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NOTES

Comparison of the Complement Fixation Test and Counterelectrophoresis Test for the Detection of Antibodies in Chagas Disease

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Sera from 452 patients and blood donors were tested for antibodies to *Trypanosoma cruzi*. The complement fixation test and a counterelectrophoresis test were used. The results showed that both tests agreed in 92% of the sera. However, the counterelectrophoresis test was not as sensitive as the complement fixation test.

The recovery of *Trypanosoma cruzi* from blood is easily accomplished during the acute phase of Chagas disease but is very difficult during the chronic phase of the disease (6). Therefore, serological tests, especially the Machado-Guerreiro complement fixation test (CFT), are the main laboratory tests used in the diagnosis of chronic Chagas disease. The CFT employs, as an antigen, an alcohol extract of the cultured forms of the trypanosome (2). Cross-reactions have been reported with sera from patients with leishmaniasis, malaria, and leprosy (8). In addition, the many technical difficulties encountered in performing the CFT are limiting factors for its use for mass screening of sera. Other diagnostic tests that have been employed in detecting *T. cruzi* infections are the indirect fluorescent-antibody test (4), hemagglutination tests (1), and the direct agglutination test (1). This investigation was conducted to compare the CFT with a new simple test that uses counterelectrophoresis (CEP): the Chagen CEP system (Hyland, Cosa Mesa, Calif. 92626).

Sera were obtained from 392 patients with and without suspected Chagas disease and from 60 blood donors at the Professor Egard Santos Hospital, Federal Medical School of Bahia, and the Fundacao Goncalo Moniz of Salvadore, Brazil. There was not enough information available to determine how many of the 392 patients had clinical manifestation of Chagas disease. The CEP test was performed with the Chagen CEP system. Electrophoresis was performed at 50 mA for 1 h with agarose plates containing paired wells sufficient in number for 22 specimens and 1 control, plus the test antigen. The antigen, predominently the tryptomastigote of *T. cruzi*, was prepared by exposing the organisms to ultrasound and lyophilizing the resulting cell extract. The test antigen was rehydrated by adding distilled water. Studies with the rehydrated antigen showed that it retained its antigenic properties for at least 10 days at −4 C. After electrophoresis, the plates were immediately rinsed with distilled water, incubated for 18 h at room temperature, and then examined for the presence or absence of bands of precipitate between the antigen and serum wells. The CFT was performed by standard techniques (2).

Figure 1 shows a positive and a negative re-

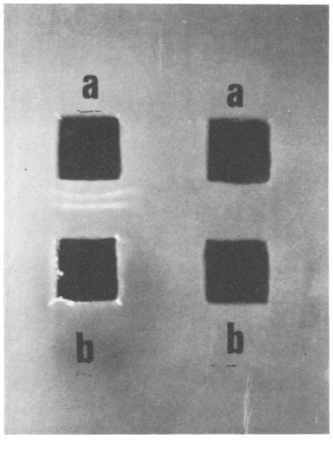

FIG. 1. *Positive and negative CEP reactions. On the left is a positive reaction. Many positive sera produced more than one band of precipitate. On the right is a negative reaction. Antigen (a), serum (b).*

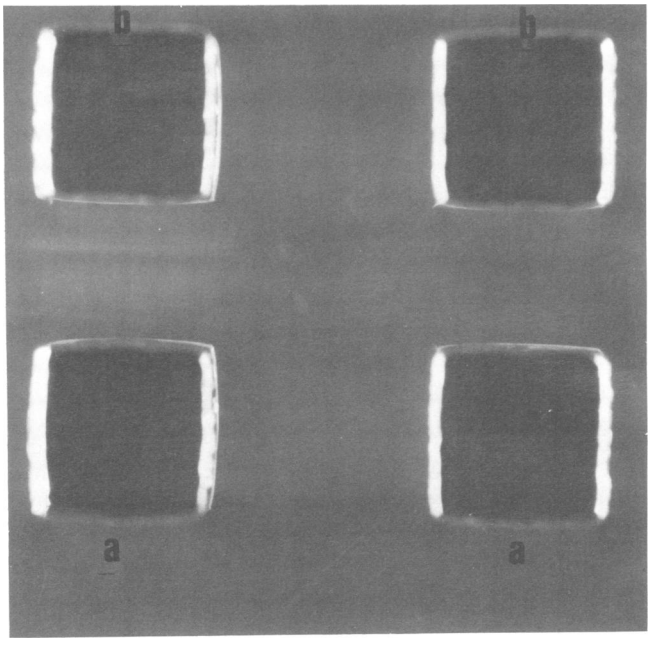

FIG. 2. *Typical false-positive reaction (on the left). On the right is the same serum retested after heating at 56 C for 30 min. Antigen (a), serum (b).*

action by CEP. It should be noted that many sera produced more than one band of precipitate, as illustrated in this figure. Figure 2 shows the nonspecific precipitates that form in the gel. These reactions were fairly common but could be distinguished from the specific reaction by the haziness and increased breadth and curvature of the precipitates formed. The false-positive reactions could also be eliminated by heating the serum at 56 C for 30 min.

Table 1 presents the data obtained on 452 sera. It is evident from the data in Table 1 that the two tests gave the same results on the majority of the specimens tested (92%). Comparison of all sera that produced a positive result with either or both tests (103) shows that CEP detected only 79 (77%) of the positive sera, compared with 98 (95%) with the CFT. CEP tests were reproducible: the 103 sera were retested with the same results.

The meaning of a positive CEP test and a negative CFT is not known. Some patients with demonstrated leishmanial bodies in their mycocardia have been reported to have a negative CFT (5). The positive CFT and a negative CEP test may indicate that the CFT has a greater sensitivity than the CEP tests or that some of the positive CFTs are false-positive reactions due to other diseases (e.g., leishmaniasis, malaria, or leprosy). Knierim (4) found that 3.4% of patients with a positive xenodiagnosis had a negative CFT, which she believed represented acute disease, before antibodies were produced. In her group of "normal" control sera, 2% produced positive CFTs and an additional 5% gave suspicious results. She did not explain these reactions. Allain and Kagan (1) found that 20% of normal sera had low-titer antibody and that patients with *Leish-*

TABLE 1. *Comparison of the CEP test and CFT in detecting serum antibodies in Chagas disease*

Test results	No. of sera	%
CFT positive, CEP positive	74	16.4
CFT negative, CEP negative	341	75.4
CFT positive, CEP negative	24	5.3
CFT negative, CEP positive	4	0.9
CFT unsatisfactory,[a] CEP negative	7	1.5
CFT unsatisfactory,[a] CEP positive	1	0.2
CFT positive, CEP unsatisfactory	0	0.0
CFT negative, CEP unsatisfactory	1	0.2

[a] Anticomplementary or serum contaminated with bacteria.

mania brasiliensis or *L. donovani* infection showed low-titer antibody.

Considering the possibility of false-positive CFTs and the 2% of serum specimens that are anti-complementary and therefore prevent performance of the CFT, it would appear that the CEP test may have an application for mass screening of sera. However, the apparent lower sensitivity of the CEP test would decrease its usefulness as a screening test. Further clinical, pathological correlations must be performed to determine if all patients with *T. cruzi* antibodies are infected with *T. cruzi*.

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