Coagglutination and Counter Immunoelectrophoresis in the Rapid Diagnosis of Typhoid Fever

NANDINI P. SHETTY, M.B., B.S., HIRESAVE SRINIVASA, M.D., AND PREMA BHAT, PH.D.

The efficacy of two methods—coagglutination (COAG) and counter immunoelectrophoresis (CIE)—in the rapid diagnosis of typhoid fever was studied in parallel with blood and clot cultures on 114 clinically suspected cases. Retrospective analysis showed that only 58 eventually were discharged and had typhoid fever. Antigen detection on their sera was done by both methods, concomitant with antigen detection on culture supernates by CIE. Sera from 50 controls were subjected to both tests. Agglutinating anti-serum being unsatisfactory in the CIE system, anti-serum to the LPS fraction of Salmonella typhi "O" 901 was used in both tests after absorption with Escherichia coli and Salmonella paratyphi A. Analysis of data with reference to retrospectively confirmed typhoid cases show that S. typhi was isolated in 58.6% and 58.3% of blood and clot cultures; antigen detection by CIE in their supernates was 81.1% and 79.2%, respectively. This correlated closely with serum COAG (81.0%) in contrast to serum CIE (5.7%). Thus, COAG was superior to CIE for serology. However, CIE done on culture supernates precludes such tedious procedures as absorption of staphylococcal agglutinins and the confirmatory blocking test. (Key words: Typhoid fever; Serum coagglutination; Counter immunoelectrophoresis; Lipopolysaccharide) Am J Clin Pathol 1985; 84: 80-84

TYPHOID FEVER is endemic in developing countries, and there is need for a rapid, inexpensive test for accurate diagnosis. So far, the most widely accepted method for conclusive diagnosis in the early stages of the disease is the demonstration of Salmonella typhi by blood culture. This is costly and time consuming. Serologic diagnosis by the Widal test is of limited value. Recently, there have been a few reports using coagglutination (COAG) and counter immunoelectrophoresis (CIE) as rapid tests in typhoid fever. The present study is aimed at the detection of soluble antigen of S. typhi in the serum samples of patients in the acute stage of the disease and in overnight bile broth cultures of whole blood as well as crushed clots, using these two technics, i.e., COAG and CIE. Their relative efficacy also has been evaluated.

Materials and Methods

Clinical Material and Clinical Information

A total of 114 patients suspected to have enteric fever who were admitted to the Departments of Medicine and Paediatrics, St. John's Medical College Hospital, Bangalore, was studied. We also collected blood from 50 voluntary blood donors as normal controls. At the end of the study, it was found that only 55 of the above 114 cases were discharged with a diagnosis of typhoid fever. Information on time of blood collection in relation to onset of fever was also noted in all cases.

Serum Samples for COAG/CIE. Serum was obtained from these 114 patients 8–15 days following onset of fever. Repeat samples could be obtained from 15 patients, five to eight days after the first sample. In addition, serum samples from the 50 normal controls were collected. All the samples were stored at −20 °C until tested.

Blood Cultures. A single blood culture was done on each of the 114 patients, concurrently. Cultures were done in bile broth using standard procedures. When possible, cultures were done prior to antibiotic therapy. Blood cultures were not done on the 15 repeat specimens.

Clot Cultures. Clots from 46 blood samples received for COAG/CIE were saved. They were cultured after they were manually ground by means of a pestle and mortar.

Following isolation of the organisms from the above cultures, S. typhi was identified by standard biochemical and serologic methods.

Culture Supernates for CIE. Supernates of 76 blood cultures and 46 clot cultures were tested for the presence of S. typhi antigen by CIE.

COAG/CIE. For conducting these technics, the following procedures were adopted:

Preparation of Lipopolysaccharide Antigen from S. typhi "O". The lipopolysaccharide (LPS) antigen of S.
**typhi** “O” was prepared according to the method described for other enterobacteria, using the reference strain 901 (National Salmonella Reference Centre, Kasauli).

Preparation of *S. typhi* “O” and “H” Anti-Sera. Two types of “O” anti-sera were raised in rabbits. One was immunized with LPS extract of *S. typhi* “O,” and the other with whole bacterial cell (WBC) suspension of the same. The anti-sera were absorbed with *Salmonella paratyphi A* and *Escherichia coli*.

On a CIE system, the absorbed LPS anti-serum was titrated and the working dilution for CIE determined. Similarly, the lowest concentration of the LPS antigen detectable by this anti-serum also was determined.

With the use of the standard tube agglutination test (STS), the titer of WBC “O” anti-serum was estimated. Likewise, “H” anti-serum was prepared in a third rabbit against reference strain of *S. typhi* “H” 901 obtained from the same center. To rule out cross-reactions in both test systems, the reagents were tested against other gram-negative bacilli like *E. coli*, *S. paratyphi A*, and *Pseudomonas aeruginosa*.

### Method for CIE

The method for CIE was essentially that described by Tsang and Chau, with certain modifications. Wells on a slide were filled to the brim either with patient's serum, control serum, or the supernatant of overnight broth cultures on the cathodal side. The LPS anti-serum was placed on the anodal side. Electrophoresis was done for one hour. Slides were examined for precipitation lines immediately and an hour later. They were left overnight at room temperature and reexamined. When anti-serum to WBC was used as neat or diluted, no lines of precipitation were noted in either test or control samples.

### Procedure for COAG

The Cowan I strain of *Staphylococcus aureus* was used. Preparation of stabilized cells and their sensitization were as described by Rajagopalan and John, with minor modifications. The cells were grown in Todd-Hewitt broth with the use of a shaker waterbath (100 rpm) for 18 hours at 37 °C. The three types of anti-sera mentioned earlier were used to sensitize the stabilized staphylococcal cells.

The test was performed as follows:

Each serum was tested neat and also in 1/10 and 1/100 dilutions to exclude the prozone phenomenon.

---

*Received from the Department of Microbiology, Christian Medical College Hospital, Vellore, India.*

---

<table>
<thead>
<tr>
<th>Organism</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. typhi</em></td>
<td>34</td>
<td>29.8</td>
</tr>
<tr>
<td><em>S. paratyphi A</em></td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Klebsiella-enterobacter</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>3</td>
<td>2.6</td>
</tr>
<tr>
<td>Negative for facultative anaerobes</td>
<td>73</td>
<td>64.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>114</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Care was taken to absorb staphylococcal agglutinins wherever indicated. Initially the tests were done in parallel with the use of cells sensitized with both types of “O” anti-sera. Since the results were comparable, subsequent tests were done with cells sensitized to the LPS anti-serum. To detect *S. typhi* “H” antigen, randomly selected serum samples of culture-positive and culture-negative patients were tested with “H” anti-serum sensitized cells. Confirmatory blocking tests were done on a panel of 25 *S. typhi*-antigen-positive sera, 12 from culture-positive and 13 from culture-negative patients. The serum samples from 50 controls were also screened. The test, thus, took approximately 2.5 hours with the steps of absorption of staphylococcal agglutinins and blocking for completion.

### Results

Results of conventional blood cultures done on 114 cases are shown in Table 1. *S. typhi* could be isolated in 34 cultures. Of the 73 that were negative on culture, only 11 were clinically diagnosed as typhoid fever. Likewise, of the 46 clot cultures done, 14 alone were positive for *S. typhi*, and, of the 26 clot cultures that were negative, only 4 were clinical cases of typhoid.

In Table 2 described below, the specimens are grouped

---

<table>
<thead>
<tr>
<th>Group*</th>
<th>No.</th>
<th>I (late)</th>
<th>II (early)</th>
<th>III (or more)</th>
<th>Clinical Diagnosis of Typhoid Fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>34</td>
<td>16</td>
<td>16</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>B†</td>
<td>13</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>1</td>
<td>7</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>D‡</td>
<td>56</td>
<td>9</td>
<td>28</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>114</strong></td>
<td><strong>31</strong></td>
<td><strong>54</strong></td>
<td><strong>25</strong></td>
<td><strong>55</strong></td>
</tr>
</tbody>
</table>

* A, culture-positive and antigen-positive cases; B, culture-negative but antigen-positive cases; C, culture and antigen negative; clinical cases of typhoid; D, culture and antigen negative; nontyphoid cases.
† Three cases discharged as PUO.
‡ Four cases time not mentioned.
Table 3. Detection of Circulating Antigen of *S. typhi* by Serum COAG and CIE in Three Different Groups of Patients

<table>
<thead>
<tr>
<th>Group*</th>
<th>Sera Tested</th>
<th>Serum COAG No.</th>
<th>Serum CIE No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>34</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>47 (81.0%)</td>
<td>3 (5.7%)</td>
</tr>
</tbody>
</table>

* A. culture-positive and antigen-positive cases; B. culture-negative but antigen positive cases; C. culture and antigen negative: clinical cases of typhoid.

Table 4. Comparison of *S. typhi* Antigen Detection by CIE on Blood Culture Supernates and Serum COAG Performed on 37 Clinical Cases of Typhoid

<table>
<thead>
<tr>
<th>Group*</th>
<th>Sera Tested</th>
<th>Broth CIE No.</th>
<th>Serum COAG No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>22</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>30 (81.1%)</td>
<td>32 (86.5%)</td>
</tr>
</tbody>
</table>

* A. culture-positive and antigen-positive cases; B. culture-negative but antigen-positive cases; C. culture and antigen negative: clinical cases of typhoid.

Findings on antigen detection have been tabulated in Tables 3, 4, and 5, and percentages have been calculated with respect to Groups A, B, and C only; D being the nontyphoid group has not been included. Table 3 compares the rates of detection of circulating antigen of *S. typhi* in the patient's serum by serum COAG and serum CIE. Antigen detection was possible in 47 out of 58 clinical cases (81%) by serum COAG and 3 out of 58 (5.7%) by serum CIE. Serum COAG and CIE were done on 15 repeat samples. Of the eight that were initially positive by COAG, six continued to be positive, whereas all were negative by serum CIE.

Presence of staphylococcal agglutinins was noted in 86% of all the serum samples tested and such samples needed to be absorbed. Results of the blocking test done on the panel of sera drawn from Groups A and B were negative, confirming the presence of *S. typhi* antigen. The findings on the 50 control sera tested revealed the presence of false-positive reaction in two of them.

The randomly selected ten culture-positive and ten culture-negative sera tested for circulating "H" antigen were also negative.

Information on antigen detection by CIE in supernates of the overnight broth cultures of blood and clots is presented in Tables 4 and 5, respectively. From Table 4 it is seen that antigen detection was possible in 30 of 37 (81.1%) clinically diagnosed cases. So, also among the clot broths subjected to CIE 19 of 24 (79.2%) had detectable antigen in them. Results have been compared with the corresponding serum COAG findings. A close correlation between antigen positivity by CIE in culture supernates and serum COAG was found.

Assessment of the relative rapidity of diagnosis by antigen detection/culture shows that conventional cultures take between three to seven days before positive.
results are seen. CIE on culture supernates, however, can be done after overnight incubation of the broth, and the test time for serum COAG is only 2.5-3 hours.

In summary, 58 cases out of a total of 114 studied included 55 patients who were discharged with a diagnosis of typhoid fever and 3 cases of PUO positive for circulating antigen. *S. typhi* could be isolated in 58.6% and 58.3% of these cases by conventional blood and clot cultures, respectively. Antigen detection in their supernates was 81.1% and 79.2%, respectively. Circulating antigen of *S. typhi* could be detected in 81% of the sera tested by COAG and only 5.7% by CIE.

Characterization of the Anti-Sera Used

Following absorption with *S. paratyphi A* and *E. coli*, the LPS anti-serum used in CIE/COAG showed a reciprocal titer of 64 on a CIE system. The lowest concentration of antigen detectable using this anti-serum in CIE was at a dilution of 1/32. The reciprocal titers of the *S. typhi* "O" and "H" absorbed anti-sera were 320 and 5,120, respectively, by STS.

Discussion

Although demonstration of *S. typhi* in blood culture is considered the most conclusive test in the diagnosis of acute-phase of typhoid fever, there are several factors that limit the recovery of the organism in culture.16

We have attempted to show that both serum COAG and CIE on supernates are far superior to conventional cultures in both rapidity and accuracy of diagnosis. Besides, these technics were analyzed in reference to the total number of cases finally diagnosed as typhoid fever by the clinician. In this context, when conventional methods could confirm only 58.6% of cases, antigen detection made it possible to diagnose an average of 80.4% cases.

The COAG test with its preliminary steps to remove staphylococcal agglutinins and the confirmatory blocking test is completed within two to three hours. Earlier workers have used COAG to detect circulating antigen in culture proven cases only.12 In our study, all the culture-proven cases, either from blood/clot, were positive by serum COAG. In addition, we have shown that 13 out of 58 clinically diagnosed cases (22.4%) missed on culture could be diagnosed by serum COAG. The blocking test done on randomly selected sera from culture-positive (Group A) and all culture-negative (Group B) cases confirmed the presence of *S. typhi* antigen. False positives were also eliminated by the above test. Results of the 15 paired serum samples by COAG test showed that antigen could be detected up to five days after the first sample.

No circulating "H" antigen could be detected. It would thus seem that the circulating antigen detected was a somatic component like LPS.

Even though the LPS and WBC anti-sera were found to be equally effective in serum COAG, we opted to use LPS anti-serum as it was found useful in the CIE system also, unlike WBC (agglutinating) anti-serum. Hence, antigen detection by CIE in our study required specific anti-sera with satisfactory precipitin titers. In the rapid detection of soluble bacterial antigen in body fluids by CIE, Jones7 also has recommended the use of such anti-sera rather than the agglutinating anti-sera.

Detection of *S. typhi* antigen in patient's serum with the use of CIE has been attempted by several workers.5,13 However, in our study, CIE for antigen detection in patient's serum was not useful. Nevertheless, CIE performed on overnight blood/clot culture supernates yielded results comparable to serum COAG (Tables 4 and 5). Our failure with serum CIE was probably due to antigen concentrations being less than 1/32 in the test serum samples. An increase in antigen concentration, as in culture supernates, facilitated precipitation making this an equally sensitive test.

In Group B (Table 4), nine broth cultures, although negative for *S. typhi*, showed the presence of *S. typhi* antigen by CIE. COAG was positive on the sera of all these cases. It is tempting to speculate that the organism, although present, was missed on culture, but the antigen in the culture supernate was of a concentration adequate for visible precipitation by CIE. Had there been a prolonged attempt to recover the organism by repeated subculture, this probably could have yielded positive results. This incongruity between the presence of antigen in broth cultures and absence of viable organisms has been reported by Sanborn and associates11 while attempting recovery of *Salmonella oranienberg* from enrichment cultures of stool.

Our results show that the rate of recovery of *S. typhi* from clot cultures is almost identical to blood culture. This is of advantage in situations where it is not always possible to get adequate amounts of blood for culture, as in children. If the clots could be saved and cultured, they would provide valuable material for both recovery of the organism as well as detection of antigen.9

In conclusion, we have shown that optimally sensitized Protein A containing staphylococcal cells were more sensitive than CIE for detecting circulating antigen. However, CIE done on overnight culture supernates was almost as sensitive as serum COAG and precluded such tedious procedures as absorption of staphylococcal agglutinins and serum dilutions. Both technics showed no cross-reaction with *S. paratyphi A* or other gram-negative bacilli, thereby demonstrating their specificity. Thus,
COAG and CIE qualify as being comparatively more rapid and economical for diagnosing typhoid fever, besides being more sensitive than conventional cultures.

Acknowledgments. The authors thank the Departments of Medicine and Paediatrics for their help in the collection of clinical samples for this study. They also gratefully acknowledge Dr. G. M. Mascarenhas, Dean, St. John’s Medical College, for his encouragement and the staff of the Department of Microbiology. They thank L. Menon and J. Kattaiah for their secretarial assistance.

References