Clinical Application of a Dot Blot Test for Diagnosis of Enteric Fever Due to *Salmonella enterica* Serovar Typhi in Patients with Typhoid Fever from Colombia and Peru

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Clinical application of a dot blot test to detect immunoglobulin G (IgG) (88% sensitivity and specificity) and IgM (12.1% sensitivity and 97% specificity) against flagellar antigen from *Salmonella enterica* serovar Typhi was performed in Peruvian and Colombian patients with typhoid fever. This test can be used as a good predictor of serovar Typhi infection in regions lacking laboratory facilities and in field studies.

One hundred and two volunteers older than 15 years with typhoid fever confirmed by bacteriological isolation of *Salmonella enterica* serovar Typhi from blood culture were included in this study. They were treated at the Instituto Colombiano de Medicina Tropical (ICMT) (Medellín and Apartadó, Colombia) or Hospital Cayetano Heredia (Lima, Perú) in 1997 and 1998. When typhoid fever was suspected, blood samples were collected for triplicate cultures (7, 8, 11, 17), and one serum sample was collected for dot blot analysis. Once diagnosis of typhoid fever was confirmed by bacteriological isolation, patients were included in the study and were dot blot tested on days 1, 7, and 15.

Serovar Typhi 1991-ICMT (isolated from the blood of a patient at the ICMT) was used to obtain the flagellar antigen. Antigen isolation and purification was carried out following procedures previously described (10). Briefly, a colony obtained from a 37°C overnight culture in nutritive agar was subcultured into 400 ml of brain heart infusion broth (Becton Dickinson BBL) at 37°C for 16 h. Bacteria were centrifuged at 300 × g for 30 min, the pellet was resuspended in 150 ml of saline, and pH was adjusted to 2. The suspension was centrifuged at 3,000 × g for 30 min, the supernatant was recovered, and the insoluble material was isolated by centrifugation at 40,000 × g for 1 h at 4°C. Two volumes of ammonium sulfate (2.67 M) were added to the recovered supernatant (pH 7.2), and the solution was stored at 4°C overnight. Polymerized flagellar protein was then recovered from the pellet after 15 min of centrifugation at 15,000 × g at 4°C, was dissolved in 5 ml of distilled water, and was dialyzed with a 50,000-pm-pore-size filter into distilled water for 18 h at room temperature and then for 18 h at 4°C into 500 ml of distilled water containing 20 g of activated carbon. Purified antigen was stored at −70°C. Antigen protein concentration was 0.7 mg/ml as determined by the Lowry method (14). Antigen purity was checked in a 10% polyacrylamide gel, and the purified flagellar protein mass was determined to be 45 to 66 kDa (10). Dot blot analysis was carried out by using nitrocellulose paper (1, 5; Bio Dot microfiltration apparatus instruction manual, Bio-Rad Laboratories, Richmond, Calif.). The paper was first soaked with Tris-buffered saline (TBS), then the membrane was placed into a Bio Dot microfiltration apparatus (Bio-Rad Laboratories). The membrane was rehydrated by using 100 μl of phosphate-buffered saline per well, which was later vacuum extracted. This was followed by the fixation of 100 μl of flagellar antigen to each well (14; Bio Dot instruction manual, Bio-Rad). Blocking solution, fetal calf serum, and 1% TBS were added (200 μl per well) and left to gravity filter. The membrane was vacuum washed with a solution of Tween and TBS. Serum samples were added in 1:50, 1:100, and 1:200 dilutions and were left to filter by gravity.

Each membrane had six serum blank wells in order to detect any unspecific color and positive and negative controls in 1:50, 1:100, and 1:200 dilutions (1, 14). After Tween-TBS washing, 100 μl of goat anti-human immunoglobulin G (IgG) or IgM (Jackson Immuno Research Laboratories) was added and gravity filtered. Finally, 4-chloronaphthol (Sigma Chemicals Co.) was added for 20 min to develop the reaction. The membrane was washed with distilled water to stop the reaction. The membrane was allowed to dry prior to the final color visualization reading (1, 14; Bio Dot instruction manual, Bio-Rad).

A total of 102 patients with typhoid fever were studied: 48 Colombians and 54 Peruvians. Difficulties arose in monitoring outpatients who had been released 7 days before the second control was scheduled. Table 1 summarizes the number of patient samples studied.

Percentages of patients with typhoid fever positive for IgG and IgM against the flagellar antigen of serovar Typhi on the day of evaluation are shown in Table 2.

On the first day of evaluation, 69 patients were tested: all of them were IgM positive and 66 had titers for IgG. Twelve patients had three serum controls (days 1, 7, and 15): 11 patients were IgM positive at the three time points tested and one patient was positive only in one. Seven patients were positive for IgG in all the periods, and three were positive in at least one. Fourteen patients were evaluated on the 1st and 7th days, and all of them were positive for IgM at both time points and 10 were IgG positive at both time points. Eight patients were evaluated on the 15th day only: seven were IgM positive and six were IgG positive.

The ideal test for an early diagnosis of typhoid fever must be sensitive, specific, rapid, simple, and inexpensive. However, conventional laboratory tests based on cultures of serovar
The development of a diagnostic test, a dot blot to detect IgG and IgM against flagellar antigen of Serovar Typhi, with a sensitivity and specificity of 88% for IgG detection and a sensitivity of 12.1% and 97% of specificity to detect IgM (1) was obtained. The dot blot test can be a diagnostic alternative in detecting typhoid fever, is an easy-to-read visual test, does not require complex laboratory facilities or training, and would be useful in rural areas where microbiology laboratory resources are difficult to obtain or are unavailable (1, 6, 16). This test can be used for the detection of IgG and IgM antibodies against serovar Typhi flagellar antigen at any of the evaluated time points during the course of typhoid fever (days 1, 7, and 15).

Serology and longevity of antibody response to several antigens from serovar Typhi had been evaluated (3, 4, 12, 13, 15). Choo et al. (4) found that the average persistence was 2.6 months, and the IgG persisted, on average, for 5.4 months. These results indicated the presence of IgG and IgM from the 1st to the 15th day of the study.

The dot blot test to detect IgG and/or IgM against flagellar antigen of Serovar Typhi can be used in laboratories in rural areas of developing countries where typhoid fever is endemic, as these regions lack the necessary facilities for isolation of bacteria. This test can also be used in field research.

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**TABLE 1.** Number of samples tested from typhoid fever patients

| Country of sample origin | No. of samples tested on: | Total |
|--------------------------|--------------------------|-------|
|                          | Day 1 | Days 1 and 7 | Days 1, 7, and 15 | Day 15 |
| Colombia                 | 38    | 6            | 4                | 48     |
| Peru                     | 31    | 7            | 8                | 54     |
| Total                    | 69    | 13           | 12               | 102    |

**TABLE 2.** Number of patients with positive antibody titers (range, 1:100 to 1:200) at three periods of evaluation

| No. of patients testing positive on* | Day 1 | Days 1 and 7 | Days 1, 7, and 15 | Day 15 |
|------------------------------------|-------|--------------|-------------------|-------|
| IgG                                | 66/69 | 10/14        | 10/12             | 6/8   |
| IgM                                | 69/69 | 14/14        | 12/12             | 7/8   |

* Given as number testing positive per total number tested.