SLIDE COAGGLUTINATION FOR *SALMONELLA TYPHI* ANTIGENS

IN BROTHS INOCULATED WITH FECES FROM TYPHOID FEVER PATIENTS

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SLIDE COAGGLUTINATION FOR *SALMONELLA TYPHI* ANTIGENS IN BROTHS INOCULATED WITH FECES FROM TYPHOID FEVER PATIENTS

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INTRODUCTION

The method of bacterial coagglutination was developed by Kronvall for serotyping *Streptococcus pneumoniae* (1973). Since that time, it has been used to successfully identify *Haemophilus influenzae*, *Neisseria gonorrhoeae, N. meningitidis* and Lancefield groups A, B, D and G streptococci (Danielsson and Kronvall, 1974; Edwards and Larson, 1974; Thirumoorthi and Dajani, 1979). The method can also be used to detect homologous soluble bacterial antigens in body fluids (Suksanong and Dajani, 1977). This coagglutination method has been applied in the detection of *S. typhi* D, Vi and d antigens from the urine of patients with suspected typhoid fever and have been found to be reliable, allowing early presumptive diagnosis of typhoid fever (Lesmana et al., 1980).

The present study was designed to determine if antigens of *S. typhi* could be detected in an enrichment broth before either bacteriologic or serologic confirmation of typhoid fever was available.

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the Infectious Disease Hospital and cultured the same way as the patients.

**Bacteriological isolation:** MacConkey (MAC), deoxycholate citrate lactose sucrose (DCLS) and Salmonella-Shigella (SS) agar plates were inoculated with 1 swab from the Amies medium. The swab was then put into 5 ml MSB. The second swab cultures were incubated 18-24 hours at 37°C and aliquots from the MSB and DSB used to inoculate MAC, DCLS and SS media which were then incubated overnight.

Salmonella-like colonies from the plated media were subcultured to Kligler iron agar (KIA), lysine iron agar, motility indole ornithine medium and urea agar slants. Growth was taken from the KIA, in the 4 tube screen that gave a presumptive *Salmonella* biochemical profile and used for serogrouping to confirm *S. typhi* (Ewing and Martin, 1974).

Blood cultures were subcultured daily to MAC, DCLS and SS and any suspicious Salmonella-like colonies identified with the 4 tube screen and serogrouping. All agar media came from a commercial source (Difco).

**Stabilized Staphylococcus preparation:** The Cowan 1 strain *Staphylococcus* was grown confluently on Mueller-Hinton agar medium for 18 hours at 37°C without added carbon dioxide. Cells were harvested by emulsifying in 5 ml phosphate buffered saline (PBS: 0.03M phosphate, 0.12 M NaCl, pH 7.2) with a glass rod. Washing and heat treatment were performed according to the method of Edwards and Larson (1974). Briefly, the harvest was washed 3 times with PBS suspended in 0.5% formaldehyde in PBS for 3 hours, again washed 3 times with PBS and made up to a final 10% suspension in PBS. The suspension was then heated at 80°C for 1 hour with constant stirring, washed 3 times in PBS and stored at a 10% stabilized suspension in PBS at 5°C until coupled to the antiserum. Staphylococcus-antibody reagent: The method of Kronvall was used to coat the cells (1973). One ml of the 10% *Staphylococcus* preparation was mixed with 0.1 ml each of monovalent *Salmonella* D, Vi and d antisera (BBL). The individual mixtures were left at room temperature for 3 hours and gently agitated at 1/2 hour intervals. Prior to use, 1 ml of the sensitized suspension was diluted with 9 ml PBS. This suspension was used as the final coagglutination reagent and called D-COAG, Vi-COAG and d-COAG.

Coagglutination procedure: Aliquots were withdrawn from the 3 and 5 ml MSB and DSB enrichment cultures 4 and 18 hours after inoculation with the rectal swabs. One drop (50 µl) each was added to 4 ringed areas on a glass slide. Equal volumes of D-, Vi-, and d-COAG were then added to each of the first 3 drops and stabilized *Staphylococcus* only to the fourth drop to serve as a negative control. The drops were mixed with an applicator stick, the slide rotated by hand for a maximum of 5 minutes and the time noted when visible agglutination appeared. The magnitude of the agglutination reaction was also noted and judged as 1+ (weak), 2+ (moderate), 3+ (good) or 4+ (strong).

**RESULTS**

*Salmonella typhi* was isolated from the first blood and/or Amies rectal swab cultures taken on admission from 60 out of 88 patients. The bacterium was also isolated from 60 MSB and 50 DSB media inoculated at the bedside from the same 60 patients.

The D-, Vi-, and d-COAG reactions were optimally positive, giving a 2-3 + reaction in 0.5-1 minute when 5 ml of MSB or DSB broth were used that had been incubated for 18 hours. However, only a 1 + reaction occurred in 2-3 minutes at the early testing period after 4 hour incubation. When the 3 ml volume of
the 2 media was tested, optimum agglutina-
tion occurred following the 4 hour incubation
period and extending the incubation period to
18 hours did not noticeably increase the
intensity of agglutination. All agglutination
reactions with the 3 ml volumes were distinct
giving 2-3 + reactions in 0.5-1 minute. There
were no instances when the 3 COAG reagents
failed to react with the same positive culture.
The negative controls were never equivocal
but in 10 instances testing with the DSB
resulted in a very light granularity following
18 hour incubation. However, there was a
definite difference between the minimal
granularity observed and the true appearance
doagglutination. No such granularity was
apparent at the 4 hour testing period.

Positive coagglutination also occurred with
inoculated MSB and DSB from 16 patients
with negative bacteriological findings. Twelve
of these patients later developed four-fold or
greater titers of Salmonella O antibody. The
remaining 12 patients from whom the bacte-
erium was not isolated and the coagglutina-
tion test was negative also failed to demon-
strate seroconversion. None of the negative
control group gave positive MSB or DSB
doagglutination results.

DISCUSSION

Typhoid fever is endemic in Indonesia and
other developing nations of the world (An-
derson et al., 1976). The lack of appropriate early
treatment may be associated with high morta-
dity due to severe toxemia, gastrointestinal
hemorrhage and/or perforation. Prompt
treatment significantly shortens the febrile
course and reduces mortality (Woodward and
Smadel, 1964; Butler et al., 1977). Since the
introduction of effective chemotherapeutics in
the late 1940's, e.g., chloramphenicol,
mortality from typhoid fever has been signifi-
cantly reduced. However, in developing
countries with substandard nutrition and
poor medical facilities, the mortality in un-
selected hospitalized patients may exceed
15% in spite of the absence of chlorampheni-
col resistance.

Currently, two methods form the basis for
the confirmation of typhoid fever in an indi-
vidual with a compatible clinical syndrome.
Direct bacteriologic confirmation resulting
from the isolation of S. typhi from clinical
sources represents the preferred means. This
method is frequently not available in areas of
high endemicity, however, due to economic
and logistic reasons. The Widal test is de-
pendent upon the demonstration of a four-fold or
greater rise in agglutinins to both O and H
antigens of S. typhi between acute and con-
valvescent specimens. However, the convales-
cent specimen is rarely obtained. Single
specimens obtained during the acute phase of
illness are frequently collected and reliance on
the results of a single Widal test is common
even though the reliability of this method may
be disputed. Certainly, the Widal test, by
itself, can never provide more than a pre-
sumptive diagnosis of typhoid (Woodward
and Smadel, 1964).

The standard approach for hospitalized
patients with suspected typhoid in Indonesia
and elsewhere in Southeast Asia is to obtain
blood and stool cultures and administer
specific therapy without waiting for culture
results. Patients seen in satellite clinics infre-
cantly have cultures performed and are often
treated with inappropriate antibiotics given
for an inadequate duration resulting in in-
creased morbidity and ultimate hospitaliza-
tion. Since the drug of choice for typhoid
fever (chloramphenicol) carries a risk of
potentially serious toxicity, even though it is
rarely observed, the need for a rapid, sensitive,
and specific confirmatory test for typhoid
would be of great assistance to the clinician
faced with an uncertain diagnosis. Similarly
the exclusion of typhoid fever from diagnostic
consideration is equally important since the
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The clinical presentation of other etiologic agents may resemble the appearance of typhoid fever.

The three coagglutination reagents used in this study successfully identified one hundred percent of the MSB and DSB fecal cultures incubated only 4 hours after inoculation from which S. typhi was isolated. In addition, 16 patients with a compatible typhoid fever clinical syndrome also gave positive COAG results when the bacterium was not cultured. Twelve of these later demonstrated seroconversion to Salmonella O antigen. Widal agglutination performed during the acute phase of illness were non-diagnostic from 11 of these 12 patients. Therefore, these patients with typhoid fever were successfully identified by the method of bacterial coagglutination long before they would have been confirmed by other means.

The coagglutination test, as demonstrated in this study, has potential to detect major S. typhi characterizing antigens, D, Vi and d in MSB and DSB enrichment media inoculated with feces from patients with typhoid fever.

SUMMARY

Salmonella typhi antigens D, Vi and d were readily detected, by slide coagglutination, in mannitol selenite (MSB) and dulcitol selenite (DSB), Salmonella enrichment broths 4 hours after inoculation with feces from 60 patients with bacteriologically confirmed typhoid fever. Positive coagglutination also occurred using MSB and DSB inoculated with fecal specimens obtained from 16 patients from whom S. typhi was not cultured. Twelve of these later seroconverted to Salmonella O antigen. None of the MSB or DSB inoculated with feces from 50 healthy control subjects, gave a positive coagglutination test. The coagglutination method appears to have potential as a rapid test for the detection of antigens of S. typhi in MSB and DSB broths inoculated with feces from patients with suspected typhoid fever.

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REFERENCES


# Slide Coagglutination for Salmonella typhi Antigens in Broths Inoculated with Feces from Typhoid Fever Patients

## Abstract

Salmonella typhi antigens D, Vi, and O were readily detected by slide coagglutination, in mannitol selenite (MSB) and dulcitol selenite (DSB), Salmonella enrichment broths 4 hours after inoculation with feces from 60 patients with bacteriologically confirmed typhoid fever. Positive coagglutination also occurred using MSB and DSB inoculated with fecal specimens obtained from 16 patients from whom S. typhi was not cultured. Twelve of these later seroconverted to Salmonella O.

### Key Words
- Coagglutination
- Salmonella typhi
- Antigens
- Typhoid fever

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