PEROXIDASE ANTIBODY TEST FOR MUCOCUTANEOUS LEISHMANIASIS SEROLOGY.
PERFORMANCE INDEXES AND COMPARISON WITH
A FLUORESCENT ANTIBODY TEST.

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SUMMARY

Performance indexes of the peroxidase antibody test were compared to that of the fluorescent antibody test. The peroxidase antibody test had a statistically higher sensitivity and negative predictive value and a higher efficiency than the fluorescent antibody test but its specificity and positive predictive value were within the 95% confidence limits for the values found for the fluorescent antibody test. Such differences did not change when Chagas' disease and visceral leishmaniasis sera were included in index calculations. Statistical analysis showed that the two tests have a substantial degree of agreement but the immunofluorescent test had a specificity index and a positive predictive value equal to 100.0% when Chagas' disease and visceral leishmaniasis sera were not included in the calculations of the performance index; in this instance, a positive test result equals a disclosure of the disease attribute due to the inexistence of false positive results. The enzyme/protein ratio of the peroxidase conjugate, resulting in heavy or light-labeled conjugates may pose technical problems to its use in serology tests.

KEY WORDS: Mucocutaneous leishmaniasis serology; Performance index; Peroxidase antibody test; Fluorescent antibody test.

INTRODUCTION

The peroxidase antibody test (IP) is considered to be an alternative to fluorescent antibody test (IF) because it does not need a costly equipment such as a fluorescence microscope and requires a lesser degree of training of lab technicians. The test was shown to display essentially the same titers as the ones found by IF tests for the diagnosis of such diseases as Chagas's disease4 or schistosomiasis3. Besides such considerations, for a test to replace another is necessary to investigate performance indexes such as sensitivity, specificity, predictive values, positive and negative and, efficiency in order to assess if the new test will be able to disclose as many true positives and true negatives as the previous one.

The present assessment of the peroxidase antibody test performance indexes was undertaken in order to check if the test could be used as an alternative to the fluorescent antibody test in mucocutaneous leishmaniasis serology.
MATERIALS AND METHODS

ANTIGEN — Seven days old L. major like promastigotes (MHOM/BR/71 49) grown in LIT culture medium were used as the antigen for IP tests. The flagellates were washed twice in 0.01M phosphate-buffered 0.15M NaCl, pH 7.2 (PBS) and fixed in 2% formalin overnight; the cells were washed twice in PBS and suspended in enough PBS and placed onto glass slides as to allow 20-25 promastigotes per microscope field.

SERAS — Ninety two sera were used to standardize the test and comprised leishmaniasis and control sera: in this second category there were sera from non-diseased individuals and sera from non-leishmaniasis individuals. Their number and diagnosis are shown in table 1. Sera were chosen according to a previous assessment of positivity or negativity by a fluorescent antibody test and from epidemiological data indicative or not of exposure to the etiological agent.

PEROXIDASE ANTIBODY TEST — 20 μl of doubling PBS dilutions of each sera were placed onto each microscope slide area. After incubation with serum dilutions the flagellates were incubated with 20 μl of an optimal dilution (1:200) of a goat IgG anti-human immunoglobulin conjugated to horseradish peroxidase (Cappel Lab., Cochranville, Pa., USA) followed by incubation with 20 μl of a solution containing 6 mg of diaminobenzidine, 10 ml of 0.05M Tris-HCl buffer, pH 7.8 and 1 ml of 0.1% H₂O₂ (all chemicals from Sigma Chemical Co., St Louis, Mo, USA). All incubations were carried out at 37°C for 30 minutes in a moist chamber and between each incubation slides were washed twice for 10 minutes each in PBS. After the last step the slides were washed in distilled water for 5 minutes and mounted. Tests were read in a Zeiss binocular optical microscope (Carl Zeiss, Oberkochen, West Germany). In all tests a positive and negative standard sera were included. Titer of each serum was considered as the last dilution to give a brownish-yellow color darker than the color developed in control sera.

FLUORESCENT ANTIBODY TESTS — All sera were submitted to an fluorescent antibody test using anti-IgG fluorescein iso-thiocya-
The specificity index of IP or IF tests (Chagas disease sera and visceral leishmaniasis included) were different from a random test not associated with disease attribute but were equal one to the other, in the sense that they were able to disclose the same "true negative" results, as seen from its 95% confidence limits in table 2. The same line of thought applies to the positive predictive value.

The peroxidase antibody test is more sensitive and has a higher negative predictive value than the fluorescent antibody test regardless of the inclusion of Chagas' disease or visceral leishmaniasis serum titer results or not in the calculation of such indexes, as shown in table 2. Because of this the test presents itself as more able to disclose positives to the test among diseased people than the fluorescent antibody test does; this capacity is defined as sensitivity by GALEN & GAMBINO, 1975.

The indexes for specificity and positive predictive value did not differ for any of the tests, as shown in table 2 by means of the overlapping of the 95% confidence limits; but as mentioned, a positive predictive value of 100% for the IF test with a consequent disclosure of true positives among all individuals displaying a positive test result is what makes the IF a better test than IP.

The statistic used to investigate the agreement between tests has shown that the kappa statistic value changed from 57.0 to 62.4, increasing from a moderate to a substantial strength of agreement between peroxidase antibody test and fluorescent antibody test depending on whether Chagas' disease and visceral leishmaniasis serum sera were included or not in the calculation. In other words, the difference in sensitivity and negative predictive value between tests is not of such magnitude as to overcome the agreement between them.
The peroxidase antibody test has been thought of as a substitute for the fluorescent antibody test because it dispenses with an expensive piece of equipment such as the fluorescent microscope and with specially trained personnel, as a matter of fact such a trial is being conducted in malaria (A. W. Ferreira, personal communication). In the case of mucocutaneous leishmaniasis serology, although the performance indexes accrued indicate that it may so be considered (with the exception mentioned earlier), the substitution may pose some problems depending on the conjugate used to perform IF tests. Although conjugates are used maximally diluted in order to overcome non-specific staining, due to specifics of an enzyme-labeled conjugate, if a heavily marked conjugate is used it may be necessary to include an endogenous peroxidase blocking step to avoid non-specific color development, if a lightly marked conjugate is used, tests may develop a very light color making very difficult to discriminate between positive and negative results. In the first case a more complex test procedure ensues, in the second a skilled technician will be needed making the peroxidase antibody test a less attractive alternative than the fluorescent antibody test. It is to be reminded that persons using antibody labeled conjugate, whether fluorescein or enzyme labeled, do not prepare their own but use the ones commercially available which not always have an optimum labeling agent antibody ratio. But even if a standardized peroxidase conjugate was available the IF test would have the advantage of presenting a maximum positive predictive value.

ACKNOWLEDGEMENTS

This work was supported by PIDE VI, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant 403608/84 and UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, grant 84053.

REFERENCES


Recebido para publicação em 24-6-1988.