The Paracoccidioidomycosis is caused by inhalation of the fungus Paracoccidioides brasiliensis (P. brasiliensis, Pb03 and Pb18) or Paracoccidioides lutzii (P. lutzii, Pb01). Currently, diagnosis involves microscopic identification of pathogen in sputum or detection of specific antibodies in serum. However, these techniques present problems such as cross-reactivity, high false negatives or false positives. The highly sensitive, non-cross-reactive methods require many reagents and expensive equipment, such as Enzyme Linked Immunosorbent Assay. Nanotechnology may provide cheaper, sensitive and specific alternative method of medical diagnosis. Among other applications, gold nanoparticles were successfully conjugated with Deoxyribonucleic acid (DNA) probes for complementary DNA sequence detection with promisor results. For diagnostic purpose, the color change from red to blue is determinant, which helps identify the positive and negative tests. The current study aims to detect the presence of P. brasiliensis using gold nanoparticles (22 nm diameters) combined to pathogen specific GP43 DNA sequences. We evaluated around 200 samples, composed by 50 % of positive and 50 % of negative samples, and the tests results achieved 100 % sensitivity and 96 % specificity using this technique. This comparatively less-time consuming and cost effective label-free gold nanoparticles based colorimetric methodology may have great impact on P. brasiliensis molecular identification and diagnosis in remote endemic areas.