

NOVEL ELISA FOR THE DIAGNOSIS OF HISTOPLASMOSIS

Toscanini María Agustina^{1,2*}, Brito Devoto Tomás^{2†}, Iovannitti Cristina², Nusblat Alejandro David¹, Cuestas María Luján².

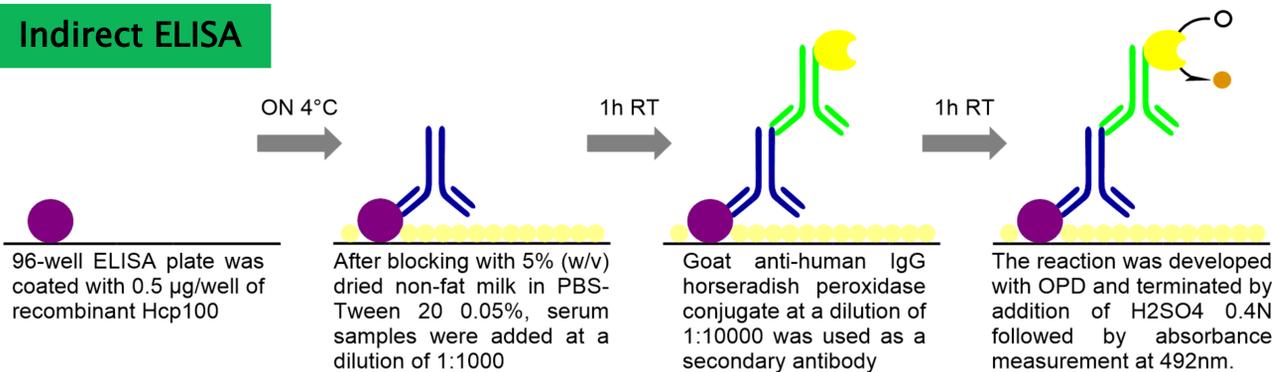
1. Universidad de Buenos Aires, CONICET, Instituto de Nanobiología (NANOBIOTEC), Argentina
2. Universidad de Buenos Aires, CONICET, Instituto de Investigaciones en Microbiología y Parasitología Médica (IMPAM), Argentina.
*ma.toscanini@conicet.gov.ar

OBJECTIVES

Histoplasmosis is a systemic and endemic mycosis caused by *Histoplasma capsulatum*. The significance of histoplasmosis results from its worldwide distribution, its ability to mimic other serious disease entities and its propensity to cause serious disseminated infection in immunocompetent patients. Definitive diagnosis involves direct visualization of the fungus in tissue and/or by culture, which may take up to 4 weeks and lacks sensitivity. The detection of antibodies offers a rapid alternative to microbiological means of diagnosis, and their detection by immunodiffusion (ID) and complement fixation (CF) is often used. Histoplasmin, the standard serodiagnostic reagent used is not ideal because of the presence of cross-reactive carbohydrate components which lead to a detrimental effect on the specificity and sensitivity of the CF and ID tests, respectively, particularly in the early acute stage of the disease. Moreover, current methods for its production are time-consuming and problematic. An alternative approach to immunodiagnosis is to detect fungal antigens in urine or serum, which is particularly useful in cases of disseminated disease. In this study, the application of a novel antigen, the 100kDa protein of *H. capsulatum*, Hcp100, for the development of an ELISA for the early diagnosis of histoplasmosis

METHODS

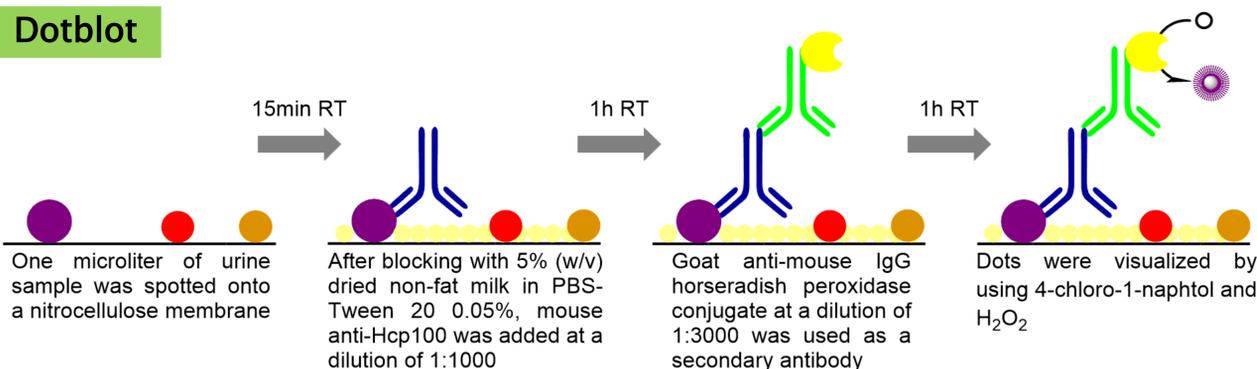
Indirect ELISA



Serums samples

- 5 Chronic histoplasmosis
- 10 Disseminated histoplasmosis + AIDS
- 1 Paracoccidioidomycosis
- 1 Coccidioidomycosis
- 5 Cryptococcosis
- 1 Tuberculosis
- 1 PJ pneumonia
- 2 Sporotrichosis
- 100 Healthy donors

Dotblot



Urine samples

- 1 Chronic histoplasmosis
- 5 Disseminated histoplasmosis + AIDS
- 3 Healthy donors

The diagnosis of histoplasmosis was confirmed either by culture or histopathology. Samples were generously given by Hospital General de Agudos Dr. Juan A. Fernández, Hospital Nacional Prof. Alejandro Posadas and Centro de Micología, UBA.

RESULTS

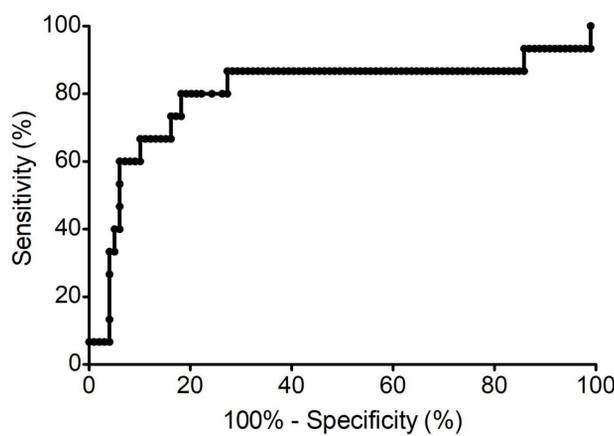
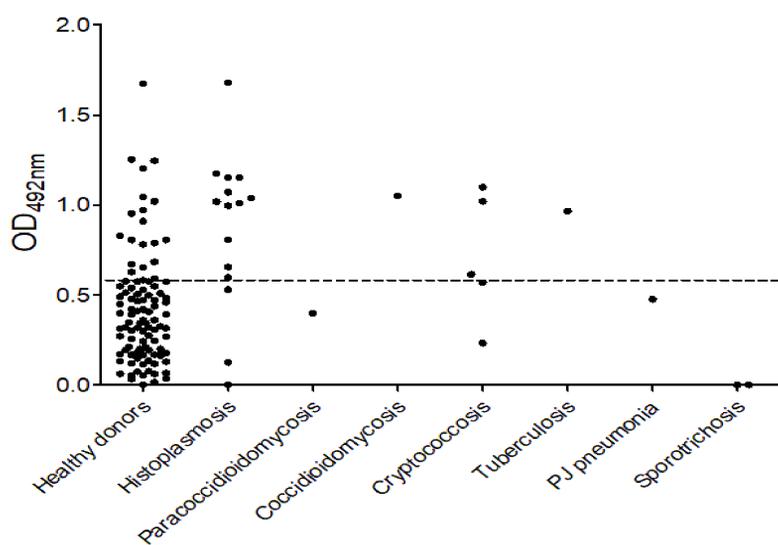


FIGURE 1. Indirect ELISA. ROC analysis determined the optimal cutoff for Hcp100 antibody detection at which point the sensitivity was 80% and the specificity was 76%. Noteworthy, 8 out of 10 AIDS patients tested positive by ELISA whilst none were positive by ID.

	Immunodiffusion	Indirect ELISA	Dotblot
Chronic histoplasmosis	5/5	5/5	1/1
Disseminated histoplasmosis + AIDS	0/10	8/10	5/5

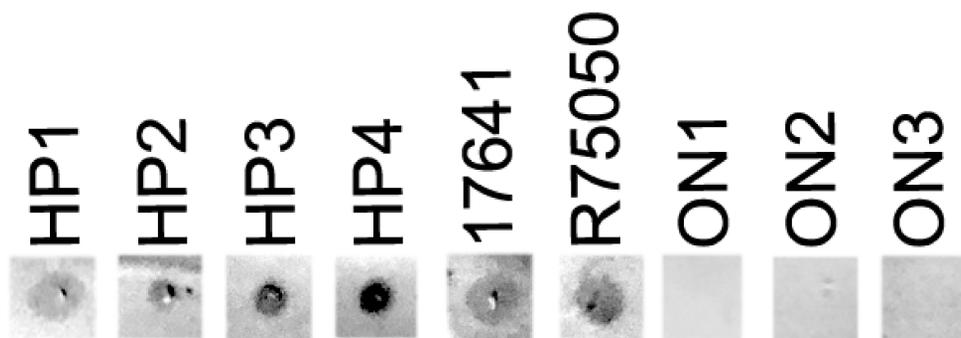


FIGURE 2. Dotblot. Urine samples from patients with chronic histoplasmosis (17641, R75050) and with disseminated histoplasmosis and AIDS (HP1, HP2, HP3, HP4) tested positive for Hcp100 while urine samples from healthy donors tested negative.

CONCLUSIONS

Detection of antibodies to Hcp100 has the potential to aid in the diagnosis of histoplasmosis, identifying cases that are falsely negative by ID. The ELISA developed herein can easily be adapted for use in-house in regional centers. Selected peptide epitopes will be useful in the development of more sensitive and specific diagnostic tests.

The new recombinant antigen Hcp100 proved to have great potential to be used in histoplasmosis diagnosis. A larger number