23. Done, A. K., Clin. Pharmacol. Therap., 1961, v2, 750.
24. Hirsch, F. G., Arch. Environ. Health, 1964, v8, 622.
25. Sayre, J. W., Kaymakçalan, S., N. Engl. J. Med., 1964, v270, 1113.
26. Dall, J. L. C., Hannah, W. M., Brit. Med. J., 1964, v2, 33.

Received June 23, 1965. P.S.E.B.M., 1965, v120.

An Indirect Fluorescent Antibody Technique Using Soluble Antigens for Serodiagnosis of *Trypanosoma cruzi* Infection. (30653)

ANDRE J. TOUSSAINT, CARL J. TARRANT AND ROBERT I. ANDERSON
(Introduced by Earl H. Fife, Jr.)
Department of Serology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D.C.

Recently described(1) methodology for an indirect fluorescent antibody technic employing a soluble somatic protein schistosome antigen and an artificial matrix has certain advantages over the normally employed whole organism tests. The advantages include: 1) objective selection of the antigen; 2) precise fluorometric reading of test results; 3) elimination of fade-out of the reaction during reading of tests; and 4) correction of nonspecific fluorescence contributed by substances in the patient's serum (*e.g.*, therapeutic drugs) and/or by free fluorescein in the conjugated antiserum.

The present study was conducted to determine whether the soluble antigen fluorescent antibody (SAFA) technic could be employed for the serorecognition of infections with *Trypanosoma cruzi* and to ascertain if antigens other than somatic schistosome protein could be used with the artificial matrix.

Materials and methods. Serum specimens. Sera from 82 individuals who had clinical, and/or serological evidence of Chagas' disease were used to evaluate the sensitivity of the SAFA technic. The specificity of the procedure was determined with sera from 54 healthy persons and 10 patients with each of the following infectious diseases—syphilis, schistosomiasis, amoebiasis, malaria, trichinosis, and filariasis. In addition, 9 sera from patients with leishmaniasis were tested. The sera had been collected and preserved under various conditions: some were preserved with either merthiolate or sodium azide whereas others were collected and shipped under aseptic conditions without a preservative. Upon receipt in the laboratory, all sera were stored at —20°C. When required for tests the sera were thawed at room temperature, and tested without heating.

Antigens. Three antigens of *T. cruzi* (exo, somatic protein and somatic carbohydrate) were employed in initial studies. The exoantigen was prepared by published methods (2) and was a pH 4.6 soluble fraction of the dialyzed, organism-free supernates from cultures of *T. cruzi* grown inside cellulose sacs. The somatic protein and somatic carbohydrate antigens were separated by chloroform-gel fractionation(3) of the trypanosomes recovered from the same cultures used to produce the exoantigen. All 3 antigens had been previously studied(2,3) in a complement fixation test.

SAFA technic. Tests were performed as previously described(1). Briefly, the method consisted of incubating each serum (0.2 ml of a 1:2 dilution in Tris buffered saline) with a cellulose acetate filter paper disc (¼″ dia., 0.45 μ porosity) impregnated with test antigen optimally diluted in 1% bovine serum albumin (BSA). A control for each serum was tested concurrently with a 1% BSA impregnated disc. Following the incubation period (18 hours at 3°-6°C) the discs were washed in saline and incubated for 30 minutes at room temperature in an optimal dilution of fluorescein tagged antihuman globulin. The discs were again washed and

TABLE I. Ability of the Artificial Matrix to Retain Chemically Distinct Antigens.

Serum	Exo	Somatic protein	Somatic carbohydrate
T. cruzi 1	R	R	R
2	R	R	—
3	R	R	R
Normal 1	—	—	—
2	—	—	—

R = reactive and (—) = nonreactive.

mounted on the adhesive side of masking tape which was then fastened to the paper chromatogram door of a fluorometer (G. K. Turner Associates, Palo Alto, Calif.). The fluorometer was equipped with a 4-watt far ultraviolet lamp, a primary filter transmitting 254-420 mμ, and a secondary filter system containing 2 filters. The latter consisted of a sharp cut filter (passing >510 mμ) and the other a 1% neutral density filter. The fluorometer was adjusted to zero using the control disc for each serum as a blank and the fluorescent dial reading (FDR) was obtained with the corresponding test disc. The following FDR readings, previously ascertained in the schistosomiasis study, were employed to interpret the test results: 0-20 FDR = nonreactive (—); 21-24 FDR = weakly reactive (wr); >25 FDR = reactive (R).

Results. Preliminary investigations indicated that basic modifications in the previously described SAFA technic for schistosomiasis were not required to convert the method to Chagas' disease. In addition, it became readily apparent that the somatic protein antigen, somatic carbohydrate antigen, and the exoantigen (presumably glycoprotein) all adhered to the matrix in the presence of 1% BSA (Table I). The somatic protein and exoantigens were selected for additional evaluation.

The sensitivity of both the somatic protein and exoantigens proved to be very satisfactory (Table II). Of the 82 Chagas' disease sera tested, 78 showed a reaction with each antigen.

The specificity also was excellent with both antigens. Sera from all 54 healthy persons were uniformly nonreactive. In the category of diseases other than Chagas' disease, a total of 69 sera were tested. All were nonreactive in tests with the exoantigen and 68 were nonreactive with the somatic protein antigen; the single serum that reacted with the somatic protein antigen was from a patient with leishmaniasis.

Discussion. The relative ease of converting the SAFA technic from schistosomiasis to Chagas' disease was not unanticipated. On theoretical grounds, one would expect that ability to change the procedure from one disease to another would depend primarily upon the sensitivity and specificity of the antigen selected, and upon the ability to fix the antigen onto the matrix; the role of the control discs in the procedure would remain the same regardless of the disease being studied. For example, the control disc would normally be of the same material, contain 1% BSA, be incubated in test serum, exposed to the optimal dilution of tagged antiserum, and read under the same fluorometric conditions. These factors set the guidelines for zeroing in the fluorometer and therefore when compared against the test disc would compensate for nonspecific fluorescence readings attributed to: 1) nonspecific adherence of test serum to the disc, 2) conditions in any one serum (*e.g.*, chemotherapeutic drugs that might fluoresce under test conditions), or 3) free fluorescein in the tagged antiserum. Since autofluorescence in any experimental antigen preparation could be easily determined, only the antigen-test serum relationship remained

TABLE II. SAFA Tests Performed with Sera from Humans with Chagas' Disease, Other Infectious Diseases, and Healthy Persons.

Diagnostic category	No. of sera tested	Results* obtained with the given antigen: Exoantigen R	wr	—	Somatic protein R	wr	—
Chagas' disease	82	74	4	4	78	0	4
Syphilis	10	0	0	10	0	0	10
Schistosomiasis	10	0	0	10	0	0	10
Amoebiasis	10	0	0	10	0	0	10
Malaria	10	0	0	10	0	0	10
Trichinosis	10	0	0	10	0	0	10
Filariasis	10	0	0	10	0	0	10
Leishmaniasis	9	0	0	9	1	0	8
Healthy persons	54	0	0	54	0	0	54

* R = reactive, wr = weakly reactive, and (—) = nonreactive.

to be accounted for in changing the SAFA technic from one disease to another. It was of considerable interest, therefore, to determine whether antigens other than the previously employed schistosome somatic protein would adhere to the disc under conditions of the test. The fact that *T. cruzi* somatic protein, somatic carbohydrate, and exo antigens all adhered to the matrix in the presence of 1% BSA (Table I) indicates that antigens from other organisms would undoubtedly adhere. The preliminary studies performed with the *T. cruzi* antigens indicated that the carbohydrate antigen was less sensitive than either the somatic protein or exoantigens. An example is shown in Table I where it may be seen that the somatic carbohydrate antigen could yield a negative result on a serum that was reactive with both the somatic protein and exoantigens in the SAFA test. This finding was not surprising, however, as the carbohydrate antigen had been shown previously to be both less sensitive and less specific than the protein antigen in complement fixation (CF) tests(3). Since the primary purpose of the 1% BSA was to block nonspecific fluorescence caused by the natural adherence of test serum to the untreated matrix(1), it was not determined whether the 1% BSA assisted in the fixation of antigen to the disc.

Of the 123 non-Chagas' disease sera tested, the only apparent false reaction occurred between the somatic protein antigen and the serum from a patient with *Leishmania brasiliensis* infection. This finding was in agreement with previous results with CF tests(2,3). On the other hand, no reaction was obtained with this serum and the exoantigen. This also was in agreement with previous CF test findings(2). Sensitivity of the SAFA procedure in the Chagas' disease sera tested was excellent; 80 (98%) of the 82 sera showed reactivity with one or the other antigen. In addition, it is noteworthy that discrepancies between the antigens occurred with only 4 sera; 2 sera were reactive with the exoantigen and nonreactive with the somatic protein antigen where as the other two were reactive with the somatic protein and nonreactive with the exoantigen. The findings obtained with the somatic protein and exoantigens indicate that the SAFA test is both a sensitive and specific procedure for the serorecognition of Chagas' disease.

Summary. A soluble antigen fluorescent antibody (SAFA) technic for the serorecognition of American trypanosomiasis is described. The technic has the following advantages over the conventional whole organism indirect fluorescent antibody tests: 1) permits the investigator to objectively select the antigen; 2) provides for mechanical reading of test results; 3) eliminates the problem of fading; and 4) accounts for nonspecific fluorescence contributed by the patient's serum (*e.g.*, drugs) and/or by free fluorescein in the conjugated antiserum. Preliminary investigations revealed that exo, somatic carbohydrate and somatic protein antigens from *T. cruzi* would adhere to the artificial matrix. The sensitivity and specificity of the somatic protein and exoantigens were evaluated. The findings indicate that the SAFA technic with either of these 2 antigens should yield excellent results.

1. Toussaint, A. J., Anderson, R. I., Appl. Microbiol., 1965, v13, in press.

2. Tarrant, C. J., Fife, E. H., Jr., Anderson, R. I., J. Parasitol., 1965, v51, 277.

3. Fife, E. H., Jr., Kent, J. F., Am. J. Trop. Med. and Hyg., 1960, v9, 512.

Received July 1, 1965. P.S.E.B.M., 1965, v120.