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THE INTERPRETATION OF THE VI-TESTS*

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In South Africa the Vi-agglutination test is widely used in attempts to trace chronic carriers of *Salmonella typhi*. Although it is often deplored that *S. typhi* is seldom isolated from the Vi-reactors, some 20,000 tests are requested each year of this Institute.[1] To determine the significance of a positive Vi-reaction, it was decided to investigate a number of sera by the Vi-test and check the reactors a second and a third time. Individuals who showed Vi-agglutinins on 3 occasions were to be considered definite reactors, and their excreta were to be subjected to repeated bacteriological examinations (50 if necessary) to detect carriers. The plan broke down twice owing to circumstances beyond our control.

Of 465 persons examined for Vi-agglutinins, 410 (88·2%) were negative in the first dilution of 1:10; only 8 (1·7%) were positive on 3 or more occasions, but their titres fluctuated between 1:40 and less than 1:10. Three of them refused further cooperation, whereas the remaining 5 had their excreta examined from 2 to 31 times; *S. typhi* was not isolated. These disappointing results once more raise the question of the value of this test. Equally important, but often conveniently ignored, is the problem of the frequency with which a positive Vi-reaction casts suspicion on a non-carrier.

Since the discovery of the Vi-antigen by Felix and Pitt,[2] the tube agglutination technique for demonstration of Vi-agglutinins has remained fundamentally unchanged, although individual workers have introduced certain modifications, particularly in antigen preparations and incubation periods. As a modification of the original technique, Saint-Martin and Desranleau[3] devised a rapid micro-method, and Spaun[4] converted the test into a haemagglutination reaction by coating human erythrocytes with dissolved Vi-antigen.

On the value of the tests for tracing chronic typhoid carriers, opinions differ. Some authors[5,6] agree with Felix's statement[7] that it is 'an aid to the detection of chronic typhoid carriers', but acknowledge the occurrence of Vi-agglutinins in healthy individuals, and their absence from a certain proportion of carriers.

A second group of authors is more enthusiastic. Saint-Martin and Desranleau[3] think that it is an efficient screening test. Pijper and Crocker[8] regard all Vi-reactors as carriers. Rische and Rohne[9] suggest that all individuals with a Vi-titre of 1:20 or more should be prevented from handling food, and Lewin,[10] discussing the application of the Vi-test, says: 'Obviously, serological tests (Vi) must be continued as a routine measure; by preventing all positive Vi-reactors from handling milk and foodstuffs many typhoid carriers would be eliminated, but it must be realized that many "normal" reactors would be penalized'.

The third group doubts its value. Davis[11] does not deny that some of his sera with low Vi-titres were possibly derived from carriers, but he thought that they were frequently of no significance. Radowsky[12] agreed that low titres did not necessarily indicate the existence of the carrier state. Spaun[13] considered the haemagglutination test unsuitable for the detection of chronic carriers, since at least 12% would be missed. Joe *et al.*,[14] in Indonesia, found that 14·7% of 'normal' people were Vi-reactors and concluded that as a screening test it was unreliable. In the experience of Manson-Bahr,[15] the Vi-test was of little practical value in areas with a high endemicity of typhoid.

Thus, the usefulness of the Vi-test in detecting chronic typhoid carriers is not universally accepted and deserves close examination.

PUBLISHED METHODS

From the abundant literature on the Vi-test those publications have been selected in which the test has been carried out quantitatively on clearly defined groups. The carriers must be bacteriologically confirmed; the controls shown to be free of *S. typhi*. Eleven papers fulfilled these criteria, but the techniques varied.

Felix[7,16] used the Watson strain, alive or formalinized, incubating 2 hours at 37°C followed by 24 hours at room temperature. Standard serum was incorporated as a control.

Horgan and Drysdale[17] used the same technique, except that the living antigen suspension was prepared from the Bhatnagar strain, Vi I.

Klein[18] incubated the tests for 24 hours at 37°C, but otherwise followed the method described by Horgan and Drysdale.[17]

Lewin, Bersohn and Hogg[5] prepared a formalinized antigen suspension from strain Vi I Bhatnagar. The tubes were incubated for 2 hours at 37°C and then centrifuged at 3,000 r.p.m. for 2 minutes. Standard serum was incorporated.

Ruhnke[6] prepared his, apparently living, suspension from strain 965 (Rauss). The incubation period was 3-4 hours at 37°C followed by 18 hours at room temperature. Standard serum was not mentioned.

Rische and Rohne[9] used strain 58907 (Robert Koch Institute, Berlin). The sera were inactivated and incubated with a presumably living suspension for 3 hours at 37°C. Standard serum was not mentioned.

Ortel[19] worked with a living suspension of strain 965 (Rauss). The sera were inactivated. He incubated for 1 hour at room temperature, followed by 2 hours at 37°C. Standard serum was not mentioned.

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Saint-Martin and Desranleau[3] used a glycerolated antigen prepared from the Bhatnagar, Vi I. In contrast to the above-mentioned authors, they employed a slide technique, in which the slide was agitated for 4 minutes and then left for 30 minutes at room temperature. The test was read under a stereoscopic microscope at a magnification of 72. Standard serum was not mentioned.

Staack and Spaun[20] coated erythrocytes of human type O with a Vi-antigen extract of strain Vi I.[4] The treated erythrocytes were used in a haemagglutination test which was incubated with inactivated serum for 1 hour at 37°C. Standard serum was not mentioned.

Landy and Lamb[21] used a similar haemagglutination technique, with a purified Vi-antigen prepared from *E. coli* 5396/38, acetone-killed and dried. The test was incubated at 37°C for 2 hours. Standard serum was incorporated.

PUBLISHED RESULTS

Table I records some authors' Vi-results among chronic carriers of *S. typhi*. Using the tube agglutination method, Klein[18] and Rische and Rohne[9] found approximately 10% of the sera were negative in the lowest dilution, 1:5. At a 1% probability level (Fisher's exact probability test) their results do not differ from those of Felix[7] and Ortel.[19] By contrast, there is a significant difference between the percentage of sero-negative typhoid carriers observed by Felix[7] (0%) and by Ortel[19] (33%). This is not due entirely to a difference in antigenic sensitivity, since several authors incorporated standard sera, and yet arrived at different results. Several other variables influence the reaction and to achieve a comprehensive picture of the kinetics, each one should be evaluated separately.

TABLE I. VI-AGGLUTININS IN PROVEN TYPHOID CARRIERS

	Tube Agglutination						*Slide Aggl.*	*Haemaggl.*	
Author Year	*I '38*	*II '43*	*III '45*	*IV '50*	*V '51*	*VI '52*	*VII '51*	*VIII '53*	*IX '53*
No. of sera	56	41	4	71	63	24	26	58	20
Neg. react. in lowest dil.	0	4	2	11	7	8	0	7	2
End titre of pos. sera:									
1 : 2 ..	—	—	—	—	—	—	4	—	—
1 : 5 ..	18	2	0	9	0	0	5	4	2
1 : 10 ..	18	12	0	12	4	2		12	2
1 : 20 ..	11	13	0	19	18	5	17	16	4
1 : 40 ..	4	6	0	11	23	5		9	5
1 : 80 ..	3	3	1	9	9	3		3	4
1 : 160 ..	1	1	1		1	0		5	1
1 : 320 ..	1				1	1		2	

I. Felix.[7] II. Klein.[18] III. Lewin, Bersohn and Hogg.[5] IV. Ruhnke.[6] V. Rische and Rohne.[9] VI. Ortel.[19] VII. Saint-Martin and Desranleau.[3] VIII. Staack and Spaun.[20] IX. Landy and Lamb.[21] — = not done

Notwithstanding certain justifiable objections to the pooling of the results, the general trend may be reflected by combining the first 6 columns of Table I (Table II). It is noteworthy that an average of 12·4% of the typhoid carriers are Vi-negative in the 1:5 dilution. If a positive reaction at a serum dilution of 1:10 is accepted as the lowest diagnostic titre—and this is often done—then 23·6% or almost one-quarter of the carriers will go undetected. Thus, if the test is to be of practical value, a positive reaction of 1:5 must, following Felix,[7] be accepted as diagnostic but, in spite of this, 10 - 15% of the carriers will have eluded detection by the Vi-agglutination test.

TABLE II. VI-TITRES AMONG 259 PROVEN CHRONIC TYPHOID CARRIERS (COMBINED RESULTS)

	Final ser. dil.	*Number*	*Per cent.*
Negative	1 : 5	32	12·4
Positive	1 : 5	29	11·2
,,	1 : 10	48	18·5
,,	1 : 20	66	25·5
,,	1 : 40	49	18·9
,,	1 : 80	28	10·8
,,	1 : 160	4	1·5
,,	1 : 320	3	1·2

The results in Table III show that sera from a number of apparently healthy people give a positive Vi-reaction. On the whole the titres are lower than those found in typhoid carriers. The proportion of 'false positive Vi-reactions' varies between 46·5% and 3·2%[17,19]. The differences are significant at the 1% probability level. In keeping with the previous arguments we may also combine the results of Table III (Table IV). It is seen that of 1,589 sera, 181 (11·4%) were positive, and that the proportion of those giving false positive reactions decreases rapidly with increasing titre. In this control material there is not a single sero-reactor of 1:80 or higher.

TABLE III. VI-AGGLUTININS IN ALLEGED NON-TYPHOID CARRIERS

	Tube Agglutination				*Slide Aggl.*	*Haem-aggl.*
Author Year	*X '40*	*III '45*	*V '51*	*VI '52*	*VII '51*	*VIII '53*
No. of sera ..	200	103	204	1,082	9,192	243
Neg. react. in lowest dil. ..	107	87	167	1,047	9,082	225
End titre of pos. sera:						
1 : 2 ..	—	—	—	—	59	—
1 : 5 ..	55	8	15	14	22	
1 : 10 ..	37	2	15	14		
1 : 20 ..	1	6	2	5	29	18
1 : 40 ..			5	2		

III. Lewin, Bersohn and Hogg.[5] V. Rische and Rohne.[9] VI. Ortel.[19] VII. Saint-Martin and Desranleau.[3] VIII. Staack and Spaun.[20] X. Horgan and Drysdale.[17] — = not done.

TABLE IV. VI-TITRES OF 1,589 APPARENTLY HEALTHY INDIVIDUALS (COMBINED RESULTS)

	Final ser. dil.	*Number*	*Per cent*
Negative	1 : 5	1,408	88·6
Positive	1 : 5	92	5·8
,,	1 : 10	68 } 181	4·3 } 11·4%
,,	1 : 20	14	0·9
,,	1 : 40	7	0·4

The percentages given in Tables II and IV may be rearranged so that the cumulative total at each serum dilution is obtained (Table V). There is a higher percentage of

TABLE V. CUMULATIVE PERCENTAGES OF VI-REACTORS AT VARIOUS SERUM DILUTIONS AMONG TYPHOID CARRIERS AND CONTROLS

	Final ser. dil.	*Typhoid carriers*	*Controls*
Negative	1 : 5	12·4%	88·6%
Positive	1 : 5	87·6%	11·4%
,,	1 : 10	76·4%	5·6%
,,	1 : 20	57·9%	1·3%
,,	1 : 40	32·4%	0·4%
,,	1 : 80	13·5%	
,,	1 : 160	2·7%	
,,	1 : 320	1·2%	

Vi-reactors among typhoid carriers than among the non-carrier group. Moreover, the Vi-reaction becomes increasingly specific as the titre rises. For example it appears to be almost specific when the titre is 160, but at that level 97% of the carriers are non-reactive. On the other hand, if the sensitivity is to be increased so that the reaction detects 87% of the carriers (1:5 dilution) one has to forfeit specificity. At this serum dilution, 11·4% 'false positive reactors' would occur among the general population, which is similar to the findings elsewhere.[14,22] In most centres relying upon the Vi-test in tracing typhoid carriers, it is usual to compromise and accept a Vi-titre of 1:10 or 1:20 as diagnostic. If 1:10 is accepted, one-quarter of the carriers would be missed and 5·6% of the non-carriers would be positive; if 1:20 is considered diagnostic, almost half of the carriers would be considered free of typhoid and 1·3% of the non-carriers would give false positive results.

In a given population the percentage probability that a Vi-positive result signifies a typhoid carrier may be expressed as:

$$\frac{\text{(No. of Vi-positive carriers in the population)} \times 100}{\text{Total no. of Vi-reactors in the same population}}$$

Ames and Robins[23] calculated statistically that there were 2,490 typhoid carriers in New York in 1940, or 1 carrier to 2,500 non-carriers, of whom only 17% were known to the health authorities. They quoted the carrier rate in Missouri as 1:3,500, but pointed out that these were minimum figures. Assuming that the carrier rate is considerably higher, e.g. 1:1,000, then there would be 100 carriers in a population of 100,000. At a 1:5 level the carriers would provide 88 Vi-reactors (Table V), whereas the non-carriers at the same level would produce 11,389 reactors. The probability of any one of the reactors being a carrier would then be $88 \times 100 \div 11{,}477 = 0{\cdot}8\%$. The probability varies with the actual carrier rate,

TABLE VI. THE PROBABILITY OF DETECTING TYPHOID CARRIERS BY MEANS OF THE VI-REACTION

Vi-titres	*Assumed carrier rates*			
	1 : 500	1 : 1000	1 : 2000	1 : 4000
1 : 5	1·5%	0·8%	0·4%	0·2%
1 : 10	2·6%	1·3%	0·7%	0·3%
1 : 20	8·2%	4·3%	2·2%	1·1%
1 : 40	14·0%	7·5%	3·9%	2·0%

the Vi-titre, and the proportion of 'false positive' Vi-reactors in the population. The first two factors are considered in Table VI, the last being regarded as a constant. By plotting the values on semi-logarithmic paper (Fig. 1) we obtain a family of graphs. The positions of the first points on the graphs support the existence of curves rather than straight lines. The relatively small number of high-titred Vi-sera obscures the continuation of the graphs, but it is possible that they will asymptotically approach the parallels to the abscissa.

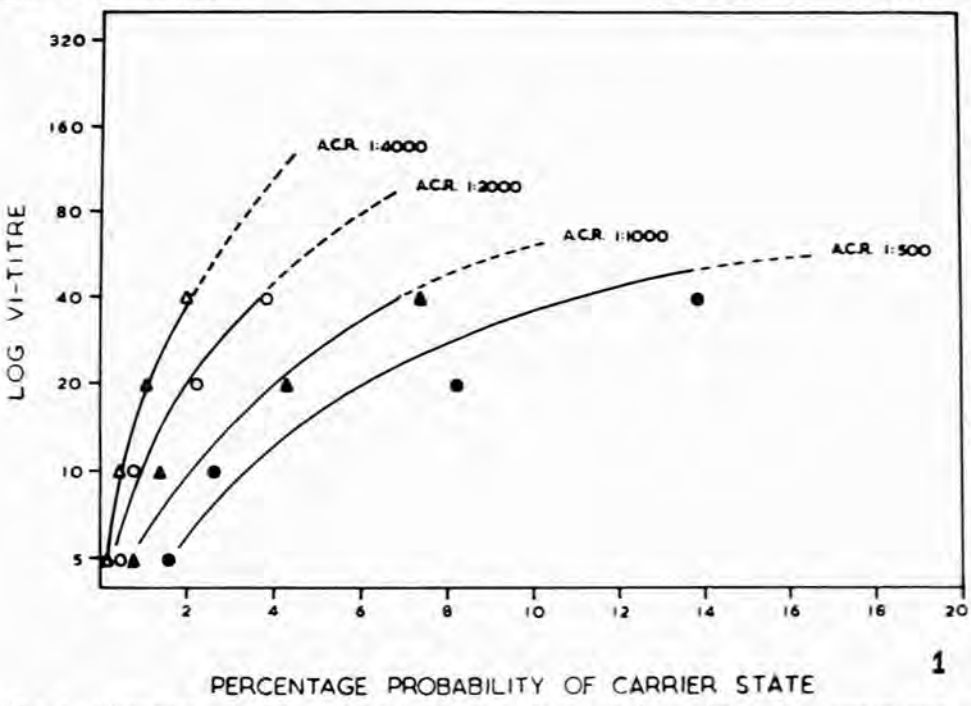

Fig. 1 Diagnostic value of Vi-agglutination titres in populations with various carrier rates. (A.C.R.=assumed carrier rate.)

It is evident from these graphs that if the carrier rate is 1 : 1000 and the criterion chosen is a serum titre of 1 : 10, then there is a 1·4% probability that such a serum will originate from a typhoid carrier, but 98·6% likelihood that it is drawn from a non-carrier. On the other hand, with a carrier rate of 1 : 4000 the probability of detecting a carrier from sera of the same titre (1 : 10), would be 0·3%, that is to say, more than 300 normals would be included for every carrier so detected. Increasing the diagnostic titre to 1 : 40 would again narrow

the field to 50 normals per carrier, while still excluding two-thirds of all carriers. These examples illustrate that the diagnostic value of a given Vi-titre varies proportionately with the prevalence of typhoid carriers.

The probability of a titre being evidence of the carrier state is given in Table VII, which is based on an assumed carrier rate of 1:1,000, but may, of course, be adapted to suit any other rate.

TABLE VII. THE SIGNIFICANCE OF INCREASING VI-TITRES (ASSUMED CARRIER RATE 1 : 1000)

Vi-titre	*Probability of Vi-reactors being carriers*	*Probability of Vi-reactors being non-carriers*	*Probability of missing carriers (100—sensitivity%)*
1 : 5	0·8%	99·2%	12·4%
1 : 10	1·4%	98·6%	23·6%
1 : 20	4·3%	95·7%	42·1%
1 : 40	7·4%	92·6%	67·6%

A satisfactory compromise for a diagnostic titre appears unattainable. The test may yet have an application in the search for a carrier known to be present in a closed community, since it may narrow the field of cultural examination. Should the carrier not be found among the Vi-reactors, it must be remembered that Vi-negative carriers occur in a percentage which increases with the stringency of the titre employed.

The conclusion to be drawn is that it is unrewarding to search at random for typhoid carriers by the Vi tube agglutination technique; on the other hand, if it is reasonable to assume that there is a carrier in a small group of 10 - 50 individuals, the test may be of some assistance, particularly if its limitations are realized.

The question arises whether a better result can be expected from slide or haemagglutination tests. Saint-Martin and Desranleau[3] found that all typhoid carriers gave a positive slide agglutination reaction with their Vi-antigen, though 16% were very weak (Table I). The specificity was 98·8% (Table III). Assuming a carrier rate of 1:1000, this gives an 8·3% probability of a Vi-reactor in the lowest dilution being a carrier and, of course, increased probabilities with higher titres. Theoretically, this test appears to be more satisfactory than tube agglutination, but other workers like Richter[24] and Rische and Rohne[9] have been unable to confirm the advantages of the slide agglutination.

The haemagglutination test[20,21] has practically the same sensitivity and specificity as the tube agglutination test. Accordingly, in its present form it offers no advantage over the classical method.

It should be pointed out that sensitivity, specificity and probability are not the only factors interfering with the interpretation of the Vi-results; for example, the degree of reproducibility as well as the influence of prophylactic immunization are relevant factors, but the assessment of these is beyond the scope of this paper.

The findings and arguments in this paper are the results of combining the observations made by a number of individual workers. The pooling, it may be argued, would be unjustified if all the tests were unsatisfactory, or if one or more tests were superior to the others. In the former case further discussion would be futile, while in the latter, the better methods should be promoted and the unsatisfactory tests abolished. The figures set out in Tables I and III do not indicate the superiority of any particular modification.

CONCLUSION AND SUMMARY

From the literature it appears that a wide range of views are held on the practical value of the Vi-test. Some authors regard it with disfavour, others think that it may have some value, and a number, more enthusiastic, consider it to be of great importance in detecting typhoid carriers.

The sensitivity and specificity of the tests have been analysed and, taking the prevalence of typhoid carriers into account, it has been possible to calculate the probability of a Vi-reactor being a typhoid carrier.

The figures presented show that the Vi-test should not be used indiscriminately in screening populations for typhoid carriers, but may have limited usefulness in an attempt to trace suspected carriers in a closed group of individuals. A Vi-reactor must be followed up by bacteriological investigation, but the chance of a reactor being a carrier is very small. The demonstration of a carrier among the reactors does not exclude the possibility of another carrier occurring among the non-reactors.

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