2. Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1962, v109, 390.

3. Chase, H. B., Montagna, W., Malone, J. D., *Anat. Rec.*, 1953, v116, 75.

4. Carruthers, C., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 259.

5. Stoffel, W., Chu, F., Ahrens, E. H., Jr., *Anal. Chem.*, 1959, v31, 307.

6. Luddy, F. E., Barford, R. A., Riemenschneider, R. W., *J. Am. Oil Chemists' Soc.*, 1960, v37, 447.

7. Carruthers, C., *Cancer Research*, 1962, v22, 294.

Received November 7, 1962. P.S.E.B.M., 1963, v112.

A Plasma Card Test for Rapid Serodiagnosis of Schistosomiasis (SPC). (28016)

E. H. SADUN, R. I. ANDERSON, AND M. J. SCHOENBECHLER

Walter Reed Army Institute of Research, Department of Medical Zoology, Washington, D. C.

Diagnosis of schistosomiasis by examination of the feces or urine is frequently unsatisfactory in light and chronic infections and in cases which are therapeutically affected. To obviate this difficulty numerous immuno-diagnostic tests have been developed. One of these, a fluorescent antibody technic(1) makes possible the use of dried blood collected by finger puncture, instead of serum. This permits rapid collection of specimens under adverse conditions and allows easy transport of specimens(2-3). In spite of these recent advances, the need still exists for an efficient field testing procedure which would permit the prompt collection of data without the time required to mail specimens to a central laboratory and to receive results. This need is particularly acute in many of the endemic areas where health officers frequently lose contact with infected people before they receive the results of serological examinations.

Recently Brewer developed a commercially available plasma collection slide(4). A card test with plasma obtained with the Brewer's collection slide was subsequently developed for field tests for syphilis and other treponematoses(5). This provided the opportunity to attempt the development of a similar technic for rapid field diagnosis of schistosomiasis, utilizing a washed antigen-cholesterol-lecithin complex developed by one of us(6) for the cercarial slide flocculation test.

This report describes the technic for the schistosomiasis plasma card (SPC) test. The procedure was evaluated with patients tested under field conditions and the findings compared to those obtained with the fluorescent antibody (FA) test performed on dried blood samples obtained from the same patients. Additional tests were performed in the laboratory with unheated serum specimens.

Materials and methods. Antigen. Schistosoma mansoni cercariae from experimentally infected *Australorbis glabratus* snails (Puerto Rican strain) were used. The cercariae were obtained by exposing infected snails to artificial light and were concentrated by sedimentation at 3 to 6°C. Following sedimentation and washing in distilled water, the cercariae were quickly shell frozen and dehydrated under vacuum from the frozen state. Nonspecific antigenic lipids were removed as described previously(6). An antigen emulsion was prepared by adsorbing the cercarial antigen onto cholesterol lecithin crystals, centrifuging and resuspending the sediment with a solution containing charcoal(5).

Sources and handling of the blood samples tested. Finger blood from 189 well documented cases of schistosomiasis was collected in endemic areas from persons in whom the diagnosis had been established by demonstrating the eggs in the stools or urine. To determine the specificity of the reaction samples were tested from individuals living in endemic areas without clinical or parasitological evidence of schistosomiasis and from individuals with prolonged history of hyper-

TABLE I. Results Obtained with the Fluorescent Antibody Test (FA) and the Schistosome-Plasma-Card Test (SPC) in Different Areas.

Location	Race	Diagnosis	Schistosomiasis endemicity	No. persons tested	Results of FA			Results of SPC		
					Pos	Doubt	Neg	Pos	Doubt	Neg
Geneva, Switz.	W	Healthy	None	11	ND*	ND	ND	0	0	11
Rome, Italy	W	Hypersens.	"	25	2	2	21	0	1	24
Witkoppen, S. A.	N	Schistosomiasis	High	34	34	0	0	31	3	0
Idem	N	No schisto.	"	10	2	0	8	1	0	9
Johannesburg, S. A.	W	" "	None	7	ND	ND	ND	0	0	7
Shalpm Manne, S. A.	N	" "	"	57	2	12	43	1†	4	52
Barberton, S. A.	N	Schistosomiasis	Very high	78	78	0	0	78	0	0
Idem	N	No schisto.	" "	2	0	0	2	0	0	2
Bukandwe, Tang.	N	Schistosomiasis	" "	77	77	0	0	77	0	0
Idem	N	No schisto.	" "	3	2	0	1	2	1	0
Arusha Chini, Tang.	N	Unknown	High	63	33	9	21	50	8	5
Total				367	230	23	96	240	17	110

* Not done.

† Also positive by FA and intradermal tests—admitted going swimming in endemic area.

sensitivity (asthma, eczema, etc.). A finger tip was cleansed with an antiseptic solution and punctured with a sterile lancet. Three-four drops of blood (approximately 0.15 ml) were allowed to fall freely onto the Brewer Plasma Collection slide coated with an anticoagulant and a lectin which are stable at room temperature and which cause both red and white blood cells to agglutinate, leaving the plasma free to flow down into the collection slot. After approximately 2 minutes the plasma was removed for testing in the unheated state with an especially provided capillary tube calibrated to 0.03 ml. Additional drops of blood were obtained from the same individuals on calibrated filter paper and mailed to the central laboratory for testing with the FA technic.

Performance and reading of the SPC test. Using the capillary tube, 0.03 ml of unheated plasma was placed on a card especially designed by Brewer for 10 separate samples. One drop of antigen suspension (approximately 1/60 ml) was distributed to each sample with a specially designed needle attached to a plastic dispensing bottle. Using a separate toothpick for each test sample, the plasma and antigen suspensions were mixed and spread until they filled the entire test surface. The card was then rocked by tilting to and fro for 4 minutes and read immediately by inspection with the naked eye. Specimens showing characteristic clumping were reported as positive and those showing no clumping at the end of the 4 minutes were reported as negative. Those showing some intermediate reactions were referred to as doubtful. Because of the extreme dryness and the presence of dust in many of the African areas where the field tests were performed, it was necessary to place the collection slides in a specially designed portable humidifying device, and to shake the cards for 4 minutes inside of a flat plastic box. If a permanent record is desired, the cards should be allowed to air dry, care being taken not to shake them until they are completely dried. The antigen suspension was tested with controls of known reactivity prior to performing the tests.

Results. The preliminary results obtained with the 2 serological tests are summarized in Table I. The findings with specimens from schistosomiasis patients illustrate the relative sensitivity of the 2 procedures. The findings with specimens from allergic patients and from healthy individuals provide an index of the relative specificity of the 2 tests. No significant difference in sensitivity and specificity of the 2 tests was observed. As indicated in Table II, a close correlation of results was obtained with the 2 tests.

The SPC test was also performed at the Walter Reed Army Institute of Research laboratories on serum from blood obtained from venipuncture which is maintained in a serum bank for comparative evaluation of testing procedures. The 102 serum samples

TABLE II. Correlation of Results Obtained with Fluorescent Antibody Test (FA) and Schistosome-Plasma-Card Test (SPC).

		Results of FA		
		Pos	Doubt	Neg
Results of SPC	Pos	*221*	7	12
	Doubt	3	*5*	9
	Neg	6	11	*75*

used for this comparison were from several diagnostic categories as described (Table III). The results indicate that the SPC test has a satisfactory sensitivity and specificity with unheated serum. Attention should be called to the fact that serum specimens from filariasis and leishmaniasis patients were obtained in areas of high endemicity for schistosomiasis, and that all tests described for serodiagnosis of schistosomiasis usually react with trichinosis antisera(1,6,7).

Antigen stability studies were also conducted. Aliquots of the antigen were distributed in several 1 ml vials which were sealed and stored at room temperature (22-27°C). Once each month for a total of 4 months one vial of antigen was opened and used to test 10 sera of known positive reactivity and 10 of known negative reactivity. In each test the 10 reactive sera gave positive results and the 10 nonreactive sera gave negative results.

Discussion. The SPC test makes use of the slide flocculation test antigen suspension in which, after centrifugation, the sediment was resuspended with a solution containing charcoal powder as a visualization agent(5). The tests are performed on plastic coated cards, which if allowed to dry properly, may be filed for future reference. The practical advantages of this test primarily designed for use as a screening test in the field are obvious. It is rapid, economical, and does not require any of the usual laboratory equipment; all materials employed are disposable, supplies and apparatus for performing 100 tests are included in a kit which occupies less than one square foot of desk space and weighs less than one pound, and the cards provide permanent records for future reference. In field surveys among school children, approximately 40 tests were conducted in one hour. For individual tests less than 10 minutes are required for collection of the sample and performance of the test.

The effectiveness of the SPC test in providing serologic support for diagnosis of schistosomiasis is apparent from the results given in Table I. However, the SPC has been tested principally in areas of high endemicity and the results may not necessarily apply to other areas. The results in Table II demonstrate the generally good agreement between the results of the SPC and the FA tests, and those presented in Table III indicate that the SPC card test has a satisfactory reactivity level with unheated serum.

Only in the group of individuals without a definite diagnosis of schistosomiasis (Arusha Chini) did the two tests not show a close agreement. In this category the number of reactors with the SPC card was considerably greater than that obtained with the FA test. In the absence of other data, it is difficult to arrive at a better understanding of the reactivity pattern in this group. If the SPC were less specific than the FA, it might be expected that this would also be noted with specimens of noninfected individuals. However, Table I shows that this was not the

TABLE III. Results Obtained with SPC Test on Serum Bank Specimens.

Diagnostic status	No. of specimens	Results of SPC			Source
		Pos	Doubt	Neg	
Schistosomiasis	29	27	0	2	Brazil; Puerto Rico
Trypanosomiasis	4	0	0	4	Uganda
Onchocerciasis	4	0	0	4	"
Syphilis	22	0	0	22	USA
Trichinosis*	12	8	0	4	"
Healthy	22	0	0	22	"
Filariasis	7	6	0	1	Pernambuco, Brazil
Leishmaniasis	2	1	0	1	Minas Gerais, "

* Biologic false positive reactor with *Schistosoma* antigens.

case. It is possible that qualitative differences between plasma and serum may be responsible for this apparent discrepancy. In other words, whereas in areas of very high endemicity such as Nelspruit and Mwanza, the antibody patterns may be such as to be detected equally well by tests on plasma and serum, in areas of lower endemicity such as in Arusha Chini, some people may have a low reactivity which is detectable by testing the plasma and not the serum. This possibility has been pointed out by Portnoy, *et al.*(5) when comparing tests employing plasma and serum among individuals which they defined as "cluster untreated" *i.e.*, individuals who have been suspected to be related to persons with known infectious syphilis.

One of the main drawbacks of this test is that several samples can be read only with some difficulty, since an atypical pattern of clumping occurs in the cards after 4 minutes. These reactions were read as doubtful and the percentage of them decreased as the person performing the tests acquired a greater degree of experience. However, to minimize the occurrence of doubtful reactions it is necessary to take strict precautions to avoid the deposition of dust particles on the cards during the test, the transferring of minute clumps of cells from the Brewer Plasma Collection slide onto the diagnostic card, and the shaking of cards beyond the 4-minute limit, when the plasma-antigen mixture has increased in viscosity due to evaporation.

Summary and conclusions. Use of a schistosomiasis card test as an indicator of antibody is described. This test is performed by using a device for obtaining plasma from 3 drops of finger blood in a rapid and simple manner, a stable antigen suspension containing charcoal, and a plastic coated card surface. Preliminary findings contained in this report suggest that the SPC test possesses adequate sensitivity and specificity. Because of the economy, rapidity and simplicity of this test, and the fact that it provides a permanent record for future reference, the procedure may be particularly useful as a screening procedure for field epidemiologic investigations.

The authors wish to express their appreciation to Drs. J. H. Brewer and G. S. Warner, Hynson, Westcott & Dunning, Inc., for suggestions which led to development of the technic and for providing generous supplies of Brewer's Plasma Collection slides and diagnostic cards. Thanks are due to Dr. N. Ansari, Chief, Parasitic Diseases Section, Division of Communicable Diseases, World Health Organization, for interest, encouragement, and support of the field evaluation. Without the assistance and cooperation of the following individuals the field tests would not have been possible: Dr. J. H. S. Gear, Director of So. African Inst. for Med. Research, Johannesburg; Dr. R. J. Pitchford, Officer in Charge, Bilharzia Field Unit, Council for Scientific and Industrial Research, Nelspruit; Dr. P. Jordan, East African Inst. for Med. Research, EASCO, Mwanza; Dr. I. Hansen, Tanganyika Planting Co., Arusha Chini; Dr. Guido Agostinucci, Director of Laboratories, Clinica Dermatologia dell'Annunizata, Rome.

1. Sadun, E. H., Williams, J. S., Anderson, R. I., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 289.

2. Anderson, R. I., Sadun, E. H., Williams, J. S., *Exp. Parasit.*, 1961a, v11, 111.

3. Sadun, E. H., Anderson, R. I., Williams, J. S., *ibid.*, 1961, v11, 117.

4. Brewer, J. H., Harris, A., presented at meeting of Maryland Branch, Am. Soc. for Microbiol., April 1962.

5. Portnoy, J., Brewer, J. H., Harris, A., *Public Health Reports*, 1962, v77, 645.

6. Anderson, R. I., *Am. J. Trop. Med. and Hyg.*, 1960, v9, 299.

7. Liu, C., Bang, F. B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 68.

Received November 12, 1962. P.S.E.B.M., 1963, v112.