JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2007, p. 246–247 Vol. 45, No. 1
0095-1137/07/$08.00+0 doi:10.1128/JCM.01403-06


# Comparison of Serological Test Kits for Diagnosis of Typhoid Fever in the Philippines[▽]

Razel L. Kawano,* Susan A. Leano, and Dorothy May A. Agdamag

*National Reference Laboratory for HIV/AIDS, Hepatitis, and other STDs, STD/AIDS Cooperative Central Laboratory, Manila, Philippines*

Received 7 July 2006/Returned for modification 21 August 2006/Accepted 18 October 2006

**We evaluated four recent antibody-detection kits for typhoid fever by using 177 febrile patients from our hospital, in 75 of whom *Salmonella enterica* serotype Typhi grew. TUBEX performed best, achieving 94.7% sensitivity and 80.4% specificity. Typhidot, SD Bioline Typhoid, and Mega Salmonella were less specific and, in most cases, less sensitive.**

Typhoid fever is still rampant in the world, with an annual incidence of 17 million cases (7). In the Philippines alone, where the disease is endemic, the case fatality incidence in 2005 was 1.8 per 100,000 people (8). Accurate diagnosis is crucial to the management of the disease, but typhoid fever can be clinically confused with other febrile diseases, such as dengue fever and malaria. Culture of the causative organism, *Salmonella enterica* serotype Typhi, is most definitive for diagnosis, but this is expensive, takes up to a week of work, and does not always produce appropriate results (6). Serological diagnosis is thus widely employed, but this is fraught with specificity problems due to, for example, background levels of immunoglobulin G (IgG) antibodies to serotype Typhi in the regions of endemicity. Four serological test kits for typhoid fever which addressed the IgG problem were recently introduced to the Philippines. For improved specificity, these kits also used subunit antigens rather than whole organisms as employed in the long-standing and widely used Widal test.

As a national reference laboratory for blood-borne infections (with a primary focus on AIDS, viral hepatitis, and sexually transmitted diseases), we undertook evaluation of the four serological test kits, all using the same set of test sera within a specified period of time. Two medical technologists, oblivious to the status of the test sera or the results of any foregoing tests, carried out the testing. When necessary, tests were repeated by two technologists independently and a consensus conclusion was reached. The instructions provided by each test kit manufacturer were followed. The kits examined were SD Bioline Typhoid (Standard Diagnostics, Kyonggi-do, Korea), TUBEX (IDL Biotech, Sollentuna, Sweden), Typhidot (Malaysian Biodiagnostic Research, Bangi, Malaysia), and Mega Salmonella (Mega Diagnostics, Los Angeles, CA). SD Bioline Typhoid uses an immunochromatographic method to visually and qualitatively detect IgM and IgG antibodies to serotype Typhi antigens (unspecified) that are indirectly labeled with colloidal gold (via an antibody). The antigen-bound antibodies are captured by anti-IgM or anti-IgG antibodies immobilized on the test strip. TUBEX uses particle separation to detect IgM antibodies from whole serum (5) to the serotype Typhi O9 lipopolysaccharide antigen. The patient's antibodies inhibit the binding between indicator particles coated with an anti-O9 monoclonal antibody and lipopolysaccharide-coated magnetic particles. It is not clear why only IgM and not IgG antibodies are inhibitory, although the latter can bind to the antigen-coated particles (5). Results are read visually and semiquantitatively against a color chart. Scores of $\leq 2$ are considered negative. With Typhidot, antibodies are detected visually in this qualitative dot blot enzyme-linked immunosorbent assay (ELISA) to a 50-kDa outer membrane protein developed using peroxidase-labeled anti-IgM (Typhidot-IgM) or anti-IgG (Typhidot-IgG) antibodies. With Mega Salmonella, patient antibodies bind to *Salmonella* antigens (unspecified) insolubilized on microplates and are quantitatively detected by ELISA with IgM-specific (Mega-IgM) or IgG-specific (Mega-IgG) peroxidase-labeled reagents. The results are read in a microplate ELISA reader. The cost in US dollars per test of the various kits sold in the Philippines in government tertiary hospitals was \$4.90 for SD Bioline, TUBEX, and Mega and \$6.86 for Typhidot; while in private tertiary hospitals, the cost per test was \$51.68 for SD Bioline, TUBEX, and Mega and \$23.52 for Typhidot.

In terms of turnaround time, TUBEX is most rapid. The tests in order from the most rapid to the least rapid are as follows: TUBEX (5 min) > SD Bioline (15 to 30 min) > Mega (2.5 to 3.0 h) > Typhidot (2.5 h). TUBEX and SD Bioline are also the simplest, involving two steps.

In the study, 177 cases (mean age, 2.5 years; 46.3% female) (Table 1) were randomly selected for the study from febrile patients suspected of having typhoid fever in the San Lazaro Hospital, an infectious disease hospital. From each patient, 10

TABLE 1. Demographics of the study cohort

| Age group (yr) | No. of males | No. of females |
|---|---|---|
| <10 | 6 | 5 |
| 10–20 | 34 | 29 |
| 21–30 | 33 | 28 |
| 31–40 | 12 | 12 |
| >40 | 10 | 8 |

* Corresponding author. Mailing address: National Reference Laboratory for HIV/AIDS, Hepatitis and Other STDs, STD/AIDS Cooperative Central Laboratory, Manila, Philippines. Phone and fax: 632-309-9528. E-mail: rzlkawano@yahoo.com.

▽ Published ahead of print on 25 October 2006.

TABLE 2. Comparative performance of the four serological test kits[a]

| Test kit | Sensitivity % (95% CI) | Specificity % (95% CI) | PPV % | NPV % |
|---|---|---|---|---|
| TUBEX | 94.7 (86.2–98.3) | 80.4 (71.1–87.3) | 78.0 | 95.3 |
| SD Bioline | | | | |
| IgM | 69.0 (55.3–80.1) | 79.3 (69.4–86.8) | 67.8 | 80.2 |
| IgG | 70.7 (57.1–81.5) | 76.1 (65.9–84.1) | 65.1 | 80.5 |
| Typhidot | | | | |
| IgM | 54.7 (42.8–66.1) | 64.7 (54.6–73.7) | 53.2 | 66.0 |
| IgG | 73.3 (61.7–82.6) | 46.1 (36.3–56.2) | 50.0 | 70.1 |
| Mega | | | | |
| IgM | 90.7 (81.1–95.8) | 49.0 (39.1–59.1) | 56.7 | 87.7 |
| IgG | 96.0 (88.0–99.0) | 39.2 (29.0–49.4) | 53.7 | 93.0 |

[a] For SD Bioline, the number of serotype Typhi culture-positive subjects examined was 58, and the number culture-negative subjects was 92; for all other tests, the number of culture-positive subjects was 75, and the number of culture-negative subjects was 102. PPV, positive predictive value; NPV, negative predictive value.

ml of blood was obtained and cultured in tryptone soy broth medium for 3 to 7 days both by BACTEC (model no. 9120; Becton Dickinson, Franklin Lakes, NJ) and by conventional methodology (7). In both methods, growth was examined further by Gram stain, culture on selective medium, and biochemical testing. Of the 177 specimens, 75 grew serotype Typhi by both methods. A total of 102 specimens were culture negative and considered nontyphoidal for the purpose of statistical computation (using EPInfo6 software [Centers for Disease Control]).

A single blood sample (3 to 4 ml) was obtained from each patient for the serological testing. The results of the various tests conducted are summarized in Table 2. As shown, in terms of test sensitivity, Mega-IgG was most sensitive. The tests in order from the most sensitive to the least sensitive are as follows: (i) Mega-IgG (96.0% [72/75]; 95% confidence interval [CI] = 88 to 90), (ii) TUBEX (94.7% [71/75]; CI = 86.2 to 98.3), (iii) Mega-IgM (90.7%; CI = 81.1 to 95.8), (iv) Typhidot-IgG (73.3%; CI = 61.7 to 82.6), (v) SD Bioline-IgG (70.7%; CI = 57.1 to 81.5), (vi) SD Bioline-IgM (69.0%; CI = 55.3 to 80.1), and (vii) Typhidot-IgM (54.7%; CI = 42.8 to 66.1).

In terms of specificity, TUBEX was most specific. The tests in order from the most specific to the least specific are as follows: (i) TUBEX (80.4%, [82/102]; CI = 71.1 to 87.3), (ii) SD Bioline-IgM (79.3%, [73/92]; CI = 69.4 to 86.8), (iii) SD Bioline-IgG (76.1%; CI = 65.9 to 84.1), (iv) Typhidot-IgM (64.7%; CI = 54.6 to 73.7), (v) Mega-IgM (49.0%; CI = 39.1 to 59.1), (vi) Typhidot-IgG (46.1%; CI = 36.3 to 56.2), and (vii) Mega-IgG (39.2%; CI = 29.0 to 49.4). Since it is unlikely that our laboratory was successful in isolating serotype Typhi from all of the patients, the specificity of the serological tests could be underestimated. Fourteen of the culture-negative subjects were antibody positive by TUBEX and the other three IgM tests (data not shown). If these were considered true typhoid patients, then the corrected specificities for TUBEX and SD Bioline-IgM, for example, would be 93.2% (82/88) and 89.7% (70/78), respectively.

Other organisms grew in 22 cases (21%) from the serotype Typhi culture-negative group. The most common (11 isolates) of these was coagulase-negative staphylococcus. Other organisms were *Bacillus* spp. (four isolates), *Pseudomonas* spp. (three isolates), *Acinetobacter* spp. (two isolates), and *Klebsiella pneumoniae* (two isolates). However, less than half of the patients infected by these organisms were positive according to the TUBEX or SD Bioline (IgM or IgG) result in each case. Since there is also no known antigenic cross-reactivity between these organisms and serotype Typhi, we suspect that the serological reactivities observed are unrelated to these infections.

It is apparent from Table 2 that tests which detect IgM antibodies are less sensitive but more specific than those of the same kind that detect IgG antibodies and vice versa. For a serological test to be useful for routine application and as an alternative to culture, it must perform well (>75%) in both sensitivity and specificity, not just in one alone, and have acceptable (>75%) positive and negative predictive values. TUBEX meets these criteria, scoring best among the four kits. SD Bioline-IgM came second. The findings for TUBEX accord well with previous observations made in Vietnam, where good sensitivity (80 to 85%) and specificity (75 to 80%) were also found (2, 4). Surprisingly, Typhidot performed poorly in contrast to previous findings (1, 4), although an earlier study by Membrebe and Chua (3), also carried out in the Philippines, had found very similar results to ours (72% sensitivity; 52% specificity). Mega appeared to be overly sensitive, resulting in poor specificities (39.2 to 49.0%). Sensitivity, on the other hand, may be affected for SD Bioline due to the competition between disease-specific and other antibodies present in whole serum for the capture antibody.

## REFERENCES


1. **Choo, K. E., S. J. Oppenheimer, A. B. Ismail, and K. H. Ong.** 1994. Rapid serodiagnosis of typhoid fever by dot enzyme immunoassay in an endemic area. Clin. Infect. Dis. **19:**172–176.
2. **House, D., J. Wain, V. A. Ho, T. S. Diep, N. T. Chinh, P. V. Bay, H. Vinh, M. Duc, C. M. Parry, G. Dougan, N. J. White, T. T. Hien, and J. J. Farrar.** 2001. Serology of typhoid fever in an area of endemicity and its relevance to diagnosis. J. Clin. Microbiol. **39:**1002–1007.
3. **Membrebe, F. A., and J. A. Chua.** 1999. The clinical utility of typhidot in the diagnosis of typhoid fever. Philipp. J. Microbiol. Infect. Dis. **28:**1–4.
4. **Olsen, S. J., J. Pruckler, W. Bibb, T. M. Nguyen, M. T. Tran, T. M. Nguyen, S. Sivapalasingam, A. Gupta, T. P. Phan, T. C. Nguyen, V. C. Nguyen, D. C. Phung, and E. D. Mintz.** 2004. Evaluation of rapid diagnostic tests for typhoid fever. J. Clin. Microbiol. **42:**1885–1889.
5. **Tam, F. C. H., and P. L. Lim.** 2003. The TUBEX typhoid test based on particle-inhibition immunoassay detects IgM but not IgG anti-O9 antibodies. J. Immunol. Methods **282:**83–91.
6. **Wain, J., T. S. Diep, V. A. Ho, A. M. Walsh, T. T. Nguyen, C. M. Parry, and N. J. White.** 1998. Quantitation of bacteria in blood of typhoid fever patients and relationship between counts and clinical features, transmissibility, and antibiotic resistance. J. Clin. Microbiol. **36:**1683–1687.
7. **World Health Organization.** 2003. Background document: the diagnosis, treatment and prevention of typhoid fever (WHO/V&B/03.07). World Health Organization, Geneva, Switzerland. http://www.who.int/vaccines-documents/DocsPDF03/www740.pdf.
8. **World Health Organization.** 2005. Environmental health country profile, Philippines. World Health Organization, Geneva, Switzerland.