A STUDY OF THE Vi AGGLUTINATION TEST FOR THE DETECTION OF TYPHOID CARRIERS

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INTRODUCTION

The routine examination of waterworks employees for the detection of carriers is an essential part of the control of water-borne typhoid fever. The performance of such examinations upon all who, by the nature of their work, might contaminate the supply became the policy of the Metropolitan Water Board in 1937, when it was decided that the most reliable of existing methods would be the examination of specimens of dejecta from each individual upon not less than three different occasions.

In addition to these routine examinations, it was necessary to test any employee who had been incapacitated as a result of an infective intestinal complaint such as enteric fever, dysentery or gastro-enteritis, in order to ascertain whether he might safely be permitted to return to work. It was also necessary to test special groups of men who, although not normally employed in direct association with the water, might at times be called upon to work in wells or in the laying of new mains.

Bacteriological analyses of this kind place a heavy burden on a laboratory and, when some thousands are involved, the rapid performance of the necessary tests may well be beyond the capacity of the staff. Despite the excellent laboratory facilities provided, it soon became evident that it would not be possible to carry out the number of examinations required for the Metropolitan Water Board in any reasonable time, and it therefore became necessary to find some method which, while sacrificing little or nothing in reliability, would reduce the amount of work required and generally speed up the procedure.

Felix, Krikorian & Reitler (1935) had suggested that the estimation of the Vi agglutinin might prove to be a suitable means for detecting carriers of the organisms of typhoid fever. This view was supported by Pijper & Crocker (1937) and by Felix (1938). There thus appeared some justification for a belief that Vi agglutination would provide a satisfactory means for 'sorting' the population preparatory to the use of cultural methods for the detection of typhoid carriers. Lt.-Col. R. F. Bridges, in charge of the Oxford Standards Laboratory of the Medical Research Council, was approached in this matter and very kindly offered to advise as to the technique to be adopted and to collaborate as far as possible in the tests.

The following is a report embodying the results of some four years' experience of the test during which over a thousand healthy employees have been examined.

PROCEDURE

The Vi agglutination test is set up with dilutions of the serum under test of 1:5, 1:10, 1:20 and 1:40 made up to 1 ml., to which is added 1 drop of agglutinable suspension (Oxford Standards Laboratory) in a round-bottomed agglutination tube $\frac{1}{2}$ in. diameter. The tubes are shaken and incubated for 2 hr. at 37° C. and are then left for a further 22 hr. at room temperature, protected from convection currents which may be set up by proximity to fires, radiators or gas burners. In each case a control consisting of the agglutinable suspension in 0·85 % saline is also set up.

The method by which the degree of Vi agglutination is estimated may be summarized as follows:

The negative reaction in the control tube produces a small pearly grey circle or 'blob' of unagglutinated cells deposited in the centre of the base of the tube. This circle has a clean-cut margin and the supernatant fluid remains turbid. Incomplete agglutination is characterized by an irregularity of the central 'blob' with clumps of agglutinated bacteria surrounding it. As might be expected, the size of the 'blob' varies with the degree of agglutination; when this is slight the 'blob' is relatively large; when agglutination is complete the 'blob' does not form and the whole base of the tube is covered with clumped organisms, the supernatant fluid being clear. In strongly agglutinating sera the deposit may take the form of a thin pellicle spread over the whole concave surface of the base of the tube; its edge may be overlapped or the margin may be seen floating in the fluid.

A positive reaction is recorded when complete or almost complete agglutination is noted in the 1:5

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or 1:10 dilutions, but if a lesser degree of agglutination is noted in these dilutions and is present also in higher dilutions, it is considered to be significant.

In addition to the Vi agglutination test, serum in a dilution of 1:15 is tested with agglutinable suspensions of *Bact. typhosum* O and H and *Bact. paratyphosum* BH. These tests are set up in Dreyer's tubes, incubation, in the case of H agglutination, being for 2 hr. and of O for 22 hr. at 52° C. *Bact. paratyphosum* A and C have not been included because these types of enteric fever are rare in this country. The commencing dilution of 1:15 is used in accordance with the recommendation of the Ministry of Health (1939*b*). Any serum which gives a positive reaction to the O or H suspensions in this dilution is re-examined and the agglutination estimated to its full titre.

Positive reactors to the Vi agglutination test or those showing significantly high O or H titres are, unless the latter can reasonably be attributed to preventive inoculation, subjected to stool and urine examinations and are not allowed to work in the neighbourhood of filtered water unless these have been negative upon at least three separate occasions at intervals of not less than 1 week.

The bacteriological examination of stools consists of the direct plating of faecal suspensions on MacConkey agar, Hynes desoxycholate medium (1942) and Wilson and Blair agar; urine is plated on MacConkey agar and, in addition, both faecal suspensions and urine are examined by enrichment methods in selenite broth (Mackenzie, 1938) followed by plating on MacConkey agar.

RESULTS

The data in Table 1 have been obtained from an extended trial of the Vi agglutination test on a sample of adult population who, by the terms of their employment, may be considered as somewhat above the average in health.

Table 1

Total no. of persons examined	1040
No. showing Vi agglutination	38 (3·7 %)

All the positive reactors have been subjected to at least three bacteriological examinations of stools and urine and one to a duodenal juice test in addition. No enteric organisms have been recovered.

Close collaboration has been maintained throughout with the Oxford Standards Laboratory, and on many occasions, particularly at the outset, specimens of serum have been sent to Colonel Bridges for his opinion. A comparison of the results obtained in the two laboratories, each from the independent examination of the same serum, is of interest, in that it indicates a high degree of conformity in a test which may, at times, be somewhat difficult to interpret.

In all, 168 specimens have been sent to Oxford with the results shown in Table 2.

Experience revealed two forms of deposition which, to the unpractised eye, might be considered as positive agglutination or, in any case, might render the test more difficult to read.

First, there was the occurrence of a fair-sized central 'blob' with clean-cut edges, but surrounded by scattered clumps of bacteria with a supernatant fluid which might show some degree of clearing. This type of reaction has occurred more frequently in the higher dilutions, and in such cases the control tube has sometimes shown the same effect.

Secondly, agglutination has manifested itself in the early stages as a diffuse scattering of clumps of organisms with no central 'blob'; later these clumps coalesced into a central mass not unlike a true 'blob', but on closer examination this was seen to have a less regular margin and a granular or coarse texture, with a dirty grey rather than pearly grey colour. In contrast, the picture of true agglutination, once formed, retains its characteristic appearance for many days.

Table 2

Both laboratories agreed negative	127
Both laboratories agreed positive	32
Oxford negative, M.W.B. positive	7
Oxford positive, M.W.B. negative	2

These reactions aroused suspicion because they sometimes occurred in uninoculated persons not exhibiting O and H agglutinins. Re-examination of these sera with freshly prepared batches of suspension gave clean negative reactions, and it was concluded that, on occasion, a suspension may become supersensitive as it ages. As a result of these reactions, the Oxford Standards Laboratory has reduced the time limit within which the suspension should be used. Reactions of the nature of those described above accounted for a number of false results which have not been included in Tables 1 and 2.

The following additional points arising from this investigation are deserving of notice.

Persistence of the Vi agglutinin

Of 492 inoculated persons examined 33 gave a history of T.A.B. inoculations within 5 years. All of the 33 showed some persistence of O and H agglutinins, but only 6 (18 %) showed Vi agglutination. The figure for positive reactors amongst recently inoculated persons is significantly higher than that for the whole series, and this suggests

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that there is some degree of persistence of the Vi agglutinin after inoculation, although it undoubtedly disappears more rapidly than O and H agglutinins.

Typhosum *O agglutination*

An analysis of O agglutination amongst inoculated and uninoculated is shown in Table 3.

It will be seen that 56·1 % of the inoculated and 40·1 % of the uninoculated persons showed O agglutination in 1:15 or higher dilutions and, while the difference is statistically significant at 0·05 level of significance, the fact remains that a high percentage of the uninoculated gave a positive reaction. The four uninoculated cases which exhibited O agglutinins in dilutions of 1:160 or more were subjected to cultural tests with negative results. It is of interest to note that the *typhosum* H reaction in each of these cases was negative at 1:15, although there was a positive Vi reaction in one case.

Table 3

	Neg. 1:15	Pos. 1:15	Pos. 1:20	Pos. 1:40	Pos. 1:80	Pos. 1:160	Pos. 1:200	Pos. 1:400
Typhosum O:								
Inoculated 492	216	276	189	84	32	11	3	1
Percentage	43·9	56·1	38·4	17·1	6·5	2·2	0·6	0·2
Uninoculated 548	328	220	140	60	14	4	1	0
Percentage	59·9	40·1	25·5	11·0	2·6	0·7	0·2	—

Table 4

	Neg. 1:15	Pos. 1:15	Pos. 1:20	Pos. 1:40	Pos. 1:80	Pos. 1:160	Pos. 1:200	Pos. 1:400	Pos. 1:800
Typhosum H:									
Inoculated 492	66	426	377	328	216	110	40	20	7
Percentage	13·4	86·6	76·6	66·7	43·9	22·4	8·1	4·1	1·4
Uninoculated 548	526	22	10	9	6	1	1	1	0
Percentage	96·0	4·0	1·8	1·6	1·1	0·2	0·2	0·2	—
Paratyphosum BH:									
Inoculated 492	197	295	230	176	103	49	17	7	3
Percentage	40·0	60·0	46·8	35·8	20·9	10·0	3·5	1·4	0·6
Uninoculated 548	533	15	9	5	2	0	0	0	0
Percentage	97·3	2·7	1·6	0·9	0·4	—	—	—	—

Typhosum *H agglutination*

An analysis of H agglutination amongst inoculated and uninoculated is shown in Table 4.

Here, in contrast, is found a marked difference between the two groups. Of the inoculated 86·6 % showed *typhosum* H and 60 % showed *paratyphosum* BH agglutination. On the other hand, of the uninoculated 4·0 % showed *typhosum* H and 2·7 % showed *paratyphosum* BH agglutination. If due regard be given to the fact that a history of inoculation depended only on a man's statement, it may well be that some of the titres obtained in the apparently uninoculated actually resulted from forgotten injections during the first Great War. In the same way, there may be some apparently inoculated individuals included in the wrong category as a result of injections of vaccines other than T.A.B. It should be recorded, however, that the greatest care has been taken throughout to elicit as accurate a history as possible.

DISCUSSION

The Ministry of Health (1939*a*), referring to waterworks administration, states that 'Every new man proposed to be employed on any part of the works where there is risk of his contaminating the water should be examined by means of a Widal test of his blood in order to ascertain whether or not he is likely to be a typhoid carrier'. The Ministry of Health (1939*b*) also states that, when it is necessary to examine large numbers of people for the typhoid-carrier state, cultural methods may be impracticable from the point of view of time and expense. It considers that the Vi test could not (at the time of publication) be performed under routine conditions and, while making no definite recommendation in regard to the use of other agglutination tests, it says that it is 'common practice to do agglutination tests and to disregard those whose serum shows no trace of typhoid agglutinin'. It states that all those showing either H or O agglutinin in titres of 1:15 or higher are 'under suspicion

and should have their faeces and urine examined on three or more occasions...'.

The experience here recorded after examining over a thousand men indicates that such a procedure does not materially assist in carrying out large numbers of examinations. The results indicate a considerable frequency of O agglutinins in the case of uninoculated subjects, an occurrence to which others have drawn attention. Almost half the persons, fairly evenly distributed between inoculated and uninoculated, reacted to *typhosum* O suspension in 1:15. The value of the O test is therefore doubtful and, if its use is to continue in the manner suggested, a dilution of 1:15 is certainly too low. With regard to H agglutination, the results illustrate the long persistence of the flagellar agglutinins after inoculation and indicate that the use of this test is of little value for the detection of carriers if a considerable proportion of those examined have been inoculated, even although many years may have since elapsed. This will constitute an important factor in considering the value of the test after the present war, when the majority of the men who have served will exhibit H agglutination. Browning, Coulthard, Cruickshank, Guthrie & Smith (1933) say of subsidiary tests, including H agglutination, 'while positive results are significant, these tests in general do not give reliable information, since carriers not infrequently yield negative reactions'.

In the present series, over 1000 individuals were examined by the Vi agglutination test, and of these only 3·7 % gave a positive reaction. These were distributed as follows: of 492 inoculated there were 22 (4·5 %) and of 548 uninoculated 16 (2·9 %) of positive reactors. Each of the positive reactors was subjected to searching cultural tests of faeces and urine on at least three occasions, but no typhoid bacteria were recovered. This series probably constitutes the largest single collection of individuals examined in England up to the present. All have been tested by a standard technique, which has developed from the pioneer work of Felix. Credit is due also to Bridges for his introduction of a killed and preserved suspension of good sensitivity and sufficient stability to make practicable the widespread use of the test.

The incidence of positive reactors (3·7 %) in the series under consideration indicates that the test is of the utmost value as a means for sorting those under examination and, when large numbers are involved, makes the detection of carriers a practical proposition. Its value for this purpose during the occurrence of epidemics has been demonstrated by the two cases investigated by the late Dr W. M. Scott and described by Felix (1938).

An observation made by Pijper & Crocker (1943) during an extensive investigation of the test in South Africa is worthy of attention. They found that, in populations where enteric incidence is high, a high percentage of those examined exhibited Vi agglutinins. Thus 26 % of those in the immediate surroundings of enteric patients were positive reactors as compared with only 5·3 % amongst the general population. The figure of 3·7 % in the present series therefore indicates a low incidence in London, which is borne out by the Registrar-General's returns, for the notification rate for London in 1942 was less than 20 per million living.

It is unnecessary again to consider in detail the literature relating to the reliability of the Vi test for the detection of typhoid carriers, for this has already been admirably summarized by Pijper & Crocker (1943). Suffice it to say that a negative reaction does not necessarily mean freedom from specific infection, for Felix (1938) found that, in a dilution as low as 1:5, some proved carriers escaped detection, but this is also true of the more laborious cultural method, as is shown by the case of Pijper & Crocker (1943) in which they recovered typhoid bacteria from the discharge from a gall-bladder fistula, but failed to isolate the organism from the stools.

The same authors recorded, in their review of the literature, Vi-negative results in 11 (8 %) of 142 proved carriers, but in many of these cases the serum was not tested in the lowest dilutions, and Felix (1938) found that, of 56 proved carriers, all showed agglutination at a dilution of 1:5, but 18 of these were negative in higher dilutions. It would be idle to speculate what might be the percentage of 'missed cases' with the dilutions now recommended, for there is not as yet sufficient evidence to justify an opinion. The examination of a sufficient number of proved carriers by the Vi test using the latest technique would provide the answer to this important question.

It is known that the excretion of typhoid bacteria may be intermittent, or that they may be so scanty that they cannot be detected by existing methods. It is known, also, that typhoid osteitis may provide the necessary antigenic stimulus, and Felix (1938) reports a Vi-positive case in which the typhoid infection existed in an empyema. He implies that positive results which cannot be confirmed culturally may be accounted for by similar 'closed' infections. In the present series, however, the most careful investigation failed to elicit any explanation of the Vi-positive results, and it would appear that a small proportion of any community may be positive reactors in the absence of any specific stimulus.

The problem thus arises whether a positive reaction to the Vi test alone should be accepted as indicating the carrier state, with possible serious consequences to the individual, and if not, what

should be the scope of subsequent examinations which may be permitted to overrule the result of this test. Felix (1938) considers that the Vi test 'only serves as a preliminary to the subsequent bacteriological examination of faeces, urine or duodenal juice'. Allison (1943) also considers that the duodenal juice, in addition to the dejecta, should be examined in the case of all positive reactors. This is a counsel of perfection, but would be likely to lead to practical as well as administrative difficulties in a community in which submission to the test of both blood and dejecta is entirely voluntary. The collection of specimens of stool and urine, however, can be performed with little inconvenience to the examinee, and in the present series not one objector has been encountered.

The Ministry of Health (1939*a*) states, in considering the Widal test, 'If a positive result is obtained which is not attributable to preventive inoculation, he should not be employed unless bacteriological examination of his excreta on at least three occasions shows negative results as regards the presence of pathogenic bacteria.' It would appear that this authoritative guidance may justifiably be held to apply also in the case of the Vi test, and it has therefore been the policy of the Laboratories of the Metropolitan Water Board to pass positive reactors if the result of the Vi test is not confirmed by three cultural examinations, except in certain cases in which there are indications that more complete investigation is advisable, but this policy is under constant review in the light of increasing experience.

CONCLUSIONS

In the search for the best method for detecting typhoid carriers amongst a healthy population it is necessary to assess the amount of work to be carried out and the inconvenience to employees against the probable yield in results. Multiple cultural examinations of the faeces and urine of the whole population would be an immense undertaking in time, labour and expense, and the question arises: Is there a test which is sufficiently reliable to justify its use as a means for sorting the community into those who require further examination and those who do not? The requirements of such a test are:

(1) That it should give a reliable indication of the carrier state. If any substantial proportion of carriers who might be detected by other means is likely to be missed, the test would be invalidated.

(2) That it should be of such a nature that it could be economically applied to large numbers.

(3) That it should not give an undue number of false positive results.

The Vi agglutination test satisfies these requirements.

In regard to (1), since no carrier has been detected in the present series, nothing has been contributed to confirm or disprove the reliability of the test, and opinion in regard to this must, of necessity, be based upon the results of other investigations in countries where typhoid fever is more prevalent or which have been devoted particularly to the examination of known carriers. On the whole it may be said that such evidence as exists indicates that the Vi test is at least as reliable a means for the detection of carriers as any other known test. In regard to (2), the present series shows that the Vi test has made practicable the examination of large numbers, and in regard to (3), it shows that, at least in a country where typhoid fever is not generally prevalent, the number of positive results which cannot subsequently be confirmed by cultural tests is not excessive, but that a positive result may be expected from a small percentage of individuals who are not apparently the hosts of typhoid bacteria.

These opinions are given with reserve, for experience of some aspects of the test is as yet insufficient to permit of dogmatism.

SUMMARY

A total of 1040 individuals has been examined for the typhoid carrier state by the O, H and Vi agglutination tests. Some observations have been made upon the technique of the Vi test.

Estimation of the O and H agglutinins proved unsuitable as a preliminary test owing to the high proportion of positive reactors. The Vi test, in contrast, gave a positive result in only a small percentage of those tested and brought the cultural work subsequently required within practical limits. All positive reactors were examined by cultural methods, and in no case were the organisms of enteric fever recovered.

The relative merits of the various tests available for the detection of carriers are discussed and certain tentative conclusions are expressed.

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REFERENCES

ALLISON, V. D. (1943). *Mon. Bull. Minist. Hlth E.P.H.L. Service*, **2**, 88.

BROWNING, C. H., COULTHARD, H. L., CRUICKSHANK, R., GUTHRIE, K. J. & SMITH, R. P. (1933). *Spec. Rep. Ser. Med. Res. Coun., Lond.*, no. 179.

FELIX, A. (1938). *Lancet*, **2**, 738.

FELIX, A., KRIKORIAN, K. S. & REITLER, R. (1935). *J. Hyg., Camb.*, **35**, 421.

HYNES, M. (1942). *J. Path. Bact.* **54**, 193.

MACKENZIE, E. F. W. (1938). *Ann. Rep. Director Water Examination, Met. Water Bd, Lond.*, no. 33, p. 24.

MINISTRY OF HEALTH (1939*a*). *Memo.* 221, His Majesty's Stationery Office, Lond.

MINISTRY OF HEALTH (1939*b*). *Memo.* 225, *Med.*, His Majesty's Stationery Office, Lond.

PIJPER, A. & CROCKER, C. G. (1937). *J. Hyg., Camb.*, **38**, 332.

PIJPER, A. & CROCKER, C. G (1943). *J. Hyg., Camb.*, **43**, 201.

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