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A New Diagnostic Method of *Salmonella* antibodies by Micro Titer Plate Wells and Comparison with Different Diagnostic Methods

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ABSTRACT

Background: Typhoid is one of the infectious diseases, slide and tube agglutination will remain not relevant as a diagnosis tool for typhoid fever in the poor countries, slide and tube agglutination have poor sensitivity and specificity, the results should be confirmed by a new diagnostic method. **Objective:** This study aimed to find a new diagnostic method that is more sensitive to typhoid fever. Therefore, micro-titer plate assays are inexpensive and do not require special equipment. **Material and methods**: In the current study, the blood samples were collected from 1000 clinically suspected cases of typhoid fever attending outpatients and inpatients clinic of "Faquos Fever Hospital" from Dec. 2016 to Dec. 2019, and laboratory investigations were blood culture, micro-titer plate, slide and tube agglutination test. **Results**: Among (1000) clinically suspected cases. Regarding sex 56% were male, and females (44%). Clinically suspected fever cases with fever 5 days, the age of the studied cases ranged from 8 to 94 years with a mean of 35.55± 19.65 years, and there is no infection by paratyphoid A and Co**nclusion**: Diagnosis of salmonella antibodies by micro-titer plate wells are reliable, this method is simple, highly sensitive, specific, and quantitative in diagnosing typhoid fever when compared with different diagnostic method.

INTRODUCTION

Typhoid fever is one of the most prevalent diseases worldwide. More than 16 million infections occur worldwide each year. Even so, serological testing (slide and tube agglutination) is still required in the form of a Widal reaction (Ismail *et al.,* 1991). Typhoid disease remains an important preventable public health problem in developing countries including Egypt. Typhoid is found all over the world, most typhoid infections and deaths occur in children of school age or younger (World Health Organization, 2014). Laboratory investigation depends on the blood, bone marrow and stool cultures are the most reliable diagnostic methods for typhoid fever but they are expensive techniques and some bacterial culture facilities are often unavailable (Olopoenia, 2000). Micro-Titer plate assays have the added advantage of processing multiple samples at once, with high sensitivity and accurate typhoid diagnosis, they do have some limitations. Micro-titer plate assays require expensive arid special equipment which restricts their use in large hospitals and laboratories (Pang *et al.,* 1998).

One of the major challenges in the diagnosis of typhoid fever is the lack of an appropriate gold standard. Given that there is no licensed vaccine against *Salmonella*, this diagnostic gap leads to inappropriate use of antibiotics, thereby enhancing antimicrobial resistance (Hatmal *et al.*, 2021). In the field of infectious diseases, typhoid fever is a life-threatening bacterial infection that remains a global health problem. The infection is associated with a significant morbidity and mortality rate, resulting in an urgent need for specific and rapid detection tests to aid and the management of the disease (Najib *et al.*, 2021).

Diagnosis of typhoid by slide agglutination is practically unexplainable unless the sensitivity and specificity and the slide agglutination test can be used to screen for negative samples, a positive slide agglutination test expression is not always helpful in diagnosing enteric fever. These agglutinins are often extremely variable and can rise as a nonspecific response to other infections (Sahastrabuddhe *et al.*, 2013). Slide agglutination as a screening test without confirmation of results by tube agglutination may lead to a false diagnosis of typhoid fever, which is not inherent to the test and is influenced by the prevalence of the disease (Andualem *et al.*, 2014).

On the other side, the diagnosis of typhoid by tube agglutination test is a widely used laboratory test for the diagnosis of typhoid fever, especially in resource-limited countries where blood cultures are not routinely available, which may lead to a false diagnosis of typhoid fever (Clegg *et al.*, 1994). Some methods are commonly used for serological identification of *Salmonella* typhi, but they are often expensive and give a false result compared to bacterial cultures, involving time-consuming, complex sample pretreatment protocols. The immunoassay reactions to detect *Salmonella* had mainly focused on O, H antigens of *Salmonella* spp (Sanderson *et al.*, 2015).

Finally, infectious diseases remain a major source of human morbidity and mortality. All deaths were due to poor diagnosis. This poor performance is further exacerbated by a large amount of test-to-test variability (World Health Organization, 2019).

Aim of the study: a new diagnostic method more sensitive to typhoid fever. Therefore, micro-titer plate testing is not expensive and no special equipment.

MATERIALS AND METHODS

Study Design and Selection of The Patients:

Sample Collection: five ml venous blood samples were obtained from thousand (1000) consenting participants after the administration of a structured questionnaire; samples were collected in plain tubes. Samples were centrifuged at 1000 rpm (revolution per minute) for 10 minutes. The Sera were harvested using Eppendorf tubes and stored at -20°C.

Inclusion Criteria: Patients of all age groups and both sexes attending the inpatient and outpatient in Faquos Fever Hospital, with clinical presentation suggestive of enteric fever, continuous high fever for more than 7 days with abdominal discomfort. Patients presented with fever symptoms, all patients were Egyptians and they came from rural areas.

Exclusion Criteria: Non-compliant patients, very severely ill patients suffering from other non-enteric diseases and those who were found to be diagnosed with patients on antibiotics and those who were found to be diagnosed with other diseases such as malaria, hepatitis and dengue fever.

The Material Provided in The Micro-Titer Test:

Reagent: distilled water, sodium chloride, sodium citrate, HCL (conc.). All chemicals product from (El Nasr Pharmaceutical Chemicals Co.). *Salmonella* antigens suspensions had commercially available in 5ml. Linear, Cromotest, Spectrum and Spain reacts. All antigens' products give the same result.

Other requirements: Working reagents contain dissolved (2.2 g NaCl), (sodium

citrate: 8.9 g), (300 µl HCl), 100 ml. D.W. Sterile test tube's micropipettes, tips and micro-titer plate wells.

Procedures of Different Methods:

Blood Culture Technique: MacConkey agar is used to identify *salmonella***.** When incubated at 37°C, small colonies 1 to 2-mm in diameter, are visible on MacConkey agar after 24 to 48 hours. At 72 hours, colonies have grown.

Micro-Titer Plate Technique:

Step 1: Bring all reagents and serum samples at room temperature before testing. Shake well and mix antigen well before dispensing. Add 100 µl of working reagent in well A, add 50 µl of working reagent in wells B, to E, add 15 µl of serum sample in the well A, mix and serial dilution in the A well, transfer 50 µl from the well A into well E, and discard 50 µl out.

Step 2: Shake the Salmonella antigen suspension well before use and add 20 µl of this suspension to each well (A-E).

Step 3: Mix the material of the plate very carefully by stirring laterally on the side of the micro-titer plate. Keep the plate stationary at room temperature (20-25 °C) in a flat position away from sources of vibration, stirring. Read the result after (3) hours, providing that the plate remains motionless, protected from vibration.

Interpretation And Reading of The Results:

- Positive reaction: Formation ring at the bottom of the well.
- Negative reaction: No ring at the bottom of the well.
- As shown control positive and negative in figure 2.
- As shown in figures 3 & 4.

Slide Technique: One drop each of undiluted patients' serum samples for the four antigens is placed on the circled card and one drop of each of the four *Salmonella* antigens is added separately and gently rotated for one minute. The appearance of agglutination gives qualitative results. To know the titer for each of the antigens, the test is repeated with dilutions of serum.

Tube Technique: Set up a row of seven tubes and add 0.4mL of saline to tubes 2 and 7. Add 0.2mL of 1/5 antiserum to tubes 1 and 2. Mix the contents of tube 2 and perform doubling dilutions to tube 6 and then discard 0.2mL instead of adding it to tube 7. Add 0.2mL of the respective bacterial O or H suspension to each tube. The positive reaction is characterized by agglutination. Incubate at 37°C for 24 hours. The agglutination appears as a "matt" or "carpet" at the bottom.

Method Validation: Micro-titer plate procedures were considered for a number of reasons. Reduce technical workload, increase efficiency, reduce reporting time and lower cost per test. Furthermore, random error is both positive and negative relative to the observed mean value of replicate determinations. These tests are replicated from a single sample to illustrate the distribution around the mean. And the accuracy of the diagnosis by (micro-titer plate) can be described in relative to the best available estimate of the "true" value, the agreement between the "measured" values, the "true" value (Koch,1999).

Ethical Approval: Our study had approved by the institutional review board of the faculty of "Medicine, Zagazig University, Egypt", approval number (ZU-IRB #6272/6-9-2021).

Statistical analysis: This "quick start" guide shows you how to carry out Cohen's kappa using SPSS for Windows, version 21.0. Software. Statistics, **,** as well as interpreting and reporting the results from this test (Sim**,** 2005)**.**

RESULTS

In Figure (1), results show, the blood culture method of *typhi 'O'* and *typhi 'H'* were 64.2% and 62.9% respectively, microtiter plate method of *S. typhi 'O'* agglutinin and *S. typhi 'H'* agglutinin were 63% and 61.8% respectively. And slide agglutination methods of *S. typhi 'O'* and *S. typhi 'H'* were 24.8% and 28.9%. Finally, by tube agglutination of *S. typhi 'O'* and *S. typhi 'H'* were 29.2% and 33.1%. The competitive of infection by different used methods. Table (1) shows that in *S. typhi 'O'* agglutinin microtiter plate had a sensitivity of 95.3%, specifics of ty 94.9%,

accuracy of 95.2%, while slide agglutination had sensitivity 23.2%, specificity 72.3%, accuracy 40.8%. Finally, tube agglutination had a sensitivity of 27.6%, specificity 67.9%, and accuracy 42% of the patients. Table (1) shows that in *S. typhi 'O'* agglutinin microtiter plate had a sensitivity 94.6%, specificity 93.8%, the accuracy 94.3% while slide agglutination had a sensitivity 28.8%, specificity of 70.9%, and accuracy 44.4%. Finally, tube agglutination had a sensitivity of 32.6%, specificity of 66%, and accuracy 45% of the patients. Also, Table (1) shows in *S. typhi 'O'* agglutinmicrotiterter plate had sensitivity, specificity and accuracy is higher than *S. typhi 'H'* agglutinin microtiter plate in all patients.

Table (2) shows that in *S. typhi 'O'* culture and micro-titer plate agreed on 612 +ve cases and 340 –ve cases only and differ in 18 detected -ve by microtiter and +ve by micro-titer plate and 30 detected +ve by micro-titer and –ve by culture. In *S. typhi 'H'* culture and micro-titer plate agreed on 595 +ve cases and 348 –ve cases only and differ in 23 detected -ve by micro-titer plate and +ve by micro-titer plate and 34 detected +ve by micro-titer and –ve by culture. A high significant agreement was found between the two methods (Kappa =0.9 in *Typhi O* and 0.8 in *Typhi H*). Also, in Table (2) the agreement between culture and both slide and tube agglutination in the detection of +ve cases in *S. typhi 'O'* agglutinin and *S. typhi 'H'* agglutinin. A weak statistical non-significant agreement was found between the methods. (Kappa =0.04 in *Typhi O* and 0.04 in *Typhi H*).

Table (3) shows that in *S. typhi 'O'* agglutinin microtiter and slide agglutination agreed on 178 +ve cases and 271 –ve cases only and differ in 111 detected -ve by slide agglutination and +ve by micro-titer plate and 440 detected +ve by micro-titer plate and –ve by slide agglutination. In *S. typhi 'H'* agglutinin microtiter plate and slide agglutination agreed on 149 +ve cases and 271 –ve cases only and differ in 99 detected -ve by slide agglutination and +ve by micro-titer plate and 481 detected +ve by micro-titer plate and –ve by slide agglutination. Weak statistical non-significant agreement was found between the two methods. (Kappa =0.03 in *Typhi O* & 0.02 in *Typhi H*). Table (3) shows that in *S. typhi 'O'* agglutinin microtiter plate and tube agglutination agreed on 199 +ve cases and 250 –ve cases only and differ in 132 detected -ve by tube agglutination and +ve by micro-titer plate and 419 detected +ve by micro-titer plate and –ve by tube agglutination. In *S. typhi 'H'* agglutinin microtiter plate and tube agglutination agreed on 175 +ve cases and 253 –ve cases only and differ in 117 detected -ve by tube agglutination and +ve by micro-titer plate and 455 detected +ve by micro-titer plate and –ve by tube agglutination. A weak statistical non-significant agreement was found between the two methods (Kappa =0.03 in *Typhi O* & 0.02 in *Typhi H*).

Table 1: Validity of sensitivity, specificity, positive predictive value, negative predictive value and accuracy of different methods in diagnosis of *S. typhi 'O'* agglutinin and *S. typhi 'H'* agglutinin in comparison to blood culture.

Variable / **Validity**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**	**p**
Micro- titer plate O	95.3 %	94.9 %	97.1 %	91.9 %	95.2 %	**
Slide agglutination O	23.2 %	72.3 %	60.1 %	34.4 %	40.8 %	NS
Tube agglutination O	27.6 %	67.9 %	60.6 %	34.3%	42 %	NS
Micro-titer plate H	94.6 %	93.8 %	96.3 %	91.1 %	94.3 %	**
Slide agglutination H	28.8 %	70.9 %	62.6 %	37 %	44.4 %	NS
Tube agglutination H	32.6 %	66 %	61.9 %	36.6 %	45 %	NS

Kappa: Cohn's kappa test, PPV: positive predictive value, NPV: negative, predictive value**.** NS: non-significant (P>0.05) **high significant.

Table 2: Validity of different methods in diagnosis of *S. typhi 'O'* agglutinin and *S. typhi 'H'* agglutinin in comparison to culture as a gold standard test.

Variable		**Blood culture**		**Total**	**Kappa**	**P**
		+ve	***-ve***			
Micro- titer plate O	*+ve*	612	18	630	0.90	<0.001 **
	-ve	30	340	370		
	Total	642	358	1000		
Slide agglutination O	*+ve*	149	99	248	0.04	0.19 NS
	-ve	493	259	752		
	Total	642	358	1000		
Tube agglutination O	*+ve*	177	115	292	0.04	0.13 NS
	-ve	465	243	708		
	Total	642	358	1000		
Variable						
Micro- titer plate H	*+ve*	595	23	618	0.88	<0.001 **
	-ve	34	348	382		
	Total	629	371	1000		
Slide agglutination H	*+ve*	181	108	289	0.04	0.19 NS
	-ve	448	263	711		
	Total	629	371	1000		
Tube agglutination H	*+ve*	205	126	331	0.04	0.13 NS
	-ve	424	245	669		
	Total	629	371	1000		

Kappa: Cohn's kappa test NS: non-significant (P>0.05) **high significant.

Table 3: Agreement between micro titer plate and slide agglutination and tube agglutination in diagnosis of infectious diseases among the studied cases.

Variable		Micro - titer plate O		Total	Kappa	P
		+ve	-ve			
Slide agglutination O	*+ve*	178	111	289	0.03	0.93 NS
	-ve	440	271	711		
	Total	618	382	1000		
Slide agglutination H	*+ve*	149	99	248	0.02	0.27 NS
	-ve	481	271	752		
	Total	630	370	1000		
Tube agglutination O	*+ve*	199	132	331	0.03	0.92 NS
	-ve	419	250	669		
	Total	618	382	1000		
Tube agglutination H	*+ve*	175	117	292	0.02	0.26 NS
	-ve	455	253	708		
	Total	630	370	1000		

Kappa: Cohn's kappa test NS: non-significant (P>0.05)

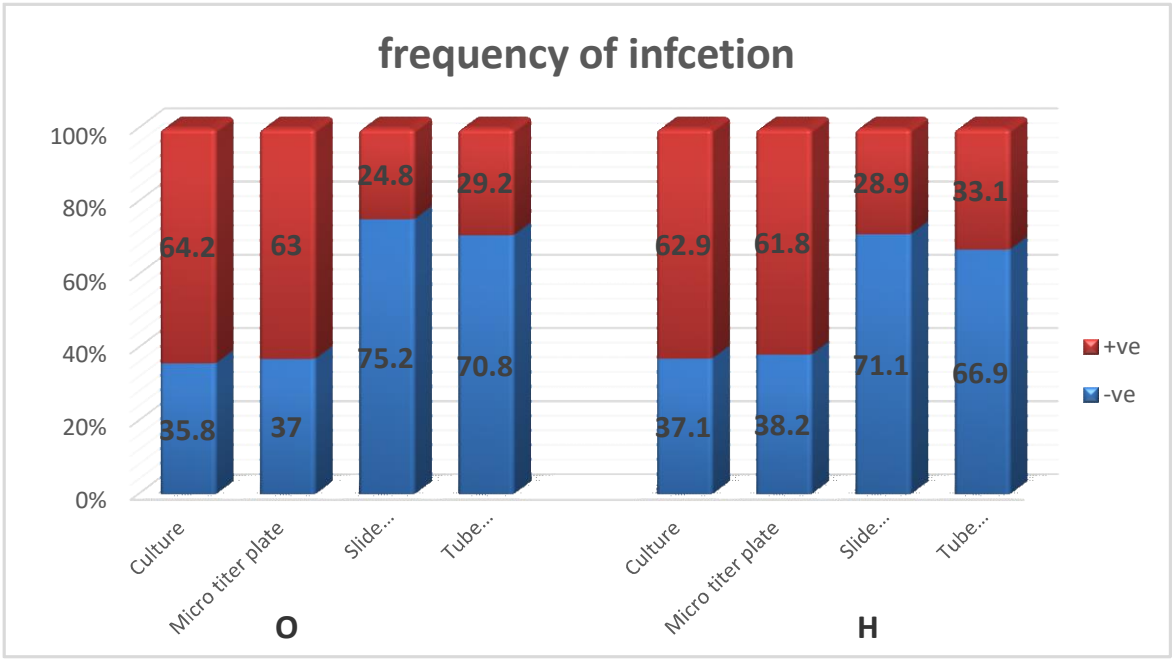

Fig. 1: Frequency of infection among the studied cases by different used methods (blood culture, micro-titer plate, slide agglutination and tube agglutination).

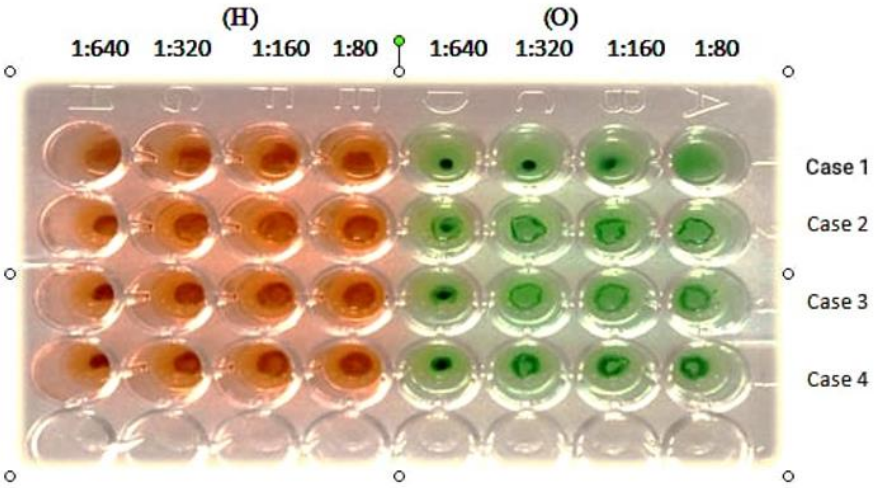

Fig.2: Determination of S. *typhi 'O'* agglutinin and S. *typhi 'H'* agglutinin by using buffer solution in micro titer plate wells (control): case 1, S. *typhi 'O'* and S. *typhi 'H'* (negative). And case 2,3,4. S. *typhi 'O'* and S. *typhi 'H'* (positive).

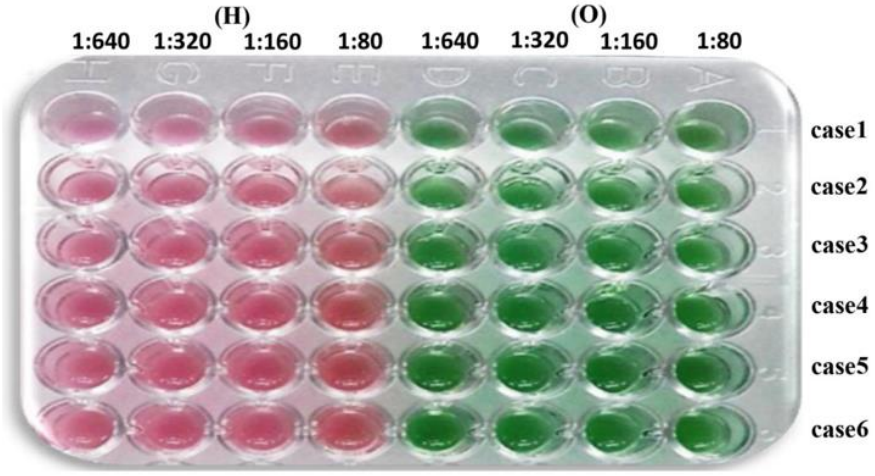

Fig.3: S. *typhi 'O'* agglutinin and S. *typhi 'H'* agglutinin by using buffer solution and micro titer plate wells (before the reaction).

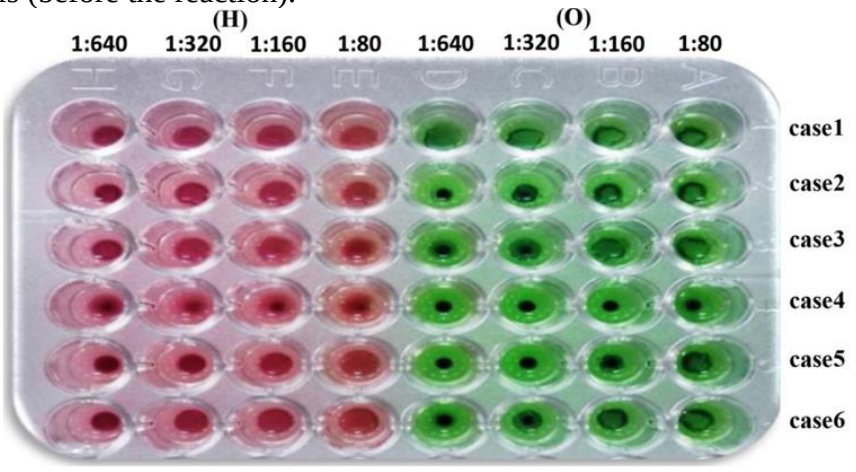

Fig.4: Determination of *S. typhi 'O'* agglutinin and *S. typhi 'H'* agglutinin by using buffer solution and micro titer plate wells (after the reaction): case 1 *S. typhi 'O'* and *S. typhi 'H'* 1:640, case 2 *S. typhi 'O'* 1:160 and *S. typhi 'H'* 1:640, case 3 *S. typhi 'O'* 1:320 and *S. typhi 'H'* 1:640, case 4 negative, case 5 *S. typhi 'O'* 1:160 and *S. typhi 'H'* 1:160, case 6 *S. typhi 'O'* 1:320 and *S. typhi 'H'* 1:320.

DISCUSSION

In our work, demonstrated a new diagnostic method of *Salmonella* antibody by micro-titer, this method is more sensitive when compared with different methods.

In our study, the micro-titer plate *S. typhi 'O'* agglutinin compared with blood culture *typhi 'O'* in the table (1) performed well as a test (P<0.001) since we observed PPV (97.1%). However, the high sensitivity was more so when *S. typhi 'O'* agglutinin micro-titer plate alone was considered, if the NPV was (91.9%). Also, in table (1) for the micro-titer plate *S. typhi 'H'* agglutinin compared with blood culture *typhi 'H'* the (P<0.001) and, PPV (96.3%), and NPV (91.1%). This clearly demonstrates that while the micro-titer plate test is useful in screening, the positivity it expresses always contributes to the correct diagnosis of disease.

Diagnosis of *Salmonella* should always be confirmed by micro-titer plate testing. Several studies have investigated the reasons for the low sensitivity of blood cultures. Traditional methods are generally inexpensive and simple, but these methods can be time-consuming because they depend on the ability of microorganisms to grow in different media, such as pre-enriched media, selective enrichment media, and selective plating media (Zhao *et al.,* 2014).

Typically, conventional methods take 2 to 3 days for initial identification and more than a week to confirm the type of pathogen (Laam *et al.,* 2014). Also, the classical methods are laborious as they require the preparation of culture media, inoculation of plates, and colony counting (Mandal *et al.,* 2011). Laboratory diagnosis by microtiter and the results obtained in the present study had incompatible with slide and tube agglutination, for slide and tube lack the sensitivity, and specificity to be reliably used at the diagnosis, where typhoid fever is diffuse; therefore, they have only a minor role in the serological confirmation of typhoid fever (Mather *et al.,* 2019).

In this study slide agglutination is a commonly used rapid screening test for this purpose, the technique still requires further developments to improve its diagnostic accuracy for typhoid fever. Comparison to the tube agglutination test is however also; this study found that the slide agglutination test performed the worst. It had very poor sensitivity, specificity, and low PPV. A weak statistical non-significant agreement was found between the two methods between the micro-titer plate and slide agglutination tests in the detection of *S. typhi 'O'* agglutinin, antigen was found in the table (1) shows *typhi 'O'* agglutinin (Kappa = 0.03) and (P>0.93) and *typhi 'H'* agglutinin (Kappa = 0.02) and (P>0.27). These results had in agreement with; enteric fever is an endemic disease in India and warrants rapid and affordable diagnosis. There are several difficulties associated with the evaluate of the slide agglutination test (Ujjwala *et al.,* 2014).

This is consistent with (Gaikwad *et al.,* 2014), who studied, diagnosis of typhoid by slide agglutination test cannot be used alone in the diagnosis of typhoid fever because a good number of patients will be falsely diagnosed positive and the doctor will submit them to antimicrobial treatment against typhoid fever. Consequently, result in the development of resistance by these bacteria to antibiotics, which suggests that these tests would not be useful in routine diagnostic situations.

Diagnosis of Widal by tube agglutination performed poorly as a diagnostic test using the method determined by the Widal test. Therefore; tube agglutination test should not be used as a diagnostic tool (Martin *et al.,* 2019). Tube agglutination would have performed better than the other typhoid rapid antibody tests, but it did no**t**, in vitro agglutination assay requires a greater technical effort. However, since it takes 18-24 hours to get results; in practice, a diagnosis is usually made based on slide agglutination results available within minutes. Slide agglutination as a screening test without confirming the results by tube agglutination may lead to a false diagnosis of enteric fever (Olopoenia *et al.,* 2000).

Globally, each year salmonella is a common cause of morbidity and mortality, especially in developing countries, where slide and test-tube agglutination are commonly used as diagnostic tools to rule out the disease. The diagnostic power of the Widal test is questionable because the test has low sensitivity and specificity of the test. Therefore, the technology still needs to be further developed to improve its diagnostic accuracy for typhoid fever (Hylemariam *et al.,* 2017). Weak laboratory control techniques may also lead to the spread of *Salmonella* in endemic areas. In addition, most endemic countries use serological tests with low sensitivity and low specificity, making the diagnosis unreliable (Ajibola *et al.,*2018). Accordingly, it was recommended that developed countries are often faced with the challenge of making treatment decisions on the basis of compatible clinical symptoms alone or a combination of clinical symptoms and Widal results obtained from acute-result from one patient. These factors contribute to a high rate of inaccurate diagnosis of typhoid because poor of diagnosis (Wasihun *et al.,*2015).

CONCLUSION:

Diagnosis of salmonella antibodies by micro-titer plate wells are reliable, this method is simple, highly sensitive, specific, and quantitative in diagnosing typhoid fever when compared with different diagnostic method.

Recommendations:

1- Early diagnosis of *salmonella* antibodies by micro-titer plate help to decrease possible complications and avoid false results.

2-This method can be generalized to diagnose some types of bacteria and viruses.

3 - Manufacturing a new type of ELISA dish suitable for the diagnosis of *salmonella*.

Competing interest: None.

REFERENCES

Ajibola O, Mshelia MB, Gulumbe BH, Eze AA. 2018. "Typhoid Fever Diagnosis in Endemic Countries": A Clog in the Wheel of Progress? *Medicina,*54(2), 23.

Andualem G, Abebe T, Kebede N, Gebre-Selassie S, Mihret A, and Alemayehu H. 2014. "A comparative study of Widal test with blood culture in the diagnosis of fever in febrile cases". *BMC Research Notes,* 17; 7:653.

Clegg AM, Passey M, Omena K, Karigifa, Suve N. 1994. "Reevaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea". *Acta Tropica,* 57(4):255-63.

Gaikwad U N, Rajurkar M. 2014. "Diagnostic efficacy of widal slide agglutination test against widal tube agglutination test in enteric fever". *International Journal of Medicine, Public Health*,4:6-9.

Hatmal MM, Al-Hatamleh MAI, Olaimat AN, Hatmal M, Alhaj-Qasem DM, Olaimat TM. 2021. "Laboratory diagnosis of paratyphoid fever: opportunity of surface plasmon resonance". *Vaccines (Basel),* 26; 9(6):556.

Hylemariam Mihiretie Mengist, Kelemu Tilahun. 2017. "Diagnostic Value of Widal test in the Diagnosis of Typhoid Fever": A Systematic Review. *Journal* of *Medical Microbiology* & Diagnosis, 6: 248.

Ismail A, Kader ZS, Kok-Hai O. 1991. "Dot enzyme Immunosorbent assay for the sero-diagnosis of typhoid fever". *Southeast Asian Journal* of *Tropical Medicine* and *Public Health*, 22(4): 563-6.

Koch DD, Peters T.1999. "Selection, evaluation of methods". In: Tietz Textbook of Clinical Chemistry, 3rd ed, CA. BURTIS, ER. ASHWOOD (Eds.), W.B. *Saunders Company, Philadelphia.* 320-335.

Laam Li, Nilmini Mendis, Hana Trigui, James D. Oliver , Sebastien P. Faucher. 2014. "The importance of the viable but non-culturable state in human bacterial pathogens". *Frontiers Microbiology*, 2;5:258.

Mandal PK, Biswas AK, Choi K, Pal UK. 2011. "Methods for rapid detection of foodborne pathogens": an overview. *American Journal* of *Food Technology*, 6, 87–102.

Martin E. Ohanu, Michael O. Iroezindu, Uzoamaka Maduakor, Obinna D Onodugo, Harish C Gugnani.2019. "Fever among febrile Nigerian cases: Critical, diagnostic performance of the Widal test, antibiotic multi-drug resistance". *Malawi Medical Journal*, 31 (3): 184-192.

Mather RG, Hopkins H, Parry CM. 2019. "Redefining typhoid diagnosis": what would an improved test need to look like? *BMJ Global Health,* 29:453–8.

Najib MA, Mustaffa KMF, Ong EBB, Selvam K, Khalid MF, Awang MS.2021. "Performance of Immunodiagnostic Tests for Typhoid Fever": A Systematic Review and Meta-Analysis. *Pathogens*, 13; 10(9): 1184.

Olopoenia LA, King AL.2000. "Widal agglutination test - 100 years later: Still plagued by controversy". *Postgraduate Medical Journal*, 76(892):80-4.

Pang T, Levine MM, Ivanoff B, Wain J, Finlay BB. 1998."Typhoid fever important issues still remain". *Trends Microbiology*, 6(4): 131-3.

Sahastrabuddhe S, Carbis R, Wierzba TF, Ochiai RL. 2013."Increasing rates of Salmonella paratyphi A, the status of its vaccine development". *Expert Review of Vaccines,* 12(9):1021-31.

Sanderson KE, Liu SL, Tang L, Johnston RN. 2015. "Salmonella Typhi and Salmonella Paratyphi A. In Molecular Medical Microbiology"; Elsevier BV: *Amsterdam, the Netherlands.* Pp 1275-1306.

Sim J, Wright CC. 2005."the Kappa Statistic in Reliability Studies: Use, Interpretation, and Sample Size Requirements". *Physical Therapy,* 85 (3):257-268.

Ujjwala, N. Gaikwad, Monali Rajurkar. 2014. "Diagnostic efficacy of Widal slide agglutination test against Widal tube agglutination test in enteric fever". *International Journal of Medicine, Public Health*,4:227-30.

Wasihun AG, Wlekidan LN, Gebremariam SA, Welderufael AL, Muthupandian S, Haile TD.2015."Diagnosis and treatment of typhoid fever and associated prevailing drug resistance in northern Ethiopia". *The International Journal of Infectious Diseases*,2015; 35:96–102.

World Health Organization. 2019." World Health Statistics Overview". WHO, Switzerland, available at/.1/.https:// apps.who.int/iris/bitstream/handle/10 665/311696/WHO-DAD-.1eng.pdf.

World Health Organization.2014. "Water-related diseases". Available at http://www.who.int/ water-_sanitation -_health/ diseases/typhoid/en/.

Zhao X, Lin CW, Wang J, Oh DH. 2014. Advances in rapid detection methods for foodborne pathogens. *Journal of Microbiology and Biotechnology*,24, 297–312.