FLUORESCENT ANTIBODY METHODS IN THE DETECTION AND CONTROL OF VENEREAL DISEASES*

A BIBLIOGRAPHICAL REVIEW OF THE LITERATURE

BY

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The purpose of this review is to bring together selected abstracts from the world literature on fluorescence methods in the diagnosis of venereal disease, to stress their value and importance, and also, with regard to syphilis, to indicate the rate of progress in various countries.

GONORRHOEA

U.S.A.

The fluorescent antibody (FA) method for the detection of gonorrhoea was first described by Deacon, Peacock, Freeman, and Harris (1959). The results of their studies were: a species-specific antigen associated with freshly isolated, inagglutinable gonococcal cultures was recognized; the antigen appeared to possess characteristics similar to Vi antigens of *Salmonella typhi* or the K antigens of the *Escherichia* group and is fully developed only in freshly isolated cultures of infectious exudates. Deacon, Peacock, Freeman, Harris, and Bunch (1960) described the results of diagnostic studies made by his group. They examined 150 females, named by male contacts suffering from acute gonorrhoea. Specimens were collected from the urethra, cervix, and vagina. By direct smear staining the FA detected 25.5 per cent. of the individuals harbouring the gonococcus. The delayed FA method increased the percentage of positive results to 58 per cent. The direct method of examination required one hour and the delayed method 20 to 24 hours. In comparison, the conventional culture procedure, including isolation and fermentation studies, required 3 to 10 days.

Deacon's results were confirmed by Shapiro and Lentz (1963), who studied 148 women named as the sexual partners of men infected with acute gonorrhoea. Specimens were obtained from the urethra, vagina, cervix, and endocervix. Seventy patients (47.3 per cent.) had positive reactions. This

compares favourably with the results obtained by conventional cultural methods; moreover, the FA method gave results in about 16 hours, whereas full identification by cultures would take up to 10 days.

Deacon's technique was adopted by the Communicable Disease Center in Atlanta (U.S.A.). Brown, Copeloff, and Peacock (1962) made the following statements:

- (1) Gonococci are detected in asymptomatic promiscuous teenagers;
- (2) The delayed FA technique is capable of detecting the gonococcus, when the Gram-stain smear is negative;
- (3) The delayed FA technique gives results comparable to good culture methods; and
- (4) The vagina, cervix, urethra and anus should all be tested to determine the presence of the gonococcus.

Pariser, Farmer, and Marino (1964) corroborated these results, and found that the male can harbour the organism of gonorrhoea without signs or symptoms of infection; he may acquire an asymptomatic infection or may harbour the organism after treatment, despite the disappearance of signs or symptoms. One can only postulate that in this asymptomatic state sufficient viable organisms are present to transmit the disease. Clinically and epidemiologically transmission by an asymptomatic male seems probable. Cure cannot be determined solely on the basis of the disappearance of signs and symptoms. Post-treatment examinations of urine and prostatic secretions by FA tests and culture provide the most accurate gauge of cure.

In spite of these findings the FA method has not yet been widely used in practice.

Cherry and Moody (1965), in a general review of the method and its applications, stated:

The delayed FA test and the cultural procedure gave positive results in the same percentage of patients, although the delayed FA test revealed more positive sites.

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They mentioned the work of Harris, Deacon, Tiedemann, and Peacock (1961), who successfully used the delayed FA technique to determine the incidence of *N. gonorrhoeae* in 213 female prisoners, none of whom had clinical symptoms of infection; 20.6 per cent. of these women gave positive results when specimens from the cervix, urethra, and vagina were examined. Cherry and Moody subsequently reported the results of Price (1964): five service laboratories examined 88,000 specimens obtained from 9,000 females, applying both FA methods and the cultural method.

There was a great deal of variation in the relative efficiency of the cultural and FA procedures among participating laboratories.

Price attributed this to a lack of uniformity in performing cultural procedures. Cherry and Moody summarize this part of their article as follows:

If the recommendations of the authors of the test are followed, immunofluorescence appears to be a highly specific and sensitive tool for the identification of *N. gonorrhoeae*.

The hesitation in accepting the FA technique as a standard method was due to a certain extent to difficulties caused by the nonspecific staining of background material. White and Kellogg (1965) employed Evans blue as a counterstain, eliminating the nonspecific background staining and increasing the reliability of the direct FA procedure. Peacock and Thayer (1964) used flazo-orange as a counterstain; nonspecific background fluorescence was quenched without obscuring points of reference, and they found that 56 per cent. of 156 women examined were positive by culture and 54 per cent. by the FA smear with flazo-orange counterstain.

GREAT BRITAIN

Fry and Wilkinson (1964) made important comparative studies. They carried out FA tests on 100 male and 94 female patients, and compared the results with those of Gram-stained smears and cultures. A higher proportion of positive results was obtained by the FA methods than by either smears or cultures, and they concluded that:

The new technique should prove useful as an ancillary to conventional cultural methods in the investigation of patients who are likely to have gonorrhoea, but in whom existing methods fail to show the presence of gonococci.

Gallwey, Nicol, and Ridley (1967) applied the direct staining FA technique for the diagnosis of gonorrhoea to evaluate it as part of a general busy clinic routine as opposed to a reference laboratory or research technique. For this purpose commercially-available fluorescent conjugated anti-

serum was used. Tests were performed on smears from 61 female patients attending a special treatment centre as contacts of males with gonorrhoea. The method was shown to be more sensitive than conventional staining techniques or cultures (seven patients were found positive by the FA method alone).

FRANCE

Siboulet, Galistin, and Huriez (1962) set forth the advantages of the immunofluorescent technique for the diagnosis of inclusion blennorrhoea.

THE NETHERLANDS

Mouton (1966) evaluated the direct FA technique and compared it with conventional culture techniques in 45 females and 55 males. Results definitely positive for gonorrhoea were obtained in 19 women and 32 men by the FA technique; in 6 women and 23 men by culture; and in 8 women and 30 men by Gram-staining. In the majority of cases showing a discrepancy between culture and FA results there was other evidence to support a diagnosis of gonorrhoea.

SWEDEN

Danielsson (1963) reported the results of comparative studies. 75 males were tested 114 times. Gonococci were detected in 59.6 per cent. of the cases by direct microscopy, in 63.5 per cent. by direct, and 76.9 per cent. by delayed FA tests, and in 71.2 per cent. by culture tests. Gonococci were detected in 19.4 per cent. of cases of treated gonorrhoea by culture tests and in 30.6 per cent. by delayed FA tests.

Danielsson (1965 a, b) compared different techniques—adsorption, one-step inhibition, and counterstaining—for the elimination of cross-reactions. He warned that in applying delayed FA tests to clinical specimens, it is necessary to take precautions against nonspecific reactions. This could be done by adsorption of the fluorescein isothiocyanate-labelled anti-gonococcal globulin with a strongly reacting staphylococcus strain or by the addition of rhodamine-labelled antistaphylococcal globulin. The latter procedure was found to be preferable, as it also afforded a counterstaining effect. In males, delayed FA test and culture were found to be of equal value for the diagnosis of gonococci. In females, delayed FA tests gave a somewhat higher yield than cultures.

SYPHILIS

U.S.A.

In the original FTA method, sera were tested at a dilution of 1 in 5, but this was found to give an

undue number of nonspecific positive results. This led to the adoption of a serum dilution of 1 in 200—the FTA-200 test (Deacon, Freeman, and Harris, 1960).

Hunter, Deacon, and Meyer (1964) further improved the FTA test. They found that a major cause of nonspecificity might be the occurrence of common or group-antigens shared by both pathogenic and saprophytic treponemes. The finding that the corresponding nonspecific group antibodies could be removed by appropriate absorption procedures suggested the application of principles that have resulted in an improved FTA test designated the FTA-Absorption procedure (FTA-ABS). Its sensitivity and specificity were determined by testing selected human sera, using several FTA test procedures and the *Treponema pallidum* immobilization (TPI) test. The results indicate that the FTA-ABS procedure is more than twice as sensitive as the FTA-200 test and that its specificity equals that obtainable by the TPI test.

After the demonstration of the persistence of treponemes in patients with treated late syphilis, FA methods were used to study this problem by Yobbs, Rockwell, and Clark (1964), who screened 102 prisoners who had reactive TPI tests. All those included in the study were required to give a complete history of their illness and its treatment, and had to be willing to undergo lumbar puncture and the surgical excision of an inguinal lymph node. 46 men were selected, all of whom had been treated for syphilis at various stages of the disease. Five nodes were shown to contain treponemal forms and in two cases these proved to be virulent for rabbits. After a course of treatment under careful supervision, the findings were completely negative in a node removed subsequently. The authors stated that the possibility of re-infection had to be absolutely ruled out before the finding of living spirochaetes could be interpreted as demonstrating treponemal survival in humans after adequate penicillin treatment. They discussed reliability of the VDRL test in comparison with the TPI and FTA tests.

Miller, Whang, Boak, and Carpenter (1964) studied technical problems associated with the FTA test and its efficacy, especially as an aid in the serological diagnosis of latent syphilis. They stated that the complexities were overcome by using a specially treated and preserved strain of treponemes. The conclusion of their studies is guarded:

This test is satisfactory as a screening aid; however, greater experience is necessary before the test can be used effectively as a routine procedure.

Sherris (1963) was also cautious enough in his review. After an exposition of the FA technique described by Deacon, Falcone, and Harris (1957) and its modification he concluded:

The antitreponemal test has approximately the same degree of specificity as the Treponemal Immobilization Test (TPI) and because of its greater simplicity, will prove to be a useful adjunct to the sensitive but somewhat less specific lipoidal antigen or RPCF tests.

The most complete exposition of the development is that of Cherry and Moody (1965). Beginning with its first use by Deacon and others (1959), they described the results of the comparative serological tests sponsored by the Venereal Diseases Branch of the Communicable Disease Center in 1957 and ended with this statement:

The FTA test possessed a specificity and sensitivity exceeding that of other treponemal tests.

They later recapitulated the results of Harris, Bossak, Deacon, and Bunch (1960), who concluded that:

If the FTA, TPI, and Kolmer-Reiter Protein tests are measuring the same antibody in spinal fluids, the FTA test is the most sensitive.

Bradford, Bodily, Ketterer, Puffer, Thomas and Tuffanelli (1965) also compared the FTA-200, FTA-ABS, and TPI tests.

Knox, Short, Wende, and Glicksman (1966) working in Houston, Texas, found that the improved FTA-ABS test . . . studied in 1,033 patients . . . was as sensitive as, or more sensitive than the TPI test in all stages of syphilis. In patients with primary syphilis, the test was reactive more often than the TPI and as often as the VDRL test. In other stages the FTA-ABS test was reactive as often as the TPI test. Okey (1966) reported the results of studies with the FTA-ABS test; after a short description of the routine methods previously used, he stated:

The Fluorescent Treponemal Antibody-Absorbed test (FTA-ABS) is the latest in a series of treponemal tests developed for use in syphilis serology. It has supplanted the FTA-200 test in this laboratory because of the greater sensitivity and specificity it affords. Present indications based on impressive evaluation statistics are that the FTA-ABS will become the generally used treponemal test because of its ease of performance and it may eventually replace the *Treponema Pallidum* Immobilization test (TPI) in all but a minority of problem cases.

Mescon and Grots (1963) reviewed the use of fluorescence microscopy in dermatology. They reported the results of their own studies of over

400 cases of syphilis in various clinical stages, and stated:

There seems to be general agreement with the results of other workers as far as the high degree of both sensitivity and specificity of the FTA-200 procedure is concerned. The latter test correlated best with the TPI procedure and it is far more sensitive than the RPCF (Reiter protein complement fixation) test which has been used as a treponemal screening test in Massachusetts.

Neblett, Merriam, Burnham, and Fine (1964) found reactive FTA tests in patients with systemic lupus erythematosus whose sera produced nuclear immunofluorescence upon tumour imprints; none had historical or clinical evidence of syphilis. Routine VDRL and Kolmer tests were reactive, but Reiter protein complement-fixation, cerebrospinal fluid examination, and immobilization tests were negative. The reactive FTA results were rendered negative by absorption with human tumour homogenates; they were diminished partially by normal tissue homogenate absorption, but remained unaffected by animal tissue powder absorptions. Patients furnishing such sera were considered non-syphilitic, although four had reactive lipoidal antigen tests, presumably false positive. False positive FTA test results may indicate the presence of an auto-immune disorder.

Stevens, Boylan, and Memoli (1967) made other comparative studies. Their introductory statement is characteristic:

The FTA-ABS procedure has been suggested as a laboratory tool with a sensitivity greater and a specificity equal to the TPI test for syphilis. Because the TPI test has generally served as a standard of reference in evaluating serologic tests for syphilis, a brief comparative study of the diagnostic utility of these procedures in our laboratory was initiated. . . The results suggest that the FTA-ABS is superior in sensitivity and specificity.

Bradford, Tuffanelli, Puffer, Bisset, Bodily, and Wood (1967) reported the results of their examinations of sera from 177 patients having past or presently reactive Standard Tests for Syphilis (STS) or diseases and conditions frequently associated with biological false positive reactions (BFP). They employed the VDRL, the TPI, and the FTA-ABS tests. Agreement between FTA-ABS and TPI tests was obtained in 64 per cent. of 44 patients with clinical, historical, or epidemiological evidence of late or latent syphilis. These authors concluded that:

The results of this study show a high level of agreement between the FTA-ABS and the TPI tests in a group of patients under study as biologic falsely

positive reactors. There was extremely good agreement between TPI and FTA-ABS in diseases or conditions other than syphilis, in that results of both tests were predominantly nonreactive. The greater sensitivity of the FTA-ABS over the TPI was significant in the group of individuals diagnosed as having syphilis. These results suggest the value of the FTA-ABS tests as an aid in the diagnosis of the more difficult problem cases.

Wood, Inouye, Argonza, Bradford, Jue, Jeong, Puffer, and Bodily (1967) made a comparison of the FTA-ABS and TPI tests on sera from 1,182 diagnostic problem cases. Comparable results were obtained in 92 per cent. of the sera tested, the fluorescent procedure showing greater reactivity than the immobilization test. They described modifications in the preparation of reagents and performance of the fluorescent test, which facilitate its routine use by the average laboratory.

The problem of biological false positive tests for syphilis in connexion with auto-immune diseases was studied by Tuffanelli, Wuepper, Bradford, and Wood (1967) with special reference to the FTA-ABS procedure. They investigated a group of 347 patients with a previous diagnosis of false positive reactions to tests for syphilis, and 176 of the 347 patients originally diagnosed as chronic false positive reactors on the basis of a single non-reactive TPI test were re-studied clinically and serologically. Indeterminate reactors (TPI-negative and FTA-ABS-positive) were not uncommonly seen and it was considered that such patients should be treated as having syphilis. It was found that 39 of 50 patients with chronic false positive reactions had systemic disease. The authors concluded that the FTA-ABS test was the most sensitive of the treponemal tests and should be performed, when possible, to distinguish cases of syphilis from false positive reactors.

Nicholas (1967) pointed out the special difficulty of the proper interpretation of serological tests for syphilis in the aged. It requires not only a basic knowledge of serology, but also the ability to recognize and diagnose the various phases of syphilis. In cases of questionable biological false reactions, the TPI and/or FTA tests should be employed. These tests will also help to establish the diagnosis of syphilis in patients with clinical manifestations of late syphilis whose serum shows no reaction by routine reagin tests.

Stevens and others (1967) concluded that the FTA-ABS test was superior in sensitivity and specificity to the TPI, FTA-200, and FTA-5 tests.

A comparison of the FTA-ABS test with the TPI test was made by Beam, Dedeaux, and Humes

(1967). Both tests were performed on 716 sera from patients suspected of having syphilis, and toxic TPI reactions occurred with 26.4 per cent.; 527 specimens were available for comparison and conflicting results were obtained in 40. Statistical analysis of the data revealed excellent correlation between the FTA-ABS and TPI tests, but favoured the FTA-ABS procedure as a laboratory aid in the diagnosis of syphilis.

Wells and Smith (1967) first used the FA technique to demonstrate *Treponema pallidum* in the eye. They stated that this stain provided a rapid method for demonstrating spirochaetes in tissue. It possessed the advantage over the darkfield in the relative permanency of the preparation (a few months rather than a few minutes) and also allowed photomicrographs to be made, because with the fluorescent antibody stain the organisms were non-motile.

Smith and Israel (1967) reported the detection of treponemes which stain with fluorescein tagged anti-*Treponema pallidum* globulin, in the aqueous humour, cerebrospinal fluid, and liver of patients with late sero-negative ocular and neurosyphilis. Spirochaetes have been found in the aqueous humour from eyes with no biomicroscopical abnormality and in cerebrospinal fluids which had normal cell counts, protein levels, and reagin and colloidal gold tests. Several patients were found to have negative findings in darkfield examinations of aqueous humour and cerebrospinal fluid, but a later study with the fluorescent antibody technique revealed morphologically typical spirochaetes which stained with anti-*T. pallidum* globulin.

GREAT BRITAIN

Wilkinson (1961) published a preliminary report on the use of the FTA-200 test on the sera of 164 presumed normal individuals, on 138 patients with treated or untreated treponemal disease (25 early, 92 latent, 41 late), and on 144 problem sera. After describing the technique, he concluded that:

It is relatively simple to perform and the preliminary results obtained suggest that it has a high level of sensitivity and specificity.

In a more detailed study (Wilkinson, 1963), sera were tested at a dilution of 1 to 200, because "it was found that many normal non-syphilitic sera would give positive reactions when tested at low dilution, in some cases up to 1 in 25". He referred to Deacon and Hunter (1962) who had suggested that this might be due to the presence of low titred antibody from commensal spirochaetes, such as are normally present in the mouth, which share the common

group antigen with *Treponema pallidum*. His conclusion was that:

The FTA test is technically much simpler to perform and can give valid results with sera unsuitable for use in the TPI test.

Wilkinson and Rayner (1966) gave the results of FTA-200 tests carried out on 3,862 sera sent for TPI testing during 1964 to the Venereal Diseases Reference Laboratory (the London Hospital). The two tests showed an overall agreement in 89.5 per cent. of sera. In tests on 381 sera from patients whose clinical details were known, the incidence of positive FTA tests which were not confirmed by positive TPI tests or clinical evidence or history of treponemal infection was 0.8 per cent.

Wilkinson (1967) modified the earlier method of removing the group antibody from the sera in order to improve the sensitivity and specificity of the FTA procedure. He tested 394 selected problem sera by the TPI test, the FTA-200 test, and the inhibition test based on the ability of sera containing anti-treponemal antibodies to block specific staining of *T. pallidum* by fluorescein-conjugated syphilitic antibody globulin. The inhibition test was found to be more sensitive than the FTA-200 test on this material and to give fewer unexplained positive results. Tests on 144 presumed normal sera were all negative.

AUSTRALIA

Garner, Collins, and Robson (1967) compared the results of the FTA-200 and TPI tests on 689 problem sera sent to the Institute of Clinical Pathology and Medical Research in Sydney, Australia, for TPI testing. "Problem" specimens were those in which other laboratories had obtained equivocal results to routine tests for syphilis, or specimens from patients in whom clinical findings, history, and serological test results conflicted. The TPI test was chosen as a comparison for the FTA-200 test because of its general acceptance as the most specific serological test available. Overall agreement between the two tests was found in 88.1 per cent. of sera.

FRANCE

The opinion of French authors is best illustrated by Dulong de Rosnay and Boineau (1962):

It seems that this method of detection (*i.e.* that of Coons and Deacon) represents the most important progress since complement-fixation was detected by Bordet.

Thivolet, Kratchko, and Sepetdjian (1963) demonstrated the diagnostic value of the immunofluorescence method in general.

Vaisman and Hamelin (1963) declared that it was a very sensitive method and seemed to have the same specificity as the Nelson test; it was the first of the treponemal tests to become positive, even before the classical serological tests. These authors also reported on the practicability of the method using dried blood, and investigated 150 specimens of blood absorbed on blotting-paper. The serological reactivity of FTA, TPI, and lipoidal antigen tests was also examined on venous blood from the same individuals. The variations found in sensitivity, specificity, and reproducibility of the blotting paper disc FTA-100 procedure were not significant, and the results were practically the same as those obtained independently on sera from the same subjects. No degradation of the antibody was observed after storage of the blotting paper discs for up to 60 days at 20 to 25°C. The advantages of finger puncture blood-sampling are obvious. The ease of transport by mail of dried blotting-paper discs in small plastic bags to a competent laboratory for FTA testing is also obvious, and avoids the risk of haemolysis, infection, breakage, etc., associated with the postal transmission of glass containers or vials.

Most important, however, is the authors' comparison of the sensitivity of the different methods:

The precocious appearance of fluorescent antibodies in the serum in primary syphilis prior to TPI antibody and reagins was observed in some cases. The sensitivity of the TPI test, on the other hand, was confirmed as being superior to that of the FTA technique (and to classical reagin serology) in comparative examinations of old treated syphilitic infections.

Vaisman, Hamelin, and Guthe (1963) tested 930 samples of whole blood absorbed on roundels of blotting paper (Canson No. 435) and dried; the results were identical in sensitivity and specificity.

Guthe, Vaisman, and Hamelin (1964) investigated the possible effects of shipment by airmail to tropical countries and of the storage of the blotting paper discs at temperatures ranging from 21 to 43°C. for 32 to 80 days without examination. Transport and storage were not found significantly to affect the results.

A very notable contribution from France was a study by Niel and Fribourg-Blanc (1963), who had carried out comparative tests over a period of three years on some 12,000 sera, using the FTA test, cardiolipin reaction, and the TPI test. They concluded:

Provided that the FTA test is carried out with scrupulous care, it has proved to be highly sensitive,

easily reproducible, and sufficiently specific. Its simplicity allows it to be used as a routine test for case-finding and evaluation of treatment. It is also a test that allows of the quantitative expression of results.

Although it is not so highly specific as the TPI test, the FTA technique has the important advantage of revealing syphilitic infection earlier than either the cardiolipin reactions or the TPI test, positive results being obtainable almost at the same time as the appearance of the primary lesion. The authors emphasize that "strict standardization of the test procedure, of the reagents used and of the manner of recording results is essential".

Niel and Fribourg-Blanc (1965) published a study of 5,169 sera, and described modifications of their technique. They perfected their optical instruments and developed strictly standardized reagents. They noted the limited value of qualitative expressions such as ++ or ±. They insisted that it was essential to follow the technique to the letter in every instance, and that the reagent should be of the highest quality so as to permit testing over a wide range of titres (from 150 to 108,000). In primary syphilis the FTA test was usually positive earlier than the cardiolipin test, and in long-standing treated syphilis it was exceptional for sera to be both FTA-negative and TPI-positive. They concluded that the efficiency of the FTA test as a case-finding technique had been amply demonstrated by their results; they considered that its simplicity, reproducibility, and sensitivity, justified its systematic use in syphilis serology.

Colombani and Ripault (1964) studied FTA reactions in 3,156 sera in parallel series with the TPI test and complement-fixation methods. FTA tests showed identical reactions with the TPI in 87.26 per cent. of cases, and with the serum complement method in 83.39 per cent. They observed that:

The FTA test is positive alone principally in the course of primary syphilis at an early stage (first week of the chancre). It can persist during the course of early syphilis for one or two years after the start of treatment. In rare cases, it can be negative when the TPI test is positive in tertiary and in all forms of syphilis after treatment. The possibility of carrying out the FTA test quantitatively makes it a useful method for the control of the serological evolution in the infected subject.

Besides these enthusiastic partisans of the FTA method there are also some sceptics. Daguet (1964), summarizing the two divergent opinions, said:

For some workers the FTA is the ideal test for the diagnosis of syphilis. In short, the FTA is called in to replace the cardiolipin reactions, such as the Nelson test. This highly optimistic notion supported by

Deacon, by Fribourg-Blanc and Niel, and by Vaisman and Hamelin, is not altogether shared by other investigators such as Nielsen, Thivolet, and ourselves.

Fribourg-Blanc and Niel (1966) first gave a very exact and clear analysis of the basic principles of immunofluorescence and a review of its diagnostic application in clinical bacteriology. Secondly, they reported the results of their own researches on 10,193 sera during a four-month period. They declared that the quality of the reaction in the FTA procedure depended on the quality of the technique and the reagents used; there were no standardized treponemal antigens and fluorescent antiglobulin conjugates. They considered immuno-fluorescent procedures did not have the absolute specificity of the TPI test.

ITALY

The FA method has been thoroughly studied and evaluated in Italy. Panti and Ulivi (1963) investigated 1,500 sera in Florence. Some were from healthy people; some from individuals in various stages of infection, treated and untreated; and some were "problem" sera. The FTA and all the classical methods and also the TPI test were employed. They concluded that the FTA test gave very sensitive and specific reactions and could profitably be used at a remarkably early stage of the infection.

Anselmi and Carlizza (1962) had carried out similar studies in Rome, emphasizing the significance of recrudescence of luetic infection. They pointed to the increased frequency of biological false positivity demonstrated by the usual sero-reactions, and expressed the opinion that the FTA method was more specific and easier to perform than the TPI test.

Vignali (1962) reported twelve cases of syphilis examined in Parma by both immunofluorescent and classical methods. In each case the date when the infection had been contracted was known. By the FA method positive results could be obtained even 1, 2 or 3 days after infection; even at a dilution of 1 in 200.

Bellone and Leigh (1964) carried out FTA tests at dilutions of 1 in 5, 1 in 50, and 1 in 200 and made comparative tests with quantitative Reiter protein complement-fixation and qualitative TPI tests on 281 patients in different stages of syphilis and many control subjects. Under a chosen experimental condition, at 1 in 50 serum dilution, it was possible to show that the FTA test was highly specific in untreated primary syphilis and that it remained reactive after treatment for a longer time than other serological tests including the TPI.

Garbin and Piacentini (1964) investigated 400 sera in Verona and compared the results with those of the standard serological tests. The FTA was always negative in non-luetic sera, even those which gave positive results by other methods. On the other hand, it was always positive in syphilitic sera, even when other reactions had become negative, except in five elderly patients under treatment, who should thus be considered to be completely cured.

Longhi (1964) in Bologna observed that tests made at various stages of treponemal infection showed clearly that the FTA was the most sensitive in primary syphilis, even in the very early stages when the TPI test and the classical serological methods gave negative results, and was, therefore, very important in the diagnosis of dubious cases.

Midana (1966) discussed the practical value of the FTA test in the diagnosis and prognosis of syphilis. After analysing the disadvantages of the standard serological tests and the points for and against the TPI procedure, he set out the advantages of the FTA method as follows: rapidity and facility of execution; stability of antigenicity, exclusion of risks of contagion to laboratory staff; sensitivity and high specificity; suitability for testing blood samples on filter paper when the specimen is to be stored and sent by mail.

GERMANY

Petzoldt (1964) declared that the results of the FTA test in primary syphilis were equal to or better than those of the TPI test. The latter gave positive results at the end of the primary stage or not until the beginning of the secondary stage, but anti-treponemal antibodies were detectable very early by the FTA technique. In secondary syphilis the results are positive by both techniques. He emphasized the value of the FTA method in checking false positive results of standard serological tests.

Meyer-Rohn (1964) tested 463 cases in Hamburg and found complete agreement between the FTA and the classical sero-reactions in 256 negative cases and 207 positive cases. In three sero-negative cases of primary syphilis the FTA test gave positive results. Agreement was complete in 64 undoubted cases of secondary syphilis, except one in which the FTA test was positive. In all cases of latent syphilis but one, the FTA test gave positive results.

Naumann (1964) tested 200 sera by the FTA and TPI methods. The results agreed in 82.5 per cent. In 6 per cent, there were discrepancies and 11.5 per cent. did not permit a clear interpretation.

Fegeler and Schössler (1965) examined 238 sera by the TPI and FTA methods; the results were

largely in agreement, and they considered that the FTA was an important complement to the TPI test. The different behaviour of the FTA test in primary, secondary, late, treated, and untreated syphilis was discussed and illustrated statistically.

Petzoldt and Tupath-Barniske (1965) tested 361 sera by both the FTA and the TPI tests. There was good agreement, but they found positive FTA and negative TPI results more often in primary syphilis; this was explained by the fact that immobilizing antibodies could be found by the TPI test only towards the end of the primary stage. Otherwise the results agreed in 97.2 per cent. When the results were discrepant the authors found it very difficult to decide which of the two tests had given a false result.

Gregorczyk (1966) studied experimental syphilis in inoculated rabbits to compare the reliability of the TPI and FTA methods. Disagreements were explained either by the higher sensitivity of the FTA test (especially in early or early-treated syphilis), or by the fact that the FTA test detects not only type-specific but also group antibodies; furthermore, it may give false reactions with sera from non-syphilitic patients with raised macroglobulin levels. He noted that sera from treated syphilitic patients, still positive in the TPI test, gave negative reactions in the FTA when absorbed with non-pathogenic Reiter treponemes. This disagreement, not to be explained by a higher degree of sensitivity or by non-specific reactivity of the FTA, led to the presumption that the antibodies causing reactivity in the TPI tests differ from those detected by the fluorescent procedure.

FINLAND

Discussing a previous investigation of problem cases, Lassus, Mustakallio, Aho, and Putkonen (1966) stated that:

A negative result of the *Treponema pallidum* immobilization (TPI) test has been relied upon as the most important single criterion for biologic false positivity (BFP) in the serological tests for syphilis. In a recent report, eleven out of 101 sera from patients with clinically verified late syphilis were non-reactive in the TPI tests but reactive in the fluorescent treponemal antibody-absorption (FTA-ABS) test in which the serum is first absorbed with Reiter treponemes to remove the group specific treponemal antibodies.

They re-examined sera from 57 patients with supposed biological false positive results by both the TPI and the FTA-ABS methods; fifty of the 57 sera non-reactive to TPI were also negative by the FTA-ABS test. One serum was reactive (+ +) and six were weakly reactive (+).

HUNGARY

Király, Jobbágy, and Mecher (1965) tested 477 "problem" sera:

- (a) sera showing unexpected serological reactivity;
- (b) negative sera from patients who had received adequate treatment;
- (c) negative sera from patients without any specific history, but clinically suspected of latent syphilis.

Two-thirds of the sera belonged to the first two categories, and one-third to the last. They considered the conflicting reports on the value of fluorescence methods in serum tests for syphilis to be due mainly to differences in technique and especially to variations in the quality of the conjugate. They concluded that the FTA-50 test performed with their own conjugate gave better agreement with the TPI test than any of four other serological methods with which it was compared.

Király, Jobbágy, and Kováts (1967) later found in a few cases that non-syphilitic human sera were reactive in the FTA test even after absorption with Reiter treponemes. They examined the possibility of treponemal group antibodies not reacting with *T. reiteri*.

LUXEMBOURG

Betz (1966) found that:

The immunofluorescent antibody reaction is certainly of great value in the sero-diagnosis of lues, because of its rapid and easy performance and good reproducibility. Its sensitivity is certainly higher than that of the TPI test in very recent or very old syphilitic infections and its specificity seems to be very high.

POLAND

Ruczkowska (1965) developed a new method for the detection of treponemal antibodies in serum and cerebrospinal fluid which is based on the following immunological phenomenon: fluorescence of an antigen induced by labelled antibody can be inhibited or markedly diminished by first exposing the antigen to specific unlabelled antibody. After investigating various factors which affect the phenomenon, a qualitative and quantitative technique of the fluorescent treponemal inhibition test was developed. With this test 1,500 sera and 100 cerebrospinal fluids were examined, and the results were compared with those obtained with TPI test and FTA indirect test. The elaborated FTA inhibition test was found to be as sensitive and specific as the FTA indirect method and TPI test. Its advantages were cheapness, ease of performance, and speed in dealing with large numbers of sera.

SWITZERLAND

Delacrétaz and Frenk (1963) considered the FTA test could be used in an earlier stage of the disease than other tests and that it was more sensitive; its use was especially indicated:

- (a) if it was suspected that the patient had received penicillin treatment after the primary infection or some local treatment which might have caused the treponemes to disappear from the lesion;
- (b) if the specific antibodies were scanty in late or congenital cases.

U.S.S.R.

Ovchinnikov, Bednova, Lurie, and Sasonova (1966) compared the FTA with other serological tests and found that the sensitivity of the immunofluorescent test exceeded that of the TPI and complement-fixation tests in all forms of syphilis. The FTA test could be used to diagnose false positive results obtained by the standard serological tests for syphilis.

Vasiliev and Bednova (1966) presented data on the behaviour of the immunofluorescent test in sera from 1,007 syphilitic patients after treatment with long-acting penicillin preparations; in particular, bicillin in combination with pyrogenal. A positive result was observed in 363 patients. The earlier the treatment was started, the more frequent were the negative findings after properly conducted therapy.

JAPAN

Matuhasi, Mizuoka, and Usui (1966) investigated the immunological character of syphilitic antibodies active in the FTA test by the use of various fluorescent antiglobulin reagents. The results are in some points similar to the observations on blood group antibody production; that is, in the early stages of syphilitic infection, only IgM antibody may be detected, but sera from six of twenty cases of this disease reacted fairly strongly to the fluorescent anti-IgA reagents.

CHINA

Li Huan Ying (1964) examined 793 clinically diagnosed cases of syphilis; 97 per cent. were positive by the FTA test, 75 per cent. by the Kolmer Wassermann reaction, and 77.7 per cent. by the Kahn test. He concluded that:

The sensitivity and reproducibility of the FTA test are both superior to that of the routine serological tests and the specificity is comparable with that of the TPI test.

Summary

The history, development, and practice of fluorescent methods in the diagnosis of gonorrhoea and syphilis are described in the form of a detailed bibliographical review to indicate progress in various parts of the world.

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Les méthodes fluorescentes appliquées aux anticorps dans la détection et la répression des maladies vétérinaires

Une revue bibliographique de la littérature

RÉSUMÉ

L'historique, le développement et la procédure des méthodes fluorescentes dans le diagnostic de la blennorragie et de la syphilis sont décrits sous forme de revue bibliographique détaillée afin d'indiquer le progrès effectué dans divers pays du monde.