Evaluation of OIdx *Histoplasma* Urinary Antigen EIA

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Short Report

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Abstract

A sandwich enzyme immunoassay (EIA) for the detection of *Histoplasma* antigens (Ag) in urine, developed by Optimum Imaging Diagnostics (OIDx) was evaluated. A verification using a standardized reference panel of urine samples found sensitivity of 92%, specificity of 32% and accuracy of 51%. In this study, the OIDx *Histoplasma* urinary Ag EIA displayed high sensitivity, however, in non-histoplasmosis cases this EIA displayed false-positive results in 68% of specimens tested.

Report

In 2019 the World Health Organization (WHO) included *Histoplasma* antigen (Ag) testing in the second model list of essential *in vitro* diagnostics. In 2020, the first WHO guidelines for the diagnosis and management of disseminated histoplasmosis among people living with HIV recommended Ag testing to diagnose histoplasmosis (1, 2). Within the last decade, commercial assays for the detection of *Histoplasma* Ag have become available, providing the potential for transfer of this technology to resource-limited countries where histoplasmosis is endemic and the capacity to diagnose histoplasmosis is limited (3–10).

Optimum Imaging Diagnostics (OIDx; Scarborough, Maine, USA) is a diagnostic company founded in 2015 that has developed a sandwich enzyme immunoassay (EIA) using a rabbit monoclonal antibody for the detection of *Histoplasma* galactomannan Ag in urine. This product is commercially available under Conformité Européenne (CE). The goal of this letter is to briefly report results obtained in our laboratory using this assay.

A standardized reference panel of urine samples were collected as part of a prospective study and stored at -80°C before use in this evaluation (11). This panel has been used previously for the validation of other *Histoplasma* antigen detection assays (3, 10, 11). A total of 78 urine samples from Colombian people living with HIV were used in this study. We tested 25 urine samples from histoplasmosis cases and 43 urine samples from non-histoplasmosis cases. Included in the non-histoplasmosis group were patients diagnosed with paracoccidioidomycosis (n = 3), cryptococcosis (n = 10), candidiasis (n = 1), aspergillosis (n = 1), pulmonary *Pneumocystis* disease (n = 3), tuberculosis (n = 23) and toxoplasmosis (n = 2). In addition, 10 urine samples from people without HIV living in an area endemic for histoplasmosis were analyzed.

The OIDx *Histoplasma* Urinary Antigen EIA was performed following the manufacturer's instructions, which included the incorporation of positive and negative controls. Samples were tested in triplicate. Results were evaluated by comparing the optical density (OD) value obtained from each patient's urine to a cutoff value derived from the product of the negative control (NC) multiplied by 1.33. Specimens with an OD below the NC OD were considered negative. Samples with OD greater than NC OD and lower than 1.33 times the NC OD were considered low positive, and finally, samples with OD greater than 1.33 times the NC OD were considered positive.

Results from the OIdx *Histoplasma* Urinary Antigen EIA identified as positive 23 of the 25 proven histoplasmosis cases (92% sensitivity; 95% confidence interval [95%CI] 74–99). Of the 53 non-histoplasmosis cases, the EIA correctly classified 17 urine specimens as negative (32% specificity; 95%CI 20–46). Overall, the OIdx EIA was able to correctly classify 40 of the 78 urine samples tested (accuracy of 51%; 95%CI 40–63), and we observed a positive predictive value of 39% (95%CI 34–44) and a negative predictive value of 89% (95%CI 68–97). To determine if adjusting the cutoff value of the assay could improve the results, a receiver operating characteristic (ROC) analysis was performed (ROC area 0.79; 95%CI 0.67–0.90). A cutoff value of 0.306 was chosen based on the best accuracy (78%) derived by the ROC analysis. Using this cutoff, the ROC analysis determined the sensitivity and specificity to be 60% and 87%, respectively.

These standardized samples, which include a larger number of control patients with other infectious diseases, have provided accurate results when evaluating other commercially available assays for the diagnosis of histoplasmosis (3, 10). During the time of this study, these specimens were concomitantly used in an evaluation of another commercial assay and yielded high sensitivity and specificity (data not shown), indicating that the specimens were still viable.

In this study, the OIdx *Histoplasma* Urinary Antigen EIA displayed excellent sensitivity for the diagnosis of histoplasmosis. However, of the non-histoplasmosis cases, 68% displayed false-positive results with the currently used cutoff, and thus the EIA assay was able to correctly classify only half of the samples tested. Based on this experience, care must be taken when using the OIdx *Histoplasma* Urinary Antigen EIA, particularly in low incidence regions where false positive results are more likely generated.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. This product evaluation was done under an agreement between OIdx and the CDC's Mycotic Diseases Branch (material transfer agreement # D-760-18).

References

1. PAHO/WHO. Guidelines for Diagnosing and Managing Disseminated Histoplasmosis among People Living with HIV 2020. Available from: https://iris.paho.org/bitstream/handle/10665.2/52304/9789275122495_eng.pdf?sequence=1&isAllowed=y.
2. World Health Organization (WHO). Second WHO Model List of Essential In Vitro Diagnostics. 2019. Available from: https://www.who.int/medical_devices/publications/Standalone_document_v8.pdf?ua=1.
3. Caceres DH, Samayoa BE, Medina NG, Tobon AM, Guzman BJ, Mercado D, et al. Multicenter validation of commercial antigenuria reagents to diagnose progressive disseminated histoplasmosis

- in people living with HIV/AIDS in two Latin American countries. *J Clin Microbiol*. 2018;56(6).
4. Martínez-Gamboa A, Niembro-Ortega MD, Torres-González P, Santiago-Cruz J, Velázquez-Zavala NG, Rangel-Cordero A, et al. Diagnostic accuracy of antigen detection in urine and molecular assays testing in different clinical samples for the diagnosis of progressive disseminated histoplasmosis in patients living with HIV/AIDS: A prospective multicenter study in Mexico. *PLoS Negl Trop Dis*. 2021;15(3):e0009215.
 5. Torres-Gonzalez P, Niembro-Ortega MD, Martinez-Gamboa A, Ahumada-Topete VH, Andrade-Villanueva J, Araujo-Melendez J, et al. Diagnostic accuracy cohort study and clinical value of the Histoplasma urine antigen (ALPHA Histoplasma EIA) for disseminated histoplasmosis among HIV infected patients: A multicenter study. *PLoS Negl Trop Dis*. 2018;12(11):e0006872.
 6. Samayoa B, Aguirre L, Bonilla O, Medina N, Lau-Bonilla D, Mercado D, et al. The Diagnostic Laboratory Hub: A New Health Care System Reveals the Incidence and Mortality of Tuberculosis, Histoplasmosis, and Cryptococcosis of PWH in Guatemala. *Open Forum Infect Dis*. 2020;7(1):ofz534.
 7. Medina N, Alastruey-Izquierdo A, Mercado D, Bonilla O, Pérez JC, Aguirre L, et al. Comparative performance of the laboratory assays used by a Diagnostic Laboratory Hub for opportunistic infections in people living with HIV. *Aids*. 2020;34(11):1625–32.
 8. Falci DR, Monteiro AA, Braz Caurio CF, Magalhaes TCO, Xavier MO, Basso RP, et al. Histoplasmosis, An Underdiagnosed Disease Affecting People Living With HIV/AIDS in Brazil: Results of a Multicenter Prospective Cohort Study Using Both Classical Mycology Tests and Histoplasma Urine Antigen Detection. *Open Forum Infect Dis*. 2019;6(4):ofz073.
 9. Bansal N, Sethuraman N, Gopalakrishnan R, Ramasubramanian V, Kumar DS, Nambi PS, et al. Can urinary histoplasma antigen test improve the diagnosis of histoplasmosis in a tuberculosis endemic region? *Mycoses*. 2019.
 10. Caceres DH, Gomez BL, Tobon AM, Chiller TM, Lindsley MD. Evaluation of a Histoplasma antigen lateral flow assay for the rapid diagnosis of progressive disseminated histoplasmosis in Colombian patients with AIDS. *Mycoses*. 2020;63(2):139–44.
 11. Caceres DH, Scheel CM, Tobon AM, Ahlquist Cleveland A, Restrepo A, Brandt ME, et al. Validation of an enzyme-linked immunosorbent assay that detects Histoplasma capsulatum antigenuria in Colombian patients with AIDS for diagnosis and follow-up during therapy. *Clin Vaccine Immunol*. 2014;21(9):1364–8.