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SOME OBSERVATIONS ON THE TPI TEST
ASSESSMENT OF RESULTS ACCORDING TO THE CLINICAL DATA
INFLUENCE OF SOME VARIABLES ON PARTIAL SPECIFIC IMMOBILIZATION* †

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We were very interested in the recent paper by Sequeira and Wilkinson (1955) and some confirmatory findings are put forward below. Fuller details will be found in the thesis of one of us (Hardy, 1956).

Distribution of Results of Qualitative TPI Tests in a Consecutive Series of 922 Subjects

Although we often emphasize to physicians who send us sera for examination that the TPI test is primarily useful in the diagnosis of untreated syphilis, we receive almost as many sera from treated cases. It occurred to us that it would be interesting to study the distribution of positive, doubtful, and negative results in the two groups. As might be expected the distribution is different, as is shown by Table I and by the Figure (overleaf).

TABLE I
SPREAD OF TPI RESULTS IN 922 CONSECUTIVE SUBJECTS

Percentage Specific Immobilization	Overall Results	Cases sent for Diagnosis	Treated Patients
100	373	204	169
100 to 90	27	14	13
90 to 80	18	2	16
80 to 70	16	1	15
70 to 60	11	2	9
60 to 50	18	4	14
50 to 40	18	6	12
40 to 30	15	0	15
30 to 20	12	0	12
20 to 10	31	12	19
10 to 0	383	224	159
Total	922	469	453

(*a*) In cases for diagnosis, the classification is, on the whole, clear cut, and the sera are either positive or negative. However, out of 469 cases, specific immobilization (SI) in the doubtful zone occurred in six. Taking into account the circumstances (prostitutes, African subjects, etc.), the possibility of an old syphilis cannot be excluded in all these six cases, which gave results in the 40 to 50 per cent. SI range.

(*b*) In the treated cases the striking general tendency is towards negativity; 178 sera out of 453 gave an SI below 20 per cent. As all these patients had probably had an SI of 100 per cent. before treatment, it is interesting to note this trend towards negativity. Contrary to the findings in the cases for diagnosis—and this is easily understood—one finds a scatter of the results in all the partial SI zones.

Influence of Duration of Incubation on Reproducibility of the TPI Test

Since the original publications of Nelson and his colleagues, the influence of the duration of incubation on the degree of SI has been emphasized; unfortunately it has not yet been possible to reach agreement on the optimum duration of incubation between the laboratories doing the TPI test. Each laboratory has its preference, without even mentioning the technique of pre-sensitization. For our part we think that the optimum is 22 hrs at 35° C., but other laboratories may well have chosen different figures (Thioglycollate: × 1·5; complement 0·15 ml.).

When the tests are done consistently in the same laboratory the results are comparable, but sometimes medical practitioners are confused when they use two different laboratories for TPI tests on the same patient: if the sera are clearly positive or clearly negative, they will be found at 100 or at 0 per cent., even if the duration of incubation is a little different; on the other hand, where sera in the zone of partial SI between 80 and 20 per cent.

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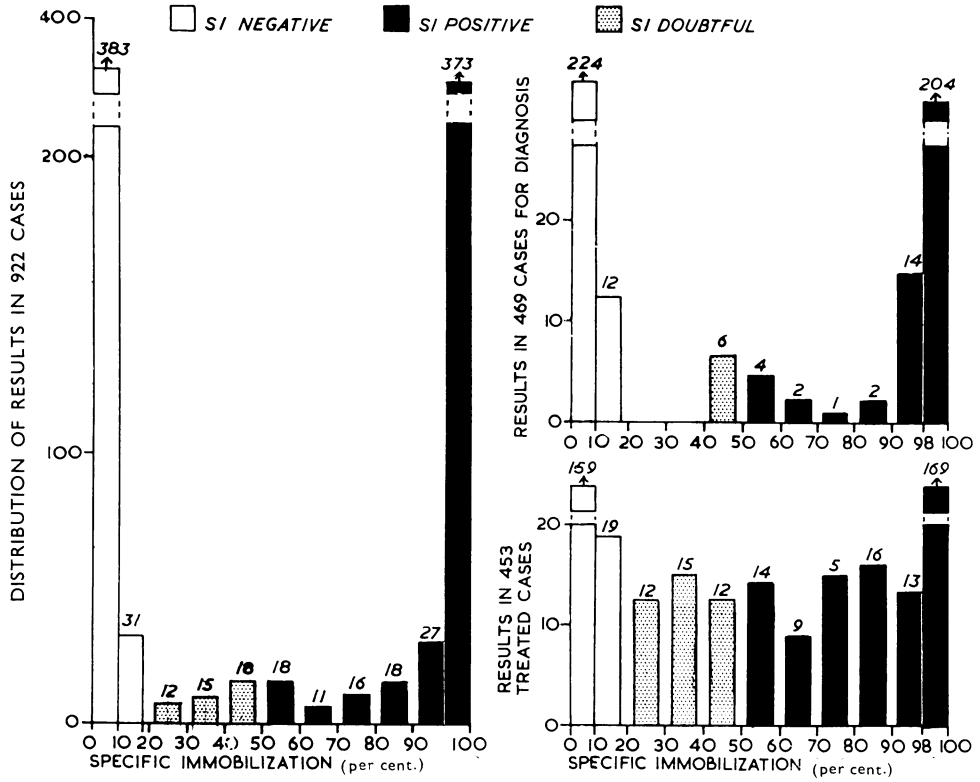

FIGURE.—Results of TPI test in a series of 922 sera. Graphs show clear-cut distribution into positive and negative for the cases for diagnosis, and scattering of results in treated cases of syphilis.

for example, are concerned, the duration of incubation will have a marked influence and the results will be different. In France this has often led to the belief that there is a defect in reproducibility, when, in fact, the disparity is the result of different technical conditions.

Table II illustrates the importance of this factor and shows the desirability of laboratories indicating clearly the duration of incubation.

Influence of Unequal Distribution of Antibody on Percentage of SI

This is a real variable which can affect the reproducibility of the test. By always choosing sera with an SI about 50 per cent. (or by diluting a positive serum so that it falls within this range) the possibilities of variations are revealed at their maximum. Table III (opposite) shows the figures obtained by distributing such a serum in twenty tubes : it can be seen that there are differences from one tube to the other, but these differences hardly interfere with the classification of the serum under consideration.

TABLE II

PERCENTAGE SPECIFIC IMMOBILIZATION ACCORDING TO DURATION OF INCUBATION

Reading after 17 hrs			Reading after 22 hrs		
Tube No.	Per cent. SI	Mean Percentage	Tube No.	Per cent. SI	Mean Percentage
1	36		21	10	
2	28		22	46	
3	10		23	48	
4	32		24	75	
5	24		25	40	
6	32		26	62	
7	40	SI	27	46	SI
8	36	34	28	58	56
9	32	per cent.	29	67	per cent.
10	40		30	62	
11	49		31	54	
12	24		32	67	
13	32		33	56	
14	24		34	50	
15	49		35	67	
16	53		36	50	
17	21		37	62	
18	28		38	71	
19	26		39	46	
20	32		40	50	

The same serum was distributed in the forty tubes. This Table shows the sampling error : 21 → 40 and 40 → 75. The influence of duration of incubation may be important : 21 → 75 per cent.; or not : 40 → 40 per cent.

TABLE III

PERCENTAGE SPECIFIC IMMOBILIZATION ACCORDING TO DISTRIBUTION

Tube No.	C	T T	Percentage SI	Tube No.	C	T T	Percentage SI
1	50	22	56	11	48	17	65
2	—	29	*42*	12	—	24	48
3	—	15	70	13	—	25·22	50
4	—	21	58	14	—	16	67
5	—	22	56	15	—	14	71
6	—	23	54	16	—	18	62
7	—	20	60	17	—	21	56
8	—	6	*88*	18	—	22	54
9	—	15	70	19	—	23	52
10	—	25	50	20	—	13·12	73

Mean SI : 60·2 per cent.

C = Control tube. T T = Test tube. Fifty *T. pallida* counted. The serum and the dilution chosen had previously been found to give about 50 per cent. SI and were the ones which offered the maximum of variations in our experience.

Repetition of Test and Reproducibility

We have just spoken of the variable introduced by the unequal distribution of antibodies. There are others which we cannot go into here : sensitization of *T. pallidum* by the rabbit, strength of the *T. pallidum* suspension used, etc.

These different variables, in practice, compensate for one another : they add or subtract and their effect can be seen by repeating the TPI in series on the same serum.

To make the variations more evident we have studied three dilutions (Table IV) : it can be seen, as always, that those which give SIs about 50 per cent. can show fairly appreciable differences from one test to the other : however, here again the observed differences do not affect the classification of the serum under consideration.

TABLE IV

DIFFERENCES OBSERVED IN A SERIES OF FOURTEEN CONSECUTIVE TPI TESTS ON THE SAME SERUM

Dilution	Percentage													
	September		October						November					December
	21	26	14	17	19	21	26	28	4	7	16	18	25	3
46	96	100	100	82	100	100	100	100	100	91	98	91	100	100
100	88	88	52	52	83	56	83	*89*	88	*49*	62	—	74	68
215	38	27	16	13	27	10	20	24	33	17	31	22	4	13

Dilution = $\sqrt[3]{10}$. Incubation : 22 hrs. Fifty *T. pallida* counted. The final dilution which gives the higher variations is 1 in 100 for this serum. This dilution gave SI around 50 per cent.

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Summary

The TPI used diagnostically gives frankly positive or negative results. When the figure found for SI is about 50 per cent., one is in a zone of high sensitivity which can be influenced by various factors. In this zone of partial SI, the greater or lesser duration of incubation can markedly affect the results. If the physician who gets the laboratory report is not aware of this, he may imagine, in error, that there is a failure in reproducibility. It is therefore important that TPI laboratories should make known the incubation time they have chosen.

As an example of one variable, the influence of unequal distribution of antibody can be mentioned : in actual fact the effect of this is limited.

The whole of the variables which may intervene can be appreciated by the observation of different SIs found during successive tests. These variables, in practice, offset one another, and even in choosing a dilution giving an SI of about 50 per cent. the differences are not very important. In practice they do not affect the reproducibility of the qualitative test which is very satisfactory.*

REFERENCES

Hardy, N. (1956). Thèse de Paris.

Sequeira, P. J. L., and Wilkinson, A. E. (1955). *British Journal of Venereal Diseases*, **31**, 134.

* In this short paper we have not mentioned the quantitative test ; when carried out carefully we think the reproducibility is excellent, of the order of one dilution.