

## COMPARISON OF THE VDRL SLIDE, TPI, AND FTA-ABS TESTS IN EXPERIMENTAL SYPHILIS IN RABBITS\*†

BY

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The first serological test for syphilis, developed by Wassermann, Neisser, and Bruck (1906), was a complement-fixation test for reagin activity. Many tests for reagin activity have been developed since then, including the Kline, Kahn, Hinton, Kolmer, Mazzini, and VDRL slide tests. However, none of these is absolutely specific for syphilis. The VDRL slide test (Harris, Rosenberg, and Riedel, 1946), which utilizes a cardiolipin-lecithin antigen, is that most widely used in the United States. The *Treponema pallidum* immobilization (TPI) test, developed by Nelson and Mayer (1949), was the first serological procedure utilizing the causative organism *Treponema pallidum* as the test antigen. Although several treponemal tests of various sensitivities and specificities have subsequently been developed, the TPI test is, at present, the most widely known verification test for diagnostic problem cases, but it is not easily performed and is available in only a few reference laboratories.

The most promising procedure for TPI replacement is the fluorescent treponemal antibody-adsorption (FTA-ABS) test (1965), which incorporates the sensitivity and specificity of immunofluorescence techniques. This test is a modification of the original procedure (Deacon, Falcone, and Harris, 1957), in which a sorbent prepared from the nonpathogenic Reiter treponeme (Stout, Kellogg, Falcone, McGrew, and Lewis, 1967) is used to remove the non-specific immunofluorescence due to several factors, including antibody to the common (group) antigen. The FTA-ABS is a less complicated procedure and is easier to perform than the TPI test.

In previous evaluations confined to human patients, the FTA-ABS test compared favourably with other serological methods (Hunter, Deacon,

and Meyer, 1964; Deacon, Lucas, and Price, 1966) in sensitivity and specificity in the detection of current or previous syphilitic infections. This paper reports a retrospective study comparing results of FTA-ABS, TPI, and VDRL slide quantitative testing of sera of normal rabbits and those with experimental syphilis or cuniculosis.

### Material and Methods

Sera from four groups of rabbits were tested: normal animals, those exposed to infection with *Treponema cuniculi*, those infected with *Treponema pallidum* (Nichols strain), and those infected with *T. pallidum* by intracutaneous inoculation and then treated with varying amounts of five different antibiotics.

**Animal Groups** Of the 536 normal rabbits—recent additions to our colony from one or two suppliers—none showed clinical or serological evidence of cuniculosis or other disease during an initial minimum observation period of 4 weeks.

The cuniculi group consisted of twenty animals exposed to *T. cuniculi* infection either by natural transmission through cohabitation and/or contact at birth or experimentally by tissue transfer. Infection was proved in seven of the twenty by the development of typical darkfield-positive lesions, the conversion of TPI and FTA-ABS tests to reactive, and/or the infection of recipients by transfer of tissue.

The untreated syphilitic group consisted of 78 animals infected by inoculation of Nichols strain *T. pallidum* into the skin of the back or into the testicle.

The treated group comprised 32 rabbits with darkfield-positive lesions from intracutaneous inoculation of Nichols *T. pallidum* which were treated with several schedules of nafcillin (1), potassium penicillin G (2), ancillin (3), cephaloridine (4), or colymycin (5) to determine the curative effects of these antibiotics. Treatment was started 7 days after the animals were inoculated and continued for 5 to 7 days.

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(1) Wyeth Laboratories, Philadelphia, Pa.  
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(3) Smith, Kline, and French Laboratories, Philadelphia, Pa.  
(4) Eli Lilly and Co., Indianapolis, Ind.  
(5) Warner-Chilcott Laboratories, Morris Plains, N.J.

**Specimens** In the normal group, only one was drawn from each animal. These were tested by the VDRL slide quantitative test and the FTA-1:5 procedure. Only those sera showing reactivity by either of these procedures were routinely tested in the FTA-ABS procedure. A random selection of 158 sera non-reactive in the VDRL slide and FTA-1:5 procedures was tested by the FTA-ABS method. TPI testing was performed on all sera showing reactivity by the VDRL slide test, some of which were also reactive in the FTA-1:5 test. TPI testing was also performed on a chance selection of 61 sera non-reactive in the VDRL, the FTA-1:5, and the FTA-ABS tests.

In the experimentally-infected groups, specimens were drawn before inoculation and at intervals after inoculation and/or treatment. Since this was a retrospective study using sera collected from animals in other studies, the intervals between testing were not always optimal for the present study. Sera were frozen and stored at  $-20^{\circ}\text{C}$ . until tested. All sera from these groups were tested by the VDRL slide quantitative test and the FTA-1:5 procedure; those showing reactivity in the FTA-1:5 procedure were then tested in the FTA-ABS procedure. Some of the reported VDRL and TPI tests were performed in accordance with the protocols of these other studies; when indicated, additional testing was performed in this study if suitable sera were available. TPI testing generally was not performed before 6 months after inoculation, except in the cuniculus study. Results of all tests were compared with those of FTA-ABS testing.

**Tests** The TPI and VDRL slide tests were performed according to the techniques described in the manual "Serologic Tests for Syphilis" (U.S. Public Health Service, 1964).

In the FTA-1:5 procedure, 0.03 ml. of a 1:5 dilution of heat-inactivated serum in phosphate buffered saline (pH 7.2) is placed on a pre-fixed slide containing 0.005 ml. *T. pallidum* antigen and the slide is incubated for 30 min. at  $37^{\circ}\text{C}$ . After two 5-min. wash periods, 0.03 ml. diluted fluorescein-labelled anti-rabbit globulin is added and the slide is incubated and washed as before. Controls included known reactive rabbit serum, moderate and minimal reactive rabbit serum, non-specific staining and non-specific serum, as outlined by procedure requirements.

The FTA-ABS test was performed according to the "Provisional Technique for the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test" (1965), with the exception that fluorescein-labelled anti-rabbit globulin was used instead of fluorescein-labelled anti-human globulin.

The fluorescein-labelled anti-rabbit globulin\* used in the FTA-1:5 and FTA-ABS tests was checked tested for quality and standard reactivity before use, as suggested in the provisional technique previously cited. It was necessary to remove non-specific staining from some lots by two to three absorptions of globulin with liver or

bone-marrow powder (100 mg. powder per ml. antibody solution).

### Results

In presenting results, weak reactors in all tests are considered reactive. Test results of the presumed normal rabbits are summarized in Table I. Of the 536 sera tested with the VDRL slide and FTA 1:5 procedures, 122 (approximately 23 per cent.) showed reactivity by one or both tests; these sera were all non-reactive in the FTA-ABS test. The 59 normal sera which were reactive by the VDRL slide test were non-reactive in the TPI test. Of 63 sera reactive in the FTA-1:5 but not in the VDRL test, thirteen were TPI-tested and found non-reactive. 158 sera were non-reactive in the VDRL slide, the FTA-1:5, and the FTA-ABS tests; the remaining 256 sera were non-reactive in the first two procedures but were not tested in the FTA-ABS procedure. 61 sera which were non-reactive in the VDRL slide, the FTA-1:5, and the FTA-ABS procedures were also TPI-non-reactive.

TABLE I  
COMPARISON OF VDRL, FTA-1:5, FTA-ABS, AND TPI  
TESTING ON PRESUMED NORMAL RABBITS  
Number Reactive/Number Tested

Test Results	Rabbits	Percentage of Total	FTA-ABS	TPI
VDRL + : FTA-1:5 -	52	10.0	0/52	0/52
VDRL - : FTA-1:5 +	63	12.0	0/63	0/13
VDRL + : FTA-1:5 +	7	1.0	0/7	0/7
VDRL - : FTA-1:5 -	414	77.0	0/158	0/61*
Total Tested	536			

Notes: + = Reactive.  
- = Non-reactive.  
\* = All 61 FTA-ABS tested.

Seven of twenty rabbits exposed to *T. cuniculi* infection were proved to be infected by at least one positive darkfield examination, by TPI or FTA-ABS conversion over an 18-month observation. The other thirteen were never shown to be infected. FTA-ABS and TPI test results were in disagreement in nine of 29 specimens tested by both procedures. One infected animal, tested at intervals of 3, 6, 9, 10, 11, 12, 18, and 24 months and tested in parallel by TPI and FTA-ABS procedures, did not show sustained reactivity by the TPI method. The TPI tests showed inconstant reactive and non-reactive results while the FTA-ABS test was reactive at 2 months and remained so throughout the 24 months observation. Another animal, infectious on tissue transfer at 6 months, was non-reactive in five TPI tests performed at 1, 7, 8, 11, and 13 months. Tests at 9 and 12 months on this animal showed weakly reactive TPI results. The FTA-ABS test became reactive at

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4 months and was still reactive on the last occasion at 13 months.

Results of testing on sera collected from untreated syphilitic animals at intervals up to 112 weeks after inoculation are summarized in Table II. VDRL slide and FTA-ABS tests were performed at 4-week intervals; however, TPI testing was not routinely performed before 24 weeks. All 78 sera were reactive in the VDRL slide, TPI, and FTA-ABS procedures at 24 weeks; 23 tested at 48 weeks were reactive; and sixteen tested at 72 weeks, as well as twelve tested at 112 weeks, remained reactive in all tests.

In only two rabbits did earlier test results show disagreement. The first was FTA-ABS-reactive and TPI-non-reactive 1 month after inoculation. By 8 weeks, however, the TPI had become weakly reactive while the FTA-ABS remained reactive. Subsequently, both tests were reactive. The other rabbit which showed disagreement was reactive in the FTA-ABS test, but only a weak reactor in the TPI test at 6 months; repeat TPI testing of the same specimen was reactive. FTA-ABS reactivity could be demonstrated as early as 1 week in most animals and persisted once established.

Nine of the 32 treated syphilitic animals were cured, and 23 were considered treatment failures on the basis of the development of recurrent darkfield positivity at the original inoculation site or in secondary lesions, the development of TPI reactivity, and/or tissue-transfer infections. As shown in Table III, all 32 rabbits were FTA-ABS-reactive when tested soon after treatment and remained reactive throughout the 6 months of testing, regardless of the adequacy of treatment.

**Discussion**

Although the FTA-ABS test has been evaluated in humans with syphilis (Deacon, Lucas, and Price, 1966) and a pattern of reactivity has been suggested, little information has previously been available on the pattern of reactivity, sensitivity, or specificity of this test in

**TABLE III**  
COMPARISON OF SEROLOGICAL TEST RESULTS IN RABBITS TREATED FOR SYPHILIS WITH ANTIBIOTICS  
Number Reactive/Number Tested

Result .. ..	Cured			Not Cured		
	VDRL	FTA-ABS	TPI	VDRL	FTA-ABS	TPI
Test .. ..						
Pre-inoculation Baseline	4/9	0/9	NT	1/23	0/23	NT
7 days after <sup>a</sup> Inoculation (pre-Rx)	3/9	4/9	NT	6/23	21/23	NT
At end of <sup>b</sup> Treatment	9/9	9/9	NT	23/23	23/23	NT
Post-Treatment	1 wk	9/9	9/9	NT	23/23	23/23
	4 wks	9/9	9/9	NT	23/23	23/23
	12 wks	5/9	9/9	NT	20/23	23/23
	24 wks	7/9	9/9	1/9 <sup>c</sup>	21/23	23/23

Notes:  
NT = Not tested.  
(a) = All animals in both groups darkfield-positive.  
(b) = All animals in cured group darkfield-negative at this time and in subsequent testing. All animals in failure group darkfield-positive at this time, continuing for 3 weeks post-Rx in 22 rabbits and 10 weeks post-Rx in one rabbit which also developed a secondary lesion at that time.  
(c) = This animal was TPI-non-reactive at 14 months and is considered cured.

infected rabbits. Such knowledge is of particular value since rabbits are the animals classically used in studying experimental syphilis.

In the present study, rabbits showed reactivity in the FTA-ABS test as early as 1 week after inoculation with *T. pallidum*. This reactivity was sustained up to the limit of our testing (28 months) in twelve untreated rabbits tested at intervals throughout the time. It seems probable from other studies (unpublished) that this reactivity may persist even longer. It is not surprising that inadequately-treated rabbits continued to be FTA-ABS-reactive, since virulent disease persisted in them as shown by relapse of primary lesions to darkfield positivity, the development of darkfield-positive secondary lesions, the development of TPI reactivity, or the infectiousness of tissue on transfer. In the cured group, treatment was completed after the development of FTA-ABS reactivity. Further dilution of the sera may well show quantitative changes in the FTA-ABS titre after adequate treatment.

**TABLE II**  
COMPARISON OF VDRL, TPI, AND FTA-ABS TESTING IN RABBITS INFECTED WITH *T. pallidum* <sup>a</sup>  
Number Reactive/Number Tested

Test	Pre-inoculation	Post-inoculation (wks)						
		1	6	12	24	48	72 <sup>b</sup>	112 <sup>c</sup>
VDRL	1/78	20/78	78/78 <sup>d</sup>	78/78 <sup>e</sup>	78/78	23/23 <sup>f</sup>	16/16 <sup>g</sup>	12/12
TPI	NT	NT	NT	NT	78/78	23/23	16/16	12/12
FTA-ABS	0/78	53/78	78/78	78/78	78/78	23/23	16/16	12/12

Notes: NT = Not tested.  
(a) = All darkfield-positive 7 days after inoculation.  
(b) = Tested at 24 wks but not at 48 wks.  
(c) = Tested at 24 wks but not at 48 and 72 wks.  
(d) = 72 showed titre ≥ 1:64. Maximum 1:512.  
(e) = 71 showed titre ≥ 1:16. Maximum 1:128.  
(f) = Highest titre 1:8.  
(g) = Highest titre 1:4.

The specificity of the FTA-ABS procedure in humans has been evaluated. While one would expect a similar specificity in other species, it has not previously been proved in the rabbit. 536 normal sera were tested. It has long been known that seemingly normal rabbits may show low-grade VDRL reactivity and that specific testing is necessary to rule out asymptomatic treponemal infection. Previously, the TPI test has been used. In this study, seventy of 536 normal animals showed reactivity in the FTA-1:5 procedure, when FTA-ABS testing was consistently non-reactive. Since the FTA-1:5 procedure is a more sensitive but less specific test than is the FTA-ABS, those non-reactive in this test were not routinely tested by the absorption procedure. 158 rabbits non-reactive in the VDRL slide and FTA-1:5 procedures were also non-reactive when tested by the FTA-ABS method.

As shown by this study, if non-reactive test results are obtained on rabbit sera in the sensitive FTA-1:5 test, results of the more specific FTA-ABS test will also be non-reactive. This has been the generally accepted pattern in human syphilitic sera and appears to be equally true in rabbits experimentally infected with Nichols strain virulent *T. pallidum*.

Since the rabbit is subjected to an endemic treponematosiis caused by *T. cuniculi*, it was most important to investigate the pattern of reactivity in animals with cuniculosis by the FTA-ABS procedure. Seven rabbits with proven infection were slower to show sustained FTA-ABS reactivity than animals in this study infected with *T. pallidum*. Two of the seven showed an irregularly alternating TPI reactivity without sequence; these two had naturally transmitted infections.

A majority of the rabbits experimentally infected with Nichols strain *T. pallidum* showed reactivity earlier in the FTA-ABS test than in the VDRL slide test. Although all animals were not TPI-tested at the same interval, those tested showed reactivity in the FTA-ABS procedure earlier than in the TPI procedure. The rapidity with which reactivity develops in experimental syphilis in rabbits is probably influenced by the size of the antigenic stimulus contained in the inoculum. This factor influences all tests, however, so that in animals inoculated with fewer treponemes, tests would be expected to become reactive in the same sequence, although the intervals between appearance of detectable reactivity may be longer.

In the present study, FTA-ABS and TPI reactivity developed earlier with *T. pallidum* infection than with *T. cuniculi* infection. This may reflect the specificity of the test as well as the size of the antigenic stimulus in these studies. FTA-ABS reactivity is better correlated with TPI reactivity as to time of appearance and persistence in cuniculosis than in experimental syphilis. Since the antigens in both these tests are suspensions of *T. pallidum*, this is not surprising, for treponemes have been reported to share general group antigens (Deacon and Hunter, 1962), and earlier studies (Turner and Hollander, 1957) suggested that strains of pallida, cuniculi, and yaws were antigenically similar. The data presented suggest that the FTA-ABS test may replace

the TPI test in serological testing in experimental syphilis because of its greater sensitivity with equal specificity and its economic advantage. Occasional instances may be expected in which, due to host variation, the TPI test will be more sensitive. The FTA-ABS test as described, like other treponemal tests, is indicative of current or previous syphilitic infection, but it does not reflect adequacy of treatment. Since reactivity is sustained once established, this test cannot be used to follow the course of treponemal infection. However, quantitated dilutions of serum in the absorption procedure may detect changes in the titre of the antibody response.

### Summary

In rabbits, the FTA-ABS procedure was more sensitive and more specific than the VDRL slide test. The FTA-ABS test also proved more sensitive and just as specific as the TPI test in experimental syphilis in the rabbit. The FTA-ABS test, once reactive, remained reactive even after curative treatment. The FTA-ABS test promises to be a valuable tool in the study of experimental treponemal infections in rabbits. The results and patterns obtained completely parallel those found in human syphilis (Deacon, Lucas, and Price, 1966).

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## ADDENDUM

As part of another study, sera from twenty *Aotus trivirgatus*—a small, New-World monkey, experimentally infected with the Nichols virulent strain *T. pallidum*—were tested by the VDRL slide, TPI, and FTA-ABS procedures. The TPI and FTA-ABS test results were well-correlated; in several animals the FTA-ABS test was the first to show reactivity. Details of studies using this species are to be reported elsewhere (Clark and Yobs, in preparation). Monkey serum from this species was equally reactive when tested with fluorescein-conjugated antiserum to either monkey or human globulin.

**Une comparaison entre les tests suivants: TPI,  
FTA-ABS, VDRL Slide dans la syphilis  
expérimentale chez le lapin**

## RÉSUMÉ

Chez le lapin le test FTA-ABS était plus sensible et plus spécifique que le test VDRL. Le test FTA-ABS a été prouvé comme étant plus sensible et pas moins spécifique que le test TPI dans la syphilis expérimentale chez le lapin. Le test FTA-ABS, une fois réactif, restait réactif même après un traitement curatif. Le test FTA-ABS promet d'être un instrument de valeur dans l'étude expérimentale des infections causées par les tréponemes chez le lapin. Les résultats obtenus sont exactement ceux observés dans la syphilis humaine (Deacon, Lucas, et Price, 1966).