Exo-antigens from *Paracoccidioides lutzii* applied to immunodiagnosis of Paracoccidioidomycosis: an report of our experience in Brazil's Midwest

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In central-west region of Brazil, where *Paracoccidioides lutzii* predominates, has been described low reactivity in immunological tests using *Paracoccidioides* standard antigen (AgB-339). Here, we aimed to evaluate the performance of exo-antigens from *P. lutzii* against patient sera from Brazilian central-west region (CWR), and to speculate their influence under PCM immunological diagnose, as well sorological follow-up of these patients. The cell-free culture antigens, known as exo-antigens, were obtained from *P. lutzii* (ATCC MYA-826), defined as PIAg, and proved by double agar-gel immunodiffusion (DID) test against 53 PCM suspect serum samples from CWR. The PCM confirmation was given in 81.13% of cases (43/53) by direct exam, biopsy specimens or culture.

FIGURE 1. Metodological fluxogram of *P. lutzii* exoantigen obtention to use in sorological tests for antibodies anti-PCM detection. The exoantigens species-specific were obtained from *P. lutzii* isolates (ATCCMYA-826).

The obtained Ag-Pl was validated by testing (ID) 43 serum samples from patients with confirmed PCM diagnosis (direct mycological, culture and/or biopsy positive results). All patients were tested using both exoantigens: the standard (Ag-339) and Ag-Pl (obtained in this study). Of these, 60.46% (26/43) was negative when tested with only AgB-339. Although the literature suggests high specificity and sensitivity to DID test (65 to 100%), we able to find only 39.53% (17/43) of reactivity using *Paracoccidioides* standard antigen. In the other hand, this percent increased to 72.09% (31/43), when we added PIAg on assay, highlighting its role to immunodiagnose.

ExoAg-Pl	32.55 % (14/43)
ExoAg-339	9.30 % (4/43)
ExoAg-Pl / ExoAg-339	30.23 % (13/43)
TOTAL (+)	72.09 % (31/43)

FIGURE 2. Venn diagram shows the percentage of ID positivity Mato Grosso do Sul (MS) patients. The figure shows the low positivity when the serum of the patients is tested with the standard antigen (Pb339) and the high positivity when tested with the *P. lutzii* antigen. While, Brazil map presents the midwest region, highlighting the MS state, due to lack of studies confirming the *P. lutzii* prevalence

From 43 PCM patients, 33 (76.74%) were positive on ID, 14 (32.5%) on direct exam, 30 (70 %) on histopathology, while culture had the worst performance with only 4/43 positive patients (9.3%).

FIGURE 3. The graphic shows the frequency of positivity to test applied to screening PCM. The comparison analysis between tests was evaluated by Cochran's Q test. Different letters indicates significant statistical differences between tests (p<0.05)

A comparative performance of double immunodiffusion (ID), direct exam, histopathology and culture from 43 PCM confirmed patients were conducted using Cochran's Q test (**Figure 3**), which revealed Q-value (X^2) of 42.43 considering $Q(3)=7.82$, $p<0.05$, indicating that there is difference in performance of tests applied to PCM screening.

Multiple comparisons two-by-two with critical value ($S\alpha$) of 2.79 indicates statistical difference between proportions of positivity of culture and ID test ($S = 5.49$), histopathology and culture ($S = 5.29$), direct exam and ID test ($S = 3.25$) and direct exam and ID test ($S = 3.46$).

Finally, when we compare the concordances between antigen from *P. lutzii* and *P. brasiliensis* (Pb-339) by ID test using kappa coefficient. Then, we found value of 0.02, reflecting a **weak** agreement.

Therefore, this **data suggest differences in specificity** of ExoAgs obtained from *Paracoccidioides* species

The molecular identification of the clinical isolates are under progress, what will help us to clarify the PCM reactivity profile at Brazilian Midwest region.

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