Xenodiagnosis

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Xenodiagnosis (XD) is a sophisticated procedure – developed by Brumpt in 1914 – which utilizes the vector, acting as a biological culture medium, for the detection of *Trypanosoma cruzi* infection in man and other mammals. In the XD non-infected laboratory reared nymphs of triatomines are used. These nymphs are fed on birds (mostly chicken) which are normally refractory to the parasite. The XD, constituted by a cylindric pot, made of wood or other similar suitable material, covered with a piece of gauze, containing 7-10 nymphs III of triatomines unfed in the previous 3-4 weeks, supported by a bracelet, is applied, during 20-30 min, to the skin surface (upper limb) of the individual to be examined. After this time the nymphs become engorged and it is advisable to keep them in entomological laboratory conditions. After about 30 days, excreta (feces and/or urine) of the insects are microscopically examined for moving *T. cruzi* trypomastigotes.

Since its introduction numerous studies on the use of XD have been carried out in several countries of the American Continent, particularly in those where Chagas disease is a major medical and public health problem. These studies – many of them ingenuous and creative – have dealt with diverse parameters of XD and can be grouped as follows:

**METHODOLOGY, STANDARIZATION OF TECHNICAL AND OPERATIONAL ASPECTS**

Since the 30’s an uninterrupted series of valuable contributions has been performed. These contributions have stressed on different aspects of it: (a) *generalities* (Dias 1934a, 1940, Mazza 1938, Talice 1944, Schenone et al. 1968a, Salgado 1969, Cerisola et al. 1974, Neal & Miles 1977, Pereira VL et al. 1989); (b) *quality and number of XD kits and species, instar and number of insects to be used*: according to the best of available information there have been neither uniform criteria for quality and number of kits nor for the instars and number of bugs to be used and duration of its application. Thus, a XD unit, considered as routine or natural XD, in summary, has consisted in one kit (one unit), commonly a cylindric pot of varied material, holding 5-10 nymphs of laboratory reared local triatomine species, applied on the skin surface of the individual for about 30 min. Time for the reading has not been well determined. In most of occasions the results have not been satisfactory in suspected cases of Chagas disease, particularly in chronic infections. Facing this situation, arose the idea of performing seriate XD [Dias (1940) practiced seriate XD in dogs infected with Venezuelan *T. cruzi* samples] similar to the techniques employed in other parasitic infections. In this way, we started using pairs of kits containing 7-10 nymphs III of *Triatoma infestans* starved for a period of 3-4 weeks; each pair of kits was applied during 25-30 min to each of both arms for three consecutive days, making a total of six kits with a total of 42 bugs. Routinely the triatomines are examined 30, 60 and 90 days after XD application. When this method was checked in a group of patients with chronic infection, demonstrated by positive serology, sensitivity obtained was 46.1% with one kit (one unit), 54.7% with one pair (two units), 61.8% with two pairs (four units) and 69.1% with three pairs (six units) (Schenone et al. 1974, 1991). To each of 109 children 0-10 year-old, with positive serology for *T. cruzi* infection who were going to receive specific treatment, a pair of XD kits was applied resulting 65 (59.6%) positive, figures that increased to 82 (75.2%) when an additional pair of kits was applied to those who had resulted negative (Schenone 1998). Several authors consider that one XD consists in the simultaneous application of 40 triatomine nymphs distributed into four containers with batches of 10 insects each, method with a good yield, but for us, consisting in the use of four XD kits simultaneously (Pereira JB et al. 1989, 1996, Pereira VL et al. 1989, Chiari et al. 1989, Coura et al. 1991, Menezes et al. 1992, Medeiros et al. 1994).
instance, authors have referred to one XD with 90 insects and one XD with 120 insects (Bronfen & Alvarenga 1991); (c) blood ingestion and mortality of insects utilized in XD: it is necessary to consider that not all the nymphs used in the XD are going to produce suitable material for the microscopical detection of T. cruzi because a proportion of them does not suck blood when are applied on the skin of patients, and another proportion dies during the laboratory maintenance periods before examinations, proportions that, in average, reach to 20.5 and 7.3% respectively (Bronfen & Alvarenga 1991). In an accurate experiment comprising 592 nymphs III of T. infestans utilized in 30 pairs of XD kits applied during 15 consecutive days (noon and midnight) to a chronic infected patient with a permanent high parasitemia, cumulative mortality of bugs observed was 0.0, 19.4, 51.9 and 87% in examinations effected 30, 60, 90 and 120 days after applications (Schenone et al. 1968a, Bronfen et al. 1989); (d) artificial XD: due to the fact that some patients present bug bite skin reaction (Mott et al. 1980). This was the reason for the introduction of this technique which has been successfully developed (Romãna & Gil 1947, Nussenzweig & Sonntag 1953, Cedillos et al. 1982b, Silva 1991). According to Santos et al. (1995), it has a higher positivity than routine or natural method. Particularly interesting is the post mortem artificial XD performed in blood collected from corpses during autopsy, with a preliminary 30% positivity in individuals whose serological tests for Chagas disease – performed previously to XD – were positive (Lopes et al. 1986); (e) technique for obtaining the substratum to be examined: abdominal compression (Schenone et al. 1968a, 1969, 1974, Pereira JB et al. 1989, Pereira VL et al. 1989, Coura et al. 1991, Silva et al. 1995); intestine dissection, grinding and homogenization (Perlowagora-Szumlewicz et al. 1982, Bronfen et al. 1989, Bronfen & Alvarenga 1991, Menezes, 1992); liquefaction of the whole insect, filtration of resultant material through cotton, and centrifugation (Maekelt, 1964, Rohwedder et al. 1970, Cedillos et al. 1982a); examination of contents: individual (Cedillos et al. 1982a, Pereira JB et al. 1989, Bronfen & Alvarenga 1991, Coura et al. 1991, Menezes et al. 1992) and pooled (Schenone et al. 1968a, Bronfen et al. 1989); (f) apparently sensitivity of XD can be improved by the so-called xenoculture which consists in sowing the intestinal contents of the triatomine nymphs used in it in a modified LIT medium (Bronfen et al. 1989); (g) reading: must be done by well trained and skilled personnel. It is highly advisable to have in mind the eventual finding of T. rangeli and/or Blastocritidia triatomae in the intestinal contents of triatomines (Cerisola et al. 1971a, Cedillos et al. 1982a); (h) interpretation of results: a positive XD in a suspected individual (clinical picture, positive serology and/or epidemiological antecedents) means T. cruzi infection and may confirm a chagasic etiology, but a negative XD no necessarily indicates absence of the parasite and the need of repeating the exam (Freitas 1952, Castro et al. 1983).

**SELECTION OF PATIENTS FOR SPECIFIC TREATMENT AND ITS EVALUATION**

Since the 70s XD has systematically been used as a reliable tool for the selection and evaluation of patients who have received specific chemotherapy for Chagas disease (Schenone et al. 1970, Cançado et al. 1973, Cerisola et al. 1977, Pereira VL et al. 1989, Schenone 1998).

**SEARCHING FOR THE MOST EFFECTIVE TRIATOMINE SPECIES**

With the aim of finding the ideal triatome for XD, different groups of investigators have undertaken trials with this purpose. Cerisola et al. (1971b) in Argentina, working with three chronic cases of Chagas disease, with reiterated positive previous XD, tested on them six species of triatomines from different countries from Central and South America and found a high positivity in T. infestans and T. pallidipennis, low positivity in Rhodnius prolixus and no positivity in T. dimidiata, Panstrongylus herreri and R. palescens. In 1996, Pereira JB et al. applied 563 XD to 563 chronic chagasic patients, between 6 and 89 years of age, from different areas of Brazil. Each XD was constituted by four boxes containing a total of 20 nymphs IV of P. megistus and 20 nymphs IV of T. infestans. The results showed that 205 (36.4%) were positive, composed by 85 (15.1%) due only to P. megistus nymphs, 44 (7.8%) to T. infestans nymphs and 76 (13.5%) to nymphs of both species, giving in consequence a total higher positivity of 28.6% in P. megistus against 21.7% in T. infestans. Perlowagora-Szumlewicz et al. (1982) working with 13 guinea-pigs with laboratory acute infection by Y strain of T. cruzi, submitted them to XD containing nymphs IV of nine species of triatomines, obtained the following rates of positivity in the corresponding bugs: P. megistus 97.8%, T. rubrovaria 95%, T. pseudomaculata 94.3%, R. neglectus 93.8%, T. sordida 84.3%, T. brasiliensis 76.9%, R. prolixus 53.1%, T. infestans 51.6% and T. dimidiata 38.2%.
**Dipetalogaster maximus**, the higher known triatomine, native from Mexico with a restricted geographical distribution in a section of Baja California – a non traditional bug for XD – became a very promising biological resource for the detection of *T. cruzi* in infected individuals. Studies carried out in Brazil by Cuba et al. (1978), Barretto et al. (1978) and Marsden et al. (1979) in significant groups of patients with chronic infection have demonstrated that nymphs IV and V of *D. maximus* are more susceptible to *T. cruzi* than the same instars of *T. infestans*, and that nymph I of *D. maximus* is as sensitive as nymph III of *T. infestans* in detecting subpatent parasitemia. On the other side, Bronfen et al. (1989) observed that nymphs III of *T. infestans* are rather more sensitive than nymphs I of *D. maximus* and that nymphs of *D. maximus* have the practical advantage of reducing the number of insects required for XD.

**EVALUATION OF PARASITEMIA, ITS RELATIONSHIP WITH CLINICAL ASPECTS AND EVOLUTION OF CHAGAS DISEASE AND ISOLATION OF STRAINS OF T. CRUZI**

Determination of parasitemia in pre-treatment trials of chronic chagasic patients permits quantification and estimates of its levels in different clinical situations and the changes through the years (Castro et al. 1983, Contreras et al. 1988, Bronfen et al. 1989, Bronfen & Alvarenga 1991, Menezes et al. 1992, Medeiros et al. 1994, Schenone et al. 1995). XD also makes possible the isolation of *T. cruzi* strains from different infected patients (Bronfen et al. 1989, Miranda et al. 1994). Pereira JB et al. (1989) proposed a classification of parasitemia based on the rate of positiveness of nymphs: I. Not detected (0%). II. Low (< 2%). III. Medium (2.1-7%). IV. High (> 7%). High parasitemia was more frequent in patients with chagasic cardiopathy. Persistent parasitemia was seen in 100% of patients of group IV, in 22.2% of group III and in none of group II. In one chronic chagasic infected patient with previous demonstration of high presence of *T. cruzi* – 67 (93.1%) out of 72 XD practiced – two series of 30 kits of XD (with 10 *T. infestans* nymphs III each) were applied at noon and midnight during 15 consecutive days, there was no circadian variation of parasitemia evaluated by the number of positive XD, 93.3% in each series (Schenone et al. 1977).

**COMPARISON OF ITS SENSITIVITY WITH OTHER PROCEDURES FOR THE DETECTION OF T. CRUZI IN THE BLOOD STREAM**

As parasitological diagnosis has a higher complexity in chronic infection than in acute infections, description will be stressed on the first.

XD performed in Chile demonstrated a positivity of 80.3, 86.4 and 49.3% in congenital, acute and chronic infections respectively (Schenone et al. 1969, 1974).

Fifty nine chronic infected patients with positive serology were simultaneously submitted to XD – with 40 nymphs of *P. megistus, T. infestans* and *D. maximus* – and hemoculture in LIT medium, resulting positive XD in 23.7, 32.2 and 42.4% with one, two and three species respectively, and hemoculture in 24.1%; these figures increased to 30.5, 33.9 and 49.4% when, according to Chiari and Brener (1966) recommendations, positivity of XD and hemoculture were combined (Bronfen et al. 1989).

In other study which included 39 chronic infected chagasic patients were examined a the same time by using XD containing 40 nymphs of *T. infestans* and hemoculture in LIT medium, results showed a positivity of 36.1 and 19.4% for each of the techniques used (Pereira VL et al. 1989).

Chiari et al. (1989) performed a study with chronic infected patients with positive serology for Chagas disease, in which XD with 40 nymphs III of *T. infestans* and hemoculture were practiced being the corresponding positivity found of 27.6 and 55.2%.

In a field survey carried out in five counties of the state of São Paulo (Brazil) by using four techniques for the detection of blood flagellates – fresh smear, stained smear, hemoculture and XD nymphs III and IV of *T. infestans, R. prolixus* and *R. neglectus* varying in number from 3-5 to 25-30 according to the size of the animal – 22 mammals out of 440 animals tested were found infected with *T. cruzi*. In these mammals (21 marsupials and one carnivore) the positivity of each technique was two fresh smears, two stained smears, four hemocultures and 22 XD (Tolezano et al. 1989).

In an experimental study with laboratory acute and chronic *T. cruzi* infected mice, hemoculture in Warren’s medium and XD with *R. prolixus* nymphs III and IV, were carried out resulting a positivity of 93 and 36% in animals with acute infection and of 31 and 14% in chronically infected animals. The authors concluded that hemoculture in Warren’s medium has shown that overall blood culture is superior to XD for the detection of *T. cruzi*, being the method of choice in the acute stage of the infection, but when the infection is in the chronic phase, the use of both methods is required to give the maximum sensitivity for detection of *T. cruzi* (Neal & Miles 1977).

**RELATIONSHIP BETWEEN CYCLE OF THE PARASITE AND THE VECTOR**

Different approaches have been studied in considering cycle of *T. cruzi* and vectors (Dias 1934, Wood 1960, Zeledon 1974, Coura 1975, Schenone et al. 1977, Bronfen et al. 1984, Alvarenga &

**Epidemiological Surveys**

By the use of XD as a tool, interesting studies have been carried out (Dias 1935, Neghme & Schenone 1962, Schenone et al. 1968b, Coura et al. 1991).

**Remarks**

XD is a diagnostic procedure which uses the vector which acts as a biological culture medium for the detection of *T. cruzi* in the blood of infected man and other mammals.

Routine or natural XD consists in the use of one cylindrical pot containing 7-10 nymphs of unfed triatomin bugs which suck blood from the skin of the individual to be examined.

After an incubation period, the abdominal contents of the utilized nymphs are examined by means of different techniques (compression of the abdomen, dissection, grinding and homogenization of the intestine, and liquefaction of the whole insect).

The yield of XD increase in direct proportion with the number of kits (units) used, for this reason seriate XD is recommended.

XD has been considered a good tool for etiological Chagas disease diagnosis, both for selection of patients for specific therapy and for its evaluation.

It has been shown useful for evaluation of parasitemia and its relationship with clinical conditions of Chagas disease.

Even though there are other efficient procedures for the detection of *T. cruzi*, XD considered an efficient diagnosis method for *T. cruzi* in the blood stream, particularly useful in chronic chagasic infection.

**References**


Schenone H, Alfaro E, Reyes H, Taucher E 1968a. Valor...


