

## Short Communication

### **Antigen detection in the serum by counter-immunoelectrophoresis for an early diagnosis of typhoid fever**

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#### **Abstract**

Twenty bacteriologically confirmed cases of typhoid fever and 23 normal healthy persons were included in this study. The convalescent sera were collected one week after the first sample. In all, 13 paired sera, 7 acute phase sera and 23 normal control samples were tested for *Salmonella typhi* antigen by counter-immunoelectrophoresis (CIE). The circulating antigen was detected in 18 of the 20 cases during acute phase. None of the convalescent sera and normal control samples showed *S. typhi* antigen. Thus, detection of antigen by CIE offers a reliable method in the early diagnosis of typhoid fever even before the appearance of antibodies in the blood at a diagnostic titre.

**Key words:** *Salmonella typhi*, antigen, counter-immunoelectrophoresis (CIE), typhoid fever, early diagnosis.

#### **1. Introduction**

Typhoid fever is still rampant in our country and accounts for a considerable number of febrile cases<sup>1,2</sup>. The diagnosis is confirmed by the isolation of the causative micro-organism, *Salmonella typhi* from the clinical samples. Since the bacteriological facilities in many of the hospitals are limited, the diagnosis is made by correlating the clinical symptoms with the significant antibody titres<sup>3</sup>. During early stages (acute phase), antibodies are not present at a significant titre and thus serological methods have a limited diagnostic value<sup>4-8</sup>. However, *S. typhi* antigen is detectable in the serum by CIE<sup>9</sup> and this may prove a useful immuno-diagnostic method for an early and rapid diagnosis of typhoid fever during acute phase.

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## 2. Materials and methods

### 2.1. Subjects

Serum samples were collected from 20 typhoid patients between 2 and 5 days after the onset of fever. In all the cases, the diagnosis was confirmed by the isolation of *S. typhi* from blood<sup>10</sup>. The convalescent sera were collected one week after the first sample from 13 of the 20 cases. Sera from 23 normal individuals served as controls. None of the subjects had been vaccinated in the preceding six months.

### 2.2. *S. typhi* antiserum

High-titre antiserum to *S. typhi* (0901) was raised in rabbits according to the method described by Cruickshank<sup>11</sup>. It was absorbed with *S. paratyphi* and *Esch. coli*. The working antiserum dilution for CIE test was determined by titration against standardized centrifuged ultrasonic lysate of *S. typhi*<sup>9</sup>. The antigenic lysate was previously standardized against *S. typhi* antiserum.\*

### 2.3. CIE test

The test was performed according to the technique described earlier<sup>10</sup>. Two rows of wells (3 mm size) were punched in the agar-gel and were charged by keeping the serum samples at cathode and *S. typhi* antiserum at anode. A constant current at a rate of 5-6 mA/cm across the slide was applied for 45 min and samples showing characteristic precipitin lines against *S. typhi* antiserum were interpreted as positives (Fig. 1). No change in the precipitin line was recorded after keeping the slide overnight at 4° C or after drying and staining with amidoblack. Positive and negative serum controls were run simultaneously in each test. Normal rabbit serum was also included to rule out the false positive reaction.



Fig 1. Exhibiting the precipitin lines between serum samples (s) and rabbit anti-*S. typhi* serum (As). (1) Positive serum control, (2) Negative serum control, (3) *S. typhi* antigen lysate, (4) Normal rabbit serum.

### 2.4. Widal test

The test was performed as described previously<sup>9</sup> using H and O antigens prepared from standard reference strain (H 901 and O 901)\*, of *S. typhi*. Positive and negative serum controls were included in each test. Significant rise in agglutinin titres to H and O antigens was considered positive. In acute sera, a titre of 1:320 or above was taken as significant<sup>9,12</sup>.

\* obtained from CRI, Kasauli.

Table I

## Comparison of antigen detection by CIE with Widal test

Sl. No.	Subjects	No. of cases	Day of sample collection	CIE <sup>+</sup> (Antigen)	Widal <sup>+</sup> (Antibody)
1.	Patients with typhoid fever:				
	(a) Acute phase	20	2nd-5th day	18	1*
	(b) Convalescent	13	9th-12th day	0	13
2.	Normal controls	23	..	0	0

\* Sample collection was made on the 5th day after the onset of fever and was negative for antigen.  
 † Positive.

### 3. Results

The results are shown in Table I. Eighteen of the 20 acute phase samples showed the presence of antigen in the sera. These serum samples were collected between 2nd and 4th day after the onset of fever. Of the remaining 2 cases, which are negative, the samples had been obtained on 2nd and 5th day respectively after the onset of fever. None of the 13 convalescent serum samples and 23 normal controls showed antigen. In contrast, only 1 of 20 acute phase samples showed antibodies at a diagnostic titre (1:320 or above) by Widal test. However in all the 13 convalescent serum samples significant rise in the titre was recorded (4-fold).

### 4. Discussion

The reliable methods exist for confirmation of typhoid fever in a patient with a compatible clinical picture: (1) isolation of *S. typhi* from the clinical materials<sup>10</sup> or (2) demonstration of a 4-fold or greater rise in antibodies to both H and O antigens of *S. typhi* by Widal test<sup>13</sup>. Culture methods take at least 48 hours or more before the diagnosis is confirmed and the demonstration of rising titre in antibodies in paired sera will be possible after the second sample has been obtained during the second week after the onset of fever (one week after the first sample). For an early diagnosis in an endemic area during acute phase, a single serum sample (Widal test) has a little, if any, diagnostic value<sup>8,9</sup>. Thus a simple, rapid and reliable test is required for an early diagnosis of typhoid fever during acute phase.

CIE has been used for detection of H. influenzae type B<sup>14</sup>; pneumococcal<sup>15</sup>, meningococcal<sup>16</sup>, gram-negative bacterial (*E. coli*, *Enterobacter* and *Protens*)<sup>17</sup> and Australia<sup>18</sup> antigens for an early and rapid diagnosis. In this study, 18 of the 20 typhoid cases have been detected by CIE, whereas with Widal test only one of the 20 cases could be confirmed during acute phase (2-5 days after the onset of fever). Moreover, none

of the 7 cases was confirmed by Widal test from which second sample (convalescent serum) could not be obtained.

In conclusion, CIE in detection of circulating *S. typhi* antigen is a simple, rapid and reliable test for the early diagnosis of typhoid fever during acute phase thus saving considerable time.

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