The Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test

Development, Use and Present Status*

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For testing problem sera, the fluorescent treponemal antibody-absorption test (FTA-ABS) is increasingly replacing other treponemal tests for syphilis (including the TPI test) in the USA. The authors review its development as a result of experience with other immunofluorescent techniques and discuss its advantages and disadvantages. Although it is a more sensitive indicator of past or present syphilitic infection than many other widely evaluated techniques, several aspects of the test are still not fully understood; in particular its specificity is not yet established and more information is needed on its reactivity in a variety of clinical diseases and in treponemal diseases other than syphilis. It is a relatively expensive technique in terms of technicians' time, reagents and equipment but there are grounds for hope that research now under way into automation of the procedure may improve the situation.

Immunofluorescent techniques today occupy a useful place in syphilis serology. In the United States of America the FTA-ABS (Fluorescent Treponemal Antibody-Absorption) test is widely used for the detection of syphilis antibodies, and a review of its status seems timely. The development and background of the test procedure, with the various evaluations that have taken place to date, are presented; areas of usefulness are indicated; and those aspects of the test which are still not well understood, or which need further investigation, are noted.

DEVELOPMENT AND BACKGROUND

The technique for the present FTA-ABS test has evolved through several developmental stages at the Venereal Disease Research Laboratory. Deacon et al. (1957) described a fluorescent treponemal antibody (FTA) test in which the indirect fluorescent antibody technique was utilized, and found that antitreponemal antibodies could be detected in human serum. The first extensive evaluation of this procedure was in the Serology Evaluation and Research Assembly (SERA) study (United States Department of Health, Education, and Welfare, 1959). In the initial report (Deacon et al., 1957) the antihuman globulin was conjugated with fluorescein isocyanate. Later, Deacon et al. (1960) reported an increase in the sensitivity of the FTA test which was apparently associated with the more intense staining (Marshall et al., 1958) obtained when antihuman globulin was labelled with the newly developed fluorescein isothiocyanate. At a 1:5 dilution of serum, 100% of syphilitics were detected, but reactivity also occurred in approximately 25% of presumed nonsyphilitics. A new version of the test procedure was described which incorporated a 1:200 dilution of the test serum, and this was known as the FTA-200 (Deacon et al., 1960). This test showed good specificity, and preliminary observations appeared to demonstrate satisfactory sensitivity. On further evaluation, the dilution of serum and, possibly, unknown reagent changes rendered the test undersensitive in the very areas in which
sensitivity was most needed—latent and late syphilis (Bradford et al., 1965; Wilkinson, 1961, 1963).1

Concurrently with the evaluation of the FTA-200 test, the nature of the reactivity of normal sera in the original test was under investigation. Deacon & Hunter (1962) reported that virulent Treponema pallidum (Nichols strain) and three cultivable treponemes seemed to share certain "common" antigens detectable by immunofluorescent techniques. They proposed the use of cultivable treponemes to remove the reactivity that many normal sera showed against T. pallidum. In this study they evolved the concept of the "common" antibody and the "specific" antibody, the latter presumably being specific for T. pallidum (and possibly other pathogenic treponemes). In a preliminary attempt to apply this information to increase sensitivity and specificity in the FTA test, Hunter (1964) used an absorption method with intact Reiter treponemes, and a blocking method in which rabbit anti-Reiter antibody was used to remove reactivity in the original test procedure.

On the basis of these findings, the FTA-ABS test was developed by Hunter et al. (1964). In their study, a "sonicate" of Reiter treponemes (one of the several treponemes which had been found (Deacon & Hunter, 1962) to share the "common" antigen with T. pallidum) was used for absorption. Later, in order to make the production of a sorbing substance as simple and as economical as possible, a new sorbing substance, called "sorbent," was described (Stout et al., 1967). Sorbent is a concentrate of the soluble clarified supernatant fluid from an autoclaved 7–9-day broth culture of Reiter treponemes. The Venereal Disease Research Laboratory and a city public health laboratory compared sorbent with sonicate for its efficacy in FTA-ABS tests on sera in several diagnostic categories (Stout et al., 1967); 300 of 303 tests agreed. The 3 disagreements were weakly reactive when sera were diluted in sorbent and nonreactive when diluted in sonicate. The data indicated that the two reagents were comparable.

A standardized technique for the performance of the FTA-ABS test was made available in provisional form by the Venereal Disease Research Laboratory in 1964. The technique was subsequently revised and published (Staff, Venereal Disease Research Laboratory, 1968).

There have been numerous reports throughout the world describing the application of the indirect immunofluorescent technique to the serological diagnosis of syphilis.

Borel & Durel (1959) conjugated antihuman globulin with fluorescein isothiocyanate and described a procedure in which a 1 : 10 dilution of the patient's serum was used. Other investigators have found that a 1 : 30 (Thivolet et al., 1960), a 1 : 50 (Mannucci & Spagnoli, 1961), a 1 : 100 (Fife et al., 1961), or a 1 : 150 2 dilution is more desirable than the 1 : 200 dilution of the serum. Manikowska-Lesinska et al. (1964) diluted 1000 nonsyphilitic sera 1 : 10, 1 : 50, 1 : 100 and 1 : 200. The authors found 2.5% nonspecificity with a 1 : 10 dilution, 0.6% with a 1 : 100 dilution, and 0.2% with the 1 : 200 dilution. Vaisman et al. (1963) described a technique for the application of the FTA test to dried blood. Niel & Fribourg-Blanc 8 indicated the need for quantitation of serum and high-titred, labelled globulin.

Griffin et al. (1961) examined the effect of altering the ratio of dye to protein in labelling antihuman globulin. Fry & Wilkinson (1963) used Evans blue as a background stain, and E. N. Fife, Jr (personal communication, 1967) has incorporated it into an FTA antigen. Thivolet & Cherby-Grosiron (1961) absorbed serum with dried rabbit testicular powder, then heated it at 62°C to remove nonspecific reactions. Király et al. (1965), in a detailed review of fluorescent treponemal antibody testing, indicated that differences in techniques and especially variations in the quality of conjugates were responsible for contradictory reports on the value of fluorescein protein tracing as a serological test for syphilis. Conjugates containing 5–7 fluorescein molecules per gammaglobulin molecule were found satisfactory for the FTA test (Király & Jobbágy, 1966). Gregorczyk (1966a), in a 75-page review, discusses the principle and method of demonstrating fluorescein-labelled antibodies and the numerous modifications of the FTA test. Evaluations of FTA procedures have involved rather large numbers of sera; for example, Manikowska-Lesinska (1966) reported on the results of 80 000 tests. Some writers have suggested that T. pallidum antigen is a primary cause of problems

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1 Also Eng., J., Nielsen, H. A. & Wereide, K. (1963) unpublished working document WHO/VDT/314; WHO/VDT/RES/29. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.

8 Niel, G. & Fribourg-Blanc, A. (1964) unpublished working document WHO/VDT/315; WHO/VDT/RES/34. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.
### TABLE 1

**COMPARATIVE REACTIVITY OF THE FTA-ABS AND TPI TESTS IN CLINICALLY DEFINED DONOR CATEGORIES**

<table>
<thead>
<tr>
<th>Category</th>
<th>Hunter et al. (1964)</th>
<th>Deacon et al. (1966)</th>
<th>Bradford et al. (1967)</th>
<th>McGrew et al. (1968)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>Percentage reactive</td>
<td>No. tested</td>
<td>Percentage reactive</td>
</tr>
<tr>
<td>Primary syphilis</td>
<td>76</td>
<td>80.3</td>
<td>36.8</td>
<td>191</td>
</tr>
<tr>
<td>Secondary syphilis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>270</td>
</tr>
<tr>
<td>Late or congenital syphilis</td>
<td>46</td>
<td>100.0</td>
<td>91.3</td>
<td>117</td>
</tr>
<tr>
<td>Latent or unclassified syphilis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>954</td>
</tr>
<tr>
<td>False positive b</td>
<td>38</td>
<td>0.0</td>
<td>0.0</td>
<td>288</td>
</tr>
<tr>
<td>Diseases other than syphilis</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>46</td>
</tr>
<tr>
<td>Presumed normal</td>
<td>110</td>
<td>0.0</td>
<td>0.0</td>
<td>384</td>
</tr>
</tbody>
</table>

1. NT: not tested; — : study did not include sera in this category.
2. The classification of a serum as "false positive" was usually based on reactivity in a routine nontreponemal test, such as the VDRL Slide, and nonreactivity in the TPI test. Thus, if the FTA-ABS test is more sensitive than the TPI test but as specific, some of the sera classified as "false positive" will, in fact, be syphilitic and will be found to have anti- *T. pallidum* antibodies detectable in the FTA-ABS test.

in reproducibility, intensity of fluorescence, and precision in testing (Lesinski, 1964; Király et al., 1965; Gregorczyk, 1966a). Matuhasi et al. (1966) investigated the immunological character of syphilis antibodies that are reactive in the FTA test by using labelled antiserum specific for the various immunoglobulin classes. Sartorius et al. (1968) have described an FTA test using saliva and have suggested that it has high specificity.

Reviews indicating that group antibody could be removed from the patient's serum by absorbing with the intact Reiter treponeme or by blocking with Reiter antiserum include procedures described by Pillot & Borel (1961) and Poetschke & Kdllisch (1959). Betz (1966) absorbed human serum with intact Reiter treponemes to remove normal treponemal antibody. Gregorczyk (1966b) quantitatively performed *Treponema pallidum* immobilization (TPI) and FTA tests (after absorbing the sera with Reiter treponemes) on sera from treated syphilitic patients and experimentally infected rabbits. Puffer et al. (1966) summarize and compare the FTA modifications with other tests for syphilis. Fribourg-Blanc & Niel (1961) compared absorption of human serum with sonicate, sorbent, and intact Reiter treponemes. Király et al. (1967) studied the multiplicity of group antibodies by absorption of human and rabbit sera with 6 treponemal strains. More recently, Király and others (1965) reported on the ratio of specific and group antibodies in the FTA test in which conjugates with different immunoglobulin specificity were used. Bredt (1967) also recently studied the group antigens shared by *T. pallidum* and Reiter treponemes. Schierz & Meigel (1968) determined that the amount of Reiter treponeme sonicate necessary in the FTA-ABS test was 200 µg per 0.05 ml of serum, with 400 µg needed for some sera. Using 1000 µg per 0.05 ml, no loss of syphilis antibody could be detected.


**SENSITIVITY AND SPECIFICITY OF THE FTA-ABS TEST**

A large number of sera have now been tested with the FTA-ABS test. Four of the various evaluations that included sera from known clinical categories are listed chronologically in Table 1. In the
evaluations by Hunter et al. (1964) and Deacon et al. (1966), a sonicate of the Reiter treponeme was used in the test procedure; in the data presented in the publications by Bradford et al. (1967), and McGrew et al. (1968), the FTA-ABS test was performed according to the provisional technique (Venereal Disease Research Laboratory, 1965), and sorbent was used. In these studies, the reactivity of the FTA-ABS test was 80%-95% in primary syphilis, approximately 100% in secondary syphilis, and 92%-100% in latent and late syphilis. Knox et al. (1966), in an interim report on their participation in a co-operative study (Deacon et al., 1966), found similar reactivities.

A number of the sera listed in Table 1 were examined with both the FTA-ABS and TPI tests. Four other published evaluations (Victor & Duffett, 1966; Stevens et al., 1967; Beam et al., 1967; Wood et al., 1967) which compare the TPI and FTA-ABS test results on diagnostic problem cases are summarized in Table 2.

The reactivity of the FTA-ABS test in treated early syphilis can be seen in the accompanying figure. Preliminary data are presented on 104 cases from a study (Lucas & Price, 1967) in which serial bleedings were taken from patients for many months after treatment of syphilis at the primary or secondary stage.

The figure shows that reactivity in the FTA-ABS test declines slowly after treatment of early syphilis. At the end of the first year of follow-up, 91% of the patients were still reactive in the FTA-ABS test. In contrast, only 40% of the patients were still reactive in the VDRL Slide test and only 38% in the TPI test.

Reactivity in the FTA-ABS test may be present 13 or more years after treatment of syphilis at the latent, tertiary or congenital stage (Atwood et al., 1968), as may be seen from Table 3. Sixty-six of 67 patients were reactive in the FTA-ABS test. Although all the patients had been reactive in a reagin test and the TPI test at the time of original diagnosis and treatment, 10% were found now to be nonreactive in the TPI test, and 27% to be nonreactive in a cardiolipin reagin test. The VDRL Slide test was nonreactive in 31% of the patients.

In untreated late latent syphilis there is evidence that the FTA-ABS test is reactive, whereas the TPI test may spontaneously become nonreactive. Rockwell et al. (1964) reported that of 93 syphilitic patients tested after 30 years or more of infection, 91% had a reactive TPI test, whereas 97% were

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**Table 2**

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Percentage of sera giving indicated reaction</th>
<th>Total no. of specimens tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPI (R)</td>
<td>TPI (N)</td>
</tr>
<tr>
<td>Victor &amp; Duffett</td>
<td>48.4</td>
<td>47.4</td>
</tr>
<tr>
<td>Beam et al. (1967)</td>
<td>31.8</td>
<td>36.2</td>
</tr>
<tr>
<td>Stevens et al. (1967)</td>
<td>47.1</td>
<td>51.0</td>
</tr>
<tr>
<td>Wood et al. (1967)</td>
<td>43.1</td>
<td>43.8</td>
</tr>
</tbody>
</table>

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* R: reactive; N: nonreactive; T or I: toxic or inconclusive.
recently, Mackey et al. (1969) studied 827 persons to determine whether reactivity to the FTA-ABS test and nonreactivity to the TPI test occurred in association with any particular disease other than syphilis. They found only 5 patients with a confirmed reactive FTA-ABS test and a nonreactive TPI test to have no clinical or historical evidence of syphilis. All 5 patients were over 50 years of age and 3 had diseases in which abnormal globulins are frequently found.

Test results on a number of patients in each of many disease categories are needed for completeness in assessing specificity. To the present, most evaluations have emphasized syphilitic patients, normal persons and patients with "false-positive" reactions in reagin tests. It would be of value to have the published reports of several evaluations in which the FTA-ABS test had been performed routinely on all patients admitted to a large teaching hospital. There is still a shortage of data concerning the reactivity of this test in such diverse clinical conditions as cancer, heart disease, kidney disease, surgical conditions, etc. Cohen and co-workers 1 in a study of reactivity among patients admitted to a large teaching hospital found 345 out of 463 with divers diagnoses to be nonreactive in the FTA-ABS test. Of the patients who were reactive in the FTA-ABS test, virtually all were found to have a previous history of syphilis.

Additional data are also needed with respect to the reactivity of the FTA-ABS test in patients infected with other pathogenic treponemes (those of yaws, pinta, bejel, etc.), and with other spiral organisms (Borrelia, Leptospira). Only one formal report in the literature indicates that serum from patients with pinta and yaws may be absorbed with Reiter treponemes and still react with T. pallidum antigens (Pilott & Borel, 1961).

There have been reports that macroglobulins, in certain collagen diseases, could cause false-positive reactions in the FTA-200 test (Fife et al., 1961; Fife, 1964), and this work has been confirmed by Wilkinson & Rayner (1966). However, this has not thus far been reported in regard to the FTA-ABS test. We have also not encountered any confirmation of the report that the factor which reacts in the FTA-200 test with the Nichols treponeme is directed against the cytoplasm of human tumour cells (Neblett et al., 1966). Several evaluations of patients with false-positive reagin tests have failed to indicate

any false-positive FTA-ABS test results attributable to either macroglobulin or antinuclear factor (Wuepper et al., 1966; Tuffanelli et al., 1967; Lassus et al., 1967; Mustakallio et al., 1967). That is not to say, of course, that it would be impossible to find false-positive reactions to the FTA-ABS test. However, no large number of patients in any particular diagnostic category has yet been brought forward as having a high incidence of false-positive reactivity in the FTA-ABS test.

CURRENT USE OF THE FTA-ABS TEST

A survey was made of 47 State health department laboratories in the USA, the District of Columbia, Puerto Rico, and the Virgin Islands to determine how the FTA-ABS test was being used. Eight of these laboratories perform FTA-ABS tests on all specimens that are reactive in routine nontreponemal antigen tests (VDRL Slide or Unheated Serum Reagin [USR] tests). Twenty-nine laboratories also perform the FTA-ABS test upon request on any specimen that is submitted. Nine of these 29 laboratories also perform this test routinely on certain categories of specimens, such as specimens from premarital and prenatal reactors, specimens with which there is a discrepancy between the results of two routine nontreponemal tests, and specimens submitted for a one-fifth volume Kolmer test with Reiter Protein (KRP) antigen. Ten States perform FTA-ABS tests only on specimens which meet specific established criteria.

In the 1968 Syphilis Serology Evaluation Study (United States Department of Health, Education, and Welfare, 1968) approximately 67 laboratories are performing the FTA-ABS test. This includes the laboratories of 47 State health departments in the USA, the District of Columbia, Puerto Rico and the Virgin Islands, and of 3 Public Health Service installations, and laboratories in 9 other countries.

The increased use of the FTA-ABS test at the State health department level has enabled those laboratories to examine the specimens from diagnostic problem cases submitted to them by private physicians, thus providing for more prompt resolution of some of these problems, and reducing the number of specimens that might still indicate the need for a TPI test.

In the fiscal year 1967 there was a noticeable decrease in the number of specimens submitted by the State health department laboratories to the Venereal Disease Research Laboratory for a TPI test. In the fiscal year 1966, an average of 475 specimens was received monthly; in the fiscal year 1967, this figure dropped to an average of 323 per month. Recently, two of the TPI testing laboratories in the USA—the US Naval Medical Center and one State health department—have discontinued the TPI test and substituted the FTA-ABS test. It is interesting to note that ophthalmologists have found the FTA-ABS test useful in evaluating patients who constitute diagnostic problems, including those in whom the nontreponemal tests are nonreactive, and those in whom intraocular treponemes have been found (Smith & Taylor, 1965; Goldman & Girard, 1967; Smith & Israel, 1967; Harner et al., 1968).

FUTURE ASPECTS OF THE FTA-ABS TEST

Despite the generally favourable acceptance thus far, additional information is desirable on several aspects of the present test.

The FTA-ABS test antigen is still obtained rather laboriously from rabbit testicular syphilomas, and it is sometimes difficult to assess the treponemes amidst debris or haze on the final slide. If *T. pallidum* could be cultivated *in vitro*, it might be possible to prepare more reproducible antigens by simpler methods.

The coating by rabbit globulins of *T. pallidum* organisms grown in rabbits (Fife et al., 1961; Fife, 1964; Wilkinson & Rayner, 1966) occurs if the organisms are left too long in the rabbit, and L. C. Logan and L. C. Norins (unpublished data) have suggested that some amount of rabbit globulin may become attached to the organisms even in the conventional 7-9-day incubation interval.

The suggestion that cultivable Reiter treponemes be used as test antigen instead of *T. pallidum* (Covert et al., 1961; Kent et al., 1962; Sasahira, 1963; Bory et al., 1963; Bredt & Tupath-Barniske, 1967) is subject to certain theoretical objections (Deacon & Hunter, 1962). Certainly a great deal of evaluation would be needed before such a substitution could be judged efficacious.

Because the action mechanism of the sorbent is not well understood, additional work is needed.

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1. Also Fribourg-Blanc, A. & Niel, G. (1967) unpublished working document WHO/VDI/RES/67.115. A limited number of copies of this document is available to persons officially or professionally interested on request to Distribution and Sales, World Health Organization, 1211 Geneva, Switzerland.
Gel precipitin lines\(^1\) have been demonstrated between sorbent and rabbit antisera to Reiter treponemes, which suggest that Reiter treponemal antigens are present in the sorbent. Recently, however, Cannefax et al. (1968) found evidence that substances not directly related to treponemes have sorbing properties. Wilkinson & Ferguson\(^2\) have recently confirmed these observations. Thus, sorbent action may involve several factors.

There have also been reports of the testing of spinal fluid with procedures of the FTA type (Harris et al., 1960; Vaisman & Hamelin, 1961; Ripault & Colombani, 1964; Nauman, 1965; Mattern et al., 1965); however, to the best of our knowledge, a standardized FTA-ABS technique for spinal fluid remains to be evolved and evaluated.

**ACKNOWLEDGEMENT**

We wish to thank Mrs Eleanor V. Price for her help in compiling the data used in the figure.

**AUTOMATION**

The FTA-ABS test has largely replaced other treponemal tests, including the TPI test, in the USA. It is usually reserved for problem cases, since, as a qualitative procedure, it does not reflect response to treatment but indicates only past or present treponemal infection. When compared with the routine nontreponemal test in current use, the FTA-ABS test is relatively expensive in terms of technicians' time, reagents, and equipment.

Automation of the procedure could have wide clinical, and even research, implications. The Space Division, Aerojet-General Corporation, working closely with the Venereal Disease Research Laboratory, has adapted the FTA-ABS test procedure to automated equipment. During the years 1966–68, 3 prototypes were studied, and the first production model is now undergoing evaluation at the Venereal Disease Research Laboratory and in several State laboratories.

**RéSUMÉ**

La mise au point de la réaction d’immuno-fluorescence après absorption (IFA) est l’aboutissement de nombreuses recherches consacrées à l’application des techniques d’immuno-fluorescence au diagnostic sérologique de la syphilis. Les auteurs décrivent les principales étapes de cette évolution: emploi pour la conjugaison de l’isocyanate, puis de l’isothiocyanate de fluorescéine, dilution du sérum d’épreuve à 1/200 (réaction IF-200), enfin absorption des sérum par le tréponème de Reiter. Une technique normalisée a été publiée par le Laboratoire de recherches sur les maladies vénériennes du Centre national des maladies transmissibles, Atlanta, Ga., Etats-Unis d’Amérique.

A ce jour, un très grand nombre de sérum ont été examinés en réaction IFA. Une revue des résultats montre que cette réaction est plus sensible que d’autres, largement utilisées, lorsqu'il s'agit de déceler une infection syphilitique en évolution ou ancienne. Sa spécificité est plus malaisée à évaluer correctement, spécialement dans les cas difficiles — cliniques ou sérologiques — et surtout si l'on choisit comme épreuve de référence le test d'immobilisation des tréponèmes (TIT). On ne dispose pas encore de données suffisantes concernant la réactivité du test IFA dans les affections non tréponémiques ou dans les tréponématoses autres que la syphilis. Il semble cependant que le nombre des réactions faussement positives ne soit pas très élevé.

La réaction IFA est actuellement d'un emploi courant aux Etats-Unis où elle a en grande partie supplanté les autres épreuves, y compris le TIT, pour l'étude des sérum difficiles. En dépit de la qualité des résultats, certains de ses aspects sont encore mal connus. L'anti-
gène spécifique est relativement long à préparer, et la culture in vitro de Treponema pallidum permettrait d’obtenir, plus simplement, des antigènes dotés de caractéristiques plus stables. Le dépôt des globulines de lapin sur T. pallidum altère parfois les propriétés de l’antigène. Le mécanisme d’action de l’absorbant est loin d’être complètement éclairci; des travaux récents indiquent que certaines substances sans rapport direct avec les tréponèmes font aussi preuve de propriétés absorbantes. L’application de la réaction IFA à l’étude du liquide céphalo-rachidien serait facilitée par l’adoption d’une technique normalisée.

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FLUORESCENT TREPONEMAL ANTIBODY-ABSORPTION (FTA-ABS) TEST


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