

Percoll-Purified *Treponema pallidum*, an Improved Fluorescent Treponemal Antibody-Absorbed Antigen

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Percoll-purified *Treponema pallidum* was evaluated as a fluorescent treponemal antibody-absorbed antigen. Borderline and false-positive reactions were essentially eliminated, resulting in sensitivity and specificity of 100 and 95.5%, respectively. The lack of background debris improved the ease and speed of reading the test.

Since the introduction of the fluorescent treponemal antibody (FTA) test for the serodiagnosis of syphilis (2), the preparation of *Treponema pallidum* antigen from infected rabbit testicular tissue has not substantially changed. This is despite the development of the hemagglutination (15, 17, 18, 22) and enzyme immunoassay (10, 14, 20) tests and modifications to the FTA test (7, 9, 11). The lack of change has been due, in part, to the difficulties associated with obtaining large quantities of purified antigen that are free of contaminating host testicular tissue and retain morphological characteristics and immunological reactivity.

Although Hunter et al. (7) standardized methodology for preparing FTA-absorbed (ABS) antigen that was free of rabbit globulin, sensitive techniques revealed that these *T. pallidum* preparations were still contaminated with rabbit testicular material (5). The development of the continuous particle electrophoresis method for separation of *T. pallidum* from testicular material (16) resulted in the availability of a purified antigen for use in the FTA-ABS test. This continuous particle electrophoresis antigen is no longer available, and its preparation was technically difficult and expensive. Furthermore, it was a low-yield procedure that resulted in nonmotile, avirulent, and ultrastructurally damaged organisms (5, 16). The possibility exists, therefore, that antigenic changes accompanying cell death were responsible for lot-to-lot variability in FTA-ABS reactivity.

A new, rapid purification technique utilizing Percoll density gradient centrifugation has been described recently (5). The organisms were shown to be ultrastructurally intact, motile, virulent, and free of detectable soluble and insoluble host testicular material. In this report we evaluate the use of Percoll-purified *T. pallidum* as an FTA-ABS antigen. The evidence presented suggests that the purity and quality of the *T. pallidum* antigen preparation may have a direct effect on the occurrence of equivocal and borderline reactions.

T. pallidum, Nichols strain, was maintained without corticosteroids and harvested in FTA-ABS buffer (CSI, Inc., Whippany, N.J.) as previously described (5). To increase bacterial yields for preparation of FTA-ABS antigen, rabbits were given cortisone acetate from days 3 to 10 postinfection (19). Soluble and insoluble host testicular tissue was re-

moved from treponemal suspensions by low-speed differential centrifugation followed by density gradient centrifugation through Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) as described elsewhere (5). Percoll was removed from the purified treponemes by centrifugation at $100,000 \times g$ for 1 h at 4°C (5), and the organisms were diluted 1:20 in lyophilization buffer (8) and maintained at 4°C for 10 days. Samples (0.5 ml) were lyophilized and stored at 4°C until used (8). Purified *T. pallidum* antigen was reconstituted in 0.5 ml of double-distilled water and adjusted to a working concentration in FTA-ABS buffer. Before use, 10-well slides (Carlson Scientific, Peotone, Ill.) were washed in 7× detergent, rinsed in water, soaked in 95% ethanol, and allowed to air dry. Ten microliters of the treponemal suspension was placed in each well and allowed to air dry in a laminar-flow hood. Antigen-coated slides were fixed in acetone for 10 min and stored at -20°C (21). Antigen testing and titration of the fluorescein-conjugated anti-human globulin (BBL Microbiology Systems, Cockeysville, Md.) were performed as described (21). The reactive control serum and a total of 122 diagnostically and serologically well-characterized sera from patients with syphilis and from patients with false-positive or nonspecific fluorescence in the FTA-ABS test were generously provided by Sandra Larsen (Treponemal Research Branch, Sexually Transmitted Disease Laboratory Program, Center for Infectious Disease, Centers for Disease Control [CDC]). The sera were selected for use on the basis of their FTA-ABS reactivity. Approximately 20 sera each were categorized as nonreactive, borderline, or 1-, 2-, 3-, and 4-plus reactive. The FTA-ABS test was performed as described (21) and interpreted in a double-blind fashion by two experienced readers. A positive test was considered to be a result of 1 to 4+. A typical 3+ reaction is shown in Fig. 1. Borderline reactions were noted but interpreted as negative (1).

The FTA-ABS test with the purified antigen (FTA-P) and the test performed at the CDC (FTA-CDC) detected 77 true positives (Table 1). False-positives occurred with both tests, but it is important to note that the false-positive results were obtained with different sera. It also appears that the ability of each test to correctly identify true negatives (normals and individuals with diseases other than syphilis) was similar.

A significant difference in the number of borderline reactions was observed, however. A total of 21 borderline reactions occurred with the FTA-CDC test (Table 1). This included 3 patients with treated latent syphilis, 1 with congenital syphilis, 3 false-positives, and 14 normal individ-

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TABLE 1. FTA-ABS testing with Percoll-purified *T. pallidum*

Diagnostic category	No. of sera by test ^a :					
	FTA-CDC			FTA-P		
	Reactive ^b	Borderline	Nonreactive	Reactive ^b	Borderline	Nonreactive
Syphilis ^c	77	4	0	77	1	3
Other diseases	0	0	20	0	0	20
Normal	0	14	0	2	1	11
False-positive	4	3	0	0	0	7

^a FTA-ABS testing performed at North Carolina Memorial Hospital with Percoll-purified *T. pallidum* (FTA-P) or by CDC with nonpurified antigen (FTA-CDC).

^b Reactive, >1+ reaction.

^c The diagnosis of syphilis was based on detailed historical, clinical, and serologic information provided to or obtained by the CDC. There were 21 patients with untreated and treated early syphilis, 52 with untreated and treated latent disease, 7 with congenital syphilis, and 1 with late cardiovascular syphilis.

uals. When these sera were tested by the FTA-P 17 were nonreactive, 2 remained borderline, and 2 became reactive. Similarly, seven sera that were diagnostically categorized as false-positives were nonreactive in the FTA-P. In contrast, one 2+, three 1+, and three borderline reactions occurred with the FTA-CDC. The sensitivity and specificity of the FTA-P were 100 and 95.5%, and those of the FTA-CDC were 98.7 and 90.9%, respectively.

All treponemal tests for syphilis utilize treponemal antigens that are contaminated with rabbit testicular material. The inability to obtain sufficient quantities of purified antigen results from the fact that only limited in vitro multiplication of the organism has been reported (3, 4, 12, 13). Furthermore, an efficient method for purification of *T. pallidum* from host tissue has not been available. Recently, however, the separation of soluble and insoluble host material from *T. pallidum* suspensions by Percoll density gradient centrifugation was described (5). The resultant organisms were biologically, morphologically, and immunologically intact. Preliminary evidence suggested that the use of Percoll-purified *T. pallidum* as an FTA-ABS antigen reduced the number of

equivocal reactions and enhanced the ease and speed of reading (P. A. Hanff, C. Fernandez, J. Waters, and J. D. Folds, Progr. Abstr. 83rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 669, 1983). This appeared to be due, in part, to the purity of the antigen and the resultant lack of background debris (5). Additionally, Percoll-purified antigen remained firmly fixed to the slides throughout the procedure. In contrast, some loss of the standard nonpurified antigen is expected (6, 21).

In an attempt to demonstrate more accurately that Percoll-purified *T. pallidum* could be effectively utilized as an FTA-ABS antigen, we tested diagnostically and serologically well-characterized sera, using lyophilized purified antigen. The sensitivity and specificity of the FTA-P and the FTA-CDC were essentially similar: 100 and 95.5% versus 98.7 and 90.9%, respectively. There was a marked difference, however, in the number of borderline reactions observed with the two tests. Of the 21 borderline results measured by the CDC, 19 (95%) were nonreactive by the FTA-P, and 2 became positive. The CDC currently recommends that borderline reactions be interpreted as negative (1). Thus, the differences observed between the FTA-P and FTA-CDC may not be clinically significant.

In contrast, the fact that all patients classified as false-positive were nonreactive with the FTA-P may have important clinical and serologic implications. We have shown that the quality and purity of the *T. pallidum* antigen have an effect on borderline and false-positive FTA-ABS results. Similar results have been obtained by comparing the reactivity of a commercial FTA-ABS antigen with nonpurified and Percoll-purified *T. pallidum* prepared from the same suspension (G. Tringali, P. A. Hanff, G. Scuderi, and G. Fragale, submitted for publication). If these results are confirmed in larger prospective evaluations utilizing FTA-ABS, hemagglutination, or enzyme-linked immunosorbent assays, it is conceivable that many of the interpretive problems encountered with the treponemal tests for syphilis and the resultant diagnostic dilemmas will be resolved.

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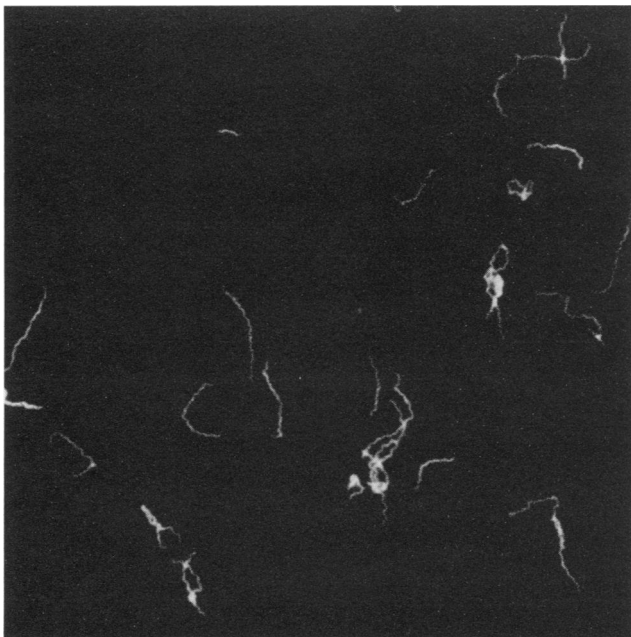


FIG. 1. Percoll-purified *T. pallidum* as an FTA-ABS antigen.

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