JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1990, p. 825–827
0095-1137/90/040825-03$02.00/0

Vol. 28, No. 4

# Rapid Diagnosis of Typhoid Fever through Identification of *Salmonella typhi* within 18 Hours of Specimen Acquisition by Culture of the Mononuclear Cell-Platelet Fraction of Blood

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Received 20 October 1989/Accepted 5 January 1990

**Detection of *Salmonella typhi* in blood by culture of the mononuclear cell-platelet layer was compared with other methods currently used for the diagnosis of typhoid fever. Colonies of *S. typhi* were present in all mononuclear cell-platelet layer-positive cultures within 18 h of plating and were identified within an additional 10 min by a coagglutination technique. In contrast, identification of all positive cultures by conventional blood culture required 3 days.**

An ideal test for typhoid fever would be sensitive, specific, rapid, and simple. It would provide data for initial clinical decision making and for epidemiological assessment of the impact of the disease. There is currently no test that fulfills these requirements.

*Salmonella typhi* can be isolated from more than 90% of patients with typhoid fever if blood, bone marrow, and intestinal secretions are cultured (1, 4–7). It is often difficult to obtain specimens of bone marrow and intestinal secretions, and in most cases only specimens of blood are cultured, reducing the sensitivity of culture diagnosis to 45 to 70% (1, 4–7). Furthermore, identification of the organism in culture may take 7 days or longer (3), markedly reducing the clinical and epidemiological usefulness of the test. A number of assays that detect *S. typhi* antibodies and antigens have been developed, and more recently a DNA probe has been described (9). None of these tests has been reproducibly shown to combine speed, simplicity, sensitivity, and specificity.

The low concentration of *S. typhi* cells in the blood of patients with typhoid fever, <15 bacteria per ml (9, 11), undoubtedly contributes to the moderate sensitivity of blood culture and antigen detection tests. We speculated that if all bacteria in a blood sample could be concentrated, this would facilitate culture of the organism and detection of bacterial antigens and DNA. We now report that when blood from typhoid patients is subjected to density gradient centrifugation, virtually all *S. typhi* cells are in the fraction containing only mononuclear cells (MNC) and platelets.

The protocols were approved by the Committee for the Protection of Human Subjects, U.S. Naval Medical Research Unit-2 (USNAMRU-2), Jakarta Detachment, and by the National Institutes of Health Research and Development, Ministry of Health, Jakarta, Indonesia. Patients older than 6 years of age with fever who were admitted to the Infectious Diseases Hospital of Jakarta, Indonesia, from mid-August through November 1987 were candidates for the first study. Data from a patient were included in the final analysis only if blood, rectal swab, and bone marrow aspirate specimens were cultured and if *S. typhi* was isolated from at least one specimen. All specimens were taken at the bedside within 30 min of each other. Bone marrow aspirate culture and rectal swab cultures were taken and processed as previously described (6, 7). Of 15 ml of venous blood collected, 7 ml was placed in a tube containing EDTA (Becton Dickinson, Rutherford, N.J.) and 8 ml was placed in a transport bottle containing 8 ml of 10% oxgall medium (Difco Laboratories, Detroit, Mich.). In the laboratory, the 16-ml blood-oxgall combination was transferred to a bottle containing 64 ml of 10% oxgall medium and was subcultured on days 1, 3, 7, 14, and 21; bacteria were isolated from oxgall by using methods described previously (6, 7).

Sepracell-MN (Sepratech Corp., Oklahoma City, Okla.) was used according to the instructions of the manufacturer. Briefly, 6 ml of well-mixed blood from the EDTA tube was transferred to a 15-ml tube containing 6.7 ml of Sepracell-MN and mixed thoroughly. The tube was centrifuged in a swinging bucket rotor (HS-4; Beckman Instruments, Inc., Fullerton, Calif.) at 2,000 × *g* at 22°C for 20 min without brake deceleration, producing a separation of the blood into three distinct layers. Sepracell-MN is a colloidal silica which forms a gradient during centrifugation; the density of each cell type determines its location within the gradient. The upper layer had an average volume of 0.6 ml and contained monocytes, lymphocytes, and platelets. The middle layer had an average volume of 9.0 ml and contained Sepracell-MN and plasma. The bottom layer had an average volume of 2.0 ml and included erythrocytes and polymorphonuclear leukocytes. Each layer was collected and transferred to a sterile tube and vortexed gently. Duplicate petri dishes containing MacConkey medium were inoculated with 100 μl of each of the three layers and incubated at 37°C. Colonies observed after overnight incubation were counted and then typed by a coagglutination assay (8). The remaining portions

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TABLE 1. Comparison of techniques for isolation of *S. typhi* in 29 patients with culture-proven typhoid fever (study 1)

| Method[a] | No. of patients with positive test results | % positive |
|---|---|---|
| MNC-P fraction | 13 | 45 |
| Blood culture | 14 | 48 |
| Rectal swab | 9 | 31 |
| Bone marrow aspirate | 25 | 86 |
| Blood culture and MNC-P | 17 | 59 |
| Blood culture and MNC-P and rectal swab | 21 | 72 |
| Bone marrow aspirate and MNC-P | 26 | 90 |
| Bone marrow aspirate and rectal swab | 28 | 97 |
| Bone marrow aspirate and MNC-P and rectal swab | 29 | 100 |

[a] *S. typhi* was isolated only from bone marrow aspirate in eight patients, only from rectal swab in three patients, and only from MNC-P in one patient.

of the plasma and erythrocyte-polymorphonuclear leukocyte layers were inoculated into oxgall medium at a 1:10 dilution and subcultured at day 7.

Phosphate-buffered saline (PBS) and blood from Americans and Indonesians with no history of typhoid were used as controls. An 18-h culture of *S. typhi* Ty2 grown in tryptic soy broth (Difco) at 37°C was serially diluted in PBS, and $10^3$ bacterial cells were added to 6 ml of anticoagulated blood or PBS. The bacteria-blood and bacteria-PBS mixtures were added to tubes containing Sepracell-MN and processed, as were patient samples.

*S. typhi* was isolated from 29 patients. A positive test was defined as the bacteriological identification of *S. typhi* from colonies subcultured from clinical specimens. Table 1 shows that bone marrow aspirate culture was positive in 86.2% (25 of 29) of patients, blood culture was positive in 48.3% (14 of 29) of patients, and rectal swab culture was positive in 31.0% (9 of 29) of patients; these results are comparable to those previously reported at this site (6, 7). The blood cultures were positive an average of 68.6 h after specimen acquisition, with a range from 48 to 86 h.

A total of 45% (13 of 29) of patients had positive cultures when the Sepracell-MN isolation method was used, and *S. typhi* was isolated from the MNC-platelet (MNC-P) layer in all 13. In 3 of these 13, *S. typhi* was also isolated from the layer containing erythrocytes and polymorphonuclear leukocytes. Each of these three patients had a greater level of bacteremia than the mean level of bacteremia for all patients. No bacteria were isolated from the plasma layer. In all Sepracell-positive cases, *S. typhi* was identified within 18 h by using a 10-min coagglutination assay on colonies that appeared on agar plates after overnight incubation. No growth was observed on any plates that were negative after 18 h. Among the 13 specimens from which *S. typhi* cells were detected in the MNC-P fraction, there were 10.9 ± 11.9 (0.5 to 34.6) organisms per ml.

To determine whether the association between *S. typhi* and the MNC-P layer was due to an artifact introduced by the density gradient centrifugation, bacteria added to normal blood or PBS were substituted for the patient specimen in the Sepracell-MN protocol. These experiments indicate that the results are not an artifact of the centrifugation. The five specimens of normal blood mixed with Ty2 had less than 1% of the organisms associated with the MNC-P layer. The majority of organisms were isolated from the plasma and the erythrocyte-plasma interface layers. None of the bacteria were isolated from the MNC-P layer in the PBS-bacteria controls, and the largest proportion of bacteria was found at the bottom of the tube.

These studies demonstrate that in patients with typhoid fever, circulating *S. typhi* cells are associated with MNC or platelets, and culture of the MNC-P fraction of the blood is as sensitive as whole blood culture, but more rapid. Our control experiments indicate that the association between *S. typhi* and the MNC-P fraction is not an artifact of centrifugation. The bacteria may be intra- or extracellular, or they may preferably associate with monocytes, lymphocytes, or platelets. Many bacteria, including *S. typhi*, have been shown to adhere to lymphocytes (10; E. Jirillo and S. Antonaci, Letter, Infection **13**:157, 1985).

Our preliminary findings were extended in a second study which was designed to determine whether or not the circulating bacteria are intracellular and to compare culture of the MNC-P fraction of blood with culture following lysis-centrifugation. Increased isolation rates and earlier recovery of a number of pathogens have been demonstrated by using the technique of lysis-centrifugation, which is based on the hemolysis of a blood sample and the subsequent centrifugation to concentrate organisms (2).

In this second study, a total of 28 ml of blood was collected from each patient; the patients had fever, were older than 14 years of age, and were admitted to the Infectious Diseases Hospital of Jakarta, Indonesia, from 27 July 1988 through 7 August 1989. The blood was processed as follows. (i) A total of 8 ml of blood was cultured in oxgall as described above. (ii) Then, 10 ml of blood was placed in an Isolator tube (Dupont, NEN Research Products, Boston, Mass.), mixed thoroughly, and centrifuged in a fixed-angle rotor at 3,000 × *g* at 22°C for 30 min without the brake. The Dupont Isolator contains agents which lyse erythrocytes and block coagulation. The top 9 ml in the Isolator tube was removed and discarded. The remaining 1-ml concentrate, containing lysed cells and bacteria from the blood sample, was vortexed and inoculated in 0.2-ml aliquots onto nutrient agar plates which were incubated at 37°C overnight. (iii) Blood (10 ml) was placed in an EDTA tube. Two 5-ml aliquots were transferred into 15-ml centrifuge tubes containing 5.5 ml of Sepracell-MN and processed as described above. The MNC-P layer of one tube was washed in PBS and resuspended in 0.6 ml of PBS, and 200-μl aliquots were inoculated on nutrient agar plates that were incubated at 37°C overnight. The MNC-P layer of the second tube was also washed in PBS and resuspended in 0.6 ml of PBS. Before plating on nutrient agar, 60 μl of a 2% solution of Nonidet P-40 (NP-40) was added to this MNC-P fraction to achieve a final concentration of 0.2% NP-40, which we determined would lyse the leukocytes without affecting the viability of the bacterial cells (unpublished results).

*S. typhi* was isolated from the blood of 31 patients. Culture of the MNC-P fraction of the 10-ml blood sample (5 ml with NP-40 and 5 ml without NP-40) and culture of the Isolator concentrate from 10 ml of blood both resulted in the isolation of *S. typhi* from 26 of these 31 typhoid fever patients (Table 2). *S. typhi* was isolated from 23 of these 31 patients when 8 ml of blood was cultured by conventional techniques. As with cultures of the MNC-P fraction, colonies were always present within 18 h of plating the Isolator concentrate. The increased speed of isolation of *S. typhi* is probably related to the concentration of the bacteria in a small volume for direct plating and the removal of serum factors that inhibit bacterial growth.

Although exposure of the MNC-P layer to NP-40 did not

TABLE 2. Comparison of different methods for the detection of *S. typhi* in the blood of 31 patients with typhoid fever (study 2)

| Patient no. | Isolator concentrate (CFU/10 ml) | MNC-P (CFU/5 ml) | | Bacteremia[a] (*S. typhi* cells/ml) | Result of blood culture[b] |
|---|---|---|---|---|---|
| | | With NP-40 | Without NP-40 | | |
| 4 | 2 | 0 | 0 | <1 | Negative |
| 19 | 8 | 0 | 0 | <1 | Negative |
| 20 | 2 | 2 | 0 | <1 | Negative |
| 22 | 1 | 0 | 1 | <1 | Negative |
| 32 | 0 | 0 | 2 | <1 | Negative |
| 42 | 9 | 4 | 0 | <1 | Negative |
| 51 | 12 | 52 | 3 | <1 | Negative |
| 68 | 182 | 126 | 148 | 22 | Negative |
| 41, 53, 60 | 0 | 0 | 0 | ND[c] | Positive |
| 3 | 182 | TNTC[d] | 72 | 17 | Positive |
| 10 | 64 | 41 | 9 | 5 | Positive |
| 11 | 18 | 32 | 42 | 4 | Positive |
| 12 | 81 | 25 | 61 | 9 | Positive |
| 21 | 0 | 3 | 0 | <1 | Positive |
| 30 | 341 | 179 | 90 | 29 | Positive |
| 36 | 1 | 89 | 2 | <1 | Positive |
| 38 | 12 | 4 | 5 | 1 | Positive |
| 40 | 103 | 129 | 69 | 11 | Positive |
| 44 | 7 | 0 | 2 | <1 | Positive |
| 47 | 9 | 5 | 5 | 1 | Positive |
| 48 | 99 | 157 | 136 | 16 | Positive |
| 49 | TNTC | TNTC | TNTC | >100 | Positive |
| 56 | 35 | 22 | 15 | 3 | Positive |
| 59 | 167 | 95 | 62 | 15 | Positive |
| 63 | 44 | 46 | 54 | 7 | Positive |
| 64 | 92 | 38 | 47 | 9 | Positive |
| 67 | 1 | 0 | 1 | <1 | Positive |
| 72 | 54 | 24 | 15 | 5 | Positive |
| 73 | 48 | 16 | 7 | 4 | Positive |

[a] Calculated as CFU in blood divided by amount of blood, combining the Isolator data and the data for MNC-P without NP-40. If no colonies were detected in MNC-P without NP-40, bacteremia was based on MNC-P with NP-40.
[b] Standard blood culture with 8 ml of blood.
[c] ND, Not determined.
[d] TNTC, Too numerous to count.

increase the frequency of *S. typhi* detection, the use of NP-40 resulted in the isolation of significantly more colonies of *S. typhi* ($P = 0.024$, $t$ test). These data suggest that many of the *S. typhi* cells in the MNC-P fraction are intracellular.

Standard laboratory equipment for aseptically handling the samples and a centrifuge (clinical type or swinging bucket rotor for use with Sepracell-MN and fixed-angle rotor for use with the Isolator) are needed for both of these nonconventional methods. On a per patient basis, the Isolator tube is more expensive than Sepracell-MN.

The current preliminary studies indicate that the combination of MNC-P culture or lysis-centrifugation culture and coagglutination can provide the clinician with a diagnosis of typhoid fever within a day of specimen acquisition, a marked improvement over conventional blood culture. Concentrating the bacteria and separating them from erythrocyte debris and serum proteins may also facilitate antigen and DNA detection in blood, and therefore this technique could be used in combination with any number of methods for bacteriological identification.

We acknowledge the encouragement and support of A. A. Loedin, Iskak Koiman, M. A. Moechtar, Fred Paleologo, Cyrus Simanjantak, Dennis Kopecko, and Richard Wistar and the technical assistance of the staffs of the Infectious Diseases Hospital of Jakarta, Jakarta, Indonesia, and the Bacteriology and Tropical Medicine Departments of USNAMRU-2, Jakarta Detachment.

This work was supported in part by the Naval Medical Research and Development Command, work unit 3M46373D807AH130, and the Army Medical Research and Development Command.

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