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Diagnosis of Typhoid Fever by Detection of *Salmonella typhi* Antigen in Urine

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A monoclonal antibody specific for group D *Salmonella* antigen 9 was used in an indirect enzyme-linked immunosorbent assay (ELISA) for detecting the antigen in urine specimens collected from patients with clinical typhoid fever in Jakarta, Indonesia. The ELISA had a sensitivity of 95% in identifying patients in whom *Salmonella typhi* was isolated from hemocultures, 73% in patients in whom *S. typhi* was isolated from stool specimens, and 40% in patients in whom the organism was isolated from bone marrow cultures. Among patients in whom *S. typhi* was isolated from blood cultures, the ELISA had a sensitivity of 65% when a single urine specimen was examined and 95% when serially collected urine specimens were examined. A dot blot immunoassay performed on a nitrocellulose filter in parallel had a sensitivity of 85%, versus 83% for the plate ELISA in which *S. typhi* was isolated from blood, bone marrow, and/or stool specimens. Since *S. typhi* antigen is intermittently excreted in the urine of patients with typhoid fever, serially collected urine from patients with typhoid should be tested for antigen 9.

A rapid, specific, and sensitive method is needed for the diagnosis of typhoid fever. Current strategies are based on detection of *Salmonella typhi* antigen(s) (12) or DNA in clinical specimens (9). DNA hybridization assays are, however, not suited for use in a clinical laboratory in a developing country where typhoid occurs sporadically. Serological assays to detect *S. typhi* antigens have been hampered by the lack of polyclonal antibody specificity (1–3, 7, 8, 10, 11, 13). Recently monoclonal antibodies directed against specific bacterial antigens have been developed (7). In 1988, we reported the use of a monoclonal antibody to detect antigen 9 of *S. typhi* in urine specimens collected from patients with typhoid fever. The test was shown to have 100% specificity and 65% sensitivity (4). In this study the sensitivity of the enzyme-linked immunosorbent assay (ELISA) to identify patients with typhoid fever by testing urine samples collected serially over time was evaluated. A more practical version of ELISA, a dot blot immunoassay performed on a nitrocellulose (NC) membrane, was also evaluated in parallel. The sensitivity and specificity of the two assays were compared.

Lipopolysacharide (LPS) was prepared from acetone-dried *S. typhi* 0901 by the hot phenol-water method (14). After three extractions, the LPS preparation showed no detectable amount of protein when assayed by the Lowry method or sodium dodecyl sulfate-polyacrylamide gel electrophoresis method (4). A rabbit weighing 2.5 kg was immunized intravenously with log-phase nutrient broth culture of *S. typhi* 0901. The first dose contained 10^4 organisms in 1 ml of normal saline. Five booster doses were subsequently given to the rabbit at 1-week intervals. Each booster dose contained 10 times the number of organisms in the previous dose. Two weeks after the last immunization, the animal was bled and the antibody titer against the LPS was determined by indirect ELISA (4). The rabbit was then exsanguinated, and the clotted blood was centrifuged at 800 × *g* for 20 min. The immunoglobulin fraction of the serum was precipitated with saturated ammonium sulfate, dialyzed, and used as the first antibody in the ELISA (Ab-1).

A monoclonal antibody specific for antigen 9 of group D salmonella was obtained by in vitro culture of the monoclone 204 D_3 (4). The ELISA titer of the monoclonal antibody preparation was determined by the indirect ELISA; one indirect ELISA unit was the smallest amount of the antibody which gave a positive indirect ELISA reaction (4).

Urine, blood, bone marrow, and stool samples were obtained from patients who were admitted to the Infectious Disease Hospital in Jakarta, Indonesia, with a clinical diagnosis of typhoid fever (6). The patients were subsequently treated with an appropriate antibiotic. Urine specimens were collected from the patients every 6 h during the first 3 days following admission. The blood, bone marrow, and stool samples were cultured as described by Hoffmann et al. (6). Four patients with dengue hemorrhagic fever in Jakarta served as negative controls. Multiple urine samples were collected from each patient.

The ELISA for *S. typhi* antigen was performed as described previously (4) except that rabbit antisalmonella immunoglobulin (Ab-1) was used instead of the rabbit anti-bovine immunoglobulin. Dot blot ELISA was performed by applying 3-μl aliquots of the urine specimen onto an NC strip (15 by 170 mm) and air drying the strip. The positive control was 3 μl of normal urine containing 1 μg of the *S. typhi* LPS; the negative control was normal urine only. The NC strip was then blocked with 0.5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.4, incubated at room temperature for 30 min, and then washed with 0.1 M Tris-HCl, pH 7.5. The strip was incubated with the monoclonal antibody (640 ELISA units/ml) on a rocking platform for 30 min, washed, and then incubated with rabbit anti-mouse immunoglobulins-alkaline phosphatase conjugate (1:1,000 in PBS, pH 7.4, containing 0.2% BSA and 0.2% gelatin). After 30 min at 37°C, the strip was washed and then

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TABLE 1. Results of bacterial cultures of 52 patients with typhoid fever from whom urines were collected

Group no.	No. of patients	Culture of:		
		Blood	Bone marrow	Urine
1	5	+	+	+
2	12	+	+	−
3	1	+	−	+
4	4	+	−	−
5	7	−	+	+
6	8	−	+	−
7	3	−	−	+
8	12	−	−	−

placed in a substrate solution (5 mg of nitroblue tetrazolium and 2.5 mg of 5-bromo-4-chloro-3-indolyl phosphate in 20 ml of 0.1 M Tris-HCl containing 0.1 M NaCl and 50 mM $MgCl_2$) for 20 min at room temperature. The reaction was stopped by washing the NC strip with distilled water, and the strip was then air dried.

Of 22 patients whose hemocultures were positive for *S. typhi* (groups 1 to 4, Table 1), 21 (95%) were positive by ELISA performed on multiple urine specimens. The sensitivity of the assay was 40% in patients who were bone marrow culture positive but blood culture negative (6 of 15 patients in groups 5 and 6) and 73% in patients who were stool culture positive but blood culture negative (8 of 11 patients in groups 3, 5, and 7). The ELISA was positive in all patients of group 8. None of the patients with dengue hemorrhagic fever was positive by the ELISA. The specificity of the assay was 100%.

A comparison of conventional culture techniques with ELISA for the detection of *S. typhi* antigen in the urine of clinically diagnosed typhoid patients is shown in Table 2. Seventy-seven percent of the clinically diagnosed typhoid patients has a positive bacterial culture, while 83% and 85% had *S. typhi* antigenuria as detected by the ELISA and dot blot ELISA, respectively. The sensitivity of the antigen detection was increased to 93% when the ELISA and dot blot ELISA were performed on the same clinical specimens.

In our previous report, specific monoclonal antibody was used in an ELISA for detecting *S. typhi* antigen 9 in the urine of patients with clinical typhoid fever and healthy controls. The test was 100% specific and could detect as little as 7.8 ng of antigen in the urine. However, the sensitivity was only 65% of the blood culture-positive typhoid cases (4). Many reasons were suggested to explain the low sensitivity of the test, including the monoepitope specificity and the poor affinity of the monoclonal antibody which was used as the second antibody in the assay, the uneven distribution and the denaturation of the antigen in frozen and thawed urine specimens, and the intermittent release of the antigen into the urine of the patients. In the present study, multiple urine samples from each patient were tested for *S. typhi* antigen by plate ELISA. The sensitivity of the test was improved significantly from 65% (when performed on a single specimen) (4) to 95% (when serially collected samples were examined). One reason for the low sensitivity of the assay observed in our previous report was the intermittent excretion of *S. typhi* antigen in the urine of patients with typhoid fever.

TABLE 2. Comparison of bacterial culture, ELISA, and dot blot ELISA for the detection of *S. typhi* infection in patients with clinical typhoid fever

Bacterial culture result (*n*)	No. (%) positive by:		
	ELISA	Dot blot ELISA	Combination of the two ELISAS
Positive[a] (40 [77%])	31 (83)	34 (85)	36 (93)
Negative[b] (12)	12	10	12

[a] Patients of groups 1 to 7.
[b] Patients of group 8.

The sensitivity of antigen detection by ELISA might be expected to increase by using a different solid phase such as the NC membrane in the enzyme immunoassay. NC has a high binding capacity for various antigens and permits the detection of lower concentrations of antigens in the sample. It binds approximately 1,000 times more protein per surface area than the microplate (5). The use of NC as the solid phase may also alleviate the problem of desorption since in this system, there is no need for coating with antibody, as in the conventional sandwich ELISA. The urine specimen can be directly dotted on the NC membrane, which would also make it easier to use in clinical practice. In this study, the sensitivity of the dot blot ELISA was similar to that of the plate ELISA and the results of the two assays correlated very well. Both immunoassays demonstrated intermittent *S. typhi* antigenuria in patients with typhoid fever.

Because most patients left the hospital soon after recovery, it was not possible to collect urines for longer than 3 days after treatment. However, the clearance of *S. typhi* antigen from the urine of most patients occurred around 16 days after the onset of the fever (3). Thus, the antigen detection assay(s) might be used not only for rapid and specific recognition of patients with typhoid fever but also for evaluation of the chemotherapeutic cure of the patients.

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