



**A Comparative analysis of VDRL, RPR, Immutrep TPHA and Instachk – TP.**

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## Introduction

Syphilis is a systemic infectious disease caused by *Treponema pallidum*<sup>[1]</sup>. It leads to overwhelming multiorgan involvement with irreversible lifelong sequelae if left untreated<sup>[2]</sup>. Diagnosis of syphilis is neither easy nor rapid. Serodiagnosis of syphilis is usually based on detection of antibodies against cardiolipin or against the causative organism<sup>[3]</sup>. New molecular tests for syphilis are unlikely to replace serology in the short term because they are expensive and require sophisticated equipment. Antibody detection by treponemal (TPHA) and nontreponemal tests (VDRL,RPR) are still regarded as the mainstay for diagnosing syphilis that are suitable for large scale use and for monitoring treatment response. Cardiolipin tests are not truly specific.<sup>[4]</sup> Fluorescent treponemal antibody absorption test is used for confirmation. An ideal serological test for syphilis should have high sensitivity and specificity<sup>[5]</sup>. This study aims to obtain a profile of serological testing for syphilis.

## Aims and Objectives

1. To study the prevalence of syphilis..
2. To compare the results of VDRL and RPR.
3. To compare the results of immutrep TPHA and instachk TP
4. To compare the specific and nonspecific test.

## STUDY PERIOD:

Dec 2010- June 2011

## SAMPLE SIZE:

Serum samples from 450 patients.

## INCLUSION CRITERIA:

Blood samples collected in Dept of venereology from patients suspected to be suffering from syphilis and from antenatal patients .

## EXCLUSION CRITERIA:

Lipemic and lysed blood samples

## Materials and Methods

This prospective cohort study was done during the period of Dec 2010 to June 2011. Four hundred and fifty serum samples were obtained. Five ml of venous blood was collected and serum was separated and subjected to VDRL, RPR, Immutrep TPHA and Instachk tests.

## METHODS

Qualitative and quantitative VDRL and RPR test were carried out on all sera using VDRL antigen obtained from Institute of Serology, Kolkata. RPR card test from AGAPPEE diagnostics. Immutrep TPHA was done using Omega diagnostics. Instachk TP was done using TRANSASIA BIOMEDICALS. All the procedures were done according to manufacturers instruction. Instachk TP is a rapid one step visual qualitative and immunochromatographic assay for detection of antibodies to Treponema pallidum antigen in blood, serum or plasma. (double antigen sandwich). The presence of two lines within fifteen minutes indicates positive result.

## Results

### AGE, GENDER DISTRIBUTION AND POSITIVITY RATE OF SEROLOGICAL TESTS .(n= 450)

TEST	16 - 30		31 - 45		46 - 60		>60		TOTAL M	TOTAL F	TOTAL POS RATE
	M	F	M	F	M	F	M	F			
NO. PTS	101	70	184	46	23	10	16	0	324	126	450
RPR +	17	0	24	1	4	1	0	0	45	2	47(10.44%)
RPR -	84	66	165	44	19	9	16	0	284	119	403
VDRL +	17	1	28	1	5	1	0	0	50	3	53(11.78%)
VDRL -	84	69	159	45	15	9	16	0	274	123	397
IMMU +	17	2	30	2	2	0	0	0	49	4	53(11.78%)
IMMU -	84	68	154	44	21	10	16	0	275	122	397
INSTA +	17	2	30	2	2	0	0	0	49	4	53(11.78%)
INSTA -	84	68	154	44	21	10	16	0	275	122	397

Majority of the patients (72%) were males in the age group of 31 – 45 yrs. Among the 450 patients VDRL and

Instachk,TPHA shows higher positivity rate of 11.78%.

### **GENDER DISTRIBUTION IN RELATION TO VARIOUS STAGES OF SYPHILIS (n=53)**

	<u>PRIMA RY</u>		<u>SEC</u>		<u>ELS</u>		<u>LLS</u>		<u>TOTAL</u>		<u>TOTAL REACTIVE</u>
	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M</u>	<u>F</u>	<u>M+F</u>
<b><u>NO OF PATIENTS</u></b>	<b><u>17</u></b>	<b><u>0</u></b>	<b><u>4</u></b>	<b><u>0</u></b>	<b><u>19</u></b>	<b><u>2</u></b>	<b><u>9</u></b>	<b><u>2</u></b>	<b><u>49</u></b>	<b><u>4</u></b>	<b><u>53</u></b>
<b><u>RPR</u></b>	<b><u>14</u></b>	<b><u>0</u></b>	<b><u>4</u></b>	<b><u>0</u></b>	<b><u>18</u></b>	<b><u>1</u></b>	<b><u>4</u></b>	<b><u>1</u></b>	<b><u>40</u></b>	<b><u>2</u></b>	<b><u>42(79.25%)</u></b>
<b><u>VDRL</u></b>	<b><u>17</u></b>	<b><u>0</u></b>	<b><u>4</u></b>	<b><u>0</u></b>	<b><u>18</u></b>	<b><u>1</u></b>	<b><u>7</u></b>	<b><u>1</u></b>	<b><u>46</u></b>	<b><u>2</u></b>	<b><u>48(90.56%)</u></b>
<b><u>IMMU</u></b>	<b><u>17</u></b>	<b><u>0</u></b>	<b><u>4</u></b>	<b><u>0</u></b>	<b><u>19</u></b>	<b><u>2</u></b>	<b><u>9</u></b>	<b><u>2</u></b>	<b><u>49</u></b>	<b><u>4</u></b>	<b><u>53(100%)</u></b>
<b><u>INSTA</u></b>	<b><u>17</u></b>	<b><u>0</u></b>	<b><u>4</u></b>	<b><u>0</u></b>	<b><u>19</u></b>	<b><u>2</u></b>	<b><u>9</u></b>	<b><u>2</u></b>	<b><u>49</u></b>	<b><u>4</u></b>	<b><u>53(100%)</u></b>
<b><u>TOTAL</u></b>	<b><u>17(32.07%)</u></b>		<b><u>4(7.5%)</u></b>		<b><u>21(39.62%)</u></b>		<b><u>11(20.76%)</u></b>		<b><u>total positives =53(11.78%)</u></b>		

out of 450 patients 53 had clinical and laboratory evidence of syphilis and the seroprevalence was 11.78%.Most of the patients were males in early latent stage (39.62%)

### **POSITIVITY RATE OF SEROLOGICAL TESTS IN EACH STAGE; (n=53)**

	<u>VDRL</u>	<u>RPR</u>	<u>IMMUTREP</u>	<u>INSTACHK</u>
<b><u>Primary</u></b>	<b><u>17(100%)</u></b>	<b><u>14(82.35%)</u></b>	<b><u>17(100%)</u></b>	<b><u>17(100%)</u></b>
<b><u>Secondary (prozone =2)</u></b>	<b><u>4(100%)</u></b>	<b><u>4(100%)</u></b>	<b><u>4(100%)</u></b>	<b><u>4(100%)</u></b>
<b><u>ELS</u></b>	<b><u>19(90.48%)</u></b>	<b><u>19(90.48%)</u></b>	<b><u>21(100%)</u></b>	<b><u>21(100%)</u></b>
<b><u>LLS</u></b>	<b><u>8(72.72%)</u></b>	<b><u>5(45.45%)</u></b>	<b><u>11(100%)</u></b>	<b><u>11(100%)</u></b>
<b><u>TOTAL</u></b>	<b><u>48(90.56%)</u></b>	<b><u>42(79.25%)</u></b>	<b><u>53(100%)</u></b>	<b><u>53(100%)</u></b>

Among the 53 syphilitic patients overall positivity rate of serological tests in the detection of syphilis is VDRL (90.56%) ,RPR (79.25%),TPHA (100%) and INSTACHK TP (100%)

**COMPARISON OF RPR and VDRL**

STAGE	UNDIL		1:2		1:4		1:8		1:16		1:32		1:64		1:128		total	
	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V	R	V
REACTIVES	0	1	3	5	2	5	23	19	11	13	6	7	2	2	0	1	47	53
BFP(5)		1	3	4	2	-	-	-	-	-	-	-	-	-	-	-	5	5
<b>TOTAL REACTIVES IN SYPHILIS</b>																	<b>42</b>	<b>48</b>
<b>REACTIVITY OF VARIOUS STAGES:</b>																		
PRIMARY	-	-	-	-	-	3	10	10	4	4	-	-	-	-	-	-	14	17
SECONDARY	-	-	-	-	-	-	-	-	-	-	2	1	2	2	-	1	4	4
ELS	-	-	-	-	-	-	9	5	6	8	4	6	-	-	-	-	19	19
LLS	-	-	-	1	-	2	4	4	1	1	-	-	-	-	-	-	5	8
<b>TOTAL</b>																	<b>42</b>	<b>48</b>

VDRL was able to detect 6 cases of low titres more frequently than RPR (11.32%) .All these 6 cases were reactive by specific tests and three of them were in primary stage and three of them were in late latent stage.RPR was one titre less than VDRL in eight cases (15.09%).biological false positives were observed in 5 cases.(1.11%) by both the tests .

**Discussion**

The seroprevalence of syphilis in this study has been observed to be 11.78%.Among the 450 patients in study 51.11% were in the age group of 31-45 years. This is in concordance with literature which says that syphilis is more common in this age group. 72% of the patients were males. This may be because males are more exposed to commercial sex workers and these females act as nidus of infection.<sup>(2)</sup> In our study 53 were diagnosed with syphilis based on clinical and laboratory evidence. Most of the patients were in the early latent stage (39.62%) followed by primary (32.07%).

VDRL, and Instachk- TP ,Immutrep TPHA were able to detect all 17 (100%) patients in the primary stage where RPR in 14 (82.35%). The patients with VDRL titres  $\leq 1:4$  and reactive by specific tests were not detected by RPR. VDRL was observed to detect low titres more frequently than RPR as the interpretation is based on microscopy in VDRL than in RPR which is based on macroscopic agglutination. All the four tests were able to detect all the 4(100%) patients in the secondary stage of the disease. Prozone phenomenon was observed in 50% of secondary stage of syphilis so quantitative analysis should be done for all suspected cases of secondary syphilis<sup>(6)</sup>.

VDRL and RPR detected 19 (90.48%) cases in early latent stage and VDRL detected 8 (72.72%) and RPR in

5 cases of late latent stage (45.45%). RPR fails to detect 3 cases in late latent stage of titres of  $\leq 1:4$  which VDRL detected. Apart from these 19 cases, both Immutrep TPHA and Instachk-TP were able to detect 2 (100%) cases additionally in early latent stage and 3(100%) cases in late latent stage of the disease which neither VDRL nor RPR could detect. The failure of nonspecific tests to detect the two patients in early latent stage is that probably the patient may be treated with antibiotics for some other reasons. Both Immutrep TPHA and Instachk-TP complement each other. Thus there is around 30% loss in the sensitivity of the non specific test during the latent stages. Generally with the use of VDRL and RPR, 30% of the cases become negative in late latent syphilis<sup>[2]</sup>. With specific tests however it remains positive for life. Its consistent with the findings of H. Young et al.<sup>(8)</sup>

Among the total of 450 patients 5 (1.11%) tested positive with VDRL and RPR respectively while the same patients tested negative with both Immutrep TPHA and Instachk-TP.. The initial positivity could thus be a biological false positive reaction seen commonly with the use of nonspecific tests. Our study has exposed the shortfall of VDRL and RPR as described in literature.<sup>(2)</sup> Among the five patients three had a history of typhoid and two of them were antenatal women. This could be biological false positive reaction commonly observed in lower titres in pregnant women.<sup>(1,7)</sup>

Among the nonspecific tests VDRL detect low titres more frequently than RPR and quantitatively they differ in titres such that RPR was one titre less than VDRL. These data shows that the RPR and VDRL tests vary quantitatively. So these tests should not be interchanged during followup of treatment and in quality control programs.

Among the specific tests though the detection rate were same the instachk test was a rapid test that can be interpreted within fifteen minutes .this can open up new avenue for early ,rapid and accurate detection of syphilis at a field level.

## Conclusion

The seroprevalence of syphilis in this study is 11.78%. Among the nonspecific tests VDRL was observed to detect low titres more frequently than RPR . Both the tests vary quantitatively and they should not be interchanged during follow up of patients and in quality control assessment .Among the specific tests though the detection rate were same the instachk test was a rapid test that can be interpreted within fifteen minutes and this can open up new avenue for early ,rapid and accurate detection of syphilis at a field level . While using nonspecific tests quantitative analysis has to be done for all suspected case of secondary syphilis to avoid prozone phenomenon which is not seen in specific tests . There is around 30% loss in the sensitivity of the

non specific test during the latent stages. Biological false positives (1.11%) were observed in non specific assays. So the present day serological tests of syphilis can thus be limited to the specific tests to exclude syphilis and the nonspecific tests to judge the need or effectiveness of antisyphilitic treatment.

### **AGE, GENDER DISTRIBUTION AND POSITIVITY RATE OF SEROLOGICAL TESTS .(n= 450)**

Test	16-30				31-45				46-60				>60				TOTAL M	TOTAL F	TOTAL POS RATE
	M	F	T	%	M	F	T	%	M	F	T	%	M	F	T	%			
<b>NO OF PTS</b>	10	70	17	38	18	46	23	51	23	10	3	7.3	16	0	16	3.5	324	126	450
<b>RPR +</b>	17	0	17	36.1	24	1	25	53.1	4	1	5	10.6	0	0	0	0	45	2	47(10.44%)
<b>VDR L +</b>	17	1	18	34	28	1	29	54.7	5	1	6	11.3	0	0	0	0	50	3	53(11.33%)
<b>TPH A +</b>	17	2	19	35.9	30	2	32	60.4	2	0	2	3.8	0	0	0	0	49	4	53(11.78%)
<b>INSTACHK +</b>	17	2	19	35.9	30	2	32	60.4	2	0	2	3.8	0	0	0	0	49	4	53(11.78%)

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