

Review Paper

How Useful is the Widal Test in Modern Clinical Practice in Developing Countries? A Review

ABSTRACT

It has been over a century since the Widal test was developed for diagnosing enteric fever. Yet, the test remains the major means of diagnosing the disease in many of the developing countries where it remains endemic. This review appraises the Widal test in regard to its performance techniques, its various drawbacks and the available alternative diagnostics methods. Extensive literature search was conducted through Google search engine and PubMed for relevant published articles in peer-reviewed academic journals. Relevant text books were also consulted. The following conclusions were reached at the end of the review: the Widal test is grossly inadequate to be relied upon as a diagnostic tool for typhoid fever in an endemic area, culture isolation of the causative organism remains the gold standard for the disease diagnosis, there is still no sufficiently reliable alternative rapid diagnostic test (RDT) for typhoid fever and the quest to develop highly effective RDTs for the disease should continue.

Keywords: *Widal test, Typhoid fever, Enteric fever, Typhoid rapid diagnostic tests*

Comment [A1]: Abstract: it is better to be divided into the following sections: Background, Methods, results and conclusion. In the methods section the type of study should be determined (non-systematic review). In the results sections the authors should illustrate the main results of the review.

1. INTRODUCTION

The Widal test was developed by Georges-Fernand-Isidore Widal, a French physician and bacteriologist, in 1896 [1,2]. It is a serological agglutination test which has been used for over a century in the diagnosis of enteric fever [2]. Despite the availability of alternative diagnostic methods, the Widal test continues to be the commonest diagnostic test for typhoid fever in many of the poor developing countries where the disease is endemic. This review, therefore, appraises the Widal test, the alternative diagnostic methods and their applicability in modern clinical practice. It is our hope that it will serve as an education material for the clinician, the laboratory worker and the general population at large.

Comment [A2]: •The introduction is too short. It does not fulfill criteria of good introduction
•Should be ended with a rationale of the study, so what is the rational for conducting the study?

2. METHODS

Extensive literature search was made through Google search engine and PubMed for relevant published articles in peer-reviewed academic journals. Relevant text books were also consulted. Keywords employed for the search were "Enteric fever", "Typhoid fever", "Widal test", "How to perform Widal test", "Usefulness of Widal test", "Alternatives to Widal test" and Typhoid rapid diagnostic tests." The references of the articles at hand were used to find other relevant references. References were stored in an electronic reference manager (Mendeley) and kept for subsequent use. Forty-eight (48) relevant articles and texts were reviewed and cited in the final draft of the review.

Comment [A3]: The type of study should be determined (non-systematic review). The methods are weak and not comprehensive: Where are the inclusion and exclusion criteria? It better to construct a table for the included and rejected papers in the methods' section
After methods start a title 'Results'

3. ENTERIC FEVER

Enteric fever, also known as typhoid fever, is a potentially fatal acute systemic illness characterized by fever and abdominal symptoms [3]. Though now rare in the industrialized countries of the world as a result of improved sanitation and food hygiene, the disease is still an important cause of morbidity and mortality in the developing countries [3].

The disease was initially called typhoid fever because of its clinical similarity to typhus fever [4]. The term "enteric fever" was proposed as a name for the disease in 1869 due to the anatomical site of the infection [4]. The two terms are, however, being used interchangeably.

Enteric fever is primarily caused by *Salmonella enterica serovar typhi* (*Salmonella typhi*). A less severe illness is caused by *Salmonella paratyphi A* and, less commonly by B and C [5]. *Salmonella typhi* is a gram-negative, non-encapsulated, facultative anaerobic, flagellated motile bacillus [6]. It has

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somatic (O), flagellar (H) and virulence (Vi) antigens [6]. The *S paratyphi* also have similar antigens denoted by AH, BH and CH.

Typhoid fever is related to rapid population growth, increased urbanization, overcrowding, inadequate human waste treatment, limited water supply and overburdened health care systems. Majority of cases are sporadic, although large outbreaks do occur commonly resulting from breakdowns in water supplies and sanitation systems [3,6]. In 2004, the WHO estimated the global burden of the disease at 21 million cases per annum, leading to an estimated 216,000–600,000 deaths annually [6]. The disease is endemic in the developing regions of the world: Indian subcontinent, South and Central America, Asia and Africa [3,6]. The world is divided into three regions according to the annual incidence of typhoid fever: high incidence (>100/100,000 cases/year) includes south-central Asia and south-east Asia; medium incidence (10-100/100,000 cases/year) includes the rest of Asia, Africa, Latin America and the Caribbean, and Oceania, except for Australia and New Zealand; and low incidence (<10/100,000 cases/year) includes Europe, North America, and the rest of the developed world. [7]

Man is the main reservoir of the organism, largely current cases and chronic carriers who disseminate the organism in their feces [6]. The disease is acquired predominantly via the feco-oral route by ingestion of contaminated food or water [5]. Less common modes of transmission include sexual transmission (oral-anal, oral-penile, and receptive anal transmission) and transmission through the use of unsterilized instruments (endoscopes, polyvinyl duodenal tube or rectal tube) [8–10]

4. THE WIDAL TEST

The Widal test is an agglutination test that measures specific antibody titers in the serum. It involves the detection of *Salmonella* antibodies in the patient's serum by the use of bacterial suspensions of *S typhi* and *S paratyphi* A and B that have been treated to retain only the O (somatic) and H (flagellar) antigens [2]. The test, therefore, operates on the premise that patients with typhoid fever have antibodies in their sera that can agglutinate homologous antigens in killed *Salmonella* suspensions. The O agglutinins which are immunoglobulin M (IgM) antibodies appear early and represent the initial serological response in acute enteric fever while the H agglutinins which are immunoglobulin G (IgG) antibodies usually develop slowly and persist for a longer duration [2,11] Killed colored suspensions of the *S typhi* O antigen, *S typhi* H antigen, *S paratyphi* AH antigen and *S paratyphi* BH antigen are routinely used for the test.

4.1 Widal test performance and methods

Two methods can be employed to perform the Widal test: the slide agglutination test and the tube agglutination test [2, 12]. Though the tube agglutination test has better accuracy, the slide test is commonly used because it is easier and faster to conduct.

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4.1.1 Slide agglutination Widal test

The slide agglutination test could either be a qualitative or a quantitative test [2].

The qualitative test is used as a rapid screening test to determine the presence of *Salmonella typhi* and *paratyphi* antibodies in the serum of patients. A drop of the patient's serum is added to an equal drop of each of the standard bacterial antigens (O, H, AH, BH) on a slide and thoroughly mixed with an application stick [2,12]. The slide is rotated gently and observed for agglutination. Physiologic saline and a previously reactive serum are used as negative and positive controls [12]. The appearance of agglutination to any of the antigens infers a positive test while non-agglutination implies a negative test. Agglutinations are visualized as clumps.

The quantitative test is usually done after the qualitative test (rapid screening test) has been performed. Only the previously detected *salmonella* antibody/antibodies in the patient's serum are then tested to determine their titers. A drop (0.03ml) of the antigen suspension which previously showed agglutination is added to aliquots of 40µl, 20µl, 10µl, and 5µl of the patient's serum on a slide and thoroughly mixed with an applicator stick. The slide is then rotated gently and observed for reactions. Tests' results are scored from 0 to 4+ [0 (no agglutination), 1+ (25% agglutination), 2+ (50% agglutination), 3+ (75% agglutination) or 4+ (100% agglutination)] [2,13]. The smallest quantity of serum that shows a 2+ or 50% agglutination is chosen as the antibody titer of the test sample [2,13]. The presence of agglutination in 40µl corresponds to 1:40, 20µl to 1:80, 10µl to 1:160 and 5µl to 1:320 titers respectively.

4.1.2 Tube agglutination Widal test

This is a more accurate but more cumbersome and time-consuming quantitative test in comparison to the quantitative slide test. It can be used to clarify ambiguous agglutination reactions gotten by the more rapid slide test [2].

Four sets of 8 test tubes are prepared for each antigen (O, H, AH, BH). The first test tube of each antigen set is then filled with 1.9ml of physiological

saline [12]. Next, 1ml of physiological saline is added to the other tubes (tubes 2-8). To the first tube, 0.1ml of the test sample (serum) is added and properly mixed. From tube 1, 1ml of the diluted sample is transferred to tube number 2 and mixed properly. The dilution is continued serially up to the 7th tube in each set of the four antigens. From tube 7 of each set, 1ml of diluted serum is discarded. Hence, the dilution of the serum sample from tubes 1-7 respectively in each antigen sets are: 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280 [12]. Tube 8 serves as a negative control. To one set of test tubes, 50µl of the O antigen is added (i.e. tubes no 1-8). Then, 50µl of the H, AH and BH antigens are added to the 2nd, 3rd and 4th set of test tubes respectively and thoroughly mixed. The tubes are then covered and incubated overnight at 37°C for up to 20 hours [2,12]. After incubation, the sediments are dislodged and observed for agglutination. The control tubes are firstly examined and they must contain no agglutination. The results are scored from 0 to 4+ agglutinations as described above for the slide test. The highest dilution of serum to produce agglutination is taken as the titer. The titer for each of the antigens is noted and recorded.

4.2 Pitfalls of the Widal test

The Widal test is a relatively cheap and readily available test, especially in developing countries where more sophisticated tests like culture and polymerase chain reaction (PCR) are either not available or unaffordable where available. It is, however, difficult to interpret because of the various reasons that may cause either a false positive (Table 1) or a false negative result (Table 2).

Table 1: Causes of false positive Widal test

1	Vaccination against <i>Salmonella typhi</i> or <i>paratyphi</i>
2	Previous enteric fever
3	Substandard quality reagent
4	Cross-reaction with non- <i>Salmonella</i> infections like malaria
5	Cross-reaction with non-typhoidal <i>Salmonella</i> antibodies
6	Infection with other <i>Enterobacteriaceae</i>
7	Anamnestic reaction
8	Non-infectious chronic illnesses like rheumatoid arthritis and ulcerative colitis
9	Laboratory error

Table 2: Causes of false negative Widal test

1	Antibiotics use before test
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The authors had discussed well the diagnostic accuracy of RDTs (in the following sections) for typhoid fever despite the widal test is lacking such data in the review !!!!!

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2	Wrong timing e.g. test before the end of first week of infection
3	Substandard quality reagent
4	Laboratory error
5	“Hidden organisms” in bone and joints
6	Poorly immunogenic strains of infecting organism

Salmonella are grouped into different serotypes (A – E) based on the somatic O antigen (the Kaufmann-White scheme) [11,14,15] There are at least 78 organisms in the serotype D to which *S typhi* belongs. While all the 78 group D organisms have O antigen 9, about 60 including *S typhi* possess O antigen 12 [14,15] Hence, infection by any of the group D serotypes could lead to the production of antibodies that can agglutinate the O antigen used in the conduct of the Widal test. Also, because all the groups A and B organisms possess O antigen 12, they could produce O antibodies that can agglutinate the Widal test O antigen. This cross-reaction with non-typhoidal *Salmonella* antibodies significantly reduces the specificity of the test and is a cause of false positive results. Several other non-*Salmonella* infectious diseases in the typhoid endemic regions like malaria, infective endocarditis, chronic viral hepatitis, dengue, brucellosis, miliary tuberculosis etc. have been demonstrated to exhibit this cross-reactivity that increases the false positive rate of the Widal test [2]. Cross-reaction with other *Enterobacteriaceae* also exists [2].

A study was conducted by Olopoenia *et al* to determine Widal agglutinin titers among a Nigerian population who had no prior typhoid immunization [2]. Among participants who had a positive malaria smear and a negative *S typhi* culture 85% had 1:40, 12% had 1:80, and 3% had 1:160 Widal titers respectively. Whereas, of the participants that had both malaria smears and *S typhi* cultures negative results 45% had 1:40, 15% had 1:80, and 10% had 1:160 Widal titers respectively. In essence, all the participants in the former group had at least a 1:40 Widal agglutination titer while 70% of the latter group had a similar response. This study further corroborates the fact that patient with malaria fever tend to have false positive Widal agglutination reaction.

Non-infectious chronic illnesses like rheumatoid arthritis and ulcerative colitis can also give a false positive result [11]. Patients who have received vaccines against *Salmonella* may have false positive reaction but this can be differentiated from true infection by a repeat test. True untreated infection usually results in a titer rise whereas vaccinated individuals do not demonstrate any titer rise. Individuals who had suffered from repeated exposure to small inocula of *S typhi* or to other *Salmonella* species that contains type 9 or 12 O antigens could have a false positive result [2]. This

can also be differentiated from true infection by lack of any rise in titer on test repetition. It should be noted, however, that the mandatory repeat Widal test after the initial positive test is usually not done in many of the places where typhoid fever is endemic. Also, individuals who had suffered from enteric fever in the past sometimes develop anti-*Salmonella* antibodies during non-typhoidal fever states. This is termed anamnestic reaction [11].

The timing of the test is very important as the antibodies begin to rise in the first week and titers increase through the second to fourth week after which they gradually decline. Hence, the test may yield a negative result in the early part of the first week. Patients who have been treated with antibiotics in the early stages may not give positive results [2,16] Such patients may also not show any rise in titer when they initially tested positive, instead there may be a fall in the titer. Occasionally, “hidden organisms” in bones and joints and poorly immunogenic strains of infecting organism may cause false negative results [16,17].

There seems to be poor standardization in the manufacturing process of the Widal antigens as variability in quality exists. DeVillier *et al* compared the *S typhi* O antigens obtained from different manufacturers by testing them against the same serum which initially tested positive by the slide agglutination test [2,18]. The study showed a significant variability in the agglutination titers of the antigens. This kind of variability could lead to either false positive or false negative results.

4.3 Interpretation of the Widal test

While several reports from certain developing countries have suggested that a single Widal test is enough to make the diagnosis of typhoid fever [19–26], others have disputed the reliability of such a single test result [27–34]. However, it is generally agreed that the reliability of the test improves if the interpretation is made against a local cut-off titer [2,15,35].

Theoretically, a single Widal test may have some diagnostic relevance in an “unexposed” febrile patient (unvaccinated or lack of active infection). In reality, however, such a single test’s result does not have any diagnostic significance in an endemic area because repeated subclinical exposures to *S typhi* may have occurred [2]. Even in cases of extremely high single Widal agglutination titers, the causal organisms may be other species of *Salmonella* and not *S typhi* [2,36].

A review by Olopoenia and King to determine the significance of the Widal test for typhoid fever diagnosis in modern medicine concluded that the test cannot be expected to give a reliable diagnostic result in endemic regions because of its several pitfalls; hence, its use should be discouraged [2]. A study was conducted by Enabulele and Nyemike in a Nigerian population to

determine the validity of a single Widal test as a diagnostic tool for enteric fever among febrile adults using blood culture as the reference standard [37]. Malaria parasite test was also done on each sample. Of the 271 participants, 124 (45.76%) were positive for the Widal agglutination test, 60 (22.14%) blood cultures grew *salmonella* and 55 (20.30%) had a co-infection of enteric fever and malaria. Out of the 124 that were positive for Widal test, only 21(16.94%) of them had a positive blood culture. Whereas, of the remaining 147 that were negative for Widal test, 39 (26.53%) of them had blood culture confirmation of enteric fever. For Widal agglutination test, a sensitivity of 35%, specificity of 51%, positive predictive value of 17% and negative predictive value of 73% were gotten. These values are too low than for the test to be considered reliable. From the foregoing, it is obvious that a single Widal test cannot be reliably used to diagnose enteric fever in an endemic area. Disregard for this fact probably accounts for the seemingly high rate of diagnosis and treatment of typhoid fever in the endemic areas which are mainly developing countries.

It is generally considered that the Widal test is helpful in the diagnosis of typhoid fever in endemic regions only if the patient has a four-fold or more increase in the O or H agglutinin titers of serum specimens taken during the acute and convalescent period of infection (2-3 weeks apart) [2,15]. This again is a major obstacle to its use because antibiotic therapy often needs to be commenced at the early stage of the infection in order to prevent the patient from developing serious complications like gut perforation and gastrointestinal bleeding. Thence, no reasonable physician would wait for 2-3 weeks without treating a patient with suspected enteric fever. Again, once antibiotics have been commenced there may be no further rise in the agglutinin titer, rather, it may decrease.

4.4 Misuse of the Widal test

Misuse of the Widal test is rife in many of the developing countries of the world [2,15,38]. This is probably due to the fact that the test is cheap and readily available in contrast to the more reliable but sophisticated diagnostic methods. This is particularly so because patients often do not go to the appropriate medical facilities with competent hands for medical attention because of ignorance and poverty. A report from Libya shows that clinicians depend on the Widal test for the diagnosis of enteric fever despite that locally acceptable threshold titer has not been determined because their laboratories lack skilled and experienced personnel and appropriate facilities to detect and serotype *Salmonella* isolates [38]. Due to the weak nature of institutional regulation in these countries there is proliferation of quack medical and laboratory practices. The laboratories often perform the test, make the diagnosis and prescribe the antibiotics [2]. Many diagnostics centers use just a single positive result for diagnosis not taking into account

the reality of cross-reaction between *S typhi* and malaria parasites or other *Salmonella* species that are also endemic in their areas and the generally low diagnostic accuracy of the Widal test. These practitioners often disregard the fact that a negative Widal test result may be more useful than a positive one thereby contributing to unnecessary use of antibiotics.

It is imperative that laboratory personnel desist from usurping the role of the physician. Their role is to perform and report the test. It is the responsibility of the requesting physician to utilize the information supplied by the laboratory in conjunction with the clinical information at his/her disposal to arrive at appropriate diagnosis. There is a need for close communication between the requesting physician and the laboratory so as to clarify ambiguous laboratory reports before use. Laboratories should desist from reporting test results in descriptive terms (negative or positive) as this may lead to wrong interpretation of results by the physician [2]. The results should rather be reported as either 'no agglutination' or in titers (1:20, 1:40, 1:80, 1:160 etc.) if agglutination is present [2].

4.5 Alternative rapid diagnostic tests to the Widal test

As a consequence of the drawbacks attendant to the Widal test, several alternative rapid diagnostic tests (RDT) have been developed. These tests include the indirect hemagglutination assay (IHA), indirect enzyme-linked immunosorbent Assay (ELISA) for immunoglobulin M (IgM) and IgG antibodies to *S typhi* polysaccharide, indirect fluorescence Vi antibody assay, counter immunoelectrophoresis (CIEP) and monoclonal antibody against *S typhi* flagellin etc [35].

Some of the commercially available RDTs kits for typhoid include- Tubex TF (IDL, Sweden), Typhidot (Malaysian Biodiagnostic Research, Malaysia), Multi-Test Dip-S-Ticks (Panbio INDX, US), Typhidot-M (Malaysian Biodiagnostic Research, Malaysia), SD Bioline (Standard Diagnostics, Korea) and Mega *Salmonella* (Mega Diagnostics, US) etc [39] Tubex TF and Typhidot are among the most commonly used of the recent generation of RDTs for typhoid fever [39]. Mostly, these tests have not been found sufficiently superior in performance to the Widal test to be recommend as lone reliable diagnostic tests for typhoid fever in endemic areas [40,41,42,43].

Thriemer *et al* conducted a meta-analysis to determine the performance of Tubex TF and Typidot in typhoid endemic countries [39]. A total of seven studies were included per test. The meta-analysis showed average sensitivity and specificity of 69% (95%CI: 45%–85%) and 88% (95%CI: 83%–91%) respectively for Tubex TF. A formal meta-analysis could not be conducted for Typhidot because of data limitation but across the extracted studies,

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sensitivity and specificity estimates ranged from 56% to 84% and 31% to 97% respectively. It was concluded that the observed performance does not support the use of either of the RDTs exclusively as the basis for diagnosis and treatment of typhoid fever.

In another meta-analysis of 37 studies that evaluated three RDTs and their variants (TUBEX in 14 studies, Typhidot in 22 studies and Test-It Typhoid immunochromatographic lateral flow assay in 9 studies), Wijedoru *et al* obtained an average sensitivity of 78% (95%CI: 71%-85%) and specificity of 87% (95%CI: 82%-91%) for TUBEX; an average sensitivity of 69% (95%CI: 59%-78%) and specificity of 90% (95% CI 78% to 93%) for all Test-It Typhoid and prototype tests (KIT); and an average sensitivity of 84% (95%CI: 73%-91%) and specificity of 79% (95%CI: 70%-87%) for Typhidot test. The authors concluded that few of the 37 studies were at a low risk of bias, the three main RDTs and their variants had moderate diagnostic accuracy, the tests did not show any evidence of superiority to one another in terms of their average sensitivity and specificity and there is need for more robust evaluations of alternative RDTs for enteric fever [44].

However, an immunodiagnostic assay for enteric fever, called typhoid/paratyphoid diagnostic assay (TPTest), has been developed lately [45]. The test is centered around the detection of anti-Salmonella enterica antibodies secreted by activated lymphocytes in the peripheral blood of acutely infected patients. [45]. TPTest had a sensitivity of 100% compared to blood culture, and specificity that ranged from 78-97% (95% CI: 73%-100%), depending on the definition of true negative among Bangladeshi patients [45]. In a study that compared the diagnostic accuracy of TPTest to Tubex and Typhidot, the sensitivity of TPTest, Tubex and Typhidot were estimated at 96.0% (95% CI: 87.1%-99.8%), 60.2% (95% CI: 49.3%-71.2%), and 59.6% (95% CI: 50.1%-69.3%), respectively; while specificity was estimated at 96.6% (95%CI: 90.7%-99.2%) for TPTest, 89.9% (95% CI 79.6%-96.8%) for Tubex, and 80.0% (67.7%-89.7%) for Typhidot [46]. This indicates that TPTest has a superior diagnostic accuracy compared to Tubex and Typhidot. Nevertheless, it takes 24-48hrs to obtain the result of the test at the moment and moderate laboratory capacity is required to conduct it [46].

5. HOW IS TYPHOID FEVER DIAGNOSED

The gold standard for the diagnosis of enteric fever remains culture isolation of the organism [2,35]. It is considered 100% specific [2,35]. The blood, bone marrow, stool, urine, rose spot, gastric and intestinal secretions may be cultured. Culture efficacy varies with the type of specimen being tested. Bone marrow culture yield is about 90% sensitive at any point in the course of the disease to as long as five days after the initiation of antibiotic therapy [35,47]. The test is not the preferred option because the procedure is extremely

painful. The sensitivity of blood culture approaches 90% in an untreated patient in the first week of infection but the figure drops to less than 50% by the third week. Large volume (10-30ml) and multiple samples (>3 times) improve the diagnostic yield [35]. The sensitivity of stool culture alone is less than 50% while urine culture is lesser [35]. A single rectal swab during hospital admission has a sensitivity of 30-40% [35]. However, the high rate of antibiotics use by patients before hospital presentation in the developing countries tends to interfere with the isolation of infectious agents from clinical specimens, particularly blood culture. Though blood culture is currently the preferred diagnostic method, it is expensive; the result is only available after more than 48 hours; and requires elaborate laboratory equipment and rare technical expertise.

The polymerase chain reaction has varying success. The nested PCR has a sensitivity of 82.7% and a specificity of 100% when blood and urine assays are combined [48]. The test is not readily available in the endemic areas and where available, it is mostly used for research purpose because it is generally too expensive for patients.

Common nonspecific laboratory findings in patients with typhoid fever include moderate anemia, thrombocytopenia, relative lymphopenia and elevated erythrocyte sedimentation rate (ESR) [Table 3] [35]. Depending on the stage of the disease at the time of diagnosis, there could be slight elevation of the prothrombin time (PT) and activated partial thromboplastin time (aPTT), a reduction in fibrinogen levels and elevation in the levels of the fibrin degradation products. The liver transaminases and serum bilirubin values could rise to as twice the reference range.

Table 3: Nonspecific laboratory findings in patients with typhoid fever

1	Moderate anemia
2	Thrombocytopenia
3	Relative lymphopenia
4	Elevated erythrocyte sedimentation rate (ESR)
5	Elevated prothrombin (PT) and activated partial thromboplastin time (aPTT)
6	Reduced fibrinogen levels
7	Elevated fibrin degradation products levels
8	Elevated liver transaminases and bilirubin

387

388 The question then comes to mind: “How is enteric fever to be diagnosed in
389 the absence of a rapid, reliable and cost-effective diagnostic method?” The
390 fact is that accurate diagnosis of typhoid fever remains a challenge in
391 resource-poor settings because the disease does not have specific clinical
392 features. The diagnosis is often made by the exclusion of other common
393 febrile illnesses in the locality. In many of the areas where malaria is
394 endemic, typhoid fever is usually suspected whenever a febrile patient gives
395 a history of repeated standard malaria treatment without abatement of the
396 fever. Features that may suggest the development of complications such as
397 generalized abdominal tenderness with guarding and hematochezia are also
398 sought for. Apart from fever and abdominal pain, nonspecific symptoms such
399 as headache, diarrhea or constipation, anorexia, body weakness and muscle
400 pains are often associated with the disease [3]. Neuropsychiatric
401 manifestations (known as typhoid psychosis) ranging from confusion to frank
402 psychosis could occur in 5% to 10% of patients with enteric fever [3]. Rose
403 spots (red macules of 2-4 millimeters diameter) may be observed in light
404 skinned persons. It is not uncommon for physicians to commence empirical
405 antibiotic therapy while awaiting culture results once a clinical impression of
406 typhoid fever has been made.

407 6. CONCLUSION

408 The Widal test cannot be relied upon to accurately diagnose typhoid fever
409 due its glaring inadequacies. Therefore, its use should be discouraged by all
410 means. The general **population**, laboratory workers and clinicians need to be
411 adequately enlightened on the limitations of the Widal test as this will go a
412 long way to reducing the rate of unnecessary diagnosis and treatment of
413 typhoid fever in the endemic populations. The quest for the development of
414 effective typhoid RDTs should continue until they could perform at levels
415 comparable to the malaria RDT. Since culture isolation of the causative
416 organism remains the definitive diagnosis of enteric fever, there is an urgent
417 need for the provision of appropriate laboratory equipment and training of
418 more laboratory personnel on microbiology culture techniques in the
419 developing countries where the disease is endemic.

420 421 COMPETING INTERESTS

422
423 Authors have declared that no competing interests exist.

424 425 REFERENCES

- 426
427 1. Editors. Fernand-Isidore Widal: French physician and bacteriologist

Comment [A10]: Too many Old references, !!!!!
Revise all reference and follow up the vancouver
style

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Comment [A11]: Wrong refernce writing!!

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Comment [A13]: Old references, !!!!!

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