Screening and Confirmation in Chagas Disease Serology - A Contribution

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In recent years, our group reported the development of two enzyme-linked immunoassays utilizing synthetic peptides (Peralta et al. 1994) or epimastigote lysates (Teixeira et al. 1994) as antigens, and the evaluation of commercial tests designed for either screening (Oelemann et al. 1998) or confirmation (Oelemann et al. 1999) in Chagas disease serology.

In order to develop an Elisa for the specific detection of anti-*Trypanosoma cruzi* antibodies (Peralta et al. 1994), two peptides, TcD and PEP2, were used either separately or in combination. The Elisa was evaluated employing a panel of 260 sera obtained from chagasic patients (n=179) and healthy residents (n=81) living in Virgem da Lapa, Minas Gerais, where Chagas disease is endemic. In addition, 37 sera obtained from patients with other pathologies, such as leprosy, tuberculosis and leishmaniasis, were employed to assess specificity. The results showed that each peptide alone presented a sensitivity of 93.8% and 91.6% (TcD and PEP2, respectively), whereas the combined peptides showed a sensitivity of 99.4%. The respective specificities were reported to be 93%, 91% and 98.7% (Peralta et al. 1994).

In another study (Teixeira et al. 1994) we developed a protocol for a *T. cruzi*-specific Western blot using the cytosolic fraction of strain Y epimastigotes as antigen. The test was evaluated with a slightly smaller panel of sera derived from the one described above (i.e. 160 from chagasic patients, 75 from healthy individuals residing in the same area, and 37 from patients with unrelated diseases). A reaction was considered positive when at least three out of seven antigen bands (14, 19, 27, 30, 34 and 75 kDa) were present. The result was indeterminate with the appearance of only two bands, and negative with only one or no band. Using this algorithm, the test showed a sensitivity of 99.3% and a specificity of 100% (Teixeira et al. 1994).

In order to assess the diagnostic performance of commercially available screening tests we evaluated in a next step (Oelemann et al. 1998) the Abbott Chagas antibody EIA, the Biolab BIOELISACRUZI® and the BIOZIMA Chagas kit. The sera employed were obtained from patients and healthy residents of four Brazilian areas where Chagas disease is either endemic or emergent and where clinical manifestations of the disease and circulating *T. cruzi* strains vary. All three tests employ antigen preparations of *T. cruzi* strain Y obtained by different protocols. The results obtained with each kit were compared to matched in-house Elisa (using the cytosolic fraction of *T. cruzi* strain Y as antigen) and immunofluorescence data. Thus, we employed 180 positive and 81 negative sera obtained in Virgem da Lapa, Minas Gerais (see above), where the cardiac and digestive forms of the disease are common; 135 positive and 305 negative sera from Paraíba and 202 positive and 44 negative sera from Piauí where the indeterminate form of the disease is common; and 3 positive and 75 negative sera obtained in the Amazon region where Chagas disease is emergent. The complete panel consisted of 520 positive and 505 negative sera. Our results demonstrated that depending on the area the three commercial kits produced specificity values between 93.3 and 100%, sensitivity values between 97.7 and 100% and accuracies between 93.6 and 100%, with none of the kits attaining 100% in all three categories (Oelemann et al. 1998).

These findings emphasize the need of a more specific and accurate test for confirmation, which should be based on cloned antigens or synthetic peptides rather than crude parasite fractions.

A good candidate for a confirmatory test is the INNO-LIA Chagas Ab. This novel line immunoassay combines several relevant immunodominant recombinant and synthetic antigens coated in separate locations onto a single nylon membrane strip.

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This physical separation of the different antigens prevents sterical hindrance during antibody binding which may be a problem if a mixture of the antigens is used in a single well of an Elisa plate. In an optimization step (Oelemann et al. 1999) we employed 1,062 sera from patients and healthy residents of the four above-mentioned areas and compared the results obtained with the INNO-LIA Chagas Ab to those obtained with four screening tests: our in-house Elisa and the three commercial assays (see above). The data was used to establish a confirmation algorithm, which was subsequently validated with an independent panel of well-characterized samples (75 positive and 148 negative for Chagas disease). The INNO-LIA Chagas Ab showed a sensitivity of 100% and a specificity of 99.32%. Furthermore, the assay was 100% specific when evaluated with sera from 40 patients with confirmed leishmaniasis (Oelemann et al. 1999).

Today, transfusion of blood from infected donors constitutes the major route of contracting Chagas disease and an annual number of 20,000 transfusion-acquired Chagas disease cases in a total of 5 to 6 million blood transfusions have been reported for Brazil (Dias 1992). In Brazilian blood banks the screening of donors for Chagas disease by at least two tests based on different methodologies is obligatory. For donors who are positive in only one test the result is usually confirmed by a third test. However, in practice, the blood bags are discharged even if they give a negative result in this confirmatory test. Therefore, to avoid unnecessary losses of blood, the negative result obtained in an ideal test for confirmation must be sufficiently convincing to overcome a single positive result obtained in one of the screening test. In our opinion this can only be achieved by employing in an individual test as many immunodominant antigens as possible. The confirmatory test described above certainly represents an example for a new line of immunoassays, thus showing the way to go.

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