Assessment of chemiluminescence and PCR effectiveness in relation to conventional serological tests for the diagnosis of Chagas’ disease

Avaliação da eficiência da quimiluminescência e PCR em relação aos testes sorológicos convencionais para o diagnóstico da doença de Chagas

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ABSTRACT

While testing 414 sera for the diagnosis of Chagas’ disease, the conventional reactions of indirect hemagglutination, indirect immunofluorescence and the immunosorbent assay showed a sensitivity of 95.7%, 100% and 98.2% and a specificity of 98%, 98% and 96.4%, respectively, and an excellent association using Fisher’s exact test. Chemiluminescence presented 100% sensitivity and 89.6% specificity, while PCR showed 100% specificity and 1.2% sensitivity. It is believed that the three conventional serological reactions are still adequate for diagnosing Chagas’ disease.

Key-words: Chagas’ disease. Diagnosis. Serology. Chemiluminescence. PCR.

RESUMO

No exame de 414 soros, para o diagnóstico da doença de Chagas, as reações convencionais de hemaglutinação indireta, imunofluorescência indireta e o ensaio imunoenzimático mostraram, respectivamente, uma sensibilidade de 95,7%, 100% e 98,2% e uma especificidade de 98%, 98% e 96,4% e excelente associação usando teste exato de Fisher. A quimioluminescência apresentou 100% de sensibilidade, 89,6% de especificidade e a PCR 100% de especificidade e 1,2% de sensibilidade. Acredita-se que as três reações sorológicas convencionais ainda são suficientes para o diagnóstico da doença de Chagas.


Chagas’ disease or American trypanosomiasis is an endemic infection of gradual evolution caused by the intracellular protozoa Trypanosoma cruzi. Diagnosis of chagasic patients has been made through parasitological and/or serological methods like indirect hemagglutination (IH), indirect immunofluorescence (IFA) and indirect enzyme-linked immunosorbent assay (ELISA). Other reactions such as chemiluminescence¹, agglutination in gel¹², lysis mediated by complement¹³ and trans-sialidase inhibition¹⁰ can be used. Chemiluminescence has not, however, been used for the diagnosis of Chagas’ disease as it is still being standardized.

Current legislation¹¹ recommends screening tests for Chagas’ disease in blood banks, but not in laboratories, using at least two methods with different methodological principles due to the false-positive and false-negative reactions found in different methodologies. IH, IFA and ELISA are considered typical reactions and are currently used. Technical scientific advances have made new tests and methods available. The suggestion to evaluate chemiluminescence and PCR as complementary tests for introduction in laboratory routines for the diagnosis of Chagas’ disease, as well as their comparison with conventional methodologies is relevant, because of the need to find out precisely what their specificity, sensitivity and efficiency are. These three aspects are of major importance as they reflect not only on the results, but also on the prospect of clarifying doubts that have been raised by
cross-reactions with antibodies from carriers of chronic diseases and also the confirmation of indeterminate results obtained in conventional serological reactions.

A total of 414 volunteers, male and female, aged between 10 and 80 years old, residents of urban and rural areas, took part in this study, which was conducted between October 2000 and January 2002. These individuals came from the municipalities of Itabira (n = 82), ferros (n = 229) and carbonita (n = 103) in the state of Minas Gerais, Brazil. Ninety-nine individuals had characteristic symptoms of the infection, such as cardiode digestive alterations (n = 79), digestive (n = 15) and cardiodigestive alterations (n = 5), in addition to being seropositive. Thirty-three volunteers showed no symptoms, even though they were seropositive, and eighteen experienced no symptoms and their sera were classified as indeterminate. The control group was made up of two hundred and thirty-four healthy seronegative volunteers who exhibited no symptoms of Chagas’ disease and were residents of the same cities as the study group.

All samples were tested by three different serological tests: IFA, IH and ELISA, carried out according to the manufacturer’s instructions (Biolab-Mérieux SA - Rio de Janeiro, Brazil). The patient was classified as positive if at least two tests were positive; negative if three tests results were negative and indeterminate if results did not fit any of the previous criteria. In order to assess reproducibility, 48 (11.6%) samples out of a total of 414 were selected at random. The diagnostic kits were made up of the same as for previous testing. Positive and negative control sera were introduced in all the reactions. Chemiluminescence was performed using the IMMULITE® Chagas IgG commercial kit (DPC-Med-Lab - Brazil) and IMMULITE system (DPC-MedLab). DNA samples from infected individuals were subjected to PCR, with the pair of primers 121 and 122 which amplified a 330pb fragment of the conserved micro region of the parasite T. cruzi kDNA minicircles.

According to the criteria used in this work, it was observed that out of a total surveyed population of 414 individuals 236 (57%) of the samples tested negative, 161 (39%) positive and 17 (4%) were indeterminate. Chemiluminescence presented the highest number of seropositive and indeterminate individuals and the lowest number of seronegative results. In relation to sensitivity, both chemiluminescence and IFA presented the highest (100%) value, but for specificity and efficiency, they presented the lowest values in comparison with conventional methods. Analysis of the Kappa index for the reproducibility of the 48 (11.6%) samples chosen at random showed that all the reactions, IH, IFA and ELISA presented an excellent agreement. For IH, the Kappa index was equal to 1.000 and for IFA and ELISA, 0.908 and 0.905 respectively. The chemiluminescence reaction also demonstrated good agreement with the Kappa index of 0.751 (p < 0.0001).

With respect to PCR, amplification was performed for the 414 blood samples tested in this study. After amplification, out of the 161 samples which had been confirmed positive, two tested positive with a sensitivity value of 1.2% and 100% specificity.

There was a prevalence of negative individuals (57%) over those who were positive (39%). The percentage of indeterminate cases (4%) could be significant when a larger population is taken into account. It is worth highlighting that this indeterminate serological group is a real concern for blood banks. It was observed that the 95.7% sensitivity value found in IH is situated between the values of 91.2% and 97% previously described, whereas the 98% specificity found in this study is inferior to the 100% previously described. No indeterminate reaction was found in the indirect immunofluorescence, presenting 100% sensitivity, 98% specificity and 98.8% efficiency. These results are similar to those of other authors who showed 100% sensitivity and 95% specificity. With respect to ELISA, 244 samples tested negative, 105 positive and 5 indeterminate with a value of 98.2% for sensitivity and 96.4% and 97.1% for specificity and efficiency, respectively. In the present study, chemiluminescence with recombinant antigen was used (DPC-Med-lab kit). Many authors suggest the use of purified antigens of T. cruzi epimastigotes or recombinant antigens in order to reduce cross-reactivity. Chemiluminescence presented the lowest specificity and efficiency: 89.6% and 93.7%, respectively. However, it presented excellent sensitivity: 100%. This can be observed in Table 1. The use of chemiluminescence as a way to diagnosis Chagas’ disease has not been used in many studies. Results of indeterminate cases have not been found by chemiluminescence, contrary to that which can be observed with the IH, IFA and ELISA reactions, possibly due to the use of trypanostigate antigens.

For PCR, only 2 positive results were found in 162 samples of chagasic patients through traditional serology, where specificity for PCR was 100% and sensitivity was 1.2%. Some authors have found low sensitivity through the hot-start technique with positive results below 60%. It has already been demonstrated that PCR is not appropriate as the sole diagnosis method, rather as a complement to serology, particularly in the case of indeterminate patients.

The results obtained in this study show that the three traditional serological reactions are still sufficiently effective for the diagnosis of Chagas’ disease. This is due to the fact that, up to now, no other methodology has been standardized with higher rates than those found for these methods, i.e. sensitivity and specificity close to 100%. Furthermore, it was shown that IFA was the most appropriate reaction for routine diagnoses. Considering this fact, clinical laboratories should be using IFA to clarify doubtful cases, since it showed 100% sensitivity and 98% specificity.

Table 1 - Serological tests for Chagas’ disease and rates of sensitivity (S), specificity (Sp) and efficiency (Ef) in 414 human sera.

<table>
<thead>
<tr>
<th>Serological method</th>
<th>n°</th>
<th>%</th>
<th>n°</th>
<th>%</th>
<th>n°</th>
<th>%</th>
<th>%</th>
<th>%</th>
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<tbody>
<tr>
<td>IH</td>
<td>251</td>
<td>60.6</td>
<td>5</td>
<td>1.2</td>
<td>158</td>
<td>38.2</td>
<td>95.7</td>
<td>98.0</td>
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<tr>
<td>IFA-IgG</td>
<td>245</td>
<td>59.4</td>
<td>0</td>
<td>0.0</td>
<td>169</td>
<td>40.8</td>
<td>100.0</td>
<td>98.0</td>
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<tr>
<td>ELISA</td>
<td>244</td>
<td>58.9</td>
<td>5</td>
<td>1.2</td>
<td>165</td>
<td>39.9</td>
<td>98.2</td>
<td>96.4</td>
</tr>
<tr>
<td>Chemiluminescence</td>
<td>224</td>
<td>54.1</td>
<td>8</td>
<td>1.9</td>
<td>182</td>
<td>44.0</td>
<td>100.0</td>
<td>89.6</td>
</tr>
</tbody>
</table>

IH: indirect hemagglutination technique; IFA: indirect immunofluorescence reaction; ELISA: enzyme-linked immunosorbent assay.
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REFERENCES


