Chagas disease can occur in two phases: acute and chronic. Laboratory diagnosis differs according to the phase suspected. In the acute phase parasitological diagnosis is mandatory, since *Trypanosoma cruzi* is readily detectable in the circulation, particularly during the first month after infection. The first test to apply is the search for the parasite in a wet smear, by a drop of blood between slide and coverslip (22 x 22 mm) observed in a microscope with magnification of 10 x 40. Their quick movements between RBCs are easily seen, after a search for at least 100 fields. When the transmission is by transfusion or when reactivation by immunosuppression is present, parasitemia is even higher, with parasites in each field. This technique is extremely simple and inexpensive but requires some experience of the observer, as well as the clinical suspicion, which must exist in endemic regions in all cases with prolonged fever. Nowadays micro-epidemics of oral transmission by contaminated foods, where several people from the same family or community presented simultaneously with fever of several days, without diagnosis, are frequent. A simple examination of fresh blood can resolve the diagnosis. Some of these cases may have rapid evolution and lead to death. Other symptoms, such as the digestive, unusual in other transmission mechanisms, may be found.

During the late acute phase, after the first four weeks, and up to 60 days, if there is clinical suspicion, methods of concentration of the parasite should be employed. Strout method is economic and easy to implement, requiring only two centrifugations. Micro-hematocrit is indicated when small volumes of blood are available, as suspicion of congenital transmission. In this case one can identify the movements of the parasite in the plasma / erythrocyte interface in the capillary under a microscope.

Chronic phase: is found in the majority of those infected with *T. cruzi*. In them, the parasitemia has decreased to the point that they are not circulating parasites, which may remain in their intracellular cycle for days or months. For that reason, even parasite multiplication techniques, as the classic blood culture or xenodiagnosis, or the called molecular as PCR, may be negative, which does not mean that there is no infection with *T. cruzi*. Using classical techniques, positivity is obtained in 20-30% of those infected, when a single collection of blood is performed. If repeated, it may reach up to 40-50% positivity. For these reasons, you should not apply parasitological tests for diagnosing chronic phase of infection, except in special situations, as in research, when monitoring treated patients, a subject that is found in other chapters.

The diagnosis of *T. cruzi* infection to confirm (or exclude) the infection during the chronic phase, is performed by requesting serology. Search for antibodies against *T. cruzi* is usually done by the so called conventional tests (indirect hemagglutination (IHA), indirect immunofluorescence (IIF) and the ELISA immunoenzymatic test). For a correct diagnosis the World Health Organization recommends that at least two of these three techniques are performed. The results obtained may be classified as concordant, discordant or inconclusive. In over 95% of cases it is possible to obtain a matching result, that is, two reactions with positive results, where the infection is confirmed or both negative, when the infection is not present.

In a minority of sera, one test is positive and the other negative, characterizing discordant result. Cross-reactions, particularly with leishmaniasis (IIF generally positive) yield this type of result. In another small proportion both results are inconclusive (gray zone). This rare situation may be observed when there is a change in the natural history of infection in certain individuals. This could be physiological or induced by treatment. In the first circumstance, physiological, is the case of an infant born to an infected mother, at 2-4 months of the nativity, without transmission of the parasite, where maternal antibodies (IgG) received by passive transmission, are being depleted by course of the time and then disappear, about 6 to 8 months. A second blood collection, after six months will show the disappearance of these antibodies, characterizing absence of infection. In the second case, an infected individual treated with chemotherapeutic drugs to kill the parasite (see paper on this issue) may cure. In this case, there is a period between the positivity in both tests and negativity, where antibody concentrations have fallen to
such a point that tests show ambiguity and fall into the gray area. This situation is often referred to as "in the way to demonstration of cure" (Rassi A). Non conventional tests have been tested and some used in routine diagnosis. Recombinant antigens, purified molecules and synthetic peptides in different supports as polymers, gels, membranes and traditional ELISA format, have been employed. Rapid tests have also been developed. All them should be tested with a sizeable number of sera, in order to be introduced in the routine. In a recent study with 11 trades of rapid tests, some prove to be good and others not. For diagnosis, it is recommended to use two tests, and one of them should be a conventional one.

**REFERENCES**


