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SALMONELLA SEROTYPING BY THE COAGGLUTINATION METHOD USING PROTEIN A RICH STAPHYLOCOCCUS SENSITIZED WITH SPECIFIC ANTIBODY

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Summary

This paper outlines a more economical method in the serological identification of salmonella by using protein-A rich staphylococci sensitized with ***Salmonella*** polyvalent O and H antisera. Preliminary studies show that this method is simple, economical and as sensitive and specific as the original commercially prepared antisera with the exception of ***Salmonella typhi*** where there appears to be some loss of sensitivity with these strains.

INTRODUCTION

Serological confirmation of an isolate is a common procedure in any bacteriology laboratory. However in this country, antisera are expensive and difficult to obtain because they are not produced locally. Hence there is a need to reduce the cost of the commonly used antisera. In this study, instead of using the commercially prepared ***Salmonella*** polyvalent antisera in the slide agglutination test for ***Salmonella*** serotyping, a coagglutination method is adopted whereby the ***Salmonella*** polyvalent O and H antisera are adsorbed onto protein-A rich staphylococci. In this way less antisera are required for routine screening.

This method has been used successfully in the serological testing of pneumococci(1), streptococci(2), gonococci(3) and dengue virus(4). It was found to be simple, rapid and specific for all the above organisms.

MATERIALS AND METHODS

Reagent particles used are formaldehyde and heat treated Cowan I staphylococci which are subsequently coated with the relevant anti-salmonella antibodies. Specific anti-bacterial antibodies have been shown to bind the protein A on the staphylococci cell wall via the Fc portion of the immunoglobulin molecule(2). Specific antibodies adsorbed in this way will become orientated with their antigen combining Fab portions directed outwards. A suspension of such staphylococci will be agglutinated by the corresponding ***Salmonella*** antigen only.

PREPARATION OF PROTEIN A – CONTAINING STAPHYLOCOCCI

The method described here is essentially the same as that of Edwards and Hilderbrand (5). Briefly, the method is as follows:– ***Staphylococcus aureus*** Cowan I strain (NCTC 8530) is grown overnight in trypticase soya broth (Difco) with aeration. The bacteria are washed four times with phosphate-buffered saline (PBS: 0.03M phosphate, 0.12M NaCl, pH 7.3). Then the bacteria are suspended in 0.5% formaldehyde and allowed to stand at room temperature for 3 hours. The treated bacteria are washed 3 times with PBS and reconstituted to a final concentration of 10% in PBS. This suspension is heated at 80°C in a hot water bath for 1 hr. The heat treated bacteria are then washed 3 times with PBS, reconstituted to 10% in PBS with 1% sodium azide and stored at 6°C until used.

PREPARATION OF SENSITIZED STAPHYLOCOCCI

1 ml of the 10% formaldehyde and heat treated staphylococci is transferred to a test tube. A 0.1 ml amount of antiserum is added and thoroughly mixed. The mixture is allowed to stand at room temperature for 3hr with gentle hand shaking at approximately ½ hr intervals. The suspension is centrifuged at 5000 g in a refrigerated centrifuge for 30 min. The supernatant is discarded, and the pellet is reconstituted to a 1% suspension in PBS with 1% sodium azide. This is then stored at 4–6°C until used.

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ANTISERA The antisera used are ***Salmonella*** polyvalent antisera O (Group A–G) and H (specific and non-specific) obtained from Wellcome Reagents Limited.

BACTERIAL STRAINS AND CLINICAL SAMPLES

24 serotypes of ***Salmonella*** and a few other strains of the family Enterobacteriaceae were used to test for the specificity of the sensitized staphylococcal reagent. This was done by emulsifying the test strain in a loopful of saline on a glass slide and then adding a loopful of the sensitized staphylococcal reagent to it. The slide was rocked to and fro for not more than **2** min and agglutination was observed macroscopically. A control of unsensitized staphylococcal reagent was used to rule out non-specific agglutination between the staphylococci and the test strain.

54 clinical specimens obtained from the routine diagnostic section of the Bacteriology Division, Institute for Medical Research were examined using the sensitized staphylococcal reagent. These were the stool specimens that had been cultured on deoxycholate citrate agar medium giving non-lactose fermenting colonies after overnight incubation at 37°C. These colonies were then picked onto triple sugar iron agar slants and incubated. Those that gave typical reactions of acid butt, alkaline slope with or without hydrogen sulphide and gas were tested by the usual slide agglutination method with commercially obtained ***Salmonella*** polyvalent O and H antisera. The results were then compared with the slide coagglutination test using the sensitized staphylococcal reagent.

RESULTS

The reactions between the sensitized staphylococcal reagent and the **24** serotypes of ***Salmonella*** plus a few other Enterobacteriaceae are shown in Table I. Except for the failure to agglutinate with the O antigen of ***Salmonella typhi,*** the sensitized staphylococcal reagent showed excellent sensitivity and specificity when compared with the original antisera. A further **11** strains of ***Salmonella typhi*** were tested with the sensitized staphylococcal reagent and again similar results were obtained even after boiling the ***Salmonella typhi*** to destroy surface antigen. With the commercial ***Salmonella*** polyvalent O antisera there was rapid agglutination even before boiling. Hence the reason for the failure to agglutinate was not due to the masking of the O antigen of ***Salmonella typhi*** by the Vi antigen but to some other reasons which we have not investigated. The cross reaction with ***Citrobacter freundii*** also occurred with the original antisera which is a recognised phenomenon.

Of the 54 clinical specimens tested, **13** were positive while **41** were negative. These results were comparable with those done with commercial antisera. Out of the **13** positive cases **2** were persistently negative when tested with the ***Salmonella*** polyvalent O sensitized staphylococcal reagent but gave a strong positive reaction with the Salmonella polyvalent H sensitized staphylococcal reagent. Later these **2** specimens were identified as ***Salmonella typhi*** with commercial antisera.

Generally the aggregates in this method were larger but the reaction was slightly slower than the commercial antisera. However all the reactions took less than **2** minutes to become evident(5).

DISCUSSION

The main advantage of this method in our context is the saving of antisera which has not been considered by previous workers. It is noted that only **0.1** ml of antisera is required to prepare **10** rnls of the sensitized staphylococcal reagent. The method of preparation is simple and can be easily carried out in any established laboratory without entailing much extra expenses. In our country, where salmonella infection is common and where food handlers are screened for salmonellosis, it would be cheaper to use this reagent. The original antisera cost about M$40/- per **2** rnls which is sufficient for about **40** slide agglutination tests. Now from the **2** rnls of antisera we can have **200** rnls of the reagent and about **8000** slide agglutination tests can be performed. In Medical Laboratory Technologists Schools the studentscan now use this reagent to practise their slide agglutination technique which was not possible previously due to the high cost of commercially prepared antisera. We are not preparing the more specific ***Salmonella*** antisera used for serotyping as the demand is not as great as the polyvalent O and H antisera.

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REFERENCES

1. Kronvall G. A rapid slide-agglutination method for typing pneumococci ty means of specific antibody adsorbed to protein A-containing staphylococci. J Med Microbiol 1973; 6: 187–90.
2. Christensen P, Kahlmeter G, Jonsson S, Kronvall G. New method for the serological grouping of streptococci with specific antibodies adsorbed to protein A – containing staphylococci. Infect Immun 1973; 7: 881–5.
3. Danielsson D, Kronvall G. Slide agglutination method for the serological identification of Neisseria gonorrhoeae with anti-gonococcal antibodies adsorbed to protein A – containing staphylococci. Appl Microbiol 1974; 27: 368–74.
4. Chan YC, Teoh SH, Aw SE. Staphylococcal agglutination – inhibition reaction: a rapid and simple test for dengue antibodies. Singapore Med J 1975; 16: 194–5.
5. Edwards EA, Hilderbrand RL. Method for identifying Salmonella and Shigella directly from the primary isolation plate by coagglutination of protein A – containing staphylococci sensitized with specific antibody. J Clin Microbiol 1976; 3: 339–43.

TABLE I.

REACTIONS BETWEEN THE SENSITIZED STAPHYLOCOCCAL REAGENT AND THE VARIOUS ORGANISMS

Organisms		CPSO	CPSH	Uncoated
Salmonella paratyphi A	(Gp A)	+++	+++	0
S. typhimurium	(Gp B)	++	+++	0
S. paratyphi B	(Gp B)	++	+++	0
S. stanley	(Gp B)	+++	+++	0
S. heidelberg	(Gp B)	++	+++	0
S. haifa	(Gp B)	+++	++	0
S. derby	(Gp B)	+	+	0
S. oslo	(Gp C_1)	++	+++	0
S. bareilly	(Gp C_1)	+++	+++	0
S. blockley	(Gp C_2)	+++	++	0
S. bovismorbifican	(Gp C_2)	++	+++	0
S. newport	(Gp C_2)			
S. typhi	(Gp D)	0	+++	0
S. enteriditis	(Gp D)	+++	+++	0
S., emek	(Gp D)	++	++	0
S. javaiana	(Gp D)	++	++	0
S. weltevreden	(Gp E_1)	+++	+++	0
S. give	(Gp E_1)	+++	+++	0
S. london	(Gp E_1)	+++	+++	0
S. lexington	(Gp E_1)	+++	+++	0
S. newington	(GP E_2)	+++	++	0
S. senftenberg	(Gp E_4)	+++	+++	0
S. rubislaw	(Gp F)	+++	+++	0
S. havana	(Gp g)	+++	+++	0
Escherichia coli		0	0	0
Klebsiella pneumoniae		0	0	0
Proteus vulgaris		0	0	0
Edwardsiella spp.		0	0	0
Citrobacter freundii		++	+	0
Shigella sonnei		0	0	0
Shigella flexneri		0	0	0

CPSO – Staphylococci coated with Salmonella polyvalent O antisera

CPSH – Staphylococci coated with Salmonella polyvalent H antisera

+++ – rapid agglutination < 1 min

++ – moderately rapid agglutination

\+ – slow agglunation but < 2 min

O – no agglutination