

TITLE: DETECTION OF IgG AND IgG2 TO ANTIGENS OF *Paracoccidioides lutzii* AND *P. brasiliensis* IN HUMAN SERA: THE INFLUENCE OF CARBOHYDRATES

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ABSTRACT:

As of 2006, there was evidence that some isolates of *Paracoccidioides* could be of a different species and since then, much has been published about *Paracoccidioides lutzii*. The serodiagnosis of Paracoccidioidomycosis (PCM) may be compromised by the use of an inappropriate antigen. Besides, it is known that carbohydrate epitopes may hinder the diagnosis of PCM. Therefore, current research aimed to investigate the influence of carbohydrates in the detection of antibodies against *Paracoccidioides* spp. by ELISA. Plates were coated with 25 µg/mL of cell free antigens (CFA) or 1 µg/mL of high molecular mass antigen (hMM) from *P. lutzii* LDR2 and *P. brasiliensis* B339. After blocking, carbohydrates were oxidated with 10 mM sodium metaperiodate diluted in 50 mM acetate buffer pH 4.5 or just acetate buffer (1 h, in the dark, room temperature) and the reaction was interrupted with 50 mM sodium borohydride diluted in PBS (30 min, in the dark, room temperature). A pool of 39 PCM patients' sera at 1/200 dilution (2 h, 35 °C) was used as primary antibodies and later the secondary antibodies: (A) anti-human IgG-peroxidase labeled antibody (Sigma A-6029, 1/4000, 1.5 h, 35 °C) or (B) unconjugated anti-human IgG2 (Sigma I-9513, 1/2000, 1.5 h, 35 °C) + Biotin/Streptoavidin. The reaction of the substrate o-phenylenediamine (OPD) was interrupted by the addition of 4 N H₂SO₄ after 20 and 30 min for CFA and hMM, respectively, and plates were read at 492 nm. Results were expressed in optical density units (O.D.). T-test was used to compare treated and not treated samples (p-value < 0.05). In addition, 10% SDS-PAGE with CFA was stained for carbohydrates (PAS). Metaperiodate diminished the reaction of antibodies at ratios from 1.1 to 3.5 times. This demonstrates that antibodies from PCM patients' recognize *P. lutzii*'s carbohydrate epitopes, as already described for *P. brasiliensis*, and this may cause false positive results. Only IgG and IgG2 anti-hMM from *P. lutzii* had no statistical differences. Although the gel shows diffuse areas of hMM of both species, reach in carbohydrates, the antibodies against *P. lutzii* detected in ELISA seem to be directed to a protein epitope. In conclusion, ELISA to whole antigens and hMM from B339 is influenced by the presence of carbohydrates. The consequence for the diagnosis of PCM when using *P. lutzii*'s antigens needs to be further investigated.

Keywords: Cell free antigen; High molecular mass antigen; Paracoccidioidomycosis; Serodiagnosis; Systemic mycosis.

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