

Recent advances in the rapid serodiagnosis of Typhoid fever S3-1

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Abstrak

Pengembangan suatu tes diagnosis spesifik untuk tifoid memerlukan penggunaan antigen spesifik. Penemuan antigen spesifik 50 kDa pada membran luar *Salmonella typhi* membawa kepada pengembangan suatu tes cepat Dot EIA (Typhidot™) untuk mendeteksi adanya IgM dan IgG antibodi spesifik anti baktri. Tes ini mempunyai sensitivitas 95%, nilai duga negatif yang tinggi dan dapat memberikan hasil dalam waktu 3 jam. Deteksi IgM saja atau dengan IgG akan memberikan kesan tifoid akut, sementara deteksi IgG saja memberikan beberapa interpretasi seperti konvalesen atau kemungkinan infeksi ulang. Disebabkan tidak adanya kekebalan terhadap demam tifoid, pasien pada daerah endemisitas tinggi sering mengalami infeksi ulang. Pada kejadian infeksi ulang akan terjadi tanggapan kebal sekunder dengan suatu efek peningkatan tajam IgG yang bermakna, melebihi IgM yang pada akhirnya tidak dapat dideteksi. Kemungkinan strategi untuk memecahkan masalah ini adalah mendeteksi adanya IgM yang tersembunyi dengan menyingkirkan IgG total. Pengembangan suatu tes deteksi IgM 3 jam (Typhidot-M™) telah memberikan diagnosis demam tifoid akut secara tepat dan dapat digunakan untuk membedakan kasus akut dari konvalesen. Data penelitian untuk mengevaluasi sensitivitas, spesifitas dan keuntungan dari berbagai tes dibandingkan dengan metode konvensional memperlihatkan bahwa tes tersebut memberikan suatu tes alternatif yang dapat diandalkan selain tes Widal.

Abstract

Development of a specific diagnostic test for typhoid requires the use of a specific antigen. The discovery of the specific 50kD antigen in the outer membrane of *Salmonella typhi* led to the development of a rapid dot EIA test (Typhidot™) to detect for the presence of specific IgM and IgG antibodies to the bacteria. The test has a sensitivity of 95%, a high negative predictive value and could produce results within 3 hours. The detection of IgM alone or with IgG would suggest acute typhoid while the detection of IgG only posed several interpretations such as convalescence or possible re-infection. Due to the lack of immunity to typhoid fever, patients in highly endemic areas often have re-infections. In the event of current re-infection, there will be a secondary immune response with a significant "boosting" effect of IgG over IgM such that the latter could not be detected. A possible strategy to resolve this problem is to "unmask" the presence of IgM by removing total IgG. The development of a 3 hour IgM detection test (Typhidot-M™) is useful in areas of high endemicity since it could differentiate new from convalescence cases. Data from studies to demonstrate the sensitivity, specificity and advantages of the various tests compared to conventional methods showed that the tests provided reliable alternatives to the Widal test.

INTRODUCTION

Typhoid fever remains a public health problem in most developing countries. The available conventional methods remain unsatisfactory since they are too slow to allow quick decision by the clinician. Culture method may show specificity but it lacked sensitivity and speed. It produced results within 2-7 days and cases of culture negative typhoid were well recognised^{1,2}. The antibody detection test (Widal test) although widely used, lacked speed, sensitivity and specificity. For meaningful interpretation of the test, demonstration of 4 fold rise in antibody titers between acute and convalescent sera 10-14 days later was essential.

An ideal diagnostic test for typhoid should be rapid, easy to perform, sensitive as well as specific. Neither of the methods mentioned satisfied the criteria. Thus there is a need to develop a rapid and specific test. Combined with sensitive diagnosis, the test would provide for prompt and effective management of typhoid fever.

RATIONALIZATION TOWARDS DEVELOPMENT OF A RAPID ANTIBODY DETECTION TEST FOR TYPHOID

The availability of a specific antigen for *S. typhi* would allow for the development of a specific test for typhoid. We have previously determined that the 50 kD outer membrane protein is antigenic as well as specific for *S. typhi* since it only reacted immunologically with typhoid sera³. Further evaluation of the antigen using the dot EIA method revealed that the 50 kD antigen could detect the presence of specific IgM and IgG in sera from patients with acute typhoid⁴.

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This discovery led to the development of Typhidot, a dot enzyme immunoassay for the rapid detection of specific IgM and IgG antibodies to *S. typhi*.

In designing an antibody detection test, several factors need to be considered. A test that detected IgM alone would reveal acute typhoid while detection of both IgM and IgG would also suggest acute typhoid but in the middle-phase of infection. On the other hand, a test that detected either total antibody or IgG alone posed several interpretations such as convalescence or re-infection. The long presence of IgG after an infection combined with excessive exposure to the organism in highly endemic areas would significantly contribute towards apparent false positive results for such a test. In the event of current re-infection, there will be a secondary immune response with a significant "boosting" effect of IgG over IgM such that the latter could not be detected. A possible strategy to resolve this problem is to remove the IgG so as to "unmask" the presence of IgM. Creation of such a diagnostic test would differentiate new from past infections. This paper described the use of the specific antigen for *S. typhi* in the development of a rapid dot enzyme immunoassay test named Typhidot-M to detect for the presence of specific IgM in acute typhoid. The test could produce results in 3 hours.

Retrospective studies were performed to evaluate both dot enzyme immunoassays (Typhidot and Typhidot-M) with regard to their sensitivity, specificity,

positive and negative predictive values compared to Widal and culture method. Usefulness of both tests in the various clinical settings were also discussed.

MATERIALS AND METHODS

Development of a rapid IgM detection test for typhoid

In an antibody detection test, in order to detect for the presence of a specific IgM, total IgG in the serum sample must be removed or inactivated. Inactivation of IgG would remove competitive binding and allow accessibility of the antigen to the specific IgM, if present. Prior to the development of the test, the bacteria was grown, harvested and its antigens prepared as previously described³. Following this, the patient's sera and control sera were IgG inactivated and incubated with nitrocellulose that had been previously predotted with the specific antigen. A period of one hour was given for the antibody-antigen reaction to form a complex. To visualise the antigen-antibody complex, the strips were incubated with peroxidase conjugated anti-Human IgM. After a reaction time of one hour, a chromogenic substrate was added for positive antigen-antibody reaction, indicated by colour development. The results were read visually. Positive results were indicated by the development of a blue colour as intense or more intense than that of the positive control. The total assay time for the test is 3 hours. A detailed mechanism of the rapid IgM detection test called Typhidot-M is shown in Figure 1.

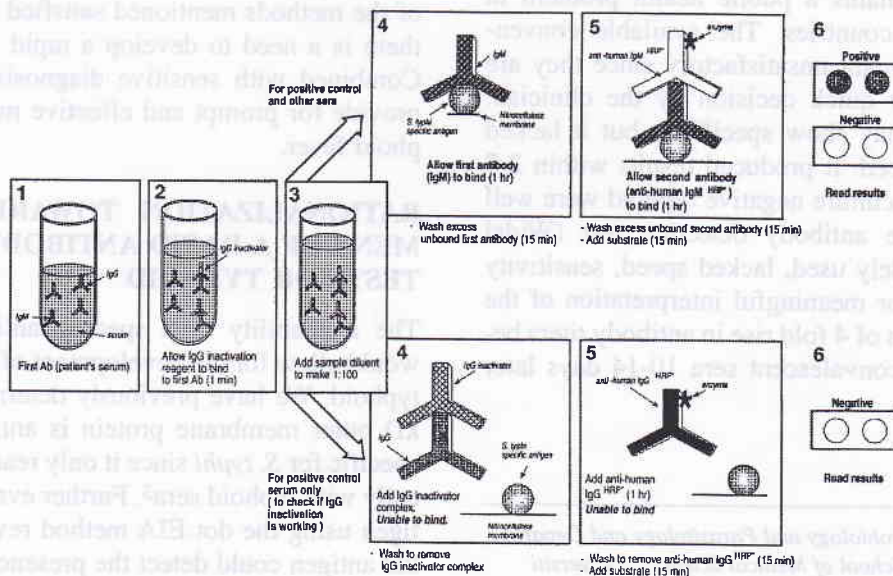


Figure 1. Assay Procedure for TYPHIDOT - M

Retrospective studies to evaluate the usefulness of Typhidot and Typhidot-M

In order to evaluate the performance of both assays, retrospective studies were done in an endemic area among febrile children admitted into the General Hospital, Kota Bharu, Kelantan, Malaysia and among the outpatients (comprising of patients of all ages) that attended the outpatient clinic or from the Accident and Emergency Unit at the Universiti Sains Malaysia Hospital, Kubang Kerian, Kelantan, Malaysia. The aim was to evaluate the performance of the tests compared to Widal and the culture method among febrile patients in a usual hospital setting.

The study among 135 febrile children were divided into 2 groups; i) those who were culture positive and had clinical features suggestive of typhoid fever (n=62) and ii) those who were culture negative and had clinical evidence of another diagnosis (n=73). The list of non typhoid included malaria, dengue, paratyphoid, scrub typhus, hepatitis, gram-negative septicaemia, and viral fevers. The criteria for clinical typhoid was as described in¹. To evaluate the performance of the tests used amongst adults and children, a second retrospective study was performed. The study comprised of 103 patients of all ages attending the outpatient and accident and emergency clinics. The criteria for division into the two groups were similar to those used for the febrile children.

RESULTS

Table 1. Comparison of culture, Widal, Typhidot and Typhidot-M to clinical diagnosis among febrile children admitted to a hospital in an endemic area.

Category	No	Culture	Widal	Typhidot	Typhidot-M
Clinically typhoid	62	28	57	56	56
Clinically non-typhoid	73	0	14	6	5
	135				
Sensitivity		45.2%	91.1%	90.3%	90.3%
Specificity		100%	80.8%	91.7%	93.1%
Positive Predictive Value		100%	80.3%	90.3%	91.8%
Negative Predictive Value		68.2%	92.2%	91.8%	91.9%

The results obtained for the retrospective study among febrile children were summarised in Table 1. The results showed very little difference in diagnostic sensitivity between Widal, Typhidot and Typhidot-M when used amongst febrile children (>90%). However, sensitivity shown by the culture technique was markedly low (45.2%). In terms of specificity, the culture technique showed 100% accuracy. Typhidot and Typhidot-M gave better results (>90% specificity) compared to the Widal test (81%). Despite being used in an endemic area, the use of Widal, Typhidot and Typhidot-M amongst children showed comparable high negative predictive values (>90%) while the culture method remained low (68%).

Table 2. Comparison of culture, Widal, Typhidot and Typhidot-M to clinical diagnosis among outpatients of all ages attending a hospital in an endemic area.

Category	No	Culture	Widal	Typhidot	Typhidot-M
Clinically typhoid	52	44	32	48	48
Clinically non-typhoid	51	0	3	7	0
	103				
Sensitivity		85.0%	77.0%	92.0%	92.0%
Specificity		100%	94.0%	88.0%	100%
Positive Predictive Value		100%	95.0%	88.0%	100%
Negative Predictive Value		86.0%	81.0%	93.0%	93.0%

When tested among outpatients of all ages, the results summarised in Table 2 showed Typhidot and Typhidot-M to have the best sensitivity profiles amongst the tests used but with little difference in sensitivity pattern between them (both at 92%). The culture technique showed an improved sensitivity of 85% compared to that obtained for the children group, while Widal showed the least sensitivity (77%). In terms of specificity, Typhidot-M proved to be equal to the culture technique at 100%. Widal showed a high specificity result of (94%) while Typhidot dropped to 88%. However, in predicting negative typhoid in an endemic area, both dot EIA tests were equally superior (both at 93%) when compared to Widal (81%) and culture (86%).

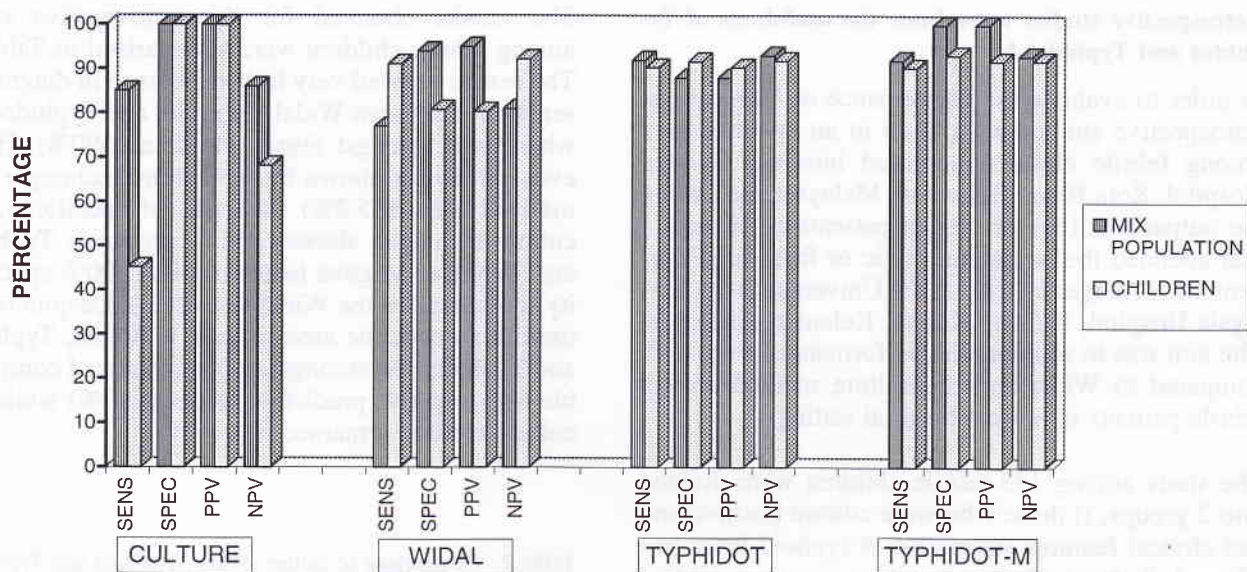


Figure 2. Comparative usefulness of the Widal, culture, Typhidot and Typhidot-M among the paediatric patients and patients of all ages. The various tests were shown in terms of their sensitivity (SENS), specificity (SPEC), positive predictive value (PPV) and negative predictive value (NPV).

In order to demonstrate the usefulness of the various tests used in the diagnosis of typhoid, results from the 2 studies were combined and compared to evaluate the significance of each test when used among paediatrics alone and amongst a mixed population of adults and children comprising of all ages (see Figure 2).

Data from Figure 2 suggested that Widal and culture showed varying performances in sensitivity and specificity patterns depending on the age group of the patient. However, both Typhidot and Typhidot-M maintained constant superior results regardless of patient age group.

CONCLUSION

It was the intention of the study to compare culture, Widal, Typhidot and Typhidot-M to clinical diagnosis since all four methods tested were simply diagnostic tools used to confirm clinical suspicion of typhoid. In the laboratory diagnosis for typhoid fever, a test regarded as the gold standard should approach 100% in terms of its sensitivity, specificity, positive and negative predictive values when used in patients, regardless of the age group. Results of the retrospective study showed that Typhidot and Typhidot-M were superior to the culture method. Although culture was regarded as the gold standard, it could not compete with Typhidot and Typhidot-M tests in terms of sensitivity, negative predictive value as well as speed.

Despite problems of accurate diagnosis associated with the Widal test, our studies showed that the test may be useful among paediatric patients. Other studies on the Widal test used among febrile children in endemic areas have also reported similar conclusions⁵. Our studies showed that Typhidot and Typhidot-M satisfied the criteria for an ideal diagnostic test for typhoid and hence could be used to replace the Widal test. Both tests which had the added advantage of diagnosis using a single serum specimen could be used in conjunction with the culture method for the rapid and accurate diagnosis of typhoid fever. The culture method despite shortfalls in speed and sensitivity is still useful for antibiotic sensitivity testing. The lack of special equipment needed to perform both dot EIA tests allowed them to be used in the field and in the small district hospitals where culture facilities may not be available. Another added advantage of the tests is its stability. Antigens predotted and preblocked on the nitrocellulose strips are stable for at least 1 year when kept at 4°C.

It is interesting to note that Typhidot and Typhidot-M have comparable high diagnostic performances when tested in Malaysia. This may be due to the fact that Malaysia has a low incidence of typhoid. In a population of high endemicity where incidence as well as relapse cases are rampant, Typhidot-M is anticipated to perform better over Typhidot in providing an accurate and reliable diagnosis of new infections. The

availability of highly specific rapid diagnostic tests for typhoid provides the avenue for clinicians to decide on prompt and effective management.

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