A preliminary report on comparison of tube and slide agglutination tests in the diagnosis of typhoid

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Summary

A comparison between the standard agglutination test (SAT) by the conventional tube test and a slide agglutination method was carried out in 11 patients with culture proven typhoid, 18 patients with a clinical and serological diagnosis of typhoid and 34 patients with non-typhoidal illnesses. 55% of patients in the first group had a negative O titre using the slide agglutination test. There was a 73% discrepancy between the 2 tests in the O titre in the first group and 56% and 50% discrepancy in the 2nd and 3rd groups respectively. The H antibody titre gave more comparable results in the 2 tests. Further validation of the slide agglutination test is required before it is recommended for clinical practice.

Key words: Standard agglutination test, tube method, rapid slide method, comparison

Introduction

The Standard Agglutination Test (SAT) is the commonest laboratory test used in the diagnosis of typhoid in Sri Lanka. The probability of errors of diagnosis using this test have been highlighted recently (1). The SAT is performed conventionally using a tube agglutination method which requires standardised antigens and overnight incubation (2). The 24 hour delay in obtaining results using this method have resulted in attempts to provide a rapid diagnostic test using a slide agglutination method (3,4). However, methods for standardisation of the reagents and validation of the slide agglutination test have not been extensively carried out. Some doubt has been cast on the accuracy of the results obtained with the slide test which compound the problem of diagnosing typhoid fever using the SAT.

The slide agglutination test has been introduced into laboratory practice in Sri Lanka, particularly in the private sector, in order to provide quick results. Antibody detecting kits from at least 5 commercial sources are available for this purpose. There is no published data locally with reference to the comparability of the slide test with the tube test. A preliminary study was therefore carried out to compare the results of one such commercial kit with the SAT performed with the conventional method.

Materials and Methods

Slide agglutination test

One kit (Citilab diagnostics, Daytonia Ltd.) sufficient for 50 tests was used between August 1991 and March 1992. The expiry date of the kit is July 1992.

The test was carried out according to the manufacturer's instructions. Clean transparent slides were used for the test. Two aliquots of 0.02 ml. of serum was pipetted onto the slide and one drop each of the *Salmonella typhi* O and H antigens from the test kit added to the serum as instructed, using the droppers provided.

The serum/antigen were mixed thoroughly and the slide rotated manually for 2 minutes. The slide was read using transillumination.

Sera which gave positive agglutination with the test antigens were serially diluted upto 1:3200, starting from a dilution of 1:50 and the test repeated as before. The final titre at which agglutination was seen was recorded.

Tube agglutination test

O and H antigens used for the SAT were prepared in the laboratory using *S. typhi* strains and methods provided by the Central Public Health Laboratory, Colindale, U.K. and checked

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for potency using antisera of known titre (Wellcome Burroughs). The SAT was performed using the standard procedure (2).

Patient sera

Sera from patients on whom an SAT was requested by clinical staff was tested using both methods. The slide agglutination test was carried out by a single investigator (VT) as described above and the tube agglutination test by a separate investigator (ATPG). The tests were done concurrently and the results compared only at the end of the study.

Results

The reagents provided in the kit were sufficient for 63 tests. Patients whose sera were tested fell into 3 diagnostic categories in relation to typhoid which are shown in Table 1.

Serological tests involving serial dilutions have an inherent variability of 1 dilution (5). Therefore, the results of the 2 tests were considered to be discrepant only when they differed by more than 2 dilutions (a fourfold difference in titre). Table 2 shows the number of sera in each group which gave such discrepant results. Table 2 and Table 3 indicates the number of patients in Group 1 with an O or H antibody titre of over 1:120 using the 2 test systems.

Table 1 : Patient groups

	No. of patients	*Diagnosis*	*Criteria for diagnosis*
Group 1	11	typhoid	positive cultures in blood, urine or faeces
Group 2	18	typhoid	clinical picture suggestive of typhoid with O titre > 1:240 (1)
Group 3	34	non-typhoidal illness	clinical + laboratory support

Table 2 : Number of sera with discrepant results for O and H antibody titre

(n)	*O antibody*	*H antibody*
Group 1 (11)	8 (73%)	1 (9%)
Group 2 (18)	10 (56%)	4 (22%)
Group 3 (34)	17 (50%)	3 (9%)

Table 3 : Number of positive SAT results (O,H > 1/120) in Group 1.

	O antibody > 1:120	*H antibody > 1:120*
Slide test	4	6
Tube test	11	5

Discussion

A preliminary study comparing 1 commercial kit used for the rapid serological diagnosis of typhoid fever with the conventional SAT showed important discrepancies between the 2 tests.

The patients whose sera was examined were divided into 3 groups depending on the final diagnosis and the criteria used for such diagnosis. In 11 patients the diagnosis of typhoid was based on the isolation of *S. typhi* from blood, urine or faeces. All patients had an O titre of > 1:120 or more using the overnight tube test. However, 6 of the 11 patients (55%) had a negative O titre using the rapid slide agglutination method. Two possible reasons could be postulated for this serious discrepancy: (a) the O antibody titre estimation using the conventional tube test requires incubation in a 37°C waterbath for 2 hours followed by overnight incubation at 4°C. O antigen detection using a rapid method would require alteration and stabilization of the antigen and it is possible that the antigen in the kit did not meet these requirements. (b) The other possible explanation is that the prozone phenomenon (2) which occurs with high titre sera could give a negative result at the screening dilution.

The H antibody titre was more comparable between the 2 test systems. However, this test is

not a very sensitive indicator of typhoid (1) as shown in the present study as well, in that 5 of the 11 patients in group 1 had H antibody titres of < 1:120. The tube agglutination test is a well standardised procedure and if proper controls are used, gives reproducible results. Although the slide agglutination test was developed to circumvent the delay in obtaining results, sufficient validation of the test has not been carried out. This preliminary study suggests that the discrepancies between the tube test and slide test could be of significance and result in misleading clinical users of this test. Further studies are necessary to compare the results of the slide tests available locally with the more established method. Until such time, caution should prevail in the use of slide tests in clinical practice.

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