Indian Journal of
Dermatology & Venereology

*(Incorporating Indian Journal of Venereal Diseases & Dermatology)*

**Vol. 30; No. 1.** **January-February 1964**

## ORIGINAL ARTICLES

# TROPENEMA PALLIDUM IMMUNE ADHERENCE (T. P. I. A.) TEST FOR SYPHILIS

by

CHACKO, C. W.* and YOGESWARI, L.**

## INTRODUCTION

The T. P. I. A. test is one of the new sero-diagnostic tests devised for syphilis using a specific virulent treponemal antigen, to detect a specific circulating antibody against T. pallidum in the patient serum. It is a practical application of a general "immune adherence" phenomenon described also by Nelson (1963) the author of the now well-established specific T. Pallidum Immobilization (T. P. I.) test for Syphilis. In the T. P. I. A. test the antigenic T. pallidum is sensitized by its specific antibody in the patients serum and adheres specifically to the surface of human red blood cells in the presence of complement. Such treponemes when centrifuged lightly go to the bottom of a tube with the deposited red cells giving a positive reaction in the T. P. I. A. test.

The T. P. I. test is technically too difficult and expensive to be performed in all laboratories, mainly because its specific antigen, consisting of virulent T. pallida, needs to be kept alive and motile in a complex survival medium under special anaerobic conditions. The T. P. I. A. test, in contrast, has been reported on by earlier workers, as a comparatively easier procedure technically, since dead or killed virulent T. pallidum are employed as the specific antigen and yet detecting a similar, if not, the same specific antibody as in the T. P. I, test in Syphilis.

In a research enquiry sponsored by the Indian Council of Medical Research to evaluate the specific worth of various Serologic tests for Syphilis using specific antigen of T. pallidum origin, the T. P. I. A. test was experimentally reproduced and evaluated in this study. Its potential specific worth in the Sero-diagnosis of Syphilis has been confirmed. Although the T. P. I. A. test was technically easier

* Dr. C W. Chacko, M. B. & B. S., D. T. M. Ph. D., Serologist & Associate Professor of Serology, Institute of Venereology, Madras Medical College, Madras-3, India.

** Dr. L. Yogeswari, M.Sc., Ph.D., Asst. Research Officer, Indian Council of Medical Research, V. D. Laboratory, Institute of Venereology, Madras Medical College, Madras-3.

to perform than the T. P. I. test, its use as a standard routine Serologic test for Syphilis, was found to be very limited, due to certain technical difficulties and other drawbacks which are discussed.

## MATERIALS AND METHODS

*T. P. I. A. Test Antigen:* The source of the antigen was the Nichol's strain of virulent T. pallidum adapted to grow in live rabbit testes tissue. The antigen was prepared as follows:—

The 2 testes of a rabbit infected with T. pallida, not more than 10 days earlier, producing primary Syphiloma of the testes, were removed and cut into thin slices with a pair of sharp scissors, taking Sterile precautions. T. pallida were then extracted from the 2 testes in about 10 ml. of a 2% sterile Sodium citrate solution, in a 50 ml. sterile conical flask, by shaking, at room temperature in a Kahn shaker. At the end of two hours of shaking, the extract contained about 100 T. pallida per dark field of the microscope. The extract was then spun with sterile precautions in a centrifuge, first lightly at 1500 RPM for 10 minutes to sediment only the testes-tissue-debris, blood cells and spermatozoa. The supernatent was then collected and centrifuged again at 10,000 RPM for 45 minutes to deposit all T. pallida. They were resuspended in the Sodium citrate solution and this differential centrifugation was repeated to wash the T. pallida free of testes-tissue as far as possible. At the final resuspension, enough sodium citrate solution was added to provide 15 to 20 T. pallida per dark field of the microscope in the antigen suspension. The suspension was then heated in the wate rbath at 65°C for 2 hours and " merthiolate " was added to a strength of 1 in 10,000 to prevent contaminants. This formed the specific antigen for the T. P. I. A. test and was preserved in the cold at 5°C.

*Complement:* The source was from fresh Serum separated from pooled blood of G. Pigs.

*Red Blood Cells:* Human "O" group red blood cells were obtained in citrated saline from the Blood Bank of the Madras General Hospital. The citrated Red blood cells were repeatedly washed clear and used in 50% suspension in normal saline.

*Serum and C. S. F. Specimens* of various clinical categories were obtained from the clinic of the Madras Institute of Venereology and other units of the Madras Government General Hospital. Specimens of blood and C. S. F. were received daily for routine standard and special serological investigation in the V. D. Laboratory from the Physicians after detailed clinical examination. The specimen of sera and C. S. F. were preserved in the cold at 5°C until ready for testing. Once a week, after inactivation in a waterbath at 56°C for 30 minutes, these specimens were tested in parallel with T. P. I. A. test against the standard Serologic test for Syphilis in routine use, namely the V. D. R. L. slide flocculation test using the

Cardiolipin antigen. This study was conducted during the course of the years 1958 and 1959.

## T. P. I. A. TEST TECHNIQUE

The procedure followed in this study was more or less similar to that described by DAGUET (1956).

*The Qualitative Technique*: The reagents described were mixed in standard Kahn test tubes as shown in the following protocol. After incubation, the tubes were spun in a centrifuge lightly to deposit only the red cells. The treponemes in the fluid phase were counted under the darkfield microscope and the percentage of them that had specifically adhered to the red cells and gone down with them was estimated given below:

### THE PROTOCOL OF THE T. P. I. A. TEST

| Tube | Purpose | STEPS | | | | | Total Volume (ml) |
|---|---|---|---|---|---|---|---|
| | | Antigen (ml.) 1 | Hanks solution (ml.) 2 | Serum or C S.F. (ml.) 3 | Complement (ml.) 4 | Red Cells (ml.) 5 | |
| 1. | Agglutination control | 0.5 | 0.45 | 0.1 (Known positive) | 0.05 | Nil | 1 |
| 2. | Sensitization control | 0.5 | 0.35 | Nil | 0.05 | 0.1 | 1 |
| 3. | Complement control | 0.5 | 0.3 | 0.1 (Known positive) | Nil | 0.1 | 1 |
| 4. | Antigen Control | 0.5 | 0.5 | Nil | Nil | Nil | 1 |
| 5. | Positive control | 0.5 | 0.25 | 0.1 (Known positive) | 0.05 | 0.1 | 1 |
| 6. | Negative control | 0.5 | 0.25 | 0.1 (Known negative) | 0.05 | 0.1 | 1 |
| 7. | Test Serum | 0.5 | 0.25 | 0.1 | 0.05 | 0.1 | 1 |

The primary incubation after the Step 3, and a secondary incubation after the Step 5 of the above protocol were each carried out in a Waterbath at 37°C for 30 minutes. The tubes were then centrifuged at 500 RPM for 5 minutes and 0.005 ml. of the supernatent was transferred to a clean slide with Kahn's pipette. Darkfield examination of the T. Pallida were done under high dry objective of the microscope and number of T. pallida were counted in 20 fields. The percentage of T. pallida that had specifically adhered to the red cells was calculated as follows:

$$\text{Specific disappearance \% of T. P.} = \frac{\text{Number in the antigen control tube minus Number in test proper tube}}{\text{Number of the antigen control tube.}} \times 100$$

The percentage disappearance above 50 was considered qualitatively "reactive" provided the controls were valid. Disappearance below 50% was considered 'non-reactive".

*The Quantitative Technique*: This was specially devised in this study. The T. P. I. A. reactive sera, which were diluted initially 1 in 5 in the qualitative test were prepared in two fold dilutions from the 1 in 5 dilution, in normal saline serially. Each dilution was then treated as individual serum and tested as in the qualitative technique.

The T. P. I. A. test titre was then calculated by a method of "50% disappearance end-point" and expressed in serum dilutions or "dils".

CALCULATION OF 50% END POINT (vide diagnostic procedures for Virus and Rickettsian Diseases, A. P. H. A. 1790 Broadway, New York, 1st Edn, 1949, page 259.

50% disappearance end point often lies between two dilutions. The exact titre at which 50% and point occurs was calculated by the following formula:

$$\frac{50 \text{ minus. Disappearance at dilution next below}}{\text{Disappearance at dilution next above. minus. Disappearance at dilution next below}} = \text{Proportionate distance,}$$

Since dilutions are increasing on a log scale, the final reading was obtained as follows:

The log of lower dilution, plus. Proportionate distance multiplied by the log of dilution factor equals the log of end point.

This is more easily done graphically. The graph on semilog paper enables the end point to be calculated for any dilution factor. The "proportionate distance" was obtained from the calculation above, and the point where the "proportionate distance" crosses the two-fold dilution line was read off. The product of this figure and the lower dilution of the two, between which the 50% end point falls, gives the final end point.

e. g. In an experiment where the dilution was two-fold, the 50% end point fell between 2560 and 5120 dils and the percentage disappearance at 2560 was 52 and at 5120, 20.4.

$$\text{Proportionate distanc} \quad \frac{50 - 20.4}{52 - 20.4} = \frac{29.6}{31.6} = .93$$

50% end point or dilution factor at .93 was 1.9 as read from the graph. Final titre was $2560 \times 1.9 = 4864.0$ i.e., 1 in 4864 dils.

The V. D. R. L. test was performed on the specimens of serum by the technique described by Harris et al (1948) and C. S. F. by the technique described by Rosenberg et al (1948) using "Cardiolipin antigen" produced at the Government Antigen production Unit "at Calcutta".

## RESULTS

TABLE 1

*Details of specimens investigated*

SERUM

| CLINICAL CATEGORY | Number Tested | |
|---|---|---|
| SYPHILIS GROUP (Untreated) | | |
| Syphilis Primary ... ... ... | 79 | |
| ,, Sedonday ... ... ... | 74 | |
| ,, Tertiary-benign ... ... ... | 35 | |
| ,, Cardio-vascular ... ... ... | 9 | |
| ,, Neuro ... ... ... | 42 | |
| ,, Congenital ... ... ... | 13 | |
| ,, Latent ... ... ... | 39 | |
| Total ... | | 291 |
| NON-SYPHILIS GROUP: | | |
| Normals ... ... ... | 116 | |
| Abnormals ... ... ... | ... | |
| Tuberculosis ... ... ... | 18 | |
| Leprosy ... ... ... | 350 | |
| Miscellaneous diseases ... ... ... | 74 | |
| Total ... | | 558 |
| Grand Total ... | | 849 |

### C. S. F.

| CLINICAL CATEGORY: Syphilis group | No. tested |
|---|---|
| Neurosyphilis ... ... ... ... | 31 |
| Non-Neuro-Syphilis symptomatic ... ... ... | 12 |
| ,, ,, latent ... ... ... | 21 |
| Sero-reactive Mental Diseases ... ... ... | 27 |
| Non-syphilis Group (normals) ... ... ... | 13 |
| Total ... | 104 |

TABLE 2

*Result of Paraliel Tests for Syphilis, Correlated with Clinical Diagnosis.*

**Sera From Syphilis Group.**

T. P. I. A. Vs. V. D. R. L. Sensitivity

| TPIA | VDRL | Primary | Secondary | Tertiary | Latent | Congenital | C.V.S. | Neuro-Syphilis | Total cases | Agreement % | Disagreement % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | 61 | 73 | 35 | 39 | 10 | 9 | 41 | 268 | 98.3 | |
| − | − | 16 | 1 | − | − | 1 | − | − | 18 | | |
| + | − | 1 | − | − | − | 2 | − | − | 4 | | 1.7 |
| − | + | 1 | − | − | − | − | − | − | 1 | | |
| Total | | 79 | 74 | 35 | 39 | 13 | 9 | 42 | 291 | | |
| Sensitivity % TPIA | | 79.7 | 98.6 | 100 | 100 | 92.3 | 100 | 100 | 93.5 | | |
| Sensitivity % VDRL | | 79.7 | 98.6 | 100 | 100 | 77 | 100 | 97.6 | 92,4 | | |

In the Table 2, the results obtained with the T. P. I. A. test are checked against clinical diagnosis of Syphilis in various clinical stages and against the results of the V. D. R. L. test for Syphilis, with reference to comparative sensitivity, and agreement and disagreements between them. It may be particularly noted in this table that the T. P. I. A. test for Syhilis has 93.5 percent sensitivity, while the V. D. R. L. test has 92.4 in 291 cases of known Syphilis. There is 98.3 percent negative and positive agreements in their resuts in Syphilis. The disagreement between the clinical diagnosis and Serologic tests results and disagreement between the 2 test resuls are mainly in primary Syphilis stage.

TABLE 3

*Results of Paraliel Tests for Syphilis Correlated with the Clinical Diagnosis Sera from Non-Syphilitic normal and abnormals.*

T. P. I. A. Vs. V. D. R. L. Specificity

| TPIA | VDRL | Normals | Tuberculosis | Leprosy | Miscellaneous diseases | Total Non-Syphilis | Agreement % | Disagreement % |
|---|---|---|---|---|---|---|---|---|
| + | + | ... | 3 | 57 | 2 | 62 | ... | ... |
| − | − | 116 | 14 | 287 | 52 | 469 | 95.2 | ... |
| + | − | .0 | .0 | .0 | .0 | .0 | ... | ... |
| − | + | ... | 1 | 6 | 20 | 27 | ... | 4.8 |
| Total | | 116 | 18 | 350 | 74 | 558 | | |
| Specificity % TPIA | | 100 | 83.3 | 83.7 | 97.3 | 85.9 | | |
| Specificity % VDRL | | 100 | 77.7 | 82 | 70.3 | 79.8 | | |

In the Table 3, the "Specificity" of the T. P. I. A. test for syphilis in Non-syphilitic normal and abnormal conditions is checked against clinical diagnosis and against the "specificity" of the VDRL test. It is to be particularly noted that both the tests have 100 percent "specificity" and agreement among themselves in 116 normal cases. In each of the group non-syphilitic abnormal cases of various diseases, the specificity of the T. P. I. A. test has been low but comparatively higher than that the VDRL test. Both the tests have been falsely reactive mainly in 57 out 350 cases of leprosy. In a total of 558 Non-syphilitic cases, the T. P. I. A. test has obtained only 85.9 percent specificity. However this is against 79.8 percent specificity for the VDRL test with which it has a total agreement of 95.2 percent only.

TABLE 4

*A List of Non-Syphilitic Cases Possibly with "Biologica Faise Positive" Reactions to the VDRL Test, Verifled So with the T. P. I. A. Test.*

| Serial No. | Non-syphilitic abnormal cases with their clinical diagnosis | TPIA | VDRL |
|---|---|---|---|
| 1. | Leprosy ... ... | Non-reactive | Reactive 1 dil |
| 2. | ,, ... ... | ,, | ,, ,, |
| 3. | ,, ... ... | ,, | ,, ,, |
| 4. | ,, ... ... | ,, | ,, ,, |
| 5. | ,, ... ... | ,, | ,, ,, |
| 6. | ,, ... ... | ,, | ,, 4 ,, |
| 7. | Not yet diagnosed ... | ,, | ,, 1 ,, |
| 8. | ,, ,, ... | ,, | ,, 1 ,, |
| 9 | ,, ,, ... | ,, | ,, ,, |
| 10. | ,, ,, ... | ,, | ,, ,, |
| 11. | ,, ,, ... | ,, | ,, 8 ,, |
| 12. | ,, ,, ... | ,, | ,, 2 ,, |
| 13. | ,, ,, ... | ,, | ,, 8 ,, |
| 14. | ,, ,, ... | ,, | ,, 2 ,, |
| 15. | ,, ,, ... | ,, | ,, 2 ,, |
| 16. | Rheumatoid arthritis ... | ,, | ,, 8 ,, |
| 17. | ,, ,, ... | ,, | ,, 1 ,, |
| 18. | ,, ,, ... | ,, | ,, 4 ,, |
| 19. | Optic neuritis ... | ,, | ,, 4 ,, |
| 20. | Ophthalmoplegia, chronic progressive | ,, | ,, 2 ,, |
| 21. | Macular changes eye, with tuberculosis | ,, | ,, 1 ,, |
| 22. | Macular changes in the eye | ,, | ,, ,, |
| 23. | Cataract ... ... | ,, | ,, ,, |
| 24. | Eosinophilia ... ... | ,, | ,, 32 ,, |
| 25. | Pregnancy ... ... | ,, | ,, 4 ,, |
| 26. | Rhinosporidiosis ... | ,, | ,, 1 ,, |
| 27. | Thyrotoxicosis ... ... | ,, | ,, 4 ,, |

TABLE 5.

*Low Titred Quantitative VDRL Test Vs. Quantitative TPIA Test, A List of Abnormal Conditions from which Syphilis Could Not Be Established or Excluded.*

| Sl. No. | Diagnosis | VDRL | TPIA |
|---|---|---|---|
| 1. | Tuberculosis | Reactive 1 dil | Non-reactive |
| 2. | Tuberculosis | ,, 4 dils | Reactive 1 dil |
| 3. | Leprosy | ,, 1 dil | Non-reactive |
| 4. | ,, | ,, 1 ,, | ,, |
| 5. | ,, | ,, 1 ,, | ,, |
| 6. | ,, | ,, 1 ,, | ,, |
| 7. | ,, | ,, 1 ,, | ,, |
| 8. | ,, | ,, 4 dils | ,, |
| 9. | ,, | ,, 1 dil | Reactive 1 dil |
| 10. | ,, | ,, 1 ,, | ,, 1 ,, |
| 11. | ,, | ,, 1 ,, | ,, 1 ,, |
| 12. | ,, | ,, 1 ,, | ,, 1 ,, |
| 13. | ,, | ,, 1 ,, | ,, 1 ,, |
| 14. | ,, | ,, 1 ,, | ,, 1 ,, |
| 15. | ,, | ,, 1 ,, | ,, 1 ,, |
| 16. | ,, | ,, 1 ,, | ,, 1 ,, |
| 17. | ,, | ,, 1 ,, | ,, 1 ,, |
| 18. | ,, | ,, 1 ,, | ,, 1 ,, |
| 19. | ,, | ,, 1 ,, | ,, 1 ,, |
| 20. | ,, | ,, 1 ,, | ,, 1 ,, |
| 21. | ,, | ,, 2 dils | ,, 1 ,, |
| 22. | ,, | ,, 2 ,, | ,, 1 ,, |
| 23. | ,, | ,, 2 ,, | ,, 1 ,, |
| 24. | ,, | ,, 2 ,, | ,, 1 ,, |
| 25. | ,, | ,, 2 ,, | ,, 1 ,, |
| 26. | ,, | ,, 2 ,, | ,, 1 ,, |
| 27. | ,, | ,, 2 ,, | ,, 1 ,, |
| 28. | ,, | ,, 2 ,, | ,, 1 ,, |
| 29. | ,, | ,, 2 ,, | ,, 1 ,, |
| 30. | ,, | ,, 4 ,, | ,, 1 ,, |
| 31. | ,, | ,, 4 ,, | ,, 1 ,, |
| 32. | ,, | ,, 4 ,, | ,, 1 ,, |
| 33. | ,, | ,, 4 ,, | ,, 1 ,, |
| 34. | ,, | ,, 4 ,, | ,, 1 ,, |
| 35. | ,, | ,, 4 ,, | ,, 1 ,, |
| 36. | ,, | ,, 4 ,, | ,, 5 ,, |
| 37. | ,, | ,, 4 ,, | ,, 5 ,, |
| 38. | Miscellaneous diseases | ,, 1 dil | non-reactive |
| 39. | ,, | ,, 1 ,, | ,, |

TABLE 5 (*Contd.*)

| Sl. No. | Diagnosis | VDRL | | TPIA | |
|---|---|---|---|---|---|
| 40. | Miscellaneous diseases | Reactive | 1 ,, | Non-reactive | |
| 41. | ,, | ,, | 1 ,, | ,, | |
| 42. | ,, | ,, | 1 ,, | ,, | |
| 43. | ,, | ,, | 1 ,, | ,, | |
| 44. | ,, | ,, | 1 ,, | ,, | |
| 45. | ,, | ,, | 1 ,, | ,, | |
| 46. | ,, | ,, | 1 ,, | ,, | |
| 47. | ,, | ,, | 2 dils | ,, | |
| 48. | ,, | ,, | 2 ,, | ,, | |
| 49. | ,, | ,, | 2 ,, | ,, | |
| 50. | ,, | ,, | 2 ,, | ,, | |
| 51. | ,, | ,, | 2 ,, | ,, | |
| 52. | ,, | ,, | 2 ,, | ,, | |
| 53. | ,, | ,, | 4 ,, | ,, | |
| 54. | ,, | ,, | 4 ,, | ,, | |
| 55. | ,, | ,, | 4 ,, | ,, | |
| 56. | ,, | ,, | 4 ,, | ,, | |
| 57. | ,, | ,, | 1 dil | Reactive | 1 dil |
| 58. | ,, | ,, | 1 ,, | ,, | 1 ,, |
| 59. | ,, | ,, | 1 ,, | ,, | 1 ,, |
| 60. | ,, | ,, | 1 ,, | ,, | 1 ,, |
| 61. | ,, | ,, | 1 ,, | ,, | 1 ,, |
| 62. | ,, | ,, | 2 dils | ,, | 5 dils |
| 63. | ,, | ,, | 2 ,, | ,, | 1 dil |
| 64. | ,, | ,, | 4 ,, | ,, | 5 dils |
| 65. | ,, | ,, | 4 ,, | ,, | 5 ,, |
| 66. | Diagnosis not established | ,, | 1 ,, | Non-reactive | |
| 67. | ,, | ,, | 2 dils | ,, | |
| 68. | ,, | ,, | 2 ,, | ,, | |
| 69. | ,, | ,, | 4 ,, | ,, | |
| 70. | ,, | ,, | 1 dil | Reactive | 1 dil |
| 71. | ,, | ,, | 1 ,, | ,, | 1 ,, |
| 72. | ,, | ,, | 2 dils | ,, | 1 ,, |
| 73. | ,, | ,, | 2 ,, | ,, | 1 ,, |
| 74. | ,, | ,, | 2 ,, | ,, | 1 ,, |
| 75. | ,, | ,, | 2 ,, | ,, | 1 ,, |
| 76. | ,, | ,, | 4 ,, | ,, | 5 ,, |
| 77. | ,, | ,, | 4 ,, | ,, | 5 ,, |
| 78. | ,, | ,, | 4 ,, | ,, | 5 ,, |
| 79. | ,, | ,, | 4 ,, | ,, | 5 ,, |

It may noted in the table 5, that certain number of results obtained with quantitative T. P. I. A. test in this particular experiment has been expressed as " Reactive I dil " which means Reactive only in the undiluted serum. Since the qualitative T. P. I. A. test has been originally devised to be performed in an initial dilution I in 5 of the serum, " T. P. I. A. test reactive I dil " obtained in this study can be expected to be " non-reactive " in the qualitiative T. P. I. A. test. In this group of sera, only 30 out of 79 cases were actually non-reactive in this experiment with undiluted serum, while about 40 more would have been non-reactive by the T. P. I. A. test as it has been devised. This has shown that there may be defects in the tecnnique which need more extensive study and standardization.

TABLE 6.

*Quantitative Results of the T. P. I. A. and VDRL Tests Compared in 130 Cases of Syphilis.*

| Dilution Units | Number of Syphilis Cases | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Undiluted ( I dil ) | 5 | 10 | 20 | 40 | 80 | 160 | 320 | 640 | Total |
| TPIA TEST | 18 | 10 | 10 | 11 | 15 | 17 | 26 | 20 | 3 | 130 |
| Dilution Units | Undiluted ( I dil ) | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | Total |
| VDRL TEST | 7 | 9 | 15 | 11 | 22 | 22 | 31 | 10 | 3 | 130 |

In the table 6, the distribution of the quantitative Serologic reaction in "Serum dilution units" of the TPIA test is checked against that of the VDRL test for Syphilis. The qualitiative, TPIA test has been done with initial dilution I in 5 of the neat Serum, while the VDRL test is done qualitatively on the undiluted serum. In the quantitative TPIA test technique doubling Serial dilution is made from an initial I in 5 dilution of the serum. In this experiment quantitative TPIA test has been done beginning from the undiluted serum. The 2 techniques may not be strictly comparable and yet they may be noted to follow similar trends when tested quantitatively as shown in this table.

TABLE 7.

T. P. I. A. Vs. V. D. R. L.

*Sensitivity and Specificity Compared.*

ON C. S. F.

| TESTS | Neurosyphilis | | | Non-Neuro Syphilis | | | Latent Syphilis | | | Sero Reactive Mental Disease | | | Nonsyphilis Group | | | Specificity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. | Pos. | Sensitivity | No. | Pos. | Neg. | No. | Pos. | Neg. | No. | Pos. | Neg. | No. | Pos. | Neg. | |
| TPIA | 31 | 28 | 90.3% | 12 | 0 | 12 | 21 | 1 | 20 | 27 | 0 | 27 | 13 | 0 | 13 | 100% |
| VDRL | 31 | 30 | 97% | 12 | 0 | 12 | 21 | 1 | 20 | 27 | 0 | 27 | 13 | 0 | 13 | 100% |

## DISCUSSION

The diagnostic value of the T. P. I. A. test for Syphilis using a specific antigen consisting of dead virulent T. pallida has been assessed in this study by estimating its percentage " sensitivity " or " Reactivity " in well-established cases of Syphilis in its various stages and its percentage of " specificity " or " Non-reactivity " in known cases of Non-Syphilitic abnormal and normal cases, in parallel test with the routine standard V. D. R. L. slide flocculation test for Syphilis using the non-specific purified tissue extract " Cardiolipin " antigen.

The T. P. I. A. test has been found to agree with the clinical diagnosis in 272 compared with 269 for the V. D. R. L. test in 291 cases of Syphilis, giving 93.5 and 92.4 percent " sensitivity ", respectively for these 2 tests as seen in the table 2. The difference between them in " sensitivity " is not considered significant statistically, and they have agreed in their results in 286 or 98.3 percent of 291 cases.

The disagreements with the clinical diagnosis, of both the tests have been mainly confined to " primary Syphilis ". Both tests were non-reactive in 17 cut 79 dark field positive primary Syphilis cases. This may be expected from them. In that earliest stage of Syphilis, the elaboration of antibodies against T. pallidum takes time. The level of the antibody in the Serum may not have reached the reactive thresh-hold level at which, the serologic tests are usually set or standardized with due regard to their combined sensitivity and specificity, to detect them at least in 17 cases in this study at the time investigated. It is generally admitted that the standard serologic tests for Syphilis may be expected to be reactive in primary syphilis only about a week after the appearance of the primary chancre and a certain percentage of primary Syphilis cases may be expected to be sero-negative. Under these circumstances the ' sensitivity ' of the T. P. I. A. test for Syphilis in this study may be considered satisfactory.

With reference to the " specificity ", a more important quality of the test under check in this study, the T. P. I. A. test has confirmed the clinical exclusion of Syphilis in 116 normal persons giving it 100 percent " specificity ". But then, the T. P. I. A. test has not shown any advantage in this regard, over the routine standard V. D. R. L. test for Syphilis which has 100 percent agreement with its results on specificity in this particular group as seen in table 3.

However, in the non-syphilitic abnormal conditions, including 18 cases of tuberculosis, 350 cases of leprosy, and 75 other miscellaneous diseases, the T. P. I. A. test has appeared to have an apparent advantage in ' specifity" over the V. D. R. L. test. But its overall specificity had come down to 85.9 percent of 558 cases when the abnormal 442 cases were added to 116 normal cases. A specificity of 85.9 percent for the T. P. I. A. test is obviously better than 79.8 percent obtained for the V. D. R. L. test. In fact the T. P. I. A. test has shown comparatively higher specificity over the V. D. R. L. test in each of the non-syphilitic disease group and yet

the specificity of the T. P. I. A. test has not been high enough in these non-syphilitic diseases. In this connection it may be mentioned that the sure exclusion or establishment of Syphilis, particularly in its latent from clinically alone, in 350 leprosy patients included in this study has not been possible. Leprosy patients belonged to the same socio-economic strata as Syphilis patients and epidemiologically liable to exposure and infection to Syphilis just as to leprosy, Therefore 83.4 per cent specificity only obtained for the T. P. I. A. test in this particulrr leprosy group may not be put seriously against it.

In the table 4, is listed 27 out of 258 abnormal conditions in which past or present treponemal infection has been reasonably excluded and in which the T. P. I. A. test has been non-reactive while the V. D. R. L. test has been reactive. These 27 cases may be included in the group of the so-called " Biologic false positive " (B. F. P.) reactions, for Syphilis to the V. D. R. L. test and varified so with the help of the T. P. I. A. test with its apparent advantage in specificity.

The physicians are often confronted with the problem of interpretation of sero-reactions in lower titres of " 1 to 4 dils " obtained with the routine standard tests like the V. D. R. L. test, in relation to Syphilis infection, past or present. With a view to find out if the T. P. I. A. test with its apparent potential specific worth would be of help to verify whether these low titre reactions were true or false, the T. P. I. A. test had been specially performed on a sera of 78 cases in which Syphilis could not be established or excluded clinically alone in this study. As shown in the table 5, 30 out 79 cases had become non-reactive to the T. P. I. A. test suggesting the possibility that it may be used in the differentiation of " latent Syphilis " diagnosed so on the strength of low titre reactions obtained with the routine V. D. R. L. test from the false positive reactions for Syphilis.

Earlier report of workers on the T. P. I. A. test have been only on the qualitative aspect of it. In this study a quantitative technique has been experimentally devised for it and parallel tests have been performed with the quantitative V. D. R. L. test on a limited number of V. D. R. L. test positive reactors. It would appear from the results shown in the table 6 that the 2 tests have similar quantitative trends, in 130 cases of Syphilis investigated. This study on the quantitative aspects of the T. P. I. A. test has shown that it is not too easy technically and yet it can be applied to this new test according to Standard Serologic methods to get the full significance from its results.

In this connection it may be noted that, in the performance of the qualitative T. P. I. A. test as it has been devised, the serum has to be diluted 1 in 5 initially in saline. In fixing an " optimal proportion " for the antibody and the antigen, the authors of the T. P. I. A. test may have had preliminary evidence and fear of " false positive " reactions in its results, if undiluted serum is used. To check on this, in the experimental trial of a quantitative technique for the T. P. I. A. test, undiluted sera were also investigated with it in a limited number of cases as shown in the

tables 5 and 6. In the group of 79 cases ( table 5 ) from which Syphilis could not be established excluded and in which the V. D. R. L. test had been reactive in low titre, T. P. I. A. test was found reactive in the undiluted serum only or reactive in 1 dil in 40 instances, They would have been non-reactive to the qualitative T. P. I. A. test if performed on the serum, initially diluted 1 in 5 and thus the V. D. R. L. test reaction would have been considered " false " in such cases. In another group of 130 cases of Syphilis investigated quantitatively with T. P. I. A. test, 18 cases had been reactive in the undiluted serum only and possibly they would have been missed by the qualitative T. P. I. A. test performed with an initial dilution of the sera 1 in 5 in saline as seen table 6. Therefore, it is felt that the technique of the T. P. I. A. test would need more extensive experimental study before the optimal proportion of its antigen and antibody is finally fixed in the qualitative test and the test standardized.

The T. P. I. A. test has been adapted and applied in this study to investigate the " cerebrospinal fluid " with a view to check on its comparatice value to diagnose neuro-syphilis specifically. From the results shown in the table 7 it has appeared that the T. P. I. A. test using a specific treponemal antigen may not have special advantage of " sensitivity " in neuro-syphilis cases, or advantage of " specificity " in non-syphilis cases, over the standard V. D. R. L. test in current use in tests for Syphilis on C. S. F.

The T. P. I. A test with its apparent specific value on serum specimen, was specially tried in this study on specimens of C. S. F, from 27 cases of mental diseases which were reactive to the V. D. R. L. test only in the serum, with a view to establish, if possible, a syphilitic etiology of these psychiatric conditions. The T. P. I. A. test has been non-reactive just as the V. D. R. L. test, in all the 27 specimens of C. S. F. iuvestigated as shown in table 7.

An earlier investigator on the T. P. I. A. test, DAGUET (1956) has shown by absorption experiments that the antibody detected with this test is distinct from the " Wassermann " type of the non-specific anti-lipoidal antibody detected in Syphilis sera, with all the Standard Serologic tests using more less purified tissue lipid antigen. Further the antibody concerned in " immune adherence " is considered to be closely allied to the specific " immobulizing antibody " of the T. P. I. test, in view of Daguet's finding that in 382 proved non-syphilitic sera not reactive to the T. P. I. test, one specimen alone gave the immune disappearance reaction, and in 307 T. P. I. reactive sera of untreated Syphilitics, only 7 failed to react to the T. P. I. A. test. Miller ( 1957 ) also reported that, in 25 serum specimens from treated cases reactive to standard Serologic tests, he found his T. P. I. test reactive in 20 and T. P. I. A. test reactive in 22. In all the 23 samples of sera from normal cases, both the T. P. I. and T. P. I. A. tests were non-reactive, while in 53 patients with no history of Syphilis but with serum reactive to standard tests, 52 were non-reactive in both the T. P. I. and T. P. I. A. tests on " one " alone was reactive to T. P. I. A. test.

The T. P. I. test could not be established in the Madras V. D. Laboratory because of the technical difficuities and lack of the complicated equipments and materials indicated. However in order to check the value of the T. P, I. A. tess against the known specific T. P. I. test in this study, 288 specimens of sera from well-established cases of leprosy, with no clinical evidence of Syphilis were specially investigated. Split samples of those leprosy sera were sent to Copenhagen State Serum Institute by air, and parallel tests were performed on them with the T. P. I. A. test at Madras, and the T. P. I. test at Copenhagen. Of the 288 samples, 234 were non-reactive, 48 were reactive, to both the tests, while 6 were reactive by the T. P. I. A. test alone.

It would appear from the results obtained in this study and reports of earlier workers, that the T. P. I. A. test for Syphilis using the Virulent treponemal antigen has tendency to follow serologically the reaction of the T. P. I. test more closely with regard to the " specificity " than that of the standard Serologic tests using the non-specific tissue extract antigen in current use in the routine diagnosis of Syphilis. Although it has been demonstrated by absorption experiments on Syphilis Sera with " cardiolipin " antigen that the T. P. I. A. test antibody is distinct from the V. D. R. L. test antibody, the complete identity of the antibody concerned with immune adherence, with the antibody concerned with Immobilization of virulent T. Pallida, has not yet been shown conclusively. This is due to the fact that the reverse absorption experiments on Syphilis sera with virulent T. pallidum antigen have proved technically difficult. It seems possible that the 2 antibodies involved on the two tests may not be quite indentical in view of the fact that, in the T. P. I. test, intact live and virulent T. pallida are used as the antigen, while in the T. P. I. A, test, the virulent T. pallida are killed with heat thereby, possibly, disturbing its constitution and antigenicity. Probably that is why there has been a lack of hundred percent agreements in all the results reported on T. P. I. and T. P. I. A. tests to-date. It is also quite possible that during the course of Syphilis infection a number of differently reacting antibodies against the several antigenic constituents which are known to be present in the T. pallidum of Syphilis, may appear in the patients serum with varying degree of specificity as has been demonstrated in various serologic test procedures for Syphilis.

The T. P. I. A. test has been found to provide certain technical advantages over the T. P. I. test in the fact that dead or killed, preserved and a stable preparation of the virulent T. pallida can be used, resulting in less expense and possibly more easy technical application of the test in most of the serologic laboratories. But there are several technical points involved in the T. P. I. A. test still needing extensive experimentation for its general application. For instance, the counting of the treponemes under darkfield microscope, their spontaneous non-specific disappearance, the antigen preparation in quantity and quality a standardized technique and a criteria of reactivity and nonreactivity and a determination of end-point in quantitation. Therefore, the T. P. I. A. test is considered not too easy

to be performed as a specific routine test in all laboratories. As a "Reference verification test" for false positive reactions for Syphilis obtainable with currently used standard tests, the T. P. I. A. test has appeared to have a useful role to play.

## SUMMARY & CONCLUSION

The T. P. I. A. test for Syphilis employing a specific virulent T. pallidum antigen, was evaluated in this study, with reference to its comparative value, with the standard V. D. R. L. tests for Syphilis, in the specific diagnosis of syphilis. It had been shown to have a satisfactory "sensitivity" of 93.5 percent, against 92.4 percent for the standard V. D. R. L. test for Syphilis in 291 cases of Syphilis. The T. P. I. A. test produced 100 percent specificity in 116 normal cases but then the VDRL test too had the same degree of specificity in this group. In non-syphilitic diseases of various categories, the T. P. I. A. test had shown only 85.9 percent specificity in 558 cases but showed an apparent advantage of the VDRL test with 79.8 percent "specificity" in the same group.

Evidence has been given from the reports of earlier workers and to a very limited extent from this study that the T. P. I. A. test has tendency to follow more closely the serological reaction of the known specific T. P. I. test for Syphilis rather than that of the currently used standard tests. A list has been given of non-syphilitic diseases in which it had been possible to verify with the T. P. I. A. test, whether the sero-reaction obtained with the VDRL test has been true or biological false positive for Syphilis.

The T. P. I. A. test has been found to be technically easier to perform than the T. P. I. test and yet it has been found to have limitations and draw-backs so that it may not be used as a routine test in the specific diagnosis or syphilis in all laboratories. It may be applied as a reference test for Syphilis to verify whether the sero-reactions obtained with the current standard test is true or false. The various aspects of the T. P. I. A. test has been discussed.

The investigation was carried out under the Indian Council of Medical Research. The financial assistance and the encouragement given by the I. C. M. R. is gratefully appreciated. The authors acknowledge the facilities provided for this study in the V. D. Laboratory of the Institute of Venereology of the Madras Medical College, by the Government of Madras through the Director of Medical Services and the Dean, Madras Medical College. The Director of the Institute of Venereology and his clinical staff are thanked for the clinical materials and advice on them. Finally, the authors are thankful for the technical assistance provided by the assistants and other technical personale in the V. D. Laboratory in this study.

## REFERENCES.

1. DAGUET, G. L. (1956): T. pallidum Immune adherence and haemagglutination BRIT. J. VEN. Dis. 32 96.
2. HARRIS, A. ROSENBERG, A. A. & DEL VECCHIO, R. (1948): The V. D R. L. slide flocculation test for Syphilis.
3. MILLER, J. N., BOAK, R. A., & CARPENTER, C M. (1957): T. pallidum Immune adherence (T. P. I. A.) test in the diagnosis of Syphilis, J. A. M. A. 163:112.
4. NELSON, R. A. (1953): The Immune adherence phenomenon. SCIENCE 118
5. ROSENBERG, A. A., HARRIS, A, & HARDING, V. L. (1948,: A macroflocculation spinal fluid test employing Cardiolipin beethin antigen J. VEN Dis INFOPM 29.359.