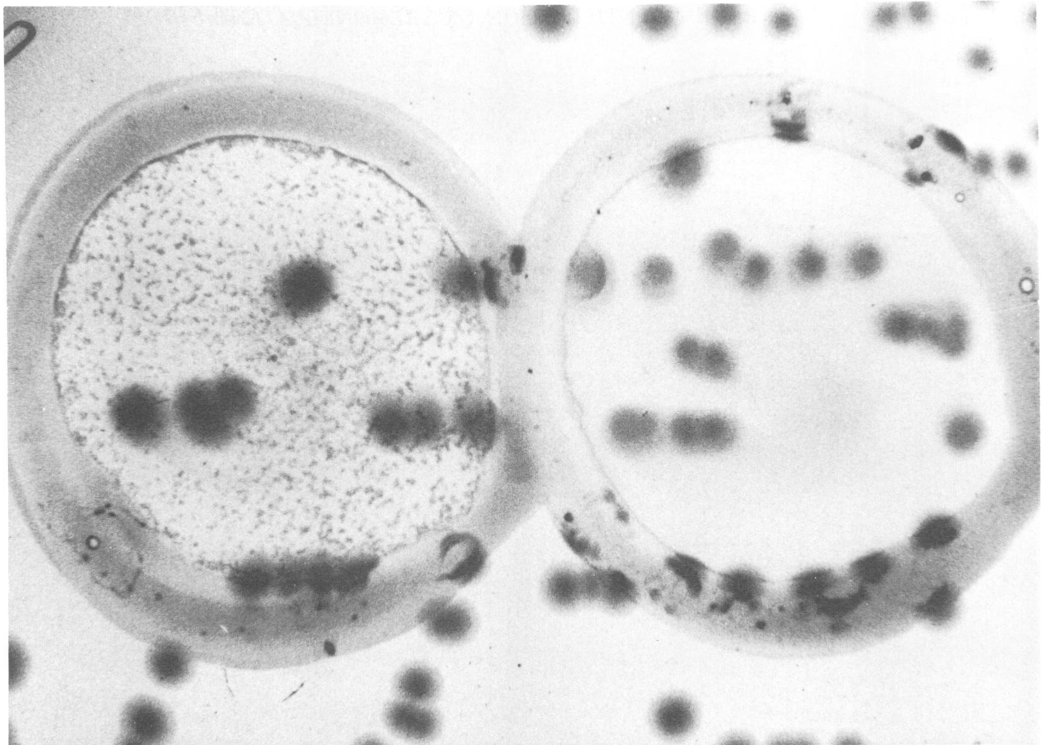

FIG. 2. *Enlargement (S. typhi) of a typical reaction using a polyvalent Salmonella antiserum-sensitized staph reagent (left). (Right) Nonsensitized staph reagent.*

TABLE 1. *Reactions of sensitized staph reagent tested against a battery of different Enterobacteriaceae that may be found in normal or pathological rectal stool cultures*[a]

Organisms under test	*S. aureus*-sensitized reagent (antiserum)[b]							
	Polyvalent *Salmonella*	*Salmonella* group A	*Salmonella* group B	*Salmonella* group C_1	*Salmonella* group C_2	*Shigella* group B	*Shigella* group D	Polyvalent *Shigella* groups B & D
Escherichia coli	−	−	−	−	−	−	−	−
Enterobater aerogenes	−	−	−	−	−	−	−	−
Enterobacter cloacae	−	−	−	−	−	−	−	−
Enterobacter hafniae	−	−	−	−	−	−	−	−
Proteus mirabilis	−	−	−	−	−	−	−	−
Citrobacter freundii	−	−	−	−	−	−	−	−
Klebsiella pneumoniae	−	−	−	−	−	−	−	−
Citrobacter diversus	−	−	−	−	−	−	−	−
Streptococcus liquefaciens	−	−	−	−	−	−	−	−
Arizona hinshawii	±	−	±	−	−	−	−	−
Salmonella munich	2+	±	±	−	4+	−	−	−
Salmonella typhi	3+	−	−	−	−	−	−	−
Salmonella group A	2+	3+	2+	−	−	−	−	−
Salmonella group B	4+	2+	4+	−	−	−	−	−
Salmonella group C_1	3+	−	−	4+	−	−	−	−
Salmonella group C_2	2+	−	−	−		−	−	−
Salmonella cholerae-suis	2+	−	1+	−	4+	−	−	−
Shigella group B	−	−	−	−	−	4+	−	2+
Shigella group D	−	−	−	−	−	−	4+	3+

[a] Reactions were from a single strain with the following exceptions: *Arizona hinshawii,* 2 strains; *Salmonella typhi,* 3 strains; *Salmonella* A, B, C_2, 3 strains each; *Salmonella* C_1, 5 strains; *Shigella* B, 5 strains; *Shigella* D, 4 strains.

[b] Reactivity was rated from 1+ to 4+: few small aggregates (1+) to large coarse aggregates (4+).

ment and epidemiological studies can be instituted immediately.

The area that needs further study is the minimum time required for enough antigen to develop after plating to be identified by this method and whether rectal swabs could be emulsified in a small amount of saline resulting in adequate antigen to give a positive test. It is not inconceivable that secretions and residual fecal material that lodge in the lower large bowel might contain significant amounts of antigen, which would cause aggregation of the sensitized staphylococci. In determining the time required for antigen development of a "stab" transfer of *Salmonella* serogroup B, it was found that adequate antigen was transferred by the "stab" to be immediately identified by the sensitized staphylococci. This suggests that very little antigen is required for identification. Such sensitivity suggests that identification of either *Salmonella* or *Shigella* might be made either directly from rectal swabs or within several hours of plate inoculation.

Further investigations to answer these questions are required.

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