A SLIDE AGGLUTINATION TEST FOR THE EXCLUSION AND DIAGNOSIS OF TYPHOID FEVER

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Both the qualitative receptor analyses and the classical Widal reaction can only be performed in well-equipped bacteriological laboratories; a sensitive and simple method (for diagnostic agglutination) practicable under primitive conditions is lacking. We have, therefore, endeavoured to work out a method which could be used both for diagnosis at the patient's bedside and for rapid and exact examination of sera in a laboratory.

For simplicity, we use the method of slide agglutination (S.A.) with undiluted serum. To be of practical significance, the test must be a sensitive indicator of O as well as H agglutinins.

The suspension is prepared roughly as follows: A single colony of Felix's very sensitive strain H 901 is tested for its motility, agglutinability and smoothness. If these are satisfactory, it is then sown on to ordinary nutrient 2% agar in three Roux bottles. A fourth Roux bottle is sown with the non-motile variant O 901. After 48 hr. the growth is washed off the surface of the agar with about 150 c.c. of physiological saline to which is added 0·6 % of formol. This suspension is then diluted to contain 9000 million bacilli per c.c., 0·2 % of a 10 % alcoholic solution of crystal violet is then added. After 24 hr. the suspension is sterile and ready for use. It can be stored at room temperature and kept for a long time.

The suspension is tested against the serum of a patient suffering from typhoid fever, which must have an O titre of 1 : 80 and the same or a somewhat lower H titre. With this serum, the reaction must be clearly positive. With serum taken from a healthy person, with titre 1 : 20, no reaction must take place.

The technique of the test is very simple, the time needed for its performance and for examination of the results being less than 3 min. A drop of the serum to be examined is placed on a clean slide and a similar-sized drop of the antigen is added. The antigen must be well shaken before use. With the corner of another slide the two drops are well mixed. The reaction can be examined immediately by holding the slide against a light background. If the reaction is positive, opaque blue coloured spots appear against the background. If negative, the drop remains homogeneous and clear. Examination of the test occupies a minute. Positive reactions appearing after 2 or 3 min. are very rare. The reaction can be seen with the naked eye.

Sera should be collected without the use of an anti-coagulant. Haemolysis does not interfere, and coloured sera give as satisfactory results as do clear ones.

The utility of this slide agglutination method has been tested on 544 sera, 130 of which came from patients under clinical observation at the hospital in Mukačevo, while 414 were sent to our laboratory for a Widal test. The Widal test was performed with a well-agglutinable formolized broth suspension. When the Widal reaction was negative or weak, and in doubtful cases, living cultures of H 901 and O 901 or killed H and O suspensions were used.

Table I shows results of experiments comparing the Widal and slide agglutination reactions. The table shows that all sera with a Widal titre of 1 : 80 or more gave a positive S.A. reaction. This is important, because Gregory & Atkinson have shown that only 0·6 % of sera from healthy persons have an H titre of 1 : 80, and only 2·3 % have this O titre.

Table I. *Comparison of Widal and slide agglutination reactions performed on* 414 *sera sent to the laboratory for diagnosis*

Widal titre	No. of cases	No. of S.A. positive cases	No. of S.A. negative cases
0–20	214	3	211
40	40	26	14
80	54	54	—
160 or higher	106	106	—

Of forty sera with a Widal titre of 1 : 40, 26 gave a weak (gradually appearing) positive S.A. reaction. Unfortunately, we have not been able to ascertain whether all of these cases were connected with typhoid fever. In only four of them was a blood culture positive; two others were persons recently vaccinated. Of the three cases with positive S.A. reaction and Widal titre between 0 and 1 : 20, one only had a positive blood culture. No particulars concerning the remaining cases could be discovered.

The advantage of the S.A. method over the classical Widal titration appears plainly when one considers those cases which showed no Widal titre, but reacted with a living H 901 culture in a dilution of 1 : 80 or higher and showed a positive S.A. reaction. We collected twenty-six such cases.

Among sera sent to our laboratory (for diagnosis) we found that in every case in which there was a suspicion of typhoid fever, the S.A. was positive. Unfortunately we were unable to learn very much about the history of these patients, so that it was necessary to try the S.A. method in a hospital where patients could be kept under strict clinical observation. This we were able to do in the hospital at Mukačevo, where typhoid fever is a very common disease.

The diagnosis of all cases was made on the basis of typical symptoms, of clinical progress and of bacteriological examination. Widal test results were recorded, but diagnosis was not based on them.

We examined 100 typhoid fever cases, the majority of which were brought to hospital during the second week of their sickness or later. With two exceptions, these cases all showed a positive S.A. reaction. In these two cases, the S.A. was still negative in the third week, but became positive later. In both of them the Widal reaction was negative also. In them it is probable that antibodies were scarce and produced late.

A few of the cases admitted in the first week already showed a positive S.A. reaction, while the Widal was still negative.

A slowly developing S.A. reaction was seen in only one case of typhoid in the first week; and in this case, the S.A. became positive within 1 min. during the second week.

Of thirty cases of dysentery, malaria, meningitis, etc. who had no recollection of having had typhoid fever, twenty-eight gave negative S.A. reactions. In two cases (one malaria, one dysentery), the S.A. was positive after 2–3 min. Agglutination tests with the H 901 culture revealed a titre of 1 : 40 in one only of these two sera. Both of these cases came from a heavily infected environment, and it is possible that they had had typhoid fever without recognizing it.

Table II. *Comparison of Widal and slide agglutination reactions performed on* 100 *cases clinically diagnosed as typhoid fever, and on thirty control cases (dysentery, malaria, meningitis, etc.)*

Illness	Result of the S.A.	Widal titre	No. of cases
I. Typhoid fever	3rd week negative later positive	None	2
	Positive	None	24
	Positive	1 : 20–1 : 40	6
	Positive	1 : 80	12
	Positive	1 : 160 or higher	56
II. Control cases	Negative	None	28
	Late positive	None	1
	Late positive	1 : 40	1

Four other cases recently convalescent from typhoid fever but coming to hospital on account of other febrile diseases gave a positive S.A. reaction.

Of eight paratyphoid cases, seven were S.A. positive. This can (probably?) be explained by the fact that *Bact. typhosum* and *Bact. paratyphosum* B share an O antigen (XII).

In those cases in which the S.A. is slow, we recommend that the test should be repeated after a few days. If the case is one of typhoid, the S.A. becomes faster as the antibody titre rises.

The probable error of the S.A. test judged on our clinical cases, two of which were negative in the second week, is therefore 3 %, and the limits of error are 0 and 7 %. On the other hand, the probable error of the classical Widal test as revealed by our clinical cases was 32 %, which is in agreement with other investigators (Delbove & Brison, 1938; Gardner *et al.* 1930).

From this we conclude that the Widal reaction ought to be used in the form of a qualitative receptor analysis using the living strain H 901 or killed O and H suspensions (Felix, 1930), or, when this is not possible, then our S.A. method, employing the antigen we describe, should be used. Our method also makes possible a rapid differentiation of positive, doubtful and frankly negative sera.

The manner in which our suspension acts is explained thus. It is made of the well-flagellated strain H 901 and the non-flagellated strain O 901, in the proportion of three parts of the former to one of the latter, mixed with a

relatively large amount of formol. This might suggest that it reacts mainly with H agglutinins, which would of course diminish its value. O agglutinins appear regularly in the first days of typhoid fever and are very important guides to diagnosis, in spite of their occasional appearance in healthy persons and their non-specific (group) character. The importance of O agglutinins was revealed by the qualitative receptor analysis, and explains the superiority of this method over the traditional Widal test (Pijper, 1930). Any method must therefore detect the O agglutinins. The antigen suspension which we describe shows a clear reaction with pure O sera of titre 1 : 80 or higher; with the slide agglutination method O agglutination appears more readily than does H agglutination. We made frequent observations and found that most *Salmonella* sera would react with bacteria possessing similar O antigens but different H antigens. Such reactions were often obtained at serum dilutions higher than the test-tube titre of the serum for O antigen. The addition of crystal violet and formol to the suspension seems to be of some importance for the sensitivity of the reaction. Other stains were tried, but they did not give such good results.

SUMMARY

A sensitive antigen suspension is described for use with a simple slide agglutination method which makes possible a serological diagnosis or exclusion of typhoid fever without recourse to a laboratory. The method has been tested on 414 sera sent to our laboratory; it detected all cases with a titre of 1 : 80 or more, and most of those with a titre of 1 : 40. The method was further tested on 130 clinically observed cases, in which it gave satisfactory results. The S.A. method gave a positive result with 98 out of 100 sera from patients with typhoid fever, whereas the classical Widal reaction gave a positive result with 68 of them only.

The intensity and rapidity of the slide agglutination reaction provide a rough measure of the titre of a serum. A quick and distinct agglutination indicates a titre of 1 : 80 or more and is diagnostic of typhoid fever. A slow and indistinct result is obtained when the titre of the serum is about 1 : 40. A negative test indicates with great probability that a diagnosis of typhoid fever may be excluded.

We think the method succeeds because the nature of the suspension employed and the peculiar behaviour of slide agglutinations permit the detection of O agglutinins as well as H agglutinins.

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