people can complete this graft technic in 15 to 25 rats in one hour. The adherence of the skin, hair and outer dessicated layers of the skin to the tape allows an unobstructed view of the viable graft and permits detection of minor changes, as compared with the control site. When the graft disc was placed without an overlying tape, or when the cast and tape were removed by the animal, the superficial dessication and hair prevented the early changes of slight edema, faint brownness in color, and reduction of graft diameter, from being seen or recognized as the early onset of the homograft reaction.

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## Fluorescent Treponemal Antibody Test Using the Reiter Treponeme.* (26456)

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The fluorescent treponemal antibody (FTA) test(1,2) for syphilis requires *Treponema pallidum* of the virulent Nichols strain as antigen, and as a result is expensive and limited in availability. This suggested an attempt to substitute the Reiter strain of treponeme, which is antigenically related and easily cultivated *in vitro*.

*Materials and methods.* Sera were from a collection of the *T. pallidum* immobilization (TPI) testing unit of the Division of Laboratories and Research; they were from persons verified as syphilitic or non-syphilitic on the basis of clinical or anamnestic evidence, supplemented by a critical test(3,4) for TPI antibody; many of the non-syphilitic individuals presented diagnostic problems in that they reacted serologically with cardiolipin antigens. Normal sera were from healthy members of both staffs and from blood donors. All sera were stored at –25°C. *T. pallidum* of the Reiter strain was cultivated at 35°C in screw-capped tubes containing 25 ml NIH thioglycollate broth† to which heated (2 hours at 56°C) rabbit serum had been added to give a final concentration of 10%. Growth was satisfactory at 2 days, the cultures remaining acceptable for antigen preparation from the 2nd to 5th day. Two ml of culture were removed aseptically from the undisturbed upper stratum and delivered into a 15× 100 mm pyrex tube containing 2 ml 0.002% sodium hypochlorite (NaClO)‡ in 0.85% NaCl solution. The NaClO was essential, the treponemes otherwise clumping, becoming atypical in morphology, and failing to adhere to slides. They were mixed well by gentle swirling, then sedimented by spinning 15 minutes at 1,000 g. The supernate was decanted, and the organisms were washed once by resuspending in 4 ml 0.85% NaCl solution, centrifuging, and decanting as before. They were suspended finally in enough 0.85% NaCl solution to make the count 10-15/430 × microscopic field, *i.e.*, about 2 × $10^6$/ml. *T. pallidum* of the Nichols strain was available as suspensions eluted from fresh or frozen(5) syphilomatous rab-

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† No. 0257-01, Difco Labs., Inc., Detroit, Mich.

‡ B.K chlorine-bearing liquid bactericide; active ingredient sodium hypochlorite 5.25%. Pensalt Chemicals Corp., Phila. 2, Pa. A 1:2,625 dilution was made in 0.85% NaCl solution.

bit testes in Nelson's medium(6) containing streptomycin and penicillinase(7); they were used on the day of elution or after 1-15 days' storage at 2-4°C. For FTA tests they were adjusted with Nelson's medium to contain 40-50 treponemes/430 × microscopic field, a count 3 to 4 times that of the Reiter suspension. This compensated for reductions in number/field that occurred during treatment of the films with serum and fluorescent antibody; ordinarily 10-15/field remained for final examination. No such losses occurred with the NaClO-treated Reiter treponemes. New precleaned microscope slides (75 × 25 × 1 mm) and cover slips (22 × 22 mm, thinness No. 1) were used: two 1-cm circles were inscribed on the former with a template and diamond stylus. Slides and cover slips were soaked 1 hour in 95% ethanol, then wiped smooth with clean gauze followed by disposable tissue.§ They were stored in dust-free containers. The technic of fluorescent antibody testing was essentially that given (1,2) for the FTA test. Aliquants (0.01 ml) of an antigen suspension were delivered into each of the circles on a slide and spread to fill the circles with a glass rod. The films were air-dried, after which the slides were immersed 10 minutes in acetone (Certified Reagent), and again air-dried. Sera to be tested were heated 30 minutes at 56°C, and a 0.1-ml sample diluted 1:200 stepwise by adding 1.9 ml phosphate buffered saline solution of pH 7.2 (PBS),‖ mixing well, transferring 0.10 ml to a tube containing 0.9 ml PBS, and again mixing well. The 1:200 dilution sufficed for qualitative tests; for quantitative testing, further serial 2-fold dilutions were made in PBS. In the test proper, 0.03-ml aliquants of diluted serum were added to 2 films each of the Reiter and Nichols treponemes, and the slides rotated¶ 30 minutes at 100 rpm in the incubator (37°C); they were protected from evaporation by lids containing moistened blotting paper. The slides were then rinsed in PBS, soaked for 5 minutes in each of 2 changes of PBS, and pressed gently on filter paper to remove excess moisture. Fluorescein-labeled horse antihuman globulin** diluted optimally (1:30) in PBS containing 2% Tween 80†† was then added, 0.03 ml being distributed over each film, and the slides again rotated 30 minutes at 100 rpm in the incubator. They were washed twice in PBS as before and blotted gently on filter paper. Finally, a drop of buffered glycerol (9 parts glycerol + 1 part PBS) was placed on each reaction site and surmounted with a cover slip. Slides were examined by darkfield microscope,‡‡ using low fluorescence immersion oil§§ between condenser and slide. Fields containing treponemes were located first by visible light, and the treponemes then examined for fluorescence by ultraviolet light. Degree of fluorescence was estimated, and the sera designated reactive (2+ to 4+ fluorescence) or non-reactive (− to 1+). Reactive specimens were diluted serially in PBS and retested. Reactivity restricted to the 1:200 dilution was represented as a titer of 1; reactivity extending to higher dilutions (1:400, 1:800, etc.) was represented similarly as 1/200th the reciprocal of the highest reactive dilution (titer = 2, 4, etc.).

§ Kimwipes, Kimberly-Clark Corp., Neenah, Wis.

‖ One liter of aqueous solution contained 6.8 g NaCl, 1.48 g $Na_2HPO_4$, and 0.43 g $KH_2PO_4$. The formula differed slightly from that prescribed for FTA tests (Bacto Hemagglutination Buffer No. 0512, Difco Labs., Inc., Detroit, Mich.), but had better buffering capacity and did not affect FTA results. It was substituted primarily because it was regularly available from laboratory stocks(8).

¶ Rotating apparatus, electric, Cat. No. 3623, A. H. Thomas Co., Phila., Pa.

** Sylvana Chemical Co., Orange, N. J.

†† Hill Top Laboratories, Inc., Cincinnati, Ohio.

‡‡ American Optical Co., Model 2; objective, achromatic, 43X; condenser, C234, wide angle, with darkfield stop plate. Illumination, Reichert "Fluorex" with power supply. Subocular filter OG-1 used continuously to exclude visible blue and residual UV light; filter BG-12 interposed for the switch from unrestricted to UV illumination. Intensity of illumination controlled by Weston Master III universal exposure meter inverted on window of Reichert illuminator; adjustment to meter reading "800" made with illuminator diaphragm.

§§ Type A, R. P. Cargille Laboratories, Inc., New York, N. Y.

TABLE I. Fluorescent Treponemal Antibody (FTA) Tests of 51 Syphilitic Persons Using Reiter (R) and Nichols (N) Strains of *Treponema pallidum.*

| | Numbers of persons yielding the given titers | | | | | | |
|---|---|---|---|---|---|---|---|
| FTA(R) titer | 16 | | | | | 1 | |
| | 8 | | | | | 3 | |
| | 4 | | | 4 | 3 | 1 | |
| | 2 | | 7 | 10 | | | |
| | 1 | 4 | 14 | 3 | | | |
| | <1 | | 1 | | | | |
| | | <1 | 1 | 2 | 4 | 8 | 16 |
| | | | FTA(N) titer | | | | |

*Results and discussion.* The relative sensitivities of the RFTA (Reiter Fluorescent Treponemal Antibody) and FTA tests were determined by parallel titration of syphilitic sera; aliquants of the same serum dilutions served for both tests. RFTA and FTA titers of 51 individuals are represented in Table I. They were the same in most instances. However, the RFTA titer was twice the FTA in 16 instances, and one-half the FTA in 5 instances. To determine whether a significant difference existed, RFTA and FTA titers were transformed to logarithms and the differences between the logarithms calculated. An analysis(9) of these differences, based on Student's t, yielded a value of 2.53, which would be expected less than 2% of the time if the tests were the same. The RFTA test thus appeared slightly but significantly more sensitive for syphilitic antibody than the FTA test. Relative specificity for syphilis was evaluated by testing qualitatively sera from 1) 103 healthy individuals, and 2) 38 persons with non-treponemal infections or non-infectious disorders who were negative in a critical 40-hour test(3,4) for TPI antibody; the majority (35) of the latter reacted in standard tests with cardiolipin antigens, and so provided a more critical test of specificity than sero-negative individuals. None of the healthy persons yielded RFTA or FTA reactions. One of the "non-specific" seroreactors reacted (titer = 1) in the RFTA test but was negative in the FTA. The test using Reiter treponemes requires further evaluation. However, present indications are that it may substitute effectively for the FTA test. The possibility of substituting an organism simply cultivated *in vitro* for one requiring *in vivo* maintenance holds advantages in simplicity and economy for the physician and diagnostic laboratory.

*Summary.* A fluorescence test for treponemal antibody using the *in vitro* cultured Reiter treponeme compares favorably in sensitivity and specificity for syphilis with one requiring the virulent Nichols strain of *T. pallidum* which must be cultivated *in vivo.*

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