Original Article

Evaluation of a newly developed ELISA against Widal, TUBEX-TF and Typhidot for typhoid fever surveillance

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Abstract

Introduction: Typhoid fever is endemic in many parts of the world and represents a major cause of acute febrile illness (AFI). Rapid and accurate laboratory methods for diagnosis of this disease are needed for both patient care and surveillance situations.

Methodology: Serum samples were collected from AFI patients and used to evaluate the performance of a newly developed ELISA assay that uses a mixture of somatic and flagellar antigens to detect the total antibody response against *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) infection. The levels of Ig isotype response (IgG, IgM and IgA) were also evaluated, and results were compared to those of TUBEX-TF and Typhidot commercial kits.

Results: Of 234 culture-confirmed typhoid patients, the total Ig ELISA diagnosed 93% compared to 71% using Widal test. This sensitivity level (93%) is higher than that observed for the individual Ig ELISAs (IgG 75%; IgM 79%; IgA 57%) and the commercial tests TUBEX-TF (75%), Typhidot IgM (63%) and Typhidot IgG (28%). An agreement of 78% was achieved between the total Ig ELISA and Widal test. The average specificity of the ELISA was 96%. Using ELISA, up to 200 samples can be tested per run with cost per test at US$0.20.

Conclusions: The developed ELISA shows superior sensitivity and specificity, when compared to Widal, TUBEX-TF and Typhidot assays, is more cost effective and allows higher throughput. This method is highly recommended for active surveillance studies or outbreak investigations of typhoid fever.

Key words: typhoid; serological diagnosis; ELISA; surveillance

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Introduction

Typhoid fever, resulting from infection by *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi), is a major cause of morbidity and mortality worldwide. Recent surveillance studies have indicated that infection by *S.* Typhi causes 21 million illnesses and 200,000 deaths annually [1,2]. In Egypt, the incidence rate of *S.* Typhi infections ranges between 13 and 59 per 100,000 persons per year [2,3]. This range is among the highest reported incidence ranges in northern African countries (10-100 per 100,000 per year) [4].

Laboratory diagnosis of typhoid fever in developing or underdeveloped countries is primarily achieved either by bacterial culture, considered to be the gold standard for diagnosis, or detection of serum antibodies using the Widal test, which is currently the standard serological method for typhoid diagnosis [5]. However, both methods are time-consuming with relatively low sensitivities and specificities, and culture methods require basic laboratory facilities [6-8]. Efforts to develop faster and more sensitive and specific serological assays for the diagnosis of typhoid fever have led to the commercialization of the TUBEX-TF and Typhidot kits. Both kits have been evaluated in several studies with somewhat variable results [5,6,9,10].

The continued development and use of accurate diagnostic tests in disease surveillance studies provides critical information to public health decision makers with respect to clinical management, disease prevention, and infection control strategies [11]. In response to this need, an in-house ELISA was developed for the diagnosis of acute typhoid fever in an endemic area during surveillance activities. The performance of this ELISA was evaluated against bacterial culture, the Widal test, and the commercial-available serologic tests, TUBEX-TF and Typhidot (IgG and IgM).

Methodology

Patient enrollment and sample collection

Patients presenting with acute febrile illness (AFI) from five Egyptian fever hospitals, located in distinct geographical regions, were enrolled in a surveillance study between June 2003 and May 2007. Informed consent was obtained from all adult participants and from parents or legal guardians of minors.

Admission was based on a standard AFI case definition per World Health Organization recommendations: any individual with fever lasting for at least two days or temperature on admission of ≥ 38.5°C; age 4 years with no identified cause of fever, such as diarrhoea or pneumonia; or clinically suspected of having typhoid fever or brucellosis [12,13]. Blood for culture and serum samples were collected on admission and sent to the US Naval Medical Research Unit No. 3 (NAMRU-3) laboratory for diagnostic testing, confirmation, and archiving.

Study serum samples

A total of 2897 serum samples collected from AFI patients and healthy subjects were included in this work. Based on culture and/or serological results, specimens were divided into three groups: typhoid-positive (n = 332), typhoid-negative (n = 2525) and healthy controls (n = 40). Typhoid-positive samples included 234 culture-confirmed cases, and 98 cases with positive Widal test results (antibody titer > 320). The typhoid-negative group included samples collected from patients with AFI of known (n = 730) and unknown (n = 1795; negative for all tests) etiologies. Serum samples collected from AFI patients of known etiology included three subgroups: brucellosis (n = 290) confirmed by culture and/or serology, rickettsiosis (n = 290) diagnosed serologically by ELISA (antibody titer > 400) and leptospirosis (n = 150) confirmed by culture and/or the microscopic agglutination test (MAT).

To compare the newly developed typhoid immunoglobulin IgM, IgG and IgA ELISAs to the commercially available TUBEX-TF IgM and Typhidot IgM and IgG kits, a subset of serum samples (388/2897, 13%) was used. This subset included 165 typhoid-positive (67 culture- and 98 Widal test-confirmed cases) samples, 216 typhoid-negative samples from febrile patients with known and unknown etiology (73 and 143, respectively) and seven healthy patient control samples.

Laboratory methods

Blood cultures were performed using BACTEC culture media (Beckton-Dickenson, Cockeysville, MD, USA) per the manufacturer's instructions. *Leptospira* cultures were performed as previously described [13]. Widal tube agglutination (TA) tests were performed for all cases enrolled in the study according to the manufacturer's instructions (Beckton-Dickenson and Company, Sparks, Maryland, USA) using BD-Difco *Salmonella* flagellar d (TH), BD-Difco *Salmonella* O group D and *Brucella abortus* antigens. Agglutination titers > 320 were considered positive [14,15]. TUBEX-TF (IDL Biotech, Bromma, Sweden) and Typhidot (Malaysian Biodiagnostic Research, Bangi, Malaysia) serological tests were performed according to the manufacturers' recommendations. Other serological methods used in this study included ELISAs for diagnosis of brucellosis [15], rickettsiosis [16] and leptospirosis (Panbio Limited, Brisbane, Australia).

Typhoid ELISA development

Standard checkerboard titrations [17] were performed to determine the optimum conditions for loading *S*. Typhi somatic (TO) and flagellar (TH) antigens (the same ones mentioned previously for tube agglutination) onto ELISA plates using positive and negative anti-*S*. Typhi antisera. The TO and TH antigens were mixed in a 1:3 ratio and loaded onto the plates at 2.5 µl/ml in carbonate buffer. Flat-bottom polystyrene ELISA plates (Thermo Labsystems, Franklin, MA, USA) were coated by adding 100 µl of the diluted antigen to each well and incubating overnight at 4°C. After decanting the antigen solution, the plates were washed three times with PBST (phosphate buffer saline [Sigma-Aldrich, St. Louis, MO, USA] containing 1% Tween 20). The coated plates were then blocked by adding 200 µl/well of blocking buffer (carbonate buffer with 1% w/v bovine serum albumin, BSA [Sigma-Aldrich, St. Louis, MO, USA]) and incubating for one hour at 37°C. After decanting the blocking solution, the plates were washed three times with PBST as before or stored at -20°C for future use (validated for up to one year). Each serum sample tested was diluted in PBST with 0.1% w/v BSA at 1:160 and 100 µl was added to each well (in duplicate). For each plate, positive and negative serum controls diluted in PBST buffer were also added. After incubation for one hour at 37°C, plates were washed as described above and 100 µl/well peroxidase-labeled goat anti-human (total Ig) conjugate (Sigma-Aldrich, St. Louis, MO, USA), diluted 1:20,000 in PBST with 0.1% BSA, was added. For detection of antibody isotypes IgG, IgM

Table 1a. Sensitivity and specificity of the Typhoid ELISA (total Ig). Patient samples tested were culture-confirmed typhoid, probable typhoid (by Widal test), febrile patients with known non-typhoidal etiology (brucellosis, rickettsiosis and leptospirosis), febrile patients with unknown etiology and healthy controls.

Study group (n)	ELISA Positive Number (%)	Sensitivity (%)	Specificity (%)
Culture-confirmed typhoid (234)	217 (93)	93	N/A*
Widal-confirmed typhoid (culture-negative) (98)	76 (78)	78	N/A*
Brucellosis (290)	8(3)	N/A*	97
Rickettsiosis (290)	20(7)	N/A*	93
Leptospirosis (150)	6(4)	N/A*	96
AFIs of unknown etiology (1795)	70(4)	N/A*	96
Healthy samples (40)	0(0)	N/A*	100

*N/A: Not applicable

and IgA, the following respective conjugates were used: anti-human IgG and IgM (Sigma-Aldrich, St. Louis, MO, USA); anti-human IgA (Jackson Immuno Research Laboratories, West Grove, PA, USA). The optimum dilution for each conjugate was determined as 1:15,000 for IgG, and 1:10,000 for both IgM and IgA. Plates were incubated for 30 minutes at 37°C, then washed three times as described above. O-phenylene diamine (OPD) substrate solution (SigmaFast, Sigma-Aldrich, St. Louis, MO, USA) was prepared according to the manufacturer's instructions, 100 µl was added to each well, and the plates were incubated for 30 minutes at ambient temperature (approximately 21 to 24°C) in the dark. The reaction was stopped by adding 50 µl of stopping solution (1 to 2 M H2SO4) to each well. Color intensities were read using a BioTek microplate reader (Labsystems, Vantaa, Finland) at 490 nm.

Absorbance (A490) values obtained from the testing of serum samples from laboratory-confirmed non-typhoid and healthy control groups (18) were used to establish cut-off values: 0.3 for total Ig, IgG and IgM; and 0.25 for IgA. Thus any test sample that generated an A490 value greater than or equal to the cut-off value at a 1:160 dilution was considered initially reactive, while those that did not were considered non-reactive and reported as negative for anti-*S*. Typhi antibodies.

Initially reactive samples were serially diluted from 1:160 to 1:5,120 and tested to determine titer values. Samples giving titer values greater than 320 were considered reactive and reported as positive for anti-*S*. Typhi antibodies.

Sensitivities of the different methods used in this study were evaluated using culture-positive typhoid patients as true positives while the specificities were evaluated using typhoid-negative and healthy samples as true negatives. Assay sensitivity and specificity calculations were determined as reported previously [15,19].

Results and discussion

Comparison of ELISA to the Widal test

Using sera from culture-positive patients, the newly developed total Ig ELISA showed improved sensitivity (93%, Table 1a) compared to that of the Widal test (71%, Table 1b) or previously published ELISAs [5,18,20]. Additionally, among these culture-positive samples, the total Ig ELISA detected 75% (50/67, Table 1b) of those not detected by the Widal test, while the Widal test detected only 1% (1/67, Table 1b) of culture positive samples testing negative for the total Ig ELISA.

This difference in sensitivity may be due to the detection limit of each assay: ELISA may be sensitive to antibody concentrations as low as 0.05 ng, while agglutination tests such as Widal may require concentrations greater than 500 ng [20].

Within the probable typhoid patient samples (culture-negative, Widal-positive), the total Ig ELISA detected 78% (Table 1a and 1b). This sensitivity level, although lower than that observed for culture-confirmed typhoid samples, is still comparable to or higher than those previously reported for the Widal-

Table 1b. ELISA total Ig and Widal test results for typhoid patients confirmed by culture (n = 234) and by Widal only (n = 98).

	Typhoid (n=332)		
	Culture diagnosed (n = 234)		Widal diagnosed (n = 98)
	Widal positive (n = 167)	Widal negative (n = 67)	Widal positive (n = 98)
ELISA positive	166 (99%)	50 (75%)	76 (78%)
ELISA negative	1 (1%)	17 (25%)	22 (22%)

Table 2. Sensitivity and specificity values of different ELISAs and the TUBEX-TF, Typhidot IgM and Typhidot IgG tests, when using typhoid samples confirmed by culture (n = 67), Widal (n = 98) and typhoid-negative samples collected from febrile patients of known etiology (n = 73), unknown etiology (n = 143) and healthy controls (n = 7).

Assay	Sensitivity (%)		Specificity (%)		
	Culture-confirmed typhoid (n = 67)	Widal-confirmed typhoid (n = 98)	Fever of known etiology (n = 73)	Fever of unknown etiology (n = 143)	Healthy (n = 7)
TUBEX-TF	75	78	85	88	100
Typhidot IgM	63	62	95	97	100
Typhidot IgG	28	28	99	99	100
ELISA total Ig	93	78	95	94	100
ELISA IgG	75	65	95	96	100
ELISA IgM	79	78	95	95	100
ELISA IgA	57	64	96	97	100
IgG+IgM ELISAs	88	84	91	92	100
IgG+IgA ELISAs	84	73	93	95	100
IgM+IgA ELISAs	88	85	91	94	100
IgG+IgM+IgA ELISAs	90	86	90	92	100

diagnosed typhoid [18,22,23]. Alternatively, these observed data may be interpreted to indicate poor specificity of the Widal test, rather than poor sensitivity of the total Ig ELISA. To investigate this, the Widal-positive samples were further analyzed according to their titer values and evaluated against the total Ig ELISA. A direct correlation was seen between Widal test antibody titer and total Ig ELISA positivity. ELISA total Ig detected 58%, 79%, 89% and 100% of Widal-positive typhoid patients with antibody titers of 320, 640, 1280 and 2560, respectively. The Widal test has been modified over the years, particularly regarding the determination of antibody titers considered to be clinically relevant [24]. Titers against the somatic (TO) and flagellar (TH) antigens were set at values ranging from 1:20 [25], 1:40 [26], 1:80 [27] to 1:160 [28]. Accordingly, we may recommend that interpretation of Widal test results with low antibody titers (< 320) should be made with caution. For example, studies using the Widal test in typhoid endemic regions may be problematic due to repeated exposure to *S.* Typhi or cross-reactivity with other diseases (e.g., malaria), making it difficult to establish a steady-state baseline titer for the population [29].

The specificity values of the total Ig ELISA, using sera from patients with laboratory-confirmed brucellosis, rickettsiosis, leptospirosis, fever with unknown etiology and healthy subjects, were 97%, 93%, 96%, 96% and 100%, respectively (Table 1a). Taken together, the specificity of the total Ig ELISA (96%) was superior to that of the Widal test {76% [6]; 80% (this study; data not shown); 85% [9]} and previously published ELISAs [5,18,20,22]. Although both tests utilize the *Salmonella* somatic (TO) and flagellar (TH) antigens, antigen-antibody interactions in the ELISA are more specific, compared to the whole cell agglutination reactions detected in the Widal test. In addition, the difficult subjective nature of reading agglutination reactions may contribute to the Widal test's lower specificity.

Comparison of ELISA to other commercially available tests

The newly developed ELISA was further optimized for detection of the individual antibody isotypes (IgG, IgM and IgA) produced against *S.* Typhi infection and evaluation of the distribution of the different antibody isotypes among acute typhoid patients. Among the individual isotype ELISAs, IgM was found to be the most sensitive (79%) while IgA the least (57%). Our results indicate that no antibody isotype is predominant, with a combination of two or three antibody isotypes detected in more than 85% of the samples tested (Table 2). Previous reports for other AFI of bacterial origin (e.g., brucellosis) indicate that the textbook immune response (initial IgM response followed by class-switching to IgG) may not occur. Also, acute and chronic stages of bacterial AFI may not be distinct, leading to the presence of more than one antibody isotype at the time of testing [15,30].

Table 3: Sensitivity and specificity of the Typhidot and TUBEX-TF tests for different sample populations from different geographical areas

Test	Location	Sensitivity %	Specificity %
Typhidot*	Current study**	63	92
	Bangladesh [32]	67	54
	India [33]	92	98
	Vietnam [6]	79	89
	Malaysia [23]	98	77
	Pakistan [11]	85	77
TUBEX-TF	Current study**	75	87
	Bangladesh [32]	60	58
	Bangladesh [34]	91	82
	Vietnam [6]	79	89
	Poland [35]	93	95
	Philippines [10]	95	80
	Vietnam [5]	87	76

* Typhidot IgM testing
** Rates using confirmed typhoid cases

The performance of the total Ig and each individual isotype ELISA was then compared to the commercially-available TUBEX-TF and Typhidot IgM and IgG test kits (Table 2) using a selected subset of serum samples (388/2897, 13%; see Methodology for subset details). Considering first the culture-confirmed samples, the total Ig ELISA showed superior sensitivity (93%) when compared to all other tests.

In addition, sensitivity values of the individual isotype ELISAs were comparable to TUBEX-TF and Typhidot IgM and higher than that of Typhidot IgG. When results from the IgG, IgM and IgA ELISAs were combined, the sensitivity of detecting anti-typhoid antibodies improved significantly, approaching that of the total Ig ELISA and congruent with that of a previous study [31].

The specificities of the different methods are also indicated in Table 2. TUBEX-TF showed the lowest specificities (85% and 88% for AFI of known and unknown etiologies, respectively), while the other assays varied between 94% and 99%. All assays were 100% specific when testing healthy control samples. The specificity levels of the different methods evaluated in this study are encouraging when compared to that of the Widal test.

The total Ig ELISA showed strong correlation (r = 0.85) with culture isolation. In comparison, TUBEX-TF and Typhidot (combined IgG and IgM) showed more moderate correlation (r = 0.65 and 0.69, respectively). Typhidot correlated moderately with ELISA and TUBEX-TF (r = 0.43 for each), while

Table 4: Practical bench-top comparison between ELISA, TUBEX-TF and Typhidot.

	ELISA*	TYPHIDOT**	TUBEX-TF
Kit	Not in kit format, but all items are standard and commercially available	Some needed equipment for analysis not provided in the kit - reagents not sufficient for the 112 test strips - each plate is enough for 7 samples only	All analytical equipment needed for analysis were provided with sufficient reagents
Specimen used (volume and type)	1.6 µl of clear serum	2.5µl – specimen type not specified	45µl of clear serum
Procedure preparation	Two reagents only; sample/conjugate diluent and substrate buffer	Tedious preparation procedure	Easy preparation procedure
Antibodies detected	May detect total antibody response or individual antibody isotypes IgG, IgM and IgA	May detect IgG or IgM	Detects IgM only
Results interpretation	Standard digital format (optical density) with pre-determined cut-off values	Colored spots evaluated using the naked eye	Colored scale evaluated using the naked eye.
Throughput***	400	60	120
Run time***	Three hours	60 minutes	30 minutes
Cost per test	$ 0.20	$ 2.20	$ 6.00

* ELISA total Ig, IgG, IgM or IgA.
** Typhidot IgM or IgG.
***In one ELISA run up to 200 samples could be tested and their results could be reported. For Typhidot, each kit has reagents enough for 30 samples and the total test time is almost one hour. However, using TUBEX-TF and Typhidot kits, we found difficulties running more than10 samples per run

correlation between ELISA and TUBEX-TF was poor (r = 0.2).

Past studies have shown that the use of TUBEX-TF and Typhidot yields highly variable sensitivity and specificity profiles, depending on the country and/or geographical region, study population, and nature of the study (Table 3). This has created difficulties in comparing results between studies and setting worldwide standards for typhoid fever diagnosis. One study revealed that the TUBEX-TF and Typhidot tests were no better than the Widal test [9], underlining the need for a more efficient diagnostic test for typhoid fever, especially during the acute stage of the disease.

Practical aspects

Practical aspects of operating each of the assays in this study were also considered as part of the evaluation (Table 4). The total Ig ELISA was relatively easy, fast and cost-effective, costing 11 and 30 times less per sample compared to the cost of the TUBEX-TF and Typhidot kits, respectively. Of the three assays, the total Ig ELISA was also capable of the highest throughput; this is of particular value when processing large batches of samples during surveillance studies or outbreak events. For example, each operator can test up to 200 ELISA samples per run compared to less than 15 samples per run for the other assays. We also believe results interpretation to be superior for ELISA, as digital formats with preset cut-off values are used to differentiate between positive and negative reactions, not requiring subjective evaluations for color intensities of spots or bands produced.

Conclusion

Typhoid fever continues to be a major public health problem worldwide. Surveillance studies provide critical information for guiding public health decisions related to the disease. Due to the nature of these surveillance studies, participating laboratories receive large numbers of samples that are tested in batches. Though current clinical diagnostic tests such as Widal, TUBEX-TF and Typhidot are more suitable and convenient than ELISA for bedside testing and in small-scale, inadequately equipped or field laboratories, an effective ELISA is superior in routine surveillance studies or outbreak investigations where high throughput is important. This study demonstrates the development of a total Ig ELISA, with superior sensitivity and specificity rates to existing tests, that is more cost-effective when testing large numbers of samples.

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References

1. Crump JA, Luby SP, Mintz ED (2004) The Global burden of typhoid fever. Bull WHO 82: 346-353.
2. Srikantiah P, Girgis FY, Luby SP, Jennings G, Wasfy MO, Crump JA, Hoekstra RM, Anwer M, Mahoney FJ (2006) Population-based surveillance of typhoid fever in Egypt. Am J Trop Med Hyg 74: 114-119.
3. Crump JA, Youssef FG, Luby SP, Wasfy MO, Rangel JM, Taalat M, Oun SA, Mahoney FJ (2003) Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. Emerg Infect Dis 9: 539-544.
4. Ghenghesh KS, Franka E, Tawil K, Wasfy MO, Ahmed SF, Rubino S, Klena JD (2009) Enteric Fever in Mediterranean North Africa. J Infect Dvl Ctries 3: 753-761.
5. House D, Wain J, Ho VA, Diep TS, Chinh NT, Bay BV, Vinh H, Duc M, Parry CM, Dougan G, White NJ, Hien TT, and Farrar JJ (2001). Serology of Typhoid Fever in an Area of Endemicity and Its Relevance to Diagnosis. J Clin Microbio 39: 1002-1007.
6. Olsen SJ, Pruckler J, Bibb W, Nguyen TM, Tran MT, Nguyen TM, Sivapalasingam S, Gupta A, Phan TP, Nguyen TC, Nguyen VC, Phung DC, Mintz ED (2004). Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol 42:1885-1889.
7. Hamze M, Naboulsi M, Vincent P (1998). Evaluation of the Widal test for diagnosing typhoid fever in Lebanon. Pathol Biol (Paris) 46: 613-616.
8. Shukla S, Chitnis DS (1997). Haemagglutination system for the simultaneous detection of LPS and anti LPS antibodies of *S*. Typhi. Indian J Med Sci 51: 265-269.

9. Dutta S, Sur D, Manna B, Sen B, Deb AK, Deen JL, Wain J, Von Seidlein L, Ochiai L, Clemens JD, Kumar Bhattacharya S (2006) Evaluation of new-generation serologic tests for the diagnosis of typhoid fever: data from a community-based surveillance in Calcutta, India. Diagn Microbiol Infect Dis 56: 359-365.
10. Kawano RL, Leano SA, Agdamag DM (2007) Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. J Clin Microbiol 45: 246-247.
11. Bhutta ZA, Dewraj HL (2006) Current concepts in the diagnosis and treatment of typhoid fever. BMJ 333:78-82.
12. Afifi S, Earhart K, Azab MA, Youssef FG, El Sakka H, Wasfy M, Mansour H, El Oun S, Rakha M, Mahoney F(2005) Hospital-based surveillance for acute febrile illness in Egypt: a focus on community-acquired bloodstream infections. Am J Trop Med Hyg 73: 392-399.
13. Parker TM, Murray CK, Richards AL, Samir A, Ismail T, Fadeel MA, Jiang J, Wasfy MO, Pimentel G (2007) Concurrent infections in acute febrile illness patients in Egypt. Am J Trop Med Hyg 77: 390-392.
14. Memish ZA, Almuneef M, Mah MW, Qassem LA, Osoba AO (2002) Comparison of the Brucella standard agglutination test with the ELISA IgG and IgM in patients with Brucella bacteremia. Diagn Microbiol Infect Dis 44: 129-132.
15. Fadeel MA, Wasfy MO, Pimentel G, Klena JD, Mahoney F, Hajjeh R (2006) Rapid enzyme-linked immunosorbent assay for the diagnosis of human brucellosis in surveillance and clinical settings in Egypt. Saudi Med J 27: 975-981.
16. Richards AL, Soeatmandji DW, Wi dodo MA, Sardjono TW, Yanuwiadi B, Hernowati TE, Baskora AD, Roebiyoso, Hakim L, Soendora M, Rahardjo E, Putri MP, Saragih JM, Strickman D, Kelly DJ, Dasch GA, Olson JG, Church CJ, Corwin AL (1997) Seroepidemiological evidence for murine and scrub typhus in Malang, Indonesia. Am J Trap Med Hyg 57: 91-95.
17. Crowther, J. R. 2001. Methods in Molecular Biology, vol. 149: The ELISA guidebook, chapter 4, pages: 83-113. Humana Press, Totowa, New Jersey, USA.
18. Sippel J, Bukhtiari N, Awan MB, Krieg R, Duncan JF, Karamat KA, Malik IA, Igbal LM, Legters L (1989) Indirect immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays (ELISAs) and IgM capture ELISA for detection of antibodies to lipopolysaccharide in adult typhoid fever patients in Pakistan. J Clin Microbiol 27: 1298-302.
19. Daniel TM and Debanne SM (1987) Serodiagnosis of tuberculosis and other mycobacterial diseases by enzyme-linked immunosorbent assay. Am Rev Respir Dis 135: 1137-1151.
20. el Fattah MM, Hassan EM, Shaheen H, Awad RA, Girgis N (1991) Evaluation of enzyme-linked immunosorbent assay for detection of *Salmonella* Typhi antigen and antibodies. J Egypt Public Health Assoc 66: 145-157.
21. Butler J (2000). Antibody-antigen and antibody-hapten reactions. In: Maggio ET, editor. Enzyme-Immunoassay. Boca Raton, Florida: CRC Press Inc. 5-52.
22. Sippel JE, Hanafy HM, Diab AS, Prato C, Arroyo R (1987) Serodiagnosis of typhoid fever in pediatric patients by anti-LPS ELISA. Trans R Soc Trop Med Hyg 81:1022-1026.
23. Gopalakrishnan V, Sekhar WY, Soo EH, Vinsent RA, Devi S (2002) Typhoid fever in Kuala Lumpur and a comparative evaluation of two commercial diagnostic kits for the detection of antibodies to *Salmonella* typhi. Singapore Med J 43: 354-358.
24. Chart H, Cheasty T, de Pinna E, Siorvanes L, Wain J, Alam D, Nizami Q, Bhutta Z (2007) Serodiagnosis of *Salmonella enterica* serovar Typhi and *S. enterica* serovars Paratyphi A, B and C human infections. J Med Microbiol 56: 1161-1166.
25. Hoffman SL, Flanigan TP, Klaucke D, Leksana B, Rockhill RC, Punjabi NH, Pulungsih SP, Sutomo A, Moechtar MA(1986) The Widal slide agglutination test, a valuable rapid diagnostic test in typhoid fever patients at the Infectious Diseases Hospital of Jakarta. Am J Epidemiol 123: 869-875.
26. Levine MM, Grados O, Gilman RH, Woodward WE, Solis-Plaza R, Waldman W (1978). Diagnostic value of the Widal test in areas endemic for typhoid fever. Am J Trop Med Hyg 27: 795- 800.
27. Buck RL, Escamilla J, Sangalang RP, Cabanban AB, Santiago LT, Ranoa CP, Cross JH (1987) Diagnostic value of a single, pre-treatment Widal test in suspected enteric fever cases in the Philippines. Trans R Soc Trop Med Hyg 81: 871-873.
28. Pang T, Puthucheary SD (1983) Significance and value of the Widal test in the diagnosis of typhoid fever in an endemic area. J Clin Pathol 36: 471-475.
29. Olopoenia LA, King AL (2000) Widal agglutination test - 100 years later: still plagued by controversy. Postgrad Med J 76: 80-84.
30. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D (2003) Laboratory-based diagnosis of brucellosis- a review of the literature. Part II: serological tests for brucellosis. Clin Lab 49: 577-589.
31. Shaheen HI, Girgis NI, Rodier GR, Kamal KA (1995). Evaluation of the response of human humeral antibodies to *Salmonella* typhi lipopolysaccharide in an area of endemic typhoid fever. Clin Infect Dis.; 21: 1012-1013.
32. Naheed A, Ram PK, Brooks WA, Mintz ED, Hossain MA, Parsons MM, Luby SP, Breiman RF (2008) Clinical value of TUBEX-TF and Typhidot rapid diagnostic tests for typhoid fever in an urban community clinic in Bangladesh. Diagn Microbiol Infect Dis 61: 381-386.
33. Jesudason MV, Sivakumar S (2006) Prospective evaluation of a rapid diagnostic test Typhidot for typhoid fever. Indian J Med Res 123: 513-516.
34. Rahman M, Siddique AK, Ta m FC, Sharmin S, Rashid H, Iqbal A, Ahmed S, Nair GB, Chaignat CL, Lim PL (2007) Rapid detection of early typhoid fever in endemic community children by the TUBEX-TF O9-antibody test. Diagn Microbiol Infect Dis 58: 275-281.
35. Oracz G, Feleszko W, Golicka D, Maksymiuk J, Klonowska A, Szajewska H (2003). Rapid diagnosis of acute *Salmonella* gastrointestinal infection. Clin Infect Dis 36: 112-115.

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